Study on the Pit of Wood Cells Using Scanning Electron Microscopy

Report 1. An Observation of the Vestured Pit in Black Locust, *Robinia pseudoacacia* LINN.

By

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走査型電子顕微鏡による木材細胞の膜孔に関する研究

第1報　ハリエンジュのベスチャード膜孔についての一観察

石田茂雄*・大谷　諄**

CONTENTS

Introduction

In wood anatomy much attention has been paid to the pit of wood cells because of its role in the movement of liquids within wood, not only in a living tree, but also in the case of the treatment of wood before use, such as drying, penetration of preservatives, etc. Valuable informations for understanding pit structure obtained with both the light and electron microscopes have been reported.

Since the scanning electron microscope is considered to be an excellent tool for the study of three dimensional structure, the authors have tried to apply it to the study of wood which is particularly complicated in its three-dimensional structure.

During the course of study on tylosis development in black locust, *Robinia pseudoacacia* LINN., the authors have observed the pits, especially vestured pits, of

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vessel walls in this species, and their structure has been recorded with micrographs from the scanning electron microscope. This paper presents brief observations of vestured pits in black locust, as a preliminary study on "vestured pits" of hardwood.

Experimental

Small wood blocks containing the outermost growth rings were cut off at the breast height from black locust trees grown in Tomakomai College Experiment Forest, Hokkaido University. Immediately after removal the sample material was placed in a fixative, F.A.A., and left there for a week. The fixed material was then washed in running water for 24 hrs. The surfaces to be observed were prepared with a microtome steel knife. The finished specimen was subjected to gradual drying; first it was placed in normal room condition for 24 hrs. and then in a desiccator with silica gel for 24 hrs. These specimens were mounted onto specimen stubs with silver paste and coated with gold in a vacuum of 5×10^{-5} torr. The scanning electron microscope used was model JSM-2 made by Japan Electron Optics Laboratory Co., Ltd. The specimen stage was inclined 45° from the horizontal and 25 kV accelerating voltage was used to examine the specimen surfaces.

It is well known that black locust normally has a high content of tyloses. Only the two outer growth rings of the material taken in July were found to have no mature tyloses in their early-wood vessels (shown in photo 1). In order to facilitate the examination of pit structure from both the inner and outer surfaces of a vessel wall, the test specimens should be taken from the two outermost rings mentioned above. Those vessels examined were thought to be completely matured.

Examination was made of; 1) pits (vestured or unvestured?), their shape and distribution on the wall of both earlywood and latewood vessels, 2) localization of vestures within a given pit, and the size and shape etc. of the vestures.

Results

A face view of the inner wall surface of an earlywood vessel element is given in photo 2, showing intervessel (V-V) pits at the left and vessel-ray (V-R) pits or vessel-axial parenchyma (V-P) pits at the right. As shown in this photo, the distribution, shape and size of the pit on the wall of the vessel element vary markedly depending on its adjacent cells. V-V pits are crowded in a regular arrangement, and uniform in size and shape, while V-R or V-P pits are rather irregular in their distribution and in their size as shown in the photo. Pit apertures of the V-V pit vary in shape from circular in photo 3 to oval in photos 4 and 5. Some of the adjacent pits are found to be confluent with each other at their inner aperture as shown in photo 5. Such combination of pits appears to occur in the case of thicker vessel wall. Separation of two vessel elements laterally contacted with each other was often observed. Both the inner surface of one vessel element and the correspoding outer surface of the vessel element of the opposite side are shown in photo 6. Photo 7 also illustrates an outer surface of a vessel element, and shows V-V

pits at the right and V–R or V–P pits at the left.

It was found that vestures principally existed in V–V pits. They are variable in their shape, size and growth in clusters, as shown in photos 10–20 which are all photographs taken on the outer surface of the vessel element. Within a given vessel element there are fewer differences in vesture morphology among pits, as shown in photos 8, 10, 13 and 15 which were taken from four different vessel elements. In these photos a polygonal shape of the V–V pit and its regular arrangement on the wall can be clearly seen. Details of vestures in photo 10 are shown enlarged in photos 11 and 12. One vestured pit in photo 13 is also enlarged in photo 14. Vestures shown in these photographs are unbranched (dot and massive) and fewer in number on the wall of each pit chamber. On the other hand, vestures in photos 15 and 16 are much more complicated in their morphology. Most of them are branched like a coral. When vestures are remarkable, the pit aperture becomes quite narrow as shown in photo 19, or is actually closed as a result of some additional incrusting substances deposited on the surface as shown in photo 20. As illustrated in the photographs above, vestures of the V–V pit usually grow at the outer pit aperture but also occur even on the pit chamber wall (mainly in dot-shape) becoming smaller toward the pit annulus. No vestures growing into lumen of the vessel were found in the present study. On the outer surface of a vessel wall some pit membranes remain and some do not (photos 9 and 10). In case of the two pits at the lower left in photo 10, no pit chambers can be seen because pit membranes are not removed, while in photo 21 a part of the vestures and the aperture is visible through the pit membrane which is aspirated on the surface of the pit chamber and covers over a vesture cluster developed at the outer pit aperture. The membrane seems to have a net-like structure composed of fibrils.

Photos 22–25 show V–R pits of the earlywood vessel wall (photos 22 and 23 are from the inner surface and 24 and 25 from the outer surface) and photos 26 (from the inner surface) and 27 (from the outer surface) show V–P pits of the earlywood vessel wall. Both vestured and unvestured pits are found in these photographs although the pit aperture was generally larger than the V–V pit (e.g., photos 3, 4 etc.). The pit border was narrower than that shown in photos 11, 17 etc. which were taken on the outer surface wall of the vessel. No vestured pits are found in photos 22 and 24, while in photos 23, 25, 26 and 27 there are found vestured pits that show both branched and unbranched vestures even within a given pit.

In the latewood vessel wall there are spiral thickenings as shown in photos 28, 30, 32, etc. In photo 28 mainly the inner surface view, and in photo 29 the outer surface view are illustrated at low magnification. As shown in photos 30 and 31 vestured pit was always found in the V–V pit similar to that of the earlywood vessel, while no vestured pit was generally found in V–R (photos 32 and 33) and V–P pits (photo 34). In very rare cases of this study, however, vestures were observed in the V–P pit as shown in photo 35.

