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Welwitschia mirabilis: Fine Structure of the Germinating Seed V. The Quiescent and Imbibed Gametophyte Interface

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With 22 figures

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Summary

Gametophyte cells in the gametophyte-embryo interface zone contain less densely packed reserves and smaller protein bodies (mean diameter 1.2 μm) as compared with contiguous and deeper-lying cells. They have more residual cytoplasm, numerous amyloplasts, mitochondria, few microbodies, fragments of ER, but no dictyosomes. Upon imbibition the protein bodies degrade rapidly, starch reserves decline and disappear within 24 h, microbodies increase in number and the ER develops longer single profiles.

After 3 days protein bodies start to fuse, microbodies multiply, the ER becomes extensive and sheetlike, and dictyosomes are observed. Between days 4 and 6 ER-derived minivacuoles are autophagically active, accumulating membrane-bound fibrillar material and engulfing lipid bodies; the cell walls have thickened; and mitochondria exhibit a close spatial relationship with lipid bodies. The outer cells of the mature feeder are crushed against the gametophyte. With imbibition physical wetting of outer gametophyte cells precedes that of interface cells, yet nuclear activation and subsequent cellular activity is accelerated in the latter and stand in sharp contrast to the slower metabolism of the former.

Key words: Welwitschia, seed, gametophyte-embryo interface.

Introduction

In two earlier studies the ultrastructural characteristics of the outer quiescent and outer imbibed cells in the megagametophyte of the *Welwitschia mirabilis* seed were reported on (BUTLER et al., 1979 a, b). It was noted that the volumes of these cells are occupied largely by protein and lipid reserves, and that the vestiges of cytoplasm contain very little by the way of organelles. Upon imbibition however, membranes and membranous structures increased in clarity and in quantity, and there was a proliferation in organelles such as microbodies, mitochondria, amyloplasts and dictyosomes concomitant with degradation of the reserve materials. Microbodies and

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lipid bodies became sequestered into protein bodies. Approximately 6 days after imbibition, at the time when the hypocotyl withdraws the cotyledons from the gametophyte, the protein bodies fused to form large aqueous vacuoles.

This study deals with the interface zone of the gametophyte and embryo, and in particular with the 4 to 5 layers of cells of the gametophyte that border on the geotropically positive side of the developing feeder, a structure which in the course of 6 days extends from an initial collar-like bulge to a 5 mm-long wedge-shaped projection and remains closely appressed to the gametophyte.

Materials and Methods

Please refer to BORNMAN et al. (1979) for a full account of the material and methods employed.

Results

Quiescent Tissue

In contrast to outer gametophyte cells, the 4 to 5 layers of cells surrounding the embryo are smaller (about 170 × 70 μm) and flattened in transection. Their appearance in the dry state is qualitatively similar to that of the deeper lying gametophyte cells, but differs quantitatively in structural components. Nuclei have a familiar lobed appearance with nucleoplasm and nucleoli anastomosed by fibrous inclusions.

Protein bodies in interface cells are relatively sparse and very small (mean diameter 1.2 μm) with densely staining contents. In contiguous gametophyte cells they are larger (mean diameter 2.5 μm) and contain a more diffuse granular matrix. Material interpreted as globoid remnants occur rarely in internal cavities of protein bodies. Lipid bodies also are not as compactly arranged as in the outer gametophyte tissue. In interface cells they are randomly scattered with the exception of those bodies in close apposition to the plasmalemma. A more characteristic pattern of arrangement around protein bodies and the cell periphery is found in the cells adjoining the gametophyte interface cells.

Reduced protein-carbohydrate and lipid reserves results in more residual cytoplasm being detectable in interface and the immediate surrounding cells than in deeper dry gametophyte tissue. One of the striking features of this 4- to 5-cell zone of gametophyte tissue surrounding the embryo is the random distribution of numerous large starch-laden amyloplasts. The prominent starch reserves in this zone are easily recognised at the light microscope level and are in marked contrast with the paucity of starch grains in deeper cells. While microbodies are rare, mitochondria are quite common. Short fragments of ER are seen infrequently in a cytoplasm studded with ribosomes. No polysome configurations are apparent. Dictyosomes are absent, a common feature of dry tissue (VARNER and SCHIDLOVSKY, 1963; PALEG and HYDE,

1964; YATSU, 1965; PAULSON and SRIVASTAVA, 1968; ABDUL-BAKI and BAKER, 1973). However, numerous small vesicles occur in the cytoplasm.

The interface cells are thin-walled except those in contact with the embryo, which have slightly thickened walls. While plasmodesmata commonly occur in the thin walls, linking neighbouring gametophyte cells, the outer walls are devoid of them.

