Study of Tyloses by the Scanning Electron Microscopy

Report 1. Some Preliminary Observations of Tyloses, mainly in Haru-nire, *Ulmus* sp.

By

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走査型電子顕微鏡によるチロースの研究 (第1報)

主としてハルニレのチロースを試料としての予備的観察

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Introduction

There have been reported many informatoins about tyloses because of their importance in tree physiology and wood technology; the cause and mechanism of tyloses formation, their occurrence and gross structure, effect on physical properties of wood etc. have been investigated mainly by the ordinary light microscopy. Electron microscopy has been used recently efficiently for the study on the ultrastructure of the tyloses wall and also on the process of formation of them…, and thus the results have brought out many of new evidences. In electron microscopic study of tyloses, however, when the ultra-thin sectioning

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method and replica technique are employed for specimen preparation, the needs of special skill and time are required and then the scope of specimens to be examined is compelled to limit.

As compared with the transmission type electron microscope, the scanning type electron microscope used in this study has advantages of simple specimen preparation and large depth of focus, and some additional unique applicabilities. For further informations of the scanning electron microscope itself readers should refer to the papers by COSSLETT[1] or KIMOTO and HONJO[6] and so on.

Since the scanning electron microscope, JSM-2 (photo 1) was put into operation at our laboratory of wood structure and properties recently, the writers have tried to apply the microscope for studying some of the basic research subject in wood and wood base materials.

Reported here is a result obtained on tyloses during the course of a study as a preliminary work where mainly the possibilities of application of this microscope to wood were examined, using tyloses and some others as materials for the purpose. The writers have studied for these years on Haru-nire as well as Yachi-damo which are wet-heartwood species[9]. These species of wood also have a characteristic property concerning the permeability of water into wood which may depend mainly on the tyloses morphology in the wood. This is the reason why Haru-nire etc. were chosen here as the main materials.

Materials and methods

Three species of hardwoods were chosen as follows for this study;

Tree species	Diameter B.H. (cm)	Height (m)	Age (year)
Haru-nire (*Ulmus davidiana* PLANCH. var. *japonica* NAKAI)	27	15.8	56
Yachi-damo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.)	27	19.0	55
Hari-giri (*Kalopanax pictus* NAKAI)	30	18.0	83

The sample trees grown in Tomakomai College Experiment Forest were felled in winter and a disk was cut from each tree trunk, resp., at about two meter height from the ground level.

Small blocks of wood were cut, in turn, from the outermost to the pith of the disk and then the blocks were converted into small pieces of 7 mm (fiber direction) × 7 mm (radial dir.) × 3 mm (tangential dir.) in size. After the specimens were air-dried, their longitudinal radial (or tangential, transverse) surface to be examined was coated uniformly with a conductive layer of gold to get a better representation of the surface topography, rotating the specimen under the gold piece being heated and evaporated in a vacuum of ca. 5×10^{-5} torr. The test specimen stuck with silver past on a copper block was inserted into the specimen

chamber of the microscope being placed on the specimen holder. The specimen surface topography was displayed on a cathod ray tube and the images were there photographed. It should be noted that all the photographs shown in this paper represent the surface view seen at an angle of 45° to the specimen, because of the principal structure of this type of electron microscope. Thus, there is need for a brief note to see the photographs correctly that magnification in the horizontal direction is lower than that in vertical, rating 0.7 (horizontal): 1.0 (vertical), as can be seen from photo 2. There are no horizontal scales for photos 3 through 43.

Results

1. Morphological differences of tyloses of sap- and heartwood in general.

a) Haru-nire

Photos 2 and 3 show the typical being state of tyloses in earlywood vessels of sap- and heartwood, resp. Generally in sapwood, excluding the youngest 2–3 rings, it is often observed that tyloses blockade the vessel lumen completely at irregular intervals in it. On the other hand, in heartwood, tyloses are often collapsed or wrinkled and then crumbled. Their morphological differences between sap- and heartwood tyloses are recognizable obviously. As shown in photo 4, in sapwood numerous fine wrinkles are found on the tylosis wall surface, while in heartwood their surface exhibits very complex and irregular appearance, as shown in the photo 5.

Of this species, tylosis changes in its morphology from the outermost inward will be discussed later in detail.

b) Yachi-damo

Photos 6 and 7 show the cross sectional view of sap- and heartwood, respectively. From these photos, it will be seen that tylosis in Yachi-damo is observed less than in both Haru-nire and Hari-giri, and also there found less difference in morphology between sapwood and heartwood. Tyloses with which a vessel lumen is filled completely are scarcely observed in this species unlike Haru-nire. Tyloses in the species usually lie loosely on the inner vessel wall as shown in photo 8 or adhere to the surface as shown in photo 9.

c) Hari-giri

Photos 10 and 11 show the typical existing state of tyloses in the earlywood vessels in sapwood and in heartwood of this species. The development of tyloses in Hari-giri is remarkable, esp., in the heartwood. In sapwood, the tyloses usually are large enough to blockade the vessels completely as shown in photo 11, while in heartwood such tyloses are also observed but some of them are collapsed. Tylosis wall in Hari-giri seems to be more thicker and harder compared with that of sapwood in Haru-nire as shown in photos 12 and 13.

Concerning the surface structure of tylosis wall, there were some differences

between sapwood and heartwood (photos 14 and 15). There are many spherical prominences on the tylosis wall in heartwood (photo 15) but in sapwood (photo 14). Their diameter is about 1 μ.

2. Morphological changes of tyloses from the outermost inward in a Haru-nire trunk.

Illustrated in photo 17 shows that an initial bud tylosis arises from contiguous parenchyma into a vessel through the pit. Another young tylosis shown in photo 18 was understood as a little more grown than that in photo 17. It can be seen as a bowl-shaped in the photograph, probably due to an artifact, i.e., to drying (photo 19). There exist some prominences on its surface. In the following stage of the development, tylosis grows up the balloon-shaped one as shown in photo 20, until it contacts with the inner vessel wall or with another tylosis adjacent to it. At this stage of development, there can be observed a number of fine wrinkles oriented in irregular direction, and also prominences on the surface of tylosis under the scanning microscope as shown in photo 21. As a result of its successive growth, tylosis becomes large enough to occupy the entire vessel cavity and a part of tylosis wall adheres to the vessel wall (photo 22). When two tyloses come into contact with one another, they appear to form a tight double tylosis wall (photo 23). Photo 24 shows the inner vessel wall covered tightly with thin wall of tylosis. The arrangement of pits which can be seen through tylosis wall indicates that the contiguous of the vessel is a ray tissue. It may be possible to think that the tylosis grew out through the opening—pit aperture—in the center of the photo, from the contiguous ray parenchyma cell.

Furthermore, it is shown in photo 25 that both tylosis wall and vessel wall adhere so tightly that the border line is difficult to detect under the microscope. The authors often observed double wall of tylosis adhering to vessel wall as shown in photo 26. This is resulted from a folding down of overarched part of a balloon-shaped tylosis. Pohto 27 shows the detail of a part of photo 26. From the photograph, the writers can observe a separated place of wall where both tylosis and vessel wall had been contacted each other. On the wall surface of tylosis, wrinkles, gross or fine, were often found, esp., in sapwood clearly.

Photo 28 and photo 29 which is a enlarged view of the former show a number of such wrinkles distributed on the wall surface. The wrinkles shown here were randomly oriented. In photos 31 and 32, however, the gross wrinkles appear to be oriented in a direction, while fine wrinkles do not. Some of the surface texture such as wrinkles seems to have become visible by some artificial treatment such as drying.

Photos 33 and 34 show the being state of tyloses at the 13th and 14th annual rings, resp., counted from the bark. It is obvious that they begin to collapse. Photo 35 shows their characteristic appearance which is often observed at the boundary zone of sap- and heartwood, so-called intermediate wood. 13th

and 14th rings stated above are corresponding to the intermediate wood.

In heartwood, morphological changes of tyloses are found more obviously compared with sapwood: most of them are collapsed, wrinkled and finally remarkably crumbled as shown in photos 36 and 37. Photo 38 shows a general appearance of the inner surface of vessel wall which is usually observed in heartwood. No tyloses show there its ordinary appearance; perhaps part of the tylosis wall would move away somewhere and the other part of it is still adhered to the vessel tightly. Photos 39 and 40 show the detail of a part in photo 38. A double part of tylosis wall and part of the inner vessel wall appear to be deposited with some fine substances.

In photo 40, vessel-ray pit apertures are covered by tyloses wall which has become more or less rough in its structure. In the final stage near the pith, it is often observed that the remains of tylosis walls are crowded in some places, e.g., near the perforation plate of the vessel as shown in photos 42 and 43.

Discussion and conclusion

It was found clearly that the existing state of tyloses in earlywood vessel in sapwood was different from that in heartwood. In Haru-nire, many tyloses in sapwood blockade the vessel lumen completely, but in heartwood. In heartwood, they are more or less collapsed and wrinkled, and often remarkably crumbled near the pith. In Hari-giri, tyloses in both sap- and heartwood are observed more thicker and harder than that of Haru-nire. Some of them in the heartwood, however, are more or less collapsed. Also in heartwood, the occurrence of tyloses is more remarkable than in sapwood of this species.

It is well known that in hardwood vessels play a main role of liquid penetration into wood and thus tyloses in vessels have the most important meaning for the phenomenon. During the course of study on the longitudinal permeability of water for sapwood and heartwood in some hardwoods, the authors[4] have found that in Haru-nire as well as Yachi-damo the permeability of heartwood is more or less higher than that of sapwood, while in Hari-giri the permeability for sapwood is obviously higher than heartwood. HAYASHI and NISHIMOTO[2] also reported that heartwood in Hari-giri is not permeable. Differences of permeability of water in sap- and heartwood of these species, may be understood easily from the point of view of the being state of tyloses in vessels described above.

