DEMONSTRATION OF MYELIN FIGURES IN UNFIXED, FREEZE-ETCHED FUNGUS SPORES

GARRY T. COLE and HENRY C. ALDRICH. From the Department of Botany, University of Florida, Gainesville, Florida 32601

INTRODUCTION

With increased use of glutaraldehyde fixatives (15), membrane whorls of myelin figures have been noted in many tissues prepared for electron microscopy (e.g., 3, 7, 9, 10, 14, 16, 17). Some workers have considered them artifacts produced by glutaraldehyde fixation (5, 12, 13). We observed myelin figures in glutaraldehyde-osmium tetroxide–fixed conidia of *Scopulariopsis brevicaulis*, a member of the Fungi Imperfecti, while following conidiogenesis (4). This report describes studies undertaken to determine whether these myelin figures were artifactual.

MATERIALS AND METHODS

Unfixed *Scopulariopsis brevicaulis* conidia were treated for 8 hr at room temperature with 30% glycerol containing one drop of Teepol detergent per 10 ml solution (8). Cells were then centrifuged, frozen in liquid Freon 22 using gold-nickel specimen holders, and freeze-etched with a Balzers BA360M machine, following the techniques of Moor and Mühlethaler (11). Conidia for sectioning were fixed with glutaraldehyde followed by OsO_4 as previously described (4).

RESULTS

Glutaraldehyde-fixed conidia usually contained large membrane whorls or myelin figures adjacent to the single nucleus (Fig. 1). Frequent association of this myelin figure with endoplasmic reticulum (ER) and with glycogen storage areas led the authors to speculate regarding its role in glycogen secretion. Unfixed, glycerinated, freeze-etched conidia also contain frequent myelin figures (Figs. 2, 3, 4). Spacing of the lamellated membranes in freeze-etched material was very regular (Figs. 2, 3) compared to the fixed material. The irregular spacing in fixed material could have been caused by the fixation or dehydration procedures. These observations suggest that myelin figures in *S. brevicaulis* spores are not artifactual, but reflect the true cellular condition, although the remote possibility that the myelin figures in the freeze-etched material were induced by the glycerol or by the Teepol detergent cannot be ruled out. Similarly, myelin figures noted by other workers might be regarded as real structures. Furthermore, the membrane surfaces of the fungal myelin figures exposed by the freeze-etch process (Fig. 4) are devoid of the particles common to many freeze-etched membranes (2). This lack of particles mimics the situation in freeze-etched myelin sheath (1) and could reflect a low level of metabolic activity in the membranes involved. Certainly the membranes are likely composed mainly of lipid, since freeze-etched lipids exhibit similar smooth surfaces (6).

Received for publication 26 April 1971, and in revised form 9 August 1971.

REFERENCES

1. BRANTON, D. 1967. *Exp. Cell Res.* **45**:703.
2. BRANTON, D. 1969. *Annu. Rev. Plant Physiol.* **20**: 209.
3. CARBONELL, L. M., and L. POLLAK. 1962. *J. Bacteriol.* **83**:1356.
4. COLE, G. T., and H. C. ALDRICH. 1971. *Can. J. Bot.* **49**:745.
5. CURGY, J. J. 1968. *J. Microsc.* **7**:63.
6. DEAMER, D. W., R. LEONARD, A. TARDIEU, and D. BRANTON. 1970. *Biochim. Biophys. Acta.* **219**: 47.
7. FLAKS, B. 1968. *J. Cell Biol.* **36**:410.
8. HESS, W. M., M. M. A. SASSEN, and C. C. REMSEN. 1968. *Mycologia.* **60**:290.
9. HOHL, H. R. 1967. *J. Bacteriol.* **90**:755.
10. MCKEEN, W. E., N. MITCHELL, and R. SMITH. 1967. *Can. J. Bot.* **45**:1489.
11. MOOR, H., and K. MÜHLETHALER. 1963. *J. Cell Biol.* **17**:609.
12. OLLAH, I., and J. ROLLICH. 1966. *Acta Biol. Acad. Sci. Hung.* **17**:65.
13. RASH, J. E., M. K. GEY, and G. O. GEY. 1970. *J. Cell Biol.* **47**(2, Pt. 2):167 a. (Abstr.)
14. ROBARDS, A. W. 1968. *Protoplasma.* **65**:449.
15. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. *J. Cell Biol.* **17**:19.
16. SMITH, D. G., and R. MARCHANT. 1968. *Arch. Mikrobiol.* **60**:340.
17. THOMAS, P. L., and P. K. ISAAC. 1967. *Can. J. Bot.* **45**:1473.