1-Day-Imbibed Tissue

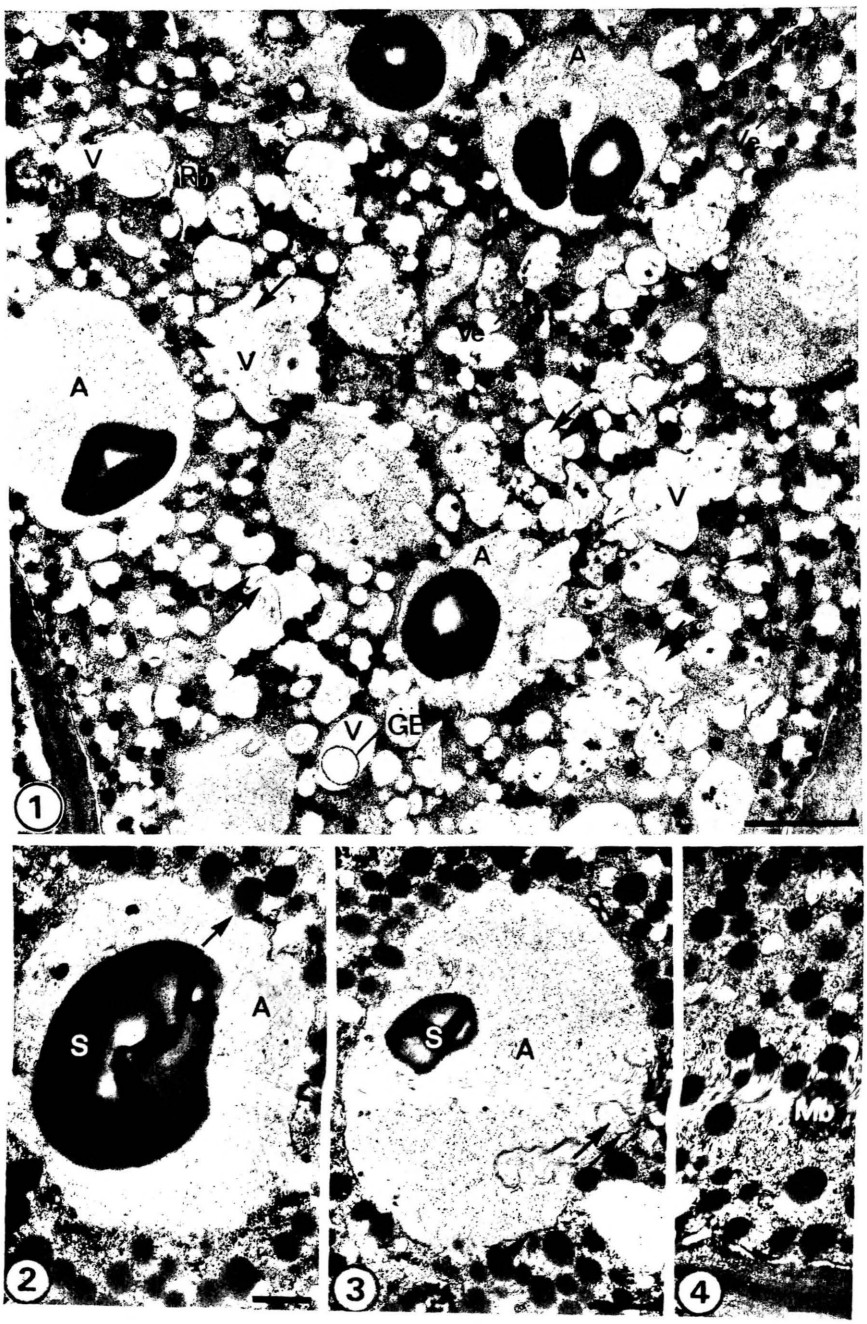
Imbibition results in a clearer fixation image. Elongated lobed nuclei have lost their initial crystalline inclusions. Enlarged nucleoli, associated with increased cell activity (JORDAN and CHAPMAN, 1971), are prominent; they are spherical in transection consisting of a fibrillar core surrounded by a well-defined granular zone (SWIFT, 1966). While dense packing makes the fibrils difficult to observe, the granular region is easily distinguished due to its looser nature. There is less condensed chromatin in the nucleoplasm of imbibed cells. It occurs scattered throughout the nucleoplasm and is located near the nuclear envelope. Small granules are dispersed throughout the nucleoplasm. They are the size of ribosomes, but do not stain as intensely.

Interface cells are relatively poorly supplied with protein body reserves. Upon hydration these extremely small protein bodies swell and begin to degrade almost immediately. The disappearance of their protein-carbohydrate reserves is a speedier process than that of their deeper-seated counterparts. The internal pattern of degradation results in rapid fragmentation and the formation of irregular clusters of material. This is in direct contrast to the more deeply-situated protein bodies which, as discrete entities, become gradually riddled with ever-increasing, enlarging and coalescing digested pockets. Ring-like globoid envelopes are occasionally seen in interface protein bodies. They enclose either electron-transparent areas or globoid remnants. The protein bodies themselves appear to become autophagically active.

Lipid bodies have decreased slightly in number and their dispersion in interface cells remains diffuse, bearing no marked relationship with protein bodies. Randomly distributed mitochondria are common and display more internal organisation at this stage than their deeply-situated counterparts. They vary in size and shape, some being greatly swollen. The cristae are short and sparse while the matrix is still largely electron-transparent containing fibrils, ribosomes and an occasional osmiophilic granule.

Some 24-hour-imbibed interface cells show a more rapid metabolic activation than others, Compact protein-carbohydrate reserves degrade, leaving numerous aqueous vacuoles. The vacuoles (Fig. 1) are not completely devoid of contents and may contain globoid envelopes and various unidentified membranous fragments (Fig. 1, arrow). The vacuoles and abundant smaller vesicles found in dry tissue fuse and appear to be engaged in intense autophagic activity (Fig. 2, double arrow).

Upon hydration starch reserves decline rapidly and are greatly depleted after 24 h imbibition (Figs. 1–3). Ribosomes and osmiophilic droplets are visible but lamellae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are largely absent and often disorganised. Some amyloplasts are invaded by protein vacuoles (Fig. 1), others begin to form surface depressions (Fig. 3. double arrow).

Microbodies have increased in number and are usually seen in the vicinity of lipid bodies as in Fig. 4. The ER displays longer single profiles which are distributed throughout the cytoplasm, often shadowing the contours of the nucleus. Inflation of the ER is quite common and it is likely that the many small vesicles in Fig. 1 (24-hour-imbibed) are localised ER dilations. Ribosomes occur attached to the ER and free in the cytoplasm (Fig. 1). As yet, no dictyosomes are seen.

3-to-6-Day-Imbibed Tissue

In 3-day-imbibed interface cells nuclei retain their polymorphic profiles. Protein vacuoles, now devoid of reserves, converge and begin to fuse at one end of the cell; lipid stores are drastically reduced. As lipid bodies are degraded, starch reserves reappear causing amyloplasts to swell and take on a circular appearance in cross section. Frequently the reserves reform into many small closely-packed starch grains. Mitochondria are numerous; they occur near the plasmalemma and scattered throughout the cytoplasm, being found in close association with amyloplasts, lipid bodies, microbodies and ER. Microbodies have increased in number and have enlarged in size. Dictyosomes, present but not profuse, are seen for the first time.

The ER has developed extensively and now consists of long sheet-like elements that ramify throughout the cytoplasm of interface and neighbouring gametophyte cells. These elements may occur singly, but are usually in parallel alignment with one or several ER profiles. The ER becomes associated with amyloplasts and may partially or completely enclose mitochondria and dictyosomes. Ribosomes fail to appear in tissues fixed with permanganate, but when comparable tissue fixed with osmium is examined, the cytoplasm is seen to be filled with ribosomes. These occur both free and attached to the ER, some being present as polysomes.