Haru-nire and Yachi-damo have been termed so-called "wet-heartwood tree species" by YAZAWA and ISHIDA[9]; in these species, heartwood moisture content in green is always higher than that of sapwood. This is a speciality of the species. Also the heartwood of the two species shows high water permeability in fiber direction comparable with the sapwood; this is another speciality of these species.

According to the morphological study of tyloses at some different stages of

their development there were found things about the structure of tylosis itself and its being state in vessels. The writers have observed the following; the initial or first stages of tylosis i. e., bud tylosis which was displayed as a ball or bowl-shaped (photos 17, 18, 19 and 20), overarching of tylosis wall in vessel lumen (photo 22), contacting of walls of two individual tyloses with one another (photo 23), tight adhering of tylosis wall to the inner vessel wall covering pit apertures (photos 24 and 25), tyloses which were collapsed or curled (photos 33, 34 and 36), crowding of the crumbled remains of tyloses at the final stage of them (photos 42 and 43) and so on. Examinations about some of these items will be continued in detail making the best use of the scanning electron microscopy.

Results of conventional electron microscopic study by using of ultra-thin sectioning method has been reported by KÓRÁN and CÔTÉ[7] or MEYER[8]. Although some fine structures of tyloses observed in this study correspond to some of the electron micrographs by them, there is a limitation to the study on the fine structure of tyloses walls because of the rather low resolving power of this type of electron microscope.

As pointed out by KÓRÁN and CÔTÉ[7] and other investigators,[3),5),8)] tylosis has a lamellar and fibrillar structure. Fine striations on the tylosis wall at early stages of its development, and also fine or gross wrinkles on the surface at the succeeding stages stated above, these may be related more or less to the fibrillar structure of the wall.

In Hari-giri, many spherical prominences on heartwood tyloses wall, in diameter of about 1 μ, are often observed. KATŌ and KISHIMA[5] reported that there were double kinds, the small on the large, of prominent warts on the surface of tylosis wall in Japanese chestnut. Such prominences on tylosis wall may be a characteristic structure for some species.

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要　　約

走査型電子顕微鏡 (JSM-2, photo 1) により，ハルニレ，ヤチダモ，ハリギリの春材道管中のチロースの存在状態を辺・心材別に一般観察を行ない，さらにハルニレについてチロースの発達段階の形態を年輪を追って観察した。得られた結果を要約すれば次の通りである。

1. ハルニレ，ハリギリはチロースの発達が著しく，辺材と心材ではそれらの春材道管中のチロースの存在状態及びチロース膜表面について明らかな相違が認められた (photo 2, 3, 4, 5, 10, 11, 12, 13, 14, 15, 16)。

2. ヤチダモはチロースの発達が少なく，辺・心材における相違も，本観察ではあまり顕著でなかった (photo 6, 7, 8, 9)。

3. ハルニレについては，チロースのそれぞれの発達段階における種々の形態が観察された。

即ち，辺材では，チロースが道管膜孔から発生し始めた状態 (photo 17)，やや生長し，道管内腔に突出している状態 (photo 18, 19, 20)，道管内腔をみたし，道管膜及び膜孔を被覆し凝着している状態 (photo 22, 24, 25)，チロースが互に接触し二重の膜を形成している状態 (photo 23)，一度凝着した後，剝離した状態 (photo 26, 27)，さらには膜表面の形態 (photo 21, 28, 29, 30, 31, 32) などが観察された。

移行材では，チロースが剝離崩壊しはじめた状態 (photo 33, 34, 35) が観察された。

心材では，チロースが剝離崩壊した状態 (photo 36, 37)，チロースの一部が道管内壁に強度に附着し，さらにある種の沈着物質と考えられるものにチロースが被われている状態 (photo 38, 39, 40, 41)，チロースの遺体とも云うべきものが穿孔板附近にかたまって存在している状態 (photo 42, 43) などが観察された。

本報告は，走査型電子顕微鏡の木材研究への適用性を検討する段階におけるチロースについての一つの予備的研究の成果であるが，チロースのように材中で特異な立体構造をもつものの研究には，本走査型電子顕微鏡の適用価値がかなり高い事がわかった。このような観点からチロースの研究を今後進めてゆく予定である。

Explanation of photographs 1~43

Photo 1. General view of the scanning electron microscope used for this study, made by the Japan Electron Optics Laboratory, JSM-2. Electron optical column and evacuation system at left, operation and display system at right and power supply unit at right bottom.

Photo 2. Haru-nire (*Ulmus davidiana* PLANCH. var. *japonica* NAKAI). Longitudinal tangential section of sapwood showing the typical existing state of tyloses in earlywood vessels.

Photo 3. Haru-nire. Longitudinal tangential section of heartwood showing the typical existing state of tyloses in earlywood vessels.

Photo 4. Haru-nire. Striations on the surface of a sapwood tylosis.

Photo 5. Haru-nire. Typical wall surface of heartwood tyloses.

Photo 6. Yachi-damo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.). Tyloses in some pores in earlywood of sapwood.

Photo 7. Yachi-damo. Tyloses in some earlywood pores in heartwood.

Photo 8. A part of photo 6 enlarged, showing tyloses more or less crumbled.

Photo 9. Yachi-damo. Wall surface as seen from the lumen of a heartwood vessel segment covered with tylosis wall.

Photo 10. Hari-giri (*Kalopanax pictus* NAKAI). Longitudinal tangential section of sapwood showing the existing state of tyloses in earlywood vessels.

Photo 11. Hari-giri. Longitudinal tangential section of heartwood vessels showing the existing state of tyloses.

Photo 12. A tylosis shown in photo 10 enlarged.

Photo 13. A part of photo 11.

Photo 14. A part of photo 12, showing tylosis wall surface in detail.

Photo 15. A part of photo 13, showing detail of a tylosis wall with some prominences.

Photo 16. A part of photo 15. Two different types of prominences on the tylosis wall surface.

Photo 17. Haru-nire. 11th annual ring from the bark. Budding tylosis at the first stage of its development.

Note: **Shown in photos 17 to 43 are photographs for Haru-nire of which 17 to 35 for the sapwood and 36 to 43 for the heartwood.**

Photo 18. A bowl-shaped bud tylosis found in the 9th annual ring. The shape of bud tylosis might be effected by the experimental treatment.

Photo 19. Detail of a part of photo 18 showing some prominences on the surface.

Photo 20. A balloon-shaped tylosis in earlywood vessel, found in the 10th annual ring.

Photo 21. A part of photo 20 showing many prominences and numerous fine wrinkles on tylosis wall.

Photo 22. A typical, well developed tylosis adhering the inner surface of the vessel found in the 4th annual ring.

Photo 23. Two tyloses contacting and adhering tightly each other in a vessel lumen.

Photo 24. Surface of tylosis adhering tightly to the inner vessel wall in earlywood. It may be possible to think that the tylosis has come out from the pit shown in the center of the photograph.

Photo 25. Tightly jointed tylosis and vessel wall. No border line is recognizable in the photograph.

Photo 26. A double wall of a tylosis at the lower half of the photo.

Photo 27. A part of photo 26 showing a exfoliation of tylosis wall from the vessel surface.

Photo 28. Numerous wrinkles distributed on the surface of a tylosis wall.

Photo 29. Detail of a part of photo 28 showing a number of gross and fine wrinkles randomly oriented.

Photo 30. A tylosis developing into the vessel lumen.

Photo 31. A part of photo 30.

Photo 32. Detail of a part of photo 30 showing the surface structure of a tylosis.

Photo 33. Tyloses beginning to collapse or to crumble in the large vessel. At right two tyloses can be seen in a small vessel cavity in the 13th annual ring.

Photo 34. Tyloses beginning to collapse found in the 14th annual ring at the boundary zone of sap- and heartwood.

Photo 35. Detail of a part of photo 34 showing a complex appearance of tyloses wall.

Photo 36. Crumbling of tyloses like paper wast in a earlywood vessel in heartwood, found in the 16th annual ring.

Photo 37. Detail of photo 36.

Photo 38. An appearance of heartwood vessel frequently observed in its older part of a trunk. The photograph was taken in the 32nd annual ring.

Photo 39. A part of photo 38.

Photo 40. The inner surface of a vessel wall showing its rough and irregular appearance due to the remains of tylosis wall and, perhaps, some other fine substances deposited on them.

Photo 41. Tyloses wall adhering to the inner surface of the vessel wall, found in the 32nd annual ring.

Photo 42. The remains of tyloses crowded next to the perforation plate in a vessel cavity near the pith.

Photo 43. Detail of a part of photo 42.

