ELECTRON MICROGRAPHS OF PLANT FIBERS

by

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INTRODUCTION

The electron microscope permits a much more detailed study of the fine structure of plant fibers than could hitherto be made with the aid of polarization microscope and the methods of X-ray diffraction. As a result, more than eighty years after NÄGELI[1] published his important micellar theory (1858), it has now become possible to visualize the elementary particles he postulated.

Electron micrographs (RUSKA AND KRETSCHMER)[2] of plant fibers were first published eight years ago, but these early photographs yielded little information that was new about the size, form and systematic arrangement of these submicroscopic particles. Neither has the work done since answered in conclusive fashion questions concerning the development of fibrils of a size near the resolution of the light microscope. These disappointing results should not, however, be laid to the microscope itself, but rather, to inadequate methods of specimen preparation. The commonly employed methods of splitting cellulose structures, such as crushing in a ball mill, splitting with ultrasonic waves or disintegration with chemicals have damaged the natural texture to such an extent that new results concerning the construction of the cell wall have not been apparent in the electron micrographs. We have, in large measure, evaded these difficulties and obtained photographs of unaltered cell wall structures by using a simple, new method of dissection and by metal shadowing the specimen preparations before microscopy.

EXPERIMENTAL

A Waring Blendor has been used to prepare satisfactory fiber sections. To do this the fibrous material was first cut to about one cm lengths, suspended in distilled water and then stirred in the blendor for five minutes. Stirring of the liquid by the blades of the blendor rotating at very high speed tears the suspended fibers to a loose pulp.

The shreaded fibers were next washed in distilled water and the heavier particles eliminated by sedimentation in a glass cylinder. One drop of finely dispersed material from the supernatant was finally dried on the usual collodion-covered grid and shadowed with either chromium or palladium.

RESULTS

Optical microscopic and polarization-optical observations have shown that plant cell walls consist of two layers that differ in their structure. The primary wall is deposited

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on the middle lamella, which consists mainly of pectin and provides the cementing substance between individual cells. This wall is formed during the growth of cells and its thickness amounts to only ca 0.5 μ. As soon as the cell has reached its final state of growth, deposition of the secondary wall sets in and continues until the cell lumen is almost completely filled.

Under ordinary circumstances the fine structure of these walls cannot be seen with the light microscope, but after staining with Congo red the primary wall appears in the polarizing microscope to be a criss-cross fibrous structure. FREY-WYSSLING[4] has used the polarizing microscope to demonstrate the arrangement of the cellulose in the secondary wall. He has found that in contrast to the primary wall, the secondary wall has its fibrils arranged in parallel layers. The cross-sectional fiber structure he postulates is shown in Fig. 1. According to this scheme the fibrils could either be completely separate, as shown in Fig. 1 (A), or they could merge into neighbours (Fig. 1 (B)), and it has hitherto been impossible with the indirect methods available to decide between these alternatives.

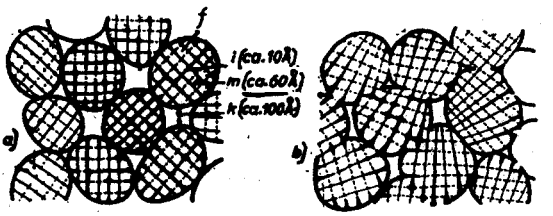

Fig. 1. Schematic cross section of the submicroscopic fiber construction according to FREY-WYSSLING. f = submicroscopic microfibrils interspersed with intermicellar spaces (i); m = schematic sketch of undistorted micelles projected into a plane; k = submicroscopic capillaries between microfibrils. In Fig. 1 (A) the microfibrils are completely separate. In Fig. 1 (B) the structure of the microfibrils merge into neighbouring fibrils. (According to FREY-WYSSLING, *Protoplasma*, 27 (1937) 372–411).

The present electron micrographs throw light on this question and reveal fibrils having a fairly constant diameter of 250–400 Å. A detailed discussion of the structure of the fibrils will, however, be deferred to a later paper to be published on this subject.

If fibers are swelled with zinc chloride for optical examination, the secondary walls clearly show in cross section an arrangement of layers. With a cellulose dye, successive layers are differently coloured and the double refraction also varies between successive lamellae. Tests made on growing cotton fibers showed that each lamella reflects the daily deposition of cellulose, the thickness of these newly formed fiber layers varying between 0.1 and 0.5 μ, according to external conditions.

We propose to investigate, with the help of the following photographs, to what extent the conceptions prevailing today correspond to actual facts.

Ramie

Though ramie is not one of the important textile fibers of commerce, the excellent arrangement of its cellulose and the absence of large quantities of non-cellulose substances have made it an especially desirable material for scientific studies.

