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THE OUTER SECONDARY WALL
1. ITS STRUCTURE

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Abstract

This paper is chiefly a review of work published by the author and others on the structure of the outer secondary wall (S1) of softwood tracheids and hardwood fibres. There is now considerable evidence that the fibrils of S1 form more than one helix. Two counter-rotating and symmetrical helices have been demonstrated and these have a helix angle of $\pm 55°$ *to* $\pm 75°$ *in the tracheids of the softwoods that have been examined so far. In hardwood fibres, the helix angle is probably significantly smaller. The nature of longitudinal features associated with S1 is discussed, together with the probable thickness of this wall. Micrographs are shown of S1* in situ *in a wood tissue.*

Introduction

THE lamellate form of the wall of mature wood tracheids and fibres, as described by Kerr and Bailey,[1] is now widely known. Within a thin integument (primary wall, P), there exists a series of coaxial layers that are characterised by the close lateral packing and consequent parallel alignment of their fibrils. To these layers, the term secondary wall is collectively applied. From studies with the polarising light microscope, it is clear that the fibril direction is not the same throughout the secondary wall, but that in the outermost and innermost layers the fibrils have a direction that is

nearly transverse, whereas those of the middle layers are almost longitudinal. For this reason, Kerr and Bailey applied the terms outer (now commonly designated S1), middle (S2) and inner (S3) secondary walls to the three systems of fibrils. In this paper, we are concerned only with the structure of the outer secondary wall. Furthermore, only wood tracheids and fibres are discussed.

Methods of observation

Until recently, the use of the light microscope to study S1 was, in the main, limited to two methods. Either the birefringent properties of transverse sections were observed or the fibres were subjected to gross swelling agents and examined in normal light. Neither of these methods however, is entirely satisfactory. The polarising microscope gives information only about the degree of orientation and the mean direction of the aligned structural elements; the considerable disturbance of the fibre wall to which gross swelling gives rise may well vitiate the results obtained by this technique.

The electron microscope has also been widely employed to elucidate the structure of plant cell walls, but, until recently, the three conventional ways of overcoming the inability of the electron beam to penetrate the comparatively thick cell wall have not been entirely satisfactory.* The three methods in question are the cutting of thin sections, disintegration of the fibre into thin fragments and the preparation of thin replicas of the surface. Although it has been used, the first of these is not a particularly informative way of investigating the structure of an individual layer of the cell wall. This has the form, more or less, of a cylindrical annulus so that even a longitudinal section, which is necessarily plane, intersects it over only a limited area. Disintegration has been widely used, but has the obvious drawback that it is difficult to relate the fragment to the structure as a whole; in many cases, that is, it is not possible to state with confidence the part of the cell wall from which an observed fragment originated. The last method, that of surface replication, has in the past been open to the same objections, because reliable methods of replicating extensive areas of rough surfaces were wanting. This difficulty has recently been overcome by improved techniques, such as those due to Page [2] and Hunger. [20]

Innovations in the field of specimen preparation for light microscopy have also been introduced, notably the application of metal shadowing and of metal-shadowed plastic solid replicas. The use of metal shadowing was

* Even if the cell wall were sufficiently electron transparent to transmit the beam, the resulting image would convey information about the several overlying fibril layers and, without recourse to stereomicroscopy, the interpretation of such micrographs would be difficult.

first proposed by Williams and Wyckoff[3] for the electron microscope and later[4] for light microscopy. It has been extensively used and advocated by the present author and his colleagues.[5] Furthermore, a method has recently been devised[6] of making plastic solid replicas, which, after metal shadowing, permit surfaces to be studied without the disturbance of the image caused by optical heterogeneities present in translucent specimens such as paper and wood sections. Examples of the application of these methods to the study of S1 accompany this text.

A characteristic of the image given by metal-shadowed specimens of plant material in the light microscope is the striations prominent in most cases. Such striations can also be observed, although with far less clarity, in unshadowed fibres that have been stained and crushed. They are not, of course, the actual microfibrils that make up the cell wall, the width of which is beyond the limit of resolution of this instrument. They are, however, generally accepted as indicating the presence of a system of microfibrils aligned in the same direction. That this is a reasonable assumption can be seen from Fig. 1, which shows the striations observed in the light microscope (inset) and the microfibrils of the same cell wall fragment revealed in the electron microscope.

A negative contrast system is obtained when a metal-shadowed specimen is viewed in transmitted light; the regions that have been protected from the evaporating metal — that is, the 'shadows' — have a higher transmissivity and therefore appear brighter than those parts exposed to metal. The importance of studying images of correct contrast, however, has been emphasised elsewhere;[6] accordingly, the contrast has been reversed to give a positive effect in all the accompanying micrographs. Furthermore, when interpreting micrographs of fibrillar patterns that have been metal-shadowed, due allowance must be made for the direction of shadowing. As a rule, this is readily established from the shadow cast by protuberances or, failing these, by small particles of dirt. When the fibrils are aligned almost in this direction (or, more correctly, the projection of this direction upon the slide), they cast shadows of negligible length and the fibril system will not be so clearly revealed as when they are perpendicular to the shadowing beam. Thus, it is possible for one set of fibrils in a crossed fibrillar pattern to appear with less prominence than the other.

Crossed fibrillar structure

Owing largely to the appearance of transverse sections in the polarising microscope, it was for long believed that the fibrils of S1 all spiral about the longitudinal axis of the cell in the same sense. Doubt was first thrown on

this assumption when, in 1950, Hodge and Wardrop[7] observed that, following the mechanical disintegration of tracheids, fragments of cell wall were obtained that exhibited a crossed fibrillar structure. Because of the method of specimen preparation employed, these workers were not able to determine from which secondary wall — or secondary walls — these fragments originated, but, by elimination of other possibilities, they were thought to have derived from either S1 or S3. Subsequently, Bosshard,[8] studying fibres of ash, found two counter-rotating fibrillar systems, which he attributed to the wall S1. A similar structure was found by Meier[9] in fibres of birch that had been subjected to degradation by wood-rotting fungi. In the case of spruce, on the other hand, Meier failed to recognise a crossed fibrillar structure, because, it would appear, of the limited areas of S1 that he obtained by this method. In each of these cases, the fibrillar pattern was observed in the electron microscope.

Using the light microscope, Emerton and Goldsmith[10] found a tendency in pine tracheids that had been pulped by the kraft process for an outer wall to part cleanly and readily from the rest of the cell wall over extensive areas (Fig. 2 — 5). This wall, which was believed to be S1, was seen to consist of two counter-rotating sets of striations that were symmetrically disposed about the axial direction. Using the same technique to study Cross and Bevan pulp tracheids from *Pinus radiata* and kraft-pulped eucalypt fibres, Wardrop and James [11] had found evidence of a crossed fibrillar pattern inconclusive, perhaps because their pulp had, for the most part, been beaten.

The observations of Emerton and Goldsmith were supported by the work of Frei, Preston and Ripley,[12] who studied thin sections of *Pinus radiata* tracheids in the electron microscope. They found in S1 two lamellae, the fibrils of which formed relatively flat helices of similar pitch, but of opposite sense.

Considerable further evidence of a crossed fibrillar structure for S1 in gymnosperm tracheids has been obtained in the author's laboratory.[13] Examples of this are illustrated in Fig. 6, 7 and 12 — 15.

Helix angles

The values of the angle between the tangent to the spiralling fibrils and the axial direction (helix angle) quoted in earlier work or measured from published micrographs were tabulated by Emerton and Goldsmith[10] This showed that, for the limited data available for hardwood fibres, the helix angle is $\pm 35°$ to $\pm 55°$. In softwood tracheids, it is greater and ranges $\pm 55°$ to $\pm 75°$. The mean of 62 determinations on 16 different tracheids of *Pinus*

patula and *P. caribea*[10] was $\pm 63°$. Since that summary was made, Frei, Preston and Ripley[12] have given for a single example of *Pinus radiata* an angle of about $\pm 65°$.

It is not to be expected, of course, that the helix angle will be invariable. Indeed, it has been shown[10] that, in some cases (such as Fig. 5), the angle may vary slightly from one face to another. Local variations in the direction of the striations can also be seen in Fig. 1, 12, 14 and 15.

