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The Distribution of Lignin in Sprucewood as Determined by Ultraviolet Microscopy

By B. J. FERGUS, A. R. PROCTER, J. A. N. SCOTT and D. A. I. GORING,
Montreal, Quebec

Abstract

The distribution of lignin in black spruce has been determined quantitatively by the study of 0.5 μm transverse sections in a UV microscope. The average lignin concentration in the compound middle lamella was about twice that in the secondary wall. The lignin concentration of the middle lamella at the cell corners of adjacent tracheids was nearly four times that in the secondary wall but the volume of the secondary wall was much greater than the volume of the middle lamella. Thus, for earlywood, 72% of the total lignin was in the secondary wall leaving only 28% in the compound middle lamella and cell corner middle lamella regions. The corresponding values for latewood were 82% and 18% respectively. Use of oblique longitudinal sections of 0.1 μm thick permitted the resolution of the compound middle lamella. The lignin concentration in the true middle lamella was found to be equal to that in the cell corner middle lamella and the primary wall lignin content to be about twice that in the secondary wall.

Zusammenfassung

Die Verteilung des Lignins in Fichtenholz wurde quantitativ durch Untersuchung von 0,5 μm dicken Querschnitten unter dem UV-Mikroskop bestimmt. Die mittlere Ligninkonzentration war in der Mittelschicht etwa doppelt so hoch wie in der Sekundärwand. Die Ligninkonzentration der Mittelschicht war in den an die Tracheiden anstoßenden Zellecken annähernd viermal höher als in der Sekundärwand, wogegen das Volumen der Sekundärwand wesentlich größer war als das der Mittelschicht. Dagegen befand sich beim Frühholz 72% des gesamten Lignins in der Sekundärwand und nur 28% fanden sich in der Mittelschicht selbst und in ihren Zelleckbereichen. Die entsprechenden Werte für Spätholz betragen 82% bzw. 18%. Die Anwendung von schrägen Längsschnitten von 0,1 μm Dicke erlaubte die Auflösung der Mittelschicht. Die Ligninkonzentration in der Mittellamelle war gleich groß wie in der in den Zellzwickeln befindlichen Mittellamelle und der Ligningehalt der Primärwand war etwa doppelt so groß wie derjenige in der Sekundärwand.

Introduction

Although the anatomy and structural organization of wood have been well documented [JANE 1956; WARDROP 1962, 1963, 1964], there remains some uncertainty on the quantitative distribution of lignin in the cell wall. One of the first to study this distribution was RITTER [1925], who concluded that 75% of the lignin in wood was located in the middle lamella, the secondary wall containing the residual 25%. By use of a micromanipulator, BAILEY [1936] isolated the middle lamella of Douglas fir, and by chemical analysis found a lignin content of approximately 71%. An essentially similar result was obtained by LANGE [1954, 1958], who used UV-microscopy and calculated the weight concentration of lignin in the secondary wall and compound middle lamella to be 16% and 73% respectively. LANGE proposed a continuous decrease in lignin concentration from the middle lamella to the lumen, but FREY [1959] also using UV-microscopy, claimed that the lignin distribution across the secondary wall was fairly uniform.

Similar uniformity of distribution in the secondary wall was reported by RUCH and HENGARTNER [1960] who had studied jute fibres. They questioned Lange's techniques on the basis that conical light had been used and that no correction was applied for the diffraction at the primary wall/secondary wall interface. However, it now seems likely that the main cause for the poor definition in LANGE's photographs was the use of a section thickness of several microns [SCOTT et al. 1968] which was the minimum generally available twenty years ago.

The question of lignin distribution has been critically appraised by BERLYN and MARK [1965], who pointed out that the Bailey-Lange result was diametrically opposed to that of RITTER. Noting that the volume fraction of the compound middle lamella in coniferous wood is only 10 ... 12% of the wood tissue volume, BERLYN and MARK showed that even if the middle lamella region was composed of 100% lignin, it could not contain more than 40% of the total lignin in wood.

The present investigation deals with the measurement of the distribution of lignin in the black spruce cell wall. The techniques used were a combination of ultraviolet microscopy and densitometric analysis of UV photomicrograph negatives. A recent appraisal of these techniques [SCOTT et al. 1968] has indicated that, provided certain experimental conditions are met, the lignin concentration in various parts of the wood tissue can be determined with reasonable accuracy.

Experimental

Small chips of dry black spruce (*Picea mariana* Mill.) wood containing the whole of the 56th or 64th annual ring of a 68-year old log were embedded and sectioned by the method described earlier [SCOTT et al. 1968]. Either methacrylate or Epon was used as the embedding medium. Transverse sections were usually 0.5 μm thick, but a cut of 0.1 μm was used for oblique longitudinal sections.

The ultrathin sections were examined under a Leitz UV-microscope. The condenser had a numerical aperture of 0.60, and the objective had a magnification of 300 : 1 and an aperture of 0.85. Photographs were taken on Kodak Spectrum Analysis No. 1-35 mm film. The wavelengths used were 280 nm or 240 nm. The former wavelength was used for quantitative measurements, the latter mainly to obtain photographs for display purposes.

The photomicrograph negatives were analysed densitometrically by means of a Joyce, Loebl Mark III CS recording microdensitometer to give the relative amounts of lignin in the various morphological regions of the wood [SCOTT et al. 1968].

Results and Discussion

An example of a UV photomicrograph of a black spruce tracheid wall, with the corresponding densitometer trace, is shown in Fig. 1. The compound middle lamella stands out as a narrow highly lignified layer with a considerably smaller but uniform lignin concentration across the secondary wall. The compound middle lamella observed here probably consists of the two primary walls and the true middle lamella.

Quantitative analysis of the negative revealed differences in the UV absorbance of the various morphological regions. As found by WARDROP [1963], the ab-

sorbance in the middle lamella at the cell corner was considerably greater than the measured absorbance of the compound middle lamella between cell corners. Similarly the radial middle lamella, on the average, exhibited a stronger absorbance than the tangential middle lamella. An unexpected result was that the radial secondary wall showed a stronger absorbance than the tangential secondary wall.

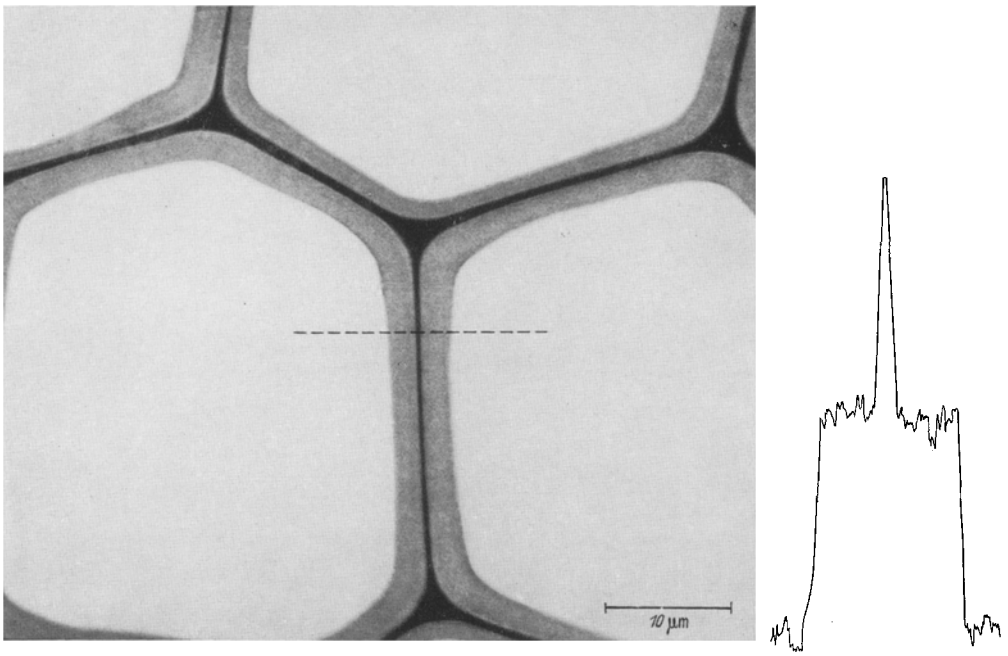

Fig. 1. Cross section of Epon embedded tracheids of black spruce earlywood photographed in ultraviolet light of wavelength 240 nm. The densitometer tracing was taken across the tracheid wall on the dotted line.

