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THE DISTRIBUTION OF THE CHEMICAL CONSTITUENTS THROUGHOUT THE CELL WALL

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Synopsis

This paper is mainly a review of the work of Lange and co-workers [1] *on the distribution of cellulose, hemicellulose and lignin in the cell wall of Swedish spruce* (**Picea excelsa**), *birch* (**Betula verrucosa**) *and cotton* (**Gossypium herbaceum**). *Some views on optical methods for micro-analysis of the cell wall and future problems in cell wall chemistry are also included in this article.*

THE PROBLEM

"Although wood . . . is an exceedingly heterogeneous material, its analysis has for the most part followed methods already developed in other branches of chemistry where greater homogeneity obtains. When a wood sample is reduced to powder of a specified degree of fineness and analysed for various constituents, it is not possible to determine the chemical nature of the various layers of the cell wall or even of the different tissues which comprise this very complicated organic structure. It is becoming increasingly apparent that the best chemical utilisation of wood is dependent not only upon a knowledge of the *amounts* of certain substances (lignin, cellulose, pentosans, etc.) in wood, but also upon *where they are located in the cell wall* and how

the complicated cell aggregate (wood) behaves when chemically treated with various reagents."

With this excellent formulation, Harlow introduces the main problem in the chemistry of the cell walls of wood.[2] This problem was studied with the limited aim of investigating cellulose, hemicellulose and lignin in the cell wall of Swedish spruce (*Picea excelsa*) on the microscale with a resolution of 0.5 – 1.0μ. Some investigations were also made on birch (*Betula verrucosa*) and cotton (*Gossypium herbaceum*).

POSSIBLE METHODS

The difficulties met with here are due chiefly to the small dimensions of the object and consequently to the very small amounts of matter (the magnitude of 10^{-10} — 10^{-12} g.). In principle, there seem to be two different ways of solving this problem — (*1*) to isolate a sufficiently large amount of a certain cell structure with a micromanipulator or a similar instrument and then to analyse it by common chemical methods or (*2*) to study the components directly in the fibre wall by physical methods. The first alternative has been tried [3] and seems to involve many difficulties. Among the physical methods, the optical ones are to be preferred, partly because they leave the material under investigation in its original state and partly because, in general, they can be applied in a strictly specific manner and elaborated in such a way as to give quantitative results.

Microphotometry with visible and ultra-violet radiation and histo-radiography were used to study the problem formulated above. Thus, lignin could be studied with ultra-violet absorption techniques [4] and cellulose and hemicellulose with cytophotometric methods in the visible range.[5] The total mass (lignin + carbohydrates) per unit area across the cell wall could be determined by measuring the absorption of soft X-rays in different parts of the cell wall.[6]

It should be emphasised that the errors are considerable when *absolute* amounts of matter are measured by histochemical methods, since there are at present no easy methods for a very exact determination of the thickness of histological preparates such as a cross-section of wood [7] (*see*, however, other work[8]). It seems therefore that the micro-optical methods at present are best applied for the *relative* determination of radiation absorbing substances — that is, for comparison of chemical composition in different parts within a single cell or neighbouring cells. The *concentration* of a component

to be measured, however, should not be less than about 1 per cent. All measurements in the work reviewed here on carbohydrate and lignin distribution in the woody cell were relative. In a very restrictive sense, 'absolute' distribution of matter in the cell wall was determined.

Since the measurements are made within *one* cell, it is obvious that a great number of cells need to be investigated in order to obtain significant results on differences in the composition of cells. The number of replications must be very great if small differences are to be investigated. Since such an investigation may be very time-consuming, only fibres of great difference in carbohydrate compositions were investigated. This meant, among other things, that no attempts were made to investigate, for example, the localisation of the different components of hemicellulose in the fibre wall. Furthermore, there is a great difference in lignin content between the compound middle lamella and the secondary wall, which makes it suitable to study this difference with microspectrographic methods in the ultra-violet radiation range.

The intrumentation for cytophotometric measurements in the optical spectral region consists of light source, monochromator (or filter), microscope and detector (photographic or photoelectric). In principle, the measurements are made in the same way as in a spectrophotometer for macroanalysis. The procedure, however, differs in two important respects—(*1*) the light path through the microscope and the specimen (conical illumination) is complicated and must be carefully controlled and (*2*) the heterogeneous structure of the preparate (owing to its biological origin) and its form give rise to non-absorptive light losses. The influence of (*1*) and (*2*) were treated in detail by Lange. [4, 9, 10]

The absorption of X-rays in matter follows simple laws. In historadiographic work, the photoelectric absorption gives the main contribution to the total attenuation. The influence of scattering is negligible. There are several ways of making an X-ray image of a biological specimen. One is to make an absorption image in the scale 1:1 and magnify it optically in a microscope.

For more details about analytical cytology, the reader is referred to the literature.[11–22]

The author has also applied a new technique, interference microscopy, to the study of the cell wall components.[59] Since this work is not yet

finished, the principles of the method and some preliminary results are given in an appendix to this paper.

The nomenclature for indicating the different parts of the cell wall is that of Bailey and Kerr.[23]

THE DISTRIBUTION OF LIGNIN IN THE CELL WALL

Native lignin was studied with microspectrographic methods by Lange, [24, 25] who showed that it was of an aromatic nature.

Lignin has a selective absorption of ultra-violet radiation in the wave length range 2 500 – 3 300 Å, where the carbohydrates have no absorption. Hence, it is possible to study the lignin directly in the woody cell wall in presence of the carbohydrate components with microspectrographic methods in the ultra-violet range.

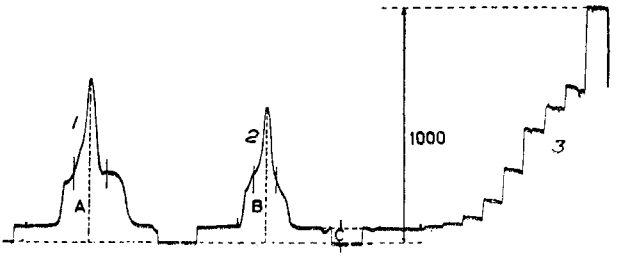


Fig. 2 — The curves *1*, *2* and *3* show the result of the photometric measurements along the lines *1*, *2* and *3* of the plate in Fig. 1. The photometric deflection is measured from the dotted line at the bottom of the figure (the position of the galvanometer spot, when no light falls on the thermocouple)
The distance between this line and the upper dotted line corresponding to an unexposed part of the plate (log $I_0/I = \infty$) is arbitrarily set at 1 000
The vertical dotted lines *A* and *B* are the maximum photometer deflections for the middle lamellae in Fig. 1

The experimental procedure was the following. A cross-section of wood (2 – 10μ thick) was embedded in glycerol, placed between quartz glasses and photographed in an ultra-violet microscope with monochromatic illumination (within the wave length range 2 750 – 3 300 Å). A rotating sector with several absorption steps was placed immediately in front of the photographic plate. Fig. 1 shows the images of the cross-section and the rotating sector. With the aid of a microdensitometer, the photographic density of the different parts of the cell wall is compared with that of the absorption steps of the sector (Fig. 2). From this comparison, the relative distributions of lignin across the cell wall is computed.

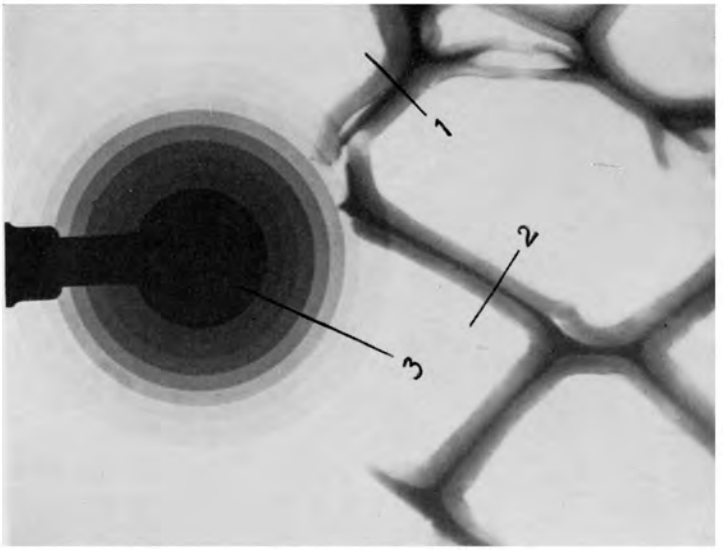


Fig. 1 — Cross-section of *Picea excelsa*, photographed at 2 800 Å together with the rotating sector
The plate (the negative of this figure) was subjected to photometric measurements along the lines indicated in the figure (about ×1 000)

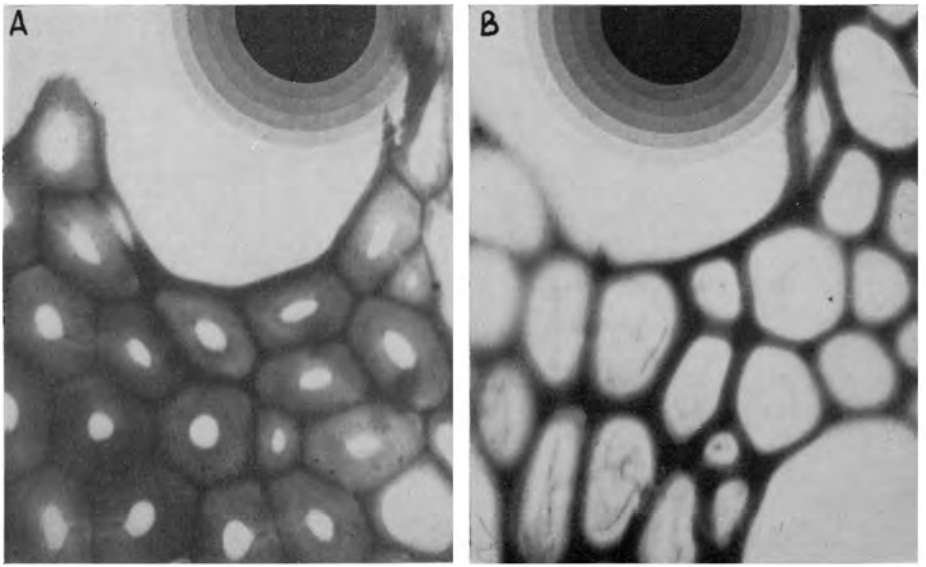
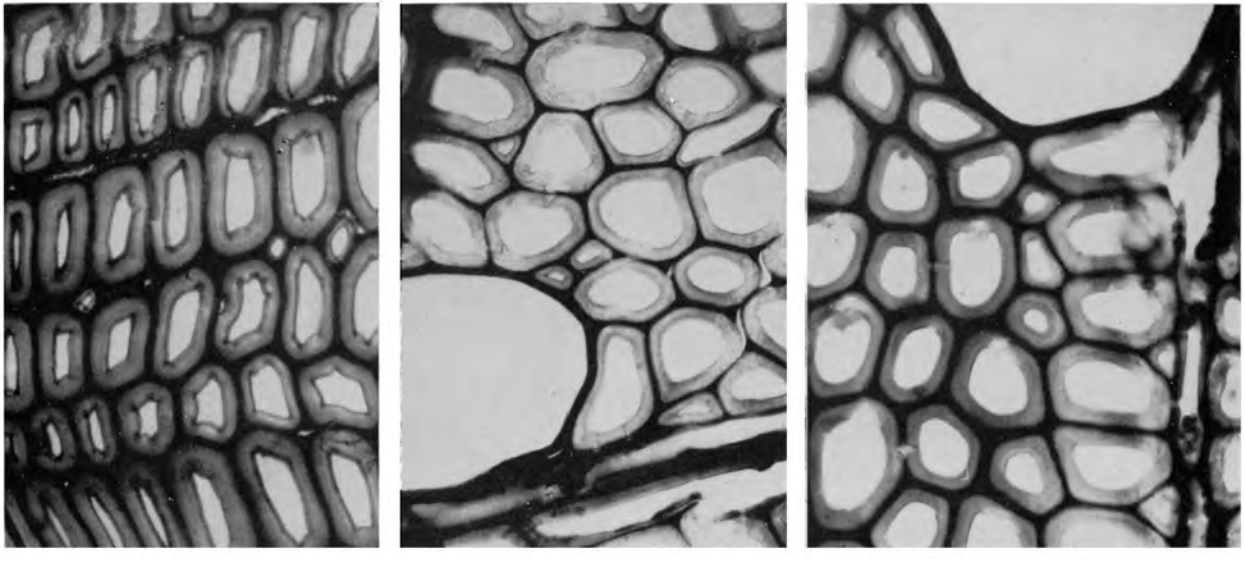


Fig. 7 — Cross-sections of normal (*A*) and tension wood (*B*) of red beech (2 750 Å) — the secondary walls of tension wood are practically free from lignin

Fig. 8 — Photographic plate of a cross-section of a fibre together with monochromator slit and rotating sector



Spruce *Birch* *Aspen*

Fig. 3 — Photomicrograph (2 750 Å) of cross-sections of spruce, birch and aspen (about ×500)

For the microspectrographic determination of the distribution of lignin in the plant cell wall, the following factors are of importance — (*1*) the absorption coefficient of lignin, (*2*) the non-absorptive light losses in wood, (*3*) the thickness of the wood specimen (cross-section) and (*4*) the aperture of the illuminating incident light.