As shown in photo 36, it was observed that a thin layer in places covered the inner surface of the earlywood vessel and the pit aperture. The layer was often

broken at the pit aperture as shown in photo 37. Such a thin broken layer can be seen through the pit aperture from the outside of the vessel shown in photo 38.

Discussion and Conclusion

A brief observation of vessel element pits, V–V, V–R, and V–P pits, in black locust was carried out. They were examined on both the inner surface and the outer surface of vessel wall. The V–V pit is easily distinguished from both V–R and V–P pits on the basis of the high regularity of its distribution on the wall, and generally smaller size of the pit apertures in both earlywood and latewood. On the other hand, V–R and V–P pits were very difficult to separate from each other only on the basis of their appearance on the inner or outer surfaces of vessel wall.

No attention was given to vessel-fiber pits in this study because the vessel was thought to be scarcely contiguous to wood fiber.

There are many varieties of vesture morphology, i.e., shape, size, number and distribution in each pit. There were often seen unbranched vestures which were dot-shape (up to 1 micron in diameter), or relatively simple rod-shape. Branched, thicker rod shape vestures with some small thin or thick branches on their tips (photo 30) or a coral form (photo 17) were also found. Although the morphology of vestures within a given pit chamber varies considerably it will be noticed that vestures only grow at the marginal zone of the outer pit aperture when their development is not remarkable, while they tend to be distributed widely on the pit chamber wall when they grow remarkably, becoming smaller in size and in number and less complicated in shape toward the pit annulus. According to Bailey[1], Côté[2] and Schmid[3], individual vestures are classified into several types, but their proposal does not apply well to black locust of this study. The present authors think that further investigations are required to classify reasonably the vestures or vestured pits based on their general morphology.

As shown in photos 36–38, a thin layer covering the inner vessel wall was often observed. The profile of this broken layer at the pit aperture might lead to a misunderstanding that the broken layer is made of vestures. The nature of this layer is not evident, but the authors think that it might be a dried up residue of a degenerated membranous plasma lining.

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2) CÔTÉ, W. A., Jr. and DAY, A. C. (1962): "Vestured pits-Fine structure and apparent relationship with warts." Tappi 45: 906–910.

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要　　約

ハリエンジュの道管膜の膜孔について，走査型電子顕微鏡により若干の観察を行なった。得られた結果を要約すれば次の通りである。

1) 道管膜の膜孔の分布は複雑であり，膜孔の種類により異なる。即ち道管一道管間の膜孔は，規則正しく配列しているが，道管一放射柔細胞，道管一軸方向柔細胞間の膜孔は不規則に分布している (photo 2)。

2) 早・晩材部とも道管膜の道管一道管間の膜孔には，常にベスチャーが存在する。ベスチャーの発達は極めて変化にとんでいる (photo 3～5, 8～20, 30, 31)。

3) 早材部の道管膜の道管一道管間の膜孔に認められるベスチャーの存在状態は，一道管要素内では，ほぼ一定している (photo 3, 4, 5, 8, 10, 13, 15, 20)。

4) 早材部の道管膜の道管一放射柔細胞，道管一軸方向柔細胞間の膜孔には，ベスチャーが存在する膜孔と存在しない膜孔が観察された (photo 22～27)。

5) 晩材部の道管膜の道管一放射柔細胞，道管一軸方向柔細胞間の膜孔には，一般にベスチャーは存在しない。しかし極めてまれにベスチャーの存在する膜孔も観察された (photo 32～35)。

Explanation of photographs (1–38)

Photo 1. Transverse surface of the material obtained in July, showing the outer three rings. The outermost ring is only earlywood of this current year. Vessels in the third ring are blocked with mature tyloses.

Photo 2. Inner surface wall of an earlywood vessel element. Intervessel pits at the left have regular arrangement while vessel-ray or vessel-parenchyma pits at the right are larger than the former and arranged more irregularly.

Photo 3. Intervessel pits on the inner surface wall of the earlywood vessel. There can be seen almost circular pit aperture where vestures grow from the margin uniformly.

Photo 4. Intervessel vestured pits on the inner surface wall of the earlywood vessel, showing typical oval shape pit apertures and their regular distribution on the wall.

Photo 5. Intervessel vestured pits on the inner surface wall of the earlywood vessel showing their oval shape aperture laterally longer. Some of them are confluent with each other in their inner aperture.

Photo 6. Separation of a vessel element (in this side) from the corresponding vessel element (in the opposite side) through the middle lamella. There can be seen regular distribution of the bordered pit both on the inner surface and on the outer surface of the vessel elements.

Photo 7. Outer surface of a vessel element, showing V–V pits regular in distribution at the right and V–R or V–P pits with less regularity in their distribution at the left.

Photo 8. Intervessel vestured pits. The photo shows vestured pits on the inner surface wall at the right and on the outer surface wall of the other opposite vessel element at the left.

Photo 9. Pit membranes partly removed and partly remained. No openings of the membrane are visible in this photograph.

Photo 10. Intervessel vestured pits on the outer surface wall showing relatively less development of vestures on them. In the photo, on the surface of the vessel wall, the pits are arranged with a regularity like a honeycomb. Each pit shows polygonal shape. Pit membranes remain in the two pits at the under left of the photo.

Photo 11. An intervessel vestured pit on the outer surface wall of the earlywood vessel. Vestures grow as simple dot-shape from the margin of the aperture into pit chamber.

Photo 12. An intervessel vestured pit on the outer surface wall of the earlywood vessel. Some of the vestures grow a little more than that shown in photo 11, and they are merely branched.

Photo 13. Intervessel vestured pits on the outer surface wall of the earlywood vessel. There are several, so-called "massive" vestures in each pit.

Photo 14. A part of photo 13. There can be seen relatively smooth surface of the vesture in the photo, highly magnified. Whether the smooth surface is the original structure of the vesture or it is due to a substance deposited on the surface in

the living tree, or due to an artifact such as coating medium are not clear.

