ULTRASTRUCTURE OF LIPID DEPOSITS AND OTHER CONTENTS IN FREEZE-ETCHED COLEOPTILE CELLS OF UNGERMINATED RICE GRAINS

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Abstract

Freeze-etching has been used to avoid exposure to aqueous media in studying ultrastructure in coleoptile cells of ungerminated rice grains. Results differ in some respects from those obtained after chemical fixation and examination of thin sections. For instance, proliferations of the plasmalemma, shrinkage of protoplasts from cell walls and endoplasmic reticulum profiles surrounding protein bodies, reported from thin-section studies, have not been substantiated. New structures observed are inclusions within lipid bodies, a lipid-rich complex at the plasmalemma, small tubules in the cytoplasm adjacent to protein bodies, and globoid inclusions within some protein bodies.

I. INTRODUCTION

Structure within scutellum cells of ungerminated wheat grains appears to be modified considerably during exposure of the tissue to water for only a few seconds (Swift and Buttrose 1972). Changes equally great may occur before chemicals in aqueous fixatives can act. By using the technique of freeze-etching, contact with aqueous media can be avoided or shortened, and the results obtained can be compared with and extend those of thin-sectioning studies. This comparison has been made for storage tissues of cereal seeds (Buttrose 1971; Swift and Buttrose 1972; Barlow *et al.* 1972) and for pea cotyledons (Swift and Buttrose 1973). Öpik (1972) has used thin-sectioning techniques to describe ultrastructure in an embryo tissue which subsequently enlarges at germination, namely the coleoptile of rice grains. This paper reports a freeze-etch study of this tissue.

II. MATERIALS AND METHODS

Grains of rice (*Oryza sativa* L. cv. Calrose and Kulu) were obtained from Yanco Research Station, New South Wales Department of Agriculture. Coleoptiles from dry grains were examined in section by light microscopy following preparation according to Feder and O'Brien (1968), and by electron microscopy following fixation in 3% glutaraldehyde in phosphate buffer (0·02M, pH 7·0), post-fixation in OsO_4, and embedding in a low-viscosity epoxy resin. For freeze-etching, coleoptiles were dissected from either dry grains or grains after germinating for 2 days. Coleoptiles from dry grains were placed in the well of a gold specimen support either in a drop of 100% glycerol as a non-aqueous medium and frozen as quickly as possible (within 8 s), or in a drop of water and frozen quickly or after 2 hr imbibition. Coleoptiles from germinating grains were placed in water droplets on a specimen support and frozen. Specimens were frozen in liquid Freon 22 cooled by liquid nitrogen, and freeze-etched in a Balzers apparatus. Preparations were routinely etched for 2 min at −100°C.

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III. Results

(*a*) *Microscopy of Sections*

The meristematic parenchyma cells from the apical region of the coleoptile (Fig. 1) contained lipid bodies, protein bodies, plastids, and mitochondria embedded in the ribosome-rich cytoplasm (Fig. 2). Sections of plastids normally enclosed regions of cytoplasm, and showed internal membranes and osmiophilic globules (Fig. 2). Protein bodies could contain spherical inclusions (Fig. 3), and occasionally profiles of the endoplasmic reticulum were seen adjacent to protein bodies (Fig. 3). Mitochondria were simple (Fig. 4). The plasmalemma was frequently identifiable at the cell wall (Fig. 4), and had numerous lipid bodies immediately adjacent to it (Fig. 2).

(*b*) *Freeze-etching*

Lipid and protein bodies together accounted for much of the volume of parenchyma cells (Figs. 5, 6, 7). These bodies were recognized on the basis of their size and numerical frequency in comparison with thin-section data and on the basis of their characteristic textures and internal simplicity in cross-fracture. Plastids could be recognized on the basis of internal membranes (Fig. 5), but mitochondria were difficult to identify. Extended profiles of endoplasmic reticulum were extremely rare. Many very small (0·1 μm) vesicles were present throughout the cytoplasm (Fig. 5).

