FINE STRUCTURAL STUDIES ON DIFFERENTIATION AND DEDIFFERENTIATION IN THE HIGHER PLANT

BY

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VOLUME I

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TO MY PARENTS

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VOLUME II

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Abbreviations

ABBREVIATIONS

a : amyloplast
ab : abaxial
ad : adaxial
cc : cortical cell
ch : chloroplast
cr : chromatin
cp : cell plate
cw : cell wall
d : dictyosome
ec : epidermal cell
EM : electron microscope
fb : fibre
g : grana
is : intercellular space
lg : lipid globule
LM : light microscope
ly : lysosome
m : mitochondrion
mb : microbody
mm : multi-membranous body
mt : microtubule
n : nucleus
ne : nuclear enclave
ni : nuclear inclusion
nl : nucleolus
np : nuclear pore
nv : nuclear vacuole
p : plastid
pb : protein body
pc : protein crystal
ph : phloem
pp : p-protein
pr : polyribosome
pt : pit
pv : provascular
r : ribosome
rer: rough endoplasmic reticulum
sg : starch grain
sec: swollen epidermal cell
SEM: scanning electron microscope
ser: smooth endoplasmic reticulum
sp : sieve plate
st : storage cell
t : tonoplast
tc : transfer cell
te : tracheary element
TEM: transmission electron micr.
v : vacuole
ve : vesicle
vb : vascular bundle
wb : wall body
x : xylem

TERMINOLOGY I (general)

abaxial: directed away from axis.

acropetal: from the base towards the apex.

adaxial: directed towards the axis.

anticlinal: at right angles to the surface of the organ.

basipetal: from the apex towards the base.

distal: situated away from the point of attachment.

oblique: semi transverse/longitudinal.

paradermal: tangential longitudinal.

periclinal: parallel to the surface of the organ.

proximal: situated near or towards the point of attachment of the organ.

radial longitudinal: longitudinal section coinciding with a radius of a cylindrical body, such as stem.

tangential longitudinal: periclinal (usually applies to epidermal cells).

transverse: across the long axis of the organ.

TERMINOLOGY II (*Phaseolus vulgaris* L.)

Day 0: mature air-dried seed.

Day 1: after 24 hours of soaking in water.

Day 2,3,4,...: days of *in vitro* culture, or germination.

Established callus tissue: callus derived from one cotyledon explant after 8-10 sub-cultures.

TERMINOLOGY III (*Linum usitatissimum* L.)

Day 0: mature air-dried seed (or embryo).

Day 1,2,3,...,9: stages of germination in the greenhouse after sowing at Day 0.

Day 10: fully elongated hypocotyl (this is the average age; elongation growth of the hypocotyl ceases between 8 and 12 days).

Day 11,12,13,...: days since sowing of intact (non-decapitated) control seedlings, with fully elongated hypocotyls.

Day 10+1: 11 days after sowing and 1 day after decapitation of the seedling (just beneath the cotyledons). Similarly, Day 10+2 indicates 12 days after sowing and 2 days after decapitation...

SUMMARY

The storage cells in the cotyledons of air-dried Phaseolus vulgaris seeds contain large quantities of polysaccharides, protein and to a lesser extent, lipids. A single multilobed nucleus occurs in the centre of each storage cell surrounded by large starch grains, small plastids and mitochondria whilst the cytoplasm occupies the narrow channels between the various organelles. The cell walls are composed of two layers, the outer, cellulosic, and inner, non-cellulosic, and the latter serves as a polysaccharide store. After hydration for 24 hours the storage cells respond to in vitro culturing, on solid medium (Murashige and Skoog, 1962) containing 2,4-D, kinetin and coconut milk, by producing a callus tissue which, when routinely subcultured, continues to grow for a potentially indefinite period. No organogenesis was observed in the callus cultures during the one year of cultivation. The callus appears on the adaxial side of the cotyledon (which is in contact with the medium) and, initially, mainly developes from the storage cells in the vicinity of the adaxial provascular strands and progressively spreads towards the abaxial surface. The nuclear divisions observed are mainly amitotic with mitoses apparently being confined to storage cells which have already undergone nuclear fragmentation. Free-wall formation occurs frequently in amitotically dividing cells and plays an active role in nuclear fragmentation. Large labyrinthine wall bodies are abundant in the dedifferentiating storage cells, sometimes apparently free in the cytoplasm or more often forming on the cell walls. Wall bodies also occur, along with freely-forming walls, in the established callus. A comparison is also made between in vivo

and *in vitro* development of the cotyledon; *in vitro* food digestion proceeds slowly and it is faster on the adaxial side, in comparison to relatively fast food digestion when *in vivo* which occurs more uniformly in the storage tissue. Neither cell division nor organelle replication occur during germination

The air-dried *Linum usitatissimum* hypocotyl serves as food store (along with the rest of the embryo) during seed dormancy, with the main food stores being lipid, protein and polysaccharides; during germination protein bodies are digested and give rise to vacuoles. The hypocotyl reaches its full height at about Day 10; by that time stomata are fully differentiated and in addition to the normal epidermal cells and guard cells, "swollen" cells occur at various sites in the epidermis; all epidermal cells contain a spindle-shaped nuclear inclusion. Decapitation of the Day 10 hypocotyl results in the appearance of 5-20 *de novo* developed adventitious buds which become macroscopically visible at the upper end of this organ by about Day 10+8. The majority become dormant but one bud eventually becomes dominant and grows into a replacement shoot. The initiation of a bud is characterised by several transverse divisions in the axially elongated, normal-type epidermal cell, followed by radial and tangential longitudinal ones. The cortical cells, in the case of a non-dormant bud, appear to dedifferentiate more-or-less contemporaneously with the epidermal cells and a provascular connection becomes established between the bud and the axial vascular tissue of the hypocotyl. In the dormant bud this connection appears to occur later when leaf primordia are already visible and only some discontinuous tracheary elements differentiate in contrast to continuous strands in the non-dormant bud. Dedifferentiation of epidermal and

cortical cells is apparently accompanied by the appearence of some highly irregular chloroplast profiles indicating some kind of chloroplast replication. After decapitation the hypocotyl becomes greener, its top swells, the cell walls of cortical cells become thicker, cortical chloroplasts develop massive starch grains and transfer cells appear in the vascular tissue connecting the adventitious bud with the axial vascular tissue as well as in the latter itself.

1. INTRODUCTION

In the case of the higher animal, cell differentiation is usually final, whereas on the contrary a differentiated but living plant cell, e.g. parenchyma, collenchyma, lignified cells, fibres and even young sieve tubes or vascular elements, can dedifferentiate to a more meristematic type and eventually redifferentiate to provide new cells that are likely to exhibit various other types of specialization. If cellular differentiation is considered to result from a blocking or deflection of certain encoded cytoplasmic activities, it must be assumed that this blocking or deflection, which is permanent in the animal cell, is only temporary in the plant cell (D'Amato, 1977; Gautheret, 1966). This totipotency of the plant cell has been exploited for centuries by horticulturists for vegetative propagation by means of grafting, rooting and or budding of cuttings. In nature, partial dedifferentiation may occur in the intact plant in the formation of the interfascicular vascular cambium and cork cambium during secondary thickening. It may also result from the interference of microorganisms and insects (in such cases a callus or gall may be produced, Bloch, 1965; Brown, 1969) or result from mechanical wounding often leading to callusing of the exposed surface. In other cases organogenesis (with or without prior callusing) may occur, e.g. the production of adventitious shoots from root stumps of *Taraxacum officinale*, *Crambe maritima* and (Bowes, 1971, 1976).

The first suggestions about the possibilities of the *in vitro* culture of plant tissues were made by Haberlandt in 1902 (translation by Krikorian and Berquan, 1969). But it was only

considerably later in the 1930's when technical cevelopments introduced by a number of workers (White, 1934, root tips of Lycopersicum esculentum; Gautheret, 1937, 1938, cambial tissue of Salix carpaea; Nobecourt, 1937, 1938a and b, parenchyma tissue of Daucus carota root and tuber cells of Solanum tuberosus) led to the succesful prolonged aseptic culture of plant cells. With the various in vitro techniques now available (as listed in the following paragraph) it is often possible to induce cellular dedifferentiation - sometimes followed by organogenesis - in plant tissue which would normally remain quiescent in the intact or wounded plant grown in vivo.

Today the following types of aseptic cultures of plants may be distinguished (Street, 1973; Butcher and Ingram, 1976); (a). Organ cultures; these are cultures (derived from root tips, stem tips, leaf primordia, primordia of immature parts of flowers and immature fruits) which in culture persist growing in an organised manner. (b). Embryo cultures; these are cultures of isolated mature or immature embryos. (c). Callus (or tissue) cultures; these are tissues arising from the dedifferentiation of cells derived from segments (explants) of plant organs. Callus cultures are usually grown as a mass of cells on a solid medium. (d). Suspension cultures; these consist of isolated cells (e.g. separated mesophyll cells derived from the leaf of Nicotiana tabacum) and very small cell aggregates remaining dispersed as they grow in agitated liquid media. Suspension cultures are sometimes called cell cultures on the grounds that they represent a lower level of organisation than callus cultures. Isolated single cells can sometimes be induced to dedifferentiate and proliferate into a cellular aggregate in which embryogenesis

or organogenesis occurs, leading to the production of new plants from single, differentiated but totipotent plant cells (Reinert, 1959; Pilet, 1961, complete plantlet arising from a single cell of Daucus carota). (e). Protoplast cultures; these are derived from single cells from which the cell walls have been removed chemically. Viable protoplasts normally regenerate walls and then divide to form aggregates which may sometimes regenerate whole plants (Smith, 1974, production of somatic hybrid plants from Nicotiana glauca and N. langsdorffii leaf mesophyll protoplasts).

In the current study the processes of cellular differentiation and dedifferentiation in the higher plant have been followed in two contrasting systems. Firstly, the normal germination changes (leading to rapid cellular senescence) in the mature cotyledons of Phaseolus vulgaris have been contrasted with the in vitro development of the isolated cotyledon; the latter dedifferentiates into callus tissue which is potentially capable of unlimited growth but without organogenesis. Secondly, the differentiation changes occurring in the epidermal and cortical tissues of the hypocotyl of Linum usitatissimum have been followed during germination. After elongation of this organ these tissues remain quiescent until eventually sloughed off with the expansion of the axis consequent on formation of secondary vascular tissue and the cork cambium. However, the decapitation of the young but fully elongated hypocotyl leads to the dedifferentiation of epidermal cells at various positions on the upper region of this organ. At such sites the adjacent epidermal and cortical cells also become dedifferentiated and soon the meristematic mass of cells (in contrast to the dedifferentiated

cells of P. vulgaris) becomes organised into an adventitious bud primordium.

In order to follow from the early stages the differentiation of the cotyledons of P. vulgaris and hypocotyl of L. usitatissimum the organs had to be examined in the air-dried seeds. The least explored stage, ultrastracturally, in the life cycle of the seed is the mature (air-dried) stage; this is presumably due to two major technical difficulties. The first problem is the generally unsatisfactory standard of specimen preservation resulting from the use on dried seeds of the fixatives and embedding agents normally employed for the preparation (for electron microscopy) of the much more commonly investigated hydrated, botanical specimens; the second problem is that dry tissues are likely to undergo varying degrees of imbibition with the use of aqueous fixatives, and so perhaps alter the structural state of the cytoplasm (Abdul-Baki and Baker, 1973; Paulson and Srivastava, 1968; Swift and Buttrose, 1973). However, many workers have attempted to overcome these difficulties by soaking in water the air-dried seeds of various species (for periods varying from a few minutes to several hours) and have assumed that no major ultrastructural changes occur in such a short period of hydration prior to fixation (Bechtel and Pomeranz, 1977, 1978a and b; Brierty et al., 1970; Buttrose, 1963; Horner and Arnott, 1965, 1966; Jones, 1969; Młodzianovski and Włowska, 1975; Młodzianovski, 1978; Neudrop, 1963; Nougarède and Pilet, 1964; Öpik, 1966; Paulson and Srivastava, 1968; Rost, 1972; Smith, 1974; Webster and Leopold, 1977; Yoo, 1970). Another method used for the study of the air-dried seeds is the freeze-etching technique, but employing 100% glycerol as an anti-freeze

agent (Swift and Buttrose, 1972, 1973) instead of the 20% aqueous solution which is normally used with this procedure (Moore, 1964). Chemical fixation of air-dried tissues by employing OsO_4 vapours as a non-aqueous fixative has also been attempted (Perner, 1965; Simon, 1974; Swift and O'Brien, 1972; Yatsu, 1965). However, comparative studies of such tissues fixed with either OsO_4 vapours, aqueous fixatives or with the freeze-etching technique (Simon, 1974; Swift and Buttrose, 1972, 1973; Swift and O'Brien, 1972) show that, while modifications may be effected at the molecular level, gross structural changes are not usually induced by aqueous fixation.

Öpik (1966) in an investigation of the changes occurring in the P. vulgaris cotyledons during germination was apparently unsuccessful in investigating the air-dried seeds and described only the epidermal cells of such material. She stated that these cells generally resemble the 24 hour hydrated ones but sometimes contain shrunken protoplasts and that the shape of all the organelles is more angular than in the hydrated tissue. She also reported in the air-dried epidermal cells the absence of endoplasmic reticulum and suggested that the latter is replaced by numerous small vesicles 0.02-0.22μm in diameter.

An extensive fine-structural description of the freshly hydrated P. vulgaris cotyledon is given by Öpik (1965, 1966) and at the light microscopic level by Smith (1974). According to these authors, the cotyledon of P. vulgaris consists of three systems of tissues: the storage tissue, the vascular bundles and the epidermal and sub-epidermal cells whilst a well developed intercellular space system occurs between the cells.

(a). The storage tissue.

The size of the storage cells varies from about 30μm in diameter around the vascular bundles, 50-80μm under the epidermis and up to 160μm in the central regions furthest away from the vascular bundles and from the surface. The chief food reserve occurs in the starch grains (which are up to 50μm in diameter) and between them lie numerous protein bodies. These are more-or-less round, delimited by a distinct single membrane and measure 2-22μm in diameter. The cell walls are lined with lipid globules, which are also found deeper in the cytoplasm, and measure approximately 0.1-1.5μm in diameter. The cytoplasm occupies the narrow spaces left by the storage bodies, it initially lacks vacuoles but contains all the usual organelles. The nucleus appears lobed (ameboid) and it measures up to 40μm. The greatly thickened walls of the storage cells are not lignified; pits and corner thickenings are conspicuous under the light microscope and under the electron microscope the outer wall is seen to be composed of fibrillar elements, whilst the inner thickened wall contains in addition tubules of circular cross section. The pits are pierced by groups of plasmodesmata.

(b). The vascular bundles.

In a transverse section from the middle of the cotyledon, 20 to 25 vascular bundles can be seen and the number of cells in a vascular bundle varies from about 50 to 250 cells. These are closely packed, elongated and they are all thin walled and in the ungerminated seeds, the bundles consist of procambial cells but later vascular elements differentiate. Occasionally central lysiginous spaces occur in the large vascular bundles.

(c). Epidermis and sub-epidermis.

The whole cotyledon is surrounded by the epidermis (with a very thin cuticle) whilst a distinct sub-epidermal layer is only present under the abaxial epidermis. The cells of the adaxial epidermis are elongated parallel to the long axis of the cotyledon, they are about 10-30μm wide, up to 100μm long and 10μm deep. The cells of the abaxial epidermis are fairly regularly polygonal in shape, up to 30μm in diameter and 10μm deep. Stomata are not differentiated at Day 1 in either epidermal layers. Protein bodies are present in both the epidermal and sub-epidermal cells but starch grains occur only in the sub-epidermal cells and these grains are smaller than in the storage cells.

(d). Intercellular space system.

At every storage cell wall junction a triangular to circular space is visible in section and these spaces are interconnected to form a complex network which ramifies throughout the storage zone and occupies about 16% of the volume of the cotyledon. However, such spaces are not present in the vascular bundles. At the adaxial side of the cotyledon the spaces terminate below the epidermis, but at the abaxial side they penetrate between the epidermal cells and into the cuticle. While most spaces evidently contain air, some are partially or completely blocked with a proteinaceous material.

The main structural changes occurring during germination, as they have been described by Öpik (1966) and Smith (1974), are as follow:

(a). Storage cells. In the mitochondria, the matrix darkens, the cristae swell and the mitochondrial ribosomes disappear,

the rough endoplasmic reticulum becomes conspicuous and free ribosome helices disappear. Digestion of the food reserves commences in cells remote from the vascular strands; the protein bodies begin to swell and fuse to form a vacuolar system, the lipid globules disappear and starch digestion begins. Between the fourth and the eighth day, the protein bodies and starch grains are digested and at the same time the cytoplasm disintegrates. The DNA levels of nuclei of the Day 1 material varies from 2C to 32C but has an average value of 16C and it decreases greatly after a few days.

(b). The epidermal and sub-epidermal cells. The protein bodies are digested and the cells become highly vacuolated; but unlike the storage cells, epidermal and sub-epidermal cells survive until the cotyledons drop off and in the sub-epidermal cells (and those surrounding the vascular bundles) chloroplasts develop. No changes occur from the 2C (epidermal) and 4C (sub-epidermal) levels of DNA at Day 1.

(c). The vascular bundle cells. The mitochondria become more numerous and more highly cristate and ribosome helices disappear by Day 1-4. Between Day 3 and 5 protein bodies and lipid globules are digested, small vacuoles are formed and some vascular elements differentiate. In some cells mitochondria darken and their cristae swell whilst parallel arrays of endoplasmic reticulum become prominent. Between Day 5-8 the mitochondrial darkening and swelling of the cristae increase whilst the endoplasmic reticulum forms concentric figures and the vacuoles become extensive.

Neither Öpik (1965, 1966) nor Smith (1974) reported cell divisions occurring during germination in the cotyledons of P.

vulgaris and these organs after losing their food stores, undergo rapid senescence followed by abscission from about Day 16 onwards. Thus normally the epigeal cotyledon in this species is an evanescent organ whose essential function is concerned with the early nutrition of the seedling before the latter becomes autotrophic. However, previous investigations (Frame *et al.*, 1976) have shown that immature cotyledons of this species can be induced to proliferate *in vitro* producing a callus. The current investigation concerns the *in vitro* culture of mature cotyledons of this species and has established that callus cultures derived from the latter source can be routinely sub-cultured and continue to flourish although organogenesis is absent, over a prolonged, potentially indefinite, period. Hence growth and development *in vitro* of mature cotyledonary tissue of *P. vulgaris* strongly contrasts with its normal fate in the germinating seedling. Somewhat surprisingly, neither the procambium nor epidermis appear to divide *in vitro*, and the present investigation primarily concerns certain of the changes in the storage cells of the explant, which occur during their dedifferentiation and callusing. In these cells the highly polyploid nuclei (Smith, 1974) become reactivated and mainly divide amitotically, whilst profuse development of freely-forming walls and large, labyrinthine wall bodies occur in the adjacent cytoplasm.