Photo 15. Intervessel vestured pits on the outer surface wall of earlywood vessel. The pits are regular in their arrangement on the wall like in photo 10. The vestures in every pit are uniform in shape, size and the being state.

Photo 16. A part of photo 15.

Photo 17. An intervessel vestured pit on the outer surface wall of earlywood vessel, the same type as shown in photo 16. Generally, vestures larger in size are branched, while smaller are unbranched. Some of the branched and all of dot-shape vestures arise from the pit chamber wall in this photo as well as in photo 16. Although in photo 16, the middle lammella is remained, no pit membrane can be seen; it is thought that the membrane had been aspirated to the pit border of this side which was removed.

Photo 18. An intervessel pit on the outer surface wall of earlywood vessel showing a typical shape of "coralloid".

Photo 19. An intervessel vestured pit on the outer surface of earlywood vessel. The development of vestures is so remarkable that pit cannal has become narrower than, e.g., the former.

Photo 20. Intervessel vestured pits on the outer surface wall of earlywood vessel. The development of vestures is very remarkable and additional incrusting substance deposits on them. The pit apertures are closed.

Photo 21. A vestured pit with pit membrane which has been aspirated on the surface of border and also has covered vestures cluster. A net-like structure of the membrane is evidently different from the case of photo 9 etc.

Photo 22. Vessel-ray pits on the inner surface wall of the earlywood vessel. No vestured pits are found there.

Photo 23. Vessel-ray pits on the inner surface of the earlywood vessel. Some pits (smaller) have vestures and some (larger) don't. It can be seen that development of vestures is changeful in them.

Photo 24. Vessel-ray pits on the outer surface of the earlywood vessel. There can be found no vesture at all. Illustrated in this photo is corresponding to the back side view of the pits in photo 22. The larger aperture and narrower pit border are characteristic compared with intervessel pit.

Photo 25. Vessel-ray pits on the outer surface of the earlywood vessel. Vestures, branched and unbranched, are found, growing at the edge of the pit border.

Photo 26. Vessel-parenchyma pits on the inner surface wall of the earlywood vessel. Both vestured pits and unvestured pits are found.

Photo 27. Vessel-parenchyma pits on the outer surface wall of earlywood vessel.

Photo 28. Distribution of spiral thickening and pits on the inner surface of the latewood vessel elements. The thickening were observed always running from lower left to upper right in such a photograph, thus it means so-called S type.

Photo 29. Vessel elements showing the outer surface where pits distribute in a regular arrangement. Some pits have pit membranes and some don't.

Photo 30. Intervessel vestured pits on the inner surface of the latewood vessel with spiral

thickenings. In each pit, there are several thick bodies of vestures with thin branches on their top.

Photo 31. Another sample of detail of the surface of a latewood vessel element, which is a little different about spiral thickenings in comparison with the former.

Photo 32. Vessel-ray pits on the inner surface wall of the latewood vessel. No vestures in all of the pits and complicated spiral thickenings are found.

Photo 33. Vessel-ray pits on the outer surface of the latewood vessel. No vestures in all of the pits are found. There are some unilaterally compound pittings of vessel. There can be seen spiral thickenings and pits on the opposite inner surface of the vessel across the lumen through this side pit apertures.

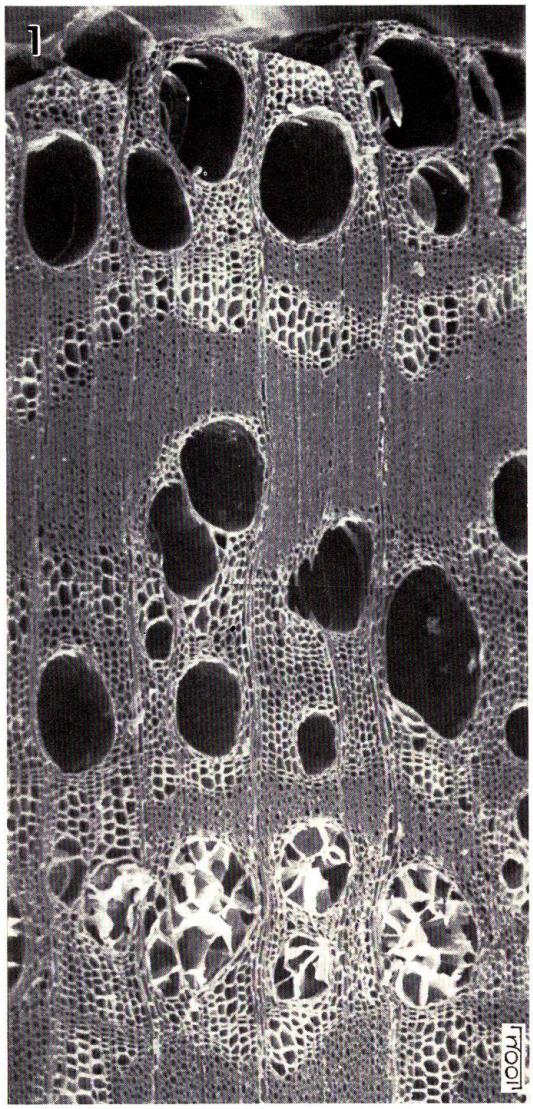
Photo 34. Vessel-parenchyma pits on the inner surface wall of the latewood vessel. No vestures are found. Spiral thickenings are complicated like in photo 32.

Photo 35. Vessel-parenchyma pits on the inner surface wall of the latewood vessel showing small vestures marked with V. It is very rare to see such a vesture in the vessel-parenchyma pit.

Photo 36. Inner surface wall of the earlywood vessel. It can be found that inner wall surface is covered continuously with a thin layer.

Photo 37. A thin layer covering the inner surface wall of vessel has often been broken at pit apertures.

