ULTRASTRUCTURAL AND HISTOCHEMICAL CHANGES IN THE BASIDIOMYCETE DOLIPORE SEPTUM ASSOCIATED WITH FRUITING

Thesis for the Degree of Ph. D.
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STANLEY LEWIS FLEGLER
1975

This is to certify that the

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ULTRASTRUCTURAL AND HISTOCHEMICAL CHANGES
IN THE BASIDIOMYCETE DOLIPORE SEPTUM
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presented by

Stanley Lewis Flegler

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William D. Fields

Major professor

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ABSTRACT

ULTRASTRUCTURAL AND HISTOCHEMICAL CHANGES IN THE BASIDIOMYCETE DOLIPORE SEPTUM ASSOCIATED WITH FRUITING

By

Stanley Lewis Flegler

An ultrastructural study of dolipore septa in Psilocybe mexicana, Volvariella bombycina, Amanita muscaria, Favolus alveolaris, Lycoperdon perlatum, Hericium coralloides, and Dacrymyces stillatus showed closed pore occlusions in the vegetative mycelium and open or absent pore occlusions in the hymenium. Dolipore septa in basidiocarp initials, stipes, and lamellae of P. mexicana had open pore occlusions. Septa in vegetative mycelium of D. stillatus had parenthesomes with no pores while the septa in the hymenium had a single pore in the parenthesome. An ultrastructural histochemical study of the dolipore septum in vegetative hyphae of P. mexicana showed digestion of the pore occlusion with trypsin and chymotrypsin. A periodic acid, thiosemicarbazide, and silver proteinate stain for polysaccharide produced no staining of the pore occlusion. Nucleic acid extraction with perchloric acid produced no changes in the pore occlusion. High magnification electron micrographs showed the pore occlusion to have a granular substructure. Microfilaments in the pore canal showed points of attachment at regions of electron dense material within the pore canal and at the pore occlusion. Some microfilaments passed out through the parenthesomes. Serial sections demonstrated that electron dense regions within the pore canal were

rings which did not block the canal. An ATPase localization procedure showed deposition of reaction product on the electron dense rings of septa in vegetative mycelium and within the pore canal of septa in vegetative mycelium and lamellae. The data and literature indicated a possible method and function of the dolipore septum in controlling protoplasmic streaming for fruiting.

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By

Stanley Lewis Flegler

A THESIS

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INTRODUCTION

The fruiting process of the mushrooms and other fungi of the class basidiomycetes is not well understood. Of the many thousands of species of wild mushrooms, only a few dozen have ever fruited artificially in culture. Because of the economic importance of mushrooms to the food and drug industry, considerable study has gone into this problem. The result of the studies have been discouraging in that no major unifying concepts have been discovered and little success has been had in inducing fruiting in other species of mushrooms.

In my ultrastructural investigations of vegetative mycelium and fruitbodies of mushrooms, I observed differences in the dolipore septum, a specialized septal structure first reported by Girbardt (1958) which is found in the class basidiomycetes. In vegetative mycelium, the septum appeared to be blocked by structures called pore occlusions. The fruitbodies had septa that were not blocked. This phenomenon had never been reported before, because no in-depth ultrastructural studies had been performed on both vegetative mycelium and fruitbodies of the same species. I decided to do comprehensive ultrastructural and histochemical studies on dolipore septa to determine if the septa could be of importance in the fruiting process.

METHODS AND MATERIALS

Mycelial cultures of Amanita muscaria (Fries) S. F. Gray, Hericium coralloides (Scop. ex Fries) S. F. Gray, Favolus alveolaris (D. C. ex Fries) Quel., Dacrymyces stillatus Nees ex Fries, Volvariella bombycina var. bombycina (Shaeff. ex Fries) Singer and Lycoperdon perlatum Pers. were obtained by tissue culture of the basidiocarps collected in the field and grown on B agar (17 g. corn meal agar, 2 g. glucose, 3 g. sucrose, 1 g. yeast extract, and 1000 ml. water). Basidiocarps of all the above except V. bombycina were fixed directly after collecting. Mycelial cultures and basidiocarps of Psilocybe mexicana Heim were obtained by mating single spore cultures on B agar. Basidiocarps of V. bombycina for fixation were produced on half-strength B agar. All mycelial cultures produced clamp connections except D. stillatus and V. bombycina, both of which do not normally produce these structures (McNabb, 1973; Shaffer, 1957).

Tissues were prepared for electron microscopy by a modification of the methods of Hess (1966). All material was fixed 2 hrs. in 3% glutaraldehyde-3% acrylic aldehyde (acrolein) followed by 2 hrs. in 1% osmium tetroxide. Both solutions were buffered at pH 7.2 with 0.1 M sodium cacodylate. Fixed tissues were dehydrated in a graded ethanol series and embedded in an ERL epoxy resin mixture (Spurr, 1969). All procedures through dehydration were carried out at ca. 4° C. Thin sections were stained with 1% uranyl acetate in 100% methanol-70%

ethanol (1:3) and subsequently in 1% lead citrate in water and examined in a Philips 300 transmission electron microscope.

Tissues selected for enzyme digestion in thick sections were cut into 200-300 µm. sections and prepared as above with a 4 hr. incubation in 0.3% trypsin (Worthington Biochemicals Corp., code TRL, 197.3 units/mg.) in the buffer at 37° C. preceding the osmium tetroxide. Controls were incubated in the buffer only.

Tissues selected for polysaccharide staining, nucleic acid extraction, and thin section enzyme digestion were fixed 2 hrs. in 2.5% glutaraldehyde followed by 2 hrs. in 1% osmium tetroxide. The osmium step was omitted in material for thin section enzyme digestion, using modifications of the procedures of Shepard (1968). Both solutions were buffered at pH 7.2 with 0.1 M sodium cacodylate. Fixed tissues were dehydrated in a graded ethanol series followed by a transition to propylene oxide and embedded in an epon-araldite resin mixture (Anderson and Ellis, 1965). All procedures through dehydration were carried out at ca. 4° C.

Staining for polysaccharide was carried out using modifications of the methods of Anderson and Personne (1970), Courtoy and Simar (1974), and Thiery (1967). Thin sections were collected on titanium grids and stained 45 min. in 1% periodic acid, rinsed and soaked in two changes of water for 20 min., then stained 45 min. in 1% thiosemicarbazide in 10% acetic acid. Subsequently the grids were rinsed and soaked in two changes of 10% acetic acid for 20 min. followed by a rinse and soak for 5 min. in each 5% and 1% acetic acid and finally in distilled water. The grids were then stained 30 min. in 1% silver proteinate in the dark, followed by a rinse in water. All solutions were used at ca. 24° C.

Control grids were stained either in the uranyl acetate and lead citrate as previously described or in the above procedure with the thiosemicarbazide step or with the periodic acid step omitted, or in the silver proteinate only.

