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ULTRASTRUCTURE OF THE ASCOSPORE WALL IN PEZIZALES (ASCOMYCETES)—IV

Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, and Sarcoscyphaceae. General discussion

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(With Plates 1–12, two Tables, and one Text-figure)

The development of wall layers and ornamentation of ascospores, plus specialized plasmic structures, are studied with the electron microscope in members of the Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, and Sarcoscyphaceae; additional results in the Pyronemataceae are given. The findings are compared with those of former studies (Merkus, 1973, 1974, 1975). A general discussion on the origin and development of the different structures concludes the series. A classification based upon species representing typical developments of all the species of the four studies is made.

INTRODUCTION

Following former publications on the subject (Merkus, 1973, 1974, 1975) this electron microscopy study completes the series of papers on the ultrastructure of the ascospore wall in Pezizales. The present paper includes the results in species belonging to the Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, and Sarcoscyphaceae. It also gives additional results in the Pyronemataceae sensu Eckblad, including those in *Pyronema omphalodes*, *Coprobia granulata*, *Geopyxis carbonaria*, *Mycolachnea hemisphaerica*, and *Octospora musci-muralis*.

In general, Eckblad's classification of the taxa (1968) has been followed. *Thecotheus* spec. (an eight-spored, ornamented species) and *Iodophanus carneus* are provisionally classified in the Thelebolaceae; e.g. Eckblad placed *Iodophanus* in the Pyronemataceae. According to Eckblad a member of the Otideaceae, *Geopyxis carbonaria*, is transferred provisionally to the Pyronemataceae sensu Eckblad.

The paper concludes with a general discussion on the subject.

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REVIEW OF EARLIER WORK

Only a few of the species studied by Le Gal (1947) could be examined under the electron microscope. Le Gal's light microscopy of these species is briefly reviewed. Some of the names she used (see footnotes) have been changed in accordance with the 'International Code of Botanical Nomenclature'.

Rhizina undulata Fr. per Pers.[1] and the related *Discina perlata* (Fr. per Fr.) Fr. develop a simple spore ornamentation that is formed between the primary wall and its covering layers. The ornamentation consists of callose and pectine and is of sporal origin. The primary wall is covered by an "assise sous-périsporique" and a "pellicule membranaire". The "assise sous-périsporique" is formed before the ornamentation develops. The "pellicule membranaire" is termed "coque interpérisporique", as it is formed at the same time as the ornamentation and is penetrated by the substance of ornamentation; the "coque interpérisporique" and the substance of the ornamentation both grow into one, the 'coque interpérisporique' also consisting of callose and pectine. During the development of the ornamentation a "périspore" is present on the outside of the ascospores; this "périspore" remains.

Helvella atra Holmskj. per Fr.[2], *Helvella elastica* Bull. per St.-Am.[3], *Helvella acetabulum* (L. per St.-Am.) Quél.[4], *Helvella macropus* (Pers. per S. F. Gray) P. Karst.[5] and some related species, all members of the Helvelleae of Le Gal (1947: 284), which are now placed in *Helvella* L. per St.-Am., normally develop smooth ascospores. But in a few cases the species show an ornamentation on the ascospores, consisting of coarse folds or of connected or isolated warts. According to Le Gal this ornamentation is false and formed by a substance situated in a "couche sous-épisporique", which deforms the epispore locally.

Unfortunately, no material of the species of the Sarcoscyphaceae studied by Le Gal was available. Instead *Sarcoscypha coccinea* (Scop. per S. F. Gray) Lamb. and *Desmazierella acicola* Lib., which also belong to this important taxon, could be studied. Le Gal's descriptions of the ornamentation patterns of the species involved resemble those of *Rhizina undulata* and *Discina perlata*. The "périspore" is permanent in all species; the ornamentation, which does not contain callose and pectine, penetrates its covering layers in *Cookeina sulcipes* (Berk.) O. K., *Cookeina tricholoma* (Mont.) O. K. and *Cookeina insititia* (Berk. & Curt.) O. K., and remains within these layers in *Aurophora dochmia* (Berk. & Curt. apud Berk.) Rifai[6], *Phillipsia domingensis* Berk., *Plectania campylospora* (Berk.) Nannf. apud Korf[7], and *Plectania platensis* (Speg.) Rifai[8].

1 *Rhizina inflata* Schaeff. per P. Karst.
2 *Leptopodia atra* (Fr. ex König) Boud.
3 *Leptopodia elastica* (Bull.) Boud.
4 *Helvella sulcata* Afz.
5 *Macropodia macropus* (Pers.) Fckl.
6 *Phillipsia dochmia* (Berk. & Curt. apud Berk.) Seaver.
7 *Sarcosoma sarasini* (P. Henn.) Boud.
8 *Urnula platensis* Speg.

MATERIALS AND METHODS

Fresh material was collected in the Netherlands, Belgium, Turkey, Papua New Guinea, Indonesia, Kenya, Uganda, and Canada; some species were provided in pure culture by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The following list gives more details about the specimens and their origins: '*Ascophanus*' *coemansii* Boud. (a species of *Coprotus*) — *van Brummelen*, culture on cow dung, 21.VI.1973 (L); — *Piepenbroek*, on cow dung, near Welsum, Olst, Overijsel, The Netherlands; *Ascozonus woolhopensis* (Berk. & Br.) Boud. — *van Brummelen*, several cultures isolated from rat dung, on oatmeal agar enriched with horse dung decoction, Ekeren, Belgium (L); *Coprobia granulata* (Bull. per Mérat) Boud. — *van Brummelen 3562*, culture on cow dung, Hollandse Rading, Maartensdijk, Utrecht, The Netherlands (L); — *van Brummelen*, culture on cow dung, Bathmen, Overijsel, The Netherlands, 6. VIII. 1973 (L); *Desmazierella acicola* Lib. — *J. Daams*, on fallen needles of *Pinus*, "Spanderswoud", Hilversum, North Holland, The Netherlands, 11.V.1973; *Geopyxis carbonaria* (Alb. & Schw. per Pers.) Sacc. — *van Brummelen & Piepenbroek 4082*, on burnt soil, 't Joppe, Gorssel, Gelderland, The Netherlands, 6.VIII.1973 (L); — *Piepenbroek 783*, on burnt soil, 't Joppe, Gorssel, Gelderland, The Netherlands (L); *Gyromitra esculenta* (Pers. per Krombh.) Fr. — *Tjallingii & van Brummelen*, among rubbish in a garden, Wageningen, Gelderland, The Netherlands, 2.IV.1974 (L); *Helvella crispa* Scop. per Fr. — *van Brummelen 4075*, under *Pinus*, Bergen, North Holland, The Netherlands, 3.XI.1973 (L); — *van Brummelen 4506*, under deciduous trees beside a road, Diepenveen, Overijsel, The Netherlands, 15.VII.1974 (L); *Octospora musci-muralis* Graddon — *Piepenbroek 720*, among mosses on an old wall in a garden, between Deventer and Twello, Voorst, Gelderland, The Netherlands, 12.XI.1973 (L); *Iodophanus carneus* (Pers. per Pers.) Korf apud Kimbr. & Korf — *van Brummelen*, culture on sheep dung, Elspeet, Gelderland, The Netherlands, 7.XI.1972 (L); — *van Brummelen*, culture, Vogelenzang, North Holland, The Netherlands (L); — *Prof. Necat*, culture on oatmeal agar enriched with horse dung decoction, Kaskaloglu, Bornova, Izmir, Turkey; *Lasiobolus monascus* Kimbr. — *van Brummelen 3563*, several cultures isolated from porcupine dung, on oatmeal agar enriched with horse dung decoction, Mt. Suckling, Papua New Guinea, 6.IX.1972 (L); *Lasiobolus pilosus* (Fr.) Sacc. — *van Brummelen*, culture on sheep dung, Elspeet, Gelderland, The Netherlands, 7.XI.1972 (L); — *van Brummelen 4077*, culture on horse dung, Overveen, Bloemendaal, North Holland, The Netherlands, 14.V.1973 (L); — *van Brummelen*, culture on hartebeest (=*Alcelaphus caama*) dung, zoological garden, Amsterdam, North Holland, The Netherlands (L); *Morchella esculenta* L. per St.-Am. — *C. Bas 6003*, on sandy soil, Meyendel, Wassenaar, South Holland, The Netherlands, 16.IV.1974 (L); — *C. Bas*, on sandy soil-peaty soil, under *Populus* and *Crataegus*, Voorschoten, South Holland, The Netherlands, 22. IV.1975 (L);

Mycolachnea hemisphaerica (Wigg. per S. F. Gray) Maire — *Piepenbroek 769*, on sandy soil, Epserbos, Epse, Gelderland, The Netherlands, 21.VII.1974 (L); *Pyronema omphalodes* (Bull.) per St.-Am.) Fckl. — *van Brummelen 4081*, on burnt soil, 't Joppe, Gorssel, Gelderland, The Netherlands, 6.VIII.1973 (L); — CBS 373.62, several cultures on cornmeal agar (L); *Rhizina undulata* Fr. per Pers. — *van Brummelen 4076*, on burnt soil, 't Joppe, Gorssel, Gelderland, The Netherlands, 6.VIII.1973 (L); — *Piepenbroek 761*, on burnt soil, "de Poll" between Wilp and Voorst, Gelderland, The Netherlands, 20.VII.1974 (L); *Sarcoscypha coccinea* (Scop. per S. F. Gray) Lamb. — of unknown (probably Central European) origin (comm. Mr. G. D. Swanenburg de Veye), 8.III.1974 (L); *Thecotheus* spec. — culture on rhinoceros dung, P. Pentjang, W. Java, Indonesia, 28.X.1972 (L); *Thelebolus crustaceus* (Fckl.) — CBS 713.69, culture isolated from carnivore dung, on oatmeal agar enriched with horse dung decoction, *R. F. Cain & al.*, Mt. Speke, Ruvenzori Mts, Uganda, 23.VII.1969 (L); *Thelebolus stercoreus* Tode per Steudel — CBS 717.69, culture isolated from deer dung, on oatmeal agar enriched with horse dung decoction, *D. Malloch*, Simcoe Co., Ontario, Canada, 13.V.1968 (L).

As not all field collections contained ripening apothecia some specimen were kept moist in conditioned growth chambers, in alternating light and darkness; *Lasiobolus pilosus*, *Iodophanus carneus*, and *Thecotheus* spec. on their own substrate for a few days at 12 °C; *Lasiobolus monascus*, *Ascozonus woolhopensis* and another strain of *Iodophanus carneus* on oatmeal agar enriched with horse dung decoction for a few days or more at 12 °C. Of the strains received from the Centraalbureau voor Schimmelcultures, *Thelebolus stercoreus* and *T. crustaceus* were grown on oatmeal agar with horse dung decoction; *T. crustaceus* also on cornmeal agar; for at least 14 days at 12 °C, in alternating light and darkness. *Pyronema omphalodes* was cultured on cornmeal agar for 5–7 days at 23 °C, in constant light with an intermittent ultraviolet period of eight hours after two days.

A suitable procedure for fixation based on previous experiments was chosen: 1–1.5% $KMnO_4$ for 30 minutes at room temperature or 1–3.25% glutaraldehyde for 3–4 hours at 4 °C as primary fixatives, both followed by postfixation with 1% OsO_4 for 60 minutes at 4 °C; generally the lowest possible percentages were applied. At the beginning of this study the apothecia were fixed only in 1–1.5% $KMnO_4$ for 60–150 minutes at room temperature; rarely, they were fixed only in 1% OsO_4 for 60 minutes at 4 °C.

For further treatment the standard methods described in the previous papers were applied, including the occasional use of the Spurr embedding medium. All sections were cut with a diamond knife.

OBSERVATIONS

MORCHELLACEAE

MORCHELLA ESCULENTA—Pls. 1A–D; 2A, B

Fixatives: $KMnO_4$-OsO_4, glutaraldehyde-$KMnO_4$ and glutaraldehyde-OsO_4.

The apical parts of the asci are filled with compact masses of glycogen that enclose small parts of membranous material; just beneath the glycogen numerous vacuoles with contents that resemble glycogen occur. After glutaraldehyde-$KMnO_4$-fixation the glycogenic contents of the vacuoles are floccose or more granular and regularly spread over the vacuoles, in a few cases partly clustered to larger electron-dense globules. After $KMnO_4$-OsO_4 or glutaraldehyde-OsO_4-fixation the vacuoles are rather empty and only a thin layer of tiny glycogenic globules is found against the tonoplast (Pl. 1A).

The basal parts of the asci are also filled with large compact concentrations of glycogen and with vacuoles like those found in the apices.

In the one-eight-nuclear stages the subapical parts of the asci contain normal ascoplasm in which the nuclei and the tubular and vesicular elements of the endoplasmic reticulum as well as larger vesicles that show low electron density and may originate from the endoplasmic reticulum itself, scattered glycogen, plus small vacuoles of the same type as described above; apart from scattered glycogen larger accumulations also exist (Pl. 1A, B). Globular structures, which are generally electron-transparent after the three types of fixation, are also found (Pl. 1A–D). Some of them are scattered over this part of the ascoplasm but the greater part of them are concentrated in groups, almost all around the glycogen. They sometimes have a moderately electron-dense or completely electron-dense aspect after the two fixations with $KMnO_4$ (Pl. 1D).

Electron-dense and irregularly shaped membranous structures may occur in the larger vesicles with poor electron density and, more abundantly, in the smaller vacuoles, where they seem to arise from invaginating membranes; they are also found in the glycogen (Pl. 1A, B).

At later stages of development eight ascospores are delimited around the nuclei in the subapical ascoplasm. They soon have a regular, ellipsoid shape and the sporoplasm contains all the ascoplasmic elements present in this part of the ascus; they are plurinucleate and in each nucleus a nucleolus can be distinguished. The remaining epiplasm abounds in globular structures. Delimitation of small ascoplasmic fragments without nuclei also occurs.

The primary wall ($KMnO_4$-OsO_4 300–450 nm, glutaraldehyde-$KMnO_4$ 250–350 nm, and glutaraldehyde-OsO_4 about 250 nm thick) is normal in appearance and shows practically no internal differentiation (Pl. 1C).

The development of the secondary wall starts during a more extensive vacuolization

and/or glycogen formation in the epiplasm; the secondary wall material is fairly electron-dense and slightly floccose (Pl. 1C, D). As it proceeds further the endospore ($KMnO_4$-OsO_4 300–400 nm, glutaraldehyde-OsO_4 about 250 nm thick) and the epispore ($KMnO_4$-OsO_4 50–55 nm, glutaraldehyde-OsO_4 about 40 nm thick) differentiate in the primary wall. The endospore may show a more electron-dense layer in the inner parts, while the epispore consists of two electron-dense layers between which an electron-transparent layer is found (Pls. 1D; 2A, B).

The latest stages of development available in the material show a loss of epiplasmic structures; most of the globular structures have also shriveled and disappeared; those remaining cluster around the two poles of the ascospores (Pl. 2B). The secondary wall has also disappeared and left a fairly electron-transparent and vaguely structured layer around the epispore (Pl. 2B). The sporoplasm has increased in electron density and contains a few small oil drops; the epiplasm consists of glycogen and empty vacuoles.

HELVELLACEAE AND RHIZINACEAE

HELVELLA CRISPA—Pls. 2C–F; 3A, B
RHIZINA UNDULATA, GYROMITRA ESCULENTA, AND G. INFULA—Pls. 3C–G; 4A–D

Fixatives: $KMnO_4$-OsO_4 for all four species, glutaraldehyde-OsO_4 for *Helvella crispa* only.

Like in *Morchella esculenta*, vacuoles and glycogen are found in the apical parts of the asci of all these species. In *H. crispa*, *G. esculenta*, and *G. infula* the glycogen exist as compact masses in the apices, but in *R. undulata* only a small amount of glycogen is found as a thin layer or, even more, as a cap just inside the ascus wall at the apex (Pl. 2C). Vacuoles with what seem to be glycogenic contents become abundant towards the subapical parts of the asci of *H. crispa*, *G. esculenta*, and *G. infula*, with the normal ascoplasm extending in its central parts, while in *R. undulata* only a few vacuoles develop that are more randomly distributed over the ascoplasm of the apical parts.

Small to large vacuoles, varying from electron-transparent to almost entirely filled with glycogen, and masses of glycogen fill the basal parts of the asci.

In all the species the subapical parts of the asci are very much like those in *M. esculenta* in the one-eight-nucleated stages. Present are the nuclei, the tubular and vesicular elements of the endoplasmic reticulum, larger and almost electron-transparent vesicles probably originating from them, small vacuoles with traces of glycogen, and scattered or more clustered glycogen. Furthermore, scattered globular structures, which are electron-transparent to more moderately electron-dense after both types of fixation, occur all over the subapical ascoplasm in all four species but they abound in the lowermost and, even more, in the uppermost part in *H. crispa* and *R. undulata* (Pl. 2C).

The smaller vacuoles contain the same electron-dense and irregularly shaped membranous structures as in *M. esculenta*.

Later stages of development show the formation of ascospores in the subapical ascoplasm. As a result of the capricious course of the delimiting membranes the ascospores are at first very irregular. They obtain their eventual shape only when the primary spore wall is complete, so that the primary wall material must have great plasticity at these stages. The sporoplasm has a regular appearance and does not yet contain many globular structures. As in *M. esculenta*, delimitation of small parts of ascoplasm without nuclei is not infrequently found in all four species.

The primary wall is homogeneous and electron-transparent (*H. crispa:* $KMnO_4$-OsO_4 75–150 nm thick; *R. undulata:* 250–400 nm thick; *G. esculenta:* 250–450 nm thick). A notable variation in thickness arises during its development, and even formation of secondary wall material may start while primary wall development is still going on.