In a recent review, D'Amato (1978) suggests that amitosis (nuclear fragmentation) may be a common phenomenon when plant explants from various sources are grown *in vitro* on a medium lacking auxin, or where the auxin-kinetin ratio is unbalanced. Cionini *et al.* (1978) have reported on the callusing of immature cotyledons of *Vicia faba* explanted on such a medium. Their

findings (which are however confined to light microscopical observations of squashed tissue) demonstrate that in this species nuclear fragmentation dominates over mitosis during early growth of the explant, and that free wall formation occurs in association with amitotic nuclear division. The present research on P. vulgaris cotyledons investigates in detail at the electron microscopic level these and other induced modifications of normal development during in vitro dedifferentiation.

Tschirch and Oesterle (1900) described the seed of Linum usitatissimum L. and they reported that large aleurone bodies (protein bodies) occur in the cotyledons but that starch is absent. According to Mayer (1978, Table 2.2.) the three main food reserves found in the L. usitatissimum cotyledons are: protein 23%, lipid 34%, and sugar 23%. An account of the structure of the protein bodies of the 24 hour hydrated cotyledonary storage cells of the same species was given by Poux (1965) and she reported globoid and crystalloid inclusions in these bodies. Crooks (1933) gave a description of the external morphological changes in the germination of L. usitatissimum: this begins with the emergence of the radicle from the seed coat by the end of the first or second day; the hypocotyl then elongates whilst the cotyledons are still in their seed coat and the hypocotyl, which is straight at Day 0, bends to a hook shape. With the further elongation of the radicle and hypocotyl, the cotyledons are pulled out of their seed coat by about the fifth day of germination and the hypocotyl eventually straightens. The epicotyl does not commence growth until the hypocotyl has fully elongated and is about 4-6cm long. In a transverse section

the lower part of the hypocotyl is root-like in vascular arrangement and a gradual reorientation of the primary vascular tissue occurs at successively higher levels throughout the hypocotyl. In the upper part of the hypocotyl, just beneath the cotyledons,eight vascular bundles are observed. These are separated in two groups, each of which becomes a cotyledonary trace.

The development of adventitious buds on the hypocotyl of L. usitatissimum (following decapitation beneath the cotyledons) according to Crooks (1933) and Link and Eggers (1946a), was reported first by Reichard (1857) and later commented upon by Burns and Hedden (1906), Tammes (1907), Beals (1923), Adams (1924). According to Crooks (1933) plants not more than 10 days old at decapitation produce 5-20 buds on the lower part of the hypocotyl and these still develop even when most of this organ is excised to leave only a few millimeters above the ground. The adventitious buds do not develop synchronously and when the better developed ones are already macroscopically visible and have already produced small leaves, others are still in primordial stages of development. Finally one bud (which may be at any level in the hypocotyl in relation to the rest of the buds) outgrows the others. This bud is not necessarily the first to have been initiated on the hypocotyl and it gives rise to a replacement shoot which grows as an unbranched axis until the formation of a flower cluster; this matures at approximately the same time as clusters on intact plants of the same age.

The first change occurring to the hypocotyl after decapitation is the expansion of intercellular spaces in the cortical region. According to Crooks (1933) the initiation of

a bud starts with a division of a single epidermal cell and this is quickly followed by a second division to produce the so-called four-cell stage. Crooks (1933) does not specify the planes of these first divisions, although from the illustrations he provides it seems that the first division is a radial longitudinal one and the second tangential longitudinal or vice versa. In turn the neighbouring epidermal cells become meristematic, whilst the underlying cortical cells lose their chloroplasts, become less vacuolated and begin to divide; this activity of the cortical parenchyma continuous until an active zone of cells extends to the endodermis. The axis of the adventitious bud is said to be derived from the original epidermal cells. After the bud primordium bulges out from the hypocotyl two or three small leaves become visible on it and they develop protoxylem elements before a vascular connection is established between the main vascular cylinder of the hypocotyl and the new bud; this vascular connection differentiates progressively inwards from the new bud to the axial vascular tissue of the hypocotyl.

Some additional details on bud initiation in this species were given by Link and Eggers (1946a); according to these workers, following decapitation the hypocotyl becomes greener and its upper region swells due to an increase in the intercellular spaces. In the material which they investigated (the same variety used by Crooks, 1933) adventitious buds develop on both decapitated and non-decapitated hypocotyls but in the former macroscopic buds develop only on the upper half of the hypocotyl whereas on the intact hypocotyl buds are initiated on the lower hypocotyl but do not usually develop into macroscopic structures. Link and Eggers (1946a) also reported that the first division leading to

bud development is always a transverse division and that this is followed by more transverse divisions within the mother epidermal cell; the radial and tangential longitudinal divisions of the epidermal cell (as illustrated by Crooks, 1933) only occur subsequently. The initial division of an epidermal cell is not preceeded by a grossly detectable increase in its size, nor is there a marked increase of its cytoplasmic contents before and subsequent to the first divisions of the cell.

Gulline (1960) demonstrated that on decapitated hypocotyls of this species which were partly covered, bud initiation only occurs on areas of the hypocotyl exposed to light. She also noted that in the Ventnor variety of L. usitatissimum employed, buds are only initiated on the decapitated hypocotyl.

2. MATERIALS AND METHODS.

2.1. Culture of experimental material.

2.1.1. Phaseolus vulgaris L. var. Bush Blue Lake 274 (Dwarf Bean).

Seeds of *Phaseolus vulgaris* were supplied by Hurst, Gunson, Cooper, Taber Limited, Witham, Essex, and used for both *in vivo* and *in vitro* studies on cotyledon development.

For the *in vivo* observations, seeds were planted in Leavington's compost in pots in the greenhouse in early summer under natural lighting conditions; cotyledon samples were collected at two day intervals for 12 days from sowing and fixed and processed for microscopy (see section 2.2.).

For the *in vitro* experiments, seeds were soaked for 24 hours in tap water, then the testas were removed carefully without damaging the cotyledons. The two cotyledons of each seed were separated and the ends of the cotyledons between which the embryo was located, were excised and discarded. (This ensured that the subsequent callus formed *in vitro*, proliferated from cotyledonary tissue only). The remaining $\frac{2}{3}$ - $\frac{3}{4}$ of each cotyledon was then sterilised in 0.1% mercuric chloride solution for 10 minutes and washed three times in sterile water. One or two cotyledons were explanted aseptically, per 100ml Erlenmeyer flask, with their adaxial surface firmly in contact with the sterile nutrient medium (Pl. 7A). The flasks were incubated in a growth room at 25°C under continuous low intensity lighting.

In preliminary experiments on callus induction from cotyledons, media A, B, J and K were used (see section 2.1.2.) and medium J was finally chosen to be used in all subsequent experiments as it generally induced the most rapid callusing of

the explant and supported good growth of the callus cultures established from the explant. All microscopic observations were made on material cultured on this medium.

The responses of the individual cotyledon explants to *in vitro* culture on this medium were variable but within four to five weeks the callus tissue, formed *de novo*, occupied most of the surface of the nutrient medium and was in a suitable state to be subcultured (Pl. 8A). Callus tissue derived from a single cotyledon was then selected as a source of inoculum to subculture and small pieces of about $0.5cm^3$ were transferred to fresh medium. The material was subsequently routinely subcultured every four-six weeks for a year. Tissue samples from both the callusing explants and established cultures were fixed and processed for microscopy (see section 2.2.).

2.1.2. Preparation of culturing media.

The *basal* medium is derived from that of Murashige and Skoog (1962) and its constituents are listed below:

Inorganic micro-nutrients		Inorganic macro-nutrients	
$FeSO_4.7H_2O$	27.8mg/l	$MgSO_4$	370mg/l
$MnSO_4.4H_2O$	22.3mg/l	$CaCl_2.2H_2O$	440mg/l
KI	0.83mg/l	KNO_3	1900mg/l
$CaCl_2.6H_2O$	0.025mg/l	NH_4NO_3	1650mg/l
$ZnSO_4.7H_2O$	8.6mg/l	KH_2PO_4	170mg/l
$CuSO_4.5H_2O$	0.025mg/l		
H_3BO_3	6.2mg/l		
$Na_2MoO_4.2H_2O$	0.25mg/l		

Organic constituents

Sucrose	30g/l
Glycine	2mg/l
Myo-Inositol	100mg/l
Vitamin B_1	0.1mg/l
Vitamin B_6	0.5mg/l
Nicotinic acid	0.5mg/l
EDTA (disodium salt)	37.3mg/l

All media were solidified with 1% Agar and growth substances were added as indicated below:

Medium A: coconut milk 10%
kinetin 0.8mg/l
IAA 2.0mg/l

Medium B: coconut milk 10%
2,4-D 5.0mg/l

Medium J: coconut milk 10%
kinetin 0.8mg/l
2,4-D 5.0mg/l

Medium K: kinetin 0.8mg/l
2,4-D 5.0mg/l

All ingredients were dissolved in distilled water in a large conical flask, except Agar which was added last after the pH of the madium had been adjusted to a value between 5.8-6; the medium was then autoclaved for 15 minutes at 15atm. in order to dissolve the Agar and approx. 50ml was then poured into each 100ml Erlenmeyer flask; colour coded cotton bungs were used as

stoppers and the medium was autoclaved again for 20 minutes at 15atm. in order to sterilise it. The medium was normally stored in a coldroom at $4^{o}C$ in the dark.

2.1.3. Linum usitatissimum L. var. Linott.

Seeds of L. usitatissimum were kindly supplied by Dr. Ibrahim of Carleton University, Montreal, Canada.

For the investigation of the early stages of germination, seeds were placed in Petri dishes lined with filter paper, kept well moistened with tap water, and grown under natural illumination in the laboratory on a bench near a window (Day 1, 2, 3 and 4 material). Tissue for microscopic investigation of these stages of germination was selected from the hypocotyl adjacent to the cotyledons. For the examination of the hypocotyl at later stages of growth and during adventitious bud formation, seeds were planted in pots or trays of Leavington's compost about 0.5cm beneath soil level. The seedlings were grown in a heated greenhouse with supplementary irradiation supplied by Thorn 400W MBFR/U high pressure mercury vapour lamps to give a photoperiod of 16 hours throughout the year. After 10 days growth in these conditions the majority of the hypocotyls were about 5cm high above the soil level, fully straightened and had ceased elongation. At this stage seedlings were selected for uniformity. From some control specimens (Day 10), 5mm segments were excised from the upper hypocotyl (i.e. adjacent to the cotyledons) fixed, and processed for microscopy. The remaining seedlings were, however, left growing; some of these were left intact (as additional controls) but others were decapitated about 1mm beneath the cotyledons and the hypocotyl/

radicle axes allowed to continue growth. Segments from the upper hypocotyls of representative samples of both lots of material were fixed and processed for microscopy at suitable intervals until well developed adventitious buds had formed on the decapitated hypocotyls (about 20 days after germination). Segments from mature (about 40 days old) intact hypocotyls were also fixed and processed to compare with the old (Day 10+30) decapitated hypocotyl.

2.2. Processing of tissues for LM and EM observations.

2.2.1. Fixation, embedding and sectioning for LM and TEM.

1. The samples were normally dissected from specimens whilst submerged in glutaraldehyde or Karnovsky fixative (buffered as later specified) and then transferred to fresh fixative for $2\frac{1}{2}$ - 3 hours at room temperature. During the last 30 minutes in the fixative specimens were placed under vacuum to remove air from the tissues and ensure that any floating specimens became submerged. Usually, after fixation the tissues were processed immediately, but in some cases specimens were kept in the fixative for a few days under refrigeration at $4^{\circ}C$. The fixative most frequently used was 3% glutaraldehyde buffered with veronal acetate - HCl 0.1M at pH 7.2. However, a mixture of paraformaldehyde-glutaraldehyde (Karnovsky, 1965) was sometimes employed as an alternative fixative and this was similarly buffered.

2. The specimens were then washed in buffer only for 3 hours at room temperature (three to four changes) to remove excess fixative.

3. The specimens were post-fixed in 2% OsO_4 (buffered as above) for 2½ hours at room temperature.

4. The specimens were again washed as in step 2.

5. The specimens were dehydrated in a graded series of acetone at room temperature:

25% acetone for 30 minutes.

50% acetone for 30 minutes.

75% acetone for 30 minutes (or overnight).

90% acetone for 30 minutes.

100% acetone for 1 hour (three changes).

(the 100% acetone was kept dry by placing anhydrous $CuSO_4$ in the storage bottle).

6. Samples for infiltration in resin were placed in a 1:1 mixture of resin: acetone without accelerator at room temperature. The amount of acetone in the mixture was gradually decreased through evaporation by leaving the vials open overnight in the fume cupboard, and the samples were then immersed in pure resin. When infiltration of the tissue was complete the samples were transferred to fresh resin with accelerator added. This was changed three times, once every 15 minutes, and at this stage samples were placed in the oven at 60°C in order to accelerate the infiltration of the tissue with the (otherwise viscous at room temperature) resin plus accelerator. Finally, the samples were placed, together with fresh resin plus accelerator, in either gelatine capsules, Beem capsules, Beem capsule lids or small aluminium dishes; polymerization was then carried out under vacuum at 60°C for 24-72 hours.

The epoxy resins most commonly employed, (as detailed

below) were an EPON / ARALDITE mixture and the low viscosity SPURR (Spurr, 1969) resin. A few specimens were also infiltrated with EPON, ARALDITE or DURCUPAN but generally the cutting properties of these (polymerized) resins were inferior to the former. All resins were thoroughly mixed first by hand and then on a magnetic stirrer for at least 30 minutes before use.

Resin recipes:

EPON / ARALDITE

EPON (Epicote)	15.45gm	resin only	resin + accelerator
DDSA	25.50gm		
ARALDITE	10.00gm		
DMP 30	0.75gm		

SPURR

ERL 4206	10.00gm	resin only	resin + accelerator
DER 736	6.00gm		
NSA	16.00gm		
S-1	0.40gm		

Semi-thin (1 or 2µm) sections of the specimens were cut on the LKB 11800 PYRAMITOME and dried down in a drop of water on a gelatine coated glass slide. In many cases serial sections of specimens were obtained either by mounting each section or every 10th or 5th section (depending on thickness) in order on the slide. Permanent preparations were made (following staining, as in 2.2.4.) by using a drop of resin with accelerator as a mounting medium and sealing with a coverslip.

Ribbons of ultra-thin sections (silver-gold) were cut with either the LKB ULTROTOME I TYPE 4802A or the LKB 8800 ULTROTOME III using 6mm thick glass knives made with the LKB 7800 KNIFEMAKER. The ribbons were floated on to the surface of water in a bath made from silver self-adhesive tape attached to the knife. The ribbons were then flattened with chloroform and collected on copper grids (size either G50 or G75) coated with a thin collodion support film (2% cellulose nitrate in amyl-acetate).

2.2.2. Modified processing of some tissues for LM and TEM.

In order to obtain better fixation and infiltration of air dried embryonic tissues of P. vulgaris and L. usitatissimum the periods in reagents were modified from 2.2.1. as follows:

1. Fixation in glutaraldehyde - 24 hours.
2. Washing - 5 hours.
3. Post-fixation in OsO_4 - 12 hours.
4. Washing - 5 hours.
5. All dehydration times were doubled from those previously specified.
6. Specimens were left in pure resin, without accelerator, for up to one week and then embedded as previously indicated.

Satisfactory ultra-thin and semi-thin sections could be obtained only with difficulty from such specimens when freshly embedded. However, when the embedded material was left for several years at room temperature, and then put in the oven at $60^{\circ}C$ for about two hours, the ease of sectioning and section quality was much improved.

For the study of the very early stages of bud formation

in L. usitatissimum, in some specimens the epidermis of the upper hypocotyl was stripped off carefully, with the aid of a razor blade and a pair of fM tweezers, after the specimens were fixed in glutaraldehyde.

The final infiltration of these epidermal strips was also modified so that they would stay flat in the polymerized resin block in order that paradermal sections could be cut from large areas of the epidermis: A piece of epidermal tissue, immersed in a drop of resin, was placed in an aluminium dish and covered with a flat block of polymerized resin to keep the tissue flat. The dish was then placed in the oven and, when the resin drop with specimen was polymerized, the dish was filled with more resin and put back in the oven; the resulting solidified block of tissue can then be cut to a suitable size and shape for the microtome specimen holder. This method allowed satisfactory paradermal sections to be prepared for both LM and TEM.

2.2.3. Section staining for TEM.

A double staining procedure with uranyl acetate and lead citrate, was used for most specimens.

1. The grids were floated (section side downwards) on the meniscus of a solytion of saturated uranyl acetate in water (7.5%), at room temperature for 20-45 minutes. The stain was contained in an overfilled polythene cup, 6mm in diameter. The grids then were washed with distilled water and dried on Velin tissue.

2. The grids were floated on a single drop (section side downwards) of lead citrete (Reynolds, 1963) in a Petri dish.

The lid of this dish was lined with dental wax to make an air tight seal and pellets of KOH surrounded the drop of stain on the wax seal so as to absorb the CO_2 and hence prevent contamination of the specimens with lead carbonate crystals which are insoluble and extremely hydrophobic (Hayat, 1970, p. 254).

2.2.4. Section staining for LM.

1. Toluidine Blue: Most sections were routinely stained for about 15 seconds in a hot (about 60°C) 1% solution of Toluidine blue in 1% borax.

Certain histochemical stains were also employed on some specimens:

2. Aniline Blue Black (C.I. 20470) 1% in 7% acetic acid was used to locate proteins. The slides were dipped in the stain for 10 minutes at 50-60°C and then washed in 7% acetic acid to remove excess dye. Proteins were stained blue.

3. Mercuric Bromophenol Blue was also employed to locate proteins (Mazia et al., 1953). Protein deposits were stained blue.

4. Iodine (0.2% iodine in 2% potassium iodide) solution was sometimes used to locate starch. Such deposits stained a reddish-purple colour.

5. Polysaccharides were traced with the Periodic Acid / Schiff (PAS) technique (Grimstone and Skaer, 1972). Sections were oxidized in 1% aqueous solution of periodic acid (HIO_4) for 10 minutes, washed in running water for 5 minutes, stained in Schiff's solution for 10 minutes and finally washed in running water for 5 minutes. Any polysaccharides present were stained

red. For a control, the oxidation with periodic acid was omitted.

2.2.5. Preparation of cleared whole mounts for LM.

In order to follow the development of the vascular tissue in the decapitated L. usitatissimum hypocotyls, the latter were fixed in glutaraldehyde (as in 2.2.1.) washed in distilled water (several changes) and then treated with 5% NaOH for 24-48 hours at 40°C. The NaOH was washed away with distilled water (several changes); the specimens were then stained with a solution of 1% tannic acid and 0.5% sodium salicylate for 1 minute followed by 1.5% ferric chloride until black or blue black. The specimens were finally washed with distilled water and mounted in glycerine jelly on slides and covered with a coverslip (Bowes, 1960; Raju et al., 1977).

