MULTIPERFORATE SEPTATIONS, WORONIN BODIES, AND SEPTAL PLUGS IN *FUSARIUM*

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INTRODUCTION

The first report of pores in fungus septa in filamentous fungi was made by de Bary (6) in 1866. Between 1866 and about 1902, a number of papers were published dealing with fungus septations and their formation. Among these papers are reports by Woronin (23), Wahrlich (22), Ternetz (21), and Strasburger (19). Woronin (23) also reported the presence of small bodies associated with septations. This report was verified by Ternetz (21) (pp. 278, 279). Buller (4) reviewed the early literature thoroughly and confirmed the above cited observations. The limit imposed by the resolution of the light microscope did not permit a more detailed investigation until the advent of the electron microscope. Girbardt (10), in 1958, was the first to show a septum in a Basidiomycete, which differed from the classical concept of a single-pored, simple, disk-like structure. The details of the Basidiomycete septum were later carefully worked out by Bracker and Butler (3). Septations in Phycomycetes vary greatly. Some lack septa or have them only at the base of sporangia, while others appear to have fairly complex septa when observed with the light microscope. Buller (4) reports that *Rhizopus nigricans* has an imperforate septum. Barrett (1) first described and illustrated the so called "pseudo-septa" in *Allomyces* in 1912. Coker (5), in 1930, published a photograph of such a septum. Published light and electron micrographs of Ascomycete and Deuteromycete septa show no essential difference from the earlier concept of a single-pored septum in these fungi (2, 7, 8, 13, 17). Exceptions to this are a number of reports of septa with multiple pores in the fungus component of lichens by Poirault (14), Meyer (12), Kienitz-Gerloff (11), and Strasburger (20) between 1896 and 1902, and in a short review by Smith (18, p. 51).

The purpose of this light and electron microscope study is to describe multiperforate septa, in *Fusarium* hyphae, and spherical Woronin bodies which form plugs in the septal pores. These plugs apparently serve as safety valves for the fungal cells.

MATERIALS AND METHODS

The following species of *Fusarium* were used in this study: *Fusarium solani* f. *phaseoli; F. solani* f. *cucurbitae* (perfect stage: *Hypomyces*), *Fusarium rigidiuscula* (perfect stage: *Calonectria*). Both perfect stages are in the Hypocreales of the Ascomycetes.

For sectioning and observation under the electron microscope, the fungus was grown on a cellophane paper disk placed on a layer of Potato Dextrose Agar (PDA) in a petri dish. Small sections of the mycelium were fixed in two ways:

1. Cold (4°C) $KMnO_4$ for 3 minutes.
2. 6 per cent glutaraldehyde in 0.1 M phosphate buffer, pH 6.8, for 4 hours in the cold, followed by thorough washing in cold buffer and fixation in 2 per cent OsO_4 overnight.

Both fixations 1 and 2 were followed by acetone dehydration. Uranyl nitrate in 70 per cent acetone was used as a postfixation stain. The material was embedded in Epon 812. Sections were cut on a Porter-Blum microtome and viewed in an RCA-EMU-3F.

Fungus septa were isolated from mycelium and conidia in the following way: mycelium grown on PDA-agar slants was boiled in water to remove the

agar, and placed in 23 M KOH, following the technique for chitin analysis described by Fuller (9). After autoclaving the KOH mixture for 3 hours, it was washed in water, neutralized, washed with 2 per cent acetic acid, and sonicated. A drop of the diluted suspension was placed on formvar grids, dried, carbonstabilized, shadowed with uranium, and examined with the electron microscope.

Fungus mycelium and conidia were also degraded enzymatically. The enzyme preparation consisted of a sterile, crude filtrate obtained from a 7-day-old shake culture of *Streptomyces sp.* The culture was grown on a salt medium, described by Reynolds (15), to which unpurified chitin had been added as a carbon and nitrogen source. The spores and mycelium were incubated in the filtrate at 37°C for 2 to 4 days. They were then washed and placed on grids for viewing with the electron microscope.

