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

Otideaceae and Pezizaceae

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(With Plates 34–45)

The development of wall layers and ornamentation of ascospores is studied with the electron microscope in members of the Otideaceae and Pezizaceae. Primary wall, endospore, and epispore develop in the same way as in *Ascodesmis* and the Pyronemataceae; the development of the secondary wall and the formation of the patterns of ornamentation resemble that in the Pyronemataceae. Special attention is paid to specialized plasmic structures.

INTRODUCTION

In earlier papers (Merkus, 1973, 1974) I reported my electron microscopy of the ultrastructure of the ascospore wall in Pezizales and discussed the results on *Ascodesmis* and the Pyronemataceae sensu Eckblad (1968).

The present paper represents a continuation of this study and gives the results in species belonging to the Otideaceae and Pezizaceae, which are practically identical with the Otideae and Aleurieae of the Aleuriaceae of Le Gal (1947: 284).

REVIEW OF EARLIER WORK

Like in my paper on the Pyronemataceae (Merkus, 1974), a brief review is given of Le Gal's light microscopy of the ornamentation patterns of the ascospores of the Pezizaceae (1947). Though this involves duplication of some of the data, it gives a better overall insight and makes it easier to interpret new results. According to the rules of the international code of botanical nomenclature some of the names used by Le Gal (see footnotes) had to be changed.

The species of Pezizaceae studied by Le Gal develop simple spore ornamentation. In all the species a primary wall arises and ornamentation on it is present; the ornamentation consists of callose and pectine formations 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 ornamentation develops. The "pellicule membranaire" is termed "coque interpérisporique" if it is formed at the same time as the ornamentation and is penetrated by the substance of the ornamentation; the "coque interpérisporique" and the substance of the ornamentation both grow into one, the "coque interpérisporique" also consisting of callose and pectine. The "pellicule membranaire" is termed "tunique externe de l'assise" if it is formed before ornamentation develops and is not penetrated by the substance of the ornamentation.

Pulparia persoonii (Crouan) Korf & *al.* apud Korf[1] develops simple ornamentation that is formed between the primary wall and its covering layers.

Peziza succosa Berk.,[2] *P. badia* Pers. per Mérat,[3] *P. echinospora* P. Karst.,[4] *P. trachycarpa* Curr.,[5] *P. apiculata* Cooke,[6] and *P. reperta* (Boud.) Moser[7] develop simple ornamentation, the substance of which penetrates the covering layers of the primary wall and is deposited on the "coque interpérisporique". During the development of the ornamentation a "périspore" is present on the outside of the ascospores. In the first four species the "périspore" disappears in a later stage; in the other two species it remains.

Le Gal does not give a description of the ornamentation patterns of the ascospores of the Otideaceae.

Though the ascoplasm is not the main subject of the present study special attention is paid to it. It reveals the development of globular structures, like oil bodies and other specialized globules of a still unknown substance; these were also described by Guilliermond (1904, 1910, 1920), the latter as "corpuscules métachromatiques". The development of the structures and their relation to ascospore development is discussed.

MATERIALS AND METHODS

The material of the species in the present study was collected in the Netherlands, in France, and in Germany; the following list gives some data about the specimens and their localities: *Otidea alutacea* (Pers. per S. F. Gray) Mass. – *van Brummelen 73-501*, on needles of *Picea*, "forêt de Liciat", Oyonnax, Ain, France, 7.X.1973 (L); — *Piepenbroek 837*, on soil under *Quercus*, 't Joppe, Gorssel, Gelderland, The Netherlands, 12.X.1974 (L); *O. bufonia* (Pers.) Boud. — *van Brummelen 4073*, on the ground under *Betula*, Schoorl, North Holland, The Netherlands, 3.IX.1973 (L); —

1 *Plicaria persoonii* (Crouan) Boud.; syn. *Marcelleina persoonii* (Crouan) Brumm.
2 *Galactinia succosa* (Berk.) Sacc.
3 *Galactinia badia* (Pers. per Mérat) Boud.
4 *Aleuria umbrina* (Boud. apud Cooke) Gill.; not *Peziza umbrina* Pers.
5 *Plicaria trachycarpa* (Curr.) Boud.
6 *Aleuria apiculata* (Cooke) Boud.
7 *Aleuria reperta* (Boud.) Boud.

Piepenbroek 802, on soil, among grasses under Quercus, 't Joppe, Gorssel, Gelderland, The Netherlands, 28.VII.1974 (L); *O. onotica* (Pers. per S. F. Gray) Fckl. — *van Brummelen 4638*, on the ground under oaks, Koningshof, Overveen, Bloemendaal, North Holland, The Netherlands, 2.XI.1974 (L); *Peziza ammophila* Dur. & Lév. apud Dur. — collected during a field trip of the Dutch Mycological Society, sandy dunes, Hollumerduinen, Ameland, Friesland, The Netherlands, 27.X.1973 (L); *P. ampliata* Pers. per Pers. — *van Brummelen 4074*, on dead culms of *Phragmites*, Nederhorst den Berg, North Holland, The Netherlands, 12.V.1973 (L); *P. badia* Pers. per Mérat — *Piepenbroek 806*, on sandy soil, near estate "Dorth", Gorssel, Gelderland, The Netherlands, 28.VII.1974 (L); — *Piepenbroek 811*, on sandy soil, estate "Ampsen", Lochem, Gelderland, The Netherlands, 10.VIII.1974 (L); *P. badiofusca* (Boud.) Dennis — *Piepenbroek 746*, on clay soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 23.VI.1974 (L); *P. emileia* Cooke — *Piepenbroek 556*, on burnt soil, "de Bannink", Colmschate, Overijsel, The Netherlands, 3.VI.1973 (L); *P. michelii* (Boud.) Dennis — *van Brummelen 4500*, on sandy soil, Waterdijk, Diepenveen, Overijsel, The Netherlands, 15.VII.1974 (L); *P. petersii* Berk. — *Siteur*, on burnt soil, Eindhoven, North Brabant, The Netherlands, 21.VII. 1974 (L); *P. plebeia* (Le Gal) Nannf. in Lundell & Nannfeldt — *Piepenbroek 773*, on sandy soil, Epserbos, Epse, Gelderland, The Netherlands, 21.VII.1974 (L); *P. praetervisa* Bres. — *van Brummelen 4072*, on burnt soil, 't Joppe, Gorssel, Gelderland, The Netherlands, 6.VIII.1973 (L); — *Piepenbroek 794*, on burnt soil, "Klein Noordijk", Wilp, Voorst, Gelderland, The Netherlands, 27.VII.1974 (L); *P. succosa* Berk. — *van Brummelen 4511*, on soil under *Salix*, Hengforder Waarden between Olst and Wijhe, Overijsel, The Netherlands, 15.VII.1974 (L); *P. succosella* Le Gal & Romagn. — *van Brummelen & Piepenbroek 4510*, on soil under *Salix*, Waterdijk, Diepenveen, Overijsel, The Netherlands, 15.VII.1974 (L); *P. trachycarpa* Curr. — *Piepenbroek 807*, on soil among mosses, near estate "Dorth", Gorssel, Gelderland, The Netherlands, 28.VII.1974 (L); *P. vesiculosa* Bull. per St-Am. — *van Brummelen 4080*, on soil in hot-house, Kortenhoef, North Holland, The Netherlands, 28.I.1974 (L); *Pulparia persoonii* Korf & *al.* apud Korf — *Piepenbroek 575b*, on loamy soil, Duursche Waarden near Fortmond, Overijsel, The Netherlands, 19.VIII.1973 (L); — *Piepenbroek 738*, on loamy soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 23.VI.1974 (L); *van Brummelen 4515*, on loamy soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 15.VII.1974 (L); *Pustularia cupularis* (L. per Fr.) Fckl. — *Piepenbroek 772*, on sandy soil, Epserbos, Epse, Gelderland, The Netherlands, 21.VII.1974 (L); *Sowerbyella radiculata* (Sow. per Fr.) Nannf. — *Haas*, under *Picea*, Kreis Heidenheim, Schwäbisch Alb, Germany, 4.X.1973 (L); — from exposition, Oyonnax, Ain, France, 22.X.1973 (L).

After collection from their substratum in the field, the apothecia were placed in the fixative. Further treatment of the apothecia followed much the same procedure as described in former studies but a few additional remarks are called for.

In addition to the former percentages used, 1% $KMnO_4$ or 1% glutaraldehyde was applied as primary fixative before postfixation with OsO_4 to improve results.

In order to solve the problem of inadequate impregnation of the embedding medium in the relatively hard material, in some cases the usual Epon embedding medium according to Luft (1961) was replaced by a low-viscosity embedding medium according to Spurr (1961), with the additive dibutyl phtalate (Clemençon, 1973). The material was transferred to the Spurr embedding medium via an ethanol series, 100% ethanol, 100% acetone, and mixtures of acetone and the Spurr embedding medium; polymerization lasted 12 hours at 60 °C.

The components of the Spurr embedding medium were used at a rate of 10.0 g ERL–4206, 6.0 g D.E.R. 736, 26.0 g nonenylsuccinic anhydride, 0.4 g dimethylaminoethanol and 0.8 g dibutyl phtalate.

All sections were cut with a diamond knife.

OBSERVATIONS

The species studied so far all showed practically the same ultrastructure and a general description could be given.

A review of the present data reveals that the ultrastructure of species of the Otideaceae and Pezizaceae answers to this description. This holds particularly for the younger stages of development; the delimitation of ascospores in the ascoplasm by two delimiting unit membranes, the form and structure of organels and their distribution in epiplasm and sporoplasm, and the development of the characteristic appearance of the epiplasm and sporoplasm accord completely.

The occurrence of globular structures in the ascoplasm distinguishes the ultrastructures of a number of species. Though specific details of these globular structures will be given farther on for each species separately, some general observations can be made about them first. After use of the glutaraldehyde-OsO_4-fixative all of them are electron-transparent and look alike. After use of the permanganate-OsO_4-fixative they can be ranked as oil bodies that are more or less electron-transparent and homogeneous; or else as other specialized globules of a still unknown substance, which are electron-dense or more moderately electron-dense and have a slightly granular structure (often with an unfixed center). In future this description is followed. The globular structures with moderate electron density mostly occur with both other types and it is not impossible that they represent intermediate forms; they are not found frequently.

It is not impossible that all three types of globular structures are formed in a very early stage of ascus development; at any rate for a number of species it has been found that they are present before meiosis and mitosis take place; the sites of their first formation then seem to be the zones of ascoplasm above and beneath the nucleus. Their formation probably continues during the further stages of ascus development since they abound particularly after meiosis and mitosis, during the delimitation of the ascospores; in that case the zones of ascoplasm between the nuclei are also involved in their formation. After spore delimitation they are found in both the young ascospores and the remaining epiplasm.

Though in some species the organization of the ascoplasmic zones above and beneath the nucleus before meiosis and mitosis is somewhat different from that of the zones between the nuclei after meiosis and mitosis, all of them are essentially similar in structure in all species; this changes during ascus development in the same way.

The globular structures and the ascoplasmic zones in which they arise largely predominate in the ascoplasm and, by the time spores have developed, also in the epiplasm.

In a good many species the ascoplasm also forms a large amount of the same granular or more flocky material as was found in the Pyronemataceae. There it was supposed that both types of material are related; the granular material was compared with the glycogen particles in *Ascodesmis microscopica* and *A. nigricans*. The results of the present study seem to confirm this, which has led to the use of the term glycogen for both the granular and the more flocky material. In very young stages of development glycogen is found scattered in the ascoplasm. When the asci develop further and the ascospores arise it abounds in clusters just inside the ascoplasmalemma and also appears in large quantities around the spores; even more than in the Pyronemataceae, it may finally form the ground mass of the epiplasm. Moreover it may form a large plug just beneath the ascus top, and may fill the basal part of the ascus completely. It is also present in small quantities in the sporoplasm.

The types of fixative applied determine the appearance of the glycogen. Permanganate-OsO_4 seems to preserve it adequately and gives it a fairly electron-dense, granular or more flocky structure. Glutaraldehyde-OsO_4 (or OsO_4 only; Merkus, 1973) gives poorer overall results, as was also found by Schrantz (1968); it makes the glycogen less electron-dense (to electron-transparent) and it seems to change the location of the glycogen, sometimes not fixing it at all or else concentrating it at a few places, thereby causing large vacuoles in the epiplasm.

