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DISCONTINUITIES IN THE MICROSCOPIC STRUCTURE OF WOOD FIBRES

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Summary

A discontinuous structural organisation characterises biological cells such as wood fibres. The correlation between discontinuities in the microscopic fibre structure and some physical and chemical features of the fibres are discussed. The co-axial lamellar structure, a longitudinal discontinuity, is of particular importance in fibre technology. Considering morphological phenomena during maceration of wood fibres, we came to the conclusion that a real discontinuity exists between the secondary wall and its inner layer, the so-called tertiary wall. The special morphological and individual character of the tertiary wall (S3) is demonstrated in a number of micrographs and colour slides. Information is given of the structure of the tertiary wall and the helix angles of the fibrillar pattern are indicated for 8 species of softwood. As the colour slides are not published in this paper and the number of micrographs has had to be reduced, some abridgements of the paper were necessary.

Wood fibres and native cellulose fibres are an extremely complex material. They are not of a uniform composition and the distribution of the various components is heterogeneous. The substances of high molecular weight

are present in the form of a solid body and we have to take into consideration all peculiarities of the structural arrangement of crystalline and amorphous materials. In addition, there is the discontinuous structural organisation that characterises all biological cells. We have to study the changes of the structural principles within a very small range. Discontinuities can be found in the amicroscopic, in the sub-microscopic and in the microscopic field.

The discontinuities of the *microscopic* fibre structure are morphological features of various nature and form. In order to classify them, we consider the fibre as a longitudinal cylinder. This permits us to group the discontinuities according to their location into those lying in a (*1*) transversal, (*2*) radial or (*3*) longitudinal direction.

Firstly, I will discuss a special case of a *transversal* discontinuity. It appears that the even flow of the fibrillar structure also shows discontinuities when a larger section of the fibre is examined.

Examination of the structure through crushing the fibres

The fibrillar structure of wood and native cellulose fibres may be observed microscopically when they are allowed to swell by soaking in water or in a dilute alkaline solution and then crushed. Using this procedure, Herzog[1] has compared the arrangement of the fibrillar structure of various textile fibres. Bucher and Widerkehr[2] used this method for the examination of papermaking pulp fibres of different qualities.

Fig. 1 is a crushed fibre, in which the wall has been broken down into fibril bundles of varying thicknessess. Here, the natural structure of the fibre has been considerably deformed. Although crushing is at best a crude process, it nevertheless enables the research worker to make interesting comparisons between various types of fibre. The microscopic examination of crushed fibres furnishes worthwhile information on structural peculiarities. This picture of a crushed fibre tip, for instance, shows quite clearly that the fibrils do not terminate abruptly, but rather that the bundles turn again into the fibre. This reversal of direction (looping) occurs in stages; it is thus that the tip is formed.

In this illustration, the fibrils are evenly spaced and the fibril bundles are loosely arranged. This is typical for the so-called soft pulp fibres that have been cooked slowly at high temperatures.

Hard fibres, on the other hand, separate into compact, comparatively thick bundles that are widely spaced from each other.

Fig. 1 illustrates what is meant by *microscopic continuity* — that is, characteristic and unaltering morphological features observed within the microscopic range.

Some crushed fibres show alternating zones where the fibrillar structure is distributed evenly and zones where a distinct division has taken place, leaving large gaps. A Rosaniline-stained fibre was crushed and the soft zones appeared slightly tinted, whereas the hard zones were deeply coloured (Fig. 2).

The fact of the periodic alternation of hard and soft zones is essential to our interpretation. We have here a structural discontinuity and not an accidental phenomenon.

The question naturally arises whether or not these transversal zones correspond to discontinuities in the structure of native, untreated fibres. The evidence hardly seems to support this view. The transversal discontinuities described here should more probably be ascribed to periodic variations in the digestion process, irregular diffusion during impregnation or topochemical influences. From a practical point of view, however, the existence of such zones in pulp fibres is naturally of considerable importance.

As a *natural transversal* discontinuity, I wish to mention the existence of dislocations and slip planes. Thorough morphological and structural examinations have been made on that subject by Wardrop and Dadswell,[3] Frey-Wyssling,[4,5] Kisser[6] and several earlier authors.

Pits as a structural discontinuity

Probably the best known disturbance in the structure of the fibre wall are the pits. They have a *radial* orientation.

A number of bordered pits are often grouped in single or double rows close to the fibre tips (Fig. 3). When such a fibre is being crushed, it appears that these particular pit zones resist deformation much better than does the remainder of the fibre wall. The wall is fibrillated and squashed; the pit zone, however, remains unchanged. As a whole it forms a discontinuity in the longitudinal direction.

Discontinuities in the longitudinal direction

In dealing with the morphological discontinuities running parallel to the longitudinal axis of the fibre, it is important to consider several features that can only be understood when the chemical delignification of wood is viewed morphologically.

In investigations we made several years ago and which were published in 1947,[2] cross-sections of wood were cooked under carefully controlled conditions and changes checked under the microscope at regular intervals during the process. It was then ascertained that the inter-cellular layer, the so-called middle layer, was the first to be attacked. Gaps appeared between the fibres, the wood tissue held perfectly together, however and resistant lignified parts were noticed especially at the corners (Fig. 4).

When finally the tissue disintegrated into single cells, some of this resistant lignin from the intercellular layer remained on the fibre surface. If formed a ridge running along the surface of the isolated fibres. These ridges can be noticed when the fibres are stained with basic dyes such as Malachite Green. Dolmetsch[7] was the first to refer to this phenomenon in dealing with the morphology of fibres. He interpreted the ridges as a reinforcement of the angles with resistant lignin. More recently, this feature has been thoroughly investigated by Jayme and Harders-Steinhauser[8] in connection with the study of irregularities during sulphite pulping.

Malachite Green also stains the partially delignified membranes intensely and it is not possible for this reason to study the process of delignification of specimens dyed with this staining agent.

A finely graded differentiation of more or less incrusted membranes was obtained by staining specimens with Chicago Blue (Fig. 5). The incrusted parts are best to be seen when digestion is fairly well advanced. Darker zones in the secondary wall become quite plainly visible at the fibre angles. These zones, however, are not sharply delineated and their form and extent varies with the degree of delignification. These observations indicate that the angles of isolated wood fibres are reinforced by the deposition of incrusting substances in the normal structure of the cellulose fibres.

Longitudinal discontinuities and beating action

Fig. 6 illustrates a strong, unbleached sulphite fibre that was beaten for a prolonged period in the Lampén mill and that shows a transverse split in the wall. This picture reveals clearly the phenomena just discussed of strongly incrusted longitudinal zones giving great rigidity to the unbleached pulp fibres. The dark lines are built into the secondary wall and lie under the primary wall and exist even where the primary wall has been torn off and only where the secondary wall has suffered considerable injuries are they damaged and exposed.