2.2.6. Preparation of hypocotyl segments of L. usitatissimum for SEM.

1. The specimens were fixed and washed as in 2.2.1. (steps 1-2)
2. The specimens were dehydrated in a graded series of acetone at room temperature as in 2.2.1. (step 5).
3. The specimens were dried in a Polaron E3000 Critical Point dryer by using CO_2 and warm water to provide the appropriate critical point conditions.
4. The dried specimens were mounted on aluminium studs with the use of silver conducting stain.
5. the specimens were coated with a thin layer of gold and palladium in a Polaron sputter-coater by producing an argon glow

discharge.

2.2.7. Microscopes.

For LM observations a ZEISS PHOTOMICROSCOPE was used with either a bright or phase contrast setting.

The initial two years TEM studies were made on an AEI 6B in the Botany Department and about 25% of the electron micrographs result from this work. Subsequently, facilities on a Philips 300 instrument became available in the Department of Microbiology; the majority of the later studies was carried out on this instrument, although some use has also been made of the Philips 301 electron microscope recently installed in the Botany Department.

The SEM photographs were taken with a CAMBRIDGE S600 of the Anatomy Department of Glasgow University.

2.2.8. Specimens examined by microscopy.

In the course of this study, section (for either LM or EM observations) were cut from approximately 300 blocks of processed tissue.

The photographs illustrated in this thesis were selected from approximately: 2600 transmission electron micrographs, 850 light micrographs, 20 scanning electron micrographs (a number of the specimens examined were not photographed since they did not show appropriate stages of bud development) and 60 macro-photographs. All negative material (except for colour) was processed and subsequently printed in the Electron Microscopy Laboratory of the Botany Department at Garscube.

During this investigation three different electron

microscopes were used and these were each calibrated (in the magnification range used) with a 2160l/mm standard specimen. However, variations of up to $\pm$10% may be present from the print magnification listed due to the inevitable errors occurring due to inaccuracy of the enlargers and rounding off of the print magnifications.

3. RESULTS.

3.1. The mature cotyledon of Phaseolus vulgaris L. (Dwarf Bean): its normal structure and development and modifications induced in it by in vitro culture.

3.1.1. General remarks concerning the cotyledon.

The structure of the freshly hydrated mature cotyledon of P. vulgaris has been investigated by Öpik (1965 and 1966) and Smith (1974); however, neither of these previous workers studied the air dried seed of this species. A detailed description of the fine-structure of the non-hydrated cotyledon (in comparison with the freshly hydrated organ) is therefore given here. However, this account is limited to observations of material fixed with aqueous fixatives (see sections 2.2.1 & 2) and it is possible that some hydration changes occured in the air dried tissue before fixation was completed (see Discussion). For unknown reasons the Day 1 cotyledonary tissue observed (taken from several separately processed batches of material) generally shows lower contrast and usually poorer preservation of the membranes than at Day 0.

3.1.2. Anatomy of the cotyledon at Day 0-1.

Plate 1B illustrates diagramatically a transverse section through the mid region of the cotyledon whilst Plate 9A shows the histology of a small sector of this organ. A single-layered epidermis is present and, on the abaxial side only, a single-layered sub-epidermis occurs; the ground (food storage) tissue makes up the bulk of the cotyledon and about 20-30 (mainly

longitudinally running) procambial strands are embedded in it, with their distribution more-or-less paralleling the slightly concave adaxial and markedly convex abaxial surfaces. These procambial strands are relatively inconspicuous and their diameter usually approximately coresponds to that of the larger storage cells (Pl. 9A). Within the storage tissue and the abaxial sub-epidermal layer large, intercellular spaces exist and the latter comprise about 16% of the cotyledon volume after hydration (Smith, 1974). In transverse section of the cotyledon the storage cells vary from about 30μm or more in width in the vicinity of the procambial strands to 150μm elsewhere, the procambial cells are about 5-15μm wide, the abaxial sub-epidermal cells are approximately 15-25μm wide whilst the epidermal cells are about 10μm wide.

3.1.3. Fine-structure of the storage tissue at Day 0-1.

The cell walls of the storage tissue serve as food stores (Smith, 1974). In the unpitted areas they are about 5μm thick (plasmalemma to plasmalemma) in the Day 0 cotyledon (Pl. 1A and 2A) and show two regions (Pl. 2B). The outer, denser part (i.e. the region on either side of the middle lamella) is cellulosic according to Smith (1974); it is about 2.5μm thick and consists of microfibrils orientated parallel to the plasmalemma ; the inner regions of the wall (i.e. adjacent to the protoplasts) consist of non-cellulosic polysaccharide (Smith, 1974) are less densely staining and composed of more loosely arranged microfibrils and scattered vesicular structures. Numerous pits (up to 1μm wide, Pl. 2A) traverse the thickened cell walls and the former are penetrated by plasmodesmata. Plate 2C shows the

cell wall after 24 hours of hydration; the inner wall is greatly swollen, although the outer wall appears unchanged. Compared with Day 0 (Pl. 2B), very many more vesicles, of about 50nm diameter, are present in the swollen inner wall region at Day 1 (Pl. 2D) as well as more what seem to be microfibrils, and these are more densely stained.

The Day 0 nucleus (Pl. 3A-B and 4A) is multilobed and measures approximately 10-15um in diameter. It is usually centrally located in the cell and its lobes penetrate between the surrounding large starch grains and numerous protein bodies (Pl. 3A-B and 4A). The nucleus is enclosed in a double membraned envelope and pores can sometimes be seen penetrating the latter (Pl. 3B). A single prominent nucleolus is usually evident (Pl. 3A) and highly condensed chromatin occupies most of the nucleoplasm (Pl. 3A) except for a narrow zone, up to about 0.5μm wide, adjacent to the envelope which is more-or-less (Pl. 3A-B) free of chromatin. Plate 4B shows the Day 1 nucleus which is similar in shape and size to Day 0 but the densely staining condensed chromatin is now generally more peripherally distributed (c.f. Day 0, Pl. 3A-B).

In the Day 0 cell numerous protein bodies (Pl. 1A, 2A, 3A-B, and 4A) are also present as food reserves and these occupy most of the remaining cytoplasm which is not taken up by the nucleus and starch grains. The protein bodies vary considerably in size (as seen in section) from about 0.1 to 5.0μm in diameter and they are rather angular in outline. Their contents stain uniformly (Pl. 1C and 4A) and they appear to be delimited by a single membrane. Plates 2C and 4B illustrate the effect of the 24 hours hydration on the protein bodies; these are now somewhat

swollen and appear rounder and more closely packed than in the Day 0 cotyledon.

The other main food reserve of the Day 0 storage cells is contained in the numerous large starch grains which are 10-20μm in diameter (Pl. 3A). Occasionally vacuolated protrusions occur from the margins of the starch grains; these regions are less dense than the adjacent cytoplasm (Pl. 6A) and sometimes appear to be bounded, together with the starch grains, by membranes (Pl. 6A, arrow). It is considered likely that such a protrusion represents the remains of the original amyloplast stroma within which the single large starch grain developed. Several of the starch grains in Plate 3A show some slight evidence of concentric zonation, which possibly reflects successive layers of polysaccharide deposition during their development. (This zonation disappears after 24 hours of hydration). The more readily apparent, densely staining radial lobes on these starch grains are of very common occurrence (Pl. 1A and 3A) and are considered to be artifacts resulting from either folds in the sections or uneven thickness of the latter (see discussion). Such artifacts also occur in hydrated starch grains (Pl. 4B and 5B).

Smaller plastids (as distinct from the large starch grains) are usually observed grouped around the Day 0 nucleus (Pl. 3B and 4A). Their outline is angular and rather irregular; they measure 1-5μm along their longest axis and are apparently surrounded by an envelope. These plastids show thylakoid membranes in the stroma and deposits of what appears to be phytoferritin occur (Pl. 3B). Occesionally small starch grains, up to approximately 2μm in diameter, and electron translucent

vesicles (Pl. 1C) are present. Lipidic inclusions, similar to the lipid globuli in the adjacent cytoplasm are sometimes also visible in the plastid stroma (Pl. 3B).

The plastids are not well preserved in the Day 1 tissues examined,as is evident in Plate 4B; however the majority of the plastids in this Plate now show the presence of starch grains.

Numerous lipid globules are found in the Day 0 cells surrounding the plastids, the large starch grains and lining the cell walls (Pl. 1C and 2A). They are densely stained and measure approximately 50nm in diameter. However, after 24 hours hydration, the lipid globules stain much less densely (Pl. 2C and 4B, arrows). Plate 5B illustrates this feature in greater detail; a small starch grain is surrounded by a layer of closely packed, lightly stained "honeycomb-like" vesicular structures which are considered to be lipid globules being digested.

The mitochondria at Day 0 measure up to 0.5µm in diameter and they are normally located along with the plastids around the nucleus, although some mitochondria are occasionally found in the outer cytoplasm between protein bodies (Pl. 1C). The mitochondrial envelopes are poorly defined but the internal membranes can sometimes be distinguished. The poor preservation of these organelles at Day 1 prevents a comparative examination of their fine-structure.

In the Day 0 storage cell the narrow channels between various organelles described above (Pl. 3A) are occupied by ground cytoplasm in which numerous free ribosomes occur (Pl. 3B). Neither endoplasmic reticulum nor dictyosomes have been observed in these tracts of cytoplasm but Öpik (1968) reported their presence at early stages of development in the immature

seed. In the Day 1 material, due mainly to the hydration of the protein bodies, the cytoplasmic tracts are reduced in size (Pl. 2C and 4B) and the endoplasmic reticulum and dictyosomes are apparently absent. However, during later germination these organelles again become abundant (Pl. 11B); perhaps arising de novo.

3.1.4. Fine-structure of the epidermal, sub-epidermal and provascular tissue at Day 0-1.

Superficial tissues composing the cotyledon generally show better preservation of their fine-structure at Day 0, possibly due to the more rapid penetration of fixatives and their lack of the large starch grains and the lesser concentration of protein bodies, compared to the storage cells. Although at Day 0 the cell walls in these tissues are relatively unthickened (Pl. 1A and 5A) they possess some pits (Pl. 1A, small arrows) which, however, are not as prominent as in the storage cells (c.f. Pl. 2A). The external wall of the epidermal cells is about 2.5μm thick (Pl. 1A) and is covered with a very thin cuticle; their other walls are only about 2μm thick (plasmalemma to plasmalemma). An uncommon feature of the epidermis can be seen in Plate 1A (large arrow) where the anticlinal wall is incomplete between what appear to be two distinct neighbouring epidermal cells each one of them containing its own nucleus.

The nuclei are lobed, and in sections measure up to 5μm, in the epidermal, and up to 10μm in the sub-epidermal cells. Plate 5A shows a typical Day 0 epidermal cell containing circular protein bodies up to 5μm in diameter; they appear more-or-less translucent but with some dense granular patches near

their bounding membrane and contrast with the uniformly dense appearance of these bodies in the storage tissue (Pl. 1C). The plastids (Pl. 5A) have round profiles and occasionally contain membraneous structures (arrow) and deposits of what appear to be phytoferritin in their stroma. The lightly staining mitochondria have circular or ellipsoid profiles. The ground cytoplasm is more abundant than in the storage cells and mainly contains free ribosomes and electron transparent vesicles (Pl. 5A, small arrows) and the latter measure 0.1-0.2μm in diameter. Plate 5C shows detail from a Day 0 abaxial sub-epidermal cell; several pores can be seen in the nuclear envelope and free ribosomes pack the cytoplasmic ground substance. Protein bodies are present and in their close proximity several tracts of what appears to be endoplasmic reticulum are present (Pl. 5C, small arrows).

The Day 0 provascular cells (Pl. 6C) are apparently uniform (with no evidence of vascular differentiation) and their structure is similar to the sub-epidermal cells but the intercellular spaces occurring in the latter are absent. Occasionally the provascular cells possess highly shrunken protoplasts (in which, however, the various organelles are visible) but it is not known whether this shrinkage is a fixation artefact. Plate 6B shows detail of a provascular cell after 24 hours hydration. In this particular cells several crystals are visible in the dense cytoplasm, and in one the plane of section reveals the lattice sub-structure. These crystals resemble the p-protein commonly observed in the phloem of this and other Leguminosae species (Esau, 1978; Lawton, 1978) and it is possible that this feature is an early indication of the future

differentiation of this cell into a phloem element.

3.1.5. The external morphology of the in vitro callusing cotyledon.

Even though all material was cultured identically, there was often considerable variation in the speed of callusing of individual cotyledon explants within a batch. The following account of callus development (both macro and microscopic) is therefore primarily based on the extent of proliferation from an explant rather than its length of time in culture.

Plates 7A-B and 8A-B illustrate successive stages of the callusing process. During the first two to three days of culturing, the cotyledon explants sometimes develop green pigmentation on their abaxial surfaces and this may persist in later stages of development (Pl. 8A). However, other explants (Pl. 7A-B and 8B) retain the pale yellow colour of the cotyledon when first inoculated. The initial sign of callus development is normally observed in the region where the margins of the slightly concave, adaxial surface of the explant is embedded in the surface of the nutrient medium (Pl. 7A, arrows). Subsequently the epidermis in this area becomes broken by the extrusion of the callus proliferating from the internal tissue (Pl. 7B, specimen a, small arrows).

Plates 7B (specimen b) and 8A-B show more advanced stages, where a considerable amount of callus tissue has already developed but in which the abaxial surfaces seem inactive. In Plate 7B (specimen b) slight callus development has also occurred in the vicinity of the abaxial vascular bundles (large arrow) which were cut across during excision of the explant.

Plate 8A shows a rapidly proliferating explant after only ten days culture and callus tissue seems to be developing from the whole of the cotyledon apart from its abaxial epidermis. In such explants which proliferate rapidly, the original cotyledons are hardly recognizable after several weeks from inoculation and Plate 8B shows the final stage of callusing generally attained after 4-6 weeks in culture. The abaxial surface remnants (arrows) of the two cotyledon explants have been lifted some distance from the surface of the agar medium by the vast expansion of the callus forming from the internal tissues. The medium itself has become somewhat dried out and its original nutrients greatly depleted, so that in order to induce further growth of the callus, sub-culturing onto fresh medium would be urgently required.

3.1.6. Histological and fine-structural observations on the development in vitro of the cotyledon.

Plate 9A shows a transverse section of a portion of a cotyledon explant, at the time of its inoculation, which is cut through the margins where ab- and adaxial surfaces merge and Plate 10 illustrates the same region from an explant but after three days in culture (c.f. Pl. 7A). Compared to Day 1 the storage cells near the adaxial surface in Plate 10 are no longer isodiametric (c.f. Pl. 9A) but have become elongated in a plane at right angles to the nutrient medium with which the adaxial epidermis was in contact. The walls of the elongated cells are now much thinner (about 2μm thick, plasmalemma to plasmalemma) and the pitted areas are correspondingly less distinct at the LM level. Distinct protein bodies are no longer present in the

callusing storage cells, but as their contents become digested these bodies progressively enlarge and join together to form one large vacuole. Neverthless, some granular material, presumably proteinaceous, is usually still visible in the vacuoles (Pl. 9B, 10, 12A and 13A). Numerous large starch grains remain clearly evident in these vacuolated storage cells (Pl. 9B and 10). Most of the adaxial epidermal cells in Plate 10 are senescent and have collapsed; at the margins the epidermal cells appear healthy but have undergone vacuolation and are now larger (c.f. Pl. 9A). Adjacent to the callusing tissue of Plate 10 the sub-epidermal storage cells remain relatively quiescent but they are now more rounded and slightly swollen compared with to the Day 1 cotyledon (Pl. 9A). In some of the sub-epidermal storage cells of Plate 10 (arrows) crystalline deposits have formed within the limits of the membranes demarcating the protein bodies (Pl. 11A). Such crystals have not been observed elsewhere in the explant nor in any tissue of the germinating cotyledons investigated in the current study; whilst neither Öpik (1965, 1966) nor Smith (1974) described their presence in the latter material. Plate 11A shows in more detail the variety of crystal profiles observed in these <u>in vitro</u> cultured cells and up to 10 crystals may appear in a section of a single protein body. Their size varies from 2-10μm along their longest axis (although in some occasions crystals of up to 50μm long and 5μm wide are observed) they are very densely stained, by both LM and EM techniques, in contrast to the lighter staining surrounding matrix of the sub-epidermal protein bodies. The higher resolution detail in Plate 11B reveals their lattice structure visible in some favourably

orientated sections.

Some of the elongated explant storage cells in Plate 10 have (as shown by thinner newly-formed walls within them) already undergone one or several divisions. In early callus development such proliferating (as distinct from vacuolating) cells tend to be located near the adaxial vascular bundles. The nuclei of proliferating cells appear to divide mainly amitotically (see details later in section 3.1.9.) and freely-forming walls occur in abundance in the cytoplasm. The amitotic divisions of the mother (storage) cells usually produce unequally sized cells and the orientation of the new walls appears to be random (Pl. 9B and 10). Plate 12A shows a typical example of a storage cell situated adjacent to an adaxial vascular bundle in an explant at an early stage of callus development. The cell has already become divided into five daughter cells of different shapes and sizes. Most of the newly-formed walls meet in the centre of the mother cell in a massive wall body, which in this section has stained densely blue with toluidine blue. (The detailed structure of wall bodies is considered in section 3.1.7.). In some of the daughter cells of Plate 12A (arrows) freely-growing walls are also present.

Fine-structural detail of a branched tract of freely-forming cell wall is shown in Plate 12B. (This would probably correspond to the early stage of growth of these walls such as occur in the area indicated by the left-hand arrow in Plate 12A). The development of the new wall in Plate 12B is uneven; in some regions it is relatively thick (up to 1μm wide) whilst elsewhere (large arrows) is very thin (about 50nm) and may be

discontinuous. The frequent close association, or sometimes apparent continuity, between the endoplasmic reticulum and the early new cell wall (Pl. 12B-C, small arrows), suggests that the rough endoplasmic reticulum might participate in the formation of the new wall. Plate 13A shows cytoplasmic detail from another storage cell in which the rough endoplasmic reticulum is profusely developed and its looped arrangment greatly resembles that of the new wall / endoplasmic reticulum complex in Plate 12B. It is possible that such endoplasmic reticulum elements as in Plate 13A demarcate the sites of new cell walls, with wall material accumulating within the lumens of the endoplasmic reticulum cisternae. Free-wall formation occurs only within the ground cytoplasm; no conclusive evidence is available as to whether in these highly vacuolated cells the new wall formation proceeds only through pre-existing cytoplasmic strands or whether these strands may also develop contemporaneously with the new walls. In Plate 13B (see also the smaller area shown in greater detail in Plate 14A) the new cell wall lies very close to a large vacuole and at one point the free-ending of this wall (arrow) protrudes into a vacuole, suggesting the potential location of a future cytoplasmic strand. Plates 14B and 15 show further examples of freely-forming walls but also demonstrate that these new walls are associated with apparently fragmenting nuclei (see section 3.1.9.).

Most freely-forming walls are of uneven thickness and their growing ends are often thickened (Pl. 13B, 14B and 15, large arrows). The thickenings on newly-formed walls may later develop into extensive wall bodies.