By the fifth to sixth day of imbibition, nuclei in gametophyte interface cells have usually assumed a more exaggerated invaginated appearance. As a result of their more pronounced convolutions they begin to resemble their deeper-seated counterparts. By comparison with 1-day-imbibed tissue nucleoli in 5-day-imbibed material have shrunk and become very dense, consisting almost entirely of compactly

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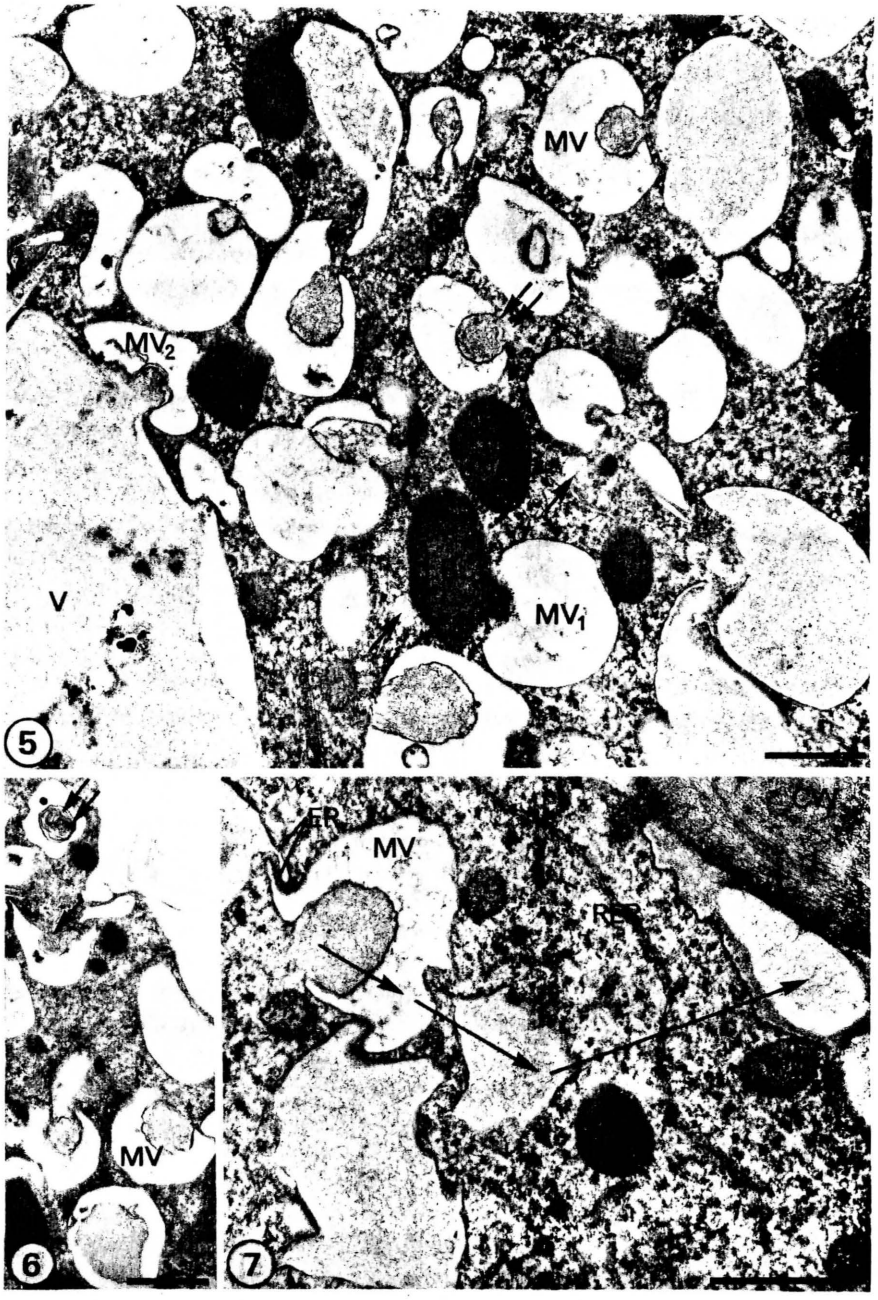
Figs. 1–4: Gametophyte interface cells imbibed for 24 h. – Fig. 1: Protein-carbohydrate reserves have been consumed leaving numerous aqueous vacuoles (V) containing unidentified membranous fragments (arrow) and globoid envelopes (GE). Vacuoles and smaller vesicles (Ve) engage in autophagic activity (double arrows). Amyloplast (A) starch reserves decline rapidly. Rb = ribosome. – Fig. 2: Amyloplast (A) with degrading starch grain (S). Note double-unit plastid membrane (arrow). – Fig. 3: Amyloplast (A) with degrading starch grain (S). Arrow indicates surrounding double unit membrane. As starch disappears little or no internal organization is laid bare. Amyloplast begins to form surface depressions (double arrow). – Fig. 4: Microbody (Mb) in vicinity of lipid bodies (LB). Bars represent 2.5 μm (Fig. 1), 0.5 μm (Figs. 2, 4), 1 μm (Fig. 3).

arranged fibrillar material. No loosely-packed outer granular zone is evident and no granules can be seen invading the fibrillar zone. Nucleolar organising regions of nucleolar chromosomes (SWIFT, 1959; JORDAN, 1971) are often peripherally embedded in the nucleolus, and nucleolar lumina are scarce. The spherical, particulate structure frequently found in a satellite position to the nucleolus is presumed to be a karyosome (HYDE, 1967). Condensed chromatin remains scattered through the nucleoplasm.

At the 4-, 5- and 6-day-stages large numbers of mini-vacuoles are frequently found clustered in limited cytoplasmic areas of the interface cells. Although some might be small protein vacuoles, the majority appear to be localised dilations of the ER. Serial sectioning revealed that mini-vacuoles often surrounded a much larger vacuole which was presumably formed by the confluence of reserve-depleted protein bodies. This vacuole is not voluminous, as original protein bodies were small and comparatively sparse. On closer inspection (Fig. 5) the mini-vacuoles appear to be autophagically active. Fibrillar material dispersed in the cytoplasm (Fig. 5, arrows) accumulates and is engulfed by the vacuoles (Figs. 5–7). The vacuoles flow around the accumulation of fibrils in an amoeboid manner and the fibrils, enclosed in a membrane, move into the vacuole (Fig. 6). Presumably the membrane then disintegrates relasing the fibrils to become freely dispersed throughout the vacuole (Figs. 5, 7). The entire process appears to be very selective as no ribosomes enter the vacuole intermingled with the fibrils. Autophagic activity is not confined to fibrillar material, however, and in Fig. 5 a mini-vacuole (MV_1) can be seen engulfing a lipid body (LB) while another (MV_2) is apparently pinching off part of the main vacuole (V). Cellular debris also makes its way into these small vacuoles, as in Fig. 6 (double arrow indicates unidentified membranous material).