The lignin ridges that, remaining from the incompletely dissolved middle lamella, run along the fibre edge, show a different behaviour. In a

carefully cooked wood fibre that was stained before beating one can see such longitudinal lignin ridges on both sides. Under mechanical treatment, the ridges are separated from the fibres with little difficulty. Where one of these ridges was previously attached, a colourless zone appears in the fibre membrane.

Although the lignin ridges do not cling strongly to the fibre surface, they are able to affect the mechanical properties of the fibre. When beaten, it was noticed on a few occasions that a fibre was cut at several points. A clean separation occurred and the two parts remained united only by one of these resistant lignin ridges.

At one place, the fibre was cut right through and the ridges also were completely broken (Fig. 7). This fibre has a row of pits and it may be seen how this pit zone, the more resistant part of the wall, holds the two broken pieces together.

As mentioned earlier, those zones of the wall bearing pits are generally more resistant to mechanical action.

We have been discussing a few discontinuities of the microscopic fibre structure and have shown some examples of visible or derivable correlation between such discontinuities and the mechanical properties of the fibres. Especial consideration must be given to the means used to make observations when discussing morphological problems. A structural discontinuity is not always a structural part of the native fibre. In certain cases, it may appear only with physical or chemical action. Moreover, morphological observations must be interpreted. It is possible therefore that structural phenomena can receive quite different interpretations, depending on how they are viewed.

The lamellar structure of fibres

We shall now deal with the finer microscopic structure of the fibre wall, which is composed of co-axial lamellae. This longitudinal discontinuity is of considerable importance in fibre research and technology.

The lamellar structure is a result of the development and growth of the cell wall. On this basis, Kerr and Bailey[9] have clarified fibre wall terminology.

The first layer to be formed is the primary wall. It is reinforced by secondary deposits that become the secondary wall. From the ontogenetic point of view, there is discontinuity therefore between the primary and the secondary wall.

Examination of morphology and fine structure have also shown a changeover in structure between the primary and the secondary walls.

Working with the electron microscope, Frey-Wyssling, Mühlethaler and Wykoff[10] found that the primary wall has a mesh-like structure, whereas the fibrils of the secondary wall were found to run parallel to each other.

There are a number of features reflecting the difference in structure between successively formed layers of the cell wall. I shall give my own observations on the subject.

In a section of spruce, the intercellular layer was dissolved, part of the tracheids were already removed and the tissue was completely macerated. The primary wall became separated from the secondary wall. (Fig. 8*a*).

This separation of an outer membrane during digestion of the wood becomes quite noticeable when processing the fibres.

It happens quite frequently that, when the fibres are being refined, an exterior layer is loosened. The closing membranes of the pits are still connected to this layer. (Fig. 8*b*). One can conclude therefore that this is the primary wall or a combination of the primary and secondary layers.

When fibres are subjected to the action of swelling and cooking agents, a lamella often loosens itself in the shape of a ribbon. That is why Schramek[11] called it *Oberhautband* (ribbon of the outer skin) and Klauditz[12] *Zellwand-Aussenschicht* (exterior layer of the cell wall). At that time, it was still difficult to classify the fibre layers with certainty. Carpenter and Lewis[13] found that the primary wall is removed during digestion and the transversely wound outer layer of the secondary wall is exposed.

Today, it is still not always possible in swelling investigations to ascertain whether these ribbons are of the primary wall or the outer layers of secondary wall or a combination of both.

Protection of the cell wall during digestion

On digestion, the cell wall is mainly exposed to the effects of the cooking liquor from the lumen side. According to Lange,[14] the lignin is mostly concentrated in the intercellular layer. In the secondary wall, the lignin content drops sharply. Dadswell and Ellis[15] thoroughly studied the behaviour of the intercellular layer of eucalyptus during digestion. They reached the conclusion that the maceration of the fibres only takes place when delignification of the intercellular substances occurs, as well as a removal of non-lignin material. Later investigations by Wardrop and Dadswell[16] showed that this non-lignin material of the intercellular substance must be as resistant as Cross and Bevan cellulose. Very vigorous chemical attack on the cellulose of the cell wall would therefore be expected.

However, it was observed during the digestion of thin-walled specimens of spruce wood in bisulphite solutions that the middle lamella is dissolved long before any change is to be noted in the wall of the fibre. The extremely thin springwood fibres are completely isolated without losing their original shape. It is only towards the end of cooking that these membranes gradually become softer; they first become wrinkled, then collapse completely.

The tertiary wall of conifer tracheids

Considering all these morphological changes during maceration of wood fibres, we come to the conclusion that the secondary wall opposite the lumen must be protected in a special way. Even several years ago, it seemed necessary for us to get more precise information on the nature, the morphology and the structure of the innermost layer of the fibre wall in order to clarify the chemical problems of wood and of isolated wood fibres.[17]

When macerating sections of conifers with strong acids and alkalis, we repeatedly noticed how a thin lamella came off the fibre wall inside the fibre (Fig. 9). It was obvious to consider the possibility that the innermost layer of the wall had a much more individual character than was then admitted in the literature on fibre anatomy and that a real discontinuity exists between the secondary wall and its inner layer. However, the experimental methods to examine this question critically and to form an opinion did not exist at the time.

I suppose that many of you present know of our efforts to identify the special nature of the innermost layer of the fibre wall. Thanks to a special staining method, it finally became possible to free this layer and to study its character. It was revealed that this inner layer has a very definite and individual character, that by its colour reactions and its swelling properties it differs completely from the secondary wall and that it possesses a distinct structure. It was practically impossible to describe the behaviour of the innermost layer of the membrane in an appropriate and satisfactory manner unless the assumption was made that it constituted a particular and distinct section of the wall. In describing these observations, we therefore termed this part the *tertiary wall*, in conformity with the earlier terminology and, in this way, differentiated it in our work from the secondary wall. In our investigations, no doubt was left as to classification and identification. In metachrome staining with Victoria Blue in an alkaline swelling reagent, the blue of the primary and tertiary walls was easily to be distinguished from the red of the secondary wall.[18]

In the first place, I want to present a number of black and white pictures to show some of the shapes that the tertiary wall has in different coniferous woods.

In Fig. 10 you see the swollen form of a tracheid of *Pinus radiata*. The primary wall of this wood species is very strongly developed. It ruptured during swelling and lies now as an open sheath next to the fibre, to which it still remains attached at certain points. The secondary wall shows balloon-swelling. In the centre, the tertiary wall can be recognised. Please keep in mind that here the primary wall, as well as the tertiary wall inside the fibre, are stained blue. The other parts are stained red. The outer layer, forming the constrictions, is of a slightly deeper red than the heavily swollen balloons of the main part of the secondary wall. During swelling, the primary wall is usually rapidly destroyed.