3.1.7. Structure and development of wall bodies in cotyledons grown in vitro.

In the survey light micrograph shown in Plate 16A a number of conspicuous (up to 50μm in diameter) wall bodies are present (large arrows). These are mainly developed in storage cells located close to the adaxial vascular bundles and these bodies occur both on the mother cell and newly-developed walls (Pl. 12A and 16A). The wall body shown in Plate 16B demonstrates the labyrinthine nature of such bodies, with projections extending from the margins for up to 15um into the adjacent cytoplasm, whilst further out in the cytoplasm (dense arrow) apparently isolated fragments of wall are present and many of these are probably parts of the same body.

Plate 17 shows part of what appears to be an early stage of a developing wall body; it seems that its development is initiated by the formation of labyrinthine wall projections into the cytoplasm which progressively become thickened by the deposition of more wall material; during this process, however, parts of the cytoplasm together with various organelles become trapped (Pl. 17, large arrows) and are finally amalgamated into the wall bodies.

Wall material deposition seems to be related to the activity of the dictyosomes which are abundant near the margins of the developing wall bodies (Pl. 17 and 18A-B). Plates 18A-B illustrate what appear to be very active dictyosomes with straight or slightly curved cisternae and numerous attached vesicles. Other such vesicles occur free in the cytoplasm (small arrows) or fusing to the margins of the wall body (dense arrows) and ribosomes appear mainly in clusters or coiled

polyribosomes.

Some examples of what are interpreted as mature wall bodies are illustrated in Plates 15 and 19A-D. These bodies (which are often extensively flecked, Plates 19A and C) are always surrounded by concentrations of mitochondria, small plastids and cisternae of rough endoplasmic reticulum, whilst the ground cytoplasm contains abundant free ribosomes. However, dictyosomes (Pl. 19A) are very rarely seen in such locations, in contrast to their abundance in the developing wall bodies (Pl. 18A-B), suggesting that wall material deposition has ceased and the wall bodies have probably reached their maximum size. However, vesicles (which sometimes contain dense inclusions) may occur attached to the margins of such wall bodies (Pl. 20A-B) and many others are found free in the adjacent cytoplasm; but these vesicles do not seem to be related to dictyosomes. The rough endoplasmic reticulum elements, may still show connections to the projections from the labyrinthine margins of the wall body and several examples are visible in Plate 21A (arrows).

Plate 20B illustrates higher resolution detail of a wall body in which three areas are visible. In the external layer fibrillar material predominates, with the microfibrils apparently orientated more-or-less parallel to the plasmalemma. Internally vesicular and membraneous inclusions (corresponding to the flecked areas seen in Plates 19A and C) are enmeshed in a fibrillar framework; these inclusions are possibly remains of cytoplasmic organelles engulfed in the labyrinthine periphery (Pl. 20A) of the wall body as it expands within the cytoplasm (c.f. Pl. 17). Finally, if the wall body is developed on an already existing but unthickened cell wall, the latter is

visible as a compact central layer (Pl. 19B and D and 20B).

Plate 21B shows the margins of a wall body with some vesicles attached to its margins (small arrows) and in the cytoplasm in the vicinity of this wall body smooth as well as rough endoplasmic reticulum is apparently present.

3.1.8. Digestion of the food reserves in the storage tissue in the in vitro explant compared to the normally germinating cotyledon.

The overall digestion of reserve food in the explant cotyledon during callus development is slow compared with the germinating cotyledon. Neverthless, by about Day 3 (Pl. 10) the in vitro mobilization of food reserves may be already quite marked and is localised in the storage tissue near the adaxial surface contacting the medium (the latter containing several sources of exogenous growth substances). Subsequent digestion of these reserves progresses, somewhat unevenly, through the storage tissue towards the abaxial cotyledon surface. By contrast in germination, digestion (which is already advanced by Day 3) occurs throughout the cotyledon but commences in the storage cells most remote from the vascular bundles (Pl. 32A). It then progressively involves storage tissue nearer the bundles until , at about Day 10, the remaining living elements are confined to the vascular tissue and its ensheathing cells, the sub-epidermal and epidermal cells (Pl. 32B). From about Day 16 onwards abscission of the cotyledon occurs.

The digestion of reserves both in vitro and in vivo mainly involves the inner, unevenly deposited, non-fibrillar areas of the storage cell walls (Pl. 2A and B), the protein

bodies and the starch grains. In the freshly hydrated (Pl. 2C) and germinating cotyledon (Pl. 34A) a considerable expansion of the innerregion of the storage cell wall is observed and later numerous, extensive elements of rough endoplasmic reticulum are present paralleling the plasmalemma (Pl.35). In the explant the initial swelling of the inner region of the wall on hydration is rapidly followed by the overall thinning of the wall as the cell undergoes vacuolation growth (c.f. Pl. 9A at Day 1 and Pl. 10 at Day 3). It is possible that the mobilized wall material is incorporated in the expanding walls (whereas in the germinating cotyledon the storage cells undergo little expansion) and an excess of such material might possibly be a factor contributing to the proliferation of freely-forming walls and wall bodies.

Starch digestion occurs similarly (but proceeds at different rates) in both *in vivo* and *in vitro* material. In many cells undergoing digestion of storage products (especially those near the vascular bundles) starch grains are frequently partially fragmented (Pl. 22D) and contain prominent central cavities with channels leading to the exterior (Pl. 33A). The cavity is filled with cytoplasm containing various organelles (Pl. 33B and 34A) including small polymorphic plastids. Starch digestion presumably occurs progressively inwards from all the fragmented surfaces exposed to the cytoplasm. The origin of these cytoplasmic intrusions might perhaps be related to a starch "explosion" such as occurs during hydration of the cotyledon of *Pisum sativum* (Swift and Buttrose, 1972 and 1973). However, in Day 1 cotyledon of *P. vulgaris* the starch grains do not appear to be fragmented. In later stage material the fragmented starch grains only occur in the vicinity of the

vascular tissue and in the more remote storage tissue their digestion seems to proceed progressively from the outside of the intact grain towards the inside. However, in neither cases is there accumulation around the grain of endoplasmic reticulum, dictyosomes or vesicles which might be indicative of localised enzymatic activity or the transportation of hydrolysed products through the cytoplasm (Pl. 33B).

Large starch grains, which are still seemingly intact, are sometimes observed in highly vacuolated cells of the explant (Pl. 9B and 16A), and may occur in the callus resulting from the first sub-culture.

In the germinating cotyledon both the epidermis and the abaxial sub-epidermis remain alive until the organ is ready to abscind (Pl. 32A-B), whilst their protein bodies retain their amorphous deposits. By contrast in the <u>in vitro</u> development the adaxial epidermal cells normally collapse soon after inoculation, whilst in the sub-epidermal cells protein crystals are formed within the protein bodies (Pl. 10).

In the storage cells of both the germinating and explant cotyledon, the digestion of the protein is accompanied by enlargment and vacuolation of the protein bodies; these coalesce and eventually form a large central vacuole (Pl. 34A).

3.1.9. The nuclear behaviour during callusing of the explant cotyledon.

As it has been mentioned before neither Öpik (1965 and 1966) nor Smith (1974) observed any cell divisions in the germinating cotyledon. The storage cell nuclei at Day 1 are highly polyploid (8-32C, according to Smith, 1974) but there is

a progressive decrease in the DNA level of the nuclei from an average of 16C in the Day 1 material until complete senescence after about 16 days. No changes during germination were noted from the 2C epidermal and 4C sub-epidermal levels at Day 1 (Smith, 1974).

The present study confirms the absence (as observed from observations of sectioned material) of nuclear divisions in germinating cotyledons, but when *in vitro*, the storage tissue is induced to undergo abnormal growth and proliferate, and it is evident that both mitotic and amitotic divisions occur. Similar phenomena have also been observed (but only at the LM level) in callusing explants from immature cotyledons of *Vicia faba* and other sources (D'Amato, 1978; Cionini *et al.*, 1978, see discussion).

Examination of numerous sections from various explants at both LM and EM level, indicated that in the dedifferentiating storage cell the first division, at least, is normally amitotic. Thus amitotic division stages are much more commonly encountered than mitotic figures in the young explant whilst mitotic divisions appear to be confined to daughter cells. Plate 26F (detail of the cell indicated by the dense arrow in Pl. 16A), illustrates, however, a mitosis proceeding in a daughter cell only partially demarcated by the freely-growing cell wall; in this plane of section, a nucleus is not visible in the other daughter cell.

3.1.10. Amitotic nuclear divisions in the in vitro grown storage cells.

As digestion of the protein reserves proceeds the

resultant vacuoles progressively expand and the storage cells increase in volume. The nuclei either migrate (from their central position at Day 0) to near one of the cell walls, or remain suspended by cytoplasmic strands in the centre of the cell (Pl. 10). The nucleus also increases considerably in size (up to 60μm in diameter) and at the same time changes in its outline are observed. Plates 22A-D, 23A-B and 24B illustrate a selection of nuclei from activated cells; their envelopes are highly convoluted and deep cytoplasmic channels intrude into the nuclei (Pl. 22A, 23B and 24A-B). The nucleoli are generaly enlarged (being up to 25μm in diameter) and most nuclei are now multinucleolate (Pl. 22B-C and 23A-B). Almost all activated nucleoli contain vacuoles, some of which are very large (Pl. 22C), and there are indications that these may be connected to the nucleoplasm (Pl. 25A, arrow), perhaps resulting in a multinucleolate condition. Plate 26A shows a nucleolus from another activated cell; one large central vacuole (measuring approx. 3μm in diameter) and several smaller ones are present. These vacuoles have a granular appearance (see detail in Pl. 26B) whilst the surrounding nucleolar material consists of very dense (□) and somewhat less dense (△) zones. These possibly correspond respectively to the fibrillar and granular zones described by Gunning and Steer (1975) for nuclei of other species. However, Plate 26B shows higher resolution of these two zones but even at this magnification it is not possible to visualise clearly a fibrillar component, although the granular zone is quite apparent. (The same reservations concerning the so-called fibrillar and granular zone applies to other publications, e.g. Plate 19 of Gunning and Steer, 1975, and

Fig. 1 of Feldman and Torrey, 1977).

Plate 23A is considered to illustrate an early stage in fragmentation in a now multinucleolate nucleus of a storage cell. The position of the highly convoluted nuclear envelope is indicated by the dotted line and a deep cytoplasmic enclave (△) is present within the nucleus; Plate 23B shows fine-structural detail of a similar situation. In both cases it seems that a multiple fragmentation is in progress eventually leading to the production of several unequally sized nuclei. Plate 24B shows what is interpreted as a later stage of nuclear fragmentation where only one narrow connection (arrow) links the potential daughter nuclei (in this plane of section). In the cytoplasmic channels running within the nucleus, the densely stained mitochondria are prominently evident. Nuclear fragmentation is also observed in older explants in the adaxial sub-epidermal cells (Pl. 25A).

As it was mentioned in section 3.1.6. segments of freely-forming wall are sometimes visible with their free ends in proximity of a nuclear isthmus (Pl. 14B, 15, and 22D). In Plate 22D the ends of four freely-growing cell walls (arrows) nearly touch the nucleus and one of them is located against a nuclear constriction (solid arrow). A similar situation can be seen at a higher resolution in Plate 14B and it is apparent that the free end (arrow) of the freely-growing wall is not actually touching the nucleus but there is a thin layer of cytoplasm separating them. In Plate 15 the freely-growing wall segments (large arrows) adjacent to the nuclear isthmus are also isolated from the latter by a thin layer of cytoplasm. Similar nuclear constrictions have been reported at LM level by Cionini *et al.*

(1978) and D'Amato (1978) (see discussion) and they consider these to be advanced stages of nuclear fragmentation.

It is not known whether the apparently intruding free ends of such walls play an active role in the constriction of the nucleus; it might be that such walls are always associated with advanced stages of nuclear fragmentation but are not necessarily revealed in the plane of the section observed (e.g. Pl. 24B).

From Plates 14B and 15 it is evident that no phragmoplast microtubules are present at the ends of the freely-forming walls, although these are present during cytokinesis (Pl. 29B).

3.1.11. Mitotic divisions in the daughter cells of the in vitro grown explant.

Plate 26C-E shows serial sections of a metaphase nucleus in which a large number of chromatids occur whilst the telophase nuclei of Plate 26F also shows numerous chromosomes. At higher resolution (Pl. 27A) of a metaphase plate sectioned longitudinally, kinetochore microtubules (plus endoplasmic reticulum) can be seen in several regions and one is attached to a chromatid (Pl. 28A, arrow). The nuclear envelope has disintegrated and the area which it formerly demarcated also contains a number of rough endoplasmic reticulum profiles and numerous free ribosomes. Plate 28B shows the fine structural features of what seems to be a face view of the metaphase plate; here it is difficult to distinguish individual chromatids and, at least partly due to the thinness of the section, their number is far less than revealed from the serial sections of Plate 26C-E. The other feature which needs to be considered is the likelihood that,

following fragmentation, the daughter nuclei which undergo mitoses have highly irregular chromosome complements.

Cytokinesis closely follows mitotic nuclear division and Plate 27B illustrates the developing cell plate lying between the newly-formed, flattened, daughter nuclei. Each nucleus contains numerous nucleoli and a concentration of endoplasmic reticulum, small plastids and mitochondria are evident in the vicinity of the nuclei; Plate 27C shows a similar situation at higher resolution. Plate 29B shows in detail the margin of a centrifugally developing cell plate which consists of numerous aggregated membraneous vesicles whilst some endoplasmic reticulum elements occur in close association with the cell plate (arrows). Numerous phragmoplast microtubuli are visible in the cytoplasm running obliquely towards the cell plate. In the first-formed central region of the cell plate the microtubuli are no longer present (Pl. 27C). Plate 29A illustrates the vesicular structure of this plate; the strands of endoplasmic reticulum which are sometimes apparently connected with it may possibly demarcate the sites of plasmodesmata. The presence of several dictyosomes, and their vesicles, in the vicinity of the cell plate suggests that they may be concerned in the formation of the latter.

3.1.12. Cytoplasmic enclaves in the nuclei of cotyledon explant cells.

Both LM and TEM observations reveal the presence of cytoplasmic enclaves in the multilobed nuclei of the callusing storage cells (Pl. 22A, 23A, 25A and 31A-B). These enclaves (which also occur in the established culture, Pl. 37A) have circular profiles measuring up to approximately 5μm in diameter

and no nuclear pores have been observed in the envelope which delimits them (Pl. 31C, arrows). Elsewhere, however, in the nuclear envelope pores are abundant (Pl. 24, large arrows; see also Pl. 38). Several of the enclaves in Plate 31A-B contain a large number of osmiophilic bodies, measuring 0.1-0.5μm in diameter, together with a number of vesicles, segments of rough endoplasmic reticulum and some free ribosomes (Pl. 31B). No other organelles have been observed here thus making these enclaves distinct from, transversely sectioned, cytoplasmic channels in which mitochondria are usually abundant (Pl. 24B).

Some similar cytoplasmic enclaves have been observed in suspensor cell nuclei of P. vulgaris (Schnepf and Nagl, 1970) and they suggest that these represent lysosomes.

In Plate 31A the enclaves marked with the symbol △ are electron transparent or contain a loose fibrillar deposit and appear similar to vacuoles. These enclaves might thus be larger cytoplasmic channels with vacuolar extensions traversing them; this interpretation is supported by the presence of a thin cytoplasmic-like layer lining them which is apparently delimited on the inside by a single membrane.

3.1.13. The fine-structure of the dedifferentiating cotyledon storage cells cultured in vitro.

A progressively increasing proportion of the volume of dedifferentiating storage cells is occupied by cytoplasm. Certain cytoplasmic features of such cells have previously been described but this section details features not directly related to the wall and nucleus.

In Plate 14B a circular enclave (lysosome) is seen

in the peripheral cytoplasm of a dedifferentiating cell and it presents a somewhat different appearance from the main cytoplasm. The latter contains numerous endoplasmic reticulum elements, dictyosomes, free ribosomes, clusters of "tailed" plastids (with small starch grains) and mitochondria. Plate 30A shows, at higher magnification, the whole of the large enclave (△) seen in Plate 14B, plus an additional smaller enclave. These enclaves are delimited by double membranes, which in places are widely separated, from the main cytoplasm (Pl. 30A, large arrows). Further detail of these enclaves is shown in Plate 30B-C; they measure approximately 3-15μm in diameter and contain osmiophilic deposits (similar to the cytoplasmic enclaves in the nucleus) and the smaller enclave (Pl. 30C) is mainly occupied by vesicles measuring approximately 0.5-1.0μm in diameter, although what appears to be a dictyosome is also present. In the larger enclave of Plate 30A endoplasmic reticulum membranes (with very few attached ribosomes, see detail in Pl. 30B) and profiles resembling mitochondria occur. The presence of organelles interpreted as senescing mitochondria or dictyosomes, and also the presence of osmiophilic bodies, which are probably lipid globules, as well as the generally different appearance of the contents of the enclaves from the main cytoplasm suggest that these might represent autophagic lysosomes (Matile, 1974).

In the present case it seems possible that during dedifferentiation, a part of the cytoplasm becomes invested by a sheet of endoplasmic reticulum to eventually form a compartment isolated from the rest of the cytoplasm. Plate 13A possibly shows an early stage of this process where the areas marked with a △ seem to have been almost completely surrounded by

endoplasmic reticulum elements and separated from the main cytoplasm. It is possible that the large enclave in Plate 30A represents a later stage of this autophagic process in which distinct senescing organelles e.g. endoplasmic reticulum and mitochondria, are still recognisable, whilst the smaller one shows an even more advanced stage where the electron lucent vesicles might represent the various, already partly autolysed, organelles. In Plate 14B the vacuole, which is delimited by a single membrane, could be considered as a lysosome at its final stage. Possibly the external membranous element of the envelope originally surrounding the cytoplasmic enclave, now forms the tonoplast of the vacuole whilst the other (internal) membrane has been autolysed. The solid arrow in Plate 14B indicates some darkly stained vesicular and membranous structures which might represent some of the end products of this autophagic process. Similar autophagic phenomena in other species have also been reported by Villiers (1971), Coulomb and Buvat (1968) and Mesquita (1972).

The enclaves observed in the cytoplasm (Pl. 30A-C) appear to be very similar to the cytoplasmic enclaves within the nuclei of the explants as previously described (c.f. Pl. 31A-B), but the former contain smaller osmiophilic deposits and more recognisable organelles (e.g. mitochondria or dictyosomes) than the latter. Although cytoplasmic enclaves were observed in the nuclei of the cells of the established callus no evidence of them was found in their cytoplasm. No enclaves were observed in either the nuclei or the cytoplasm of the <u>in vivo</u> germinating cotyledon.

In the cytoplasm of dedifferentiating storage cells in the *in vitro* grown cotyledon many more plastids are usually observed than at Day 0-1. The profiles of these proplastids and amyloplasts vary considerably in size from about 0.5 to 6µm and they may vary from circular to rod-shaped, as seen in sectioned material (Pl. 14B and 15). The stroma is usually granular and uniformly stained and occasionally contains some thylakoids (Pl. 20A, small arrows). The amyloplasts are easily recognisable due to the several starch grains, measuring from 0.5 to 3µm in diameter, which they contain. However, the smaller proplastids are difficult to distinguish from mitochondria (Pl. 14B, 15 and 21B). The amyloplasts and plastids may occur more-or-less isolated or in clusters which are normally located near the nucleus (Pl. 15). Usually the proplastids and amyloplasts are aggregated in clusters (△), are smaller than those occurring singly in the cytoplasm; the former sometimes show "tails" (Pl. 14B and 15) which are orientated towards the centre of the cluster.