Thin sections for enzyme digestion were mounted on copper grids and incubated in either 0.3% trypsin (Worthington, code TRL, 197.3 units/mg.) or 0.3% alpha-chymotrypsin (Worthington, code CDI, 58.5 units/mg.) in distilled water adjusted to pH 6.8 with 0.01 N NaOH at 37° C. for varying lengths of time. Control grids were incubated in the same solution minus the enzymes. The grids were then stained in uranyl acetate and lead citrate as previously described.

Nucleic acid extraction was performed according to a modification of the procedures of Leduc and Bernhard (1961) and Douglas (1970). Thin sections were mounted on copper grids and incubated in 10% perchloric acid at 37° C. for periods of up to 18 hrs. Control grids were incubated in water adjusted to pH 1.5 with 0.1 N HCl for the same time periods. The grids were then stained in uranyl acetate and lead citrate.

Localization of ATPase activity was performed according to the procedures of Gilder and Cronshaw (1973).

RESULTS

The vegetative mycelium of Psilocybe mexicana produced dolipore septa (Figs. 1, 2) similar to those described by Moore and Marchant (1972) for Polyporus biennis. The appearance of septa was similar in vegetative mycelium both before and after fruiting. The basidiocarp initials (1-2 mm. diameter) produced dolipore septa (Fig. 3) in which the pore occlusion no longer appeared to block the pore channel as it did in the vegetative mycelium. The dolipore septa in the stipe of mature basidiocarps (Fig. 6) also showed an open pore occlusion and in addition the parenthesomes usually were indented on the side of the hyphae toward the culture medium. The pore occlusions in the septa of the lamellae were also open (Figs. 4, 5).

The vegetative mycelium of Volvariella bombycina produced septa (Fig. 7) with a definite, though irregular, pore occlusion while septa in the lamellae (Fig. 8) had no pore occlusion. The vegetative mycelium of Amanita muscaria produced septa (Fig. 9) with a well defined electron transparent pore occlusion while the occlusion of septa in the lamellae (Fig. 10) was perforated. Favolus alveolaris vegetative mycelium (Fig. 11) septa exhibited closed pore occlusions while the septa in the hymenium had no occlusions (Fig. 12). Lycoperdon perlatum vegetative mycelium (Fig. 13) had septa with electron transparent pore occlusions while septa in the gleba (Fig. 14) had open occlusions or no occlusions. Hericium coralloides vegetative mycelium had septa (Fig. 15) with large electron dense occlusions while septa of the hymenium (Fig. 16) had open

Figure 1. Longitudinal section of a dolipore septum of vegetative mycelium of Psilocybe mexicana showing the parenthesome (P) and the closed pore occlusion (O).

Figure 2. Transverse section of a dolipore septum of vegetative mycelium of Psilocybe mexicana showing a closed pore occlusion.

Figure 3. Longitudinal section of a dolipore septum of a basidiocarp initial of Psilocybe mexicana showing an open pore occlusion.

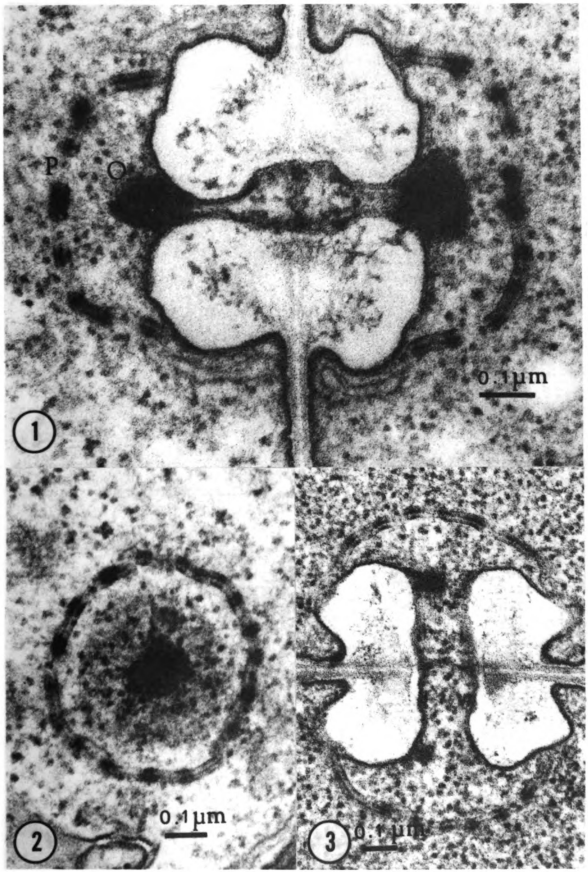

P
O
0.1μm
1
0.1μm
2
0.1μm
3

Figure 4. Longitudinal section of a dolipore septum of lamellae of Psilocybe mexicana showing an open pore occlusion.

Figure 5. Transverse section of a dolipore septum of lamellae of Psilocybe mexicana showing an open pore occlusion.

Figure 6. Longitudinal section of a dolipore septum of the stipe of Psilocybe mexicana showing an open pore occlusion and an indentation in the parenthesome.

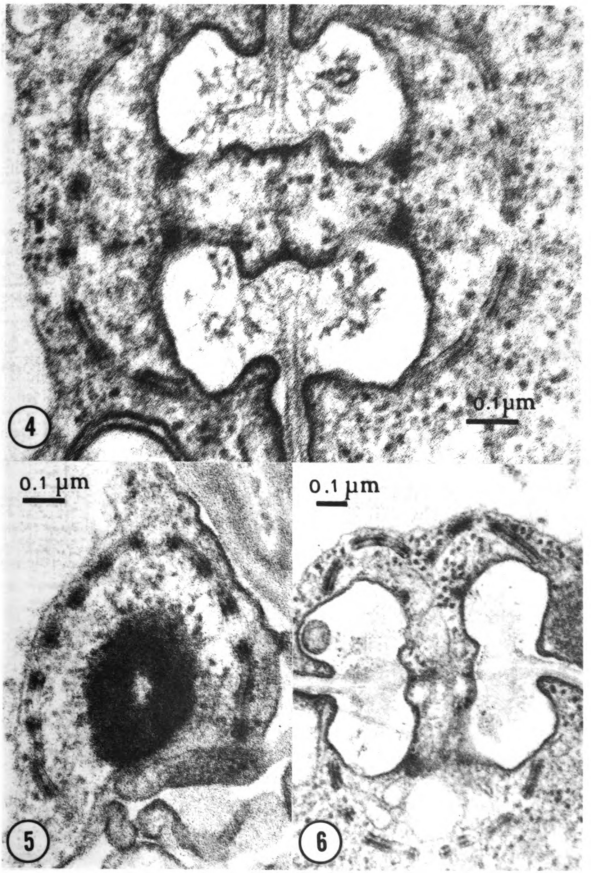

4
0.1 µm
0.1 µm
5
0.1 µm
6

Figure 7. Longitudinal section of a dolipore septum of vegetative mycelium of Volvariella bombycina showing a closed pore occlusion.