In order to facilitate the presentation and discussion of the data, it was convenient to represent the principal morphological regions by certain symbols. These are listed in Table 1.

The easiest parameter to measure is C_{ML}/C_S which is given by the ratio of the height of the compound middle lamella peak to the secondary wall plateau in the densitometer trace and is independent of section thickness and film developing conditions. However, it is possible to measure C_{ML} and C_S separately, if the precautions described in an earlier report [SCOTT et al. 1968], are taken. The densitometer traces also yield cell wall dimensions, the two most important being WT and MT (see Table 1). These dimensions are measured at the half-height of the corresponding absorption step in the densitometer trace.

Variation of UV Absorbance Across the Annual Ring

The UV absorbance of the tangential secondary wall and compound middle lamella was measured from the densitometer traces for each tangential cell wall across two different samples of the 64th annual ring embedded in methacrylate.

The results are shown in Fig. 2. The annual ring contained 55 cells. Each point on Fig. 2, and subsequent Figs. 3 and 4, represents the average of determinations on at least three adjacent cell walls at the same cell number position in the annual ring. No absorption measurements were made on the radial walls as these walls were wrinkled and heavily pitted. In the case of the tangential walls, the cell dimensions WT_t and MT_t were obtained directly from the densitometer traces. The radial and tangential tracheid diameters and WT_r were measured on the photomicrograph negatives.

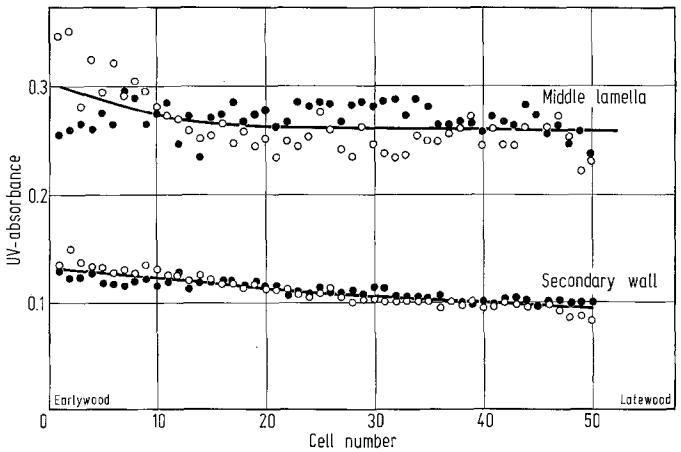

Fig. 2. Variation of the UV-absorbance of the tangential compound middle lamella and secondary wall across the annual increment. Cell no. 1 is the first earlywood cell, cell no. 55 the last latewood cell. The filled and empty circles refer to two different samples of the same annual ring.

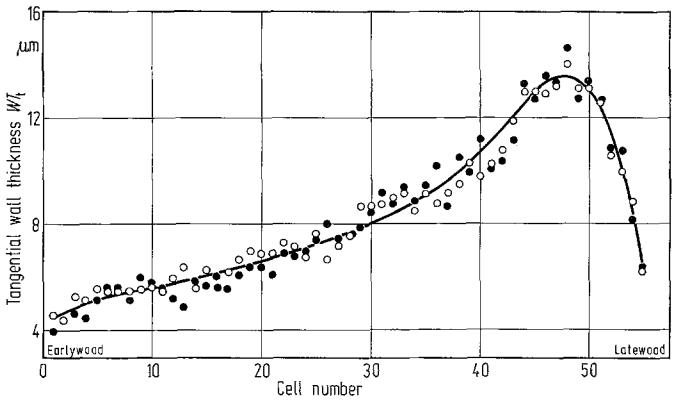

Fig. 3. Variation of the tangential double cell wall thickness across the annual ring. The filled and empty circles refer to two different samples of the same annual ring.

From Fig. 2 it can be seen that there was a steady decrease in the UV-absorbance of the tangential secondary wall in the transition earlywood to latewood. The UV-absorbance of the secondary wall of the 45th cell was only 78% of that of the 10th cell. Apart from some irregularity in the first 10 earlywood cells the UV-absorbance of the tangential compound middle lamella remained fairly constant over the remainder of the annual ring.

The variations of the various cell wall dimensions across the annual ring are shown in Figs. 3 ... 5. In Fig. 3, WT_t increased steadily from a value of about 5 μm for the first earlywood cells, reached a maximum value of about 14 μm in the cell interval 45 ... 50 and then decreased rapidly over the last five latewood cells. The variation of WT_r (Fig. 4) was similar to that of WT_t but the size of the change in dimensions, 4.5 μm ... 17 μm, was slightly greater. Furthermore, there was no marked decrease in WT_r over the last five latewood cells. Interestingly, the ratio WT_t/WT_r was fairly constant at an average value of 1.16, with arithmetic

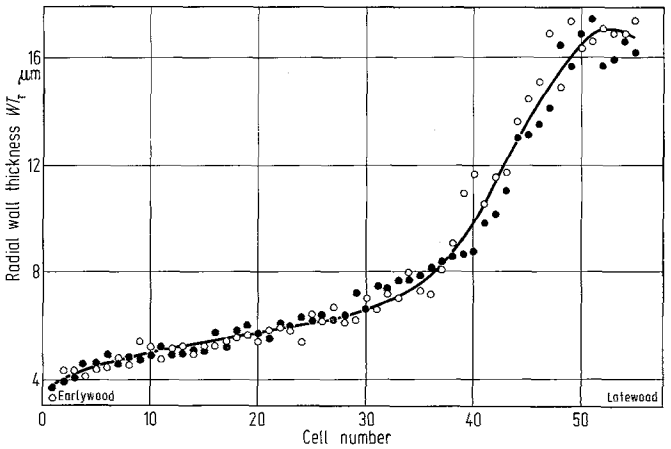

Fig. 4. Variation of the radial double cell wall thickness across the annual ring. The filled and empty circles refer to two different samples of the same annual ring.

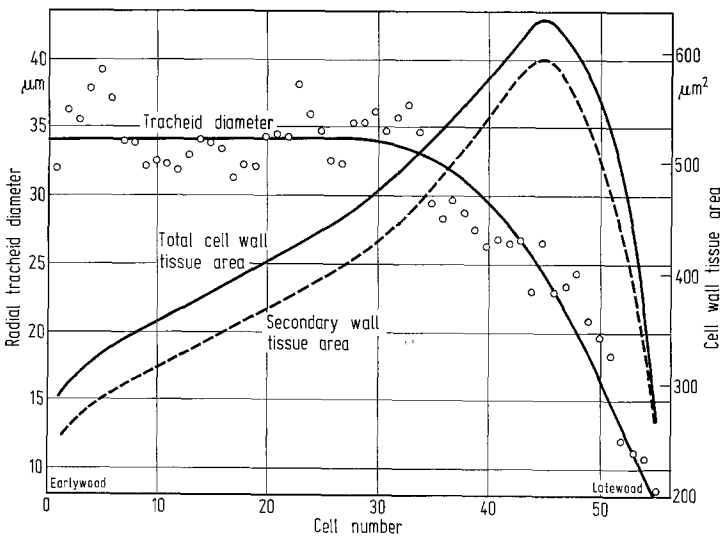

Fig. 5. Variation of radial tracheid diameter and the tissue area of the cell wall across the annual ring.

deviation of ± 0.05, for the first 40 cells but then decreased rapidly to 0.38 for the last latewood cell.