The influence of (*1*), (*3*) and (*4*) on the determination of the lignin distribution can be calculated.[9] However, there are no possibilities of treating the influence of (*3*) theoretically. The problem must therefore be approached empirically.

Summarising the four points above, there seem to be two ways of obtaining knowledge of the lignin distribution in the woody cell wall. The first is to apply microspectrographic methods (conical illumination) on *thin* cross-sections of wood at wave lengths (about 2 800 Å) where the absorption of lignin is high. The lignin distribution measured has to be approximately corrected for non-absorptive light losses in the inner regions of the cell wall, with the aid of the results obtained from the distribution measurement at 2 750 Å and 3 300 Å on thick cross-sections (Fig. 4). (For details, *see* Lange.[4]) The second way is to use parallel incident light of long wave length on fairly thick cross-sections (in order to obtain an extinction $>$ 0.05). In this way, the influence of non-absorptive light losses and the thickness of the cross-section is reduced. Only the results of the first method are discussed here.

Fig. 3 represents photomicrographs of three cross-sections embedded in glycerol from spruce, birch and aspen, respectively, taken at 2 750 Å, showing the strongly absorbing, highly lignified compound middle lamella, as well as the weaker absorbing secondary cell walls. As is easily seen, lignin is mainly deposited in the compound middle lamella. Hence, the average concentration of lignin in the secondary walls must certainly be considerably below the average concentration of lignin in wood — for spruce about 30 per cent. and for birch about 20 per cent.

As is seen from the photomicrographs in Fig. 3, there is a very rapid change in lignin concentration in the outer regions of the secondary cell wall. In order to obtain further information about the position in the cell wall where the change is most rapid, photomicrographs in the ultra-violet (U.V.) range (2 750 Å) and in polarised visible light of identical cross-sections of Swedish spruce were compared. In the U.V. photomicrographs, the distance (*A*) across the most heavily U.V. absorbing part (the compound middle lamella) was measured. In the photomicrographs taken in polarised visible light, two distances along the same path, as in the U.V. photomicrograph, were

measured — the width (*B*) of the dark non-birefringent part (the middle lamella and the two adjacent primary walls) and the greatest distance (*C*) between the birefringent outer layers of the secondary wall. All distances were referred to the same scale of magnification. The results for a few cross-sections are shown in Table 1.

TABLE 1

Comparison of the thickness of the compound middle lamella measured on U.V. *microphotographs* (*A*) *and the shortest and greatest distance between the birefringent outer layers of the secondary wall measured on photomicrographs in polarised light* (*B and C*)

Dimensions in μ (*see text*)

A	*B*	*C*
1.3	0.7	2.8
1.6	0.8	2.1
1.3	0.8	2.9
2.4	2.4	3.9
1.3	0.5	2.1
1.6	0.5	1.8
1.6	0.8	2.6
1.3	0.5	1.8
1.6	1.3	2.6
1.6	0.8	1.8
1.8	0.5	2.1

The results of the measurements show that the great change in lignin concentration in the cell wall of Swedish spruce takes place within the outermost parts of the secondary wall. On the outer side of this layer, the cell wall can be considered as composed mostly of lignin. On the inner side (which is the major part of the cell wall), on the other hand, the carbohydrates are the dominating components. This result indicates that it is highly probable that there is a great change in fibre properties in the narrow range of the outermost part of the secondary wall. The secondary wall is roughly the carbohydrate part of the fibre and the compound middle lamella the lignin part.

Fig. 2 shows two arbitrarily selected distribution curves for spruce photometrically recorded together with the records of the transmittance of the sector steps. The thickness of the cross-sections varied from about 3μ to about 5μ. With these dimensions, the ratio between the cross-section thickness and the width of the cell wall is around 0.5 or somewhat greater.

This implies that the measured values of the relative lignin distribution are about 20 per cent. too high in the secondary cell wall owing to the convergent light.[9] From experiments, [4] it is known that the relative concentration of lignin (in comparison with that in the compound middle lamella) in the regions around the lumen is about 50 per cent. too high, owing to non-absorptive light losses. It should be observed, however, that this estimation is based on experiments on fairly thick cross-sections. Since very thin cross-sections (3–5μ) have too low absorption (extinction < 0.05 at 3 300 Å) in the regions around the lumen, it is not possible to make the estimation of light losses direct on the thin cross-sections.

Thus, the distribution curve (measured as extinction) is first corrected in such a way that the values around the lumen and in the main part of the secondary wall are reduced by about 30 per cent., the correcting values gradually decreasing towards the middle lamella. This corrected curve is further adjusted for the influence of the convergent incident light (Table 10)[9] in such a way that the relative extinction values in the secondary wall are reduced by 20 per cent. The procedure is illustrated in Fig. 4. Further, the relative lignin distribution curves are transformed into 'absolute' ones in the following way. The area below the relative lignin distribution curve represents a measure of the amount of lignin in wood. If the material (carbohydrate + lignin) concentration is assumed to be approximately constant across the cell wall, the problem is to determine this value so that the area under the lignin distribution curve will be 28 per cent. (for spruce) of the total area under the total material distribution curve (*cf.* Fig. 4). The concentration of lignin determined in this way is 10 – 20 per cent. around the lumen and 60 – 90 per cent. in the middle lamella. From about fifty measurements on thin cross-sections selected from some 500, the average values are respectively 73 per cent. and 16 per cent. These values are valid for spruce. Bailey found 71 per cent. lignin in the compound middle lamella of Douglas fir.[3] For birch, the values of the lignin concentrations in the secondary cell wall are lower, which is also the case for other kinds of hardwoods such as ash, aspen, beech, birch and elm (Fig. 3).

It is supposed that lignin gives wood certain mechanical properties. If so, differences between the lignin distribution in normal wood and reaction wood (that is, compression wood and tension wood) could be expected. For the study of this problem, compression wood of Swedish spruce and tension wood of red beech were compared with respect to lignin distribution with 'normal' specimens of Swedish spruce and red beech, respectively. In the case of compression wood, the differences in lignin distribution compared

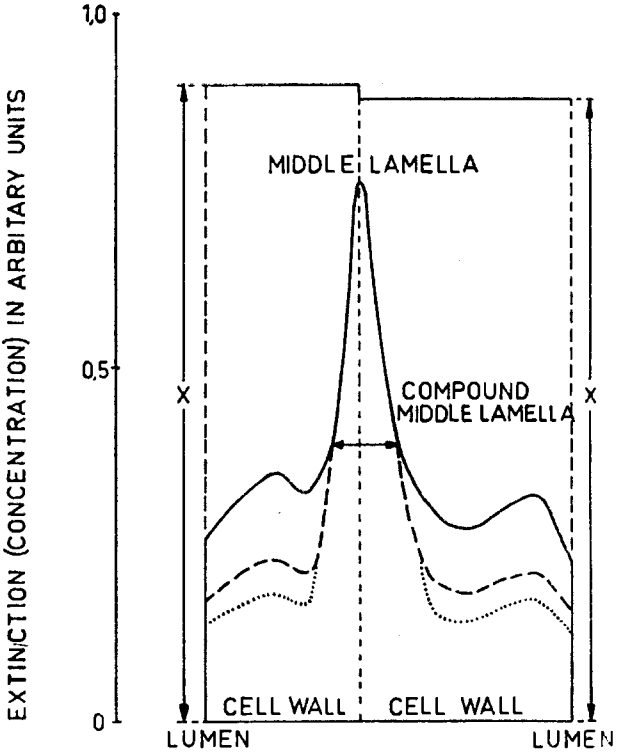


Fig. 4 — Illustration of the procedure for conversion and correction of the measured extinction distribution to lignin concentration distribution.

The solid curve is the measured extinction at 2 750 Å across two cell walls of spruce. The ratios between the ordinate value (extinction) in the middle lamella and those of points on the unbroken line in the regions of the secondary cell wall are increased by 30 per cent. in order to correct approximately for the influence of non-absorptive light losses (*cf.* text p. 149). These corrections, which are computed for the inner half of the cell wall, give a 'true' distribution of lignin extinction in this part of the cell wall. The extinction values are plotted (dotted line) and extrapolated towards the compound middle lamella. This curve is, in its turn, corrected for the convergence of the incident light (Table 10 in the paper by Lange[4]). The ratio of the ordinate values for points in the inner parts of the cell wall and that of the middle lamella are reduced by 20 per cent., a value based on the knowledge of the angular aperture (50°) and an estimated value of the cross-section thickness. The final curve (points) is extrapolated towards the compound middle lamella. Thus, the lowest curve represents approximately the relative lignin distribution. In order to transform the relative lignin distribution to an 'absolute' distribution, a value of x (total material concentration assumed to be constant across the whole cell wall) is computed in such a way that the area below the lignin distribution curve will be 28 per cent. (for spruce) of that below the constant value (x) found for the material distribution. The value of x is computed for the two cell walls (upper solid lines). In the case illustrated in the figure, the concentration of lignin in the middle lamella is about 85 per cent. and around the lumen about 15 per cent.

with normal wood are easily seen from Fig. 5, showing the lignin distribution from one lumen across the cell walls to the next lumen. Between the secondary cell walls, there is a narrow region containing a relatively large amount of lignin. This region seems to form a network that can be described as a system of adjacent hollow tubes with relatively thin walls containing much lignin. Within this system of 'lignin tubes', the major part of the cell wall is localised. The outer regions of these cell walls have the highest lignin concentration of all parts in the compression wood. The region around the lumen contains very small amounts of lignin, if any (Fig. 6).

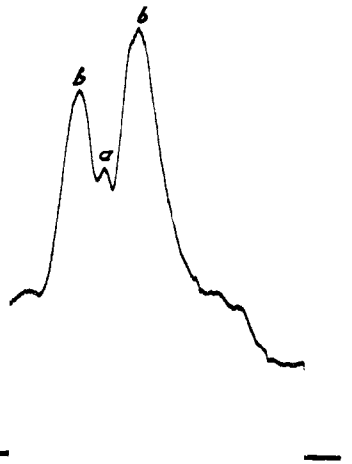


Fig. 5 — Recorded light transmission across two cell walls of compression wood of spruce — the lignin distribution is different from that in normal wood (*cf.* Fig. 6)

A comparison between tension wood and normal wood from red beech is shown in Fig. 7, which represents two photomicrographs taken at 2 750 Å with the image of the rotating sector to show that the photographs are printed under comparable conditions. It is clearly seen that the cell walls of normal wood contain more lignin than those of tension wood. In the latter case, the lignin is deposited almost entirely in the compound middle lamella with the cell walls practically free of lignin (*cf.* Wardrop and Dadswell[26]).