The perimeter of lipid bodies, which apparently invariably cross-fractured, did not stand out distinctly in glycerol-frozen preparations (Fig. 6), but did in preparations exposed to water for 8 s (Fig. 7). Lipid bodies were not spherical in these preparations, but were almost so after 2 hr imbibition (Fig. 5) and quite spherical after 2 days germination (Fig. 10). They were associated with the cell wall region (Figs. 5, 8), and also were distributed throughout the cytoplasm. A corrugated structure with fracture faces lacking membrane particles (compare with areas of plasmalemma fracture faces) occurred between lipid bodies located at the plasmalemma (Fig. 8). Many cross-fracture profiles of lipid bodies in tissue frozen either in glycerol or immediately in water showed spherical depressions within them (Figs. 6, 7). These depressions were absent after 2 hr imbibition and 2 days germination.

Protein bodies changed in shape from irregular in glycerol-frozen preparations (Fig. 6) to spherical after only a few seconds in water (Figs. 5, 9, 12). Occasionally protein bodies had a spherical inclusion (Fig. 12). The small vesicles distributed in the cytoplasm (Fig. 5) were often close to protein bodies and often elongated or tubular (Fig. 9).

A search was made for concentric whorls of endoplasmic reticulum (Öpik 1972), but without success. The structure illustrated in Figure 11 could be interpreted to be such a whorl, but there are other interpretations. In all cases where the

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Fig. 2.—Electron micrograph of a thin section of a rice coleoptile cell. *CW*, cell wall; *L*, lipid body; *M*, mitochondrion; *PL*, plastid; *P*, protein body.

Fig. 3.—Protein body (*P*) with inclusion, and some endoplasmic reticulum profiles, from a thin section.

Fig. 4.—Edge of a cell in thin section, showing plasmalemma (*PM*) and structures (encircled) which may correspond to vesicles of freeze-etch replicas. *L*, lipid body; *M*, mitochondrion.