Secondary wall formation in *H. crispa* is regular but not extensive. The secondary wall material is fairly electron-dense and homogeneous at all stages after $KMnO_4$-OsO_4-fixation (Pls. 2D; 3A), more floccose after glutaraldehyde-OsO_4-fixation. It disappears when the ascospores mature and an endospore and epispore have developed in the primary wall; remnants of the investing membrane persist for some time (Pl. 3A, B). The endospore ($KMnO_4$-OsO_4 75–100 nm thick) does not have any internal structure; the epispore ($KMnO_4$-OsO_4 30–60 nm thick) shows the basic pattern of two electron-dense layers separated by an electron-transparent layer in which a thin, extra electron-dense layer can sometimes be distinguished (Pl. 3B).

During the ascospore development in *H. crispa*, the globular structures in the epiplasm and sporoplasm grow out distintly to large oil drops; a variable but generally large number of new and smaller oil drops seem to be added also, finally occupying the greater part of the epiplasm and sporoplasm (e.g. Pl. 2D, E). In the remaining part of the epiplasm, glycogen and vacuoles with floccose contents predominate.

A remarkable differentiation in the primary wall distinguishes *H. crispa* both from all species previously described (Merkus, 1973, 1974, 1975) and also from all those to be discussed in this study. As Le Gal (1947) already observed with the light microscope, the primary wall material grows out locally and forms thickenings on the outside that seem to be permanent. The outgrowths (young ascospores: $KMnO_4$-OsO_4 20–50 nm thick; mature ascospores: glutaraldehyde-OsO_4 about 85 nm thick) occur in both the inner and outer parts of the primary wall and are fairly electron-dense after both fixations (Pl. 2E, F).

As in *H. crispa*, secondary wall formation in *R. undulata* regularly passes off; it is far more extensive at the two poles of the ascospores, the secondary wall material is permanent. Differentiation of the primary wall into an endospore and an epispore follows the normal pattern. In the main the epispore (30–40 nm thick) has the same structure as that in *H. crispa*; the outer electron-dense layer becomes somewhat thicker in the latest stages (Pl. 3F, G) and internal striation can be seen in the intermediate electron-transparent layer (Pl. 3F). The endospore (200–300 nm thick) does

not remain homogeneous like in *H. crispa* but differentiates further in its outer parts, where two fairly electron-dense layers join the epispore; its remaining inner parts remain homogeneous and electron-transparent (Pl. 3F, G).

The secondary wall material of *R. undulata* is homogeneous and fairly electron-dense in its first stages of development but it soon becomes more electron-dense. At first some dots (Pl. 3C) and very thin layers (Pl. 3D) appear at a slight distance from the epispore. These soon form a continuous layer (Pl. 3F) which increases in thickness particularly near the two poles of the ascospores (Pl. 3G). The compact ornamentation that is finally formed at the two poles of the ascospores varies in thickness from 1200 nm up to 3000 nm and forms rounded spines or more cup-shaped projections, one at each pole, containing some large ellipsoid holes up to 700 × 300 nm in section (Pl. 3E). At the sides of the spores a smooth layer of secondary wall material of about 100 nm thick is present (Pl. 3F).

In *R. undulata* the globular structures in the epiplasm do not enlarge as much as in *H. crispa*, so that the epiplasm remains more intact, particularly in the layer just inside the ascus wall. The epiplasm develops glycogen and larger vacuoles with floccose contents. The sporoplasm increases in electron density and each spore generally develops two large oil drops, together with a number of smaller ones.

The development of the ascospores in *G. esculenta* and *G. infula* could not be studied extensively since only the younger stages of *G. esculenta* were present while the final stages of *G. infula* were absent. But some points should be mentioned. The differentiation of the primary wall into an endospore (*G. infula* 200–250 nm thick) and an epispore (*G. infula* 30–40 nm thick) and the formation of a secondary wall is regular in both species (Pl. 4A–D). The epispore of *G. infula* markedly resembles that of *H. crispa* and particularly that of *R. undulata*; the endospore of *G. infula* is like that in *R. undulata*, it shows differentiation in the outer parts, and even the inner parts are no longer homogeneous (Pl. 4D). The secondary wall material of *G. infula* condenses as floccose electron-dense material on the epispore but it is doubtful whether it persists (Pl. 4C, D); the mature ascospores are smooth. Both in the epiplasm and sporoplasm of the two species the oil drops increase in number and size; in the epiplasm the amount of glycogen increases and also occurs against the ascus wall in compact masses.

THELEBOLACEAE

"ASCOPHANUS" COEMANSII, THELEBOLUS CRUSTACEUS AND T. STERCOREUS—Pl. 5A–G

Fixatives: $KMnO_4$-OsO_4 for all three species, for *T. crustaceus* and *T. stercoreus* also glutaraldehyde-OsO_4. The apothecia of *T. stercoreus* are uniascal but each ascus is multispored; those of *A. coemansii* and *T. crustaceus* multiascal and all asci are multispored. As for the development of the ascospores in the ascoplasm, it is apparently rather simple in all three species.

Both fixatives show that the primary wall is homogeneously electron-transparent before further differentiation, sometimes with a slight increase in electron density in the inner parts; in *A. coemansii* it is 350–550 nm thick, in *T. crustaceus* 250–450 nm thick, and in *T. stercoreus* 300–550 nm thick after $KMnO_4$-OsO_4-fixation (Pl. 4E). It gives rise to an epispore and endospore that remain simple through all stages of development, each having the same appearance after the two different fixations.

The epispore is about 10 nm thick in all three species and seems to consist of only one electron-dense layer; this is homogeneous. Pictures of *T. crustaceus* and *A. coemansii* however suggest that the fairly electron-transparent layer just inside the electron-dense layer also belongs to the epispore. Nevertheless it is believed that this layer belongs to the endospore. The endospore is fairly electron-transparent, also without much internal differentiation; in *A. coemansii* 450–750 nm thick; in *T. crustaceus* 400–600 nm thick ($KMnO_4$-OsO_4 and glutaraldehyde-OsO_4); in *T. stercoreus* 300–550 nm ($KMnO_4$-OsO_4) and 200–350 nm (glutaraldehyde-OsO_4) thick. In *T. stercoreus* some concentration of spore wall material is present in the middle layers of the endospore while, as mentioned above, in *T. crustaceus* and *A. coemansii* endospore material seems to separate from the inner layers and join the epispore (Pls. 4F; 5A, C–G. Pl. 5F, G from *A. coemansii* are not representative of this development but added only for the epiplasmic structures).

The secondary wall starts to develop during primary wall differentiation. It is found all around the ascospores but it remains restricted to a thin layer (Pls. 4E, F; 5A). After $KMnO_4$-OsO_4-fixation the secondary wall material is fairly electron-dense and homogeneous, after glutaraldehyde-OsO_4 more floccose. It condenses on the epispore at later stages of development, where it forms a smooth and homogeneously electron-dense layer about 40 nm thick (Pl. 5C–G).

The epiplasm is fairly normal in appearance. Apart from large quantities of glycogen with scattered organels it has developed a few globular structures (electron-dense after $KMnO_4$-OsO_4-fixation; fairly electron-dense after glutaraldehyde-OsO_4-fixation), and small and electron-transparent vacuoles that do not seem to fuse at older stages. In *A. coemansii* and *T. stercoreus* the glycogen disappears as the ascospores mature; in *T. crustaceus* its electron density increases and clusters appear on the secondary wall. Furthermore, peculiar structures are found in the epiplasm of *A. coemansii* and *T. stercoreus*. In *A. coemansii* they are formed by clews of electron-dense material that probably arises from epiplasmic membranes (Pl. 5F); they show further growth during ascal development but fade into large masses of fairly electron-dense material as the ascospores mature (Pl. 5G). In *T. stercoreus* the structures are formed by large globules that occur particularly along the ascus wall and that evidently consist of a network of plaited units, each unit about 35 nm thick and made up of one broad and electron-transparent layer with a thin and electron-dense layer in the middle and bounded by electron-dense layers at both sides; loose units are possibly found in the epiplasm; the globules seem to be membrane-bounded (Pls. 4F; 5A, B). The sporoplasm is normal and contains some small vacuoles.

As the ascospores mature all the organels in the epiplasm disappear but the in-

vesting membrane is still present in the latest stages. The sporoplasm becomes electron-dense but does not develop any oil drops. The mature spores are smooth; it is not known whether the secondary wall material disappears; it could persist as an extra, smooth layer on the primary wall.

ASCOZONUS WOOLHOPENSIS—Pl. 6A–C

Fixatives: $KMnO_4$ and glutaraldehyde-OsO_4. The apothecia are multiascal and each ascus is multispored.

The development of the ascospores is as simple as it is in "*Ascophanus*" *coemansii*, *Thelebolus crustaceus*, and *T. stercoreus*. With both fixatives a homogeneous, electron-transparent and 150–250 nm thick primary wall is visible. It differentiates into a simple epispore, about 10 nm thick and consisting of a single electron-dense layer, and an endospore, which is 150–250 nm thick and increases in electron density but does not show any further differentiation (Pl. 6A–C).

Like in the three preceding species the secondary wall develops all around the ascospores but it may show a further increase in thickness (Pl. 6A). The secondary wall material is rather floccose after both fixations and does not condense; it disappears when the ascospores mature (Pl. 6B, C). The investing membrane remains present in the latest stages of development.

The development of the epiplasm could not be studied thoroughly. It contains the usual organels and much glycogen is present. Typical circular membranous units are found at some distance from the investing membrane; their origin remains unknown (Pl. 6B). Connections between the secondary wall and the epiplasm have been found in some instances along the investing membrane.

In the latest stages of development the sporoplasm has increased in electron density but neither oil drops nor vacuoles have developed. The epiplasm has lost its original structure and become vague and empty; some vacuoles are found after glutaraldehyde-OsO_4-fixation. The mature ascospores are smooth.

LASIOBOLUS PILOSUS—Pls. 6D–I; 7A, B

Fixatives: $KMnO_4$-OsO_4 and glutaraldehyde-OsO_4, the latter particularly for the older stages of development. The species has the habitual eight-spored asci, in multiascal apothecia. The youngest stages present show the beginning of secondary wall formation.

The primary wall is homogeneously electron-transparent and 600–1000 nm thick ($KMnO_4$-OsO_4). The secondary wall has started to form around the primary wall; its contents are homogeneous and fairly electron-transparent. The epiplasm is normal in appearance and shows the development of small vacuoles with somewhat floccose contents and of glycogen. Globular structures that are electron-dense after $KMnO_4$-OsO_4-fixation are also present and seem to be scattered over both the epiplasm and sporoplasm; their formation could not be studied.

In the next stages of development the secondary wall grows further into the epiplasm and increases in electron density. Endoplasmic reticulum clusters along the investing membrane and contact places between the epiplasm and the secondary wall material as well as complete invaginations from the epiplasm into the secondary wall arise in large quantities on the investing membrane; some of the invaginations enclose part of electron-dense globular structures (Pl. 6D, G). Simultaneously with the intensification of these phenomena the amount of glycogen in the epiplasm increases and the vacuoles fuse, the primary wall differentiates into an epispore and endospore and the secondary wall material condenses on the primary wall as granular material regardless of the fixatives (Pl. 6D–G).

The differentiation of the primary wall is regular and, in contrast to the preceding species of the Thelebolaceae, complex. The epispore is about 45 nm thick after $KMnO_4$-OsO_4-fixation and shows the usual pattern of two electron-dense layers, separated by an intermediate and fairly electron-transparent layer (Pl. 6E) that soon presents fine striation at later stages (Pl. 6F, H, I). After the same fixation the endospore is 500–1000 nm thick and starts to form an electron-transparent layer just beneath the epispore (Pl. 6D, E). At later stages of development this electron-transparent endospore layer is separated further from the inner and fairly electron-dense parts of the endospore by the formation of a thick, electron-dense layer which is sometimes coarsely striate, while on its outer side a fairly electron-dense and finely striated layer that probably results from endospore material develops (Pl. 6F–I).

As already mentioned, the contents of the secondary wall start to condense as granular material but in the latest stages of development they form a compact electron-dense layer on the epispore (Pl. 7A, B). It is not certain whether this layer persists or disappears with the remaining epiplasm as the ascospores mature. The mature ascospores are smooth; the sporoplasm is electron-dense, without any oil drops.

LASIOBOLUS MONASCUS—Pl. 7C–G

Fixative: $KMnO_4$-OsO_4. Like in *Thelebolus stercoreus* the apothecia contain one ascus and each ascus is multispored.

Only a few pictures of the advanced stages of development are available which show that secondary wall formation extends largely into the epiplasm (Pl. 7C–E), so that the remaining epiplasmic membranes are pressed together. Glycogen is found near the ascus wall. The secondary wall material is fairly electron-transparent and floccose and disappears in the final stages, as does the epiplasmic material (Pl. 7C–G).

Furthermore, the development of the epispore and endospore in the primary wall is difficult to trace. Comparing the pictures with those of *Lasiobolus pilosus*, it seems that also here a layer differentiates between the epispore and the endospore; the origin of the layer seems to be found in the outermost endospore layer (Pl. 7E, F).

An increase in electron density in the outer part of the endospore also occurs (Pl. 7F, G). The sporoplasm becomes electron-dense when the ascospores mature and it develops some small vacuoles but no oil drops. The mature ascospores are smooth (Pl. 7G).

IODOPHANUS CARNEUS—Pls. 7H, I; 8A, B

Fixative: $KMnO_4$-OsO_4. The apothecia are multiascal; each ascus had eight ascospores.

In the uninuclear asci small vacuoles with somewhat floccose contents are present in those parts of the ascoplasm that border the central ascoplasm containing the nucleus. The remaining apical and basal parts of the asci are completely filled with glycogen; only the uppermost parts of the asci still contain some normal ascoplasm. Small electron-dense globular structures are scattered over the ascoplasm around the nucleus. During meiosis and mitosis and the first stages of ascospore development the glycogen in the epiplasm increases in amount and appears partly as membrane-bounded globules of varying sizes and electron density, particularly around the ascospores. At the same time the vacuoles increase in number and size (Pl. 7H).

Later stages reveal the development of the ascospore walls. The primary wall is homogeneous and electron-transparent and 400–800 nm thick. It differentiates into an epispore and an endospore that both remain fairly simple. The epispore is 35–50 nm thick and reveals the usual pattern of two electron-dense layers separated by an electron-transparent layer; only the outer layer thickens somewhat more at later stages. The endospore is 300–900 nm thick and remains fairly electron-transparent; some slight increase in electron density becomes visible in its outer parts and at about 100–150 nm from the sporoplasmalemma a thin electron-dense layer arises (Pls. 7I; 8A).

Even before differentiation begins in the primary wall the investing membrane separates almost entirely from it. The secondary wall formed between the primary wall and the investing membrane consists of fairly electron-dense material that shows a close resemblance with the surrounding glycogen. Contact places along the investing membrane between the secondary wall and the epiplasm have been found; the investing membrane runs rather irregularly (Pl. 7I).

The secondary wall material starts to condense as a thin electron-dense layer on the primary wall (Pl. 7I). While the primary wall is differentiating the secondary wall is condensed further and transformed into an ornamentation that consists of warts (Pl. 8A, B). The warts vary in height from 150–600 nm and are spread irregularly over the ascospore surface; they are connected by a smooth layer that is about 30 nm thick (Pl. 8B). During the formation of the ascospore walls the glycogen in the epiplasm becomes more floccose and vacuoles with the same electron-dense coarse-floccose contents develop in between; they seem to be in contact with the glycogen and fill the basal parts of the asci completely.

When the ascospores mature, the epiplasm slowly disappears and the investing membrane is no longer recognizable; the remnants of the secondary wall material that have not condensed remain longest. The sporoplasm of the mature spores has increased in electron density and developed some small vacuoles.

THECOTHEUS SPEC.—Pl. 8C–G

Fixative: $KMnO_4$. As in *Lasiobolus pilosus* and *Iodophanus carneus* the apothecia are multiascal and each ascus has eight ascospores.

The young ascoplasm is regular. At the uni-nuclear stage a large vacuole with somewhat floccose contents occurs in the basal parts of the asci, while the apical parts of the asci contain large masses of glycogen. A few electron-dense globular structures are scattered over the ascoplasm. Once meiosis and mitosis have occurred and the formation of ascospore walls has started the amount of glycogen in the epiplasm increases further, particularly along the ascus wall, and vacuolization in the epiplasm begins; the vacuoles have the same flocosse contents as those in the basal parts of the asci.

The primary wall is about 500 nm thick at the two poles of the ascospores, elsewhere about 1000 nm thick. It is homogeneously electron-transparent at first but soon after its formation electron density increases in the inner parts (Pl. 8C); its differentiation products are the epispore (60–65 nm thick) and the endospore (at the two poles of the ascospores about 500 nm thick, elsewhere about 1000 nm thick). The epispore probably consists of a single fairly electron-dense layer when it has just been formed; at later stages an outer layer with an increased electron density differentiates. The endospore is fairly electron-dense and only its outermost part remains electron-transparent for any length of time; at the latest stages of development a rather diffuse electron-dense layer differentiates in the outermost part (Pl. 8D, F).

Before differentiation in the primary wall starts, the secondary wall develops; it is found all around the ascospore, particularly at the two poles. The investing membrane runs irregularly and shows connections between the secondary wall and the surrounding epiplasm. The secondary wall material is fairly electron-dense and floccose; it contains inclusions from the epiplasm (Pl. 8C).