Photo 38. Thin layer described above can be visible through the pit from the outer surface of the vessel.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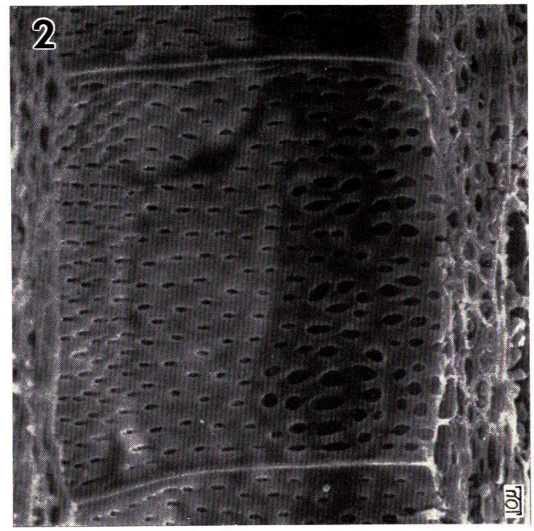
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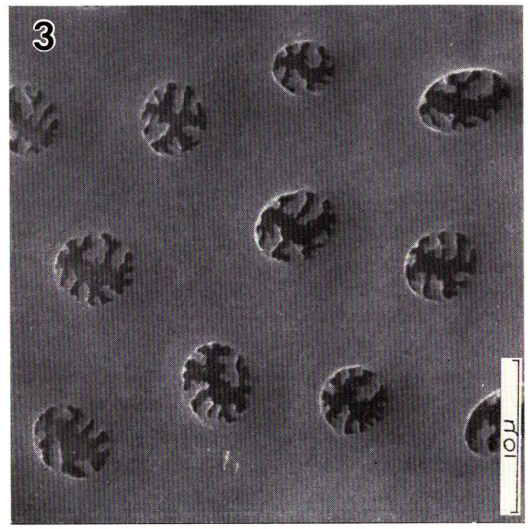
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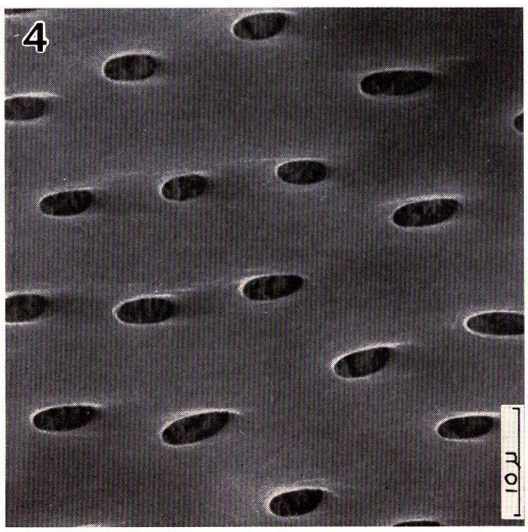
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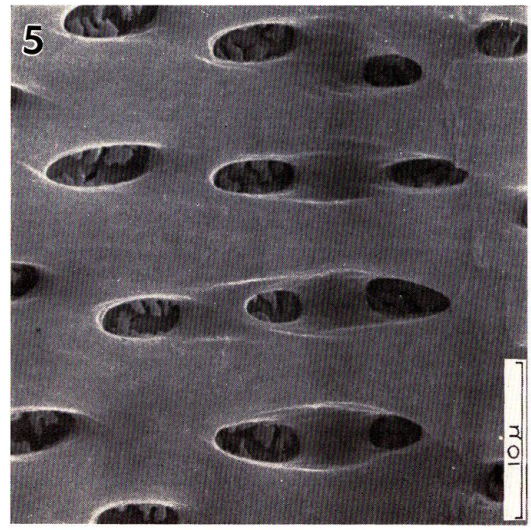
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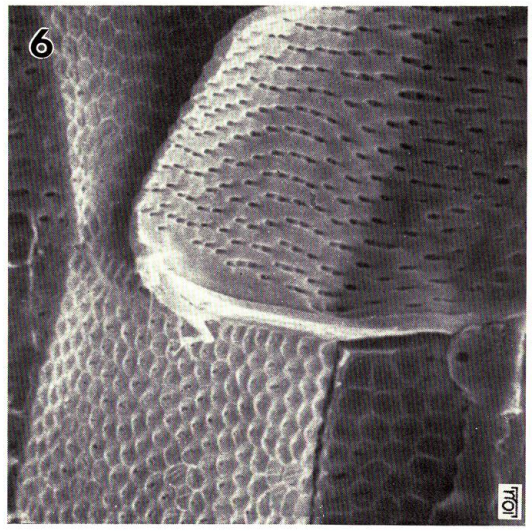
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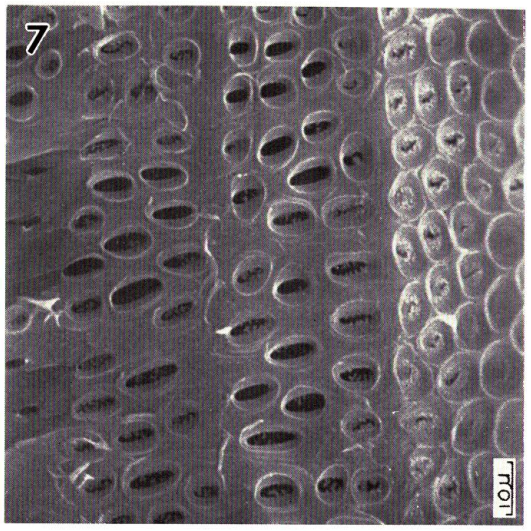