Probability of discrete layers

When considering the feasibility of a crossed fibrillar pattern, Wardrop[14] drew attention to two effects that at first would appear to preclude it. Such a structure has not been recognised when cells that have been crushed and stained are studied in the light microscope. This is negative evidence that may, perhaps, reflect the limitations of this technique. More serious, on a first consideration, is the behaviour of cells subjected to strong swelling agents. The beads or balloons of S2 that result are often apparently surrounded by torn helical ribbons of S1, but counter-rotating helices are not observed. This observation does not necessarily disprove the existence of crossed fibrils, but indicates rather that, if two helices exist, their fibrils are not interwoven but form discrete layers. For S2 to swell out into a balloon, it would then first be necessary for one set of fibrils to be disrupted, following which the fibre would twist freely, in such a way that the fibrils of S2 become more nearly longitudinal[15] as the balloon is formed. It seems likely that it is ribbons of this second set that are sometimes observed on fibres treated in this way.

There is experimental support for the hypothesis that the two helices are in distinct layers. Emerton and Goldsmith[10] drew attention to local regions in their micrographs where one set of striations was missing or where one set clearly overlay the other. Subsequent work has shown that, from macerated tracheids and fibres, fragments of a thin layer are often found that exhibit very marked parallel striations in one direction only and at an angle to the axial direction consistent with that of the S1 helices. These striations may arise, partly at least, from folding during drying, but it may reasonably be supposed that they reflect the alignment of the fibrils. Examples of this are shown for macerated pine tracheids (Fig. 8) and for birch fibres (Fig. 9, 10). The existence of such fragments has also been reported and illustrated by Wardrop and James.[11]

According to Meier, [9] one of the two counter-rotating helices in birch predominates over the other. Frei, Preston and Ripley[12] have argued that,

since only a single striation direction is observed in the light microscope, one helix must predominate. The thickness of S1 (*see* below) is such that there must be several microfibrils in its transverse section; clearly, the fibrils spiralling in one sense could, in theory, outnumber those in the opposite helix. In our own work we have found no clear evidence in support of this.

Whatever the form and degree of association of the two helices of S1, there is a marked looseness between S1 and S2, particularly after delignification. This was stressed by Meier[9] and is further emphasised by the accompanying micrographs, which show the predisposition of the two walls to separate when the tracheids are mechanically handled. Furthermore, it has been pointed out that, when tracheids are subjected to balloon swelling, S1 ruptures here and there and actually glides over S2 to form prominent gatherings at the nodes between the balloons (Fig. 11), notwithstanding the strong outward pressure of S2 against S1.[16]

Yet again, it has been observed that when wood that has been treated with alkali is broken under a tensile load failure occurs mainly between S1 and S2.[21] It has earlier been shown that untreated wood often fails in this way.[22, 23]

The following interesting property should help to establish whether the sense of the respective helices is unique or whether the outermost helix is sometimes right- and sometimes left-handed. If a series of parallel lines is drawn on the upper surface of a piece of paper and a similar series is drawn on the under surface in such a way that, when the paper is held to the light, the two sets appear crossed, it will be found that, irrespective of which side of the paper is uppermost, the lines always lie in the same direction. The sheet may even be turned in its own plane through 180° without changing this. The direction is determined by that arbitrarily chosen for the first set of lines. Thus, provided that we can be certain of viewing the surface uppermost on the slide, the sense of the outermost helix of S1 will be revealed, regardless of whether it is the inner or outer surface of the wall that is uppermost.

Longitudinal features

A striking feature of many of the accompanying micrographs are the markings that lie in the axial direction. There is evidence that these occur chiefly at the edges of the cells. They were originally interpreted[10] as bundles of fibrils. The possibility exists, however, that, notwithstanding their apparent intertwining (Fig. 3 and 9), these longitudinal features are folds and creases that result from drying or are strings of resistant lignin.

It may be noted that, if S1 consists of two sets of fibrils symmetrically disposed about the longitudinal direction with which they make an angle exceeding 45°, then the component of these two fibril sets in the transverse direction will exceed that in the longitudinal direction. It would therefore be expected that, on swelling, such a system would tend to increase in length longitudinally and, on drying, to shrink in this direction. Any folds resulting from drying would then lie perpendicular to this shrinkage direction — that is, transverse.

In certain cases where an isolated fragment of S1 has dried down in such a way that it can be observed from both sides (such as Fig. 13), a longitudinal feature appears to lie mainly in or on one surface of the wall. As we have seen, however, it is not possible to distinguish between the inner and outer of two surfaces each of which consists of a set of parallel lines that are crossed with respect to one another simply on the evidence of the direction of the lines, so that it cannot be said in these cases whether the longitudinal feature is on the inner or the outer surface.

There is one small piece of evidence in support of the original supposition. Frei, Preston and Ripley[12] observed, in addition to two counter-rotating helices at $\pm 65°$, a third fibrillar orientation more nearly parallel to the cell axis. This was limited to one electron micrograph and, as these authors emphasised, the observation provides little more than an indication of the possible existence of longitudinal fibrils. More recent work in the author's laboratory, with the light and electron microscopes, has been inconclusive on this point and the matter must for the time being be left open.

It is noteworthy that, in certain cases, fragments of S1 are observed that have dried down with a single twist so that both sides may be observed. In some such cases, the longitudinal features are equally clear from whichever side they are viewed (Fig. 12). In others (Fig. 13), they appear to be located mainly on one side.

Thickness

The thickness of S1 after delignification and drying has been determined from the length of shadow cast by the wall after it has been dried down flat on a glass slide and metal-shadowed at a known angle. For accuracy, this should be carried out with a microdensitometer. An approximate determination has been obtained[10] by means of a travelling microscope. Nine determinations on various parts of six different pine tracheids yielded a mean thickness of 1 440 Å. The standard deviation for a single determination was 340 Å and this took account not only of the spread of measured shadow

lengths, but also of a possible error of $\pm 2°$ in the shadowing angle (nominally 15°). It is not easy, however, to determine the length of shadow precisely and it is considered that this value is, if in error, somewhat high.

Fracture lines

It is to be expected that, when a cell wall is ruptured, failure will occur to a large extent *between* the parallel fibrils, thereby indicating their orientation. In the case of a wall consisting of two sets of counter-rotating fibrils, the resulting fracture would often be dentate. Examples of this were shown by Emerton and Goldsmith[10] and can be seen in Fig. 4, 14, and 15. Such a symmetrically indented fracture line is characteristic of S1 and is quite distinct from that obtained by failure of the middle secondary wall, which can be seen in Fig. 16 and further examples of which have been given elsewhere.[17] By means of this specific failure, it has been possible to identify S1 in replicas of the cut surface of wood. In Fig. 17, for example, S2 of pine can be seen underlying S1, the presence of which is indicated chiefly by its cut edge. A radial surface of spruce is shown in Fig. 18. This has been cut in the region of the middle lamella. Structureless material is conspicuous on the right half of the picture; the edge of this is remarkably similar to that of, say, Fig. 14. Furthermore, although the underlying fibrillar pattern is largely obscured by encrusting material, there are indications, particularly in the upper part of the tracheid on the right, of the fibrils of S2 passing from lower left to top right. These observations suggest that the amorphous materials of the middle lamella encrust the cellulose fibrils of S1 and penetrate to the outer layers of S2.

Although fractures are commonly dentate, they are not invariably of this form. Many examples are found where they are more or less straight and in an axial direction. In such cases (as Fig. 3 and 6), it is believed that the wall may have torn along the longitudinal features referred to above.

S1 as transitional between P and S2

It is established that, in certain elongated cells, the widely separated fibrils of the primary wall are somewhat dispersed, but have a preferred orientation that is mainly longitudinal on the outside of the wall and predominantly transverse on the inside. It has been found[18] that the fibrils are first laid down in a more or less transverse direction, but that, as the cell extends in length, they become disoriented in a longitudinal direction. There is now some evidence that this is also the case in the tracheids and fibres of wood.[19] Thus, although the close packing of the fibrils of S1

support its classification as part of the secondary wall, in one sense its two flat helices bear more relation to the somewhat dispersed transverse fibrils of the inner surface of the primary wall than to the single steep helix of the middle secondary wall. This characteristic of S1, of being transitional between P and S2 with each of which it is contiguous, has led Meier[9] to propose the name *Übergangslamelle* for this wall. Wardrop[19] has raised the question whether the crossed helices may not be related to the persistence of surface growth of the cell during the formation of S1.

