ULTRASTRUCTURE OF THE GERMINATING *WELWITSCHIA MIRABILIS* SEED.

by

VALERIE BUTLER.

M. Sc. (Natal)

Thesis (Ph.D.; Botany) - University of Natal, Pietermaritzburg, 1975. Degree conferred 1976

Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Botany, University of Natal.

Thesis, (Ph.D), Natal, 1975.

December 1975.

CONTENTS

PREFACE

The research described in this thesis was done in the laboratories of the Botany Department of the University of Natal, Pietermaritzburg, under the direction of Professor C.H. Bornman. The whole thesis, except where it is specifically indicated to the contrary in the text, is the candidate's own original work.

ABSTRACT

The structure and chemical composition of quiescent *Welwitschia mirabilis* (Hooker fil.) seeds and resultant changes occurring during the first 7 days of germination were studied. Research was focussed on the megagametophyte and its interrelationship with the non-vascularised outgrowth produced by the embryo. This outgrowth was given the name "feeder" by Bower in 1881 who postulated that it functions as an absorptive organ. However the possibility existed that it merely fulfilled the mechanical role of anchorage.

Following hydration activation of embryonic collar cells precedes that of gametophyte cells whose rate of activation is governed by relative distance from the embryo. This sequence of activation is suggestive of a stimulatory factor diffusing from the embryo into the gametophyte. Starch, protein and lipid reserves in the collar and developing feeder are consumed within 36 to 48 h. As a consequence the rapidly developing seedling is probably largely dependent on nutrient material in the gametophyte until the plumule emerges, after approximately 5 to 6 days germination. Ventral feeder cells in contact with gametophyte tissue apparently act as transfer cells, developing numerous small wall projections invested with plasmalemma which result in a much greater absorptive surface area. The large numbers of mitochondria occurring in these cells might suggest active uptake of nutrients. At the 3- to 4-day-stage the feeder and gametophyte adhere firmly. While this adherance

probably facilitates translocation of nutrients it could also have the secondary function of anchoring the feeder in the gametophyte, thus providing the emerging plumule with a firm base. The apparent root cap origin of ventral feeder cells might explain the positive geotropism of the feeder, as recent work *inter alia* by Wilkins and Wain (1975) has shown that root cap cells may be geoperceptive.

Cytochemical methods used at the light and electron microscope level suggest that reserve material within protein bodies of the embryo and gametophyte might exist as a protein-carbohydrate complex and that globoid origin might be cytoplasmic. The immediate digestion of protein body reserves in the embryo and gametophyte interface zone argues the presence of pre-existing hydrolytic enzymes laid down within the protein bodies prior to quiescence. However the enzymes responsible for reserve breakdown in deep gametophyte tissue seem to be synthesised *de novo*. Protein hydrolysis precedes lipid digestion which possibly indicates that some of the resulting free amino acids might be used in the *de novo* synthesis of lipases. Lipid bodies, microbodies, mitochondria and amyloplasts encircled with ER seem to form a complex. Fatty acids resulting from lipase action in the lipid bodies (Ching 1968) are probably converted by microbodies (glyoxysomes) to succunate (Breidenbach and Beevers 1967) which is converted to sucrose by the action of mitochondria (Cooper and Beevers 1969a, b). Excess sucrose is probably converted to starch and stored in the amyloplasts. In 5 days the mean dry mass of the gameto-

phyte decreases by approximately 47% during which time the total amount of lipid decreases by 76.5% and protein by 14%. Although some of the hydrolysed fatty acids and amino acids are no doubt utilised in the gametophyte it is suggested that the majority of fatty acids are probably converted to sugars which, together with free amino acids (and possibly simple peptides) are transported to and absorbed by the embryo via the feeder whence they are utilised for seedling growth.

INTRODUCTION

Welwitschia mirabilis (Hooker fil.) has been the subject of controversy and speculation ever since it was first recorded in 1860. Even its species designation has become a topic of debate. It was originally described by the Austrian naturalist Frederic Welwitsch (Hooker 1863) near Cabo Negro in Angola. Welwitsch wrote to Sir William Hooker describing his rare find, suggesting the generic name of *Tumboa*, a derivation of its native name "tumbo" meaning stump. Nine months later this plant was quite independently found by the artist and explorer Thomas Baines (Hooker 1863) near Haikamchab in Damaraland in South West Africa. Baines' sketch and material reached Kew first. The material was useless as it was in a bad state of decay, but the sketch was incorporated by Hooker (1863) in his description of the plant which he named *Welwitschia mirabilis*. It has since been suggested (Benson 1970) that the plant be called *Welwitschia bainesii* to honour both discoverers. The opinion of botanists remained divided on this issue (Benson 1970) until recently when the case was submitted by Dyer and Verdoorn (1972) to the Standing Committee on the Stabilization of Specific Names. The official ruling published in Taxon 24 (1) Feb. 1975, is that the specific name *Welwitschia mirabilis* Hooker takes priority over *W. bainesii*.

Welwitschia mirabilis is a monotypic species usually grouped with the genera *Ephedra* and *Gnetum* under the order Gnetales

(Foster and Gifford 1959). The three genera of this order possess advanced angiosperm-like anatomical and morphological features; features that have been used to show that Gnetalean plants could be the ancestors of flowering plants and could represent a link between angiosperms and gymnosperms (Foster and Gifford 1959; Sporne 1965). Unfortunately the Gnetales have virtually no fossil record (Sporne 1965) and so such theories remain purely speculative. Today the Gnetales are regarded as highly specialised off-shoots in gymnosperm evolution which bear no close relationship to any present day gymnosperm (Foster and Gifford 1959).

Welwitschia is endemic to a narrow coastal belt which traverses extremely arid desert country along the coast of South West Africa and Angola (Rodin 1953a). In this desolate area, where rainfall is practically non-existent, *Welwitschia* is an enigma. It survives and thrives even though it has few of the adaptive features usually associated with drought-tolerant plants. It is an anomaly in that it grows in a severe desert but displays such non-xeromorphic characteristics as enormous leaf surface areas, numerous stomata, both ab-and adaxially, and a relatively thin cuticle (Bornman 1972). Its survival in the Namib Desert indicates that it must have specially efficient mechanisms for water uptake. Recent work by Bornman *et al.*,(1973) has shown that the plant's water requirements might be met by utilization of fog condensate.

In appearance *Welwitschia* is pure science fiction. Its grotesquely stunted stem bears two giant foliage leaves that grow

continuously throughout its life, which may exceed 2 000 years (Herre 1961). The leaves are shredded, sprawling in an untidy tangle over the desert gravel. It is dioecious with strobili-bearing branches arising from a series of transverse ridges, parallel to the leaf bases. It has been speculated that it might have originated under tropical conditions (Bornman 1972) but managed to adapt itself during its evolutionary development by remaining in a permanently arrested seedling form to survive the changing conditions culminating eventually in the severe desert environment found on the West Coast of Southern Africa.

Although this unique plant possesses angiospermous features, it has a preponderance of gymnospermous characteristics. Its seeds are naked, each containing an embryo surrounded by megagametophyte tissue. Little is known of *Welwitschia* embryogeny (Sporne 1965) probably as a direct result of inaccessibility of material at progressive stages of embryo development.

Very few seedlings and young plants (1 - 5 yrs) are encountered in the "*Welwitschia* belt" of the desert. In any one area, e.g. on the Welwitschia Fläche, the plants exhibit wide ranges in age. It is evident that only a few seeds germinate, survive and establish themselves about every 5 to 10 years. The *Welwitschia* colony produces a vast number of seeds a large percentage (99%) of which are either sterile or rendered non-viable (Bornman *et al.*, 1971). Of the viable seed, few germinate, and germinability falls off fairly rapidly with

age. The seeds are unexpectedly plump for a desert plant, with a high fat content.

The seed has been examined closely from the point of view of its structure, chemical composition and the resultant changes occurring during germination. A large part of this study is devoted to the inter-relationship between the embryo (sporophyte) and its encompassing nutritive tissue (megagametophyte) which, incidentally, is erroneously referred to as endosperm throughout most of the literature e.g. Bower 1881a; Pearson 1929; Martens 1971.

The degradation of storage products in the megagametophyte, their mobilization and fate are investigated. Research is centred on the possible transferance of nutritive material from the gametophyte to the embryo, and the presence of transfer cells (Pate and Gunning 1972) is investigated.

The germinating embryo produces a protuberant outgrowth which remains embedded in the seed. This non-vascularized emergence was given the name "feeder" by Bower in 1881 although he had no evidence that it functions as a suctorial organ. It could merely fulfil the mechanical role of anchorage. If it is an absorptive organ, its closest parallel in morphology and function with other plants is to be found in *Selaginella*.

The apparent positive geotropism of the feeder was also investigated. Recent work *inter alia* by Wilkins and Wain (1975) has shown that root cap cells may be geoperceptive. This is

of interest as the ventral cells of the embryonic feeder appear to be of root cap origin.

In the lay-out of this thesis the first section of the study deals with observations and discussion of the gross morphology and anatomy of the germinating seed, thus providing the requisite background information. For the sake of clarity the gametophyte and embryo of the germinating seed are described and discussed separately and are considered *in toto* in the concluding chapter. The section devoted to the gametophyte is divided into two subsections, viz: deeply-situated gametophyte cells (i.e. cells situated far from the embryo - Fig. 9, box 1) and gametophyte interface cells, which are in close proximity to the collar and developing feeder (Fig. 9 box 2). As it was impossible to study the entire embryo ultrastructurally, research was focussed on the salient features of collar and developing feeder.

Relatively little electron microscopical work has been carried out on gymnospermous seed (Ching 1965, 1972; Durzan, Mia and Ramaiah 1971; Simola 1974). In the case of *Welwitschia* investigations have been restricted to light microscopical investigations (Martens and Waterkeyn 1964; Martens 1971). Most in depth work on seeds has been confined to the angiosperms, the literature of which is voluminous.

Welwitschia mirabilis occupies a unique position among vascular plants. Apart from its bizarre habit, its ability to

survive under harsh Namib Desert conditions make it a plant of interest. Its seeds must germinate readily and rapidly to forge the vital link between reproduction and survival. An intimate knowledge of seed structure and germination could also form a basis upon which at least part of the drought tolerance of this species can be evaluated.

MATERIALS AND METHODS.

The seeds used in this investigation were collected from plants growing on the Welwitschia Fläche, about 48 km east of Swakopmund, S.W.A. They were carefully selected for plumpness, surface sterilised in 1% Cetavlon, denuded of outer seed coat and germinated on moist filter paper in an incubator at 25 ± 1° C.

Light Microscopy

Material was fixed in 10% water-soluble acrolein, dehydrated and embedded in Tissue mat (Jensen 1962). Transverse and longitudinal serial sections of material ranging from quiescent seeds to 8-day-old seedlings were obtained with a rotary microtome. Sections were stained with the periodic acid - Schiff (PAS) reaction (Jensen 1962), mercuric bromphenol blue (Pearse 1961), toluidine blue (Jacobsen *et al.*, 1971) and Sudan IV (Jensen 1962). Photographs were taken with a Zeiss photomicroscope.

Electron Microscopy

Diced tissue pieces were fixed in 6% glutaraldehyde buffered with 0.05 M sodium cacodylate for 6h at 4° C and post-fixed in 2% osmium tetroxide likewise buffered with 0.05 M sodium cacodylate for 15h also at 4° C. The pH of all the buffered solutions was 7.2. After washing the tissues for 90 min with three changes of 0.05 M cacodylate buffer, the material was dehydrated in either a graded alcohol or graded acetone series. Alcohol dehydration was followed with three changes of

propylene oxide and infiltration with an Araldite - Epon - DDSA (1 : 1 : 3) mixture. During the graded acetone dehydration series the tissues remained overnight in 70% acetone containing 1% uranyl nitrate. These tissue pieces were then embedded in Epon 812 (Luft 1961). The material was sectioned with a diamond knife and the sections were stained on copper grids with 2% uranyl acetate (aqueous) followed by lead citrate (Reynolds 1963). Sections were viewed in Zeiss EM 9A and Hitache HU 11E electron microscopes.

Cytochemical Procedures

Acid Phosphatase - Berjaks (1968) modified Gomori method (1952). Tissue pieces were fixed in 6% glutaraldehyde buffered with acetate at pH 7.2, followed by three washes in acetate buffers of descending pH, namely 7.2, 6.0 and 5.0. The tissues were then incubated in Gomori medium at 37° C for 1h, washed in pH 5.0 acetate buffer for 10 min, 2% acetic acid for 1 min and acetate buffer (pH 7.2) for 5 min. Post-fixation was carried out in Luft's permanganate buffered with phosphate at pH 7.2 and dehydration in a graded alcohol series followed by propylene oxide. The tissues were again embedded in an Araldite - Epon - DDSA (1 : 1 : 3) mixture. As a control, material was processed in a similar manner but with 0.42% sodium fluoride added to the Gomori medium as an enzyme inhibitor. Sections were stained with lead citrate (Reynolds 1963).

Silver hexamine

The silver hexamine test for carbohydrate was performed as

described by Rambourg (1967). Material conventionally prepared for electron microscopy was sectioned and ribbons of thin sections floated on 1% periodic acid for 20 min. After three short rinses, sections were stored overnight on distilled water. They were then stained on 3% silver hexamine for 30 min at 60° C. After rinsing on distilled water they were transferred to a second bath of 3% silver hexamine for another 25-30 min also at 60° C. The sections were then rinsed on distilled water and floated on 5% sodium thiosulphate for 5-10 min. Following final rinsing the ribbons were picked up on grids and observed with a Hitachi HU 11E electron microscope. Controls were prepared by staining sections without previous hydrolysis on periodic acid.

Scanning Electron Microscopy

The seed wing was coated lightly with gold palladium in a vacuum evaporator and viewed in a Hitachi SSM-2 scanning electron microscope.

Biochemical Analyses

Welwitschia seed were imbibed on moist filter paper in Petri dishes in the dark at 25° C for periods of 2 and 5 days. The embryos of unimbibed dry seed and of the 2-day and 5-day treatments were removed and the gametophyte dried to constant weight in an oven at 100° C. The decrease in gametophyte mass over the experimental period was calculated and the material was ground prior to chemical analyses.

Ether extract

Fats were quantitatively extracted from gametophyte tissue with diethyl ether in a Soxhlet apparatus.

Protein and free amino acids

Free amino acids in fat extracted material were dissolved in 70% ethanol and quantitatively separated from the protein fraction by ultrafiltration through an Amicon DIAFLO conical filter. The material containing the protein fraction was quantitatively removed from the conical filter and hydrolysed *in vacuo* with 6N HCl for 24h at 110° C. The amino acids from both the free amino acid fraction and the protein hydrolysate were separated on a Beckman Model 120B amino acid analyser, using the two-column accelerated system of Speckman (1963).

Free fatty acid determination

The determination of fatty acid content and composition of fat extracted from the gametophyte at all three stages (0, 2 and 5 days) was carried out by the South African Bureau of Standards (SABS).

MORPHOLOGY AND ANATOMY OF GERMINATING SEED

INTRODUCTION

Although accounts of the morphology and anatomy of germinating *Welwitschia* seeds have appeared over the past century (Hooker 1863; Bower 1880, 1881a,b; Naudin 1882; Sykes 1910a,b; Hill and de Fraine 1910; Pearson 1929; Rodin 1953b; Martens and Waterkeyn 1964; Bornman *et al.*, 1972; Butler *et al.*, 1973), it seems advisable here briefly to sketch their habit of germination as a background to a fuller understanding of the ultrastructural and biochemical changes that take place.

RESULTS

Germination

Welwitschia is dioecious with reproductive branches arising from meristematic tissue adjacent and approximately parallel to the leaf bases. The "inflorescence" branches in a dichasial manner, each branch terminating in a regular strobilus (Fig. 1) with opposite and decussately arranged megasporophylls or bracts. The mature seed (Fig. 2) is subtended by a bract and consists of an embryo surrounded by gametophyte tissue which, in turn, is enclosed by two envelopes. The thin inner envelope or integument extends at one end into a tubular micropyle. The thick outer envelope or perianth (Martens 1959) forms a strong seed coat which is expanded laterally into a thin papery wing. The wing is fibrous (Fig. 3) and hygroscopic.

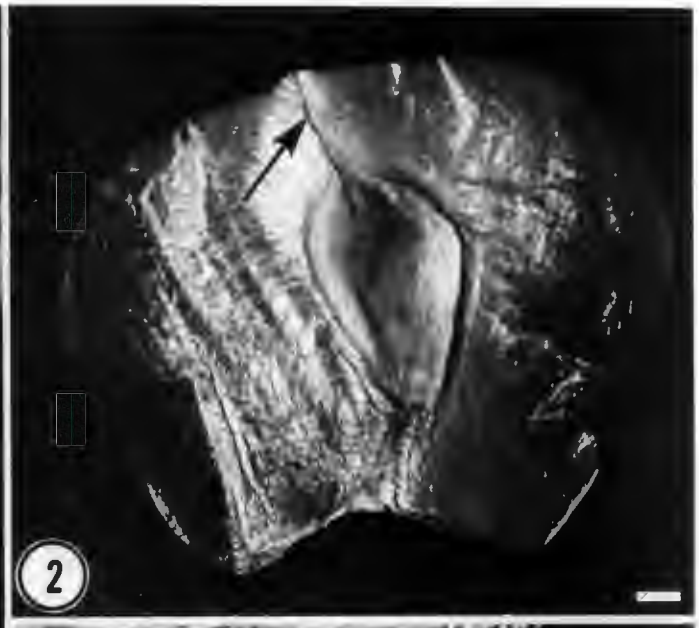
Prior to germination the seed is hard and dry (Fig. 4). It has a great capacity for imbibition. When supplied with water

PLATE 1.

Fig. 1. Megastrobilus with opposite and decussately arranged megasporophylls. Droplets represent an exudate.—Fig. 2. Mature seed. Inner integument extends into tubular micropyle (arrow). Outer seed coat expands into wing.—Fig. 3. Scanning electron micrograph of portion of seed wing torn to expose fibres.—Figs. 4-8. Seed coat removed.—Fig. 4. Dry seed.—Fig. 5. Fully imbibed seed.—Fig. 6. Germinating seed showing emergence of radicle.—Fig. 7. Looped emergence of hypocotyl.—Fig. 8. Surface view of emerging hypocotyl showing splitting of gametophyte tissue.

Bars represent 4cm (Fig. 1), 0,2mm (Fig. 3), 2mm (Figs. 2,4-8).


1

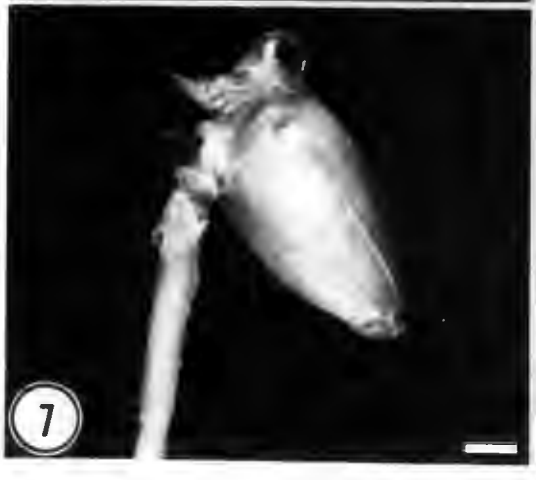

2

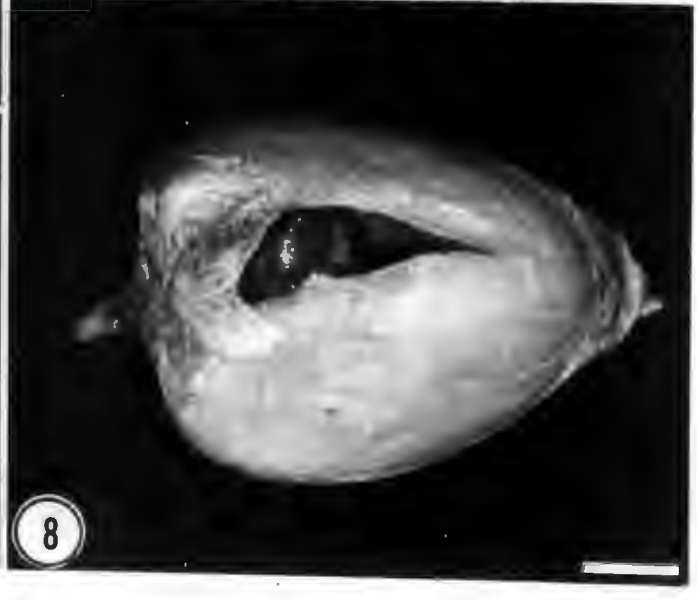

3


4


5


6


7


8

it imbibes rapidly (Fig. 5), swelling and more than doubling its initial weight within 7 h (Bornman *et al.*, 1972). Once germination is initiated the radicle elongates rapidly, splitting the outer envelope on the lower surface beneath the wing. The radicle does not emerge through the micropyle, the base of which is visible in Fig. 6. Germination is epigeous. The looped hypocotyl appears directly above the point of emergence of the root (Fig. 7). Occasionally, as shown in Fig. 8, it emerges in a central position causing the upper gametophyte tissue to split. As the hypocotyl progressively straightens, the two cotyledons are progressively withdrawn.

Feeder Development.

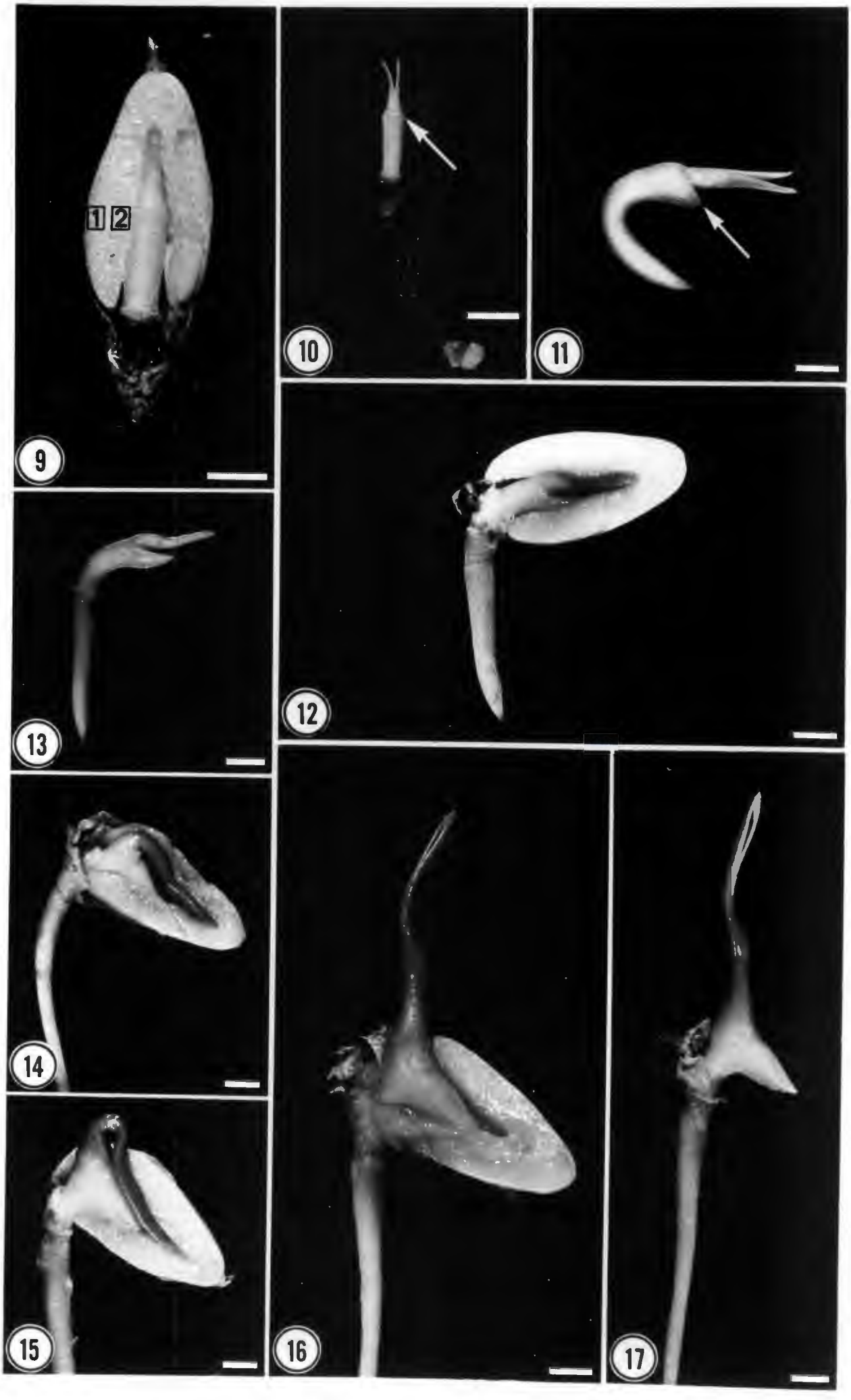
In the dry state the embryo is shrunken and brittle and often separated from the surrounding gametophyte by a narrow space. When fully imbibed (after 7 h at 25°C), the embryo becomes swollen and pressed against the now moist, spongy gametophyte (Fig. 9). Figure 10 shows the embryo in Fig. 9 after removal of the gametophyte tissue. The embryo consists of a long radicle and a short hypocotyl separated by a uniform bulge, the so-called collar (Bower 1881a). The shoot apex is situated between two laterally compressed cotyledons. The tip of the radicle is enclosed in a loose-fitting coleorhiza-like cap of dead cells to which a coiled suspensor is attached.

As germination rates varied greatly, times cited are average. Soon after germination is initiated the collar forms a protuberance or lateral process on the lower surface of the embryo.

PLATE 2.

Figs. 9-17. Seedling development.—Figs. 9,12,14-16. Seedcoat and portion of gametophyte removed.—Figs. 10,11,13,17. Seedcoat and gametophyte removed.—Fig. 9. Mature embryo in fully imbibed seed. Box 1 = deep gametophyte tissue studied. Box 2 = interface gametophyte tissue studied.—Fig. 10. Embryo of Fig. 9 after removal of gametophyte. A uniform swelling or collar (arrow) separates cylindrical radicle and hypocotyl which bears two cotyledons.—Fig. 11. Excised embryo after 2 days germination. A feeder (arrow) has begun forming laterally from the collar.—Fig. 12. Embryo 3 days after germination.—Fig. 13. Excised embryo of Fig. 12. Note wedge-like shape of feeder.—Fig. 14. Embryo 4 days after germination. Hypocotyl has lengthened and is beginning to arch.—Fig. 15. Seedling 5 days after germination showing looped emergence of plumule.—Fig. 16. Seedling 6 days after germination. Plumule is erect.—Fig. 17. Seedling of Fig. 16 after removal of gametophyte. Feeder is mature.

Bars represent 2mm.(Figs. 9-17).


1 2
9
10
11
12
13
14
15
16
17

This process was called "feeder" by Bower (1881a) who ascribed to it an absorptive function. By the time the radicle reaches a length of 10 mm (2 days after germination) the feeder is already conspicuous (Fig. 11, arrow). Growth of the feeder is rapid. Figure 12 shows the relative sizes of root, plumule and feeder 3 days after germination. The wedge-like shape of the feeder is shown by Fig. 13. As the feeder continues its growth the hypocotyl lengthens and as shown in Fig. 14 (4 days after germination), is just beginning to arch upwards prior to its looped emergence from the seed.

The close contact between feeder and gametophyte is depicted in Fig. 15 (5 days after germination). Force is required to separate them as they tend to become firmly cemented together. The hypocotyl has now emerged and the cotyledons are in the process of emergence. At the 6-day-stage the cotyledons are completely withdrawn from the seed (Fig. 16). The mature feeder (Fig. 17, excised seedling of Fig. 16) is about 5 mm long with a convex lower surface, a flatter upper surface and a curved edge. The feeder has been variously described as wedge-shaped (Bower 1881a) or footlike or spadelike (Coulter and Chamberlain 1910).

DISCUSSION

Germination of *Welwitschia* seeds has often been difficult in the past with many seedlings dying of fungal infection (Rodin 1953; Bornman *et al.*, 1972; Rowley 1972). In the desert whole strobili are commonly infected with *Aspergillus niger*.

This fungal infection that heavily parasitises the seeds is closely related to the activity of a large sucking Hemipteran, *Probergrothius sexpunctatis* (Bornman *et al.*, 1972). In this investigation the problem was largely overcome by selecting seeds for plumpness, removing the outer envelope, and surface-sterilizing the seed. When held at room temperature, *Welwitschia* seeds display widely differing rates of germination. Therefore, seeds were germinated in an incubator at 25° C. As different rates were still recorded, all germination times cited are purely average. Imbibition is rapid and appears to be affected by temperature. When supplied with water seeds are fully imbibed after 7 h at 25° C. Higher temperatures induced faster germination but provided ideal conditions for the development of *Aspergillus niger*.

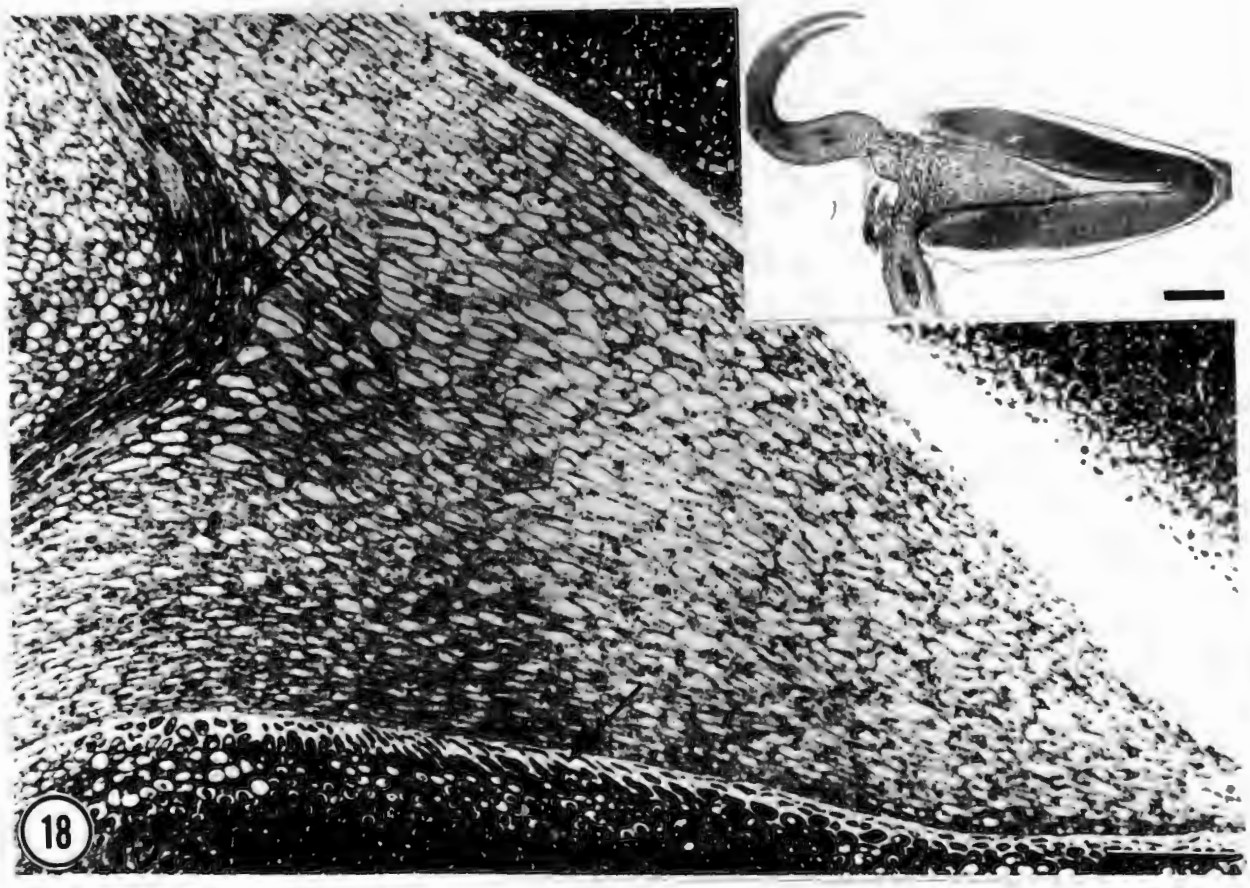
The feeder invariably develops from the lower surface of the collar irrespective of the side on which the laterally compressed seed is placed (Bower 1881a). Even when seeds are placed on edge the feeder forms on the lower surface. If seeds are germinated on a vertical rotating wheel the feeder grows from any position on the collar. When they are germinated vertically with the blunt end of the seed projecting upwards the feeder grows downward from the collar like a frill. This suggests that the feeder responds positively to geotropic stimulus.

Morphologically the feeder is regarded as an emergence (Bower 1881a). It results from elongation and division of cortical tissue in the collar region (Bower 1881a, Martens 1971) (Fig. 18). It is non-vascularised, consisting of elongated thin-

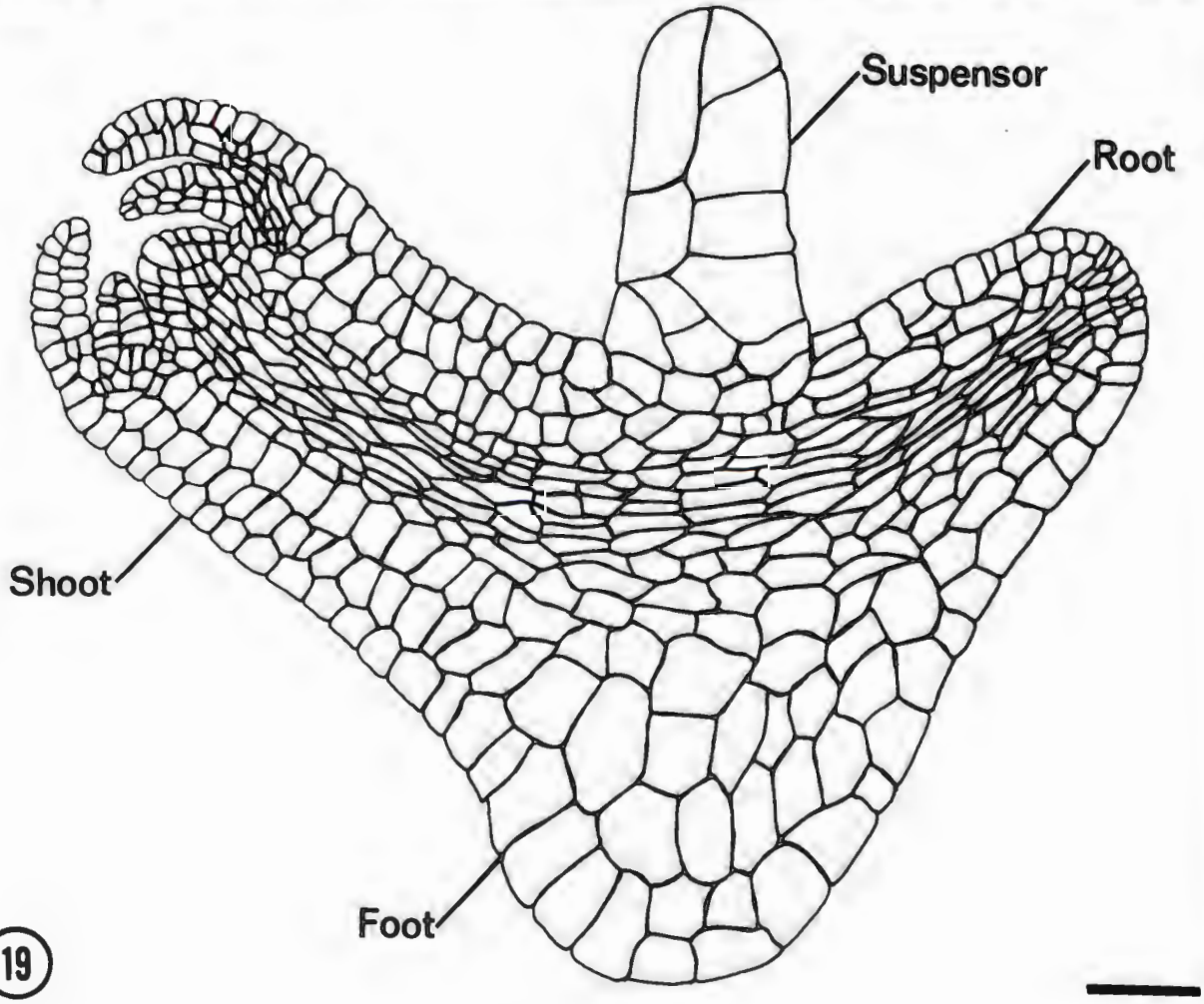
PLATE 3.

Fig. 18. Light micrograph of L.S. of 6-day-old feeder showing its non-vascularised nature and the close contact between its ventral surface and the gametophyte. Arrow indicates gametophyte interface cells studied. Hypocotylary vascular bundles nearest the feeder curve toward its base (double arrow) but do not enter it. Inset: low magnification of L.S. through 6-day-old seedling.—Fig. 19. Embryo of *Selaginella martensii* (redrawn from Bruchman, Flora 99 : 12, 1909).

Bars represent 0.5mm (Fig. 18), 2mm (Inset), 0.05mm (Fig. 19).



18



19

walled cells. The four vascular bundles of the hypocotyl enlarge as they traverse the collar zone, especially the two nearest the feeder which curve outward toward it but do not enter it (Fig. 18). The curvature seems to be the result of cell division in this area. Serial transverse and longitudinal sections reveal a slight increase in amount of phloem in all four bundles in the collar zone. Xylem increase is dramatic and is greatest in the region opposite the feeder (Butler, M.Sc. thesis 1970). If the feeder in fact is an absorptive organ it might be responsible not only for uptake of nutritive material but also of water. By chance a week-old seedling was left unwatered and in darkness for two weeks. When it was discovered, the filter paper was dry and the radicle had withered but the seedling was still alive, though etiolated. When the seed was slit open the gametophyte, which had shrunken against the feeder, was remarkably moist. It seems that the gametophyte can retain water for long periods. Possibly this is an adaptive feature which aids the establishment and survival of young seedlings under desert conditions.

During early germination (Figs. 11 - 15) the plumule separates the upper surface of the feeder from the gametophytic tissue. When the cotyledons have emerged (Fig. 16), a narrow space is left between upper feeder and gametophyte. Presumably most absorption, if it occurs at all, is via the lower surface of the feeder which remains in close contact with the gametophyte (Fig. 16).

In the seeds of other higher plants, vascularised reduced cotyledons (e.g. Palmae, Gramineae, *Cycas*, *Ginkgo*) or haustoria which develop from the suspensor (e.g. *Tropaeolum*) do act as absorptive organs (Bower 1881a). A haustorial-like structure similar to the feeder of *Welwitschia* occurs in some members of the Cucurbitaceae (Flahault 1877). Although morphologically it is equivalent to the feeder of *Welwitschia*, it is strictly speaking not an absorptive organ as the seeds are exalbuminous. Flahault (1877) ascribes to it the mechanical function of aiding the escape of the cotyledons from the testa. He also describes a similar structure in *Mirabilis jalapa* which produces albuminous seed. However, even here, he attributes to it a mechanical function only (Fahault 1877). The feeder of *Welwitschia* could very effectively fulfil a mechanical role in aiding cotyledon escape, as the feeder and gametophyte become firmly welded together providing the plumule with a firm base during its withdrawal from the seed. However, if the feeder of *Welwitschia* is also an absorptive organ, the closest parallel in morphology and function with other plants would appear to be found in *Selaginella*. The non-vascularised foot of *Selaginella* (Fig. 19) is also produced by lateral extension and division of cortical tissue of the hypocotyledonary stem.

GAMETOPHYTE.

DEEPLY-SITUATED GAMETOPHYTE CELLS.

INTRODUCTION.

A vast amount of information on the fine structure of germinating seeds has accumulated in recent years. Most authors have concentrated on metabolic and ultrastructural changes in storage organs and the embryo proper of angiospermous seed; species from Gramineae and Leguminosae being particularly well researched (Rost 1972). In contrast to this and with a few notable exceptions (Ching 1965 1972; Durzan *et al.*, 1971; Simola 1974), relatively little work has been done on gymnospermous seed. No previous ultrastructural and/or biochemical investigation has been made on germinating *Welwitschia* seed.

Due to wide variations in germination rates and often large differences among neighbouring cells with regard to rate of reserve product degradation and vacuolation, developmental changes in this study are not always expressed in terms of seed germination times. When times are quoted they are purely average figures.

RESULTS.

DAY ZERO.

The general appearance of deeply-situated gametophyte cells (Fig. 9, box 1) in dry quiescent *Welwitschia* seed is shown in Fig. 20. The cells are large (200 x 130 μm) and the walls thin and traversed by numerous plasmodesmata (Fig. 20). Perhaps these cells most striking feature is their massive store of reserve materials (Figs. 20, 21). Much of the cell's volume is taken up by protein bodies (protein grains, aleurone grains). Almost the entire remainder of the cell is filled with lipid bodies (lipid/oil droplets, spherosomes). The lipid bodies line the plasmalemma and occur in the intervening spaces between the protein bodies in such abundance that the cytoplasm is almost completely obscured. Glimpses of cytoplasm are rare and little detail can be resolved. Few organelles (Figs. 34-36) are discernible at this stage. The occasional amyloplasts (Figs. 20, 34) are probably the most immediately obvious due to the large starch grains they possess. The nucleus (Fig. 22) is grotesquely misshapen. The distorted, scalloped effect may be due to the pressure of reserve materials against its surface, squeezing it out of shape.

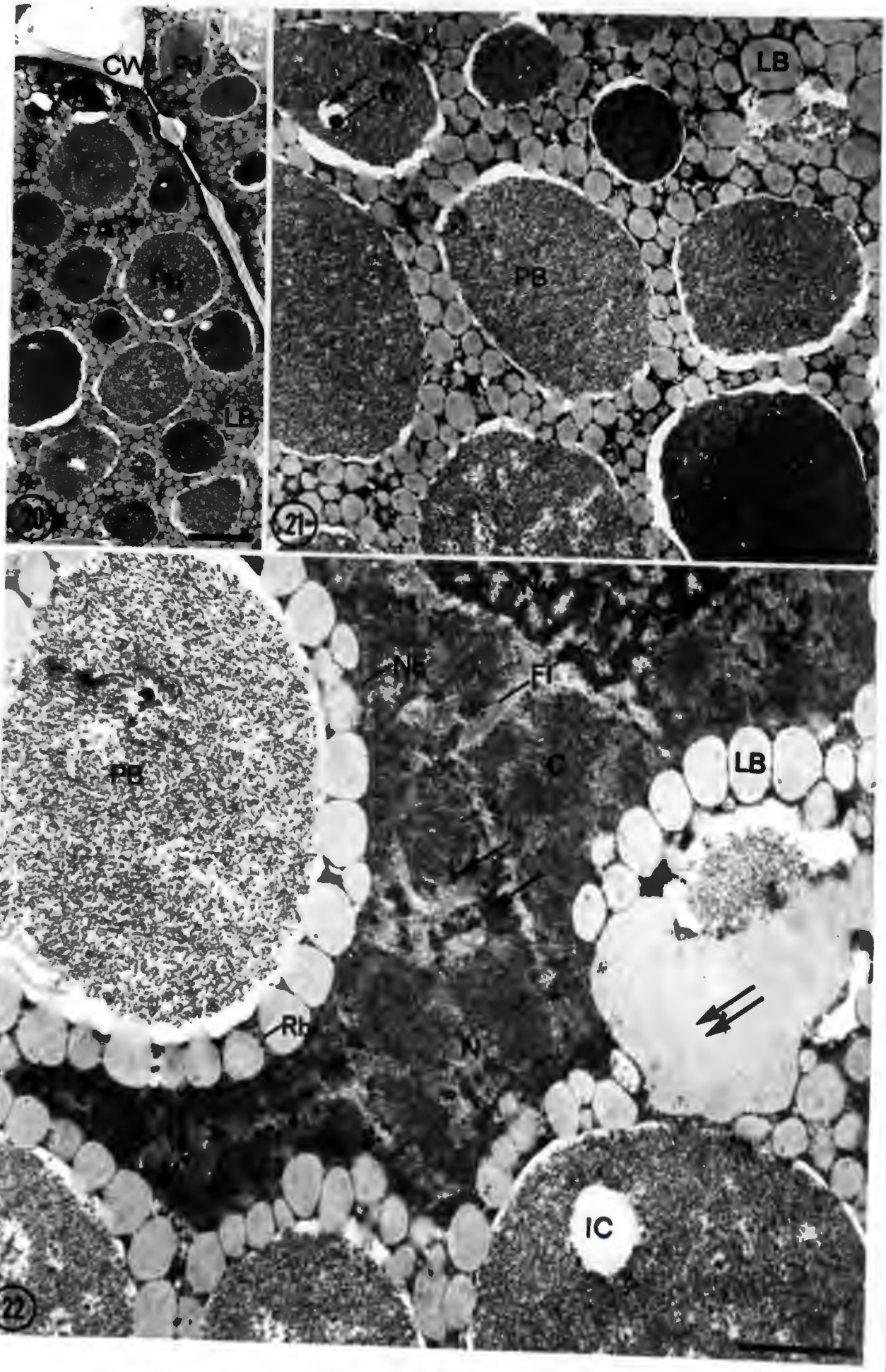
Nucleus

The lobed, convoluted nucleus (Fig. 22) is compact and dense. Ramifying between the electron-dense chromatin are pale fibrous inclusions that are possibly proteinaceous, as they appear to stain with mercuric bromphenol blue (Pearse 1961). These inclusions anastomose throughout the nucleoplasm and nucleoli (Fig. 22). The nucleoli are vacuolate, elongate ovals in longitudinal section, circular in transection; their vacuoles are packed with fibrous inclusions. Coarse, darkly-

PLATE 4.

Figs. 20-22. Deeply-situated gametophyte cells in dry, quiescent seed. —Fig. 20. Gametophyte cells with massive stores of reserve materials. Cytoplasm is almost completely obscured by closely packed protein bodies (PB) and lipid bodies (LB). Amyloplasts (A) are rare. Cell walls (CW) are thin with numerous plasmodesmata (Pd).—Fig. 21. Portion of dry gametophyte cell. Note non-uniform texture of granular matrix of protein body (PB). Globoids (G) may occur in electron-transparent cavities (arrow) of protein body matrix. LB = lipid body.—Fig. 22. Portion of dry, lobed nucleus (N) showing fibrous inclusions (FI) anastomosing throughout nucleoplasm and nucleolus (Nu). Darkly staining areas (arrows) similar in texture and density to nucleolar material often surround nucleoplasm inclusions. Ribosomes (Rb) are present in cytoplasm as unaggregated monosomes. Lipid bodies (LB) occasionally coalesce (double arrow). C = chromatin; IC = internal (globoid) cavity; NE = nuclear envelope; PB= protein body.

Bars represent 2.5μm (Figs. 20, 21), 1μm (Fig. 22).


CW
PM
LB
20
LB
PB
21
PB
Nb
Fl
C
LB
Rb
N
IC
22

staining areas similar in texture and density to nucleolar material frequently surround the inclusions in the nucleoplasm. The double nuclear membrane is faintly discernible. Nuclear pores were indistinct and rarely observed.

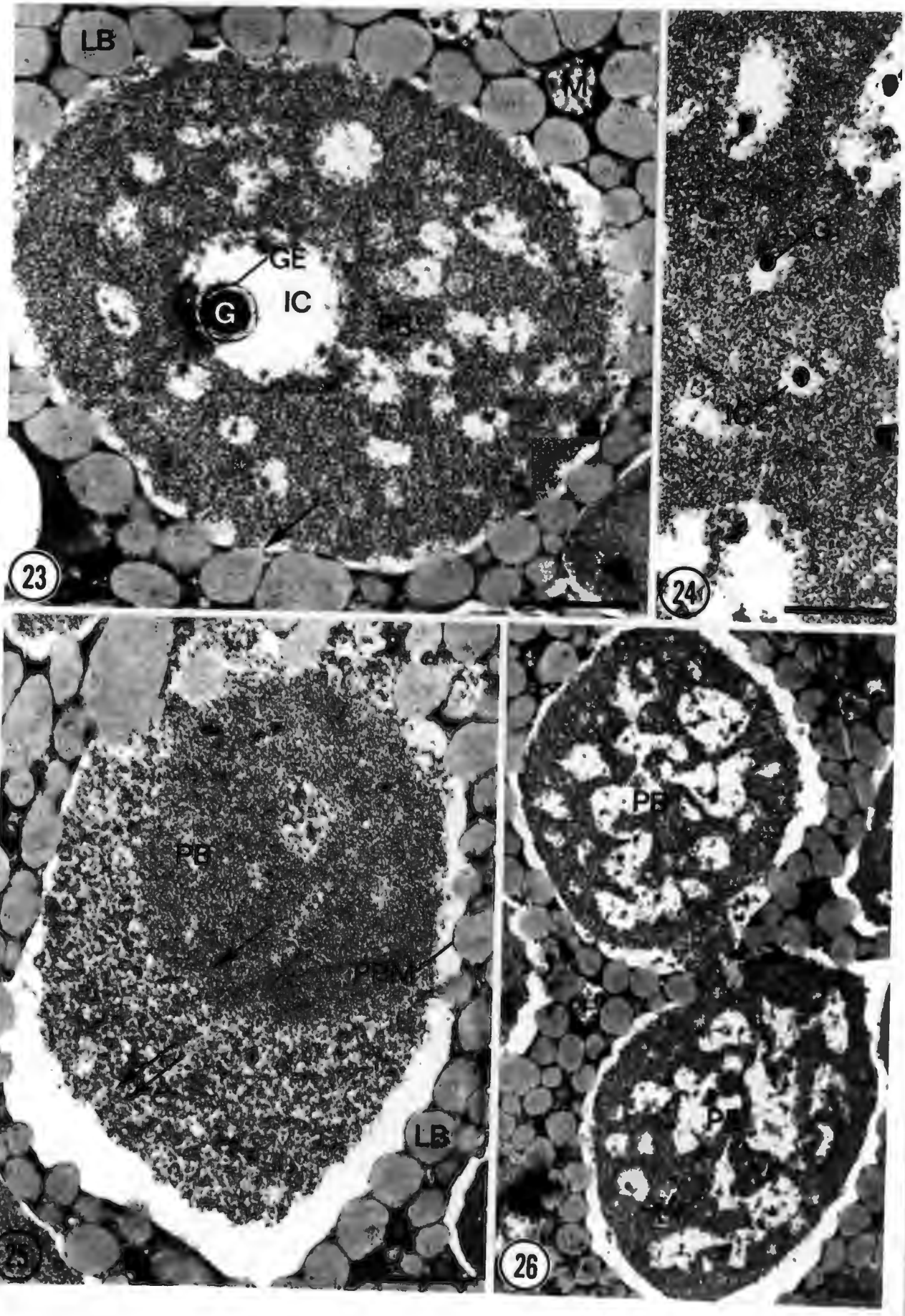
Protein Bodies

Protein bodies may be roughly spherical to ovoid in shape (Fig. 20) or may possess an irregular outline (Fig. 41). They vary considerably in size, perhaps due to plane of sections, with maximum diameter ranging from 1 μm to 9 μm. Commonly they are 4 μm to 5 μm. Typically, as illustrated in Fig. 23, each protein body consists of a granular matrix enclosed by a single unit membrane (Ching 1972; Rost 1972). The granular texture of the matrix is not uniform in all protein bodies. Some are dense (Fig. 21), others less compact (Fig. 22), while others are mottled with electron-transparent areas (Fig. 23). Infrequently highly electron-dense inclusions (globoids) may occur in the electron-transparent cavities (internal or globoid cavities) of a protein body as shown in Figs. 21, 23, (Pfeffer 1872; Poux 1963; Sobolev 1966; Lui and Altschul 1967; Jacobsen *et al.*, 1971). Some protein bodies contain a number of small globoids (Fig. 24), others a single large globoid (Figs. 21, 23). The large osmiophilic globoid in Fig. 23 seems to be membrane bound (Poux 1965; Khokhlova 1971) but the fine structural characteristics of a unit membrane could not be resolved (Jones 1970; Jacobsen *et al.*, 1971; Lott and Vollmer 1973b. Most globoids are devoid of outer bounding envelopes (Figs. 21, 24) and many have a pitted appearance (Fig. 24).

PLATE 5.

Figs. 23-26. Protein bodies in deeply-situated dry gametophyte cells. —Fig. 23. Each protein body (PB) contains a granular matrix enclosed by a single unit membrane (arrow). The matrix may be mottled with internal electron-transparent cavities (IC), frequently called globoid cavities, in which globoids (G) may occur. Note outer bounding envelope of globoid (GE). LB = lipid body; M = mitochondrion.—Fig. 24. Numerous small globoids (G) within internal electron transparent cavities (IC) of protein body matrix.—Fig. 25. Non-uniformity of granular texture is rare within a single protein body (PB). Note finely granular core (arrow) almost surrounded by less dense coarsely granular matrix (double arrow). LB = lipid body; PBM = protein body membrane. —Fig. 26. Dumb-bell shaped, fused protein bodies (PB).

Bars represent 1μm (Figs. 23-26).


LB
M
GE
G
IC
PB
23
24
PB
PBM
LB
25
PB
P
26

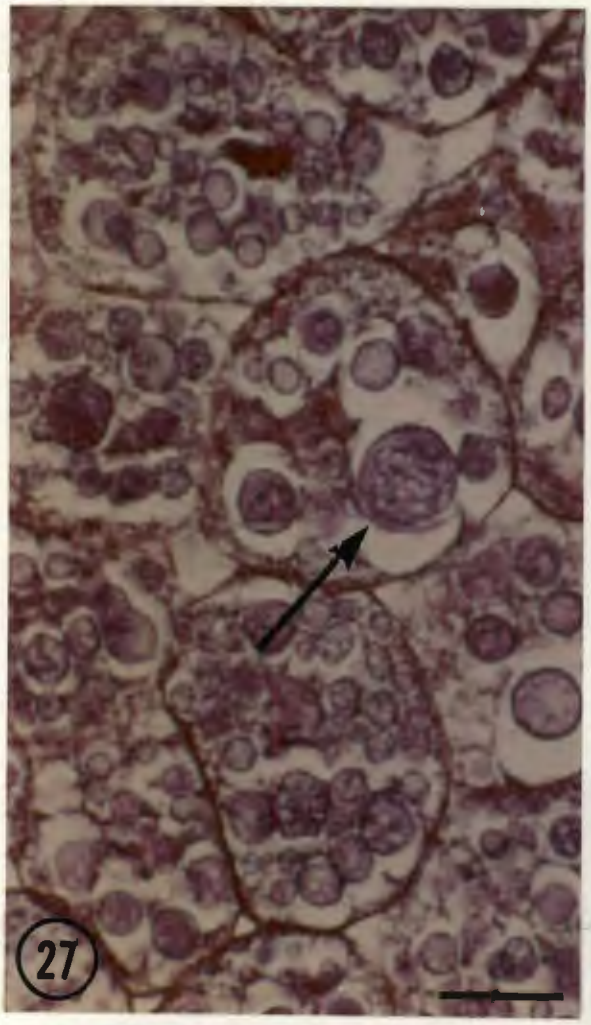
Non-uniformity among protein body matrices is quite obvious in Fig. 20. Rarely, non-uniformity of granular texture is apparent within a single protein body (Fig. 25). In Fig. 25 a compact finely granular core is almost surrounded by a less dense coarsely granular matrix. Unit membranes surrounding protein bodies are not very distinct (Fig. 25, arrow) - a common phenomenon in dry material. Electron-transparent areas between reserve deposits and bounding membranes (Fig. 25) probably occur as a result of hydration during fixation. Occasionally dumb-bell shaped, fused protein bodies are seen in dry material (Fig. 26).

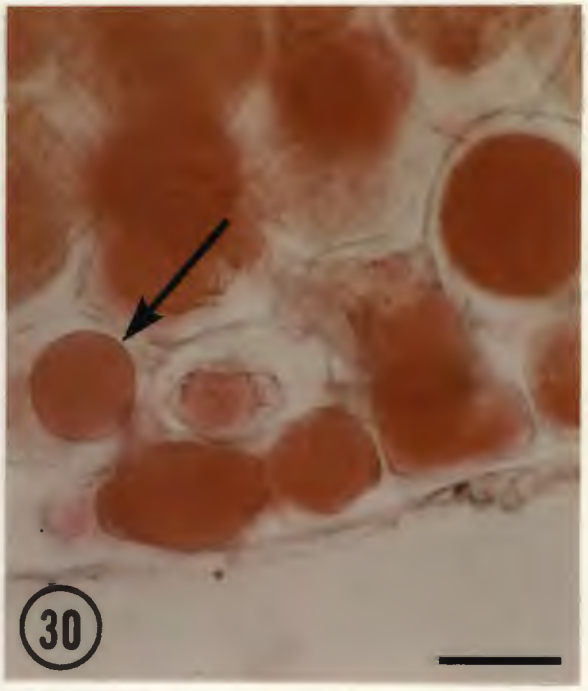
Cytochemical methods at the light microscope level (Jensen 1962; Pearse 1961; Jacobsen *et al.*, 1971) were used to confirm the nature of reserve substances within the protein body. Cells stained with mercuric bromphenol blue, a quantitative total protein stain (Pearse 1961), are shown in Fig. 27. As expected the protein bodies stained a definite blue indicating that the reserves are proteinaceous. Surprisingly the same bodies also stained with periodic acid-Schiff reagent (Fig. 28), a stain for total carbohydrates (Jensen 1962). In 1971 Jacobsen *et al.*, reported that small protein-carbohydrate crystalloids embedded in aleurone grains (protein bodies) of barley stain a greenish colour with toluidine blue. In *Welwitschia* the entire protein body stained a greenish colour (Fig. 29) with toluidine blue. This seemed to suggest that the entire reserve may exist as a protein-carbohydrate complex.

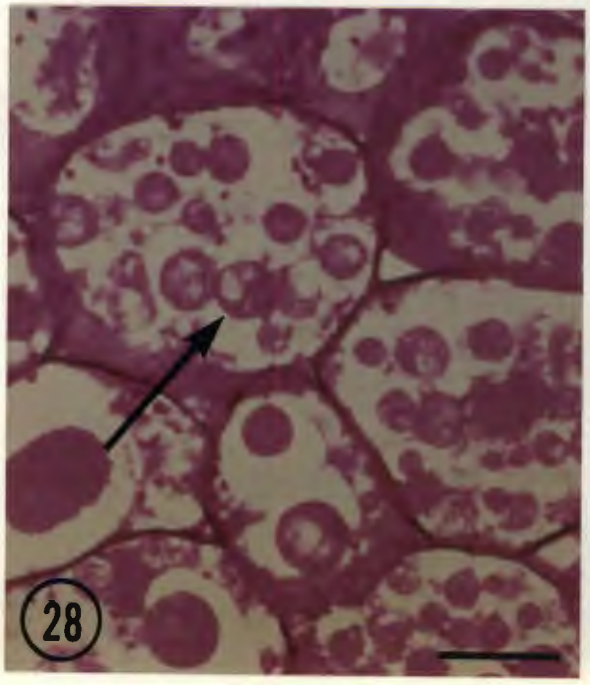
PLATE 6.

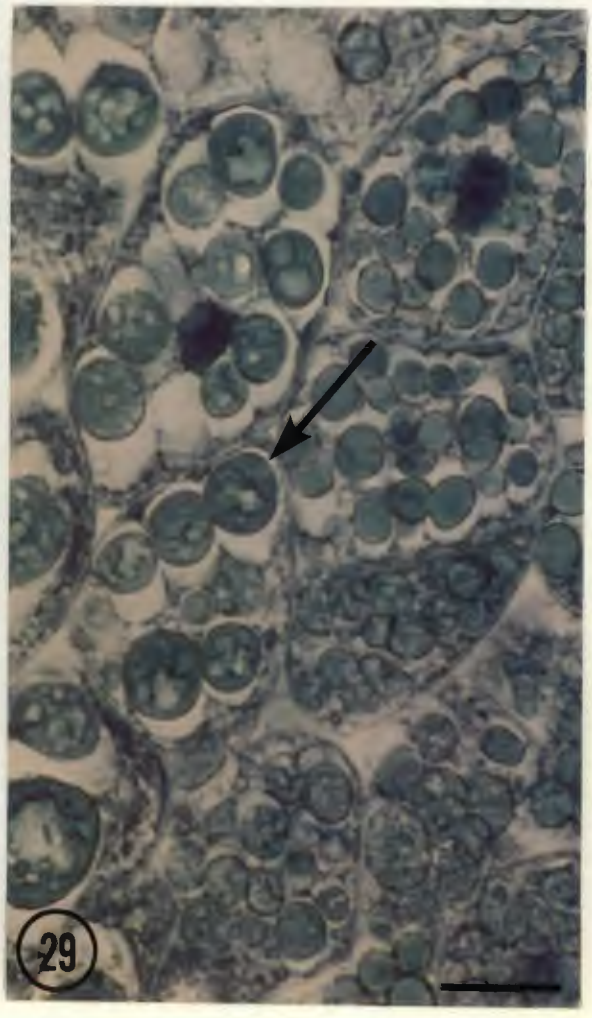
Figs. 27-30. Cytochemical staining of gametophyte tissue at light microscope level.—Fig. 27. Stained with mercuric bromphenol blue. Arrow indicates protein body.—Fig. 28. Stained with periodic acid - Schiff reagent. Arrow indicates protein body.—Fig. 29. Stained with toluidine blue. Arrow indicates protein body.—Fig. 30. Fresh free-hand section stained with Sudan IV. Arrow indicates lipid pool.

Bars represent 25 μm (Figs. 27-30).