Figure 8. Longitudinal section of a dolipore septum of lamellae of Volvariella bombycina showing an open pore occlusion.

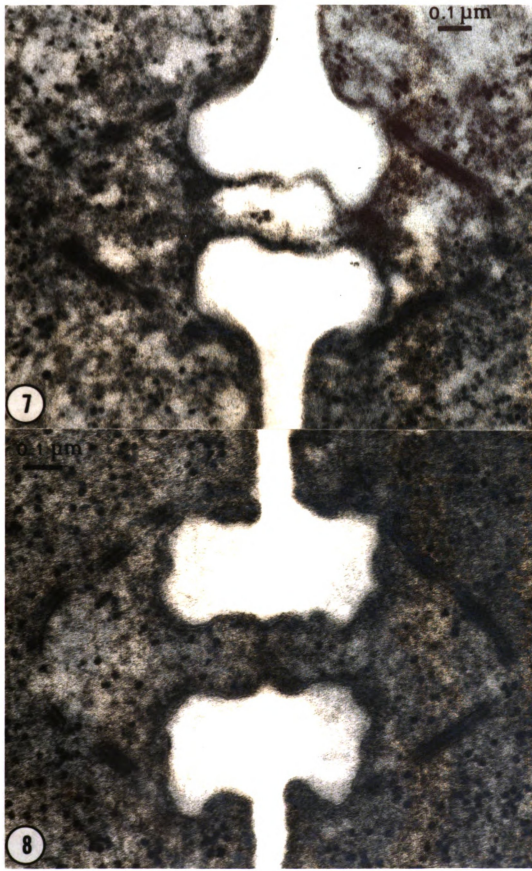
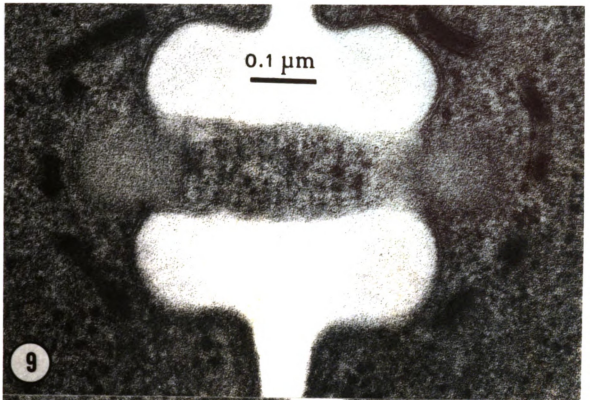

0.1 µm
7
0.1 µm
8

Figure 9. Longitudinal section of a dolipore septum of vegetative mycelium of Amanita muscaria showing a closed electron transparent pore occlusion.

Figure 10. Longitudinal section of a dolipore septum of lamellae of Amanita muscaria showing an open pore occlusion. The left side of the section was not perfectly median.


0.1 µm
9

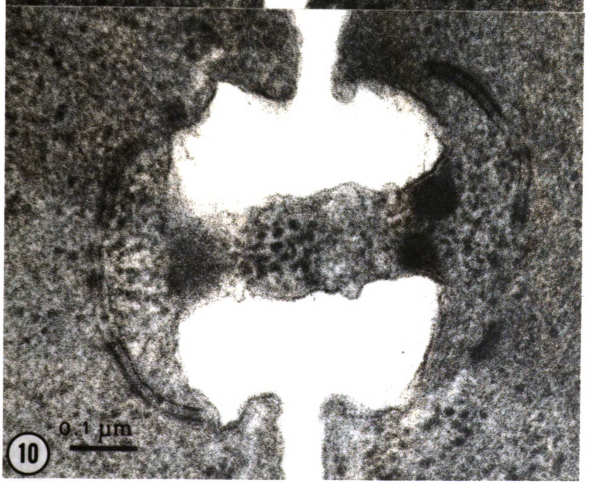
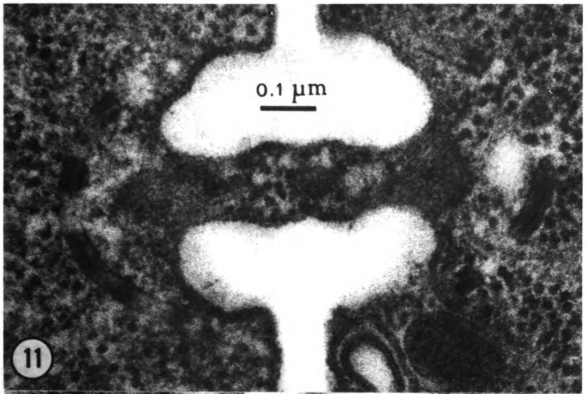

0.1 µm
10

Figure 11. Longitudinal section of a dolipore septum of vegetative mycelium of Favolus alveolaris showing a closed pore occlusion.

Figure 12. Longitudinal section of a dolipore septum of the hymenium of Favolus alveolaris showing an open pore occlusion. The right side of the section was not perfectly median.


0.1 µm
11

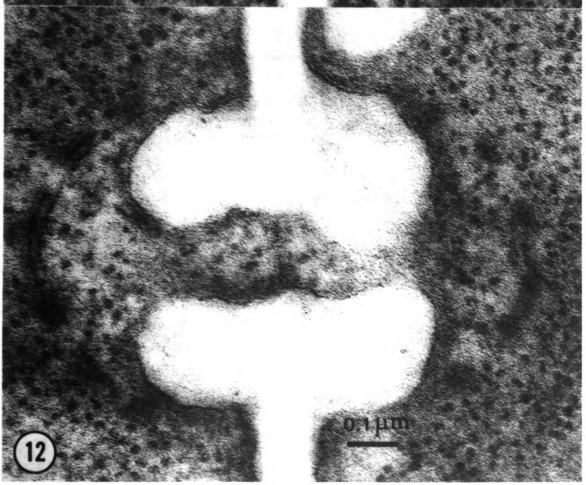

0.1 µm
12

Figure 13. Longitudinal section of a dolipore septum of vegetative mycelium of Lycoperdon perlatum showing a closed electron transparent pore occlusion.

Figure 14. Longitudinal section of a dolipore septum of the gleba of Lycoperdon perlatum showing an open pore occlusion.