The development of the spore walls is much the same as in *Ascodesmis* and the Pyronemataceae; between the two delimiting unit membranes wall material successively forming the primary and secondary walls is deposited; at first the primary wall is homogeneous in appearance but in a later stage of development it seems to differentiate into an outer epispore and an inner endospore; the secondary wall arises between the primary wall and the outer delimiting membrane that is now called investing membrane; the inner delimiting membrane becomes the sporoplasmalemma. Primary wall, epispore, and endospore show exactly the same development and ultrastructure as those in *Ascodesmis* and the Pyronemataceae. For a general description compare my earlier studies. The development and the ultrastructure of the secondary wall have much in common with those in the Pyronemataceae; they will be described in detail for each species separately.

As was found in *Ascodesmis* and the Pyronemataceae the epiplasm gradually loses the organels originally present; apart from the formation of glycogen, large vacuoles that often have flocky contents develop in the epiplasm of many species. When the spores mature the epiplasm disintegrates almost completely; only a thin layer of original plasm just inside the ascoplasmalemma may remain. In the sporoplasm all

organels remain present; apart from a general increase in electron density its total appearance changes in many species through the development of large oil drops.

OTIDEACEAE

OTIDEA ALUTACEA, O. BUFONIA, AND O. ONOTICA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. Of the three species *Otidea bufonia* showed the best fixation; the description of the younger stages of development applies only to this species; the mature spores at any rate do not differ.

Young asci before meiosis and mitosis show a normal ascoplasm without any special unusual structures; clusters of glycogen have already been formed. In the same stage however changes are found in the endoplasmic reticulum; at some places it widens slightly, becomes electron-transparent internally and may form circular structures (Pl. 34A); it may also widen further and form small vacuoles with vesicles of varying sizes (Pl.34B); finally a local increase in electron density may be found (Pl. 34A, B); a relation with the electron dense globules, evidently arising in this stage, is not impossible (Pl. 34A, B). Whether there is any connection between these changes and the presence of glycogen is not clear.

Following stages of development show further structural changes in the ascoplasm and formation of the spores. The epiplasm forms increasing amounts of glycogen, and vacuoles with fairly electron-dense flocky contents; the organels disappear. The sporoplasm increases in electron density and develops oil drops. The primary wall (permanganate-OsO_4 350–450 nm, glutaraldehyde-OsO_4 250 nm thick), the epispore (25 nm thick), and the endospore (permanganate-OsO_4 250 nm, glutaraldehyde-OsO_4 200 nm thick) are normal in appearance; apart from the normal striation in the epispore the endospore shows some internal differentiation (Pl. 34C, D, G). The development of the secondary wall proceeds regularly; it consists of homogeneous or more flocky material and varies from 100–1000 nm in thickness; inclusions from the epiplasm are sometimes present (Pl. 34C, D).

The permanganate-OsO_4-fixed material could not be observed in older stages. In all three species it has been found in the glutaraldehyde-OsO_4-fixed material that part of the contents of the secondary wall concentrates on the epispore. Here it forms two succeeding smooth layers that each have a thickness of about 70 nm; though connected, they remain clearly distinguishable (Pl. 34F, G). The structures that have been found in the secondary wall of *Otidea onotica* and, to some extent, also in that of *O. alutacea* possibly represent transitional forms in the development of the two layers (Pl. 34E).

By the time the spores have matured the epiplasm and the rest of the secondary wall have disappeared; the condensed layers on the epispore seem to remain.

PUSTULARIA CUPULARIS

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4.

The youngest stages present show a primary wall (permanganate-OsO_4 100–200 nm, glutaraldehyde-OsO_4 50–100 nm thick) that has a thin inner layer of an obviously loose structure and a homogeneous outer layer which appears in the slides as an uninterrupted, circular band along the spores. Separation of the investing membrane has occurred along the whole primary wall. The intermediate space, which varies in thickness from 100–1500 nm, has been filled up with fairly electron-dense flocky material forming the secondary wall; it also contains inclusions like vesicular structures that must have been derived from the epiplasm or from the investing membrane, which in both fixatives runs irregularly. Epiplasm and sporoplasm are normal in appearance (Pl. 35A).

From this stage of development on, the epiplasm disintegrates slowly; it appears loose and flocky, like the contents of the secondary wall; the organels in it disappear. Little glycogen seems to be formed. At the same time the sporoplasm increases greatly in electron density and develops oil drops. In the glutaraldehyde-OsO_4-fixed material some concentration of secondary wall material is found near the primary wall or distributed in the secondary wall (Pl. 35B).

In succeeding stages an epispore and an endospore evolve; the epispore is 30 nm thick and has normal striation; as in *Sepultaria*, the endospore (permanganate-OsO_4 150–250 nm, glutaraldehyde-OsO_4 100–150 nm thick) may show a broad electron-dense outer part and a thin and sometimes interrupted fairly electron-dense layer in the innermore parts (Pl. 35C, D).

In the mature asci the epiplasm has dissolved completely and almost disappeared, leaving only a small zone at the inner side of the ascus wall. The secondary wall has also been broken down; the remnants of the investing membrane remain the longest. The mature spores are smooth (Pl. 35D).

SOWERBYELLA RADICULATA

Fixative: glutaraldehyde-OsO_4. Though from the pictures available nothing can be said about the development of the ascoplasm and, in later stages, of the epiplasm, a few remarks can still be made about the spores.

The first stages of development show that the structures of the primary wall (250–400 nm thick) and the sporoplasm do not differ from the general description; the sporoplasm develops some oil drops. In following stages an epispore (25 nm thick) and an endospore (150–200 nm thick) have differentiated and the secondary wall has been formed between the investing membrane and the primary wall.

The secondary wall material has a granular electron-dense structure, though it is sometimes found to be more homogeneous. During further spore development the secondary wall material concentrates on the primary wall, where ornamentation crops up. Perpendicular to the primary wall an internal striation of the elements

of ornamentation is evident in the younger stages before their electron density has increased that far (Pl. 35E). This may agree with fibrous structures that have been found in the secondary wall (Pl. 35F).

The mature spores have tapering or more rounded spines about 50–250 nm high, at the ends of the spores up to 700 nm high. The spines occur at regular intervals and are connected by a smooth layer of about 10 nm thick; growing together is also to be found (Pl. 35G).

PEZIZACEAE

PEZIZA AMMOPHILA AND P. PRAETERVISA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4.

In these two species the ascoplasmic zones above and beneath the nucleus, in which the globular structures start to arise, are more or less partitioned in an early stage of development; the central ascoplasm is subdivided into a kind of membrane enveloped plasmic compartments between which glycogen is found, and the more superficial ascoplasm contains much glycogen gathered in clusters (Pl. 36A).

The organels in the compartments are: elements of the endoplasmic reticulum, which appear as tubular or vesicular structures; larger vesicles that usually show poor electron density and may be derived from the endoplasmic reticulum; and membranous structures which are globular and seem to consist of a moderately electron-dense center that might be glycogen and that is wrapped up in a varying number of membranes, possibly invaginating in the center. At many places the membranes enveloping the compartments are diffuse in appearance. The vesicles with poor electron density are also found in the superficial ascoplasm.

The globular structures, which are electron-dense in these species, are particularly evident in the glycogen of the superficial ascoplasm but are also found between the compartments in the central ascoplasm (Pl. 36A).

In later stages of development, in which meiosis and mitosis take place and spore delimitation starts (Pl. 37A), the compartments enlarge considerably and grow into large vacuoles with a flocky basic substance (Pl. 36B). Simultaneously the ascoplasm between the nuclei becomes essentially similar in appearance, whereby the glycogen and the electron-dense globular structures are conspicuous. Large vacuoles however are not found here; the elements of the endoplasmic reticulum and the membranous structures cluster in small vacuoles (Pl. 36C).

Once the spores are formed the development of the plasm proceeds in the epiplasm. Here the amount of glycogen proceeds to increase; the glycogen also fills the basal part of the ascus completely.

Both the primary wall and the sporoplasm are regular in appearance. In *Peziza praetervisa* the primary wall (permanganate-OsO_4 400–500 nm, glutaraldehyde-OsO_4 200–300 nm thick) is electron-transparent but sometimes shows a thin and fairly electron-dense intermediate layer. In *Peziza ammophila* the primary wall

(permanganate-OsO_4 400–600 nm, glutaraldehyde-OsO_4 300–500 nm thick) is completely homogeneous (Pl. 37C). In the sporoplasm both the electron-dense globular structures and an abundant endoplasmic reticulum are present; the electron density has increased (Pl. 37B, C, D).

Separation of the investing membrane occurs along the whole primary wall, in some places becoming fairly conspicuous; the investing membrane runs straight. The secondary wall that is formed between the primary wall and the investing membrane has fairly electron-dense and homogeneous contents (Pl. 37C, D).

In *Peziza praetervisa* the amount of glycogen increases in this stage so much that only a small strip of epiplasmic structures remains between the glycogen and the secondary wall. In *Peziza ammophila* large vacuoles with flocky electron-dense contents develop around the spores; but for the most part these vacuoles disappear again and are replaced by large clusters of glycogen. Once the secondary wall has reached its ultimate thickness (locally 200 nm in *Peziza ammophila* and 500 nm in *P. praetervisa*) all organels in the epiplasm will have disappeared; the epiplasm then consists only of glycogen, in which the electron-dense globular structures are no longer conspicuous; they shrivel and finally disappear. In the sporoplasm two oil drops develop; the electron-dense globular structures remain.

During the development of the secondary wall an epispore and an endospore evolve; the epispore (*Peziza praetervisa*: 35–40 nm thick; *P. ammophila*: 35–50 nm thick) shows a normal pattern of differentiation; the endospore (*Peziza praetervisa*: permanganate-OsO_4 300–400 nm, glutaraldehyde-OsO_4 150–250 nm thick; *P. ammophila*: permanganate-OsO_4 500–1500 nm, glutaraldehyde-OsO_4 350–500 nm thick) sometimes has a thin layer with increased electron density in the innermost part (Pl. 37B, D, E, F).

When the spores mature the contents of the secondary wall concentrate on the epispore in both species (Pl. 37D, E). In *Peziza ammophila* this results in a smooth electron-dense layer about 70 nm thick, in which internal differentiation can be distinguished; it shows subtle striation that is surrounded by a heavier layer (Pl. 37F). In *Peziza praetervisa* ornamentation is formed consisting of a fairly smooth electron-dense layer about 40 nm thick, at regular intervals punctuated by small rounded warts approximately 300 nm high; the elements of ornamentation do not show any internal structure (Pl. 37B). In both species the epiplasm and the rest of the secondary wall disappear.

PEZIZA VESICULOSA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4.

The appearance of the ascoplasm of this species agrees fairly well with that of the two preceding species; the structures are similar but not so abundant as in *Peziza ammophila* and *P. praetervisa*; the ascoplasm above and beneath the nucleus shows subdivision into compartments in the initial stages of development, particularly in the upper part of the ascus (Pl. 38A, B). In this species both the basal part and some

of the superficial part of the ascoplasm consist of glycogen in this stage, the basal part clearly containing the remnants of the original ascoplasm.

After delimitation of the spores, subdivision into plasmic compartments becomes more evident in the epiplasm beneath the lowermost spore. The membranes forming the compartments may show numerous invaginations; they may also separate locally and have larger areas with glycogen; in the glycogen the globular structures are found (Pl. 38C). In contrast to those in *Peziza ammophila* and *P. praetervisa* these develop as fairly electron-transparent structures with somewhat flocky contents (Pl. 38B) and become only in a later stage more electron-dense (Pl. 38C). The epiplasm between the spores develops an essentially corresponding structure, as does that in *Peziza ammophila* and *P. praetervisa.*

In following stages a primary wall (permanganate-OsO_4 500–900 nm thick) is formed (Pl. 39A, B) which seems to differentiate into an epispore (35–50 nm thick) and an endospore (permanganate-OsO_4 500–2000 nm thick) (Pl. 39C, D, E, F); the secondary wall arises between the primary wall and the investing membrane, its local thickness amounting to about 700 nm (Pl. 39B, C). The appearance of the primary wall and, at this stage, also of the secondary wall accords with those in *Peziza ammophila* and *P. praetervisa.* The structures of the epiplasm and the sporoplasm are also similar to those in *P. ammophila* and *P. praetervisa*; in the epiplasm vacuolization and formation of large clusters of glycogen are found; in the sporoplasm no oil drops are formed.