27


30


28


29

As a further check dry gametophyte tissue was stained using Rambourg's (1967) silver methenamine technique for the detection of carbohydrates at the EM level. In the experiment (Fig. 31) the protein bodies and cell walls stained consistently with silver methenamine. The control (Fig. 32) in which the sections were not oxidised on periodic acid prior to staining, was comparatively clean. Lipid was often dissolved from the light bodies during the treatment (Figs. 31, 32).

These cytochemical results seem to indicate that, in the case of *Welwitschia*, the "protein bodies" are actually protein-carbohydrate bodies. However, to avoid changing nomenclature I shall continue to refer to the body as a whole as a protein body, and to its contents as protein-carbohydrate.

Globoids were rarely seen at the light microscope level. The few observed seemed to stain red with toluidine blue. Apparently this is characteristic of globoids (Vazart 1960). The red colour is thought to be due to the presence of phytin and, perhaps, free phosphate (Jacobsen *et al.*, 1971). Another distinguishing feature of the globoids was their association with high acid phosphatase activity as shown in Figs. 113-115; (Poux 1965; Villers 1971).

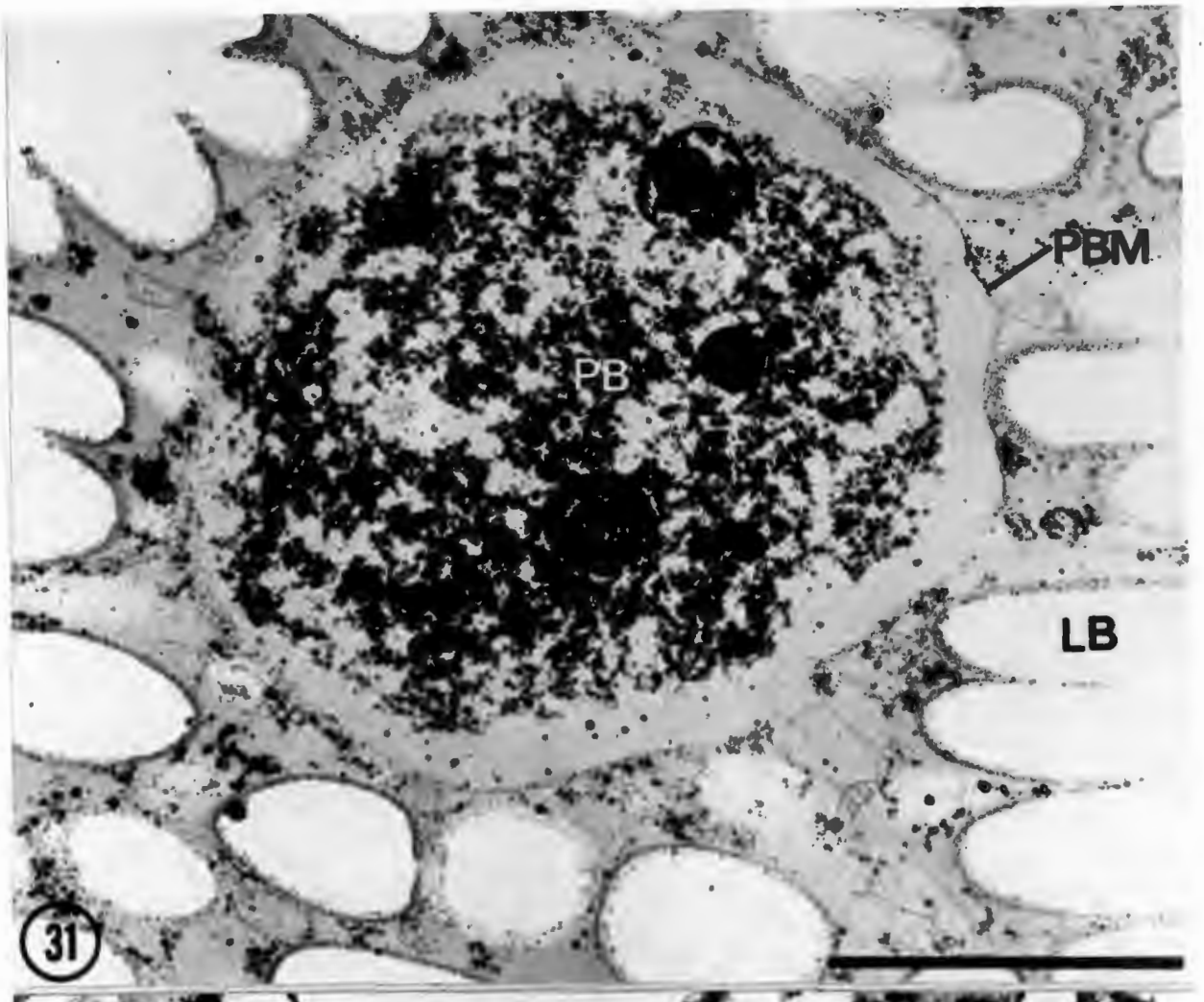
Lipid Bodies

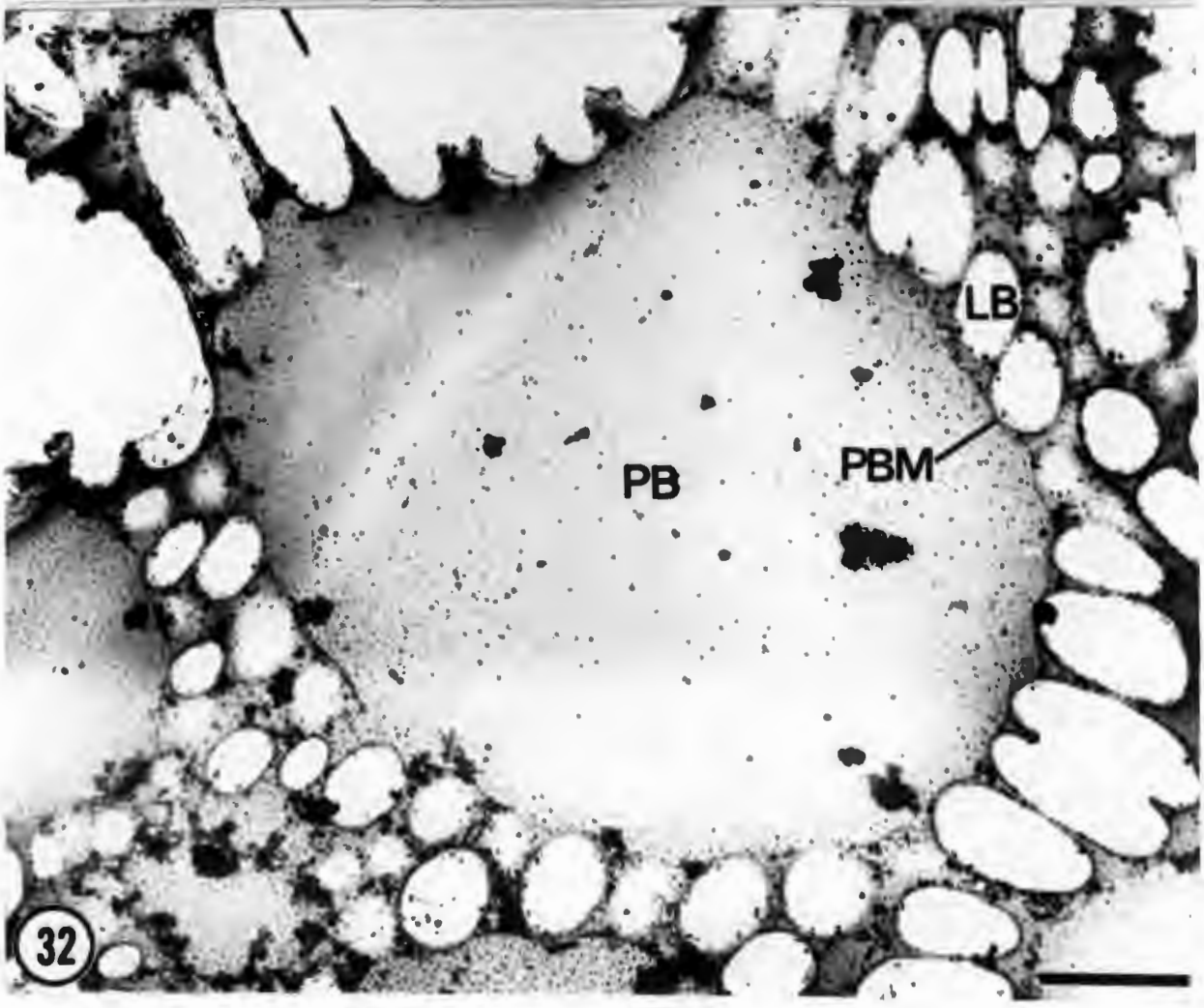
Fresh free-hand sections were used to localise lipids in dry material, as immersion in an alcohol dehydration series removed them. Cytoplasmic lipid was very mobile and accumulated

PLATE 7.

Figs. 31,32. Silver hexamine staining for detection of carbohydrates at electron microscope level.—Fig. 31. Protein bodies (PB) stain consistently with silver methenamine. Lipid is often dissolved from the lipid bodies (LB). PBM = protein body membrane.—Fig. 32. Protein bodies (PB) in control sections not oxidised on periodic acid prior to staining remain comparatively clean. LB = lipid body; PBM = protein body membrane.

Bars represent 1μm (Figs. 31,32).


PBM
PB
LB
31


LB
PB
PBM
32

in pools on sectioning. The lipid stained with Sudan IV (Fig. 30) and Sudan Black - all saturated solutions in 70% ethanol (Jensen 1962). The cytoplasmic staining (Fig. 30) was presumably due to coalescing lipid bodies (Fig. 22). These bodies are numerous. They are aligned along the cell walls and surround the protein bodies, being in intimate contact with the plasmalemma (Fig. 20) and protein body membranes (Figs. 20-23). Lipid bodies are distributed throughout the entire remainder of the cell (Fig. 20). Consequently very little residual cytoplasm is detectable. In the dry state lipid bodies are in contact with the nuclear membrane (Fig. 22) due to the close packaging of reserve materials.

Lipid bodies are spherical and smooth (Fig. 22) with diameters 0.2 µm to 0.7 µm and an average diameter of 0.5 µm. They are homogeneous in structure, stain grey and possess a thin peripheral electron-dense line. Close packaging commonly produces a "honeycomb" effect (Fig. 33). Despite compression the bodies usually maintain their discreteness, although occasionally they coalesce to form a lipid pool (Figs. 22, 33).

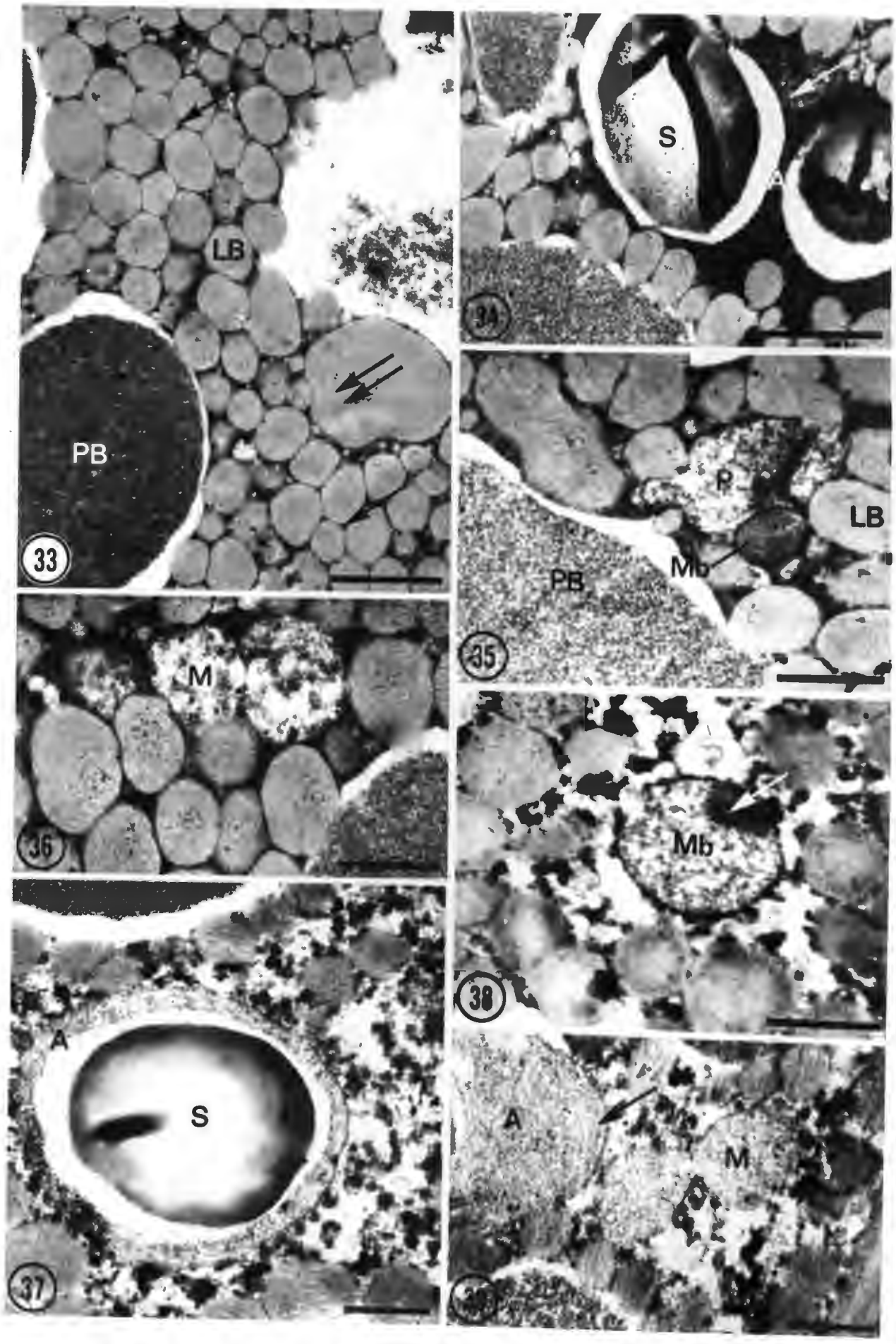
Organelles in residual cytoplasm

Amyloplasts (Figs. 20, 34) are present but not profuse in deep gametophyte tissue. In the dry state they are poorly defined with indistinct bounding membranes and an absence of internal lamellae (Paulson and Srivastava 1968). Much of the internal volume is occupied by a single or several large starch grains. The plastid matrix is often dense (Fig. 34) and little detail

PLATE 8.

Figs. 33-36. Dry gametophyte cells.—Fig. 33. Close packaging of lipid bodies (LB) produces a "honeycomb" effect (arrows). Lipid bodies usually maintain their discreteness but occasionally coalesce to form a lipid pool (double arrow). PB = protein body.—Fig. 34. Amyloplast (A) with dense matrix (arrow), indistinct bounding membranes and absence of internal lamellae. Starch grains (S) occupy much of internal volume.—Fig. 35. Oval microbody (Mb) with finely granular matrix bounded by single-unit membrane. LB = lipid body; P = plastid; PB = protein body.—Fig. 36. Mitochondria (M) with electron-translucent regions and no cristae. Mitochondrial matrix contains ribosomes, osmiophilic granules and fibrils.—Figs. 37-39. Gametophyte cells imbibed for 24 h.—Fig. 37. Amyloplast (A) showing finely granular matrix and surrounding double-unit membrane. S = starch grain.—Fig. 38. Microbody (Mb) containing an inner electron-dense amorphous area (arrow) in contact with bounding unit membrane.—Fig. 39. Dividing mitochondrion (M) with short vesiculate cristae. Note amyloplast (A) with single lamella (arrow).

Bars represent 1μm (Figs. 33,34), 0.5μm (Figs. 35-39).


LB
PB
33
S
34
P
LB
Mb
PB
35
M
36
Mb
38
A
S
37
A
M

can be resolved. Very few microbodies (Fig. 35) are observed, possibly due to their small size and the obscuring effect of reserve material. They are oval in profile with a moderately electron-dense, finely granular matrix surrounded by a single unit membrane. Mitochondria (Figs. 23, 36) are more numerous. They are spherical organelles with electron-translucent regions and little apparent development of internal structure (Paulson and Srivastava 1968). No cristae are seen but the mitochondrial matrix contains ribosomes, osmiophilic granules and fibrils presumed to contain DNA (Bisalputra and Bisalputra 1967) (Figs. 23, 36). No endoplasmic reticulum (ER) or dictyosomes are evident in the unimbibed gametophyte. Ribosomes are present (Fig. 22, arrow) as unaggregated monosomes. No polysome configurations are detected in the residual cytoplasm.

DAY 1.

After 24h imbibition the nucleus and cytoplasmic organelles of gametophyte cells show increased clarity of membranes. Internal structure, at first poorly defined, is more clearly observed. On hydration nuclear fibrous inclusions disperse, completely disappearing within 24h in freshly harvested seed. The nucleus, with its variable number of nucleoli, retains its irregular, invaginated shape. The nucleus of a 1-day-imbibed gametophyte cell is superficially similar to the 3-day-imbibed nucleus depicted in Fig. 40. The only noticeable difference

is that the nucleoli are smaller and denser in the 1-day-imbibed state. Condensed chromatin appears dispersed throughout the nucleoplasm.

The nuclear membranes and protein body membranes can be clearly resolved. The double-unit membranes surrounding the amyloplasts at first indistinct (Fig. 34), are now evident (Fig. 37). The matrix is finely granular, containing ribosomes and osmiophilic droplets (Kislev *et al.*, 1965). Few lamellae are seen (Fig. 39, arrow). Most amyloplasts contain one, possibly two, starch grains. Microbodies (Fig. 38) are now seen to consist of a granular matrix with an inner electron-dense amorphous area in contact with the bounding unit membrane (Vigil 1970). The double-unit structure of bounding mitochondrial membranes is obvious (Fig. 39). Although clarity is still imperfect, cristae seem to become organised and the mitochondrial matrix is no longer translucent. Mitochondrial constrictions, as seen in Fig. 39, were interpreted as representing dividing organelles (Schulz and Jensen 1973).

The cytoplasm does not appear homogeneous but consists of randomly scattered darkly staining areas on a light, finely granular background (Figs. 37 - 39). ER, polysomes and dictyosomes, at this stage, are unobserved.

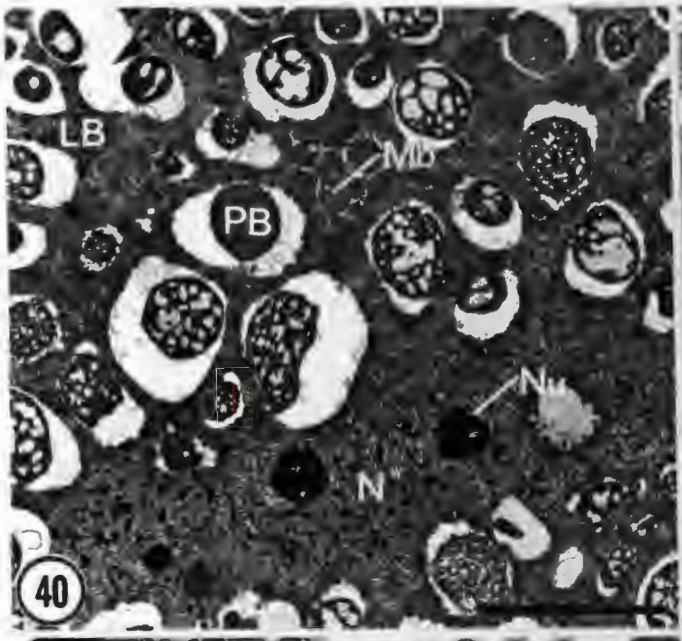
DAY 3 - 6.

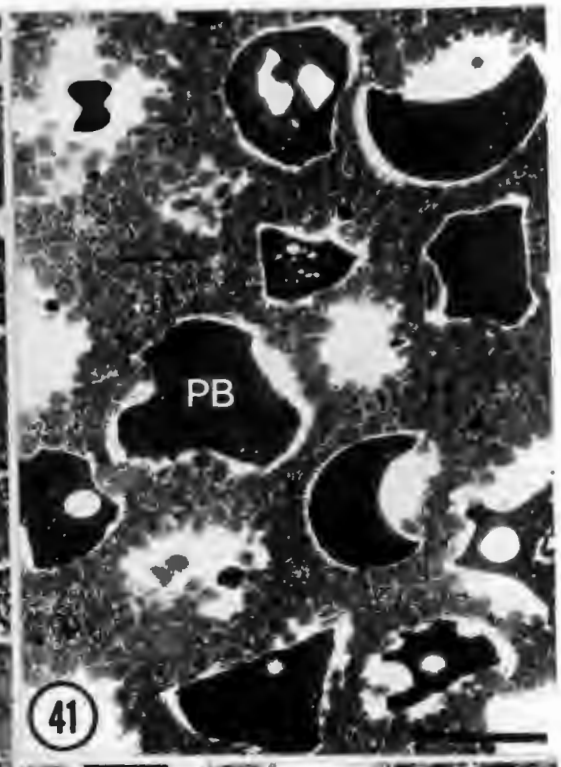
Fig. 40 is a micrograph at low magnification of a 3-day-im-

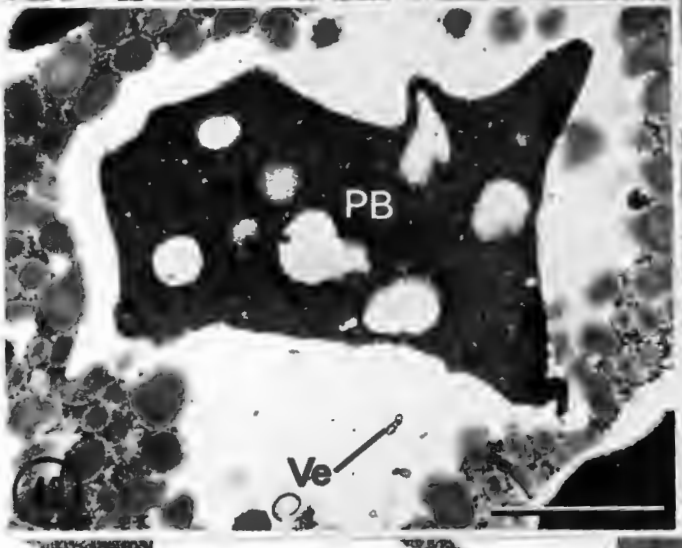
PLATE 9.

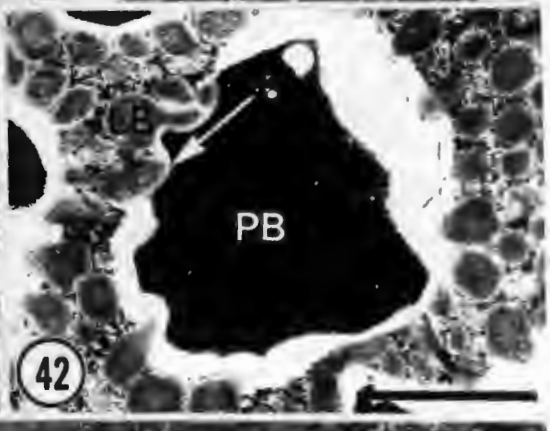
Figs. 40-45. 3-day-imbibed gametophyte cells.—Fig. 40. Low magnification of portion of gametophyte cell showing invaginated nucleus (N) with vacuolate nucleoli (Nu) and protein bodies (PB) in various stages of degradation. Note coalescence of protein bodies (arrow). Lipid bodies (LB) have declined in number. Mb = microbody.—Fig. 41. Angular shaped protein body (PB) reserves.—Fig. 42. Protein body (PB) reserves with surface indentations (arrows) caused by appression of lipid bodies (LB).—Fig. 43. Protein body (PB) with small internal digested pockets giving reserves a fenestrated appearance. Ve = vesicles.—Fig. 44. Portion of protein body (PB) reserves showing coalescence of digested pockets.—Fig. 45. Spherical protein body (PB) reserves. LB = lipid body; M = mitochondrion; PBM = protein body membrane; Ve = vesicle.

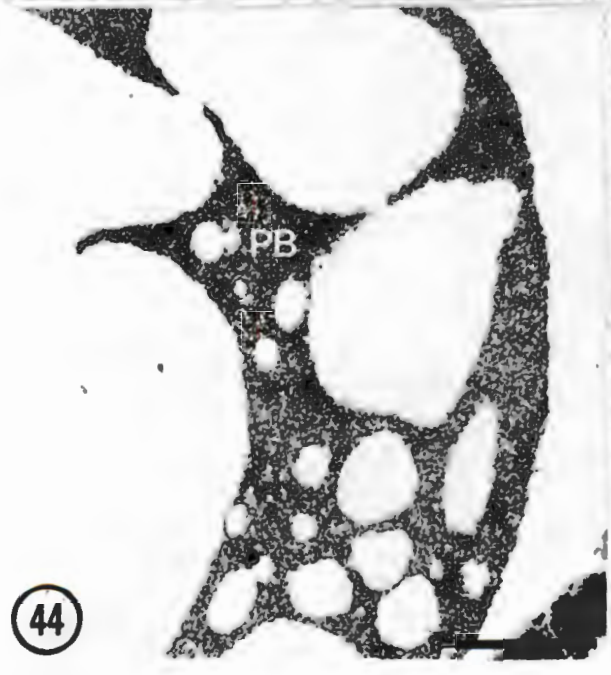
Bars represent 10μm (Fig. 40), 2.5μm (Figs. 41-43), 1μm (Figs. 44,45).

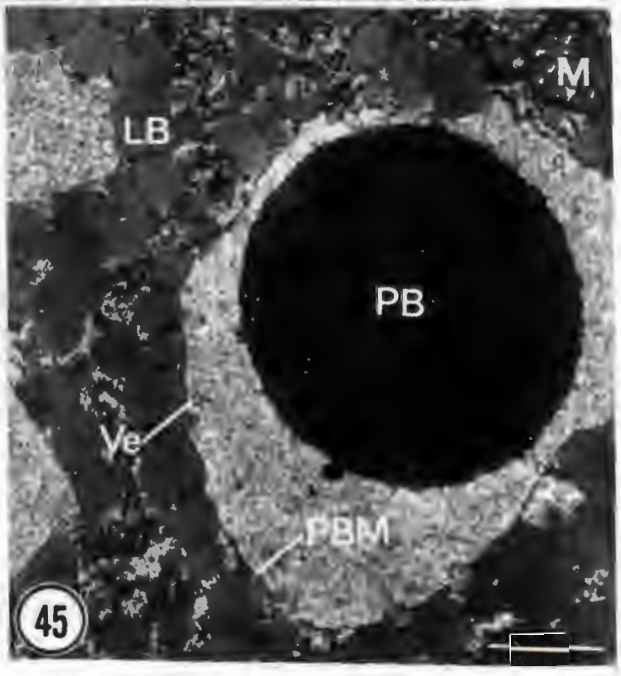

LB
Mb
PB
N
N
40


PB
41


PB
Ve
43


LB
PB
42


PB
44


M
LB
PB
Ve
PBM
45

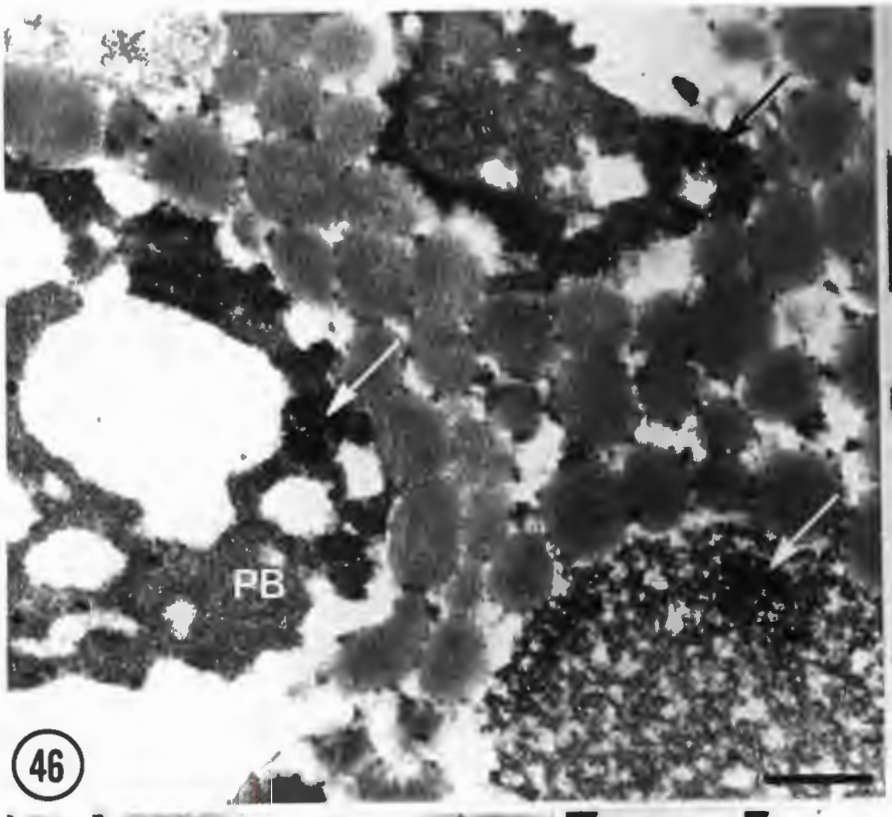
bibed gametophyte cell. Nucleoli within an invaginated nucleus appear large, vacuolate and active (Lowary and Avers 1965; Hyde 1967; Fowke and Setterfield 1968; Jordan and Chapman 1971; Villiers 1971) and there is no trace of nuclear and nucleolar crystals. At this stage there are slightly fewer lipid bodies and they are no longer in contact with the nuclear membrane. Cytoplasmic organelles are more frequently visualised and membrane definition is clearer.

Increased hydration has resulted in the swelling of protein bodies (Fig. 40) (Srivastava and Paulson 1968; Briarty *et al.*, 1970; Rost 1972; Abdul-Baki and Baker 1973). The large electron transparent spaces between reserve deposits and bounding membranes expanded as hydration progressed. Protein bodies in Fig. 40 are in various stages of degradation. Digestion seems to begin from within the protein-carbohydrate deposits. Small, internal digested pockets give the reserves a fenestrated appearance (Figs. 40, 43). As digestion proceeds the pockets coalesce (Figs. 40, 44). Adjacent protein bodies also fuse (Fig. 40). Most but not all protein bodies are spherical (Figs. 40, 45). Some, by virtue of their angular shape (Fig. 41) suggest that digestion begins from the periphery (Rost 1972). Some peripheral digestion is possible, but in *Welwitschia* the usual digestion pattern is one of internal degradation. It is suggested that close packing of lipid and protein bodies during dehydration of the ripening seed is the major cause of peripheral pitting (Fig. 42). As a protein body in a germinating seed hydrates, its membrane is separated from its reserve material but the surface indentations caused by

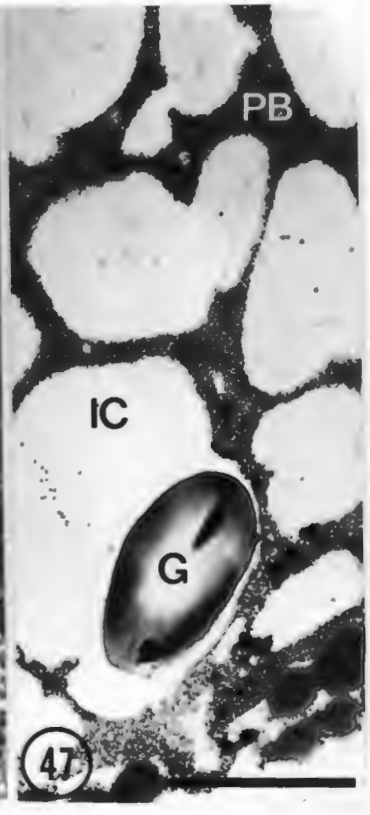
PLATE 10.

Figs. 46-49. 3-day-imbibed deep gametophyte cells.—Fig. 46. Protein bodies (PB) with small spherical particulate crystals (arrows) embedded in protein-carbohydrate reserves.—Fig. 47. Large globoid (G) within internal cavity (IC) of protein body (PB) reserves.—Fig. 48. Solid osmiophilic crystals (Cr) embedded in protein-carbohydrate reserves of protein body (PB). Note crystal (arrow) in contact with protein body membrane.—Fig. 49. Large osmiophilic crystal (Cr) apparently migrating from cytoplasm into protein body (PB).

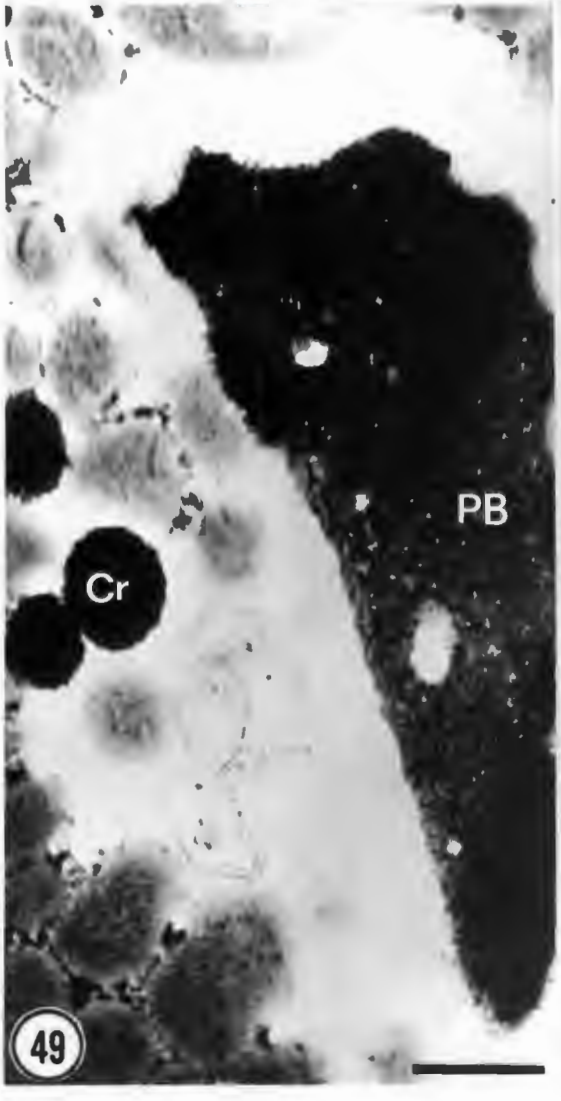
Bars represent 0.5 μm (Figs. 46, 48, 49), 2.5 μm (Fig. 47).



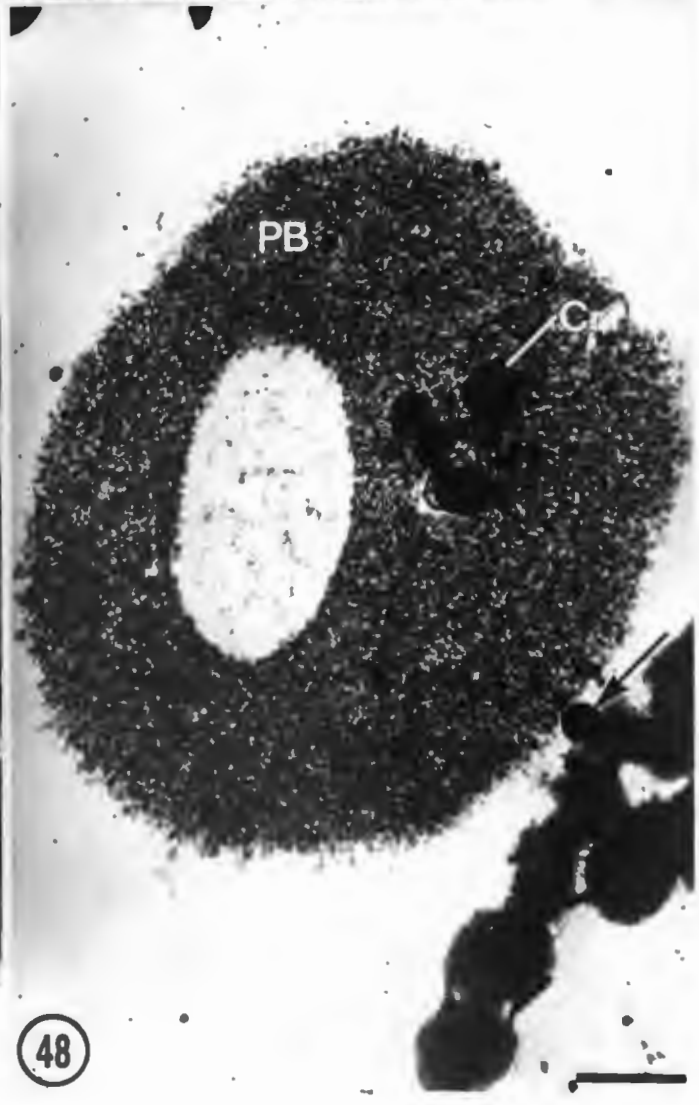
46



47



49



48

the appression of lipid bodies remain. Commonly undulations of the protein body membrane coincide with those of the reserves (Fig. 42). Some protein bodies are so compact it is difficult to discern their granular nature (Figs. 42, 45). This is especially true for angular protein bodies usually found in the most deeply-situated gametophyte cells (Fig. 41).

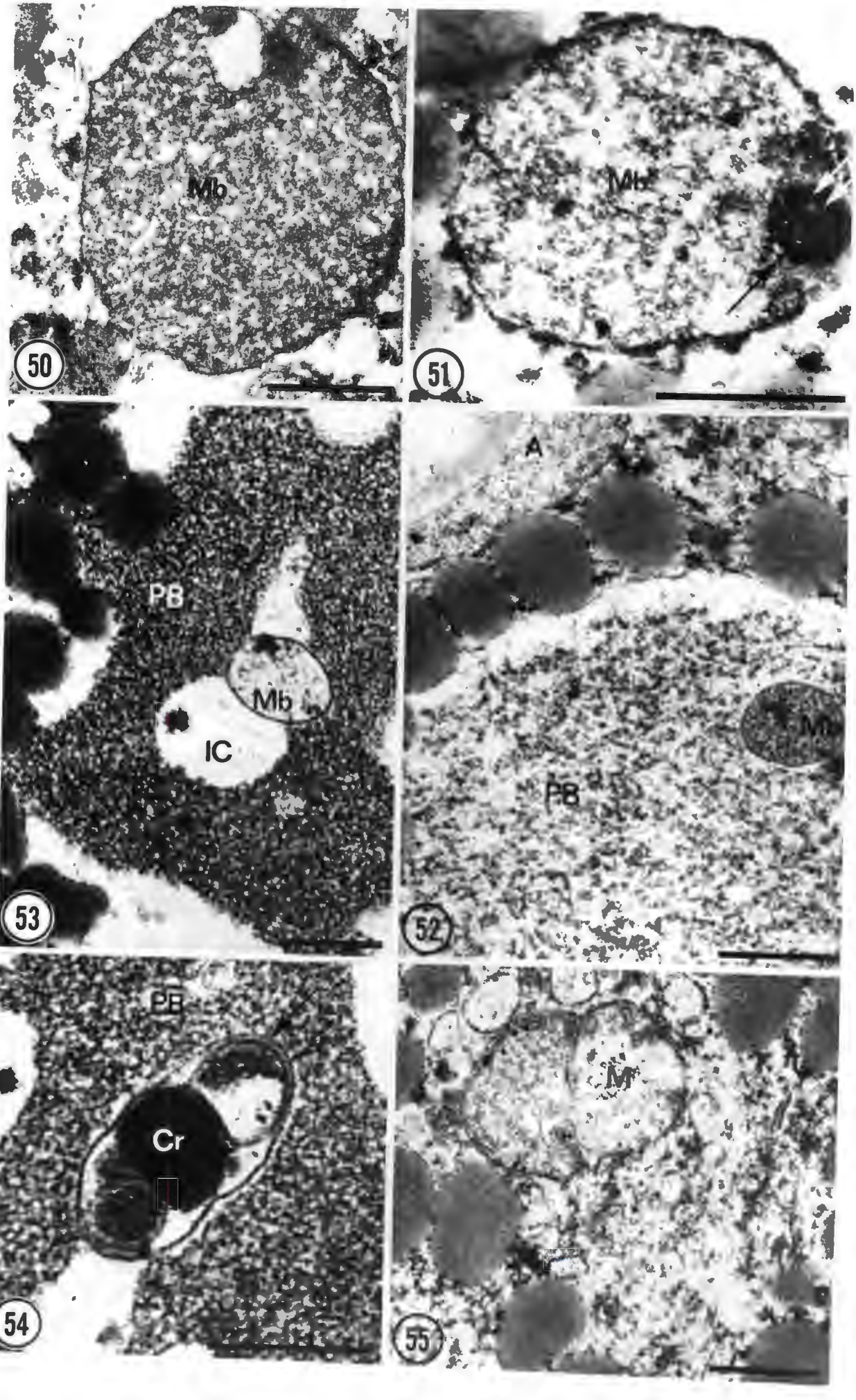
Crystalline inclusions corresponding to the globoids described in seed literature (Pfeffer 1872; Poux 1963; Sobolev 1966; Lui and Altschul 1967; Jacobsen *et al.*, 1971) stained red with toluidine blue (Jacobsen *et al.*, 1971) and also reacted positively for acid phosphatase (Poux 1965; Villiers 1971). They occur infrequently in gametophyte protein bodies, the majority being similar to those depicted in Figs. 21, 23 and 24. They appear either as a single large crystal or several small ones within the internal cavities of reserve deposits. They may be electron-dense or pitted and may or may not be surrounded by a bounding envelope.

The preceding description does not cover all crystal-like inclusions observed. Frequently small, spherical, particulate crystals of a uniform size (diameter ca. 0,3 µm) are seen apparently embedded within protein-carbohydrate reserves (Fig. 46). The crystal type in Fig. 47 is rarely seen. Superficially it looks suspiciously like a starch grain but has the globoid characteristics of being associated with high acid phosphatase activity (Figs. 113-115) and always appearing within internal cavities of the reserve material. Solid osmiophilic crystals are sometimes observed in contact with

PLATE 11.

Figs. 50-55. 3-day-imbibed gametophyte cells.—Fig. 50. Large microbody (Mb) with cup-shaped depression.—Fig. 51. Microbody (Mb) containing spherical inclusion (double arrow) within amorphous electron-dense region. Note small ribosome-like particles (arrow).—Fig. 52. Intact microbody (Mb) sequestered in protein body (PB). A = amyloplast.—Fig. 53. Sequestered microbody (Mb) within an internal cavity (IC) of protein body (PB), reserves.—Fig. 54. Two degenerating microbodies flanking osmiophilic crystal (Cr) within protein body (PB). The three bodies are enclosed within a membrane (arrow).—Fig. 55. Dividing mitochondrion (M).

Bars represent 0.5 μm (Figs. 50-55).


Mb
50
Mb
51
PB
Mb
IC
53
A
PB
52
PB
Cr
54
M
55

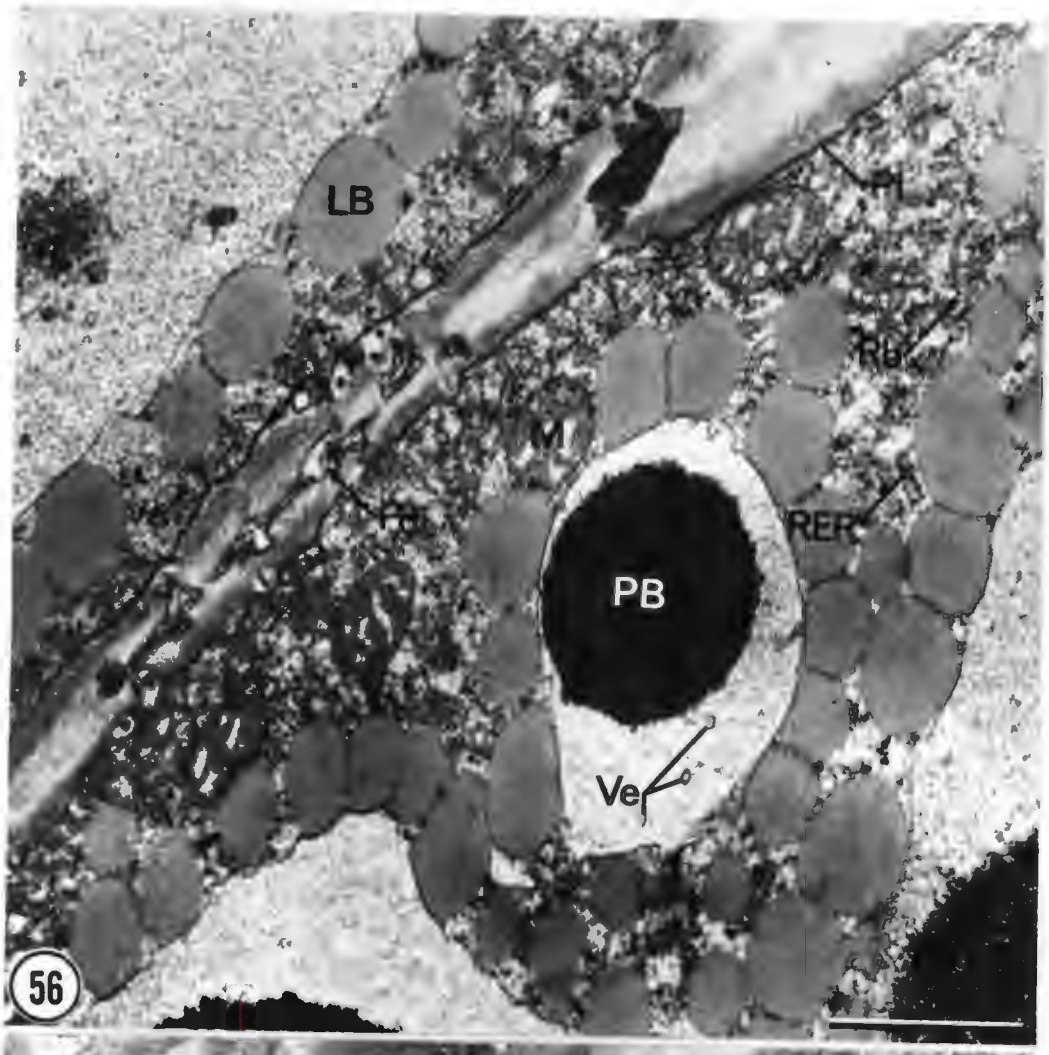
protein body membranes (Figs. 48, 49) and within protein-carbohydrate reserves (Fig. 48). Their distribution suggests that the crystals might be migrating from the cytoplasm into the protein bodies (cf. Figs. 93, 94). The crystals in Fig. 48 might be moving towards the internal cavity (where crystalline inclusions are usually located in a protein body). One of the crystals seems to have a containing envelope. At present I have no evidence to indicate that all the crystals seen within the protein bodies are globoids. However, due to their superficial appearance, distribution and staining properties (Poux 1965; Jacobsen *et al.*, 1971) it seems that the majority of crystals as depicted in Figs. 21, 23 and 24 are globoids.

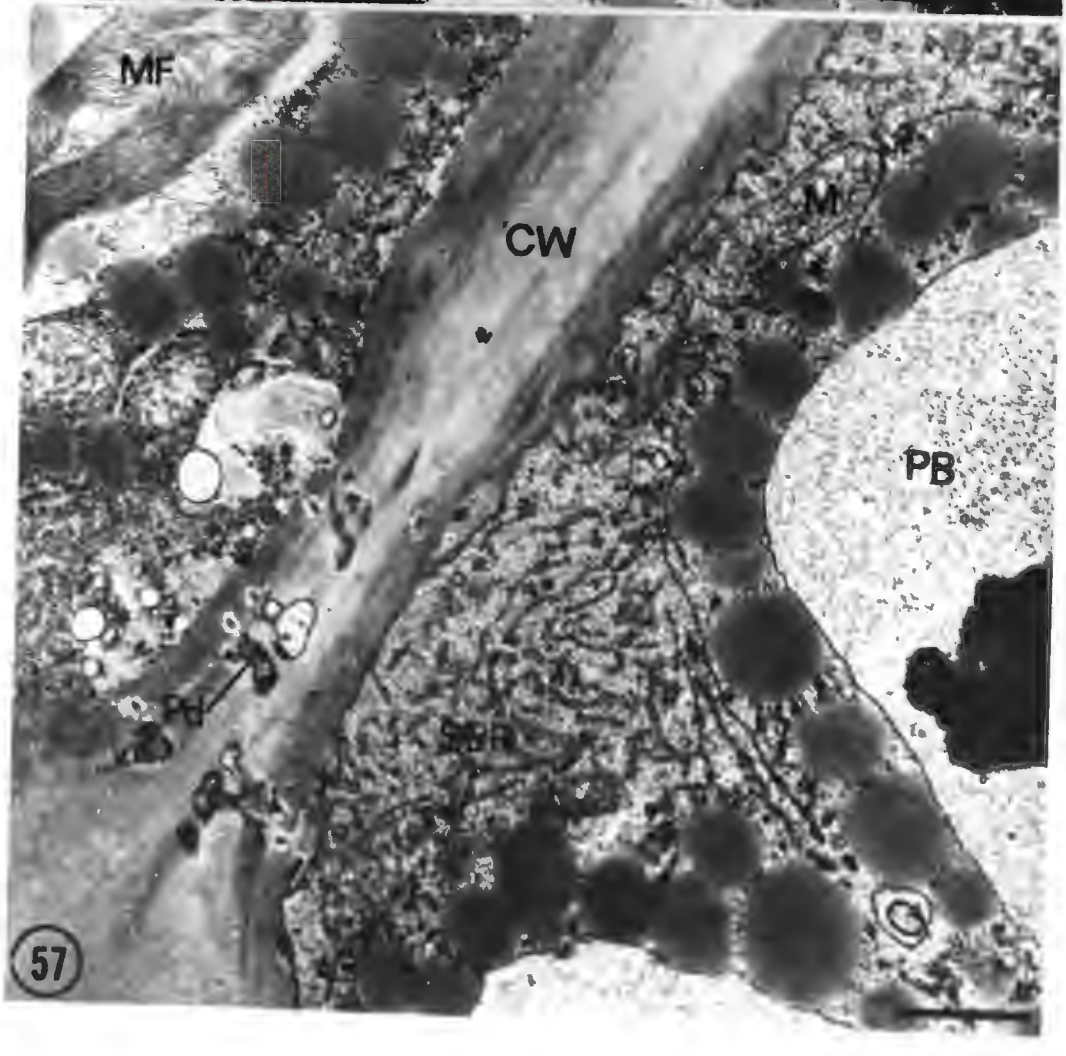
At the 3-day-imbibed stage microbodies (Fig. 50) show a striking increase in size and number. They are often extremely large (1,3 x 1,5 µm) and may develop cup-shaped depressions (Fig. 50). Some microbodies contain a spherical inclusion (diameter ca. 0,3 µm) within the amorphous electron-dense region which is usually located along the enclosing membrane (Fig. 51). Small particles, the size of ribosomes (ca. 20 nm) are sometimes seen in this dense area. The matrix remains granular. Sequestration of microbodies into protein bodies is a common occurrence (Figs. 52-54). Superficially the sequestered microbody in Fig. 52 appears normal with a granular matrix, dense eccentric core and an intact unit membrane. Some microbodies enter compact reserve deposits via the fenestrations (Fig. 53). In Fig. 54 two microbodies flank an osmio-

PLATE 12.

Figs. 56, 57. 4-day-imbibed gametophyte cells.—Fig. 56. Small vesicles (Ve) are commonly observed within protein bodies (PB) apparently migrating toward and adhering to the compact reserves. Lipid bodies (LB) characteristically surround the protein bodies but no longer line the plasmalemma (Pl). The cytoplasm contains numerous mitochondria (M) and short vesiculate pieces of rough ER (RER). Pd = plasmodesmata; Rb = ribosomes.—Fig. 57. Smooth ER (SER) in close association with protein bodies (PB) and plasmodesmata (Pd). CW = cell wall; M = mitochondrion; MF = myelin figure.

Bars represent 1 μm (Figs. 56,57).


LB
M
PB
RER
Ve
56


MF
CW
M
PB
Pd
SER
57

philic crystal deep within protein-carbohydrate reserves. An intriguing feature is the apparent enclosure of the three bodies by a membrane. These microbodies are in a state of degeneration.

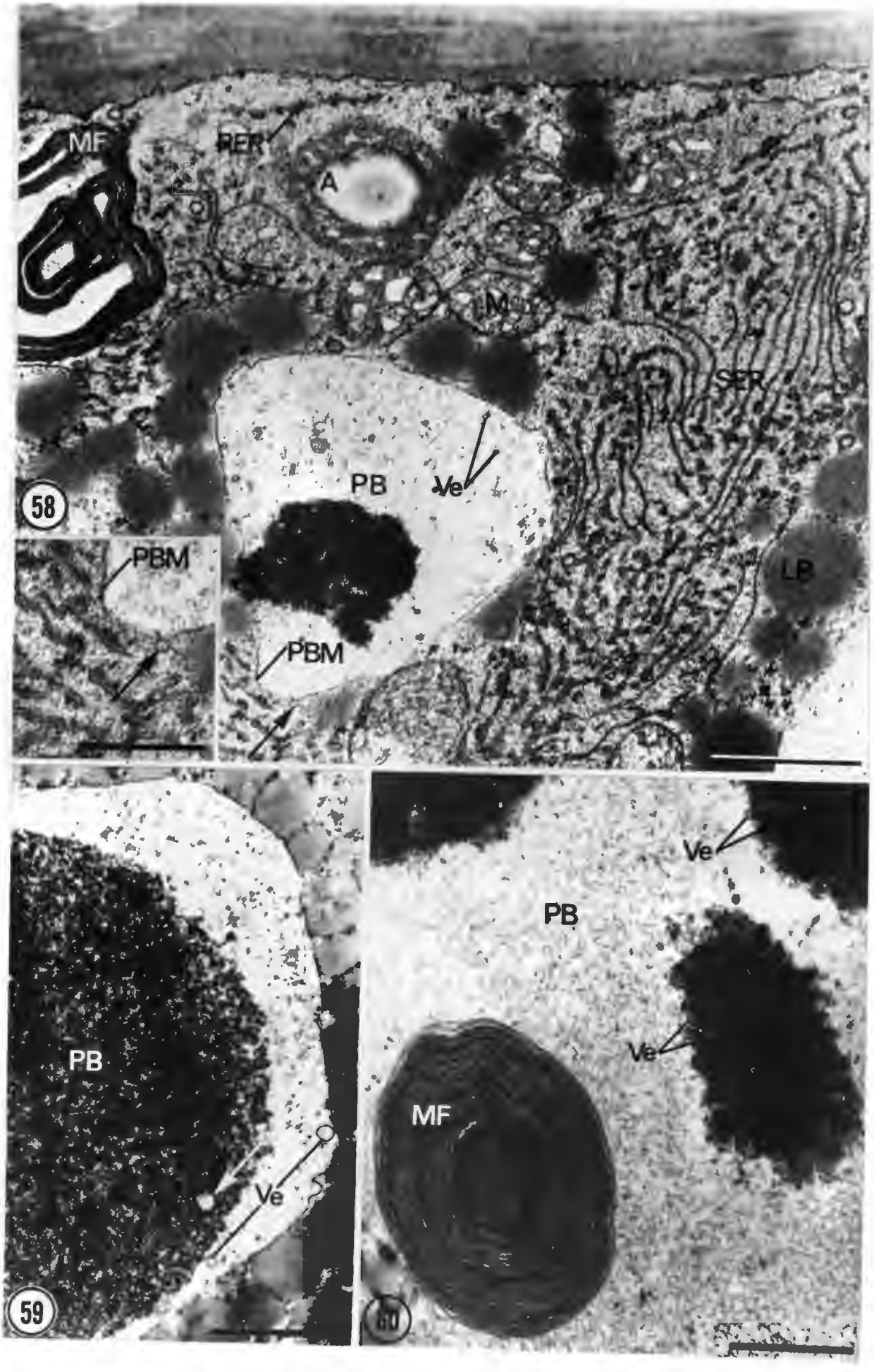
Cytoplasmic organelles have become increasingly apparent and active. Mitochondria seemingly divide by both fission (Fig. 39) and partitioning of the organelle by a single continuous crista (Fig. 55) (Tandler *et al.*, 1969). By the 4th day of imbibition numerous mitochondria are randomly distributed throughout the cytoplasm. They are spherical to oval (Fig. 56) with a dense matrix and vesiculate cristae. Ribosomes are more noticeable in the cytoplasm and occur both free and attached to the ER. There has been a sharp decline in lipid reserves. The remaining lipid bodies no longer line the plasmalemma but still display a distinct pattern of arrangement around the protein bodies. Small vesicles are repeatedly observed in protein bodies (Fig. 56). They seem to migrate toward the compact reserves and adhere to them.

As the protein-carbohydrate reserves are degraded more ER is produced (Fig. 57). A great deal of it is smooth ER and it is often found in close association with the protein bodies and plasmodesmata (Fig. 57). The plasmodesmata commonly occur in aggregates (Figs. 56, 57) and often appear to be interconnected in the region of the middle lamella (Fig. 50). This narrow cavity (Fig. 50) is reminiscent of the larger median cavity in sieve areas of *Welwitschia* leaf phloem, as described by Evert *et al.*, 1973).

PLATE 13.

Figs. 58-60. 5-day-imbibed gametophyte cells.—Fig. 58. Portion of gametophyte cell. Proliferation of ER and mitochondria (M) occurs concomitant to disappearance of protein body (PB) reserves. Amyloplasts (A) increase in number as lipid bodies (LB) are degraded. Note vesicles (Ve) within protein body and blebbing of ER. An ER-derived vesicle (arrow, inset) can be seen in contact with protein body membrane (PBM). MF = myelin figure; RER = rough ER; SER = smooth ER.—Fig. 59. Protein body (PB). Note vesicles (Ve) apparently migrating toward and entering (arrow) protein body reserves. —Fig. 60. Myelin figures (MF) are frequently encountered in protein bodies (PB). Note vesicles (Ve) adhering to and entering reserve material.

Bars represent 1 μm (Figs. 58-60), 0.5 μm (Fig. 58 inset).


MF
RER
A
M
SER
PB
Ve
LB
PBM
58
PBM
PB
Ve
59
Ve
PB
Ve
MF
60

In Fig. 58 (5-day-imbibed) the cellular contents appear to be engaged in intense metabolic activity. Proliferation of ER and mitochondria occurs concomitant to the disappearance of protein body reserves. Plastids containing starch grains are readily observed and show a sharp increase in number as lipid bodies are degraded. Sparse dictyosomes (Fig. 58) and myelin figures (Figs. 57, 58) are observed for the first time. The ER which has increased dramatically blebs profusely (Fig. 58), proliferating many small vesicles. Some of these ER-derived vesicles are often seen in contact with protein body membranes (Fig. 58, inset). They appear to pass into the protein body and move toward the compact reserves (Fig. 58) as originally noted in Fig. 56. The vesicles adhere to and actually enter the reserves (Figs. 59, 60). At this time myelin figures are frequently encountered in protein vacuoles (Fig. 60).

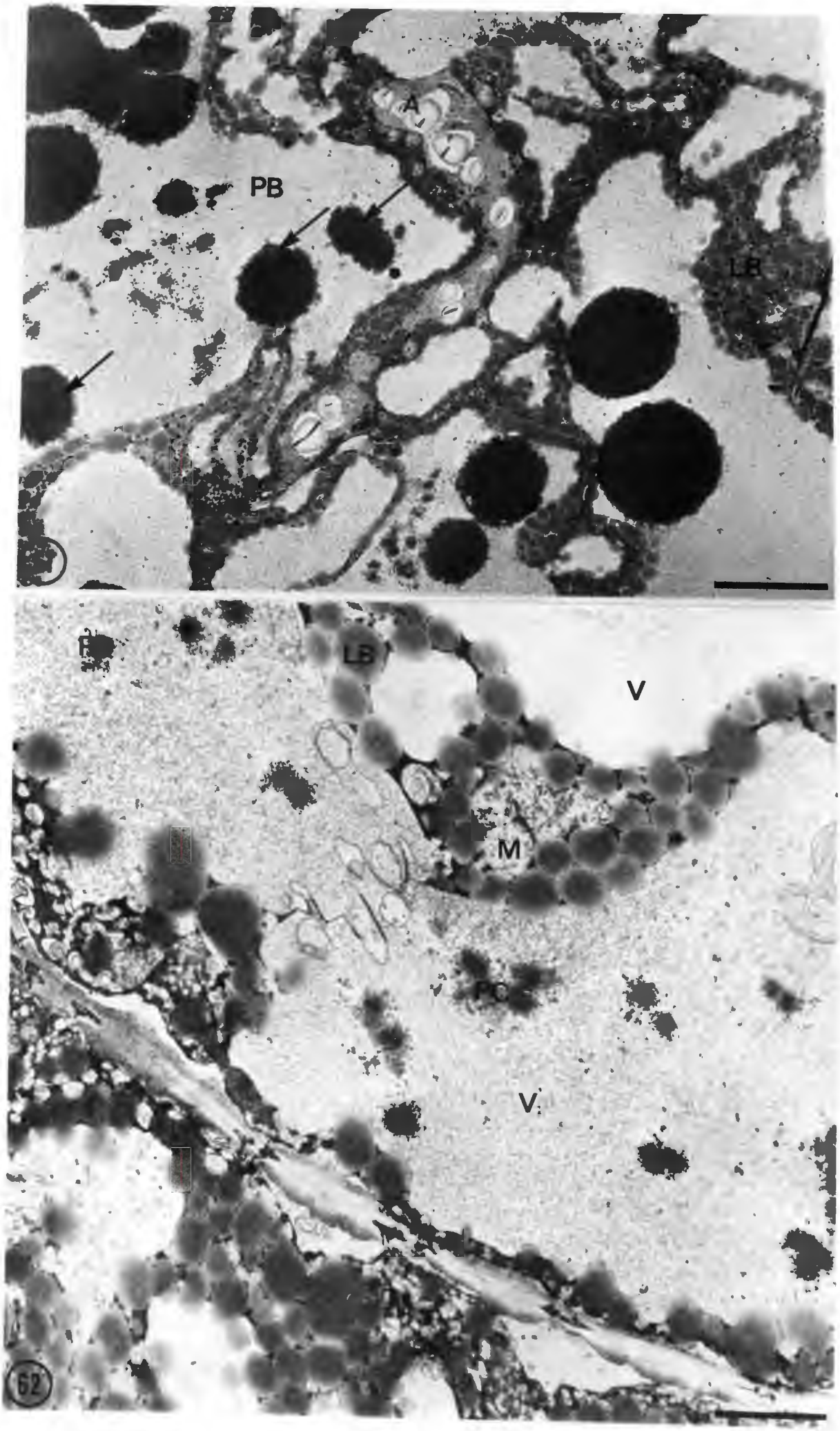
Usually by the end of the 6th day of imbibition the plumule has emerged from the seed (Fig. 16), and is soon green and photosynthetic. Gametophyte cells situated farthest from the embryo still contain a fair proportion of reserve materials (Fig. 61). Adjacent protein bodies have fused and the remaining compact protein-carbohydrate reserves are peripherally distributed in the vacuole (Fig. 61). Lipid reserves have been depleted and starch-laden plastids are common (Fig. 61).