Light microscope pictures were taken with a Zeiss automatic photomicroscope, using oil-immersion, phase contrast lenses. Both live hyphae as well as isolated septa were examined. The latter were stained with acid fuchsin in lactic acid for greater contrast.

RESULTS

MULTIPERFORATE SEPTATIONS: The classical Ascomycete and Deuteromycete septum is a simple disk with a central pore. The septum is formed as an ingrowth from the lateral wall. Fig. 1 is a longitudinal section of a *Fusarium* hypha showing the pore. In Fig. 3 isolated single-pored septa from macroconidia are shown, and the pore is seen in face view. Figs. 6, 9, and 10 show similar septa in conidia where they are still attached to the lateral walls. The thickened outer rim and the central pore area are also visible under the light microscope (Fig. 7). The diameter of these septations can vary greatly depending on the part of the conidium from which they originated.

In the sectioning of large hyphae, one often finds septa of the type shown in Figs. 2 and 17. Here the cross-wall is perforated a number of times. The pores are located toward the edge of the septum (Fig. 17). Figs. 4 and 5 show an isolated multiperforate septum and a septal fragment. It is interesting to note that the dense outer rim found in the single-pored septum is missing. The diameter of the pores in multiperforate septa is about the same as that of the pore of single-pored septa. The absence of the heavy rim thickening can also be seen under the light microscope (compare Fig. 7 with 8). Fig. 11 is believed to be a multiperforate septum in a large hypha which is branching. One septum and its hyphal branch are visible to the upper left coming from the larger, main hypha below. On the upper right of this main hypha one can see the face-view of a number of septal pores with the outline of the septal rim around it. The second branch hypha has been digested away above the septum by the KOH treatment. The distinct thickening of the septal rim, as in the left septum and in the septa found in conidia shown in Fig. 3, 6, 7, 9, and 10, is missing. From isolated septa it appears that in both the single-pored and multiperforate septations the area immediately surrounding the pore is somewhat more electron opaque or thicker.

WORONIN BODIES: In the species of *Fusarium* which we have sectioned, we have found an electron-opaque body (Woronin body) which is round or oval in shape. It is generally associated with septa and especially septal pores (Figs. 1, 2, 14 to 17). We have also found these bodies in *Phymatotrichum omnivorum*. From two to four of these bodies are close to each septal pore in electron micrographs. The number visible depends on the

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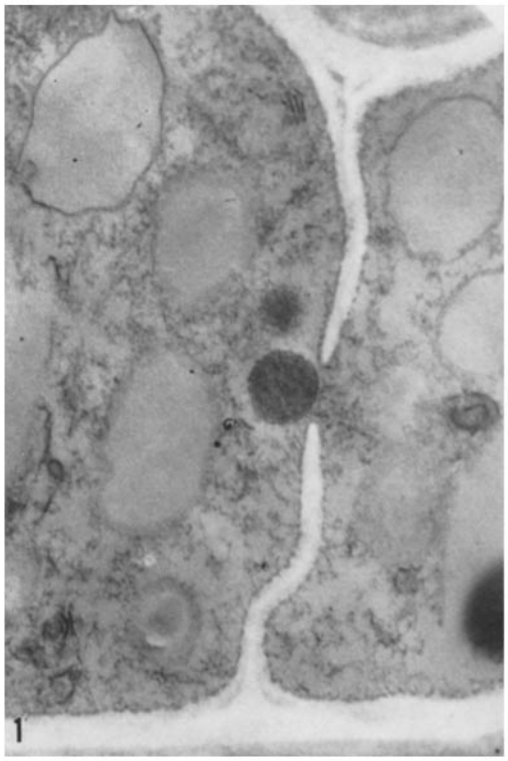
All illustrations are from *Fusarium solani* f. *phaseoli*. The magnifications indicated are approximate.