The last stages of development of the spores are the same as in *Peziza ammophila.* The concentration of the secondary wall material on the epispore (Pl. 39C, D) results in a rather smooth electron-dense layer (about 100 nm thick), which shows a similar internal structure, subtle striation surrounded by a heavier layer (Pl. 39E, F); the epiplasm and the rest of the secondary wall disappear.

PEZIZA MICHELII, P. PLEBEIA, P. SUCCOSA, AND P. SUCCOSELLA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. These four species have so much in common that they can be described together.

Young asci before meiosis and mitosis show a rather vague partition of the ascoplasm above and beneath the nucleus into a central part and a more superficial part, the latter consisting mainly of normal ascoplasm; poorly electron-dense endoplasmic vesicles and glycogen are scattered over both parts. The appearance of the central part is practically the same as that of the internuclear ascoplasm in *Peziza ammophila* and *P. praetervisa*; the elements of the endoplasmic reticulum together with numerous membranous structures cluster in small vacuoles. In *Peziza succosa* and *P. succosella* the membranous structures are globular; in *P. michelii* and *P. plebeia* reniform or dumb-bell-shaped structures are also present (Pls. 40A, B, C; 41A, B, C).

Characteristic of the four species is that the numerous globular structures prove to be more or less electron-transparent oil bodies, which are present all over the ascoplasm. Though not always clearly visible in this young stage of development the

oil bodies are embedded in the glycogen; in later stages, when the amount of glycogen increases, this becomes more evident (Pls. 40C; 41B, C). Though young asci were difficult to find it appeared that in *Peziza michelii* and in *P. plebeia* all the structures are present in asci that have just been formed (Pl. 41A).

After delimitation of the spores the epiplasm between the nuclei will have developed similar structures, and the amount of both the oil bodies and the glycogen will have largely increased. The glycogen is found in clusters all over the ascus, particularly in the apical and basal part; in the latter it has completely replaced the epiplasm. The sporoplasm contains numerous oil bodies. In *Peziza michelii* and *P. plebeia* the epiplasm does not change further in a succeeding stage of development; in *P. succosa* and *P. succosella* real vacuoles may arise.

The spores develop normally. The primary wall (permanganate-OsO_4 150–350 nm, glutaraldehyde-OsO_4 100–200 nm thick) does not show any internal differentiation before the endospore (permanganate-OsO_4 150–250 nm, glutaraldehyde-OsO_4 100–200 nm thick) and the epispore (*Peziza michelii* and *P. plebeia*: 45–55 nm thick; *P. succosa* and *P. succosella*: 50–60 nm thick) evolve. The endospore is practically homogeneous in appearance; the epispore shows the ordinary layered structure (Pls. 42; 43). The secondary wall is formed between the investing membrane and the primary wall. It has flocky, fairly electron-dense contents and may vary widely in thickness; the investing membrane runs almost straight (Pls. 42A, B; 43A). In this stage of development the epiplasm slowly disintegrates; just inside the ascus wall it remains the longest. Here the oil bodies become elongated and orientated perpendicular to the ascus wall; they sometimes develop a somewhat flocky content. Invaginations from the epiplasm into the secondary wall have been found with both types of fixative (Pl. 43A, C). The sporoplasm develops large oil drops and a sometimes conspicuous endoplasmic reticulum.

Before the differentiation of the primary wall the contents of the secondary wall concentrate into electron-dense globules, flattened globules or more continuous layers on the primary wall (Pls. 42B; 43C, F). In following stages the secondary wall material gradually concentrates, and warts and ridges connected by a smooth thin layer arise on the primary wall at regular intervals. During further maturation of the spores both the epiplasm and the rest of the secondary wall disappear.

In *Peziza succosa* and *P. succosella*, the elements of ornamentation at first show internal differentiation. In the permanganate-OsO_4-fixed material the basal parts of the elements are fairly electron-dense and maintain a rather loose structure, the condensed material that borders the epispore showing subtle striation all over the spore surface; the upper parts are fairly electron-dense and homogeneous and seem to have been added separately as a kind of cap (Pls. 42C, D; 43B). In the glutaraldehyde-OsO_4-fixed material the loose structure of the basal parts may show striation perpendicular to the spore surface; the upper parts are electron-dense and may be covered by an electron-transparent layer (Pl. 42F, G).

In later stages the internal differences in the elements of ornamentation finally disappear in the permanganate-OsO_4-fixed material and the ornamentation becomes

electron-dense (Pl. 42E). In glutaraldehyde-OsO_4-fixed material of *Peziza succosella* the electron-transparent material remains present and seems to be surrounded by an electron-dense layer (Pl. 42H).

In *Peziza succosa* and *P. succosella* ornamentation is rather similar. In *P. succosa* warts 600–800 nm high, which are sometimes slightly broadened or may have grown into ridges, are found together with smaller warts about 150–300 nm high; in *P. succosella* warts and ridges are connected by a smooth layer about 50 nm thick (Pl. 42E, H).

In *Peziza michelii* and *P. plebeia* the concentration of secondary wall material on the epispore seems to have increased more regularly since separate parts cannot easily be distinguished; only less solid parts seem to remain and form permanent gaps (Pl. 43D, E). In *P. plebeia* ornamentation develops in exactly the same way as in *P. michelii*; glutaraldehyde-OsO_4-fixed material could not be studied in the latest stages of development. The ornamentation consists of rounded warts, which vary in size from 200–700 nm and may have broadened or grown together; the connective layer varies in thickness from 60–100 nm.

PEZIZA BADIA

Fixative: permanganate-OsO_4.

Though this species closely resembles *Peziza michelii*, *P. plebeia*, *P. succosa*, and *P. succosella* in all stages of development, it has a particular amount in common with the first two species mentioned. In an early stage of ascus development differences are found in the fact that the membranous structures are almost never globular, but reniform, dumb-bell-shaped or in other ways irregular (Pl. 44A). In a somewhat later stage large clusters of glycogen take the place of the organels in the epiplasm.

The aspects of the primary wall (300 nm thick), the epispore (45–55 nm thick), and the endospore (150–200 nm thick) agree with the general description, as does the aspect of the secondary wall. Like in *Peziza michelii* and *P. plebeia* the condensation of secondary wall material proceeds fairly regularly; the different parts in the developing elements of ornamentation, clearly present in *P. succosa* and *P. succosella*, are not formed.

The ornamentation is surrounded by an electron-dense layer that is evidently formed by its outermost part; it consists of warts and ridges (100–400 nm high), which may form an incomplete network and which are connected by a smooth layer about 40 nm thick (Pl. 44B).

PEZIZA BADIOFUSCA, P. EMILEIA, AND P. PETERSII

Fixative: permanganate-OsO_4. As an incipient spore formation is present in the youngest stages studied, it could not be concluded what kind of development the ascoplasm undergoes.

The structures of the epispore (*Peziza badiofusca* and *P. petersii*: 60 nm thick; *P. emileia*: 40–50 nm thick) and the endospore (*P. badiofusca*: 200–250 nm thick; *P. emileia*: 500–800 nm thick; *P. petersii*: 250–350 nm thick) do not differ from the general description (Pls. 44C, D, E, F; 45A, B). The endospore sometimes has a thin electron-dense layer in the innermore parts; in *P. petersii* several electron-dense layers may be present (Pl. 44F).

The secondary wall has also developed; it varies largely in thickness along the surface of a single spore; its contents are fairly electron-dense and homogeneous, sometimes slightly flocky (Pl. 44C). The investing membrane runs almost straight. The epiplasmic organels have nearly all disappeared and have been replaced by glycogen; the remainder is found close to the ascus wall. Here they may occur together with the same electron-dense globular structures as those found in other species, which at this place may be elongated and orientated perpendicular to the ascus wall. The sporoplasm has an increased electron density and has developed oil drops; it often shows a rather abundant endoplasmic reticulum.

The development of spore ornamentation resembles that in preceding species. In the first stages of redistribution of the secondary wall material internal differences divide the elements of ornamentation in various parts. In *Peziza badiofusca* the basal parts of the elements are electron-dense and homogeneous, the intermediate parts have a fairly electron-dense and loose structure, and the upper parts are fairly electron-dense and homogeneous (Pl. 45A). In *P. emileia* and *P. petersii* the developing elements of ornamentation are homogeneous in structure, the upper parts being less electron-dense than the lower parts, in which in *P. petersii* subtle striation is sometimes distinguishable (Pl. 44C, D, E, F). In some places in *P. petersii* the internal structure of the elements is loose, which causes permanent "gaps" (Pl. 44E). In later stages all the internal differences disappear and the elements of ornamentation become completely electron-dense in all three species (Pl. 45B).

In the mature spores of *Peziza badiofusca* ornamentation is formed by rounded, isolated warts, 300–700 nm high and spread regularly on the spore surface; they are connected by a rather smooth layer about 50 nm thick in which small warts are sometimes found (Pl. 45B). In *P. emileia* ornamentation is formed by slender, isolated warts (500–800 nm high), which are connected by a smooth layer (about 30 nm thick) and occur at regular intervals (Pl. 44D). In *P. petersii* warts and ridges (300–500 nm high) are also found at regular intervals along the spore surface and are connected by a smooth layer (60–70 nm thick); in the oldest stages of development the tops of the warts may enlarge further and grow out laterally, the warts sometimes fusing (Pl. 44F).

Like in *Peziza badia*, ornamentation in *P. badiofusca* and particularly that in *P. petersii* is surrounded by a marked electron-dense layer (Pls. 44E, F; 45B). In all three species the epiplasm and the rest of the secondary wall have disappeared when the spores are mature.

PEZIZA TRACHYCARPA

Fixative: permanganate-OsO_4. In the youngest stages discernible the development of the primary wall is complete and a secondary wall has already started to form. Therefore, and as a result of relatively poor preservation, no conclusions could be drawn about the first stages of the ascoplasm.

The primary wall is 600–800 nm thick and has a normal aspect; separation of the investing membrane from the primary wall has made formation of the secondary wall possible; this has homogeneous and fairly electron-dense contents. In the remnants of the epiplasm glycogen is found and the same oil bodies occur as in several other species. The sporoplasm has fairly electron-dense contents with an extensive endoplasmic reticulum; it has developed one large oil drop, sometimes accompanied by smaller ones.

In a following stage of development a normal epispore (60 nm thick) and endospore (200–250 nm thick) differentiate and, though in most places the investing membrane has gone, it can be seen that locally the secondary wall increases further in thickness. The primary wall becomes extremely wavy in outline, which must be seen as an artefact.

At the same time the secondary wall material concentrates on the epispore. Like in *Peziza emileia* and *P. petersii*, at first the basal parts of the developing elements of ornamentation are electron-dense and homogeneous, while the upper parts are fairly electron-dense and homogeneous (Pl. 45C). A locally loose structure of the elements may cause permanent "gaps", as was also found in *P. petersii*. In later stages the elements of ornamentation become completely electron-dense. Together with the epiplasm, the rest of the secondary wall material disappears.

When the spores mature the upper parts of the elements of ornamentation grow out laterally, no longer forming warts, but umbrella-shaped structures (1200–1500 nm high) that sometimes fuse at the edges. In between the epispore is covered by a smooth layer (50 nm thick). Finally, their temporary "hairy" appearance is worth mentioning; this is not found in any of the other species studied (Pl. 45D).

PULPARIA PERSOONII

Fixative: permanganate-OsO_4. Preservation of this species appears to be difficult; this made a complete study of it impossible.

As regards the contents of the ascoplasm and their distribution over the ascus, young stages show close similarity to *Peziza badia*, *P. michelii*, *P. plebeia*, *P. succosa*, and *P. succosella*; particularly the first three species agree in that the membranous structures are globular or more irregularly formed. A noteworthy difference with these species is the absence of oil bodies and the presence of electron-dense globular structures (often with an unfixed center, as sometimes found in other species) resembling those in *Peziza ammophila*, *P. praetervisa*, and others.

The eventual development of vacuoles in the epiplasm is uncertain; glycogen is present in large quantities and takes the place of most of the original epiplasm. In the sporoplasm large oil drops develop. The development of the spore walls is normal; the epispore (50 nm thick) and the endospore (250–350 nm thick) seem to differentiate from the primary wall (300–400 nm thick); the secondary wall develops between the primary wall and the separating investing membrane.