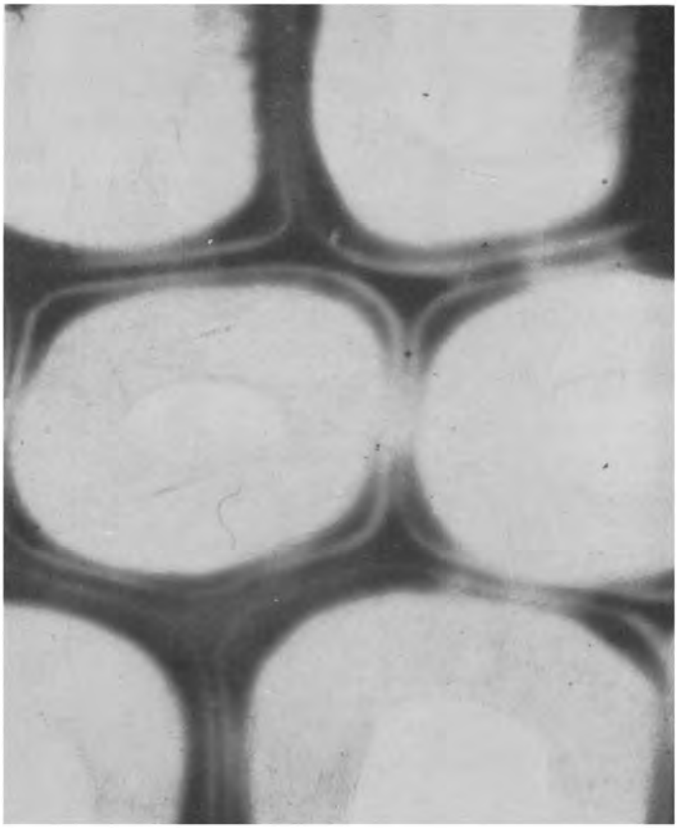


Fig. 6 — Cross-section of compression wood of spruce (2 750 Å) The secondary walls are practically free from lignin and the boundary line between lumen and cell wall is difficult to see (about $\times 1\,000$)

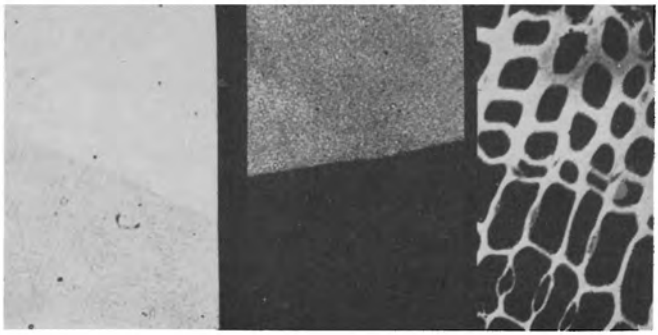


Fig. 25 — A microradiogram of *Picea excelsa* and the corresponding step wedge

THE DISTRIBUTION OF CARBOHYDRATES (CELLULOSE AND HEMICELLULOSE) IN THE CELL WALL

It is not possible to study the carbohydrates in the plant cell wall by microspectrographic methods in the presence of lignin. In order to investigate the distribution of carbohydrates in lignified cell walls, the lignin must first be removed. In addition, since the carbohydrates have no selective absorption in the wavelength range easily accessible for experimentation (2 000 – 7 000 Å), they must be combined in some way with a light absorbing component to allow microspectrographic studies.

The combination between the light absorbing component and the carbohydrates should be carried out in such a way that —

(*1*) The magnitude of the light absorption is a quantitative measure of the carbohydrates in the fibre, which means that they should react quantitatively with the light absorbing component.

(*2*) The fibre structure is left intact and

(*3*) If possible, the characteristic light absorption is situated in the visible wavelength range, where losses due to light scattering in the fibre wall are less than in the ultra-violet range.

There are many colour reactions for carbohydrates reported in the literature.[27] Common for all is that the carbohydrate material is degraded by hydrolysis and reacts with a light-absorbing component, resulting in a more or less characteristically coloured solution or at least in a coloured fibre with a partially destroyed structure. It is obvious from condition (*2*) indicated above that this method cannot be used for microspectrographic investigations on the carbohydrates in the plant cell wall.

Conditions (*1*) — (*3*) could be fulfilled by using *p*-phenylazobenzoyl chloride as a light-absorbing component. The reaction product between this substance and the carbohydrates of the plant cell wall is an orange red intact fibre.[28]

In order to eliminate the influence of non-absorptive light losses due to the heterogeneous structure of the esterified fibre, the cross-section should be embedded in pyridine during the microspectrographic measurement. However, the fibre cross-sections swell considerably in pyridine and corrections for changes of dimensions must be considered. The elimination of non-absorptive light losses and corrections for changes in fibre dimensions due to swelling in pyridine were treated in detail.[10, 29] The distribution of the light absorption across the swollen cell wall is also a measure of the distribution of carbohydrate material across the cell wall.

The degree of esterification was, for most of the fibre materials, confirmed only by the nitrogen content. In Table 2, the values of the pentosan content

and nitrogen content are shown for different fibre materials containing various amounts of pentosans. The nitrogen content varies in agreement with the calculated values, within the limits of accuracy for the analytical method used.

TABLE 2

Nitrogen content and pentosan content (in per cent.) for different fibre esters

	Nitrogen found	*Pentosans*	*Nitrogen calculated*
Holocellulose, spruce	10.87	9[30]	10.64
Holocellulose, birch	10.45	32[30]	10.52
Sulphite pulp, spruce	10.46	6[31]	10.65
Sulphate pulp, pine	10.88	11[31]	10.61
Standard cotton	10.65	—	10.68
Hexosans			10.68
Pentosans			10.22

Since the accuracy of microspectrographic methods is less than that obtained with conventional analytical methods for the macroscale and since there are no methods available at present for a selective differentiation of cellulose and hemicellulose of the cell wall, the conventional methods — that is, alkaline and hydrolytic treatments or combinations of both — have been applied to obtain a rough differentiation between the cellulose and hemicellulose fractions. Thus, the concept of hemicellulose as applied in this work is more related to the carbohydrate material that is dissolved from the various kinds of carbohydrate fibres obtained through alkaline or acid treatment than to the more or less unknown properties of the complex mixture of polysaccharides included in the term hemicellulose.

Following is a short description of the different fibre samples investigated.

A. *Fibre samples, laboratory preparations*

1. Holocellulose from Swedish spruce (*Picea excelsa*) was prepared by Jayme's method. [32] Yield from wood 70 per cent. Nitrogen content of the fibre ester[27] 10.87 per cent.
2. Hot alkali treated holocellulose. Holocellulose (sample 1) was treated with sodium hydroxide (2 per cent.) at 100°C for 2 hr. Nitrogen content of the fibre ester 10.70 per cent. Yield from holocellulose 75.7 per cent.
3. Hot alkali treated cross-sections of holocellulose. The chemical treatment was identical with that above; however, the holocellulose fibre material was in the form of cross-sections. This type of material was prepared in order to obtain information about differences between intact holocellulose fibres and cross-sections that may occur due to alkali treatment.

4. Cold alkali treated holocellulose. Holocellulose (sample 1) was treated with sodium hydroxide (11 per cent.) at 0°C for 1 hr. The alkali-treated fibres were then washed with successively weaker solutions of sodium hydroxide. Yield from holocellulose 65.0 per cent. Nitrogen content of the fibre ester 10.72 per cent.
5. Partially hydrolysed holocellulose. Holocellulose (sample 1) was treated by a modified Nickerson method. This modification (shorter reaction time) was necessary, since it is difficult to prepare cross-sections from holocellulose fibres that have been hydrolysed during the longer reaction time commonly used. The reaction conditions are — 3.5 M hydrochloric acid + 0.6 M ferric chloride, 95°C, 0.5 hr. Nitrogen content of the fibre ester 10.59 per cent.
6. Prehydrolysed, cold alkali treated holocellulose. Holocellulose (sample 1) was first hydrolysed with 1.25 M sulphuric acid at 90°C for 5 min. The product was then swollen in sodium hydroxide (17.8 per cent.) at 20°C for 1 hr. Yield from holocellulose 61.0 per cent. Nitrogen content of the fibre ester 10.81 per cent.
7. Holocellulose from birch was prepared by Jayme's method.[32] Yield from wood 79.5 per cent. Nitrogen content of the fibre ester 10.45 per cent.
8. Hot alkali treated birch holocellulose was prepared from sample *7* analogous to sample *2*. Yield from holocellulose 63.0 per cent.
9. Standard cotton was prepared by the method recommended by American Chemical Society. [33] Nitrogen content of the fibre ester 10.65 per cent.
10. Hot alkali treated standard cotton was prepared under the same reaction conditions as used for samples *2* and *8*. Yield from standard cotton 95 per cent.
11. Regenerated cellulose in the form of rayon fibres.
12. Spruce sulphite rayon pulp, 90.1 per cent. alpha-cellulose (according to the Swedish Standard Method CCA 7). Yield from wood 44.6 per cent., viscosity 28.5 cP. Nitrogen content of the fibre ester 10.68 per cent.

B. *Fibre samples from commercial pulps*

13. Spruce sulphite acetylation pulp, 95.0 per cent. alpha-cellulose. Yield from wood 33.4 per cent., viscosity 28.5 cP. Nitrogen content of the fibre ester 10.76 per cent.
14. Pine sulphate kraft pulp, estimated yield from wood 42 per cent. Nitrogen content of the fibre ester 10.88 per cent.
15. Pine prehydrolysed sulphate pulp, estimated yield from wood 34 per cent. Nitrogen content of the fibre ester 10.71 per cent.

It should be pointed out that the various fibre samples were selected from a big batch of fibres. Thus, no differentiation was made, for example, between fibres of different age or from different localities in the trunk of the tree. Vessels were, however, excluded from the fibres from birch.

Fig. 8 shows the images of the object (a cross-section of a fibre ester swollen in pyridine) and the monochromator slit together with the image of the rotating sector.

Fig. 9 shows the photometrically recorded transmission curves measured on a photographic plate across two cell walls of a cold alkali treated spruce holocellulose after a short prehydrolysis. The lumen is to the left. Fig. 8 also shows the recorded light transmission for the different steps of the reference system — that is, the rotating sector.

In order to obtain an average material distribution curve characteristic for the fibre material investigated, an average curve for a cross-section was computed from individual distribution curves in different directions across the cell wall. The distance from lumen to the outermost layer was arbitrarily set alike for all fibres. The light absorption (measured as extinction from the transmission distribution curve) was computed for characteristic points on the transmittance curve across the cell wall in order to obtain the 'saw character' of the light absorption (10 – 40 points). The points representing the arithmetic means were connected giving an average material distribution

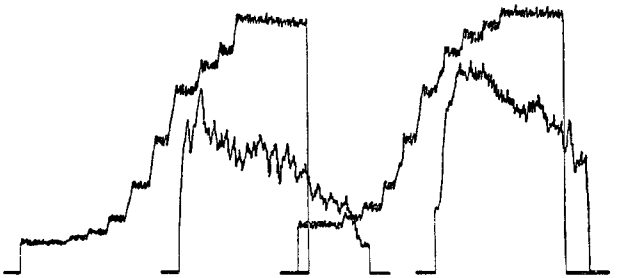


Fig. 9 — Transmission curves for cross-sections of cold alkali treated spruce holocellulose after a short prehydrolysis

curve for the fibre cross-section (Fig. 11). The highest value of concentration within the cell wall was arbitrarily set at 100 per cent. In the same way, an average material distribution curve was determined for the whole fibre sample from the average distribution curves for the different cross-sections. Such a material distribution curve gives fairly reliable information on the average relative concentration of material in different layers in the cell wall. Thus, the shape of material distribution curves are comparable for different kinds of fibres. As a consequence, however, small variations in material distribution (for instance, owing to the layering of lamellae) are smoothed out. These types of variations must be studied in the individual material distribution curves.

For many types of fibre, the average material distribution curve can be represented by a straight line.