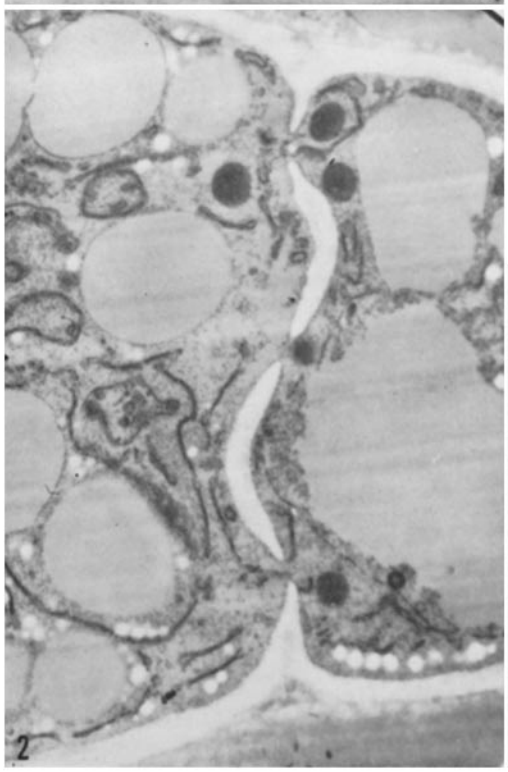
FIGURE 1 Longitudinal cross-section through septal pore and one Woronin body. $KMnO_4$. × 40,000.

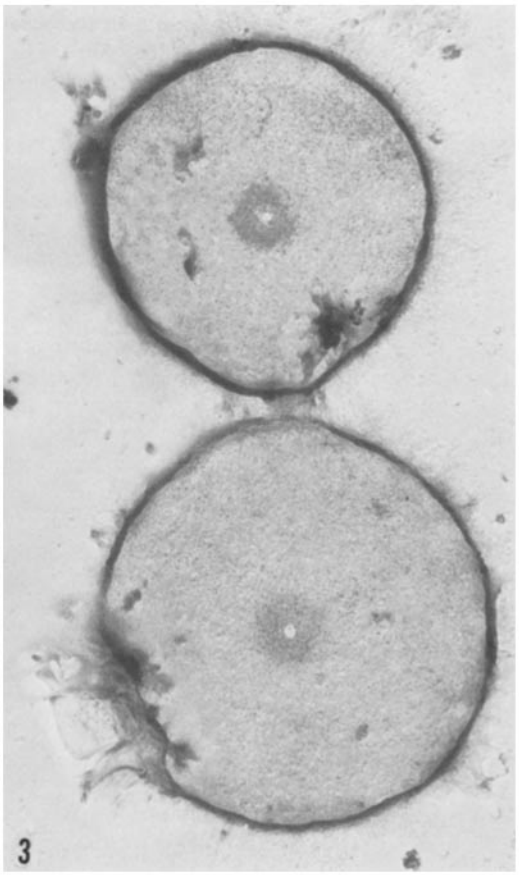
FIGURE 2 Same as Fig. 1. Three septal pores can be seen. $KMnO_4$. × 21,000.

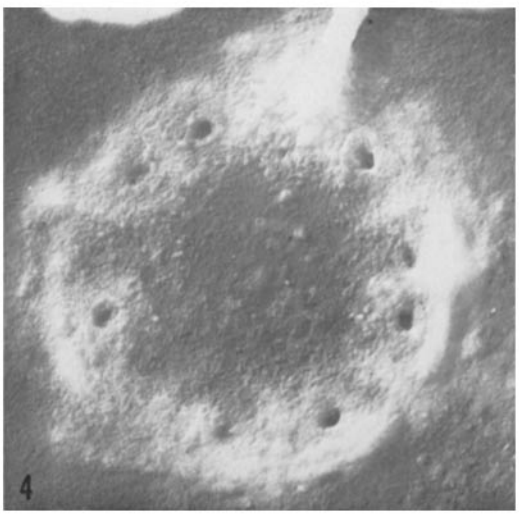
FIGURE 3 Isolated septa from macroconidia. Note central septal pore and dense outer rim of septum. Uranium shadow. × 12,000.