At first the secondary wall material is homogeneous and fairly electron-dense. When its redistribution starts, electron-dense spots arise at regular distances from each other on the spore surface. As they gradually enlarge, the remaining contents of the secondary wall seem to be restructured into fibrous elements; these easily join and give the developing elements of ornamentation a freakish appearance (Pl. 45E). It is not clear whether these structures result from normal development or are caused by incomplete fixation. In the mature spores ornamentation is formed by irregular warts (400–800 nm high) that maintain a fibrous outer layer (Pl. 45F).

DISCUSSION

On many points the results of this electron microscopy confirm those of earlier studies. Not only does the general ultrastructure of each species agree with what is already known of Ascomycetes but the way in which the ascospores start to develop — delimitation of the spores in the ascoplasm by two delimiting unit membranes and the formation of spore walls between these two delimiting unit membranes — bears out similar developments in *Ascodesmis* (Merkus, 1973), in the Pyronemataceae (Merkus, 1974), and in other Ascomycetes (Reeves, 1967; Carroll, 1966, 1967, 1969; Delay, 1966; Wells, 1972; Schrantz, 1966, 1970).

Later stages of development reveal a strong structural resemblance to both *Ascodesmis* and the Pyronemataceae. The different appearance of two successively formed spore wall layers has again led to the use of the terms primary and secondary walls; and the processes involved in the formation of these spore walls, the differentiation of the primary wall into the epispore and the endospore, the internal differentiation of the epispore and the endospore, the various reactions of the spore wall layers on the fixatives applied, together with the structural changes of the epiplasm and sporoplasm, also agree. Perhaps, therefore, it will suffice to refer to previous discussions on these subjects.

A marked difference with the results obtained thus far is the appearance of the young ascoplasm of the genera *Otidea*, *Peziza*, and *Pulparia*. Globular structures, like oil bodies and other specialized globules, are present in most of the species; the more or less electron-transparent oil bodies are restricted to *Peziza*; the other specialized globules, which are electron-dense or more moderately electron-dense, prove to exist in *Otidea* and *Pulparia* and are found in a number of species of *Peziza*. The genus *Sowerbyella* could not be adequately studied on this point. The genus *Pustularia* is more usual in its internal structure and lacks the globular structures.

It is difficult to decide what processes are involved in the development of the

globular structures, which are often found embedded in glycogen. It is not impossible that the membranous structures in the central ascoplasm may play a role, though intervention of the poorly electron-dense vesicles derived from the endoplasmic reticulum must also not be overlooked. More knowledge about their chemistry might reveal something more about these processes. In this connection the development of the ascoplasm of species of *Otidea* seems to represent a simple form of those in the other species.

The globular structures are probably a food reserve; this was also assumed by Guilliermond (1904, 1910, 1920). They do not seem to play an active role in the formation of the spore walls. In the epiplasm they slowly lose their contents and disappear when the spores mature, not by being absorbed by the spores (Guilliermond) but by shrinkage or shriveling. In the sporoplasm they seem to persist; the oil bodies join to large oil drops, like in *Peziza badia*, *P. michelii*, *P. plebeia*, *P. succosa*, and *P. succosella*; the other specialized globules maintain their original form. When oil bodies are absent in the ascoplasm the spores may still develop oil drops in later stages, like in *Otidea alutacea*, *O. bufonia*, *O. onotica*, *Peziza ammophila*, *P. badiofusca*, *P. emileia*, *P. petersii*, *P. praetervisa*, *P. trachycarpa*, *Pulparia persoonii*, and *Pustularia cupularis*. In *Peziza vesiculosa* no oil drops are formed at all.

A food reserve is also provided in large quantities by glycogen. Though the possibility of chemical changes or the influence of the fixative cannot be precluded it seems as if other structural forms of glycogen exist apart from the typical glycogen particles that have been described (a.o. by Schrantz, 1968, for *Peziza plebeia*).

The aspect of the secondary wall and the structural changes it undergoes during the development of ornamentation is the same as in the Pyronemataceae. The secondary wall material is at first regularly spread in the secondary wall and is similar in appearance to the older epiplasm in most of the species. It seems to be redistributed in later stages and condenses or concentrates on the epispore, where it forms a complete ornamentation. Like in the Pyronemataceae, the processes involved in this condensation or concentration of secondary wall material are regular and characteristic for each species separately. A continuous addition of new secondary wall material during the formation of ornamentation is not impossible. The fate of the remaining secondary wall material and the investing membrane is unknown, though it is highly likely that it disappears with the epiplasm.

Like in the Pyronemataceae this development of ornamentation is found in all the species with ornamented spores, viz. *Peziza badia*, *P. badiofusca*, *P. emileia*, *P. michelii*, *P. petersii*, *P. plebeia*, *P. praetervisa*, *P. succosa*, *P. succosella*, *Pulparia persoonii*, and *Sowerbyella radiculata*. Apart from the striation in the developing ornamentation of *Sowerbyella radiculata*, the thin surrounding layer that is present in *Peziza badia*, *P. badiofusca*, and *P. petersii*, and the somewhat fibrous outer part of the ornamentation of *Pulparia persoonii*, no further internal differentiation in the ornamentation is found in any of the species.

In comparing the development of the smooth spores in the Pyronemataceae and in the species of this study it appears that similarities exist; in *Pustularia cupularis* some

condensation of secondary wall material is found but it disappears in a later stage so that ultimately the epispore forms the outermost part of the mature spores. In *Peziza ammophila* and *P. vesiculosa* however a permanent condensation of secondary wall material causes the formation of extra, smooth layers on the epispore. A similar addition of smooth layers on the epispore occurs in *Otidea alutacea*, *O. bufonia*, and *O. onotica* but it is uncertain whether these layers are permanent.

Though the terminology of the spore wall layers is somewhat different my results on the species *Pustularia cupularis* and *Peziza plebeia* agree with those of Schrantz (1966, 1970). His descriptions for both the development of two succeeding spore wall layers, the "couche primaire" (primary wall) and the "périspore" (secondary wall) in both species, and the formation of the "couche ornementale" in the "périspore" of *Peziza plebeia* are similar to those in the present study. He also found differentiation of the "couche primaire" into the outer "exospore" (epispore) and the inner "épispore" (endospore) in both species, and further development of the innermost "endospore" (not described as a particular layer by me) in *Pustularia cupularis*; and mentioned the presence of an "ectospore" (investing membrane) and "masses denses" (plasmic inclusions).

As on the one hand so many similarities prove to exist between the ultrastructure of the species of the Pyronemataceae, Otideaceae, and Pezizaceae and, on the other hand, Le Gal's observations on these taxa also agree, I can add no further details to the comparison of the two studies. To avoid further duplication I refer to previous discussions on the subject.

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REFERENCES

CARROLL, G. C. (1966). A study of the fine structure of ascosporogenesis in *Saccobolus kerverni* and *Ascodesmis sphaerospora*. Ph. D. Thesis. University of Texas, Austin [unpublished].

—— (1967). The ultrastructure of ascospore delimitation in *Saccobolus kerverni*. *In* J. Cell Biol. **33**: 218–224.

—— (1969). A study of the fine structure of ascosporogenesis in *Saccobolus kerverni*. *In* Arch. Mikrobiol. **66**: 321–339.

CLEMENÇON, H. (1973). Die Wandstrukturen der Basidiosporen. III. *Cortinarius* und *Dermocybe*. *In* Z. Pilzk. **39**: 121–144.

DELAY, C. (1966). Etude de l'infrastructure de l'asque d'*Ascobolus immersus* Pers. pendant la maturation des spores. *In* Annls Sci. nat. (Bot.) XII **7**: 361–420.

ECKBLAD, F.-E. (1968). The genera of the operculate Discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. *In* Nytt Mag. Bot. **15**: 1–191.

GUILLIERMOND, A. (1904). Contribution à l'étude de la formation des asques et de l'épiplasme des Ascomycètes. *In* Revue gén. Bot. **16**: 49–66.

—— (1910). A propos des corpuscules métachromatiques ou grains de volutine. *In* Arch. Protistenk. **19**: 289–309.

—— (1920). Sur les relations entre le chondriome des champignons et la métachromatine. *In* C.r. Séanc. Soc. Biol. **83**: 855–858.

LE GAL, M. (1947). Recherches sur les ornementations sporales des Discomycètes operculés. *In* Annls Sci. nat. (Bot.) XI **8**: 73–297.

LUFT, J. H. (1961). Improvements in epoxy resin embedding methods. *In* J. biophys. biochem. Cytol. **9**: 409–414.

MERKUS, E. (1973). Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)—I. *Ascodesmis microscopica* (Crouan) Seaver and *A. nigricans* van Tiegh. *In* Persoonia **7**: 351–366.

—— (1974). Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)—II. Pyronemataceae sensu Eckblad. *In* Persoonia **8**: 1–22.

REEVES, JR., F. (1967). The fine structure of ascospore formation in *Pyronema domesticum*. *In* Mycologia **59**: 1018–1033.

SCHRANTZ, J.-P. (1966). Contribution à l'étude de la formation de la paroi sporale chez *Pustularia cupularis* (L.) Fuck. *In* C. r. hebd. Séanc. Acad. Sci., Paris **262**: 1212–1215.

—— (1968). Ultrastructure et localisation du glycogène chez l'Ascomycète *Galactinia plebeia* Le Gal. *In* Rev. Cytol. Biol. vég. **31**: 151–154.

—— (1970). Etude cytologique, en microscopie optique et électronique, de quelques Ascomycètes. II. La paroi. *In* Rev. Cytol. Biol. vég. **33**: 111–168.

SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *In* J. Ultrastruct. Res. **26**: 31–43.

WELLS, K. (1972). Light and electron microscopic studies of *Ascobolus stercorarius*. II. Ascus and ascospore ontogeny. *In* Univ. Calif. Publs. Bot. **62**: 1–93.

EXPLANATION OF PLATES 34–45

ABBREVIATIONS USED IN PLATES. — AW, ascus wall; CM, condensed material; E, epiplasm; En, endospore; Ep, epispore; ER, endoplasmic reticulum; 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; V, vesicle; Va, vacuole.

PLATE 34

Figs. A-D. *Otidea bufonia*, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Figs. A, B. ascoplasm, before spore development, × 18,200; Fig. C. spore development, development of secondary wall, × 29,000; Fig. D. id. also showing development of epispore and endospore, × 29,000.

Fig. E. *Otidea onotica*, spore development, temporary internal structure of condensing secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 29,700.

Fig. F. *Otidea alutacea*, spore development, condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 18,500.

Fig. G. *Otidea bufonia*, detail of condensed secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 115,500.

PLATE 35

Figs. A-D. *Pustularia cupularis*, spore development, stained with lead citrate: Fig. A. beginning of secondary wall formation, fixed in 1% $KMnO_4$ and 1% OsO_4, × 29,700; Fig. B. further development of secondary wall, with condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4, × 29,700; Fig. C. development of epispore and endospore, fixed in 1% glutaraldehyde and 1% OsO_4, × 36,300; Fig. D. id.

Figs. E-G. *Sowerbyella radiculata*, spore development, fixed in 3.25% glutaraldehyde and 1% OsO_4: Fig. E. condensation of secondary wall material and development of epispore and endospore, stained with uranyl acetate and lead citrate, × 36,300; Fig. F. id. showing fibrous structure of secondary wall material, stained with lead citrate; Fig. G. advanced state in development of ornamentation, stained with uranyl acetate and lead citrate, × 29,700.

PLATE 36

Figs. A-C. *Peziza praetervisa*, ascoplasm, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. upper part of ascus before meiosis and mitosis, × 16,600; Fig. B. upper part of ascus after meiosis and mitosis, × 8,400; Fig. C. ascoplasm of the same ascus, between the nuclei, × 8,400.

PLATE 37

Figs. A, B. *Peziza praetervisa*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. young stage of spore development, just after spore delimitation, × 14,900; Fig. B. advanced state in the development of ornamentation, epispore, and endospore, × 18,200.

Figs. C-F. *Peziza ammophila*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. C. beginning of secondary wall formation, × 18,200; Fig. D. condensation of secondary wall material and development of epispore and endospore, × 9,900; Fig. E. id. × 18,200; Fig. F. id. detail, advanced state of spore development, × 36,300.