In the storage cells of the *in vivo* germinating cotyledon neither type of plastids were observed but instead chloroplasts developed in certain cells. In Plate 34B these measure approx. 5µm long and contain starch grains of up to 3µm in diameter. The stroma is granular and very densely stained and within it very closely packed grana, composed of up to 20 thylakoids, occur.

The mitochondrial profiles observed in the dedifferentiating storage cells are morphologically very similar to the ones in the Day 0-1 or the *in vivo* germinating cotyledon. These organelles, as it has been previously mentioned, are

particularly conspicuous near the labyrinthine projections of the wall bodies (Pl. 19A and 20A-B), and near nuclei undergoing mitosis. In amitotically dividing nuclei (Pl. 23B, 24B and 25A) the mitochondria are found deep in the cytoplasmic channels penetrating the nucleus. Occasionally profiles of apparently bell-shaped mitochondria are observed in the dedifferentiating storage cells (Pl. 15, dense arrows) similar to the mitochondrial profiles described by Bagshaw (1969) for the callusing tuber cells of Helianthus tuberosus.

3.1.14. Histology and fine-structure of the established callus tissue grown in vitro.

Macroscopically the established callus appears as an amorphous tissue mass (as in the callus directly derived from the explant in Plate 8A); its colour is yellow-brown, or sometimes yellow-green, which turns to brown when senescing. During the one year, in which the cultures were maintained, no shoots or roots (or their primordia) were observed. The callus is very friable, suggesting that the cells are only loosely connected. Occasionally some relatively harder groups of cells are found deeper in the callus but there is no evidence of tracheary tissue.

LM observations indicate a high degree of heterogeneity in the cells comprising the callus, with no cells from the original explant being present in the material investigated, which had been through 8 subcultures. Normally the cells near the surface of the callus are closely packed, relatively small (about 30-100µm along their longest axis) and highly irregular in shape (Pl. 36B and 37B). Their polymorphism is also visible

in fresh callus tissue examined under the dissecting microscope, thus confirming this as a real feature of the cells rather than an artefact introduced during processing.

Plate 36A is a representative area from the interior of the established callus. The highly vacuolated cells are mostly rounded and longer (up to 200μm) than the surface cells, whilst the outlines of the non-rounded cells are generally not as complexly irregular as in the surface cells. The intercellular spaces are extensively developed thus contributing to the friability of the callus tissue noted previously.

From the cytological point of view many differences are found from cell to cell (c.f. Pl. 36A and 37B); however, the individual organelles appear similar within cells. Plates 36B and 37B show meristematic-like cells in which the nuclei (each usually containing a single large nucleolus of up to 8μm in diameter) lie in dense cytoplasm, either in strands within the vacuole or lining the wall. The fine-structure of a similar cell is shown in Plate 39A, here the highly lobed nucleus is surrounded by dense cytoplasm containing free ribosomes, endoplasmic reticulum elements, mitochondria (with circular or oval profiles up to 1μm wide) and numerous small (2-5μm) "tailed" amyloplasts without grana. Plate 38 shows detail of a nucleus and surrounding cytoplasm from such a cell. As in the explant the major lobes of the nucleus are intricately sub-divided, pores are evident in the envelope (arrows) and a connection between the outer nuclear membrane and the endoplasmic reticulum is visible (small arrow). Cytoplasmic enclaves sometimes occur in the nucleus (Pl. 37A, arrows). In Plate 39C a series of parallel microtubules are evident in relation to the cell wall/

plasmalemma interface of a callus cell. In neither the proliferating explant nor germinating cotyledon have microtubules been observed in this position, although phragmoplast microtubules (Pl. 29B) occur in the explant. In Plates 38 and 39C numerous ribosomes are evident in the dense cytoplasm and many of them form in polysomes. In the highly vacuolated cells the ground cytoplasm not located in the vicinity of the nucleus appears very translucent with very few ribosomes and relatively few other organelles (Pl. 39B). The walls of the established callus cells are generally thin (measuring about 1.0μm, plasmalemma to plasmalemma) and plasmodesmatal connections through these walls have not been observed.

As in the cotyledon explant the proliferation of the established callus appears to occur by amitotic as well as mitotic divisions. Plate 38 shows a highly complexly lobed nucleus and Plate 37A illustrates what seem to be two daughter nuclei (with prominent nucleoli) which are thought to have formed amitotically. By contrast Plate 37C (detail of arrowed cell in Pl. 37B) shows a mitotic nucleus.

Freely-forming walls have been observed in the established cultures and may participate in nuclear fragmentation. Wall bodies (Pl. 37A and 40A-B) are common in these cultures and are of similar form and size range to those in the explants. However, in some cases large aggregations of myelin-like bodies occur in addition to the vesicular inclusions found in the explant.

3.2. The hypocotyl of Linum usitatissimum L.: its normal structure and development and modification induced in it following decapitation.

3.2.1. Day 0 (air dried) embryo.

The cytological characteristics of the various tissues in both the hypocotyl and cotyledons are: All cells contain (usually in copious quantities, see analysis figures on following page) protein and lipid bodies and these deposits are usually angular in outline (Pl. 42A-B). The nucleus is more-or-less spherical but with a slightly irregular, but not lobed, margin (c.f. the Day 0 nucleus of P. vulgaris, Pl. 3A). A single nucleolus (staining more densely than the general cytoplasm) usually is observed in the centre of the nucleus. In the peripheral nucleoplasm patches of material of greater density than elsewhere are commonly observed (Pl. 42, large arrows) although this could represent a staining artifact (c.f. 45B). As it is commonly the case in air dried seeds (see reviews by Abdul Baki and Baker, 1973, and Taylorson and Hendricks, 1977, and also in P. vulgaris) it is very difficult to identify other organelles within the cytoplasm; in this case neither endoplasmic reticulum nor dictyosomes apparently occur in the narrow channels occupying the spaces between the closely packed food reserves. However, the residual cytoplasm is densely packed with ribosomes and also structures interpreted as plastids, but not clearly defined by an envelope (Pl. 42A-B). The latter bodies may may contain electron dense granules, about 60nm in diameter (probably representing plastoglobuli) and small deposits about 0.3µm which could be either lipid or starch. In addition smaller

structures, tentatively classified as mitochondria, are visible but these are again not clearly defined by an envelope, possibly due to poor fixation (Pl. 42A). No plasmodesmata have been identified penetrating the cell walls, although, the "channels" observed in Plates 42A and 43B (large arrows), which are considered to be artifacts, might indicate sites where plasmodesmata occur. However, plasmodesmata are apparently absent even at later stages of development (e.g. in the cortical cells shown in Pl. 46B and 53A-B).

The three main reserve foods are protein, lipid and sugar, and on a fresh weight basis these occupy 23%, 34% and 23% respectively of the seed (Mayer, 1978, Table 2.2.). The protein bodies vary from approximately 1 to 12μm in diameter and within such a body four different areas of varying density are often observed (Pl. 42A and 43A-B). In Plate 42A these are identified as follows: (a), is a central electron transparent area, possibly corresponding to an air space; (b), a region of moderate density (usually surrounding area (a)), which sometimes contains membraneous or granular inclusions (small arrow); (c), the main bulk of proteinaceous material, denser than (b), which is peripherally situated and delimited by a single membrane from the cytoplasm; and (d), areas of greatest electron density located inside (c). Poux (1965) examined seeds of this species which had been hydrated for four hours and identified the areas (b)-(d) but not area (a).

In the present investigation, mercuric bromophenol blue (Mazia et al., 1953) and aniline blue black were employed as histochemical stains to identify proteins in 1-2μm survey sections. Only areas (b)-(d) gave positive reaction with these

stains.

The lipid bodies vary from 0.1 to 10μm in diameter, and the larger deposits seem to be composed of many smaller subunits fused together (Pl. 42A).

According to Mayer (1978) the same proportion of sugars is present in the seed as protein, however, when 2μm thick sections of seeds and hypocotyls were treated with the Periodic Acid/Schiff (PAS) technique (Grimstone and Skaer, 1972), no positive reaction occurred in the cytoplasm, although the cell walls did stain positively.

The walls vary in thickness from about 0.1μm (plasmalemma to plasmalemma) when in contact with other cells, to 0.5μm where contacting intercellular spaces (Pl. 42A and 43A-B).

3.2.2. Day 0 epidermal cells.

The epidermis consists of a single layer of uniform (apart from some variation in size) cells without differentiated stomata or even recognizable guard cell precusors (Pl. 43A, C-D). The cell measure approximately 5-15μm in the plane parallel to the long axis of the hypocotyl (their future main plane of growth), by about 15-20μm in a radial and 15μm in a tangential plane (Pl. 43C-D).

Their structure is similar to other tissues (as described in section 3.2.1.); but the protein bodies (Pl. 43D) are smaller, about 1-6μm in diameter, and show less internal elaboration, usually only consisting of dense deposits (area d) although zone (a) is sometimes visible. Spherical nuclei are located in the centre of the cells and measure approx. 5-8μm. In transverse sections the anticlinal (radial) walls sometimes appear

corrugated (Pl. 43D) and "channels" are sometimes evident in these and the transverse walls (Pl. 43A and C).

3.2.3. Day 0 cortical cells.

These are approximately cylindrical cells (Pl. 43A-B and 44A-B), measuring 15-20μm in the plane parallel to the long axis of the hypocotyl (Pl. 44B) and approximately 20-50μm in diameter but with the inner cortical cells tending to be wider (Pl. 43A).

The cortical cells lie in long rows parallel to the axis of the hypocotyl and their transverse end walls are fully in contact with the neighbouring cells (Pl. 44A-B). However, prominent intercellular spaces occur between these rows (Pl. 42A, 43A-B and 44A) forming longitudinal channels which may facilitate water penetration during seed hydration.

The nuclei are similar in size to those in the epidermis, they usually lie in the centre of the cells surrounded by lipid bodies and a number of protein bodies and the larger of the latter, regions (a)-(d) are usually evident. As in the epidermis, "channels" often occur in the cell walls (Pl. 42A, large arrows, and 42B).

3.2.4. Day 0 provascular tissue and pith.

In transverse sections of the hypocotyl the cells of the provascular tissue measure approx. 5-10μm in diameter and are comparatively small compared with those of the adjacent cortex and pith (Pl. 43A). They are fairly uniform in appearance and without signs of differentiation into vascular elements. They contain only relatively small deposits of lipid and protein. In a few specimens the provascular cells posessed shrunken proto-

plasts; this might be a feature of tissue from non-viable seeds, or a processing artifact probably reflecting a more rapid hydration of the wall than the protoplast, by the aqueous fixative.

The contents of the pith cells are very similar to those of the cortex but normally they are somewhat smaller (about 15μm in diameter), closely packed and without the intercellular spaces occurring in the latter (Pl. 43A).

3.2.5. Day 1-4; the hypocotyl during the early stages of germination.

Figures 24.14A-D of Esau (1977) illustrate stages in germination of L. usitatissimum from the radicle (A), through the development of the hypocotyl hook (B), the straightening and elongation of the hypocotyl and unfolding of the cotyledons (C-D).

With the hydration of the seed the mobilization of the reserve food commences in the embryo, accompanied by vacuolation of the tissue and elongation of the radicle and the hypocotyl. Already by Day 1 (Pl. 44C), the protein bodies have swollen, compared to Day 0, and in some cases neighbouring bodies have apparently fused (arrows); area (d) is no longer evident in the protein bodies. Compared to Day 0, the lipid bodies now appear rounded and are more widely spaced (Pl. 45B). In the Day 1 cells, membranous structures are generally better defined; thus in Pl. 45B the nuclear and plastid envelopes, dictyosomes and plasmalemma are clearly visible.

At Day 1 the radicle-hypocotyl axis is straight, but by Day 2-3, because of greater vacuolation of the protein bodies and consequent faster elongation of the epidermal and cortical cells in one side of the hypocotyl than on the diametrically opposite

side, a hypocotyl hook develops (Pl. 45A and 46A). By Day 4 the protein deposits have been digested and in the epidermal and cortical cells the vacuolation is especially prominent(Pl. 45A). These newly-formed vacuoles are bounded by the membranes originally delimiting the protein bodies. In Day 3 specimen shown in Plate 46B, granular and membranous inclusions (arrows) from the original protein bodies are sometimes still persistent. These occur in the large, central vacuoles now occupying each of the epidermal and cortical cells as a result of the fusion of the smaller vacuoles. This type of vacuolation, which is similar to the one observed in the storage cells of the P. vulgaris cotyledons, has also been described in L. usitatissimum by Poux (1965) and more recently at the light microscopic level by Dhar and Vijayaragavan (1979).

During vacuolation, in the cortical cells the nuclei move from their original central position, at Day 0, into an apparently random peripheral position in the cytoplasm lining the cell wall (Pl. 45B and 46B); whilst in the epidermal cells they migrate to the peripheral cytoplasm (Pl. 47A-B) but often close to the inner (periclinal) wall. The nuclei of both tissues have increased in size approximately 10-15μm in diameter and show a more irregular outline (but not so marked as in P. vulgaris, see Pl. 3A). They contain a single large, nucleolus measuring about 5μm and this is sometimes vacuolated (Pl. 46B). In the nucleolus of Plate 47B dense and less dense areas occur, possibly corresponding to the fibrillar and granular zones of Gunning and Steer (1975).

In the cytoplasm of the Day 3-4 hypocotyl, lipid bodies are still prominent (especially in the cortex, Pl. 46B and 47B) but occupy peripheral positions and they are fewer than at Day 0

(Pl. 42A). By Day 3-4 the cytoplasm in the epidermal cells is reduced to a thin layer lining the walls (Pl. 45A and 47A) and within it abundant free ribosomes occur, whilst in addition rough endoplasmic reticulum, numerous dictyosomes and well defined plastids and mitochondria are now visible. The plastids are up to 6μm wide and are of varied morphology; they may contain sparsely developed granae and large, rounded, electron dense inclusions (Pl. 47A). The mitochondria generally have more-or-less circular or ellipsoid profiles and show well developed cristae.

Between Day 2-4 stomata and "swollen" cells (see section 3.2.6.) develop within the epidermis of the hypocotyl; their development apparently proceeds rapidly and their differentiation from the uniform epidermis of Day 0-1 has not been followed. At the same time in all types of epidermal cells of the hypocotyl a single, crystal-like, inclusion begins to develop in each nucleus (Pl. 47A). Both mercuric bromophenol blue and aniline blue black give positive reactions with these inclusions, thus indicating their proteinaceous nature. During elongation of the hypocotyl, the epidermal cells change their squat shape and become elongated parallel to the long axis of this organ. The epidermal nuclei also become somewhat elongated in this plane and the long axis of the spindle-shaped nuclear inclusions are similarly orientated. In a cross section of the nucleus, the inclusions appear hexagonal (Pl. 47A) and are up to 2μm in diameter by 30μm in length. No crystalline sub-structure has been observed in these inclusions (c.f. P. vulgaris Pl. 11B), although in longitudinal sections at higher resolution fibrillar components can be seen (c.f. Pl. 50). A close association between the nuclear inclusions and nucleoli

is frequently apparent (Pl. 47A, see also Day 10+8 epidermis in Pl. 50B and 52).

The differentiation of the cortex follows a similar pathway to the epidermis; the principal cytoplasmic differences concern the plastids, where large starch grains and prominent granae are evident (Pl. 47B); also in the cortical cells nuclear inclusions are absent.

During early germination the provascular tissue, generally contains small quantities of protein and lipid deposits. However, at Day 1 scattered groups of three or four provascular cells occur (Pl. 48A) which apparently lack such food stores, are rich in cytoplasm and contain numerous mitochondria and dictyosomes; such cells may perhaps be destined to undergo early differentiation into vascular elements. By Day 3-4 some mature sieve and tracheary elements have already differentiated. Plate 48B shows both mature and differentiating protoxylem; in the cytoplasm lining the secondary thickened walls of these differentiating elements, numerous strands of rough endoplasmic reticulum, dictyosomes and translucent vesicles are present.

3.2.6. Day 10: the fully elongated, intact hypocotyl.

A histological description of the seedling anatomy of L. usitatissimum is given by Crooks (1933), whilst Link and Eggers (1946a and b) and Gulline (1960) give additional information on the epidermal-cortical structure of the hypocotyl. However, these workers employed the rather crude fixatives then current for light microscopy and examined relatively thick sections of wax embedded material. The cytological observations are therefore greatly limited; their descriptions concentrated

more on cell walls and planes of divisions and largely ignored the cytoplasmic contents. Thus the present description of the hypocotyl, which is based on material processed with modern (EM) fixation techniques and semi-thin or ultra-thin sections of resin embedded material, concentrates on the various features which were not considered by these previous workers.

In the epidermis four types of morphologicaly different cells are found; normal epicermal cells, "swollen" cells, stomatal guard and subsidiary cells (Pl. 49A-B and 51A). In a TS of the hypocotyl the "swollen" cells (Pl. 49A, small arrows) are wider (up to 50µm) than the normal ones and protrude from the external surface of the epidermis. In a surface view the "swollen" cells measure approx. 100µm long, are pointed at one end and flattened at the other and tend to form longitudinal rows (usually single) separated by several rows of other epidermal cells (Pl. 49A-B and 50A).

The normal epidermal cells (Pl. 50A-B) are elongated (about 150µm long by 40µm wide) and in longitudinal section may be either spindle shaped, rectangular or pointed at one end and flattened at the other. A single large vacuole occupies most of the cell volume and only a thin layer of cytoplasm lines the cell walls. An elongated nucleus, with one or two nucleoli (Pl. 50B and 52) and a nuclear inclusion, is usually observed close to the inner periclinal wall and is normally surrounded by a concentration of mitochondria, rough endoplasmic reticulum, free ribosomes and some plastids. The latter are somewhat limited in number and their fine structure is characteristic of these cells; the plastids contain a granular stroma with a few thylakoids, and a central denser body, possibly of proteinaceous nature (Pl. 47A

and 54A) No starch is normally observed in them. "Swollen" cells have a similar cytoplasmic appearance to the normal ones, apparently differing only in their shape and size from the latter.

The stomata of the hypocotyl are of the paracytic type (Pl. 49A). The stomatal guard cells measure approx. 30-40μm along their long axes and subsidiary cells 40-50μm. Their plastids are more numerous than in the other epidermal cells and unlike in the latter these plastids contain large starch grains and more numerous grana. However, nuclear inclusions are present in both guard and subsidiary cells as in the other epidermal cells.

It should be emphasised that there is no evidence in either the fully elongated intact hypocotyl or in preceeding stages of germination of any occurrence of bud primordia and there is also no cytological indication of the existence of specific epidermal cells which might later give rise to bud primordia. Likewise within the cortex the lateral procambial strands (which might later form vascular connections with such buds) are also absent.