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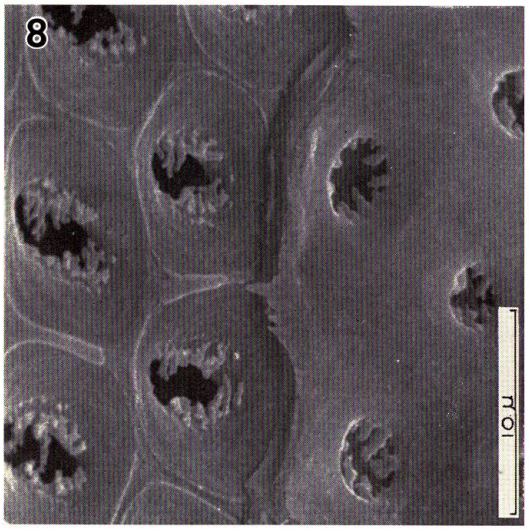
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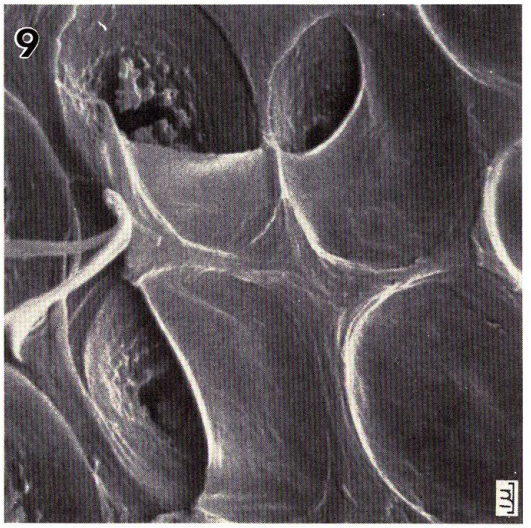
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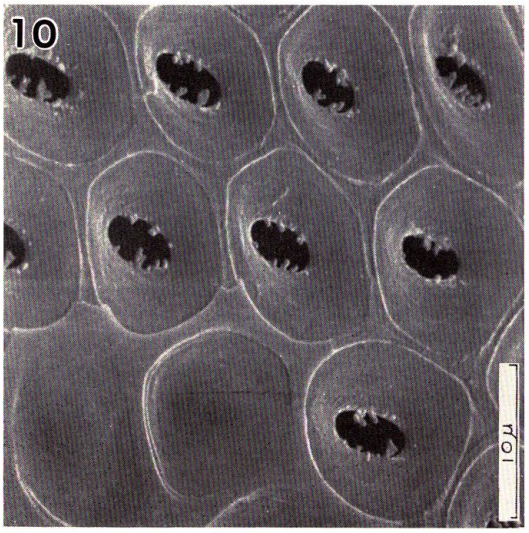
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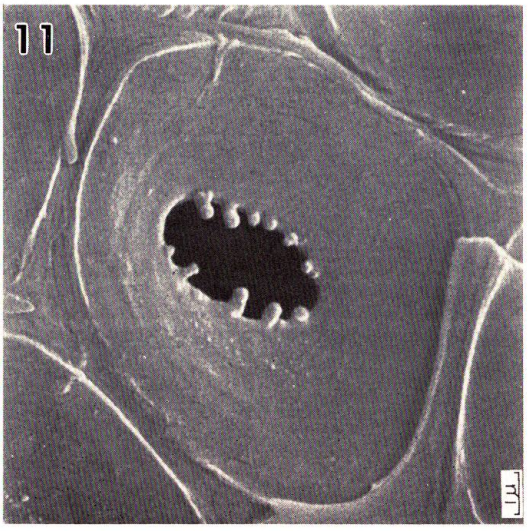
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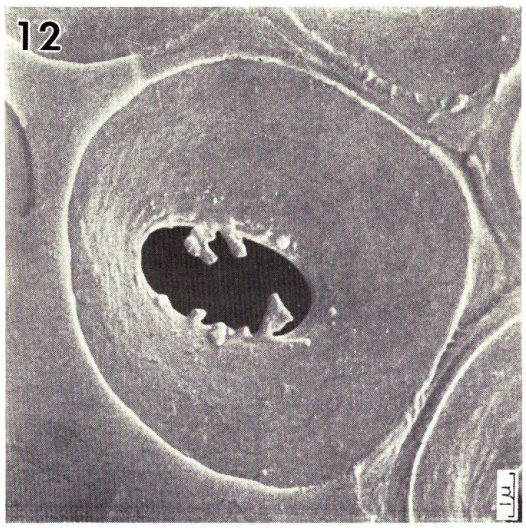
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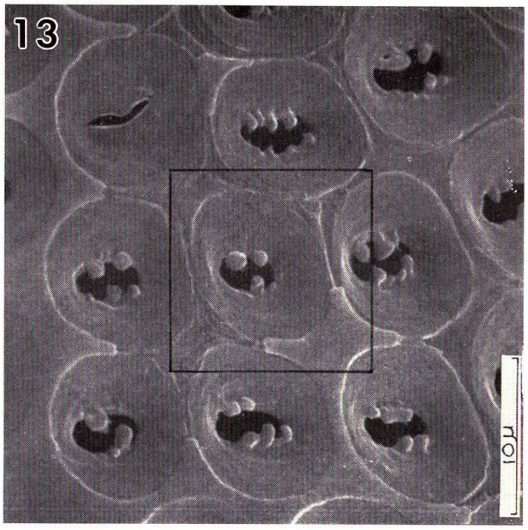