Its two wall types mentioned above, *i.e.*, primary wall (Fig. 2) and secondary wall (Fig. 3), can evidently be clearly distinguished in electron micrographs. In both layers we find the cellulose developed as completely individualized fibrils, having a thickness of about 250 Å. The arrangement of these fibrils is, however, entirely different in the two layers. As indicated by the earlier polarization-optical and X-ray investigations, the secondary wall consists of parallel layers in which the fibrils are in such close contact that it is often hard to distinguish them from one another. The fibrils of the primary

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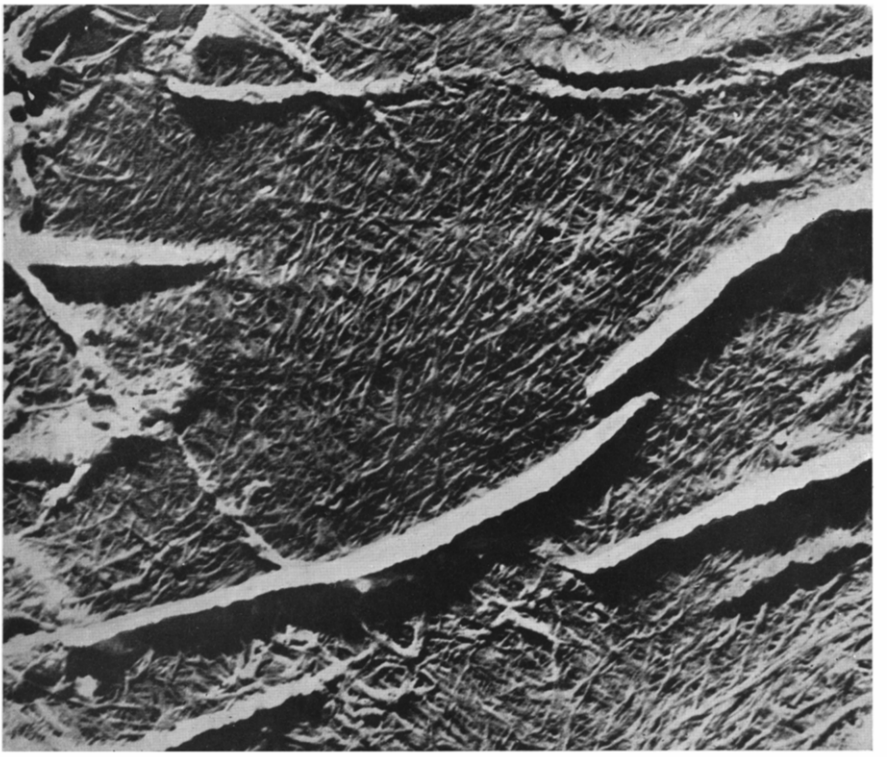
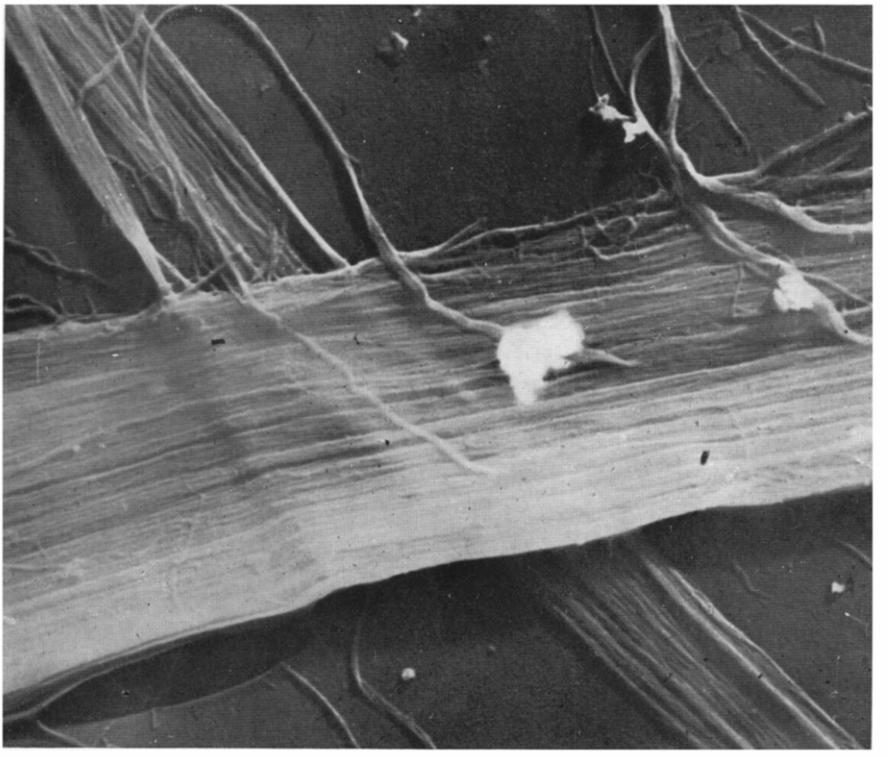


Fig. 2. An electron micrograph of the primary wall of a ramie fiber. Adhering non-cellulosic substances give to the fibers of this and certain other photographs a somewhat diffuse appearance. Magnification = 20000 ×.