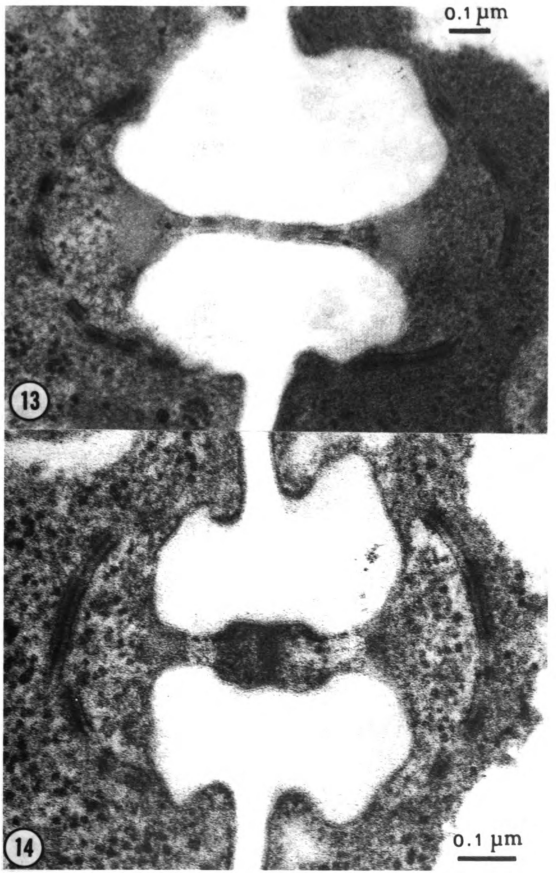
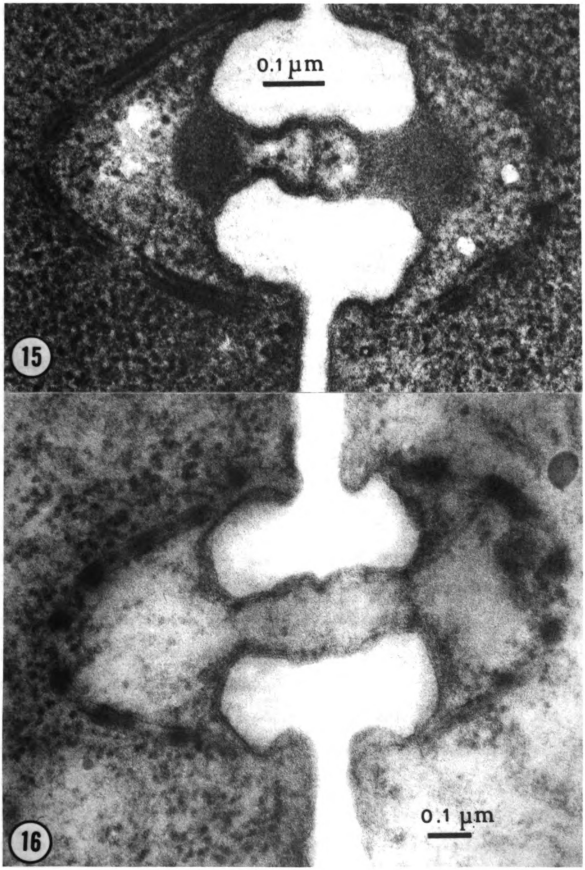

0.1 µm
13
14
0.1 µm

Figure 15. Longitudinal section of a dolipore septum of vegetative mycelium of Hericium coralloides showing a closed electron dense pore occlusion.

Figure 16. Longitudinal section of a dolipore septum of the hymenium of Hericium coralloides showing an open pore occlusion.


0.1 µm
15
16
0.1 µm

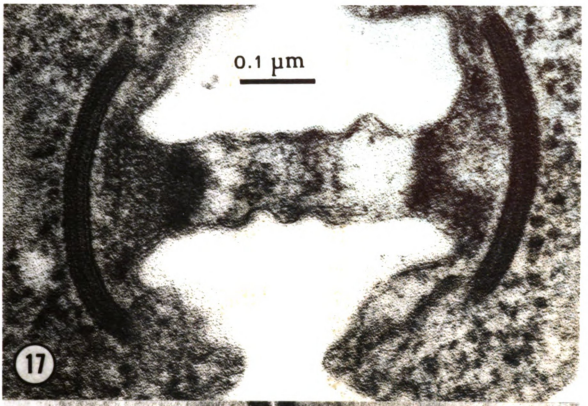
occlusions or no occlusions. Dacrymyces stillatus vegetative mycelium produced small coarse septa (Fig. 17) with dense, closed pore occlusions and parenthesomes with no openings while the hymenium (Fig. 18) produced small coarse septa with a small opening in the pore occlusions and a single hole in the parenthesome. In all cases the generalizations noted above held true for all of the septa examined in a particular vegetative or reproductive state. Most dolipore septa were of approximately the same dimensions with the exception of D. stillatus.

Septa of Psilocybe mexicana vegetative mycelium showed pore occlusions with a granular substructure (Fig. 20) and with microfilaments in the pore canal (Fig. 19) most of which appeared to terminate in the occlusions. A few microfilaments were observed passing through the occlusions and out through the parenthesomes. The microfilaments also showed an attachment to rings of electron dense material within the pore canal (Fig. 19). There usually was a distinct central ring with a less distinct ring on both sides (Figs. 19, 24, 25, 28, 30, 32, 33). The lateral rings usually showed an association with the constrictions of the septal swelling (Fig. 19). Analysis of serial sections of septa in cross-section and longitudinal section (Figs. 21-26) showed the electron dense material to be true rings and not a continuous membrane across the pore canal. Endoplasmic reticulum was often found connected to the ends of the parenthesomes (Fig. 19).

Trypsin digestion of thick sections (Fig. 27) produced septa with the pore occlusions disrupted and digested while controls showed the occlusions intact. Digestion of thin sections with trypsin (Fig. 28) and alpha-chymotrypsin (Fig. 29) showed digested pore occlusions while controls (Fig. 30) had pore occlusions present. The relatively poor fixation in these hyphae is probably the result of lack of osmium

Figure 17. Longitudinal section of a dolipore septum of vegetative mycelium of Dacrymyces stillatus showing a closed pore occlusion and an imperforate parenthesome.

Figure 18. Longitudinal section of a dolipore septum of the hymenium of Dacrymyces stillatus showing an open pore occlusion and a parenthesome with a single aperture. The right side of the section was not perfectly median.