Acknowledgements

I am indebted to my colleagues who assisted in the experimental work on which this paper is based. Mr. W. H. Hale took most of the light micrographs and Mr. D. H. Page the electron micrograph of Fig. 1. Fig. 2 — 5 were taken by Miss Valerie Goldsmith and first appeared in *Holzforschung*.[10]

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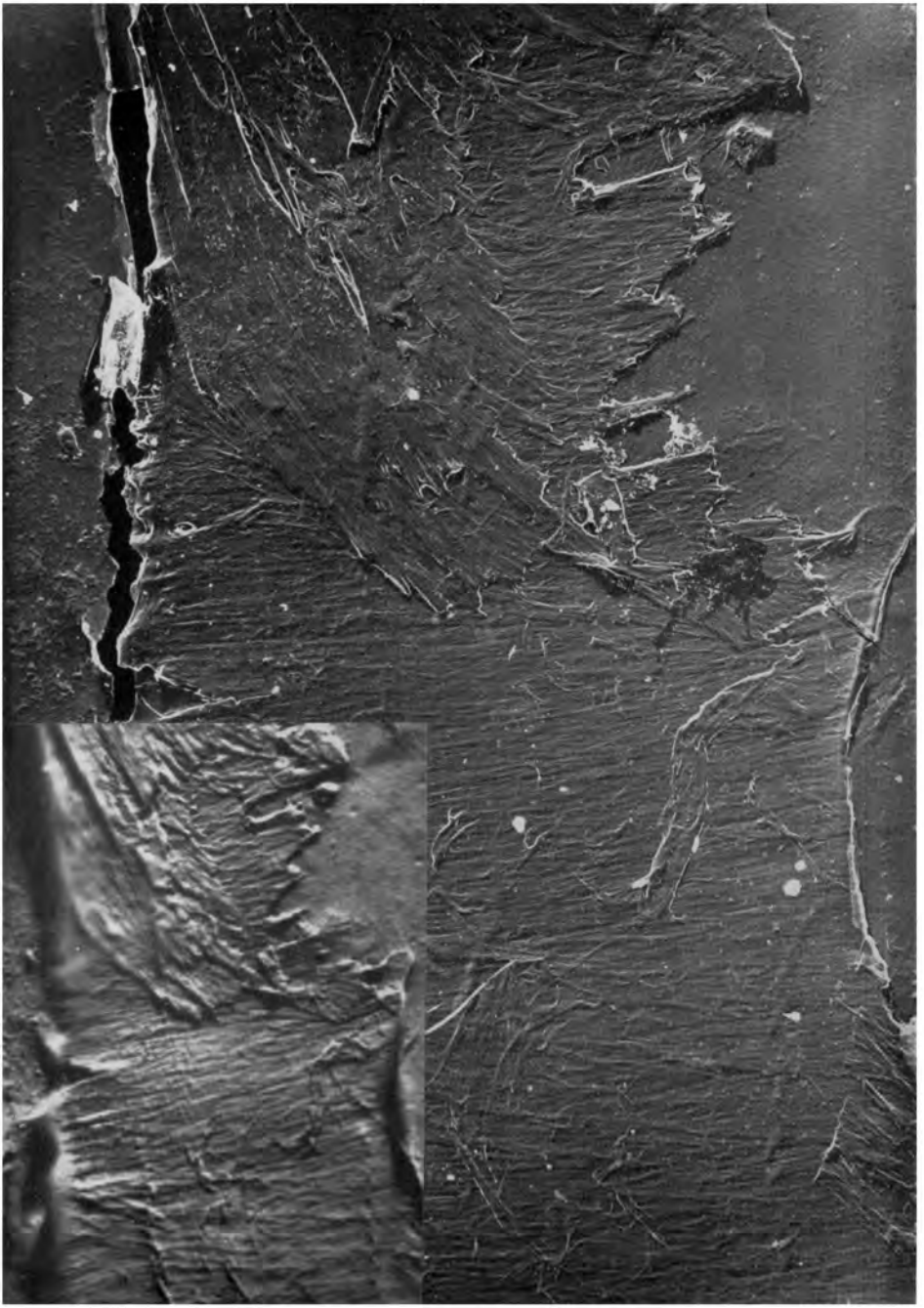


Fig. 1 — Fragment of the wall of a macerated Scots pine tracheid, showing S2 overlying S1 — the microfibrils observed with the electron microscope (×3 600) and (*inset*) the striations observed in the light microscope (×1 500)

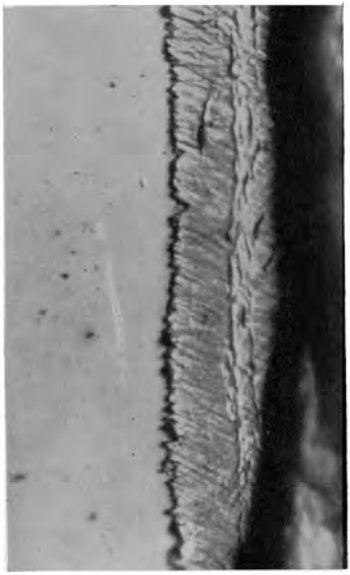
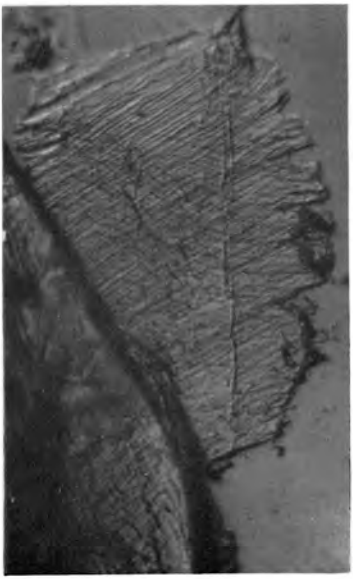


Fig. 3 — Kraft-pulped *Pinus patula* — (*below*) detail (×1 875) of above (×375)



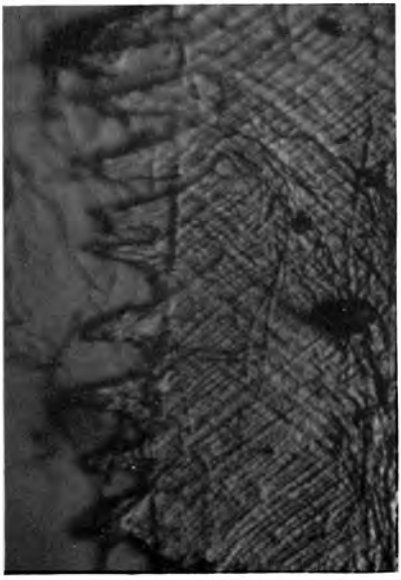


Fig. 2 (*above*) — Kraft-pulped *Pinus caribea* (×1 000)

Fig. 4 (*below*) — Kraft-pulped *Pinus patula* (×1 350)

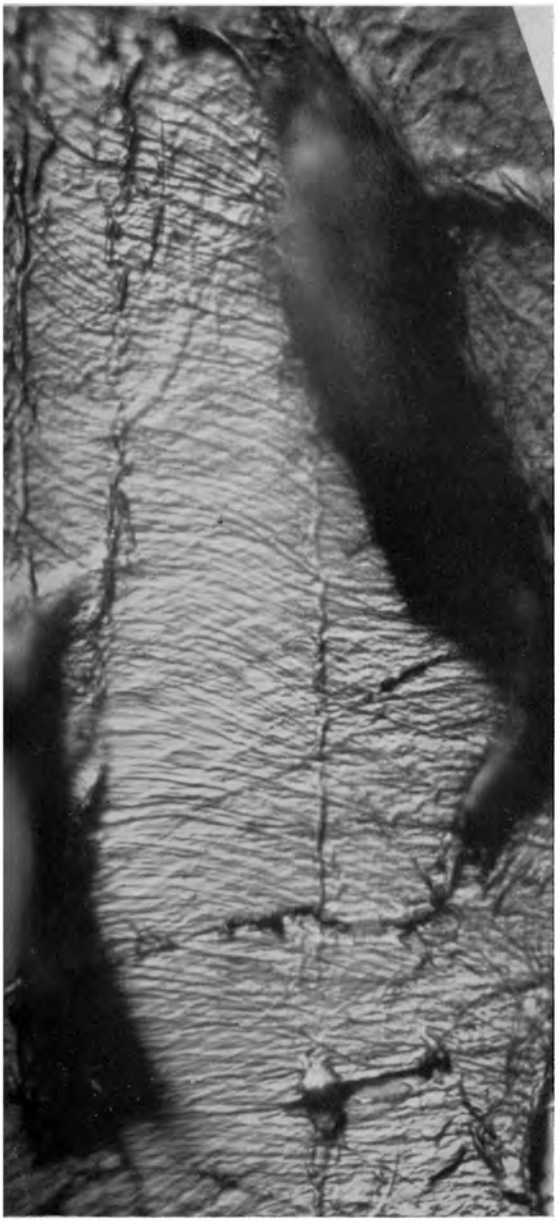


Fig. 5 — Kraft-pulped *Pinus caribea* (× 1 550)