The tertiary wall in both pine and spruce fibres has a membranous character. The tertiary walls of pine are considerably stronger and better developed than those of the various species of spruce. Like a heavy tube, the tertiary wall sticks out of the non-swollen part of the pine fibre. The tertiary wall shows no change in the width of its lumen, whether it be observed at an unswollen or dissolved site on the fibre. The swelling medium naturally also had some effect on the tertiary wall, although this wall was highly protected during our experiments. There is a loosening of the structure corresponding to the structural discontinuities of the tertiary wall. For instance, very fine lines of a spiral structure become perceptible (Fig. 11). Obviously, we have here the case of a real helix and not of an accidental fold. We can follow the turns of the helix. Please notice that in the case of *Pinus silvestris* this spiral runs almost transversally to the fibre axis.

Under the influence of the swelling agent, the tertiary wall disintegrates according to its discontinuous inner microscopic structure (Fig. 12). In our classification, from a morphological point of view, we call these structures *complex* helices. The complex helices show that there is still much work to be done in order to be able to determine and describe schematically on what principle the structure is built. We can only make a small step forward here.

It has become evident that complex helices are often divided into laminar (ribbon-like) elements. The tertiary wall of *Pinus insignis* was broken up into a system of latticed ribbons. From various observations, laminar elements seem to be a typical form of the finer structural discontinuity of the tertiary wall of many fibres. When resolving a fibre of *Pinus insignis*, the tertiary wall showed at first a folded and complex structure that finally disintegrated very clearly into a ribbon-shaped element.

The complex helices can certainly show that in morphology one must reckon with many masked structural discontinuities, which cannot be recognised as such in native fibres, but which must be taken into consideration in dealing with technical questions. The frequency of these structures shows that they are not incidental phenomena. Many of these forms also appear as a typical sign of the tertiary walls in certain species.

During swelling, the tertiary wall of Canadian spruce shows a very typical complex structure, which has been termed a fine chain structure. In this, the fibre differs from other species of spruce.

Tertiary wall and pits

The interpretation that the inner layer represents an individual layer finds its support in the behaviour at the bordered pits. The secondary wall is perforated by the pit openings. In consequence, the inner layer of the secondary wall should also be perforated.

Our investigations have clearly proved and it is photographically documented that the tertiary wall passes through the secondary wall at the pit openings and is in contact with the outer wall layers.[17, 19]

In Fig. 13, a membrane-shaped tertiary wall is connected with the primary wall. The secondary wall is heavily swollen and dissolved to a large extent. The tertiary wall obviously runs into the pits.

The connection between tertiary and primary walls, however, is generally broken when the fibre is greatly swollen.

A number of times, it was observed how the continuations of the pits are directly connected with the helical structure of the tertiary wall. One notices, for instance, how several spirals of a helix run together and pass through the secondary wall to the pit openings of the primary wall. Because of the swelling of the secondary wall, the connection is naturally tightly stretched. Often we find an entire row of pit appendices emerge from the tertiary wall corresponding to the loops of the helix. Please note in Fig. 14 (*Larix*) the pronounced helicoidal structure. The coils are clearly visible.

The helicoidal system of the tertiary wall

The helical principle in the structure of the tertiary wall had already become evident in various ways and a thorough and systematic description of these forms was given in my earlier publications. Today, we have become acquainted with some of the complexly screwed types. In the majority of cases, the fibrillar pattern is reflected in a fine striation of the membranes or in close, tight threads. Threads and striations are in my opinion forms

consequent upon fibrillar orientation. The threads are set in the membrane and their inclination does not change, even when the tertiary membranes are distorted. This would signify that the fibrils have to slide in a counter-movement and such a supposition can hardly be accepted. Actually, the angle of inclination is strikingly constant.

When examining helicoidal structures, two details are of special interest — (*a*) the sense of the winding and (*b*) the angle of inclination.

The sense of the winding was examined microscopically on a great number of tertiary walls. This is simply done by changing the projecting plane so that the lower and the upper parts of the object can be examined separately. In all the coniferae checked by us, we noticed that (with a few exceptions) all tertiary walls are wound to the left. When projecting the picture, the helix is therefore S-shaped. You see in Fig. 15 on a spruce tertiary wall the striation caused by the fibrillar pattern. The angle of inclination is 65°. When illustrating the front and back at the same time, a criss-cross pattern is produced.

The lumen of other spruce fibres is considerably narrower and the windings are obviously much steeper than in Fig. 15. A 30° angle was measured towards the fibre axis.

It is an interesting fact that the tertiary walls of fir are not only considerably more strongly developed than those of spruce, but they show a much flatter helical pattern.

The morphological determination of the helix angles

Fig. 16 gives some information about certain questions in the morphological measurement of helix angles —

(*a*) The mathematical helix angle is the angle between the tangent and the horizontal line (σ). In fibre morphology, the helix angle is usually indicated with the angle φ formed by the tangents on the windings with the longitudinal fibre axis.

(*b*) The position of the fibre axis is not always exactly determined. As long as the threads of the front and back of the cylinder can be seen, it is always more exact to measure the angle formed by both tangents.
If the fibre does not lie in the projecting plane, the apparent position of winding is changed, but the angles between the tangents do not change.

(*c*) When an object has been bent, the windings are compressed or widened. We get the correct angle of inclination only on condition that we base our measurements on the undeformed, neutral axis.

(*d*) Fig. (*d*) shows a series of lines constructed in the same manner as in Fig. (*e*). It is evident that it is impossible to indicate the exact position of the undeformed axis: therefore we cannot measure the angles on these objects.

The helix angles of the tertiary walls of the following species are listed in Table 1 — spruce, Canadian spruce, black spruce, Douglas fir, silver fir, balsam fir, larch, pine and *Pinus radiata D. Don.*

Each angle given corresponds to a single tertiary wall. The first column is the average for the group in question; the last column shows the morphological category to which the tertiary wall belongs.

In the case of spruce, two groups may be distinguished — narrow-lumened tertiary walls with steeply inclined angles of 30° and wide-lumened fine membranes with angles of inclination of 65°. Between these two types, two further groups may be clearly distinguished. They are composed by those fibres with complex and open threads and it is not possible to draw conclusions as to the inclination of the fibrillar structure from the angles.

Canadian spruce also has an inclination of 65°; black spruce and Douglas fir are somewhat less steep with 70°. The low angle of inclination of fir (80°) is striking. Larch and pine also have low angles of inclination.