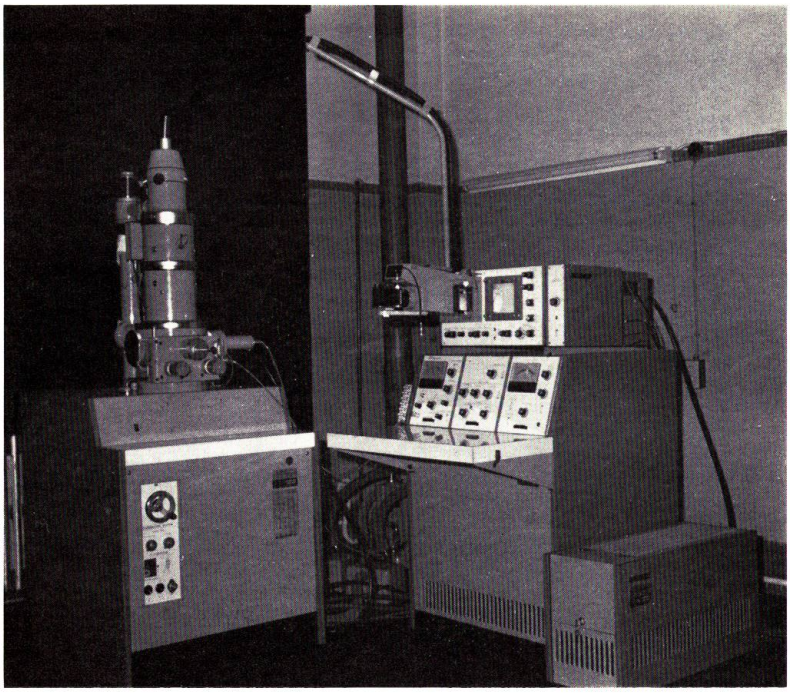
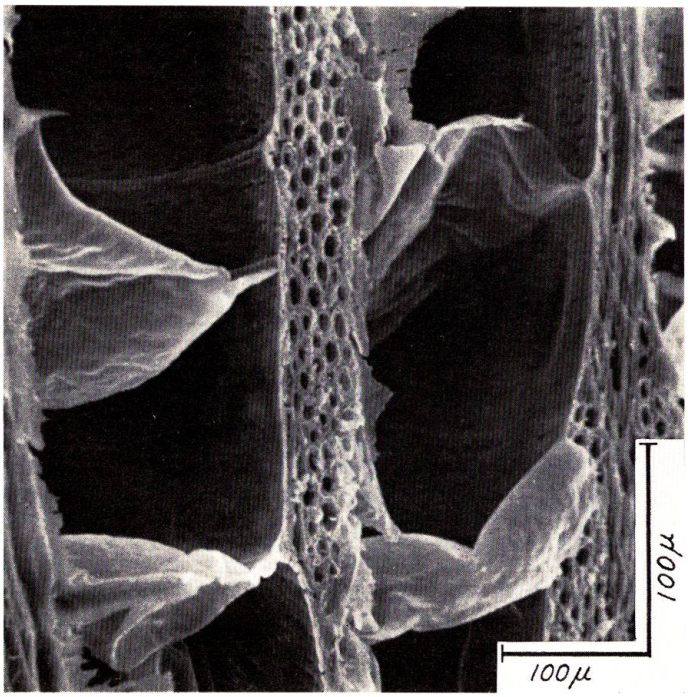


Photo 1.



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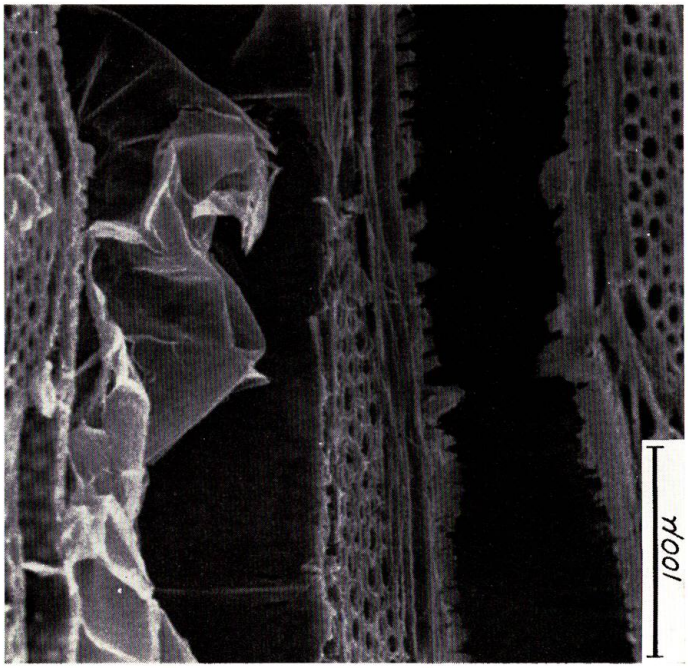


Photo 3.

Photo 4.

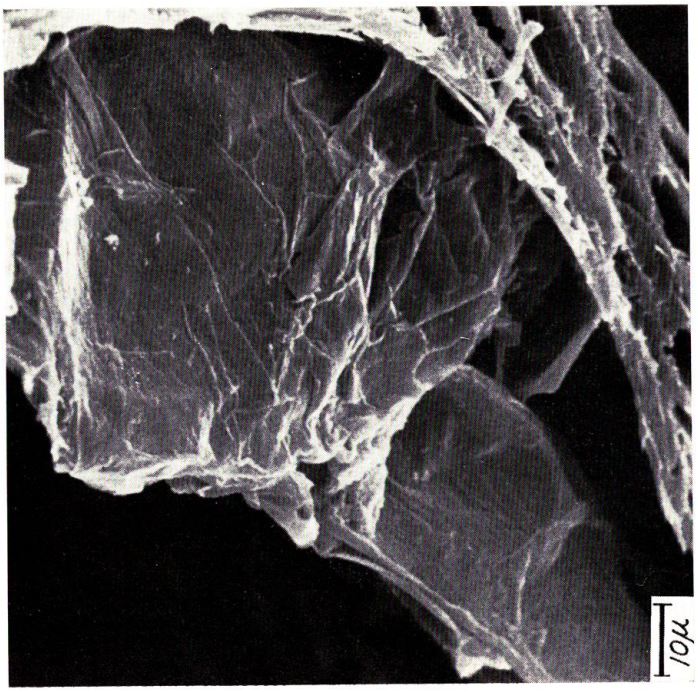
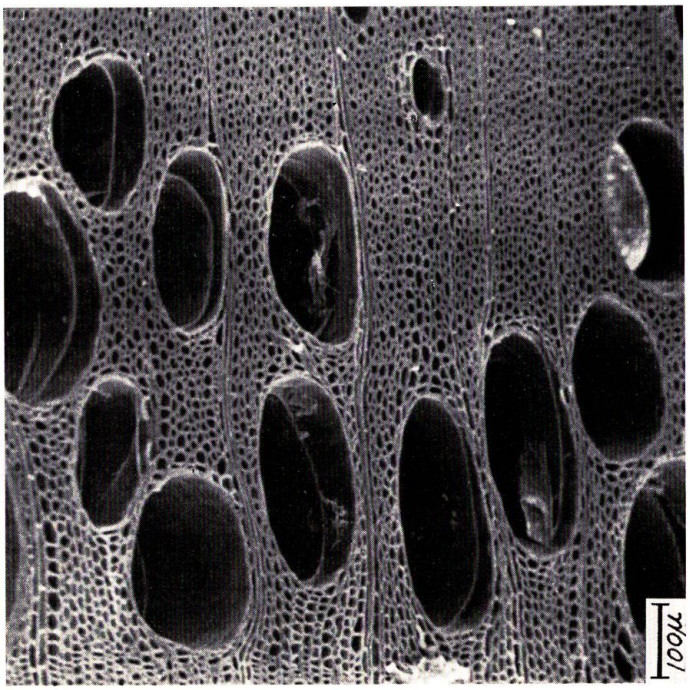


Photo 5.



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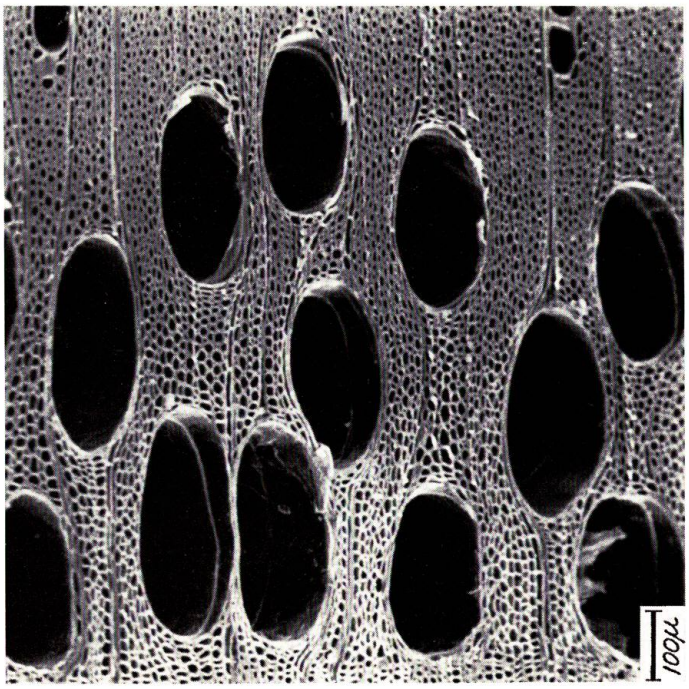