A short segment of ER is seen issuing from the mini-vacuole that is engulfing fibrillar material as seen in Fig. 7. Similar pieces of ER leading from other vacuoles (in Fig. 7) support the notion that mini-vacuoles are simply dilations of the ER. The vacuolar membranes are free of ribosomes although ER leading from the vacuoles may be rough (Fig. 7). Figure 7 shows fibrillar material being taken up by and released within the mini-vacuoles. The micrographs, though static images, give the impression that the fibrillar contents of the mini-vacuoles are added to interface cell

Figs. 5–7: 5-day-imbibed gametophyte interface cells. – Fig. 5: Autophagic mini-vacuoles (MV). Fibrillar material accumulates in the cytoplasm (arrow) and is engulfed by a mini-vacuole (double arrow). A minivacuole (MV_1) commences the engulfing of a lipid body (LB) while another (MV_2) pinches off part of the main vacuole (V). M = mitochondrion; Mb = Microbody. – Fig. 6: Mini-vacuoles (MV) engulfing cytoplasmic fibrillar material (arrow). Cellular debris, e.g. unidentified membranous material (double arrow) also makes its way into mini-vacuoles. – Fig. 7: Segments of ER can be seen issuing from mini-vacuoles (MV) which suggests that these small vacuoles are ER dilations. The fibrillar contents of the mini-vacuoles are seemingly added to the interface cell wall (CW) in contact with the feeder (arrows). M = mitochondrion; RER = rough ER. Bars represent 1 μm (Figs. 5–7).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MV2
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MV1
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MV
ER

walls in contact with the feeder. These walls then thicken rapidly in a short space of time. Although dictyosomes are present (Figs. 5, 7), they are not common and do not seem to be closely associated with the cell walls.

Five days after initial imbibition the walls of the gametophyte interface cells have thickened noticeably. Although the walls contiguous with those of the inner gametophyte cells have thickened (ca. 1.5 μm), this thickening is insignificant in comparison with the cell walls (ca. 5 μm) now adhering firmly to the embryo's feeder. Radial walls between adjacent interface cells (ca. 2 μm) become thicker than inner tangential walls, but remain narrow in comparison with outer tangential walls. Inner wall areas traversed by plasmodesmata remain thin with characteristic median cavities coalescing and enlarging in the middle lamella region. Plasmodesmata are less common between adjacent interface cells and may occur singly with no corresponding diminution in wall thickness. No plasmodesmata form in the outer wall.

Already at the 3-day-stage gametophyte interface walls in contact with the feeder had begun to thicken. Concomitantly a dark substance appeared in the space between gametophyte and feeder. This densely-staining material is apparently secreted from gametophyte and feeder cells and seems to function as an adhesive causing the two structures to adhere firmly. More wall material is added until, at the 4- to 5-day-stage, the outer wall has a distinctly layered appearance. The interface walls now become mucilaginous. The outer layers of the wall are slightly compressed while the inner layers have a spongy appearance. Radial walls between adjacent interface cells also become mucilaginous. Apart from the addition of the mini-vacuole contents to the wall (Fig. 7) some of the increased thickness could be due to wall hydrolysis with resultant swelling.

Gametophyte interface walls are thickest at the base of the embryonic feeder, becoming progressively thinner toward its tip. Gametophyte cells adjacent to interface cells also develop thickened walls. The pattern of thickening is similar to that of interface cells with the walls nearest the feeder also becoming thickest. On the 6th day most of the thickened walls have developed numerous median blind channels which apparently represent the final stage in the development of the spongy wall layers. As the plumule of the young seedling emerges from the seed, the outer cells of

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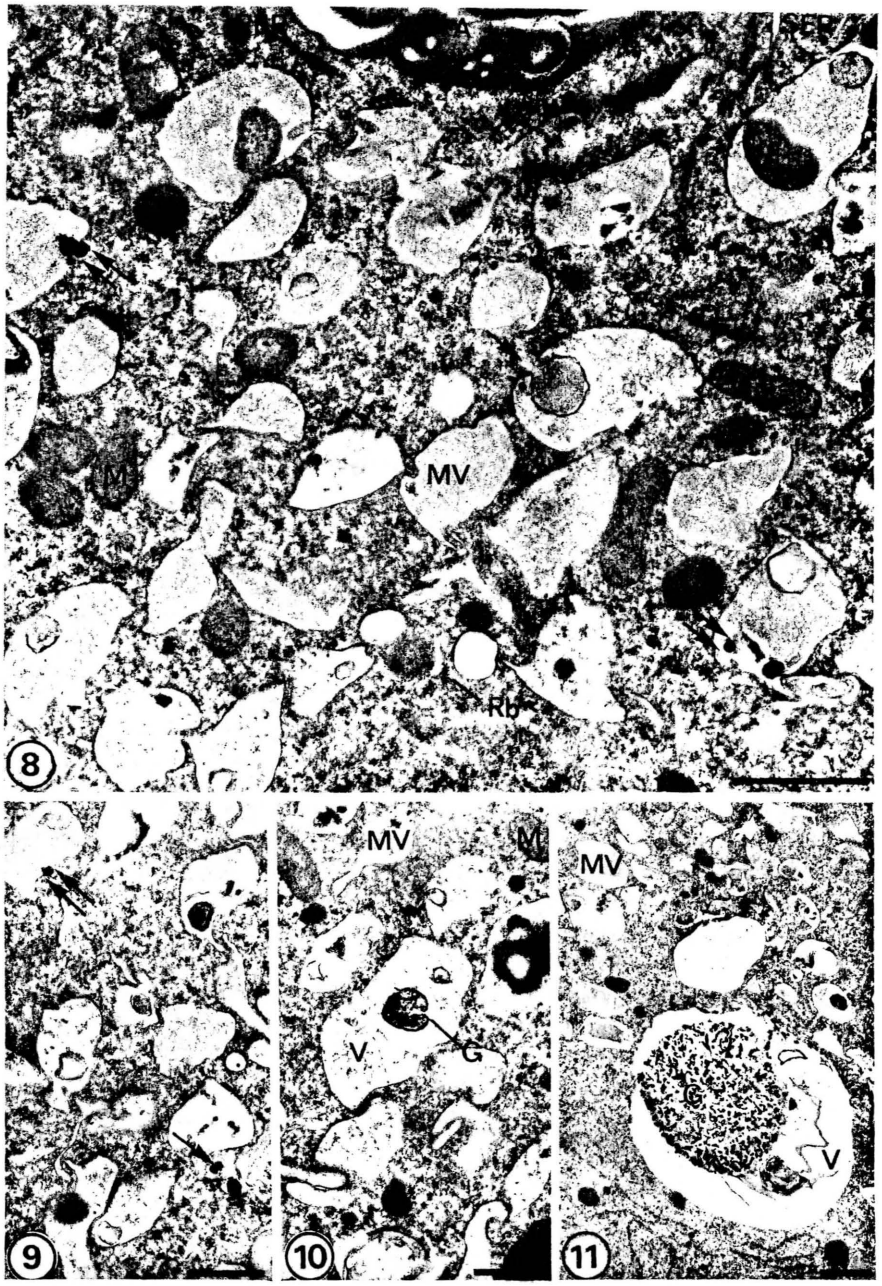
Figs. 8–11: 5-day-imbibed gametophyte cells in interface zone. – Fig. 8: Autophagic mini-vacuoles (MV) with fibrillar contents. A mini-vacuole is seen engulfing cytoplasmic fibrillar material (arrow). Small, spherical, highly electron-dense inclusions (double arrows) have moved into the mini-vacuoles. Note dictyosome (D) with vesicles containing fibrillar material. A = amyloplast; LB = lipid body; M = mitochondrion; Rb = ribosome; RER = rough ER; SER = smooth ER. – Fig. 9: Small osmiophilic crystals, free in cytoplasm (arrow), move into mini-vacuole (double arrow). – Fig. 10: Pitted crystal, interpreted as a globoid, (G) within protein body vacuole (V). M = mitochondrion; MV = mini-vacuole. – Fig. 11: Large particulate globoid (G) within protein body vacuole (V). MV = mini-vacuole. Bars represent 1 μm (Figs. 8, 11), 0.5 μm (Figs. 9, 10).