Table 1 shows that the measurements of various tertiary walls within one group are fairly constant, even when the morphological details are quite different.

TABLE 1

Tertiary wall	φ	σ	*Measured angles*	*Morphological type*
Picea excelsa	30°	60°	32°; 32°; 28°; 29°; 33°	Membranes with striation
			28°; 31°; 32°; 28°	Narrow-lumened helices
	—	—	44°; 41°	Open threads
			40°; 44°	Complex structure
			38°; 45°; 44°; 40°	Chain-like structure
	—	—	58°; 57°; 60°	Fine membranes with open threads
	65°	25°	64°; 65°; 70°; 66°	Wide-lumened membranes with striation
Picea canadensis	65°	25°	65°	Fine membranes with striations
	—	—	39°; 41°	Fine chain-like structure
Picea mariana	70°	20°	69°	Membrane with striation
Pseudotsuga taxifolia	70°	20°	70°; 71°; 68°	Membrane with threads
Abies pectinata	80°	10°	81°; 78°; 82°; 76°; 81°; 81°	Tight threads
Abies balsamea	82°	8°	82°	Membrane with striation
	—	—	71°	Helical thread
Larix europaea	89°	1°	89°; 89°; 88°	Coarse thread
Pinus silvestris	84°	6°	84°; 84°; 84°	Membrane with helical thread
	—	—	81°	Complex structure
Pinus radiata D. Don.	—	—	75°; 76°	Complex structure

It will be seen that the inclination of spruce is partly steeper, partly not very different from the inclination of the fibrils of the main layer of the secondary wall, whereas that of pine and — strikingly — also of fir, is oriented rather in a transversal direction.

This explains largely the statement by Wardrop and Dadswell[20] that the very fine inner layers of cross-sections of spruce show no double refraction as is the case with pine.

The colour differences of the tertiary wall

Changes in fibrillar organisation can be included in the considerations of the individuality of the inner layer: but they are not its only criterion. When making our investigations, we got the impression that for technical purposes the tertiary wall has to be treated as something special and we used other criteria — the special microscopical morphology and features, the different swelling capacities, the solubility, the reactions when cooking the wood and last, but not least, differential metachromatic staining. I shall not enter into explanations of the colour differentiation, but only wish to state that by such staining it was clearly defined which part of the fibre membrane we were describing.

By this colour differentiation, it was proved beyond doubt that the conception of Wardrop and Dadswell is correct, according to which the balloon swelling derives from the outer layer of the secondary wall. The primary wall has no share in it.

Conclusion

The conception that the secondary wall is bordered towards the lumen by a special layer has been abandoned more and more during recent years. To quote Bailey and Kerr[21] —

> "There are no actual discontinuities in the cellulose matrix and the three layers of the secondary wall are due to varying orientations in the successively formed parts of the wall."

This statement has had a strong influence on the interpretation of findings in cell wall research of the past years.

It seems to us that our observations in technological research on pulp fibres show that an important part of this fundamental concept should be revised — "There *is* an actual discontinuity in the constitution of the secondary wall of coniferous wood fibres."

Through this knowledge, new aspects have been opened up for observations in wood chemistry — for example, when comparing different cooking

procedures such as sulphate and sulphite cooks, during impregnation, bleaching, etc. The presence of a tertiary wall, with a distinct microscopic structure, is also of importance with regard to the questions of stability, elasticity and flexibility of the fibres. The sorption of water may be influenced by the tertiary wall, which is hard to swell. All these factors are connected with the problems of papermaking.

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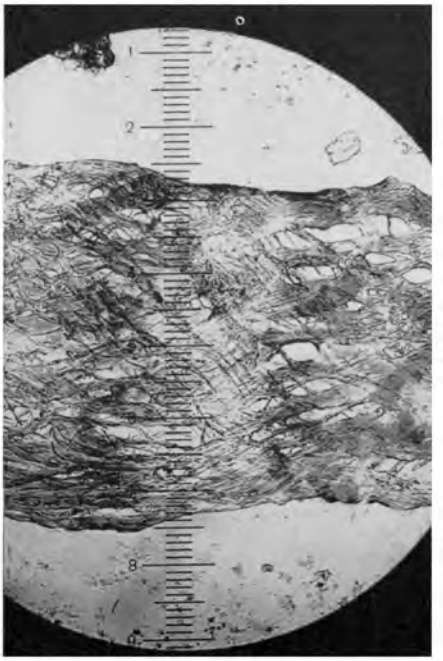
×220

Fig. 1 — Tip of a spruce fibre, crushed
The separated fibril bundles represent a 'microscopic continuity' : the fibrillar strands do not terminate at the tip, they are looped in steps

×100

Fig. 3 — Pit zone resists deformation when crushing the fibre

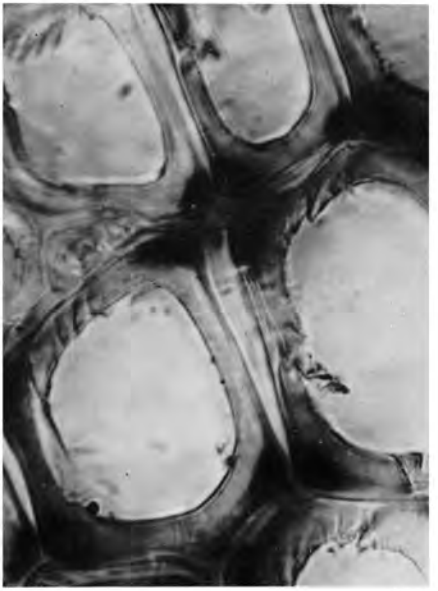


×135

Fig. 2 — Transversal discontinuities in spruce fibres
Periodic alternations of soft zones (evenly separated fibrils, slightly tinged) and of hard zones (gaps, dark colour)
(1 division of scale = 7μ)

×350

Fig. 4 — Cross-section of spruce
The middle lamella is partially dissolved and the cells become separated; resistant lignin ridges remain on the surface of the fibre edges



×1250

Fig. 5 — The corners of the secondary wall are reinforced by incrusting substances; they are seen as dark spots after staining with Chicago Blue.