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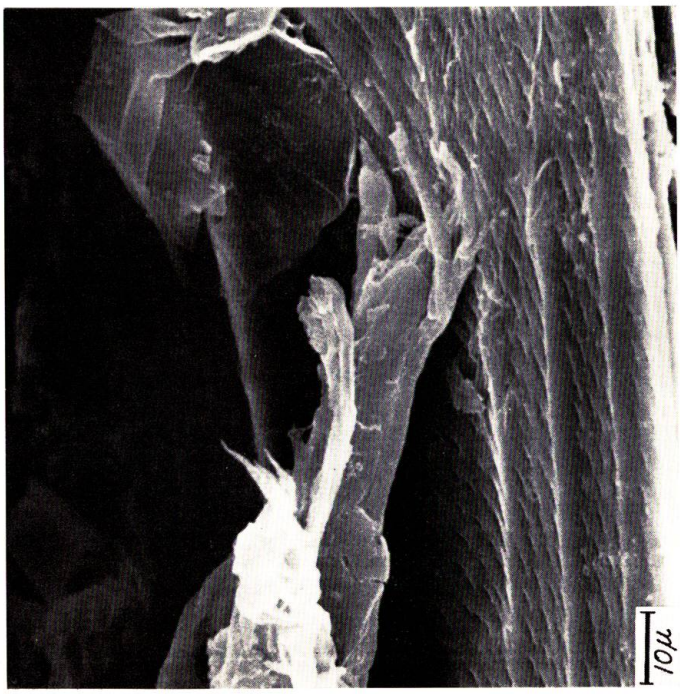

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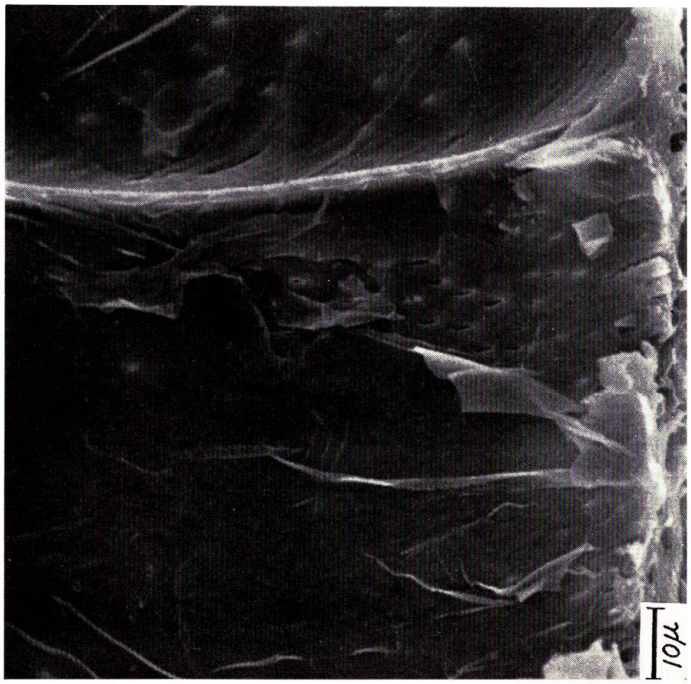

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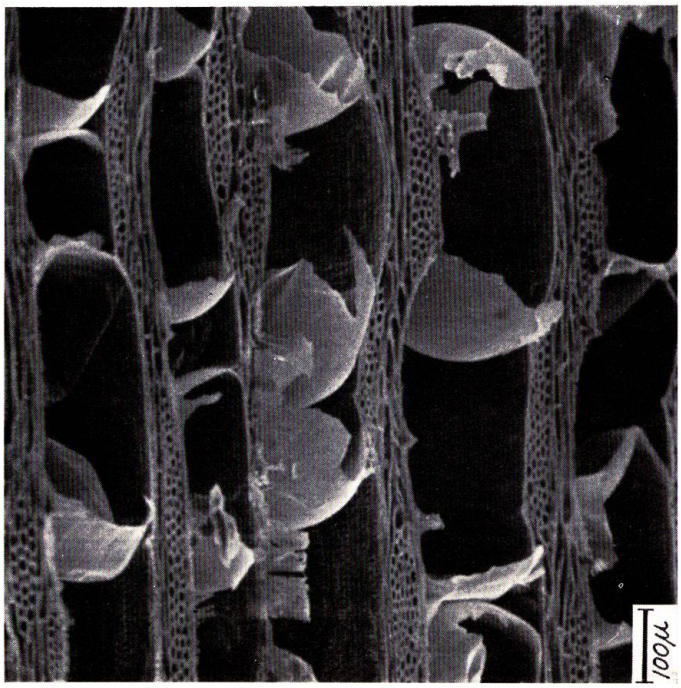

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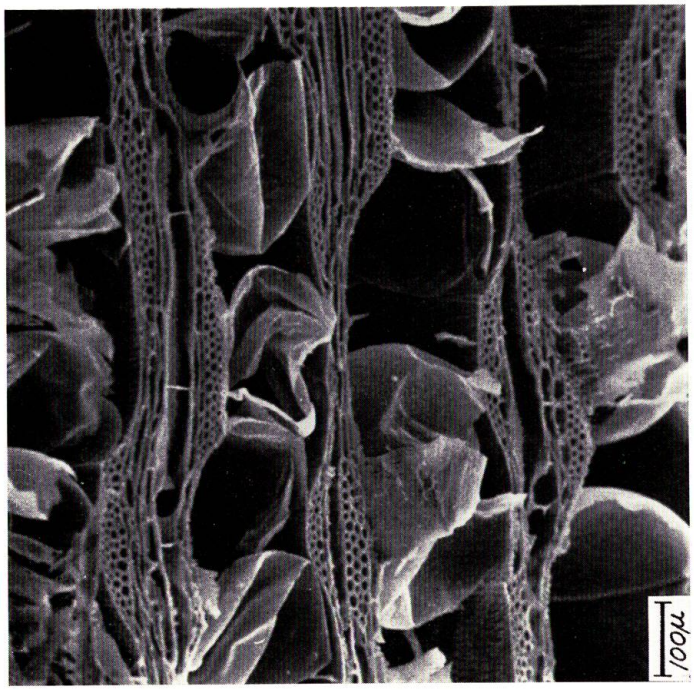

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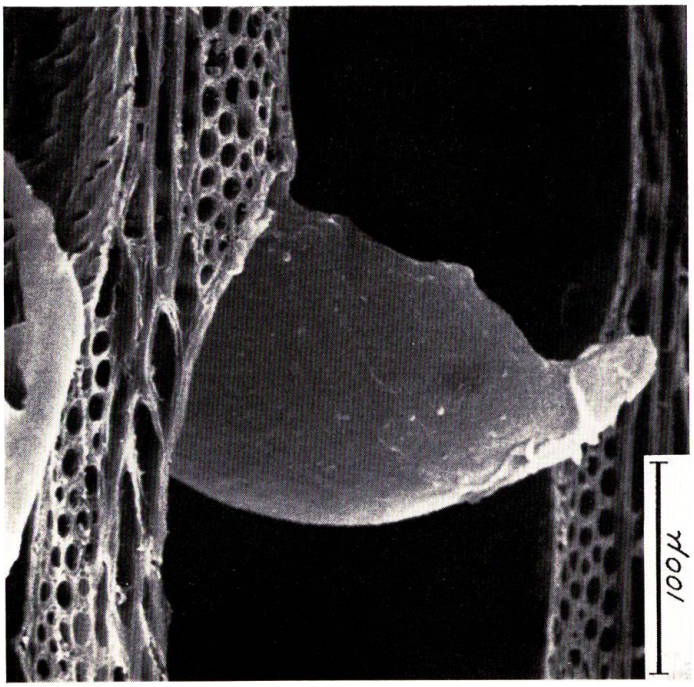

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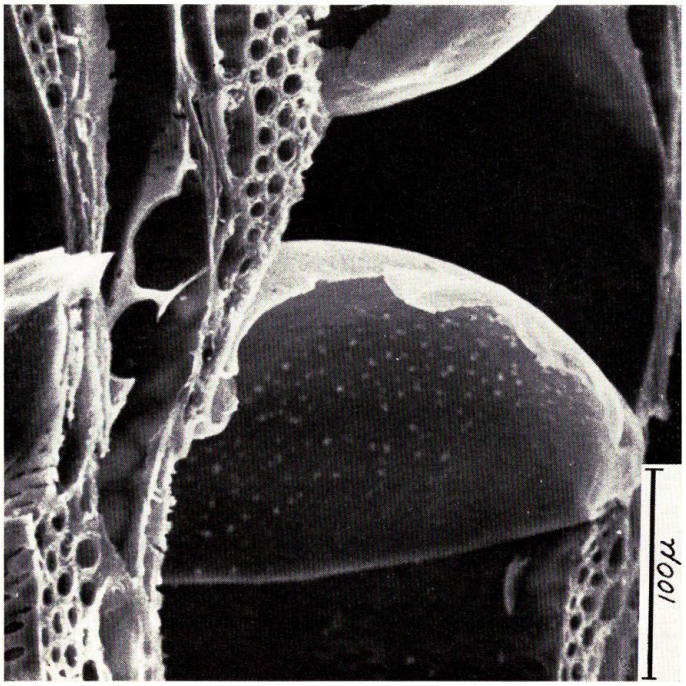
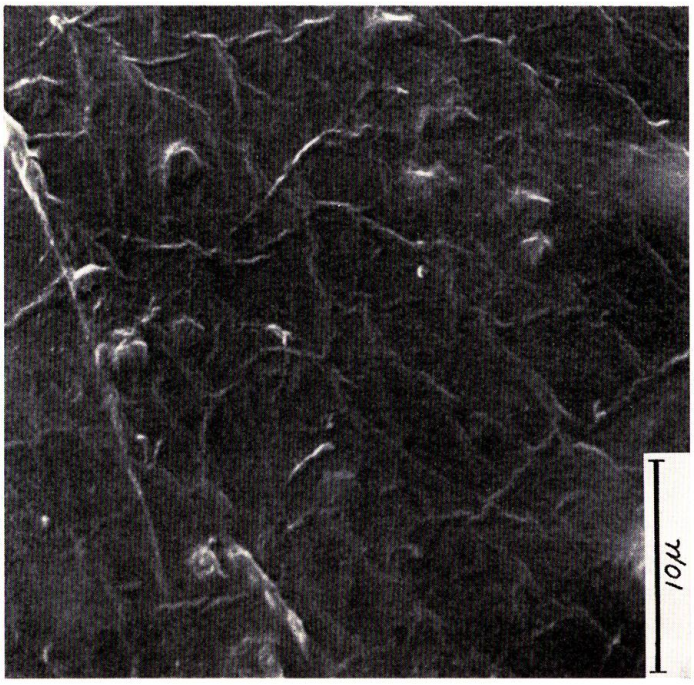


Photo 13.