During differentiation in the primary wall the secondary wall material increases in electron density and condenses (Pl. 8D). The condensation process starts throughout the secondary wall but soon concentrates on the epispore; in this way globular structures granular in appearance arise at regular intervals along the ascospore, giving ornamentation patterns of flat warts (about 200–300 nm high) (Pl. 8E, F). At the two poles of the ascospores the condensation process lags, leaving huge "blisters" which finally disappear when the ascospores mature but which create bowl-shaped ornamentation, also granular in appearance, at both poles (about 800–900 nm high) (Pl. 8E, G). During secondary wall development and formation of the ornamentation patterns the vacuoles in the epiplasm increase considerably in number and size and finally fuse to form one large vacuole. The glycogen also increases and fills up the remaining epiplasm.

At the oldest stages present in the material the sporoplasm has greatly increased in electron density and developed some small vacuoles; no oil drops are found. The epiplasm has disintegrated but the investing membrane is still present. Not all secondary wall material has condensed; its fate remains unknown.

PYRONEMATACEAE

PYRONEMA OMPHALODES—Pl. 9A–G

Fixatives: $KMnO_4$-OsO_4 and glutaraldehyde-OsO_4; the following description applies only to the $KMnO_4$-OsO_4-fixation.

Though the ascoplasm does not contain any unusual structures, it has a peculiar development in the uni-nuclear stage and in the first stages of meiosis and mitosis. In the basal parts of the asci large vacuoles with floccose electron-dense contents develop close to the nuclear material. In the apical parts of the asci the endoplasmic reticulum changes; its membranes cluster and increase in electron density, like in *Otidea bufonia* (Merkus, 1975), but they do not widen so far or form complete vesicles. In *Pyronema omphalodes*, the membranes become fairly vague locally, creating large and fairly electron-dense blurs in the ascoplasm. The same occurs with the mitochondrial membranes. Very probably the formation of electron-dense globular structures, present in these parts of the asci, is associated with the changes in membrane structure (Pl. 9A).

The young ascospores develop regularly; the primary wall ($KMnO_4$-OsO_4 250–400 nm thick) is homogeneous and electron-transparent at the younger stages of development (Pl. 9B). It increases in electron density at later stages, when a secondary wall is formed (Pl. 9C). Along the investing membrane a local and incidental attachment of secondary wall material to the primary wall is found while the latter is still developing. Both at the same time and in succeeding stages small vacuoles with floccose electron-dense material arise in the epiplasm; tubular and vesicular elements of the endoplasmic reticulum abound (Pl. 9B, C).

During the development of the secondary wall the primary wall increases in electron density and differentiates into an epispore ($KMnO_4$-OsO_4 35–50 nm, glutaraldehyde-OsO_4 35–40 nm thick) and an endospore ($KMnO_4$-OsO_4 250–450 but in extreme cases to 2000 nm thick, glutaraldehyde-OsO_4 150-250 nm thick); during this differentiation radial striation in the endospore is apparent. In the epispore an outer electron-dense layer becomes visible at first; further development reveals an inner electron-dense layer. While additional differentiation takes place the endospore becomes homogeneous (Pl. 9E, F). The secondary wall may vary in thickness along the ascospore surface. The secondary wall material is fairly electron-dense and seems to be in contact with the epiplasmic vacuoles that have meanwhile increased in volume and fused to surround the ascospores completely (Pl. 9E, F),

thereby also running out into the apical and basal parts of the asci. The remaining epiplasm around the vacuoles still contains some of the original organels, including the electron-dense globules, but for the most part it has been replaced by glycogen, particularly along the ascus wall.

At later stages the epiplasmic changes in the apical part of the asci are complete. Glycogen with scattered vesicular and membranous structures fill most of these parts of the asci; in the uppermost parts a great deal of endoplasmic reticulum occurs as tubules and vesicles and, close to the glycogen, forms clusters of typical membranous structures and electron-dense globular structures (Pl. 9D, probably also representing the "tractus" or "funiculus" described by Chadefaud (1960)). The basal parts of the asci are filled with glycogen.

By the latest stages of development the endospore has differentiated into an inner electron-transparent and an outer electron-dense part; in the latter a further striation, which joins that in the epispore, is visible (Pl. 9G). The secondary wall material disappears completely; only the investing membrane may remain for some time, together with the remnants of the epiplasm; the mature spores are smooth. The sporoplasm has increased in electron density and developed small vacuoles that are usually arranged in groups at the two poles of the oval ascospores together with electron-dense granules that line the tonoplast; no oil drops are found (Pl. 9G).

COPROBIA GRANULATA—Pl. 10A–E

Fixative: $KMnO_4$-OsO_4.

The young ascoplasm is normal in appearance. Like in *Pyronema omphalodes*, a large vacuole with floccose electron-dense contents is present at the uninuclear stage in the basal parts of the asci, and electron-dense globular structures are found scattered in the ascoplasm; their development however could not be studied. When meiosis and mitosis start vacuoles also develop near the apical parts of the asci, while in the uppermost parts typical membranous endoplasmic reticulum appears. Further stages reveal the development of the ascospores.

The primary wall is homogeneous and electron-transparent; it varies in thickness from 400 to 550 nm (but extreme values of 1000 nm are also found). When its internal differentiation starts, the inner part increases in electron density and at the boundary between the inner and more exterior parts a fairly electron-dense and somewhat radial striation becomes visible (Pl. 10A). In succeeding stages an endospore (250–500 nm, in extreme cases up to 1000 nm thick) and epispore (35–50 nm thick) arise. The inner endospore starts to take over the internal differentiation of the primary wall but this differentiation disappears at later stages and the endospore parts become more homogeneous. A thin layer in the outer endospore develops its own internal structure that immediately joins the striation arising in the inner electron-dense layer of the epispore. The outer electron-dense layer of the epispore is conspicuous and becomes rather broad at later stages. In the final stages the fairly electron-dense intermediate epispore layer has a fine radial striation that seems to continue into the inner epispore layer (Pl. 10B–E).

During the differentiation of the primary wall, changes in the epiplasm and the secondary wall take place. The secondary wall starts to develop at various places on the ascospore surface, and small vacuoles with floccose electron-dense contents that arise in the epiplasm along the ascospores fuse to form larger vacuoles in between. At first the secondary wall material consists of fairly electron-dense floccose material but when the secondary wall spreads all over the ascospore surface it encloses more homogeneous material, with vesicles and vacuoles that are clearly derived from the surrounding epiplasm (Pl. 10A). Later on it increases further in electron density, condenses internally and, together with the vacuolar material that has now fused to one large vacuole, grows so much that it fills nearly the whole ascus, leaving only a small strip of normal ascoplasm just inside the ascoplasmalemma. Here a few scattered electron-dense globular structures are still present. The contents of the vacuole have almost disappeared. Apart from some glycogen in the remaining epiplasm no further glycogen seems to be formed in the asci. In the meantime the sporoplasm has increased in electron density.

The internal condensation of the secondary wall material starts early. During differentiation of the primary wall a very thin layer of electron-dense material is formed in several places on the ascospore surface at a distance of about 30–50 nm from the primary wall. Once the layer encloses the whole ascospore more electron-dense material is apposed at regular intervals along the spore surface (Pl. 10A). Finally this results in a condensation pattern that includes globular structures at the two poles of the ascospores and more flattened structures along the sides of the spores, most of them with further internal striation of alternating electron-dense and fairly electron-dense layers (each about 15–20 nm thick) and all connected by an electron-dense layer about 15–20 nm thick (Pl. 10 B–E).

The latest stages present in the material still show the intact secondary wall with its internal condensation pattern. Since the mature ascospores are smooth it is however very probable that both will disappear when the ascospores have matured.

GEOPYXIS CARBONARIA—Pl. 10F–K

Fixative: $KMnO_4$-OsO_4.

Like in *Coprobia granulata*, the young ascoplasm resembles that in *Pyronema omphalodes* in that in the uninuclear stage one large vacuole with floccose electron-dense contents develops in the basal parts of the asci, and electron-dense globular structures are found scattered in the ascoplasm. The presence of smaller vacuoles near the apical parts of the asci during meiosis and mitosis and of an increased amount of endoplasmic reticulum in the uppermost parts at this stage also agree with the pictures of *C. granulata*. Further stages of development reflect the differences between the three species.

From the very first stages of formation the primary wall (150–200 nm thick) has somewhat radial striation that persists in the endospore, where it differentiates in the primary wall material. Separation of the investing membrane from the primary wall

starts regularly over the complete ascospore surface but secondary wall formation becomes conspicuous at only a few places on the ascospore surface; the secondary wall material is fairly electron-dense and homogeneous at these stages. In the epiplasm endoplasmic reticulum occurs around the developing secondary wall but no contact points between the secondary wall material and the surrounding epiplasm have been found (Pl. 10F).

Like in *P. omphalodes*, at later stages the epiplasm is filled with glycogen and vacuoles with some floccose contents; for the most part the glycogen fills the apical and basal parts of the asci and is present in a continuous layer inside the ascoplasmalemma; the vacuolar material fuses to one large vacuole that completely surrounds the ascospores and extends in the apical and basal parts of the asci. The same stages of development reveal a maximum of secondary wall material, now floccose, along the whole ascospore surface.

The sporoplasm increases slightly in electron density at the first stages of development and develops a large amount of membranous material that clusters mostly at one of the poles of the ascospores; electron-dense globular structures are also found. At later stages some small vacuoles are formed and the increase in electron density becomes complete; no oil drops are present.

During the plasmic developments and the formation of secondary wall material the primary wall differentiates into an endospore (200–400 nm thick) and an epispore (30–45 nm thick). The endospore develops a further radial striation of fairly electron-dense material; in the outermost part it develops a homogeneous and fairly electron-dense layer in which extra striation becomes apparent in the latest stages. Further differentiation of the epispore reveals two electron-dense layers, separated by an electron-transparent layer; in the two electron-dense layers dense striation is sometimes visible in the latest stages (Pl. 10G–K).

Finally the secondary wall material partly condenses on the epispore as a homogeneous and compact, rather smooth electron-dense 50–100 nm thick layer (Pl. 10G–K). It is not certain whether this layer remains, to form the outermost part of the mature ascospores, or disappears, together with the remnants of the epiplasm and the rest of the secondary wall material; the mature ascospores are smooth.

MYCOLACHNEA HEMISPHAERICA—Pl. 11A–E

Fixative: $KMnO_4$-OsO_4. The youngest stages available show the development of the ascospores.

The already formed primary wall is homogeneous and electron-transparent and 550–850 nm thick. Secondary wall formation starts soon afterwards. The investing membrane shows an irregular course and in some places may extend widely in the epiplasm. Connections between the secondary wall and the epiplasm, and membranous inclusions in the secondary wall are found. The epiplasm itself is rather vague and has no glycogen and few peculiarly globular structures; the sporoplasm is of normal appearance and fairly electron-dense. The secondary wall material is

fairly electron-transparent and somewhat floccose. It increases in electron density and condenses near the primary wall at the same time that the differentiation of the epispore and endospore in the primary wall starts. At first the epispore is fairly electron-dense and homogeneous, while the endospore does not change during differentiation from the primary wall (Pl. 11A).

In a succeeding stage of development the amount of condensed secondary wall material on the epispore increases to a rather smooth layer, in which globular structures with increased electron density are present. The development intensifies at further stages and gives rise to spiny elements of ornamentation consisting of clustered and electron-dense, globular structures of different sizes (Pl. 11B–E). Meanwhile the development in the primary wall has continued and a typical triple epispore and a further differentiated endospore have been formed. The epispore is 30–40 nm thick and may show extra striation in its intermediate, electron-transparent layer. The outer electron-dense layer has increased in thickness. The endospore is 500–800 nm thick, finally consisting of at least four layers that show increased electron density towards the outside of the ascospores (Pl. 11B–E).

In the mature asci glycogen is formed as a continuous layer along the inner side of the ascus wall. The original epiplasm has disappeared; remnants of the investing membrane are still apparent; no vacuoles have developed. The sporoplasm is electron-dense and contains a few large oil drops.

OCTOSPORA MUSCI-MURALIS—Pl. 11F–H

Fixative: $KMnO_4$-OsO_4.

Like in *Mycolachnea hemisphaerica*, young ascospores have already developed and at this stage some resemblance between the two species can be noted. The primary wall is homogeneously electron-transparent and 200–400 nm thick. Secondary wall formation starts along the complete spore surface but may extend here and there in the epiplasm; the secondary wall material is fairly electron-transparent and homogeneous (Pl. 11F). Inclusions from the epiplasm in the secondary wall have been found. The sporoplasm is normal; apart from many small electron-transparent vacuoles it contains oil drops that soon grow and fuse to form one or (mostly) two large oil drops; an increase in electron density is found. The epiplasm could not be adequately fixed but more glycogen develops than in *Mycolachnea hemispaerica*.

Subsequently the primary wall differentiates into an epispore and an endospore. The epispore (35–50 nm thick) has the usual structure; both electron-dense layers and the intermediate less electron-dense layer are finely striate; the outer layer further increases in thickness. At first the endospore (150–350 nm thick) is homogeneous and electron-transparent, possibly with a slight increase in electron density in the middle layer. At later stages the outer parts increase in electron density and form complete extra layers that join the epispore, while the inner parts decrease in electron density (Pl. 11F–H). All secondary wall material finally disappears, as do the remnants of the epiplasm. The mature ascospores are smooth (Pl. 11H).

SARCOSCYPHACEAE

SARCOSCYPHA COCCINEA—Pl. 12A–D

Fixative: $KMnO_4$-OsO_4. S. coccinea has long asci with eight ascospores in a single row.

Young stages of development show the asci in the one-eight-nuclear stages. The ascoplasm in the apical parts of the asci is homogeneous and fairly electron-dense, with small spots of electron-transparent material, possibly glycogen, and with thin membranous structures and tiny vesicles. The subapical and medial parts of the ascoplasm contain an extensive amount of endoplasmic reticulum that closely resembles that in *Pyronema omphalodes* and *Otidae bufonia* (Merkus, 1975). The membranes of the endoplasmic reticulum may form electron-dense blurs in the ascoplasm; they may also widen locally and form extra vacuoles with electron-dense vesicles and membranous structures of varying sizes; an increase in electron density in the membranes also seems to exist (Pl. 12A).

In subsequent stages of development the ascospores are delimited and the primary and secondary walls are formed. The basal parts of the asci develop faster than the apical parts. The epiplasm in the apical parts of the asci is still intact; it contains the usual organels and small amounts of glycogen; the structures described earlier have disappeared. The apical ascospores lie close to one another and are irregular in form; they have not yet acquired their ultimate shape, though their primary walls are almost complete. The same phenomenon was also seen in *Helvella crispa*, *Rhizina undulata* and *Gyromitra esculenta*. Here in *S. coccinea* the primary wall is also of unequal thickness along the ascospores. The sporoplasm contains some glycogen and a few small oil drops. The epiplasm in the basal parts of the asci consists of diffuse glycogen with remnants of membranes. The basal ascospores are spaced out and become ellipsoid to oblong with truncate poles; their primary walls are complete. They contain one or two oil drops of moderate size, together with some glycogen. The secondary walls have extended widely along the poles of the basal ascospores.

Electron-dense globular structures are present in the upper parts of the asci but in the lower parts they are less electron-dense to electron-transparent, particularly in the later stages. Like in *Pyronema omphalodes* and in the *Otidea* species studied they probably arise on the endoplasmic reticulum.

In later stages the development is more homogeneous throughout the ascus; the following descriptions apply to these stages. The primary wall of the ascospores is homogeneous and electron-transparent; along the lateral sides of the spores it is up to 800 nm thick (Pl. 12B). It develops an epispore and an endospore. The epispore (35–40 nm thick) differentiates into two electron-dense layers with an electron-transparent intermediate layer; the outer layer increases further in thickness, while the intermediate and inner layer are slightly striate in the latest stages (Pl. 12D). At first the endospore is homogeneous and electron-transparent; at the poles of the ascospores it sometimes develops fairly electron-dense material internally; this contains fibrous structures.

Subsequently the endospore is differentiated into a fairly electron-dense outer part (350–450 nm thick) and a lighter inner part (200 nm thick). The change from the outer to the inner part is abrupt but wall material may also become condensed in the latest stages. Just in the middle of the truncated poles of the ascospores the outermost part of the electron-dense outer endospore often increases in thickness, forming a less electron-dense knob that is covered completely by the epispore. In the innermost part of the electron-dense outer endospore this knob is bounded by a newly formed "epispore". Where the knob is formed, the complete endospore may reach a thickness of 1200 nm (Pl. 12C, D).

During fixation in $KMnO_4$-OsO_4 the outer and the inner layer of the endospore easily split at the sides of the ascospores and particularly in both polar rims of each spore, so that the complete endospore measures from 700–1000 nm at the sides of the spores to extreme widths near the polar rims. Splitting is also found just inside the epispore.

Secondary wall formation starts during primary wall differentiation; the investing membrane detaches from the primary wall; secondary wall material, which is homogeneous and fairly electron-dense, is formed in the intermediate space. It is found all over the ascospore surface but in particular at the truncate poles of each ascospore, where the secondary walls of adjacent spores may finally meet and join.

In the latest stages of development all secondary wall material mixes with the epiplasm; this has also changed and now contains much glycogen and only a few organels. Remnants of the investing membrane persist for some time. The sporoplasm is normal and apart from small vacuoles contains a large number of oil drops, the larger ones in two groups at both poles of the ascospores, the smaller ones in a thick layer against the spore wall. No ornamentation is formed; the mature ascospores are smooth.

DESMAZIERELLA ACICOLA—Pl. 12E–G

Fixatives: $KMnO_4$-OsO_4 and glutaraldehyde-OsO_4. Only a few apothecia could be sectioned.