The vacuoles formed by coalescence of protein bodies in less deeply-situated gametophyte cells (nearer embryonic feeder) are largely aqueous containing only a fraction of their original

PLATE 14.

Figs. 61,62. 6-day-imbibed gametophyte cells.—Fig. 61. Portion of deeply-situated gametophyte cell. Note fusion of adjacent protein bodies (PB) and peripheral distribution of remaining compact protein carbohydrate reserves (arrows). Lipid (LB) reserves have been depleted and starch-laden amyloplasts (A) are common.—Fig. 62. Less deeply-situated gametophyte cells. Vacuoles (V) are largely aqueous and much of lipid (LB) has been mobilised and dispersed. Cells appear senescent with electron-dense cytoplasm and swollen organelles. M = mitochondrion; PC = protein-carbohydrate reserves.

Bars represent 0.5 μm (Fig. 61), 2.5 μm (Fig. 62).


A
PB
LB
P
LB
V
M
PC
V
62

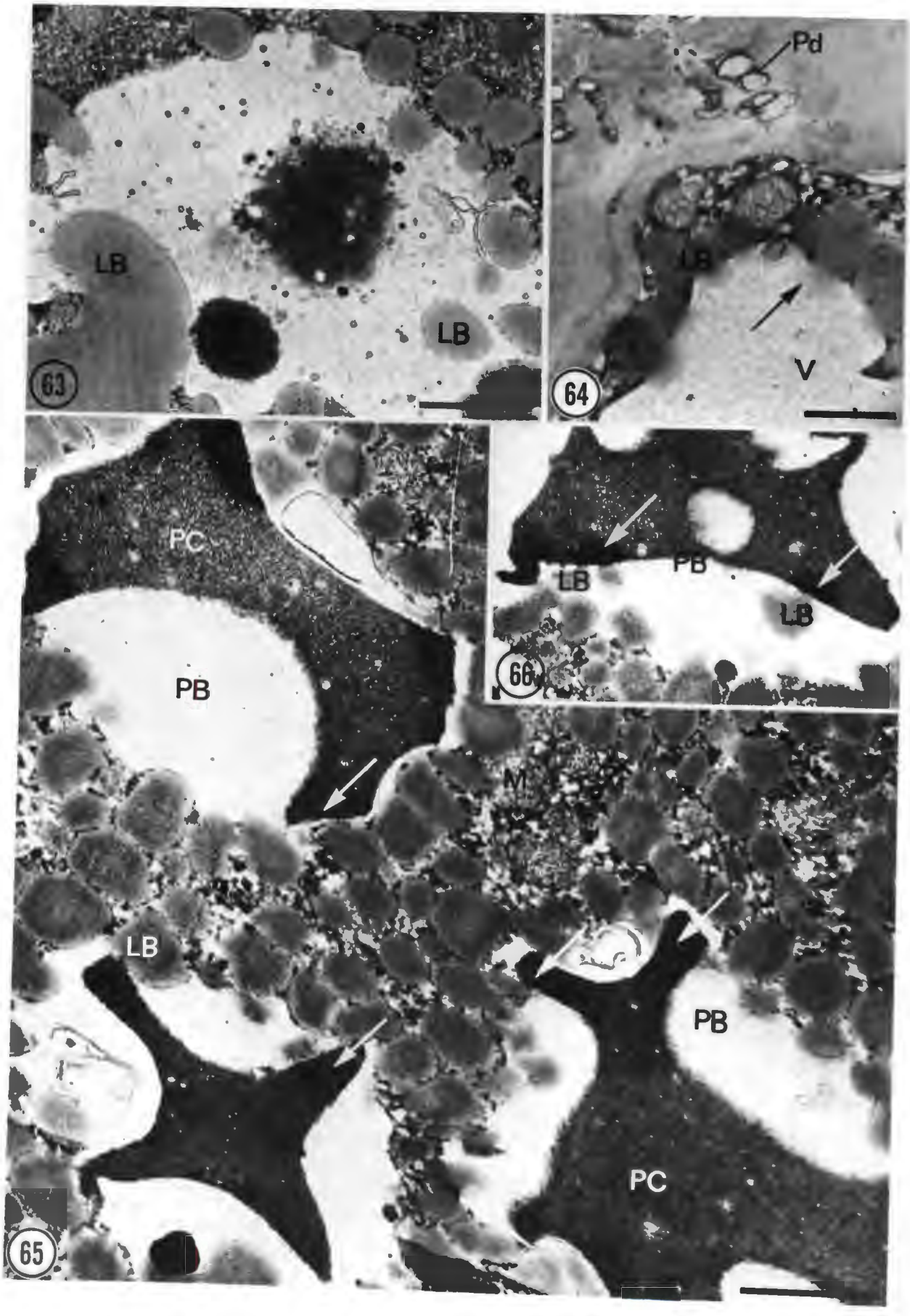
protein-carbohydrate reserves (Fig. 62). Much of the lipid has also been mobilised and dispersed. These cells often appear senescent with electron-dense cytoplasm and swollen organelles (Fig. 62). When breaks occur in vacuole membranes, cell contents are lysed.

On hydration of the gametophyte, protein body degradation always commences prior to that of lipid. Subsequently lipid digestion begins and occurs concurrently with that of protein bodies. As lipid reserves are consumed, starch reserves accumulate (Fig. 61). Migration of lipid bodies into vacuoles is a common phenomenon (Fig. 63) and is seen during early stages when vacuoles contain compact reserves (Fig. 63 - 4-day-imbibed; note vesicles), and at later stages when reserves have been dispersed (Figs. 62, 64 - 6-day-imbibed) and cells may appear senescent. Lipid bodies often seem to swell as they enter the vacuole (Figs. 62, 64) and may become less electron-dense (Fig. 64). Sometimes protein-carbohydrate reserves stain darkly in the proximity of lipid bodies (Fig. 65). This phenomenon is apparent in Fig. 65 even though most of the lipid bodies are confined outside the vacuole membrane. In Fig. 66 lipid bodies have entered a vacuole and osmiophilic patches have formed at their point of contact with the reserve material. The dark patches suggest a possible biochemical interaction.

PLATE 15.

Fig. 63. Protein body (PB) of 4-day-imbibed gametophyte cell. Note migration of lipid bodies (LB) into protein body.—Fig. 64. Portion of senescing 6-day-imbibed gametophyte cell. Lipid bodies (LB) appear to swell (arrow) as they enter vacuole (V). Pd = plasmodesmata.—Fig. 65-66. 3-day-imbibed gametophyte cells.—Fig. 65. Protein body (PB) reserves often stain darkly (arrows) in the vicinity of lipid bodies (LB). M = mitochondria; PC = protein-carbohydrate reserves.—Fig. 66. Lipid bodies (LB) have entered protein body (PB). Dark osmiophilic patches (arrows) have formed at their point of contact with reserve material.

Bars represent 1 μm (Figs. 63-66).


LB
LB
63
Pd
LB
V
64
PC
PB
LB
PB
LB
66
M
LB
PB
PC
65

GAMETOPHYTE INTERFACE CELLS.

INTRODUCTION.

In the preceding chapter, the fine structure of deeply situated (Fig. 9, box 1), dry gametophyte cells was examined in detail, together with the ultrastructural changes which occur as the seed germinates. This chapter is an extension of the previous one and focuses on the gametophyte cells in the vicinity of the embryo (Fig. 9, box 2) with special emphasis on those cells immediately in contact with the developing and mature feeder (Fig. 67, arrow), hereafter referred to as gametophyte interface cells.

RESULTS.

DAY ZERO.

In contrast to deeper gametophyte cells, the 4 to 5 layers of cells surrounding the embryo are smaller (170 x 70 µm) (Fig. 68) and flattened in transection. Their appearance in the dry state is qualitatively similar to that of deep gametophyte cells, but differs quantitatively in structural components (Fig. 69).

Nucleus

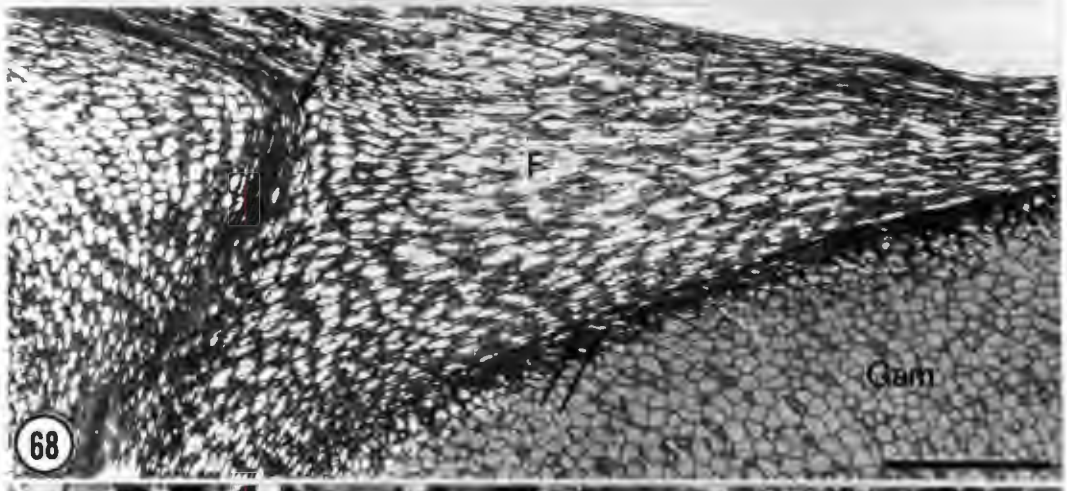
Nuclei have a familiar lobed appearance with nucleoplasm and nucleoli anastomosed by fibrous inclusions. Less dense

PLATE 16.

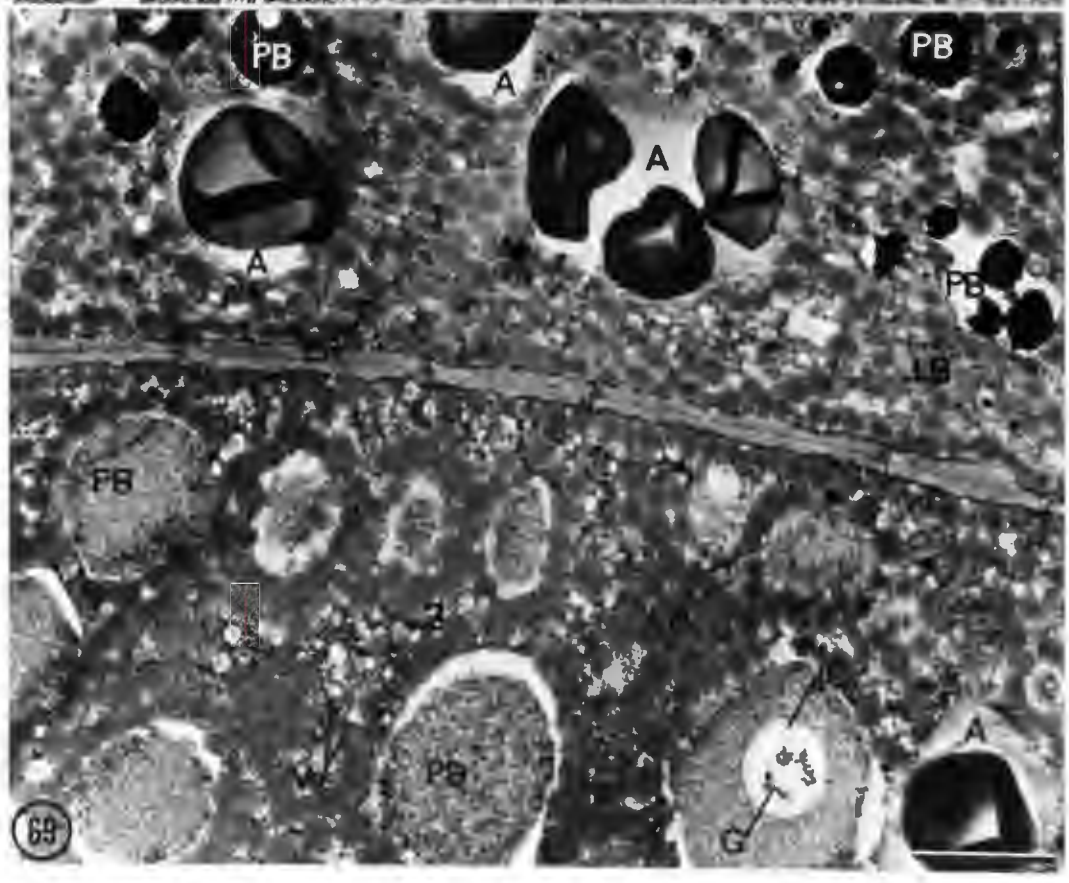
Fig. 67. Light micrograph of L.S. through 6-day-old seedling. Note close contact between ventral surface of feeder and gametophyte. Arrow indicates interface zone. Section stained in PAS.—Fig. 68. Light micrograph of feeder (F) showing attachment to gametophyte (Gam) in 5-day-old seedling. Note elongate, vacuolate cells of feeder and vascular curvature (arrow) at base of feeder. Prominent starch reserves (double arrow) are easily recognisable in the gametophyte interface zone. Deeply-situated gametophyte cells appear devoid of starch. Section stained in PAS.—Fig. 69. Dry gametophyte cells in interface zone. Residual cytoplasm is detectable and starch-laden amyloplasts (A) are numerous. Protein bodies (PB) in actual interface cell (1) are small, and sparse with dense contents. Those in contiguous cell (2) are larger with a more diffuse granular matrix. Note globoid (G) remnants in internal cavity (IC) of protein body. Lipid bodies (LB) are less compactly arranged than those of deep gametophyte cells. Lipid bodies appear scattered in actual interface cell, but show a more characteristic pattern of arrangement in contiguous cell. ER = endoplasmic reticulum; Mb = microbody; Ve = vesicle.

Bars represent 1 mm (Figs. 67,68), 2.5 μm (Fig. 69).

67



68



69

packaging of reserve materials in these cells (Fig. 69) could minimise their distorting effect on nuclei which consequently are not as severely malformed as in deep gametophyte cells (Figs. 22, 40).

Protein bodies

Protein bodies in interface cells are relatively sparse and very small (mean diameter 1.2 μm) with densely staining contents (Fig. 69, cell 1). In contiguous gametophyte cells they are larger (mean diameter 2.5 μm) containing a more diffuse granular matrix (Fig. 69, cell 2). Rarely, internal cavities of protein bodies contain material interpreted as globoid remnants (Fig. 69).

Lipid bodies

Lipid bodies (Fig. 69) are not as compactly arranged as in the deep gametophyte tissue (Figs. 20, 21). In interface cells they give the impression of being loosely scattered (Fig. 69, cell 1) 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 (Fig. 69, cell 2).

Organelles in residual cytoplasm

Reduced protein-carbohydrate and lipid reserves results in more residual cytoplasm being detectable in interface and immediately surrounding cells (Fig. 69) than in deeper dry gametophyte

tissue (Figs. 20, 21). One of the most striking features of the 4 to 5 cell deep zone of gametophyte tissue surrounding the embryo is the random distribution of numerous large starch-laden amyloplasts (Fig. 69). The prominent starch reserves in this zone are easily recognised at the light microscope level (Fig. 68) and are in marked contrast with the paucity of starch grains in deeper cells.

While microbodies are of rare occurrence (Fig. 69) mitochondria are quite common. Short fragments of ER (Fig. 69) are seen infrequently in the cytoplasm which is studded with ribosomes. No polysome configurations are apparent. Dictyosomes seem to be 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 (Fig. 69).

Cell walls

Interface cells are thin-walled (Fig. 69, cell 1) with slightly thickened outer walls in contact with the embryo. While plasmodesmata commonly occur in the thin walls, linking neighbouring gametophyte cells (Fig. 69), the outer walls are devoid of plasmodesmata.

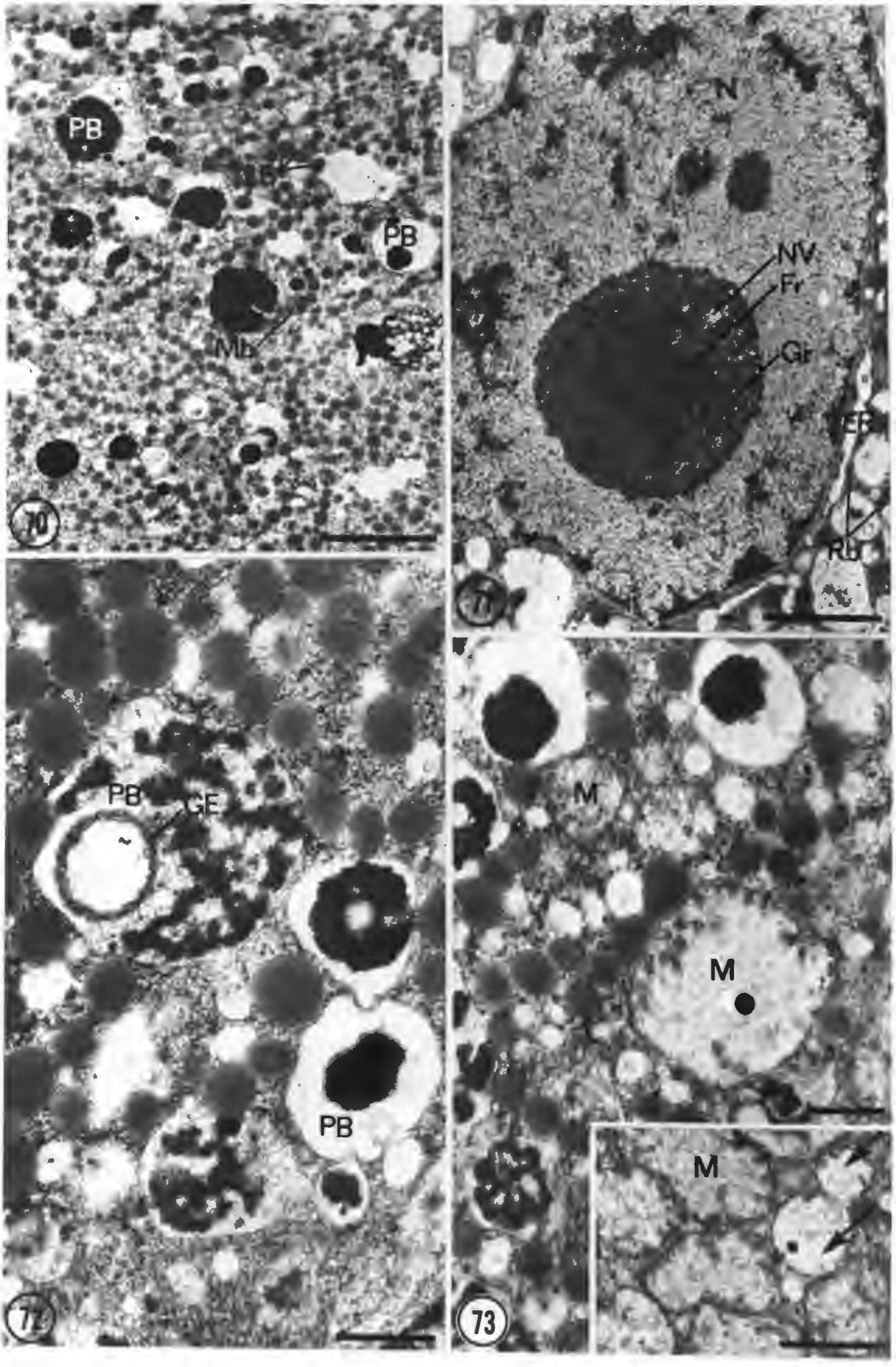
DAY 1.

Imbibition results in a clearer fixation image (Fig. 70). Figure 70 is a 24-hour-imbibed interface cell whose ultrastructural features reflect awakened metabolic activity. Elongated

PLATE 17.

Figs. 70-73. Gametophyte interface cells imbibed for 24 h.—Fig. 70. Portion of gametophyte interface cell showing degrading protein bodies (PB). LB = lipid body; Mb = microbody.—Fig. 71. An elongated, lobed nucleus (N) with enlarged nucleolus (Nu) consisting of fibrillar core (Fr) and surrounding granular zone (Gr). The granular region is beginning to intersperse in the fibrillar zone. C = chromatin; ER = endoplasmic reticulum; NV = nucleolar vacuole; Rb = ribosome.—Fig. 72. Degrading protein bodies (PB). An internal pattern of degradation results in rapid fragmentation of protein-carbohydrate reserves. Ring-like globoid envelopes (GE) are occasionally seen. They enclose either electron-transparent areas or globoid remnants.—Fig. 73. Mitochondria (M) are common, variable in shape, and may be greatly swollen with short sparse cristae. The largely electron-transparent matrix contains fibrils, ribosomes and an occasional osmiophilic granule. Inset: arrowed mitochondria appear to be engaged in autophagic activity.

Bars represent 2.5 μm (Figs. 70,71), 0.5 μm (Figs. 72,73), 1 μm (Fig. 73 inset).


PB
PB
Mb
70
N
NV
Fr
Gr
EB
RB
71
PB
GE
PB
72
M
M
M
73

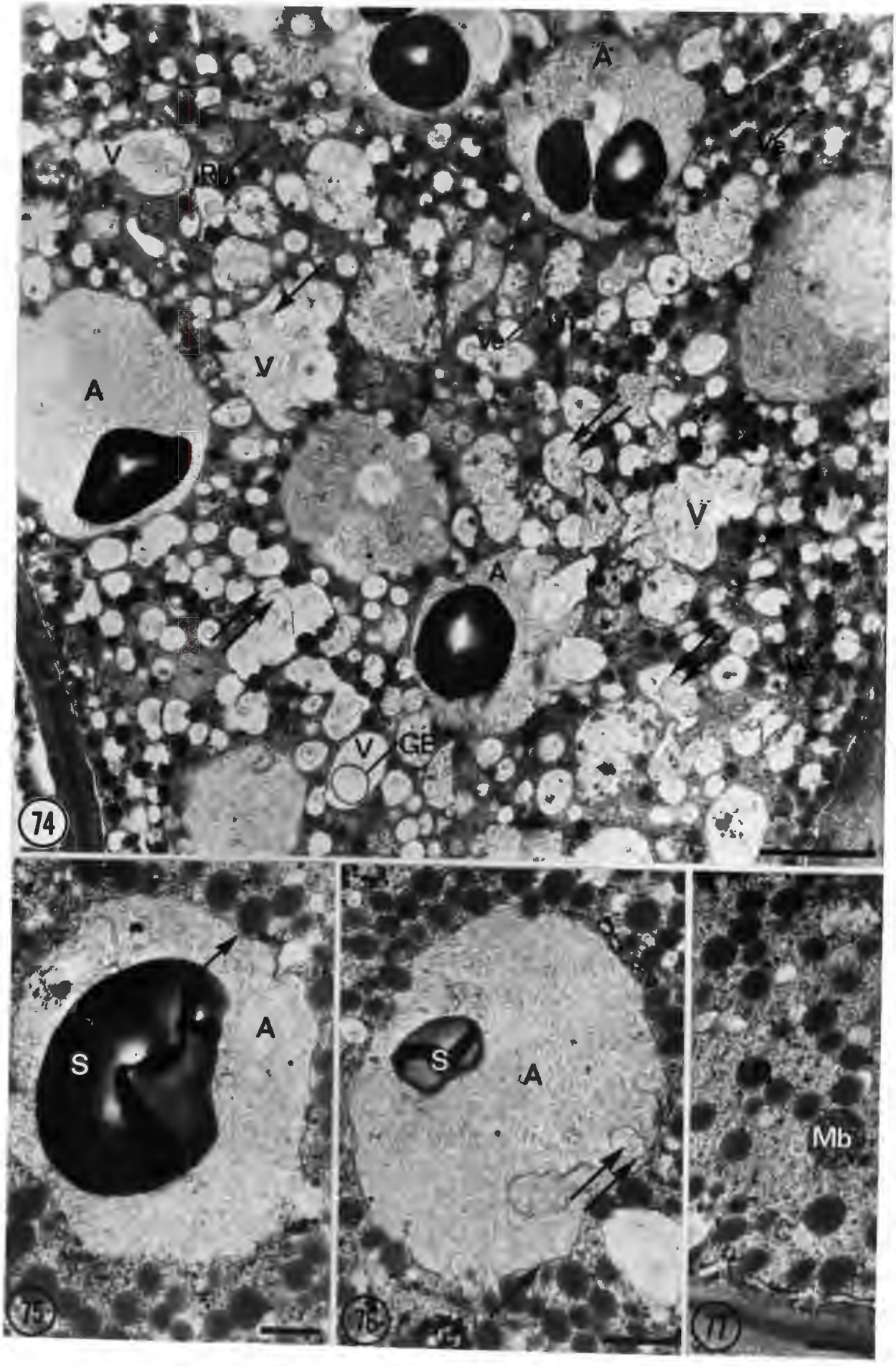
lobed nuclei (Fig. 71) have lost their initial crystalline inclusions. Enlarged nucleoli, associated with increased cell activity (Jordan and Chapman 1971), are prominent. They are spherical in transection consisting of a fibrillar core surrounded by a well-defined granular zone as shown in Fig. 71 (Swift 1966). While dense packing makes the fibrils difficult to observe, the granular region is easily distinguished due to its looser nature. In Fig. 71 the granular region is beginning to intersperse in the fibrillar zone, and nucleolar vacuoles (Swift 1966) become apparent. The enlarged granular zone indicates an increase in ribosomal precursor-ribonucleoprotein particles (Jordan and Chapman 1971). There is less condensed chromatin in the nucleoplasm of imbibed cells. It exists scattered throughout the nucleoplasm and is located near the nuclear envelope (Fig. 71). Small granules are dispersed throughout the nucleoplasm. They are the size of ribosomes, though not as intensely staining.

It is obvious from Fig. 70 that 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. An internal pattern of degradation results in rapid fragmentation and the formation of irregular lumps of material as in Fig. 72. 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 evelopes

PLATE 18.

Figs. 74-77. Gametophyte interface cells imbibed for 24h.—Fig. 74. 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. 75. Amyloplast (A) with degrading starch grain (S). Note double-unit plastid membrane (arrow).—Fig. 76. 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. 77. Microbody (Mb) in vicinity of lipid bodies (LB).

Bars represent 2.5 μm (Fig. 74), 0.5 μm (Figs. 75,77), 1 μm (Fig. 76).


A
V
RL
V
A
Ve
V
A
V
GE
74
S
A
75
S
A
76
Mb
77

as depicted in Fig. 72 are occasionally seen in interface protein bodies. They enclose either electron-transparent areas, as in Fig. 72, or globoid remnants. The protein bodies themselves appear to become active autophagically and extend in amoeboid-like fashion toward one another (Fig. 72).

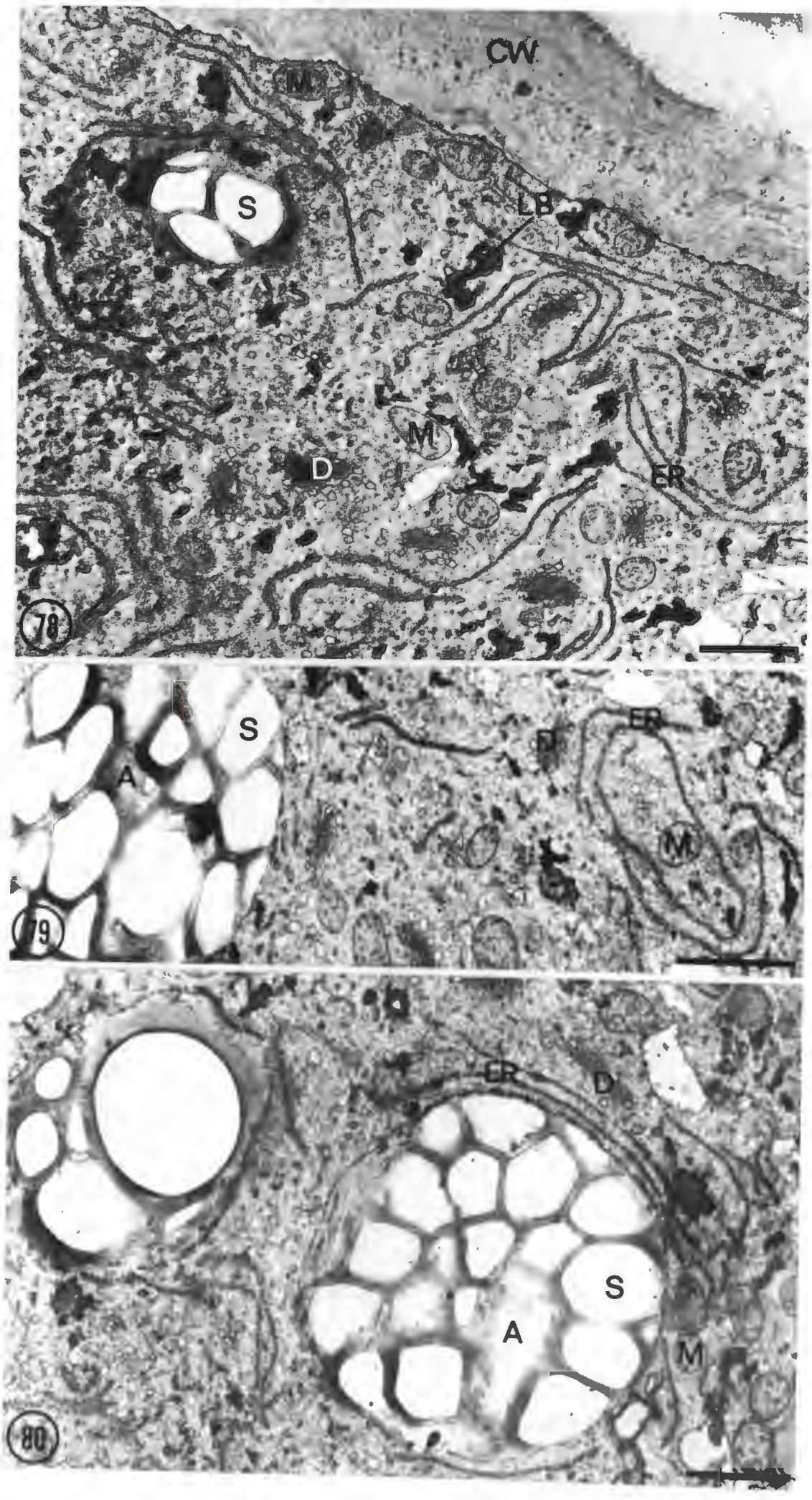
Lipid bodies have decreased slightly in number and their dispersion in interface cells as seen in Figs. 70 and 72 remains diffuse, bearing no marked relationship with protein bodies. Randomly distributed mitochondria are common and display more internal organization at this stage (Fig. 73) than deeply situated mitochondria (Fig. 39). They vary in size and shape, some appearing greatly swollen (Fig. 73). The cristae are short and sparse while the matrix is still largely electron-transparent containing fibrils, ribosomes and an occasional osmiophilic granule. The arrowed mitochondria in Fig. 73, inset, seem to be engaged in autophagic activity. However their profile may represent the division of a swollen mitochondrion.

Some 24-hour-imbibed interface cells show a more rapid metabolic activation than others. This is amply demonstrated by a comparison of Figs. 70, 72 and 74. In Fig. 70 compact protein-carbohydrate reserves have begun to degrade. In Fig. 72 reserves have fragmented, while in Fig. 74 the reserves have been consumed leaving numerous aqueous vacuoles. The vacuoles in Fig. 74 are not completely devoid of contents and may contain globoid envelopes (Fig. 74, arrow) and various unidentified membranous fragments. The vacuoles and abundant smaller vesicles

PLATE 19.

Figs. 78-80. Micrographs of permanganate-fixed (Luft) 3-day-imbibed gametophyte interface cells.—Fig. 78. Lipid bodies (LB) are drastically reduced in number. Their crenulated appearance is a common permanganate fixation artifact. Starch reserves (S) reappear as lipid bodies are degraded. The cytoplasm now contains numerous mitochondria (M) and extensive sheet-like ER. Dictyosomes (D) appear for the first time. The cell wall (CW) in contact with the developing feeder begins to acquire a spongy appearance.—Fig. 79. Amyloplast (A) containing numerous small, closely-packed starch grains (S). Note ER - enclosed mitochondrion, (M). D = dictyosome.—Fig. 80. Amyloplast (A) with numerous starch grains (S). Note association of ER and mitochondria (M) with amyloplast. D = dictyosome.

Bars represent 1 μm (Figs. 78-80).


CW
M
S
LB
M
D
ER
78
S
A
D
ER
M
79
ER
D
S
A
M
80

originally noted in dry tissue (Fig. 69) fuse and seemingly engage in intense autophagic activity (Fig. 74).

On hydration starch reserves decline rapidly and are greatly depleted after 24h imbibition (Fig. 74). As the starch grains disappear (Figs. 75, 76) little or no internal organization is laid bare. Ribosomes and osmiophilic droplets (Figs. 75, 76) are visible but lamellae are largely absent and often disorganised. In fact, if it were not for their double membranes (Fig. 76, arrow) the amyloplasts would look more like vacuoles containing starch grains than plastids. Some amyloplasts are "attacked" by protein vacuoles (Fig. 74), others begin to form surface depressions (Fig. 76).

Microbodies have increased in number and are usually seen in the vicinity of lipid bodies as in Fig. 77. The ER displays longer single profiles which are distributed throughout the cytoplasm, often shadowing the contours of the nucleus (Fig. 71). Inflation of the ER is quite common (Fig. 71) and it seems likely that the many small vesicles in Fig. 74 (24-hour-imbibed) and Fig. 69 (unimbibed) are localised ER dilations. Ribosomes occur attached to the ER (Fig. 71) and free in the cytoplasm (Figs. 71, 74) which is rich in them. No dictyosomes were seen.

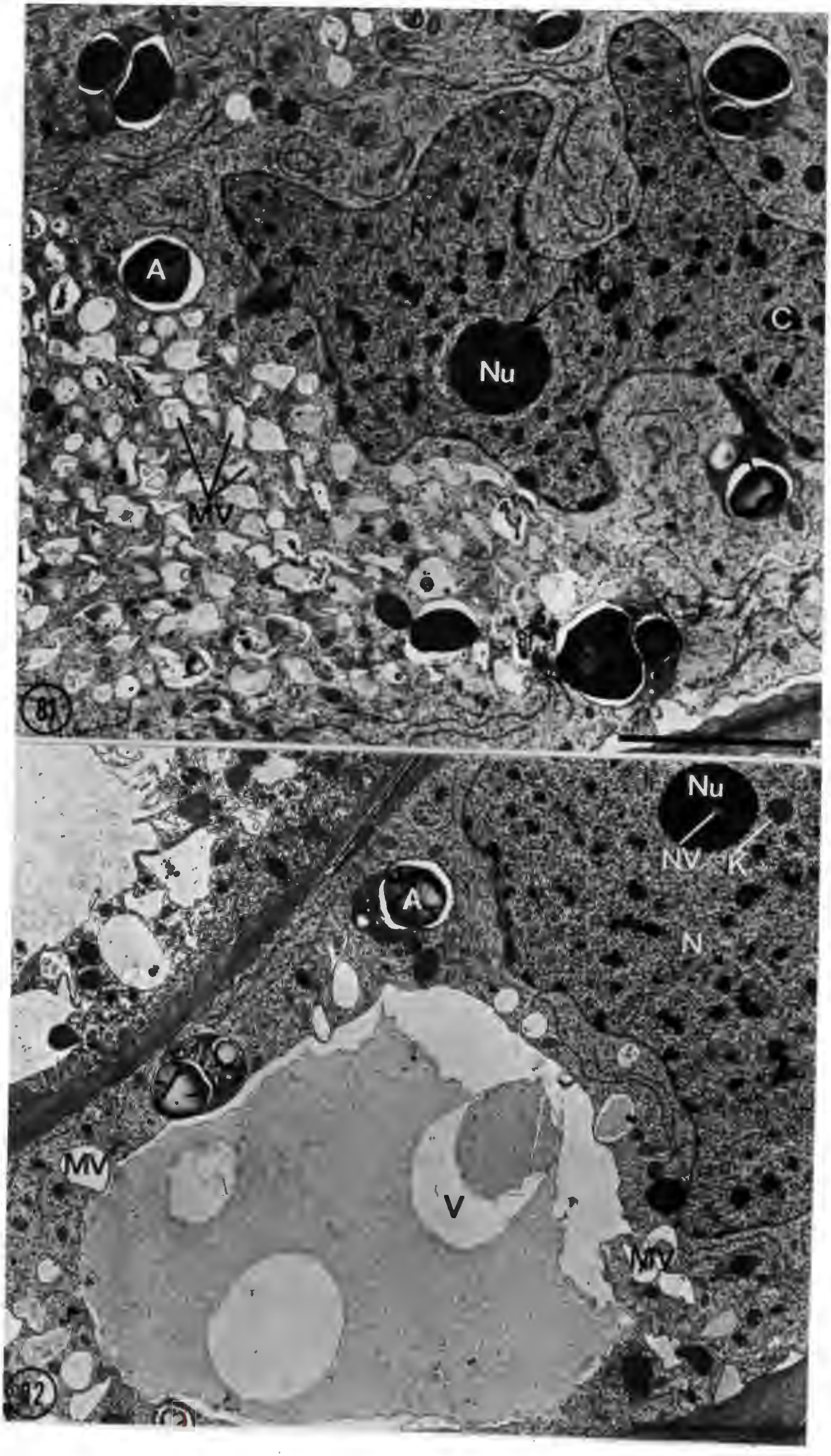
DAY 3-6

Figures 78 to 80 are micrographs of permanganate-fixed (Luft

PLATE 20.

Figs. 81,82. 5-day-imbibed gametophyte interface cells.—Fig. 81. Invaginated nucleus (N) containing nucleolus (Nu) which has become smaller and denser. Note nucleolar organizer (NO). Large numbers of mini-vacuoles (MV) are frequently observed in limited cytoplasmic areas. A = amyloplast; C = chromatin.—Fig. 82. Nucleus (N) contains karyosome (K) in satellite position to nucleolus (Nu). Note nucleolar vacuole (NV). Large cytoplasmic vacuole (V) formed by fusing protein bodies is surrounded by mini-vacuoles (MV). A = amyloplast.

Bars represent 5 μm (Fig. 81), 1 μm (Fig. 82).


A
Nu
MV
C
81
Nu
NV
K
A
N
MV
V
MV
82

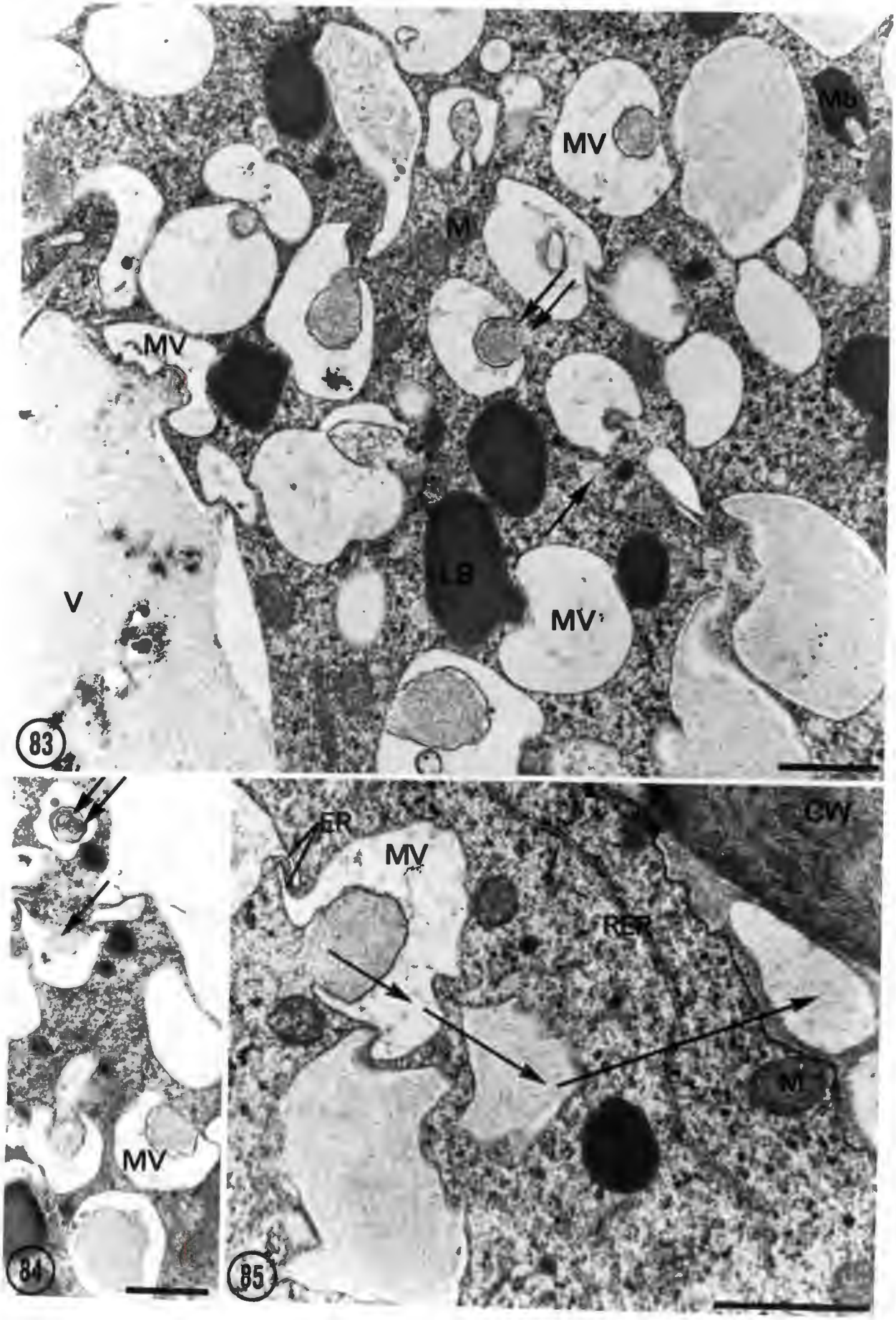
1956) 3-day-imbibed gametophyte interface cells. Artifacts arising from this procedure include the destruction of cytoplasmic particles with a high RNA content (Glauert 1967), poor fixation of chromosomal material (Glauert 1967) and apparent shrinkage of lipid bodies (Villiers 1971). Permanganate is a vigorous oxidant, oxidising and removing almost all cell inclusions except the phospholipid membranes (Mercer and Birbeck 1966) which are given high contrast making them starkly visible against the washed-out cytoplasm (Figs. 78-80). Many investigators have used permanganate as a fixative for both dry and freshly-imbibed tissue of quiescent and dormant seed (Paleg and Hyde 1964; Yatsu 1965; Villiers 1971) because of inherent difficulties in acquiring clear fixation images in the presence of large stores of lipid (Villiers 1971).

In 3-day-imbibed interface cells nuclei retain their polymorphic profiles and protein vacuoles, now devoid of reserves, converge and begin to fuse at one end of the cell. Lipid stores are drastically reduced as evidenced by Figs. 78-80. The crenulated appearance of individual lipid bodies in Figs. 78-80 is a common permanganate fixation artifact. As lipid bodies are degraded, starch reserves reappear (Figs. 78-80) causing amyloplasts to swell and take on a circular appearance in cross section (Figs. 78, 80). Frequently the reserves are rebuilt in the form of many small closely-packed starch grains (Figs. 79, 80) as opposed to the original one to three large grains (Fig. 69). Mitochondria are numerous. They occur near the plasmalemma (Fig. 78) and scattered throughout the cytoplasm (Fig. 78), being found in close association with amyloplasts,

PLATE 21.

Figs. 83-85. 5-day-imbibed gametophyte interface cells.—Fig. 83. Autophagic mini-vacuoles (MV). Fibrillar material accumulates in the cytoplasm (arrow) and is engulfed by a mini-vacuole (double arrow). A mini-vacuole can be seen attacking a lipid body (LB) while another pinches off part of the main vacuole (V). M = mitochondrion; Mb = Microbody.—Fig. 84. 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. 85. Segments of ER can be seen issuing from minivacuoles (MV) which suggests that these small vacuoles are ER dilations. The fibrillar contents of the mini-vacuoles appear to be added to contents the interface cell wall (CW) in contact with the feeder (arrows). M = mitochondrion; RER = rough ER.

Bars represent 1 μm (Figs. 83-85).


MV
M
MV
V
LB
MV
83
MV
84
ER
MV
CW
RER
M
85

lipid bodies, microbodies and ER (Figs. 78-80). Microbodies have increased in number and appear to have enlarged in size.

The ER has developed extensively and now consists of long sheet-like elements which ramify throughout the cytoplasm of interface (Fig. 78) and neighbouring gametophyte cells. These elements may occur singly, but are usually in parallel alignment with one or several ER profiles (Fig. 78). The ER becomes associated with amyloplasts (Fig. 80) and may partially or completely enclose mitochondria (Fig. 79) and dictyosomes (Fig. 78). Ribosomes fail to appear in tissues fixed with permanganate (Figs. 78-80). However 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 appearing as polysomes.

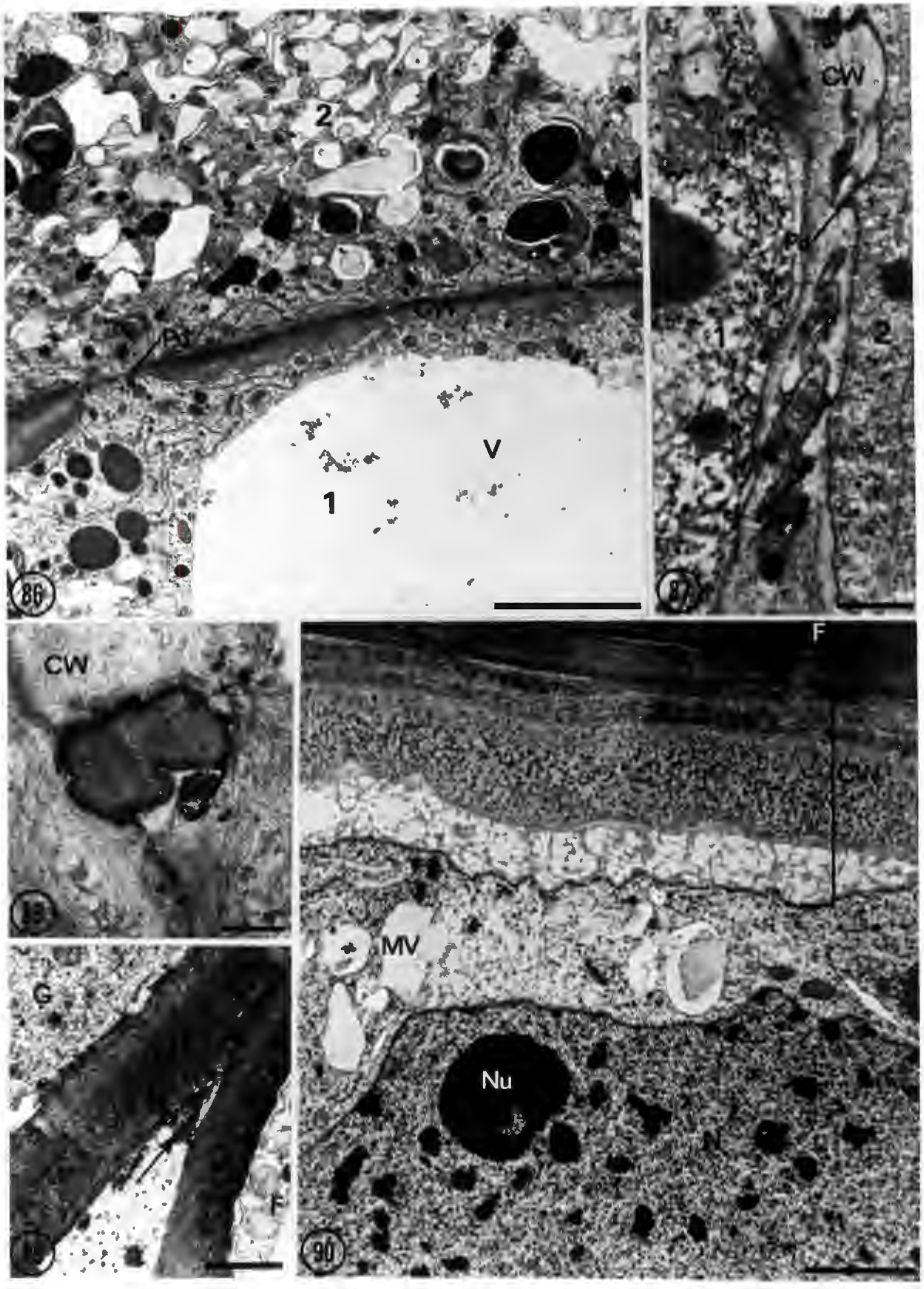
Dictyosomes are now seen for the first time (Figs. 78, 79). They are present but not profuse, and become even less common after the 3-to 4-day-imbibed stage. The cell wall in contact with the developing feeder has thickened and is beginning to acquire a "spongy" appearance (Fig. 78).

By the fifth to sixth day of imbibition nuclei in gametophyte interface cells have usually assumed a more exaggerated invaginated appearance similar to that seen in Fig. 81. (5-day-imbibed). As a result of their more pronounced convolutions they begin to resemble their deeper-seated counterparts (Fig. 40). Comparing the nucleolus in Fig. 81 (5-day-imbibed, diameter 2.5 μm) with that in Fig. 71 (1-day-imbibed, diameter

PLATE 22.

Figs. 86-88. 5-day-imbibed cells in gametophyte interface zone.—Fig. 86. Cell walls (CW) between actual interface (1) and contiguous cell (2) are commonly traversed by plasmodesmata (Pd). Interface cell wall is slightly thickened. V = vacuole.—Fig. 87. Wall (CW) areas traversed by plasmodesmata (Pd) remain thin between interface (1) and inner contiguous cells (2). Note coalescing and enlarging median cavities in the middle lamella region.—Fig. 88. Thick radial walls (CW) between adjacent interface cells. Note large median cavity.—Fig. 89. 3-day-imbibed cells. The gametophyte (G) interface cell wall in contact with the embryonic feeder (F) has begun to thicken. Note densely-staining material (arrow) secreted by gametophyte and feeder interface cells. This substance apparently acts as an adhesive.—Fig. 90. Portion of 5-day-imbibed gametophyte interface cell. Outer cell wall (CW) in contact with feeder (F) is thick, many-layered and mucilaginous. Outer layers are compressed, inner layers spongy. MV = mini-vacuole; N = nucleus; Nu = nucleolus.

Bars represent 5 μm (Fig. 86), 0.5 μm (Figs. 87,88), 1 μm (Fig. 89), 2 mμ (Fig. 90).

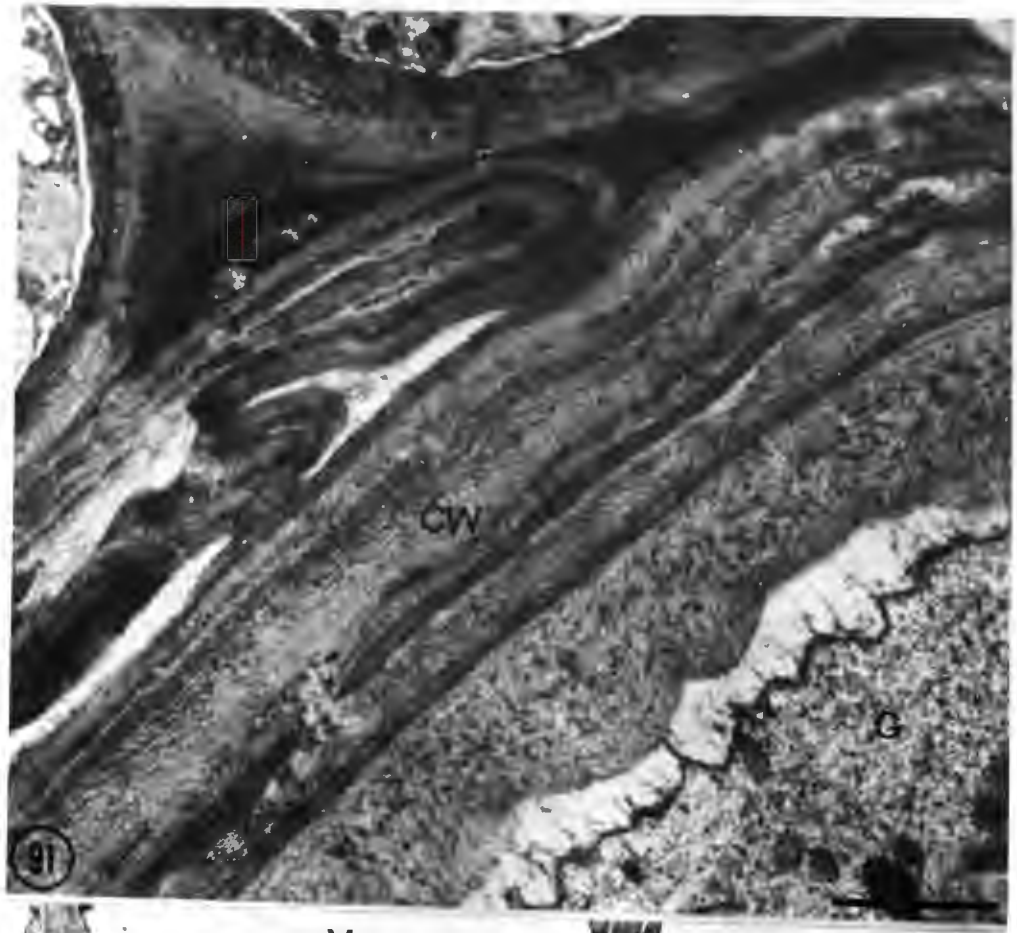

2
CW
PM
V
1
86
CW
1
2
87
CW
88
F
CW
MV
Nu
N
90
G
F

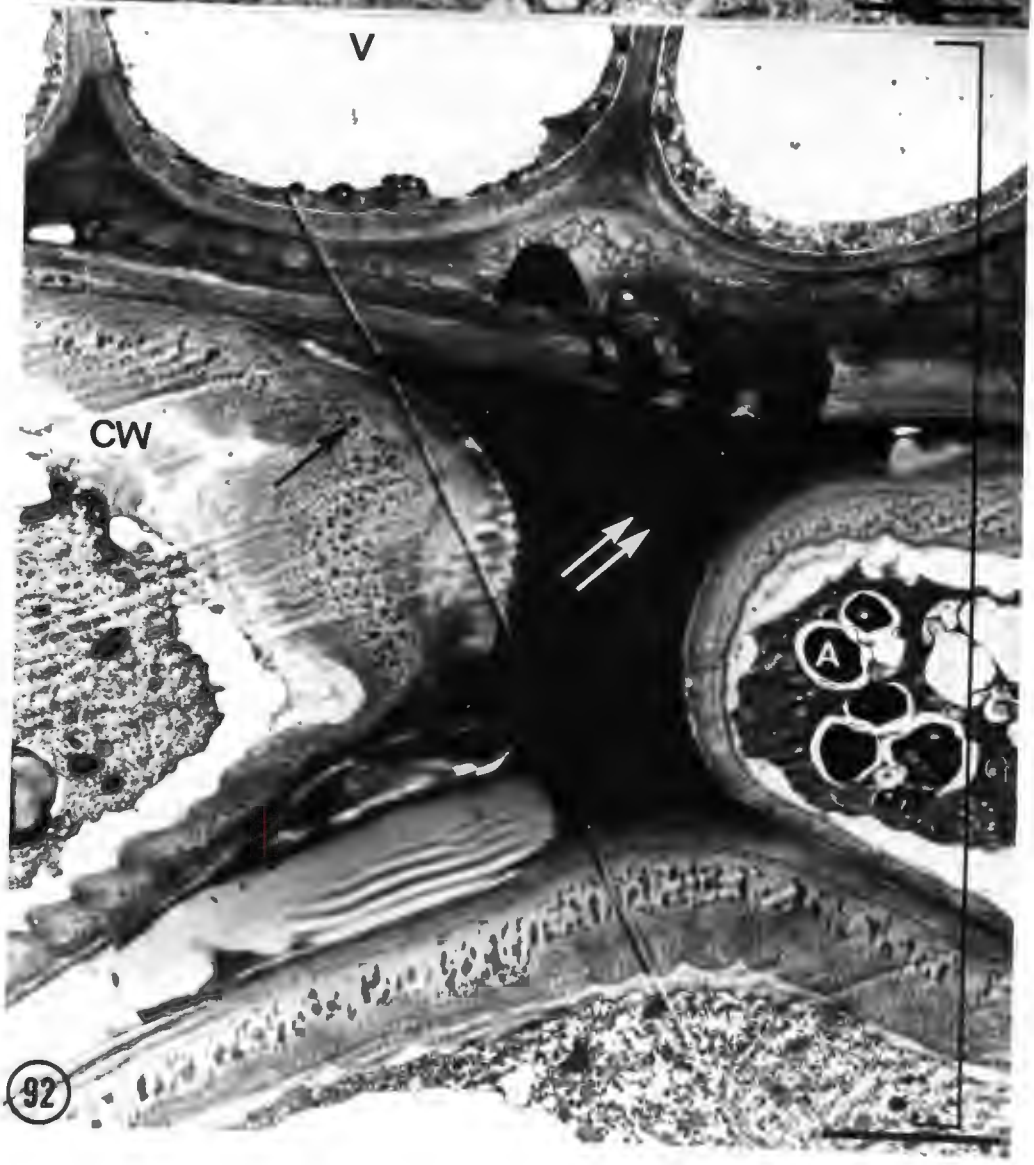
5 μm), it is obvious that the nucleolus in 5-day-imbibed material has shrunk. It has also become very dense and appears to consist almost entirely of compactly arranged fibrillar material. No loosely-packed outer granular zone is evident and no granules can be seen invading the fibrillar zone. Nucleolar organizing regions of nucleolar chromosomes (Swift 1959; Jordan 1971) are often peripherally embedded in the nucleolus (Fig. 81) and nucleolar vacuoles (Fig. 82) 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 liberally 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 (Fig. 81, 5-day-imbibed). 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 (Fig. 82) 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. 83) the mini-vacuoles are active autophagically. Fibrillar material dispersed in the cytoplasm (Fig. 83, arrows) appears to accumulate and is engulfed by the vacuoles (Figs. 83-85). The vacuoles seem to flow around the accumulation of fibrils in an amoeboid manner and the fibrils, enclosed in a membrane, move into the vacuole (Fig. 84). Presumably the membrane then disintegrates releasing the fibrils to become freely dispersed throughout

PLATE 23.

Fig. 91. Interface between gametophyte (G) and embryonic feeder (F), 5-day-imbibed. Note thick, many-layered gametophyte cell wall (CW) in contact with feeder. Outer cells of mature feeder are crushed.— Fig. 92. Interface between gametophyte (G) and embryonic feeder (F), 6-day-imbibed. Outer cell walls (CW) of gametophyte interface cells have developed numerous median blind channels (arrow). Gametophyte cells adjacent to actual interface cells may also develop thickened walls. Note electron-dense secretion (double arrow) which acts as a cementing agent between feeder and gametophyte. Ventral cells of mature feeder have become crushed. A = amyloplast; V = vacuole.

Bars represent 2.5 μm (Fig. 91), 5 μm (Fig. 92).


CW
G
91


V
CW
A
92

the vacuole (Figs. 83, 85). 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. 83 a mini-vacuole can be seen "attacking" a lipid body while another is apparently caught in the act of pinching off part of the main vacuole. Cellular debris also makes its way into these small vacuoles e.g. Fig. 84, double arrow (unidentified membranous material).