MV
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the mature feeder become crushed against the gametophyte. By the time the plumule is erect (6-day-stage) the number of crushed feeder cells has increased.

Mini-vacuoles (that is, dilated smooth ER) in interface and neighbouring gametophyte cells imbibed for approximately 5 to 6 days frequently contain small, spherical, highly elelctron-dense inclusions thought to be crystalline. Similar crystals occur free in the cytoplasm (Fig. 9. arrows) and apparently move into the mini-vacuoles (Figs. 8, 9, arrow). At present there is little evidence to indicate the origin and composition of these crystals. Larger pitted (Fig. 10) and particulate (Fig. 11) crystals interpreted as globoids are occasionally present. These inclusions are contained within protein body vacuoles which are usually larger than mini-vacuoles (Fig. 11). Non-dilated rough ER is still prolific but less randomly distributed than in the 3-day-stage. It now exists in a very close spatial relationship with amyloplasts, mitochondria, nuclei and the plasmalemma. Starch-laden amyloplasts become completely surrounded by numerous layers of rough ER. Mitochondria are often surrounded by one or two layers of ER and occasionally by many concentric coils. One to two long profiles of ER are usually arranged parallel to the nuclear envelope and the plasmalemma.

The cytoplasm remains filled with ribosomes, many of which are attached to the ER. Polysome and helical configurations are common. Dictyosomes which are sparse, produce vesicles with fibrillar contents. Amyloplasts, rich in starch, are distributed throughout the cytoplasm with occasional concentrations around the nucleus. Mitochondria are ubiquitous.

By the 5- to 6-day-stage lipid stores have been reduced. Remaining lipid bodies vary widely in size (Figs. 12, 22), perhaps reflecting different rates of digestion. It seems likely that the larger bodies in Fig. 12 and 22 are formed by coalescence. Concurrent with lipid degradation, starch reserves are built up and microbodies, presumed to be glyoxysomes (FREDERICK et al., 1968), increase in number. This microbody increase is apparently achieved by means of fission (Fig. 12); the inner electron-dense amorphous area divides, providing each daughter microbody with an osmiophilic eccentric core (arrows) prior to completion of constriction. The close