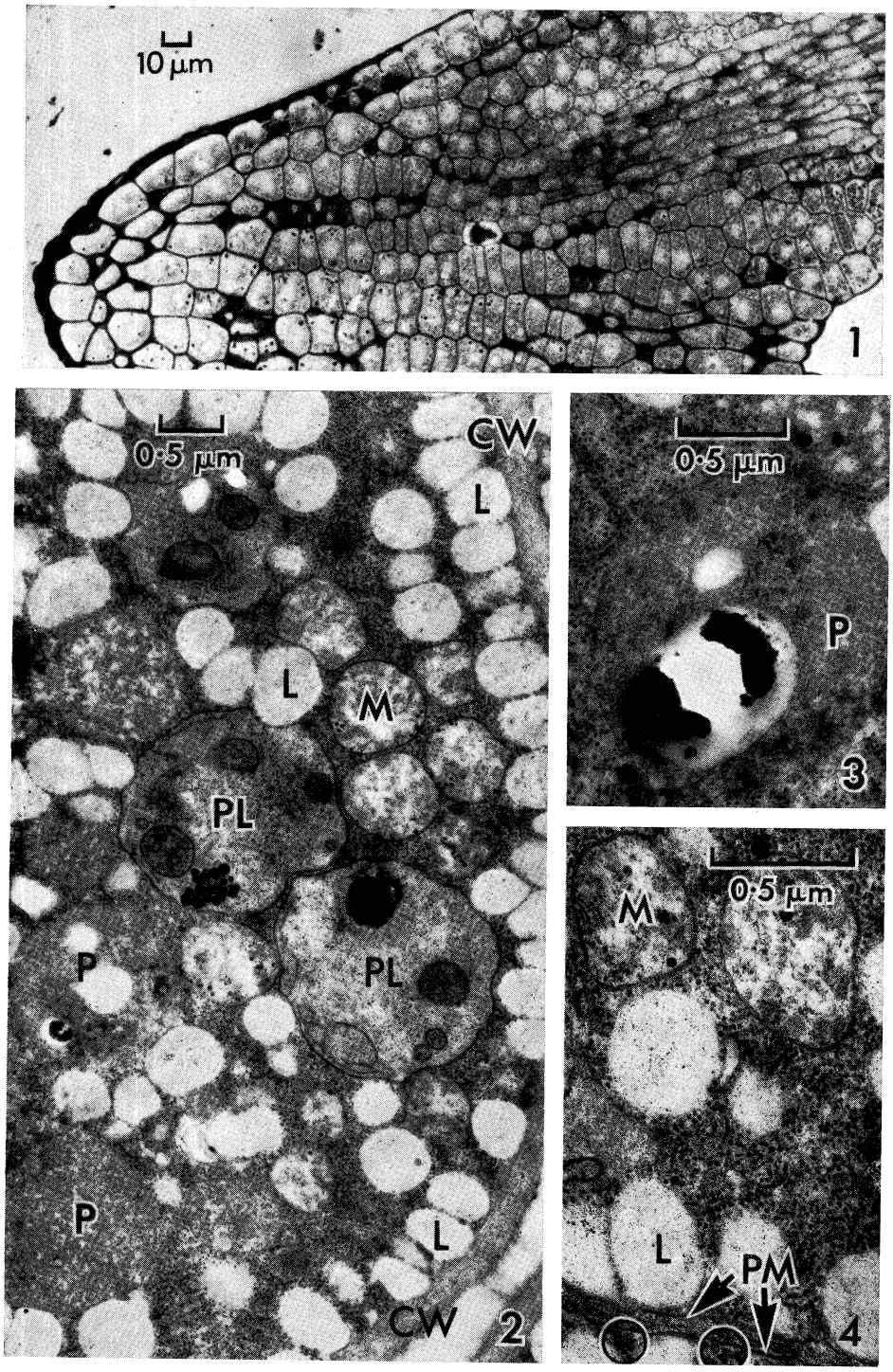
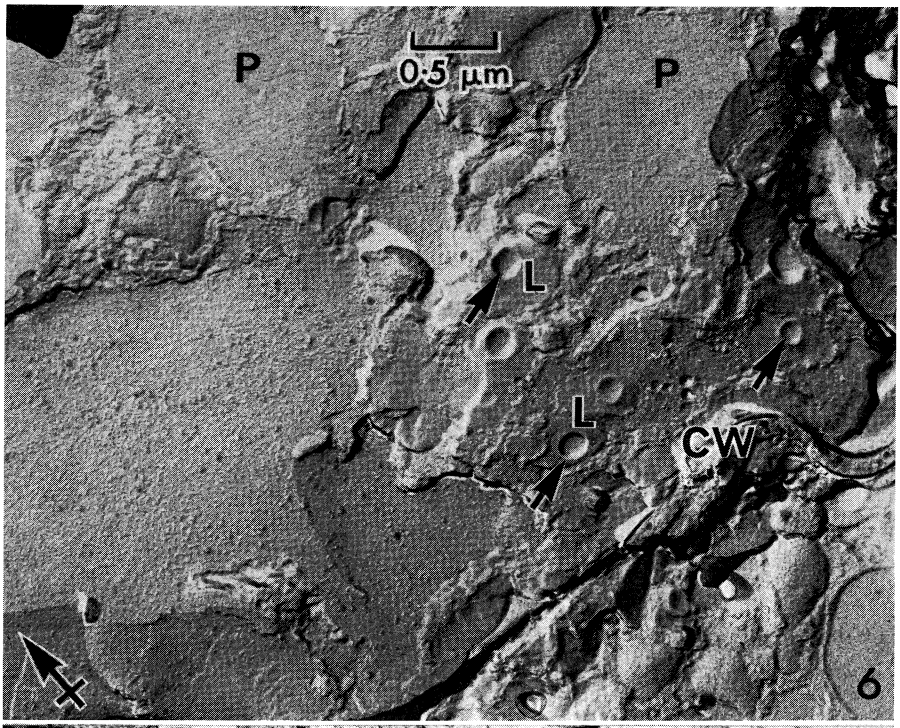


Fig. 1.—Light micrograph of longitudinal section at the apex of a rice coleoptile. PAS–toluidine blue stain.