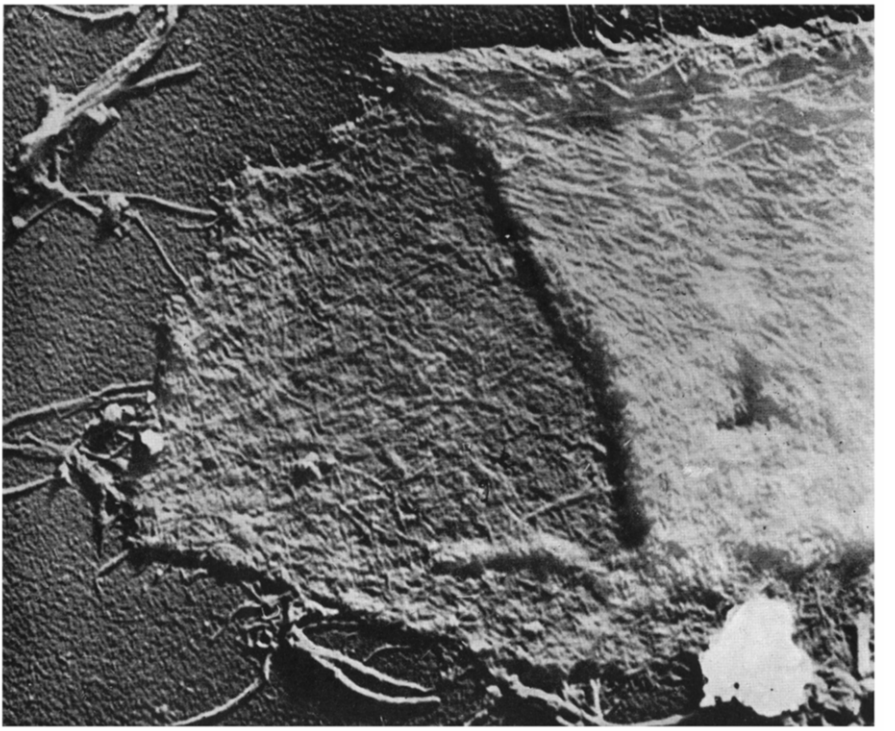


Fig. 4. An electron micrograph of the primary wall of a cotton fiber. Magnification = 20000 ×

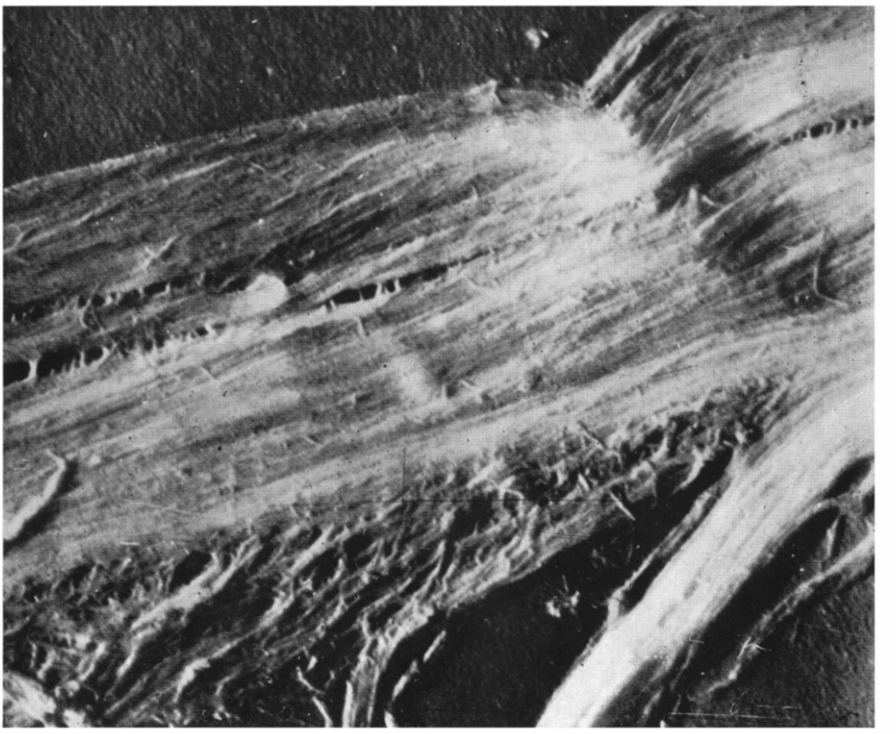


Fig. 5. An electron micrograph of the secondary wall of a cotton fiber. Magnification = 20000 ×