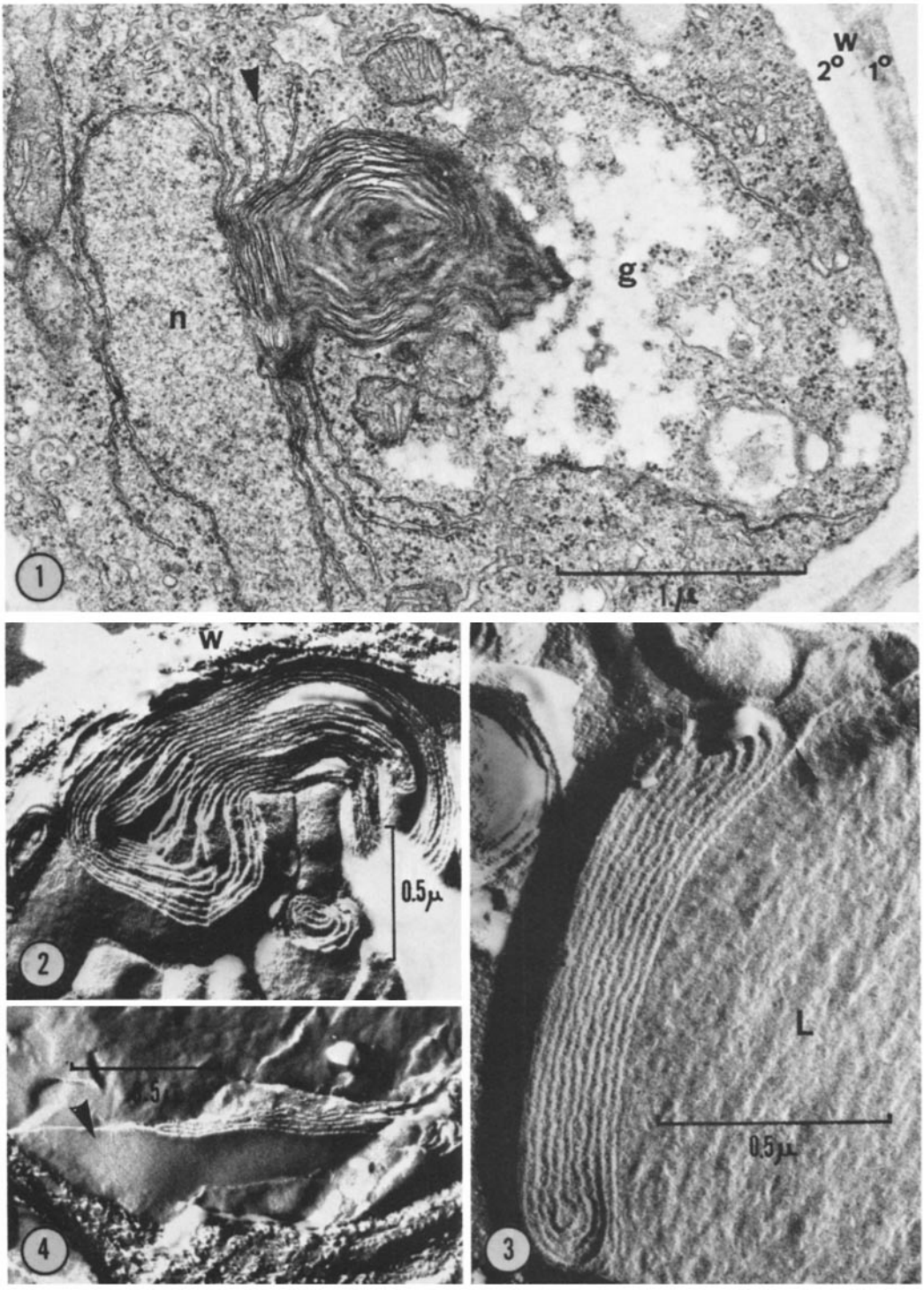

FIGURE 1 Ultrathin section of glutaraldehyde-fixed conidium of *Scopulariopsis brevicaulis*. Nucleus (*n*) at left is flattened against myelin figure, from which extend ER cisternae (arrow). *g*, glycogen. *W1°*, *W2°*: primary and secondary wall. × 31,200.

FIGURE 2 Myelin figure near wall (*W*) in an unfixed, freeze-etched conidium. × 42,000.

FIGURE 3 Cross-fractured myelin figure adjacent to a lipid droplet (*L*). Note generally equidistant spacing of lamellae in this and previous figure. Presumptive peripheral ER connection at arrow. × 63,100.

FIGURE 4 Obliquely fractured myelin figure illustrating smooth nature of the freeze-etched membrane surfaces (arrow). × 50,000.