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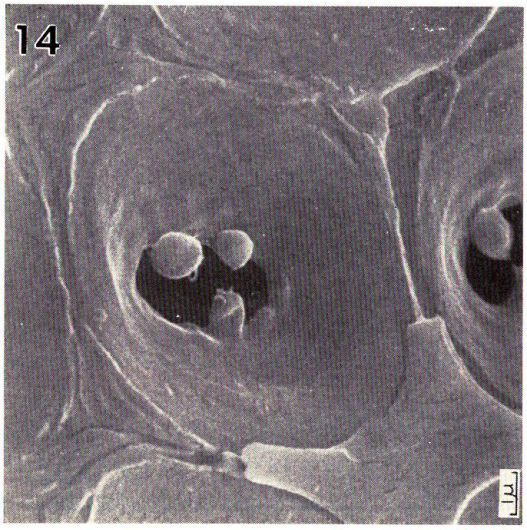
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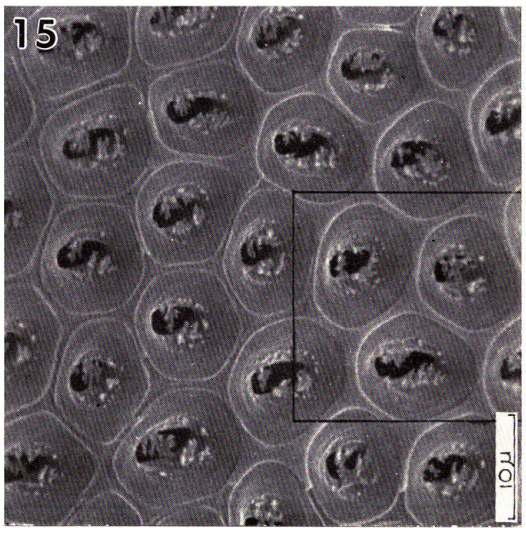
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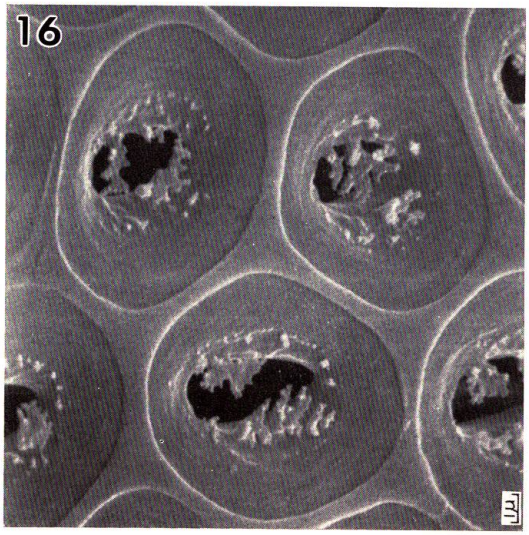
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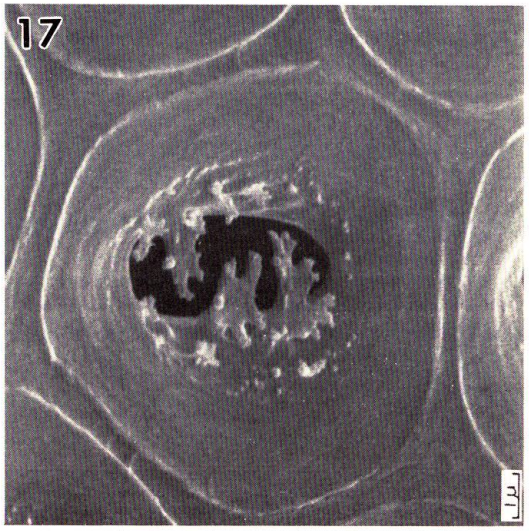
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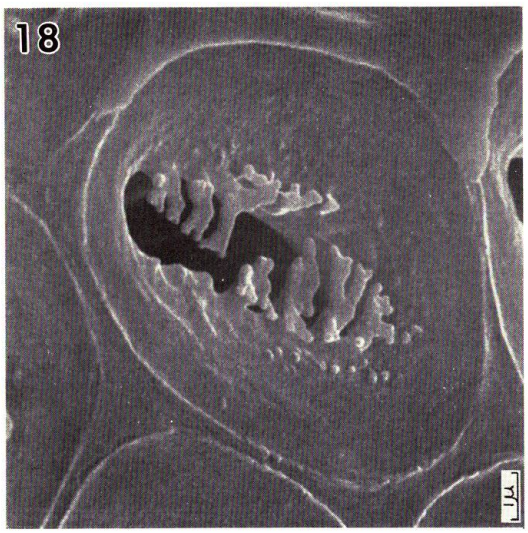
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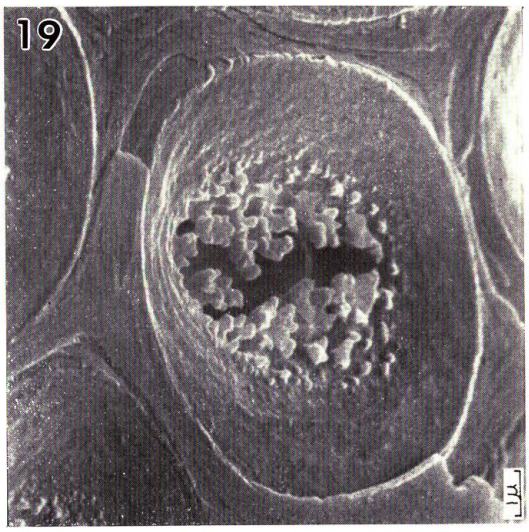
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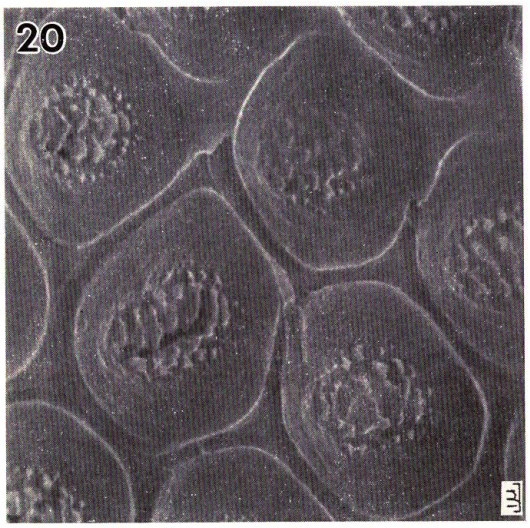