Fig. 6 — Beaten spruce fibre
The primary wall is separated: dark lines of incrusted zones are built into the secondary wall and lie under the primary wall

×270

Fig. 7 — Spruce fibre cut by beating action
The broken pieces are connected with the more resistant pit zone of the wall

×280

×290

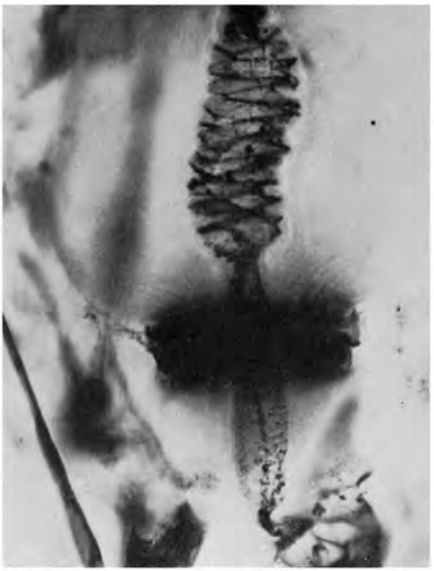
Fig. 8*b* — An exterior layer is separated when beating a fibre: it is suggested that it is the primary wall, as the closing membranes (tori) of the pits are connected with this layer

×1000

Fig. 9 — When macerating wood sections, a lamella came off the fibre inside the secondary wall (spruce)

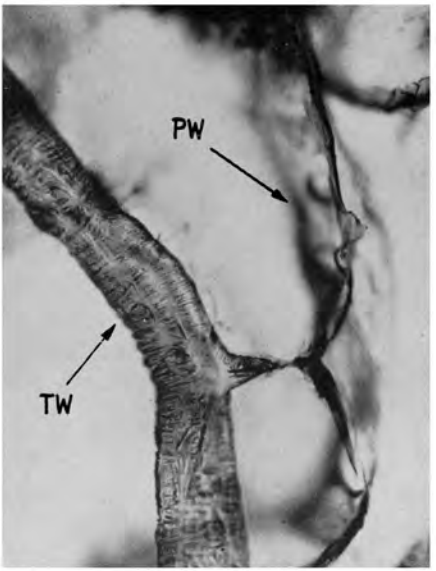
Fig. 10 — Swelling of a fibre (*Pinus radiata D. Don*)
The primary wall bursts: it forms a sheet and is still attached to the fibre at certain points; the outer layer of the secondary wall constricts the swelling wall and produces balloon-swelling ×95

Fig. 11 — Isolated tertiary wall of *Pinus silvestris*
Lines of a helical structure are perceptible in the membraneous tertiary wall ×500



×500

Fig. 12 — The membranous tertiary wall disintegrates according to its discontinuous microscopic structure and forms a complex helix



×280

Fig. 13 — The tertiary wall enters the pits: it passes through the swollen secondary wall and is connected to the primary wall

Fig. 14 — Several spirals of the helicoidal structure of the tertiary wall run together and pass through the secondary wall to the pit openings in the primary wall (*Larix europaea*)

×280

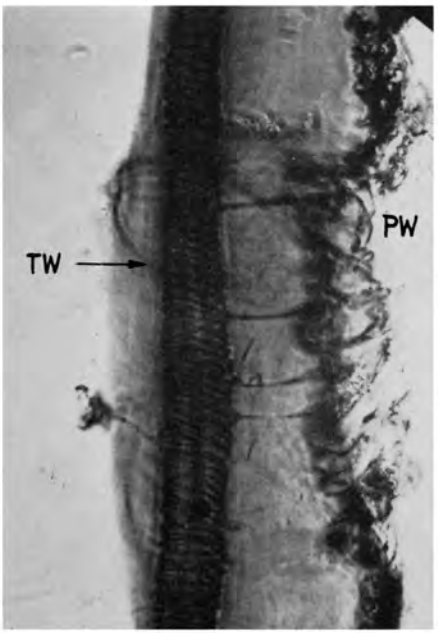


Fig. 15 — The fibrillar pattern of the tertiary wall in spruce: it has an inclination of 65° to the fibre axis

×520

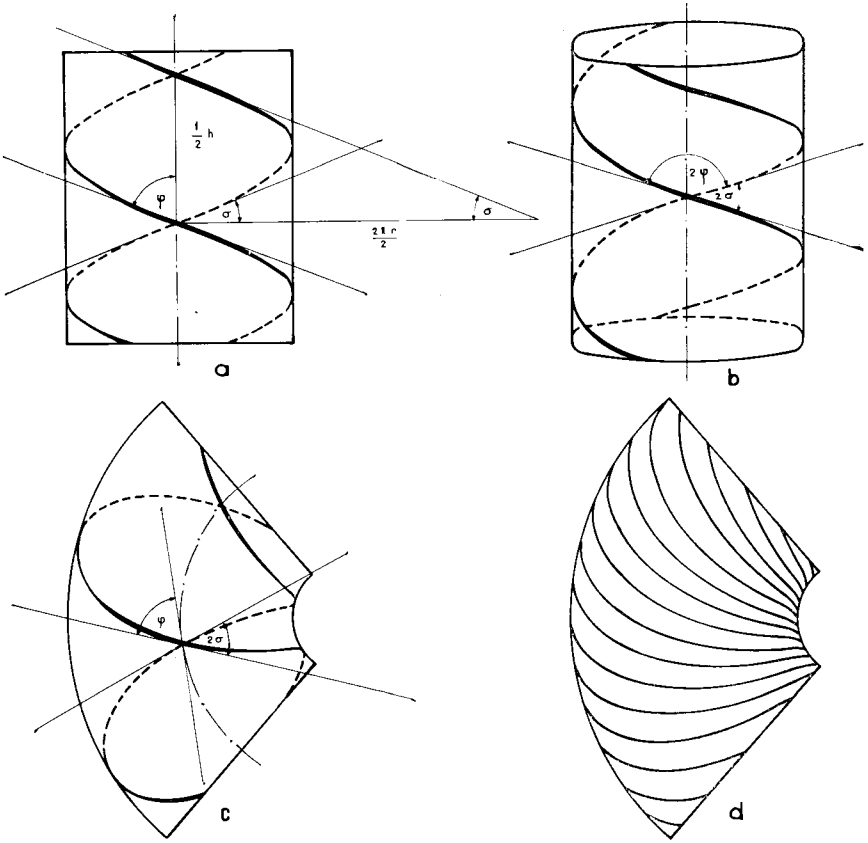


Fig. 16 — Measuring and discussion of helix angles —
(*a*) normal view; (*b*) oblique position of the fibre axis; (*c*) deformation of the helices when bending the longitudinal axis; (*d*) a series of helical threads in a bent cylinder

×1000

Fig. 17 — Fibrillar patterns of the tertiary wall of spruce
There are two sets of threads — one with transversal orientation (87°), another with an inclination of 65° (*see* discussion, page 31)

×1200

Fig. 8a — During maceration of wood, an exterior layer of the cell (primary wall?) has become separated from the fibre

DISCUSSION

DR. W. GALLAY: May I ask Prof. Frey-Wyssling why he starts with the elementary fibril as an entity? After having stated that the so-called macrofibril and the microfibril can be observed over a wide range of diameters depending on the method of preparation and therefore are not distinct entities, why not confine ourselves to the individual cellulose molecule as the only true entity?