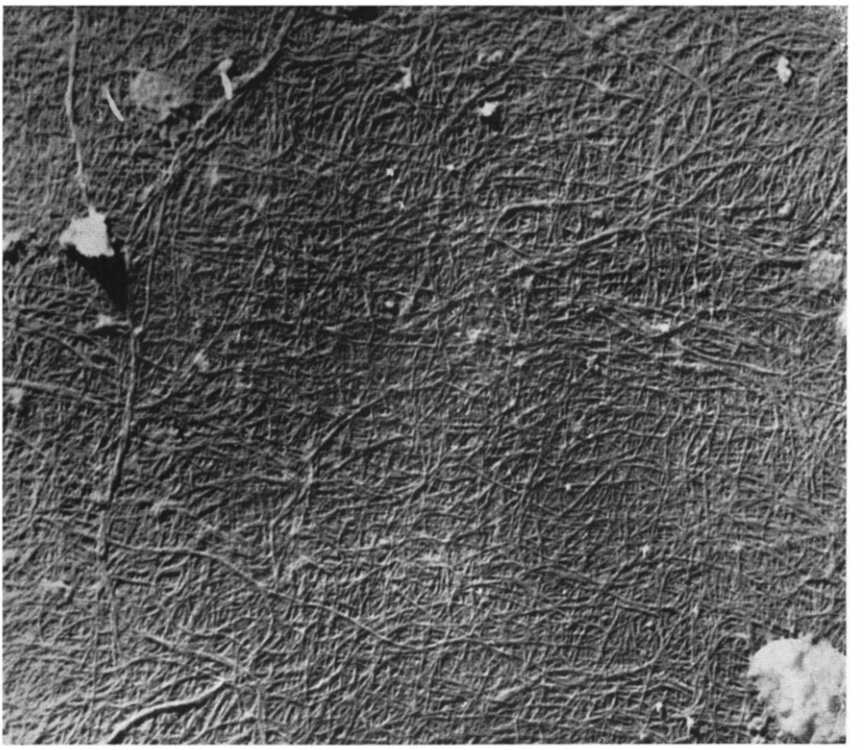


Fig. 6. An electron micrograph of the primary wall of a flax fiber. Magnification = 20000 ×.

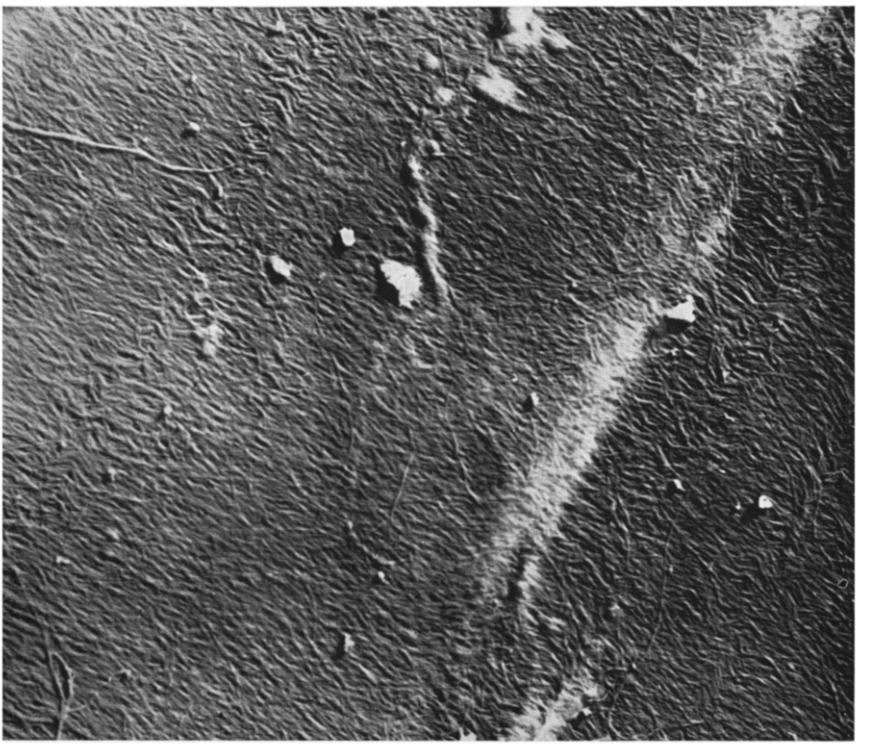


Fig. 7. An electron micrograph of the secondary wall of a flax fibre. Magnification = 20 000 ×.

Fig. 8. An electron micrograph of sisal. The interior wall is heavily impregnated with lignin Magnification = 20000 ×.