The cortical cells (Pl. 51A and 53A) are cylindrical, measuring approx. 30-40μm in diameter by 50-80μm in length; the widest ones tending to be situated in the mid cortex (Pl. 49A). Large intercellular spaces occur, especially between their longitudinal walls, forming channels along the long axis of the hypocotyl. The thin cytoplasmic layer, which lines the cell walls contains relatively few mitochondria, endoplasmic reticulum and ribosomes but chloroplasts are prominent (Pl. 51A). These contain well developed grana but only very small or no starch grains (Pl. 53A). The cell walls are very thin, about 0.2μm

(plasmalemma to plasmalemma). The nucleus (containing a single nucleolus) lies in the peripheral cytoplasm, usually about half the way along the long axis of the cell and flattened in this plane.

The topography of the vascular tissue differs in the lower and the upper parts of the hypocotyl due to the change from its arrangement in the radicle to that in the hypocotyl. In the upper part of the hypocotyl two cotyledonary traces (each consisting of a few closely associated vascular bundles, Crooks, 1933) surround the prominent pith (Pl. 49). In TS each cotyledonary trace consists of several layers of small celled tracheary elements on the inside, surrounded by a broad zone of cambium plus immature vascular elements whilst a narrow band of phloem lies to the outside.

At this stage of development of the hypocotyl fibres begin to form in the inner cortex (Pl. 49A, arrows). The fibre initials can be distinguished (before the walls thicken) by the large size of the starch grains in their chloroplasts and the numerous vesicles in their protoplasts (Pl. 54B) compared to the chlorenchymatous cortical cell (Pl. 53A). Plate 50C is an electron micrograph showing some transversely sectioned fibres; some of them appear to have empty lumens surrounded by secondary walls (which have apparently separated from the primary wall) and these are probably mature fibres; whilst others have thickened walls but contain dense cytoplasm and are presumably still differentiating. Neither Crooks (1933) nor other workers previously cited mentioned the presence of fibres in the young hypocotyl of L. usitatissimum. Esau (1943) stated that in the growing stem of this species the fibres grow by apical

intrusive growth, however this aspect of their development was not investigated in the current study.

The epidermal and cortical structure of the intact hypocotyl shows no obvious changes from about Day 10-20 but later the hypocotyl undergoes extensive secondary thickening and formation of cork cambium (see section 3.2.7.)

3.2.7. Changes occurring in the fully elongated hypocotyl after decapitation.

Within a few days of decapitation the hypocotyl has assumed a deeper green colour (than the control) and the region adjacent to the excised surface has slightly swollen (Pl. 41A). LM and TEM observations (made at various levels along the decapitated hypocotyl) show a massive accumulation of starch (Pl. 51C and 53B) inside the chloroplasts of the cortex and pith, and also in the stomatal guard and subsidiary cells but not in the other types of epidermal cells. In the upper, swollen part of the decapitated hypocotyl (Pl. 51B) the cortical cells are more irregular and with a greatly expanded intercellular space system (compared to the control material, Pl. 51A) and are often reminiscent of the spongy mesophyll cell of the leaf. The cortical cells appear to be generally wider in this region of the experimental material compared with the control (c.f. 51A-B), the cortical cell walls of the experimental material are generally thicker (0.6μm wide, plasmalemma to plasmalemma) and their cytoplasmic lining is now thicker and denser (c.f. Pl. 53A-B). Another feature of the decapitated hypocotyls is the common occurence of microbodies in the cortical cells in close association with the chloroplasts (Pl. 53B and 54C) whereas

those are rarely seen in control material (Pl. 53A).

In old, Day 10+30, decapitated hypocotyls (Pl. 55B), there are considerable differences from Day 40 (intact) hypocotyls (Pl. 55A). The epidermis remains active in experimental material and both anticlinal and periclinal divisions take place, eventually leading in places to a bilayered epidermis. By contrast the epidermal cells of the intact hypocotyl do not appear to divide after the hypocotyl has fully elongated (Day 10) and in the older hypocotyl the epidermis is becoming replaced due to the activity of the cork cambium arising in the cortex (Pl. 55A, arrow).

The cortical cells of the experimental material (at all stages) apparently retain the same number of chloroplasts as in the control but with the growth of the non-dormant adventitious bud into a large lateral shoot the cortical chloroplasts of the hypocotyl lose their massive starch grains. The development of the secondary xylem in the Day 10+30 hypocotyl is much less extensive than in the control (Day 40) hypocotyl and in the former a large pith cavity occurs (c.f. Pl. 55A-B). Decapitation also affects the development of the fibres; by Day 10+30 there are many more visible in a TS of the hypocotyl (Pl. 55B) compared to control (Day 40, Pl. 55A) material and the former fibres have thicker walls.

The decapitation of the hypocotyl also results by Day 10+10 in the development of transfer cells in relation to the main (axial) vascular strands of the hypocotyl (Pl. 55C) and such cells are not observed in comparable control material. The xylem transfer cells develop ingrowths only on the cell walls in contact with the tracheary elements (Pl. 56A) but in the phloem

transfer cells such ingrowths are apparently uniformly distributed on all walls. However, in the older material examined of both Day 40 (control) and Day 10+30 (experimental) hypocotyls, transfer cells are not visible in relation to the axial vascular tissue. This suggests that these wall ingrowths are evanescent structures, perhaps serving some temporary purpose (e.g. active transportation of solutes, see review by Pate and Gunning, 1972) and that later the ingrowths become digested.

3.2.8. The initiation and early development of adventitious bud after decapitation of the hypocotyl.

LM, TEM and SEM observations indicate that buds are initiated from the normal epidermal cells but not from the stomatal guard cells, subsidiary cells or "swollen"cells. By about Day 10+6 to 10+10, 5 to 20 adventitious buds at different stages of development are visible under the dissecting microscope and these are usually confined to the upper half of the hypocotyl (Pl. 41B). Examination of epidermal strips from various hypocotyls of these ages (in which a large number of epidermal cells from the same hypocotyl can be observed with a 10x objective lens, see section 2.2.2.) shows, however, that numerous smaller bud primordia, at the stage when only one or two epidermal cells are involved, are also present.

In one experiment, when seeds were planted under the same experimental conditions, but in spring instead of winter, buds developed mainly on the lower half of the hypocotyl (Pl. 41C). However, observations on bud development have been confined to those originating in the upper hypocotyl.

Within 3-4 days of decapitation (Day 10+3 to 10+4) changes

occur in certain normal epidermal cells which are detectable only by electron microscopy and this initial dedifferentiation concerns mainly the plastids and nuclei.

In non-activated epidermal cells the plastids have more-or-less round profiles and are up to 5μm in diameter with the same appearance as in the control. Plate 52 shows a longitudinal section of the nucleus of what it is considered to be an activated epidermal cell; the nuclear outline appears as slightly convoluted with occasionally some cytoplasmic invaginations (thin arrow) whilst the nucleolus has several vacuoles and dense and less dense (granular) zones. The plastids have developed some starch grains and in addition they contain some dense bodies with irregular outlines (large arrows) together with some granular in appearance (small arrows) and smaller circular or oval (solid arrows) bodies in their stroma. In the cytoplasm of such cells endoplasmic reticulum elements and dictyosomes are frequently seen together with some microbodies. No changes, however, are yet observed in the fine-structure of the nuclear inclusion.

In some activated (but as yet undivided) epidermal cells of the experimental material (Pl. 56B) the plastids now appear different and have developed one to several starch grains measuring up to 2μm, more thylakoids, and one or more osmiophilic bodies up to 2μm in diameter compared to the plastids in the control epidermis. The plastids are now polymorphic and in Plate 56B the plastid on the right has developed a long tail and might represent a stage in the replication of this organelle.

The longitudinally sectioned nucleus shown in Plate 56B is now considerably shorter (about 12μm long in the plane of this section compared to about 30μm long in the Day 0 hypocotyl).

However, it has increased in width to about 10μm (compared to about 5-7μm in the control) and is as wide as the epidermal cell itself. The nuclear inclusion is also much shorter (about 10μm long compared to 30μm long in the control) and it now lies transverse rather than parallel to the long axis of the epidermal cell. The daughter nuclei resulting from this first mitosis (Pl. 59A) no longer contain inclusions and it seems that the latter are digested just prior to this process.

Prior to mitosis in an activated epidermal cell the nucleus migrates from its peripheral position and comes to lie in the centre of the cell, suspended in the vacuole by cytoplasmic strands connected to the peripheral cytoplasm. The mitotic spindle of the first mitosis is normally orientated so that the equator lies transversely, or somewhat obliquely, to the long axis of the mother cell (Pl. 57A-B). Subsequent centrifugal development of the cell plate (apparently resulting from increased activity of dictyosomes and endoplasmic reticulum elements, Pl. 57C) divides the epidermal cell into two (usually equal) still highly vacuolated daughter cells. Closely following the first division of the mother cell there follow more transverse divisions in the daughter cells and the derivative cells then further divide by tangential and radial longitudinal divisions. Plate 57A shows a paradermal section of the Day 10+8 epidermis where a normal epidermal cell (arrow) has divided transversely into six daughter cells which are still highly vacuolated. These first transverse divisions were missed by Crooks (1933) who describes the radial and tangential longitudinal divisions as occurring first but Link and Eggers (1946) describe the same sequence of divisions in the epidermis

(as currently observed) and comment on the misinterpretation made by Crooks (1933). At this early stage of the bud initiation the daughter cells are still retained within the confines of the mother cell wall and vary in appearance from densely cytoplasmic cells with prominent nuclei (primary meristematic cells, Gautheret, 1966) to still considerably vacuolated cells (Pl. 58A-B).

Although initial activation of hypocotyl occurs in isolated epidermal cells, the cells adjacent to these loci subsequently become involved. The sequence in which the neighbouring cells undergo dedifferentiation varies: most commonly the next epidermal cells to differentiate are those contacting either the proximal, distal or both ends of the original mother cell, whilst the lateral cells become involved later (Pl. 58A-B); or all the epidermal cells surrounding the initiating cell undergo dedifferentiation more or less simultaneously (Pl. 58D). Finally more peripheral epidermal cells also become involved, so that a prominent bud primordium (bulge stage, Crooks, 1933) develops around the original epidermal locus. The SEM micrographs (Pl. 63A-C) show surface views of different stages of development of the adventitious bud primordium; Plate 63A shows a very early stage where one epidermal cell has apparently undergone several transverse divisions whilst in Plate 63B a few radial longitudinal divisions appear to have occurred and in Plate 63C the latter divisions are more abundant.

Plate 59A illustrates a daughter epidermal cells from a bud initial (c.f. Pl. 58B-C); several varying sized vacuoles are present and plastids still contain comparatively large (up to 1.5μm wide) starch grains. The nucleus is about 6μm wide and is

slightly convoluted in outline; the large vacuolated nucleolus (which is about 4μm wide and reminiscent of that of the callusing cells of P. vulgaris, Pl. 26A) shows some dense (possibly fibrillar) and less dense (granular) zones. In Plate 59A the cytoplasm contains abundant free ribosomes but rough endoplasmic reticulum is sparse. The mitochondria have oval or circular profiles and the plastids have irregular outlines; the plastid indicated by an arrow shows a tail which lacks starch.

After a number of divisions have occurred within the limits of the mother cell (Pl. 58C) the smaller daughter cells (Pl. 59B and 61A) are characterised by an increasing proportion of cytoplasm and smaller vacuoles with granular contents. The denser cytoplasm contains mainly free ribosomes whilst endoplasmic reticulum and dictyosomes are less common. The plastids are more-or-less similar in shape and size to those previously described, but with smaller starch grains of up to 0.5μm in diameter. The nuclei are of similar size to Plate 59A but are now rounded and contain a single non-vacuolated nucleolus (Pl. 59B). This contains dense and less dense zones, whilst some isolated patches of densely stained material are observed in the nucleoplasm near the nuclear envelope. The arrows indicate the mother cell walls (original cell walls of the epidermal cell) which are thicker than the newly-formed cell walls. The latter are penetrated by plasmodesmata (small arrows).

Following the activation of the epidermal cells to form a bud initial further divisions in various planes in both the epidermis and the cortex give rise to the bud primordium on which normally the first two leaf primordia arise on either side of the apex (Pl. 63A) proximally and distally in relation to the long

axis of the hypocotyl. However, some apparently aberrant, oversized bud primordia are occasionally observed; Plate 63B shows a face view of one of these buds and Plate 66 is a RLS of a similar bud. It seems possible that these buds are formed by the synchronous development of several bud primordia very close to each other thus producing these teratomatous buds. The oversized bud observed in Plate 66 seems to be a dormant one since no provascular tissue is observed (see section 3.2.9.) within the cortex.

From the large number of specimens investigated it is apparent that initiation of the adventitious bud occurs only from normal epidermal cells, but "swollen" cells become involved later and divide like normal epidermal cells (Pl. 62C, arrow and 63A). The stomatal guard cells, however, do not dedifferentiate even at later stages of bud development; intact stomata can be distinguished in Plate 63B (arrows) on the bulging bud primordium.

3.2.9. The later development of the adventitious bud and its vascular connection with the main hypocotyledonary vascular tissue.

According to Crooks (1933) and Link and Eggers (1946a) the adventitious bud develops exclusively from the epidermal cells. However no clear evidence of the exclusion of cortical participation in bud development is produced in either publications. On the contrary, in the present study, careful examination of transverse, tangential and paradermal serial sections indicates that both cortical and epidermal derivatives participate in the development of the bud (Pl. 67B-D). However, after dedifferentiation begins in the cortex, it is difficult to

unambiguously distinguish the derivatives of the outer cortex from those of epidermal origin. One distinction might be based on the presence of chloroplasts (similar to the ones present in the cortical cells) or plastids (similar to the ones present in the epidermal cells) but it is likely that some chloroplast containing cells may also originate from the epidermis e.g. in the potential mesophyll of the leaf primordium (Pl. 67B-E); in the deeper cortex of the hypocotyl the origin of the provascular tissue from cortical derivatives is very clear (Pl. 67B-F and 68).

Plate 57B is a low power electron micrograph (of a paradermal section of the hypocotyl) showing the base of a transversely sectioned bud primordium at a stage similar to the one shown in Plates 63A and 68. The cells illustarated in this Plate are at different stages of dedifferentiation. The cells at the top have undergone one or more divisions but the longitudinal outlines of the mother cortical cells are still quite distinct; these daughter cells contain large chloroplasts which, however, have lost their massive starch grains and seem to be dividing (see section 3.2.10.). These cells are still highly vacuolated but with the nucleus occupying a central position instead of being peripherally located. The cells in the mid-left of Plate 57B (detail in Pl. 61B) are closely packed and without intercellular spaces between them. They are still prominently vacuolated and contain chloroplasts with well developed thylakoids, although these plastids are smaller than in the control cortical cells; some small agranal plastids (Pl. 61B, arrow) are also present in these cells. In one of the cells shown in Plate 60B, a circular chloroplast profile can be seen (large arrow) and it seems that this is undergoing changes

leading to the formation of a modified plastid characteristic of sieve tube phloem elements (c.f. Pl. 70A-B, large arrows) thus indicating that this cell might be a potential sieve tube element. The cells on the mid-right of Plate 60B are more highly dedifferentiated, containing dense cytoplasm, small vacuoles, small agranal plastids (Pl. 61A, arrows) and large nuclei with more-or-less circular profiles, each usually containing one prominent nucleolus. The cytoplasm in these cells (Pl. 61A) is occupied by closely packed free ribosomes and within it lightly staining mitochondria occur; multi-membraneous myelin-like bodies are also commonly observed.

Crooks (1933) briefly mentions the vascularisation of the adventitious bud but only describes the development of xylem; he suggested that protoxylem elements are differentiated in the first leaves of the new bud before cell division has begun in the inner layers of the cortex of the hypocotyl. The differentiation of the vascular tissue proceeds inwards from the bud to the endodermis of the hypocotyl with the formation first of many isolated tracheary elements which later join into continuous strands. However, Crooks (1933) does not mention if there is any difference between dominant adventitious buds (those giving rise to a replacement shoot) and dormant (arrested) buds, in relation to the development of their vascular connection. In the present work this particular problem was studied by employing both serially sectioned and cleared specimens, since the examination of only a few randomly cut sections is likely to be misleading. Careful observation of cleared decapitated hypocotyls shows that sometimes tracheary elements can be found running obliquely in the cortex towards the epidermis at sites where no obvious bud

primordia are present (Pl. 64D). On the other hand, sometimes buds which have already reached an advanced stage of development may either have no tracheary elements, or only a few, which, do not form continuous strands (Pl. 64A). Plate 65 shows a hypocotyl where the dominant adventitious bud has already developed into a replacement stem about 5cm high and it has a massive vascular connection with the main cylinder, whilst the other two dormant buds (arrows) have only developed several discontinuous tracheary elements. The axial vascular tissue of the hypocotyl above the point of the attachment of the dominant bud ceases development after decapitation.

The timing of protoxylem differentiation, as indicated by cleared specimens, depends on whether the bud is dormant or not. In the former (Pl. 64A) this process is relatively slow and occurs after the bud primordium is already visible; however, in the latter (Pl. 64B) this process seems to be more-or-less synchronous with the bulging of a bud primordium or even occurs before this swelling is visible. In the radially longitudinally sectioned specimens the cell plates of dividing cortical cells are formed in oblique planes corresponding to the orientation of the future procambial strands (Pl. 68, large arrows) and these are accompanied by radial (Pl. 67) and tangential longitudinal divisions. The first divisions seem to occur more-or-less simultaneously throughout the cortex and no evidence was found in the present study of a basipetal dedifferentiation and vascularisation as described by Crooks (1933). However, the latter conclusion might appear to be correct if only isolated transverse sections through the upper part of a bud primordium were observed (c.f. Pl. 67A-B) or a few random radial longitudinal

sections examined.

The degree of dedifferentiation of the cortical cells varies considerably depending on their future. Some daughter cells, even after several divisions remain vacuolated and similar in contents to the mother cortical cells, whilst others dedifferentiate further into provascular cells and then redifferentiate to phloem and tracheary elements (Pl. 69A) of the vascular strands which (at least in the dominant bud) eventually connect the adventitious bud with the axial vascular tissue of the hypocotyl. Examination of serial sections (at the EM level) of the cortex in regions where provascular stands are located, indicates that the first vascular elements which redifferentiate are phloem, recognizable by their sieve plates and modified plastids (Pl. 60B, large arrow, and 69B). This is followed by the development of phloem transfer cells and trachcary elements. Serial transverse sections through the base of a dominant bud (paradermal section of the hypocotyl, Pl. 72B-F) reveal that about 15 provascular strands develop (giving an oval outline in section) connecting the bud with the axial vascular tissue of the hypocotyl. Observations made at both LM and EM levels indicate that within these provascular strands, transfer cells differentiate only in relation to the phloem elements. The transfer cells may vary considerably in shape and size; Plate 69A illustrates some of these cells and how they are related topographically to phloem and tracheary elements and Plates 70A-B and 71 show more details of their fine structure. These cells contain the characteristic tortuous finger-like cell wall ingrowths, which extend deep inside the cytoplasm and are surrounded by an electron translucent area (which may represent

slight plasmolysis) delimited by the tonoplast. Their cytoplasm is packed with free ribosomes and contains some rough endoplasmic reticulum elements (Pl. 71). Some chloroplasts, measuring up to 4μm long, are observed and these contain well defined grana and plastoglobuli in their stroma. Mitochondria are numerous and mainly located in between the wall ingrowths; these mitochondria measure up to 1μm in diameter, their profiles are either circular or oval and they contain well defined cristae; however, some of them posess translucent matrices and this could represent either an artifact or a sign of senescence.