FIGURE 4 Isolated multiperforate septation printed as a negative to show pores in higher contrast. Note absence of outer rim of septum which has been lost during isolation. Uranium shadow. × 20,000.









way in which the section passes through the pore and its adjacent areas. These bodies show little internal structure, and a distinct membrane surrounds them (Figs. 15, 16). Woronin bodies can also be readily seen with the phase-contrast microscope in young, non-vacuolated hyphae (Figs. 12, 13). The size of these dark structures under the light microscope corresponds approximately to the size of the structures found in electron micrographs. Woronin bodies, although much less discernible, are also visible in the light microscope without phase-contrast. They rarely move about and are closely associated with the septum. From one to six of these bodies are seen, under phase, adjacent to a septum. No attempts were made to stain them with nucleophilic stains as did Ternetz (21) and Schrantz (16). Two other types of bodies, of similar density, were observed with the phase-contrast microscope. One is of the same size as the Woronin body and moves within a cell of the hypha, occasionally coming in contact with the cell's septa. The other is twice as large in diameter, and also moves around, rarely associating itself with the septum. A number (six to eight) of bodies which are of the same size as Woronin bodies were observed around newly forming septa in hyphal tip cells.

SEPTAL PLUGS: In hyphae which have degenerated or are in the process of degeneration, or which have been damaged, a plug forms in the septal pore. This plug is of the same electron density as the Woronin bodies and has a single membrane surrounding it (Figs. 14, 16).

DISCUSSION

MULTIPERFORATE SEPTA: The reports of multiperforate septa in the fungal components of lichens at the turn of the century (11, 12, 14, 20) have largely been ignored, except in reviews by Smith (18) and Buller (4). The pores in multiperforate septations which we have found in *Fusarium* are arranged in a circular pattern near the outer edge of the septum, with one or more pores in the center. The pore sizes of septa isolated by three separate methods (sonication alone, enzymatic digestion, and KOH-treatment) were approximately the same, regardless of the method of isolation. The size of pores in multiperforate septa coincides with that of pores of single-pored septa.

The majority of the single-pored septa which we have found seem to have come from conidia. These septa are less readily degraded by the various isolation procedures than are the multiperforate septa. We think this is due to the fact that the conidia, from which many single-pored septa originate, are much more solidly constructed than hyphae. This may be the reason that the isolated multiperforate septum which originates in the hypha is in a much more advanced state of disintegration after isolation than the single-pored septum. Furthermore, this explains

FIGURE 5 Fragment of isolated multiperforate septum, printed as a negative to show pores better. Uranium shadow. × 33,500.

FIGURE 6 Phase micrograph of macroconidium containing folded septations, and three isolated septa (arrows). × 1,300.

FIGURE 7 Phase micrograph of isolated single-pored septum. Note dense rim of septum. × 850.

FIGURE 8 Phase micrograph of isolated multiperforate septum. Note absence of rim around septum, similar to electron micrograph in Fig. 4. × 1,700.

FIGURE 9 Part of macroconidium showing folded septations with dense rims. × 2,300.

FIGURE 10 Part of macroconidium containing septations and isolated septa. × 2,600.