The radial and tangential tracheid diameters were measured for one sample across the annual ring. The tangential tracheid diameters of adjacent cells across

the annual ring were approximately constant at a value of about 36 μm. The radial tracheid diameter was fairly constant for the first 25 cells at a value of about 34 μm, thereafter it decreased rapidly to a value of 7.7 μm for the last latewood cell (Fig. 5). Each point on Fig. 5 represents the average of determinations on at least five adjacent tracheids at the same cell number position in the annual ring.

The distributions shown in Figs. 3 ... 5 are similar to those obtained by MURRAY and THOMAS [1961] for four softwood species.

By a consideration of the various cell wall dimensions it was possible to calculate the cell wall tissue area for a rectangular tracheid (see later). Fig. 5 shows the variation of secondary wall tissue area and total cell wall tissue area (i.e. secondary wall plus the compound middle lamella) with the position of the tracheid within the annual ring.

Table 1. *Relative Lignin Concentrations and Cell Wall Dimensions*

Wood	Embedding medium	Annual Ring	$\frac{C_{ML}}{C_S}$ (t)	$\frac{C_{ML}}{C_S}$ (r)	$\frac{C_{ML}}{C_S}$ (cc)	WT_t μm	WT_r μm	MT_t μm	MT_r μm
Earlywood	Methacrylate	56 th	2.11	2.47	3.82	5.3	4.7	0.40	0.55
Earlywood	Methacrylate	64 th	2.12	2.47	3.88	5.6	4.5	0.37	0.45
Earlywood	Epon	64 th	2.03	2.24	3.60	5.5	4.5	0.43	0.47
Mean for Earlywood (approx. cells no. 1 ... 20)			2.08	2.33	3.77	5.5	4.6	0.40	0.49
Mean for Latewood (approx. cells no. 41 ... 50)		64 th	2.7	—	4.5	13	14	—	—

C_{ML} Volume concentration of lignin in the compound middle lamella (g/cm³)
C_S Volume concentration of lignin in the secondary wall (g/cm³)
WT Double cell wall thickness (μm)
MT Compound middle lamella thickness (μm)
r Refers to the radial direction in the wood
t Refers to the tangential direction in the wood
cc Refers to the cell corner region of the middle lamella.

Distribution of Lignin in Spruce Tracheids

The values of C_{ML}/C_S for the different morphological regions of the earlywood cell walls of the 56th and 64th annual rings are given in Table 1. The earlywood cells correspond approximately to cells no. 1 ... 20 in Fig. 2. The agreement between the two annual rings is good. Use of Epon as an embedding medium gave equivalent results. For the methacrylate embedded 64th annual ring, the standard deviation in (C_{ML}/C_S) (r) and (C_{ML}/C_S) (t) was $\pm$ 0.20 and $\pm$ 0.16 respectively.

The ratio was highest at the cell corner region and decreased in the order

$$\frac{C_{ML}}{C_S}\ (cc) > \frac{C_{ML}}{C_S}\ (r) > \frac{C_{ML}}{C_S}\ (t)\,.$$

The values of (C_{ML}/C_S) (r) and (C_{ML}/C_S) (t) are similar to those reported for jute, where $C_{ML}/C_S = 1.9$ from fluorescence microscopy and 2.2 from UV microscopy. RUCH and HENGARTNER [1960] ascribed this difference in ratios to an increase in C_{ML} caused by the diffraction present at the primary wall/secondary wall interface.

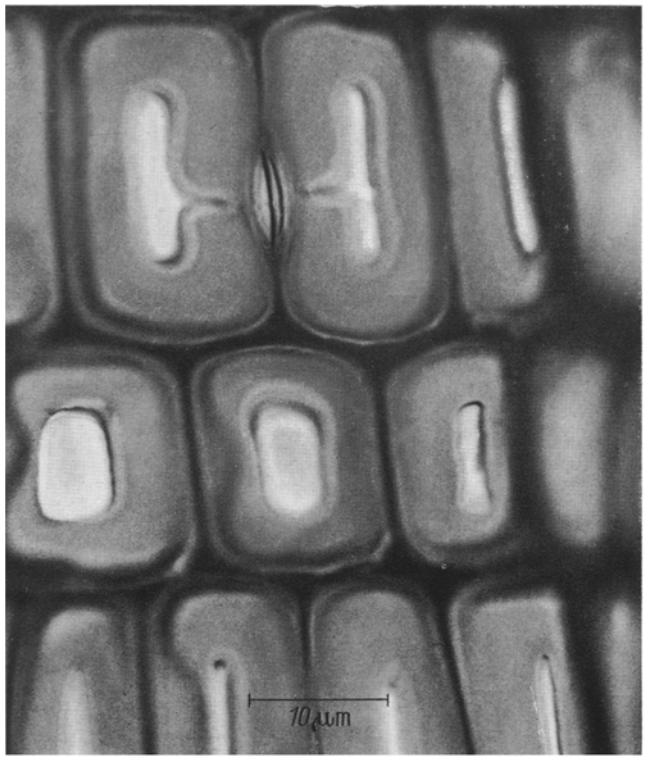

Fig. 6. Cross section of black spruce latewood, embedded in methacrylate and photographed at 280 µm.

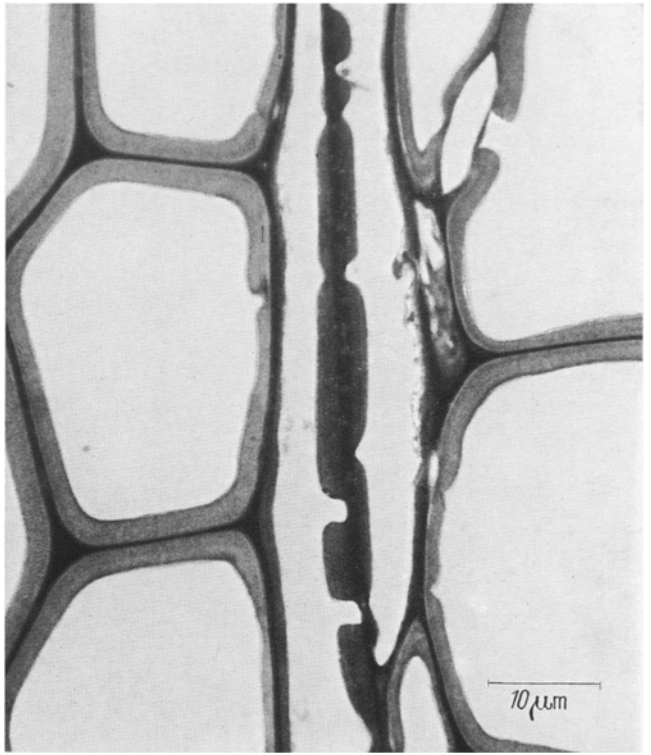

Fig. 7. Cross section of an Epon embedded ray parenchyma cell. Note the high UV-absorption of the ray cell wall when compared to the tracheid secondary wall. $\lambda = 240$ nm.

A UV photomicrograph of spruce latewood is shown in Fig. 6. Comparison with Fig. 1 shows the poorer quality of the latewood print. Considerable effort towards improving the photomicrographs of latewood met little success. For this reason the latewood cells were not studied as extensively as the earlywood cells and hence the latewood data in Table 1 are not as numerous as the earlywood data. However, the measurements show that C_{ML}/C_S increases for latewood both in the tangential middle lamella and the cell corner region. This result was expected in view of the trends in absorbance shown in Fig. 2.

The appropriate cell wall dimensions are also listed in Table 1. The standard deviations in the values of MT_t and MT_r for earlywood of the methacrylate embedded 64th annual ring were $\pm$ 0.03 μm and $\pm$ 0.04 μm respectively (23 samples each). For the same samples the standard deviations in WT_t and WT_r were $\pm$ 0.6 μm and $\pm$ 0.7 μm respectively.