Asci are found that show the same differences between the apical and basal parts during ascospore development as in *Sarcoscypha coccinea*, though not to the same extent. The apical parts of the asci have an intact epiplasm; the primary walls of the ascospores, which are in a single row close to one another and do not yet have their ultimate shape, are still developing. The basal parts of the asci have an epiplasm consisting almost entirely of diffuse glycogen; the secondary walls of the oval ascospores, which are spaced out, are also still developing. Globular structures and glycogen are present throughout the asci, both in the epiplasm and sporoplasm. The globular structures occur particularly around the ascospores in the epiplasm; they are electron-dense to electron-transparent after $KMnO_4$-OsO_4-fixation, electron-transparent only after glutaraldehyde-OsO_4-fixation. Like in *S. coccinea* later stages of development show a more homogeneous pattern in the apical and basal parts of the asci.

The primary wall of the ascospores is homogeneous and electron-transparent; after $KMnO_4$-OsO_4-fixation 400–600 nm thick along the whole circumference of a spore (Pl. 12E). It develops into an epispore ($KMnO_4$-OsO_4 35–40 nm, glutaraldehyde-OsO_4 40 nm thick) and an endospore ($KMnO_4$-OsO_4 700–1000 nm, glutaraldehyde-OsO_4 about 600 nm thick) that closely resemble that in *S. coccinea*. After $KMnO_4$-OsO_4-fixation the epispore shows the same differentiation into two electron-dense layers separated by an intermediate electron-transparent layer, with the outer layer still increasing slightly in thickness. After glutaraldehyde-OsO_4-fixation (which was not applied to *S. coccinea*) the epispore consists of an electron-dense inner layer and a fairly electron-transparent outer layer in which denser striation can be discovered. At first the endospore is homogeneous and electron-transparent in both types of fixatives but it soon differentiates. After $KMnO_4$-OsO_4-fixation the same electron-dense outer part as in *S. coccinea* and the same electron-transparent inner part become apparent. After glutaraldehyde-OsO_4-fixation the endospore is delimited by a fairly electron-dense layer against the plasmalemma, from where radial striation runs to the outer surface of the ascospores (Pl. 12F, G).

The secondary wall material is deposited between the primary wall and the investing membrane (Pl. 12E). After $KMnO_4$-OsO_4-fixation it is homogeneous and fairly electron-transparent and, at later stages of development, slightly concentrated as electron-dense material on the epispore. After glutaraldehyde-OsO_4-fixation (which was applied to later stages of development only) it becomes fibrous-floccose throughout the secondary wall. It is not possible to say whether the secondary wall develops around the entire ascospore wall or only at the two poles of the ascospores as in S. coccinea; its fate is also unknown (Pl. 12F, G).

In the later stages of development the epiplasm loses its organels. The globular structures remain longest and are clustered along the borders of the secondary wall; finally they also disappear. The sporoplasm then contains small vacuoles and many smaller and larger oil drops. The mature ascospores are smooth.

DISCUSSION

From the assembled results of this study and a comparison of them with those of previous studies (Merkus, 1973, 1974, 1975) it may be concluded that throughout the Pezizales the ultrastructure and development of the ascospores show considerable agreement.

The general ascal ultrastructure is the same for all species and corresponds with the usual descriptions such as those in the summaries of Hawker (1965) and Bracker (1967). I have already discussed the basic plasmic organels in detail (Merkus, 1973) but unusual individual structures need further comment.

The occurrence of globular structures (indicated as GS on the Plates) was reported in a great many species belonging to the genera *Otidea* and *Peziza* as well as in the single species *Pulparia persoonii* (Merkus, 1975). They have been found in all the

species discussed in the present study and judging by earlier results they may also occur in other species. Being less relevant they have not been mentioned elsewhere (Merkus, 1973, 1974). Their appearance depends on the species and on the fixative applied, differing particularly after $KMnO_4$-OsO_4 and glutaraldehyde-$KMnO_4$. Intermediate forms are not frequently found; they could depend on the stage of development.

As stressed earlier (Merkus, 1975), the origin of the globular structures is not clear. Evidence that the electron-dense globular structures found after the $KMnO_4$-OsO_4-fixative are derived directly from endoplasmic reticulum has been discovered in *Otidea bufonia*, *Pyronema omphalodes*, and *Sarcoscypha coccinea*, which may represent simpler forms of ascoplasmic development. The presence of additional, more electron-transparent globular structures in *Sarcoscypha coccinea*, and particularly the more complicated ascoplasm in *Gyromitra esculenta*, *G. infula*, *Helvella crispa*, *Morchella esculenta*, *Peziza ammophila*, *P. badia*, *P. michelii*, *P. plebeia*, *P. praetervisa*, *P. succosa*, *P. succosella*, *P. vesiculosa*, and *Rhizina undulata*, with the occurrence of either electron-dense or less electron-dense to electron-transparent products after use of the $KMnO_4$-OsO_4 and glutaraldehyde-$KMnO_4$-fixatives, however, suggests that the development of the globular structures is more complicated and that other organels, like endoplasmic vesicles and membranous structures, or glycogen (indicated on the Plates as EV, MS, and G or Gr, respectively) could be involved.

All the globular structures are formed in an early stage of development, before or during meiosis and mitosis, particularly in the more apical parts of the asci; in the genera *Peziza* and *Pulparia* formation in the more basal parts of the asci is also extensive. When the ascospores are delimited in the ascoplasm, the globular structures seem to spread at random over the epiplasm and sporoplasm; formation in the epiplasm could continue for some time, so that here their number possibly increases. The role of the globular structures has been discussed (Merkus, 1975). The electron-dense globular structures are the same as the "corpuscules métachromatiques" of Guilliermond (1904, 1910, 1920) or "globules métachromatiques" of Le Gal (1947).

In some species with a more complex ascoplasm, viz. *Gyromitra esculenta*, *G. infula*, *Helvella crispa*, *Morchella esculenta*, *Peziza badia*, *P. michelii*, *P. plebeia*, *P. succosa*, *P. succosella*, and *Rhizina undulata*, the globular structures grow out to true oil bodies that persist for some time in the epiplasm during ascospore development and that are permanently present in the sporoplasm, where they fuse to form larger oil drops. The ascoplasm of *Peziza trachycarpa* could not be studied adequately but apparently its further development proceeds in the same way. In *Desmazierella acicola* and *Gyromitra esculenta*, where the ascoplasm is less complex, this is also found. When present in the other species, the globular structures do not enlarge further but maintain their original form; they shrivel and disappear in the epiplasm but remain in the sporoplasm.

Independent development also causes oil drops to arise in the sporoplasm of a large number of the species, viz. *Aleuria aurantia*, *Anthracobia melaloma*, *Boudiera echinulata*, *Octospora musci-muralis*, *Lamprospora crec'hqueraultii*, *L. dictydiola*, *Melastiza*

chateri, *Mycolachnea hemisphaerica*, *Neotiella ithacaensis (Rehm) Schweers sensu Schweers*[9], *Otidea alutacea*, *O. bufonia*, *O. onotica*, *Peziza ammophila*, *P. badiofusca*, *P. emileia*, *P. petersii*, *P. praetervisa*, *P. trachycarpa*, *Pulparia persoonii*, *Pustularia cupularis*, *Scutellinia armatospora*, *S. scutellata*, *Sepultaria arenosa*, *S. tenuis*, *Trichophaea abundans*, and *T. woolhopeia*. Oil drops in the sporoplasm have not been found in *Ascodesmis microscopica*, *A. nigricans*, "*Ascophanus*" *coemansii*, *Ascozonus woolhopensis*, *Cheilymenia pulcherrima*, *Coprobia granulata*, *Iodophanus carneus*, *Geopyxis carbonaria*, *Lasiobolus monascus*, *L. pilosus*, *Peziza vesiculosa*, *Pyronema omphalodes*, *Sowerbyella radiculata*, *Thecotheus spec.*, *Thelebolus crustaceus*, and *T. stercoreus*.

Special plasmic structures other than those discussed above have been found in "*Ascophanus*" *coemansii*, *Ascozonus woolhopensis*, and *Thelebolus stercoreus*. The function of these structures could not be determined; those in "*Ascophanus*" *coemansii* are probably identical with the reticulate structures Boudier (1869) described for this species, and, together with those in *Ascozonus woolhopensis*, could result from endoplasmic reticulum.

For *Ascobolus furfuraceus* Pers. per Hook.[10] Wells (1972) mentioned the occurrence of numerous lipid globules on the surface of the perispore sac (=secondary wall) and in the sporoplasm during the latter stages of ascospore development; at maturity these disappear from the margin of the perispore sac. They may be identical with the electron-transparent globular structures of other species.

Glycogen is formed in a large number of the species. Its varying appearance and its role as a supplier of food were discussed earlier (Merkus, 1975); it will be mentioned later in relation to the development of secondary wall material.

Ascospore development follows a common line in the Pezizales (Delay, 1966; Carroll, 1966, 1967, 1969; Schrantz, 1966, 1970; Wells, 1972; Merkus, 1973, 1974, 1975); details of this line have been discussed (Merkus, 1973, 1974, 1975). The main points are the following.

All the ascospores in a single ascus are formed simultaneously through delimitation by an outer and inner delimiting unit membrane; in nearly all species they also develop simultaneously in later stages of wall formation. By contrast the ascospores of *Sarcoscypha coccinea* and *Desmazierella acicola* develop more rapidly in the basal parts of the asci than in the apical parts, as does the remaining epiplasm in these species.

Though interesting, the origin of the two delimiting membranes is not under discussion here. When the ascospore walls arise between them, the inner membrane becomes the sporoplasmalemma, which is permanent and may have a function in primary wall development. The outer membrane becomes the investing membrane, which may play a role in both primary and secondary wall formation; by the time the ascospores are mature it may have disappeared or else remain as an adhesive film on the outside of the ascospores when they leave the ascus.

The primary wall is formed first; it is homogeneous and electron-transparent.

[9] Khare (1975), who studied the type of *Humaria ithacaensis* Rehm, found that the name was missapplied by Schweers. For Schweers' fungus a correct name is not yet available.

[10] *Ascobolus stercorarius* (Bull. per St. Amans) Schroet.

In most of the species the ascospores are rounded off before the primary wall is formed but in *Desmazierella acicola*, *Gyromitra esculenta*, *G. infula*, *Helvella crispa*, *Rhizina undulata*, and *Sarcoscypha coccinea* the ascospores round off during primary wall development. The thickness of the primary wall not only varies according to the species; it also depends on the treatment, the fixatives with glutaraldehyde giving either no swelling or at least less swelling than the fixatives with $KMnO_4$, which might produce abnormal results. Conclusions based on thickness are therefore unreliable. In *Helvella crispa* the primary wall grows out internally and forms thickenings; these were also observed by Le Gal (1947).

The secondary wall is formed next. In a few rare instances secondary wall formation was found to start on a limited scale before primary wall formation was finished. In general however secondary wall formation is found as a separate process that starts when the primary wall is complete. The secondary wall is layed down between the primary wall and the investing membrane. The formation may start immediately all over the ascospore surface, or at first more locally, either remaining so or only at later stages spreading out. In *Ascodesmis microscopica* and *A. nigricans* the secondary wall is internally differentiated; in all other species the $KMnO_4$-OsO_4-fixative gives the secondary wall material a fairly homogeneous aspect, while the glutaraldehyde-OsO_4-fixative makes it look more flocky; it is always more electron-dense than the primary wall material. To judge from their structural similarities it is highly probable that parts of the epiplasm are incorporated in the secondary wall and make up its ground substance; glycogen or derivations of it are possibly involved; the investing membrane may play an active role in the process. Though the presence of an investing membrane is not mentioned by Bellemere & Melendez-Howell (1976), these authors stress the active role of the epiplasm during the formation of the material of ornamentation. It is most unlikely that the sporoplasm has a function in secondary wall formation as was supposed by Le Gal (1947), Moore (1963, 1965), Reeves (1967), and Lynn & Magee (1970). The apposition of extra wall material on the inner side of the primary wall is very unusual and found only in *Trichophaea woolhopeia*. Secondary wall formation remains rather limited in "*Ascophanus*" *coemansii*, *Ascozonus woolhopensis*, *Thelebolus crustaceus*, and *T. stercoreus*.

All the species have a secondary wall outside the primary wall and its fate determines the ultimate aspect of the ascospores. Different groups of development can be distinguished (see also Fig. 1).

1. Without further changes all secondary wall material loses its original structure and disappears in: *Ascozonus woolhopensis*, *Helvella crispa*, *Octospora musci-muralis*, *Lasiobolus monascus*, *Morchella esculenta*, *Pyronema omphalodes*, *Sarcoscypha coccinea*, and *Trichophaea woolhopeia*.

2. Secondary wall material condenses at random for a short time and finally all of it disappears in: *Anthracobia melaloma*, *Pustularia cupularis*, *Sepultaria arenosa*, and *S. tenuis*.

3. Secondary wall material condenses to form a complete ornamentation pattern on the epispore but finally all of it disappears in: *Coprobia granulata.*

4. Secondary wall material condenses to form a permanent smooth and uniform layer on the epispore in: "*Ascophanus*" *coemansii, Geopyxis carbonaria, Lasiobolus pilosis, Otidea alutacea, O. bufonia, O. onotica, Peziza ammophila, P. vesiculosa, Thelebolus crustaceus,* and *T. stercoreus.*

5. Secondary wall material condenses to form a permanent ornamentation on the epispore in: *Aleuria aurantia, Boudiera echinulata, Cheilymenia pulcherrima, Iodophanus carneus, Lamprospora crec'hquerault, L. dictydiola, Melastiza chateri, Mycolachnea hemisphaerica,* "*Neotiella ithacaensis*", *Peziza badia, P. badiofusca, P. emileia, P. michelii, P. petersii, P. plebeia, P. praetervisa, P. succosa, P. succosella, P. trachycarpa, Pulparia persoonii, Rhizina undulata, Scutellinia armatospora, S. scutellata, Sowerbyella radiculata, Thecotheus* spec., and *Trichophaea abundans.*

6. Secondary wall material is penetrated by pigment granules (Wells, 1972, for *Ascobolus furfuraceus* or a violet pigment (Carroll, 1969, for *Saccobolus glaber* (Pers. per Pers.) Lamb.[11]) from the surrounding vacuoles, which finally condense on the epispore and possibly from the ultimate ornamentation in: *Ascobolus furfuraceus* and *Saccobolus glaber.*

7. Secondary wall material immediately constitutes the ornamentation patterns in: *Ascodesmis microscopica* and *A. nigricans.*

Because the series of pictures is incomplete not all species can be placed definitively in any of these groups. *Desmazierella aciocla, Gyromitra esculenta,* and *G. infula* belong to group 2 or 4; the position of *Otidea alutacea, O. bufonia, O. onotica,* and *Lasiobolus pilosus* in group 4 is also uncertain, the species might be transferred to group 2.

Internal differentiation of the ornamentation is found in *Ascodesmis microscopica, A. nigricans,* and *Lamprospora crec'hquerault ii,* and possibly also in *Sowerbyella radiculata.* Slight striation of the innermost parts of the ornamentation is found in "*Neotiella ithacaensis*". The different structures in the ornamentation of most of the *Peziza* species and of *Pulparia persoonii* is difficult to interpret and may be present only temporarily.

The formation of the epispore and the endospore is a special problem and opinions about it differ (Merkus, 1973, 1974). Though it is not recognizable in all species, it is most likely that the primary wall differentiates into an outer epispore and an inner endospore through the redistribution of primary wall material (in this my conclusion agrees with that of Delay, 1966). The redistribution of endospore material in *Sarcoscypha coccinea,* which leads to the formation of a "second epispore' inside the

[11] *Saccobolus kervernii* (Crouan) Boud.

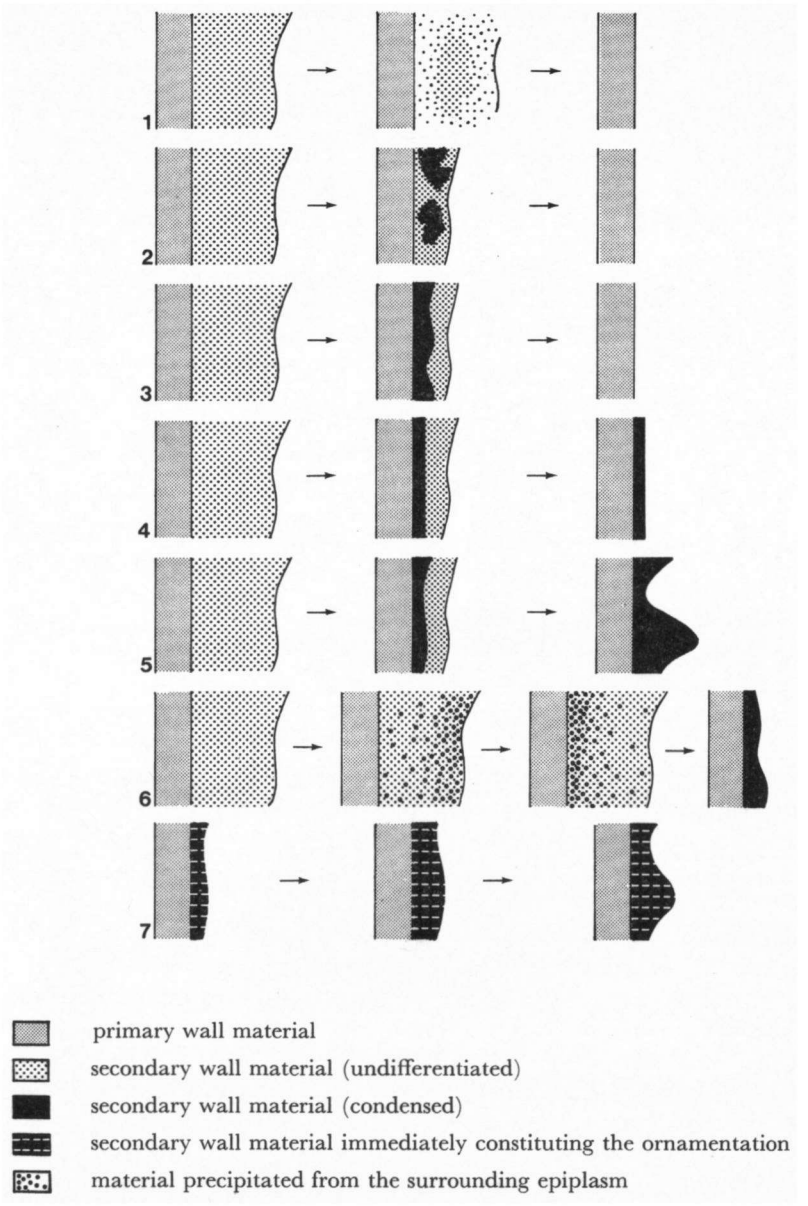

Fig. 1. Diagrammatic schemes of different groups of secondary wall development. The numbers refer to the different units.

endospore, strongly supports this view. After $KMnO_4$-OsO_4-fixation the epispore in most species shows a basic patern that consists of two electron-dense layers, separated by a less electron-dense to electron-transparent intermediate layer. In a few species the epispore is simpler and seems to consist of only one electron-dense layer (*"Ascophanus" coemansii, Ascozonus woolhopensis, Thelebolus crustaceus*, and *T. stercoreus*), sometimes with an increase in electron density in the outer parts (*Thecotheus* spec.). Glutaraldehyde-OsO_4 may give different appearances, often with layers on the outside that are less electron-dense. Further differentiation in the epispore and endospore leading to the formation of fine or coarse striation has been found in many species. Most of the striation is fine and found in the epispore, parallel to the ascospore surface. In a number of species it is also present in the outer parts of the endospore; in that case it largely inhibits delimitation of both layers; for this there are various explanations. A general increase in electron density and coarse striation in the endospore, perpendicular to the ascospore surface, is sometimes found, particularly after glutaraldehyde-OsO_4-fixation.