PROF. A. FREY-WYSSLING: It is not possible to disintegrate elementary fibrils by mechanical means. You must treat them with cellulose solvents that destroy all the hydrogen bonds in order to obtain individual cellulose chains. Morphologically, therefore, it is not really the chain that is the element; it is the elementary fibril.

MR. L. G. COTTRALL: How does Prof. Frey-Wyssling envisage that the strength of a lignin-free fibre is derived? We are given to understand that the cell wall is made up of masses of microfibrils oriented in different directions in the various layers. There must be some attachment between these small elements, either a mechanical attachment (an entanglement) or else a bonding — alternatively, a combination of both. How does Prof. Frey-Wyssling conceive that this attachment between the microfibrils occurs? If there is any bonding, has he any idea of the proportions to which these elements take part in the bonding? — what is the number of bonds per unit area?

PROF. FREY-WYSSLING: The situation is this. We do not really know the length of the elementary fibrils, but, compared with their diameter, they are practically of indefinite length. If they are side by side and there is mutually only very weak bonding — sometimes a hydrogen bond here and there — this very long surface makes an even stronger bonding than the sum of the covalent bonds of the molecule chain in the cross-section of the elementary fibril. The longitudinal area of contact is indefinitely much higher than the cross-section, so the number of bondings per unit area can be small. Then, of course, if there is hemicellulose left between the fibrils, much stronger lateral bonding results, exceeding many times the tensile strength of the elementary fibrils.

DR. B. G. RÅNBY: Last time we met (in 1954), we discussed the dimensions of the sub-microscopic fibrils and debated the existence of the 250 Å fibrils, already in question at that time. Today, we find that a famous member

of the Royal Society has joined the '100 Å school'; we are pleased therefore not to have to discuss these matters any more. We agree that the fibrils are about 100 Å wide and frequently aggregated. We must be a little cautious about how elementary the fibrils are and how well defined they are, because there are indications that the crystallisation takes place after the chains have been formed and are deposited. At least, we have been able to show that in the case of a *Dictyosteleum* slime mould. The reason the dimension 100 Å is preferred seems to be related to an interplay between different (thermodynamic, kinetic) forces, which make the cellulose chains crystallise to fibrils that happen to be about 100 Å wide for wood fibre cell walls. Somewhat wider fibrils have been found in animal and algal cellulose (in tunicates and *Valonia* algae, respectively).

I should like Prof. Frey-Wyssling to discuss the difference between the 100 Å fibrils in the primary wall and the fibrils of the same dimension in the secondary wall, because they do not seem to be identical, according to some reports I recall.

Another point is that the slip planes in the cell walls which were first discussed by Prof. Frey-Wyssling and later by Dr. Bucher, seem to me to be related to slip planes in the cellulose lattice of the fibrils. Such slip planes in the lattice would cause irregularities (disorder in the hydrogen bonding) and thus make the cellulose chains more susceptible to chemical attack — for instance, to heterogeneous hydrolysis or oxidation.

My third point is what proof is there of an 'interwoven' fabric of fibrils in the primary wall? I am not convinced by the evidence put forward in support of it. It would be interesting to have it discussed further.

PROF. FREY-WYSSLING: I will start with the last item first — the question of weaving. If you look carefully at the electron micrographs, you will find an arrangement like that in Fig. A. I would like to discuss this situation in terms of a familiar game — Pick-up-sticks. You have a bundle of long sticks (the microfibrils), you let them fall and then the game is to take away one stick after another. The man who wins can take all of them away without disturbing the others. Those who play this game know very well that, if they get to the position where the sticks are entangled as in Fig. A, they have lost the game. In one respect, I think the criticism of Dr. Rånby is right. The weaving is not like the warp and weft weaving of a cloth, as we thought in the very beginning, but it is an entanglement. Its origin can be explained as follows. If the layer where the microfibrils grow is not just of the dimension of the diameter of the microfibrils, but is somewhat thicker,

they will not grow strictly parallel to the surface, but at a slight angle to it and an entanglement, as illustrated is possible.

At the discussion on microfibrils at the Cell Biology Symposium in St. Andrews some weeks ago, evidence was put forward that there is bipolar tip growth of the elementary fibrils in bacterial cellulose. If it is permitted to transpose this to the cytoplasm, these fibrils will grow into an entanglement.

In the electron microscope you find a distortion in the fibrils (*see* Fig. B). My argument on these slip planes is the following. Solubility, staining and so on are functions of the fibre's density. If the fibrils are separated by such distortion, a place exists where easier penetration is possible for chemical agents. I cannot imagine that the grid has been broken or attacked by the mechanical forces involved. It is more important that the surface of the fibrils, formerly densely packed, has been freed.

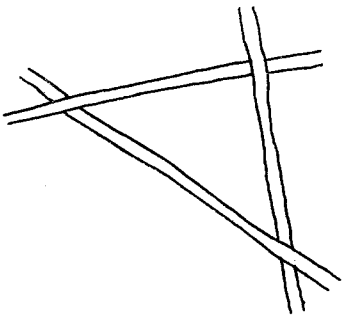


Fig. A

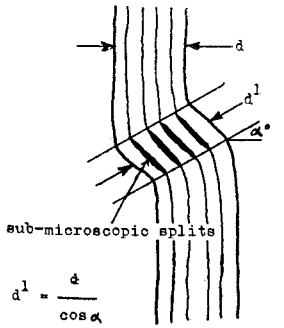


Fig. B

DR. RÅNBY: I think that is one effect, but the question is — how much bending can a lattice take before the lattice itself slips?

PROF. FREY-WYSSLING: We have measured these angles; they are about 30°. In an X-ray pattern of such a bend, you would find no difference from a straight fibre. On the other hand, the loosening of the parallel texture is quite obvious.

There is only a small chemical difference between the fibrils in the primary and secondary walls. The cellulose of the parallel-textured secondary wall may be crystalline to 70 per cent., that of the disperse-textured primary wall to 40 per cent. or 50 per cent. — that is, a similar crystallinity as in bacterial cellulose. In the first case, degrees of polymerisation of 3 000 – 5 000 are found; in the second case, it is slightly less — 1 000 – 2 000. Thus, there is no real difference in the chemical properties of these two celluloses.

As to the diameter of the sub-microscopic fibrils, they are rather coarse in the primary wall. As a rule, they range about 250 Å and are well individualised: this is why I insisted on the existence of microfibrils of this size. It was only recently that we could disintegrate the primary fibrils into somewhat twisted elementary fibrils.