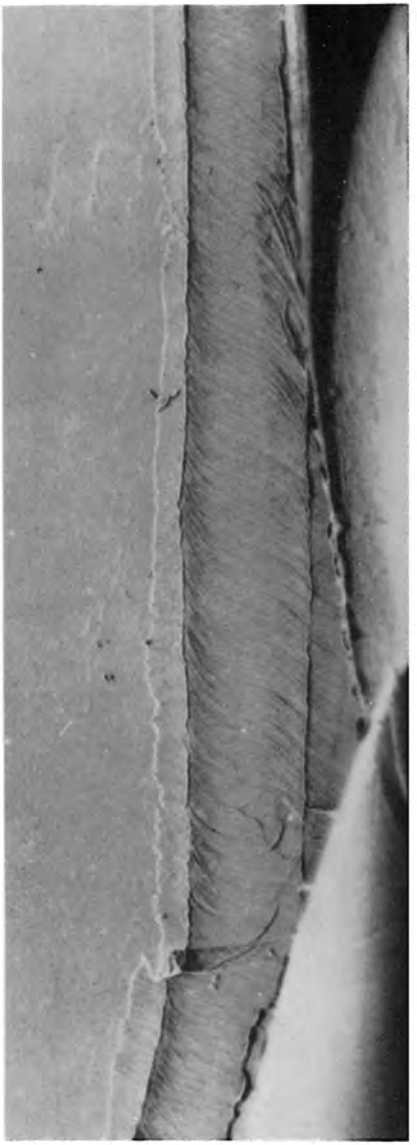


Fig. 6 — Macerated Scots pine (*Pinus sylvestris*) (× 575)

Fig. 7 — Macerated Scots pine (×385)

Fig. 8 — Macerated Scots pine (×260)

Fig. 9 — Macerated birch (*B. papyrifera*) (×725)

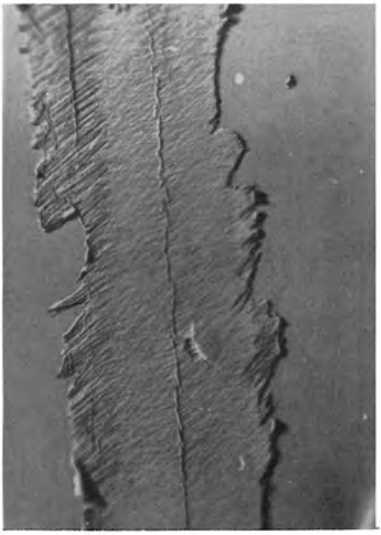


Fig. 10 — Macerated birch (×725)

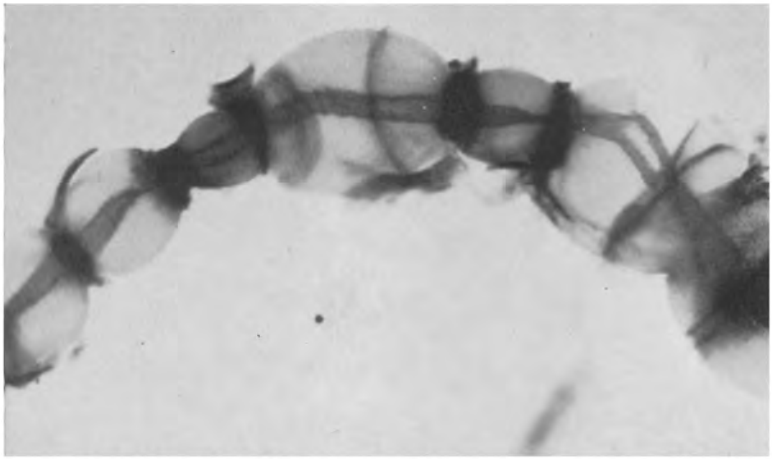


Fig. 11 — Pine tracheid swollen in phosphoric acid (×155)

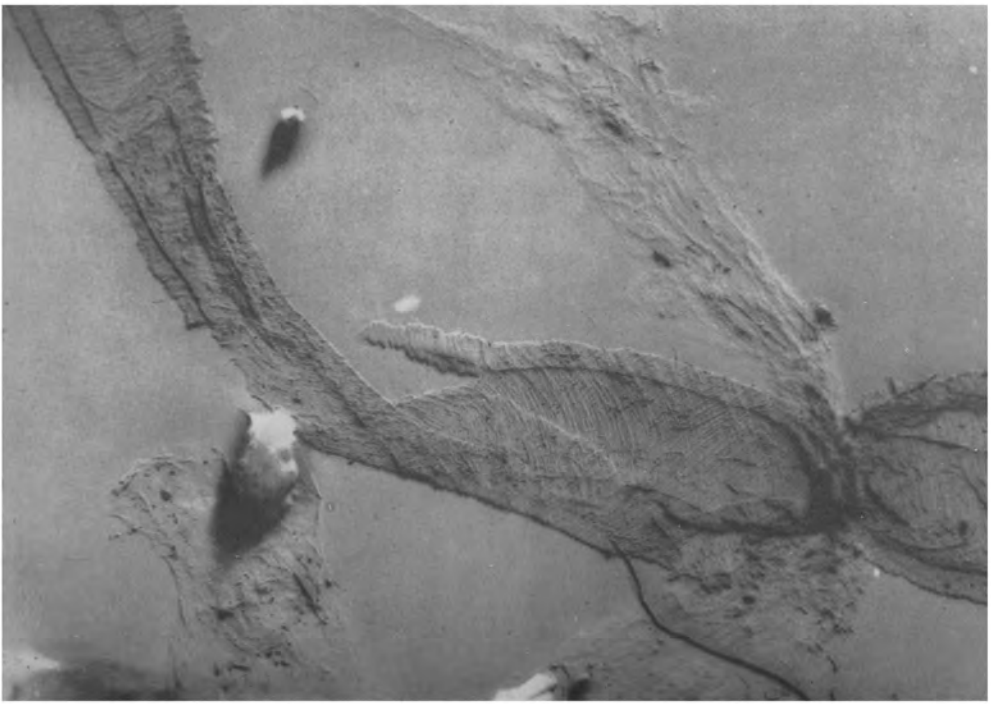


Fig. 12 — Macerated European spruce (*Picea excelsa*) (×500)