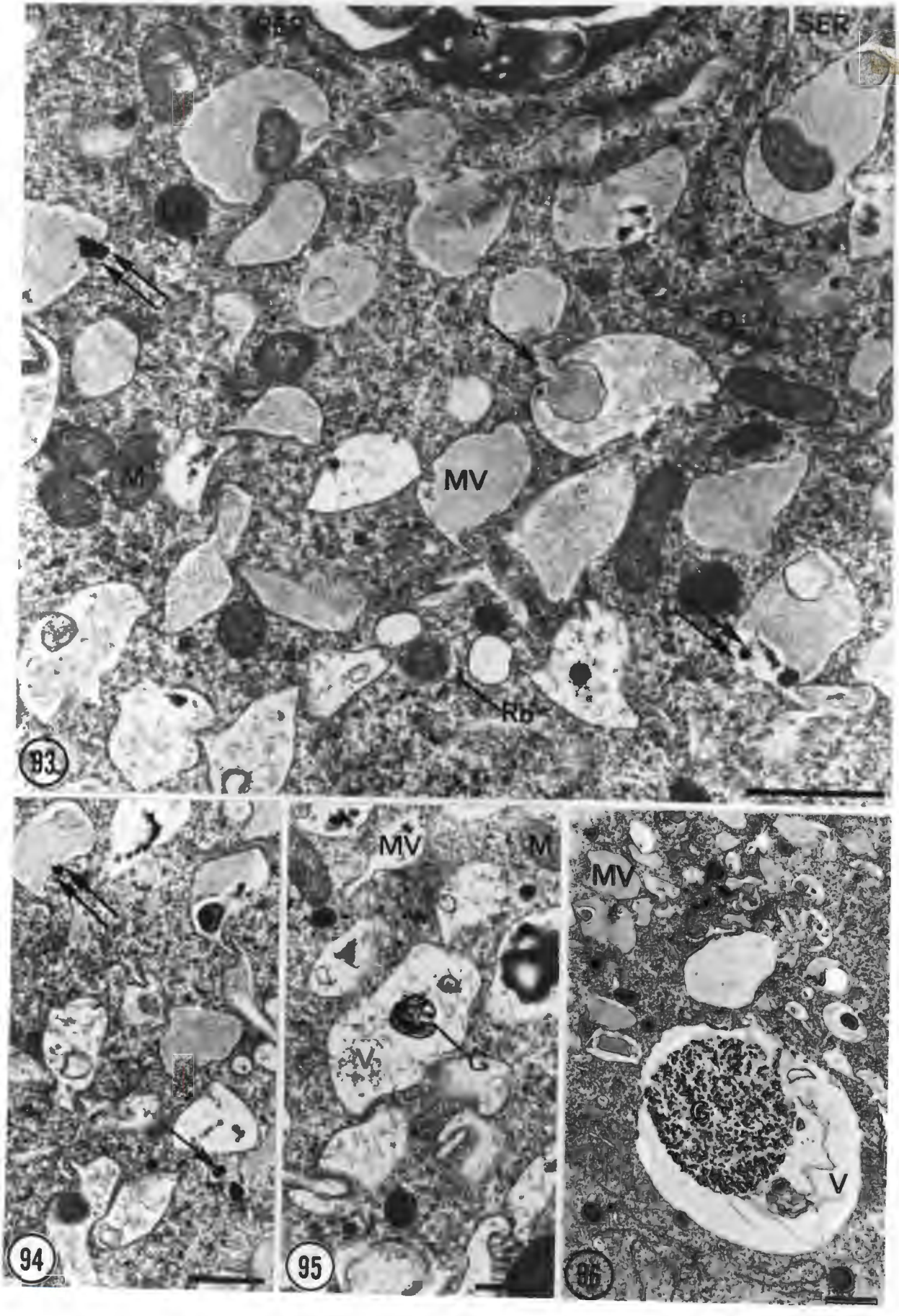
A short segment of ER can be seen issuing from the mini-vacuole engulfing fibrillar material in Fig. 85. Similar pieces of ER leading from other vacuoles in Fig. 85 confirm the idea that the mini-vacuoles are simply dilations of the ER. The vacuolar membranes are free of ribosomes although ER leading from the vacuoles may be rough (Fig. 85). Fig. 85 shows fibrillar material being taken up by and released within mini-vacuoles. The micrographs studied, though static images, give the impression that the fibrillar contents of the mini-vacuoles are added to interface cell walls in contact with the feeder (Fig. 85). These walls thicken rapidly in a short space of time. Although dictyosomes are present (Figs. 83, 85), they are not common and do not seem to be closely associated with the cell walls.

Five days after initial imbibition gametophyte interface cells have noticeably thicker walls. Although the interface walls contiguous with inner gametophyte cells have thickened (Fig. 86, 1.5 μm) this thickening is insignificant in comparison with outer walls (Fig. 90, 5 μm) now adhering firmly to the

PLATE 24.

Figs. 93-96. 5-day-imbibed gametophyte cells in interface zone.—Fig. 93. Autophagic mini-vacuoles (MV) with fibrillar contents. A mini-vacuole can be seen engulfing cytoplasmic fibrillar material (arrow). Small, spherical, highly electron-dense inclusions (double arrows) seem to be moving 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. 94. Small osmiophilic crystals free in cytoplasm (arrow) and moving into mini-vacuole (double arrow).—Fig. 95. Pitted crystal interpreted as a globoid (G) within protein body vacuole (V). M = mitochondrion; MV = mini-vacuole.—Fig. 96. Large particulate globoid (G) within protein body vacuole (V). MV = mini-vacuole.

Bars represent 1 μm (Figs. 93,96), 0.5 μm (Figs. 94,95).


SER
MV
M
Rb
93
MV
M
V
94
95
MV
V
96

feeder. Radial walls between adjacent interface cells (Fig. 88, 2 μm) become thicker than inner tangential walls (Fig. 86) but remain narrow in comparison with outer tangential walls (Fig. 90). Inner wall areas traversed by plasmodesmata remain thin (Fig. 86) with characteristic median cavities coalescing and enlarging in the middle lamella region (Fig. 87). Plasmodesmata are less common between adjacent interface cells (Fig. 88, note large median cavity) and may occur singly with no corresponding diminution in wall thickness. No plasmodesmata form in the outer wall.

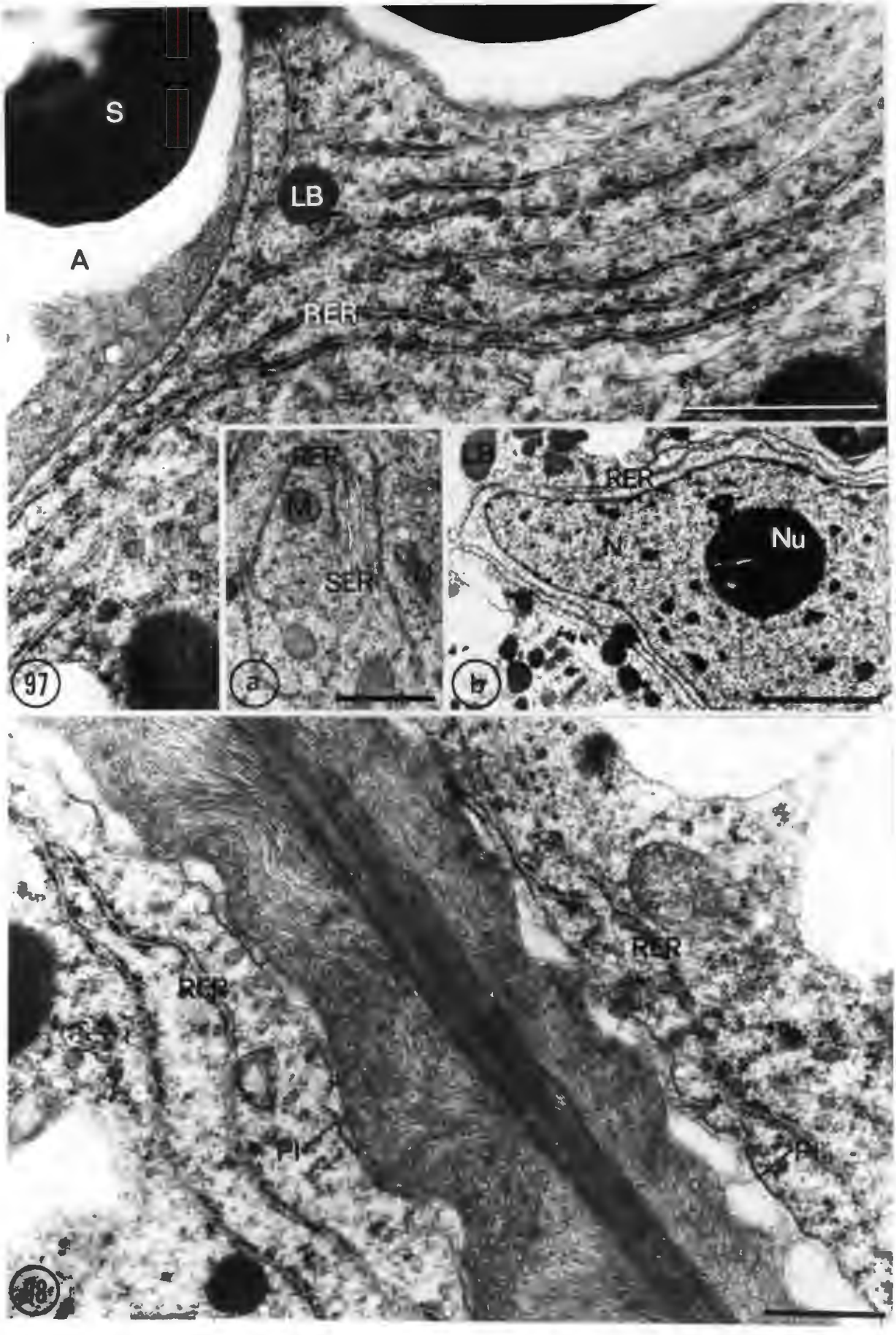
At the 3-day-stage gametophyte interface walls in contact with the feeder have begun to thicken (Fig. 89). Concomitantly a dark substance appears in the space between gametophyte and feeder (Fig. 89). This densely-staining material is apparently secreted from gametophyte and feeder cells and seems to function as an adhesive causing the two structures to adhere firmly. As time passes more wall material is added until, at the 4-to 5-day-stage the outer wall has a distinctly layered appearance (Fig. 90). Meanwhile the interface walls have become mucilaginous. The outer layers of the wall are somewhat compressed while the inner layers have a spongy appearance (Figs. 90, 91). Radial walls between adjacent interface cells also become mucilaginous (Fig. 88). Apart from addition of mini-vacuole contents to the wall (Fig. 85) some of the increased wall thickness might be due to wall hydrolysis with resultant swelling.

Gametophyte interface walls are thickest at the base of the

PLATE 25.

Figs. 97,98. 5-day-imbibed gametophyte cells in interface zone. —Fig. 97. Starch-laden amyloplasts (A) are surrounded by numerous layers of rough ER (RER). Inset: a, mitochondria (M) are often enclosed by ER; b, several long profiles of rough ER are usually arranged parallel to nuclear envelope. D = dictyosome; LB = lipid body; N = nucleus; Nu = nucleolus; SER = smooth ER; S = starch. —Fig. 98. Long profiles of rough ER (RER) run parallel to plasmalemma (Pl).

Bars represent 1 μm (Fig. 97, inset a,b; Fig. 98).


S
A
LB
RER
97
RER
M
SER
a
LB
RER
N
Nu
b
RER
RER
PI
PI
98

embryonic feeder e.g. Fig. 91 (5-day-imbibed), becoming progressively thinner (Fig. 90, 5-day-imbibed) toward the tip of the feeder where they may be as thin as the wall depicted in Fig. 89. Gametophyte cells adjacent to interface cells may also develop thickened walls (Fig. 92). Their pattern of wall thickening is similar to that of interface cells with walls nearest the feeder becoming thickest. On the 6th day (Fig. 92) 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. The previously mentioned electron-dense secretion (Fig. 89) which seems to act as a cementing agent between feeder and gametophyte has accumulated (Fig. 92).

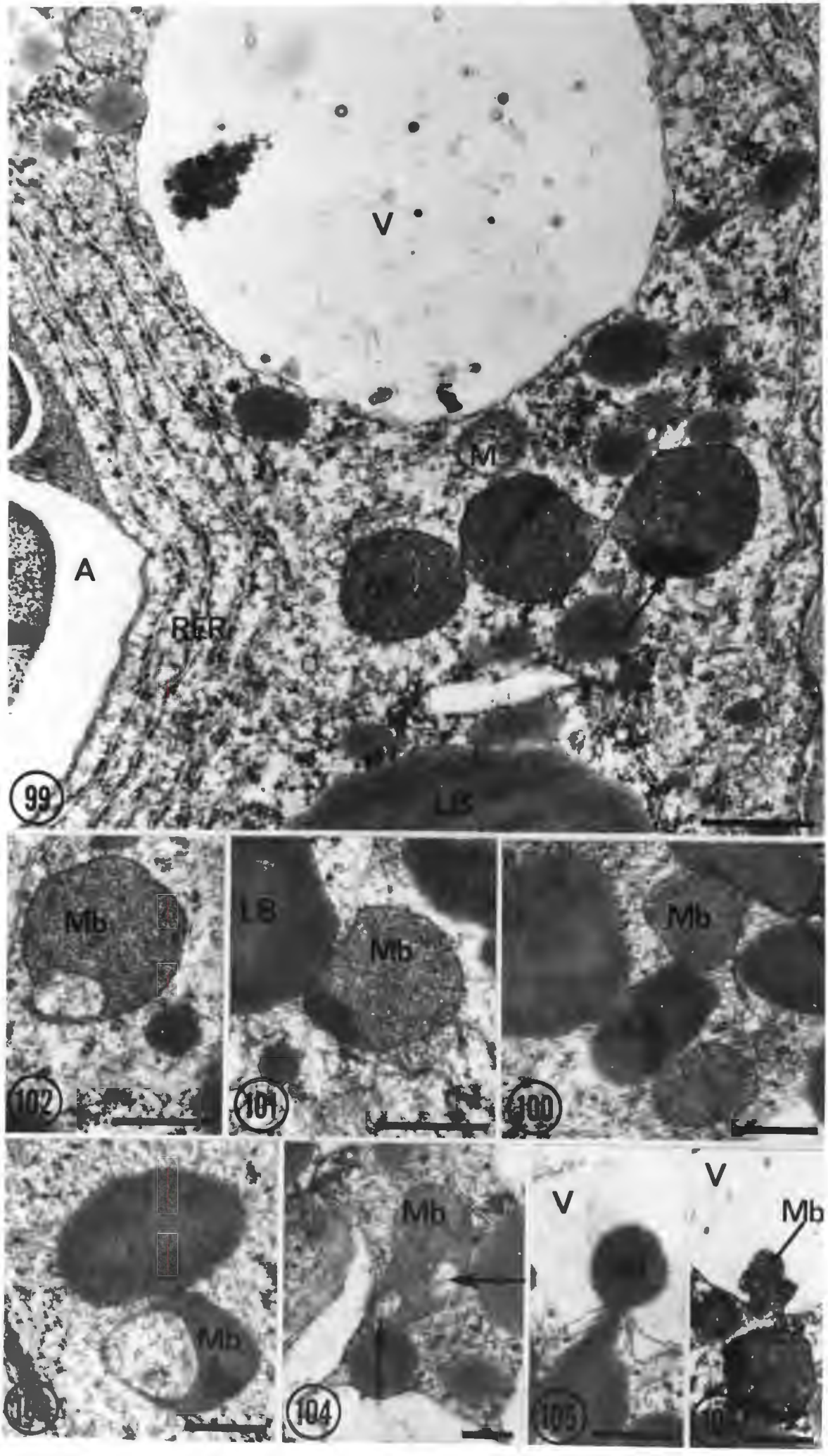
As the plumule of the young seedling emerges from the seed (5-day-stage - see Fig. 15), the outer cells of the mature feeder become crushed against the gametophyte (Fig. 91). By the time the plumule is erect (6-day-stage - see Fig. 16) the number of crushed feeder cells has increased (Fig. 92).

Mini-vacuoles (dilated smooth ER) in interface and neighbouring gametophyte cells imbibed for approximately 5 to 6 days frequently contain small, spherical, highly electron-dense inclusions (Fig. 84) thought to be crystalline. Similar crystals occur free in the cytoplasm (Fig. 94) and have been seen apparently moving into mini-vacuoles (Figs. 93, 94, arrow). At present there is no evidence to indicate the origin and composition of these crystals. Larger pitted (Fig. 95) and particulate (Fig. 96) crystals interpreted as globoids are occasion-

PLATE 26.

Figs. 99-106. Microbodies and lipid bodies in 5 to 6-day-imbibed gametophyte cells of interface zone.—Fig. 99. Mitochondria (M), lipid bodies (LB) and microbodies (Mb) become 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. 100. Microbodies (Mb) in close association with lipid bodies (LB).—Fig. 101. Microbody (Mb) associated with lipid body (LB). Dense inclusion (nucleoid) of microbody is characteristically located along bounding membrane.—Fig. 102. Microbody (Mb) with cup-shaped depression.—Fig. 103. Cup-shaped microbody (Mb) associated with lipid body.—Fig. 104. Cup-shaped depressions of microbody (Mb) often form directly opposite a lipid body (arrows).—Fig. 105. Lipid body (LB) passing into vacuole (V).—Fig. 106. Microbody (Mb) entering vacuole (V).

Bars represent 0.5 μm (Figs. 99-103, 105, 106), 1 μm (Fig. 104).


V
M
A
RER
LB
99
Mb
102
LB
Mb
101
Mb
100
Mb
Mb
104
V
105
V
Mb
10

ly seen. These inclusions are contained within protein body vacuoles which are usually larger than mini-vacuoles (Fig. 96). Non-dilated rough ER (Figs. 97, 98) is still prolific but less randomly distributed than in the 3-day-stage (Figs. 78-80). It now shows a very close spatial relationship with amyloplasts, mitochondria, nuclei and the plasmalemma (Figs. 97, 98). Starch -laden amyloplasts become completely surrounded by numerous layers of rough ER (Fig. 97). Mitochondria (Fig. 97, inset) are often enclosed by one or two layers of ER and may occasionally be surrounded by many concentric coils. One to two long profiles of ER are usually arranged parallel to the nuclear envelope (Fig. 97, inset) and the plasmalemma (Fig. 98).

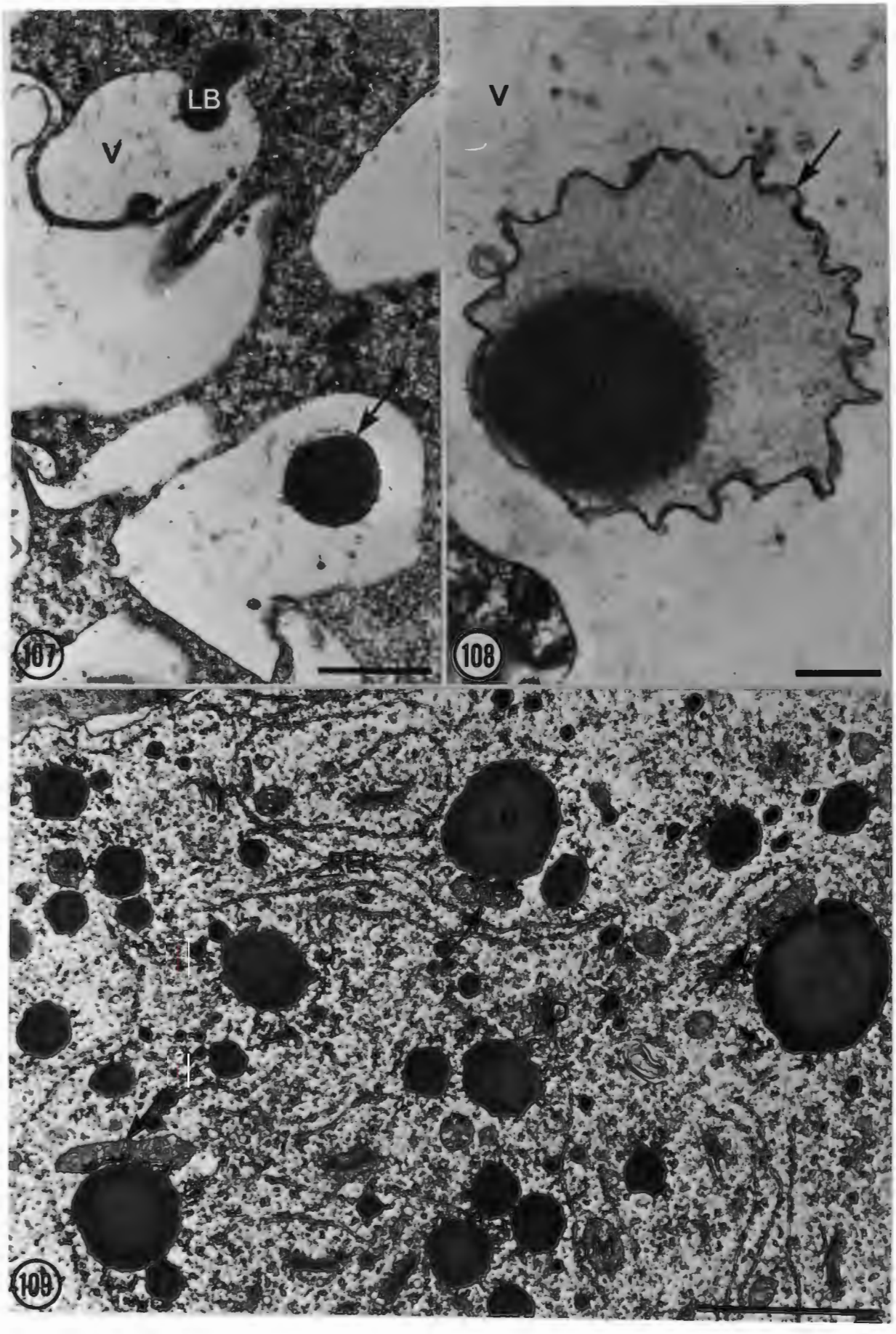
The cytoplasm remains filled with ribosomes, many of which are attached to the ER (Figs. 93, 97). Polysome and helical configurations are common. Dictyosomes (Fig. 97, inset), which are comparatively rare, produce vesicles with fibrillar contents (Fig. 93, 97 inset a). Amyloplasts, rich in starch, are distributed throughout the cytoplasm with occasional concentrations around the nucleus as in Fig. 81. Mitochondria seem to be ubiquitous.

By the 5-to 6-day-stage lipid stores have been drastically reduced. Remaining lipid bodies show a wide variation in size (Figs. 99, 109) perhaps reflecting different rates of digestion. It seems likely that the larger lipid bodies in Fig. 99 and 109 are formed by coalescence. Concurrent to lipid degradation, starch reserves are built up and micro-

PLATE 27.

Figs. 107-109. Lipid bodies in 5 to 6-day-imbibed gametophyte cells of interface zone.—Fig. 107. Migration of lipid bodies (LB) into vacuoles (V). Note apparent membrane (arrow) surrounding sequestered lipid body.—Fig. 108. Sequestered lipid body (LB) apparently degrading while still surrounded by membrane (arrow). V = vacuole.—Fig. 109. 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. 107, 108), 5 μm (Fig. 109).

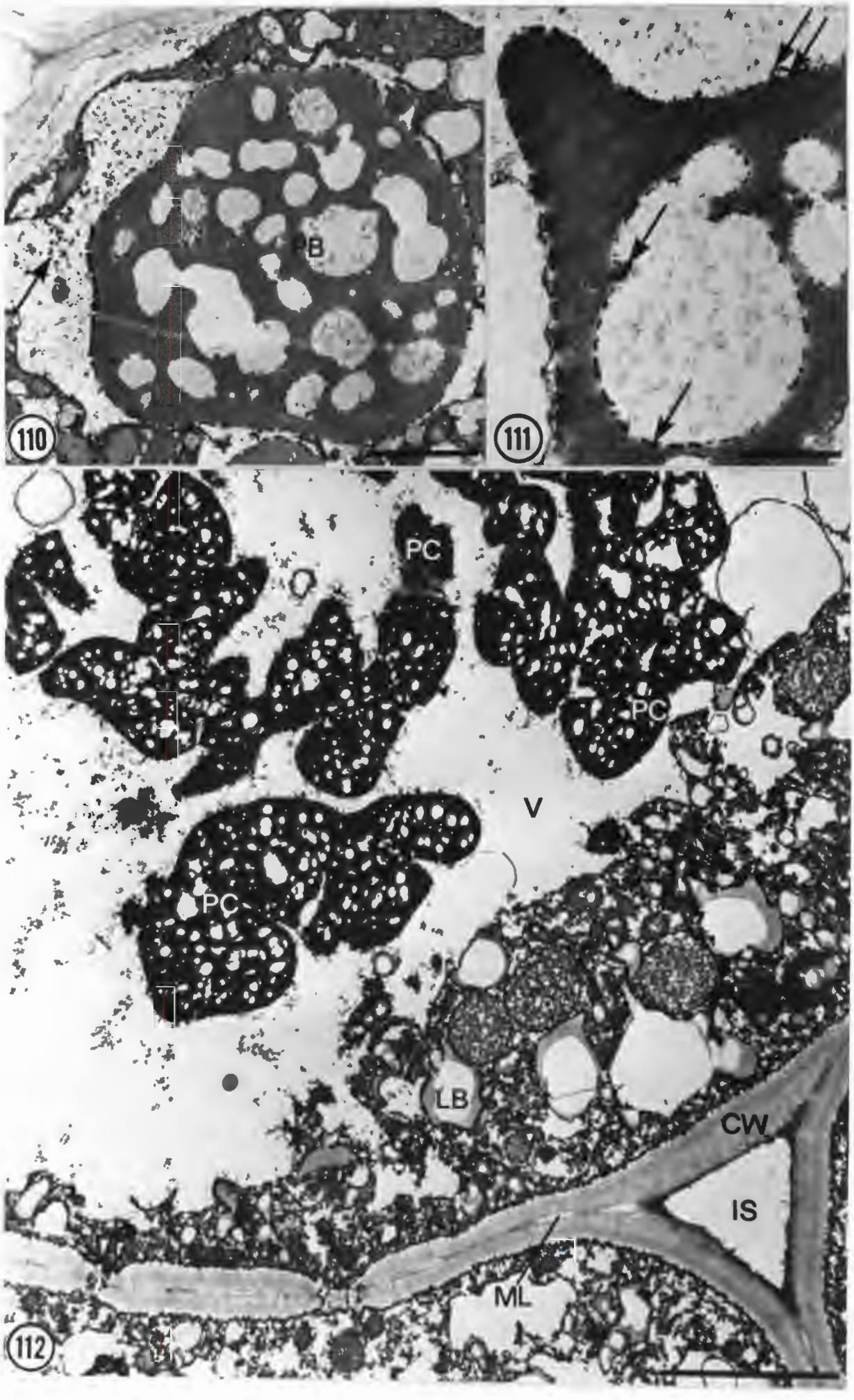

LB
V
V
107
108
109

bodies, presumed to be glyoxysomes (Frederick *et al.*, 1968), increase in number (Fig. 99). This microbody increase is apparently achieved by means of fission (Fig. 99). The inner electron-dense amorphous area seems to divide providing each daughter microbody with an osmiophilic eccentric core prior to completion of constriction (Fig. 99). The close association of microbodies, lipid bodies and mitochondria with starch-laden and rough ER surrounded amyloplasts as seen in Fig. 99 becomes a common spatial relationship occurring too frequently to be the result of mere chance. Microbodies are often observed in intimate association with lipid bodies (Figs. 100, 101), some microbodies showing a close relationship with more than one lipid body (Figs. 100, 104). Characteristically the dense inclusion of the microbody is usually located along the boundary membrane (Figs. 99, 101, 103, 106). A large number of microbody profiles were observed and it was noticed that the section of the membrane bearing the amorphous inclusion rarely comes in close contact with a lipid body as in Fig. 99. The microbodies vary in size and shape. Most are roughly spherical to oval but some develop cup-shaped depressions (Figs. 102-104). In a number of cases these depressions seemed to form directly opposite a lipid body. As is the case in deeply-situated gametophyte tissue (Figs. 53, 54, 63, 64) lipid and microbody migration into vacuoles (Figs. 105, 106) becomes a fairly commonly observed phenomenon in the interface zone. Occasionally a lipid body entering a vacuole appears to retain a surrounding membrane derived from the tonoplast (Fig. 107). In some instances these lipid bodies seem to degrade while

PLATE 28.

Figs. 110-112. Localization of acid phosphatase in cells of deep gametophyte using Gomori reaction (1952) modified by Berjak (1968). —Fig. 110. Vesicles (arrow) entering protein body (PB) give positive reaction for acid phosphatase.—Fig. 111. At later stage lead precipitation (arrows) indicating acid phosphatase activity is localised within pockets of reserve material. Apparently the vesicles (double arrow) release their contents on contact with the reserve material.—Fig. 112. Later entire protein-carbohydrate reserves (PC) stain darkly indicating the diffusion of acid phosphatase throughout the reserves. CW = cell wall; IS = intercellular space; LB = lipid body; ML = middle lamella; V = Vacuole.

Bars represent 2.5 μm (Fig. 110), 1 μm (Fig. 111), 5 μm (Fig. 112).


PB
110
111
PC
PC
PC
V
LB
CW
IS
ML
112

still surrounded by the membrane (Fig. 107, arrow; Fig. 108). Frequently mitochondria are seen in very close association with lipid bodies (Fig. 109).

Acid phosphatase activity

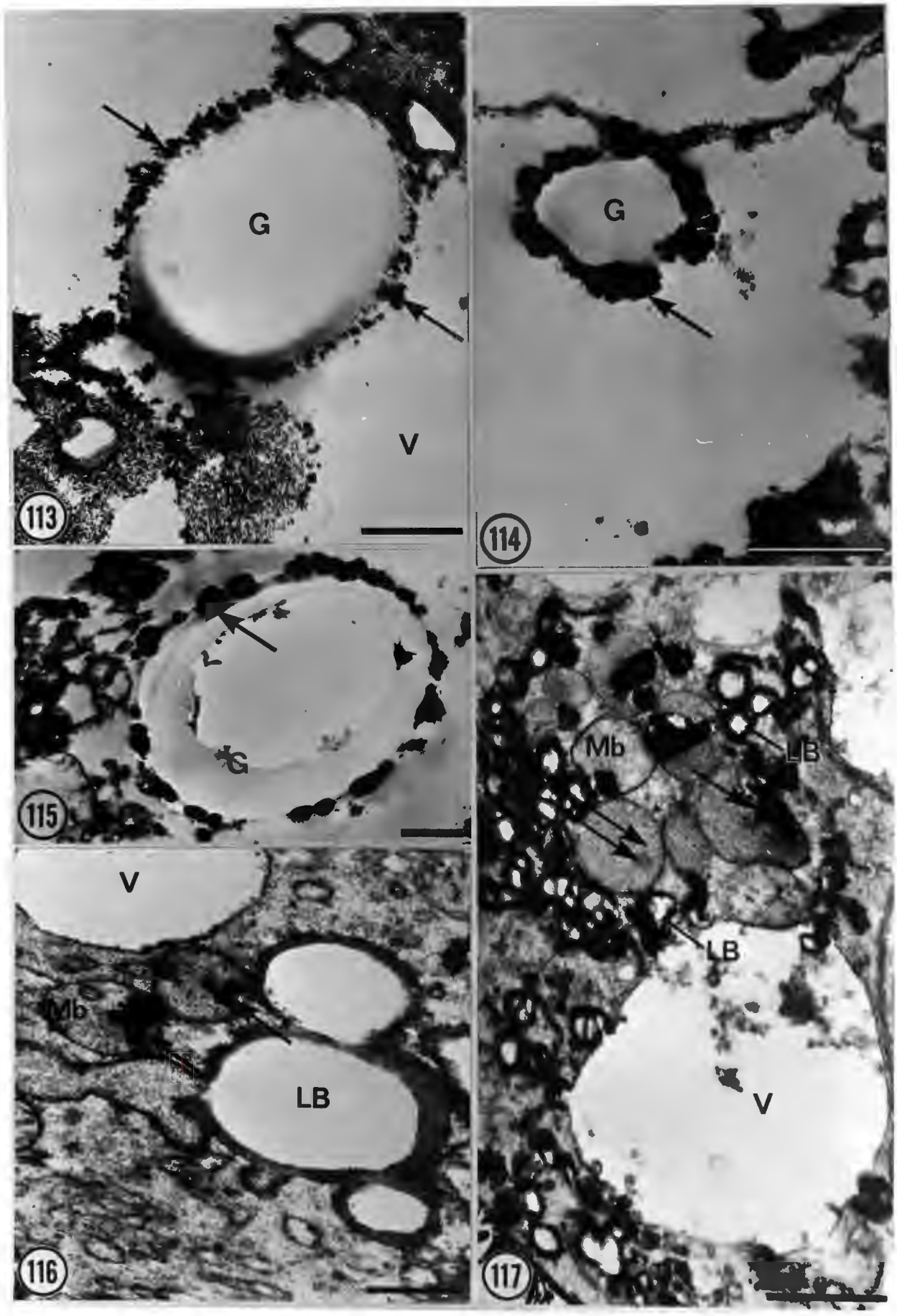
Using the Gomori reaction (1952) modified by Berjak (1968) acid phosphatase activity was localised in gametophyte and feeder tissue. On studying deeply-situated gametophyte cells it became apparent that the numerous vesicles commonly seen entering the protein bodies give a positive reaction for acid phosphatase (Fig. 110). At a slightly later stage lead precipitation, indicating acid phosphatase activity, is localised within the pockets of the compact reserves (Fig. 111). Apparently the majority of the vesicles release their contents on contact with the reserve material (Fig. 111, double arrow). Later the entire protein-carbohydrate reserves stain darkly indicating the diffusion of acid phosphatase throughout the reserves (Fig. 112). Protein body globoids in both gametophyte (Fig. 113) and feeder tissue (Figs. 114, 115) are characteristically associated with acid phosphatase activity (Poux 1965; Villiers 1971). Occasionally the entire globoid stains intensely but in most cases the lead precipitate forms at the periphery of the globoid (Figs. 113-114).

Strong localised positive reactions for acid phosphatase are found in microbodies of gametophyte (Fig. 116) and feeder (Fig. 117). In most cases lead precipitation is confined to the amorphous eccentric core of the microbody (Fig. 117). However, not all microbodies show a positive reaction for acid phospha-

PLATE 29.

Figs. 113-117. Localization of acid phosphatase using Gomori reaction (1952) modified by Berjak (1968).—Fig. 113. Protein body globoid (G) in gametophyte characteristically associated with acid phosphatase activity (arrows). PC = protein-carbohydrate reserves; V = vacuole.—Fig. 114. Globoid (G) in protein body of feeder tissue. Note peripheral localization of acid phosphatase (arrow).—Fig. 115. Globoid (G) in protein body of feeder tissue. Acid phosphatase activity is localised at periphery of globoid (arrow), the central portion of which is torn out.—Fig. 116. Gametophyte microbodies (Mb) showing localised positive reactions for acid phosphatase (arrows). LB = lipid body; V = vacuole.—Fig. 117. Feeder microbodies (Mb) displaying localised positive reactions for acid phosphatase. Lead precipitation is generally confined to the amorphous eccentric core of the microbody (arrow). However not all microbodies show a positive reaction (double arrow). Note acid phosphatase activity localised in lipid bodies (LB), cf. Fig. 116. V = vacuole.

Bars represent 0.5 μm (Figs. 113-116), 1 μm (Fig. 117).


G
V
113
G
114
G
115
V
Mb
LB
116
Mb
LB
LB
V
117

tase (Fig. 117).

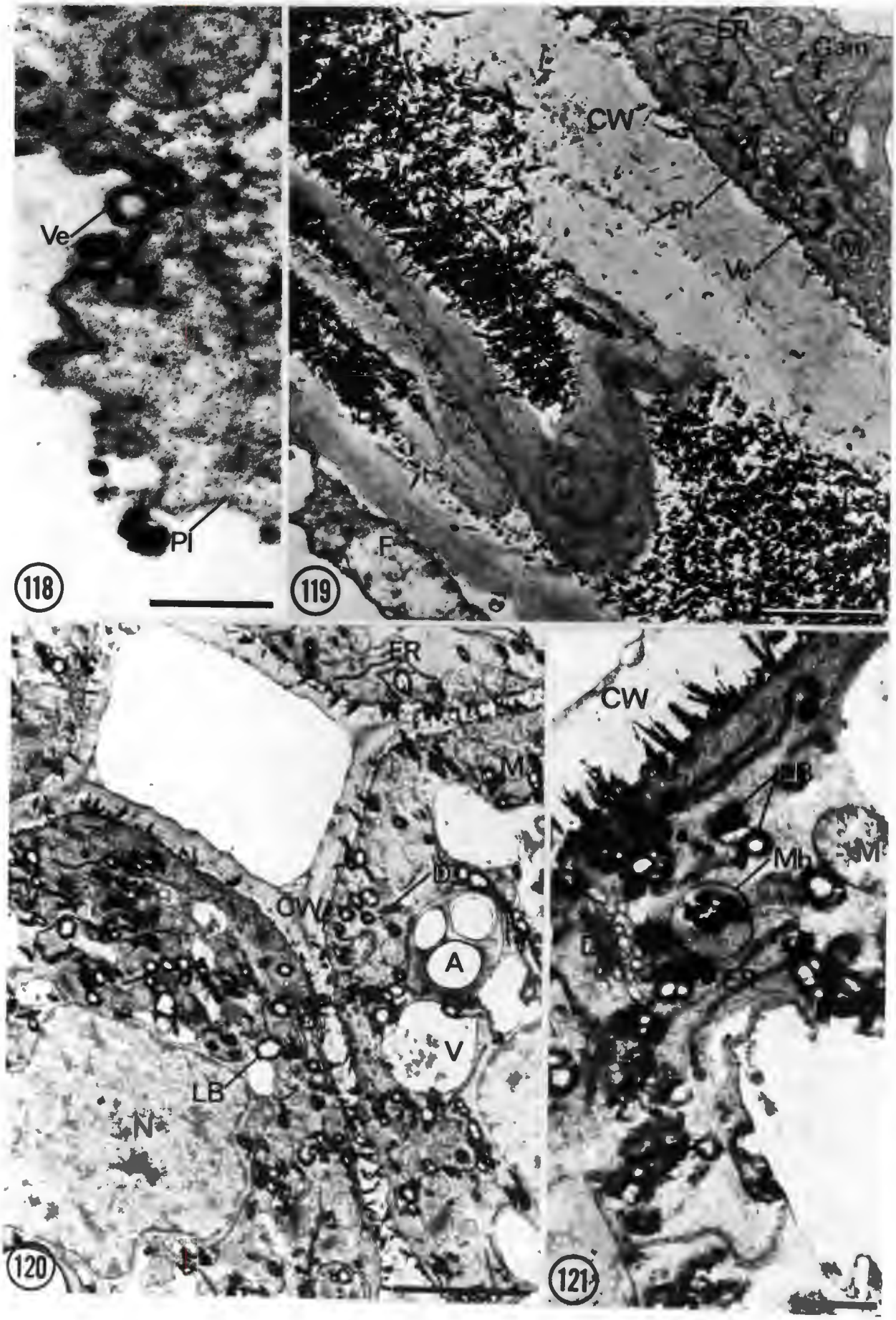
Acid phosphatase activity in gametophyte walls is largely confined to the corners of the cells where intercellular spaces develop (Fig. 112). This activity gradually extends along the middle lamellae between adjacent cells as they age. Vesicles apparently transporting acid phosphatase into the space between wall and plasmalemma were frequently observed in 3-day-imbibed gametophyte interface cells (Figs. 118, 119). On the whole acid phosphatase activity appears to be more extensive in feeder than in gametophyte cells. Lead precipitation in feeder walls is apparent at a very early stage (Figs. 120, 121 - 2-day-imbibed) with greatest activity occurring in the inner wall zone adjacent to the plasmalemma. By the 3rd to 4th day outer feeder cells, beginning at the base of the emergence, senesce and are crushed against the gametophyte (Fig. 119). Strong acid phosphatase activity is seen throughout these cells (Fig. 119). Gametophyte cells show diffuse acid phosphatase activity in the cytoplasm at a later stage. This may occur anytime between the 5-to 8-day-imbibed stages as senescence occurs asynchronously in neighbouring gametophyte cells.

Lipid bodies in gametophyte tissue incubated in Gomori medium do not stain darkly (Fig. 116). However those of the feeder showed a dark staining reaction (Figs. 117, 120, 121) which seems to indicate that some acid phosphatase activity is localised in feeder lipid bodies. ER and dictyosomes of gametophyte (Fig. 119) and feeder (Fig. 121) showed no acid phospha-

PLATE 30.

Figs. 118-121. Localization of acid phosphatase using Gomori reaction (1952) modified by Berjak (1968).—Fig. 118. 3-day-imbibed gametophyte interface cell. Note vesicles (Ve) apparently transporting acid phosphatase into space between wall and plasmalemma (Pl).—Fig. 119. Interface between gametophyte (Gam) and feeder (F) at base of feeder in 3-day-imbibed seed. Outer feeder cells are crushed and show strong acid phosphatase activity. Note vesicles (Ve) transporting acid phosphatase into space between wall (CW) and plasmalemma (Pl). D = dictyosome; M = mitochondrion, ER = Endoplasmic reticulum.—Fig. 120. Feeder cells from embryo germinated for 2 days. Lead precipitation indicating acid phosphatase activity in cell walls (CW) occurs in the inner wall zone adjacent to plasmalemma. Acid phosphatase is also localised in lipid bodies (LB). A = amyloplast; D = dictyosome; ER = endoplasmic reticulum; M = mitochondiron; N = nucleus; V = vacuole.—Fig. 121. Portion of feeder cell from embryo germinated for 2 days showing localization of acid phosphatase activity in inner wall zone (CW), in microbodies (Mb) and lipid bodies (LB). ER, dictyosomes (D) and mitochondria (M) showed no acid phosphatase activity.

Bars represent 0.5 μm (Figs. 118, 121), 2.5 μm (Figs. 119, 120).


Ve
Pl
118
ER
Golgi
CW
Pl
M
Ve
F
119
ER
M
D
CW
A
V
LB
N
120
CW
LB
Mb
M
121

tase activity. Other organelles e.g. mitochondria and plastids were also negative.

DISCUSSION.

As desiccated seed tissue is difficult to fix, embed and section many authors have resorted to the practice of soaking their material for varying lengths of time before fixation (Setterfield *et al.*, 1959; Bagley *et al.*, 1963; Nieuwdorp 1963; Varner and Schidlovsky 1963; Horner and Arnott 1965, 1966; Bain and Mercer 1966b; Klein and Ben-Shaul 1966; Abdul-Baki and Baker 1973). It has been suggested that presoaking causes no significant organelle changes (Bagley *et al.*, 1963; Klein and Ben-Shaul 1966; Abdul-Baki and Baker 1973), but it should be borne in mind that pre-soaked material cannot reflect the true structure of dehydrated cells. Due to the technical difficulties involved, relatively few investigators have fixed air-dry seed material (Paulson and Srivastava 1968).

In all the abovementioned studies, aqueous fixatives were used. This is not ideal for the study of dry tissue as imbibitional changes most certainly are induced e.g. swelling of organelles (Yoo 1970; Swift and Buttrose 1973), with the result that an accurate picture of unimbibed material is not obtained. Perner (1965) fixed radicles of dry *Pisum sativum* seed with osmium tetroxide (0s04) vapour. To acquire satisfactory results he extended fixation time from several weeks to months. His micrographs, in which organelles appear shrunken, membranes intact and ER unexpectedly prominent, probably reflect orga-

nelle structure in dry seed tissue very closely. Yatsu (1965) used Perner's vapour method of fixation on the cotyledons of dry *Gossypium hirsutum*. Apart from these two studies this method of fixation has not been widely used. The extended, time-consuming period of fixation required has probably acted as a deterrent. Results too are not always satisfactory (Paulson and Srivastava 1968). Freeze-etching should provide natural preservation of cell ultrastructure in the air-dry state (Mollenhauer and Totten 1971). Yet, despite this, freeze-etching has not enjoyed the wide usage it seems to merit and the number of investigations of this type are relatively few (Buttrose 1971; Lott *et al.*, 1971; Swift and Buttrose 1972, 1973; Lott and Vollmer 1973a,b).

In this study on *Welwitschia* seed aqueous fixation methods were used (see Methods). Material described under Results as dry or unimbibed was fixed air-dry. Owing to difficulty in acquiring clear fixation images of dry and/or freshly imbibed tissue of quiescent and dormant seed by osmium fixation many investigators have preferentially used permanganate as a fixative (Paleg and Hyde 1964; Yatsu 1965; Villiers 1971; Jones 1969). With permanganate fixation membrane definition is increased and organelles are more clearly seen owing to lipid body shrinkage, but cyto- and nucleoplasmic details are obliterated. For this reason all micrographs of quiescent seed material in this study are osmium-fixed. Although *Welwitschia* seeds were not subjected to a pre-soaking period, the aqueous fixatives used must have induced slight changes. With this in mind the general appearance of "dry" *Welwitschia*

gametophyte cells is qualitatively similar to numerous other descriptions of "dry" seed tissue. The cells are characterised by massive stores of reserve materials which tend to have an obscuring effect on residual cytoplasm. Embedded in this cytoplasm are organelles recognisable as mitochondria, amyloplasts and microbodies despite poor internal organization. The unit structure of membranes surrounding nuclei and organelles is typically poorly defined and cannot be resolved.

In agreement with the results of many other authors, dictyosomes appear to be missing (Varner and Schidlovsky 1963; Paleg and Hyde 1964; Yatsu 1965; Paulson and Srivastava 1968; Abdul-Baki and Baker 1973), ER is fragmentary and of infrequent occurrence (Yatsu 1965; Bain and Mercer 1966; Paulson and Srivastava 1968; Yoo 1970; Hallam *et al.*, 1972; Abdul-Baki and Baker, 1973) and ribosomes are present as unaggregated monosomes (Paulson and Srivastava 1968). Yoo (1970) and Hallam *et al.*, (1972) have reported stacks of flattened vesicles in dry, unimbibed tissue of *Pisum sativum* and *Secale cereale* respectively. Yoo interprets them as prodictyosomes and Hallam *et al.*, as dictyosomes. Although most studies have indicated that ER is poorly represented or absent in dry seed tissue, Perner (1965) has demonstrated a well-defined system of rough and smooth ER in dry *Pisum sativum* radicles using his osmium vapour fixation technique. Klein and Ben-Shaul (1966) have also reported conspicuous ER in dry embryonic axes of *Phaseolus vulgaris*. While the majority of investigators have recorded that polysomes are missing in dry tissue (Paulson and Srivastava 1968; Deltour and Bronchart 1971; McCarthy *et al.*,

1971), there is some evidence that polysomes do exist in dry seeds (Dure and Waters 1965), but are damaged mechanically (Hallam *et al.*, 1972) or chemically (Loening 1968) during isolation or fixation.

It seems that failure to recognise dictyosomes, polysomes and a well-developed ER system in electron micrographs of dry seed tissue fixed according to standard procedures does not necessarily imply that they do not exist (Yatsu 1965; Yoo 1970; Abdul-Baki and Baker 1973). Yatsu (1965) has suggested that dictyosomes become so compressed in the dehydrated state that they are unrecognizable and Abdul-Baki and Baker (1973) speculate that dictyosome and ER membranes become very fragile in dry tissue and are consequently more difficult to preserve.

Seeds of *Welwitschia mirabilis* do not appear to be subjected to, or require, a period of dormancy. In the dry condition the seeds remain in a state of quiescence, merely requiring water for the initiation of germination. While quiescent, dry seed tissues display minimal rates of metabolic activity and are highly resistant to adverse environmental conditions (Paulson and Srivastava 1968). However deleterious changes related to vigour loss are known to occur during the dry state (Villiers 1972; Abdul-Baki and Baker 1973). In *Welwitschia mirabilis* germinability declines fairly rapidly. With increasing age seeds undergo a proportionate delay in observable germination and most are non-viable within 3 to 4 years. Apparently, aging seeds accumulate extensive membrane damage and require a period in which the cytoplasm can regain its

normal appearance before extension growth of the embryo occurs (Villiers 1972). Freshly harvested, dry seed was used in this investigation to ensure increased viability, prompt germination and lack of age-induced damage.

Following rapid uptake of water the cells of the embryo and gametophyte hydrate, expand and become more active. Apparently many of the biochemical and ultrastructural changes that take place on hydration are the reverse of those occurring during seed maturation (Hallam *et al.*, 1972). In conformity with other studies, the changes accompanying transformation from a dry, quiescent to a hydrated, active state include increased clarity of membranes, progressive depletion of reserve materials, development and proliferation of pre-existing organelles e.g. mitochondria, plastids, microbodies and ER and the appearance and increase of dictyosomes and polysomes (Marcus and Feeley 1964 1966; Srivastava and Paulson 1968b; Yoo 1970; Abdul-Baki and Baker 1973).

Nucleus

Nuclear polymorphism appears to be a characteristic of *Welwitschia* gametophyte tissue. The conspicuously lobed, convoluted nature of nuclear profiles, initially seen in dry tissue, persists in the active imbibed state. Nuclear invaginations noted in other work has been variously attributed to quiescence (Yeoman and Street 1973), high metabolic activity (Clowes and Juniper 1968) and senescence (Berjak and Villiers 1970). In the quiescent state *Welwitschia* gametophyte nuclei display

obvious polymorphism. Deeply-situated gametophyte cells with compact reserves possess grossly distorted nuclei while interface cells with fewer, looser-packed reserves contain nuclei which are correspondingly less misshapen. It would seem that much of the distortion is caused by pressure of reserve materials against the nuclear surface. On hydration gametophyte cells are activated and reserve degradation commences. Despite progressive loss of reserve materials, nuclei retain their irregular form. Interface cells rapidly achieve a high degree of metabolic activity and stand in sharp contrast to the much slower metabolism of deeper-seated cells. The nuclei of interface cells e.g. Fig. 81, gradually assume a more exaggerated extensively lobed appearance as metabolic activity intensifies. It has been postulated that irregularity of the nuclear outline indicates intense activity between nucleus and cytoplasm by increasing the boundary area through which the substances can pass (Clowes and Juniper 1968). Lobing of the nucleus has also been connected with senescence of cells (Berjak and Villiers 1970) and seems to accompany increased cell activity leading to the climacteric phase just prior to cell death (Gilliland 1973).

The abundant fibrous inclusions which pervade both nucleolus and nucleoplasm probably constitute the most striking feature of quiescent nuclei in *Welwitschia* gametophyte tissue. Histochemical staining (mercuric bromphenol blue - Pearse 1961) indicates that the inclusions have a proteinaceous nature. It has not been established whether they also contain ribonucleic acid (RNA). Occurrence of proteinaceous inclusions in plant

cell nuclei has been well documented. Wergin *et al.*, (1970) recognise four distinct types viz: amorphous, fibrous, paracrystalline and crystalline. Evert (1970) in *Tilia americana* reports nuclear P-protein (slime) inclusions in sieve elements. The anastomosing 3-D network of nuclear inclusions in *Welwitschia* differs from all previous descriptions of nuclear inclusions. Their uniqueness lies firstly in their vast profusion and secondly in their presence within the nucleolus. There have been repeated reports of nuclear inclusions in close proximity or actual contact with nucleoli (Wergin *et al.*, 1970) but nucleolar "invasion" has not been previously noted. In *Antirrhinum majus* Wergin *et al.*, (1970) noted that the granular material forming the peripheral zone of the nucleolus becomes irregularly distributed at the point of contact with an inclusion, with most of the ribosome-like particles aggregating at the interface between the nucleolus and inclusion. In *Welwitschia* the branching nucleoplasmic inclusions are frequently surrounded by coarse, granular material of a similar staining intensity to nucleolar material.

In some species of plants e.g. *Camplyoneuron phyllitidis, Antirrhinum majus, Coleus blumei, Asplenium nidus, Galtonia candicans* and *Dahlia variabilis* a developmental inverse size relationship exists between nucleoli and nuclear inclusions (Wergin *et al.*, 1970). Wergin *et al.*, (1970) postulate that the two structures are associated and interconvertible.

It is known that the nucleolus is responsible for the DNA-depen-

dent synthesis of ribosomal precursors (Jordan 1971) which pass into the cytoplasm. As actinomycin D is an antimetabolite which suppresses DNA-dependent RNA synthesis (Reich *et al.*, 1961), by binding to DNA (Kirk 1960) its affect on the nucleolus is of interest. Although diverse results have been recorded (Hyde 1967), treatment in most cases caused segregation of the nucleolus into discrete components (Wergin *et al.*, 1970). In some instances it induced the appearance of nuclear inclusions (Lane 1969). Lane (1969) treated oocytes of the newt *Triturus viridescens* with actinomycin D and reported the appearance of strange fibrillar nuclear inclusions.

It is not known whether the mechanism producing nuclear inclusions in plants operates in an analagous manner to that induced by antimetabolites in animals (Wergin *et al.*, 1970). Perhaps the nuclear inclusions in the dry gametophye tissue of *Welwitschia* are the result of suppression of nucleolar organizer DNA. This is of course pure speculation and a great deal more information is needed for the elucidation of this subject. It is possible, however, that a metabolic association might exist between nucleolus and nuclear inclusions. In *Welwitschia* the inclusions penetrate the nucleolus and are apparently surrounded in the nucleoplasm by granular nucleolar material. The inclusions are present when the nucleolus is in the dry quiescent state, but disappear rapidly (within 24h) upon hydration and reactivation of the nucleolus.

A number of papers and reviews have been published on the

ability of viruses to induce nuclear inclusions (Esau 1967). *Welwitschia* nuclear inclusions do not conform to these descriptions. They appear too profuse for a viral infestation and there is difficulty in accounting for their rapid disappearance. For an infestation of this magnitude to decoat and disappear within 24h seems impossible. A large sample of healthy, viable seed was investigated and, in every instance, nuclear inclusions were present in dry gametophyte tissue. It is concluded that the nuclear inclusions in this study are not attributable to viruses. There have been few reports of nuclear inclusions in seed material (Villiers 1972) and this is perhaps related to the fact that many authors pre-soak their material prior to fixation. It is evident that in *Welwitschia* nuclear inclusions disperse rapidly upon hydration.

During the formation of *Welwitschia* gametophyte tissue free-nuclear mitotic divisions are followed by irregular laying down of cross walls. Resultant nuclear fusion gives rise to polyploids of varying degrees (Martens 1959, 1961, 1963). As the gametophyte is not uniformly haploid its nuclei contain a variable number of nucleoli. Similar to previous descriptions of quiescent nuclei (Hyde 1967; Jordan and Chapman 1971) the nucleoli in the dry state consist almost entirely of dense fibrillar material with no noticeable granular zone. However they contrast sharply with existing descriptions in two respects: firstly in their possession of inclusion-filled vacuoles and secondly in the peripheral arrangement of apparently nucleolar-derived granular material around nucleoplasmic inclusions. Upon hydration perhaps the most dramatic ultrastructural change observed was the prompt disappearance of nuclear inclusions.

Apart from this the most significant changes were related to the activation of the nucleolus. The transformation of the nucleolus from quiescence to a highly active state was best seen in the embryo. Consequently this process will be dealt with more fully in the discussion devoted to the embryonic feeder.

The nucleolus is widely regarded as the site of precursor rRNA synthesis. On hydration, in agreement with previous work (Hyde 1967; Jordan 1971), the volume of the nucleolus increases and is followed by an increase in the ribosomal content of the cell in preparation for protein synthesis. Polysomes, at first absent, begin to accumulate.

During initial imbibition physical wetting of deeply-situated gametophyte tissue takes place prior to that of the interface zone. Despite this, nuclear activation and subsequent cell metabolism is accelerated in interface cells and soon outstrips that of deeper-seated cells. In fact there seems to be a sequence of activation from the interface to the outermost gametophyte cells. 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 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. In the outermost gametophyte cells nuclear inclusions vanish but nucleoli tend

to remain in a semi-quiescent state. This gradation of nuclear activity in the gametophyte coupled with the fact that nuclear activation in the embryo seems to shortly precede that of contiguous gametophyte cells, is suggestive of the diffusion of an unknown factor from the embryo into the gametophyte where it possibly stimulates or enhances nuclear activity. Further biochemical studies are needed.

No cell division is observed in gametophyte tissue. Most of the dense chromatin evident in the nuclei in dry material disperses on hydration but large patches of condensed chromatin persist.

Protein bodies

Numerous papers and reviews have been written on seed proteins and protein bodies (Altschul *et al.*, 1966; Rost 1972). In fact protein bodies have probably received more attention than any other structure in quiescent/dormant and germinating seeds (Paulson and Srivastava 1968). Rost's (1972) recent paper reviews the work of a variety of authors and classifies protein bodies into three main categories. The three types consist of those with no inclusions, those with globoid inclusions only and those with globoid and crystalloid inclusions. According to Jacobsen *et al.*, (1971) the crystalloid inclusion is actually a protein-carbohydrate body.

It would appear that the protein bodies of *Welwitschia* constitute a fourth category. The results of cytochemical methods

used at the light and electron microscope level seem to suggest that the entire reserve material within the body may exist as a protein-carbohydrate complex i.e. the entire structure is a protein-carbohydrate body! However this term has been previously used by Jacobsen *et al.*, (1971) to describe inclusions within protein bodies of diverse families. To avoid the confusion of nomenclature changes the body as a whole will be referred to as a protein body, and its contents as protein-carbohydrate.

The protein bodies stain positively with mercuric bromphenol blue (a quantitative total protein stain - Pearse 1961) and also with periodic acid - Schiff reagent (a stain for total carbohydrates - Jensen 1962). Jacobsen *et al.*, (1971) have reported that protein-carbohydrate crystalloid inclusions in the aleurone grains (protein bodies) of barley stain a greenish colour with Toluidine blue. In *Welwitschia* the entire protein body and cell walls stain greenish with Toluidine blue. Similarly protein bodies and cell walls stain consistently at the E M level with Rambourg's (1967) silver methenamine technique for carbohydrate detection. The compound which causes the protein bodies to stain green with Toluidine blue is thought to be a natural component of the bodies but its identity is as yet unknown (Jacobsen *et al.*, 1971).

Protein-carbohydrate crystalloid inclusions seem to be very rich in protein (Pfeffer 1872; Jacobsen *et al.*, 1971). Jacobsen *et al.*, (1971) report that in terms of the entire protein body of barley the reserve matrix, although rich in proteins, appears

to contain less than the protein-carbohydrate inclusions. It would seem therefore that *Welwitschia* gametophyte protein bodies contain a very high concentration of protein which is also borne out by biochemical extraction results (see Table I).

There has been some confusion in the past in the identification of crystalloids (protein-carbohydrate bodies) and globoids (Jacobsen *et al.*, 1971). Some authors have mistaken globoid cavities for globoids (Paulson and Srivastava 1968), globoids for crystalloids (Eb and Nieuwdorp 1967; Jones 1969a) and vice versa (Paulson and Srivastava 1968). In *Welwitschia* there is little possibility of mistaken identity. The reserve matrices appear to be entirely protein-carbohydrate in nature and globoids are scarce. When present they exist either as a single large or several small inclusions usually within internal cavities of the reserve deposits. They exhibit characteristic globoid staining properties, staining red with Toluidine blue (Vazart 1960) due to the presence of phytin and, perhaps, free phospate (Jacobsen *et al.*, 1971) and showing typical high acid phosphatase activity (Poux 1965; Villiers 1971).

The globoid, originally described by Pfeffer (1872), is perhaps the most widely recognised protein body inclusion. Despite extensive reference in the literature little is known about them and their origin is obscure. They apparently represent sites of accumulation of phytin, the calcium, potassium and magnesium salt of myo-inositol hexaphosphate and are thought to be bound to protein (Jacobsen *et al.*, 1971; Ching 1972). On occasion

the globoids of protein bodies of some species have been reported to contain lipid (Salmon 1940).

Ring-like globoid envelopes are sometimes seen enclosing globoids or globoid remnants in *Welwitschia*. Poux (1965) and Khokhlova (1971) report the presence of a membrane around the phytin globoid in *Hordeum* and *Cucurbita* respectively. In *Welwitschia mirabilis* the fine structural characteristics of a unit membrane could not be resolved and the results of Lott and Vollmer's (1973b) recent freeze etch study on the cotyledons of *Cucurbita maxima* do not indicate the presence of a globoid membrane. However a heavy deposit of electron-dense material at the globoid interface often occurs after chemical fixation. Jones and Price (1970) have shown that in barley this electron-dense limiting envelope has a unique honeycomb-like appearance.

The globoids in *Welwitschia* are highly electron-dense and may be pitted. It is thought that the globoid serves as a localized phosphagen in the protein body (Abdul-Baki and Baker 1973) and also supplies potassium, magnesium and calcium for the synthesis of metabolites and of functional (e.g. co-enzymes) and structural constituents (Ching 1972). Pitting of the globoid probably represents part of the digestion process (Lott and Vollmer, 1973b. In *Welwitschia* after several days of germination the pitted areas increase in number and size and begin to fuse. Ultimately this internal pattern of digestion leads to the disintegration of the globoid into small fragments which may or may not remain enclosed within the apparent bounding envelope. Speed of globoid digestion is

highly variable in different gametophyte cells although there is a general tendency for globoids nearer the interface zone to degrade at a more rapid pace than their deeper-seated counterparts. In most instances globoids in actual interface cells are merely represented by remnants even in the dry state. This could perhaps be regarded as further evidence that digestion is a continuous process beginning before, and being resumed after, the period of quiescence (Rost 1972).

Apart from globoids, other osmiophilic crystalline inclusions are observed to aggregate within gametophyte protein bodies during the course of germination. Between days 3 and 6 small solid crystals are sometimes seen in the cytoplasm in contact with protein body membranes and within protein-carbohydrate reserves. Their distribution suggests crystal migration from the cytoplasm into the protein bodies. At present there is no conclusive evidence to indicate that these small, spherical electron-dense crystals are also globoids. If they are, then this would imply a cytoplasmic origin for globoids. Very little is known about the origin of globoids (Rest and Vaughan 1972). It is widely thought that they precipitate out within the vacuole (Frey-Wyssling and Mühlethaler 1965; Rest and Vaughan 1972). According to Rest and Vaughan (1972) globoids first appear within protein vacuoles of developing *Sinapis alba* seed as spherical bodies. The vacuole has long been regarded as a deposition site for waste products (Robards 1970) and it is possible that the crystals with a cytoplasmic origin in *Welwitschia* gametophyte might represent inorganic waste material e.g. calcium oxalate. However, at present there is no evidence to indicate the composition of these crystals.