3.2.10. Dedifferentiation and replication of the cortical chloroplasts.

As previously mentioned (section 3.2.7.), decapitation of the hypocotyl results within a few days in the swelling of its upper region (Pl. 41A) due both to an increase in the intercellular space system within the cortex and to some growth in the constituent cells (c.f. Pl. 51A-B). This vacuolation growth of the cortical cells is widespread and not confined to regions adjacent to bud primordia forming in the epidermis. Accompanying the increase in cortical cell size there is a general enlargment of the chloroplasts and considerable accumulation of starch. In Day 10 (control) cortical cells, the chloroplasts are approximately 5-8μm long by 3-5μm wide, they mainly line the longitudinal cell walls and are generally orientated so that their long axes parallel these walls and contain little or no starch (Pl. 53A). Within a few days of decapitation the chloroplasts have approximately doubled in size to about 15μm in length by 8μm in width and within the stroma of each plastid

one to several starch grains occur (Pl. 53B and 54C). The starch grains tend to be oval in outline and generally their long axes co-incide with those of the plastids containing them. When only a single starch grain is visible in a chloroplast (Pl. 53B) the grain is often very large (up to 5μm long by 3μm wide for the densely staining core or 7μm long by 5μm wide, if the translucent zone is also included), whereas when multiple grains occur these are smaller (Pl. 54C and 73). It is possible that the translucent peripheral region of the starch grains may represent a shrinkage artifact, possibly occurring during fixation or later during processing. On the other hand similarly translucent areas sometimes occur within the densely staining core of the grain (Pl. 75A). Whithin the dense core of the starch grain more densely staining bands are usually visible. These may represent artifacts of similar origin to those already described from the callusing cotyledon of P. vulgaris (see discussion).

The period of starch accumulation within the majority of cortical chloroplasts appears to be relatively short-lived. Sections of hypocotyls on which a new lateral shoot has become established from the outgrowth of an adventitious bud (Day 10+15 or older), reveal that the chloroplasts in cortical tissue situated at some distance from the new axis now posess only small starch grains, or none are present, and that the chloroplasts are similar in dimensions to those in cortical material. The general fine-structural features of these cells also resemble those of the control cortical cells. By contrast, however, cortical cells which are involved in the development of the provascular strands show marked dedifferentiation changes accompanying cellular division. In relation to the plastids of these dedifferentiating

cells two main types are evident: chloroplasts (Pl. 74A, on the left) and smaller plastids with a dense stroma which lack thylakoids or starch grains but contain a number of translucent vesicles (Pl. 74A, on the right). The chloroplasts are most common in the larger (but already sub-divided) cortical cells, whereas the other type of plastid represents most of the population in the smaller cells resulting from numerous divisions of an original cortical cell. Thus accompanying the dedifferentiation and division of the cortical cells the original chloroplast population apparently undergoes replication and the progeny become progressively modified. Plate 74A shows a daughter cell with several chloroplasts present. These contain prominent starch grains and are generally similar to those previously described, which are encountered throughout the cortex soon after decapitation of the hypocotyl, and show large translucent areas surrounding the dense core of the starch grains. However, the three large chloroplasts in the centre of Plate 74A are apparently connected through narrow constrictions (large arrows) with each segment containing its own thylakoid system, starch grains and plastoglobuli. Chloroplast profiles showing constrictions of this type are commonly encountered in these cells (Pl. 74A and 75B). Although there are inevitably considerable problems concerning the correct interpretation of dynamic cellular processes as reconstructed from the evidence of static pictures of the cell, it is nevertheless considered that such profiles represent late stages in the approximately equal division of a mature chloroplast. The three potential daughter chloroplasts in Plate 74B measure about 5μm by 3μm and chloroplast sections of similar dimensions are of frequent occurrence (e.g. smaller

chloroplasts in Plate 74B). In other apparently dividing chloroplasts (Pl. 75A-B) the silhouette and internal morphology of the parent chloroplasts may be much less regular than described above. Thus in Plate 75A the isthmus connects a smaller (as seen from a single section) component with a much larger (partner) chloroplast. In Plate 75B the outline of the large plastid is highly "amoeboid". Each main segment contains thylakoid membranes but their orientation differs; the grana in the central segment (■) are apparently seen in approximately face view whereas the grana are mainly transversely sectioned in one of the other segments of the mother chloroplast (● or ▲). Plate 76A shows a nearby section of this same chloroplast with the orientation of the thylakoids in the central lobe the same as in the previously detailed illustration. But one of the terminal lobes (▲), which in Plate 75B is attached to the other chloroplasts, lies apparently detached from the mother plastid in Plate 76A. Plate 76B shows (from another section) a large area of the cell containing the mother cell shown in Plates 75B and 76A; the terminal lobe (●) of this plastid is seen at the upper left of Plate 76B. In the latter plate another two polymorphic chloroplasts are also visible.

Plate 77A illustrates a chloroplast with a long "tail" and in this latter region neither thylakoids nor starch are present. Plate 78A shows a group of apparently separate plastids (possibly all parts of a single plastid complex); one of these chloroplasts shows an agranal tail and several similar, but isolated, profiles around it. Another of the chloroplasts posesses a swollen agranal end (arrow), which contains a large cytoplasmic enclave (bounded by the plastid envelope) which is of similar appearance

to the ground cytoplasm. The chloroplast in Plate 77B shows an irregularly outlined agranal region at its base and a section cut along the plane indicated by the dotted line would demonstrate an area of cytoplasm bounded by the plastic envelope (c.f. Pl. 78A, cytoplasmic enclave in agranal end of plastid).

The agranal "tails" (Pl. 77A) or otherwise irregular blebs (Pl. 77B) projecting from chloroplasts are considered to represent an alternative mode of plastid replication giving rise in this case to small proplastids, containing few or no membranes within the stroma (Pl. 74A and 78A-B) instead of the small chloroplasts predominantly formed in less dedifferentiated tissues.

4. DISCUSSION

In connection with the ultrastructural examination of air dried seeds, there are two major problems. The first is the generally unsatisfactory standard of specimen preservation resulting from the use, on air dried seeds, of the fixatives and embedding agents normally employed for the preparation (for electron microscopy) of the more common hydrated, botanical specimens. The second problem is that dry tissues are likely to undergo varying degree of imbibition with the use of aqueous fixatives, so perhaps altering the structural state of the cytoplasm (Abdul Baki and Baker, 1973; Paulson and Srivastava, 1968; Swift and Buttrose, 1973).

However, many workers have soaked air dried seeds of various species, for periods varying from a few minutes to several hours, and have assumed that no major ultrastructural changes occur in such short periods of hydration (Briarty et al., 1970; Buttrose, 1963; Horner and Arnott, 1965, 1966; Jones, 1969; Młodzianowski and Włowska, 1975; Młodzianowski, 1978; Nieudrop, 1963; Nougarède and Pilet, 1964; Öpik, 1965, 1966; Paulson and Srivastava, 1968; Rost, 1972; Smith, 1974; Webster and Leopold, 1977; Yoo, 1970).

It is, neverthless, evident that such a procedure limits to some extend the validity of comparative studies of features such as membrane systems in dry and germinating seeds. This limitation is demonstrated in the studies by Swift and Buttrose (1972, 1973). They utilised the freeze-etching technique, but with the unusual procedure of employing 100% glycerol as an anti-freeze agent instead of the 20% aqueous solution which is

normally used with this procedure (Moor , 1964). Little or no etching occurs to the specimens before replication since very little water is present in the air dried seed tissue to sublimate. Comparison of specimens processed without prior hydration with tissues briefly hydrated before freeze-etching (and also with sections of chemically fixed material) demonstrated that in the scutellum of Triticum aestivum both protein bodies and lipid droplets, after only a few seconds of imbibition, changed from angular outlined bodies to rounded ones; whilst the cell walls of storage cells of Pisum sativum cotyledons changed from "straight" to "wavy" after a few seconds of imbibition.

Attempts have also been made to employ OsO_4 vapours as a non-aqueous fixative for dried seeds. In studies by Perner (1965), Swift and O'Brien (1972), Simon (1974) and Yatsu (1965), it was found that fixation with these vapours of air dried tissues frequently results in poor definition of the plasma membrane, bad infiltration of the resin and difficulties in sectioning. (According to Hayat, 1970, p. 68, the use of aqueous glutaraldehyde, now widespread in use as a fixative, generally increases the permeability of the tissues to the embedding media). However, comparative studies of such dried tissues, fixed with either OsO_4 vapours or aqueous fixatives, made by Simon (1974) and Swift and O'Brien (1972) show that while modifications may be effected at the molecular level, gross structural changes are not induced by aqueous fixation.

In the present study air dried cotyledons of P. vulgaris and hypocotyls and cotyledons of L. usitatissimum were fixed with aqueous fixatives without pre-soaking in water. The quality of sectioning of the resin blocks was very poor

immediately after the tissues were processed, but a dramatic improvement was noticed when these blocks were re-sectioned several years later. Although there appears to be little discussion of this phenomenon in the literature, it is commonly recognised amongst electron microscopists that air-curing of tissue blocks for periods ranging from a few days to several years (depending on the nature of the tissue) considerably facilitates sectioning. The quality of fixation observed in the sections of air dried tissues of the species currently investigated generally appears satisfactory as compared with previously investigated seed tissues. Organelles such as plastids, mitochondria and nuclei are easily recognised, although the tripartate fine-structure of their envelopes cannot be resolved.

Preliminary attempts were also made (in the current studies) to examine the air-dried cotyledons of P. vulgaris by the modified freeze-etching method as described by Swift and Buttrose (1972, 1973) using 100% glycerol but some problems were experienced. Firstly the 100% glycerol did not act as a very succesful adhesive when frozen, and as a result the specimens would not stay on the specimen stage during fracturing. Secondly, the air dried tissue was very hard (especially at the temperature of $-100^{o}C$ employed in this technique) and therefore the razor blade was damaged very easily during fracturing of the tissue prior to replication. In the few replicas obtained with this technique the only clearly visualisable features observed were nuclei in which the distribution of the nuclear pores was apparent. These preliminary results are encouraging and suggest that further experimentation with this procedure might be profitable.

Ö̈pik (1966) in her investigation of the germination changes in the cotyledons of P. vulgaris described the epidermal cells of the air dried seed but without producing photographic evidence. The preservation of the inner tissues of the dried seed was inadequate, so that in general her description of the non-germinated cotyledon is in fact based upon that of the 24 hour hydrated seed. According to Ö̈pik (1966) the epidermal cells appear similar in structure in both the dry and hydrated state. Similar results were obtained in the present study; the cells contain very few food stores, mitochondria and plastids are easily recognisable whilst small electron translucent vesicles are abundant in the cytoplasm. However, no shrunken protoplasts were ever observed in the epidermal cells such as described by Ö̈pik (1966). The latter might perhaps represent cells of non-viable or damaged seeds, or artifacts introduced during processing of the tissue.

The differences observed in the current study between Day 0 and Day 1 storage cells of P. vulgaris mainly concern the plastids, nuclei and food stores. Within 24 hours of hydration most plastids have developed prominent starch grains in their stroma in contrast to the rare, small starch grains observed in the plastids of the Day 0 storage cells. The most likely sources of carbohydrate for these newly-formed starch grains lie in the large starch grains and non-cellulosic parts of the walls of the storage cells Ö̈pik (1966) did not mention the occurrence of plastids in these cells, although the small starch grains, about 5μm in diameter, clustered around the nucleus of the storage cells of the hydrated cotyledon described by Smith (1974) at the light microscopic level, presumably represent the starch grains

formed in the plastids currently observed. A similar accumulation of starch has also been reported by Smith and Flinn (1967) in the plastids of the storage cells of Pisum arvense. The shape of the Day 0 and Day 1 nuclei appear rather similar although their nucleoplasm differs. By Day 1 the chromatin, which is scattered within the Day 0 nucleus, is more condensed and more peripherally distributed. The only differences observed between Day 0 and Day 1 protein bodies were a change in shape from angular to rounder and their slight expansion. The lipid globules become densely stained after 24 hours of hydration. No comparable observations were made on these various features described above by Öpik (1966) or Smith (1974). The outer, micrifibrillar region of the storage cell wall appears unchanged at Day 1 but the inner layer is considerably swollen (in comparison to Day 0) and more vesicular structures occur within it and these are now more densely stained. Öpik (1966) described the Day 1 (or older) cell walls of the storage cells of this species as being non-lignified, thickened (especially at the corners) and conspicuous with pits penetrated by plasmodesmata and that the wall consists of two layers. Smith (1974) mentioned that these walls stained fairly strongly with the periodic acid-Schiff's reagent (PAS) but the corner thickening stained only faintly.

Changes were also observed in the present study, in the fine-structure of the large starch grains which probably reflect changes in their chemical composition before and after hydration.

Preliminary examination of starch grains which had been scraped off an air dried P. vulgaris cotyledon and mounted in 100% glycerol, shows that before imbibition, starch grains are not hollow although they possess concentric zonation. However,

when water is applied on air dried starch grains under the microscope, the former "explode" as they rapidly absorb water and their diameter increases by about 30%; these hydrated starch grains appear hollow. The same "explosion" and size increase are also observed when aqueous fixative is applied instead of water and this means that the structure of the Day 0 starch grains currently observed in cotyledons processed for electron microscopy, has already changed. In in situ, however, it seems that the same changes occur in the starch grains but these take place slowly probably due to space availability and the thick walls of the storage cells which restrict an "explosion" occurring. Swift and Buttrose (1973) reported that a starch "explosion" occurred as soon as air dried cotyledonary tissue of Pisum sativum came into contact with water and the resulting eroded starch grain contained non-starch material in their cavities. Hollow starch grains were reported at the light microscopic level by Smith (1974) in ungerminated (but hydrated) cotyledons of P. vulgaris and he described them as containing air in their cavities.

Following hydration, digestion of the starch grains begins by intrusion of cytoplasm into the cavity of the grain but no evidence was found of how this occurs. Digestion then proceeds mainly from the inside towards the outside of the grain and eventually this leads to the fragmentation of the grain.

Harris (1976) noted a similar sequence of events in germinating seeds of Phaseolus aureus and he suggested that

cytoplasm initially intrudes into the eroded starch grain through a pore. Since the diameter of the partly digested grain measures approximately the same as in the intact grains, he suggested that hydrolysis proceeds mainly from the inside of the grain rather than the outside, although without explaining how he determined the size of the starch grain before digestion began.

Bain and Mercer (1966) noted that some starch grains in germinating cotyledons of Pisum arvense had a wavy outline which perhaps indicated intensive localised enzyme attack, whilst the smooth outline of other grains suggests that the enzyme attack might be taking place more evenly.

In the present study of the in vitro callusing cotyledonary storage tissue of P. vulgaris fragmentation of the starch grains was initially mainly restricted to the vicinity of the abaxial procambial strands whilst elsewhere intact looking starch grains were found (even in cells of well developed callus). In the germinating cotyledon starch fragmentation occurs to the vicinity of all the procambial strands and all starch grains digested by about Day 8-10.

Most starch grains currently observed (see also discussion on L. usitatissimum) posess some radial, darkly stained bands which are probably artifacts resulting either from folds in, or uneven thickness of the sections; it seems likely that the chemicals used for processing of the tissues for electron microscopy result in the change of the nature of starch and its sectioning properties. As a result, when sections are floated on the surface of the water bath during sectioning, the starch grains in the section expand, but when the latter are then placed on the coated grid and dried out the sectioned starch grains

become folded. Such folds become more evident in starch grains of hydrated cotyledonary tissue, where occasionally the folded expanded starch grain section overlaps parts of the area which surrounds the starch grain (c.f. Pl. 2C, 5B and dark shadow at the lower part of Pl. 22B).

Rost (1972) reviewed the literature on protein bodies and described them as being membrane bound bodies usually measuring 0.3-3.0μm in diameter and he also divided them into three categories, according to the presence or absence of inclusions: without inclusions, with globoid inclusions only, and with both globoid and crystalloid inclusions. All three protein body types are chemically similar.

The protein bodies currently observed in P. vulgaris are without inclusions. According to Öpik (1966) and Smith (1974) during germination the protein bodies first swell and their contents become more diffuse; the individual bodies then fuse to form large vacuoles occupying the one end of the cell whilst the other end is occupied by starch grains, probably due to precipitation of the latter. The current findings are in agreement with these observations. However, in the in vitro grown cotyledons, in addition to the fusion of protein bodies as occurs in vivo some apparent lysosomal activity was noticed taking part in both protein body digestion as well as digestion of parts of the cytoplasm. During this autophagic process endoplasmic reticulum elements seem to surround parts of the cytoplasm together with occasional protein bodies and isolate them from the rest of the cytoplasm; at the next stage the two unit membranes composing the cisterna separate and whilst the external one forms the tonoplast the internal one still

delimits the cytoplasmic enclave within which the various organelles are eventually being digested (together with their delimiting membrane) and thus finally leaving a vacuole with some darkly stained deposits enclosed in it. The role of protein bodies and their lysosomal activities have been summarised by Matile (1974, 1978): (a). they store certain reserve substances (e.g. phytin, metals and protein); (b). during germination they become lysosome-like and their food reserves are mobilised and (c). they may subsequently form a cell compartment (vacuole) in which cell constituents are broken down. However, these protein body derived lysosomes are normally bounded by a single membrane unlike the double membrane bounded ones currently observed. Lysosomes, initially bounded by a double membrane and hence similar to these in P. vulgaris, have also been observed by Coulomb (1968), Mesquita (1972) and Villiers (1971) and they suggest an autophagic mechanism similar to the one currently observed in the P. vulgaris in vitro grown storage cells. Matile (1974) suggested that the autophagic mechanism, described above, and the autophagic activity of vacuoles either occur simultaneously, or the former may proceed the latter; by comparing the two processes he suggested that they appear rather similar.

Occasionally some cytoplasmic enclaves (similar in fine-structure to the cytoplasmic enclaves described above of P. vulgaris) were observed in the nuclei of the callusing explants of this species and in the nuclei of the cells of the established callus tissue. These enclaves apparently contain osmiophilic deposits, mitochondria- and dictyosome-like structures, and are delimited by a double membrane which, however, does not posess nuclear pores. Similar nuclear enclaves have also been reported

by Schrepf and Nagl (1970) within the nuclei of the suspensor cells of P. vulgaris developed in vivo and they suggest that these might represent lysosome-like structures.