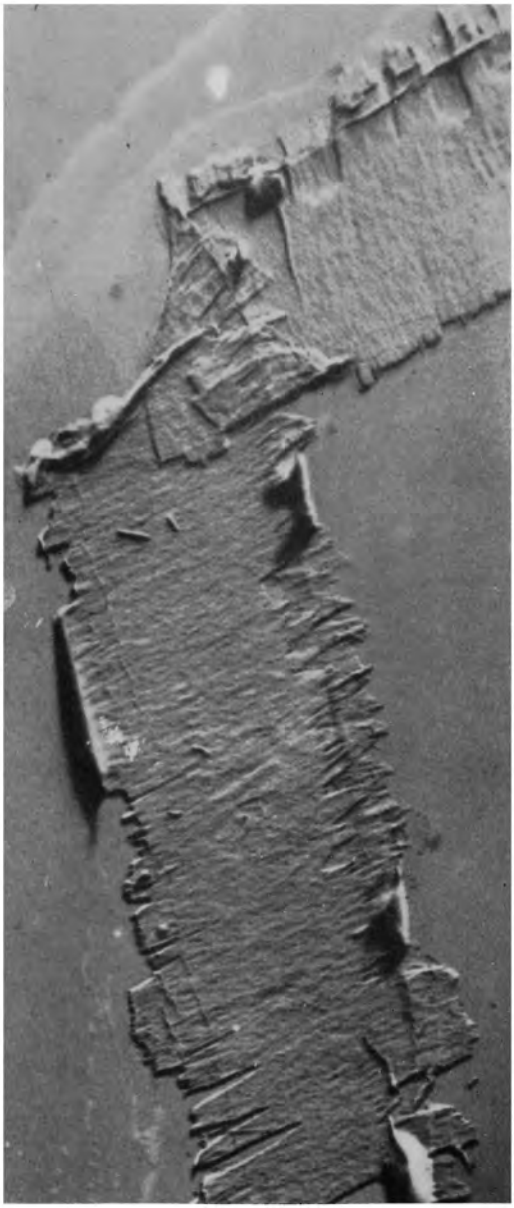


Fig. 13 — Macerated Scots pine (×1 025)

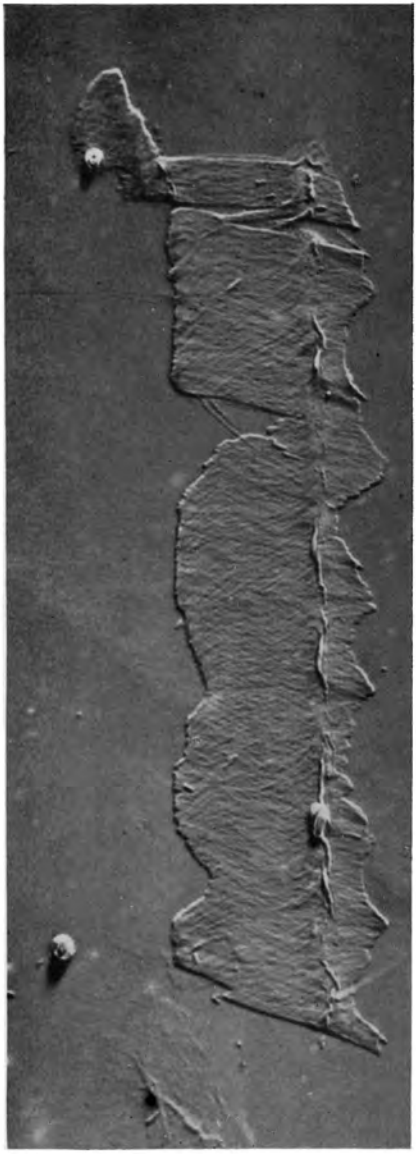


Fig. 14 — Macerated Scots pine (×675)

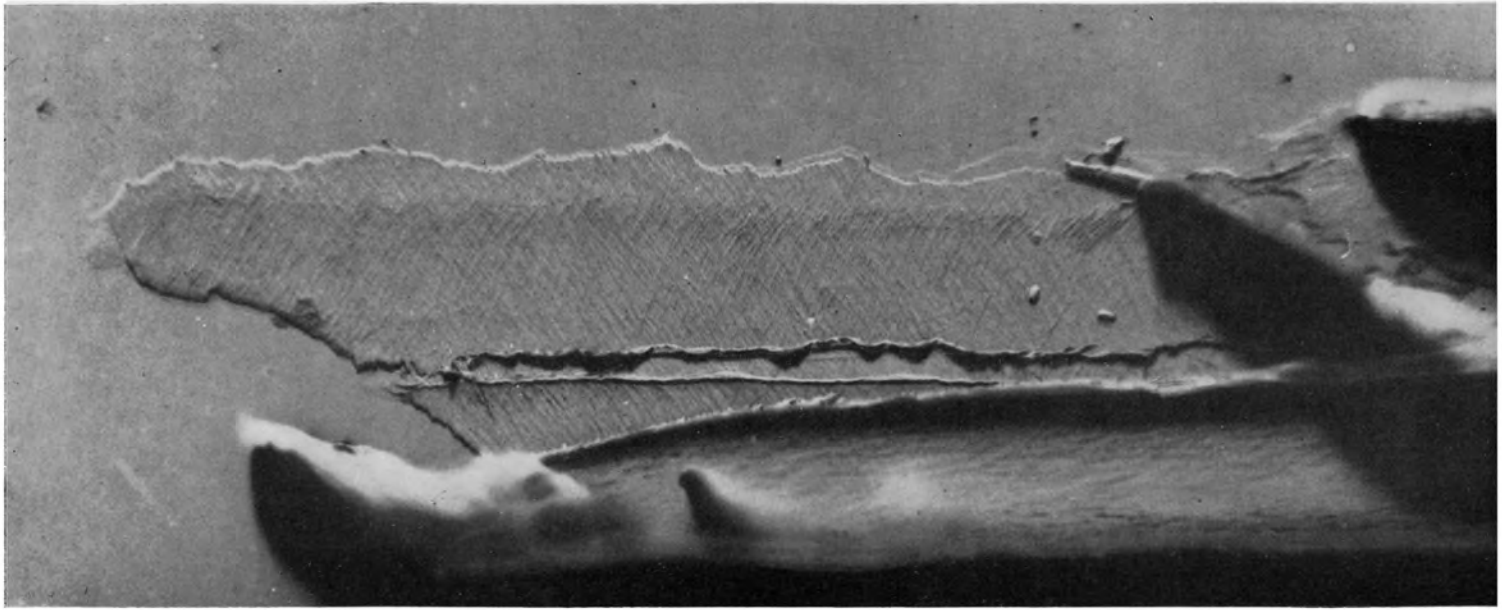


Fig. 15 — Macerated Scots pine (×775)

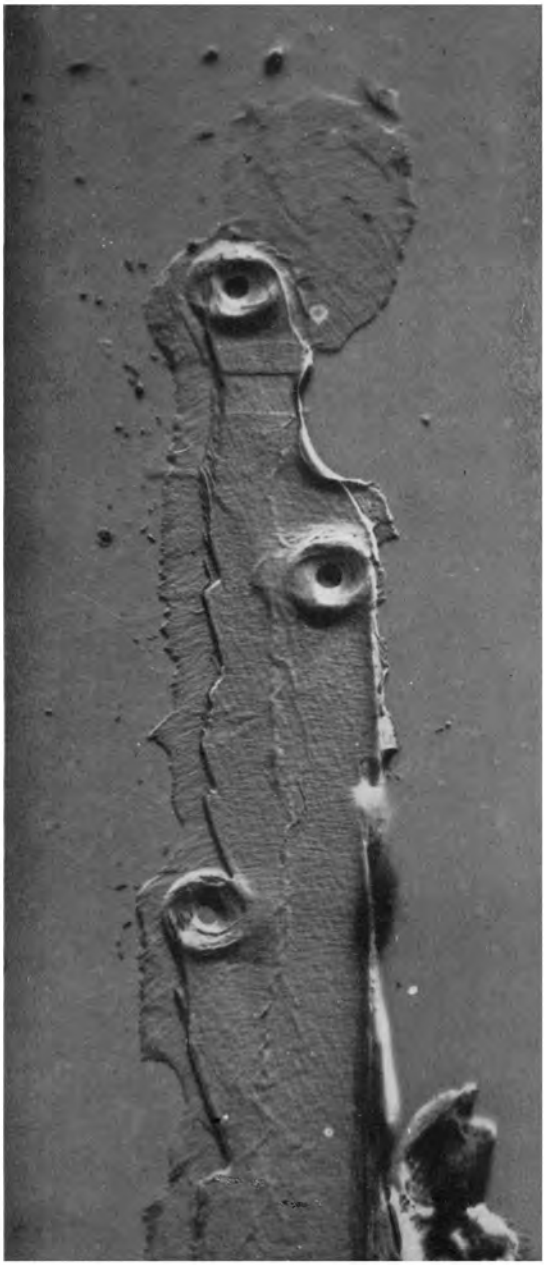


Fig. 16 — Macerated Scots pine — S3 overlies S2, the fracture line of which may be seen sweeping round the pits
Underlying S2 is S1 — the fracture line of this is quite different.
The bordered pits have collapsed during drying ($\times 650$).