DR. F. MULLER: Would Prof. Frey-Wyssling tell us how to identify the elementary fibrils with the crystalline regions assumed by so many authors in a cellulose structure?

PROF. FREY-WYSSLING: This is difficult to say. With the electron microscope, we cannot distinguish between crystalline and paracrystalline cellulose — that is, between the crystalline core of the elementary fibrils and its less orderly arranged surface layers. Formerly, this was known as amorphous cellulose; now we call it paracrystalline cellulose. The thickness of this layer can be calculated, taking 70 per cent. crystallinity, estimating how the cross-sections of this core would be shaped and then deducing the thickness of the layer of the non-crystalline cellulose. I think Dr. Rånby agrees that what is seen with the electron microscope is the border of the paracrystalline cellulose. This layer is very important, because it can make contact with neighbouring elementary fibrils and form hydrogen bonds.

DR. MULLER: I assume we must accept the concept of chain molecules running from one elementary fibril over to the other?

PROF. FREY-WYSSLING: That is difficult to say, because all our discussion bears on the cross-section; therefore, we cannot see on our diagram whether such a pair of elementary fibrils will merge into one another in other planes. Therefore, the question you have put forward cannot be answered in a positive or a negative sense. Have you ever seen branching elementary fibrils, Dr. Rånby?

DR. RÅNBY: No, in no case was it clearly branching, unless it was an aggregated thicker string. In these cases, you could see a twist of the strings, indicating a fibril bundle, but branching does not seem to be very frequent. The differences between the fibrils in the primary and secondary wall as described by Prof. Frey-Wyssling are significant and very interesting to me.

DR. MULLER: I have another question, this time for Dr. Bucher. Is anything known about the differences in chemical composition between the tertiary and the secondary wall, because the differences in their behaviour should, I think, be caused by differences in chemical composition and not only by differences in structure?

DR. H. BUCHER: I think there is a difference in chemical character. Victoria Blue has the property of an indicator — blue in acid solution, red in alkaline solution. That the tertiary wall remains blue with this stain even in alkaline swelling agent could be explained by the reaction of the dyestuff with the substance of the tertiary wall.

What is this substance? It has a special affinity for the dyestuff, but I think this is not a very exact interpretation. It probably consists of pure cellulose and hemicelluloses with acid groups. From their investigations, Meier and Yllner in Stockholm conclude that the tertiary wall might consist of xylans.

DR. H. MEIER: Do the tertiary walls with flat helices in spruce belong to springwood fibres and the tertiary walls with steeper helices to latewood fibres?

DR. BUCHER: It is difficult to identify swollen fibres. Wide-lumened tertiary walls that have windings at an angle of 65° may originate from springwood fibres, the narrow-lumened walls with 30° windings from latewood fibres.

MR. H. W. EMERTON: May I show two slides that bear on the subject discussed by Dr. Bucher? These are of the freshly cut surface of spruce (*P. excelsa*) and viewed in Fig. C across the lumen at the inner surface of the cell wall. Rather more than the width of three tracheids is shown. In the lefthand cell, the inner secondary (or tertiary) wall, S3, is present almost throughout; towards the bottom of the picture, it has probably been disturbed by the knife. The righthand cell (with the bordered pits) shows similar features. In the central tracheid, the striations of the middle secondary wall only are evident, almost axial in this case: it cannot be said whether S3 has been removed by the knife or whether it was never present. We have observed S3 in this way many times in spruce and the results obtained are supported by the electron micrographs taken by my colleague D. H. Page (Fig. D). There appears in this species to be a fibrillar helix making an angle, with respect to an axial direction, typically of approximately 65°. This helix is of the S-form. Surrounding this in the cell wall (and therefore underlying it in pictures of this kind) is at least one other layer of S3, the microfibrils of which are often observed to be almost transverse. Has Dr. Bucher any evidence of two fibril systems being present in this wall?

DR. BUCHER: Fig. 22 of our first publication in 1953 on the tertiary lamella was taken before we did our measurements on helical inclination and it shows typical striations on a spruce fibre with slight sinoid form, indicating that it is a helix. We found transverse orientation visible in one part and