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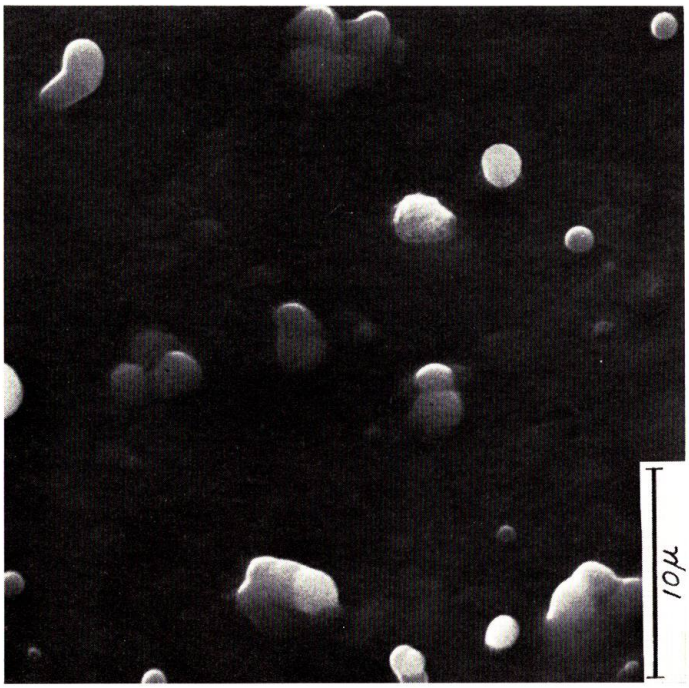


Photo 15.

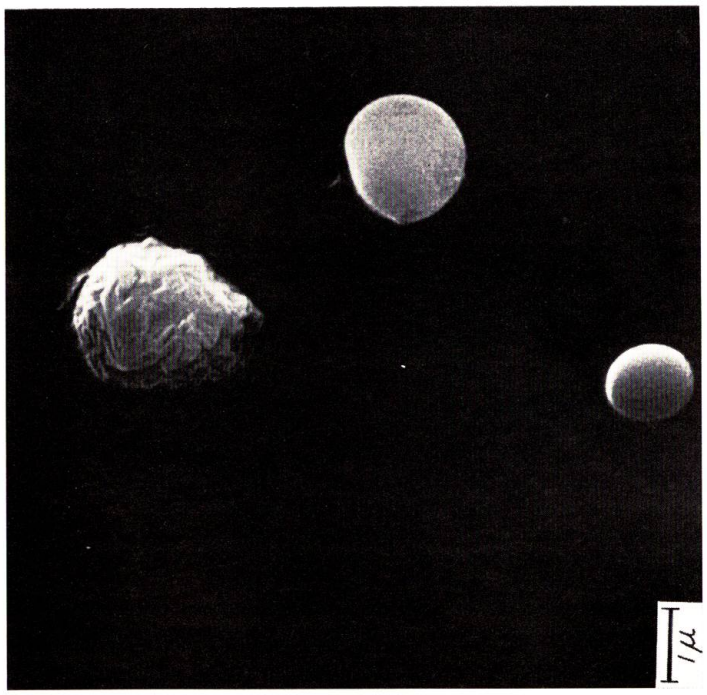
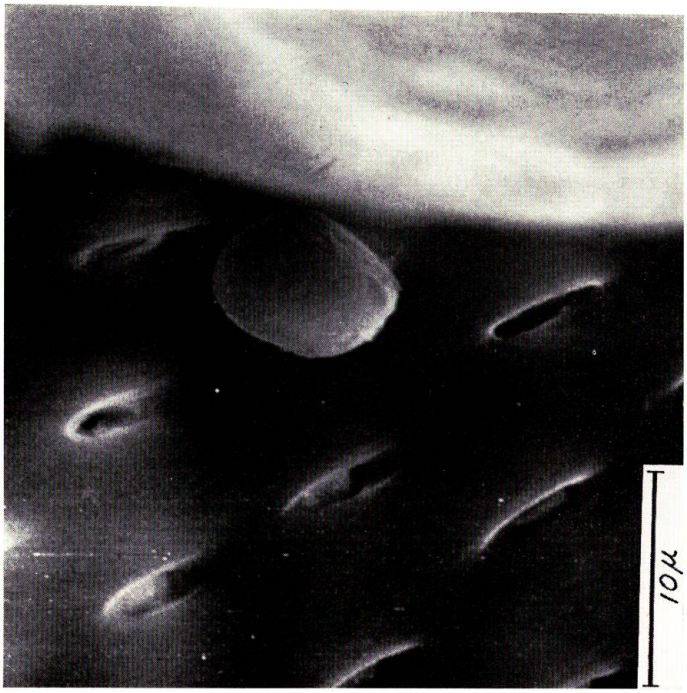


Photo 16.



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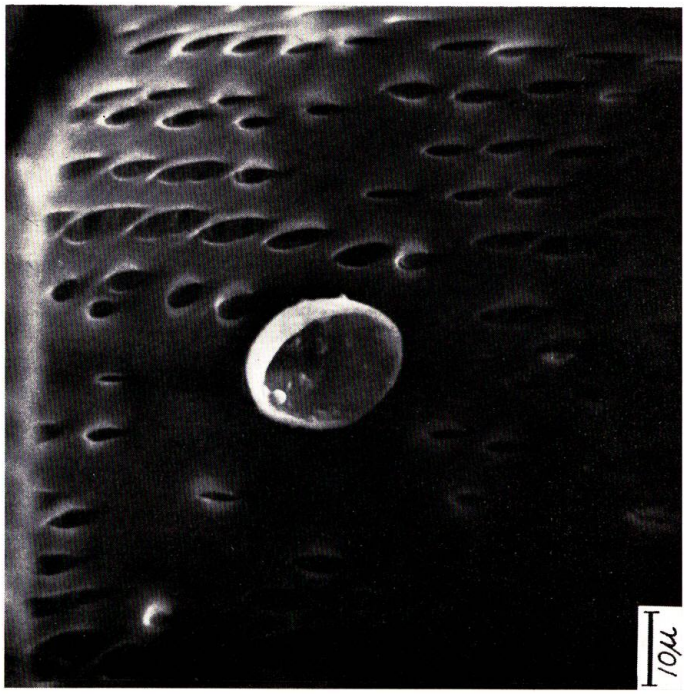

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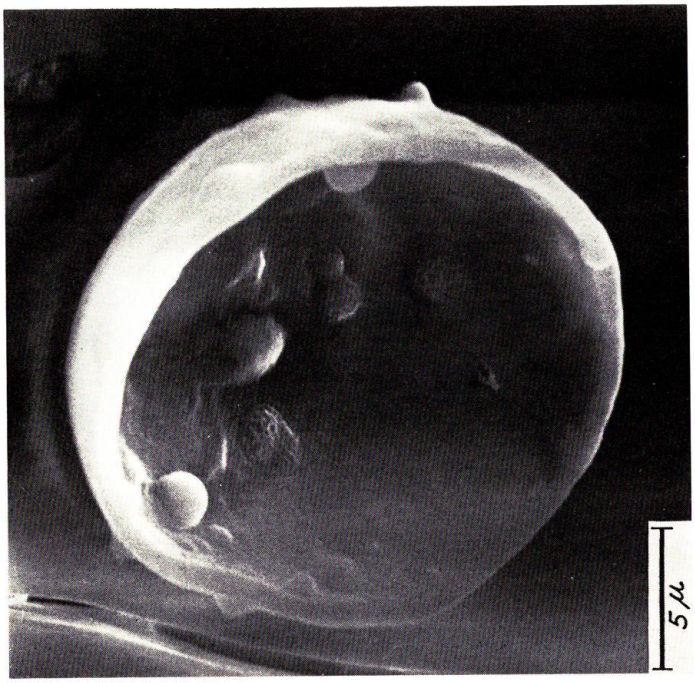

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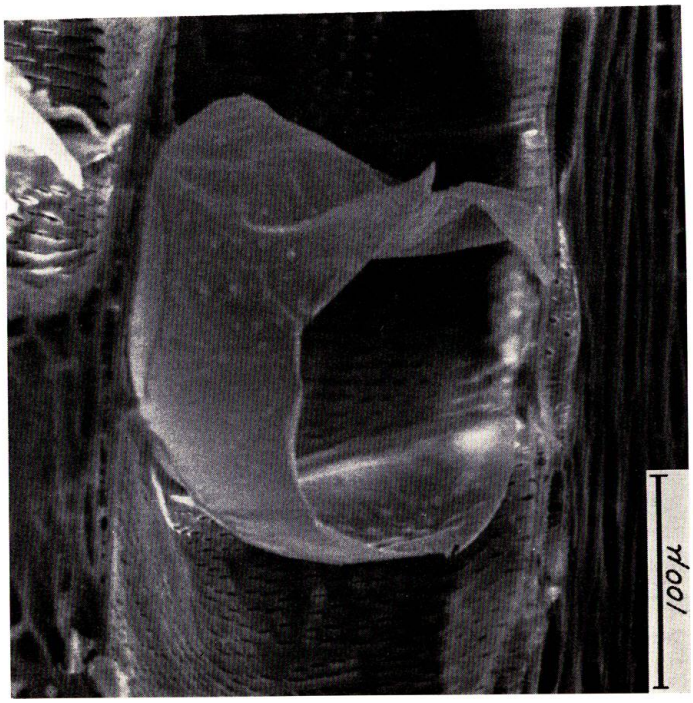