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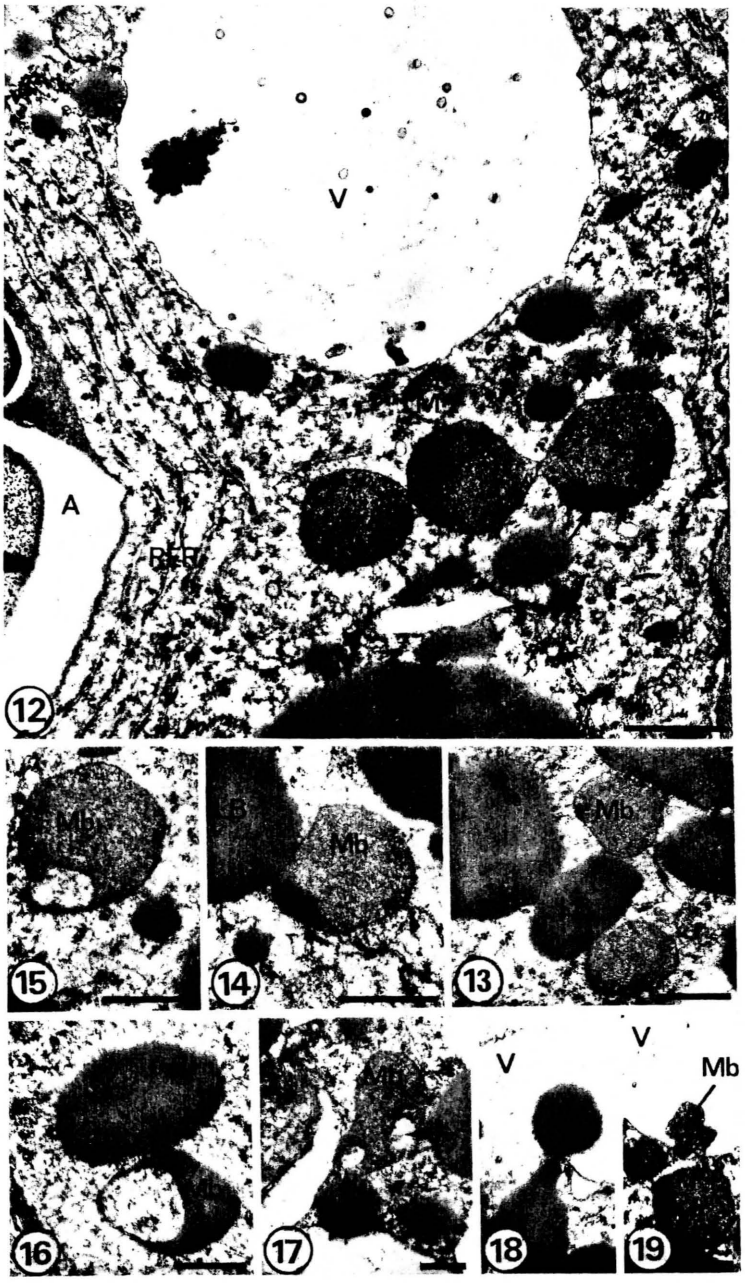
Figs. 12–19: Microbodies and lipid bodies in 5- to 6-day-imbibed gametophyte cells of interface zone. – Fig. 12: Mitochondria (M), lipid bodies (LB) and microbodies (Mb) are closely associated with starch-laden, rough ER (RER) surrounded amyloplasts (A). Note dividing microbody. Inner electron-dense amorphous area divides prior to completion of constriction, providing each daughter microbody with an osmiophilic core (arrows). V = vacuole. – Fig. 13: Microbodies (Mb) associated with lipid body (LB). – Fig. 14: Dense inclusion (nucleoid) of microbody is characteristically located along bounding membrane. – Fig. 15: Microbody (Mb) with cup-shaped depression. – Fig. 16: Cup-shaped microbody (Mb) associated with lipid body. – Fig. 17: Cup-shaped depressions of microbody (Mb) often form directly opposite a lipid body (arrows). – Fig. 18: Lipid body (LB) passing into vacuole (V). – Fig. 19: Microbody (Mb) entering vacuole (V). Bars represent 0.5 μm (Figs. 12–16, 18, 19), 1 μm (Fig. 17).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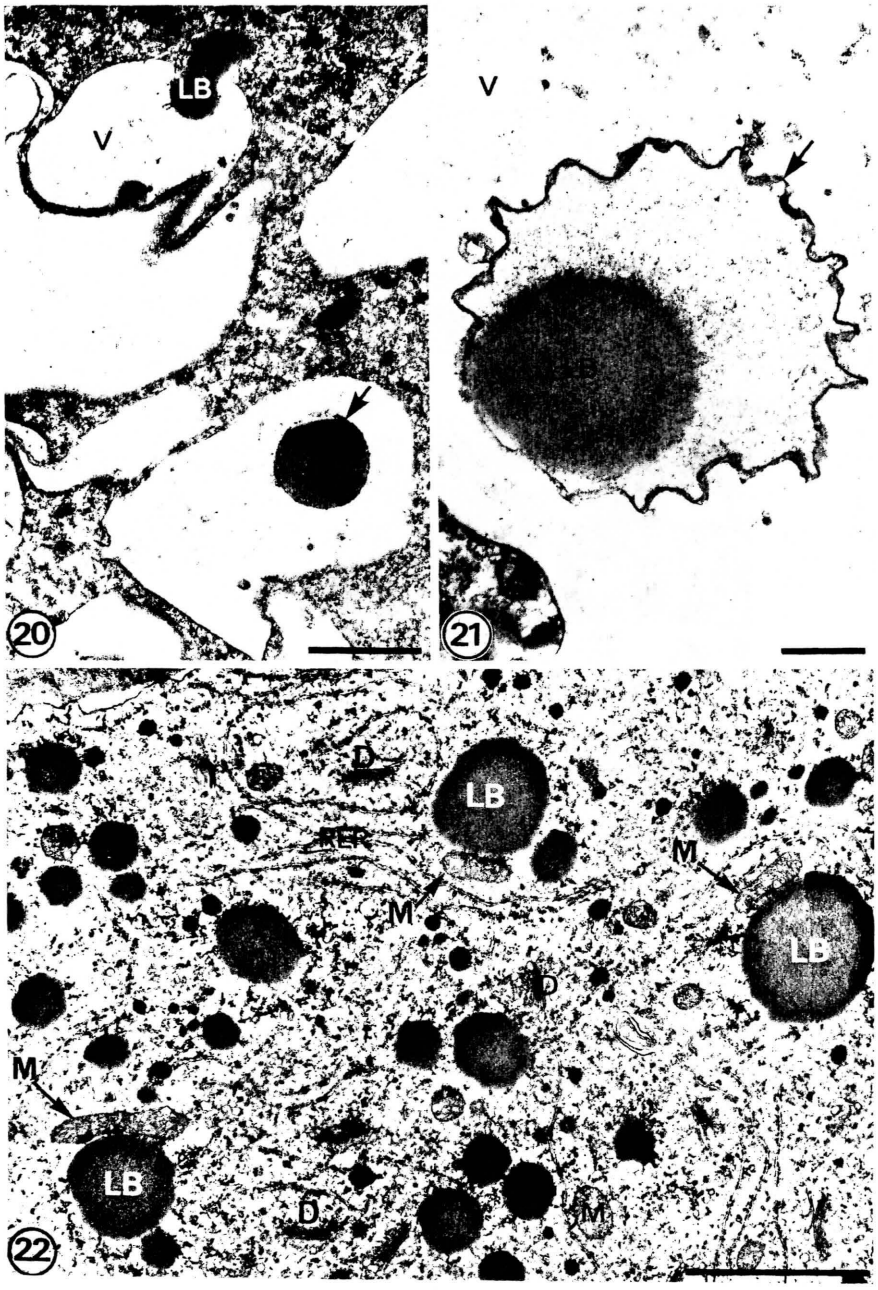
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association of microbodies, lipid bodies and mitochondria with starch-laden and rough ER-surrounded amyloplasts as seen in Fig. 12, becomes a common spatial relationship occurring too frequently to be the result of mere chance. Microbodies are often found in intimate association with lipid bodies (Figs. 13, 14), some microbodies showing a close relationship with more than one lipid body (Figs. 13, 17). Characteristically the dense inclusion of the microbody is usually located along the boundary membrane (Figs. 12, 14, 16, 19). A large number of microbody profiles were observed and it was noticed that the section of the membrane bearing the amorphous inclusion rarely comes into close contact with a lipid body (Fig. 12). The microbodies vary in size and shape; most are roughly spherical to oval, but some develop cup-shaped depressions (Figs. 15–17). In a number of cases these depressions form directly opposite a lipid body. As is the case in deeply-situated gametophyte tissue lipid and microbody migration into protein body vacuoles (Figs. 18, 19) is fairly commonly observed in the interface zone. Occasionally a lipid body entering a vacuole retains a limiting membrane derived from the tonoplast (Fig. 20). In some instances these lipid bodies degrade while still surrounded by the membrane (Fig. 20, arrow; Fig. 21). Frequently mitochondria are in very close association with lipid bodies (Fig. 22, arrows).

Discussion

During initial imbibition physical wetting of the outer or deeply-situated gametophyte tissue takes place prior to that of the interface zone. Despite this, nuclear activation and subsequent cell metabolism is accelerated in the interface cells and soon outstrip that of the outer cells. In fact there seems to be a sequence of activation from the interface to the outermost gametophyte cells.