General survey

An even relative material distribution across the cell wall is found in the fibre from which only small amounts of carbohydrates have been dissolved

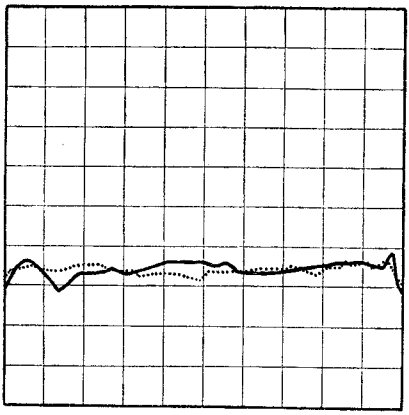


Fig. 10 — Relative material distribution (expressed as extinction) for cell walls of spruce holocellulose — lumen to the right

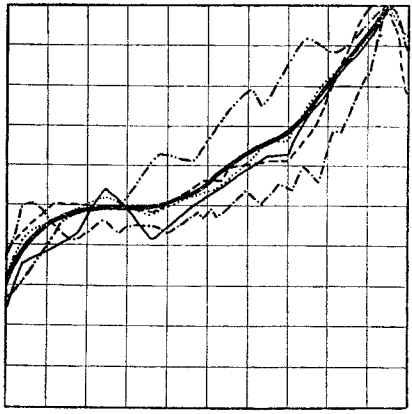


Fig. 11 — Relative material distribution curves and an average distribution curve within a single cell wall for hot alkali treated spruce holocellulose

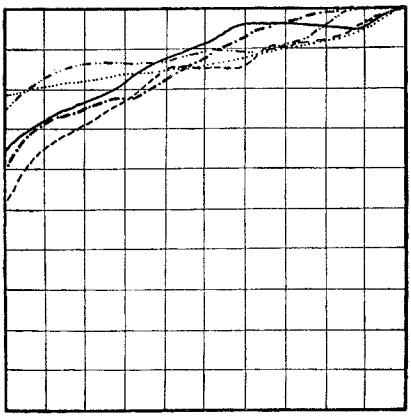


Fig. 12 — Relative material distribution curves within a single cell wall for cold alkali treated spruce holocellulose

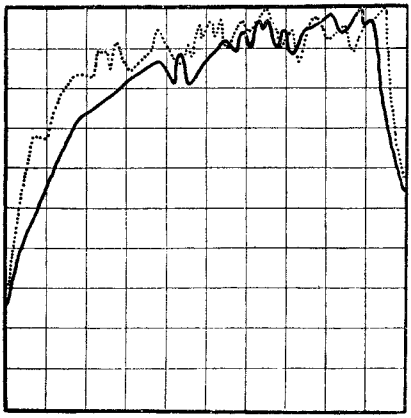


Fig. 13 — Relative material distribution curves within a single cell wall of spruce holocellulose after 0.5 hr. Nickerson hydrolysis

(that is, holocellulose from spruce, birch and cotton). Only small variations on both sides of the average value of concentration are found. Hence, the

concentration of carbohydrates is the same around the lumen as in the outermost region of the cell wall (Fig. 10, 15 and 17).

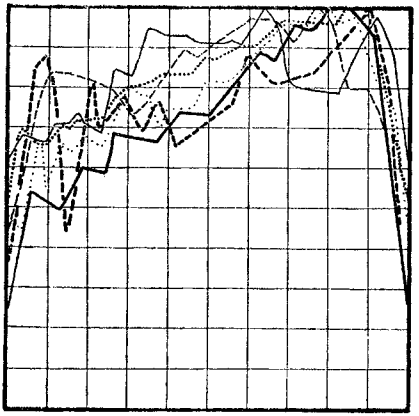


Fig. 14 — Relative material distribution curves within a single cell wall of cold alkali treated spruce holocellulose after a short prehydrolysis

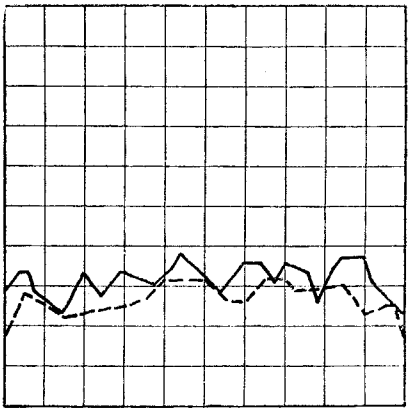


Fig. 15 — Relative material distribution curves within a single cell wall of birch holocellulose

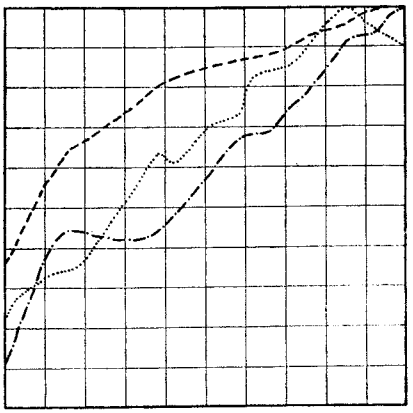


Fig. 16 — Relative material distribution curves for hot alkali treated birch holocellulose

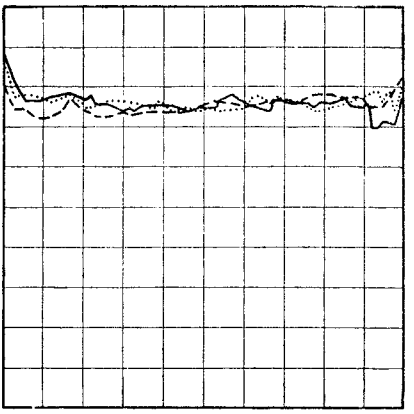


Fig. 17 — Relative material distribution curves within a single cell wall of standard cotton

The concentration of material in a regenerated carbohydrate fibre (sample *11*) is constant over the whole surface of the cross-section.

In the remaining fibre samples (*2* – *6*, *8* and *10*), greater or smaller

amounts of hemicellulose have been removed. The majority of the carbohydrate material left is cellulose. Hence, the relative distribution curves in

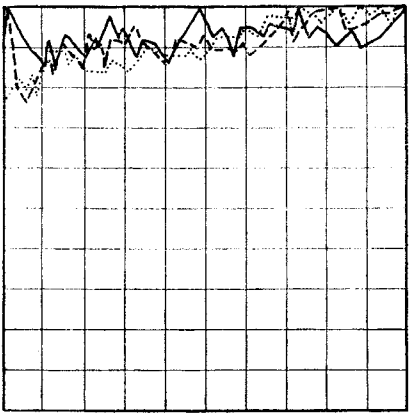


Fig. 18 — Relative material distribution curves within a single cell wall of hot alkali treated standard cotton

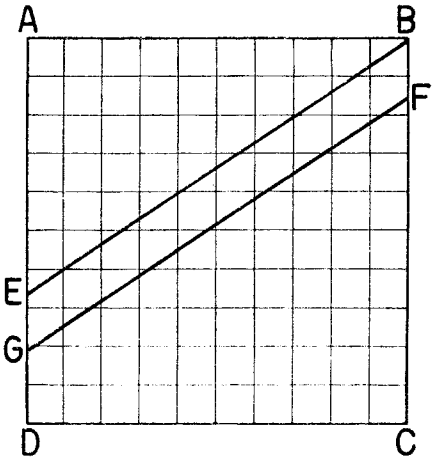


Fig. 19 — 'Absolute' material distribution curve *FG* calculated from the relative material distribution curve *BE* (*cf.* the text)

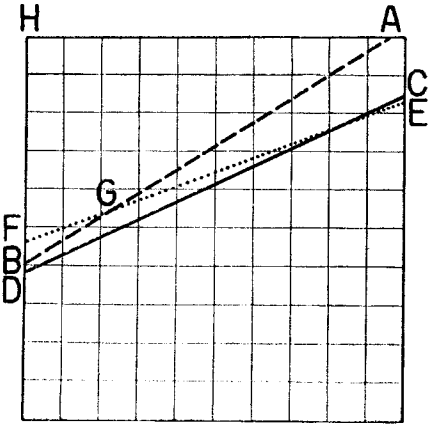


Fig. 20 — Collection of 'absolute' average distribution curves (*cf.* the text)

these latter cases give a rough picture of how cellulose is distributed across the cell wall. In all cases (*2 – 6*, *8* and *10*), the packing density of the cell wall material has its maximum around the lumen. The concentration of material

decreases uniformly towards the outer layers of the cell wall. The different distribution curves will be discussed in detail in the following sections of this paper. As a general conclusion, however, it can be stated that cellulose

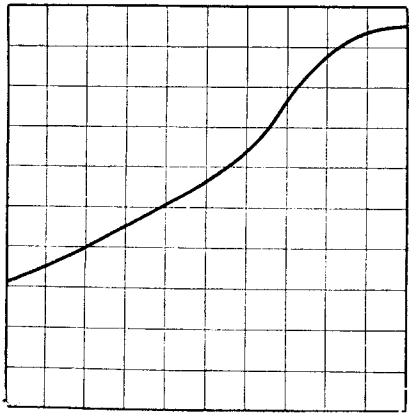


Fig. 21 — 'Absolute' average material distribution curve for sulphite rayon pulp

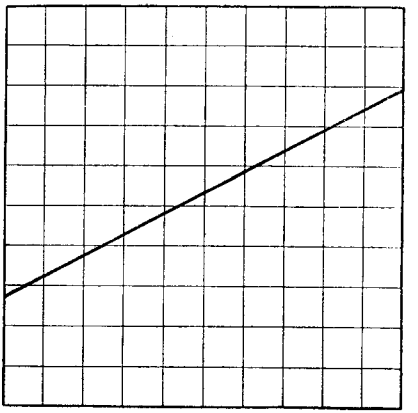


Fig. 22 — 'Absolute' average material distribution curve for sulphite acetylation pulp.

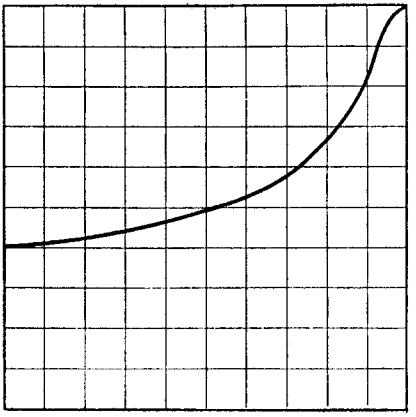


Fig. 23 — 'Absolute' average material distribution curve for pine sulphate pulp

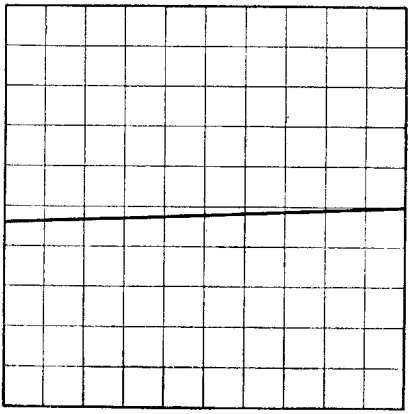


Fig. 24 — 'Absolute' average material distribution curve for pine prehydrolysed sulphate pulp

in the fibres investigated has its maximum concentration around the lumen. Information about the distribution of hemicellulose in the fibre can be obtained from the difference in material distributions between the holo-

cellulose fibres (*1* and *7*) and the hydrolysed or alkali-treated fibres (*2–6*, *8* and *10*). Most of the accessible material in the fibre wall is located in the outer layers of the cell wall, steadily decreasing in amount towards the lumen. Hence, the effect of the different treatments mentioned above is most pronounced in the outer layers of the cell wall.

The deviation from the linear tendency of the distribution curves at the boundaries of the cell wall is due to convergence of the illuminating light. An approximative correction for this is most easily made by extrapolating the distribution curve from the inner part of the cell wall towards the boundaries.

Comparison of relative material distribution in a hot alkali treated fibre and a hot alkali treated fibre cross-section

In all fibre samples investigated, the different treatments (alkali treatment and acid hydrolysis) have removed material mainly from the outer layers of the cell wall. The material that goes into solution in the different cases is located in the same regions of the fibre wall. The simplest explanation for the agreement between the different distribution curves seems to be that — (*1*) roughly the same kind of materials is removed from the fibre in the different cases and (*2*) the location of this material represents that of the accessible material in the cell wall. However, the distribution of the removed material or of the accessible material could also be interpreted simply as being due to the diffusion of the alkali or acid from the outer layers into the inner ones of the cell wall. In order to obtain some information about this possibility, the distribution curves of samples *2* and *3* may be compared. Both samples were hot alkali treated in exactly the same way (*see* above), but the latter sample was in the form of cross-sections, thus making the whole cross-area available to the alkali. The comparison shows that there are no principal differences in the distribution curves between the two samples, the curves being slightly smoother in the case of cross-sections. In the first case, the alkali must first penetrate the outer regions before reaching the inner ones; in the latter, on the other hand, the whole surface of the cross-section reacts simultaneously. Hence, the accessible material is removed regardless of the method of attack and there is no evidence of hindrance due to difficulties of diffusion through the outer regions of the cell wall.

In the following, the material distribution curves are discussed only for fibre samples when the whole fibres and not cross-sections have been

chemically treated. In addition, it is quite obvious that the treatment of whole fibres corresponds to what occurs when pulps of different qualities are produced.

Detailed information about the relative distribution curves

The influence of cold alkali treatment compared with the hot alkali treatment on holocellulose from spruce

The distribution of material in the cell wall of spruce holocellulose after cold alkali treatment shows the same type of distribution as is found for the hot alkali treated fibre samples. The carbohydrates are most concentrated in the regions around the lumen. The decrease of concentration towards the outer layers, however, is not so evident as for the hot alkali treated fibres. The distribution curve is more even and has no wave shape (Fig. 11 and 12).