Fig. 5.—Part of a freeze-etched rice coleoptile cell following 2 hr soaking of the tissue in water. *CW*, cell wall; *L*, lipid body; *P*, protein body; *PL*, plastid; *V*, vesicle. ↕, direction of shadowing (similarly indicated on Figures 6–12).



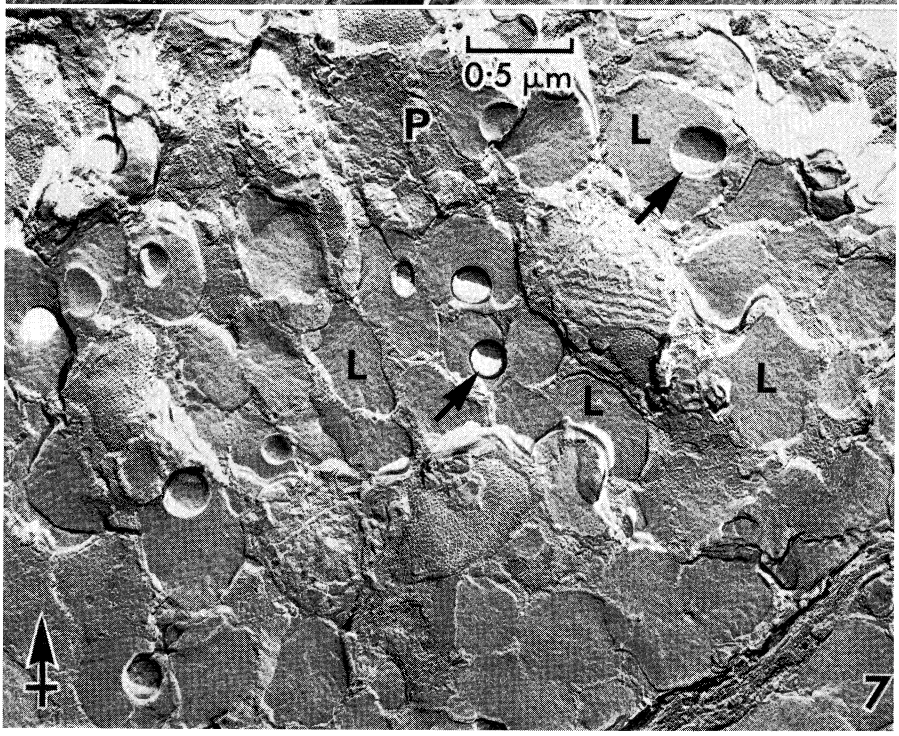


Fig. 6.—Freeze-etched rice coleoptile cell after freezing in 100% glycerol. Arrows point to depressions within lipid bodies (*L*). Note the wavy cell wall (*CW*). *P*, protein body.
Fig. 7.—Freeze-etched rice coleoptile cell after 8 s exposure to liquid water. Arrows point to depressions within lipid bodies (*L*). *P*, protein body.