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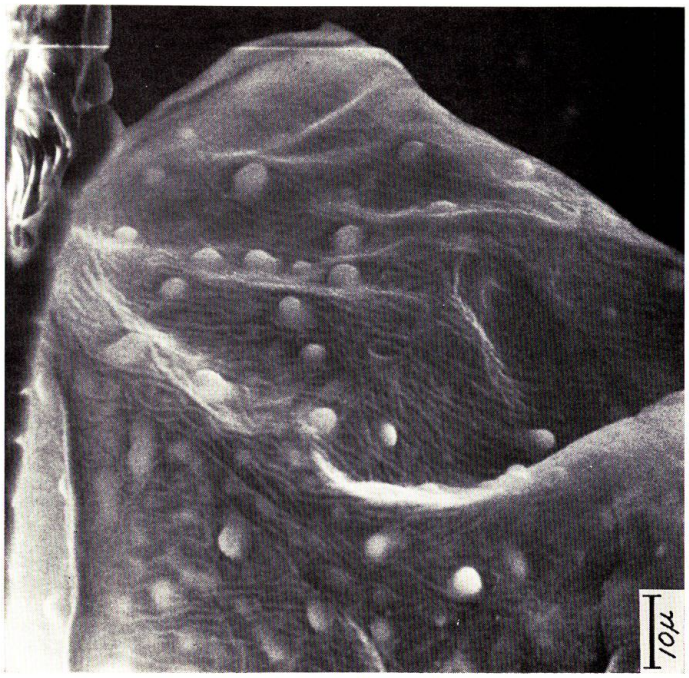

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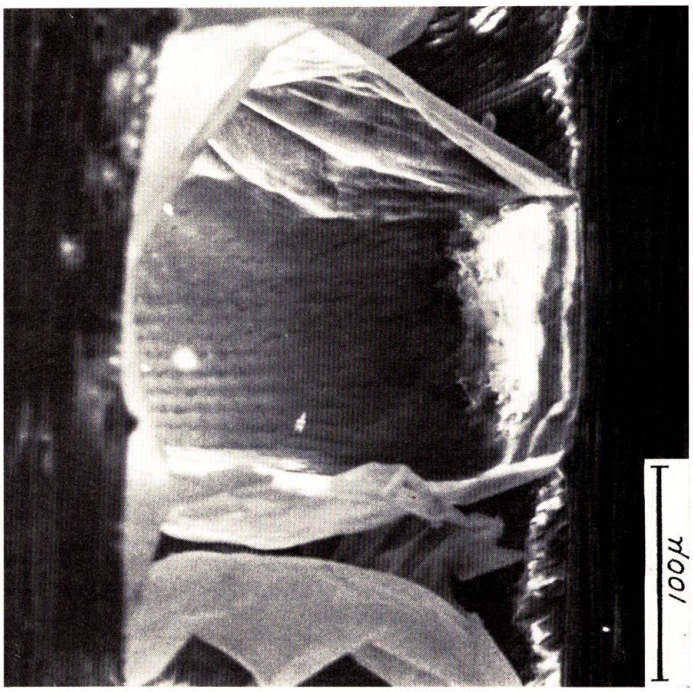

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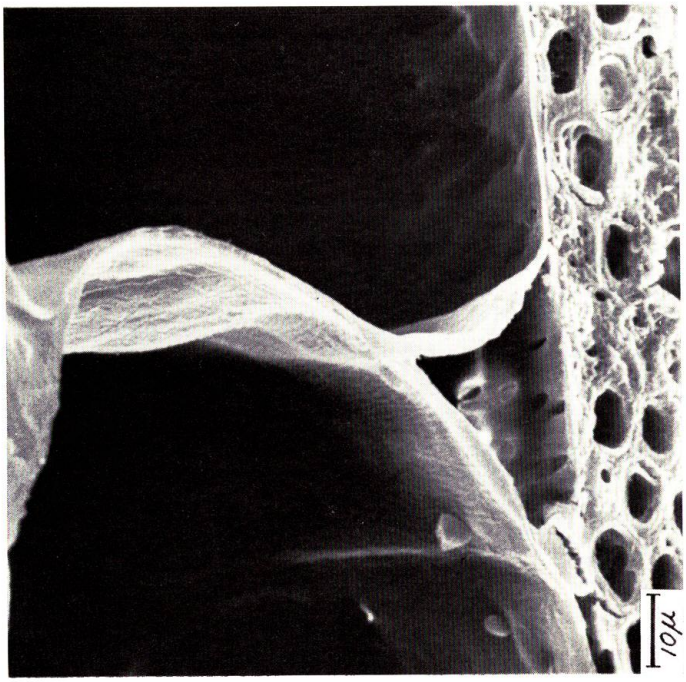

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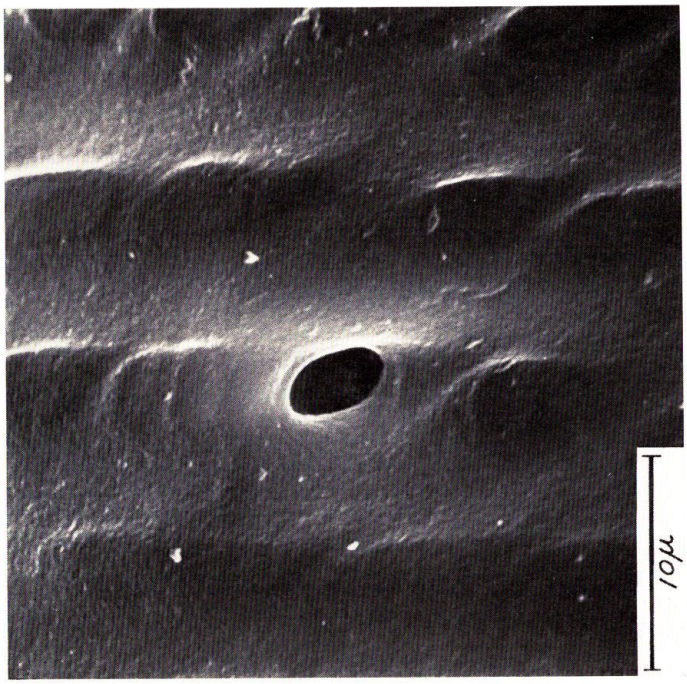

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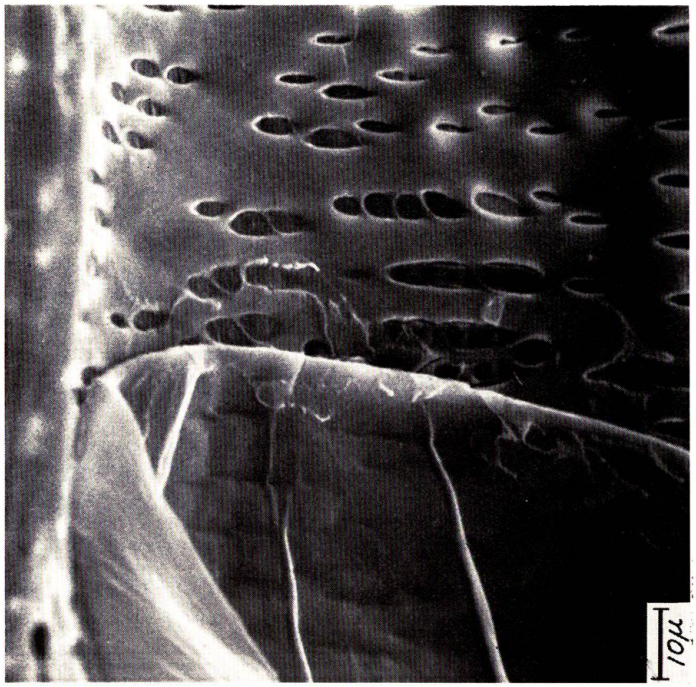

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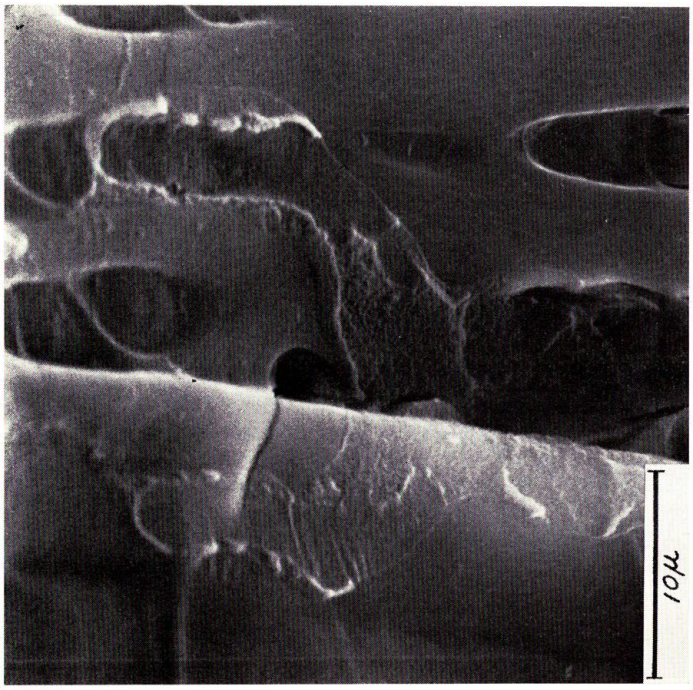