FIGURE 11 Face view of multiperforate septum in a large branching hypha. Branch hypha belonging to this septum has been digested away by KOH treatment. Note absence of dense rim around septum. Second branch hypha to the upper left with included septation (dark area). Uranium shadow. × 4,800.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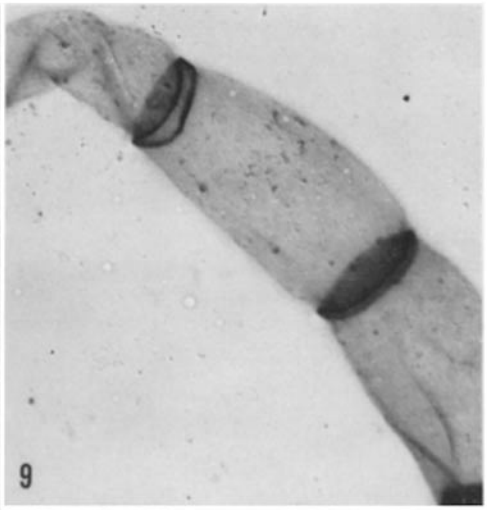
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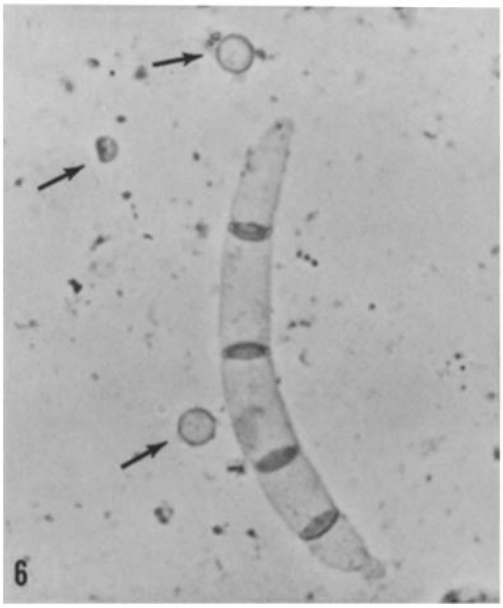
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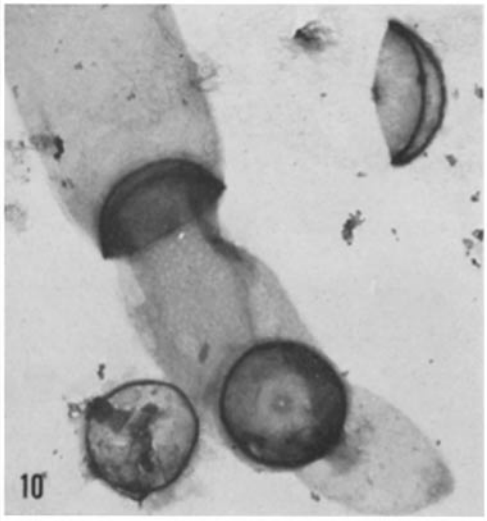
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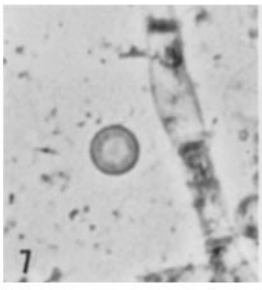
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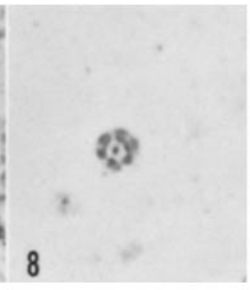
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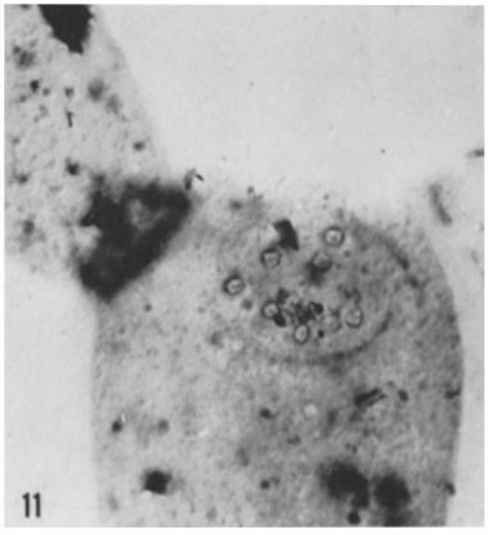
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why we have had considerable difficulty in finding isolated multiperforate septa, whereas they could be found quite easily in sectioned material. We have never observed a multiperforate septum in a conidium, either in isolated or in sectioned material.

The precise location of the multiperforate septa in the hyphal thallus is a matter of conjecture. We have only found them in large hyphae. A possible location for such multiperforate septum would be a conidiophore in a sporodochium in which a large amount of material has to be translocated for the manufacture of the large conidial spore masses. We have no indication as to how these septa are formed. The pores could be formed by a digestion process, in which parts of the septal wall are removed to facilitate greater translocation of materials. That wall digestion can take place is evidenced by the anastomoses of hyphae, and crozier formation in ascogenous hyphae. Another mode of formation could be the one described for the "pseudo-septa" in *Allomyces* by Barrett (1) in which the wall grows inward in a wagon wheel spoke-like fashion, leaving empty spaces for septal pores.