0.1 µm
17

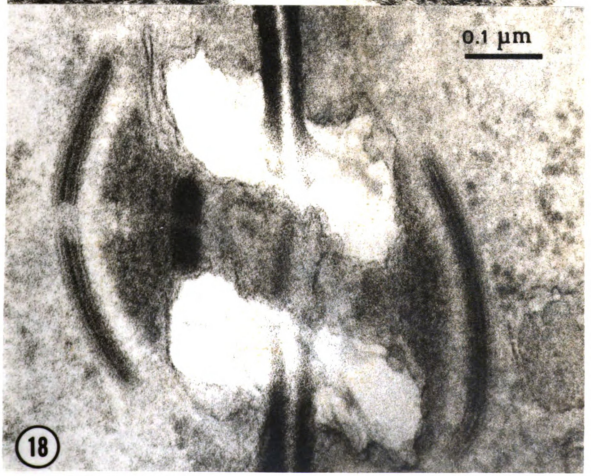
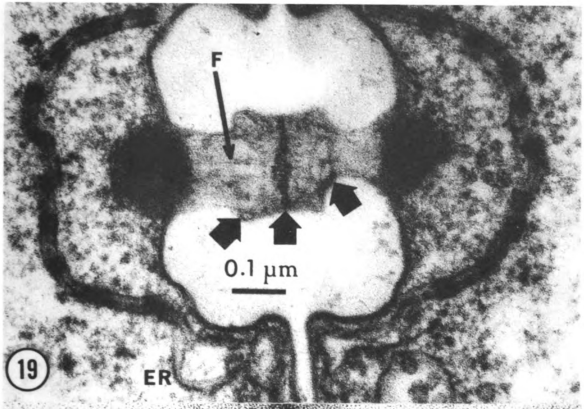

0.1 µm
18

Figure 19. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium showing the endoplasmic reticulum (er) and microfilaments (f). The arrows indicate the location of the three electron dense regions.

Figure 20. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium showing the granular nature of the pore occlusion.


F
0.1 µm
19
ER

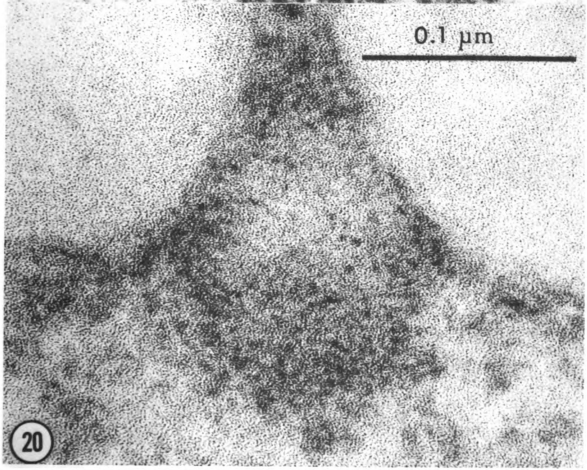

0.1 µm
20

Figure 21-23. Transverse serial sections of a dolipore septum of Psilocybe mexicana vegetative mycelium taken at the same magnification. Figure 21 was taken through the center of the dolipore at the location of the cell wall. Figure 22 was taken distal to the center of the dolipore. Figure 23 was taken at the point of constriction of the pore canal as represented by the right arrow of Figure 19. All sections show the electron dense material in the pore canal to be in the form of rings.

Figure 24-26. Longitudinal serial sections of a dolipore septum of Psilocybe mexicana vegetative mycelium taken at the same magnification. Figure 24 was taken through the center of the dolipore. The open nature of the electron dense material in the center of the pore canal is shown. Figure 25 was taken near the edge of the pore canal and shows the continuous nature of the electron dense material at this point. Figure 26 was taken distal to the pore canal.

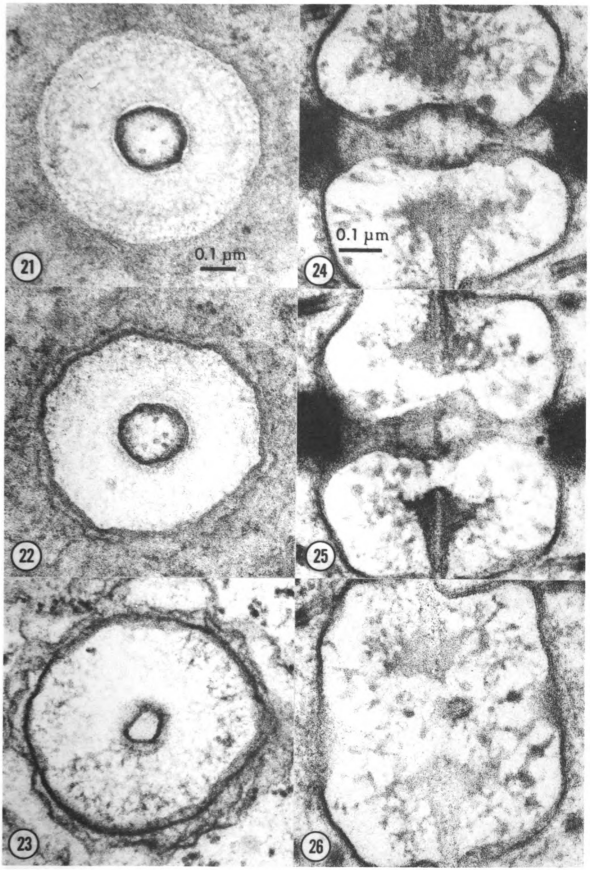
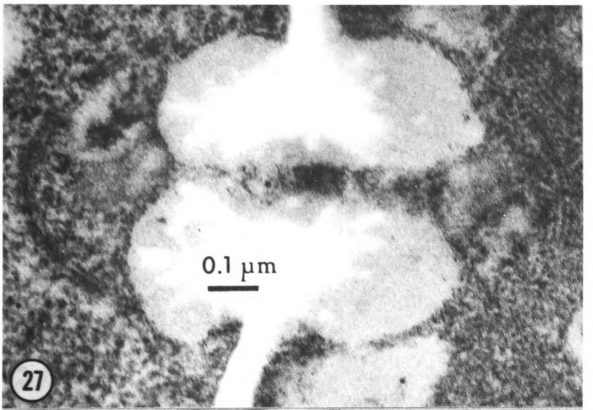

0.1 µm
21
22
23
0.1 µm
24
25
26

Figure 27. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium showing digested pore occlusions. Thick sections of the mycelium were incubated 4 hr. in trypsin.

Figure 28. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium showing digested pore occlusions. Thin sections of embedded material were incubated 90 min. in trypsin.