During the cold alkali treatment, regions in the fibre wall that are inaccessible to the hot alkali treatment are opened. It is highly probable that the inaccessible regions for the hot alkali treatment are located around the lumen. The reason for this seems to be that cellulose is most densely packed in this region. As a consequence, the distribution of carbohydrates across the cell wall is more even for the cold alkali treated holocellulose fibre than for the hot alkali treated one. On the other hand, the difference in shape of the distribution curves between hot alkali and cold alkali treated holocellulose fibres could also be interpreted as only a small dissolving effect of the cold alkali treatment in the outer layers of the cell wall, which seems improbable. The question will be discussed further in the following section.

As a consequence of the considerable swelling of the fibre wall, part of the cold alkali treated fibres are difficult to use for microspectrographic purpose. The distribution curves presented here thus give information about the distribution of matter in those fibres that still remain intact after the cold alkali treatment.

The hydrolytic treatment compared with the alkali treatment of the holocellulose fibre

The short Nickerson hydrolysis (0.5 hr.) removed carbohydrates mainly from the outer parts of the cell wall and progressively less material towards the lumen. The acid penetrates the whole cell wall, mainly attacking the easily accessible non-cellulose material, which is first dissolved from the fibre. The distribution of the material left in the cell wall should therefore resemble that of a hot alkali treated holocellulose fibre, which is also the

case, as seen from a comparison of Fig. 11 and 13. More material is removed from the holocellulose fibre during 1 hr. Nickerson hydrolysis and the hydrolysed fibre is more or less damaged. An equidistantly distributed attack on the fibre along the fibre axis is a characteristic feature of the esterified hydrolysed fibre. As a result of this attack, cross-sections of the esterified fibre are formed. It is the distribution curves for such cross-sections that are discussed here.

A short prehydrolysis followed by a cold alkali treatment

Alpha-cellulose from spruce holocellulose was prepared with 17.8 per cent. sodium hydroxide at 0°C for 1 hr. after a short prehydrolysis (sample *6*). The combined hydrolysis and alkali treatment seems to be a method of high selectivity for cellulose and hemicellulose in spruce holocellulose fibres.[34]

The zig-zag course of the distribution curve may be a sign of an effective dissolution of accessible non-cellulose material. The variations in shape between different distribution curves for the same cross-section, on the other hand, are greater than for other fibre samples investigated. The reason for this may be the short time for prehydrolysis, during which not even hydrochloric acid is able to attack the accessible material uniformly (*cf.* Fig. 14).

'Absolute' distribution curves

In the sections above, only the shape of the different distribution curves was discussed. The relative amounts of matter within different regions in the cell wall of a fibre sample can be computed fairly accurately. On the other hand, there is no possibility of comparing the absolute amounts of matter in the cell wall for different kinds of fibres with only a knowledge of the relative distribution curves. In order to do this, further knowledge of the thickness of the cross-section and of the degree of swelling when the section is embedded in pyridine is necessary. Measurements of these quantities are both difficult and time-consuming and have not been performed. The soundest way to do this seems to be with X-ray microscopic methods applied to non-esterified native cellulose fibres when these methods are available in the future or with micro-interference methods.[45–54, 59]

Even without a knowledge of section thickness and degree of swelling, it is possible to compare distribution curves for different fibre samples on a quantitative basis, provided the different kinds of fibres have been prepared from the same fibre origin. In addition, the amounts of material removed by different treatments should be known. Thus, the distribution curves for

the cell wall of spruce holocellulose and of all kinds of carbohydrate fibres from spruce can be quantitatively compared, provided the yields of the carbohydrates in the fibres are known.

The procedure is illustrated in Fig. 19. Let *AB* represent the relative distribution of carbohydrates in a holocellulose fibre. The distance *CD* represents the width of the cell from lumen to the outermost layer. The ordinate represents, as usual, relative concentration units. The area below *AB* is a relative measure of the content of carbohydrates in holocellulose. Suppose now that holocellulose is treated chemically giving a new kind of carbohydrate fibre, *H'*, with a known yield of carbohydrates. The relative distribution curve for this new fibre may be represented by *BE*. As mentioned earlier, the highest concentration within the cell wall is arbitrarily set at 100. Even if the dissolved material is mostly located in the outer layers of the cell wall, this does not necessarily mean that the concentration of material at the lumen is the same for all kinds of carbohydrate fibres obtained from holocellulose. The area below *BE* (*BEDC*) is a measure of the carbohydrate content of the new fibre. Since the yield of the second fibre is known, it is possible to draw the relative distribution curve so that the area below it will be the same fraction of the area below the holocellulose distribution curve (*ABCD*) as the yield of the fibre. Let this 'absolute' distribution curve be *FG*. It is obvious that the curve *FG* still represents the relative distribution of matter in the cell wall of *H'*. The area *ABFG* represents the amount of dissolved matter when the fibre *H'* was prepared from the holocellulose. Furthermore, the area also shows the relative amounts of matter dissolved from different regions in the cell wall of holocellulose when the fibre *H'* is prepared. As the curve *FG* is drawn in Fig. 19, about 16 per cent. of the material around the lumen is dissolved from the holocellulose fibre, about 50 per cent. from the middle and about 80 per cent. from the outermost layer, when the fibre *H'* is prepared. In this sense, the curve *FG* may be considered 'absolute'. It is obvious that distribution curves for two fibres of spruce and birch origin, respectively, cannot be compared in this way.

The 'absolute' distribution curves for holocellulose, hot and cold alkali treated holocellulose and prehydrolysed and cold alkali treated holocellulose fibres, all from Swedish spruce, are collected in Fig. 20.

The distribution for holocellulose is represented by *AH*. The distribution of material after a hot alkali treatment is represented by the line *AB*, showing that the dissolved material has been deposited in the outer layers of the fibre. It should be observed that no material has been dissolved from the region in the immediate vicinity of the lumen. The distribution of

material after a cold alkali treatment is represented by *EF*, showing a more uniform dissolution of material across the whole cell wall. However, the majority of material dissolved from the fibre is situated in the outer layers of the cell wall. These curves show that the material left in the cell wall both after hot and cold alkali treatment is to a great extent deposited towards the lumen. This material consists mainly of cellulose. During the hot alkali treatment, not only are the accessible parts of the fibre wall attacked, but the surfaces of the cellulose strings as well. Since the cellulose strings are less densely packed in the outer regions of the cell wall it can be expected that the attack on the cellulose system takes place mainly in these regions. This assumption is verified by a comparison of the distribution curves for hot and cold alkali treated holocellulose fibres. As is seen from Fig. 20, the hot alkali treatment has removed more material from the outer regions of the cell wall than has the cold alkali treatment. The difference, represented by the area *BFG*, is small, but the tendency is obvious. If, on the other hand, the material density around the lumen for the two kinds of matter is compared, a marked difference between hot and cold alkali treatment can be observed. The relatively small swelling power of the hot alkali treatment is not sufficient to open the dense fibre structure around the lumen. The non-cellulose material deposited in this region cannot be removed. The non-cellulose material is dissolved from the fibre wall only when cold alkali treatment accompanied by extensive swelling is applied. The position of the material that can be removed only after extensive swelling is represented by the area *GEA*. The distribution of material in the cell wall of the prehydrolysed and cold alkali treated spruce holocellulose is very similar to that of cold alkali treated fibres. The short prehydrolysis has attacked a small portion of material situated mainly in the outer layers of the cell wall, as judged by the area representing the difference in distribution between the two types of cold alkali treatment.

It should be observed that the above discussion of the different ways of attack on the fibre material is limited to the fibre material described here — holocellulose from spruce. For other kinds of cellulose fibres, which may differ in their properties from those of holocellulose fibres, the conclusions arrived at in this work may not be applicable.

The distribution of material in cotton is fairly even across the cell wall. During the alkali treatment of standard cotton, about 5 per cent. were dissolved from the cell wall. There are indications that the dissolved matter is mainly deposited in the outer layers of the cell wall.

In the rayon pulp fibre (Fig. 21), most of the accessible parts of the cell wall are removed. The distribution of the remaining material resembles that of cellulose reported above. The concentration of cellulose at the lumen is high. Only about half as much material is deposited in the outermost layer of the cell wall.

In the acetylation pulp fibre, part of the cellulose system has been removed since the yield is rather low. This is illustrated in Fig. 22, showing the material distribution across the cell wall of an acetylation pulp fibre. There has been severe attack even upon the material around the lumen. Across the whole cell wall the accessible material is removed together with parts of the ordered cellulose. The acetylation pulp fibre must have a rather open and porous structure in comparison, for instance, with a holocellulose fibre.

The bleached sulphate fibre (Fig. 23) shows a remarkable distribution. The region around the lumen has a very high concentration of material. If the high degree of swelling at the lumen in comparison with that of the perimeter of the fibre is taken into consideration, the relative concentration of material even exceeds the value of 100, which is theoretically impossible and must be attributed to errors in the measurement. Whether the properties of this latter layer in the fibre are native or are a consequence of the sulphate cooking treatment cannot be decided by microscopical methods.

If the sulphate fibre is prehydrolysed, the material distribution will change considerably compared with that of the untreated sulphate fibre (Fig. 24). The prehydrolysis either removed or makes it possible for the subsequent alkali treatment to remove a great part of the material around the lumen. The distribution is approximately even, with a slightly increasing concentration towards the lumen. Thus, one essential difference between the two sulphate fibre types lies in the different properties of the layer around the lumen.

It should be noted that the two sulphate fibres are manufactured from pine. No measurements of the distribution of material in holocellulose fibres from pine were made. An even distribution of material has been assumed, analogous to that obtained for the corresponding fibre from spruce.

Summary

From the microspectrographic measurement reported above it can be stated that —

1. Holocellulose is approximately evenly distributed across the cell wall in spruce and birch. In the latter case, there is perhaps a tendency towards an increasing amount of carbohydrate material towards the outer regions of the cell wall.

2. Cellulose is most densely packed around the lumen in spruce and birch with the relative packing density in the outermost layers about half of that around the lumen.
3. Hemicellulose is about half or more of the carbohydrate material in the outer regions of the cell wall of spruce and birch. Around the lumen, the percentage of carbohydrate material consisting of hemicellulose is estimated at 10–20 per cent.
4. In cotton, cellulose is approximately evenly distributed across the cell wall. A small amount of accessible material is deposited mainly in the outer regions of the fibre wall.

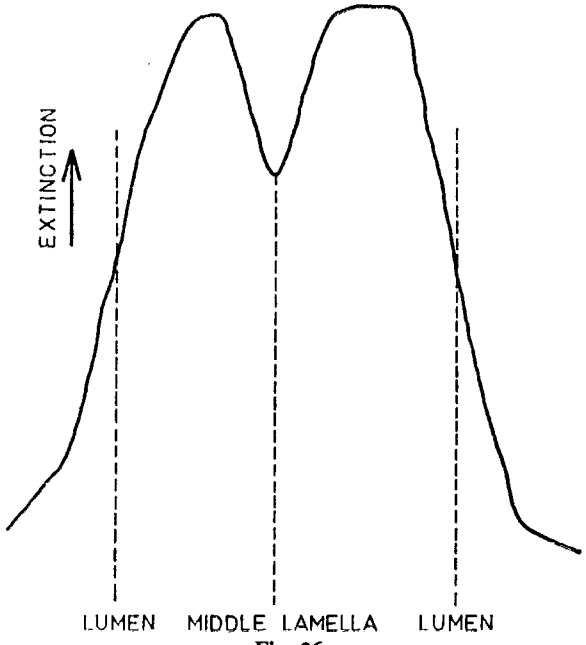


Fig. 26

MASS DISTRIBUTION IN THE CELL WALLS OF SWEDISH SPRUCE AND BIRCH

It would be of interest to connect the distribution of the carbohydrate and lignin components quantitatively — that is, to obtain information of the relative distribution of mass (dry weight) across the cell wall of wood. With the knowledge of the specific weights of the cell wall components, it would also be possible to obtain information about the relative porosity of different parts of the cell wall. Facts about the porosity of the cell wall of wood would be of some importance for the study of diffusion and penetration processes in the cell wall when wood is chemically modified to obtain different kinds of technical products.