The tangential double cell wall in earlywood was thicker than the radial wall. This trend may have been due to increased swelling in the tangential direction [BOUTELJE 1962] which could also produce the lower volume concentration of lignin in the tangential wall [SCOTT et al. 1968]. In addition, significant differences were found in the dimensions (Table 1) and UV absorbance [SCOTT et al. 1968, Fig. 4] of the tangential and radial compound middle lamellae. Such morphological detail is of considerable interest and may well prove to be a general characteristic of conifer woods. However, the present work is more concerned with establishing quantitatively the broad patterns of lignin distribution. Therefore the dimensions and lignin concentration ratios of the radial and tangential walls will be averaged in the subsequent analysis.

From the values of C_{ML}/C_S shown in Table 1, it was possible to calculate the quantitative distribution of lignin in the various morphological regions of the cell wall. If uniform density and swelling are assumed throughout the tracheid cell wall, we may write

$$a\,(\text{Volume fraction of secondary}) + a\,\frac{C_{ML}}{C_S}\,(\text{r})\,(\text{t})\,(\text{Volume fraction of the compound middle lamella}) + a\,\frac{C_{ML}}{C_S}\,(\text{cc})\,(\text{Volume fraction of cell corner middle lamella}) = \text{W} \qquad (1)$$

in which a and W are the weight concentrations of lignin in g/g of dry secondary wall and whole wood respectively.

In order to obtain a from Eq. (1) we must first know the relative tissue volumes of the morphological subdivisions. By assuming a rectangular tracheid and using the appropriate cell wall dimensions it was possible to calculate geometrically the tissue area of the secondary wall and compound middle lamella for an average spruce tracheid. For earlywood cells at approximately the cell no. 10 position the tissue areas for the secondary wall and compound middle lamella were 312 μm^2 and 31 μm^2 respectively. The tissue area of the cell corner middle lamella was obtained by measuring the corresponding areas off $8'' \times 10''$ prints (final magnification 1995×) of the photomicrograph negatives. The boundary of the cell corner was taken at the point at which the thin compound middle lamella expanded rapidly. For obvious reasons, this measurement was rather approximate. For 96 cell corner middle lamellae the average tissue area per tracheid in earlywood

was found to be 14 μm^2. The change in cell corner tissue area in the transition earlywood to latewood was negligible. The tissue area calculation was extended to each cell layer in the annual ring and the results are shown in Fig. 5.

As the sections were of constant thickness, the tissue areas were directly proportional to tissue volumes. Thus the volume fraction of each morphological region could be computed and is given in Table 2 together with the relative UV-absorbances compared with unity for the secondary wall. The volume fraction of the secondary wall is by far the largest and rises from 87% in earlywood to 94% in latewood. The compound middle lamella and cell corner regions occupy only 13% of the earlywood and 6% of the latewood tissue volumes.

A further requirement in the application of Eq. (1) is a value of W. The Klason lignin content of the black spruce sample was 27%. However, some corrections are necessary before this figure can be substituted in Eq. (1).

The ray cells were omitted in the tissue volume measurements and as these cells have been shown to possess significantly higher lignin contents than the gross wood [BAILEY 1956; HARADA, WARDROP 1956] it is necessary to correct the observed lignin content of the black spruce whole wood for that of the ray cells. Fig. 7 shows a typical UV-photomicrograph of a ray parenchyma cell. Densitometric analysis showed that the UV-absorbance of similar ray cell walls was some 50% higher than that of the tracheid secondary wall. Also, the absorbance of the ray cell walls did not change from early to latewood. Hence the ray cells can be assumed to have a lignin content that is about 50% higher than the tracheid lignin content of 27%. Thus, to a good approximation, the ray cell lignin content is (27.0 + 13.5)%, i.e. 40%. Although no data are available for black spruce, ray cells constitute from 4.0 ... 4.7% of the total wood volume of Norwegian spruce [BACK 1960]. From these figures the lignin content of the tracheids, corrected for the higher lignin content of the ray cells, can be estimated to be 26.4%.

The lignin content of 26.4% is an average of the earlywood and latewood lignin contents. WU and WILSON [1967] reported that for Sitka spruce, earlywood contained 1.5 ... 2% more lignin than latewood. Assuming a 1.8% difference for black spruce and that the average for earlywood and latewood equals the lignin content of the whole wood, we find

$$\begin{aligned} W \text{ (earlywood)} &= 0.273 \text{ g/g}, \\ W \text{ (latewood)} &= 0.255 \text{ g/g}. \end{aligned} \tag{2}$$

Substitution of these values together with the values of volume fraction and C_{ML}/C_S into Eq. (1) yields

$$\begin{aligned} a \text{ (earlywood)} &= 0.225 \text{ g/g}, \\ a \text{ (latewood)} &= 0.222 \text{ g/g}. \end{aligned} \tag{3}$$

From the C_{ML}/C_S ratios the lignin concentrations in the compound middle lamella and cell corner region can be calculated and are shown in Table 2. The proportion of lignin in each morphological region is readily derived from the volume fraction and the C_{ML}/C_S ratios.

The results in Table 2 show that the secondary wall, because of its dominant tissue volume, accounts for over 72% of the total lignin in the earlywood tracheids and 82% in the latewood tracheids. On average, less than 23% of the lignin is contained in the regions exterior to the secondary wall. This result supports the

estimate by BERLYN and MARK [1965] that less than 40% of the lignin in wood is located in the compound middle lamella region.

For earlywood, the weight concentration of lignin in the secondary wall is 22.5% which agrees rather well with a recent estimate by STAMM and SANDERS [1966] based on the density of the wood substance of the loblolly pine. These authors concluded that between 22 and 24% of the earlywood secondary wall substance was lignin.

A somewhat unexpected result was that the lignin concentration was virtually the same for the earlywood and latewood secondary walls. This is in apparent contradiction with the trend shown in Fig. 2 for the decrease in UV-absorbance of the tangential secondary wall across the annual ring. As the UV-absorbance is volume dependent, the trend in Fig. 2 could be a reflection of the increased swelling of the cell wall from earlywood to latewood. In fact, this has been observed recently by BOUTELJE [1962], who showed that latewood cells swell some 11% more than earlywood cells. It is not known whether this increased swelling arises solely from the secondary wall or extends to the compound middle lamella as well. It is important to note that the validity of Eq. (1) is not affected provided the swelling is the same in all regions of the tracheid. Failure of this condition could account for the apparent increased weight concentration of lignin in the latewood middle lamella, whereas the results in Fig. 2 indicate that the volume concentration of lignin in this region remains approximately constant.

The compound middle lamella is composed of two primary walls and the true middle lamella. Hence, the lignin content of 50% in Table 2 is probably an average value for the three layers. The lignin concentration in the cell corner middle lamella was high. Evidently this region was almost pure lignin. As the cell corner middle lamella is an extension of the true middle lamella it might be expected that the latter would have the same high lignin content. The resolution of this question is discussed in a later section of the paper.

Table 2. *The Distribution of Lignin in the Spruce Tracheid*

Wood	Morphological differentiation	Relative absorbance	Tissue volume fraction %	Lignin (% of total)	Lignin concentration g/g
Earlywood	S	1	87.4	72.1	0.225
	ML(r), (t)	2.21	8.7	15.8	0.497
	ML(cc)	3.77	3.9	12.1	0.848
Latewood	S	1	93.7	81.7	0.222
	ML(r), (t)	2.7	4.1	9.7	0.60
	ML(cc)	4.5	2.2	8.6	1.00

S Secondary wall, ML Middle lammella, r radial, t tangential, cc cell corner

The Calculation of Lignin Concentrations from the Extinction Coefficient

It was of considerable interest to check the lignin content of the earlywood secondary wall, as calculated by the tissue volume method, with a direct calculation from the UV-absorbance data.

The lignin concentration in any particular morphological region can be calculated directly from the Beer-Lambert law

$$\text{UV-absorbance} = \varepsilon \times C \times d \qquad (4)$$

where ε is the extinction coefficient, C the volume concentration of lignin and d the thickness of the section.