The small, spherical, particulate crystals of uniform size (diam. ca. 0.3 μm) which are seen apparently embedded within protein-carbohydrate reserves as in Fig. 46 appear superficially similar to the spherical inclusions (diam. also ca. 0.3 μm) of some microbodies (Fig. 51). As sequestration of microbodies into protein bodies is of common occurrence (Figs. 52 - 54) it is postulated that this might account for the origin of these crystals. Using the Gomori reaction acid phosphatase was localised in the amorphous eccentric core of many microbodies (Fig. 117). Although this core is the region to which the spherical particulate inclusions are confined within the microbody, it was not possible to ascertain whether the crystal was responsible for the positive reaction because lead precipitation was too heavy. Further studies are needed to determine the composition of these crystals. Some crystals within the protein body markedly resemble starch grains e.g. Fig. 47. However they possess globoid characteristics in their high acid phosphatase activity (Figs. 113 - 115) and occurrence within internal cavities of the reserve material.

In *Welwitschia* the protein bodies are usually roughly spherical to ovoid in shape and are typically enclosed by a single unit membrane. In very deeply-situated dry gametophyte tissue they may possess an irregular outline which possibly develops as a result of close packaging of lipid and protein bodies. Deep gametophyte cells contain protein bodies which are larger

(diam. ca. 5 μm) and more plentiful than those in the interface zone (diam. ca. 1.2 μm). The granular texture of protein-carbohydrate reserves often appears to vary from one protein body to the next (Fig. 20). Some reserve matrices are so compact it is difficult to discern their granular nature (Figs. 42-45). This is especially true for the angular protein bodies found in deeply-situated cells (Fig. 41).

Each protein body probably contains a mixture of all kinds of proteins which were synthesised at the time of formation of the bodies. In the past some authors have suggested a plastid origin for protein bodies while others have suggested a vacuolar origin (Khoo and Wolf 1970). The present climate of opinion tends to favour the latter concept. It is commonly accepted that vacuoles arise from the ER (Dianty 1968). Whether protein bodies arise directly from the ER or within vacuoles which have arisen from the ER has not yet been satisfactorily settled (Rest and Vaughan 1972). The occasional interconnections seen between protein bodies in dry gametophyte tissue (Fig. 26) suggest that they are possibly derived from the ER.

Various patterns of protein body reserve breakdown have been reported. A common pattern entails the swelling and coalescence of protein bodies followed by internal fragmentation (Bagley *et al.*, 1963; Horner and Arnott, 1965; Smith and Flinn 1967; Briarty *et al.*, 1970; Swift and O'Brien 1972). Briarty *et al.*, (1970) report that in *Vicia faba* proteolysis occurs uniformly throughout the body and suggest that latent

enzymes formed during seed maturation are responsible. The protein matrix becomes dispersed, less dense and is finally lost. Dengler (1967) reports that protein bodies in the root of *Clarkia* embryos develop peripheral and internal cavities which gradually enlarge until a vacuole is all that remains. Horner and Arnott (1965) state that in *Yucca schidigera* initial peripheral erosion is subsequently followed by internal breakdown to form a network. According to Rost (1972) the digestion pattern of protein bodies in *Setaria lutescens* radicle is one of peripheral pitting. Following the formation of peripheral cavities, vesicles appear in the digested area. Rost suggests that this invaginated pattern of digestion, with concomitant vesicle formation is indicative of controlled release of digested products. Mollenhauer and Totten (1971) attribute vesicle formation in protein bodies of 48h-soaked corn radicles to fixation artifact. However, as pointed out by Rost (1972) 48h is enough time for digestion to take place and the vesicles observed by Mollenhauer and Totten (1971) could possibly be part of the natural digestion process. Vesicles have been reported to be associated with protein body digestion in *Fraxinus excelsior* (Villiers 1971) and have been seen accompanying globoid digestion in barley (Jones 1969a). Jones (1969b) also reports apparent fusion of ER-derived vesicles with the protein bodies prior to and during initial swelling. He suggests that these vesicles might contain a protease responsible for protein reserve hydrolysis.

In *Welwitschia* the granular texture of the protein-carbohydrate

matrix is not uniform in all protein bodies. Many are mottled with electron-transparent areas (Figs. 23, 26) suggesting that digestion is a continuous process begun before the seed becomes quiescent (Rost 1972). Upon hydration the comparatively small protein bodies in the interface zone swell and begin to degrade almost immediately. Rapid internal fragmentation results in the formation of irregular lumps of material which soon disappear. Larger deeper-seated gametophyte protein bodies are degraded more slowly. It is clearly evident that their rate of digestion is governed by their relative distance from the embryo. The more deeply they are situated, the more slowly they are degraded. This sequence of protein body degradation suggests a degradative mechanism controlled by the embryo. In deep gametophyte tissue the usual protein body digestion pattern is one of internal degradation. Small, digested pockets arise within the protein-carbohydrate deposits. As digestion proceeds these pockets gradually increase, enlarge and coalesce (Figs. 40, 43, 44). The indented shapes of some protein-carbohydrate reserves, e.g. Fig. 41, seem to imply initial peripheral digestion. It is suggested that, while some peripheral digestion is possible, most of the surface indentations are caused by appression of lipid bodies during the dehydrated quiescent state. On hydration the membrane is separated from the reserve deposits. In Fig. 42 the undulations of the protein body membrane can be seen to coincide with those of the reserves.

The numerous, apparently ER-derived vesicles (Fig. 58) seen

entering the protein bodies give a positive reaction for acid phosphatase (Fig. 110). They move towards the compact reserves, adhere to and enter them (Figs. 59, 60). Using the Gomori reaction (1952) modified by Berjak (1968) lead precipitation indicating acid phosphatase activity is soon localised within the pockets of the compact reserves (Fig. 111). At a slightly later stage the entire protein-carbohydrate reserves stain darkly indicating the diffusion of acid phosphatase, and possibly other hydrolytic enzymes, throughout the reserves. It is postulated that these vesicles might contain the hydrolytic enzymes responsible for reserve hydrolysis. According to Briarty *et al.*, (1970) peripheral breakdown would be expected if the hydrolytic enzymes are produced externally. However the fact that the vesicles actually enter the reserve deposits in *Welwitschia* (Fig. 59) might account for the internal fenestrated pattern of digestion commonly observed. It would therefore seem in *Welwitschia* that the enzymes responsible for reserve breakdown in deep gametophyte tissue are synthesised *de novo*. In contrast to this, the immediate and rapid degradation of smaller protein bodies in the interface zone appears to suggest the existence of latent hydrolytic enzymes laid down within the body prior to quiescence. Morris (1968) and Briarty *et al.*, (1970) report the presence of pre-existing proteases within the protein bodies of *Pisum sativum* and *Vicia faba*. Perhaps in *Welwitschia* more than one mechanism of breakdown operates. Clearly a great deal more information is needed to fully elucidate the operative degradative systems.

The classical concept that protein bodies are actually protein vacuoles is widely accepted and is consistent with observations on the germination of *Welwitschia mirabilis* seed. In *Welwitschia* reserves are broken down leaving a series of aqueous vacuoles. During and after dissolution of the reserves, the swollen protein bodies (or protein vacuoles) often coalesce. This swelling and coalescence is probably the result of an increase in the osmotic and imbibition pressure of the bodies due to hydrolysis of their contents (Briarty *et al.*, 1970).

A number of authors (Matile 1966, 1968a, b; Matile and Moore 1968; Poux 1965; Yatsu and Jacks 1968) have shown the presence of several hydrolytic enzymes within the protein bodies of various seed species. Matile (1968) refers to the protein bodies/vacuoles as lysosomes, likening them to the lysosomes of animal cells (Matile 1969). Acid phosphatase is an enzyme which has been commonly located in protein bodies of a large number of plant species (Villiers 1971; Poux 1965) as well as in lysosomes of animal cells (Matile 1968). In *Welwitschia* acid phosphatase apparently enters gametophyte protein bodies within ER-derived vesicles (Figs. 110,111). The presence of acid phosphatase within protein bodies would tend to explain the lysosomal activity of these bodies (Villiers 1971) as it is generally accepted that this enzyme acts on the phospholipid component of the membranes causing membrane breakdown. In *Welwitschia* as in other plant species, organelles are commonly seen to be sequestered within the protein vacuoles and organelles in various stages of degradation together with unidentified membranous fragments are a frequent sight within these vacuoles

(Figs. 52-54, 60, 62). It is thought that the vacuoles function in the removal of damaged and senescent organelles and in the recycling of essential substances (Villiers 1972). When breaks occur in vacuole membranes of senescent gametophyte cells, the hydrolytic enzymes are released and the cell contents are lysed. As pointed out by Matile (1968a) it is obvious that the vacuole fulfils a number of roles. Firstly it acts as a temporary storage site for reserve substances. Secondly it becomes lysosome-like and reserves are mobilised during germination and thirdly it is responsible for the degradation of damaged or non-functional organelles. Lastly it also acts as a site for waste product deposition. (Robards, 1970).

Calculated on a dry weight basis the unimbibed gametophyte contains approximately 22.6% protein. Upon imbibition the total protein content decreases by 9% during the first two days, followed by a further 5% between the 2- and 5-day-stages. The loss of electron-dense granular material from gametophyte protein body matrices is concurrent with the decrease in protein during germination. This decrease in protein is thought to represent the hydrolysis of proteins followed by the transport of amino acids and, possibly, simple peptides (Street and Cockburn 1972) to the embryo where they are rapidly consumed in its metabolism. Not all products of protein hydrolysis are translocated as some of the free amino acids and peptides are no doubt used as an energy source and/or utilized in the synthesis of structural proteins and enzymes in the gametophyte.

Analytic data on free amino acids in the gametophyte of dry seeds and seeds germinated for 2 and 5 days respectively are given in Table I. Nineteen free amino acids were identified. In addition an unknown peak was found partly overlapping aspartic acid, probably representing hydroxyproline. During the first two days of germination the total free amino acids decreased by approximately 52.4%. A portion of this decrease is probably related to *de novo* synthesis of enzymes (Van Staden *et al.*, 1975). In *Welwitschia* enzymes responsible for protein hydrolysis seem to pre-exist in the gametophyte interface zone but are apparently synthesised *de novo* in deep gametophyte tissue. In addition as lipid body degradation begins subsequent to protein hydrolysis, it is suggested that some of the free amino acids are utilised in the *de novo* synthesis of lipases (Van Staden *et al.*, 1975). The fact that the largest amount of lipid degradation takes place on the second day of imbibition (Table II), which coincides with decreased free amino acids (Table I), apparently supports this hypothesis.

The quantitative and qualitative fluctuations in amino acids between the 0- dand 5-day-stages (Table I) could suggest differential utilization (Ching 1972) or differential translocation of amino acids. Arginine is the dominant free amino acid constituting between 40 and 60% of the total free amino acids at any given time. With a few exceptions viz: glycine, ½ cysteine, methionine and glutamine the concentration of amino acids increases after five days germination.

Table I. Free amino acid and protein content of gametophyte tissue of germinating *Welwitschia mirabilis* seed.

Amino acid	Free amino acid content µg/gametophyte.			Protein content µg/gametophyte.		
	Day 0	Day 2	Day 5	Day 0	Day 2	Day 5
Lysine	50	trace	84	986	501	435
Histidine	20	trace	41	423	249	189
Ammonia	-	-	-	256	144	117
Arginine	677	480	925	2923	1405	1184
Aspartic acid	19	52	48	1988	1012	1027
Threonine	28	5	51	817	431	404
Serine	56	13	100	1111	587	492
Glutamic acid	61	44	108	4333	2228	1537
Proline	68	42	126	984	438	550
Glycine	29	11	29	885	491	408
Alanine	53	14	69	1044	551	523
½ Cysteine	28	trace	trace	168	114	164
Valine	25	8	78	1354	797	708
Methionine	20	trace	14	270	140	162
Isoleucine	14	4	39	822	427	456
Leucine	23	6	43	1351	719	653
Tyrosine	18	11	56	759	419	363
Phenylalanine	40	26	69	1105	574	550
Asparagine	153	25	314	-	-	-
Glutamine	219	19	142	-	-	-
Total	1602	762	2337	21576	11227	9921
Percentage (dry mass basis).	1.7	1.4	4.6	22.6	20.6	19.4

Lipid bodies

In the literature lipid bodies have been referred to by a number of different names viz: oil bodies (Rest and Vaughan 1972), fat bodies (Ching 1970), lipid bodies (Yoo 1970; Villiers 1971; Rost 1972; Swift and Buttrose 1973), lipid droplets (Paulson and Srivastava 1968; Hallam *et al.*, 1972; Schulz and Jensen 1973), lipid globules (Opik 1966), oil vacuoles (Nieuwdorp 1963; Lott and Vollmer 1973b), spherosomes (Nieuwdorp and Buys 1964; Paleg and Hyde 1964; Jacks *et al.*, 1967; Vigil 1970; Jacobsen *et al.*, 1971), intercellular network (Bagley *et al.*, 1963) and osmium-dense bodies (Klein and Ben-Shaul 1966). The term lipid body is used throughout this investigation.

Similar to many previously described lipid bodies, those of *Welwitschia* gametophyte are homogeneous in structure and are spherical and smooth with a thin peripheral electron-dense line. They are most compactly arranged in deep gametophyte tissue where they are in intimate contact with the plasma membrane and protein body membranes. A large proportion of the cell's volume is taken up by protein bodies, but almost the entire remainder of the cell is filled with lipid bodies. In the dry state, due to close packaging of reserve materials, lipid bodies are often seen in contact with the nuclear membrane. In gametophyte interface cells contiguous with the embryo lipid bodies are less abundant and are apparently loosely scattered with the exception of those bodies aligned along the cell wall. In cells adjacent to the interface cells lipid bodies display a more typical pattern of arrangement, and are seen in close apposition to both protein bodies and the plasmalemma. Yoo (1970) suggests that early close packaging of seed lipid bodies against cell walls could help

prevent loss of cell moisture. In *Welwitschia* gametophyte, similar to other seed tissues investigated, the peripheral lipid bodies tend to move inward upon cell activation. The lipid bodies which surround protein bodies however retain their distinct pattern of arrangement. According to Villiers (1971) a structural connection probably exists between the lipid and protein bodies. In comparison with deep gametophyte cells, interface cells have relatively scanty reserves. Protein bodies are few and small while most of the lipid bodies show a scattered dispersion. Interface cells give a superficial impression of being slightly less dehydrated than deeper cells. This is probably advantageous in that these cell can, (and do), start to function earlier.

The lipid bodies are of roughly similar dimensions (diam. 0.5 µm). Despite close packaging and compression producing a "honeycomb" effect (Fig. 33) the bodies tend to retain their discreteness, although occasionally they coalesce to form a lipid pool. Storage fungi (Anderson *et al.*, 1970) and aging (Villiers 1972) are known to enhance loss of integrity and promote confluence of lipid bodies.

The origin of lipid bodies remains controversial. Although most investigators describe these bodies as arising from the ER (Grieshaber 1964; Frey-Wyssling and Mühlethaler 1965; Balz 1966; Semadeni 1967; Schwarzenbach 1971; Werker and Vaughan 1974), other modes of origin have been suggested (Harwood *et al.*, 1971; Rest and Vaughan 1972). Rest and Vaughan (1972) working on the developing seed of *Sinapis alba* record that the lipid

bodies seem to arise directly from the cytoplasm. They found no evidence suggesting that these bodies arise from the ER. Harwood *et al.*, (1971), working on maturing *Ricinus communis* endosperm report particulate vacuole-like inclusions within developing lipid bodies. On finding that these lipid bodies contain enzymes necessary for triglyceride synthesis, they suggest that the inclusions are the site of this synthesis. As mature *Welwitschia mirabilis* seed was used in this investigation it was not possible to observe either the absence or presence of vacuole-like inclusions in developing lipid bodies. According to Frey-Wyssling and Mühlethaler (1965) lipid bodies arise from spherosomes which originate from the ER. They report that both spherosomes and lipid bodies are bounded by a unit membrane. Other investigators (Grieshaber 1964; Paleg and Hyde 1964; Abdul-Baki and Baker 1973; Werker and Vaughan 1974) have also reported the presence of a limiting membrane surrounding the lipid body. While Schwarzenbach (1971) agrees that lipid bodies arise from the ER, he suggests that oil synthesis takes place between the layers of the unit membrane to leave a mature lipid body surrounded solely by the outer protein layer of the original unit membrane. In *Welwitschia* gametophyte cells there is no evidence of a 3-layered unit membrane bounding the lipid bodies. Jacobsen *et al.*, (1971), Swift and Buttrose (1973), Rest and Vaughan (1972) and Harwood *et al.*, (1971) in *Hordeum vulgare*, *Pisum sativum*, *Sinapis alba* and *Ricinus communis* respectively report a similar inability to resolve the fine structural characteristics of a peripheral unit membrane. Harwood *et al.*, (1971) and Jacobsen *et al.*,(1971) ascribe the thin peripheral electron-dense line

surrounding the lipid body to boundary staining artifact. Rest and Vaughan (1972) postulate that the sharp interface is formed by a monomolecular layer of phospho-lipid arranged in such a manner as to prevent confluence of adjacent bodies. Schwarzenbach's (1971) theory that lipid bodies are surrounded by a ½-unit membrane is favoured in the present investigation. Werker and Vaughan (1974) working on germinating *S. alba* seed report that the lipid bodies are surrounded by a membrane to which ribosomes are attached. They suggest that this is evidence that lipid bodies develop from the ER. Although in *Welwitschia* gametophyte few ribosomes are visible in the dry state due to the obscuring effect of reserve materials, some are seen in contact with lipid bodies (Fig. 22) (cf. lipid bodies of dry collar). However no encompassing membrane is observed. It is interesting to note that Yoo (1970) has shown in *Pisum sativum* radicles that short segments of smooth ER sometimes remain attached to lipid bodies. He postulates that lipid bodies supply lipids to the membrane systems and are especially involved in ER formation.

Upon hydration of the gametophyte, protein body degradation commences first and is shortly followed by lipid digestion. The pattern of lipid digestion in gametophyte tissue is similar to that of protein bodies in that lipid reserves are consumed rapidly in interface cells but slowly in deeper-seated cells. That is, once again there appears to be a gradation in activity away from the embryo.

Apparently reserve triglycerides stored in lipid bodies are

first hydrolysed *in situ* into free fatty acids and glycerol by the action of lipases (Ching 1968; Ory *et al.*, 1968; Ory 1969). It has been demonstrated that a certain class of microbodies, subsequently named glyoxysomes (Breidenbach *et al.*, 1968), contain the enzymes for both β-oxidation of fatty acids and the glyoxylate bypass which converts acetyl CoA units to succinate (Breidenbach and Beevers 1967). Succinate is converted by mitochondria via malate and oxalacetate to phosphoenolypyruvate and eventually to sucrose (Canvin and Beevers 1961; Beevers 1961; Cooper and Beevers 1969a, b).

In *Welwitschia* gametophyte microbodies assumed to be glyoxysomes, are frequently seen in intimate association with lipid bodies. In dry tissue microbodies are present, but of rare occurrence. Upon imbibition they increase in number dramatically by means of fission and are already fairly numerous between days 2 and 3 by which time rapid lipid degradation is under way. These observations on *Welwitschia* gametophyte microbodies are in agreement with those obtained by other authors where rapid depletion of stored lipids is parallelled by an increase in number and size of microbodies (Ching 1970; Gerhardt and Beevers 1970; Vigil 1970; Lopez-Perez *et al.*, 1974). According to some authors (Gerhardt and Beevers 1970; Vigil 1970) a concomitant decrease in microbody (glyoxysome) numbers occurs as lipid stores decline toward the end of the germination period. In deeply-situated cells of *Welwitschia* gametophyte, microbodies increase exponentially until approximately day 4 after which their rate of increase slows down and a plateau is reached. In interface gametophyte cells the

plateau is reached much sooner i.e. either on day 2 or 3. Although there is no perceptible decline in numbers many microbodies develop cup-shaped depressions during late germination which could be interpreted as an indication of senescence or quiescence (Yeoman and Street 1973). The sequestration of microbodies within protein vacuoles is of common occurrence throughout the germination period and probably accounts for the removal of damaged and/or senescent microbodies. During lipid degradation a number of organelles develop a close spatial relationship with lipid bodies. Microbodies (glyoxysomes) are intimately associated with the lipid bodies while mitochondria and rough ER - surrounded starch-laden amyloplasts are common close neighbours. The lipid bodies, microbodies, 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 probably converted by microbodies (glyoxysomes) to succinate (Breidenbach and Beevers 1969) which is in turn converted to sucrose by the action of mitochondria (Beevers 1961; Canvin and Beevers 1961; Cooper and Beevers 1969a,b). While some of the sucrose is no doubt utilised as a source of energy within the cell, it is believed that the bulk of the sucrose is transported to and absorbed by the embryo, via the feeder. Excess sucrose is probably converted to starch and stored temporarily in the amyloplasts.

In animals fatty acids are oxidised in mitochondria (Ching 1972) while in plants the enzymes responsible for β-oxidation of fatty acids are apparently exclusively associated with

glyoxysomes (Cooper and Beevers 1969a,b; Hutton and Stumpf 1969). In flax seeds α-oxidation of fatty acids occurs in addition to β-oxidation (Ching 1972) and it is thought that the enzymes involved in this α-oxidation are located in the mitochondria and supernatant (Stumpf1969; Ching 1972). Mitochondria frequently occur in very close association with lipid bodies in *Welwitschia* gametophyte, especially in the interface zone (Fig. 109). This intimate association (Fig. 109) could suggest that perhaps both α-and β-oxidation systems may be operative in *Welwitschia* as well. However biochemical studies are needed to substantiate this speculation.

Migration of lipid bodies into protein vacuoles is a frequently observed phenomenon in *Welwitschia* gametophyte tissue (Figs. 63-66, 105). Microbodies too are often seen within the protein vacuoles. Although the simultaneous presence of lipid bodies and microbodies within the same vacuole has not yet been observed, it might be possible that a certain amount of lipid digestion could occur in the vacuole. Vigil (1970), working on castor bean endosperm showed cytochemically that microbodies sequestered in vacuoles still contain a very active catalase. In other words there was no loss of enzyme activity at least prior to digestion. Most of the lipid bodies are seen entering protein vacuoles whose reserves have been dispersed, but a large number can also be seen entering vacuoles containing compact reserves. Osmiophilic patches form in the protein-carbohydrate reserves at any point of contact with lipid bodies. This is suggestive of a possible biochemical interaction.

TABLE II. Fatty acid content and composition of gametophyte tissue of germinating *Welwitschia mirabilis* seed. Results expressed as g/100g.

Day		0	2	5
Diethyl ether extract - Total lipid		27.7	11.6	6.5
Saturated				
Code	Fatty acid			
16:0	Palmitic	2.3	0.8	0.5
18:0	Stearic	0.5	0.3	0.1
Unsaturated				
Code	Fatty acid			
18:1	Oleic	1.7	0.7	3.7
18:2	Linoleic	1.3	0.5	0.3
18:3	Linolenic	6.6	2.6	1.6

Traces of palmitoleic and another unidentified fatty acid were also noted in all three samples.

For a desert plant, the gametophyte of *Welwitschia mirabilis* seed has an inordinately high lipid content (27.7% - see Table II). During germination distinct changes occur in the lipid content of the gametophyte (Table II). Within 5 days the extractable lipid decreases from ca. 27.7% in the unimbibed state to ca. 6.5% just prior to plumule emergence. The total amount of lipid decreases by approximately 58% during the first 48h. As lipid degradation begins subsequent to protein body reserve hydrolysis, most of the lipid appears to degrade on

the second day of imbibition. After day 2 the rate of lipid breakdown begins to diminish with only a further 18.5% broken down in the following 3 days. The appearance and gradual accumulation of starch accompanies lipid exhaustion.

Although there are fluctuations in different types of free fatty acids in the gametophyte from 0 to 5 days, the total amount present at any given time seems to account for ca. 44% of the total lipids present. In many higher plants oleic and palmitic acids are the most common fatty acids (Stumpf 1969). But similar to flax seeds (Ching 1972) the dominant fatty acid in *Welwitschia* gametophyte is linolenic acid (Table II). Palmitic, stearic, oleic and linoleic fatty acids are present in smaller amounts. The major reserve component, linolenic acid, appears to be preferentially utilised in *Welwitschia* gametophyte tissue (Table II) followed by palmitic, stearic and linoleic acid to lesser extents. Oleic acid, after showing an initial decline accumulates after the second day. Some of the hydrolysed fatty acids are no doubt used in the synthesis of phospholipids and glycolipids necessary for organelle formation (Ching, 1972) but it is felt that the majority are probably converted to sugars, a large proportion of which is transported to and absorbed by the embryo for seedling growth.

In 1968 Matile and Spichiger suggested that spherosomes or lipid bodies are similar to lysosomes in animal cells as they were found to contain similar hydrolytic enzymes including RNase and DNase. Vigil in 1970 speculated that their preparations were contaminated with other organelles. However,

the presence of lipase in lipid bodies has been well established (Ching 1968; Ory *et al.*, 1968; Ory 1969). 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 lipid bodies. However the lipid bodies in *Welwitschia* gametophyte showed no acid phosphatase activity when incubated in Gomori medium (1952) modified by Berjak (1968).

Amyloplasts

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 prominent 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 (Meyer and Anderson 1952), and it is suggested that the starch is used during the initial burst of cellular activity in this zone following imbibition. While the interface and neighbouring cells rapidly achieve intense metabolic activity, deeper-seated cells have a much slower metabolism. Within 2 to 3 days interface zone starch reserves are replaced as lipid is hydrolysed. Large starch reserves are rebuilt, often in the form of numerous small, closely-packed grains as opposed to several large grains. In deeply-situated cells amyloplasts gradually increase in number and starch reserves progressively accumulate as lipid is degraded. Starch-laden amyloplasts, particularly in the interface zone, become completely

surrounded by layers of rough ER and frequently develop close spatial relationships with mitochondria, microbodies and degrading lipid bodies.

Microbodies

Few microbodies are discernible in unimbibed gametophyte tissue. Following hydration they increase conspicuously in size and number and develop extremely close spatial relationships with lipid bodies. While not identified biochemically, their frequent apposition to lipid bodies coupled with a parallel increase as lipid stores decline suggests that the majority might belong to the class of microbodies called glyoxysomes by Breidenbach *et al.*, in 1968. As mentioned previously glyoxysomes contain the enzymes for β-oxidation of fatty acids and the glyoxylate cycle which converts acetyl CoA units to succinate (Breidenbach and Beevers 1967). The observed presence of mitochondria and starch-rich amyloplasts in the immediate proximity of juxtaposed lipid and microbodies indicates the possible mitochondrial conversion of succinate to sucrose (Beevers 1961; Canvin and Beevers 1961; Cooper and Beevers 1969a,b) and the storage of excess sucrose in the form of starch. Some authors (Gerhardt and Beevers 1970; Vigil 1970) report a drop in microbody population as lipid stores are depleted. In *Welwitschia* gametophyte no such decline is perceptible. After an initial exponential phase lasting for several days, a plateau is reached. The microbodies commonly develop cup-shaped depressions, especially during late germination, which in many instances form directly opposite a lipid body e.g. Figs. 103, 104. These invaginations possibly

indicate intense activity between lipid and microbody as a result of an increased surface area through which substances (e.g. fatty acids) can pass. However the possibility exists that the indented shapes might be an expression of senescence or quiescence (Yeoman and Street 1973).

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 may bud directly from the ER. No continuities between ER and microbodies were observed in the present study, although failure to find them does not necessarily imply their absence. In the main, microbody increase in *Welwitschia* gametophyte seems to be accomplished by means of fission (Fig. 99).

In the dry state gametophyte microbodies do not appear to possess the electron-dense eccentric cores called nucleoids by Vigil (1970). Upon hydration amorphous dense nucleoids become apparent within 24h and are invariably in contact with the bounding membrane of the microbody. Prior to fission nucleoids contained within microbodies seem to divide to provide each daughter microbody with a nucleoid. In view of previous reports of the presence of microbody RNA (Gerhardt and Beevers 1969; Ching 1970), the small particles occasionally seen within nucleoids (Fig. 51) may be ribosomal. Ching (1970) suggests that pre-existing microbodies in dry tissue contain stable mRNA's and ribosomes which synthesize functional enzymes when free amino acids, coenzymes and cofactors are made available during germination.

As microbodies are in extremely short supply in dry gametophyte tissue it seems evident that the bulk if not all of the glyoxysome enzymes are in fact synthesised *de novo*. Upon hydration microbodies increase in number as lipid reserves degrade.

Apparently some microbodies, termed peroxisomes (Tolbert *et al.*, 1968), contain enzymes involved in photorespiration. Vigil (1970) suggests that further biochemical studies may indicate other microbodies with specialised enzyme compositions and further postulates that the presence of inclusions may indicate organelle "differentiation". Some *Welwitschia* gametophyte microbodies contain a small spherical particulate inclusion within the nucleoid (Fig. 51) which superficially resembles certain particulate crystals of the same size (diameter ca. 0.3 µm) seen embedded within protein-carbohydrate reserves (Fig. 46). Since sequestration of microbodies within protein bodies is of common occurrence it is suggested that this could account for the origin of these protein body crystals.

Welwitschia microbody crystals differ from those described for castor bean (Vigil, 1970) which consist of dense rods arranged in a cross-band pattern. In *Welwitschia* the particulate crystals do not exhibit a latticed structure. Catalase is apparently incorporated in the microbody crystals of castor bean (Vigil 1970). Localization of catalase activity using diaminobenzidine stain (Novikoff and Goldfischer 1968) was not carried out on *Welwitschia* gametophyte tissue. However, using the Gomori reaction (1952) modified by Berjak (1968) acid phosphatase activity was frequently localised in the

nucleoids of *Welwitschia* gametophyte microbodies. Owing to very heavy lead precipitation it was impossible to determine whether the crystal was responsible for the positive staining reaction. Many crystalline inclusions within *Welwitschia* protein bodies also stain positively indicating acid phosphatase.

Mitochondria

In dry tissue mitochondria are more numerous in the interface zone than in deeper gametophyte cells. Perhaps their relative abundance in this zone is influenced by the proximity of the embryo. As previously suggested it seems likely that a stimulatory factor from the embryo initiates or enhances metabolic activity in the gametophyte with interface cells becoming more rapidly and highly activated than deeper cells.

Within 24h of imbibition, many interface mitochondria are greatly swollen. Although the enlargement appears to be a pathological condition the swelling might be a natural process preceding division of the organelle by fission or partitioning (Schulz and Jensen 1973). The arrowed mitochondria in Fig. 73, inset, could represent the division of an oversized mitochondrion. At the same time, it is possible that these organelles are fusing to give rise to a larger mitochondrion. Mitochondria proliferate and are soon ubiquitously distributed with many becoming especially associated with lipid bodies, microbodies, and amyloplasts as previously discussed.

Dictyosomes

Dictyosomes are of rare occurrence within gametophyte tissue

and do not seem to be closely associated with cell walls. The fact that no cell division occurs within the mature gametophyte might explain their sparseness. Although one would have expected dictyosomes to be involved in the dramatic thickening of outer interface walls in contact with the feeder, this does not seem to be the case.

ER

In dry gametophyte tissue ER is fragmentary in the interface zone, but with hydration undergoes an immediate and notable increase. This increase parallels the depletion of the small interface protein bodies whose reserves are no doubt partially utilised in ER formation. The prompt degradation of the contents of these interface protein bodies seems to suggest the presence of latent hydrolytic enzymes within them. The subsequent proliferation of ER and formation of polysomes are thought to reflect the increased synthesis of structural proteins and enzymes The ER develops extensively and becomes closely associated with amyloplasts, mitochondria, nuclei and the plasmalemma. Starch-rich amyloplasts become completely surrounded by numerous concentric layers of rough ER and mitochondria are often enclosed by one or two layers. The close spatial relationship between the ER and these organelles may signify a role for it either in the transport (Villiers 1971) or temporary storage of materials. It is also possible that increased ER is an indication of oxygen deficiency (Mercer and Rathgeber 1962). Using *Torulopsis* cultures Linnane, Vitols and Nowland, (1962) demonstrated that cells maintained in anaerobic conditions were rich in ER in which enzymes usually found in mitochondria were located. If increased ER in *Welwitschia* gametophyte is as a

result of partial anaerobiosis it no doubt plays a role in energy relations.

ER production in deep gametophyte tissue is not as rapid as in the interface zone but, after several days, soon becomes profuse. The ER proliferates large numbers of small vesicles, many of which are seen in contact with protein body membranes (Fig. 58, inset). The vesicles apparently pass into the protein bodies (Fig. 58) and may penetrate the reserves (Figs. 59, 60). Acid phosphatase has been localised in these vesicles and, as discussed previously, it is postulated that these vesicles might also contain other hydrolitic enzymes responsible for reserve hydrolysis, i.e. in contrast to the interface zone, enzymes degrading protein body reserves in the deep gametophyte appear to be produced *de novo*.

Inflation of the ER becomes commonplace in interface zone cells. The inflations are localised giving rise to large numbers of mini-vacuoles in limited areas of the cytoplasm. In most cases the dilated ER tends to surround the larger cell vacuole formed by protein vacuole confluence. Short segments of ER seen issuing from many mini-vacuoles confirms the ER origin of these dilations. Although deposition of protein-carbohydrate reserves was not observed within these small dilations their behaviour tends to corroborate the classical concept that protein bodies arise from ER. Similar to protein vacuoles mini-vacuoles indulge in autophagic activity and also accumulate crystals. These crystals are of cytoplasmic origin and could possibly represent waste material.

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 apparently dispersed in the cytoplasm (Fig. 83, arrow) seems to accumulate before being selectively engulfed by mini-vacuoles (Figs. 83 - 85). 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. Dictyosomes are rare and are not closely associated with the cell walls although those observed did produce vesicles with fibrillar contents (Fig. 93). The origin of cytoplasmic fibrils is unknown. Although Fig. 93 suggests that fibrils are released upon the rupture of dictyosome vesicles, this seems very unlikely as dictyosomes are extremely sparse.

Cell Walls

Part of the reserves of the gametophyte include the hemicelluloses which form the cell walls, and it is thought that besides the addition of mini-vacuole contents to the outer interface cell walls, it is possible that 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 *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, it is possible that the hydrolysed channels facilitate the diffusion of substances through the wall.

The densely-staining material that accumulates between gametophyte and feeder cells is most likely pectinaceous. Apparently secreted from both the gametophyte and the feeder, it probably functions as an adhesive between the two structures. Unlike 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.

FEEDER.

INTRODUCTION

Once germination is initiated the collar forms a wedge-shaped feeder (Bower 1881a) whose ventral surface becomes firmly anchored to the gametophyte tissue (Figs. 16, 18). The development of this structure was followed over a period of 6 days (Figs. 9-17).

RESULTS

DAY ZERO

Unimbibed collar cells are thin-walled (ca. 0,2 µm) and roughly isodiametric in shape (ca. 24 x 22 µm). Their reserve materials are scanty in comparison with gametophyte cells and, as a result, the cytoplasm is much in evidence. There is also greater clarity of membranes.

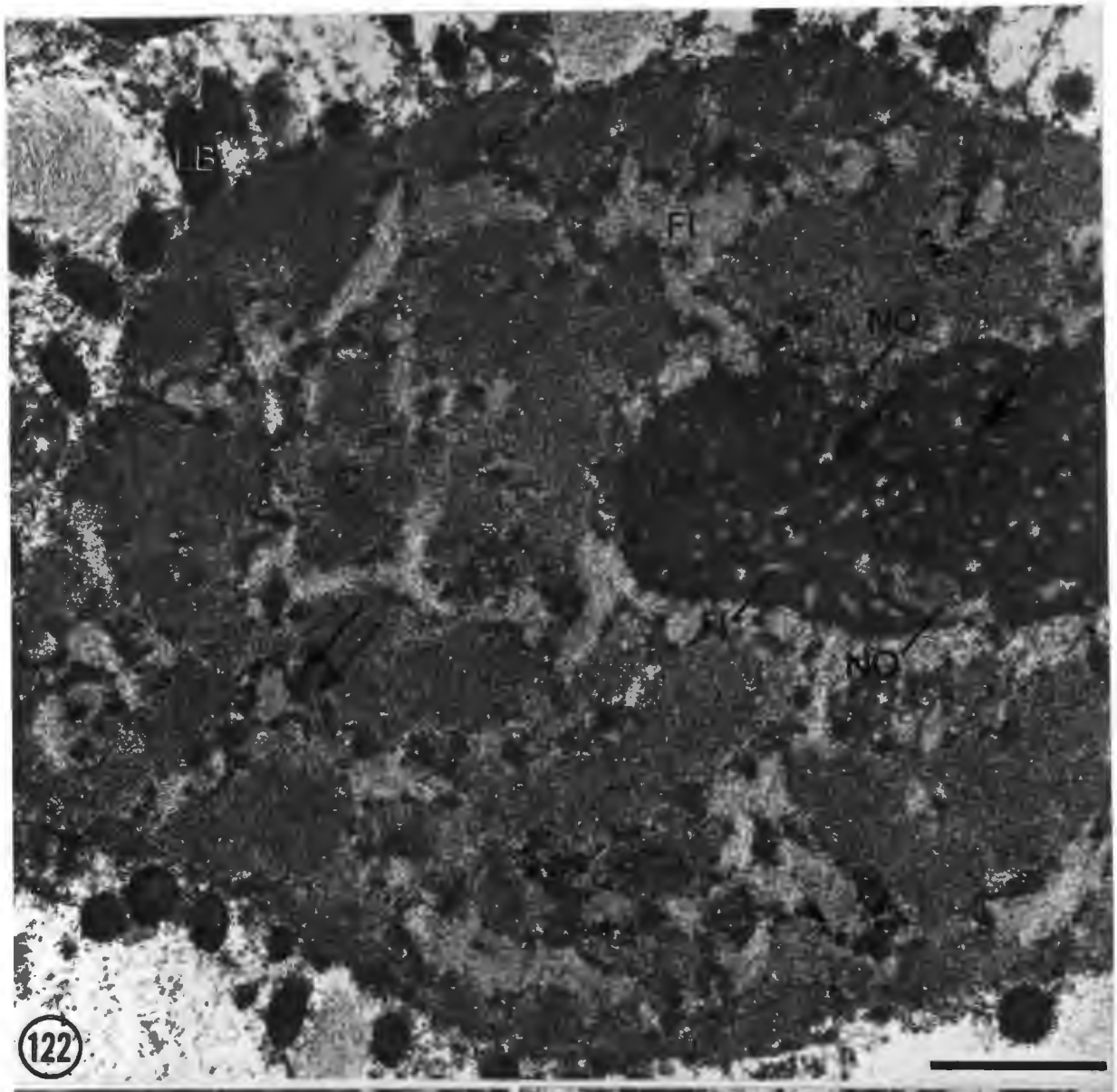
Nucleus

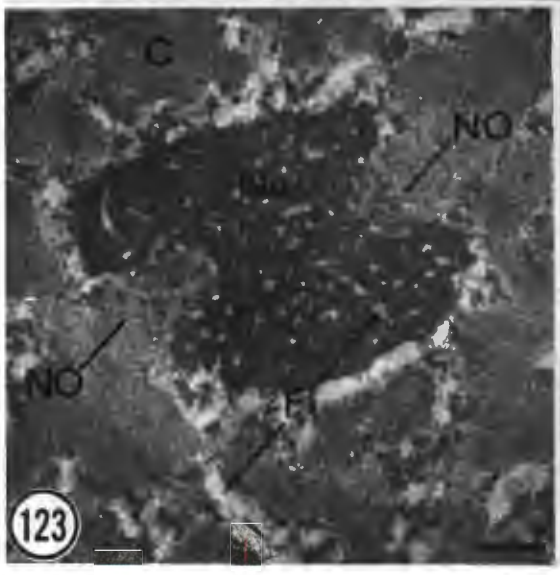
Figure 122 shows a nucleus typical of unimbibed collar cells. They are spherical to ovoid in shape and present a much clearer fixation image than the polymorphic nuclei of the dry gametophyte tissue. Apart from shape and clarity the ultrastructural features of dry collar and gametophyte nuclei are basically similar. Fibrous inclusions pervade the nucleoplasm and nucleolar vacuoles (Figs. 122-124). Nucleolar material is dense and appears to be composed almost entirely of closely-

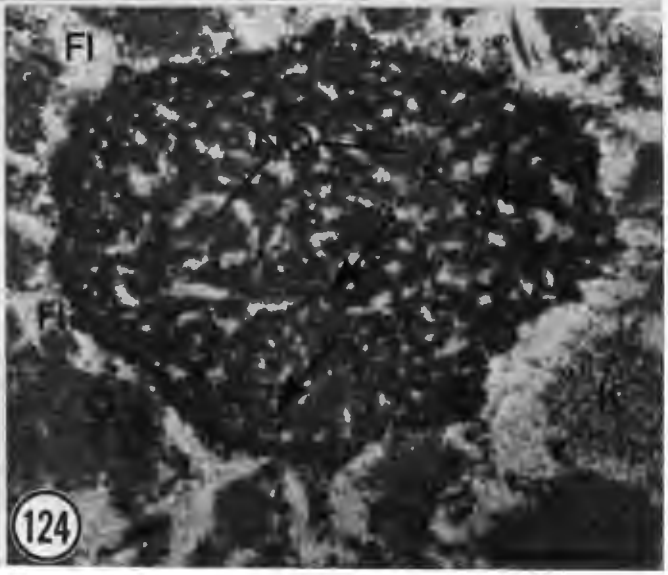
PLATE 31.

Figs. 122-124. Nuclei of unimbibed collar cells.—Fig. 122. Dry nucleus containing fibrous inclusions (FI) which pervade nucleoplasm and nucleolar vacuoles. Nucleolar (Nu) material appears dense with no noticeable granular zone, although large scattered granules (arrows) are apparent. Note chromosomal nucleolar organizing regions (NO) embedded in quiescent nucleolus. Electron-dense granular material frequently surrounds nucleoplasmic fibrous inclusions (double arrow). Cytoplasmic fibrous inclusions strongly resemble nuclear inclusions. C = chromatin; LB = lipid body; NE = nuclear envelope. —Fig. 123. Chromosomal nucleolar organizing regions (NO) embedded in quiescent nucleolus (Nu). C = chromatin; FI = fibrous inclusions. —Fig. 124. Nucleolus (Nu) and karyosome (K) in dry, quiescent nucleus. Note chromosomal nucleolar organizing regions (NO) embedded in nucleolus and large scattered dense granules (arrows). C = chromatin; FI = fibrous inclusions.

Bars represent 1 μm (Figs. 122-124).


LG
Fl
NO
NO
122


C
NO
NO
Fl
123


Fl
Fl
124

packed fibrils with no noticeable granular zone. However scattered large dense granules are apparent (Fig. 124). Chromosomal nucleolar organizing regions (Swift 1959; Jordan 1971) are commonly seen embedded in these quiescent nucleoli (Figs. 122, 123, 124). Nucleolar satellites or karyosomes (Hyde 1967) as seen in Fig. 124 consist of relatively lightly-staining granules.

The nucleoplasm presents a striking appearance with fibrous inclusions, frequently surrounded by electron-dense granular material, taking up all available space not occupied by condensed chromatin (Fig. 122).

Cytoplasmic crystals

An interesting feature of the cytoplasm is the presence of scattered, crystal-like aggregates (Fig. 125) which strongly resemble the nuclear and nucleolar fibrous inclusions (Fig. 122). They occur free, unbound by membranes and do not appear to be orientated in any special way. They vary in shape and size. Most aggregates appear roughly circular in transection (Fig. 127) but may be short and fat, long and spindle-like or serpentine in longisection (Figs. 126-129). They occur throughout the cytoplasm but are primarily concentrated in the region immediately surrounding the nucleus (Figs. 122, 126). Less commonly they are seen associated with lipid bodies (Fig. 126), protein bodies (Fig. 129), plastids and mitochondria (Fig. 130).

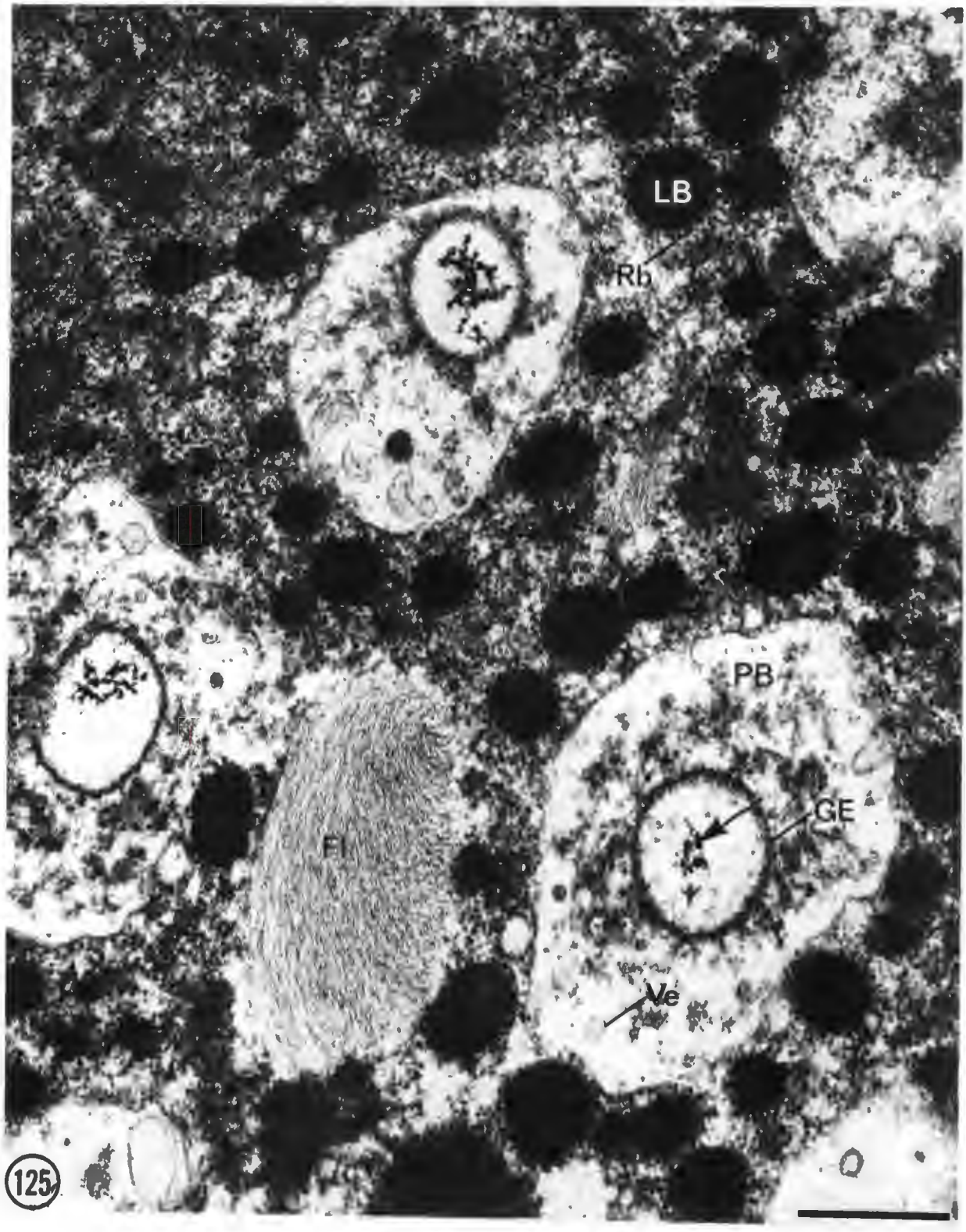
Protein bodies

The protein bodies (diam. ca. 1,8 µm) of Fig. 125 are fairly typical of dry collar cells. Reserves are meagre and vesicles

PLATE 32.

Fig. 125. High magnification of portion of dry collar cell. Cytoplasmic fibrous inclusions (FI) are morphologically similar to nuclear inclusions and occur free and unbound by membranes. Protein body (PB) reserves are meagre, but vesicles (Ve) and globoid inclusions are common. Note globoid remnants (arrow) surrounded by globoid envelope (GE). The lipid bodies (LB) are not found in close apposition to protein body membranes. They are darkly staining and their entire surface appears to be covered with ribosomes (Rb). The cytoplasm is studded with ribosomes.

Bar represents 0.5 μm (Fig. 125).



125

and globoid inclusions common. Some globoids are rounded and electron-dense but the majority are represented by fragments surrounded by a globoid envelope (Fig. 125).

Lipid bodies

Lipid bodies, unlike those of the gametophyte, stain very darkly (Fig. 125, diam. ca. 0,3 µm). They are relatively few, appearing in loose arrangements around protein bodies and not in close apposition to their membranes (Fig. 125). They also line the plasmalemma and may be found in close proximity to the nuclear envelope (Fig. 122). On close inspection ribosomes are seen attached to the periphery of the lipid bodies (Figs. 125, 132) apparently covering the entire surface.

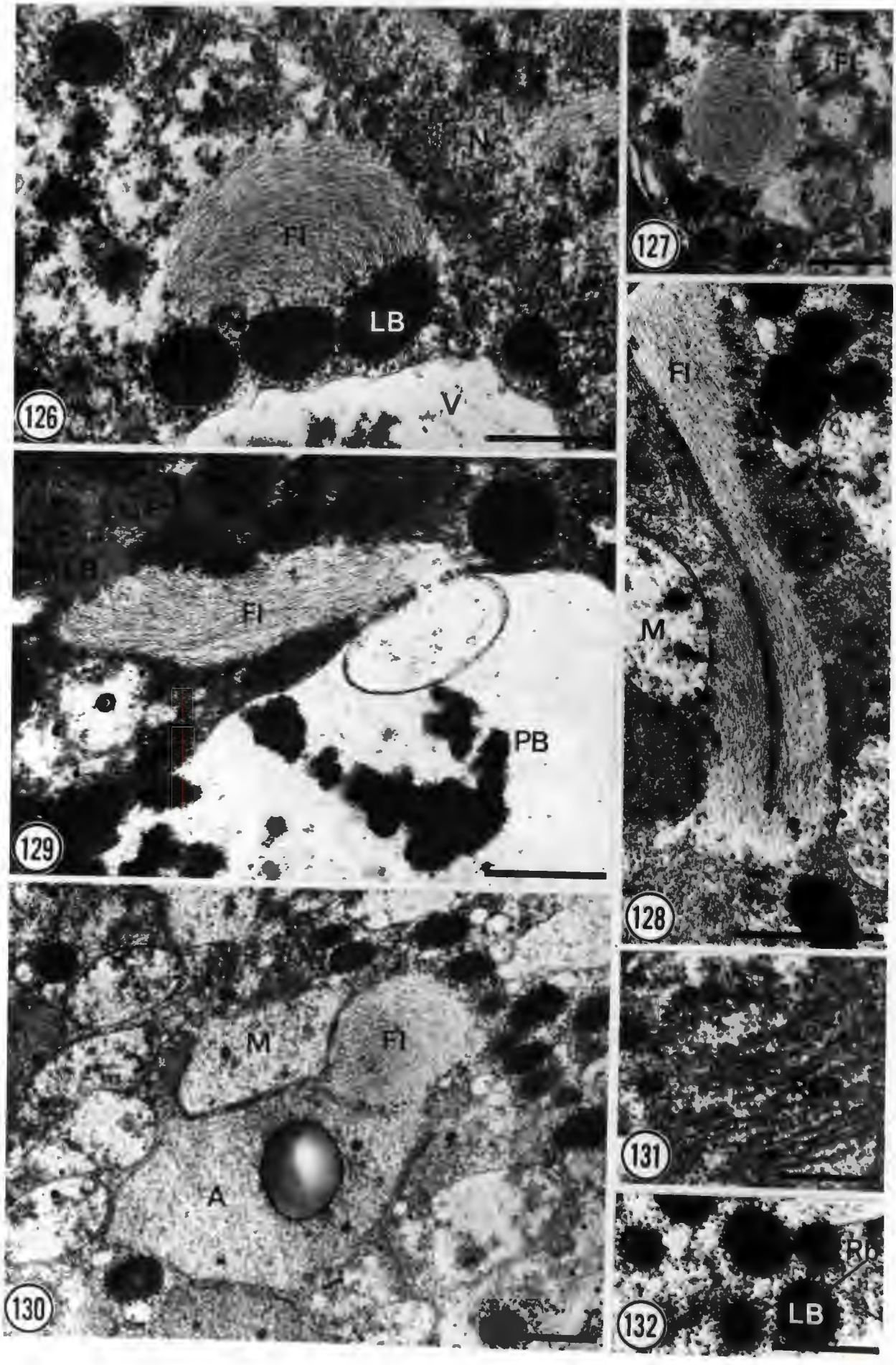
Organelles in residual cytoplasm

Amyloplasts are fairly common. Apparently free of lamellae, they usually contain a single starch grain and osmiophilic granules as in Fig. 130. Mitochondria (Fig. 130) are practically devoid of cristae and are randomly distributed in the cytoplasm. Their matrix, in which osmiophilic granules, fibrils and what appear to be ribosomes can be distinguished, is very electron-transparent. Microbodies are rare and no dictyosomes are observed. In contrast to dry gametophyte tissue, ER is evident in dry collar cells (Fig. 131). However its occurrence is sparse and usually appears as short segments of rough ER which are often arranged in stacks as shown in Fig. 131. In Fig. 125 the cytoplasm is seen to be richly studded with ribosomes which also occur attached to lipid bodies and infrequent short segments of ER (Fig. 131). No polysomes are observed.

PLATE 33.

Figs. 126-132. Dry collar cells.—Fig. 126. L.S. of cytoplasmic fibrous inclusion (FI) associated with nucleus (N) and lipid bodies (LB). V = vacuole.—Fig. 127. Cytoplasmic fibrous inclusion (FI) appears circular in transection.—Fig. 128. L.S. serpentine cytoplasmic fibrous inclusion (FI) associated with mitochondria (M).—Fig. 129. L.S. spindle-shaped cytoplasmic fibrous inclusion (FI) associated with lipid (LB) and protein bodies (PB).—Fig. 130. Transection through cytoplasmic fibrous inclusion (FI) associated with amyloplast (A) and mitochondrion (M).—Fig. 131. Stacked, short segments of rough ER (RER) are frequently evident in dry collar cells.—Fig. 132. Ribosomes (Rb) commonly occur attached to lipid bodies (LB).

Bars represent 0.5 μm (Fig. 126-132).


N
Fl
LB
V
126
Fl
127
Fl
M
128
LB
Fl
PB
129
M
Fl
A
130
131
Rb
LB
132

DAY 1

Figure 133 is a deeply-situated collar cell imbibed for 24 h. Figure 134 is a cell situated nearer the collar surface which has been imbibed for the same length of time. It is immediately apparent that the outer collar cells are at a more advanced stage than the inner cells as there is greater clarity, membranes are more distinct and cell contents reflect greater metabolic activity.

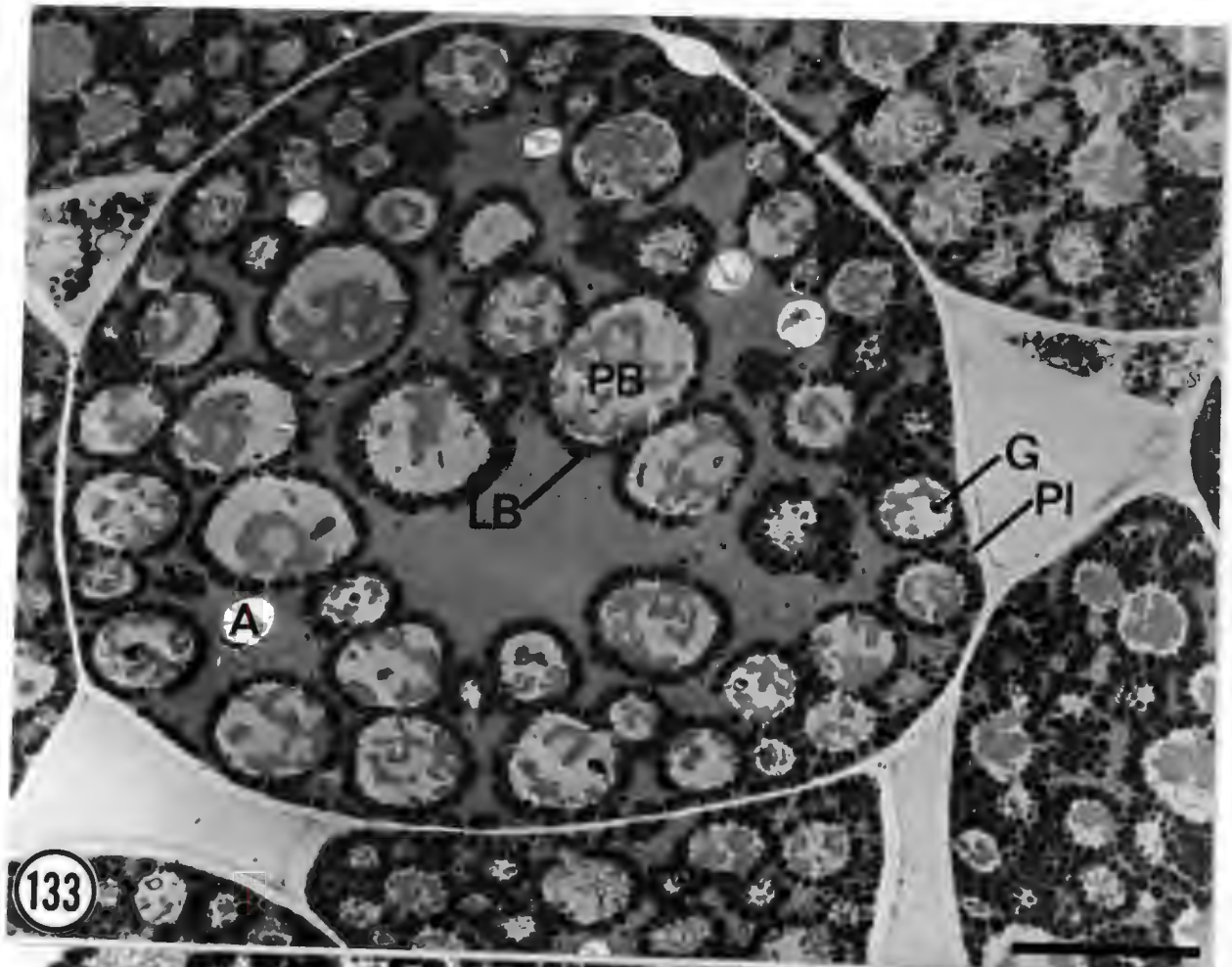
On hydration nucleolar and nucleoplasmic crystals disperse rapidly and in Fig. 134 have disappeared completely. Vacuolate nucleoli lose their compactness and now appear less electron-dense, especially in the developing outer granular zone. Chromosomal material thought to represent nucleolar organizing regions (Jordan 1971) of nucleolar chromosomes become deeply embedded in the inner fibrillar zone (Fig. 134, arrows). The granular karyosome remains apparent and the nucleoplasm retains some scattered condensed chromatin.

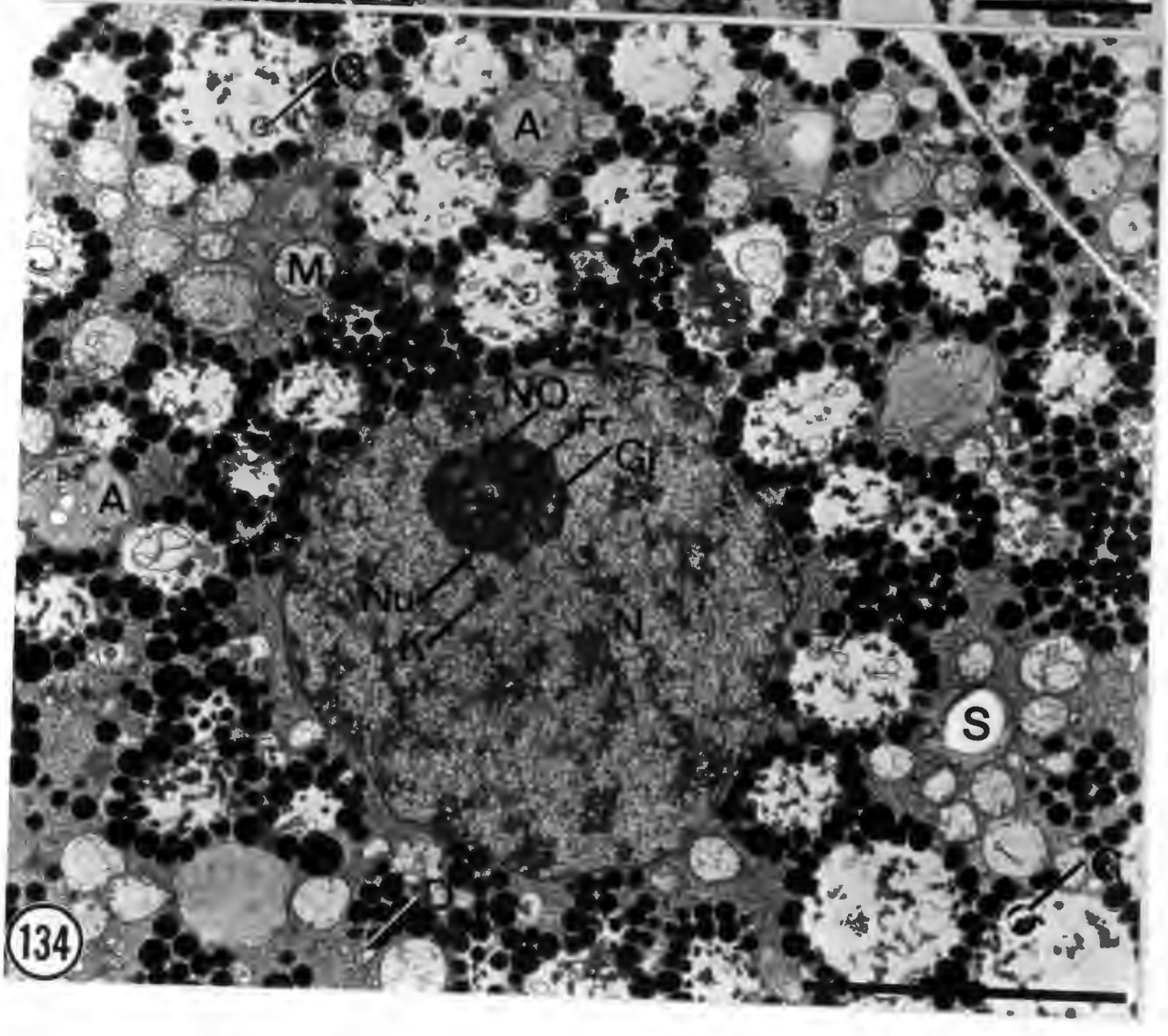
Electron-dense lipid bodies now lie closely apposed to plasmalemma and protein body membranes (Figs. 133, 134). The lipid bodies are now free of ribosomes. Protein body reserves are scanty in comparison with those of gametophyte cells. Their digestion has already begun in Fig. 133(inner collar cells) and is accompanied by protein body fusion (Fig. 133, arrow). In Fig. 134 (outer collar cell) protein-carbohydrate digestion has progressed even further. Solid and particulate globoids remain plentiful, often occurring in protein bodies practically

PLATE 34.