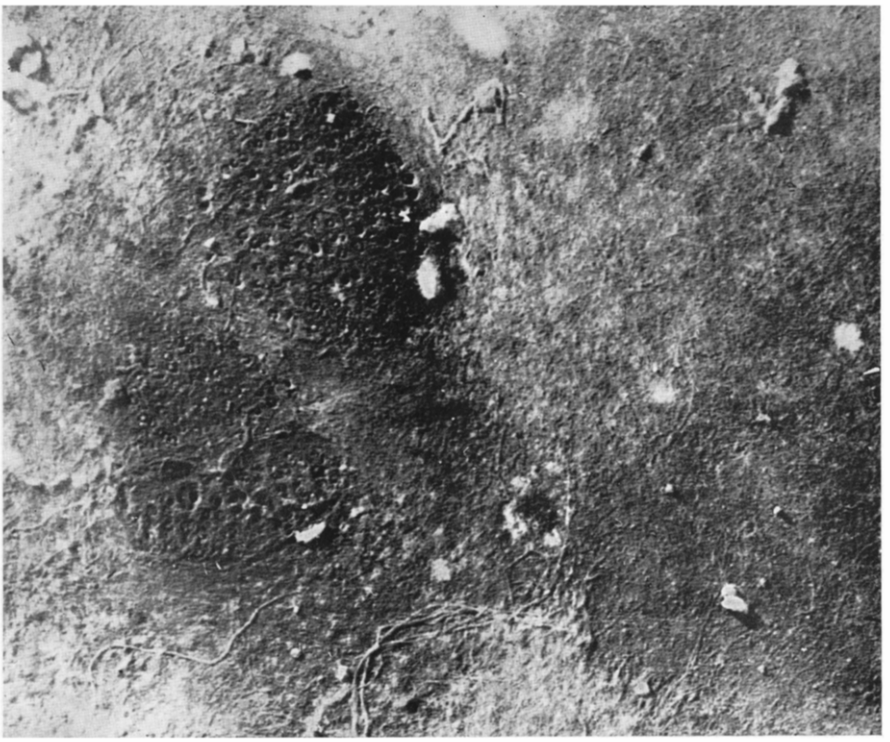


Fig. 9. An electron micrograph of sisal. A layer deposited deeper in the wall. Magnification = 20.000

wall, on the other hand, are intertwined to form a loose network. Amorphous material incrusts the fibrils of both walls. Consisting principally of wax, pectin and hemicellulose, it can be dissolved by diluted H_2O_2 or hypochlorite, without the cellulose structure being attacked.

Cotton

There are more non-cellulose ingredients in the primary wall of cotton than of ramie (Fig. 4). As HESS and his collaborators pointed out, there is in fact so much wax, pectin, etc. in young cotton fibers that the X-ray diagram of cellulose is completely masked and does not become apparent until about thirty-six days after the petals have fallen. The cellulose diagram can, however, be shown as early as five days after falling of the petals, if the primary wall is first extracted with diluted alkali and then bleached. As will be seen from Fig. 4, these amorphous substances are embedded between the microfibrils, just as in the case of ramie.

Again as with ramie the fibrils in the secondary wall (Fig. 5) are arranged in parallel layers. Seen under the polarizing microscope, these layers do not follow a direction parallel to the fiber axis, but ascend spirally. Their direction of winding may be different, as for instance one layer will assume an S-shaped direction and the next one will be twisted in the opposite sense. This gives such fibers a very high degree of elasticity.

Flax

Flax fibers show more clearly than any others studied the fine structure of the fibrils composing them (Fig. 6). In the secondary wall (Fig. 7) these fibrils are obviously much more poorly arranged than in either ramie or cotton. They proceed, more or less intertwined, in the same general direction, but they do not form the closely packed parallel aggregates seen in these other fibers.

Flax provides an especially good material for testing the idea of FARR AND ECKERSON[5] that separate fibrils result from a linear joining of microscopic cellulose particles. These particles, which they thought they had discovered in the protoplasm of young cotton fibers, were supposed to be ellipsoidal (1.1 μ thick and 1.5 μ long) and to be covered by a sheath of some non-crystalline substance. It was supposed that during cell growth the particles joined in chain-like fashion and later deposited as a whole in the cell wall. All the photographs of this paper conflict with this hypothesis. The fibrils do not have a thickness of 1.1 μ, nor is there any segmentation indicating that the fibrils consist of particles joined together. Flax fibrils too are of a uniform thickness of ca 250 Å.

Sisal

Lignin-containing fibers such as sisal, jute and wood present a picture different from that of the foregoing fibers.

Comparatively little is known as yet of the origin of lignin, of its chemical structure or the nature of its distribution in the cell wall. It is still undecided whether it occurs in a pure form or is combined chemically with other substances, but chemists, in increasing numbers, are assuming that most lignin is combined with carbohydrates. It is a fact that these substances are so intergrown with one another that on dissolving one the other still remains as a continuous cell-wall system.