Referring to the ultrastructure and the development of ascospores different types can be distinguished. The distinction is based mainly on the data resulting from $KMnO_4$-OsO_4-fixation, as only part of the species were also fixed in the glutaraldehyde-OsO_4-fixative. The *Ascobolus furfuraceus* type is based on data published by Delay (1966), Carroll (1966, 1967, 1969) and Wells (1972). De Bary "bubbles", which were described in ascospores by Dodge (1957), Kimbrough (1966), and Kimbrough & Korf (1967) could not be found after fixation.

The following types can be distinguished.—

I. *Sarcoscypha coccinea* type.

Basal parts of asci in a state of development during ascospore formation more advanced than in apical parts; ascospores acquire their ultimate shape during primary wall development; tendency to complex ascoplasm with electron-dense and electron-transparent globules, and intermediate forms; oil drops in sporoplasm derived from the electron-transparent globules; epispore without much further differentiation; endospore with marked differentiation into an electron-dense outer part that passes abruptly into an electron-transparent inner part; formation of a "second epispore" in the endospore at the apices of ascospores (not found in *Desmazierella acicola*); for secondary wall material groups 1 and 2 or 4.

Desmazierella acicola and *Sarcoscypha coccinea.*

II. *Morchella esculenta* type.

Complex ascoplasm with electron-transparent and electron-dense globules, and intermediate forms; oil drops in sporoplasm derived from the electron-transparent globules; epispore and endospore without much further differentiation; for secondary wall material group 1.

Morchella esculenta.

III. *Helvella crispa* type.

Ascospores acquire their ultimate shape during primary wall development; complex ascoplasm with electron-transparent and more electron dense globules, oil drops in sporoplasm derived from the electron-transparent globules; occasional formation of extra wall layers in primary wall; epispore and endospore without much further differentiation; for secondary wall material group 1.

Helvella crispa.

IV. *Rhizina undulata* type.

Ascospores acquire their ultimate shape during primary wall development; complex ascoplasm with electron-transparent and more electron-dense globules; oil drops in sporoplasm derived from the electron-transparent globules; epispore without much further differentiation; endospore with (slight) increases in electron density in the outermost parts; for secondary wall material groups 2 or 4 and 5.

Gyromitra esculenta, G. infula, and *Rhizina undulata.*

V. *Otidea bufonia* type.

Tendency to complex ascoplasm with electron-dense globules; independent development of oil drops in sporoplasm; further differentiation in epispore and endospore; for secondary wall material possibly group 4.

Otidea alutacea, O. bufonia, and *O. onotica.*

VI. *Peziza vesiculosa* type.

Complex ascoplasm with electron-dense and more electron-transparent globules; no oil drops in sporoplasm; epispore without much further differentiation; endospore with some fine striation in the outermost parts; for secondary wall material group 4.

Peziza vesiculosa.

VII. *Peziza praetervisa* type.

Complex ascoplasm with electron-dense globules; independent development of oil drops in sporoplasm; epispore without much further differentiation; endospore with some fine striation in the outermost parts; for secondary wall material groups 4 and 5.

Pezia ammophila and *P. praetervisa.*

VIII. *Peziza succosa* type.

Complex ascoplasm with electron-transparent globules, *Peziza trachycarpa* also with more electron-dense globules; oil drops in sporoplasm derived from the electron-transparent globules; epispore and endospore without much further differentiation; for secondary wall material group 5.

Peziza badia, P. michelii, P. plebeia, P. succosa, and *P. succosella; P. trachycarpa* possibly also belongs to this group but the ascoplasm could not be adequately studied.

IX. *Pyronema omphalodes* type.

Tendency to complex ascoplasm with electron-dense globules; generally much glycogen in the epiplasm; no oil drops in sporoplasm, epispore occasionally with

fine striation; endospore with a fairly broad electron-dense layer in the outermost parts; for secondary wall material group 1.

Pyronema omphalodes.

X. *Lamprospora dictydiola* type.

Normal ascoplasm with electron-dense globules; with or without independent development of oil drops in sporoplasm; generally much glycogen in the epiplasm, epispore occasionally with fine striation; endospore with a thin to broader electron-dense layer in the outermost parts; for secondary wall material groups 1, 3, 4 and 5.

Aleuria aurantia, Boudiera echinulata, Cheilymenia pulcherrima, Coprobia granulata, Geopyxis carbonaria, Octospora musci-muralis, Iodophanus carneus, Lamprospora crec'hqueraultii, and *L. dictydiola.*

XI. *Scutellinia armatospora* type.

Normal ascoplasm with a few or more numerous electron-dense globules; independent development of oil drops in sporoplasm; epispore with further differentiation; endospore with striation in the outermost parts; for secondary wall material groups 2 and 5.

Anthracobia melaloma, Melastiza chateri, "Neotiella ithacaensis", Scutellinia armatospora, and *S. scutellata.*

XII. *Sepultaria arenosa* type.

Normal ascoplasm with a few electron-dense globules; independent development of oil drops in sproroplasm; epispore with fine striation; endospore with an outer electron-dense and an inner electron-transparent part, also with clustered material in between; for secondary wall material groups 1, 2, and 5.

Mycolachnea hemisphaerica, Pustularia cupularis, Sepultaria arenosa, S. tenuis, Trichophaea abundans, and *T. woolhopeia.*

XIII. *Lasiobolus pilosus* type.

Normal ascoplasm with electron-dense globules; no oil drops in sporoplasm; epispore with further differentiation; endospore without much further differentiation; for secondary wall material groups 1 and, possibly 4.

Lasiobolus monascus and *L. pilosus.*

XIV. *Thecotheus* spec. type.

Normal ascoplasm with a few electron-dense globules; no oil drops in sporoplasm; simple epispore; endospore with slight increases in electron density in the outermost parts; for secondary wall material group 5.

Thecotheus spec.

XV. *Thelebolus stercoreus* type.

Normal ascoplasm with a few electron-dense globules; no oil drops in sporoplasm; simple epispore; endospore mostly with some fine striation in the outermost parts; for secondary wall material group 4; secondary wall formation strongly limited.

"Ascophanus" coemansii, Thelebolus crustaceus, and *T. stercoreus.*

TABLE I

Characteristics of the different types

type	ascoplasm	globules	oil drops	epispore	endospore	group of sec. wall formation
I[12)13)]	→ complex	ed., et., int.	← et. glob.	not. diff.	diff. in ed. outer and et. inner part	1, 2 or 4
II	complex	ed., et., int.	← et. glob.	not diff.	not diff.	1
III[13)]	complex	ed., et.	← et. glob.	not diff.	not. diff.	1
IV[13)]	complex	ed., et.	← et. glob.	not diff.	ed. in outermost parts	2 or 4, 5
V	→ complex	ed.	indep. dev.	diff.	diff.	?4
VI	complex	ed., et.	not present	not diff.	some striation in outermost parts	4
VII	complex	ed.	indep. dev.	not diff.	some striation in outermost parts	4, 5
VIII	complex	et. (+ed.)	← et. glob.	not diff.	not diff.	5
IX	→ complex	ed.	not present	diff. including fine striation	broad ed. layer in outermost parts	1
X	normal	ed.	not present or indep. dev.	diff. including fine striation	thin-broader ed. layer in outermost parts	1, 3, 4, 5

XI	normal	ed.	indep. dev.	diff.	striation in outermost parts	2, 5
XII	normal	ed.	indep. dev.	diff. including fine striation	diff. in ed. and et. inner part	1, 2, 5
XIII	normal	ed.	not present	diff.	not diff.	1, ?4
XIV	normal	ed.	not present	simple	ed. in outermost parts	5
XV	normal	ed.	not present	simple	some striation in outermost parts	4
XVI	normal	ed.	not present	simple	not diff.	1
XVII	normal	lipoid glob.	not present	diff. including fine striation	not diff.	6
XVIII	normal	ed.	not present	diff.	not diff.	7

Abbreviations: diff., differentiation; ed., electron-dense; et., electron-transparent; glob., globules; indep. dev., independent development; int., intermediate; →, tendency to; ←, derived from.

[12] Basal parts of asci in a state of development during ascopore formation more advanced than in apical parts; "second epispore" in endospore.

[13] Ascospores acquire their ultimate shape during primary wall development.

XVI. *Ascozonus woolhopensis* type.
Normal ascoplasm with a few electron-dense globules; no oil drops in sporoplasm; simple epispore; endospore without further differentiation; for secondary wall material group 1; secondary wall formation rather limited.
Ascozonus woolhopensis.

XVII. *Ascobolus furfuraceus* type.
Normal ascoplasm, in *Ascobolus furfuraceus* with lipid globules; no oil drops in sporoplasm; epispore with fine striation; endospore without further differentiation; for secondary wall material group 6.
Ascobolus immersus Pers. per Pers., *A. furfuraceus*, and *Saccobolus glaber.*

XVIII. *Ascodesmis microscopica* type.
Normal ascoplasm with a few electron-dense globules; no oil drops in sporoplasm; epispore with further differentiation; endospore without further differentiation; for secondary material group 7.
Ascodesmis microscopica and *A. nigricans.*

The different types, based only on the findings of this study, call for further remarks. The eighteen types can be grouped into six units (see Tables I and II). Though the characteristics of each unit can overlap and some are present in more than two of them, each unit clearly represents related types. While in the first two units the complex ascoplasm, or at least a tendency to one, is found, the other four units have normal ascoplasm, while the tendency to a complex ascoplasm is found in only one type (IX).

TABLE II

DISTRIBUTION OF TYPES OVER THE UNITS

first unit	second unit	third unit	fourth unit	fifth unit	sixth unit
type I–IV	type V–VIII	type IX–XIII	type XIV–XVI	type XVII	type XVIII

In the first unit the characteristic development of the asci in type I distinguishes this type from all the others; the differentiation of a "second epispore" in the endospore, which has not been found in the other species, supports this view. Type III and type IV come close to type I, as all three have in common that the ascospores have

their ultimate shape only during primary wall development; this pleads for great plasticity of the primary wall material at these stages. Regarding the other characteristics, type II is the most closely united with the three types in the first unit. In the second unit types VI and VII closely resemble each other, while type VIII comes closer to the types of the first unit. Apart from the normal ascoplasm in most of the species the third unit represents species which have in particular more differentiation in both epispore and endospore. Types IX–XII closely resemble each other and their distinction is based on the different types of endospores: type XIII lacks differentiation in the endospore and is possibly related to the fourth unit. The types of the fourth unit are similarly in having a simple epispore but otherwise also closely agree. The types of the fifth and sixth unit have been kept apart because of the characteristic development of the secondary walls, which differ completely from those of the other types.

As regards the distribution of the different species over the types, *Pustularia cupularis* is placed within the *Sepultaria arenosa* type, which represents species of the Pyronemataceae sensu Eckblad; though some characteristics agree, a closer resemblance to species of *Otidea* (a. o. mentioned by Le Gal, 1947; Dennis, 1968; Eckblad, 1968) was not revealed. Berthet (1964) also mentioned the relationship between the genera *Pustularia* and *Sepultaria*; his view is based on cytochemical reactions of the nuclei. *Iodophanus carneus* and *Boudiera echinulata*, both with amyloid ascus walls, are placed within the *Lamprospora dictydiola* type, which further contains species of the Pyronemataceae. The relationship between *Iodophanus carneus* and other members of the Pyronemataceae was given earlier by Eckblad (1968) but the relationship between *Boudiera echinulata* and species of *Ascodesmis* that he mentioned (Ascodesmidoideae; Eckblad, 1968) could not be confirmed. *Thecotheus* spec., which also has amyloid ascus walls and which was placed in the Thelebolaceae by Eckblad (1968) is a separate type that nonetheless closely resembles the types representing other Thelebolaceae. *Sowerbyella radiculata* and *Pulparia persoonii* have not been classified in the types because they are not well enough known.

In *Ascodesmis microscopica* and *A. nigricans* the term secondary wall is appropriate for the second wall as it forms a permanent and rigid ornamentation on the epispore. In all other species the term might be open to question since the second wall is a temporary and transient formation that constitutes the materials for permanent extra wall layers, smooth or forming an ornamentation and arising outside on the epispore. But despite their differences in appearances and the fate of the material of the second wall in the species, in all cases it is desirable to use the term secondary wall together with the term primary wall since the walls represent stages in development and are more natural than the complex terminology of the wall layers in Le Gal's work, not to mention the confusing use of names like endospore, epispore, and perispore by others. By using the terms primary and secondary wall for the two wall layers that are formed in succession the term endospore and epispore are reserved for the further internal differentiation products of the primary wall.

Finally it is worth while to compare Le Gal's observations (1947, 1949) and ours.

The "false ornamentation" that she describes for *Helvella crispa* probably represents the formation of extra wall material in the primary wall, which is found in this species. But about the development of ornamentation our views probably do not agree. As pointed out earlier (Merkus, 1974), no evidence has been obtained for a "complex" development of ornamentation involving "masses globuleuses". Nor can it be decided precisely what structure corresponds to the "assise sous-périsporique" or to the "périspore". In view of Le Gal's drawings it is most probable that where she found no "périspore" the secondary wall corresponds to the "assise sous-périsporique", and that where she describes a "périspore" the secondary wall corresponds to it. In the first case the investing membrane corresponds to the "pellicule membranaire", which never arises simultaneously with the ornamentation, as a "coque interpérisporique" but which is always present before ornamentation, as a "tunique externe". In the latter case the "assise sous-périsporique" must be seen either as the outermost part of the primary wall, which is also supposed by Bellemere & Melendez-Howell (1976), or as the innermost part of the secondary wall; a layer that might represent the investing membrane is then absent. Cytochemistry of the substance of ornamentation constituting the secondary wall was not carried out but might reveal more about the exact nature of the secondary wall material; Le Gal's premise that the ornamentation is mostly formed of callose and pectine could therefore not be confirmed. As stressed earlier, there is no evidence for sporal origin of the secondary wall material, as was supposed by Le Gal; in fact, all pictures produce evidence in support of the theory that it is epiplasmic in origin.

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EXPLANATION OF PLATES 1–12

ABBREVIATIONS USED IN PLATES. — AW. ascus wall; CM, condensed material; E, epiplasm; En, endospore; Ep, epispore; EV, endoplasmic vesicle; G, glycogen; GS, globular structure; IAM, inner ascospore-delimiting membrane; IM, investing membrane; MS, membranous structure; OAM, outer ascospore-delimiting membrane; N, nucleus; PI, plasmic inclusions; PW, primary wall; S, sporoplasm; SW, secondary wall; T, tonoplast; Va, vacuole.

PLATE 1

Figs. A–D. *Morchella esculenta*, stained with uranyl acetate and lead citrate: Fig. A. ascoplasm during meiosis, fixed in 1% glutaraldehyde and 1% $KMnO_4$, ×4,600; Fig. B. id. detail of basal part of ascus, ×14,900; Fig. C. ascospore development, beginning of secondary wall formation, fixed in 1% $KMnO_4$ and 1% OsO_4, ×18,200; Fig. D. id. secondary wall formation and development of epispore and endospore.

PLATE 2

Figs. A, B. *Morchella esculenta*, advanced states in ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×18,200.

Figs. C–F. *Helvella crispa*: Fig. C. ascoplasm, upper part of ascus before meiosis and mitosis, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate, ×4,600; Fig. D. ascospore development, beginning of secondary wall formation, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×23,100; Fig. E. id. detail of primary wall, ×36,300; Fig. F. id. fixed in 3.25% glutaraldehyde and 1% OsO_4.

PLATE 3

Figs. A, B. *Helvella crispa*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4, ×23,100: Fig. A. secondary wall formation and development of epispore and endospore, stained with uranyl acetate and lead citrate; Fig. B. advanced state in ascospore development, stained with lead citrate.