A serious limitation on the application of the Beer-Lambert law to wood sections is the lack of any reliable value for the extinction coefficient of lignin in wood. There is a large scatter in the value of ε_{280} for various lignins isolated from sprucewood and it is difficult to know which, if any, preparation corresponds to the protolignin. Figures quoted for ε_{280} range from 12.8 cm^{-1} 1 g^{-1} for spruce lignin sulphonate [YEAN, GORING 1964] to 18.7 cm^{-1} 1 g^{-1} for spruce kraft lignin [MCNAUGHTON et. al. 1967].

Ball milled lignin, prepared by the method of Björkman, is generally regarded as having a chemical structure near to that of the lignin in the wood. However, SARKANEN and coworkers [1967] found that when milled wood lignins from the sapwood of three softwood species were reduced with sodium borohydride the extinction coefficient at 280 mμ was perceptibly lower. From their data the average value of ε_{280} for the three softwoods was decreased from 18.9 ... 15.6 cm^{-1} 1 g^{-1} by this treatment (Table 3). The lowering of ε_{280} was attributed to the

Table 3. *Ultraviolet Absorption Data of Milled Wood Lignins*[1]

Species	Untreated lignins ε_{280} (cm^{-1} 1 g^{-1})	Borohydride reduced lignins ε_{280} (cm^{-1} 1 g^{-1})
Douglas-fir	18.8	15.8
Western red cedar	18.6	15.6
Himalayan pine	19.2	15.4

[1] Sarkanen et al. 1967

borohydride reduction of carbonyl groups conjugated with the phenyl nucleus. Conjugated carbonyl absorption contributes heavily to the lignin spectrum above 300μm. This is illustrated by the fact that the average value of $\varepsilon_{280}/\varepsilon_{305}$, for the softwoods, is 1.94 for untreated lignin and 3.39 for sodium borohydride reduced lignins. As described in more detail in a subsequent report [FERGUS, GORING in print] lignin in the spruce secondary wall yields a value of $\varepsilon_{280}/\varepsilon_{305} = 3.8$. Thus it seems reasonable to assume that spruce proto-lignin has a UV spectrum similar to that of the reduced milled wood lignins. As softwood lignins are comprised almost entirely of the guaiacylpropane unit and hence will have similar values of ε_{280}, the value of $\varepsilon_{280} = 15.6$ cm^{-1} 1 g^{-1} was used for the calculation of absolute lignin concentrations from Eq. (4). Interestingly, an ε_{280} of 15.3 cm^{-1} 1 g^{-1} can be computed from the UV spectrum published by PEW [1957] for ball milled spruce wood dissolved in aqueous lithium bromide.

The UV-absorbance of the secondary wall (average of the radial and tangential walls) for earlywood tracheids at about the cell no. 10 position, was 0.154 for a section 0.5 μm thick [SCOTT et al. 1968]. This UV-absorbance must be reduced by 3% to correct for the effect of nonparallel illumination. The corrected UV-absorbance is then 0.149. By substitution of this value and $\varepsilon = 15.6$ cm^{-1} 1 g^{-1}, and $d = 0.5$ μm in Eq. (4), the volume concentration of lignin in the spruce earlywood secondary wall is 0.191 g/cm^2. To convert to the weight concentration of lignin it is necessary to multiply this volume concentration by the specific volume of the water swollen cell wall. STONE and SCALLAN [1967] have found the specific volume of the water swollen cell wall of black spruce to be 1.07 cm^2/g.

The weight concentration of lignin in the earlywood secondary wall is thus 0.204 g/g. The tissue volume calculation yielded a value of 0.225 g/g (Table 2). Considering the assumptions and approximations involved the results obtained by the two methods of calculation are in good agreement.

Resolution of the Coumpound Middle Lamella

Although the compound middle lamella width (Table 1) is considerably greater than the limit of microscope resolution, 0.16 ... 0.20 μm [SCOTT et al. 1968], the three composite layers cannot be separately resolved. In consequence the lignin distribution within the compound middle lamella cannot be determined experimentally by the study of transverse cross sections. Furthermore, the lower limit of object size upon which accurate quantitative absorption measurements may be made is 3 λ ... 4 λ [CASPERSSON 1936]. Although the cell corner middle lamella fulfills this condition, the compound middle lamella width is usually less than 2 λ at $\lambda = 0.28$ μm.

The measured UV-absorption of the compound middle lamella is considerably lower than that of the cell corner middle lamella. Such a situation suggests two major alternatives.

1. The observed difference in lignin concentration between these two regions is real, but may be experimentally overemphasized because of the limited size of the middle lamella.

2. The observed difference between the two regions is an optical artefact caused by the very small width of the middle lamella. In reality, the lignin concentration in the true middle lamella does not change significantly between the cell corner areas of adjacent tracheids.

Careful examination of the problem revealed that by cutting ultrathin longitudinal sections at a low angle to the main tracheid axis it might be possible to obtain a greatly enlarged compound middle lamella. For a cut made at 6 ... 7° to the tracheid axis the apparent thickness of the compound middle lamella could be increased from 0.4 μm to over 3 μm. The minimum section thickness considered practical both to cut and to photograph was 0.1 μm. Accordingly, 0.1 μm sections were cut from the longitudinal tangential face of spruce earlywood tracheids embedded in Epon. The face of the block was inclined at an angle of 6° from the vertical. In order to obtain greater accuracy in absorption measurements the sections were photographed at 240 nm, a wavelength at which lignin has a higher absorption than at 280 nm.

A UV-photomicrograph of an oblique tangential double cell wall is shown in Fig. 8. A narrow, highly UV-absorbing layer is evident in the middle of the double cell wall. This layer is flanked by two others, also narrow, but absorbing less UV-light.

Evidence that the tricomposite layer in Fig. 8 is in fact a resolved compound middle lamella can be obtained by consideration of the cell wall dimensions. From several walls in the oblique section, the apparent double cell wall thickness was 46.3 μm and the tricomposite layer 3.51 μm the ratio of these layer widths being approximately 5.5 : 0.41. The double cell wall thickneses were obtained directly from the photomicrograph negatives, and the tricomposite layer thickness from the width at half-height of the corresponding absorption steps in the densitometer

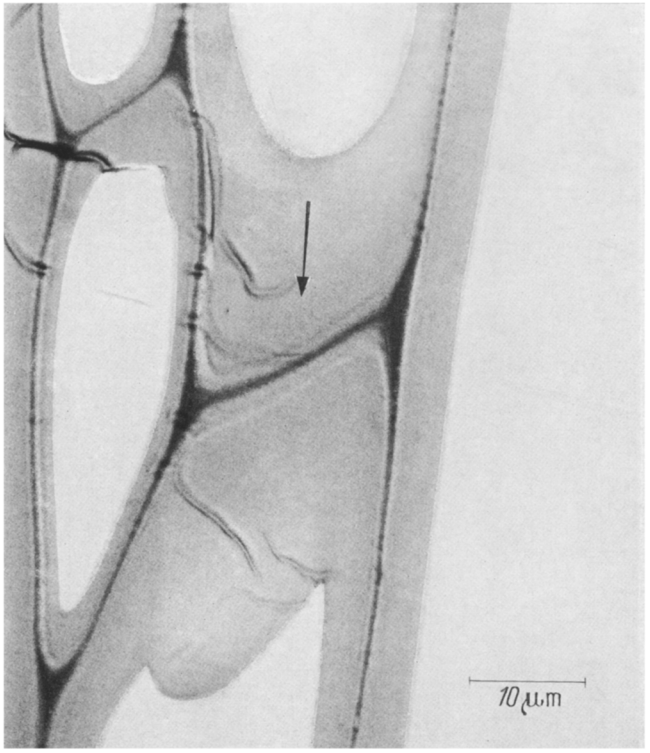

Fig. 8. Longitudinal tangential section (0.1 µm) cut at an angle of 7° from the main tracheid axis. Note the highly UV-absorbing middle layer and the two lesser absorbing layers on either side of it (see arrow) $\lambda = 240$ nm.