Electron micrographs of sisal show that, as was the case with fiber types mentioned

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above, non-cellulose substances are deposited between the micro fibrils. The interior walls are heavily impregnated with lignin (Fig. 8), the rough surface of which represents a very ragged picture. Remains of decayed protoplasm are often seen in these interior walls; in Fig. 8 they are apparent in the weakly developed fold that extends diagonally through the wall.

Layers deposited deeper in the wall (Fig. 9) are also completely filled with lignin, but their surface is smoother. As this figure indicates, the electron microscope shows hitherto unseen groups of fine pores penetrating through the cell wall. These pores undoubtedly provide the sole connection between cells after strong lignification has impeded early metabolism through their walls.

A completely intact fibrillar structure of the cellulose is apparent after extracting lignin from the walls (Fig. 10). There are no indications that cellulose had been attacked during this extraction, and the thickness of the micro fibrils is not altered. It would thus appear that the fibrils are constructed of cellulose only and that sheaths of non-cellulose substances, as postulated by FARR AND WERGIN, are absent.

A skeleton of lignin is left behind after saccharification of the cellulose with concentrated acid (Fig. 11). This lignin evidently is in sheets and must have been deposited in this way rather than in the continuous fashion heretofore postulated. Such a manner of deposition parallels the layer-like deposition of cellulose. It would appear that after the cellulose structure has formed, each new wall layer is filled in with lignin. The electron micrographs demonstrate that the cellulose and lignin form two continuous structures, but that these two structures most intimately interpenetrate. This is the same structural principle as is presented by a wall of reinforced concrete. The skeleton, corresponding to the iron bars, is furnished by cellulose fibrils while lignin corresponds to the cement. This is well demonstrated in Fig. 11 where after dissolving cellulose from the wall, the former points of penetration of its fibrils leave fine pores through the lignin. This clever combination of high tensile strength cellulose and pressure resisting material (lignin) gives a wall offering the greatest mechanical stability.

Wood fibers

Wood, like sisal, has a high content of substances other than cellulose, as the following typical dry-weight analysis indicates:

Cellulose	40–50%
Lignin	20–30%
Hemicellulose . . .	10–30%

As would be expected from such an analysis, electron micrographs show the fibrils in thick sections to be entirely covered by these non-cellulosic substances. Cellulose can, however, be seen in thinner sections (Fig. 12) and there too it consists of fibrils having a thickness of ca 250 Å. Lignin, shown in these sections, has an amorphous-granular structure that exhibits no intimate connection with cellulose since the fibrils appear unchanged by extraction (Fig. 13).

DISCUSSION

The electron micrographs of this paper indicate that in spite of their different origins (seed hairs, bast fibers, wood), all the plant cell walls examined have been

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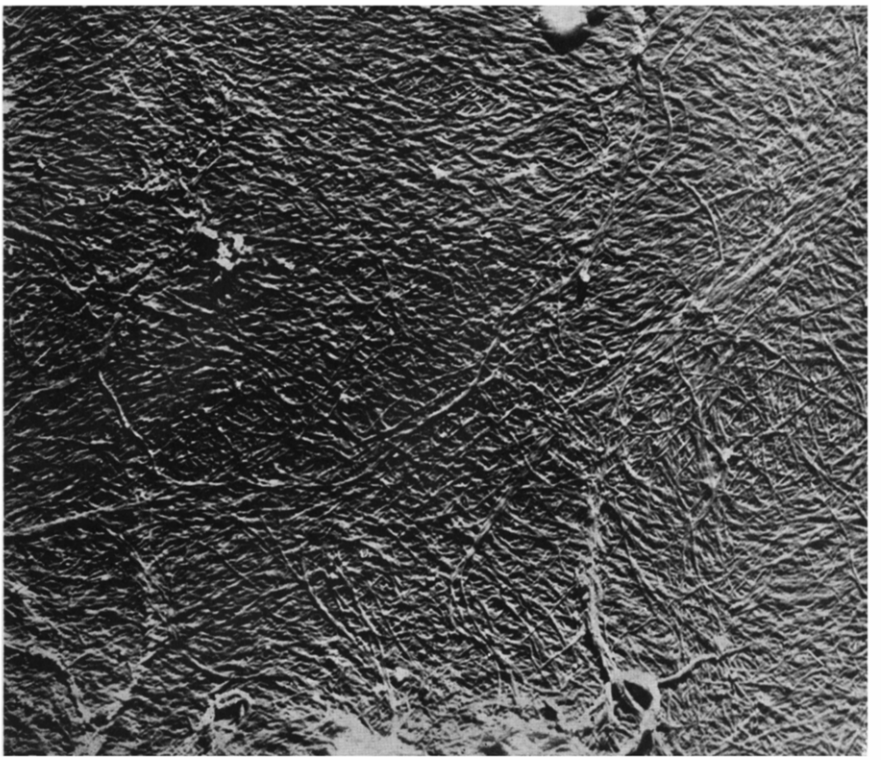

Fig. 10. An electron micrograph of sisal after extracting lignin from the wall. Magnification = 20000 ×.