Figs. 133,134. Collar cells imbibed for 24 h.—Fig. 133. Deeply situated collar cell. Electron-dense lipid bodies (LB) lie closely apposed to plasmalemma (Pl) and protein body (PB) membranes. Digestion of protein body contents has begun, and is accompanied by protein body fusion (arrow). Solid and particulate globoids (G) remain plentiful. Starch-laden amyloplasts (A) are common.—Fig. 134. Cell situated nearer collar surface showing greater metabolic activity than deeper seated cells e.g. Fig. 133. Fibrous nuclear and cytoplasmic inclusions have dispersed and dissappeared. Vacuolate nucleoli (Nu) have lost compactness especially in developing outer granular zone (Gr). Nucleolar organizer (NO) becomes deeply embedded in inner fibrillar zone (Fr). Protein-carbohydrate digestion has progressed and the many remaining solid and particulate globoids (G) often occur in protein bodies practically devoid of contents. Starch (S) reserves have declined rapidly and most amyloplasts (A) are bare of starch. D = dictyosome; K = karyosome; M = mitochondrion; N = nucleus.

Bars represent 5 μm (Figs. 133, 134).


PB
LB
G
Pl
A
133


A
M
NO
Fr
Gl
A
Nu
K
N
S
134

devoid of contents (Figs. 134, 135).

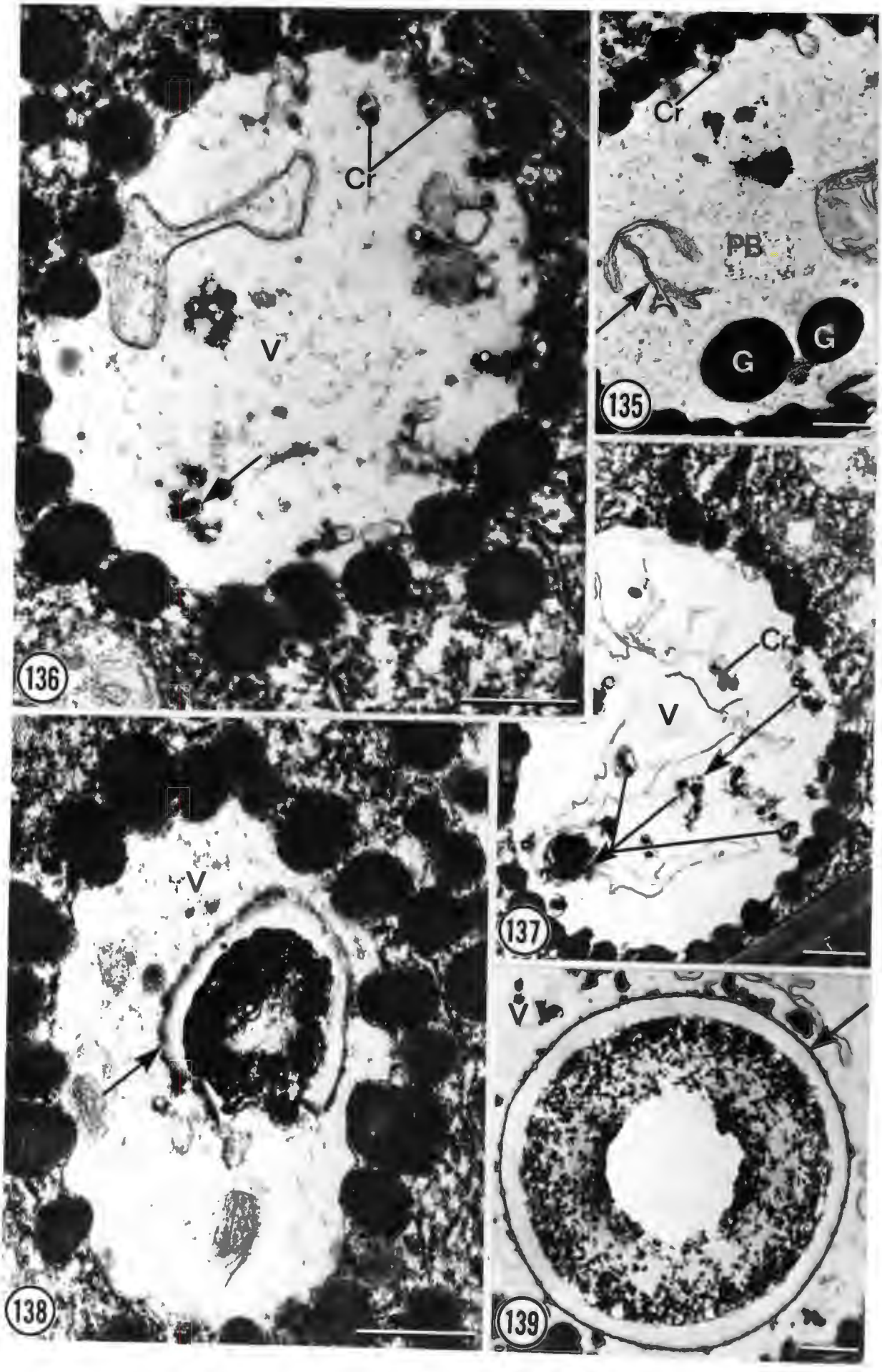
The protein body in Fig. 135 contains two solid electron-dense globoids, unidentified membranous inclusions and mere fragments of protein-carbohydrate reserve material. A small osmiophilic crystal which can be seen in contact with an invagination of the bounding membrane, appears to be on the point of passing into the protein body (henceforth called protein vacuole). Movement of small crystals from the cytoplasm into protein vacuoles has been accelerated as in Fig. 136. The majority of these crystals have an outer bounding envelope. Some micrographs, e.g. Fig. 137, suggest their movement toward and adherance to a larger crystal. In Fig. 138 an apparent agglomeration of smaller crystals is surrounded by a loose-fitting envelope. Some crystal accumulations are particulate and spongy in appearance as in Fig. 139 whose central, perhaps solid, core has been ripped out on sectioning. Not all globoids are solid as in Fig. 135. A great many of them seem to have undergone some internal digestion as evidenced by the pitted nature of the globoid in Fig. 146. These pitted regions seem to increase in size and number and eventually fuse together (Fig. 141). As this process continues it results in the fragmentation of the globoid (Figs. 142, 143). The fragments, though not enclosed by a bounding envelope, may aggregate in a spherical to oval configuration. Occasionally lighter staining reserve material is interspersed among the crystals (Fig. 143).

Comparing Figs. 133 and 134 it is obvious that starch reserves

PLATE 35.

Figs. 135-139. Collar cells imbibed for 24 h.—Fig. 135. Protein body (PB) containing electron-dense solid globoids (G) unidentified membranous inclusions (arrow) and fragments of protein-carbohydrate reserves. Note small osmiophilic crystal (Cr) in contact with invagination of bounding membrane apparently entering the protein body. —Fig. 136. The movement of small crystals (Cr) from cytoplasm into protein body vacuoles (V) accelerates. Most of the crystals seem to have a bounding envelope (arrow).—Fig. 137. The small crystals (Cr) entering the protein body vacuole (V) apparently move toward and adhere to a larger crystal (arrows).—Fig. 138. An agglomeration of smaller crystals is surrounded by a loose-fitting envelope (arrow) V = vacuole.—Fig. 139. A particulate crystal accumulation whose central, perhaps solid, core has been ripped out on sectioning. Note bounding envelope (arrow). V = vacuole.

Bars represent 0,5 µm (Figs. 135-139).


Cr
V
136
Cr
PB
G
G
135
Cr
V
137
V
138
V
139

decline rapidly as hydration progresses. In Fig. 134 most of the amyloplasts are bare of starch reserves. Mitochondria, with sparse cristae, are still fairly electron-transparent. Microbodies and ER become more apparent and dictyosomes now appear for the first time. The ribosome population remains dense.

DAY 1.5

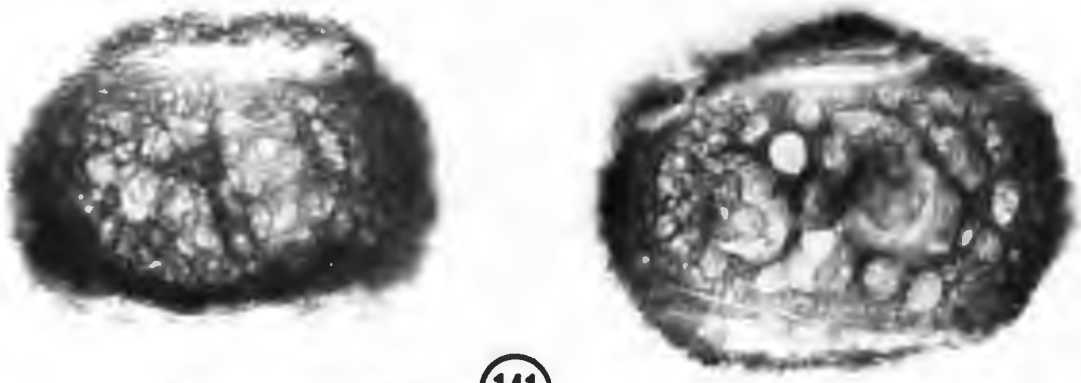
Figure 144 is a montage showing surface and deeply-situated collar cells at the 36-hour-imbibed stage. With increased hydration inner (area 2) and outer (area 1) collar cells no longer display the initial wide diversity in the apparent rate of metabolic activity first noted in Figs. 133 and 134 (1-day-imbibed).

There is a dramatic increase in cellular activity. While nuclei become slightly irregular, nucleoli increase in size and activity. In each nucleolus the peripheral granular zone enlarges further and appears to become interspersed in the central fibrillar region. Long profiles of rough ER become common and polysome configurations can often be identified. Fusion of protein vacuoles continues and is frequently more advanced in inner as opposed to outer collar cells. Lipid bodies have declined in number. They no longer line the plasmalemma and, although some remain in contact with protein vacuole membranes, the majority seem to exist in scattered clumps in the cytoplasm. Concurrent to lipid degradation, starch reserves reappear in the amyloplasts. Mitochondria have lost their

PLATE 36.

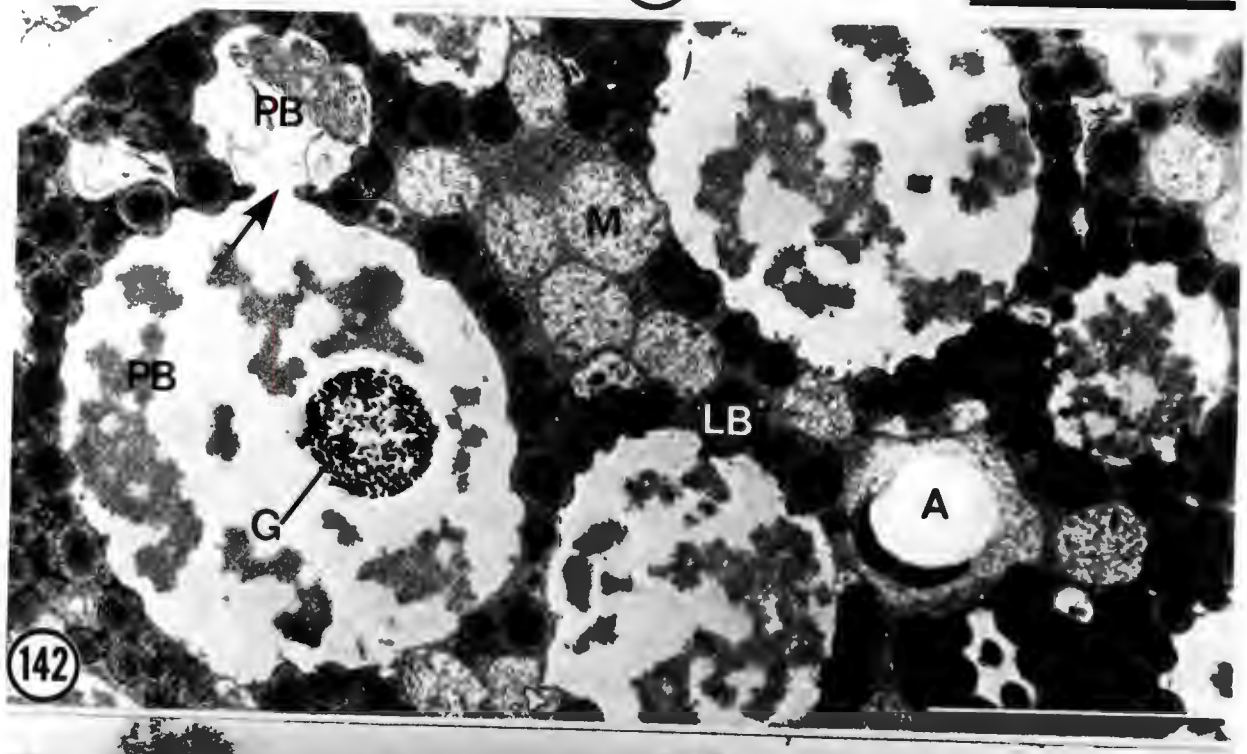
Figs. 140-143. Collar cells imbibed for 24 h.—Fig. 140. Pitted nature of globoid suggests internal digestion.—Fig. 141. As globoid digestion proceeds pitted regions increase in size and number and eventually fuse together.—Fig. 142. Internal digestion ultimately results in fragmentation of the globoid (G). Note fusion (arrow) of protein bodies (PB). A = amyloplast; LB = lipid body; M = mitochondrion.—Fig. 143. High magnification of fragmented globoid. Some protein-carbohydrate (PC) material is interspersed among the crystals.

Bars represent 0.25 μm (Figs. 140, 141, 143), 2 μm (Figs. 142).

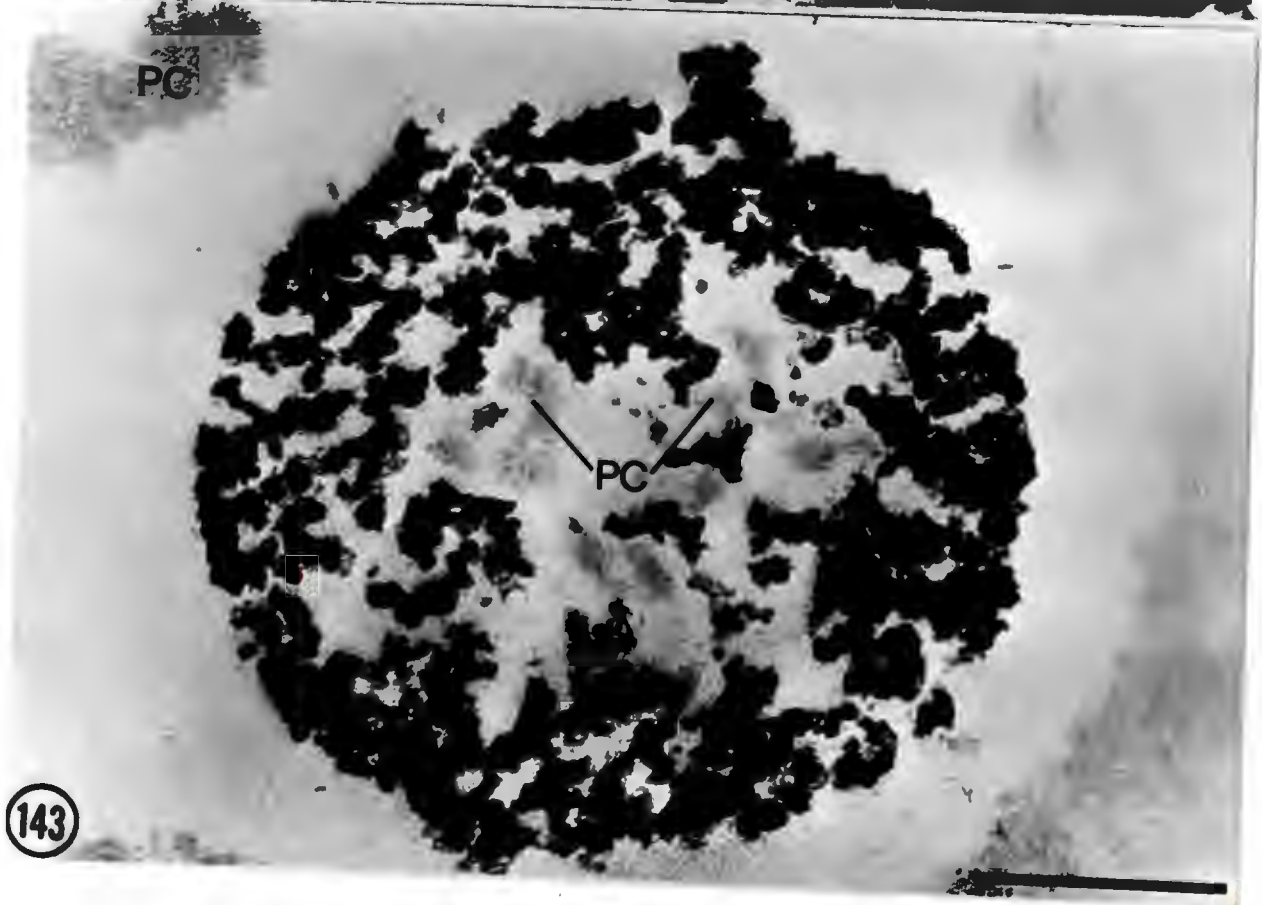


140

141



142



143

original electron-transparency and now contain numerous cristae. Dictyosomes and microbodies have increased in number.

At this point the discussion will be considered in two sections. The first will deal with surface cells of the collar (now called feeder interface cells), in the developing feeder region, which are in direct contact with the gametophyte as shown in Figs. 144, 145. The second section will concentrate on the development of more deeply-situated feeder cells (Fig. 144).

FEEDER INTERFACE CELLS

DAY 2

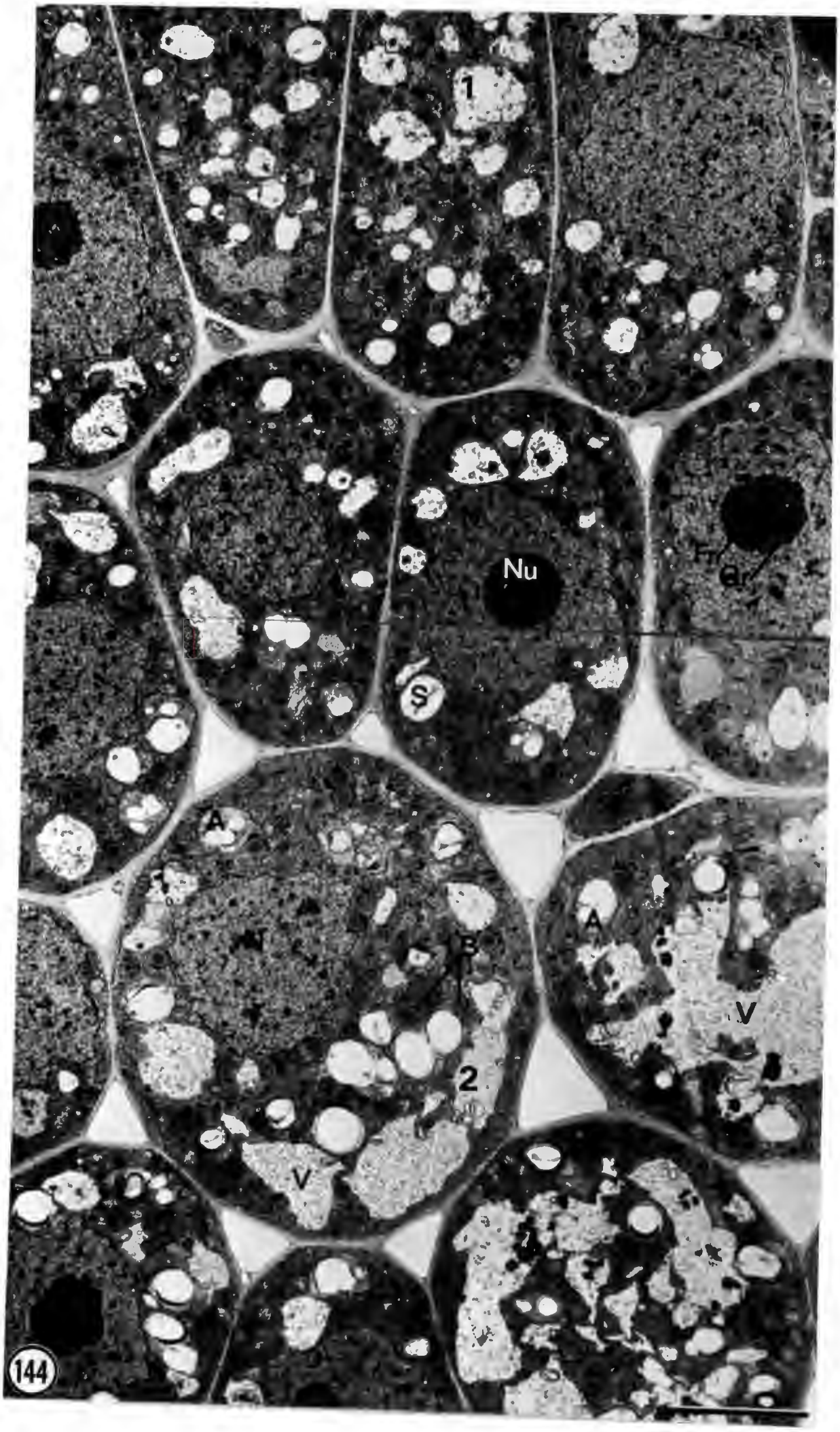
Feeder interface cells (Fig. 146) are usually elongate in transection (ca. 21 x 10 μm). Their nuclei strongly resemble those described for Fig. 144 (1.5 day) with their irregular contours and large active nucleoli. However, after two days germination, very little if any chromatin remains apparent in the nucleoplasm (cf. Figs. 122, 134, 144).

A fairly big vacuole, formed by the fusion of protein vacuoles, now occupies a large portion of the cell (Fig. 146). Characteristically it is located close to the outer interface wall. Some lipid persists, but the amount present is much less than that in Fig. 144 (1.5-day-stage). The remaining lipid bodies have a scattered distribution in the cytoplasm. Starch grains continue to occupy the amyloplasts. Large numbers of mitochon-

PLATE 37.

Fig. 144. Montage showing surface (1) and deeply-situated (2) collar cells 36 h after germination. Cellular activity has increased dramatically. Nuclei (N) become irregular. Nucleoli (Nu) have enlarged and the peripheral granular zone (Gr) becomes interspersed in the central fibrillar region (Fr). Fusion of protein vacuoles (V) continues while lipid bodies (LB) decline in number. Starch (S) reserves reappear in the amyloplasts (A).

Bar represents 5 μm (Fig. 144).



144

dria, indicative of a high respiration rate, seem to be primarily concentrated along the cell walls (Figs. 146, 147).

While microbodies decline in number, apparently active dictyosomes increase producing vesicles containing fibrillar material which is presumably contributed to the cell walls (Fig. 147). The vesicles migrate towards and fuse with the plasmalemma (Fig. 147, arrow), releasing their contents on fusion. Occasionally a few vesicles seem to pass through the plasmalemma prior to discharge of contents (Fig. 148, arrow).

Long and short ER profiles are scattered in the cytoplasm. Most are rough but some are or contain smooth segments. Smooth ER is sometimes seen to inflate and rough ER to bleb (Fig. 147). Ribosomes exist as mono- or polysomes either free or attached to ER. In the vicinity of lipid bodies, ribosomes seem to stain more darkly (Fig. 147).

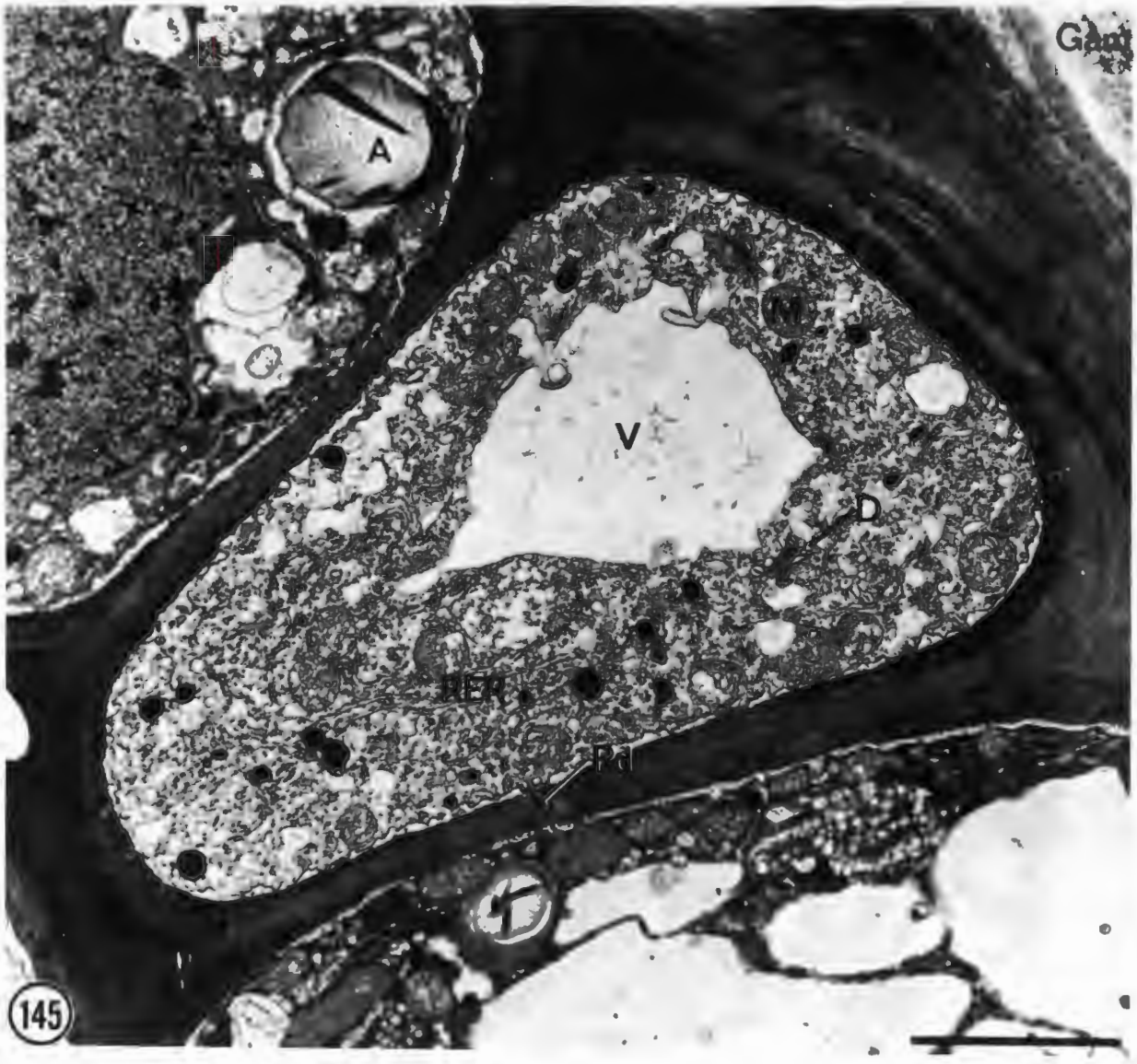
DAY 3-6

Ultrastructurally 2- and 3-day-imbibed feeder interface cells are very similar in appearance. The only significant differences are apparently to be found in cell wall structure and dictyosome activity. The outer cell walls thicken. While vesicles continue to contribute wall additives on the inner surface of the cell wall, the outer part seems to disintegrate or at least becomes less compact (Fig. 148). The beginning of this process is apparent at the 2-day-stage (Fig. 147). Dic-

PLATE 38.

Fig. 145. Surface cells of collar (now called feeder interface cells) in direct contact with gametophyte (Gam). A = amyloplast; D = dictyosome; M = mitochondrion; Pd = plasmodesmata; RER = rough ER; V = vacuole.

Bar represents 5 μm (Fig. 145).


A
V
D
RER
Pd
145

tyosome activity is prolific and the outer wall becomes very thick and mucilaginous. In the cytoplasm, minor ER dilations become more frequent (Fig. 149, arrows).

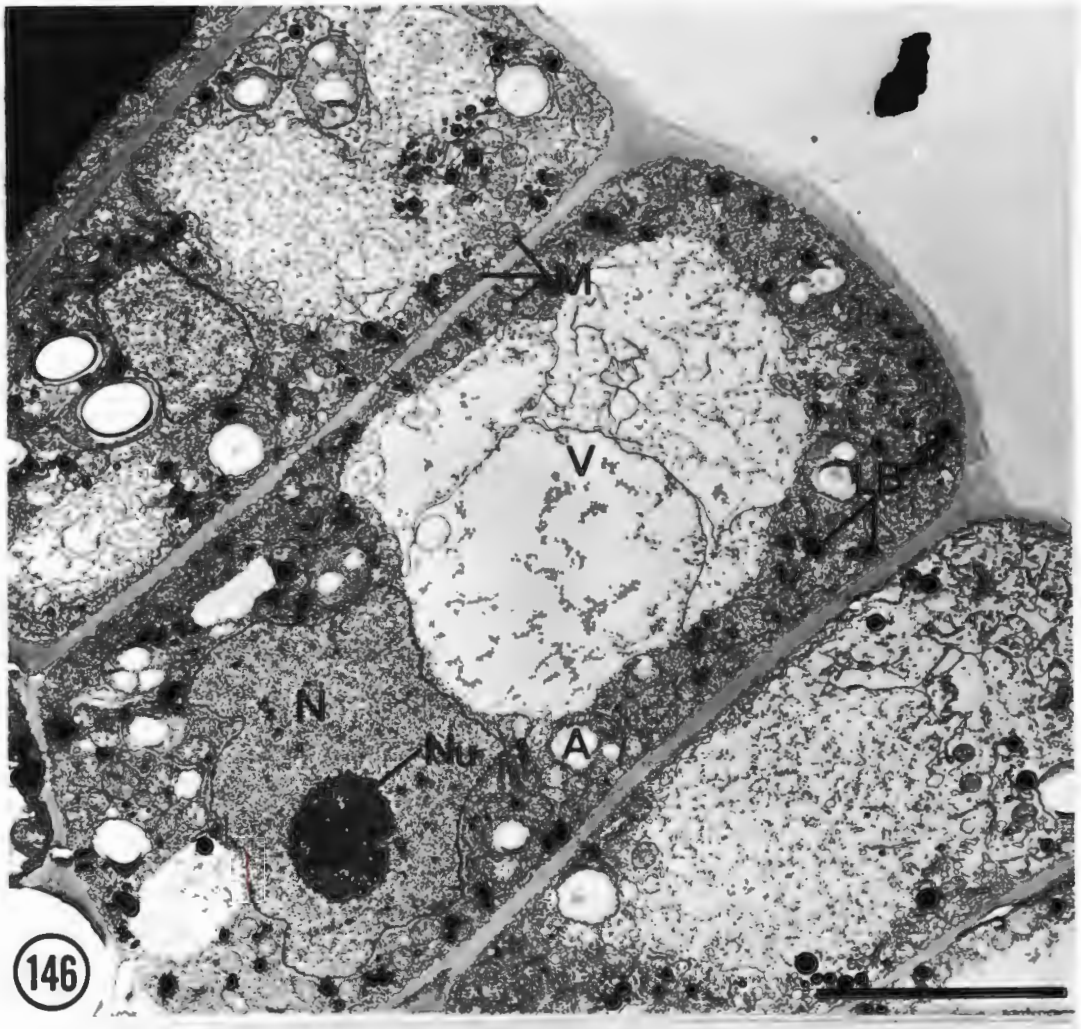
Approaching the fourth day of germination small wall projections invested with plasmalemma have begun forming along the inner surface of the outer wall. Microfibrils are often evident at right angles to the plasmalemma (Fig. 150, arrow). Nuclei remain active in appearance. Apart from a few sporadic lipid bodies, reserve materials have largely disappeared. At this stage the plumule is still within the seedcoat (Figs. 12, 14). The cytoplasm contains many mitochondria which vary in size and shape and dictyosomes which continue to actively proliferate vesicles. The dilations of smooth segments of ER become larger and more frequent and in many instances rough ER can be seen leading either into or out of the inflated areas (Fig. 150). Polysomes (Figs. 150, 151) are extremely common.

Figure 151 is a fairly typical feeder interface cell after approximately four days germination. Wall projections with associated plasmalemma convolutions have increased and the outer wall has thickened considerably assuming a mucilaginous texture. At this stage the feeder adheres strongly to gametophyte tissue by its ventral surface (Fig. 14). The peripheral interface cells of the feeder's ventral surface begin to undergo some cell wall disintegration. They have an overlapping arrangement and degradation usually begins in the middle lamella region at the tips of overlapped cells (Fig. 151) in

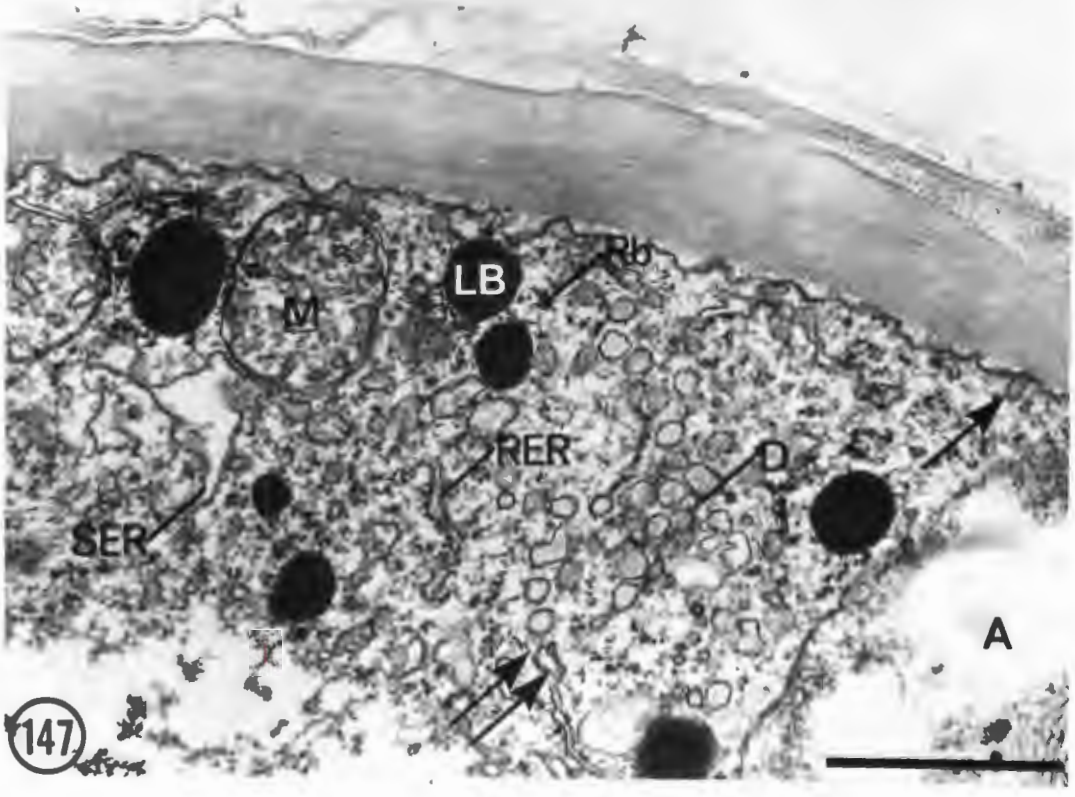
PLATE 39.

Figs. 146,147. Feeder interface cells after 2 days germination.—Fig. 146. T.S. Elongate feeder interface cells. Note irregular-shaped nucleus (N) with large active nucleolus (Nu). Vacuoles (V) are characteristically located close to surface cell wall. The remaining lipid bodies (LB) display a scattered distribution and amyloplasts (A) commonly contain starch grains. The numerous mitochondria (M) are primarily concentrated along the cell walls.—Fig. 147. T.S. portion of feeder interface cell showing outer cell wall. Mitochondria (M) and dictyosomes (D) are associated with cell wall. Dictyosome - derived vesicles containing fibrillar material migrate towards and fuse with the plasmalemma (arrow). Smooth ER (SER) sometimes inflates and rough ER (RER) may bleb (double arrow). In the vicinity of lipid bodies (LB) ribosomes (Rb) stain more darkly. The outer surface of the cell wall appears to be disintegrating. A = amyloplast.

Bars represent 5 μm (Fig. 146), 1 μm (Fig. 147).



146



147

regions coinciding with areas where acid phosphatase is localised.

The cytoplasmic features of interface cells on the fourth day of germination are similar to those described for Fig. 150 (3.5-day-stage). From the fourth day (Fig. 152) mitochondria increase dramatically in number while dictyosome activity diminishes. The entire inner wall surface now possesses wall projections and plasmalemma convolutions. These convolutions are not prominent but serve to increase the surface area of the plasmalemma (Figs. 151, 152).

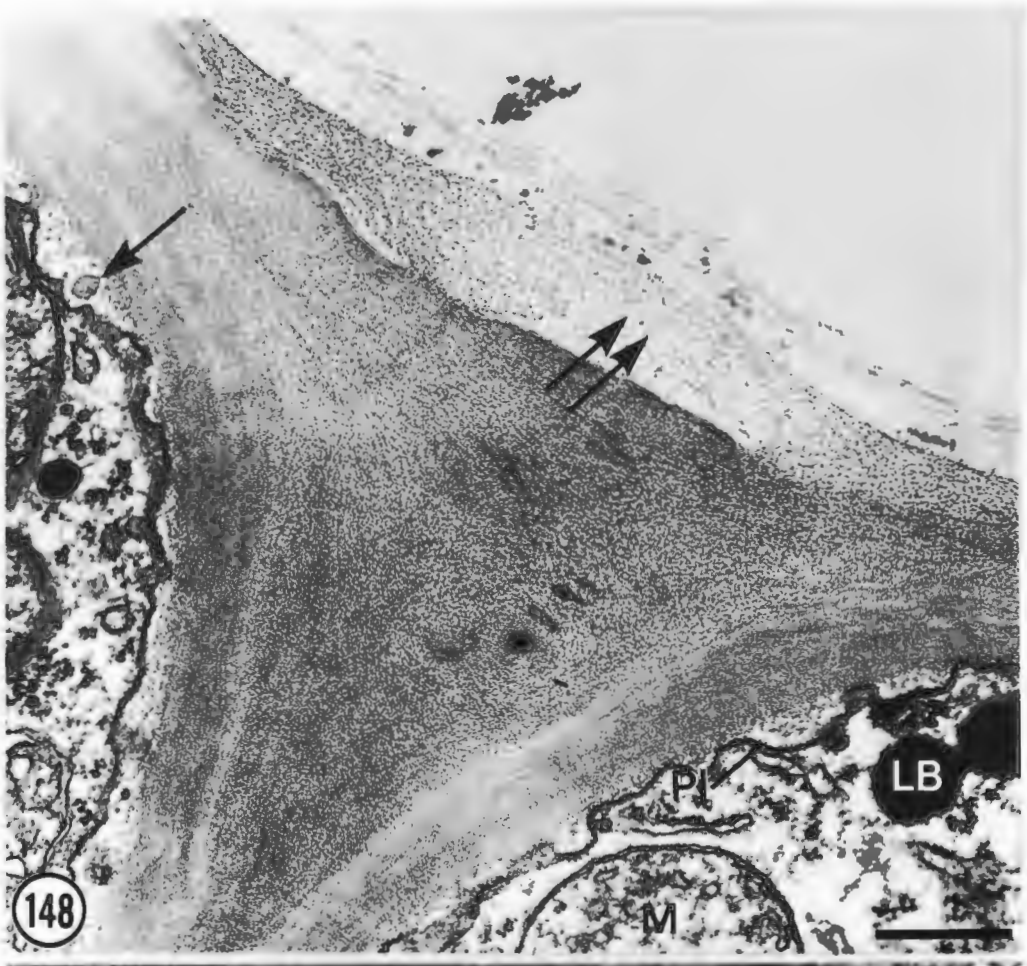
By the sixth day of germination the plumule has usually emerged from the seed (Fig. 16). Ventral interface cells near the tip of the feeder remain fairly active (Fig. 153) although many begin to show signs of senescence. Nuclei become increasingly irregular and ER continues to dilate. Polysomes are conspicuous (Fig. 153) but not profuse. Amyloplasts and microbodies are rare and dictyosome activity has waned. Mitochondria are still numerous (Fig. 153) but a large number are beginning to swell and develop electron-transparent areas. From Fig. 153 it is apparent that many small vesicles are discharged and accumulate between the plasmalemma and cell wall. They are unlike the previously described dictyosome-derived vesicles in that they do not contain fibrillar material.

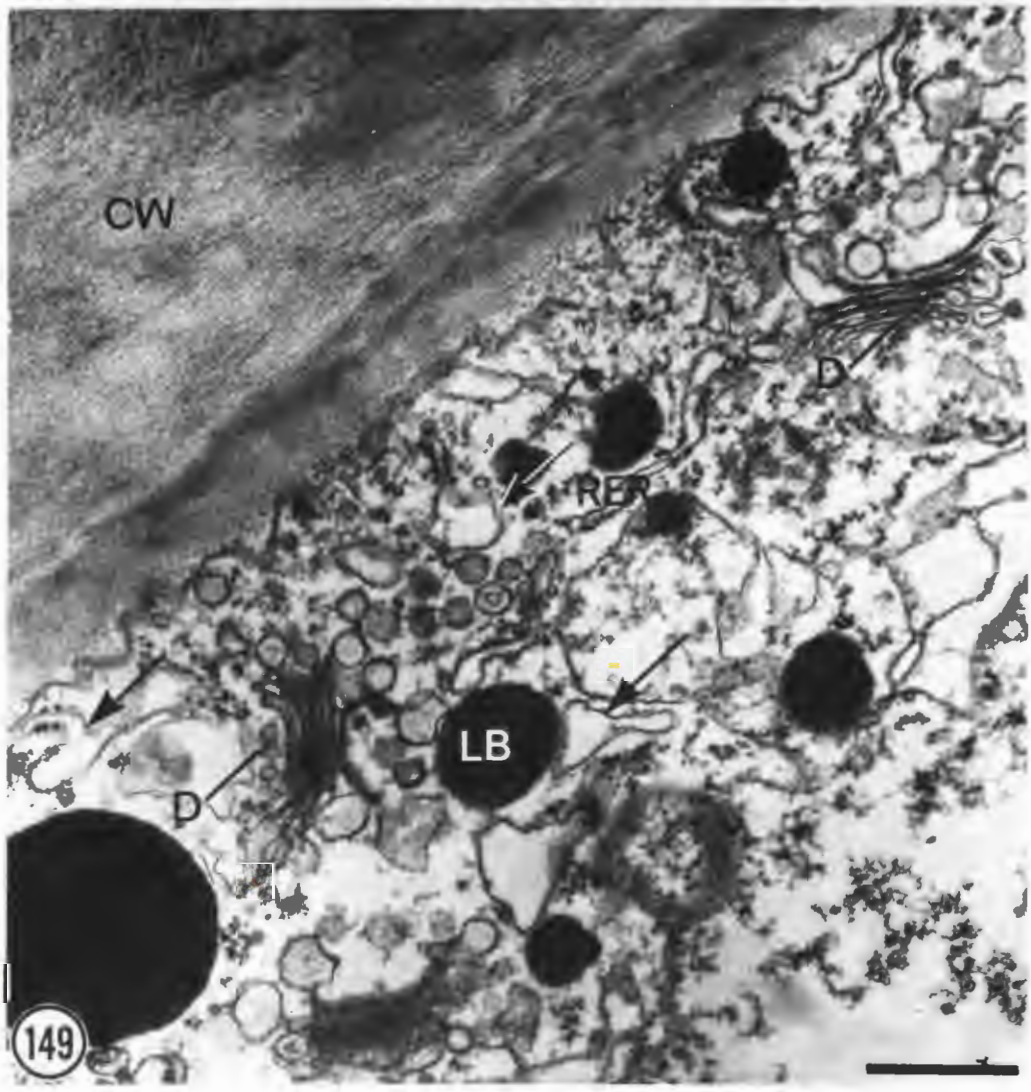
Figure 154 is of 6-day-stage interface cells situated near the base of the feeder. Comparison of Figs. 154 and 153 clearly illustrates that senescence accelerates from the tip (Fig. 153)

PLATE 40.

Figs. 148, 149. Feeder interface cells at 3-day-stage.—Fig. 148. Outer cell walls of feeder interface cells thicken. Dictyosome-derived vesicles continue contributing to inner surface of cell walls with vesicles (arrow) sometimes passing through the plasmalemma (Pl) prior to discharge of contents. At the same time the outer surface of the cell wall seems to disintegrate or become less compact (double arrow). LB = lipid body; M = mitochondrion.—Fig. 149. Prolific dictyosome (D) activity results in the outer cell wall (CW) becoming very thick and mucilaginous. Minor ER dilations (arrows) become more frequent. LB = lipid body; RER = rough ER.

Bars represent 0,5 μm (Figs. 148, 149).


PI
LB
M
148


CW
D
RER
LB
D
149

to the base (Fig. 154) of the wedge-shaped feeder. Disintegration in the middle lamella region between interface and adjacent cells first noticed in Fig. 151 (4-day-imbibed) has progressed in Fig. 154 (6-day-imbibed). The condition of the cytoplasm of these interface cells (Fig. 154) reflects senescence. Mitochondria are swollen and ER and dictyosomes are dilated. The protoplast of the lower interface cell in Fig. 154 has lost turgor and has shrunk away from the wall. The tonoplast has broken and the cytoplasm appears dilute. At the extreme base of the feeder peripheral cells have senesced completely and become crushed against the gametophyte (Fig. 155 - 6-day-imbibed). These cells, and cells in the process of degeneration, give a strong positive reaction for acid phosphatase (Fig. 119).

INNER FEEDER CELLS

DAY 3-6

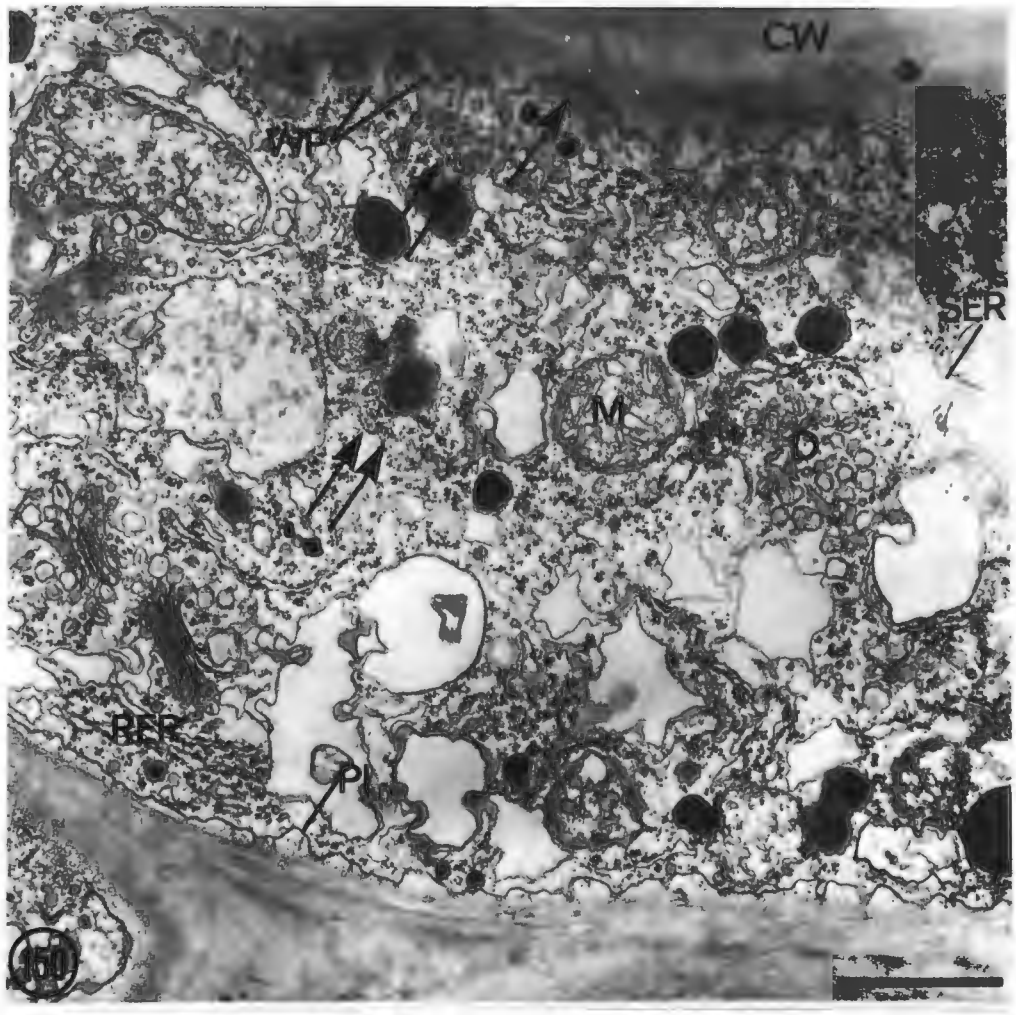
From the 1.5 day stage, as represented by Fig. 144, inner collar cells on the side of the developing feeder elongate rapidly, measuring approximately 350 x 60 µm after 3-4 days germination. Figs. 156 and 157 are fairly typical of inner feeder cells at this stage when the rapidly growing feeder is about 4 mm in length (Fig. 14).

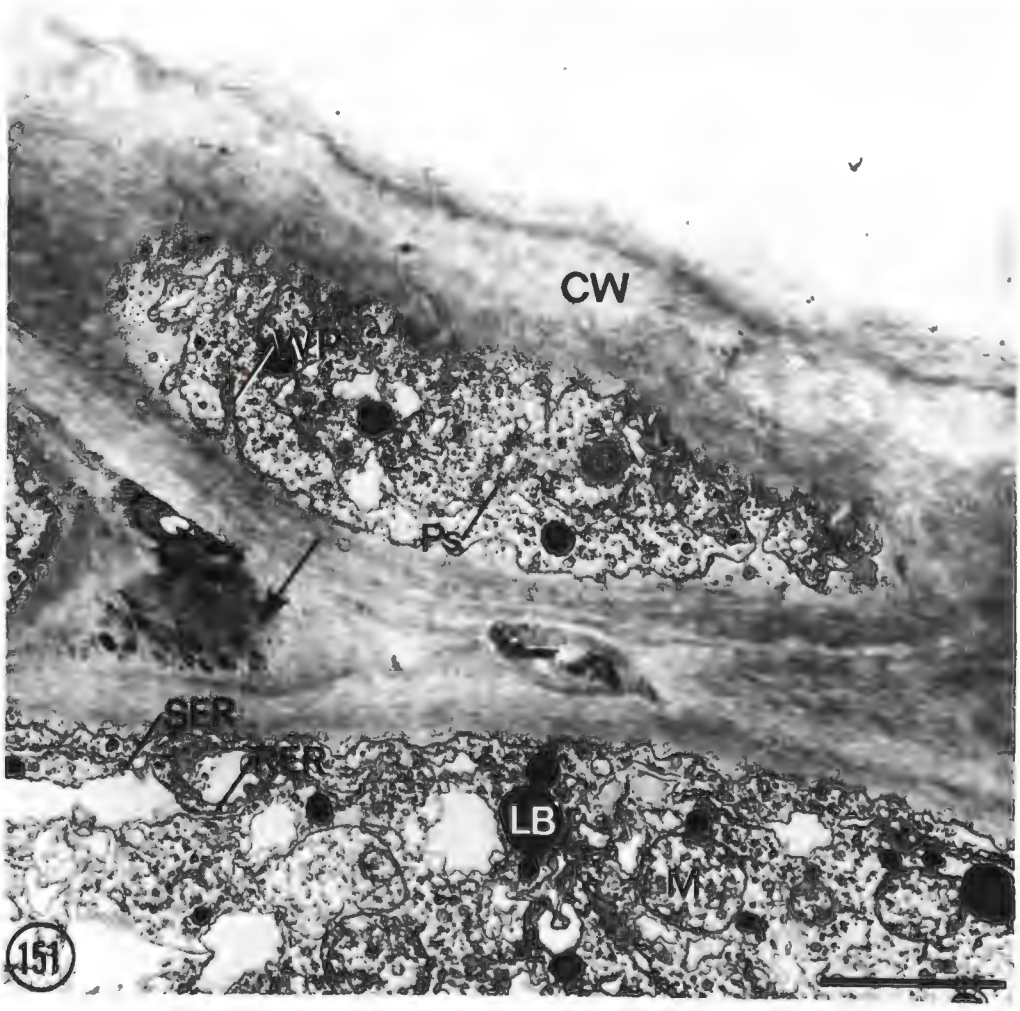
Nuclei have become elongated ovoids with slightly irregular

PLATE 41.

Fig. 150. L.S. Feeder interface cell after 3.5 days germination. Small wall projections (WP) invested with plasmalemma (Pl) have begun forming along inner surface of outer cell wall (CW). Microfibrils (arrow) are evident at right angles to plasmalemma. Reserve materials have largely disappeared. The cytoplasm contains many mitochondria (M) and active dictyosomes (D). Dilation of smooth segments of ER (SER) become larger and more frequent. Note rough ER (RER) either leading into or out of inflated areas. Polysomes (double arrows) are common.—Fig. 151. L.S. feeder interface cell after approximately 4 days germination. Wall projections (WP) with associated plasmalemma convolutions have increased. Outer cell wall (CW) is thick and mucilaginous. Peripheral interface cells have an overlapping arrangement. Some cell wall degradation begins in the middle lamella region at the tips of overlapped cells (arrow). LB = lipid body; M = mitochondrion; Ps = Polysomes; RER = rough ER; SER = smooth ER.

Bars represent 1 μm (Figs. 150, 151).


CW
VP
SER
M
D
RER
PI
150


CW
VP
PS
SER
ER
LB
M
151

contours. Nucleoli are large and active with the bulk of their volume formed by the granular zone which surrounds and interperses the fibrillar region (Fig. 157).

All protein reserves have been consumed and the remaining protein vacuoles have fused to form a large central vacuole in which there is no trace of globoids or globoid particles. The central vacuole is not visible in Figs. 156 and 157 as these sections were cut through the cytoplasm marginal to the vacuole. Lipid stores have been drastically reduced and now exist as widely dispersed lipid bodies.

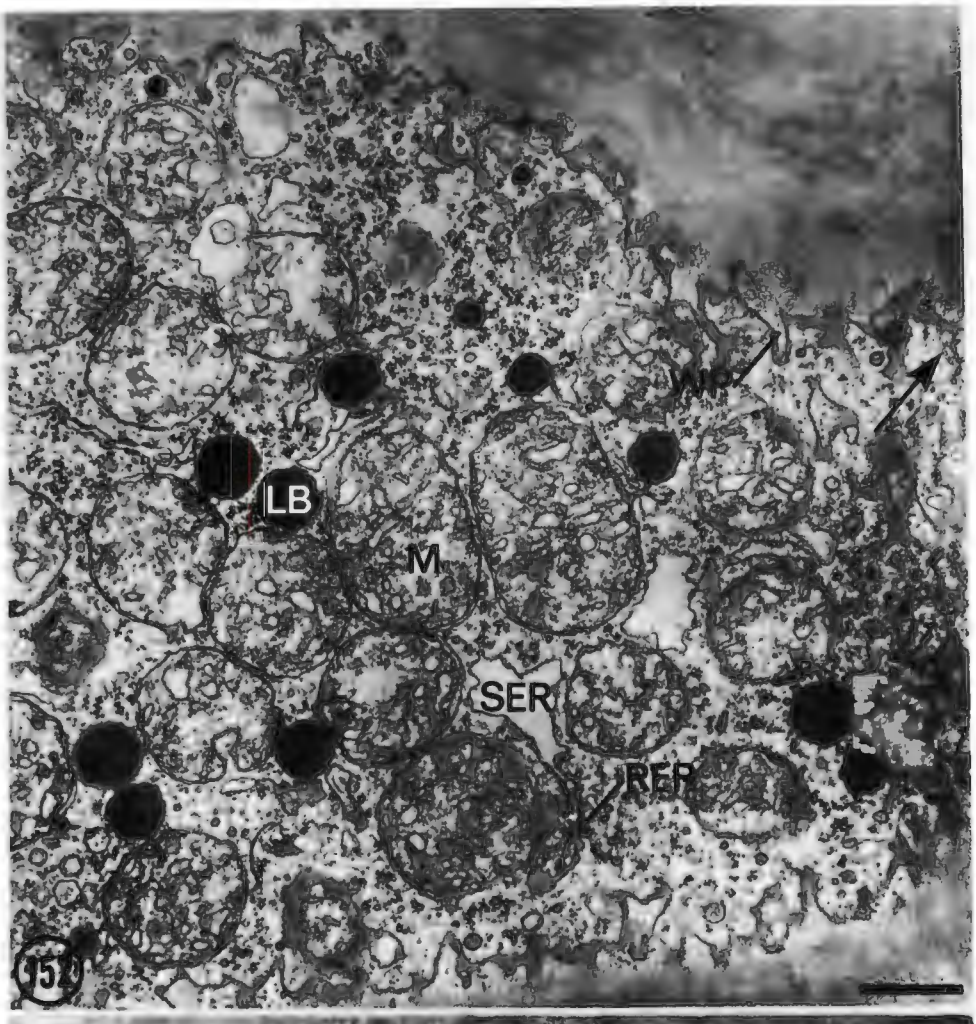
The cytoplasm appears metabolically active with numerous mitochondria, active dictyosomes and a fairly well-developed ER system which shows frequent dilations forming small clear vacuoles (Figs. 156, 157). Amyloplasts with starch grains are quite common while microbodies are comparatively rare. Polysome configurations are common and occur both free and attached to the ER. No ribosomes are attached to the localised dilations of the ER. Cell walls remain thin.

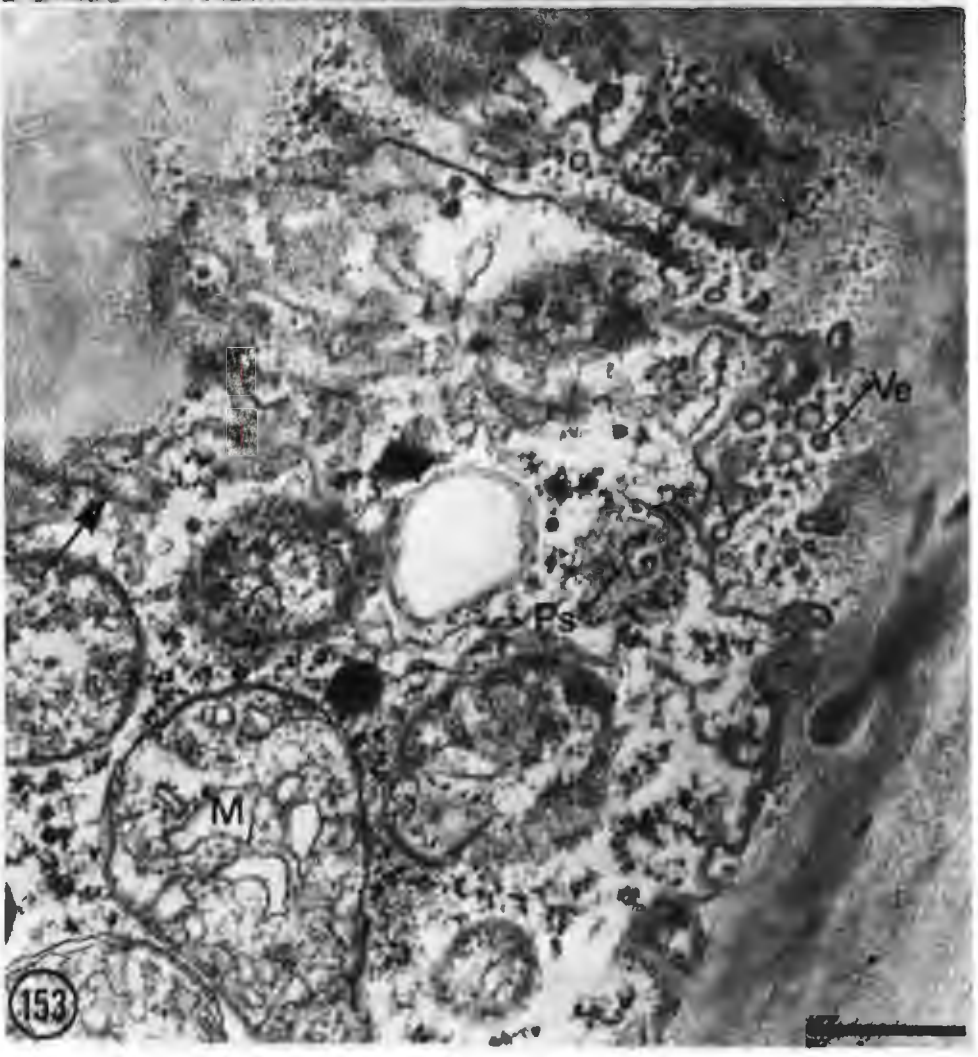
Between the fourth and fifth days of germination many of the internal feeder cells are still elongating as the feeder continues to enlarge (Fig. 15). Some cell division occurs but is apparently confined to the base of the emergence. At about this time the nuclear envelope of nuclei in deeply-situated feeder cells may appear to bleb (Fig. 158), and ER connections with the envelope are frequently seen. Some of the membrane

PLATE 42.