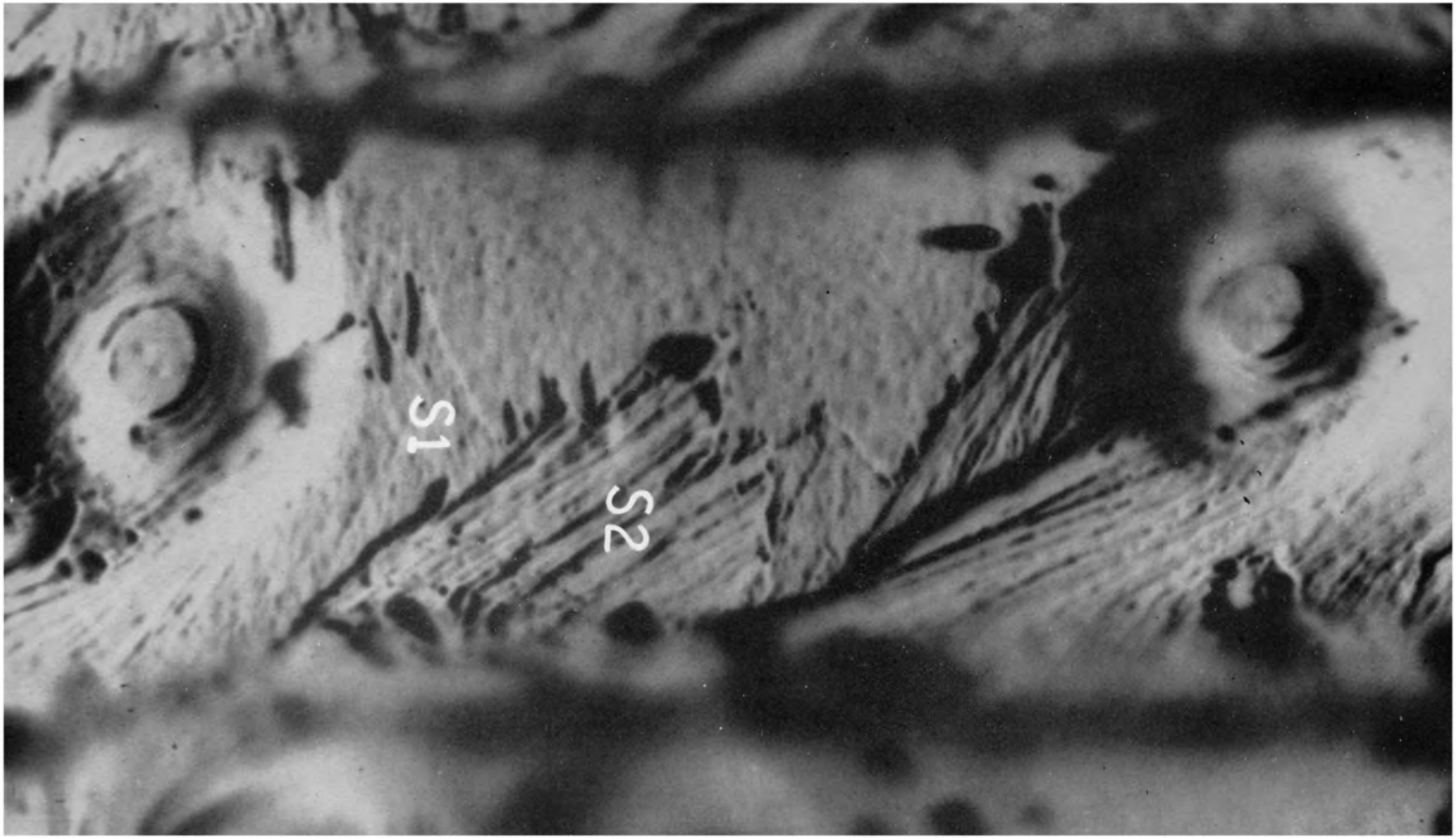


Fig. 17 — Radial surface of Scots pine showing S2 and S1 viewed from lumen side—rather more than the width of one tracheid is included (metal-shadowed plastic solid replica $\times 2\ 900$)

Fig. 18 — Radial surface of European spruce near middle lamella (metal shadowed plastic solid replica × 750)

DISCUSSION

PROF. H. W. GIERTZ: It is obvious that the surface properties of the fibres are of the greatest importance for papermaking and therefore the primary wall (P) and the outer layer of the secondary wall (S1) are of special interest. The primary wall of fibres originating from different types of pulp was studied at the Fibre Chemistry Section of the Swedish Forest Products Research Laboratory during the years 1952-55. The technique used was the same as that used by Dr. Bucher and it is described in my paper for Friday morning (*see* page 397).

At this symposium, when dealing with the surface of the fibre, interest has been concentrated on both the primary wall (Bucher, Giertz) and S1 (Emerton). Before going on to present the results of our investigation, I should like to say why I have drawn the conclusion that the skin-like formations and fragments observed when swelling fibres stained with Victoria Blue in cuprammonium solution originate from the primary wall (and not from S1) —

1. Typical remnants of the middle lamella can be seen on the undissolved skins (Fig. 3 (*b*), page 406), which shows that the skin is the outermost part of the fibre.
2. The pit opening is covered with a membrane on which the *torus* can be identified (Fig. E). If the skin belonged to S1, the pit would be an open hole.
3. If fibres that in unbeaten condition show skins are beaten, the skin is removed, but the fibre in any case swells with ballooning (which demonstrates the presence of S1) and gradually dissolves completely (Fig. 3 (*e*), Page 408).

Using this technique with swelling and partial dissolving in cuprammonium solution, it has been possible to estimate the amount of primary wall that covers the fibre (for details, *see* pages 397, 398).

The quantitative value (as a percentage of the total fibre surface) for the fibre surface area from which the primary wall has been removed is called the *exposed surface*. This exposed surface has been determined for several different kinds of pulp, for instance —

Unbleached pulps	*Exposed surface, %*
Strong sulphite	0
Medium sulphite	14
Rayon grade sulphite	68
Strong sulphate	0
Soft sulphate	0

Bleached pulps

Unbleached sulphite	14
Bleached: Cl — Alkali — Hypo — Alkali — Hypo	72
Unbleached sulphate	0
Bleached: Cl — Alkali — Hypo — Hypo — Hypo	77
Bleached: Cl — Alkali — ClO_2 — Alkali — ClO_2	41

As can be seen from this table, the primary wall is attacked during sulphite cooking. In strong pulps, all fibres are covered with the primary wall; but, in softer pulps and rayon grade pulps, it has been ruptured to a great extent. Unbleached sulphate pulps seem all to be covered. The primary wall is, as could be expected, partly torn off during bleaching, hypochlorite having a more drastic effect than chlorine dioxide.

MR. G. HUNGER: We have clearly seen these parallel features in Mr. Emerton's pictures. It is interesting that, on the inside of the primary wall on top of secondary wall No. 1, we have seen the same features, definitely being microfibrils in our case. It has already been shown by Frey-Wyssling and Muhlethaler that the primary wall goes over at the corners of the cell into microfibrils running parallel to the cell axis. The primary wall of fibres of a spruce pulp, swollen in a swelling agent, are ruptured and fall away from the fibre; only microfibril bundles running parallel to the axis remain. Thus, it seems very likely that these longitudinal features found by us on top of layer S1 may be continued *under* S1, as Mr. Emerton's micrographs have shown.

PROF. B. STEENBERG: All the micrographs have been surface replicas. The technique of Dr. Asunmaa, using transmission electron micrography, gives some further information. In these studies, we have never seen anything we could call a primary wall in accordance with Mr. Emerton's use of the term, but we can see the S1 layer quite distinctly. If you dye it with osmium, S1 differentiates from the S2 layer, the former being more osmium-impregnated. If a flake of the surface comes off, penetration of osmium is possible from both sides, but the other layer is still quite distinctly osmium-coloured, whereas the other is not. So the dyeing of S1 is not just a matter of decreased penetration from the surface inwards. Whether S1 is only two layers or whether it is a multitude of layers, I will not say. From examination of cross-sections, I would say that it is very possible that there are many more than two layers. Of course, looking from the surface as in replicas, you may see whether there is a third and a fourth layer. Cross-sections are valuable here and they indicate the existence of more than two layers.