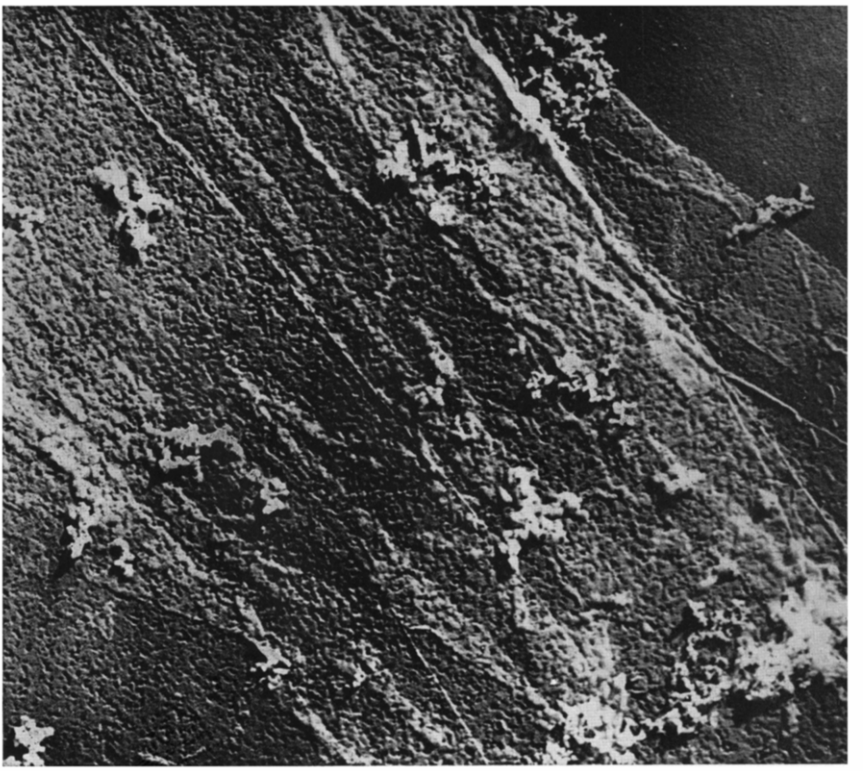

Fig. 11. An electron micrograph of a skeleton of lignin after saccharification of the cellulose with

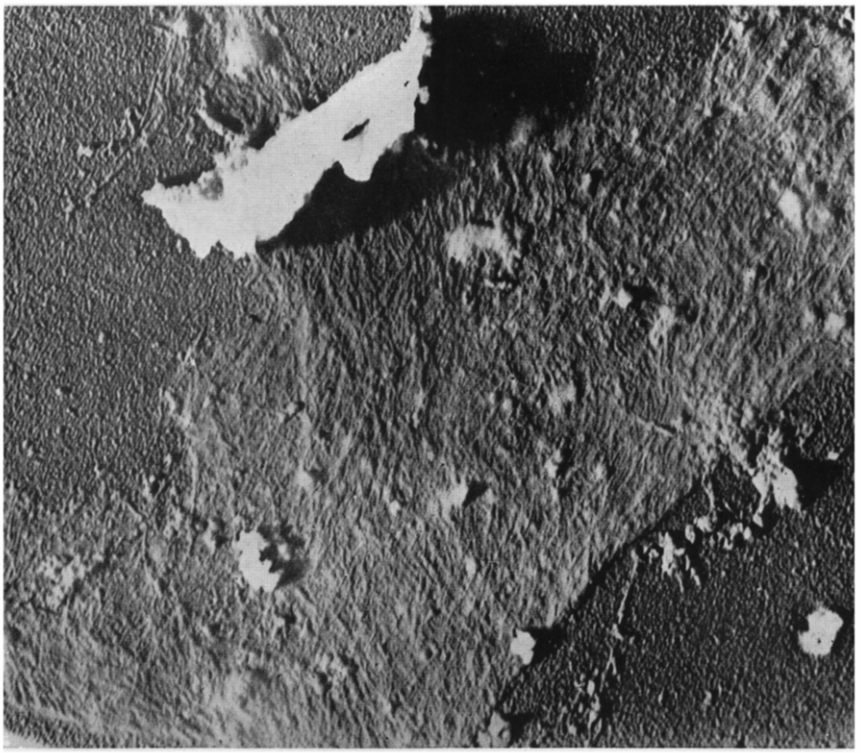

Fig. 12. An electron micrograph of a wood fiber. Magnification = 20000 ×.