0.1 μm
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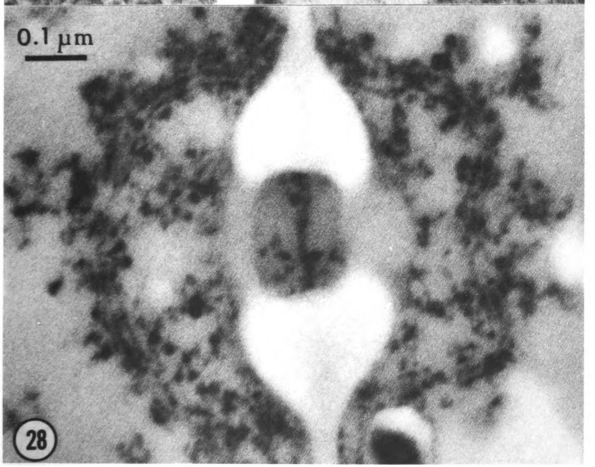
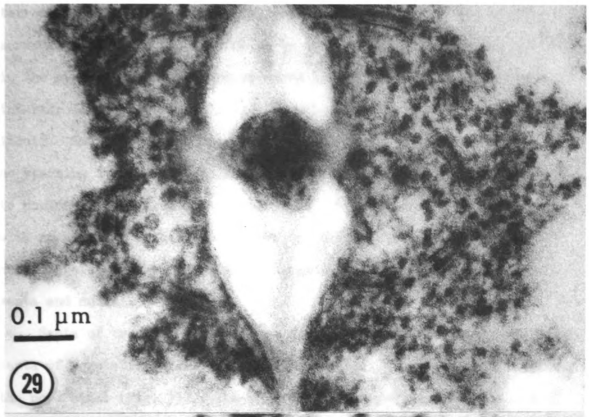

0.1 μm
28

Figure 29. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium showing digested pore occlusions. Thin sections of embedded material were incubated 6 hr. in chymotrypsin.

Figure 30. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium showing intact pore occlusions. Thin sections of embedded material were incubated 6 hr. in a control solution without enzymes.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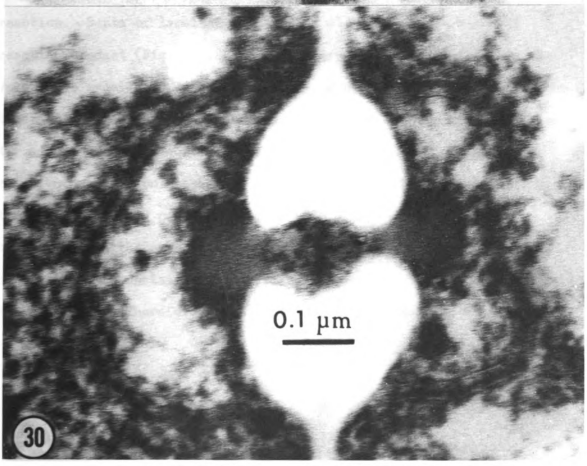
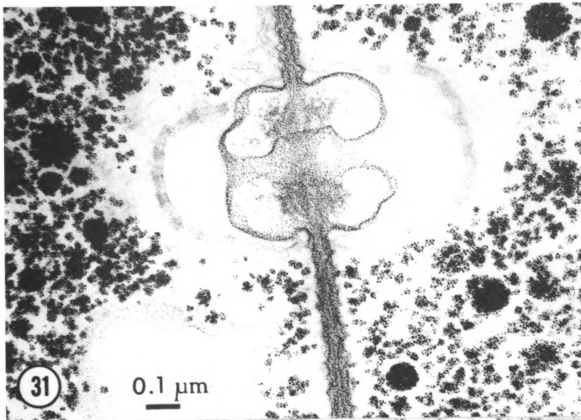

0.1 µm
30

tetroxide post-fixation. The polysaccharide stain (Fig. 31) showed accumulations of polysaccharide to be abundant and randomly distributed in the hyphae. Some staining occurred in the cell wall, plasmalemma, interior of the septal swelling, parenthesomes, and the pore canal itself. The pore occlusion showed no staining. Control sections showed no staining attributable to the polysaccharide stain. Hyphae subjected to nucleic acid extraction showed septa (Fig. 32) that appeared normal with no apparent effect on the pore occlusion. The chromatin and nucleoli of nuclei appeared to be less dense. Controls showed normal septa and nuclei.

Septa of treated hyphae in the ATPase localization procedure showed septa of vegetative mycelium to have deposits of reaction product within the pore canal (Fig. 33). The rings in the pore canal showed a strong reaction. Septa of lamellae also showed a large concentration of reaction product (Fig. 34). The rings were not as distinct as in the vegetative mycelium. In general, septa of hyphae with ATP added in the treatment showed more reaction product than did the treatments with no ATP added. Septa in control treatments with NaF added showed some reaction product, but less than the other treatments. Grids of each treatment which had not been stained with uranyl acetate and lead citrate showed the same pattern as stained grids. Because of the low contrast of the unstained grids, visualization of ultrastructural detail was difficult. Therefore, most observations were made on stained grids.

Figure 31. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium stained for polysaccharides. Deposition of reaction product within the cytoplasm, plasmalemma, cell wall, and parenthesomes is shown. No reaction product was deposited on the pore occlusions.

Figure 32. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium treated in a nucleic acid extraction procedure. There was no effect on the pore occlusions.


31
0.1 μm

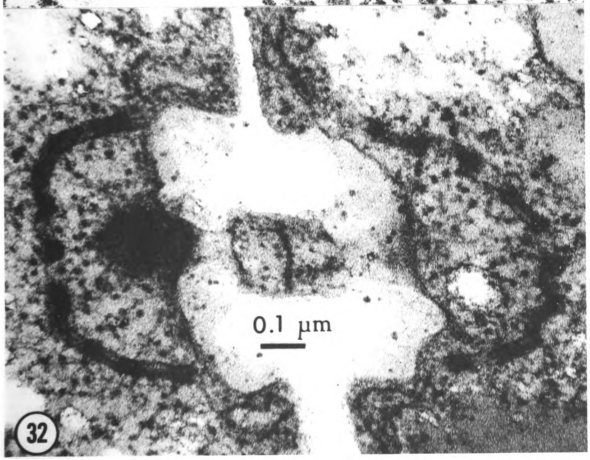
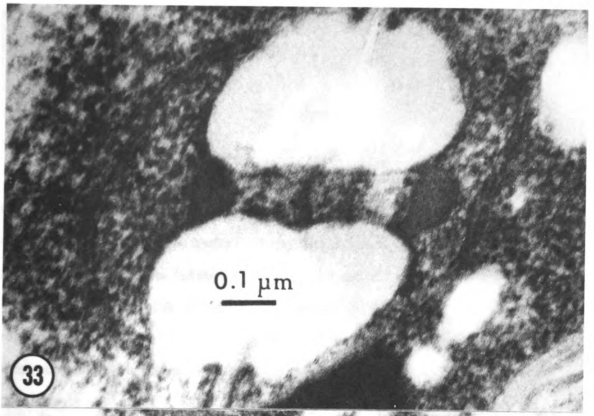

0.1 μm
32

Figure 33. Longitudinal section of a dolipore septum of Psilocybe mexicana vegetative mycelium treated in an ATPase localization procedure. Reaction product was produced within the location of the three electron dense regions within the pore canal.

Figure 34. Longitudinal section of a dolipore septum of Psilocybe mexicana from lamellae tissue treated in an ATPase localization procedure. Reaction product was produced within the pore canal.