PLATE 38

Figs. A-C. *Peziza vesiculosa*, ascoplasm, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. upper part of ascus before meiosis and mitosis, stained with uranyl acetate and lead citrate, × 9,900; Fig. B. basal part of ascus before meiosis and mitosis, stained with uranyl acetate and lead citrate, × 17,300; Fig. C. basal part of ascus after delimitation of spores, stained with lead citrate, × 17,300.

PLATE 39

Figs. A-F. *Peziza vesiculosa*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. young ascospore during formation of primary wall, stained with uranyl acetate and lead citrate, × 18,200; Fig. B. beginning of secondary wall formation, stained with uranyl

acetate and lead citrate, ×9,900; Fig. C. condensation of secondary wall material and development of epispore and endospore, stained with lead citrate, ×18,200; Fig. D. id.; Fig. E. id.; Fig. F. id. advanced state of spore development, stained with uranyl acetate and lead citrate, ×21,400.

PLATE 40

Figs. A, B. *Peziza succosa*, ascoplasm, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. upper part of ascus before meiosis and mitosis, ×7,100; Fig. B. detail, ×16,600.

Fig. C. *Peziza succosella*, id. ×5,800.

PLATE 41

Fig. A. *Peziza michelii*, ascoplasm, upper part of ascus before meiosis and mitosis, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×7,400.

Figs. B, C. *Peziza plebeia*, id.: Fig. B. ×7,100; Fig. C. detail, ×21,400.

PLATE 42

Figs. A-H. *Peziza succosella*, spore development: Fig. A. beginning of secondary wall formation, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700; Fig. B. id. condensation of secondary wall material and development of epispore and endospore, stained with lead citrate; Fig. C. id. stained with uranyl acetate and lead citrate; Fig. D. id. stained with lead citrate, ×33,300; Fig. E. id. advanced state of spore development, stained with uranyl acetate and lead citrate, ×29,700; Fig. F. condensation of secondary wall material and development of epispore and endospore, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700; Fig. G. id.; Fig. H. id. advanced state of spore development.

PLATE 43

Figs. A, B. *Peziza succosa*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700: Fig. A. beginning of secondary wall formation and some condensation of secondary wall material; Fig. B. condensation of secondary wall material and development of epispore and endospore.

Figs. C-E. *Peziza michelii*, spore development, stained with uranyl acetate and lead citrate, ×29,700: Fig. C. condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4; Fig. D. id. also showing development of epispore and endospore, fixed in 1% $KMnO_4$ and 1% OsO_4; Fig. E. id.

Fig. F. *Peziza plebeia*, spore development, condensation of secondary wall material, fixed in 1% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700.

PLATE 44

Figs. A, B. *Peziza badia*, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×23,100: Fig. A. detail of ascoplasm in upper part of ascus before meiosis and mitosis; Fig. B. condensation of secondary wall material and development of epispore and endospore, advanced state of spore development.

Figs. C, D. *Peziza emileia*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, ×29,700: Fig. C. condensation of secondary wall material and development of epispore and endospore; Fig. D. id. advanced state of spore development.

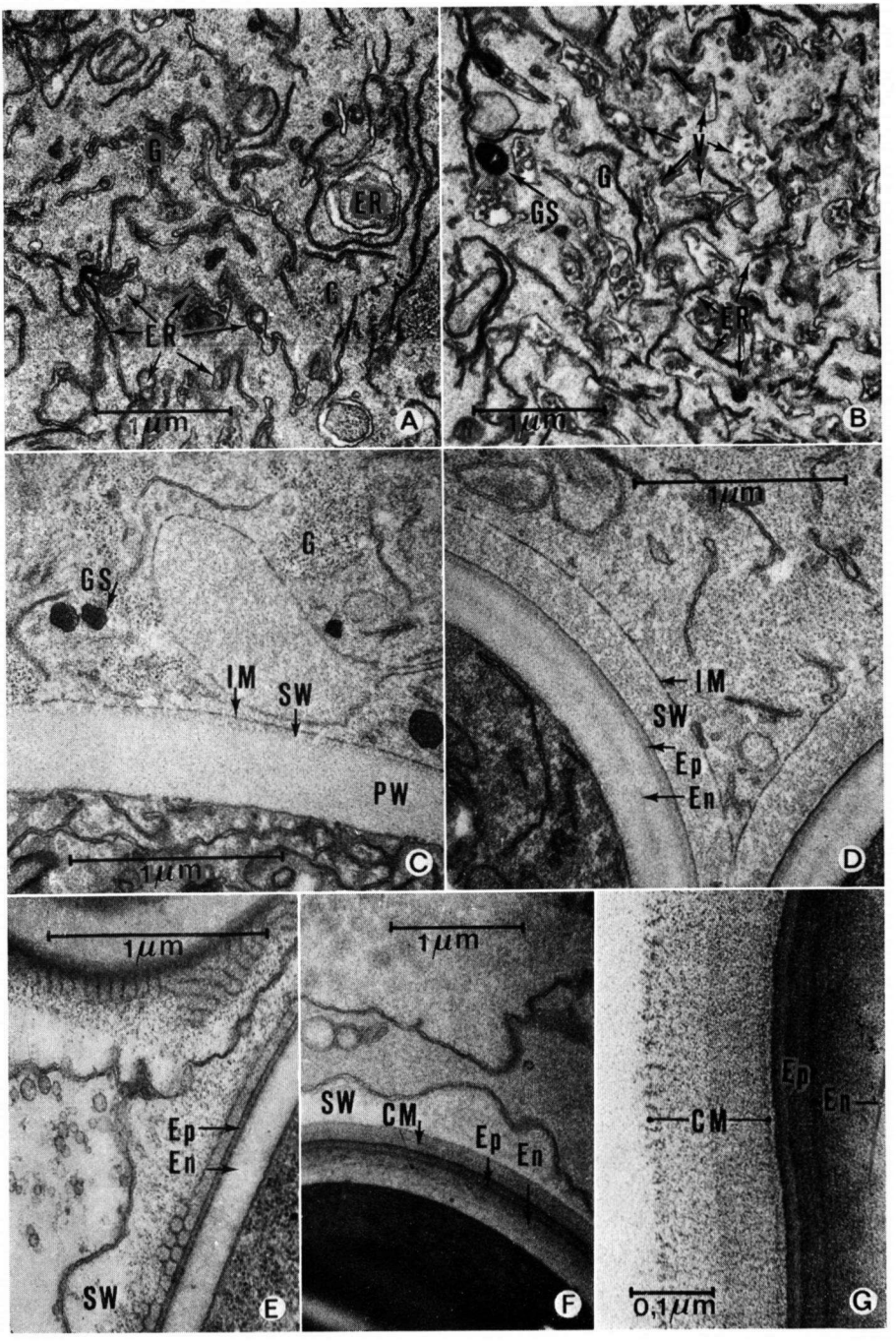
Figs. E, F. *Peziza petersii*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. E. condensation of secondary wall material and development of epispore and endospore, ×29,700; Fig. F. id. advanced state of spore development, ×33,300.

PLATE 45

Figs. A, B. *Peziza badiofusca*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. condensation of secondary wall material and development of epispore and endospore, ×29,700; Fig. B. id. advanced state of spore development, ×23,100.

Figs. C, D. *Peziza trachycarpa*, spore development, fixed in 1% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. C. condensation of secondary wall material and development of epispore and endospore, ×29,700; Fig. D. id. advanced state of spore development, ×23,100.