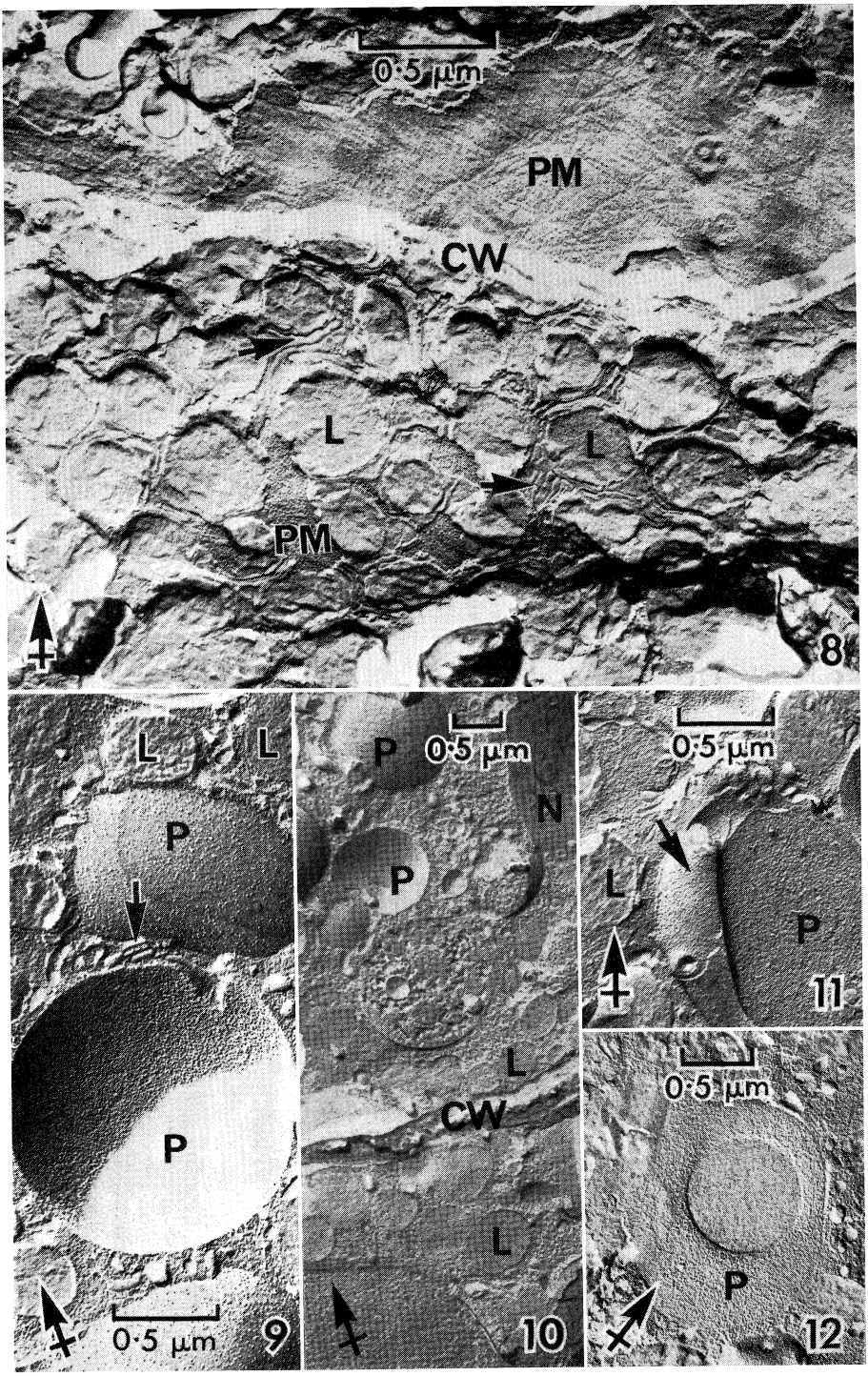


Fig. 8.—Freeze-etch replica from a rice coleoptile cell following 2 hr soaking in water. The upper half shows a fracture face of the plasmalemma (*PM*) facing towards the cell wall (*CW*), the lower half shows small areas of the complementary fracture face of the plasmalemma facing towards the cytoplasm. Arrows point to corrugated structure. *L*, lipid body.

plasmalemma was exposed by fracture, it appeared as an unfolded sheet with only minor striations, due presumably to underlying cellulose fibrils (Fig. 8). The cell walls of non-imbibed tissue had folds (Fig. 6), which were not seen after imbibition.