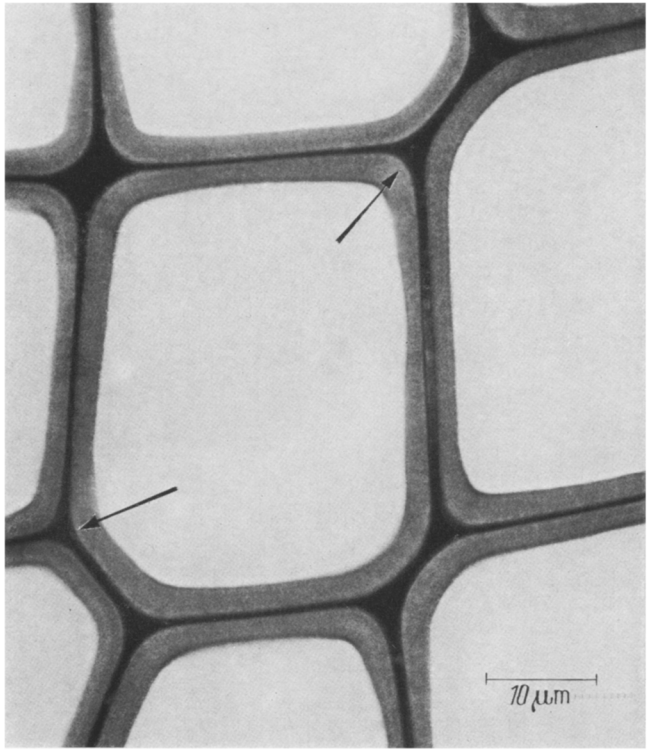

Fig. 11. Cross section of earlywood tracheids embedded in Epon. Note the medium intensity layer between the cell corner middle lamella and secondary wall (see arrows). $\lambda = 240$ nm.

traces. The ratio 5.5 : 0.41 is almost identical to the ratio $WT_t : MT_t = 5.5 : 0.40$ (Table 1) in transverse cross sections. Hence, the tricomposite layer does appear to correspond to a resolved compound middle lamella. From a comparison of the double cell wall dimensions in transverse and oblique sections it is possible to show that the sections were cut at an angle of $\sin^{-1}(5.5)/(46.3)$, i.e. 7°.

It seems reasonable to assume that the highly absorbing middle layer in Fig. 8 is, in fact, the true middle lamella flanked by the less absorbing primary walls. The average of several densitometer traces gave an apparent width of 0.73 μm for the true middle lamella which corresponded to about 0.1 μm in transverse sections. Densitometric analysis of 10 oblique tangential walls showed that the true middle lamella had an absorption 3.64 times that of the secondary wall whereas the ratio of primary wall to secondary wall absorption was 1.97.

This oblique sectioning experiment can yield unambiguous results only if the section thickness is less than the width of the true middle lamella. If the middle lamella width is smaller than the section thickness it is not possible to obtain the dimensions and absorbance of the true middle lamella. These two conditions are shown in Fig. 9 which depicts diagrammatically oblique sections, 0.1 μm in width, cut at 7° to the main tracheid axis. In Fig. 9a, the true middle lamella is 0.2 μm thick, while in Fig. 9b it is 0.05 μm. For the geometries represented in Figs. 9a and 9b, the theoretical traces yield a plateau for the middle lamella. In Fig. 9a, the absorbance of the middle lamella is given by the height of the plateau and the middle lamella thickness corresponds to the width of the plateau at half-height multiplied by sin 7°. However, in Fig. 9b, the width at half-height multiplied by tan 7° gives the section thickness and the height of the plateau corresponds to the average absorbance arising from equal layers of middle lamella and primary wall. Interestingly, it can be shown that in the case of Fig. 9b, a middle lamella thinner than 0.07 μm would require a lignin concentration greater than 1 g/g in order to satisfy the requirements of the absorbance data.

Fig. 9c represents an oblique section cut at 7° to the tracheid axis and with the true middle lamella width equal to the section width of 0.1 μm. In the theoretical trace the plateau has now been replaced by a peak, the height of which corresponds to the absorbance of the true middle lamella. As with Fig. 9a, the width of the true middle lamella is given by the width at half-height multiplied by sin 7°. An average experimental densitometer trace is given in smoothed form as a dotted line. Apart from the slight depressions between the primary wall and the middle lamella absorption peaks, the experimental trace is very similar to that proposed by consideration of the geometry of the system. However, it must be remembered that no account has been taken of the limitation of optical resolution and non-parallel illumination [SCOTT et al. 1968] in drawing Figs. 9a, b and c.

If the geometry in Fig. 9c is taken to be approximately correct then the middle peak in the densitometer trace corresponds to the true middle lamella absorbance and (C_{ML}/C_S) (t) = 3.64. Although only 10 samples were considered the results indicate that (C_{ML}/C_S) (t) is tending to the value of C_{ML}/C_S (cc) = 3.77. On the basis of these measurements it is not unreasonable to conclude that the lignin content of the true middle lamella does not differ from that of the cell corner middle lamella. This experiment also showed that the primary wall lignin content is about one-half that of the true middle lamella.

The distribution of lignin across the tangential double cell wall is depicted in Fig. 10. The cell wall dimensions are drawn to scale where $WT_t = 5.5$ μm, $MT_t = 0.40$ μm, and the true middle lamella width is approximately 0.1 μm. The experimentally obtained densitometer trace for a transverse section is also drawn on this figure (dashed trace). As can be seen from Fig. 10 the measurement of (C_{ML}/C_S) (t) in transverse sections is essentially a measurement of (C_P/C_S) (t) where C_P is the lignin concentration of the primary wall.

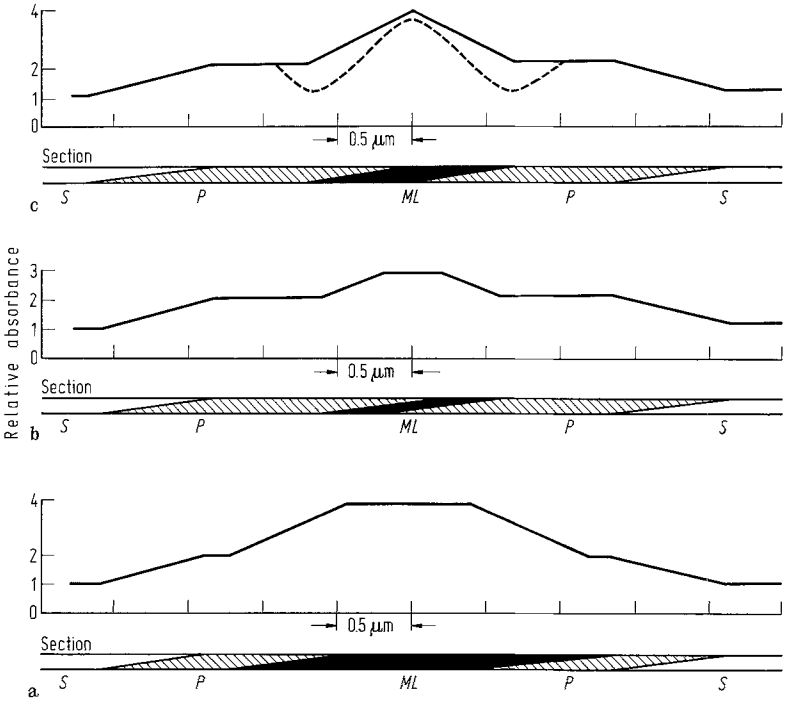

Fig. 9. Diagrammatic representation of a cross sectional view and resulting densitometer trace of an obliquely sectioned tangential compound middle lammella. The section is cut at approximately 7° from the main tracheid axis. The solid line is the predicted densitometer trace; the dotted line in Fig. 9c is the average of the experimentally obtained traces. (a) Section thickness (0.1 μm) considerably less than middle lamella width (0.2 μm). (b) Section thickness (0.1 μm) considerably greater than middle lamella width (0.05 μm). (c) Section thickness (0.1 μm) equals middle lamella width (0.1 μm).
In constructing the theoretical traces, the relative absorbances of the secondary wall, primary wall and true middle lamella were assumed to be 1:1.97:3.77.