Figs. E, F. *Pulparia persoonii*, spore development, stained with uranyl acetate and lead citrate: Fig. E. condensation of secondary wall material and development of epispore and endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4, ×29,700; Fig. F. id. advanced state of spore development, fixed in 1% $KMnO_4$ and 1% OsO_4, ×36,300.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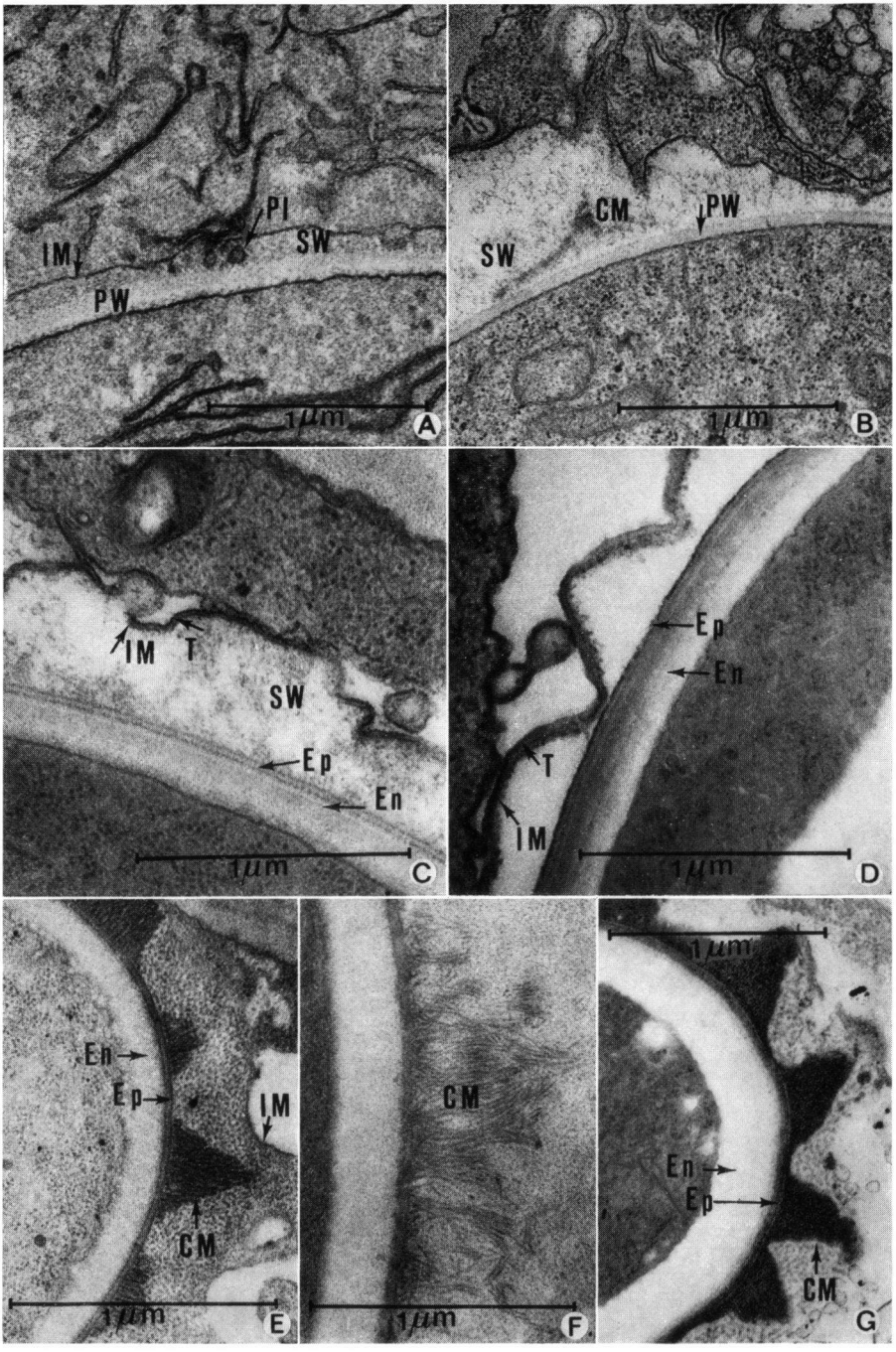