Figs. C–G. *Rhizina undulata*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. C. secondary wall formation and development of epispore and endospore, condensation of secondary wall material, stained with uranyl acetate and lead citrate, ×29,700; Fig. D. id.; Fig. E. advanced state in development of ornamentation at one of two poles of ascospore, stained with uranyl acetate and lead citrate, ×14,900; Fig. F. id. along ascospore, ×43,300; Fig. G. id. stained with lead citrate.

PLATE 4

Fig. A. *Gyromitra esculenta*, ascospore development, secondary wall formation and development of epispore and endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700.

Figs. B, D. *Gyromitra infula*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. B. young ascospore after formation of primary wall, ×23,100; Fig. C. secondary wall formation and development of epispore and endospore, condensation of secondary wall material, ×29,700; Fig. D. id. advanced state in ascospore development.

Figs. E, F. *Thelebolus stercoreus*, ascospore development, ×29,700: Fig. E. beginning of secondary wall formation, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate; Fig. F. secondary wall formation and epiplasm with particular structures, fixed in 1.5% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate.

PLATE 5

Figs. A–C. *Thelebolus stercoreus*, ascospore development: Fig. A. secondary wall formation and epiplasm with particular structures, fixed in 1.5% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700; Fig. B. detail of epiplasmic structures, fixed in 6.5% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate, × 165,300; Fig. C. advanced state in ascospore development, with epispore and endospore and condensed secondary wall material, fixed in 1.5% $KMnO_4$ and stained with lead citrate, ×36,300.

Figs. D, E. *Thelebolus crustaceus*, advanced states in ascospore development, with epispore and endospore and condensed secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate, ×36,300.

Figs. F, G. "*Ascophanus*" *coemansii*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. F. secondary wall formation and condensation of secondary wall material, and particular epiplasmic structures, ×29,700; Fig. G. advanced state in development, ×36,300.

PLATE 6

Figs. A–C. *Ascozonus woolhopensis*, ascospore development, fixed in 1.5% $KMnO_4$: Fig. A. secondary wall formation and development of epispore and endospore, stained with uranyl acetate and lead citrate, ×29,700; Fig. B. id. with particular epiplasmic structures, not stained, ×47,900; Fig. C. advanced state in ascospore development, ×46,200.

Figs. D–I. *Lasiobolus pilosus*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. D. secondary wall formation and development of epispore and endospore, condensation of secondary wall material, ×23,100; Fig. E. id. detail of epispore and endospore, ×57,800; Fig. F. id.; Fig. G. advanced state in ascospore development, ×23,100; Fig. H. id. detail of epispore and endospore, ×57,800; Fig. I. id.

PLATE 7

Figs. A, B. *Lasiobolus pilosus*, advanced states in ascospore development, stained with uranyl acetate and lead citrate: Fig. A. fixed in 1.5% $KMnO_4$ and 1% OsO_4, ×29,700; Fig. B. fixed in 1.5% glutaraldehyde and 1% OsO_4, ×57,800.

Figs. C–G. Lasiobolus monascus, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. C. secondary wall formation and development of epispore and endospore, ×18,200; Fig. D–F. id. ×36,300; Fig. G. advanced state in ascospore development, ×36,300.

Figs. H, I. *Iodophanus carneus*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×18,200: Fig. H. young state in ascospore development, just after spore delimitation; Fig. I. secondary wall formation and development of epispore and endospore, condensation of secondary wall material.

PLATE 8

Figs. A, B. *Iodophanus carneus*, ascospore development, stained with uranyl acetate and lead citrate: Fig. A. further condensation of secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4, ×18,200; Fig. B. advanced state in development of ornamentation, ×36,300.

Figs. C–G. *Thecotheus* spec., ascospore development, fixed in 1.5% $KMnO_4$: Fig. C. beginning of secondary wall formation, stained with uranyl acetate and lead citrate, ×23,100; Fig. D. id. with development of epispore and endospore; Fig. E. condensation of secondary wall material, ×14,200; Fig. F. advanced state in development of ornamentation along ascospore, stained with uranyl acetate and lead citrate, ×23,100; Fig. G. id. at one of two poles of ascospore, ×18,200.

PLATE 9

Figs. A–G. *Pyronema omphalodes*, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. ascoplasm, upper part of ascus before meiosis and mitosis, ×8,100; Fig. B. ascospore development, beginning of secondary wall formation, ×29,700; Fig. C. id. more advanced secondary wall formation, ×23,100; Fig. D. epiplasm, upper part of ascus during ascospore development, ×8,100; Fig. E. ascospore development, development of epispore and endospore, ×23,100; Fig. F. id ×29,700; Fig. G. advanced state in ascospore development; ×29,700.

PLATE 10

Figs. A–E. *Coprobia granulata*, ascospore development, stained with uranyl acetate and lead citrate: Fig. A. secondary wall formation and condensation of secondary wall material, fixed; in 1.5% $KMnO_4$, ×29,700; Fig. B. id. with development of epispore and endospore, ×46,200; Fig. C. id. fixed in 1.5% $KMnO_4$ and 1% OsO_4; Fig. D, E. advanced states in ascospore development, fixed in 1.5% $KMnO_4$, ×46,200.

Figs. F–K. *Geopyxis carbonaria*, ascospore development: Fig. F. beginning of secondary wall formation, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×23,100; Fig. G. development of epispore and endospore and condensation of secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×36,300; Fig. H. id. fixed in 1% $KMnO_4$ and 1% OsO_4; Fig. I. id. fixed in 1.5% $KMnO_4$ and 1% OsO_4; Fig. K. id. advanced state in ascospore development, stained with lead citrate.

PLATE 11

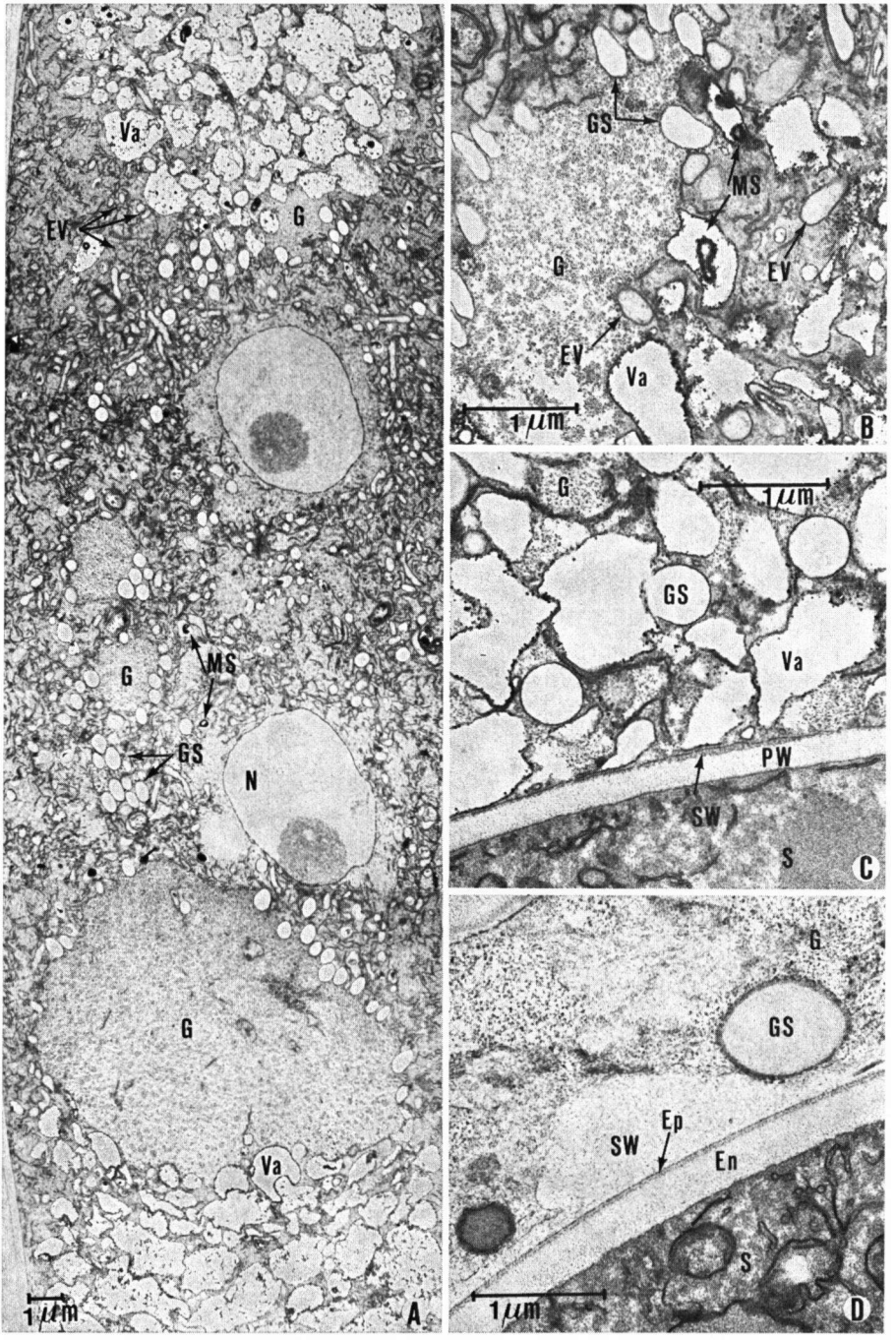
Figs. A–E. *Mycolachnea hemisphaerica*, ascospore development, fixed in 1% $KMnO_4$ and 1% OsO_4: Fig. A. secondary wall formation and development of epispore and endospore, stained with uranyl acetate and lead citrate, ×18,200; Fig. B. further states in condensation of secondary wall material and in differentiation of primary wall, stained with uranyl acetate and lead citrate; ×23,100; Fig. C. id. ×46,200; Fig. D. id. ×18,200; Fig. E. advanced state in development of ornamentation, stained with uranyl acetate and lead citrate, ×14,900.

Figs. F–H. *Octospora musci-muralis*, ascospore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. F. secondary wall formation and development of epispore and endospore, stained with uranyl acetate and lead citrate, ×29,700; Fig. G. id. detail of epispore and endospore, ×46,200; Fig. H. id. advanced state in ascospore development, stained with lead citrate.

PLATE 12

Figs. A–D. *Sarcoscypha coccinea*, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. ascoplasm, upper part of ascus before meiosis and mitoses, stained with uranyl acetate and lead citrate, ×8,100; Fig. B. ascospore development, secondary wall formation at one of two poles of ascospore, stained with uranyl acetate and lead citrate, × 12,600; Fig. C. advanced state in ascospore development, with epispore and endospore and further differentiation in endospore at one of two poles of ascospore, ×29,700; Fig. D. id. along ascospore, stained with lead citrate.