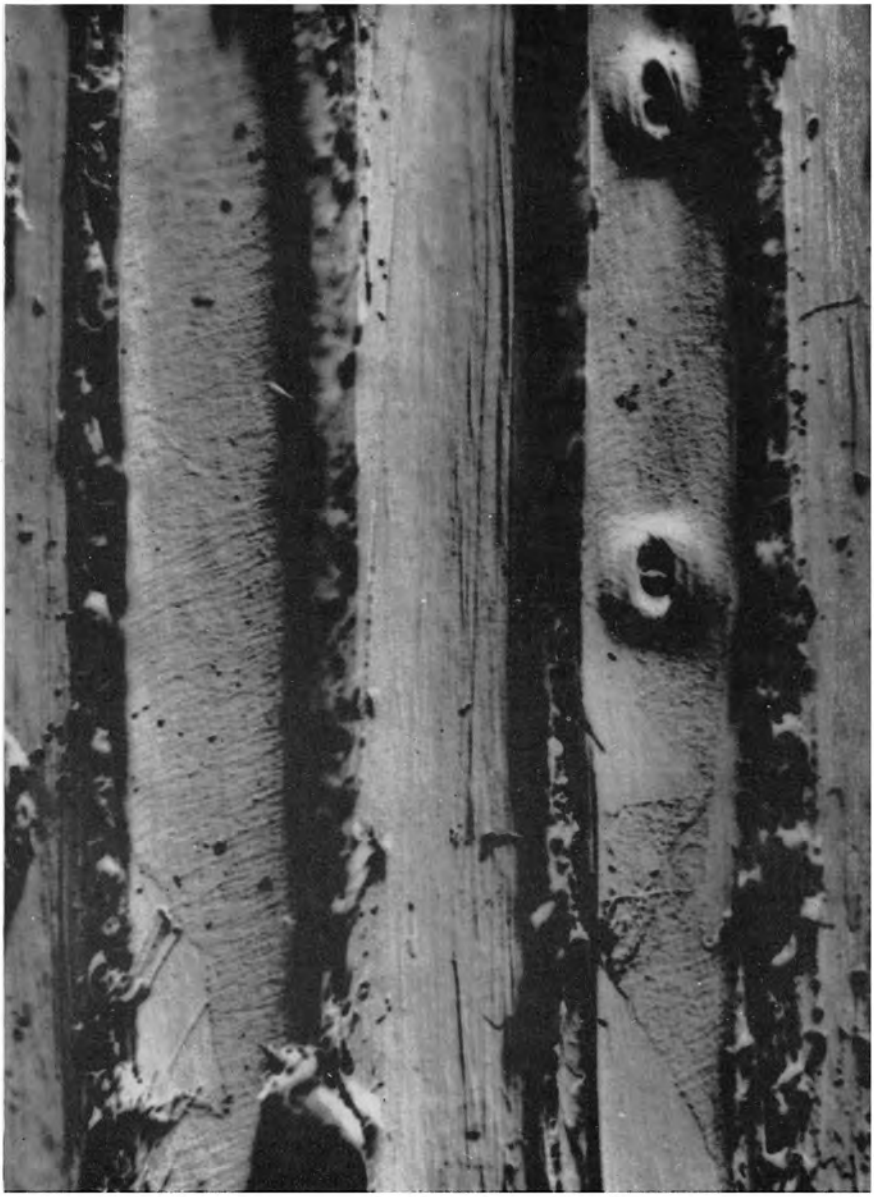


Fig. C — A radial surface of Norway spruce — the cut has passed through the lumina (light micrograph × 1020)

steeper orientation in another (65°). I showed the picture to a mathematician to have him interpret the helix that must be present. Although he had no idea what the subject was, he found that there must be *two* different helices. There exists no possibility of a *single* helical system producing winding features as seen in this picture (Fig. 17, page 26).

DR. D. ATACK: The so-called slip planes are very interesting features, since they appear to be points of initiation of failure in wood. The initiation of failure in metals is supposedly controlled by defects in the structure — is there any defect in the wood structure to give rise to the localised folding (unfortunately called slip planes) under compression, which may be identified microscopically? In this example, I do not wish to imply that the slip planes in wood are analogous to those observed in some metals. Robinson some 30 years ago called slip planes the dislocation lines that appear in wood under stress and observed them very frequently in wood from which lignin had not been removed.

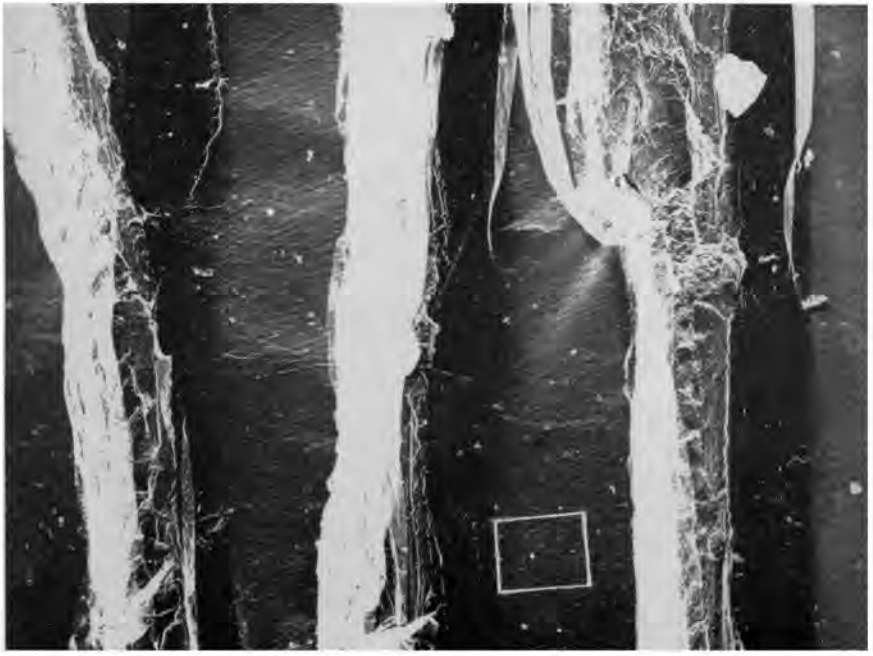


Fig. D (*i*) — As Fig. C (electron micrograph × 900)

Could either speaker suggest any particular morphological feature in the wood that may be associated with the formation or the initiation of these slip planes? Furthermore, what determines the angle of the slip planes?

PROF. FREY-WYSSLING: In my experience, the angle depends on how much the loosening takes place. If the texture is loosened very much, the angle is steeper; if the loosening is small, it is flatter. If there are two adjacent tracheids, dislocation will go through to the next wall at the same angle. As shown in the illustrations, even if there are several dislocation planes, they are all at the same angle. It is interesting that the compression necessary to produce them is about one tenth of the crushing pressure of wood. I should like to insist that these dislocation lines have nothing whatsoever to do with slip planes. In lignified fibres, they are rather rare, because it is much more difficult to produce the necessary distortions.

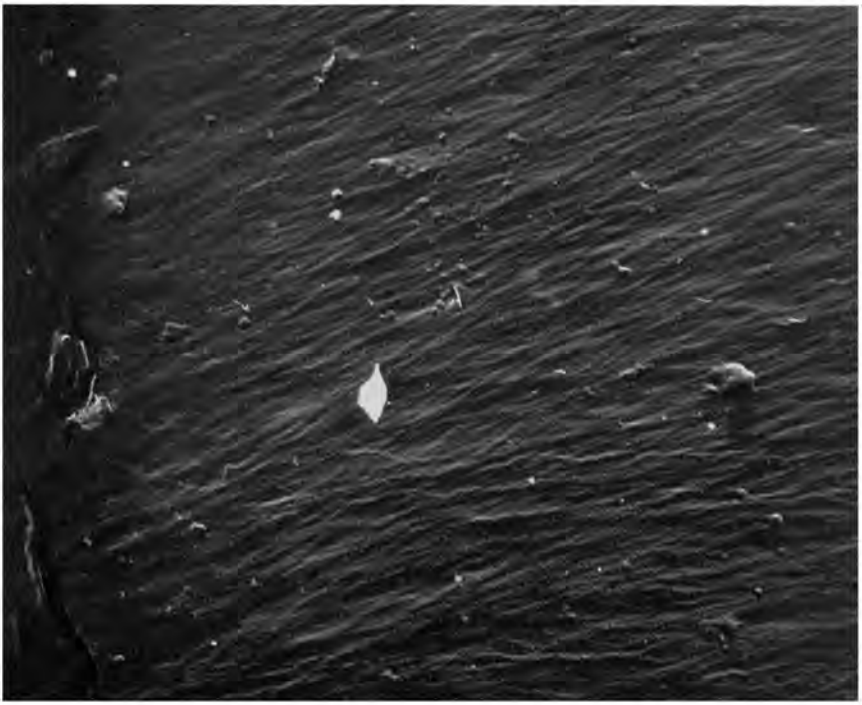


Fig. D (*ii*) — Enlargement of the inset area of Fig. D (*i*)
(electron micrograph × 9 000)