A solution to this problem is to apply quantitative microradiographic methods (for a review, *see* Lindström [35]) as described above.[6] Using polychromatic X-rays of the wavelengths 8Å and softer, the mass (dry weight) per surface unit of sections of cytologic structures (for example, a cross-section of wood, Fig. 25) can be determined.

Fig. 26 shows a typical example of the variation of the absorption (measured as extinction) of X-rays across the two cell walls from lumen to lumen. The extinction is lower in the compound middle lamella than in the secondary wall, which is also the case for birch. The ratios between the extinctions in the secondary wall and in the compound middle lamella are given in Table 3. The extinction values for the two regions represent the maximum extinction value of the secondary wall and the minimum extinction value of the compound middle lamella. The difference of extinction ratios for spruce and birch is significant. The extinctions were measured on microradiograms having the greatest resolving power and the values are selected from about one hundred microradiograms.

TABLE 3
Extinction ratios

	Spruce	*Birch*
	1.63	1.39
	1.24	1.23
	1.94	1.68
	1.42	1.63
	1.54	1.20
	1.26	1.35
	1.40	1.32
	1.54	1.32
	1.40	1.25
	1.62	1.45
	1.31	1.44
	1.85	1.60
	1.53	1.36
	1.54	1.41
	1.60	
	1.87	
	1.46	
	2.00	
	1.21	
	1.18	
	1.64	
	1.40	
	1.40	
	1.28	
	1.53	
	1.50	
	1.65	
Average	1.52	1.40

The ratio of the masses (dry weight) per surface unit for cross-sections of the two cell wall regions was calculated as follows.

The X-ray absorption in the two cell wall regions is expressed as —

$$\left(\frac{\mu}{\rho}\right)_c \cdot m_{cW} + \left(\frac{\mu}{\rho}\right)_l \cdot m_{lW} = \left(\frac{\mu}{\rho}\right)_{ref} \cdot u_W \cdot w \quad \ldots\ldots\ldots \quad (1)$$

$$\left(\frac{\mu}{\rho}\right)_c \cdot m_{cL} + \left(\frac{\mu}{\rho}\right)_l \cdot m_{lL} = \left(\frac{\mu}{\rho}\right)_{ref} \cdot u_L \cdot w \quad \ldots\ldots\ldots \quad (2)$$

where $\left(\frac{\mu}{\rho}\right)_c$, $\left(\frac{\mu}{\rho}\right)_l$ and $\left(\frac{\mu}{\rho}\right)_{ref}$ are the mass absorption coefficients for cellulose, lignin and the reference system respectively and

m_{cW} = mass of carbohydrates in the secondary wall (g./cm.²)

m_{cL} = mass of carbohydrates in the compound middle lamella (g./cm.²)

m_{lW} = mass of lignin in the secondary wall (g./cm.²)

m_{lL} mass of lignin in the compound middle lamella (g./cm.²)

u_W number of foil equivalents of the secondary wall determined by comparing the photometer deflection by the calibration curve of the step wedge [6, 35, 36]

u_L = number of foil equivalents of the compound middle lamella

w = the weight per surface unit of one reference foil. [6, 35, 36]

Dividing equation (*1*) by equation (*2*), the following expression is obtained —

$$\frac{\left(\frac{\mu}{\rho}\right)_c \cdot m_{cW} + \left(\frac{\mu}{\rho}\right)_l \cdot m_{lW}}{\left(\frac{\mu}{\rho}\right)_c \cdot m_{cL} + \left(\frac{\mu}{\rho}\right)_l \cdot m_{lL}} = \frac{u_W}{u_L} = k \quad \ldots\ldots\ldots \quad (3)$$

The values of k for spruce and birch are 1.52 and 1.40, respectively Table 3). Equation (*3*) is transformed into —

$$\frac{\left(\frac{\mu}{\rho}\right)_c \cdot x \cdot M_W + \left(\frac{\mu}{\rho}\right)_l \cdot (1-x) \cdot M_w}{\left(\frac{\mu}{\rho}\right)_c \cdot (1-y) \cdot M_L + \left(\frac{\mu}{\rho}\right)_l \cdot y \cdot M_L} = k \quad \ldots\ldots\ldots \quad (4)$$

where

$M_W = m_{cW} + m_{lW}$, the total mass in the secondary wall (g./cm².)

$M_L = m_{cL} + m_{lL}$, the total mass in the compound middle lamella (g./cm.²)

x = the weight fraction of carbohydrates in the secondary wall.

y = the weight fraction of lignin in the compound middle lamella.

Equation (*4*) may be transformed into —

$$\frac{M_W}{M_L} = \frac{1-y + \frac{\left(\frac{\mu}{\rho}\right)_c}{\left(\frac{\mu}{\rho}\right)_l}}{x + \frac{\left(\frac{\mu}{\rho}\right)_c}{\left(\frac{\mu}{\rho}\right)_l} \cdot (1-x)} \cdot k \quad \ldots\ldots\ldots\ldots \quad (5)$$

where $\left(\frac{\mu}{\rho}\right)_c = 1.076$ and $\left(\frac{\mu}{\rho}\right)_l = 908$ (8.32 Å).

From equation (*5*), it is possible to calculate the ratio M_W/M_L, the ratio of masses per surface unit (or volume unit, since the thickness is constant over the cross-section investigated) of cross-sections for the secondary wall and middle lamella as a function of x and y — that is, the composition of the two cell wall regions with respect to carbohydrates and lignin. With a knowledge of the specific gravity for carbohydrates (for cellulose about 1.55[37]) and for lignin (about 1.4[38]), it is possible to calculate an average specific gravity for the secondary wall and for the middle lamella (ρ_W and ρ_L, respectively) for different values of x and y. If the cell wall material is uniformly packed in the two cell wall regions, the ratio M_W/M_L would be equal to the ratio ρ_W/ρ_L However, for all possible values of x and y, $\frac{M_W}{M_L} > \frac{\rho_W}{\rho_L}$, which means that the middle lamella has a more porous structure than the secondary wall, which seems to be slightly more evident in the case of spruce than of birch. The results of the calculation are shown in Table 4.

The first column shows the weight fraction of carbohydrates in the secondary wall, the second column the weight fraction of lignin in the compound middle lamella. In the third column, the ratio of ρ_W/ρ_L is given and, in the fourth, the ratio M_W/M_L calculated from equation (*5*). In the fifth column, the relative packing density (RPD) of the material in the compound middle lamella is tabulated. In order to define the relative packing density of the material in the compound middle lamella, the material in the secondary wall is *assumed* to fill up this region completely. With this arbitrary assumption, the relative packing density of the material in the compound middle lamella is defined as $\frac{\rho_W}{\rho_L} : \frac{M_W}{M_L} \times 100$ per cent. With this definition, the relative packing density of the material in the secondary wall is obviously 100 per cent.

TABLE 4

	x	y	$\frac{\rho_W}{\rho_L}$	$\frac{M_W}{M_L}$	RPD,%
Spruce	0.9	1.0	1.10	1.30	84
		0.9	1.09	1.33	82
		0.8	1.07	1.35	80
		0.7	1.06	1.38	77
	0.8	1.0	1.08	1.33	81
		0.9	1.07	1.35	80
		0.8	1.06	1.37	78
		0.7	1.05	1.40	75
	0.7	1.0	1.07	1.35	79
		0.9	1.06	1.37	77
		0.8	1.05	1.40	75
		0.7	1.04	1.42	73
Birch	1.0	1.0	1.11	1.18	94
		0.9	1.10	1.21	91
		0.8	1.09	1.22	89
		0.7	1.07	1.24	87
	0.9	1.0	1.10	1.20	91
		0.9	1.09	1.22	89
		0.8	1.07	1.24	87
		0.7	1.06	1.27	84
	0.8	1.0	1.08	1.22	89
		0.9	1.07	1.24	87
		0.8	1.06	1.26	84
		0.7	1.05	1.29	82

The most probable values for x and y were discussed in detail.[6]

It should be observed that all measurements are made on cross-sections in vacuum. The influence of shrinkage was not corrected for, since reliable measurements on the shrinkage of different layers of the cell wall of spruce and birch are not available.

It would be of great interest to compare the distribution of mass (dry weight) across the cell wall with those of carbohydrates and lignin. However, since the resolution for quantitative microradiographic work had its lower limit at about 2 – 3 μ, the results from microradiographic and microspectrographic investigation on wood and cellulose fibres could not be compared quantitatively in detail. For instance, the distribution of mass within the compound middle lamella (about 2 μ) cannot be measured. It would perhaps be possible to compare the mass per unit area for the outer and inner part of the secondary wall.

Since only relative measurements have been made for the two kinds of wood in this work, it is not possible to compare the results on an absolute basis, that is, to compare the mass per unit colume in the cell walls for spruce and birch. If, however, the fact is taken into consideration that the cell wall can roughly be divided into two parts of considerably different character — namely, the compound middle lamella and the secondary wall — there may be a certain possibility of making quantitative comparisons. It is known that birch contains less lignin than spruce. Furthermore, the secondary wall is less lignified in birch than in spruce. The main difference in lignin content may be attributed to the difference in the lignin content of the secondary wall. It is not unreasonable to assume that in the compound middle lamella the concentration and the deposition of lignin is about the same for the two kinds of wood. As a consequence of this assumption, the mass (dry weight) of material per unit volume would be somewhat greater in the secondary wall of spruce than in the same region of birch. Thus, the secondary wall of spruce seems to be less porous and contains more lignin than the secondary wall of birch.

Summary

Thus, if there is no possibility of combining the results of the distribution of the components in the cell wall in minute detail, the results nevertheless give a general picture of the chemical composition of the various layers in the cell wall. The cell wall can roughly be divided into two parts — the compound middle lamella and the secondary wall. The former represents the lignin part, the second the carbohydrate part of the fibre wall. The most rapid change of the lignin distribution takes place in the outer layer of the secondary wall. The concentration of lignin in the middle lamella is estimated to be 60 – 90 per cent. and that around the lumen to be 10 – 20 per cent. (spruce). The cell walls of certain hardwoods are less lignified and those of reaction woods are practically free from lignin. In spruce, about 60 per cent. of the carbohydrate material in their outermost layer is hemicellulose, whereas only about 15 per cent. of the carbohydrate material around the lumen consists of hemicellulose. The distributions of lignin and hemicellulose follow one another quantitatively, which could be interpreted as an indication of a bond of some kind between hemicellulose and lignin. However, the similar distributions of the two components could also be considered as a consequence of the distribution of available space between the ordered cellulose strings. Cellulose is the dominating component around the lumen and the amount in the outermost layer is only about half of that around the lumen. Consequently, there is more space in the outer regions for deposition of hemicellulose and lignin.

Summarising the above points, the structure of the cell wall from the point of view of distribution can be described in the following way. Cellulose forms an ordered system of strings more densely packed around the lumen and more loosely aggregated in the outer regions of the cell wall. In between are deposited, more isotropically, hemicellulose and lignin, both of which are more concentrated towards the outer layers of the cell wall. The change of distributions of the components from the secondary wall to the compound middle lamella is not known in detail. Particularly the relative amounts of cellulose (if any) and hemicellulose in this latter region have still to be determined. The dominating role of lignin in this part of the cell wall is clearly demonstrated by ultra-violet microscopy. X-ray absorption measurements on cross-sections of wood in vacuum show that the compound middle lamella has a lower packing density than the rest of the cell wall.

Thus, the cell wall of wood seems to be highly ordered in the region around the lumen, the degree of order decreasing towards the middle lamella, which probably can be considered as an amorphous structure.

Hence, the ratio of order/disorder seems to be high around the lumen and lower in the outer regions of the cell wall, possibly giving the former part more auto-protective properties towards chemical attack than the latter regions, implying a local variation of chemical properties across the cell wall of the fibre. Assuming a constant crystalline/amorphous ratio of the cellulose strings across the whole cell wall, this ratio would vary across the cell wall of most pulp fibres, being highest in the region around the lumen.

The non-uniform distribution of cellulose and hemicellulose in carbohydrate fibres could be confirmed in an indirect way from measurements of diffusion of Congo Red in different pulp fibres. [39] The great difference in the rate of dye adsorption in the early and later stages of dyeing of the pulp fibres was attributed to a non-uniform distribution of carbohydrate material in the fibre walls.