MR. Ö. ELLEFSEN: I want to make a few comments in connection with the paper read by Dr. Rånby. He referred to work we published a few years ago on cellulose structure.* This investigation was carried out on unoriented specimens of regenerated cellulose from viscose solutions and it is quite correct that we could not find the X-ray results consistent with the well-known chain structure of cellulose suggested by Meier and co-workers.

We tried both models mentioned by Dr. Rånby (the boat form and the chain form) and neither could be interpreted by means of the X-ray results.

In a more recent investigation,† amorphous cellulose, both native and mercerised, has been subjected to the same technique and we have so far not been able to confirm the chain proposed by Meier and Misch. As the distance between the two hydrogen atoms connected with the carbon atoms on both sides of the glucosidic link in this model is only about 1.7Å, we tried to interpret our X-ray findings by means of a certain amount of twisting in the glucose units and, as Dr. Rånby mentioned, we found the best result was obtained when a twist of about 45° was introduced.

I want also to point out that, in recent work carried out by Dr. Norman‡ on oriented specimens of ramie and fortisan, neither model was compatible with the X-ray findings.

I would like also to mention that Dr. Mering and co-workers in France have found in mercerised or regenerated cellulose that the chain structure put forward today by Dr. Rånby and first mentioned by Dr. P. H. Hermans in Holland is the correct one.

Another point is Dr. Rånby's reference to the determination of crystallinity in cellulose specimens by Hermans' method. We also have tried to develop a similar method,† but the results are somewhat different from Dr. Hermans' findings. The general value he found for native cellulose is about 70 per cent. crystalline matter and 30 per cent. non-crystalline matter. With our method, the figures obtained were about 50/50. Of course, as Dr. Rånby pointed out, all these methods are just average methods and what we really need is a method to give a real order/disorder distribution in cellulose specimens. The method we have developed gives automatically a *maximum* value for the amorphous part of the specimens. It is interesting, however, that, if we start with the model of microfibrils 30 Å × 100 Å dis-

* Björnhaug, A., Ellefsen, Ö. and Tönnesen, B. A., 'Interpretation of X-ray Diagrams of Unoriented Organic Chain Polymer Substances — 3. Regenerated Cellulose': *Norsk Skogind.*, 1953, **7** (6), 171

† Ellefsen, Ö., Wang Lund, E., Tönnesen, B. A. and Öien, K., 'Studies on Cellulose Characterisation by Means of X-ray Methods: *Norsk Skogind.*, 1957, **11** (8), 284; (9), 349

‡ Norman, N., Medelelse No. 219, Universitetets Fysiske Institutt (Oslo, 1954)

cussed by Prof. Frey-Wyssling yesterday, we find that, of the 120 cellulose chains that can be present in this structural unit, 48 lie in the surface portion. These 48 chains can easily account for the high amount of non-crystalline material found by means of our method.

Before finishing, may I emphasise that I find it very promising that the problems of cellulose chain structure are being tackled by scientists in different places (Dr. Rånby referred to Japanese work in progress), as I think we really have to know the exact chain structure and arrangement in order to understand why the fundamental microfibrils have just the size we observe in the electron microscope.

DR. J. SIKORSKI: I should like to comment on the following three points—(*1*) the orientation of the crystallographic planes in the microfibrils, (*2*) the morphology of the microfibrils and (*3*) the general significance of the microfibrillar elements in cellulose and other fibres.

The original observations of the preferential orientation of the (101) crystallographic planes in the microfibrils of native cellulose were made[1] on films prepared from dried aqueous suspensions of the colloidal particles obtained from cotton, ramie and jute, using a method of acid hydrolysis similar to that of Rånby.[2] We have not been successful, however, in preparing homogeneous films (containing exclusively individual particles) and it would therefore be of some interest to enquire whether our findings were confirmed or otherwise by other workers. The difference in intensity between the (101) and ($10\bar{1}$) reflections of cellulose (in two diffraction photographs, one with the X-ray beam parallel and the other perpendicular to the surface of the films of particles) could only be interpreted to indicate that the (101) planes are preferentially oriented parallel to the surface of the film and, consequently, to the larger face of the microfibril.[1]

Estimation of the height of cellulose microfibrils involves calibration of the shadowing angle and measurement of the length of the 'shadow' in electron micrographs. With reference to the former, no indication is given of the method used by Morehead for this purpose; in our own work, latex particles were employed throughout (*see* Fig. 6[3]). Rånby suggests, however, that the figures quoted by us for the height of cellulose particles are too low, because of the reduction of the shadow length by the deformation of the supporting membranes. Our observations were not confined to a restricted area of one grid and close examination of the data for the coefficient of

1. Mukherjee, S. M., Sikorski, J. and Woods, H. J., *Nature*, 1951, **167**, 821
2. Rånby, B., *Acta Chem. Scand.*, 1949, **3**, 649
3. Mukherjee, S. M., Sikorski, J. and Woods, H. J., *J. Text. Inst.*, 1952, **43**, T 169

variation obtained in our experiments (*see* Table I[3]) indicates that wide variations in the shadowing angle could not have occurred. The other possibility, that of the formation of 'local imprints' under individual particles, is also very unlikely, as the weights of a single particle and of the equivalent area of supporting film are of the same order of magnitude. Furthermore, some experimental evidence, involving much larger microfibrils (obtained from synthetic polymers) refutes such a suggestion.

The third point, that of the significance of the microfibril, is perhaps of the greatest interest to all of us working in the field of fibrous structures. It is evident that the old concept of a two-phase system (crystalline and amorphous) in polymers requires some revision and one is now forced to accept the well-known idea of Frey-Wyssling advanced before the advent of the electron microscope. It is reasonable to regard the microfibrils as containing a greater proportion of the crystalline material[4] than mainly amorphous interfibrillar regions. Furthermore, it is necessary to accept the views of Fürth[5] who suggested that it is the basic thermodynamic restriction that limits the size of the intrinsic structure elements of all biological objects and crystalline materials in general[4] to an order of magnitude a few hundred times larger than atomic dimensions.

DR. A. MYERS: I wish to draw attention to some of the work we have been doing recently at Leeds University on the structure and composition of the microfibrillar fractions in the cell walls of algae.

In electron micrographs of chemically isolated microfibrils from *Rhodymenia Palmata* (a red alga), the microfibrils appear to be quite normal cellulose fibrils and yet, upon hydrolysis, they yield a mixture of 50 per cent. glucose and 50 per cent. xylose.

Electron micrograph examination of a piece of blended wall of another alga, *Porphyra*, has shown the microfibrils to be surrounded by an amorphous matrix, which can be largely removed by treatment with boiling water. When the wall was further treated by a cold normal solution of alkali, the microfibrils were broken down into particles of pure mannan. The polysaccharides removed during the alkali treatment yielded, on recovery and hydrolysis, a mixture of the sugars galactose, xylose and mannose. In no fraction of the cell wall was glucose detected.

I should like to draw attention to these facts, in view of the assumption often made that all microfibrils are cellulosic.

4. Balashov, V. and Preston, R. D., *Nature*, 1955, **176**, 64
5. Fürth, R., *Exp. Med. Surg.*, 1955, **13**, 17

An electron micrograph of the microfibrils from an unusual wood specimen was examined. It is typical of that obtained from present-day wood, yet the specimen is of oak recovered from the original wall around the city of York and is about 2 000 years old. Analysis shows that only 37 per cent. of the dry weight of this ancient wood is cellulose — a loss of about 15 per cent. compared with modern oak. This 15 per cent. loss has in no noticeable way changed the appearance of the microfibrils. It would be dangerous to deduce anything further from these results, but it is tempting to correlate the loss in weight with the disappearance of amorphous cellulose, which is in the same order of magnitude.

DR. B. G. RÅNBY: I will not comment about the sample of York wood, because it has had plenty of time to crystallise and deteriorate in 2 000 years. Let me say that, of course, it is not only cellulose chains that crystallise into fibrils, because nylon does it and even polyethylene does it, especially when oriented by stretching. We should not be too surprised, therefore, if we find mannans, xylans and other chain molecules forming fibrils. Cellulose has a very strong inclination to form fibrils, because its molecules are stiff, straight and probably unbranched chains.