Fig. 152. L.S. portion of feeder interface cell after 4 days germination. The entire inner wall surface possesses wall projection (WP) and plasmalemma convolutions. Note microfibrils (arrow). Mitochondria (M) have increased in number dramatically. LB = lipid body; RER = rough ER; SER = smooth ER.—Fig. 153. L.S. portion of interface cell near tip of feeder after 6 days germination. Mitochondria (M) are still numerous and polysomes (Ps) are conspicuous. Many small vesicles (Ve) have accumulated between plasmalemma and cell wall. Unlike previously described dictyosome-derived vesicles (Figs. 147,149) they do not contain fibrillar material. Note microfibrils (arrow).

Bars represent 1 μm (Fig. 152), 0.5 μm (Fig. 153).


LB
M
SER
REP
152


Ve
Ps
M
153

continuities between ER and nuclear envelope become dilated as in Fig. 159. The inflation of localised portions of the ER system continues and dilations can be seen in progressive stages of development (Figs. 160-162). Rough ER frequently leads into and/or out of the dilations, but the dilations themselves are smooth (Figs. 160-162). Nuclei continue to present an active appearance. Nucleoli remain large while the surface contours of the nucleus may become increasingly lobed as depicted in Fig. 163. Clouds of small dark granules of ribosome-like proportions are present in the nucleoplasm (Figs. 160, 163). Their distribution gives the impression that they are moving from the periphery of the nucleolus toward the nuclear envelope. Irregularity of the nucleus results in frequent glancing sections cut tangential to the nuclear membrane. These sections, e.g. Fig. 164, allow a surface view of the nuclear pores whose structure consists of an annulus composed of eight subunits and a central granule (Yoo and Bayley 1967; Jordan 1971).

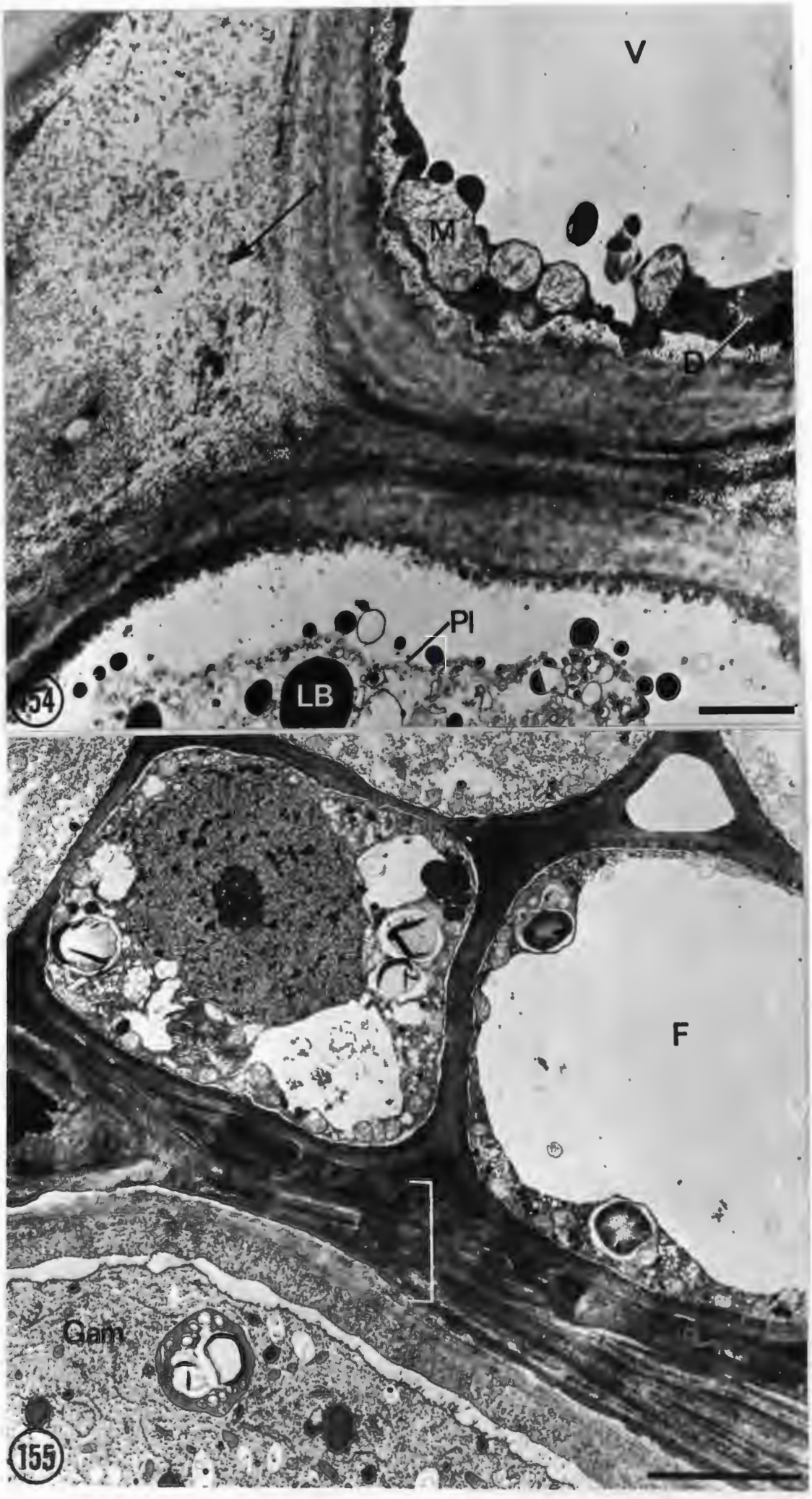
Discrete, spiral polysome configurations are rife. They are most frequently seen attached to the ER system (Figs. 163 and 165-167) but also cover the nuclear surface (Fig. 164, arrows). The majority exist as single coils containing between 13 to 18 ribosomes (Figs. 164-166) but occasionally double coils are encountered e.g. the large polysome containing 38 ribosomes in Fig. 167.

Active mitochondria, starch-laden amyloplasts and rough ER fre-

PLATE 43.

Fig. 154. 6-day-stage interface cells near base of feeder. The condition of the cytoplasm reflects senescence. Organelles are swollen, the protoplast has lost turgor and, in the lower cell, has shrunk away from the wall. The tonoplast has also broken and the cytoplasm appears dilute. Note disintegration in middle lamella region (arrow) between interface and adjacent cells. D = dictyosome; LB = lipid body; M = mitochondrion; Pl = plasmalemma; V = vacuole.—Fig. 155. Interface between gametophyte (Gam) and extreme base of feeder (F) at 6-day-stage. Peripheral feeder cells have senesced and become crushed against gametophyte (bracket).

Bars represent 1 μm (Fig. 154), 5 μm (Fig. 155).


V
M
D
PI
LB
154
F
Gam
155

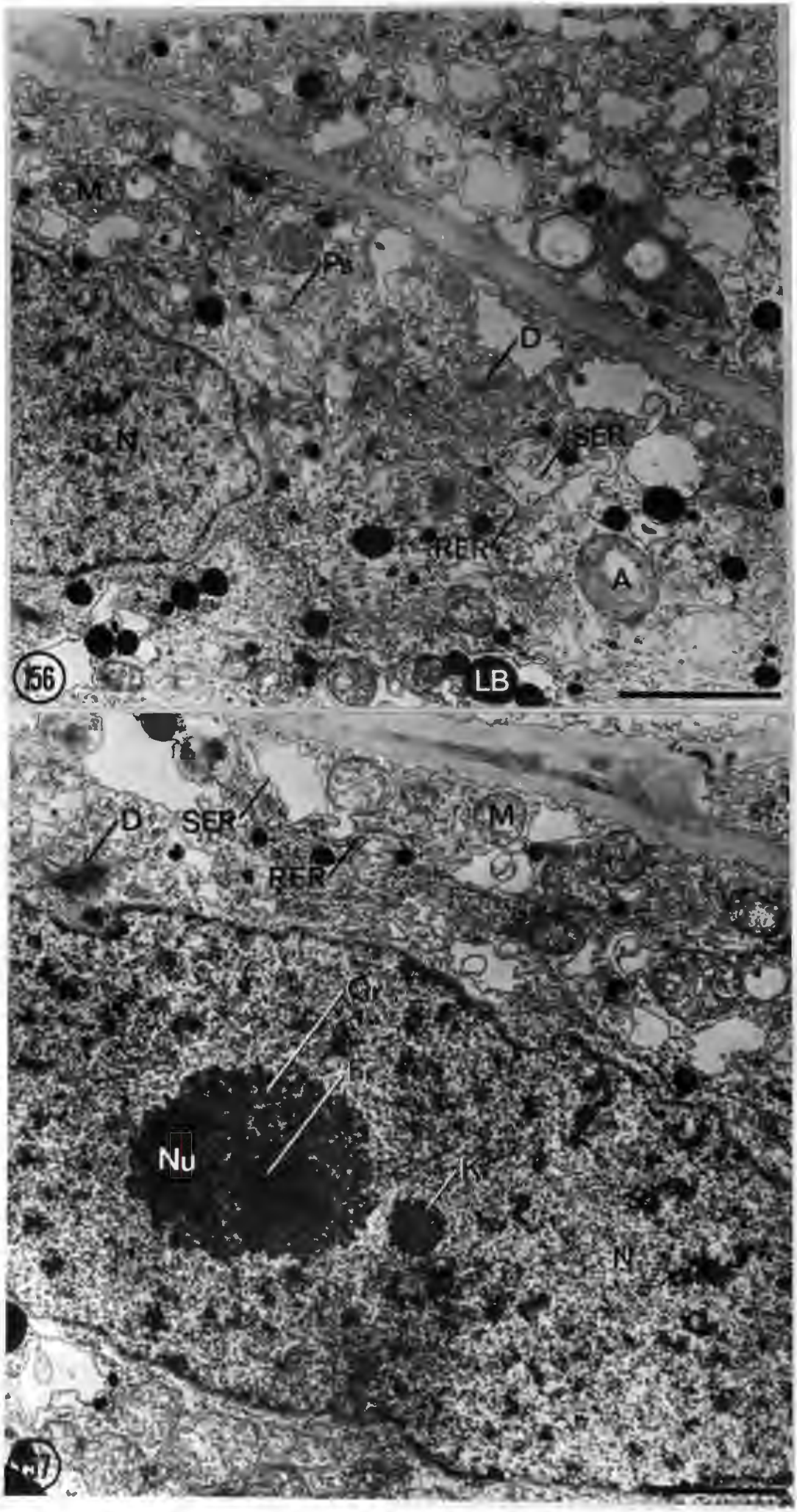
quently develop close spatial relationships with the nucleus (Fig. 163). In cells which have apparently ceased elongating scattered dictyosomes decrease in number and begin to structurally resemble the dictyosome depicted in Fig. 168. This dictyosome (Fig. 168) clearly shows the polarity typical of this organelle (Morre *et al.*, 1971) in which the proximal pole or forming face is associated with the nuclear envelope or ER while the maturing face is at the distal pole. Cells which are still in the process of elongating contain a much larger population of dictyosomes, all of which actively proliferate numerous hypertrophied vesicles (Figs. 169, 170). These large vesicles containing fibrillar material (Fig. 169) move through the cytoplasm to fuse with the plasmalemma and release their contents. Despite constant addition of wall material, the elongating cell walls remain thin (Figs. 156, 157, 169) and do not approach the thick proportions of interface cell walls (Fig. 151). The cytoplasm of these deeply-situated cells is rich in ribosomes (Figs. 169, 170).

On the fifth day of germination a small percentage of cytoplasmic organelles appear to be in states of degeneration e.g. the swollen mitochondria and amyloplast with deteriorating matrices in Fig. 171 and the "exploded" mitochondria in Fig. 172 which produce large electron-transparent extrusions. All these degenerating organelles are eventually sequestered in the central vacuole where they are degraded. However the majority of amyloplasts (Figs. 173, 174) and mitochondria (Fig. 175) retain their healthy appearance. Starch reserves fluctuate (Figs. 171,

PLATE 44.

Figs.156, 157. Inner feeder cells at 3.5-day-stage.—Fig. 156. Cytoplasm appears metabolically active with numerous mitochondria (M), active dictyosomes (D) and well-developed ER system which shows frequent dilations. Amyloplasts (A) with starch grains are common. Polysome (Ps) configurations occur free and attached to ER. Lipid bodies (LB) have been drastically reduced in number. N = nucleus; RER = rough ER; SER = Smooth ER.—Fig. 157. Elongated, ovoid nucleus (N) with slightly irregular contours. Nucleolus (Nu) is large and active with the bulk of its volume formed by the granular zone (Gr) which surrounds and intersperses the fibrillar zone (Fr). D = dictyosome; K = karyosome; M = mitochondrion; RER = rough ER; SER = smooth ER (dilated).

Bars represent 2.5 μm (Figs. 156, 157).


M
Ps
D
N
SER
RER
A
156
LB
D
SER
M
RER
Nu
N

173, 174) but are usually a constant cell feature. Dictyosomes gradually begin to decline in number and activity (Figs. 174, 176). Non-membrane bound pockets of unidentified finely granular material are sometimes seen (Figs. 174,176).

Fig. 177 is a longisection of an internal feeder cell after approximately 5 days germination. Typically it is thin-walled, highly vacuolate and well supplied with mitochondria. Fig. 178 is a similar cell cut transversely. Between the fifth and sixth days of germination most nuclei of internal feeder cells have taken up marginal positions against the cell wall as in Fig. 179. They are usually elongated (Fig. 179) with slightly to deeply lobed contours (Fig. 178). The nucleoli are still large and vacuolate and embedded chromosome material (Hyde 1967) is frequently observed (Fig. 179). However they are beginning to assume a slightly less active appearance (Hyde 1967) due to the separation of their fibrillar and granular regions into distinct zones (Fig. 179) as opposed to their previous interspersion (Fig. 178).

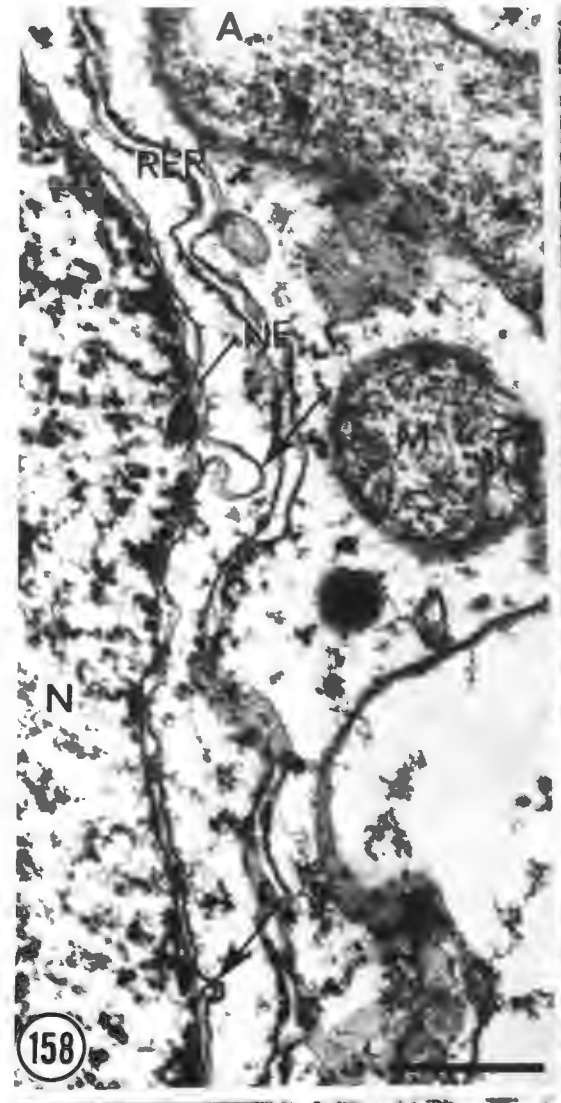
The cells are highly vacuolate and cytoplasm is usually restricted to thin layers lining the cell walls. Mitochondria are still present in large numbers (Figs. 178, 179) and amyloplasts with starch deposits are prominent. Dictyosomes, ribosomes and ER are no longer plentiful and the cytoplasm and nucleoplasm often assume a leached appearance (Fig. 180).

Plasmodesmata are more common in deeply-situated feeder cells than in surface interface cells. They are similar to those

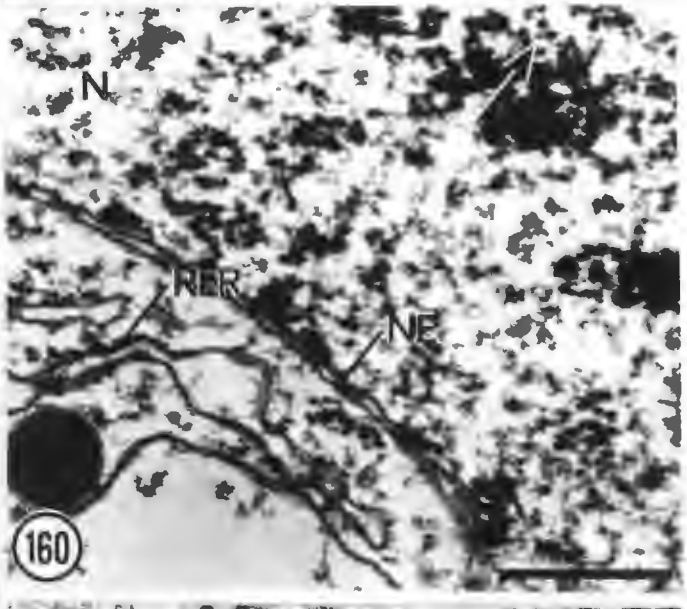
PLATE 45.

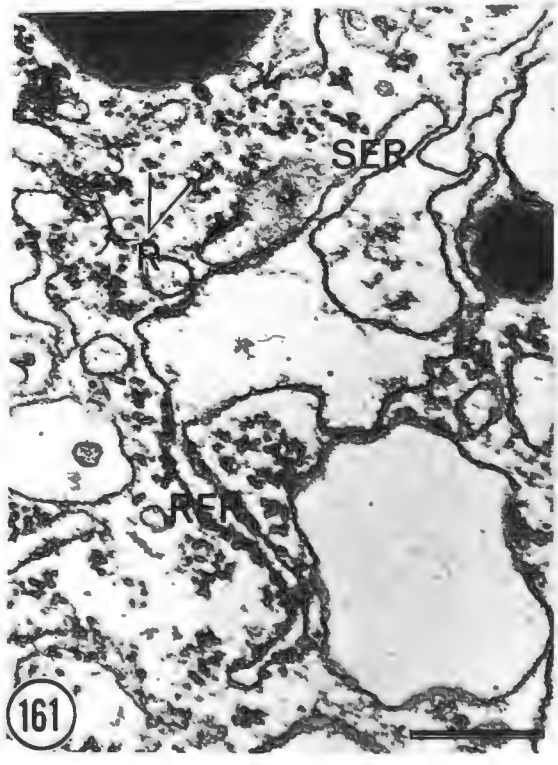
Figs. 158-162. Inner feeder cells at 4.5-day-stage.—Fig. 158. Nuclear envelope (NE) may appear to bleb (arrows). A = amyloplast; M = mitochondrion; N = nucleus; RER = rough ER.—Fig. 159. Continuity (arrow) between dilated ER and nuclear envelope (NE).—Fig. 160. Small inflation of localised portion of ER. Rough ER (RER) leads into dilation, but dilation itself is smooth. Note small granules (arrow) of ribosome-like proportions in nucleoplasm. N = Nucleus; NE = nuclear envelope.—Fig. 161. Inflation of ER. R = ribosome; RER = rough ER; SER = smooth ER.—Fig. 162. Inflation of ER becomes progressively more common. Note rough ER (RER) leading into and out of smooth dilated areas. LB = lipid body.

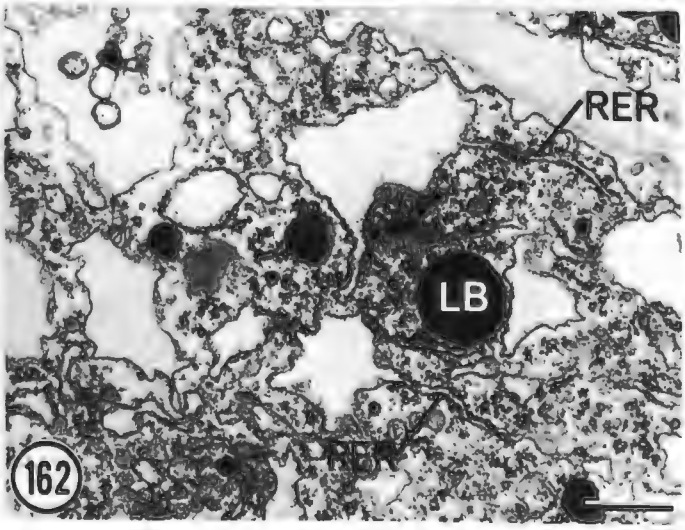
Bars represent 0.5 μm (Figs. 158, 160, 162), 0.25 μm (Figs. 159, 161).


A
RER
NE
M
N
158


NE
159


N
RER
NE
160


SER
R
RER
161


RER
LB
RER
162

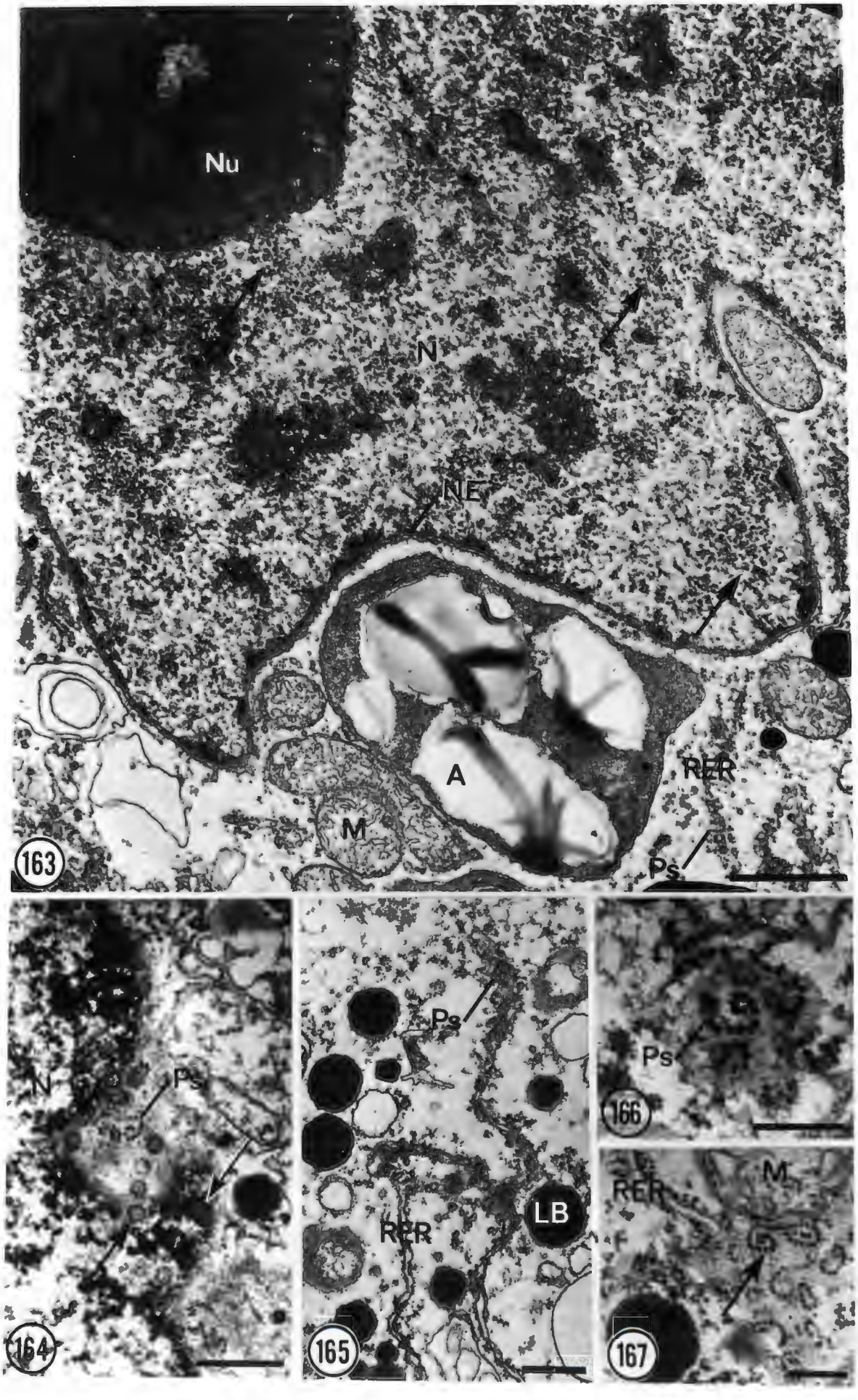
described for gametophyte tissue, and consist of plasmalemma-lined pores which are often joined in the median region (Fig. 182) where large cavities frequently form. The plasmodesmata in Fig. 181 are orientated obliquely and as a result only the median cavities can be seen. Sometimes wall projections and related plasmalemma convolutions are apparent in deeply situated feeder cells as in Fig. 183 (6-day-imbibed). However this type of wall configuration is not common.

Fig. 184 illustrates basal feeder cells in the vicinity of a vascular strand in a 6-day-old seedling. These cells, which are richly supplied with plasmodesmata, give the appearance of being metabolically more active than previously described inner feeder cells of a similar age. The central vacuole is large and autophagically active as evidenced by included membranes and degrading organelles (Fig. 184). Osmiophilic crystal remnants, possibly globoid in nature, are still apparent. The cytoplasm is well supplied with mitochondria and short segments of rough ER. Lipid bodies exist in a scattered dispersion, dictyosomes are few and plastids are devoid of starch.

PLATE 46.

Figs. 163-167. Inner feeder cells at 4.5-day-stage.—Fig. 163. Surface contours of nucleus (N) become increasingly lobed. Note masses of small dark granules (arrows) of ribosome-like proportions apparently streaming from periphery of nucleolus (Nu) to nuclear envelope (NE). Rough ER (RER), mitochondria (M) and starch-laden amyloplasts (A) develop close spatial relationships with nucleus. Ps = polysome.—Fig. 164. Glancing section cut tangential to nuclear membrane showing surface view of nuclear pores (arrows) which consist of an annulus composed of 8 subunits and a central granule. Polysomes (Ps) also cover the nuclear surface. N = nucleus.—Fig. 165. Polysome (Ps) configurations are most commonly seen attached to the ER system. LB = lipid body; RER = rough ER.—Fig. 166. High magnification of polysomes (Ps) attached to ER. Most polysomes exist as single coils.—Fig. 167. Large double-coiled polysome (arrow). M = mitochondrion; RER = rough ER.

Bars represent 1 μm (Fig. 163), 0.5 μm (Figs. 164, 165), 0.25 μm (Figs. 166, 167).


Nu
N
NE
A
M
RER
Ps
163
N
Ps
164
Ps
LB
RER
165
Ps
166
RER
M
167

DISCUSSION

Reserve materials contained within embryonic collar cells appear meagre in comparison with the massive reserves of gametophyte cells. This paucity of collar reserves could be partly responsible for the greater clarity of membranes in the unimbibed state when compared with dry gametophyte tissue. Presence of large amounts of lipid invariably adds to the difficulty of obtaining clear fixation images (Villiers 1971). Collar cells appear less dehydrated than gametophyte cells and this no doubt expedites their rapid enzymic activation upon imbibition. It is evident from the electron micrographs studied that activation of collar cells precedes that of gametophyte cells. In addition, the sequential activation of gametophyte cells from the embryo outwards gives evidence for the possible diffusion of a stimulatory factor from the embryo which induces or enhances metabolic activity in the surrounding gametophyte.

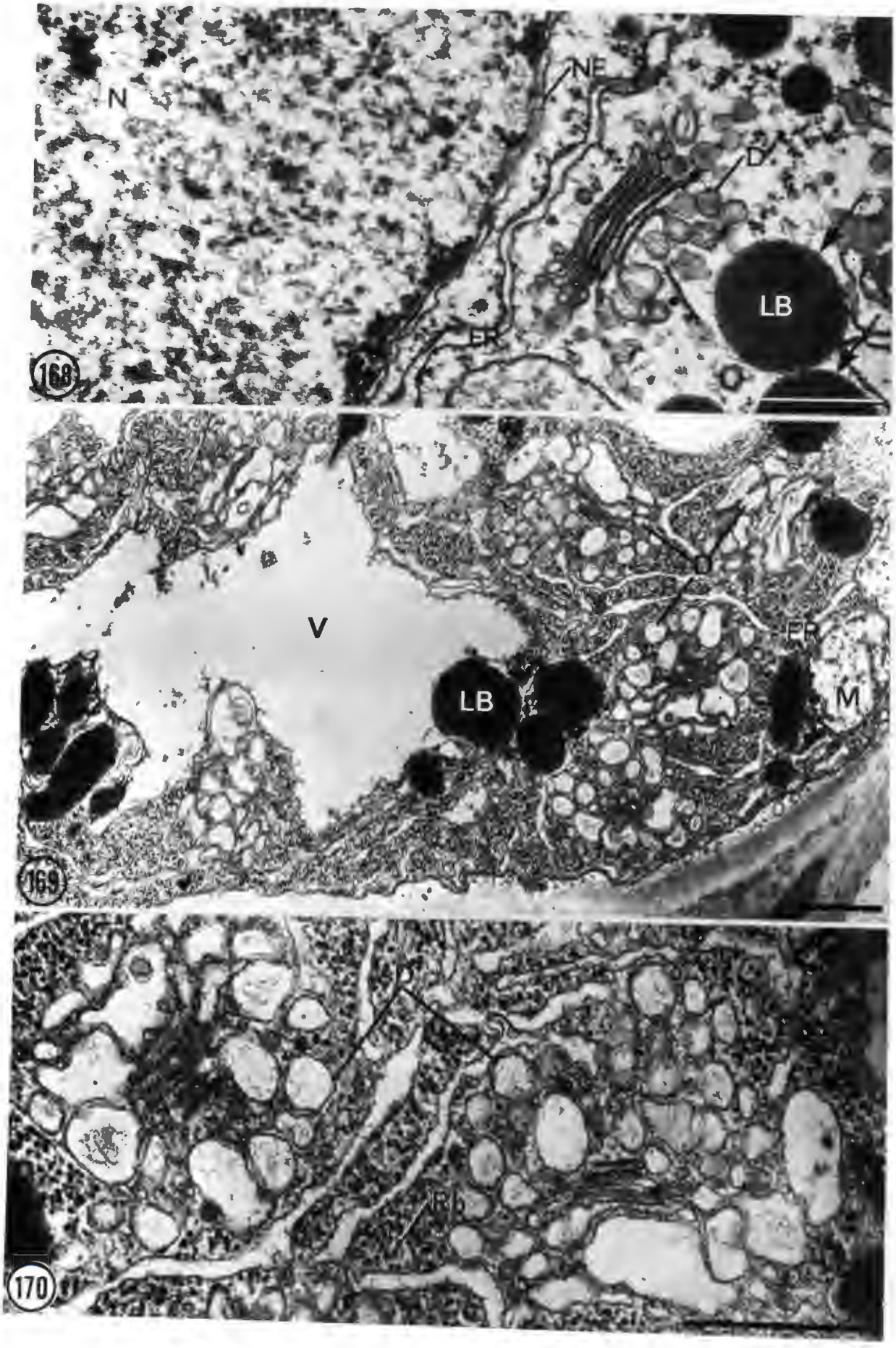
Nucleus

In contrast to the pronounced lobing common to dry gametophytic nuclei, embryonic collar nuclei are free from distortion and are spherical to ovoid in the dry, quiescent state. Their lack of deformity could be due to the scanty nature of collar reserves. The reserve materials are loosely packed and cannot exert a deforming pressure on nuclear surfaces as is apparently the case in gametophyte tissue. Meristematic nuclei are invariably rounded (Yeoman and Street 1973) and since collar nuclei are capable of further division (Bower 1881a; Martens 1971) their spherical form is not surprising. Conversely the

PLATE 47.

Figs. 168-170. Inner feeder cells at 4 to 5-day-stage.—Fig. 168. Dictyosome (D) from cell which has ceased elongating. This dictyosome shows typical polarity with the proximal pole or forming face associated with nuclear envelope (NE) or ER, while maturing face is at distal pole. Note apparent single unit membrane (arrows) surrounding each lipid body (LB). N = nucleus.—Fig. 169. Portion of cell which is still elongating containing a large population of dictyosomes (D). The dictyosomes are actively proliferating numerous hypertrophied vesicles containing fibrillar material. The cytoplasm is rich in ribosomes. ER = endoplasmic reticulum; LB = lipid body; M = mitochondrion; V = vacuole.—Fig. 170. High magnification of active dictyosomes (D) in elongating cell. Note fibrillar contents of hypertrophied vesicles. The cytoplasm is richly populated with ribosomes (Rb).

Bars represent 0.5 μm (Figs. 168-170).


N
NE
D
LB
ER
168
V
D
ER
LB
M
169
170

bizarrely misshapen gametophyte nuclei with chromosomal complements varying from haploid through different degrees of ploidy (Martens 1963; Sporne 1965) seem incapable of further division.

Upon hydration collar nuclei are rapidly activated with surface contours assuming slight irregularities as metabolic activity in these cells intensifies. While the irregularities are noticeable they are not as pronounced as the convolutions common to gametophyte nuclei. As the embryo germinates the collar forms a wedge-shaped feeder, the ventral surface of which becomes firmly attached to the gametophyte tissue. After approximately 4 to 5 days nuclei of feeder cells in contact with the gametophyte become increasingly irregular. While initial irregularity in nuclear contours is probably associated with heightened cellular activity (Clowes and Juniper 1968), ultimate accentuation of these nuclear lobes seems to be connected with senescence.

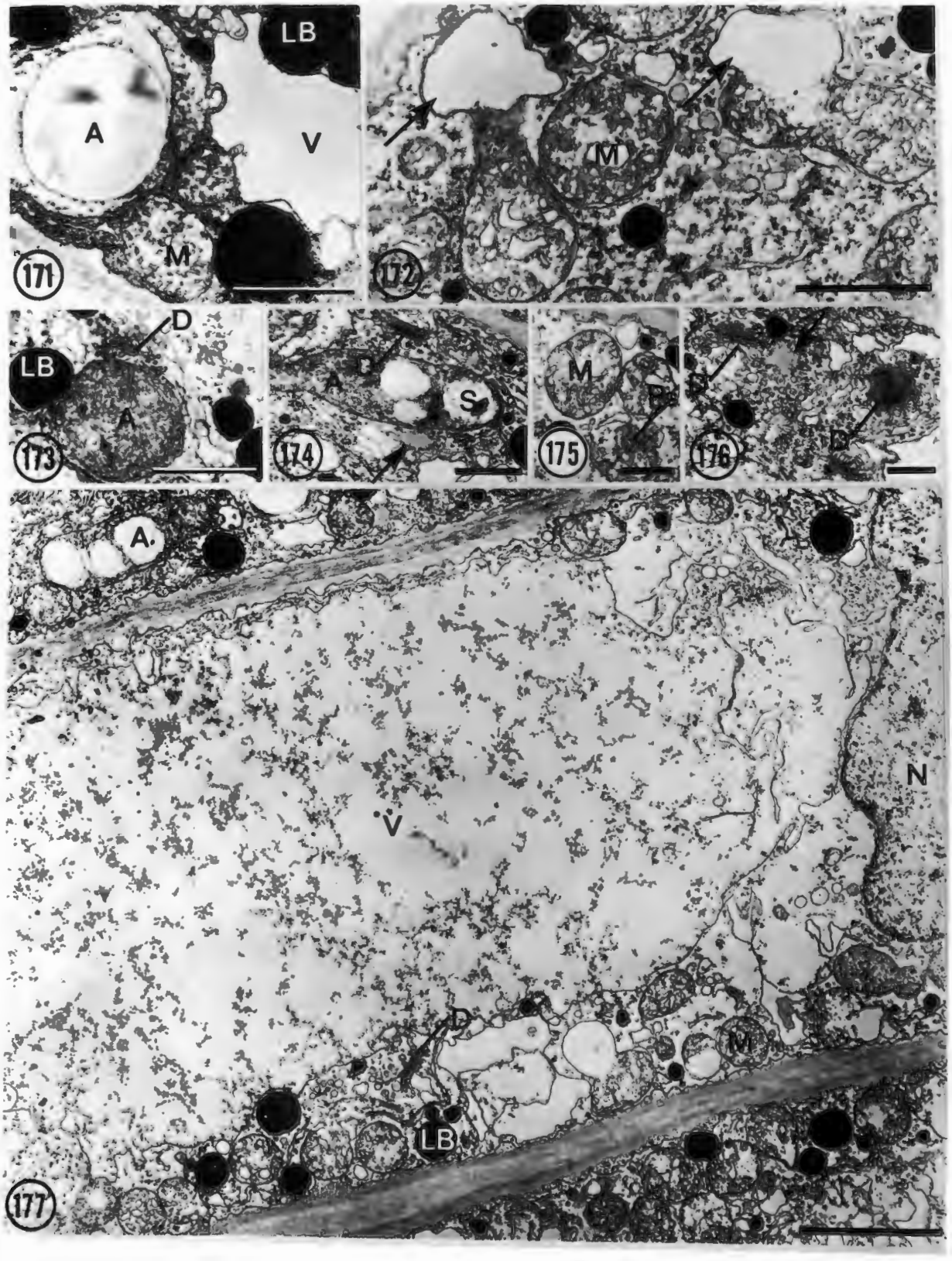
The slightly flattened ovoid-shaped nuclei within the highly vacuolate, elongate inner feeder cells also develop small surface irregularities as metabolic activity rises. The shape of these nuclei and the marginal position assumed are not suggestive of further cell division (Yeoman and Street 1973) which in fact is confined to the base of the emergence (Martens 1971).

Apart from shape and clarity of fixation dry collar and gametophyte nuclei display similar ultrastructural features. In both instances the most outstanding feature of the quiescent

PLATE 48.

Figs. 171-177. Inner feeder cells at 5-day-stage.—Fig. 171. Swollen mitochondria (M) and amyloplast (A) with deteriorating matrices. These degenerating organelles are eventually sequestered in autophagic vacuoles (V). LB = lipid body.—Fig. 172. Mitochondria (M) frequently burst and produce large electron-transparent extrusions (arrows).—Fig. 173. Amyloplast (A) that has lost its starch reserves. D = dictyosome; LB = lipid body.—Fig. 174. Healthy amyloplast (A) with starch reserves (S). Note non-membrane bound finely granular material (arrow) in cytoplasm. D = dictyosome.—Fig. 175. Healthy mitochondria (M). Ps = polysome.—Fig. 176. Dictyosomes (D) decline in number and activity. Note non-membrane bound finely granular material (arrow) in cytoplasm.—Fig. 177. L.S. portion of internal feeder cell. It is thin-walled, highly vacuolate (V) and well supplied with mitochondria (M). A = amyloplast; D = dictyosome; LB = lipid body; N = nucleus.

Bars represent 1 μm (Figs. 171-176), 2.5 μm (Figs. 177).


LB
A
V
M
171
M
172
D
LB
A
173
A
S
174
M
175
D
176
A.
V
N
D
M
LB
177

nuclei lies in the vast profusion of fibrous inclusions which anastomose throughout the nucleoplasm and nucleolus. The occurrence of nuclear inclusions in the plant kingdom is widespread and has been reported in more than 200 plant species including ferns, gymnosperms and angiosperms (Wergin *et al.*, 1970). Despite this, nuclear inclusions in *Welwitschia* are unique, differing from all previous descriptions firstly in their superabundance and secondly in their presence within the nucleolus.

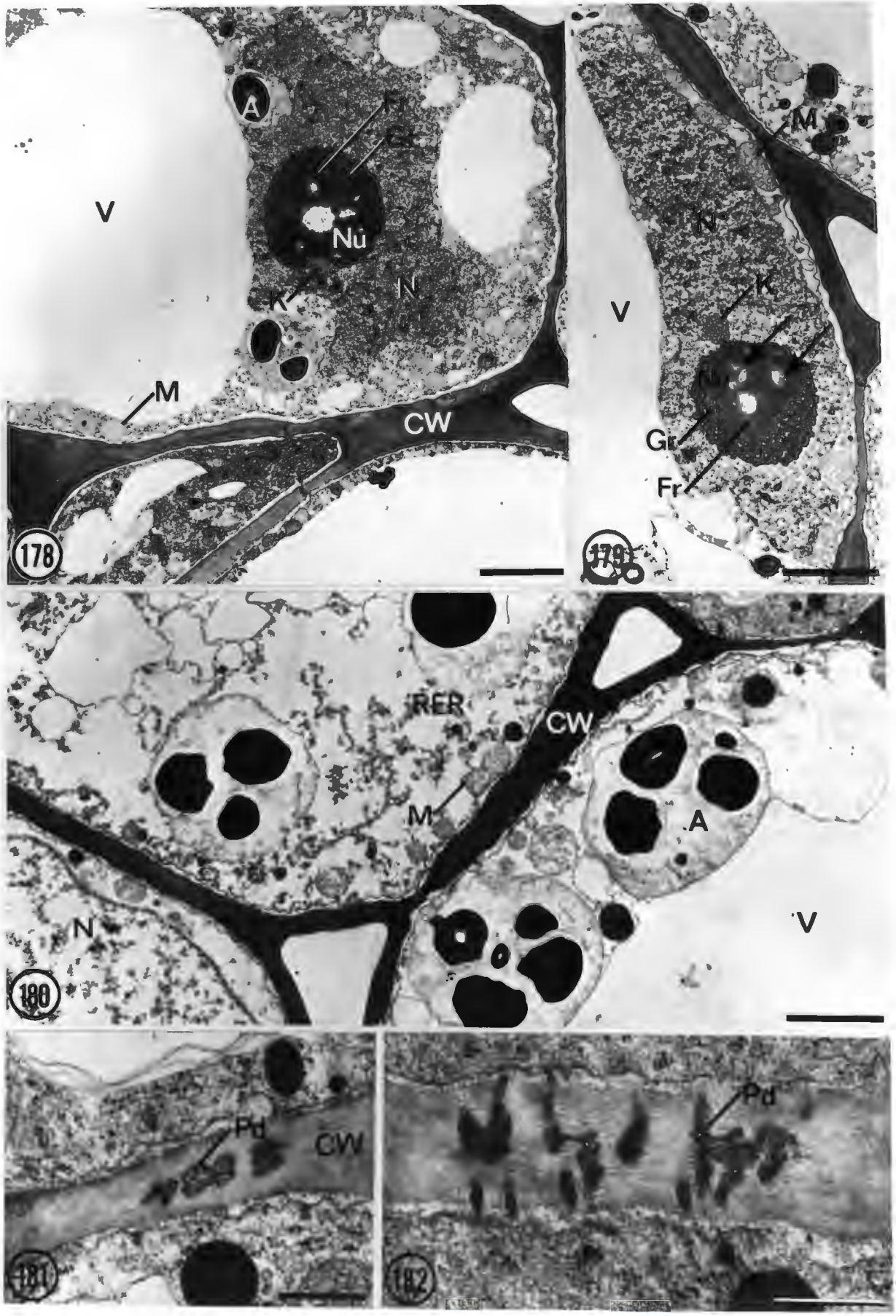
In the collar, inclusions which are morphologically identical to those seen in the nucleus are also distributed within the cytoplasm. Inability to locate similar cytoplasmic inclusions within gametophyte cells does not necessarily prove their absence. They could possibly be obscured by the massive stores of reserve materials. The occurrence of similar inclusions in both nucleus and cytoplasm has been reported for certain cell types in six other species of plant viz: *Pisum sativum* (Yoo 1970), *Pinus nigra, Coleus blumei, Glycine max, Lens culinaris* and *Phaseolus vulgaris* (Wergin *et al.*, 1970).

In some plants e.g. *Lens culinaris* inclusions have been found in the peri-nuclear space of the nuclear envelope and also in the cisternae of the ER (Lance-Nougarède 1966). In *Welwitschia* cytoplasmic inclusions of the dry collar occur free and unbound by membranes. It should however, be borne in mind that ER is apparently fragile and difficult to preserve in the dry state (Yatsu 1965; Abdul-Baki and Baker 1973).

PLATE 49.

Fig. 178. TS. inner feeder cell at 5-day-stage. Cells are thin-walled (CW) and highly vacuolate (V). Lobed nucleus (N) is moving to a marginal position. Nucleolus (Nu) is still large and vacuolate. Note interspersion of fibrillar (Fr) and granular (Gr) regions. A = amyloplast; K = karyosome; M = mitochondrion.-Figs. 179-182. TS. inner feeder cells at 5.5-day-stage.-Fig. 179. Marginal nucleus (N) is elongated and less lobed. Nucleolus (Nu) though still large and vacuolate is beginning to assume a less active appearance due to separation of fibrillar (Fr) and granular (Gr) regions into distinct zones. Embedded chromosome material (arrows) is still observed within fibrillar zone. K = karyosome; M = mitochondrion; V = vacuole.-Fig. 180. TS. highly vacuolate (V) cells in which cytoplasm is usually restricted to thin layers lining cell walls (CW). Cytoplasm and nucleoplasm often assume a leached appearance. Mitochondria (M) are plentiful and starchfilled amyloplasts (A) are prominent. N = nucleus; RER = rough ER.-Fig. 181. Plasmodesmata (Pd) in cell wall (CW). Oblique orientation results in only median cavities being visible.-Fig. 182. Plasmodesmata (Pd) are similar to those described for gametophyte tissue. They consist of plasmalemma-lined pores which are often joined in the median region.

Bars represent 2.5 μm (Figs. 171-173), 0.5 μm (Figs. 181, 182).


A
Fr
Gr
V
Nu
N
K
M
CW
178
M
N
V
K
Nu
Gr
Fr
179
RER
CW
M
A
N
V
180
Pd
CW
181
Pd
182

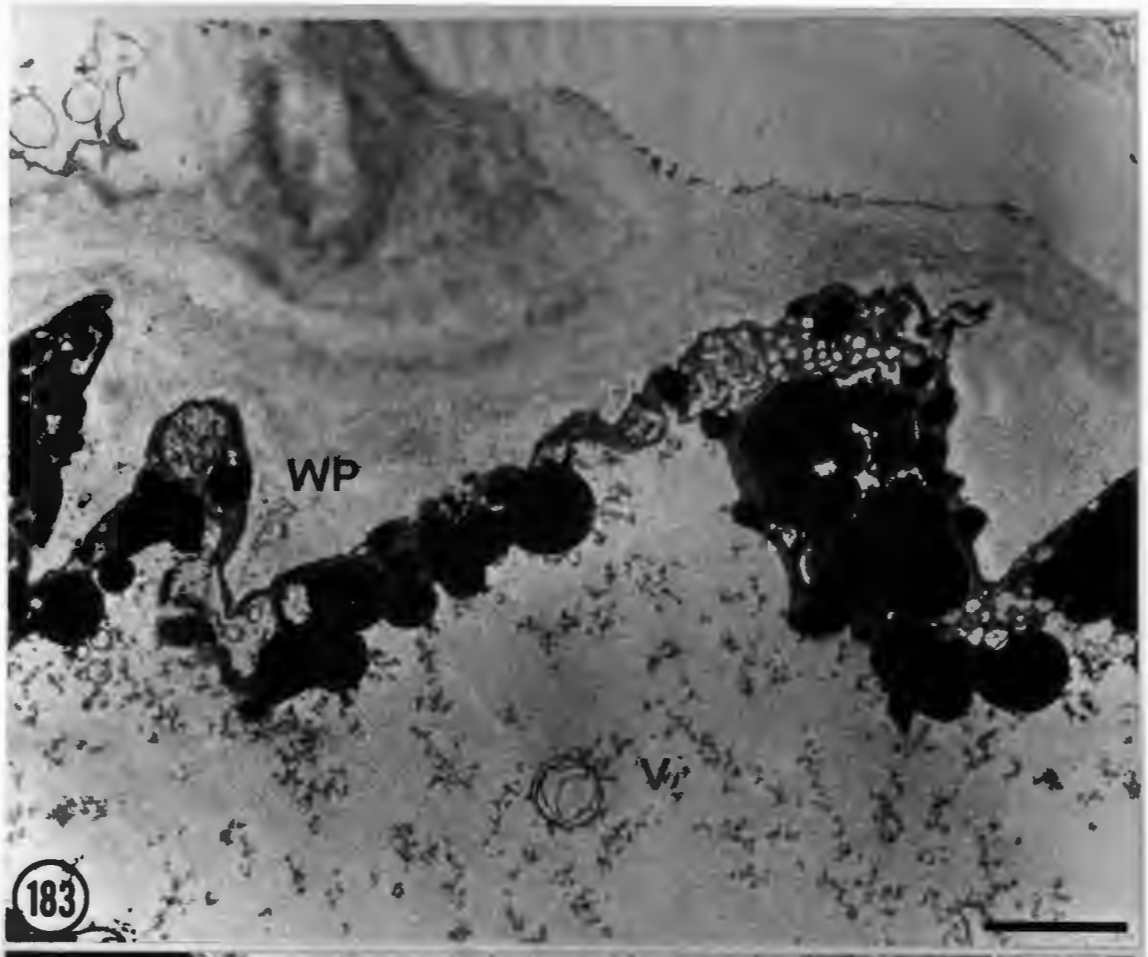
Their are three possible origins of morphologically similar nuclear and cytoplasmic inclusions (Wergin *et al.*,1970). The inclusions may originate in the cytoplasm and become incorporated in the nucleus, or arise in the nucleus and spread to the cytoplasm, or arise independently of one another in the nucleus and cytoplasm. The apparent absence of cytoplasmic inclusions in gametophyte cells coupled with their concentration primarily in the region immediately surrounding the nucleus in collar cells seems to suggest that in *Welwitschia* fibrous inclusions originate in the nucleus. Fibrous nuclear and cytoplasmic inclusions present in mature dry *Welwitschia* seeds disperse rapidly and disappear within 24h of imbibition. Consequently an ultrastructural study of developing and maturing seed might help to clarify the origin of these inclusions. Apparently the majority of previously described nuclear and cytoplasmic inclusions have been observed in hydrated cells of mature plants (Wergin *et al.*, 1970).

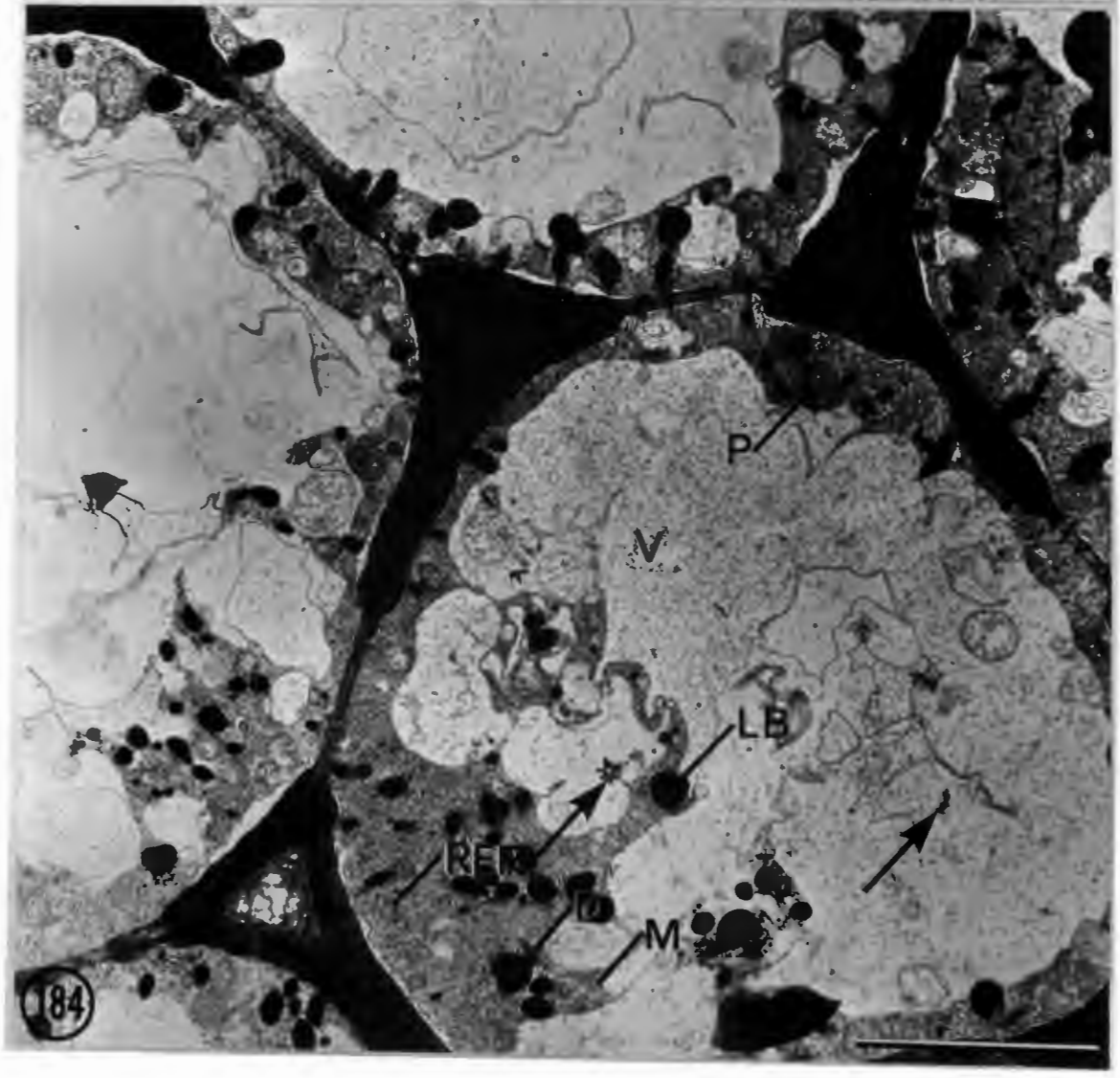
In transection cytoplasmic fibrous aggregates are roughly circular while in longisection they are variable in shape. Although they appear in section as scattered, separate entities, the possibility exists that they are connected to form a continuous anastomosing network in the cytoplasm which is similar to that found in the nucleus. To verify this, numerous serial sections will have to be cut. The cytoplasmic inclusions appear to be particularly associated with the nucleus, although sporadic associations between the inclusions and lipid bodies, protein bodies, plastids, and mitochondria do exist.

PLATE 50.

Figs. 183, 184. Inner feeder cells at 6-day-stage.—Fig. 183. L.S. portion of highly vacuolate inner feeder cell. Note wall projections (WP) and related plasmalemma convolutions. V = vacuole.—Fig. 184. T.S. basal feeder cells in vicinity of vascular strand. Central vacuole (V) is large and autophagically active containing degrading organelles, unidentified membranous material and osmiophilic crystal remnants (arrows) believed to be globoid in nature. Cytoplasm contains numerous mitochondria (M) and short segments of rough ER (RER). Lipid bodies (LB) are scattered, dictyosomes (D) are few and plastids (P) devoid of starch.

Bars represent 1 μm (Fig. 183), 5 μm (Fig. 184).


WP
V
183


P
V
LB
RER
M
184

When the fibrous inclusions were tested for the presence of protein using mercuric bromphenol blue (Pearse 1961) a positive reaction was obtained. This is in agreement with the work of other investigators (Zimmerman 1891; Barton 1967; Amelunxen and Giele 1968; Villiers 1968; Briarty *et al.*, 1970; Wergin *et al.*, 1970; Yoo 1970) who are of the opinion that similar nuclear and cytoplasmic inclusions in other plant species are also proteinaceous. The function of these inclusions is obscure. It has been previously suggested that cytoplasmic fibrous inclusions may represent either a storage form of protein (Yoo 1970) or enzymes that are activated upon imbibition (Briarty *et al.*, 1970). While nuclear and cytoplasmic inclusions in *Welwitschia* are morphologically identical, it is difficult to believe that the entire nucleus could be pervaded with stored enzymes. It has never been established whether the inclusions also contain ribonucleic acid, as the use of RNase on tissue embedded in Epon-Araldite plastics gives inconsistent results (Wergin *et al.*, 1970). Wergin *et al.*, (1970) suggest that water-soluble plastics will be required to obtain reliable results.

A very close association seems to exist between nuclear inclusions and nucleoli in *Welwitschia mirabilis*. This feature is discussed in the section devoted to the gametophyte. Recent evidence indicates the strong possibility of an inverse size relationship between nucleoli and nuclear inclusions in several plant species (Wergin *et al.*, 1970). In fact, in *Dahlia variabilis* crystals have been seen to completely replace the

nucleoli of guard cells under certain environmental conditions (Weber 1926). In the fern *Camplyoneuron phyllitidis L.* Wergin *et al.*, (1970) report that the appearance of nuclear inclusions during cell differentiation may coincide with dissolution of the nucleolus. As a result of these and other fine structural studies (Meyer 1920; Thaler 1966) the suggestion has been made that inclusions and nucleoli are interconvertible structures (Wergin *et al.*, 1970). Attempts have been made to induce nuclear inclusions by varying environmental conditions (Wever 1926; Frey-Wyssling and Mühlethaler 1965) and some success has been recorded. There are few reports of nuclear inclusions in seed material (Villiers 1968; Wergin *et al.*, 1970 .In the majority of papers the development of inclusions was followed in nuclei of hydrated, active and differentiating cells. In contrast to this nuclear inclusions of *Welwitschia mirabilis* seed are present in the dry state but disappear rapidly upon imbibition. Further discussion of nuclear inclusions appears elsewhere in the section devoted to the gametophyte.

Apart from the presence of fibrous inclusions within nucleolar vacuoles, dry collar nucleoli are similar in appearance to previously described quiescent and/or dormant nucleoli (Hyde 1967; Jordan 1971). In the dry, quiescent state they consist of densely packed darkly-staining, fibrillar material with no apparent granular zone. In contrast to gametophyte nucleoli they also possess large dense scattered granules. Hyde (1967) reports that osmium tetroxide (OsO4) buffered with phosphate

at pH 8 produces a nucleolar image containing clear dense granules while OsO4 buffered with cacodylate produces an image devoid of these granules. In this study both embryo and gametophyte tissue were fixed in cacodylate-buffered osmium. Granules appeared in dry embryonic nucleoli but not in gametophyte nucleoli. The nature of these large granules is unknown. Similar to previous observations (Jordan 1971) chromosomal nucleolar organizing regions embedded in quiescent nucleoli stain lighter than the rest of the condensed chromosomal material in the nucleus.

Upon hydration the ultrastructure of the nucleolus changes significantly. Perhaps the most outstanding change lies in the immediate disappearance of nuclear fibrous inclusions. Apart from this there is a very definite change in the size of the nucleolus. It is well-known that there is a relationship between nucleolar size and cellular metabolic activity, particularly protein synthesis (Hyde 1967). On activation the nucleolus swells in size, simultaneously becoming less compact especially in the developing outer granular zone. At the same time the chromosomal organizing regions become deeply embedded in the inner fibrillar zone. As the peripheral granular zone enlarges and becomes interspersed in the central fibrillar core there is a corresponding dramatic increase in cellular activity.

It is widely accepted that the nucleolus is responsible for the DNA-dependent synthesis of ribosomal precursors (Jordan 1971). DNA of the nucleolar organizer is transcribed into

rRNA by an RNA polymerase enzyme. Molecules of two different sizes are formed viz. 18S and 28S ribosomal RNA (rRNA) molecules and methyl groups are incorporated (Jordan 1971). Apparently the released 18S and 28S rRNA molecules coil up forming 40S and 60S ribosomal subunits. The smaller 40S subunit is processed faster than the 60S subunit and Jordan (1971) suggests that it passes straight out of the nucleolus and nucleus. It takes approximately an hour for the 285 rRNA molecule to become the large 60S subunit and it is thought that the granules of the nucleolar granular zone represent an accumulation of these particles (Jordan 1971). Little is known about the transport of ribosomal subunits from the nucleolus to the cyto-plasm. In Fig. 163 masses of dark granules, possibly 60S rRNA subunits, stream from the periphery of the nucleolus toward the nuclear envelope. They are frequently observed in contact with the inner surface of the nuclear envelope but have never been seen to pass through the nuclear pores. Structurally the nuclear pores (Fig. 164) are similar to existing descriptions (Yoo and Bayley 1967; Jorgan 1971). Each consists of an annulus composed of eight subunits and a central granule. It has been suggested that the transport of ribosomal precursor particles probably involves some unwinding as the particles pass through the nuclear pores (Jordan 1971). Once within the cytoplasm the 60S subunits give rise by combination with 40S subunits to ribosomes (Jordan 1971).