Figs. E–G. *Desmazierella acicola*, ascospore development: Fig. E. beginning of secondary wall formation fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×23,100; Fig. F. id. advanced state in ascospore development, with epispore and endospore, stained with lead citrate; Fig. G. advancea state in ascospore development, with condensation of secondary wall material, fixed in 3.25% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700.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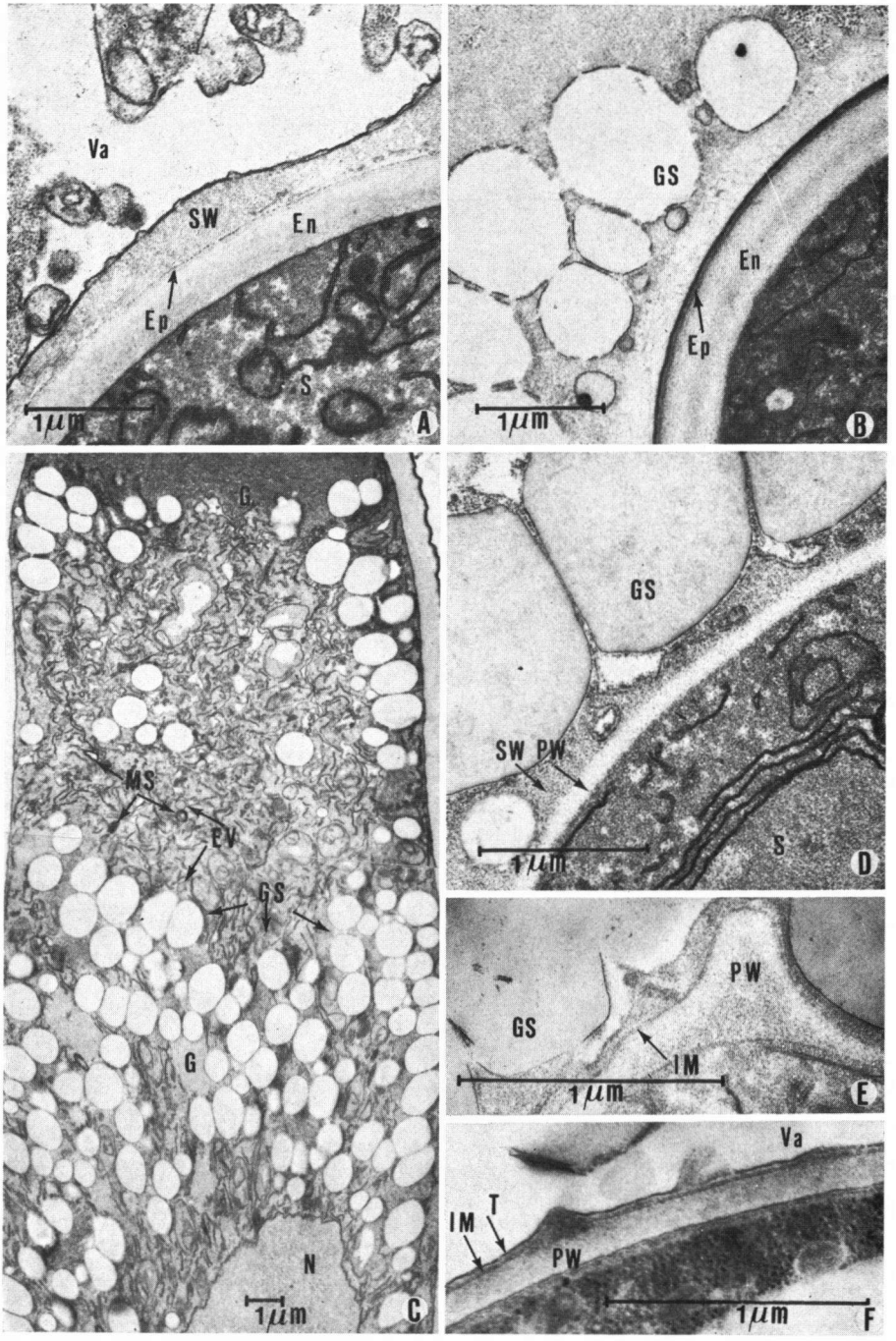
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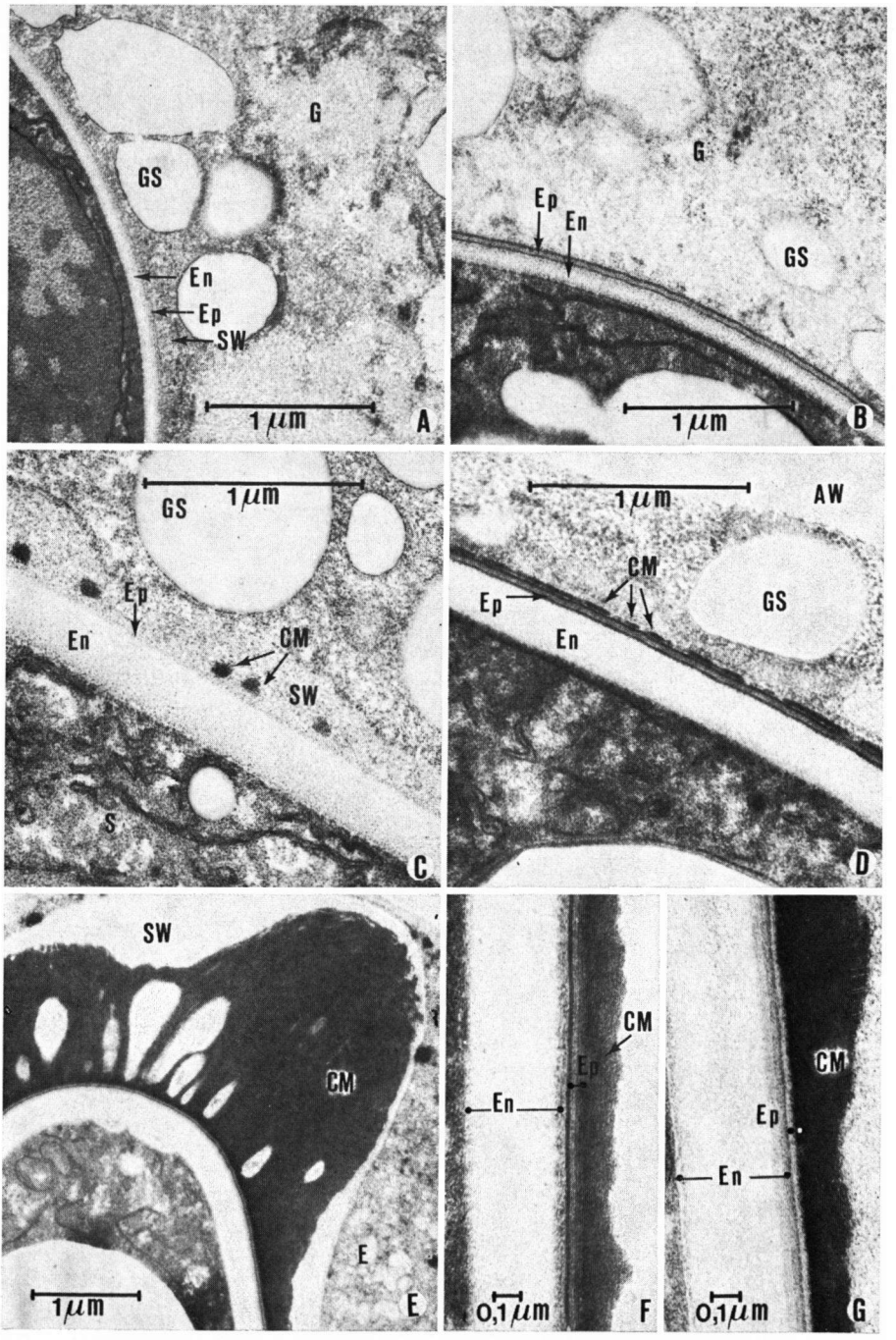
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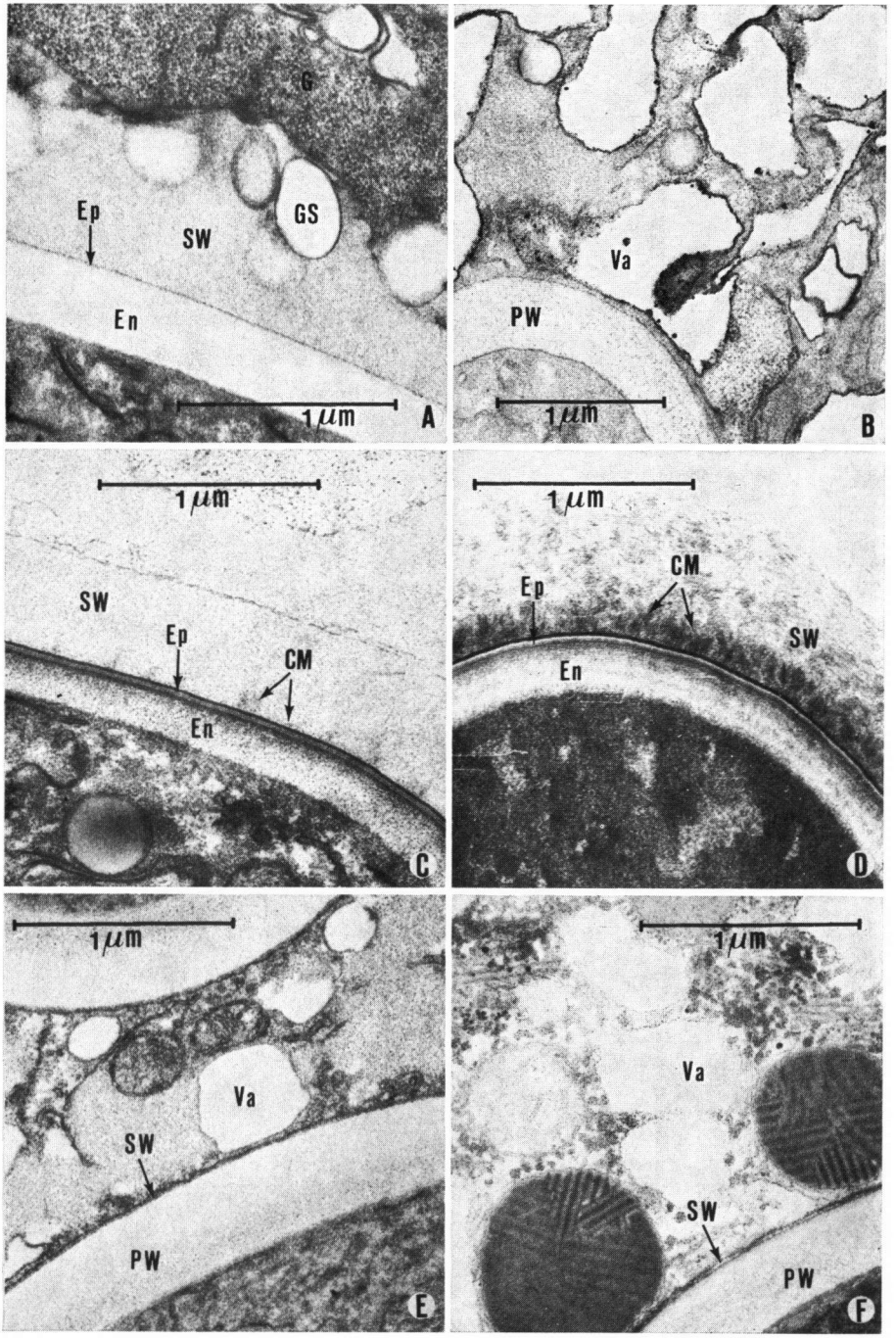
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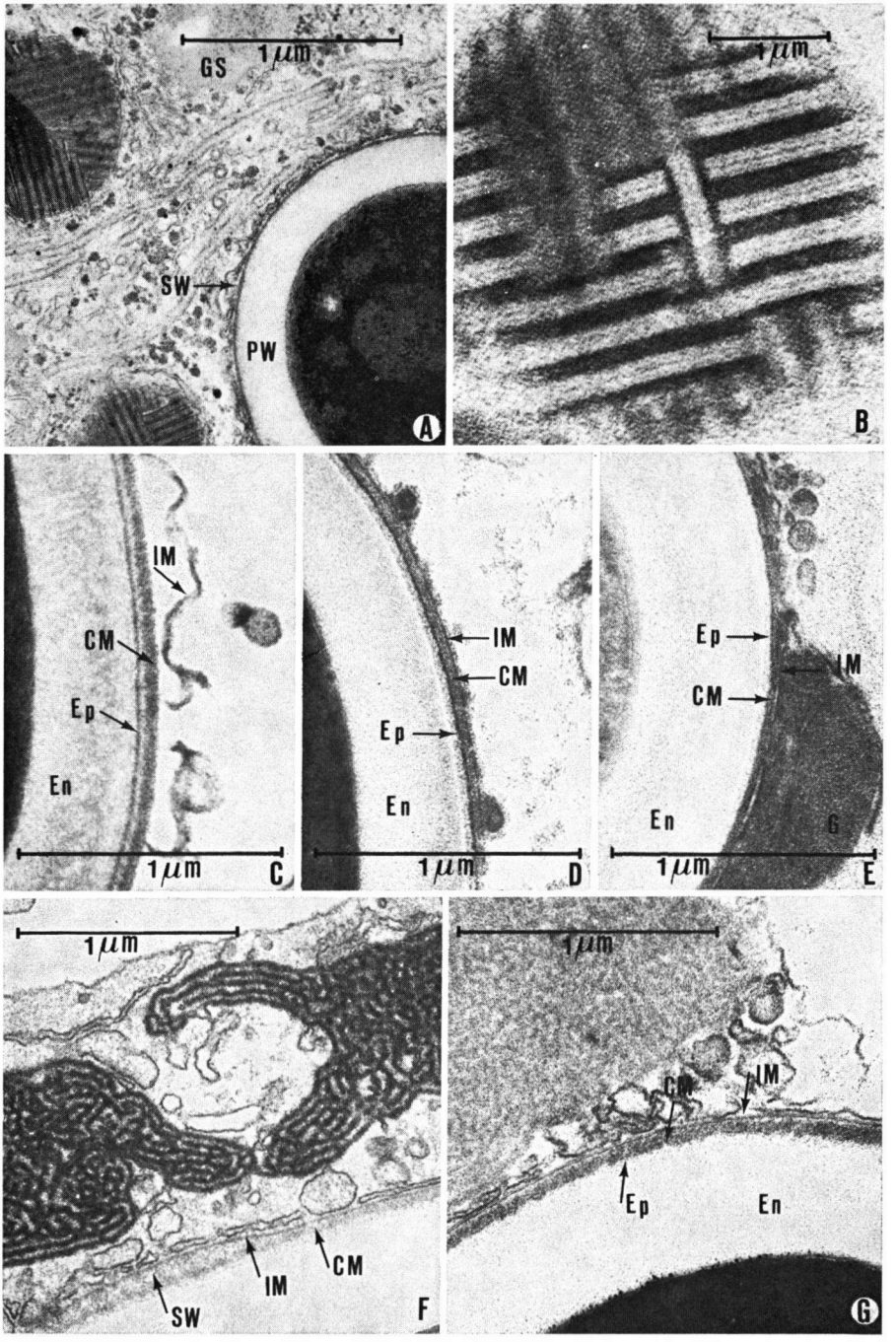