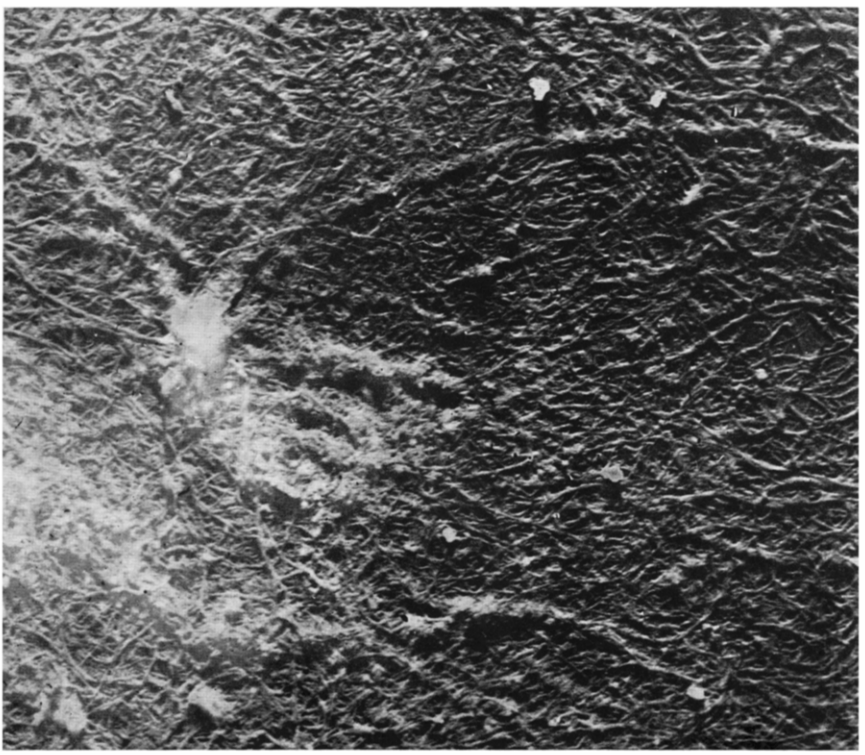

Fig. 13. An electron micrograph of a wood fiber after extracting lignin from the wall

constructed in the same general manner. Investigation of steps by which such cell walls have formed has now been carried out using the fast growing cells in coleoptiles, and these results will be published in a later paper.

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SUMMARY

Cell walls of ramie, cotton, flax, sisal and wood have been examined with the electron microscope. The Waring Blendor splits the fibers successfully and yields sections exhibiting an unaltered natural texture. In all these fibers, the cellulose consists of completely individualized micro-fibrils having a thickness of from ca 250–400 Å. In primary walls the micro-fibrils are intertwined to form a network, while in secondary walls they have a common direction and thus are arranged in a more or less parallel manner. Non-cellulose substances, such as lignin, pectin, wax and hemicellulose, are embedded between such fibrils and can be extracted from the cell walls to leave the fibrils undisturbed. Cellulose and non-cellulose each form an independent system.

RÉSUMÉ

Les parois des cellules de ramie, coton, lin, sisal et bois ont été soumises à un examen électronoptique. En employant une nouvelle méthode de coupage à l'aide du Waring Blendor, nous avons réussi à obtenir des coupures présentant une texture tout à fait naturelle. Il a été constaté que la cellulose dans toutes les fibres est formée de microfibrilles complètement individualisées et de 250–400 Å d'épaisseur. Dans la paroi primaire, ces microfibrilles sont entrelacées comme un filet, tandis que dans la paroi secondaire, elles sont toutes placées dans la même direction et arrangées plus ou moins parallèlement. Entre ces fibrilles se trouvent les substances étrangères à la cellulose, telles que lignine, pectine, cire et hémicellulose. Si on extrait ces substances de la paroi cellulaire, aucun changement des fibrilles n'apparait, ce qui veut dire que les deux substances forment deux systèmes séparés, indépendants l'un de l'autre.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurden Zellwände von Ramie, Baumwolle, Flachs, Sisal und Holz elektronenoptisch untersucht. Durch Anwendung einer neuartigen Schneide-Methode mit Hilfe des Waring Blendors, gelang es Schnitte mit völlig natürlicher Textur zu erhalten. Es zeigte sich, dass die Cellulose in allen Fasern in Form von 250–400 Å dicken, vollständig individualisierten Fibrillen ausgebildet ist. In der Primärwand sind diese Mikrofibrillen netzartig durcheinander verflochten, während sie in der Sekundärwand alle in der gleichen Richtung, mehr oder weniger parallel geordnet, verlaufen. Zwischen diesen Fibrillen sind die cellulosefremden Stoffe, wie z.B. Lignin, Pektin, Wachs und Hemicellulose eingelagert. Herauslösen derselben aus der Zellwand zeigt keine Veränderung der Fibrillen, was darauf hindeutet, dass beide Substanzen ein voneinander unabhängiges System bilden.

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