If the shaded curve in Fig. 10 is a more accurate representation of the lignin distribution across the tracheid double cell wall, the question arises as to the validity of the analysis given in Table 2. We have already seen that the dimensions of the compound middle lamella correspond almost exactly to the width of the tricomposite layer revealed by oblique sectioning. But what about the lignin concentration? The product of relative absorbance (ordinate) times cell wall dimension (abscissa) in Fig. 10 is a measure of the amount of lignin residing in a particular region of the cell wall. From Fig. 10 it can be shown that the area under the compound middle lamella as measured on transverse sections is 0.832 units, the primary walls 0.591 units and the true middle lamella 0.377 units. Thus the total quantity of lignin in the primary walls and true middle lamella, as

revealed by the oblique sections, is about 16% greater than the lignin in the compound middle lamella measured in transverse sections. This is a comparatively small error. A calculation based on the higher value of lignin content obtained from the oblique sections gives the proportion of lignin in the secondary wall, compound middle lamella and middle lamella cell corner region to be 70%, 18% and 12% respectively. There is little change from the corresponding figures in Table 2. It can therefore be claimed that although the compound middle lamella is not resolved by UV-microscopy of transverse sections, its dimensions and average lignin content are derived with reasonable accuracy by this method.

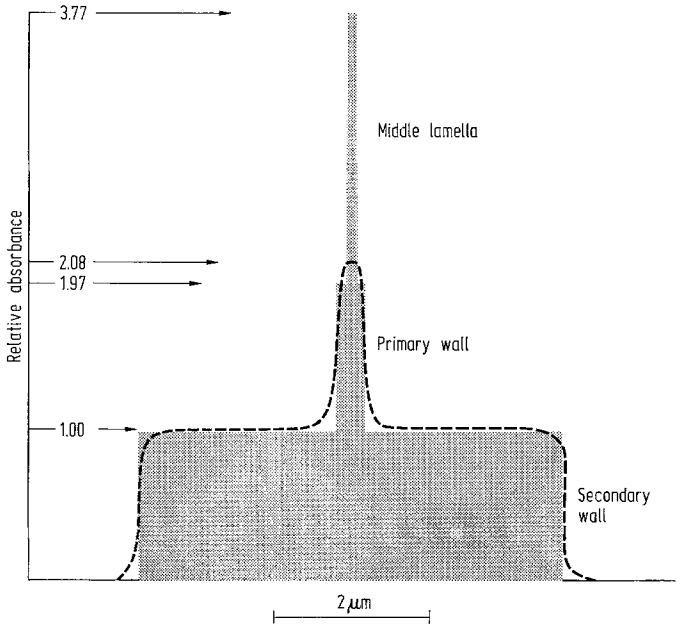

Fig. 10. Idealized distribution of lignin across the tangential double cell wall of earlywood as obtained for oblique sections (shaded area) compared with a typical experimental densitometer trace on transverse cross sections (dashed line).

In some densitometer traces of the oblique walls there was found to be an absorbing layer adjacent to the primary wall. This layer was of varying width and its absorption was about $\frac{1}{3}$ more than that of the secondary wall. This was probably the S_1 layer but on account of the uncertainty in its measurement and the lack of a sufficient number of walls no quantitative study was made. Although an S_1 layer adjacent to the compound middle lamella was usually not evident in UV-photomicrographs of transverse walls it could often be recognized as an absorbing layer slightly darker than the rest of the secondary wall around a cell corner middle lamella (Fig. 11).

Other Morphological Features

The heavy lignification in the ray parenchyma cell is apparent in Fig. 7 and a distinct lamellar structure is evident in the cell wall. A lamellar structure in the ray cell wall has also been detected by electron microscopy [HARADA 1965].

Lamellation of the cell wall was not restricted to ray cells. UV-photomicrographs at 240 μm of spruce tracheid secondary walls often revealed two or three broad lamellations parallel to the middle lamella. Lamellation of the cell wall of various hardwoods and softwoods has been reported by TRAYNARD et al. [1954] for tissue delignified by chlorine or nitric acid. PAGE and DE GRÂCE [1967] found that concentric layers were present in commercially pulped softwood fibres that had been subjected to the mechanical stresses of beating and refining. JAYME and TORGERSEN [1966], using UV-microscopy, observed occasional lamellation in some spruce latewood tracheids. The lamellations found in the present work were not very marked and could not be detected in micrographs taken at wavelengths higher than 240 nm. These lamellations were apparent in the original print of Fig. 1 and can also be seen as small peaks in the secondary wall plateau in the corresponding densitometer trace.

A layer of high UV-absorption surrounds the pits (Fig. 12, single arrow). This layer most probably corresponds to the initial pit border [WARDROP 1964]. There appears to be a progressive decrease in the UV-absorption in the initial pit border as the pit aperture is approached (Fig. 12, double arrow). The pit membrane has been shown to be unlignified [BAMBER 1961]. However, the torus, which straddles the membrane near its centre, shows a very strong UV-absorption (Fig. 13). If Epon is used as an embedding medium, the high UV-absorption of the torus disappears and a fine structure consisting of a thin absorbing layer is apparent within the torus (Fig. 14). The width of this "torus middle lamella" is about 0.10 μm. Interestingly, this is considerably less than the lower resolution limit of the microscope, which at $\lambda = 0.24$ μm is 0.14 ... 0.17 μm [SCOTT et al. 1969]. The extraction of the highly absorbing material from the torus when Epon is used for embedding can most probably be attributed to the use of propylene oxide in the solvent exchange procedure. This absorbing material is also rapidly removed by kraft and sulphite cooking liquors [PROCTER et al. 1967].

The Occurrence of Splits and Fissures

An interesting phenomenon is illustrated in Fig. 15 where splits have occurred in the region of the S_1 layer. Such splits were observed in several sections. WARDROP [1963] has pointed out that mechanical splits usually occur between S_1 and S_2 whereas chemical splits normally take place within the middle lamella. From the electron microscopy of black spruce fibre surfaces, separated by tensile failure, KORÁN [1967, 1969] found that the site of failure occurred consistently in the primary wall and S_1 wall layers. Furthermore, the type of splitting was temperature dependent. The splits observed in the present work are therefore attributed to mechanical damage of the section during its preparation, and illustrate the potentially weak area about the S_1 layer.

Concluding Remarks

The most important conclusion that emerged from this investigation was that although the compound middle lamella was highly lignified, with respect to the secondary wall, it only contained a small percentage of the total lignin in the

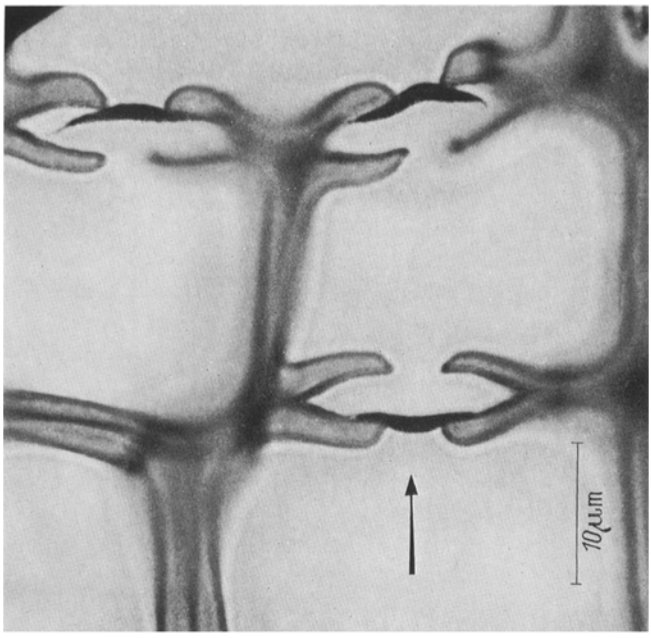

Fig. 13. Bordered pits in methacrylate embedded black spruce earlywood showing the high UV-absorption of the torus (see arrow). $\lambda = 280$ nm.