Let me take the speakers in reverse order.

When I commented on Dr. Sikorski's and Dr. Wood's work on microfibrils some years ago, I may have dealt inadequately with the methods they used.

We did not assume the imprint or sagging of the membranes because of the weight of the objects studied. The mass of cellulose micelles is of the order 10^{-18} g. and the weights involved are negligible in comparison with the surface forces at the contact area between the particles and the membrane. When Dr. Sikorski now presents width/thickness ratios of about 4, I would say this is in fair agreement with our ratio of about 3. We did not agree with your reported fibril thickness 18-16Å, but your present values of about 30Å are very close to ours.

We were very anxious to use the glass surface as membrane support to prevent these imprint or sagging effects, but, if similar results can be obtained without using a glass surface, that is excellent.

I think the evidence Sikorski and Woods brought out about the orientation of the (101) plane in deposited micelle films is very convincing, even if they did not have more than 90 per cent. particles in their specimens. The 10 per cent. aggregates, etc. would cause background scattering, but the evidence is conclusive enough.

On the question of the significance of microfibrils, we have to rely on the evidence from the electron micrographs, which agrees with earlier data from X-ray scattering and surface area measurements.

Now, I wish to comment on Mr. Ellefsen's X-ray data for the amount of cellulose in the micelle surfaces. From earlier work, we obtained an X-ray crystallinity of about 70 per cent., but only some 10 per cent. easily accessible cellulose, for example, in heterogeneous hydrolysis. The difference was explained as a micelle surface effect. With only 50 per cent. crystallinity by the Ellefsen method, we have to assume an even more extensive surface layer. With these data, too, one must accept the fibril surface layer as largely inaccessible to acid hydrolysis. These layers are indeed so well organised and ordered in cotton and ramie cellulose that constant boiling hydrochloric acid does not seem to penetrate or attack the cellulose micelles from the sides, only from the ends according to A. Sharple's interpretation. That is not the case with native wood cellulose, when some hydrolytic attack on side surfaces has to be assumed.

Then there is the question of the carboxyl groups in the wood cellulose, which no one has brought up. Do not quote me as saying that the carboxyl groups are responsible for the high rate of hydrolysis of wood cellulose in phosphoric acid. I have said that we found an increased stability with decreasing carboxyl groups content. We have still to investigate the effect of the aldehyde groups we tried to oxidise. We used sodium chlorite for this purpose and it is quite possible that we oxidised only some of the groups (probably the end groups) and others (of another type) still remained. Maybe, it was just this other type of aldehyde group that we reduced with sodium borohydride ($NaBH_4$) and that was responsible for the stabilisation effect we obtained. Again, we have to say that this is all we know, but the work is in progress. We still have these two possibilities — the carboxyl groups, against which we have evidence and the aldehyde groups, against which we have no firm evidence at the moment, because we lack the analytical data.

It was also said that the carboxyl groups are not reduced by sodium borohydride. At least, we know that the carboxyl group content as we analysed it was decreased when we carried out this reduction. Furthermore, we also tried the reaction with polybutylmethacrylate, which was reduced to about 98 per cent.

MR. H. W. EMERTON: My comments will be largely concerned with dispelling what are, I believe, a few misconceptions. First of all, with one exception, the pictures I showed this morning were light micrographs and not electron micrographs.

Then there is the question of those longitudinal features that I showed. Some people have asked me if there is any possibility that these are the same as the lignin ridges to which Dr. Bucher drew attention yesterday. I do not for one moment think that they are. At the same time, I am not denying the existence of those lignin ridges external to the fibre; both are undoubtedly present.

With regard to the location of the longitudinal features that I showed this morning, I do not say that they would never be on the outside of S1, but quite definitely in certain cases they are on the inside of this wall. In those cases when we can, with complete confidence, say that S1 is folded back so that we observe its inner surface, it can be seen that the longitudinal features are on the inner surface. We have never with certainty identified them on the outside of S1.

Prof. Steenberg mentioned that in some pictures more than two layers might be revealed in S1. I would subscribe to that possibility.

Dr. Sikorski mentioned our figure of $\pm 2°$ in the shadowing angle. These were preparations metal-shadowed for the light microscope, so that, of course, the specimens were dried on a glass slide prior to shadowing. The uncertainty in the shadowing angle is almost entirely due to a difference in the angle subtended by the metal source at the two ends of the slide.

PROF. STEENBERG: Dr. Rånby brought up the question of surface tension, which I wish to comment further upon. I should very much like any of the speakers to go a little further into the details of what effect on the final features the surface tension will have. I am quite sure that this is a very important thing. There may be effects due to surface tension against the surface on which the fibre material is dried. Would you think that it is possible to say how much of these different structural features are influenced by surface tension, especially surface tension against the surface on which the material is evaporated?

DR. RÅNBY: This is very important and I think it should be observed. I have been told by papermakers that you cannot make paper from a pulp suspended in an organic liquid. In this case, we did not put the particles on the glass surface. The collodion or formvar membrane was coated on the glass surface; the cellulose suspension was then dried on the membrane and shadowed. The membrane was then stripped from the glass and studied in the electron microscope. This technique has been developed in our laboratories by F. F. Morehead and we think it is a safe method to avoid

membrane distortion effects. The results show that we have somewhat higher values for the fibril thicknesses than have Sikorski and Woods.

Frankly, we do not know very much about contact angles or the adhesion between cellulose and collodion and even less about the surface tension between cellulose and formvar. There is a lot to be done here and I hope those who are still working with the electron microscope will do it.

PROF. STEENBERG: If you take a wet membrane from a cellulose or some cellulose derivative preparation and dry it on glass, it may stay like a membrane on the glass; if you dry it on teflon foil, you may find no membranes, but a woolly, hairy-looking material. They look like two entirely different materials. Surface tension may change the appearance of very fine particles: that is the point I feel is very important.

MR. EMERTON: If I may comment on this question of surface tension, I have three illustrations to show. Surface tension has very marked effects both upon the fibre itself and upon the membranes of microfibrils that peel away from it. When a wood fibre is dried on to a glass slide, we observe that the lumen usually collapses and the fibre is pulled down flat by surface tension. So far as pine and spruce are concerned only a small proportion of latewood fibres resist this and, as beating proceeds, these too tend to collapse.

Fibrillated sheets of microfibrils are apt to react to surface tension in two ways. They tend to be pulled into close contact with the substrate and, if this is cellulosic (or even glass), they bond firmly to it so that they are prevented from shrinking on themselves. Such membranes are usually taut, because at quite an early stage of drying they bond to the surface with which they are pulled into contact; any fibres to which they are attached continue to lose water and shrink, thereby stretching the membrane. Secondly, the contracting water envelope surrounding a membrane that is suspended between two fibres gathers up the sheet of microfibrils and it finally bonds to itself, as Steenberg said, like a curling-up leaf. These effects are illustrated in Fig. F and G.

Fig. E—The primary wall from a strong sulphite fibre after the secondary wall has been dissolved in cuprammonium solution — note the dark longitudinal formations that originate from the middle lamella and the *torus* fixed on the membrane in the pit opening

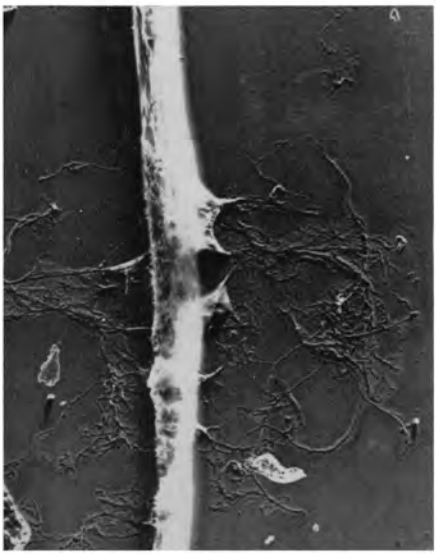


Fig. F — Bonding of fibrillated membrane to substrate and effect of surface tension (light micrograph × 250)

Fig. G — Effect of surface tension on a fibrillated membrane between two fibres (light micrograph × 250)