A similar picture was obtained by Boulton and Morton [40] from diffusion measurements on natural cellulose fibres. They found that the rate of dyeing was very rapid in the beginning, the equilibrium being attained very slowly. They attributed this behaviour to a non-uniform fine structure across the fibre wall.

The distribution of carbohydrates is confirmed in an interesting way from the work on ancient buried and decayed wood by Varossieau, [41] who made microscopic investigations of the cell wall structure of wood that had been

buried for periods varying 30 — 600 years. It was found that the deterioration began in the regions around the lumen and proceeded towards the outer layers of the cell wall. It was further found that the percentage of pentosans in the decayed wood increased with time and deterioration, [42] which may be explained from a non-uniform distribution of cellulose and hemicellulose across the cell wall in the manner reported in this work.

Part of the investigations by Haas [43] on the morphological and chemical structure of carbohydrate fibres from wood are in agreement with the results obtained in this paper.

This picture of the distribution of the main components in the cell wall, based on quantitative and semi-quantitative data, is quite in accordance with earlier, more qualitative investigations based on, for instance, micro-dissection, colour reactions and solubility tests (for review, *see* Harlow [2] and Wardrop[44]).

Future work

The further development of the microchemical work on wood and cellulose fibres is to a great extent dependent upon new micro-optical methods.

When the X-ray microscope [45–47] is available, the determination of mass will be carried out with considerably increased resolution and with greater possibility of measuring quantitatively the influence of shrinkage in different cell structures.

Ingenious methods have recently been suggested (appendix) [48–54] for the determination of the mass of cells with the aid of interference microscopy. A great advantage of these methods is that it is possible to study the object without drying it. It can, for instance, be investigated in water as the embedding medium. These methods considerably reduce the number of processes that must be applied in order to obtain the preparate suitable for investigation. The study of carbohydrates in the fibre would be possible, for instance, without esterification and subsequent swelling.

The composition of the compound middle lamella would, for example, be possible to study on a cross-section of a series of wood samples delignified to different degrees and with known carbohydrate composition. A suitable material for the study of the composition of the compound middle lamella seems to be certain types of larch wood, from which great amounts of water soluble polysaccharides can be dissolved with water. [55] It is not unreasonable to assume that the majority of the water soluble material is deposited in the compound middle lamella.

The determination of the ratio of the crystalline to amorphous regions in different layers in the cell wall seems to be possible by future study with the X-ray microscope.

There also seems to be a promising field of research in the application of microspectrographic methods in the ultra-violet radiation range to studies of lignin in the cambium layer of wood. "The physiological and biochemical processes in the cambium, in its maturing derivatives and their functioning in the living tree are imperfectly understood and progress in their investigation is relatively slow. Owing to obvious economic urges, chemists and physicists have concerned themselves largely with analyses of dead wood and with the walls of certain of its constituent cells — for example, wood fibres. Plant physiologists have concerned themselves largely with shorter lived plants, which provide easier material for controlled experimentation. Thus, there is an urgent need at present for a much broadened programme of research in tree physiology, involving more closely co-ordinated effort by workers in different scientific disciplines. It is essential in this connection that data obtained by new techniques and methodologies be interpreted in the light of data that have been accumulated in other diversified fields of research." — Bailey. [56]

The study of diffusion and penetration in the cell wall of wood will be an important field of investigation in the future, particularly for the study of technical processes in the pulp and paper industry as, for example, cooking, bleaching and refining. Investigations of an indicative character have been carried out earlier by the author. [57,58] It should be clearly pointed out, however, that this vast field is practically unexplored.

Appendix

Interference microscopy

It is possible to determine the mass per unit area (g./cm.^{2}) of the different components in a microscopic specimen (its chemical composition) with a resolving power of 0.5 — 1.0 μ. The method is based on the fact that there is a relationship between the optical path difference (o.p.d.) and the difference between the refractive index of the specimen and that of the embedding medium. The measurements of o.p.d. can be made with an interferometer microscope (for example, Dyson [49]). In order to determine the mass per unit area (or unit volume, if the thickness of the specimen is known) for n components, the o.p.d. must be measured with the specimen embedded in n different embedding media with n different refractive indices. It must be assumed that the embedding media do not alter, by interaction,

the refractive indices of the components and further that all pores in the specimen are filled with the fluid embedding medium. If this method is applied to wood, it is thus possible to determine the mass per unit area for lignin and carbohydrates in different regions of the cell wall, that is, how the chemical composition of wood changes across the cell wall. A great advantage of this method is that the mass of lignin and carbohydrates can be determined simultaneously and that the specimen is left intact for other investigations, since no chemical treatment is involved. It can therefore be stated that interference microscopy, to a certain extent, can be used instead of the micro-optical methods described above. In the following, the main background of the method is given.

As an optical model, a plane parallel plate with the geometrical thickness t is used.

1. The plate is uniformly filled with a substance with refractive index μ and density ρ. The plate is embedded in a medium with refractive index μ_m. The o.p.d. (φ_m) between rays passing the plate and those passing outside the plate can be measured with the aid of the interference microscope. The o.p.d. is given by —

$$\varphi_m = (\mu - \mu_m) \cdot t \ .$$

Now, $t \cdot \rho = m$ (mass per unit area).

$$\text{Hence, } m = \frac{\varphi_m}{\chi} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{where } \chi = \frac{\mu - \mu_m}{\rho} = \text{constant}$$

2. The plate is filled with two substances, *1* and *2*, with refractive index and density μ_1, ρ_1 and μ_2, ρ_2, respectively. It is further assumed that the substances do not completely fill the plate. A certain fraction, f, is pores. It is assumed that there is no interaction between embedding medium and the components *1* and *2*. Further, the embedding medium should permeate the pores. That is to say, the o.p.d. due to the porous plate is the same as it would be if the substances *1* and *2* were packed, with no spaces, in a plate occupying the same projected area as the original plate. Let the effective thickness (the thickness of the packed component) of the components *1* and *2* be t_1 and t_2.

Then $t_1 + t_2 = t\,(1-f)$.

The plate described above can be used as a model of a cross-section of a woody cell wall with the two components lignin and carbohydrates and a certain fraction of pores.

We now measure the o.p.d. of the plate first embedded in a medium with refractive indix μ_m and then in a medium with refactive indix μ_n. In the first case we have —

For component *1* — $\varphi_m\ (1) = (\mu_1 - \mu_m) \cdot t_1$.

For component *2* — $\varphi_m\ (2) = (\mu_2 - \mu_m) \cdot t_2$.

Further, $\varphi_m = \varphi_m\ (1) + \varphi_m\ (2)$

$$= \frac{\mu_1 - \mu_m}{\rho_1} \cdot m_1 + \left[\frac{\mu_2 - \mu_m}{\rho_2} \cdot m_2\right]$$

$$= \chi_{1m} \cdot m_1 + \chi_{2m} \cdot m_2 \qquad \ldots\ldots(2)$$

where m_1 and m_2 are the mass per unit area for component 1 and 2 respectively.

In the second case, the following relation is obtained —

$$\varphi_n = \chi_{1n} \cdot m_1 + \chi_{2n} \cdot m_2 \qquad \ldots\ldots(3)$$

From (*2*) and (*3*), m_1 and m_2 can be computed (that is to say, the chemical composition), since φ_m and φ_n have been determined experimentally (x_{1m}, χ_{2m}, χ_{1n} and χ_{2n} are constants). The effective thickness, $t_1 + t_2$, is obtained from —

$$t_1 + t_2 = t\,(1-f) = \frac{\varphi_m - \varphi_n}{\mu_n - \mu_m} \qquad \ldots\ldots(4)$$

In order to calculate f, the geometric thickness t of the specimen must be known.

The average density of the specimen is determined by —

$$\rho_{av} = \frac{m_1 + m_2}{t_1 + t_2} = \frac{(m_1 + m_2)(\mu_n - \mu_m)}{\varphi_m - \varphi_n} \qquad \ldots\ldots(5)$$

Thus, it is possible to determine the mass of carbohydrates (m_c) and that of lignin (m_l) per unit area in different regions of the cell wall simply by measureing two o.p.d. φ_m and φ_n of a cross-section of wood embedded in two different embedding media with refractive indices μ_m and μ_n, respectively. It is further possible to determine the effective thickness (and from that a relative measure of the porosity) and the average specific gravity for different parts of the cell wall.

In this way, the earlier results of Lange for the distribution of carbohydrates and lignin could be confirmed. Cross-sections of spruce (*Picea excelsa*) were embedded in benzene and bromobenzene and the corresponding o.p.d. were measured with the aid of a Dyson interference microscope. Measurements were made for the compound middle lamella and a point in the middle of the secondary wall. The results are given in Table 5.

TABLE 5

	$\varnothing_6$	$\varnothing_{brb}$	*Carbo-hydrate* %	*Lignin*, %	ρ_{av}	*teff*	RPD, %
MLI	+1.54	+0.44	37.7	62.3	1.445	10.0μ	85
SWI	+0.75	—0.54	94.4	5.6	1.589	11.8μ	
MLII	+1.22	+0.29	45.5	54.5	1.445	8.5μ	67
SWII	+0.83	—0.58	93.9	6.1	1.589	12.8μ	
MLIII	+1.43	+0.54	18.7	81.3	1.44	8.1μ	88
SWIII	+0.75	—0.26	83.3	16.7	1.561	9.2μ	

$\varnothing_6$ = o.p.d. in benzene ML = middle lamella
$\varnothing_{brb}$ = o.p.d. in bromobenzene SW = secondary wall
RPD = relative packing density in ML (in SW = 100 per cent.)

Collection of data

It is highly desirable to have equipment for collecting and handling data in connection with the micro-optical methods described above. An apparatus for that purpose has recently been constructed.[60] This scanning and computing cell analyser for quantitative purposes performs a calculation of 12 000 transmission or density values in a microscopic field. The values are stored with maintained spatial localisation. The calculation of the 12 000 values takes 4 min. An area of down to $0.2\mu^2$ for an individual measurement can be calculated if a light microscope is used.

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DISCUSSION

MR. J. BOS: I want to make some comments on the degree of polymerisation (D.P.) of reaction wood cellulose.

The occurrence of reaction wood in hardwood has been the subject of investigations from different viewpoints. The papermaking properties of pulps produced by the sulphate process from this abnormal wood have been determined especially by Jayme and his co-workers, as well as recently by Watson.

As there is also rather a quantity of poplar pulp produced by the sulphite process, we thought it useful to investigate the influence of reaction wood in poplar on this kind of pulp.

On a number of trunks of *Populus eur. serotina*, the Wood Institute T.N.O. at Delft determined microscopically the distribution of the reaction wood. From these, we selected four with heavy occurrence of reaction wood throughout their length for the pulping experiments. Under standard conditions, with a maximum temperature of 130°C, we prepared an easy bleaching paper grade sulphite pulp from the part of each trunk with reaction wood, as well as from the opposite part without reaction wood. The yield ranged 49 — 51 per cent. for the normal wood on a wood basis and 57.5 — 64.5 per cent. for the reaction wood.

The properties of handsheets of the unbeaten pulps differed considerably. As an example, the breaking length for the pulps from the normal wood was 5 000 — 6 000 m. and 2 100 — 3 250 m. for the pulps from the reaction wood.

There exists a strongly negative correlation between the strength properties of the pulps and the yield. This means that the strength of the pulp depends on the quantity of reaction wood fibres. I think it is not of much use now to mention all the other determinations we made on the wood and the pulp, but I wish to mention one observation that, to our knowledge, has not been published in the literature before. We determined the cuoxam viscosity of the pulps and found that the D.P. showed a correlation with pulp yield in the same way as the strength properties (Table 1, columns 6 and 7).

As it is possible that sulphite pulping causes degradation of the cellulose chains, especially with the reaction wood fibres, we determined also for tree number 6 (with the highest pulp yield from the reaction wood part) the D.P. of a holocellulose prepared from the wood by the acid chlorite method and of

the same after extraction with 5 per cent. potassium hydroxide in an atmosphere of nitrogen. The results are given in Table 2.

We think these figures mean that there is, indeed, a certain degree of degradation of the reaction wood by sulphite pulping, so that the difference with normal wood is accentuated; but the results with the holocellulose indicate that it is fairly certain that the D.P. of the alpha-cellulose of the reaction wood fibres is lower than that of the normal wood fibres.