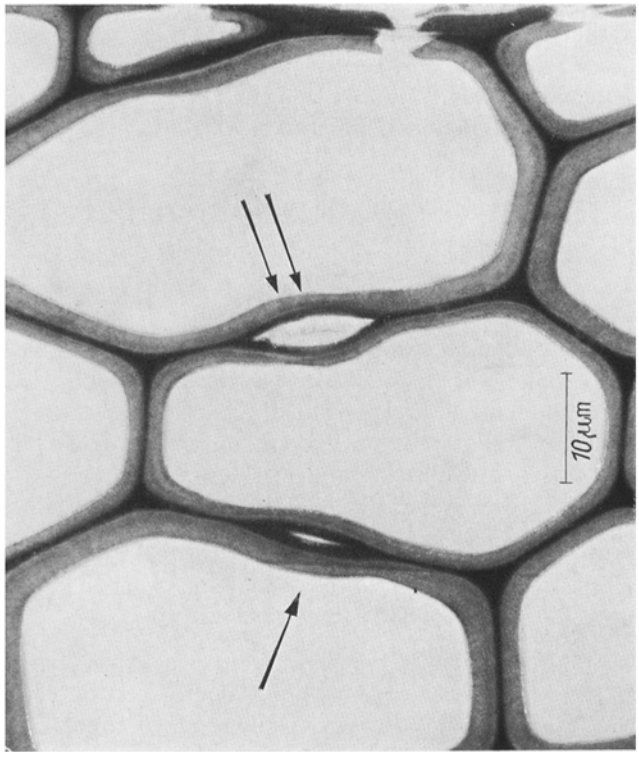

Fig. 12. Highly lignified initial pit borders in spruce earlywood. $\lambda = 240$ nm.

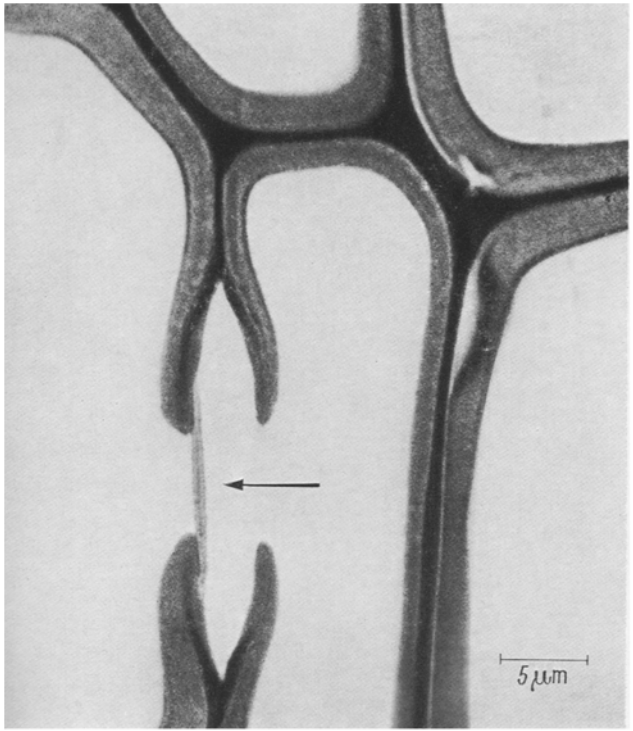

Fig. 14. Bordered pit in Epon embedded black spruce earlywood. The torus has lost all its high UV-absorption (compare Fig. 13) and a fine structure is apparent within the torus (see arrow). $\lambda = 240$ nm.

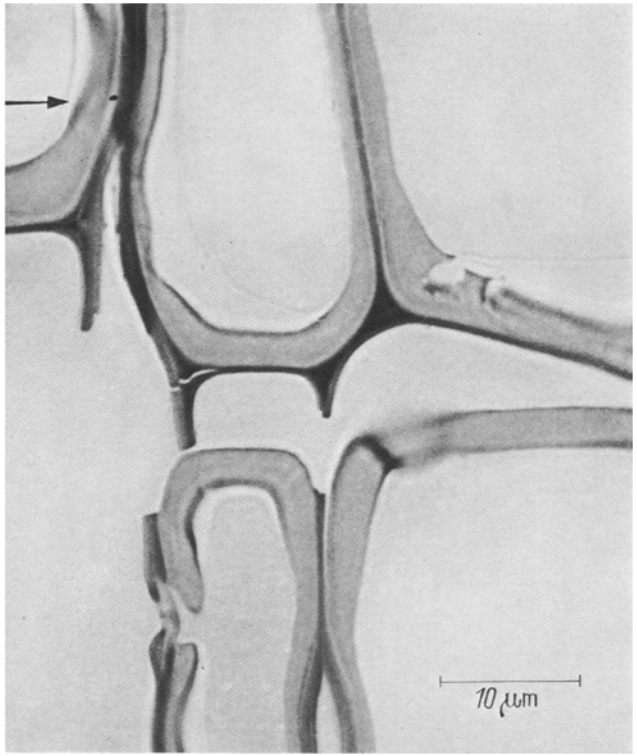

Fig. 15. Cross section of black spruce showing the location of mechanical splits. Rupture of the methacrylate embedding material from the cell wall can be seen in the lumen of the cell on the upper left (see arrow).

wood. This was predicted by BERLYN and MARK [1965] but until now experimental evidence to support this prediction has been scarce. It is interesting to note that BAILEY [1936] obtained a lignin concentration in the middle lamella not very different from the values found in the present work. It is in the estimation of the proportion of total lignin in the middle lamella that most errors have crept into the literature.

The reliability of the method is best shown by the good agreement between the two calculations of the lignin concentration in the secondary wall. The method involving relative absorbances and tissue volumes is quite distinct from the direct calculation from the UV-absorbance data utilising the Beer-Lambert relationship. A useful extension of the present work would be to check the technique by similar measurements on a wood with a lignin distribution different from that of spruce.

Although the study of ray cells and bordered pits was not the primary purpose of the present work, the micrographs revealed certain of their interesting features. The fine structure and chemical composition of the various pit types is not yet fully understood. Possibly, a detailed investigation with the UV-microscope may elucidate more fully the distribution of lignin around and within these important morphological entities.

Finally, it may be profitable to speculate on the significance of these findings to the technology of softwood pulping. The purpose of chemical pulping is either to remove as much of the lignin as possible from the wood without excessive degradation of the carbohydrate component or to weaken the middle lamella bond so that reasonably intact fibres can be obtained. The oblique sectioning experiment has shown us that the quantity of lignin in the true middle lamella is only about 5% of the weight of the wood. Thus, the logical way to pulp softwoods is to use a delignifying agent which is highly selective for the almost pure lignin in the middle lamella. The wood is then reduced to separate fibres, highly lignified, and containing over 95% of the original wood substance. This primary pulp is then used as such, or further delignified to yield the type of fibre required. However, it now appears that most of the commercially-used pulping processes work in the opposite fashion [PROCTER et al. 1967; FERGUS, GORING in print], where the lignin is preferentially removed from the secondary wall. By the time the middle lamella is attacked valuable carbohydrates have been degraded in the fibre wall. This not only decreases the fibre strength but obviates the attainment of maximum yield at a particular degree of lignin removal. The discovery of a middle lamella selective delignifying agent may not be possible but it is certainly worth a try.

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B. J. FERGUS, A. R. PROCTER, J. A. N. SCOTT and D. A. I. GORING,
Department of Chemistry, McGill University and Physical Division Pulp and
Paper Research Institute of Canada, Montreal, Quebec, Canada.