Between the fourth and sixth days of germination, surface cells of the feeder interface region begin to senesce and deve-

lop increasingly irregular nuclei. Nuclei of deeply-situated feeder cells present an active appearance as the cells elongate and vacuolate. By the time elongation has been completed, usually between the fifth and sixth days of germination, nuclei have assumed marginal positions against the wall. The nucleoli are still fairly large and vacuolate with embedded chromosomal material but appear less active due to the separation of the fibrillar and granular zones. Ultimately the nucleolus shrinks in volume and apparently reverts to a quiescent state.

The spherical body or karyosome, frequently seen either attached peripherally to the nucleolus or in a satellite position in dry and imbibed collar cells has been described by La Fontaine (1965) in three separate plant species. According to Hyde (1967) the karyosome is probably a universal component of plant meristem nuclei.

In the dry state the nucleoplasm of collar and gametophytic nuclei are very similar in appearance. The branching fibrous inclusions take up all available space not occupied by condensed chromatin and are frequently surrounded by darkly staining granular material. As in the gametophyte this coarse electron-dense material stains with a similar intensity to nucleolar material (Fig. 122).

Lobing of an active nucleus has already been discussed. In addition to this the number of membrane continuities between the ER and the nuclear envelope tends to increase and the nuclear envelope also appears to bleb.

Protein Bodies

Embryo and gametophyte protein bodies are strikingly different. While the vast majority of gametophyte protein bodies are large (diameter ca. 5 μm) and filled with reserves, those of the embryo are small in size (diameter ca. 1.8 μm) and contain meagre reserves. These reserves usually consist of scattered lumps of electron-dense protein-carbohydrate material, the larger lumps of which may be mottled with electron-transparent areas. Their appearance is further suggestive of a continuous digestive process beginning before and being resumed after the period of quiescence. However study of developing protein bodies in maturing *Sinapis alba* seeds (Rest and Vaughan 1972) have shown that protein body reserves are initially laid down in lump form. It is therefore possible that the sparse protein body reserves present in dry *Welwitschia mirabilis* embryos merely reflect the reserves built up prior to quiescence. It is evident that a study of developing and maturing seed is needed.

Digestion of protein body reserves begins almost immediately upon imbibition, followed shortly by the start of lipid degradation. Activation of embryonic collar cells precedes that of gametophyte cells in which rate of activation seems to be governed by relative distance from the embryo. As previously mentioned this sequence of activation seems to suggest a degradative mechanism controlled by the embryo, possibly by means of a diffusing stimulatory factor from the embryo.

Whereas globoids are scarce in the protein-carbohydrate rich

protein bodies of the gametophyte, they are common in the protein bodies of dry collar cells which in contrast contain scanty reserves. They exhibit characteristic globoid staining properties (Vazart 1960; Poux 1965; Villiers 1971) and apparently represent an accumulation of phytin (Jacobsen *et al.*, 1971). Because the phytin (globoid) content of the gametophyte is low while that of the embryo is high, it would seem that the embryo probably supplies the gametophyte with phosphate. In the dry state globoids are represented by solid and fragmented electron-dense inclusions. The fragmentation of some of the globoids suggests a certain amount of digestion begun before quiescence. Following hydration the digestion pattern is identical to that already described for gametophyte globoids. It entails an internal pattern of digestion leading to pitting and ultimate disintegration of the globoid.

Little is known about the origin of globoids. Although it is widely believed that globoids precipitate out within the protein body (Frey-Wyssling and Mühlethaler 1965; Rest and Vaughan 1972) Morrison *et al.*, (1972) have suggested that in wheat the globoids may have a cytoplasmic origin. In the embryo of *Welwitschia mirabilis* small crystals can be seen entering protein bodies within 24h of imbibition (Figs. 135, 136). Once within a protein body these cytoplasmic crystals appear to be attracted to one another. Figs. 136, 137, 138 show various stages of crystal agglomeration. The final crystal accumulation, as in Fig. 138, looks remarkably like a globoid even to possessing a loose-fitting envelope. This envelope

might be formed by the fusion of the outer bounding envelopes (Fig. 136) of the small agglomerating crystals. Although as yet there is no direct evidence that the small cytoplasmic crystals are globoid in nature, the final product of their apparent accumulation exhibits a characteristic globoid staining reaction with Toluidine blue (Vazart 1960; Jacobsen *et al.*, 1971) and typical globoid high acid phosphatase activity (Poux 1965; Villiers 1971). In gametophyte tissue small cytoplasmic crystals are sometimes seen apparently migrating into protein bodies between days 3 and 6. However they are not very common and do not seem to aggregate within the protein body. It is not known whether they are similar in composition to the cytoplasmic crystals of the embryo. Available evidence seems to suggest that globoid origin is cytoplasmic in the embryo and could be cytoplasmic in the gametophyte.

Upon hydration the immediate and rapid digestion of protein body reserves seems to suggest the presence of pre-existing hydrolytic enzymes laid down within the body prior to quiescence. Perhaps the many vesicles present in the dry protein bodies could contain the enzymes responsible for reserve hydrolysis. However this is mere speculation.

Protein body reserves are rapidly consumed leaving numerous small aqueous vacuoles which fuse to form a single large vacuole. In feeder interface cells this vacuole is characteristically located close to the outer interface wall (Fig. 146).

At the 3- to 4-day-stage interface cells near the base of the feeder begin to senesce. Breaks occur in vacuole membranes leading to the release of acid phosphatase and possibly other hydrolytic enzymes. Cell contents are lysed and the cells themselves eventually crushed. In contrast to surface interface cells, inner feeder cells contain much larger vacuoles with cytoplasm usually restricted to thin layers lining the cell walls. In these deeply-situated cells vacuolar membranes retain their integrity and consequently no lysis of cell contents is observed even at the 6-day-stage. The presence of included degrading organelles and membranes, however, indicates that the vacuoles are autophagically active.

Lipid Bodies

Lipid bodies of the dry embryo stain very darkly in comparison with those of the gametophyte. Several authors (Frey-Wyssling *et al.*, 1963; Rest and Vaughan 1972) studying developing seeds of various species have noticed a decrease in electron-density of lipid bodies with maturation and it is thought that the loss of stainability represents a change in the nature of the lipid (Rest and Vaughan 1972). The darkly staining lipid bodies in quiescent *Welwitschia* embryos are therefore possibly not as mature as those of the gametophyte.

In the literature lipid bodies are characteristically described as being in direct contact with the plasmalemma and protein body membranes. This is the case in the majority of gametophyte cells in *Welwitschia* with the exception of gametophyte interface cells in which the lipid bodies appear loosely

scattered with some bodies aligned along the cell wall. In the dry embryo lipid bodies line the plasmalemma and, although they appear in loose arrangements around the protein bodies, are not seen in contact with their membranes (Fig. 125). It is only upon hydration that collar lipid bodies become closely apposed to the protein body membranes (Figs. 133, 134). Perhaps this, too, is a reflection of the relative immaturity of lipid bodies in the dry embryo.

Controversy surrounds lipid body origin (see gametophyte discussion). Many investigators believe that lipid bodies arise from the ER (Grieshaber 1964; Frey-Wyssling and Mühlethaler 1965; Balz 1966; Semadeni 1967; Schwarzenbach 1971; Werker and Vaughan 1974). Supporting evidence for this suggestion is to be found in reports of limiting membranes surrounding lipid bodies in various seed species (Grieshaber 1964; Frey-Wyssling and Mühlethaler 1965; Paleg and Hyde 1964; Abdul-Baki and Baker 1973) and in the reports of ribosomes attached to lipid body membranes in *Sinapis alba* (Werker and Vaughan 1974) and ER segments attached to lipid bodies in *Pisum sativum* (Yoo 1970). However many authors (Jacobsen *et al.*1971; Harwood *et al.*, 1971; Rest and Vaughan 1972; Swift and Buttrose 1973) have been unable to resolve a tripartite peripheral unit membrane around the lipid body. Consequently other modes of origin (Hardwood *et al.*, 1971; Rest and Vaughan 1972) have been postulated (see gametophyte discussion). Schwarzenbach (1971), while agreeing that lipid bodies arise from the ER, suggests that oil synthesis takes place between

the layers of the unit membrane resulting in a mature lipid body bounded by a half-unit membrane i.e. bounded by the outer protein layer of the original unit membrane.

Lipid bodies in *Welwitschia* gametophyte possess a thin peripheral electron-dense line, but there is no evidence of a unit membrane. The thin bounding line seems to be more in the nature of a half-unit membrane, as suggested by Schwarzenbach (1971), than a three-layered unit membrane. In contrast to this, lipid bodies in the developing feeder (embryo) are sometimes surrounded by a distinct tri-partite unit membrane (Fig. 168 - arrows). Numerous ribosomes are also seen attached to the periphery of lipid bodies in dry collar (embryo) material. Lipid body membranes are not discernible in this dry material, but it is well-known that membranes are fragile and often difficult to preserve in the dry state. These facts make it tempting to conclude that lipid bodies are indeed derived from the ER. The absence of a definite three-layered unit membrane in mature lipid bodies may be due to differentiation that the membrane undergoes resulting in the half unit structure proposed by Schwarzenbach (1971).

On hydration lipid stores decline rapidly. The lipid bodies no doubt provide a source of metabolic substrates and possibly supply lipids to membrane systems, particularly the ER (Yoo 1970). Concurrent to lipid degradation starch reserves appear in amyloplasts. Approximately 3 to 4 days after germination the lipid (and protein) stores have been dramatically reduced. At this stage (Figs. 12, 14) the plumule is still

enclosed within the seed and is therefore incapable of photosynthesis. However the feeder is developing rapidly (Fig. 13) and it is believed that breakdown products (e.g. sucrose) from the lipid bodies of the gametophyte are absorbed by the feeder.

It has been previously suggested that lipid bodies (spherosomes) may be lysosomal (Sorokin and Sorokin 1966). Matile and Spichiger (1968) working on tobacco endosperm cells isolated lipid bodies in which a number of acid hydrolases (including acid phosphatase) were localised. In *Welwitschia* gametophyte and feeder (embryo) tissue were incubated in Gomori medium (1952) modified after Berjak (1968). While the lipid bodies in gametophyte tissue showed a negative staining reaction (Fig. 116) those of the embryo stained darkly (Figs. 117, 120, 121), apparently indicating the presence of acid phosphatase. However, the lipid bodies in *Welwitschia* do not appear to perform a lysosomal function.

Amyloplasts

Starch-rich amyloplasts are a common feature of dry collar cells. The starch probably forms a readily available energy source on which the germinating embryo can draw to extrude a large lateral process or feeder in a short space of time (Butler 1970). The starch is rapidly utilised during the first 24h of cellular activity following imbibition. During the next 12h much of the starch is replaced as collar lipid reserves are hydrolysed. As the feeder develops starch reserves

fluctuate but are usually a constant cell feature. It is suggested that a large percentage of starch present in the mature feeder is formed as the result of mobilisation and transport of sucrose from the gametophyte into the embryo.

Microbodies

Microbodies are rare in dry collar tissue. Upon hydration they increase exponentially for 24h but soon decline in number between 24 and 48h. The build-up of the microbody population parallels the rapid decline of lipid stores during the early stages of cellular activity following imbibition. Forty-eight hours after germination lipid stores are greatly depleted and the microbody population drops rapidly. The close association of microbodies with lipid bodies and their corresponding increase as lipid stores decline suggests that they might belong to the previously discussed class of microbodies called glyoxysomes (Breidenbach *et al.*, 1968). Glyoxysomes are known to contain the enzymes for β-oxidation of fatty acids and the glyoxylate cycle which converts acetyl CoA to succinate (Breidenbach and Beevers 1967).

Mitochondria

Randomly distributed mitochondria are present in the cytoplasm of dry collar cells. They contain an electron-transparent matrix and very few cristae. On hydration the mitochondria lose their original electron-transparency and, within 36h, have developed numerous cristae. Between the second and fourth day of germination the mitochondria increase dramatically in number especially in the feeder interface cells in contact

with the gametophyte (Figs. 146, 152). In these interface cells the mitochondria are primarily concentrated along the cell walls. At the 4-day-stage interface cell walls have usually developed many small wall projections (Figs. 151, 152). Although they are not as pronounced as the transfer-type wall projections originally described by Pate and Gunning (1972), they do result in a greater absorptive surface area. The large numbers of mitochondria, indicative of a high respiration rate, coupled with the apparent development of transfer-type wall configurations, might suggest the active uptake of nutrients from the gametophyte. At this stage, apart from a few sporadic lipid bodies, reserve materials in the feeder have largely disappeared. The plumule is still within the seedcoat (Fig. 14) and is therefore incapable of photosynthesis.

In contrast to the mitochondria of surface interface cells those of inner feeder cells, together with rough ER and amyloplasts, tend to develop close spatial relationships with the nucleus.

At the 6-day-stage the cotyledons are completely withdrawn from the seed. Once exposed to light the orange cotyledons gradually change to a bright green colour as chlorophyll is increasingly synthesised in the plastids. The seedling is now capable of photosynthesising and should soon be independent of remaining nutrient material in the gametophyte. At this time mitochondria can often be seen in various stages of senescence. They swell and develop electron-transparent areas.

Dictyosomes

In common with many previous studies (Varner and Schidlovsky 1963; Paleg and Hyde 1964; Yatsu 1965; Paulson and Srivastava 1968; Abdul-Baki and Baker 1973) dictyosomes are unrecognisable in the dry embryo. They appear initially on the first day of imbibition, increasing dramatically in number between the 2- and 3-day-stage.

In interface cells dictyosome activity is prolific between the 2- and 4-day-stages. Dictyosome-derived vesicles containing fibrillar material migrate toward and fuse with the plasmalemma. The released fibrillar contents are presumably contributed to the cell walls (particularly the outer cell walls) which, by the fourth day, have thickened considerably (Fig. 151). The outer interface walls in actual contact with the gametophyte also assume a mucilaginous appearance. From the fourth day dictyosome activity diminishes and no further increase in wall thickness is observed. Between the 4- and 6-day-stages interface cells may show signs of senescence. In senescing cells many small vesicles accumulate between the cell wall and plasmalemma. They are dissimilar to the previously described dictyosome vesicles in that they contain no fibrillar material. Their function is obscure.

The feeder results from division and elongation of cortical cells in the collar region. It is non-vascularised, consisting of elongated thin-walled cells. Inner feeder cells which are actively elongating contain a large population of dictyosomes

which produce hypertrophied vesicles with fibrillar contents. These vesicles fuse with the plasmalemma and release their contents. The vesicle membranes are apparently incorporated into the plasma membrane (Morré *et al.*, 1971). Despite addition of material to the cell walls, they remain thin. It is suggested that elongation of the cell is responsible for the constant wall thickness observed in inner feeder cells. Once the cells have ceased elongating the dictyosomes decrease in number, no longer produce hypertrophied vesicles, and structurally resemble the dictyosome depicted in Fig. 168.

ER

Most studies have indicated that ER is fragmentary and of infrequent occurrence in dry seed tissue (Yatsu 1965; Bain and Mercer 1966; Paulson and Srivastava 1968; Yoo 1970; Hallam *et al.*, 1972; Abdul-Baki and Baker 1973). In *Welwitschia* ER is present in dry collar cells as short stacked segments of rough ER. Although its distribution is sparse, it is much more plentiful than the ER of dry gametophyte tissue. Upon hydration protein body reserves are rapidly degraded. Accompanying this degradation numerous long and short profiles of rough ER appear scattered throughout the cytoplasm. Despite the rich population of ribosomes in dry tissue, no polysome configurations are apparent. They are first observed approximately 36h after initial imbibition. The prompt proliferation of ER and formation of polysomes are thought to indicate synthesis of enzymes and structural proteins. Rough and smooth ER are evident in cells of the interface zone at the 2-day-stage.

They may occur as separate profiles or may interlead. From the second day onward smooth ER is seen to inflate and rough ER to bleb. Approaching the fourth day of germination inflation of smooth ER becomes more frequent with rough ER invariably leading into and out of the localised dilations. Unidentified membranous fragments are frequently found within the inflations which apparently indulge in autophagic activity (Fig. 150). It is thought that these dilations may have a lysosomal function (Berjak and Villiers 1970). As outer interface cells senesce, the ER becomes increasingly dilated.

In deeply-situated feeder cells frequent dilations of the ER system become apparent between the 3- and 4-day-stages (Figs. 156, 157). After the fourth day the nuclear envelope is often seen to bleb (Fig. 158) and membrane continuities between the ER and nuclear envelope may dilate (Fig. 159). Some, but not all, dilations act in an autophagic manner. It is possible that the swellings may reflect senescence resulting from damage to the structural integrity of the membrane system (Villiers 1972). However minor dilations of the ER have also been observed to occur in cells which are not senescent e.g. in active cells of the developing micropylar endosperm of *Capsella* during embryogenesis (Schulz and Jensen 1974).

From the second day of germination polysome configurations become rife, occurring free and attached to the ER and nuclear surface. Between the 5- and 6-day-stages, as the plumule emerges, highly vacuolate inner feeder cells experience a

drop in ribosomal population with the cytoplasm concomitantly taking on a leached appearance. In the vicinity of lipid bodies, ribosomes frequently stain more heavily with lead and uranium salts than those in the surrounding cytoplasm (Fig. 147). It is not known whether this is due to a chemical or physical difference in the protein or nucleic acid component of these ribosomes.

Cell Walls

In the dry state collar cells are thin-walled (0.2 μm). From imbibition until the 4-day-stage feeder interface cell walls thicken, particularly the outermost walls (0.9 μm) in contact with gametophyte tissue. Approaching the fourth day of germination wall projections invested with plasmalemma begin to form along the inner surface of the outer wall (Fig. 150). After four days germination wall projections with associated plasmalemma convolutions have increased and cover the entire inner wall surface of the cell (Fig. 152). These projections, together with the previously mentioned overlapping nature of the outer cells, results in a much greater absorptive surface area. Although the wall projections are not as pronounced as the transfer-type wall configurations described by Pate and Gunning (1972) it is suggested that these cells probably function as transfer cells with the wall projections facilitating absorption and/or secretion of substances. The large numbers of mitochondria occurring in these cells (Fig. 152) might suggest the active uptake of nutrients.

Interface cell walls presumably thicken as a result of dictyo-

some activity (Fig. 149). While fibrillar material is contributed to the inner surface of the cell walls, the outer part seems to disintegrate or become less compact (Fig. 148). At the 4-day-stage the thick outer walls have assumed a mucilaginous texture. Perhaps the mucilaginous nature of these cell walls is partly responsible for the very firm adherance between the feeder and gametophyte. Considerable force is required to separate them. With the gametophyte and feeder firmly adhering together, the transfer of nutrients is probably facilitated. The adherance could also have a secondary function. The feeder is anchored in the gametophyte tissue, providing the plumule with a firm base as it emerges from the seed.

The thickness of interface cell walls was initially surprising as thin-walled cells had been expected. The overlapping of outer interface cells coupled with their relatively short life span and development of thick mucilaginous walls led one to suspect that these cells might be of root cap origin. Light microscopical investigation of a 7h-imbibed embryo revealed a root tip protected by a massive root cap (Butler 1970). This extensive root cap telescopes backwards until approximately the midpoint of the collar. This observation supports the conclusion that the lower feeder interface cells are of root cap origin. If these cells are in fact root cap cells, this might explain the positive geotropism of the feeder. Recent work *inter alia* by Wilkins and Wain (1975) has shown that root cap cells may be geoperceptive.

As previously mentioned outer interface cells are loosely overlapped. At approximately the 4-day-stage degradation of the middle lamella region can be observed starting at the tips of overlapped cells. By day 6 (Fig. 154) this disintegration has progressed markedly. The region of degradation coincides with areas where acid phosphatase is located. As the cell senesces numerous small vesicles are discharged and accumulate between the plasmalemma and cell wall (Fig. 153). These vesicles do not show a positive staining reaction for acid phosphatase. However this does not rule out the possibility that these vesicles might contain other hydrolytic enzymes.

As the feeder develops inner cells elongate, remaining thin-walled. Occasionally wall projections and related plasmalemma convolutions form (Fig. 183), but this is the exception rather than the rule. Inner feeder cells are typically highly vacuolate and well supplied with mitochondria and plasmodesmata. Some cell division occurs, but is confined to the base of the feeder (Martens 1971). At the 5- to 6-day-stage basal feeder cells in the vicinity of vascular strands are thin-walled, roughly isodiametric in shape and are particularly richly supplied with plasmodesmata. They give the impression of being metabolically more active than the elongate inner feeder cells. Although they contain no wall projections they possibly act in a transfer capacity transferring nutrients and water (see section III discussion) absorbed from the gametophyte to the vascular strands.

CONCLUSION

Results obtained in this study of the structure and chemical composition of dry *Welwitschia* seeds together with resultant changes occurring during germination supply evidence supporting the original suggestion by Bower (1881a) that the protuberant non-vascularised outgrowth produced by the embryo, the feeder, functions as an absorptive organ.

A definite interrelationship seems to exist between the embryo and gametophyte. Upon hydration activation of embryonic collar cells precedes that of gametophyte cells in which rate of activation seems to be governed by relative distance from the embryo. This sequence of activation is suggestive of the diffusion of an unknown factor from the embryo into the gametophyte where it possibly stimulates or enhances cellular activity. Biochemical studies are needed to substantiate and identify this postulated diffusing stimulatory factor.

In comparison with the massive reserves of gametophyte cells, reserve materials contained within embryonic collar cells, with the exception of starch, are meagre. Upon hydration collar protein body reserves are consumed within approximately 36h during which time lipid stores decline rapidly. The rich supplies of starch originally present in dry collar cells are utilised during the first 24h of cellular activity following imbibition. It is suggested that this starch forms a readily available energy source on which the embryo draws to

extrude a large lateral process or feeder in a short space of time. The plumule remains within the seed for 5 to 6 days before emerging and assuming a photosynthetic role. During this time the developing seedling is probably largely dependent on nutrient material in the gametophyte.

Approaching the fourth day of germination ventral cells of the feeder in contact with gametophyte tissue begin to develop numerous small wall projections invested with plasmalemma. These projections, in conjunction with the loosely overlapped nature of the outer cells results in a much greater absorptive surface area. Although the wall projections are not as pronounced as the transfer-type wall configurations described by Pate and Gunning (1972) it is suggested that these cells probably function as transfer cells, facilitating absorption and/or secretion of substances. The large numbers of mitochondria occurring in these cells might suggest the active uptake of nutrients. As the feeder develops, densely-staining material that is most likely pectinaceous accumulates between gametophyte and ventral feeder cells. It is apparently secreted from both gametophyte and feeder and probably functions as an adhesive between the two structures. By the 3- to 4-day-stage feeder and gametophyte adhere so firmly that considerable force is required to separate them. With gametophyte and feeder firmly adhering together translocation of nutrients is probably facilitated. The adherance could also have the secondary function of anchoring the feeder in the gametophyte to provide the plumule with a firm base as it emerges from the seed.

Within 5 days the mean dry mass of the gametophyte decreases by approximately 47%. During this time period the total amount of lipid decreases by 76.5% and protein by 14%. Some of the hydrolysed fatty acids are probably used as an energy source and/or utilised in the synthesis of phospholipids and glycolipids necessary for organelle formation (Ching 1972) in the gametophyte. By the same token a certain proportion of amino acids are no doubt also used in the gametophyte as an energy source and for the synthesis of structural proteins and enzymes. However, it is suggested that the majority of fatty aicds are converted to sugars and that these sugars, together with large amounts of free amino acids and, possibly, simple peptides are transported to and absorbed by the embryo via the feeder. Once within the embryo the nutrients could be utilised for seedling growth.

The feeder is non-vascularised. In the hypocotyl the four vascular bundles enlarge as they traverse the collar zone, especially the two nearest the feeder which curve outward toward it but do not enter it. All four bundles contain an increased amount of xylem and phloem in the collar region. The fact that xylem increase is dramatic and is greatest in the region opposite the feeder (Butler 1970) leads one to suspect that the feeder might be responsible not only for up-take of nutritive material but also of water. The gametophyte can apparently retain water for long periods of time and this is possibly an adaptive feature to aid the establishment and survival of young seedlings under desert conditions.

Ventral cells of the feeder appear to be of root cap origin.

Recent work *inter alia* by Wilkins and Wain (1975) has shown that root cap cells may be geoperceptive. This might explain the positive geotropism of the feeder.

The presence of fibrous proteinaceous inclusions constitutes the most striking feature of quiescent nuclei in both embryo and gametophyte tissue. They differ from all previous descriptions of nuclear inclusions, their uniqueness lying firstly in their superabundance and secondly in their presence within the nucleolus. The function of these inclusions is obscure. A metabolic association seems to exist between the inclusions and the nucleolus. The inclusions penetrate the nucleolus and are surrounded by granular nucleolar-like material in the nucleoplasm. They are present when the nucleolus is in the quiescent state, but disappear rapidly (within 24h) upon hydration and reactivation of the nucleolus. The possibility exists that nuclear inclusions may be the result of suppression of nucleolar-organizer DNA (Wergin *et al.*, 1970), but speculations of this kind need to be substantiated with biochemical studies. In the embryo morphologically identical inclusions to those seen in the nucleus are also distributed in the cytoplasm. It is thought that the fibrous cytoplasmic inclusions probably originate in the nucleus. A study of developing and maturing seed might help to clarify their origin.

The result of cytochemical methods used at the light and electron microscope level seem to suggest that the reserve material within protein bodies of the embryo and gametophyte might exist as a protein-carbohydrate complex and that

globoid origin might be cytoplasmic. The occurrence of protein bodies mottled with electron-transparent areas in the dry state might indicate that digestion is a continuous process begun before the seed becomes quiescent (Rost 1972). The digestion pattern is one of internal degradation with small digested pockets gradually increasing in number, enlarging and coalescing. Upon hydration the immediate and rapid digestion of protein body reserves in the embryo and in the gametophyte interface zone argues the presence of pre-existing hydrolytic enzymes laid down within the protein bodies prior to quiescence. However the enzymes responsible for reserve breakdown in deep gametophyte tissue seem to be synthesised *de novo*. In their case it is postulated that certain ER-derived vesicles seen entering the protein bodies might contain the enzymes responsible for hydrolysis.

In the developing feeder lipid bodies are sometimes surrounded by a distinct tri-partite unit membrane. In addition numerous ribosomes are also seen attached to the periphery of lipid bodies in dry collar cells. These facts make it tempting to conclude that lipid bodies are indeed derived from the ER as has been proposed by a number of investigators (Grieshaber 1964; Frey-Wyssling and Mühlethaler 1965; Semadeni 1967; Schwarzenbach 1971; Werker and Vaughan 1974). In the gametophyte the thin peripheral electron-dense line surrounding the lipid body seems more in the nature of a half-unit membrane (Schwarzenbach 1971) than a three-layered unit membrane. This absence of a definite three-layered unit membrane in mature

lipid bodies may be due to differentiation that the membrane undergoes resulting in the half-unit structure proposed by Schwarzenbach (1971). Lipid bodies apparently undergo a decrease in electron-density with maturation (Frey-Wyssling *et al.*, 1963; Rest and Vaughan 1972). Comparing the darkly-staining lipid bodies of the embryo with their attached ribosomes and occasional surrounding unit membranes with the grey lipid bodies which seem to be surrounded by half-unit membranes in the gametophyte, one is forced to the conclusion that the lipid bodies of the quiescent embryo are not as mature as those of the gametophyte.

Protein hydrolysis precedes lipid digestion which possibly indicates that some of the resulting free amino acids might be used in the *de novo* synthesis of lipases. These lipases apparently hydrolyse reserve triglycerides *in situ* in lipid bodies (Ching 1968; Ory *et al.*, 1968; Ory 1969) into free fatty acids and glycerol. The observed close association of microbodies with lipid bodies and their corresponding increase as lipid stores decline suggests that they might belong to the class of microbodies called glyoxysomes (Breidenbach *et al.*, 1968). Glyoxysomes are known to contain the enzymes for β-oxidation of fatty acids and the glyoxylate bypass which converts acetyl CoA to succinate (Breidenbach and Beevers 1967). In *Welwitschia* lipid bodies, microbodies, mitochondria and amyloplasts encircled with ER seem to form a complex. Fatty acids resulting from lipase action in the lipid bodies are probably converted by microbodies (glyoxysomes) to succinate

(Breidenbach and Beevers 1967) which is in turn converted to sucrose by the action of mitochondria (Beevers 1961; Canvin and Beevers 1961; Cooper and Beevers 1969a, b). Excess sucrose is then probably converted to starch and stored temporarily in the amyloplasts. The frequent intimate association of mitochondria with lipid bodies could possibly indicate that both α- and β-oxidation of fatty acids occurs in *Welwitschia*. Biochemical studies are needed to substantiate this speculation. In flax seeds α-oxidation of fatty acids occurs in addition to β-oxidation (Ching 1972) and it is thought that the enzymes involved in this α-oxidation are located in the mitochondria and supernatant (Stumpf 1969; Ching 1972).

Whereas dictyosomes appear to be actively involved in cell wall formation in the developing seedling, they are singularly rare in gametophyte tissue. Perhaps the absence of cell division might explain their sparseness. The dramatic thickening of gametophyte cell walls in contact with the feeder is apparently due to the action of ER-derived mini-vacuoles. These mini-vacuoles engulf cytoplasmic fibrillar material, fuse with the plasmalemma and release their contents to add to the cell wall. It is possible, however, that a certain amount of the increased thickness is due to wall hydrolysis with resultant swelling.

In the seeds of other higher plants reduced cotyledons (e.g. Palmae, Gramineae, *Cycas*, *Ginkgo*) or haustoria which develop from the suspensor (e.g. *Tropaeolum*) act as absorptive organs

(Bower 1881a). In addition some haustorial-like structures have a mechanical rather than an absorptive function. (Flahault 1877). From observations made in this study it would appear that, while possibly fulfilling a mechanical role, the feeder of *Welwitschia mirabilis* functions as an absorptive organ as originally postulated by Bower (1881a). This being the case, the closest parallel in morphology and function would appear to be found in *Selaginella*.

LITERATURE CITED

ABDUL-BAKI, A.A., and J.E. BAKER, 1973 : Are changes in cellular organelles or membranes related to vigor loss in seeds? Seed Sci. and Technol. 1, 89-125.

ALTSCHUL, A.M., L.Y. YATSU, R.L. ORY, and E.M. ENGLEMAN, 1966 : Seed Proteins. Ann. Rev. Plant Physiol. 17, 113-136.

AMELUNXEN, F., and T. GIELE, 1968 : Die Struktur der Eiweifskristalle in den Zellkernen von *Melampyrum nemorosum* L. Z. Pflanzenphysiol. 58, 457.

ANDERSON, J.D., J.E. BAKER, and E.K. WORTHINGTON, 1970 : Ultrastructural changes of embryos of wheat infected with storage fungi. Plant Physiol. 46, 857-859.

BAGLEY, B.W., J.H. CHERRY, M.L. ROLLINS, and A.M. ALTSCHUL, 1963 : A study of protein bodies during germination of peanut *(Arachis hypogea)* seed. Amer. J. Bot. 50, 523-532.

BAIN, J.M., and F.V. MERCER, 1966 : Subcellular organization of the cotyledons in germinating seeds and seedlings of *Pisum sativum* L. Aust. J. Biol. Sci. 19, 69-84.

BALZ, J.P., 1966 : Intracelluläre lokalisation und Funktion von hydrolytischen Enzymen bei Tabak. Planta 70, 207-236.

BARTON, R., 1967 : Occurrence and structure of intranuclear crystals in *Chara* cells. Planta 77, 203-211.

BEEVERS, H., 1961 : Respiratory Metabolism. New York : Harper Row.

BENSON, L., 1970 : The status of *Welwitschia mirabilis*. Cactus and Succulent Journal of America 40 (5), 200.

BERJAK, P., 1968 : A lysosome-like organelle in root cap of *Zea mays*. J. Ultrastruct. Res. 23, 233-242.

– and T.A. VILLIERS, 1970 : 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.

BISALPUTRA, T., and A.A. BISALPUTRA, 1967 : Chloroplast and mitochondrial DNA in a brown alga *Egregia menzieskii*. J. Cell Biol. 33, 511-520.

BORNMAN, C.H., 1972 : *Welwitschia mirabilis* : paradox of the Namib Desert. Endeavour, 31 (113), 95-99.

– C.E.J. BOTHA, and L.J. NASH, 1973 : *Welwitschia mirabilis* : Observations on movement of water and assimilates under föhn and fog conditions. Madoqua, Series II, 2, 25-31.

BORNMAN, C.H., J.A. ELSWORTHY, V. BUTLER, and C.E.J. BOTHA, 1972 : *Welwitschia mirabilis* : Observations on general habit, seed, seedling, and leaf characteristics. Madoqua, Series II, 1, 53-66.

BOWER, F.O., 1880 : The germination of *Welwitschia mirabilis* (prel. note). Nature 22, 591.

– 1881a : On the germination and histology of seedlings of *Welwitschia mirabilis*. Quart. Jour. Microsc. Sci. 21, 15-30.

– 1881b : On the further development of *Welwitschia mirabilis*. Quart. Jour. Microsc. Sci. 21, 571-594.

BREIDENBACH, R.W., and H. BEEVERS, 1967 : Association of the glyoxylate cycle enzymes in a novel subcellular particle from castor bean endosperm. Biochem. Biophys. Res. Commun. 20, 53.

– A. KAHN, and H. BEEVERS, 1968 : Characterization of glyoxysomes from castor bean endosperm. Plant Physiol. 43 : 705.

BRIARTY, L.G., D.A. COULT, and D. BOULTER, 1970 : Protein bodies of germinating seeds of *Vicia faba*. Changes in fine structure and biochemistry. J. Exp. Bot. 21 (67), 513-524.

BUTLER, V., 1970 : The morphology and vascular anatomy of *Welwitschia mirabilis* seedlings. M. Sc. thesis, University of Natal, Pietermaritzburg.

– C.H. BORNMAN, and R.F. EVERT, 1973 : *Welwitschia mirabilis* : Morphology of the seedling. Bot. Gaz. 134, 52-59.

BUTTROSE, M.S., 1971 : Ultrastructure of barley aleurone cells as shown by freeze-etching. Planta 96, 13-26.

CANVIN, D.T., and H. BEEVERS, 1961 : Sucrose synthesis from acetate in the germinating castor bean, kinetins and pathway. J. biol. Chem. 236, 988-995.

CHING, T.M., 1965 : Metabolic and ultrastructural changes in germinating Douglas fir seeds. Plant Physiol. 40, Suppl. VIII.

– 1968 : Intracellular distribution of lipolytic activity in the female gametophyte of germinating Douglas fir seeds. Lipids 3, 482.

– 1970 : Glyoxysomes in megagametophyte of germinating Ponderosa pine seeds. Plant Physiol. 46, 475-482.

– 1972 : Metabolism of germinating seeds. In : Seed Biology, Volume II. pp. 103-218 (T.T. KOZLOWSKI, ed.). New York : Academic Press.

CLOWES, F.A.L., and B.E. JUNIPER, 1968 : Plant Cells : Botanical monograph, volume 8. London : Blackwell Scientific Publications.

COOPER, T.G., and H. BEEVERS, 1969a : Mitochondria and glyoxysomes from castor bean endosperm. J. biol. Chem. 244, 3507-3513.

– – 1969b : Oxidation in glyoxysomes from castor bean endosperm. J. biol. Chem. 244, 3514-3520.

COULTER, J.M., and C.J. CHAMBERLAIN, 1910 : Morphology of Gymnosperms. Chicago : Chicago Press.

DELTOUR, R., and R. BRONCHART, 1971 : Changements de l'ultrastructure des cellules radiculaires de *Zea mays* au debut de la germination. Planta 97, 197-207.

DENGLER, R.E., 1967 : Histochemistry and ultrastructure of the embryo axis of *Clarkia* during seed maturation and germination. Dissertation : Doctor of Philosophy in Botany, Graduate Division, University of California, Davis.

DIANTY, J., 1968 : The structure and possible function of the vacuole. In : Plant Cell Organelles. pp 40-46 (J.B. PRIDHAM, ed.). London : Academic Press.

DURE, L., and L. WATERS, 1965 : Long-lived messenger RNA : evidence from cottonseed germination. Science 147 : 410-412.

DURZAN, D.J., A.J. MIA, and P.K. RAMAIAH, 1971 : The metabolism and subcellular organization of the jack pine embryo *(Pinus banksiana)* during germination. Can. J. Bot. 49, 927-938.

DYER, R.A., and I.C. VERDOORN, 1972 : Science or sentiment : The *Welwitschia* problem. Taxon 21, 485-489.

EB, A.A. van der, P.J. NIEUWDORP, 1967 : Electron microscopic structure of the aleurone cells of barley during germination. Acta Bot. Neerl. 15, 690-699.

ESAU, K., 1967 : Anatomy of plant virus infections. Ann. Rev. Phytopathol. 5, 45-76.

ESSNER, E., 1969 : Localization of peroxidase activity in microbodies of fetal mouse liver. J. Histochem. Cytochem. 17 : 454-466.

EVERT, R.F., C.H. BORNMAN, V. BUTLER, and M.G. GILLILAND, 1973 : Structure and development of sieve areas in leaf veins of *Welwitschia*. Protoplasma 76, 23-34.

– and B.P. DESHPANDE, 1970 : Nuclear P Protein in sieve elements of *Tilia americana*. J. Cell. Biol. 44, 462-466.

FLAHAULT, C., 1877 : Sur les rapports de la radicule avec la tigelle, dans l'embryon des Phanérogames. Bull. Soc. Bot. France 24, 135-141.

FOSTER, A.S., and E.M. GIFFORD, Jr. 1959 : Comparative morphology of vascular plants. San Francisco : Freeman.

FOWKE, L.C., and G. SETTERFIELD, 1968 : Cytological responses in Jerusalem artichoke tuber slices during ageing and subsequent auxin treatment. In : Physiology and Biochemistry of Plant Growth Substances. pp 581-602 (F. WIGHTMAN, and G. SETTERFIELD, ed.) Ottawa : Range Press.

FREDERICK, S.E., E.H. NEWCOMB, E.L. VIGIL, and W.P. WERGIN, 1968 : Fine-structural characterization of plant microbodies. Planta 81 : 229-252.

FREY-WYSSLING, A., E. GRIESHABER, and K. MÜHLETHALER, 1963 : Origin of spherosomes in plant cells. J. Ultrastruct. Res. 8, 506-516.

– and K. MÜHLETHALER, 1965 : Ultrastructural Plant Cytology. Amsterdam : Elsevier Publishing Co.

GERHARDT, B.P., and H. BEEVERS, 1969 : Occurrence of RNA in glyoxysomes from castor bean endosperm. Plant Physiol. 44, 1475-1477.

– – 1970 : Developmental studies on glyoxysomes in *Ricinus* endosperm. J. Cell Biol. 44, 94-102.

GILLILAND, M.G., 1973 : Abscission in the pedicel in *Hibiscus rosa-sinensis*. M.Sc. thesis, University of Natal, Pietermaritzburg.

GLAUERT, A.M. 1967 : The fixation and embedding of biological specimens. In : Techniques for electron microscopy (D. KAY ed.) Oxford : Blackwell Scientific Publications.

GOMORI, G., 1952 : Microscopic Histochemistry. Chicago : University of Chicago Press.

GRIESHABER, E., 1964 : Entwicklung und Feinbau der Sphärosomen in Pflanzenzellen. Vjschr. naturforsch. Ges. Zürich 109, 1-23.

HALLAM, N.D., B.E. ROBERTS, and D.J. OSBORNE, 1972 : Embryogenesis and germination in rye *(Secale cereale L.)* II. Biochemical and fine structural changes during germination. Planta 105, 293-309.

HARWOOD, J.L., A. SODJA, P.K. STUMPF, and A.R. SPURR, 1971 : On the origin of oil droplets in maturing castor bean seeds, *Ricinus communis*. Lipids 6, 851-854.

HERRE, H., 1961 : The age of *Welwitschia bainsii* (Hook. f.) Carr. : C14 research. L.c., 27, 139.

HILL, T.G. and E. de FRAINE, 1910 : On the seedling structure of gymnosperms. Part IV. Gnetales. Ann. Bot. 24, 319-353.

HOLCOMB, G.E., A.C. HILDEBRANDT, and R.F. EVERT, 1967 : Staining and acid phosphatase reactions of spherosomes in plant tissue culture cells. Amer. J. Bot. 54, 1204-1209.

HOOKER, J.C., 1863 : On *Welwitschia*, a new genus of Gnetaceae. Trans. Linn. Soc. London. 24 : 1-48.

HORNER, H.T. Jr., and H.J. ARNOTT, 1965 : A histochemical and ultrastructural study of *Yucca* seed proteins. Amer. J. Bot. 52, 1027-1038.

– – 1966 : A histochemical and ultrastructural study of pre- and post-germinated *Yucca* seeds. Bot. Gaz. 127, 48-64.

HUTTON, D., and P.K. STUMPF, 1969 : Fat metabolism in higher Plants XXXVII. Characterization of the β-oxidation systems from maturing and germinating castor bean seeds. Plant Physiol. 44, 508-516.

HYDE, B.H., 1967 : Changes in nucleolar ultrastructure associated with differentiation in the root tip. J. Ultrastruc. Res. 18, 25-54.

JACKS, T.J., L.Y. YATSU, and A.M. ALTSCHUL, 1967 : Isolation and characterization of peanut spherosomes. Plant Physiol. 42, 585.

JACOBSEN, J.V., R.B. KNOX and N.A. PYLIOTIS, 1971 : The structure and composition of aleurone grains in the barley aleurone layer. Planta 101, 189-209.

JENSEN, W.A., 1962 : Botanical Histochemistry. San Francisco : Freeman.

JONES, R.L., 1969a : The fine structure of barley aleurone cells. Planta 85, 359-375.

– 1969b : Gibberellic acid and the fine structure of barley aleurone cells. I. Changes during the lag phase of α-amylase synthesis. Planta 87, 119-133.

– 1974 : The structure of the lettuce endosperm. Planta 121, 133-146.

– and J.M. PRICE, 1970 : Gibberellic acid and the fine structure of barley aleurone cells. III Vacuolation of the aleurone cell during the phase of ribonuclease release. Planta 94, 191-202.

JORDAN, E.G., 1971 : The Nucleolus. In : Oxford Biology Readers. (J.J. HEAD, and O.E. LOWENSTEIN, ed.) London : Oxford University Press.

– and J.M. CHAPMAN, 1971 : Ultrastructural changes in the nucleoli of Jerusalem artichoke *(Helianthus tuberosus)* tuber discs. J. Exp. Bot. 22, 627-634.

KHOKHLOVA, V.A., 1971 : Disintegration of aleurone grains in cotyledons of germinating squash seeds. Soviet Plant Physiol. 18, 855-861. Translated from Fiziologiya Rastenii 18, 1010-1015.

KHOO, U., and M.J. WOLF, 1970 : Origin and development of protein granules in maize endosperm. Amer. J. Bot. 57, 1042-1050.

KIRK, J.M., 1960 : The mode of action of actinomycin D. Biochim. Biophys. Acta 42, 167.

KISLEV, N., H. SWIFT, and L. BOGORAD, 1965 : Nucleic acids of chloroplasts and mitochondria in Swiss chard. J. Cell Biol. 25, 327-344.

KLEIN, S., and Y. BEN-SHAUL, 1966 : Changes in cell fine structure of Lima bean axes during early germination. Can. J. Bot. 44, 331-340.

LA FONTAINE, J.G., 1965 : A light and electron microscope study of small spherical nuclear bodies in meristematic cells of *Allium cepa, Vicia faba* and *Raphanus sativus*. J. Cell Biol. 26, 1.

LANCE-NOUGARÈDE, A., 1966 : Presence de structures proteiques a´ arrangement periodique et d'aspect cristallin dans les mitochondries de l'epiderme des jeunes feuilles de lentille *(Lens culinaris)*. Compt. Rend. Acad. Sci. 263, 246-249.

LANE, N.J., 1969 : Intranuclear fibrillar bodies in actinomycin D-treated oocytes. J. Cell Biol. 40, 286-291.

LINNANE, A.W., E. VITOLS, and P.G. NOWLAND, 1962 : Studies on the origin of yeast mitochondria. J. Cell Biol. 13, 345.

LOENING, U.E., 1968 : The occurrence and properties of polysomes in plant tissues. In : Plant Cell Organelles (J.B. PRIDHAM, ed.). New York : Academic Press.

LOPEZ-PEREZ, M.J., A. GIMINEZ-SOLVES, F.D. CALONGE, and A. SANTOS-RUIZ, 1974 : Evidence of glyoxysomes in germinating pine seeds. Plant Science Letters 2, 377-386.

LOTT, J.N.A., P.L. LARSEN, and J.J. DARLEY, 1971 : Protein bodies from the cotyledons of *Cucurbita maxima*. Can. J. Bot. 49, 1777-1782.

– and C.M. VOLLMER, 1973a : The structure of protein bodies in *Cucurbita maxima* cotyledons. Can. J. Bot. 51, 687-688.

– – 1973b : Changes in the cotyledons of *Cucurbita maxima* during germination, IV Protein Bodies. Protoplasma 78, 255-271.

LOWARY, P.A., and C.J. AVERS, 1965 : Nucleolar variation during differentiation of *Phleum* root epidermis. Amer. J. Bot. 52, 199.

LUFT, J.H., 1956 : Permanganate - a new fixative for electron microscopy. J. biochem. biophys. Cytol. 2, 799-802.

– 1961 : Improvements in epoxy embedding methods. J. biophys. biochem. Cytol. 9, 409-427.

LUI, N.S.T., and A.M. ALTSCHUL, 1967 : Isolation of globoids from cotton seed aleurone grain. Arch. Biochem. 121, 678-684.

MARCUS, A., and J. FEELEY, 1964 : Protein synthesis in imbibed seeds. II Polysome formation during imbibition. J. Biol. Chem. 240, 1675-1680.

– – 1966 : Ribosome activation and polysome formation *in vitro* : requirement for ATP. Proc. Nat. Acad. Sci. U.S.A. 56, 1770-1777.

MARTENS, P. 1959 : Études sur les Gnétales - III. Structure et ontogenèse du cône et de la fleur femelles de *Welwitschia mirabilis*. La Cellule 60, 169-286.

– 1961 : Études sur les Gnétales - V. Structure et ontogonèse du cône et de la fleur mâles de *Welwitschia mirabilis*. La Cellule 62 : 5-91.

– 1963 : Études sur les Gnétales - VI. Recherches sur *Welwitschia mirabilis* - III. L'ovule et le sac embryonnaire. Les sacs embryonnaires extra-floraux. La Cellule 63 : 307-329.

– 1971 : Les Gnétophytes. Encyclopaedia of Plant Anatomy, Volume XII. Berlin : Gebrüder Borntraeger.

– and L. WATERKEYN, 1964 : Recherches sur *Welwitschia mirabilis* - IV. Germination et plantules. Structure, fonctionnement et productions du méristème caulinaire apical. (Études sur les Gnétales - VII). La Cellule 65, 5-68.

MATILE, P., 1966 : Enzyme der Vakuolen aus Wurzelzellen von Maiskeimlingen. Ein Beitrag zur functionellen Bedeutung der Vakuole bei der intrazellularen Verdauung. Z. Naturf. 21b, 871-878.

MATILE, P., 1968a : Lysosomes in root tip cells in corn seedlings. Planta, 79, 181-196.

– 1968b : Vacuoles as lysosomes of plant cells. Biochem. J. 111, 26-7.

– 1969 : Plant lysosomes. In Lysosomes in Biology and Pathology. pp 406-430 (DINGLE and FELL ed.). Amsterdam : North Holland Publishing Company.

– and H. MOOR, 1968 : Vacuolation : origin and development of the lysosomal apparatus in root tip cells. Planta 80, 159-175.

– and J. SPICHIGER, 1968 : Lysosomal enzymes in spherosomes (oil droplets) of tobacco endosperm. Z. Pflanzenphysiol. 58, 277-280.

McCARTHY, W.J., A.A. APP, and W.J. CROTTY, 1971 : The effect of calcium on *in vitro* polyphenylalanine synthesis by rice ribosomes. Biochim. biophys. Acta. (Amst.) 246, 132-140.

MERCER, E.H., and M.S.C. BIRBECK, 1966 : Electron Microscopy. A handbook for biologists. Oxford : Blackwell Scientific Publications.

– and N. RATHGEBER, 1962 : Nectar secretion and cell membranes. Fifth Int. Congr. Electron Microsc., Philadelphia, 2, WW11.

MEYER, A., 1920 : Morphologische und physiologische Analyse der Zelle der Pflanzen und Tiere, Vol. 1, p 218. Fischer, Jena.

MEYER, B.S., and D.B. ANDERSON, 1952 : Plant Physiology. Princeton : Van Nostrand.

MOLLENHAUER, H.H. and C. TOTTEN, 1971 : Studies on seeds. I. Fixation of seeds. J. Cell Biol. 48, 387-394.

MORRÉ, D.J., H.H. MOLLENHAUER and C.E. BRACKER, 1971 : Origin and continuity of golgi apparatus. In : Origin and Continuity of Cell Organelles. (J. REINERT and H. URSPRUNG). Berlin : Springer-Verlag.

MORRIS, J., 1968 : Proteolysis in seeds. Ph. D. Thesis, University of Liverpool.

MORRISON, I.N., J. KUO, and T.P. O'BRIEN, 1975 : Histochemistry and fine structure of developing wheat aleurome cells. Planta 103, 105-116.

NAUDIN, C., 1882 : Germination of *Welwitschia*. Gardeners Chr. 419, 14.

NIEUWDORP, P., 1963 : Electron microscopic structure of the epithelial cells of the scutellum of barley. Acta Bot. Neerl. 12, 295-301.

– and M.C. BUYS, 1964 : Electron microscopic structure of the epithelial cells of the scutellum of barley II. Cytology of the cells during germination. Acta Bot. Neerl. 13, 559-565.

NOVIKOFF, A.B. and S. GOLDFISCHER, 1968 : Visualization of microbodies for light and electron microscopy. J. Histochem. Cytochem. 16, 507.

OPIK, H., 1966 : Changes in cell fine structure in the cotyledons of *Phaseolus vulgaris* L. during germination. J. Exp. Bot. 17, 427-439.

ORY, R.L., 1969 : Acid lipase of castor bean. Lipids 4, 177.

– L.Y. YATSU and H.W. KIRCHER, 1968 : Association of lipase activity with the spherosomes of *Racinus communis*. Arch. Biochem. Biophys. 123, 255.

PALEG, L., and B. HYDE, 1964 : Physiological effects of gibberellic acid. VII. Electron microscopy of barley aleurone grains. Plant Physiol. 39, 673-680.

PATE, J.S. and B.E.S. GUNNING, 1972 : Transfer cells. Ann. Rev. Plant Physiol. 23, 173-196.

PAULSON, R.E., and L.M. SRIVASTAVA, 1968 : The fine structure of the embryo of *Lactuca sativa*. I. Dry embryo. Can. J. Bot. 46, 1437-1445.

PEARSE, A.G.E., 1961 : Histochemistry. London : Churchill.

PEARSON, H.H.W., 1929 : Gnetales. London : Cambridge University Press.

PERNER, E., 1965 : Elektronenmikroskopische Untersuchungen au Zellen von Embryonen im Zustand völliger Samenruhe. I. Mitteilung. Die zelluläre Strukturordnung in der Radicula lufttrockner Samen von *Pisum sativum*. Planta 65 : 334-357.

PFEFFER, W., 1872 : Untersuchungen über die Protein-Körper und die Bedeutung des Asparagius beim Keimen der Samen. Jb. wiss. Bot. 8, 529-571.

POUX, N., 1963 : Localization des phosphates et de la phosphatase acide dans des cellules des embryons de blé (*Triticum vulgare* Vill.) lors de la germination. J. Microscopie 2, 557-568.

– 1965 : Localization de l'activité phosphatasique acide et des phosphates dans les grains d'aleurone. I. Grains d'aleurone renfermant à la fois globoides et crystalloides. J. Microscopie 4, 771-782.

RAMBOURG, A., 1967 : An improved silver methenamine technique for detection of periodic acid reactive complex carbohydrates. J. Histochem. Cytochem. 15, 409-412.

REICH, E., R.M. FRANKLIN, A.J. SHATKIN, and E.L. TATUM, 1961 : Effects of actinomycin D on cellular nucleic acid synthesis and virus production. Science 134, 556-557.

REST, J.A., and J.G. VAUGHAN, 1972 : The development of protein and oil bodies in the seed of *Sinapis alba* L. Planta 105, 245-262.

REYNOLDS, E.S., 1963 : The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208-212.

ROBARDS, A.W., 1970 : Electron Microscopy and Plant Ultrastructure. London : McGraw-Hill.

RODIN, R.J., 1953a : Distribution of *Welwitschia mirabilis*. Amer. J. Bot. 40, 280-285.

– 1953b : Seedling morphology of *Welwitschia*. Amer. J. Bot. 40, 371-378.

ROST, T.L., 1972 : The ultrastructure and physiology of protein bodies and lipids from hydrated dormant and non-dormant embryos of *Setaria lutescens* (Gramineae). Amer. J. Bot. 59, 607-616.

ROWLEY, G., 1972 : Voyage into the impossible - I meet *Welwitschia*. Journal of the Royal Horticultural Society, Vol. XCVII, Part 8.

SALMON, J., 1940 : Quelques remarques sur l'état physique et le comportement histochimique des globoides. C.R. Acad. Sci. (Paris) 211, 510-512.

SCHULZ, P., and W.A. JENSEN, 1973 : *Capsella* embryogenesis : The central cell. J. Cell Sci. 12, 741-763.

– – 1974 : *Capsella* embryogenesis : The development of the free nuclear endosperm. Protoplasma 80, 183-205.

SCHWARZENBACH, A.M., 1971 : Observations on spherosomal membranes. Cytobiologie 4, 145-147.

SEMADENI, E.G., 1967 : Enzymatische Charakterisierung der Lysosomenaquivalente (Sphärosomen) von Maiskeimlingen. Planta 72, 91-118.

SETTERFIELD, G., H. STERN, and F.B. JOHNSTON, 1959 : Fine structure in cells of pea and wheat embryos. Can. J. Bot. 37, 65-72.

SIMOLA, L.K., 1974 : The ultrastructure of dry and germinating seeds of *Pinus sylvestris* L. Acta Botanica Fennica 103, 1-31.

SMITH, D.L., and A.M. FLINN, 1967 : Histology and histochemistry of the cotyledons of *Pisum arvense* L. during germination. Planta 74, 72-85.

SOBOLEV, A.M., 1966 : On the state of phytin in aleurone grains of mature and germinating seeds. Soviet Plant Physiol. 13, 177-183.

SOROKIN, H.P., and S. SOROKIN, 1966 : The spherosomes of *Campanula persicifolia* L. Protoplasma 62, 216-236.

SPECKMAN, D.H., 1963 : Fed. Proc. 22, 244.

SPORNE, K.R., 1965 : The Morphology of Gymnosperms. London : Hutchinson University Library.

SRIVASTAVA, L.M., and R.E. PAULSON, 1968 : The fine structure of the embryo of *Lactuca sativa*. II. Changes during germination. Can. J. Bot. 46, 1447-1453.

STANDING COMMITTEE ON STABILIZATION OF SPECIFIC NAMES of the International Association for Plant Taxonomy (Report of), 1975 : Taxon 24, 174.

STREET, H.E., and W. COCKBURN, 1972 : Plant Metabolism. Oxford : Pergamon Press.

STUMPF, P.K., 1969 : Lipid metabolism. In : Plant Biochemistry (J. BONNER, and J.E. VARNER, ed.). New York : Academic press.

— 1969 : Metabolism of fatty acids. Annu. Rev. Biochem. 38, 159.

SWIFT, H.H., 1959 : Nucleolar function. In : Symposium on molecular biology, pp. 266-303. Chicago : University of Chicago Press.

— 1966 : Report of the nucleolus nomenclature committee. Natn Cancer Inst. Monogr. 23, 573-574.

SWIFT, J.G., and M.S. BUTTROSE, 1972 : Freeze-etch studies of protein bodies in wheat scutellum. J. Ultrastruct. Res. 40, 378-390.

— — 1973 : Protein bodies, lipid layers and amyloplasts in freeze-etched pea cotyledons. Planta 109, 61-72.

— and T.P. O'BRIEN, 1972 : The fine structure of the wheat scutellum during germination. Aust. J. Biol. Sci. 25, 469-486.

SYKES, M.G. (THODAY), 1910a : The anatomy and morphology of the leaves and inflorescences of *Welwitschia mirabilis*. Phil. Trans. Roy. Soc. London B201, 179-226.

SYKES, M.G., 1910b : On the anatomy of *Welwitschia mirabilis* in its seedling and adult stages. Trans. Linn. Soc. London ser. 2, 7, 327-354.

TANDLER, B., R.A. ERLANDSON, A. SMITH, and E.L. WYNDER, 1969 : Riboflavin and mouse hepatic cell structure and function. II. Division of mitochondria during recovery from simple deficiency. J. Cell Biol. 41, 447-493.

THALER, I., 1966 : Protoplasmatologia 11B26γ (as quoted by W.P. WERGIN *et al.*, 1970 : Fine structural investigation of nuclear inclusions in plants. J. Ultrastruct. Res. 30, 533-557).

TOLBERT, N.E., A. OESER, T. KISAKI, R.H. HAGEMAN, and R.K. YAMAZAKI, 1968 : Peroxisomes from spinach leaves containing enzymes related to glycolate metabolism. J. Biol. Chem. 243, 5179-5184.

TSUKADA, H., Y. MOCHIZUKI, and T. KONISHI, 1968 : Morphogenesis and development of microbodies of hepatocytes of rats during pre- and postnatal growth. J. Cell Biol. 37, 231-243.

VAN STADEN, J., M.G. GILLILAND, S.E. DREWES, and J.E. DAVEY, 1975 : Changes in the food reserves of viable and non-viable *Protea compacta* embryos during incubation. Z. Pflanzenphysiol. 76, 369-377.

VARNER, J.E., and G. SCHIDLOVSKY, 1963 : Intracellular distribution of proteins in pea cotyledons. Plant Physiol. 38, 139-144.

VAZART, B., 1960 : Étude cytologique du grain d'aleurone dans le malt. Bull. Soc. Bot. France 107, 185-192.

VIGIL, E.L., 1970 : Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. J. Cell Biol. 46, 435-454.

VILLIERS, T.A., 1968 : Intranuclear crystals in plant embryo cells. Planta 78, 11-16.

– 1971 : Cytological studies in dormancy. I. Embryo maturation during dormancy in *Fraxinus excelsior*. New Phytol. 70, 751-760.

– 1972 : Cytological studies in dormancy. II. Pathological ageing changes during prolonged dormancy and recovery upon dormancy release. New Phytol. 71, 145-152.

WALEK-CZERNECKA, A., 1962 : 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.

WEBER, F., 1926 : Planta 1, 441 (as quoted by W.P. WERGIN *et al.*, 1970 : Fine structural investigation of nuclear inclusions in plants. J. Ultrastruct. Res. 30, 533-557).

WERGIN, W.P., P.J. GRUBER and E.H. NEWCOMB, 1970 : Fine structural investigation of nuclear inclusions in plants. J. Ultrastruct. Res. 30, 533-557.

WERKER, E., and J.G. VAUGHAN, 1974 : Anatomical and ultrastructural changes in aleurone and myrosin cells of *Sinapis alba* during germination. Planta 116, 243-255.

WILKINS, H., and R.L. WAIN, 1975 : The role of the root cap in the response of the primary roots of *Zea mays* L. seedlings to white light and gravity. Planta 123, 217-222.

YATSU, L.Y., 1965 : The ultrastructure of cotyledonary tissue from *Gossypium hirsutum* L. seeds. J. Cell Biol. 25, 193-199.

– and T.J. JACKS, 1968 : Association of lysosomal activity with aleurone grains in plant seeds. Arch. Biochem. and Biophys. 124, 466-471.

YEOMAN, M.M., and H.E. STREET, 1973 : General cytology of cultured cells. In : Plant Tissue and Cell Culture. Botanical Monographs, Volume 11 (H.E. STREET ed.). Oxford : Blackwell Scientific Publications.

YOO, B.Y., 1970 : Ultrastructural changes in cells of pea embryo radicles during germination. J. Cell. Biol. 45, 158-171.

– and S.T. BAYLEY, 1967 : The structure of pores in isolated pea nuclei. J. Ultrastruct. Res. 18, 651-660.

ZIMMERMAN, A., 1891 : Beiträge zur Morphologie und Physiologie der Pflanzenzelle Vol. II (H. LAUPP Jr. ed.). Tübingen.

ACKNOWLEDGEMENTS

I am grateful to Professor C.H. Bornman of the Department of Botany, University of Natal for the guidance, encouragement and constructive criticism given during the course of this research and the writing of this thesis.

I am indebted to Mr. J. Marais for free amino acid analyses (Table I) and to the South African Bureau of Standards (SABS) for the determination of fatty acid content and composition (Table II) of seed material.

Special thanks are also due to Mr. V. Bandu, Mr. D.A. Guillard and Mr. D. Tunnington for photographic assistance and advice.