IV. Discussion

It can be reasoned that the corrugated structure lying between lipid bodies at the plasmalemma is lipid-rich: the texture was similar (smooth and lacking membrane particles) to that of cross-fractured lipid bodies; and fractures, which pass through lipid preferentially (Branton 1969), passed at least as readily through this structure as through the plasmalemma. It is possible that the structures seen in "transverse" fracture as circular vesicles (Fig. 5) appear as tubular (corrugated) structures in "longitudinal fracture" (Fig. 8). This corrugated structure was similar to that ensheathing protein bodies of pea cotyledons (Swift and Buttrose 1973). The results therefore suggest that an almost continuous layer of lipid lies adjacent to the plasmalemma of coleoptile cells in ungerminated rice grains.

Convoluted elaborations of the plasmalemma in thin sections of coleoptile cells of ungerminated rice grains described by Öpik (1972) were not observed in thin sections in the present study. This discrepancy may be due to difference in cultivar. However, it is noted that the convolutions illustrated by Öpik appear to be very similar to the saccules which develop from composite lipid vesicles at the plasmalemma of cotyledon cells from dormant and germinating pea and bean seeds (Mollenhauer and Totten 1971). Our freeze-etch results suggest that, located near the plasmalemma of rice coleoptile cells, there is a system of lipid-rich saccules, which in thin section following tissue preparation according to the method of Öpik (1972) can be confused with the plasmalemma and in freeze-etch replica appears as a corrugated structure. It is possible that composite lipid bodies, or at least lipid saccules, are not confined to lipid-poor pea and bean cotyledons.

Inclusions within lipid bodies have not previously been observed. The present results do not prove that inclusions are entirely surrounded by lipid of the lipid body, or that they represent invaginations of cytoplasm. As they were always represented in replica by concave depressions it appears that their contents were either gaseous or of some very brittle material which was invariably scattered away during fracturing. The nature of these inclusions requires further investigation.

There are certain comparisons which remain to be made between the present results and those of Öpik (1972). In the present study no shrinking of protoplasts away from cell walls was observed; shrinkage observed previously in thin section could have been due to cultivar differences or to artifact. The present results have

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Fig. 9.—Area from a freeze-etch replica of a rice coleoptile cell after 8 s exposure to water. Arrow points to tubular vesicles. *L*, lipid body; *P*, protein body.

Fig. 10.—Area from a freeze-etch replica of a coleoptile cell from a rice grain after 2 days germination. *CW*, cell wall; *L*, lipid body; *N*, nucleus; *P*, protein body.

Fig. 11.—Area from a freeze-etched rice coleoptile cell following 2 hr soaking in water. Arrow points to whorled membrane complex. *L*, lipid body; *P*, protein body.

Fig. 12.—Area from a freeze-etched rice coleoptile cell after 8 s exposure to water. *P*, protein body.

confirmed that endoplasmic reticulum in parallel layers is very infrequent. They have not confirmed, however, that endoplasmic reticulum in close surrounding association with protein bodies is a common feature of the cells of rice coleoptile. However, freeze-etch results show that small tubules can be associated with protein bodies (Fig. 9), and it is possible that the parallel-row arrangement of ribosomes noted in thin section (Öpik 1972) may be due to ribosomes aligned on these tubules. Similar structures have been observed in cells of wheat scutellum (Swift and Buttrose 1972). It was surprising that the concentric complexes of rough and smooth endoplasmic reticulum observed (Öpik 1972) in thin section, especially in vascular bundle cells, were not observed in the Australian cultivars. These complexes were reported to be up to 1 μm in diameter (Öpik 1972) and should have been easily recognizable. The only structure which could be interpreted as such a concentric complex is shown in Figure 11. A structure not described by Öpik (1972) was the spherical inclusion in some protein bodies. This resembled phytin globoids found in protein bodies of barley aleurone (Buttrose 1971) and wheat scutellum (Swift and Buttrose 1972).

V. References

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