Nucleus

Following initial activation interface nucleoli enlarge over a period of several days – during which time the ribosomal population of the cells increases – before gradually shrinking and reverting to a less active state as the cell reserves become depleted. Nucleoli in deeper-seated cells show a similar but slower-paced behaviour, making it obvious that the rapidity with which the changes take place is in direct proportion to distance from the embryo. The nuclei of interface cells gradually assume a more exaggerated extensively lobed appearance as metabolic activity

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Figs. 20–22: Lipid bodies in 5- to 6-day-imbibed gametophyte cells of interface zone. – Fig. 20: Migration of lipid bodies (LB) into vacuoles (V). Note apparent membrane (arrow) surrounding sequestered lipid body. – Fig. 21: Sequestered lipid body (LB) is degrading while still confined by membrane (arrow). V = vacuole. – Fig. 22: Mitochondria are frequently seen in close association with lipid bodies (arrows). D = dictyosome; LB = lipid body; M = mitochondrion; RER = rough ER. Bars represent 0.5 μm (Figs. 21, 22), 5 μm (Fig. 22).

accelerates. It has been postulated that irregularity of the nuclear outline indicates intense activity between nucleus and cytoplasm by increasing the boundary area through which substances can pass (CLOWES and JUNIPER, 1968). However, lobing of the nucleus has also been connected with cell senescence (BERJAK and VILLIERS, 1970) and seems to accompany increased cell activity leading to the climacteric phase just prior to cell death (GILLILAND et al., 1976).

Protein and Lipid Reserves

In comparison with deeply-lying gametophyte cells, interface cells have relatively scanty reserves. Protein bodies are few and small, while most of the lipid bodies are dispersed through the cytoplasm, except in the region of the plasmalemma. Interface cells appear slightly less dehydrated than deeper cells, which suggests that these cells start to function earlier. YOO (1970) suggests that early close packaging of seed lipid bodies against cell walls could help prevent loss of cell moisture. In the *Welwitschia* gametophyte, the lipid bodies in the region of the plasmalemma move inward upon cell activation. Those few that surround the protein bodies, however, retain their distinct pattern of arrangement. According to VILLIERS (1971) a structural connection probably exists between the lipid and protein bodies. Although the migration of microbodies and lipid bodies into protein body vacuoles occurs, their simultaneous presence within the same vacuole has not yet been observed. VIGIL (1970), working on castor bean endosperm showed cytochemically that microbodies sequestered in vacuoles still contain a very active catalase, which implies that a certain amount of lipid digestion occurs in the vacuole. In our material most of the lipid bodies enter protein body vacuoles whose reserves have already been dispersed, but a number also enter vacuoles containing compact reserves. Osmiophilic regions form in the protein-carbohydrate reserves at any point of contact with lipid bodies. This suggests a possible biochemical interaction.

In 1968 MATILE and SPICHIGER suggested a similarity between spherosomes or lipid bodies and the lysosomes in animal cells since they were found to contain similar hydrolytic enzymes including RNase and DNase. VIGIL (1970) speculated that their preparations were contaminated with other organelles. However, the presence of lipase in lipid bodies is well established (CHING, 1968; ORY et al., 1968). A number of investigators (WALEK-CZERNECKA, 1962; HOLCOMB et al., 1967; SEMADENI, 1967) have reported the presence of acid phosphatase (often used as a lysosome marker) in lipids bodies. However, the lipid bodies in the *Welwitschia* gametophyte showed no acid phosphatase activity.

Residual Cytoplasm

Although there is no perceptible decline in number, the fact that many microbodies develop cup-shaped depressions during late germination could be interpreted as an indication of senescence or quiescence (YEOMAN and STREET, 1973). The sequestration

of microbodies into protein body vacuoles is of common occurrence throughout the germination period and probably accounts for the turnover of damaged and/or senescent microbodies. During lipid degradation the lipid bodies, mitochondria and amyloplasts encircled with ER seem to form a complex. Fatty acids, resulting from lipase action in the lipid bodies (CHING, 1968; ORY et al., 1968; ORY, 1969) are presumably converted by microbodies (glyoxysomes) to succinate (BREIDENBACH and BEEVERS, 1969) which in turn is converted to sucrose by the action of mitochondria. While some of the sucrose is no doubt utilised as a source of energy within the cell, the bulk is probably transported to and absorbed by the embryo, via the feeder. Excess sucrose is probably converted to starch and stored temporarily in the amyloplasts.

Mitochondria frequently occur in very close association with lipid bodies in *Welwitschia* gametophyte, especially in the interface zone (Fig. 22). This intimate association suggests that perhaps both α- and β-oxidation systems may be operative in *Welwitschia* as is the case in flax seed (CHING, 1972). However, although *Welwitschia* and flax both contain linolenic acid as dominant fatty acid, biochemical studies are needed to substantiate this speculation.

Several investigators, working on plant (VIGIL, 1970) and animal cells (TSUKADA et al., 1968; ESSNER, 1969) have reported a direct attachment between microbody and ER. This has led them to suggest that microbodies bud directly from the ER. We did not observe continuities between ER and microbodies, although failure to find them does not necessarily imply their absence.

Inflation of the ER is commonplace in interface zone cells and gives rise to large numbers of mini-vacuoles in limited areas of the cytoplasm. In most cases the dilated ER surrounds the larger cell vacuole formed by protein body confluence. Although deposition of protein-carbohydrate reserves was not observed within these small dilations their behaviour further supports the classical concept that protein bodies arise from ER. Similar to protein body vacuoles, mini-vacuoles indulge in autophagic activity and also accumulate crystals. These crystals are of cytoplasmic origin and possibly represent ergastic material.

In dry gametophyte tissue starch-rich amyloplasts are primarily concentrated in the 4 to 5 cell-deep interface zone immediately surrounding the embryo. In sharp contrast to this, amyloplast distribution in deep gametophyte cells is sporadic and meagre. On hydration the starch reserves of the interface zone are rapidly depleted within about 24 hours. It is well-known that carbohydrate is utilised preferentially before lipid and protein reserves and the starch is probably used during the initial burst of cellular activity in this zone following inhibition. While the interface and neighbouring cells rapidly achieve intense metabolic activity, deeper-seated cells appear to have a much slower metabolism. Within 2 to 3 days interface zone starch reserves are replaced as lipid is hydrolysed.

Although one would have expected dictyosomes to be involved in the dramatic thickening of the outer interface walls in contact with the embryo's feeder, this is

not the case. Dictyosomes are rare and are not closely associated with the cell walls although those observed did produce vesicles with fibrillar contents.

Apart from typical autophagic activity involving sequestration of organelles, crystals, cellular debris etc., mini-vacuoles also exhibit a more specialised type of autophagy. Fibrillar material dispersed in the cytoplasm accumulates before being selectively engulfed by the mini-vacuoles. The mini-vacuoles then apparently take on a dictyosome-like function. They fuse with the plasmalemma and release their fibrillar contents to add to the rapidly thickening interface cell walls in contact with the feeder.