TABLE 1

Properties of sulphite pulps from normal wood (N) and reaction wood (R) of four poplar trees

Tree number	*Yield, %*		*Breaking length, m.*		$\overline{DP}$	
	N	*R*	*N*	*R*	*N*	*R*
13	49.2	57.5	5 150	3 250	1 460	1 120
3	51.2	59.5	4 950	2 900	1 730	980
7	49.1	60.9	6 000	2 750	1 630	890
6	50.9	64.4	5 950	2 100	1 530	760

TABLE 2

Degree of polymerisation of holocellulose from tree No. 6

Pulp condition	$\overline{DP}$	
	Normal wood	*Reaction wood*
Original holocellulose	1 080	930
After extraction with 5% KOH under nitrogen	1 430	940

We are continuing our investigations with the determination of the molecular weight distribution curves of the two kinds of wood from the same trunk to obtain more thorough knowledge of the chemical structure of the secondary wall of the reaction wood fibre.

DR. W. GALLAY: With reference to the second paper this morning, I would like to recall to your memory what I consider to be a classical experiment reported by Hägglund several years ago. In the preparation of sulphite

pulp, he used a rotating digester, having a perforated septum down the middle, so that two separate sulphite production experiments could be carried out under the same conditions in so far as liquor, temperature and pressure were concerned. The chips were from the same source. Major differences in strength properties were obtained by varying only the extent of the filling of the digester with wood, far higher strength being obtained when chip movement was reduced to a minimum. Many of you will doubtless recall the work.

It would appear that the explanation cannot be ascribed solely to damage done by extensive defibration of wood in the digester, since the integrity of the chips was apparently well maintained. Some explanation might well be provided by considering a continued mechanical compression and relaxation of the chips. When the latter only partially filled the container, it resulted in a condition allowing undesirable features of access of acid to portions of the structure.

It is pointed out that this is the condition brought about in acid degradation of 'acid-susceptible' wood, following compression or shearing forces. Unfortunately, ordinary chipping as at present practised introduces a great deal of this acid susceptibility, with consequent serious effect on pulp strength.

I wonder in this connection whether our expert microscopists who have reported so effectively at this meeting have ever produced pictures of an actual separation at the fibre wall of the tracheid resulting from mechanical action of this kind, which in turn would allow for access of hot acid to very sensitive elements.

DR. P. LANGE: If you take out chips during different phases of a sulphite cook, make cross-sections from them and inspect them under the microscope, they are very often seen to be split along the radial middle lamella, owing to the cutting action of the microtome knife. It is possible therefore that the chips treated as Dr. Gallay described may easily be damaged in these middle lamella regions. The reason may be that the cooking acid attacks the middle lamella via the pits and, since the pits are mostly to be found on the radial surfaces, these are weakened by the hydrolytic action of the cooking acid, resulting in a decreased resistance to mechanical action.

DR. B. G. RÅNBY: If you look upon sulphite cooking as a process, you are surprised that it is at all possible to obtain a cellulose of such a high degree of polymerisation from it: the temperature is high (130° — 145°C) and the pH quite low (1.5 — 2) towards the end of the process. If you use groundwood (that is, newsprint) or even cotton fibres (which are much better

crystallised) in the sulphite process instead of the wood chips, the cellulose depolymerises very badly. The resulting pulp fibres are degraded and they give paper of low strength. I do think that the reason for this is not only a better penetration of the cooking liquid through the crushed cell walls; it lies deeper than that.

The original, undamaged pulp fibres, without sharp breaks or ruptures, have a structure with the crystalline cellulose well protected as deposited in the fibre wall together with the hemicellulose. According to Lange's measurements, the hemicellulose is distributed throughout the wood cell walls. If you remove the hemicellulose, the crystalline cellulose fibrils are left much less protected — that is, more easily accessible. Even drying the fibres after extraction of the hemicellulose considerably increases the accessibility to hydrolysis of the cellulose framework, as further described in my paper, with a lower D.P. after hydrolysis as a result. With the hemicellulose remaining in the fibres (holocellulose), while they are being dried, the cellulose fibrils are hardly affected.

In a region where a pulp fibre is crushed or sharply bent, it seems likely that cellulose fibrils will be deformed more than their crystalline lattice can take elastically, so that slip planes develop in the lattices. The lattice will be more disordered and consequently more accessible to chemical attack at these points. Such phenomena would explain why mechanically damaged wood fibres are hydrolysed so fast at the break points that they disintegrate into fragments as often observed in practice.

This interpretation would mean that the protection of the cellulose in a sulphite cook at the high acidity and the high temperature used is due to two factors — the long extended crystalline regions inside the individual cellulose fibrils and the close packing with the incrusted hemicellulose. If the cell wall layers are ruptured, regardless of whether it is done by external forces or by shrinkage in drying, accessible points in the cellulose fibrils (slip planes in the cellulose lattice) are introduced, which opens the way to chemical degradation.

From this point of view, it was very interesting to hear about the lower D.P. values found for cellulose in tension wood. Tension wood, if the term is correct, should mean wood tissue that has been under stress. Mechanical tension could introduce slip planes in the cellulose lattice, which in themselves might degrade the cellulose chains. Slip planes in particular would make the cellulose more accessible to chemical attack during its isolation. It would be interesting therefore to know the levelling off D.P. after hydrolysis of these pulp fibres with dilute mineral acid. You would expect lower values than for

normal woodpulp fibres. Are you sure, Mr. Bos, that you extracted the cellulose without further degradation?

MR. BOS: We determined the levelling off D.P. by hydrolysis of the chemical pulps with hydrochloric acid. The results should be considered as preliminary and have to be confirmed by further data.

If the D.P. is plotted against the residue, as in your paper, the slope for reaction wood is less steep than for normal wood and therefore resembles more that for cotton. The mean position of the two lines does not differ, however.

DR. RÅNBY: Unextracted holocellulose behaves differently from chemical grade pulps and cotton fibres when hydrolysed. The initial reaction brings the holocellulose to a levelling off D.P. much higher than those of chemical grade pulps and cotton, but the continued hydrolysis causes a more marked and gradual drop in D.P. of the residual hydrocellulose from holocellulose. These data suggest then that the crystalline regions of the cellulose fibrils in the original wood are longer, but of lower general lattice order than those of cotton fibres. It is a surprising finding that cellulose from compression wood behaves more like cotton cellulose.

MR. BOS: Although it was not mentioned in the paper read by Christensen, I can add that Wardrop has produced X-ray photographs of Eucalyptus fibres and they show the degree of crystallinity of the cellulose from tension wood is much higher than for normal wood. It is of the order of that for cotton.

Recalling the earlier discussion on hemicellulose and paper strength, I think it would be useful to study further the compression wood in softwoods and the tension wood in hardwoods from the papermaker's point of view. We can say that the inner structure of the cell wall is quite different. In this way, we can have a better knowledge of the influence of the structural features of the cell wall for papermaking purposes and we can achieve faster results than by studying solely pulp fibres from normal wood.

MR. F. M. CROOK: Dr. Christensen mentioned this morning that I had some further information on Wardrop's proposed structure of the S1 layer of the secondary wall. I think it is probably appropriate for me to mention it now.

I have Wardrop's electron micrographs with me. We tried them on the episcope last night, but unfortunately they did not have enough contrast to be normally visible on the screen, so I am afraid I will have to try to draw the scheme for you on the blackboard.

Under the primary wall with its disordered arrangement of fibrils, Wardrop proposes that the first appearance of the S1 layer is a series of fine microfibril bundles. Overlying that, approximately at rightangles, comes a second layer of fine microfibril bundles in the fashion of Fig. 17 in Wardrop's paper.

Wardrop calls this stage of the organisation his fine grid. Overlying this fine grid, there is a layer of coarse microfibril bundles in the same direction as one of these two. If you look at Fig. 18 in the paper, you will see something like that there.

The development of the S1 layer continues still further. Overlying this again comes a second series of coarse fibril bundles. Wardrop calls this a coarse grid system. This upper layer may continue to have bundles deposited in it to form a complete sheet. Wardrop considers that this may reconcile a good many conflicting views of the S1 layer. For one thing, it would agree with Asunmaa's finding that there were probably several layers of S1 wall thickening. It also confirms several ideas on cross-fibrillated structures. The existence of a complete sheet in one direction would explain some confusion about the existence of a cross-fibrillated structure and the observation of a single angle of extinction in the polarising microscope. Incidentally, the helical systems being symmetrically disposed about the long axis of the fibre, the angle at which they cross (measured wet in the polarising microscope) is about 80°. If only the fine grid system is present, the angle (measured dry in the electron microscope) remains at about 80°; but, if the coarse grid system is present, there is a change in angle to about 60°, presumably because the points of attachment in the coarse grid are quite firm and the distortion of the coarse grid is superimposed on that of the fine grid. This change in angles, he supposes, may correspond with the elongation of some wood on drying.

The fine fibril bundles are about 600 Å and the coarse bundles about 2 000 Å. If anybody is interested, I have Wardrop's pictures of *Pinus radiata* with me. It is rather interesting that I have found the same structure in some of my own work on other woods.

PROF. B. STEENBERG: The question raised by Dr. Rånby about hydrolytic attack is well worth further consideration. If I understood Dr. Rånby right, he was suggesting something that could be related to a sort of stress corrosion. You take a piece of metal, bend it and put it into a corrosive substance or liquid, you know that it will corrode very rapidly indeed. You may say that the forces of bending have decreased the energy of activation for the corrosion.

The important thing in this connection is that such a break is an autocatalytic process, which means that the metal will break, not in many parts, but just in two parts and, from that moment on, there will be practically no further corrosion.

Therefore, if there is bending somewhere that introduces stresses in the chain, you will certainly have very strong hydrolytic attack at that point of stress, but the action, I think we all agree, will be autocatalytic. The stressed portion will break rapidly, the stresses will be relieved and you will have made two pieces. In order to explain that it breaks down into small pieces, either you have to assume that stresses are distributed all over the molecular chain, so that there is an equal chance of hydrolytic attack at intervals roughly about the length of the resulting pieces or you can assume according to Dr. Gallay that there will be, in the course of the chemical attack, repeated flexes and bendings of the structure that, because of their random nature may introduce a stress, now here, now there. Then the hydrolytic attack sets in at one place or another.

It is difficult with Rånby's theory to see how just one bending can be so fatal.

If we use the idea of stress corrosion, we must find a way that makes it understandable why the molecules do not break at one point but at so many points, as the decrease in D.P. shows. Another assumption is that the thermodynamic activity of the whole system is changed by swelling procedures that will, of course, influence every individual part. Let us assume that the long molecules are reinforcement in the amorphous concrete. If the whole structure is swollen, stresses will be introduced in practically all the long strand of chains and they are liable to break at several points. Swelling in undamaged fibres is restricted by the S1 structure. If S1 is broken anywhere, the swelling can occur. Comparison with an osmotic cell with and without membrane failure may give a clue.

DR. RÅNBY: Prof. Steenberg has discussed the increased accessibility of native cellulose fibrils as stress corrosion. This concept seems to be useful for the understanding and description of this phenomenon. I would like to refer to electron micrographs shown yesterday of well crystallised cellulose fibrils from animal cellulose (tunicin). Before hydrolysis, the fibrils were wavy and sometimes sharply bent, indicating the presence of stress in the lattice along the fibrils. After hydrolysis, the fibrils are straightened out and practically only rodlike fibril fragments are seen with a number of sharp breaks at various intervals. This looks like localised hydrolysis at stress

points, allowing the fibrils to recrystallise and straighten out when a few chains are broken at these points.

Slip planes in the lattice of the fibrils do not necessarily mean stress. Their formation could simply mean a local disordering of the lattice — an opening of original hydrogen bonds and formation of new bonds of lower strength. Such an effect is easy to accept when you realise that practically all hydroxyl groups in solid cellulose are engaged in hydrogen bonds of various strength and regularity. Stress corrosion and local lattice disorder could explain the accessibility of cellulose fibrils to chemical degradation at certain points.

THE CHAIRMAN: Can anybody shed any light on the galactose question mentioned by Dr. Christensen?

DR. H. MEIER: We studied in Stockholm the carbohydrate composition of compression wood, but, unfortunately, I have no data on the galactose content. We found, however, that the mannose content was considerably lower in compression wood than in normal wood. Therefore, it seems that the carbohydrate composition in compression wood is rather different from that in normal wood.

PROF. L. G. STOCKMAN: Some years ago, an investigation on compression wood from spruce wood (*Picea abies*) showed it to contain 10 per cent. of galactose, which is in very good agreement with the Australian figure.