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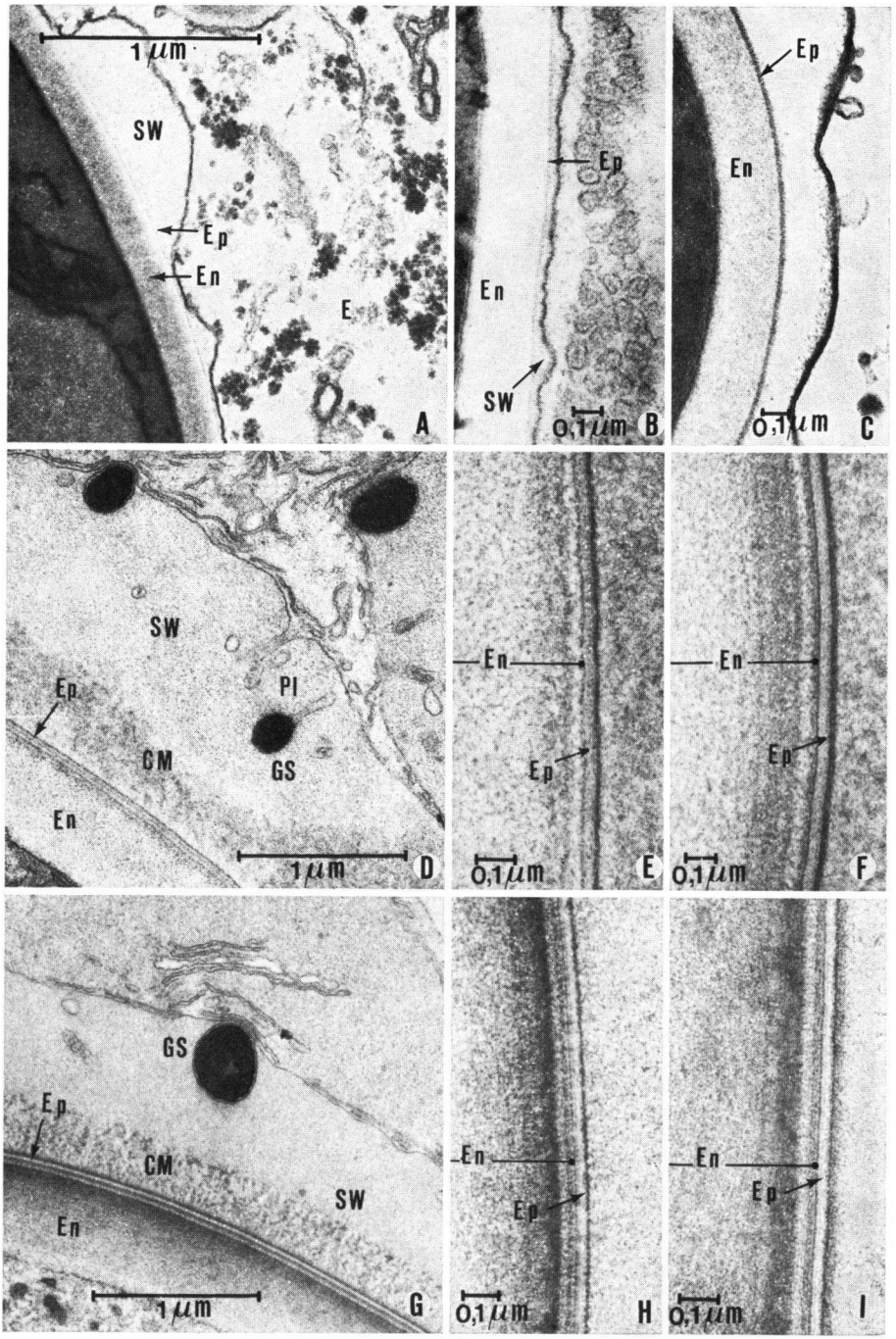
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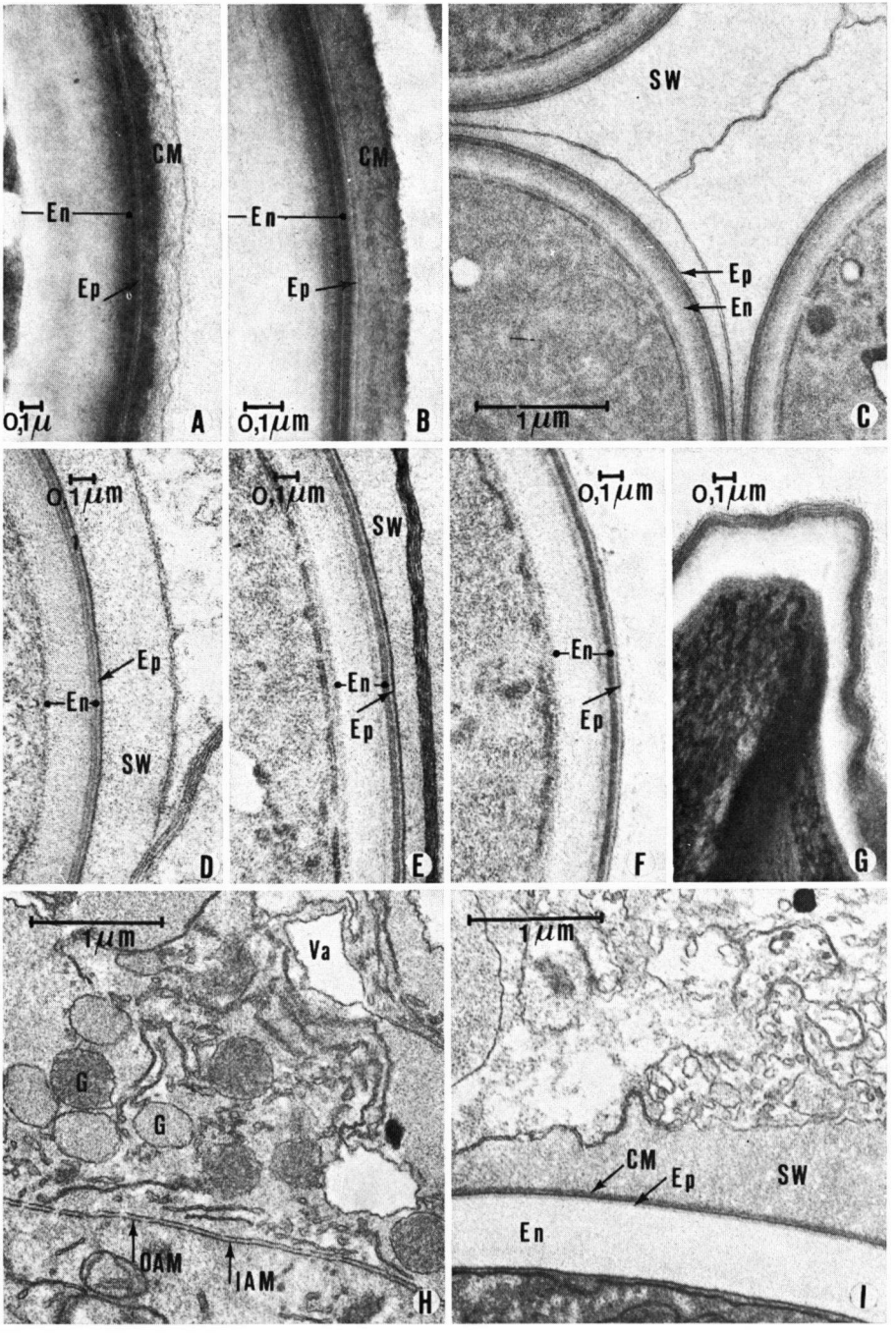
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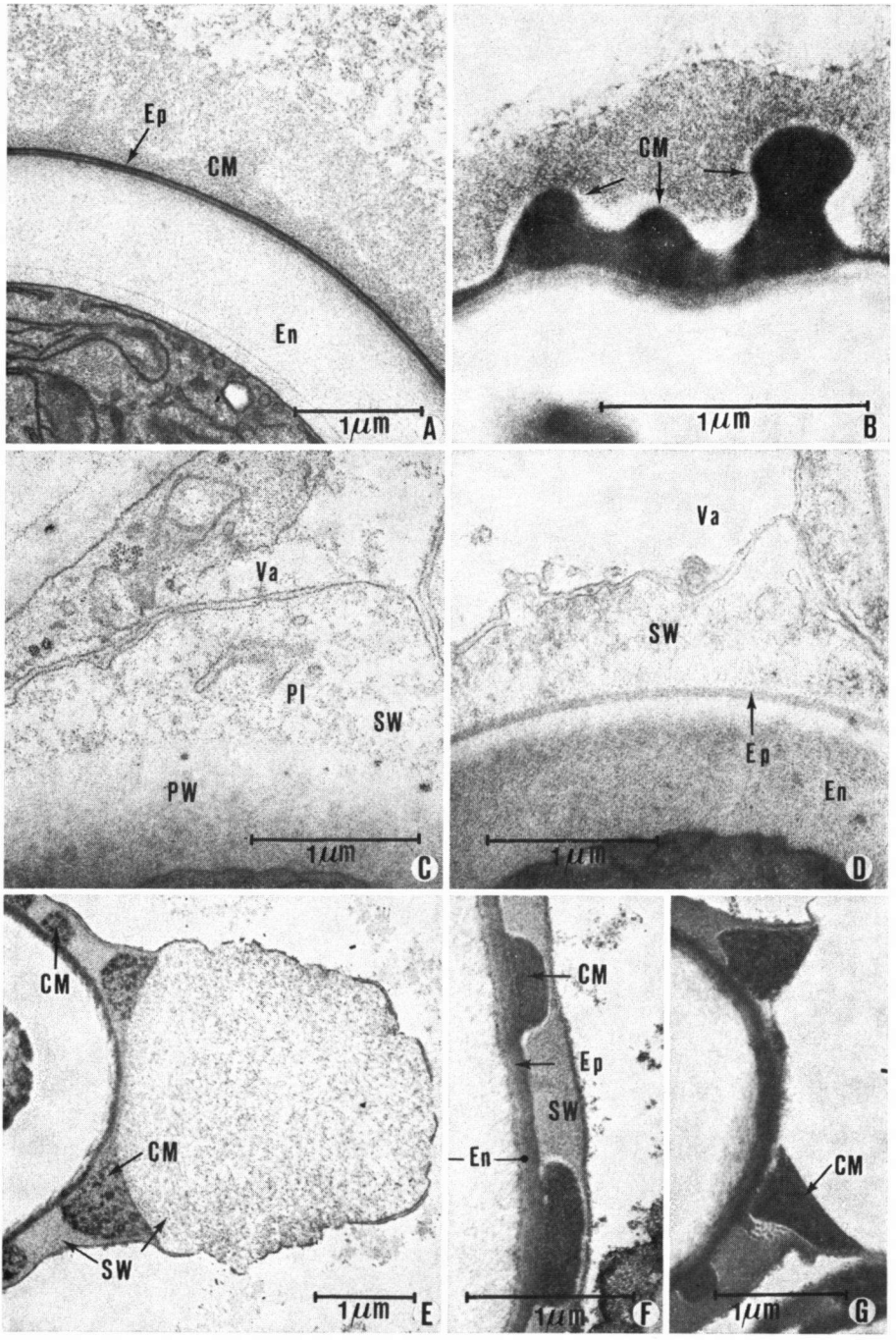
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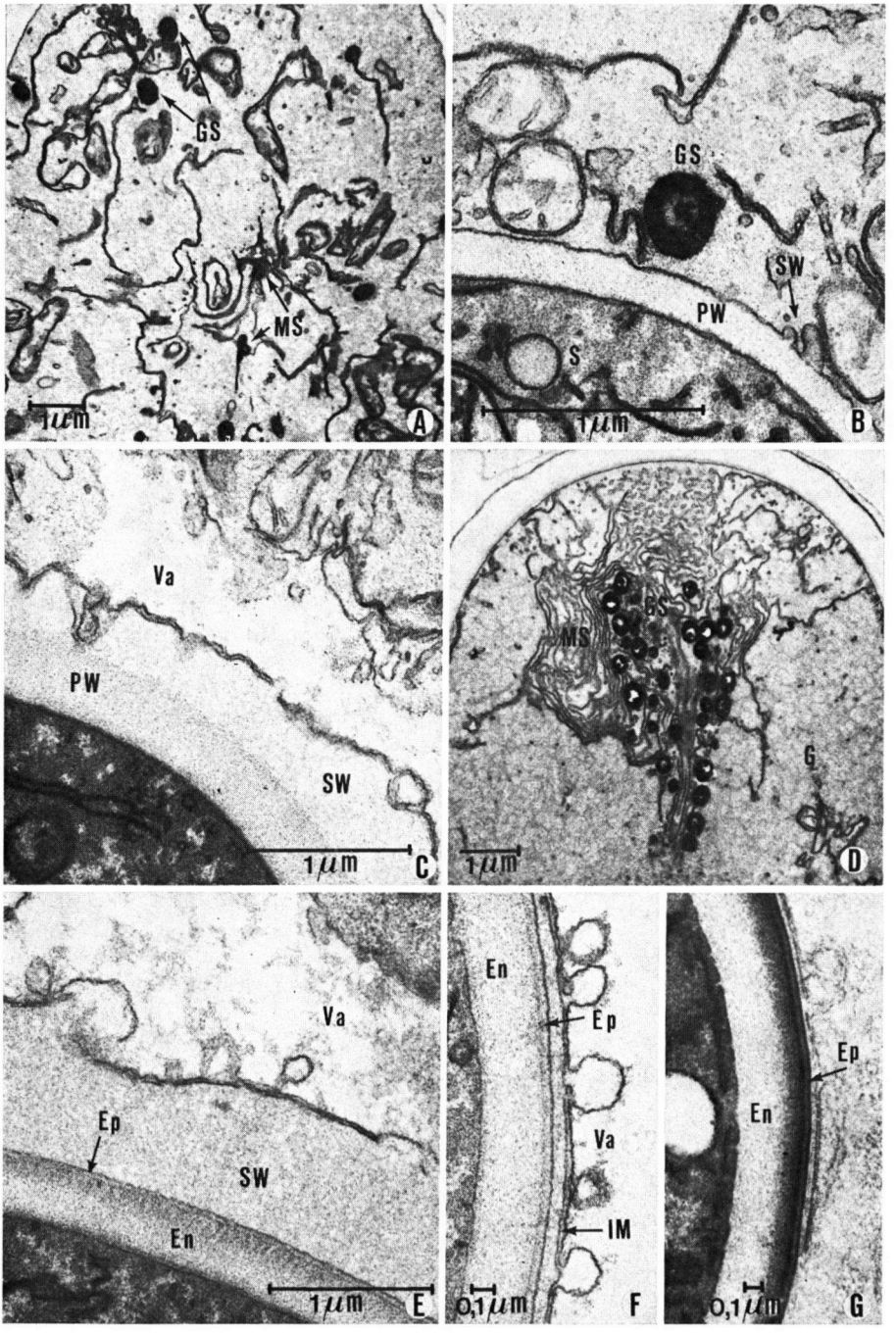
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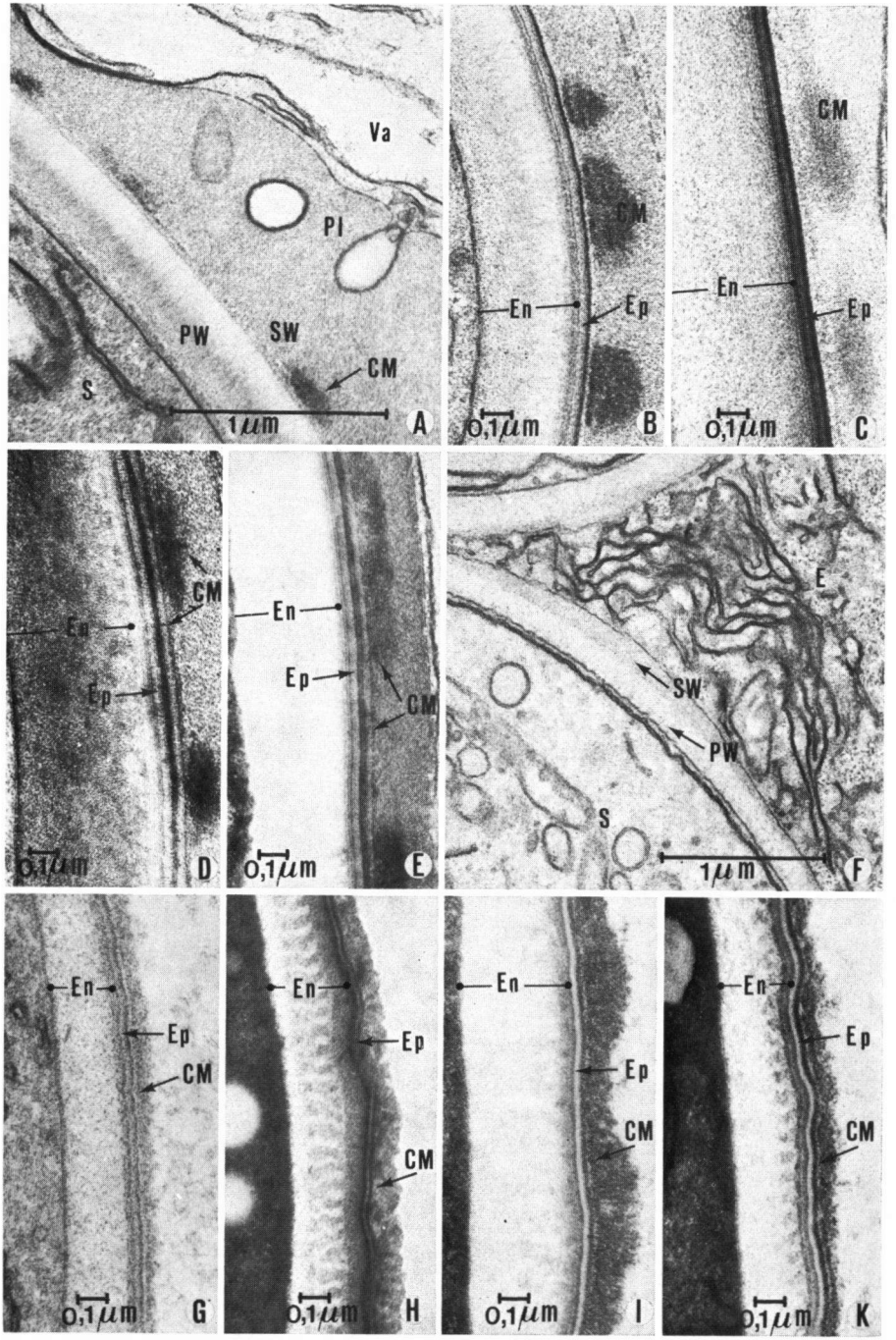
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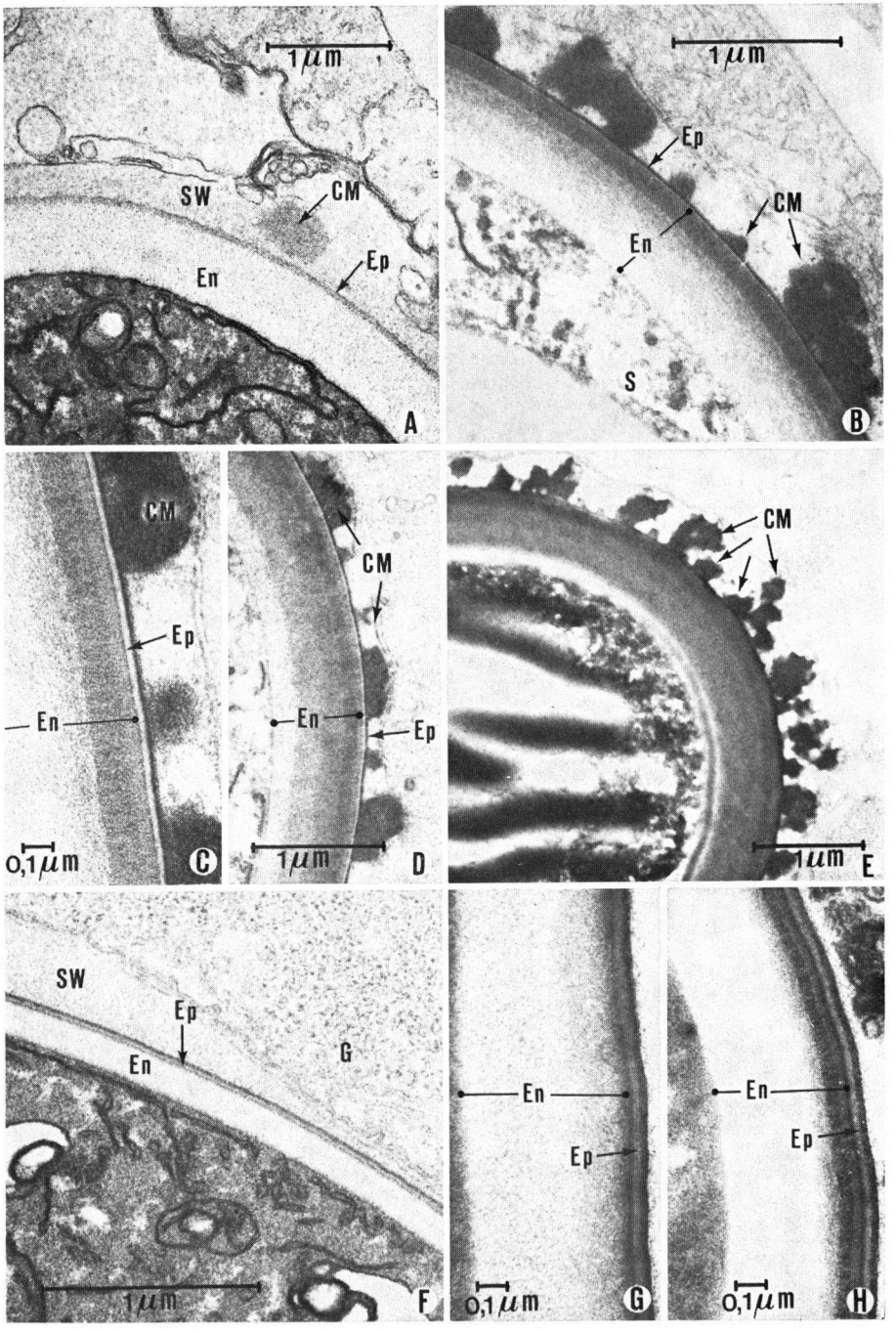
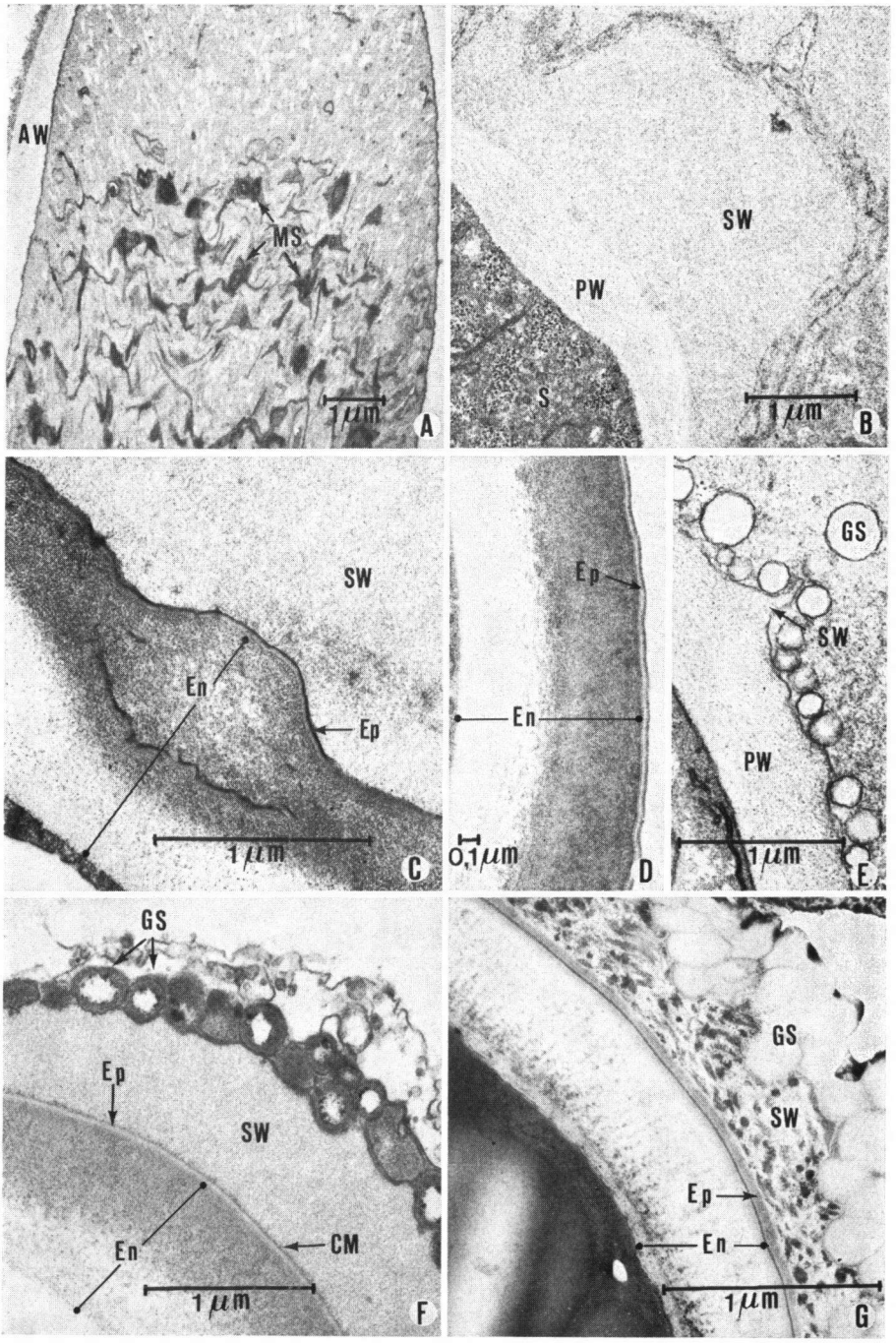

1 µm
SW
CM
Ep
En
A
1 µm
Ep
CM
En
S
B
CM
Ep
En
0,1 µm
C
CM
En
Ep
1 µm
D
CM
1 µm
E
SW
Ep
En
G
1 µm
F
En
Ep
0,1 µm
G
En
Ep
0,1 µm
H


AW
MS
1 µm
A
SW
PW
S
1 µm
B
SW
En
Ep
1 µm
C
Ep
En
0,1 µm
D
GS
SW
PW
1 µm
E
GS
Ep
SW
En
CM
1 µm
F
GS
SW
Ep
En
1 µm
G