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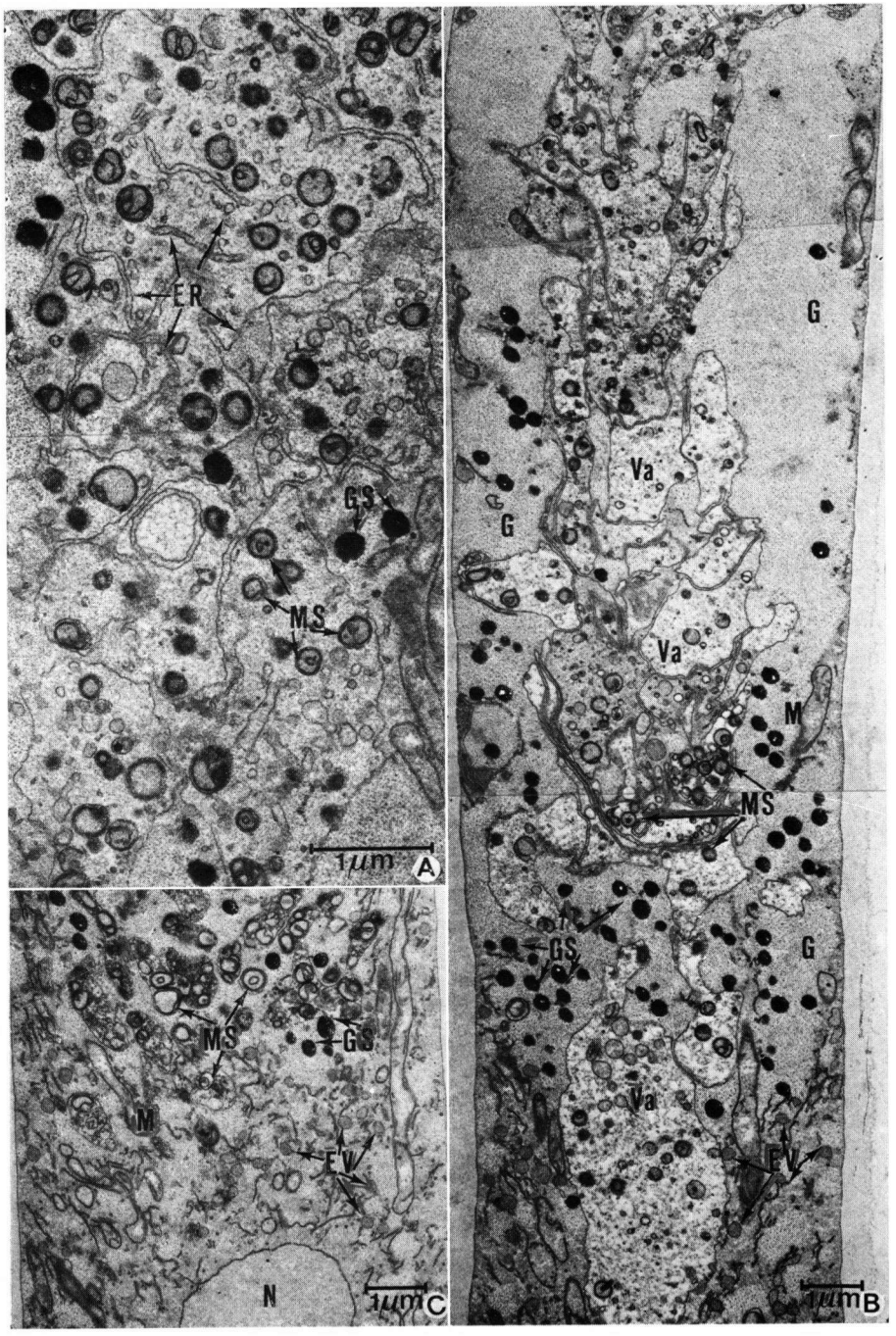
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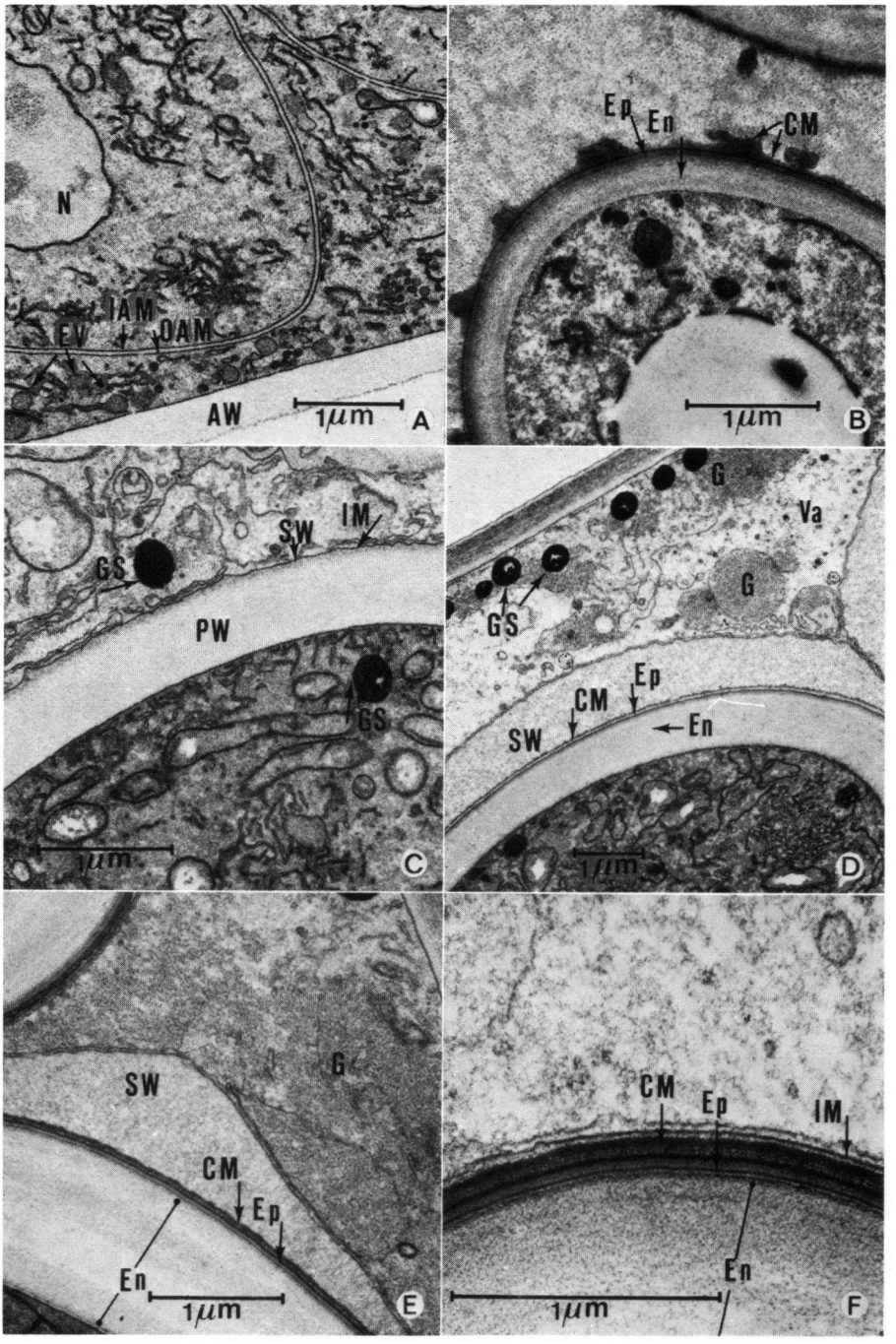
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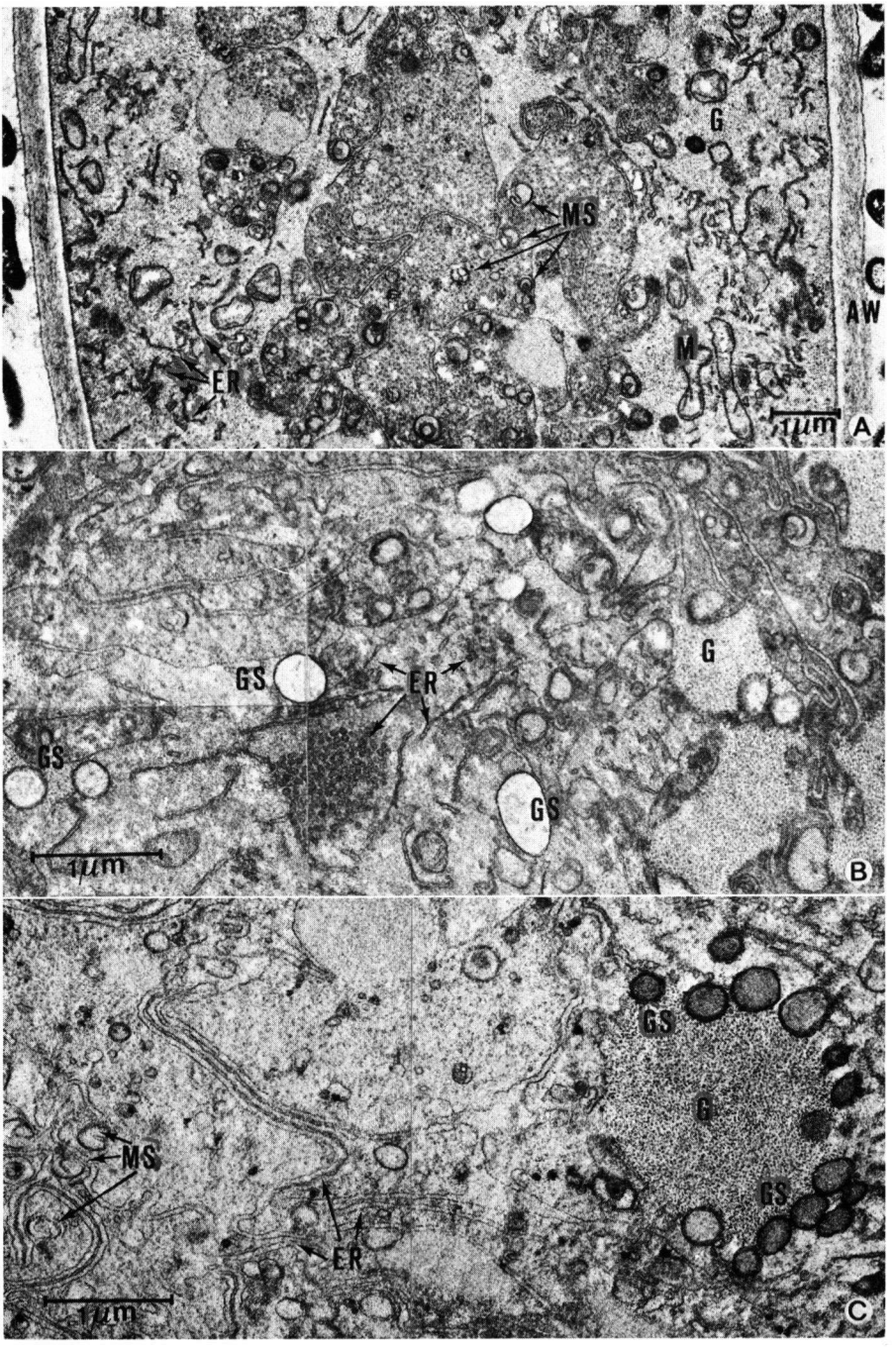
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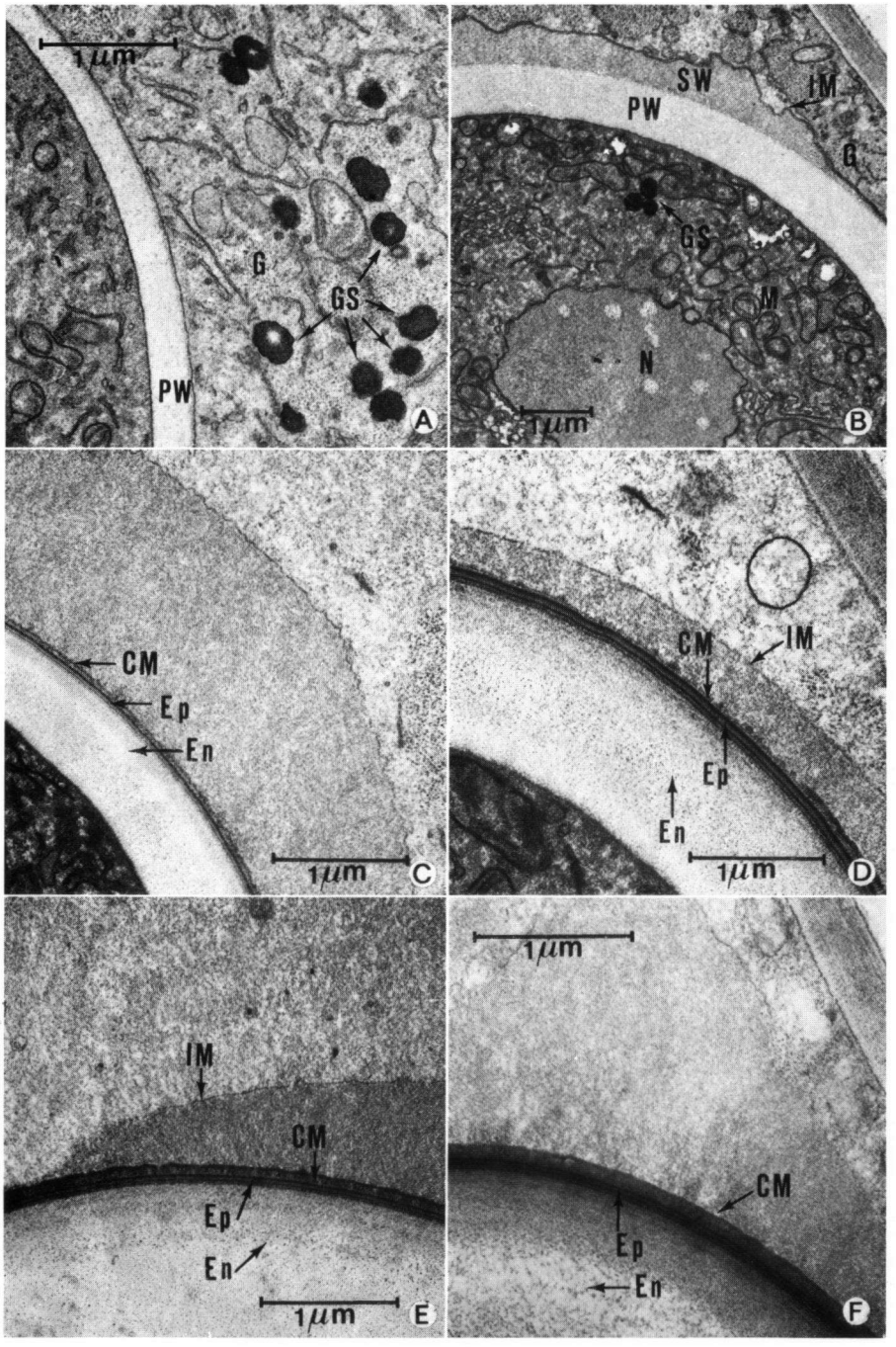
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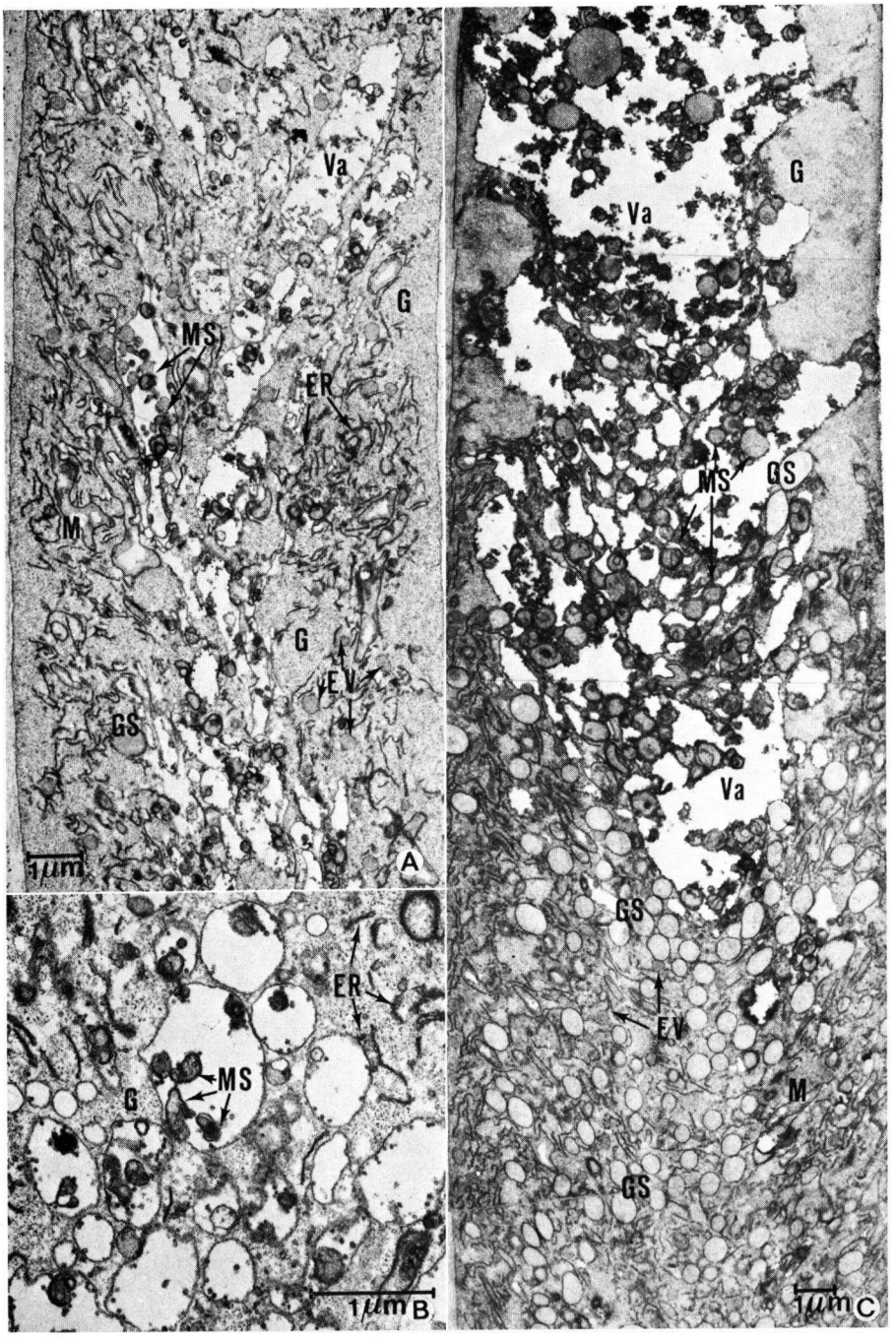
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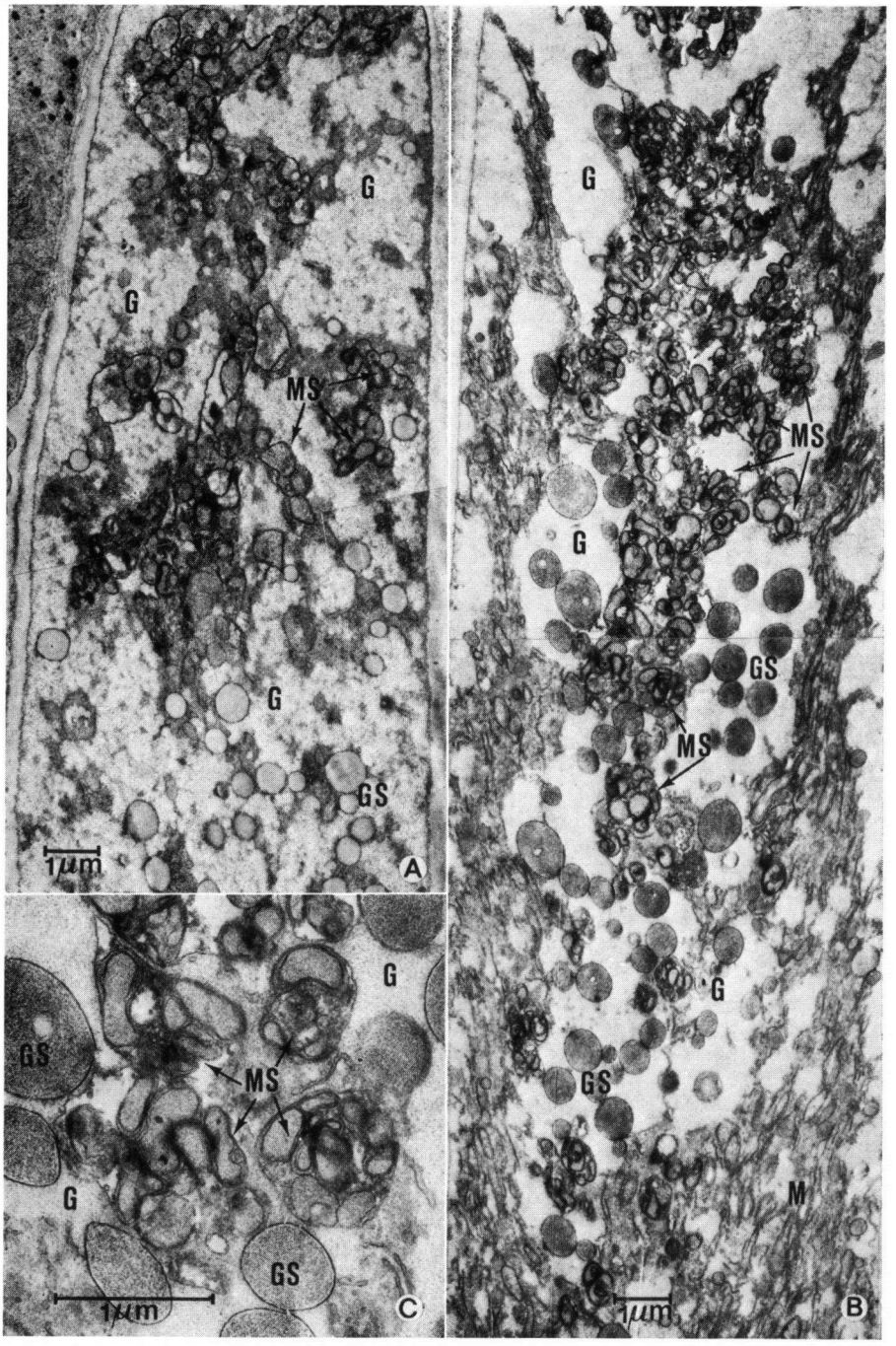