SEPTAL PLUGS AND WORONIN BODIES: Buller (4) and Zalokar (24) have demonstrated that when a hyphal cell is ruptured or otherwise disturbed its pores in the end walls will close almost instantaneously, sealing off the adjacent cells very effectively. This appears to be an important device for the self-preservation of the fungus. How this mechanism functions is not clear. The electron micrographs of Shatkin and Tatum (17), Moore and McAlear (13), and Dickson (8) show that a dark plug is formed in the septal pore. The plug appears uniformly dense and is surrounded by a membrane (Fig. 16) (Dickson, Fig. 7). The plug shown by Shatkin and Tatum (17, Fig. 7) has 5 distinct layers. Our interpretation of the picture is that the section passed through the edge of the plug and pore, skimming the edge of the septal pore slightly. This accounts for the light central area in this picture. Furthermore, this light central area is clearly continuous with the septal wall to the left of the plug (Fig. 16 shows the same thing). Median sections through plugs are uniformly dark in appearance.

Associated with almost all septa which we have found are round or oblong bodies. Similar bodies can be seen in electron micrographs of other authors (2, 7, 8, 13, 16). They have been interpreted as lipid bodies (7, 13) and as bodies of an unknown nature associated with pores and plugs (2, 8, 16). They resemble septal plugs in their density and in being bounded by a membrane (Fig. 15) (2, 8, 16). The constant close association of these bodies with pores would make them appear to be the logical source of the plugs. When an adjacent cell is ruptured, the pressure in the cell should force one of these balls into the pore, plugging it. Several balls per pore only increase the safety factor. In a recent paper Schrantz (16) has come to the same conclusion, although he

FIGURE 12 Phase micrograph of young hypha and septum of *Fusarium solani* f. *phaseoli*. Note two dark bodies (Woronin bodies) adjacent to center of septum. × 4,800.

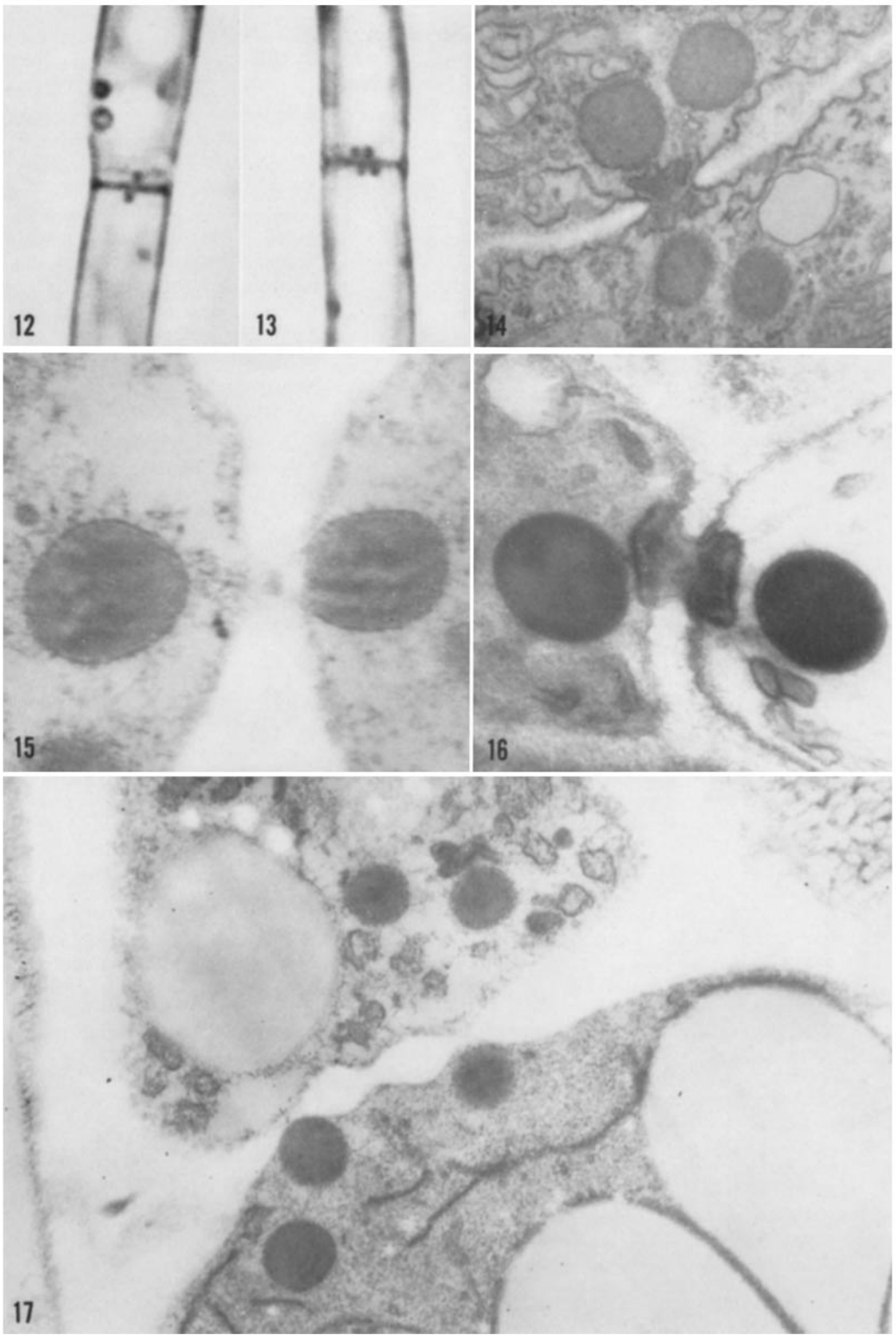
FIGURE 13 Same as Fig. 12. Showing four Woronin bodies associated with septum. × 4,200.