Cell Walls

Part of the reserves of the gametophyte include the hemi-celluloses which form the cell walls, and it is thought that besides the addition of mini-vacuole contents to the outer interface cell walls a certain amount of the increased wall thickness is due to wall hydrolysis with resultant swelling. By the sixth day of imbibition most of the thickened walls have developed numerous median blind channels whilst the outer layers remain compressed and undigested. This pattern of cell wall digestion is similar to that described for lettuce endosperm (Jones, 1974). Jones (1974) suggests that this pattern indicates that the enzymes are produced within the endosperm cells. Similarly, it seems apparent that the cellulolytic enzymes responsible for interface wall digestion in the *Welwitschia* gametophyte are produced within the interface cells and are not derived from the embryo. As no plasmodesmata form in the thick outer interface wall, the hydrolysed channels could facilitate the diffusion of substances through the wall. The densely-staining material that accumulates between gametophyte and feeder cells and which appears to function as an adhesive, is most likely pectinaceous. Unlike in the embryo, acid phosphatase activity is not very extensive in gametophyte cell walls. It is mainly confined to cell corners from whence it may extend along the middle lamella.

Interface cells rapidly achieve a high degree of metabolic activity that stands in sharp constrast to the much slower metabolism and intracellular turnover of the outer, more deeply-seated cells, despite the fact that the latter probably become hydrated first.

References

Abdul-Baki, A. A. and J. E. Baker: Are changes in cellular organelles or membranes related to vigor loss in seeds? Seed Sci. and Technol. *1*, 89–125 (1973).

Berjak, P. and T. A. Villiers: Ageing in plant embryos. I. The establishment of the sequence of development and senescence in the root cap during germination. New Phytol. *69*, 929–938 (1970).

Bornman, C. H., V. Butler, and W. A. Jensen: *Welwitschia mirabilis:* fine structure of the germinating seed I. Orientation. Z. Pflanzenphysiol. *91*, 189–196 (1979).

Butler, V., C. H. Bornman, and W. A. Jensen: *Welwitschia mirabilis:* fine structure of the germinating seed III. The quiescent gametophyte. Z. Pflanzenphysiol. *91,* 399–413 (1979 a).
– – – *Welwitschia mirabilis:* fine structure of the germinating seed IV. The imbibed gametophyte. Z. Pflanzenphysiol. *92,* 95–113 (1979 b).
Ching, T. M.: Intracellular distribution of lipolytic activity in the female gametophyte of germinating Douglas fir seeds. Lipids *3,* 482 (1968).
– Metabolism of germinating seeds. In: T. T. Kozlowski (Ed.): Seed Biology, Volume II, PP. 103–218. Academic Press, New York, 1972.
Clowes, F. A. L. and B. E. Juniper: Plant Cells: Botanical monograph, Volume 8. London: Blackwell Scientific Publications, 1968.
Essner, E.: Localization of peroxidase activity in microbodies of fetal mouse liver. J. Histochem. *17,* 454–466 (1969).
Frederick, S. E., E. H. Newcomb, E. L. Vigil, and W. P. Wergin: Fine-structural characterization of plant microbodies. Planta *81,* 229–252 (1968).
Gilliland, M. G., C. H. Bornman, and F. T. Addicott: Ultrastructure and acid phosphatase in pedicel abscission of *Hibiscus.* Amer. J. Bot. *63,* 925–935 (1976).
Holcomb, G. E., A. C. Hildebrandt, and R. F. Evert: Staining and acid phosphatase reactions of spherosomes in plant tissue culture cells. Amer. J. Bot. *54,* 1204–1209 (1967).
Hyde, B. H.: Changes in nucleolar ultrastructure associated with differentiation in the root tip. J. Ultrastruc. Res. *18,* 25–54 (1967).
Jones, R. L.: The structure of the lettuce endosperm. Planta *121,* 133–146 (1974).
Jordan, E. G.: The Nucleolus. In: J. J. Head and O. E. Lowenstein (Ed.): Oxford Biology Readers. Oxford University Press, London, 1971.
Jordan, E. G. and J. M. Chapman: Ultrastructural changes in the nucleoli of Jersualem artichoke *(Helianthus tuberosus)* tuber discs. J. Exp. Bot. 22, 627–634 (1971).
Matile, P. and J. Spichiger: Lysosomal enzymes in spherosomes (oil droplets) of tobacco endosperm. Z. Pflanzenphysiol. *58,* 277–280 (1968).
Ory, R. L., L. Y. Yatsu, and H. W. Kircher: Association of lipase activity with the spherosomes of *Ricinus communis.* Arch. Biochem. Biophys. *123,* 255 (1968).
Paleg, L. and B. Hyde: Physiological effects of gibberellic acid. VII. Electron microscopy of barley aleurone grains. Plant Physiol. *39,* 673–680 (1964).
Paulson, R. E. and L. M.: Srivastava: The fine structure of the embryo of *Lactuca sativa.* I. Dry embryo. Can. J. Bot *46,* 1437–1445 (1968).
Semadeni, E. G.: Enzymatische Charakterisierung der Lysosomenaquivalente (Sphärosomen) von Maiskeimlingen. Planta *72,* 91–118 (1967).
Swift, H. H.: Nucleolar function. In: Symposium on molecular biology, pp. 266–303. University of Chicago Press, Chicago, 1959.
– Report of the nucleolus nomenclature committee. Natn Cancer Inst. Monogr. *23,* 573–574 (1966).
Tsukada, H., Y. Mochizuki, and T. Konishi: Morphogenesis and development of microbodies of hepatocytes of rats during pre- and postnatal growth. J. Cell Biol. *37,* 231–243 (1968).
Varner, J. E. and G. Schidlovsky: Intracellular distribution of proteins in pea cotyledons. Plant Physiol. *38,* 139–144 (1963).
Vigil, E. L.: Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. J. Cell Biol. *46,* 435–454 (1970).
Villiers, T. A.: Cytological studies in dormancy. I. Embryo maturation during dormancy in *Fraxinus excelsior.* New Phytol. *70,* 751–760 (1971).
Walek-Czernecka, A.: Mise en évidence de la phosphatase acide (monophosphoesterase II) dans les sphérosomes des cellules épidermiques des écailles bulbaire d'*Allium cepa.* Acta Soc. Bot. Polon. *31,* 539–543 (1962).

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Y.: The ultrastructure of cotyledonary tissue from *Gossypium hirsutum* L. seeds. iol. *25*, 193–199 (1965).
. M. and H. E. STREET: General cytology of cultured cells. In: Plant Tissue and ture. Botanical Monographs, Volume 11 (H. E. STREET ed.) Oxford: Blackwell : Publications (1973).
: Ultrastructural changes in cells of pea embryo radicles during germination. J. . *45*, 158–171 (1970).

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