0.1 µm
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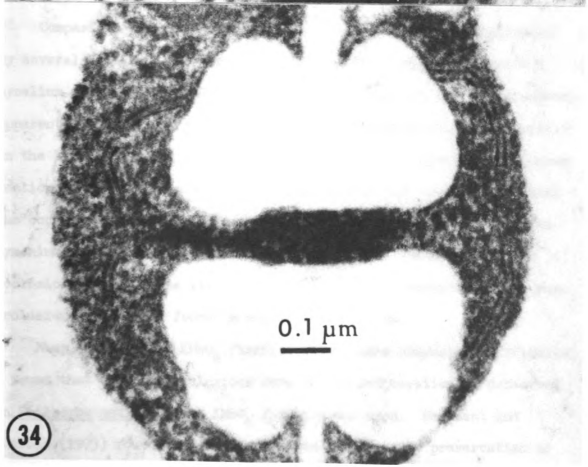
0.1 µm
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DISCUSSION

The results indicate that the dolipore septa of basidiomycete vegetative mycelium characteristically possess closed pore occlusions while occlusions with a central canal or septa lacking occlusions are characteristic of the hymenium or lamellae of all species studied. In Psilocybe mexicana the closed occlusion is characteristic of the vegetative mycelium before and after fruiting has occurred, while the open occlusion is characteristic of basidiocarp initials, stipes, and lamellae.

Comparison of these findings with those of others is complicated by several factors. Some authors did not specify whether vegetative mycelium or basidiocarp tissue was used in their studies. Other authors apparently did not observe median sections through the dolipore septa. In the latter instance it is possible to observe an open pore occlusion sectioned through the rim (Fig. 5), rather than the center thus making the occlusion appear closed. In my study some pore occlusions in the hymenium did appear closed while most were open. These closed pore occlusions appear to be attributable to non-median sections. Open pore occlusions were never found in vegetative mycelium.

Many authors used $KMnO_4$ fixation which makes comparison difficult. I found that the pore occlusions were poorly and erratically preserved in Psilocybe mexicana when $KMnO_4$ fixation was used. Marchant and Wessels (1973) found that $KMnO_4$ fixation gave erratic preservation of

the pore occlusions of Schizophyllum commune while acrolein-osmiun fixation gave much better preservation. Setliff et al. (1972b) published micrographs indicating that $KMnO_4$ gave poor preservation of the pore occlusions in Polyporus tomentosus, Poria latemarginata, and Rhizoctonia solani while glutaraldehyde-osmium gave good preservation.

Where glutaraldehyde-osmium or acrolein-osmium fixation was used, results are consistent with my findings. Setliff et al. (1972b) displayed open occlusions in the basidiocarps of Polyporus tomentosus and Poria latemarginata. Likewise Ellis et al. (1972) found open occlusions in the basidiocarps of Coprinus stercorarius. Thielke (1972a) observed a similar situation the basidiocarps of Stropharia rugosa annulata and Agaricus bisporus, and the basidiocarps of Lycoperdon perlatum (Marchant, 1969) were also reported to have open occlusions. Conversely published studies of vegetative mycelium of Coprinus lagopus (Casselton et al., 1971), Polysticus versicolor (Girbardt, 1958), and Schizophyllum commune (Niederpruem et al., 1971; Koltin and Flexer, 1969; Marchant and Wessels, 1973, 1974; and Mayfield, 1974) show the dolipore septa to be closed by occlusions.

Some exceptions to the above results appear in studies of glutaraldehyde-osmium or acrolein-osmium fixed basidiomycetes. Setliff et al. (1972a) published one micrograph of a dolipore septum in the basidiocarp of Poria latemarginata with one side of the septum completely occluded and the other side open. It appears that this section was not a perfectly median section since subsequent work by the same author (Setliff et al., 1972b) showed septa of the basidiocarp of P. latemarginata with open occlusions. Setliff et al. (1972b) discussed two types of pore occlusions in two of the three species he observed.

One type was associated with a pore rim and the other type was an electron dense occlusion with no rim. It is likely that the occlusions without a rim were open pore occlusions which had been cut thru the edge of the pore occlusion (Fig. 5). A contradictory finding was the occurrence of closed occlusions in the basidiocarps of Clathrus cancellatus (Eyme and Parriaud, 1970). There was no indication that serial sectioning of the hyphae was attempted in this study and a small channel in the occlusions would be evident only in median sections. In my study of Dacrymyces stillatus only careful examination of numerous sections revealed the consistent presence of a small channel in the pore.

Where $KMnO_4$ was used as a fixative it is possible to find dolipore septa of vegetative hyphae figured with closed occlusions (Moore and Marchant, 1972; Moore and Kreger-van Rij, 1972; Kreger-van Rij and Veenhuis, 1971, 1973; Girbardt, 1961; Mayfield, 1974; Heintz and Niederpruem, 1971; Butler and Bracker, 1970; Bracker and Butler, 1963, 1964; and Beneke, 1963 and personal communication) or with open occlusions (Berliner and Duff, 1965; Giesy and Day, 1965; Wilsenach and Kessel, 1965; and Marchant and Wessels, 1973). Manocha (1965) observed open occlusions in basidiocarps of Agaricus bisporus. It seems likely that the conflicting appearance of dolipore occlusions in $KMnO_4$ fixed tissues is an artifact of preservation. Supporting this, Marchant and Wessels (1973) saw open pore occlusions with $KMnO_4$ fixation and closed occlusions with acrolein-osmium fixation in vegetative mycelium of Schizophyllum commune. Freeze-etched material, in which fixation artifacts should be at a minimum, show closed pore occlusions in vegetative mycelium of Polyporus betulinus (Moore and Marchant, 1972).

Finally there are some studies where evaluation is not possible due to the nature of the fixation, use of non-median sections, lack of indication of whether basidiocarps of vegetative mycelium was used, etc, (e.g.: Wells, 1964, 1965; Hyde and Walkinshaw, 1966; Moore and McAlear, 1962; Jersild et al., 1967; Niederpruem and Wessels, 1969; Olah, 1973; Hashioka, 1971; Voelz and Niederpruem, 1964; Foerster et al., 1965; and Raper and Flexer, 1971).

The granular nature of the pore occlusion seen in my study has been observed in other basidiomycetes (Marchant and Wessels, 1973; Moore and Marchant, 1972). Likewise the association of endoplasmic reticulum with the parenthesomes has been observed by others (Ellis et al., 1972; Thielke, 1972a; Marchant and Wessels, 1973; Setliff et al., 1972b; Jersild et al., 1967; Braker and Butler, 1963; Hyde and Walkinshaw, 1966; Wilsenack and Kessel, 1965; Moore and Marchant, 1972; Berliner and Duff, 1965; Giesey and Day, 1965; Marchant, 1969). Ellis et al. (1972) found that in Coprinus stercorarius the endoplasmic reticulum was of the rough type. Microfilaments in the pore canal terminating in the pore occlusions or passing through the parenthesomes have also been observed by others (Moore and Marchant, 1972; Marchant and Wessels, 1973). The attachment of the microfilaments to the electron dense rings has not been reported before. Electron dense regions within the pore canal, which were described as membranes or plugs blocking the canal, were first described by Thielke (1972a, 1972b). My study positively determined that in P. mexicana the electron dense regions are not solid membranes or plugs but rings. Because the diameter of the opening in the rings is about the same thickness of thin sections, most longitudinal sections would not show the opening because one or both

edges of the ring would be included in the thin section. Only serial sections of both longitudinal and cross-sections would show the ring-like nature of the material. There was no indication in Thielke's work (1972a, 1972b) that serial sectioning was performed.