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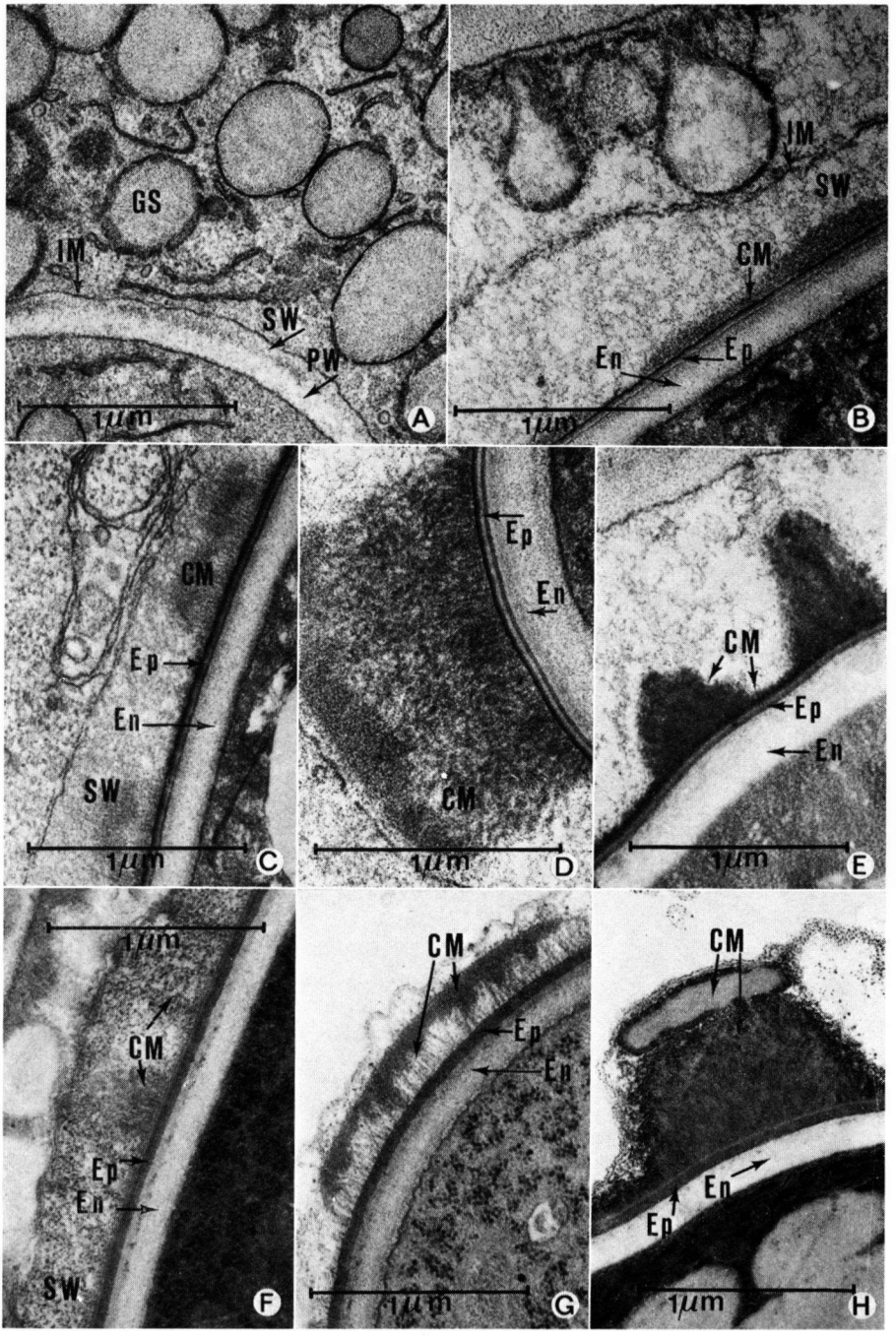
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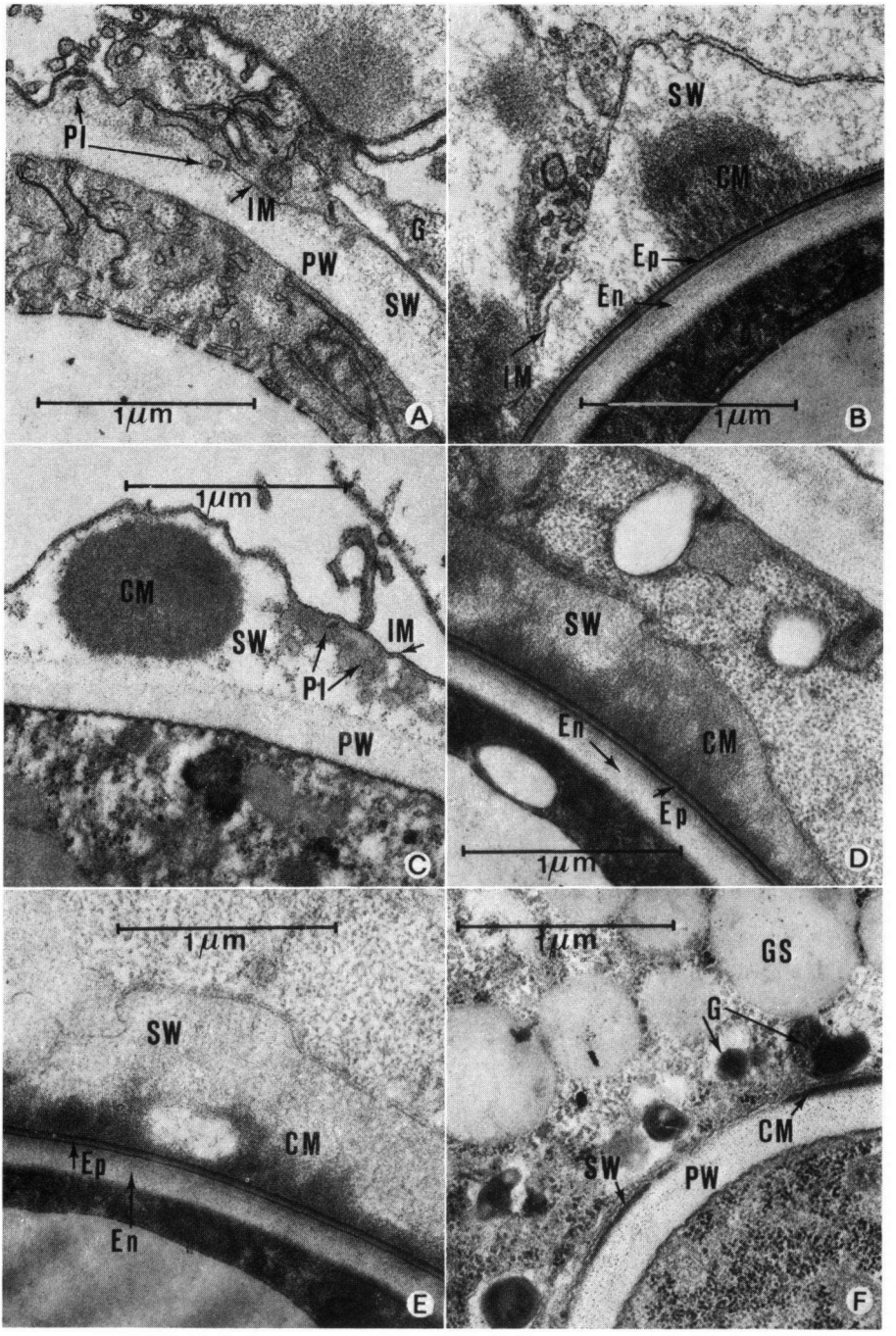
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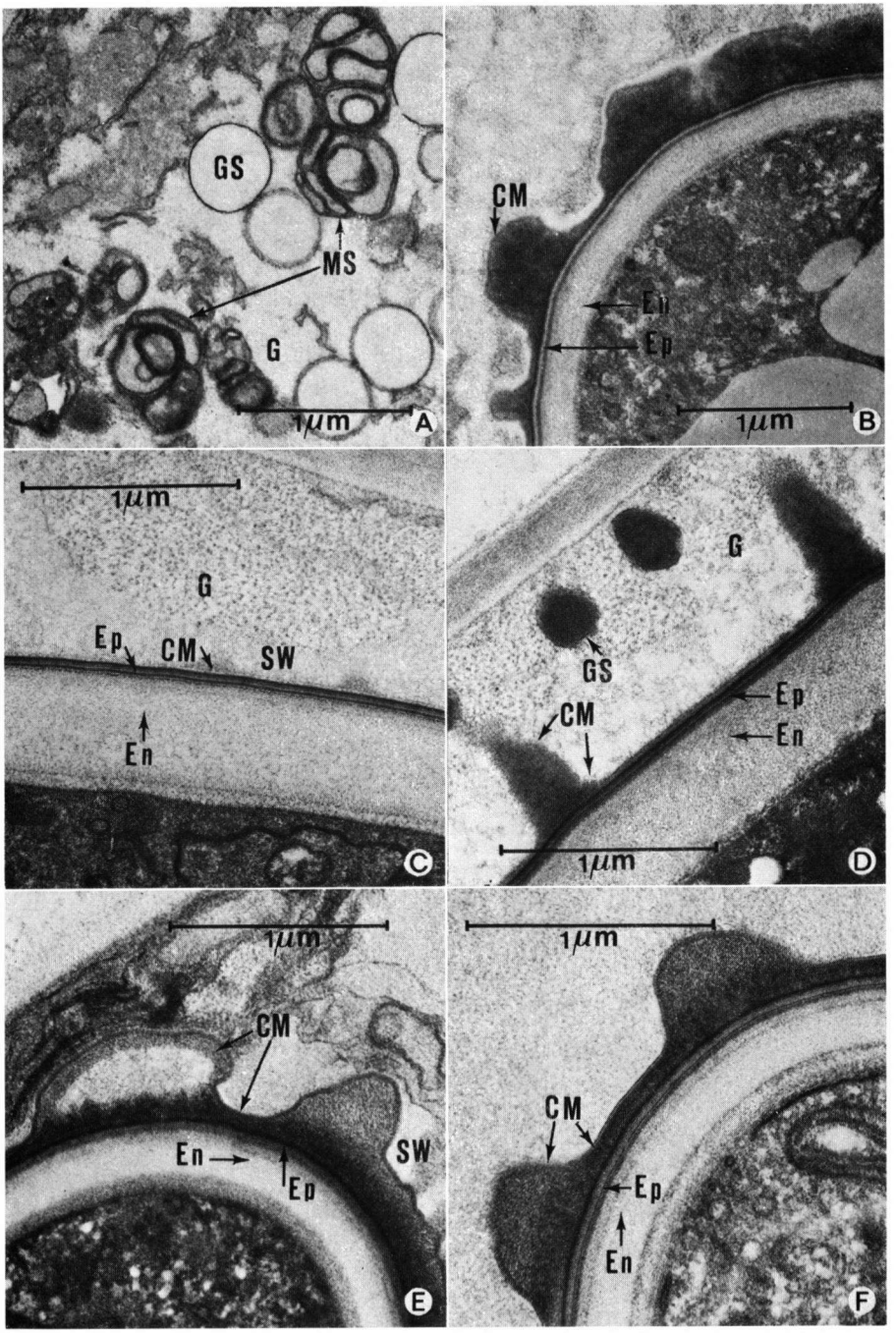
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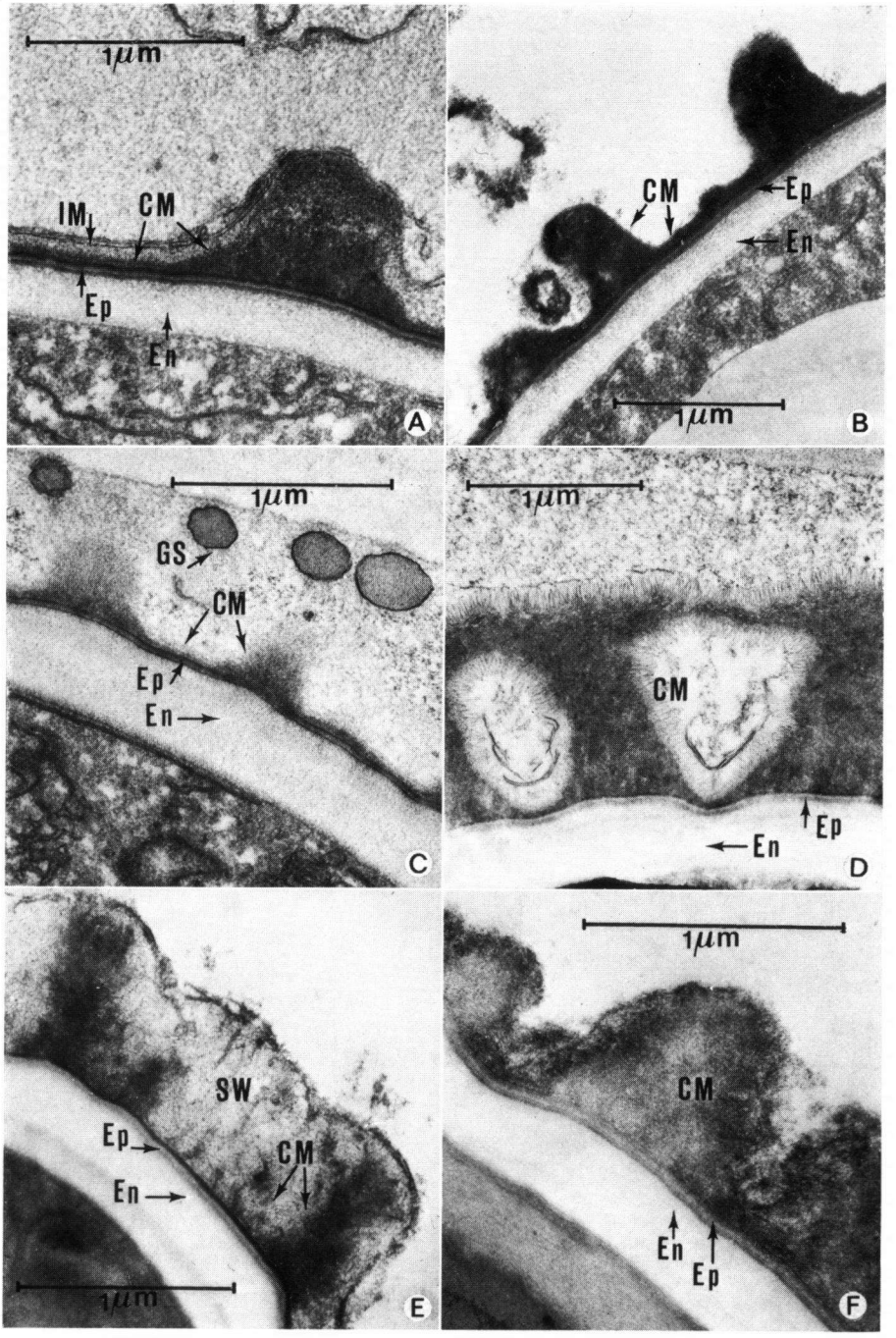
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