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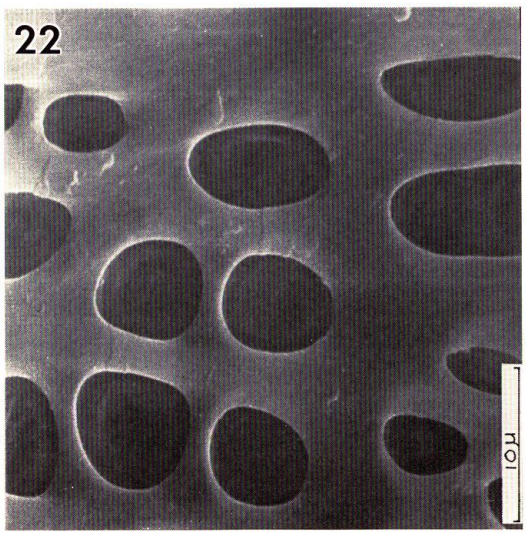
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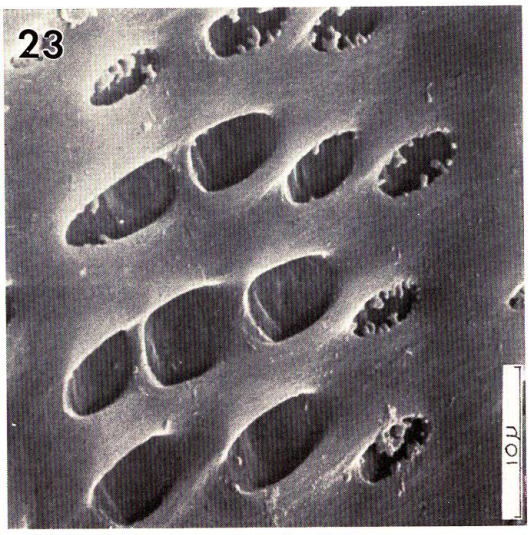
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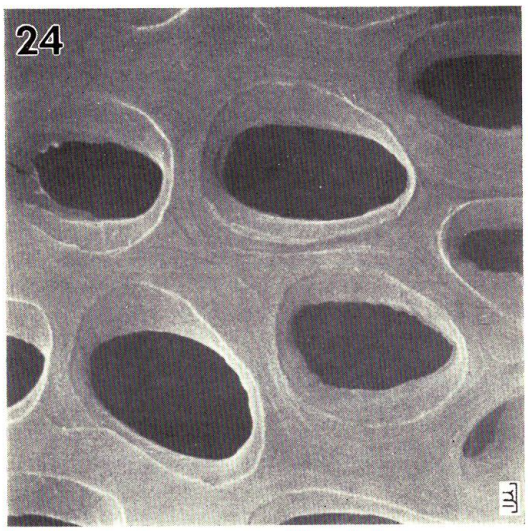
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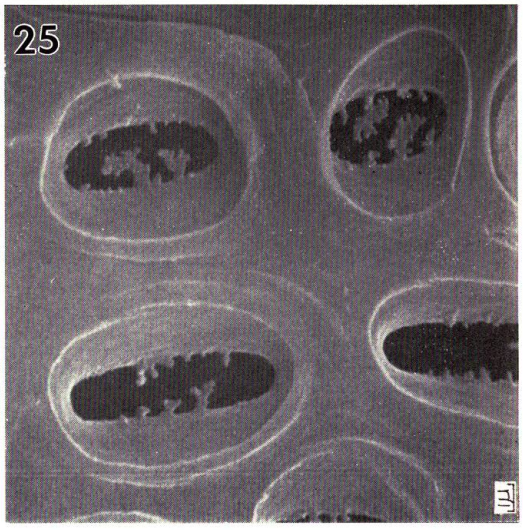
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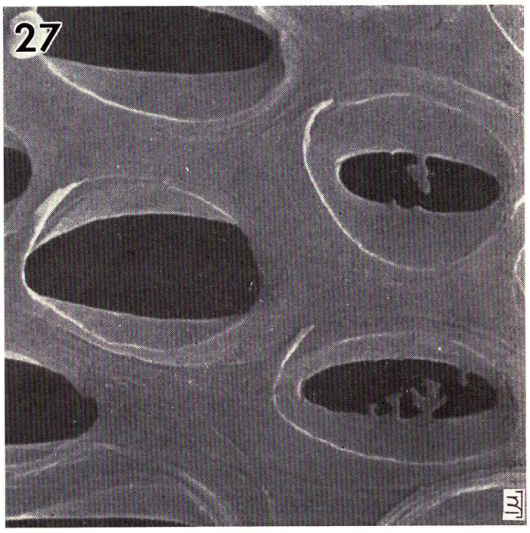
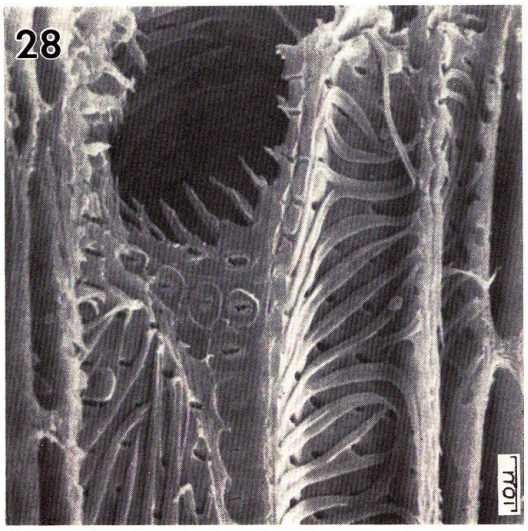
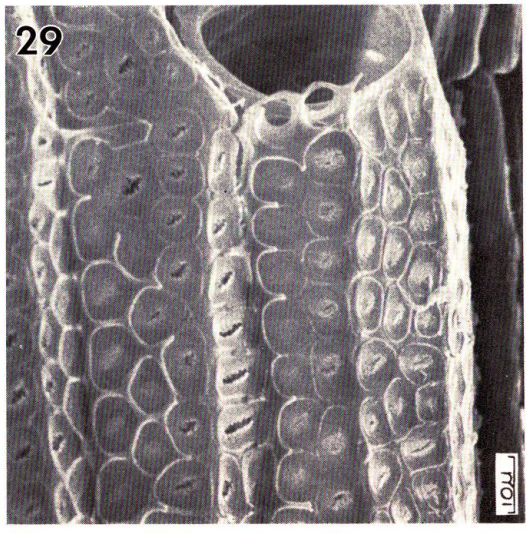
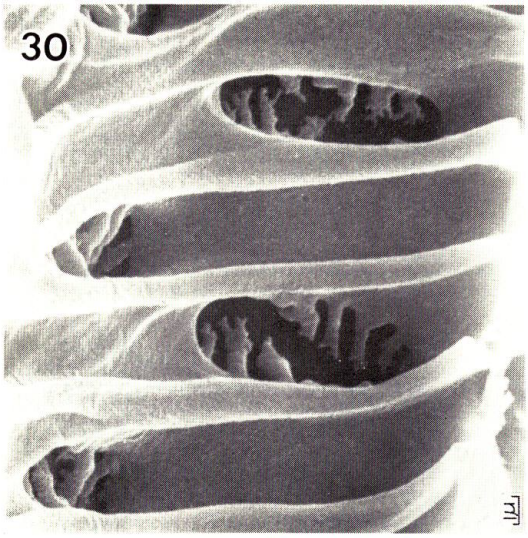
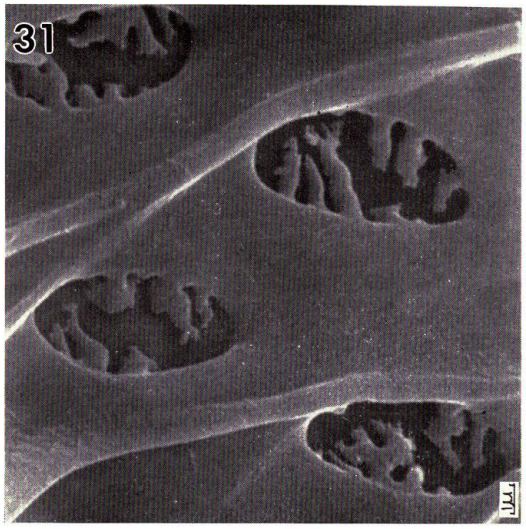