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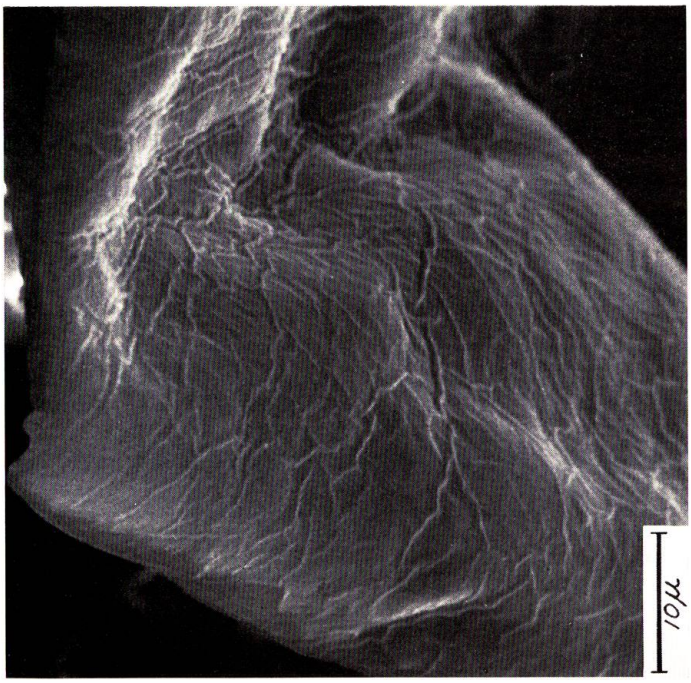

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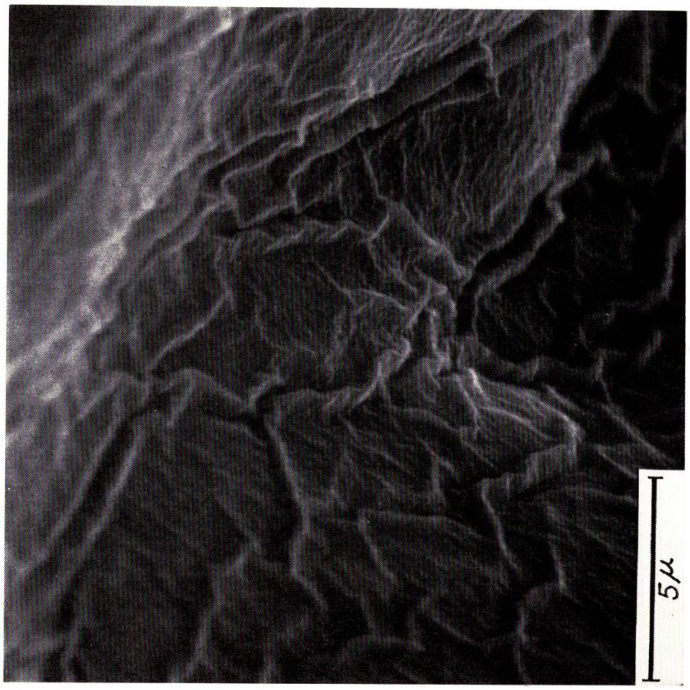

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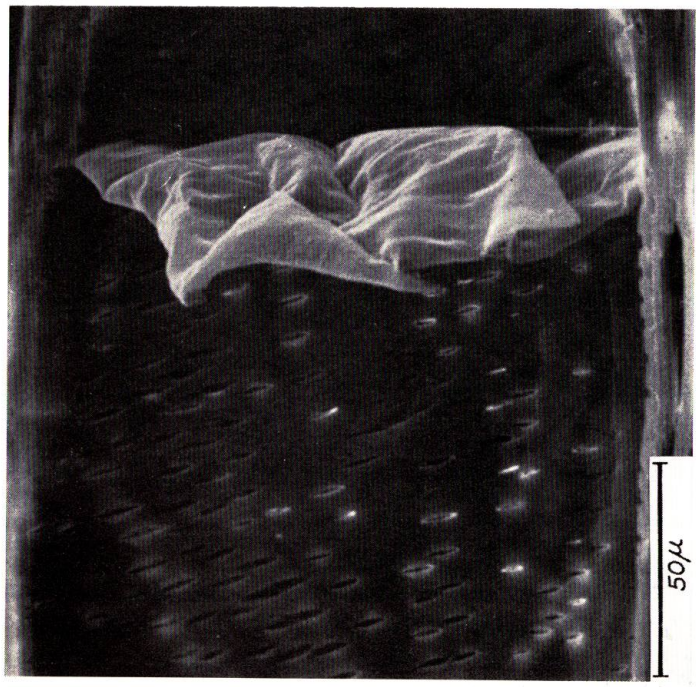

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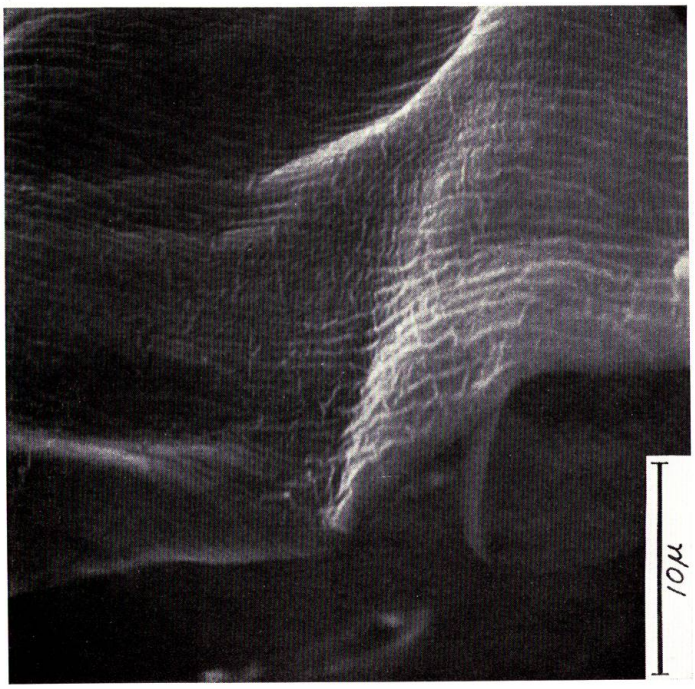

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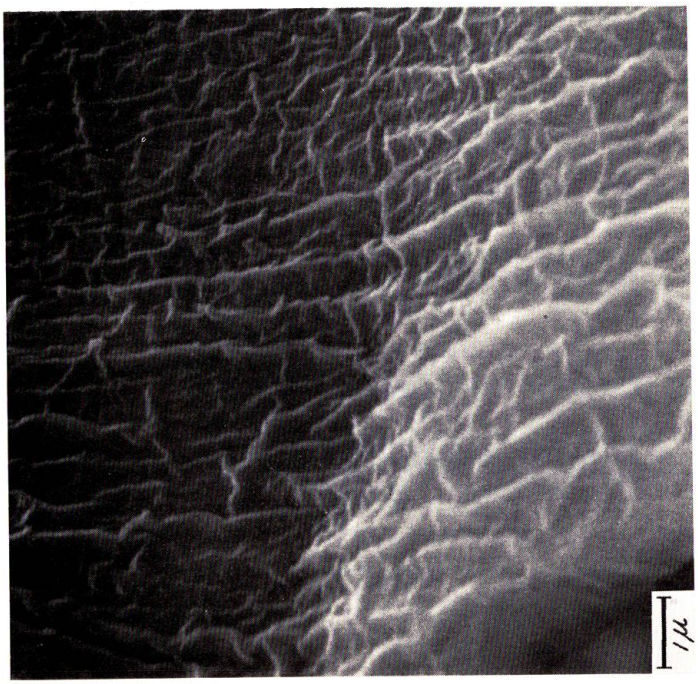