The concentration of ATPase in the pore canal probably indicates that this is a region of high metabolic activity. Schramm (1971) also reported high levels of ATPase in the pore canal. The significance of the ATPase activity and its association with the microfilaments and pore rings is not understood.

The digestion of the pore occlusion with proteolytic enzymes in both thick and thin section indicates that it is made of protein. My results are similar to proteolytic enzyme digestion of various plant and animal structures performed by others (Leduc and Bernhard, 1961; Weintraub and Ragetli, 1968; Douglas et al., 1970; Shepard, 1968; Gouranton and Thomas, 1974). Although histochemical interpretation is difficult, trypsin acts on basic amino acids while chymotrypsin acts on the aromatic neutral amino acids (White et al., 1964; Leduc and Bernhard, 1961; Pearse, 1972). The protein in the occlusion is probably of a complex mixed nature because no difference could be observed in the action of the two enzymes. The granular substructure observed in the pore occlusion may indicate a complex folded nature of the protein.

The absence of a reaction product in the pore occlusion with the polysaccharide stain indicates that it probably has little or no polysaccharide component. The reaction within the cell wall is to be expected because of the complex polysaccharides present in fungal cell walls (Janszen and Wessels, 1970; Wessels and Marchant, 1974; Vorisek et al., 1974. Similar results were observed in the cell walls of other

basidiomycetes (McLaughlin, 1974; Matthews and Niederpruem, 1973) and an ascomycete (Vorisek et al., 1974). Staining in the cytoplasm was random and similar to the pattern observed by Matthews and Niederpruem (1973) in primordia of Coprinus lagopus. Except for the absence of staining in the parenthesomes, septa of Coprinus lagopus showed a staining reaction similar to mine (McLaughlin, 1974). Since septa in the lamellae of C. lagopus have virtually no trace of a pore occlusion, comparison of this structure is not possible.

The absence of any effect of the nucleic acid extractions on the pore occlusions indicates that DNA or RNA is probably not a significant structural component of the pore occlusion. McLaughlin's studies (1972) indicated a possibility of RNA in the cytoplasm surrounding the parenthesomes of septa in the lamellae of Coprinus lagopus. No similar reaction was found in my studies.

The function of the dolipore septum has long been thought to be that of maintaining a stable nuclear condition, allowing nuclear migration, and allowing for cytoplasmic continuity between cells (Bracker, 1967; Mayfield, 1974). Possibly the function of regulating cytoplasmic streaming for fruiting should also be considered. Changes in the appearance of the pore occlusion of dolipore septa are correlated with fruiting. The pore occlusion may act as a valve to prevent or limit protoplasmic streaming in the vegetative mycelium and to allow increased protoplasmic streaming when fruiting begins. Butler and Bracker (1970) thought that in Rhizoctonia solani the pore occlusion acted as a plug for aged cells or in cells under stress. We found no support for this argument. We observed closed pore occlusions in very young hyphae and in very old hyphae of Psilocybe mexicana. The fixative

($KMnO_4$) used by these workers may have been more disruptive to young than to older hyphae thus leading them to the conclusion that young hyphae were not plugged by the pore occlusions. Setliff et al. (1972b) mentioned that open occlusions in a basidiocarp may allow for nutrient transport, although no comparison was made of vegetative mycelium and basidiocarps of the same species. Dense aggregrations of virus particles have been found in the dolipore septa of diseased basidiocarps of Agaricus bisporus (Dieleman-van Zaayen, 1972). Symptoms of the virus disease were changes in the rate of growth and gross abnormalities of the basidiocarp (Dieleman-van Zaayen, 1969). Possibly the symptoms are partially due to the virus particles blocking the pore canal and limiting protoplasmic streaming. In Dacrymyces stillatus the parenthesome may be involved in the regulation of protoplasmic streaming in that the parenthesomes in the basidiocarp have a single opening (Fig. 18) while those in the vegetative mycelium appear without openings (Fig. 17). Because the openings are very small, it is often necessary to look at many sections before they may be found. Solid parenthesomes have also been reported in Polyporus tomentosus (Setliff et al., 1972b) and Exidia glandulosa (Raper and Flexer, 1971).

The histochemical experiments show the pore occlusion probably is protein. A possibility of other components associated with the protein exists. The occlusion could be lipoprotein. No attempt was made to look for a lipid component because adequate methods do not exist (Pearse, 1972). If the pore occlusion does regulate protoplasmic streaming for fruiting, then a simple protein composition might be advantageous for that regulation. A proteolytic enzyme specific for the pore occlusion could be released when environmental and nutritional

conditions are favorable. Endoplasmic reticulum is a site of enzyme synthesis, and enzymes specific for digestion of the pore occlusion may be synthesized at the parenthesome and endoplasmic reticulum association. The microfilaments and pore rings in the septa may also have a function in conjunction with the pore occlusions in regulating protoplasmic streaming. The rings may act in providing a rigid frame to prevent collapse of the pore canal and to serve as an anchor for the microfilaments. The association of the lateral rings near the site of constrictions of the septal swelling gives some support for this theory. Functions in protoplasmic movement, intracellular transport, and maintenance of cell shape have been suggested for microtubules (Bracker, 1967). The microfilaments may function in enzyme transport, perhaps in conjunction with the endoplasmic reticulum, in the opening of the pore occlusion. The microfilaments may function in maintaining the structural integrity of the dolipore as was suggested by Moore and Marchant (1972). Perhaps in an active process requiring ATPase, they hold the pore occlusions in place. When the proper time comes they might cease to hold the occlusions in place or at least allow a portion of the occlusion to be removed, thus allowing increased protoplasmic streaming. Patton and Marchant (1975) treated vegetative hyphae of Polyporus biennis with cytochalasin B in an attempt to disrupt the microfilaments of the septum. No major changes in the septa were reported, but the authors suggested that only a portion of the microfilaments may have been disrupted. The micrograph they presented appeared to show a lack of pore occlusions in the septum, although this was not reported in the results. They did report changes in the form and growth of the hyphae. Because it is not known whether the pore occlusion is dissolved or mechanically allowed to fall away as fruiting begins, either postulated process is possible.

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