27
1μm

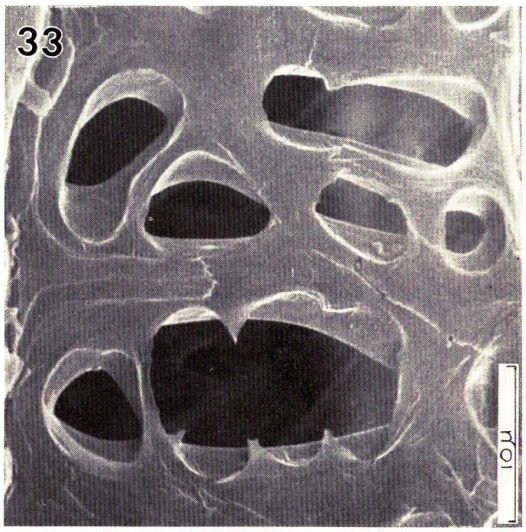

28
100μm

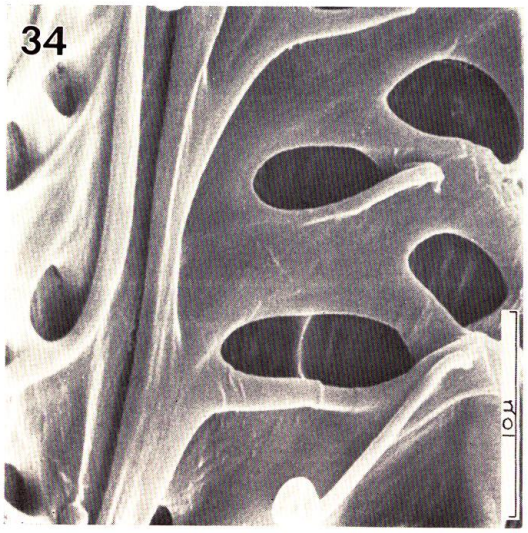

29
100μm

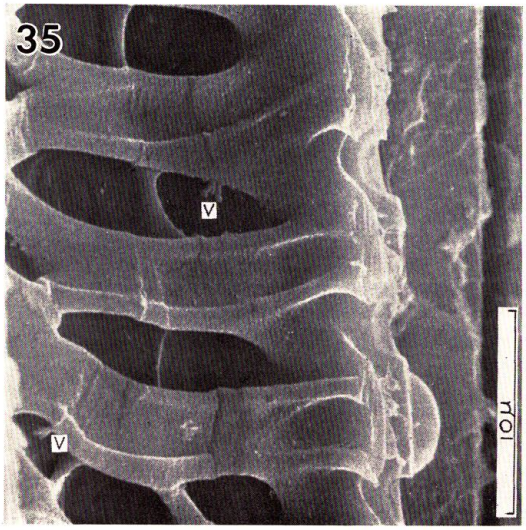

30
1μm

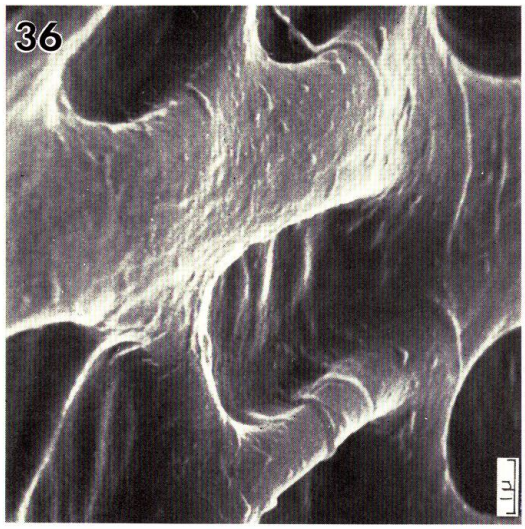

31
1μm


32
10μm

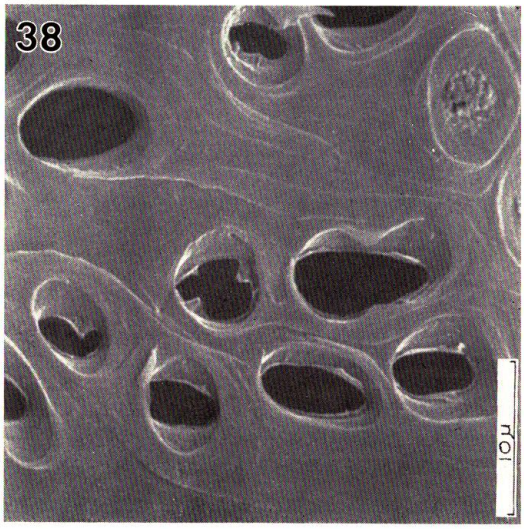

33
10μm


34
10μm


35
V
V
10μm


36
1μ


37
1μ


38
10μ