FIGURE 14 Septum showing two dense bodies (Woronin bodies) on each side of septum and a membrane-bounded central plug in the septal pore. Note central area of plug is lighter because the section probably does not pass exactly through the center of the pore. Glutaraldehyde-OsO_4. × 58,000.

FIGURE 15 Two Woronin bodies close to central septal pore. Note membrane surrounding balls. $KMnO_4$. × 67,000.

FIGURE 16 Septum showing two Woronin bodies and plug in septal pore. $KMnO_4$. × 71,000.

FIGURE 17 Longitudinal section through a septum at outer edge of a hypha. Note multiple pores in this area as well as dark Woronin bodies. $KMnO_4$. × 48,000.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shows no plugs in his pictures. Zalokar (24) reports that centrifuged hyphae of *Neurospora* recover within about 1 hour after centrifugation, resuming growth and protoplasmic streaming. It is not clear, though, from his paper whether this streaming takes place through pores formerly plugged or through newly formed septa. Bracker (2) assumes that septa in haustorial necks of *Erysiphe graminis* may be plugged at times by these balls.

We believe that the round bodies are the same structures as those first described by Woronin (23) nearly one-hundred years ago. Their location and behavior are similar. Buller (4) also observed similar structures. His description differs from our observations in that he claims that these bodies move around in the vacuole of the fungus. With the light microscope, we have observed structures of the same size and appearance as the Woronin bodies, which move in the manner described by Buller. We believe, though, that they are quite separate entities, since according to our observations the Woronin bodies remain confined to the immediate vicinity of the septum.

SUMMARY

Three structures of *Fusarium* hyphae are illustrated and discussed: multiperforate septa, Woronin bodies, and septal plugs.

Septations with multiple perforations were found in large hyphae. Multiple perforations were demonstrated in sectioned septa, as well as in chemically and enzymatically isolated septa, under the electron microscope and also under the light microscope. Woronin bodies, which are small spherical to oblong structures, are found closely associated with septa. It is suggested that they function as safety valves, which protect the hyphal cell content by the plugging of septal pores upon injury of an adjacent cell.

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