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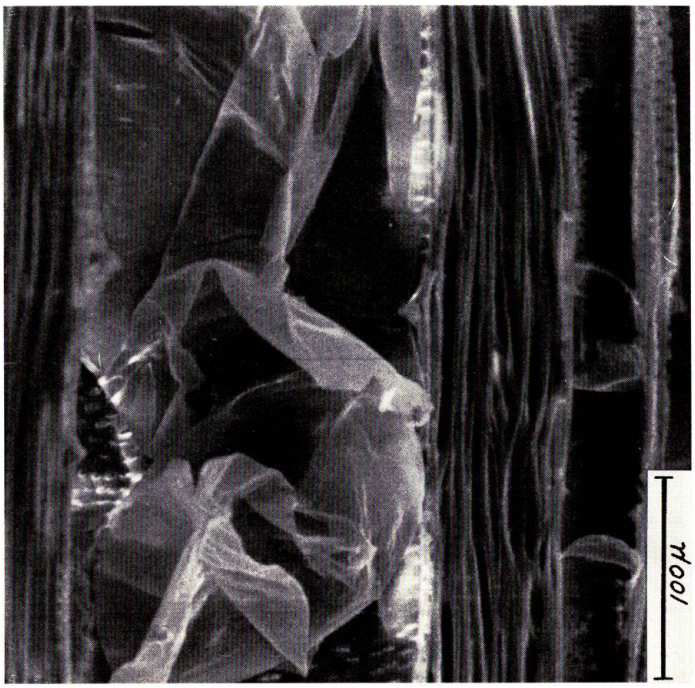
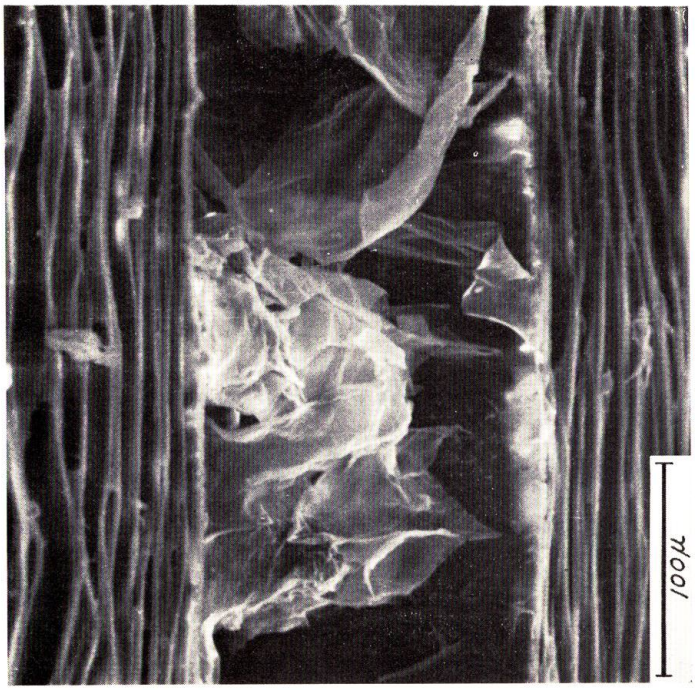


Photo 33.

Photo 34.

Photo 35.



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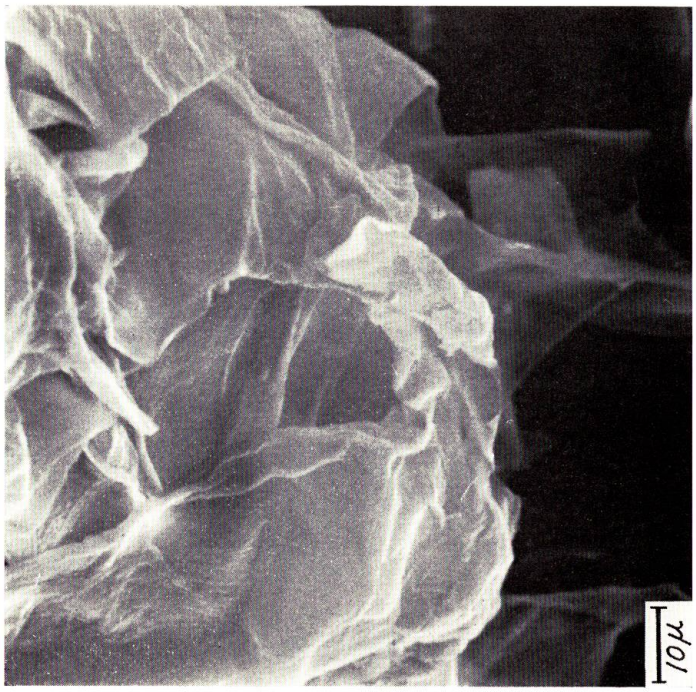

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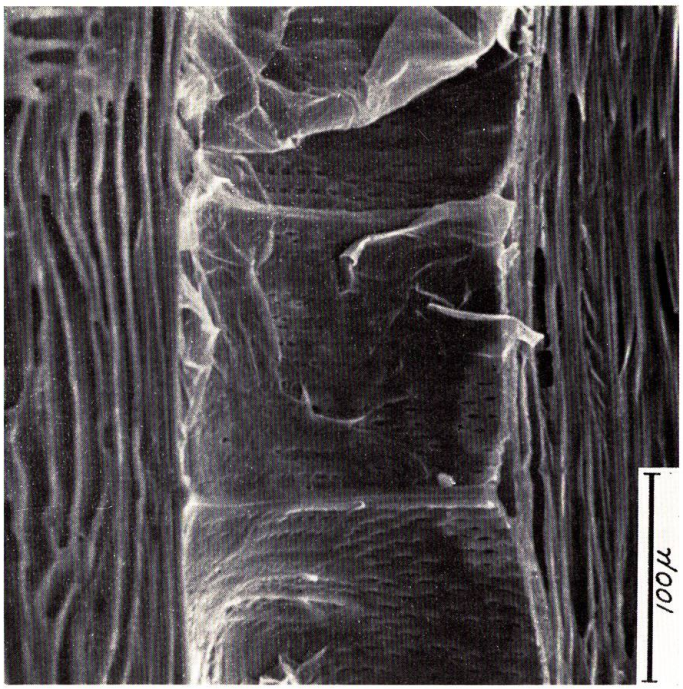

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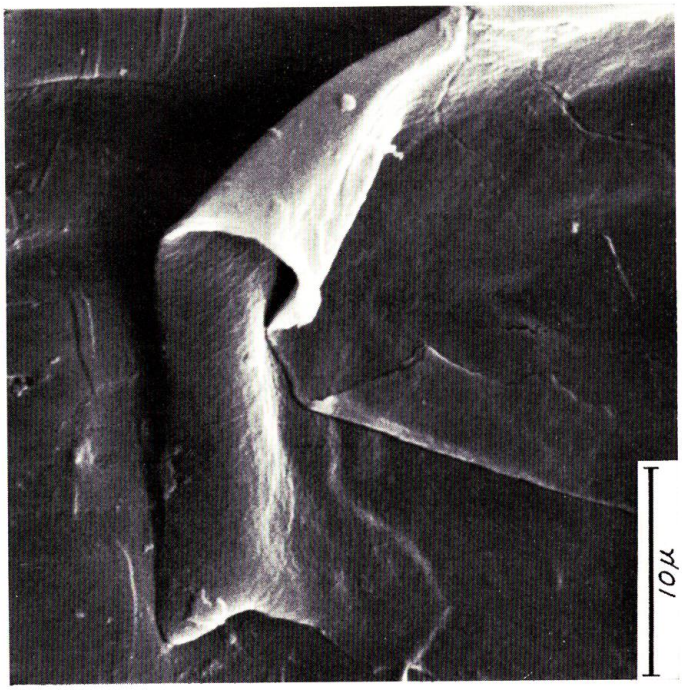

Photo 39.

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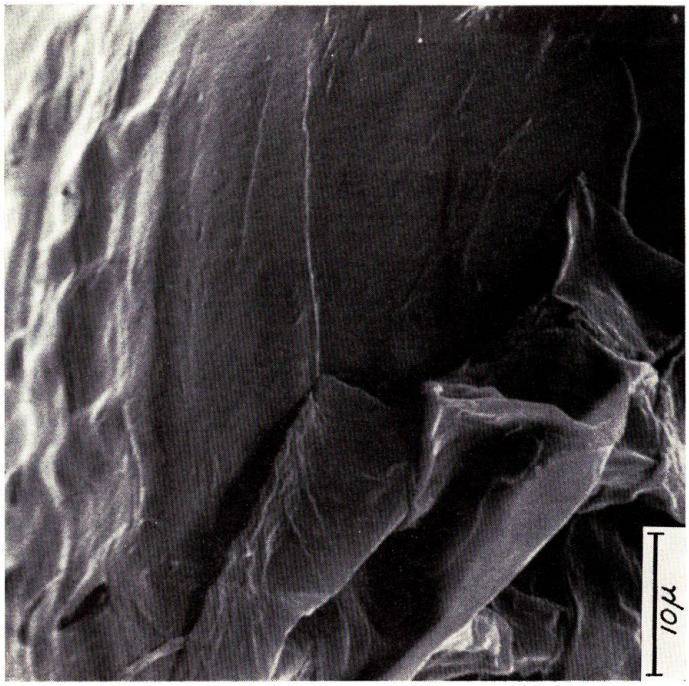

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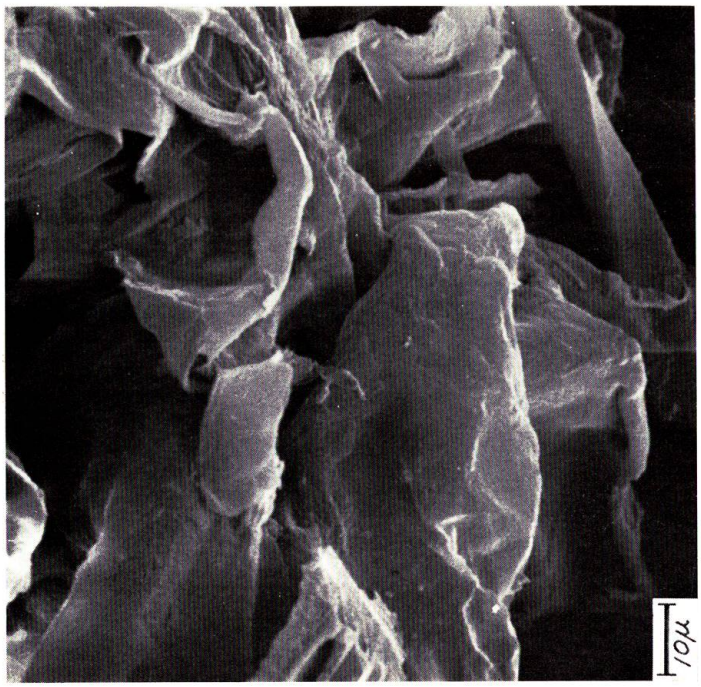

Photo 43.