Neither Öpik (1966) nor Smith (1974) in their studies on the germinating P. vulgaris cotyledon observed any protein crystals in the protein bodies; likewise such crystals were absent in the in vivo material currently studied. By contrast numerous crystals occur in some protein bodies of the sub-epidermal storage cells of the in vitro cultured cotyledon. It seems that this crystalisation of the protein is in some way affected by the presence of the nutrient medium since crystals were only observed in the sub-epidermal storage tissue of the adaxial surface of the cotyledon which is in intimate contact with the solidified surface of the medium. It would be interesting to further investigate this phenomenon, by determining whether protein crystals would form in other sites, e.g. near the excised surface of deeper located cells if placed in contact with the solidified medium, or whether this potential is confined to the adaxial storage cells only. Further experimentation might determine what role may be played by the various nutrients and growth substances within the medium. It seems from a survey of the literature that no other such experimental system has yet been studied.

Another deposit of apperently proteinaceous nature observed at Day 0-1 in the present study of the P. vulgaris cotyledon are the p-protein crystals (formerly called slime) located only in cells of the procambial strands and having square profiles in transverse sections. These crystals have been reported in sieve elements of various species (Bouck and

Cronshaw, 1965; Esau, 1971, 1978; Esau and Gill, 1971; Lawton, 1978a and b; Lawton and Newman, 1979; Wergin et al., 1970) but they have not been described in air dried seeds. In general there seems to be one p-protein crystal in each sieve element of the Papilionaceae (Lawton, 1978b), to which P. vulgaris belongs. According to Esau (1977) this protein is initially aggregated into one or more bodies but subsequently it spreads out in the cytoplasm, forming strands of a network; after the tonoplast disappears, the dispersed p-protein is found in a parietal position in the cell lumen and sieve plate pores. Esau (1978) describes the crystals as either[a] spindle-shaped structure, square when sectioned transversely, with tail-like extensions at both ends or non-tailed and consisting of tubular sub-units in more-or-less orderly aggregates. The crystals currently reported in the procambial tissue of air dried seeds of P. vulgaris have not been observed from such a source in other species and their presence is perhaps an indication that the cells containing them are destined to differentiate into sieve elements during germination.

According to Öpik (1966) and Smith (1974) during the in vivo germination of the P. vulgaris cotyledons, mobilization of food stores is relatively fast; the reserves disappear first from the procambial tissue (Day 1-3) whilst in the storage tissue mobilization of food reserves begins in the central cells remote from the vascular bundles and epidermis (Day 3-7). By Day 5-9 it has progressed to the tissue underlying the abaxial epidermis and at this stage the empty cells collapse. The reserves now become removed from the abaxial epidermis and sub-epidermis and the reserves then disappear from the cells around the vascular

bundles and near the adaxial epidermis (Day 10-16). Finally from Day 16 onwards the cotyledons abscind. Nuclear and cell division is absent during germination and the storage cell nuclei show a progressive decrease in DNA levels from the modal value of 16C at Day 1 (Smith, 1974).

Cotyledon explants have not been used very often in tissue cultures and when they are used they have either been left to germinate for several days (L. usitatissimum, Rybczansky, 1975; Ibrahim et al., 1975) or explanted immature (Cionini et al., 1978; D'Amato, 1978; Frame et al., 1976; Marks and Davies, 1979). Previous investigations (Frame et al., 1976) have shown that immature cotyledons of P. vulgaris can be induced to proliferate in vitro and in the present study it has been established that cotyledons from the mature air-dried seed of the same species will also undergo callusing, although, organogenesis is absent. Callus cultures derived from the latter source can be routinely sub-culturedand continue to flourish over a prolonged, potentially indefinite, period. Thus the growth and development in vitro of mature cotyledonary tissue of P. vulgaris strongly contrasts with its normal fate in the germinating seedling.

Neither the procambium nor the epidermis appear to divide in vitro, and in the current study certain changes concerning primarily the storage tissue have been investigated. In these cells the highly polyploid nuclei (Smith, 1974) become reactivated and mainly divide amitotically, whilst profuse development of freely-forming walls and large labyrinthine bodies occur in the adjacent cytoplasm. In his recent review D'Amato (1978) cites evidence for the common occurence of amitosis, later followed by mitosis, when explants of plant organs are grown in vitro on a

medium lacking auxin or in which the auxin-kinetin ratio is unbalanced. Evidence of amitotically as well as mitotically proliferating calli has also been found by Bennici *et al.* (1976) in callus derived from the suspensor of *P. coccineus* cultured in an hormone free medium; 88% of the divisions observed were amitotic and each amitosis was followed by mitosis of both nuclei resulting from the amitosis. In their investigation of callus induction from immature cotyledons of *Vicia faba*, Cionini *et al.* (1978) employed light microscopical and cytophotometric techniques to examine squashes (presumably mainly consisting of storage cells) and described the following phenomena: (a). From about Day 4 onwards resumption of DNA replication which increased the DNA content of the largest nuclei to 64C and higher (at excision the nuclear DNA values range from 2-32C) accompanied by extrusion into the cytoplasm of nucleoli and/or Feulgen-positive material. (b). Furrowing (lobation) of nuclei eventually resulting, at Day 10-18 of culture, in nuclear fragmentation responsible for the production of binucleate or multinucleate cells. The nuclear fragmentation was confirmed by DNA cytophotometric measurements, and in the fragmented nuclei maximum DNA levels are about 16C. In some bi- or multinucleate cells cellularization occured later on. (c). Occurrence, between Day 6 and 25 of culture of rare mitoses in both intact nuclei and nuclear fragments resulting from nuclear fragmentation. (d). Rise of the mitotic index after transfer to fresh medium of calli produced from the cotyledonary explants and rather rapid mitotic selection in favour of the euploid chromosome number.

Although the study made by Cionini *et al.* (1978) is more concerned with the genetical rather than the fine-structural

changes occuring during callusing (as currently studied in P. vulgaris) from their above description it is apparent that many of the changes occuring in the mature storage cells of the callusing cotyledons of P. vulgaris closely parallel those of the immature cotyledonary tissue of Vicia faba. In P. vulgaris the nuclei are already polyploid at explantation (ranging from 8-32C, Smith, 1974) and this accords with the situation in the latter species. However, since the DNA changes have not been currently examined, it is not known whether (as in V. faba) a resumption of nuclear DNA synthesis occurs in P. vulgaris prior to fragmentation and this would be an interesting point for further research.

There is evidence that prior to nuclear fragmentation in P. vulgaris there is a great increase in the degree of lobing of the nucleus and in the intricacy of convolutions of the individual lobes, whilst multiple nucleoli replace the original single nucleolus. In their studies Cionini et al. (1978) describe an extrusion into the cytoplasm of Feulgen-positive material and/or nuclei. However, in fact the illustrations of this phenomenon given by the above authors are from squashes and there is no convincing evidence that the small and apparently isolated patches of Feulgen-positive material (surrounding the main nucleus) represent extruded nuclear material rather than micronuclei or small lobes still attached to the main nucleus, as frequently observed in this study. Neverthless, time lapse observations on living material from Nicotiana glauca x langsdorffii callus, made by Martini and Nuti Ronchi (1977) show some evidence in certain cells of an occasional nucleoli extrusion from the multinucleolate nuclei into the cytoplasm.

Extrusion of nuclear material into the cytoplasm was not observed in the present study, although, small, apparently detached, nuclei which might represent micronuclei have been repeatedly noticed in the cytoplasm of dedifferentiating cells.

The observations on cellularisation, following nuclear fragmentation, in V. faba (Cionini et al., 1978) and N. glauca (Nuti Ronchi et al., 1973) are greatly limited due to being confined to squashed material examined with the light microscope. It is not possible, therefore, to judge from their work whether the initial development of the freely-forming walls is more-or-less contemporaneous (as in P. vulgaris) with the first amitotic division. In P. vulgaris several examples of the close proximity of the ends of the freely-forming walls to a nuclear isthmus have been observed, and it is possible that these new walls play an active role in nuclear fragmentation. However, occasionally nuclear fragmentation apparently occurs before any new wall formation is apparent and it might be that freely-forming walls are not present at early stages of activation of the nucleus when increased lobing first becomes evident. The various methods used for the examination of these phenomena (Feulgen-stained squashes of fresh material, sometimes counterstained with Light green) used by Cionini et al. (1978), Martini and Nuti Ronchi (1977) and Nuti Ronchi et al. (1973), and light and electron microscopy of fixed and sectioned material, as currently employed have both advantages and disadvantages. The squash technique suffers from lack of resolution in the specimen (e.g. details of the nuclear envelope) and possible distortion of the three dimensional structure of the cells but on the other hand is quick and easy. The observations on sections of fixed material reveal more detail,

but are in general made on random sections from which it is difficult to reconstruct the three dimensional structure.

Cytological and autoradiographic methods were used by Nuti Ronchi et al. (1973) to study the nuclear fragmentation in dedifferentiating cells of N. glauca pith tissue grown in vitro; they found that application of colchicine (which disrupts the mitotic spindle organization, Borisy at al., 1967; Taylor, 1965), completely inhibits the formation of multinucleolate cells of the explants, wheras normal mitotic divisions are resumed after recovering from this treatment. They therefore postulated that "...spindle fibers, similar to the mitotic apparatus are necessary for the nuclear (amitotic) division". In the P. vulgaris explant there is no evidence of the presence of microtubules at the margins of the freely-forming walls, although, phragmoplast microtubules are present at the margins of the cell plate developing after mitosis.

Marks and Davies (1979) reported mitoses in the cotyledons of Pisum sativum when cultured in vitro; by examining metaphase nuclei of feulgen stained squashes and sections of wax embedded proliferating cotyledons, they observed diploid, polyploid and polytene cells and these mitoses were mainly observed at the sites of procambial strands.

Freely-forming cell walls have been frequently observed in the P. vulgaris explants and more rarely in the established callus tissue. In in vitro protoplast cultures obtained from leaves of Antirrhinum majus, Prat and Poirier-Hamon (1975) observed some "embryoids" frequently posessing incomplete walls, either connected to the mother cell wall or apparently isolated in the cytoplasm. Wall invaginations, which subdivide the cells

without apparent mitoses occurring and resembling freely-forming cell walls, have also been noted in established callus cultures of Andrographis paniculata (Bowes, 1969; Butcher and Bowes, 1969) as well as in cortical explants of the radicle of Pisum sativum (Bowes and Torrey, 1976). In these examples there is no evidence of centrifugally developing cell plates as occur between the mitotically divided nuclei in the P. vulgaris explant. In the "embryoids" of A. majus, Prat and Poirier-Hamon (1975) also reported the presence of multilobed nuclei as well as cells with several nuclei, but neverthless they assumed that the nuclear divisions were mitotic.

In vivo, freely-forming cell walls have been reported during the transition from free nuclear to cellular endosperm of Triticum aestivum (Morisson and O'Brien, 1976), Stellaria media (Newcomb and Fowke, 1973) and during the development of the embryo sac of Helianthus annus after fertilization; in these cases freely-forming cell walls develop from the micropylar towards the chalazal region of the embryo sac and the mode of their development seems in many respects similar to the situation in P. vulgaris cotyledon explant. In H. annus the dictyosomes produce profuse vesicles in the vicinity of the ends of freely-growing walls and Newcomb (1973) stated that these walls are apparently formed from coalescence of the dictyosome vesicles. On the other hand Newcomb and Fowke (1973) recorded that the concentration of endoplasmic reticulum and dictyosomes is very variable in the vicinity of freely-forming walls of S. media, and they suggested that the large vesicles (of unknown origin) occurring near the ends of such walls may play a role in the growth of the latter. In T. aestivum (Morrison and O'Brien, 1976)

dictyosomes and endoplasmic reticulum are scarce near the ends of freely-forming walls in the endosperm developing in the dorsal region of the grain. However, in the ventral region, endoplasmic reticulum is abundant and the boundaries between the sheet of endoplasmic reticulum and the freely-forming walls are almost indistinguishable in some grazing sections, whilst the ends of the freely-forming walls are composed of aggregating vesicles. Morrison et al. (1978) consider that both dictyosome vesicles and endoplasmic reticulum may well be incorporated either wholly or in part into the growing wall.

Wall bodies are observed in dedifferentiating storage cells of the P. vulgaris explant grown in vitro, particularly in cells in the vicinity of the vascular tissue, and also in cells of the established callus tissue but not in the cells of the in vivo germinating cotyledons. The first description of wall bodies in cells grown in vitro was apparently given by Bowes and Butcher (1967) and Bowes (1969) in callus tissue of Andrographis paniculata. According to these authors such bodies appear as thickened regions of the wall containing numerous membrane bounded bodies, complex membranous configurations, isolated membranous elements or myelin-like bodies and hence these wall overgrowths were termed membranous wall bodies. They sometimes occur in meristematic cells but they reach their full development in highly vacuolated senescing cells. It has been suggested (Bowes, 1969) that in A. paniculata, the membranous wall bodies are formed by the activity and eventual coalescence of several adjacent lomasomes which occur in profusion at the plasmalemma/cell wall interface. The lomasomes are small, wart-like bodies (containing both membranous and

fibrillar material) which project into the cytoplasm from the wall, but are demarcated from the former by an outer membrane continuous with the plasmalemma. In the callus tissue of Taraxacum officinale (Bowes, 1979) it is also possible that wall bodies may be derived from the amalgamation of lomasomes. However, lomasomes are not observed in P. vulgaris explants or established callus tissue. In the latter species it seems likely that the labyrinthine margins of the growing bodies enclose numerous tracts of cytoplasm and it is therefore rather surprising thatentrapped membraneous elements are not commonly observed in the mature wall bodies.

The wall bodies observed in P. vulgaris reach their full development whilst the explant storage cells are still dedifferentiating and the ones observed in the established callus cells are considerably smaller. Wall bodies develop both on pre-existing (mother) walls and also on freely-forming walls and sometimes arise in isolation in the cytoplasm. Dictyosomes are abundant near the margins of the developing wall bodies and numerous vesicles which are apparently fusing with them may contain wall material and thus contribute to their growth. Endoplasmic reticulum elements are also seen attached to the wall bodies (and freely-forming walls) and it seems likely that these may also participate in wall material deposition. Wall bodies of the established callus never occur in isolation in the cytoplasm.

These observations, however, greatly differ from the observations made on wall bodies on A. paniculata (Bowes and Butcher, 1967; Bowes, 1969) and T. officinale (Bowes, 1979) where their development is associated with the presence of

lomasomes, although, the development of wall thickenings observed in Acer pseudoplatanus (Davey and Street, 1971) is associated with the presence of dictyosomes and opaque globules probably of dictyosome origin. Davey and Street, (1971) suggest that the wall thickenings observed by them may be related to the wall invaginations of the transfer cells described by Gunning, Pate and Briarty (1968), Gunning and Pate (1969), Pate and Gunning (1969) and that these wall thickenings may be involved in short distance membrane-mediated transfer of solutes within the cellular aggregates; however, transfer cells are always seen in close association with vascular elements (see also discussion on L. usitatissimum) and never isolated in parenchymatous tissue. In P. vulgaris explant wall bodies sometimes appear in cells in the vicinity of vascular bundles but these cells are not necessarily in contact with the vascular elements as this is normally the case in transfer cells.

It might be that the wall bodies currently observed are evanescent structures serving as temporary stores of excess polysaccharides originating from the non-cellulosic substance of the walls of the storage cells, the starch grains or the sucrose in the medium. These hydrolysed products of these polysaccharides are not translocated elsewhere (as in the germinating seedling) but are mainly deposited in the dedifferentiating cells occurring near the vascular tissue of the cotyledon, rather than in the intermediately situated storage cells which occur at a similar distance to the surface of the agar medium. The development of the wall bodies could also be related to the production of an extracellular polysaccharide, found in suspension cultures of cells derived from P. vulgaris cotyledons (Mante and Boll, 1975,

1976 and 1978). The optimum production of this polysaccharide requires the presence of both 2,4-D and Kinetin in the nutrient medium, and both these regulators are present in the nutrient medium used in the current investigation. The later fate of the wall bodies in the explant has not been examined and it would be interesting to find out whether these are digested during later callus development when the medium and tissue is depleted of nutrients. In the highly polyploid suspensor cells of the developing embryo in P. vulgaris (Schnepf and Nagl, 1970) wall invaginations occur which can give rise to wall labyrinths and their Figure 6 shows a proliferation of the external wall which is identical to the labyrinth margins of the wall bodies in P. vulgaris cotyledon explants. Pearce et al. (1974) reported the development of wall bodies in regenerating protoplasts of Lycopersicum esculentum, Nicotiana tabacum and Secale seriale and they describe them as internal structures often not in contact with the plasmalemma or regenerated wall. Similar results have also been obtained by Gigot, Schmitt and Hirth (1972) in Nicotiana glutinosa protoplast clusters.

In in vivo growing higher plants structures similar to wall bodies have only been observed in virus infected tissues (Spencer and Kimmins, 1970, in P. vulgaris leaves inoculated with TMV(V_1); Kim and Fulton, 1973, in P. vulgaris leaves inoculated with BPMV; McMullen et al., 1977, in Hordeum vulgare leaves inoculated with BSMV). In all virus infected plants the reaction of the cells after inoculation with virus particles appears to be quite similar: paramural bodies are developed after the infection and this is followed by the development of abnormal cell wall protrusions. However, no fine-structural

evidence has been found of a viral infection in the material currently investigated of P. vulgaris and none of the previous descriptions of wall bodies in in vitro cultures reported such an infection.

A number of morphological or fine-structural studies have been made on the initiation of callusing in an explant in comparison with the structure of the established in vitro culture. These observations have been made on parenchymatous explants derived from the root of Daucus carota (Israel and Steward, 1965, 1966; Jordan and Chapman, 1973) the Helianthus tuberosus tuber cells (Fowke and Setterfield, 1968, 1969; Yeoman et al., 1970; Bagshaw et al., 1969), Pisum sativum roots (Bowes and Torrey, 1976; Feldman and Torrey, 1977). In these parenchymatous explants, when freshly isolated, the cells contain a large central vacuole and a thin layer of cytoplasm which lines the cell wall whilst sometimes cytoplasmic strands traverse the central vacuole. Storage products are occasionally present, either in the vacuoles (e.g. sucrose) or in the plastids and chloroplasts (starch, phytoferritin). The nuclei are usually flattened against the cell wall and contain one, sometimes vacuolated, nucleolus. The respiratory activity of the fresh explant is very low (Yeoman et al., 1965) but the fine-structure of the mitochondria appears quite normal and their shape may be as simple as spherical, oval or rod shaped (P. sativum, Bowes and Torrey, 1976) to more complex structures like the cup-shaped mitochondria encounted in H. tuberosus (Bagshaw et al., 1969). Quiescent cells are usually poor in polyribosomes and most of the ribosomes in H. tuberosus tuber cells (Yeoman and Street, 1973) are not found in clusters but scattered singly in the ground substance whilst some

ribosomes may be seen attached to the endoplasmic reticulum or to the nuclear envelope. In such tissues dictyosomes are usually rare (Fowke and Setterfield, 1968; Tran Thanh Van and Chlyah, 1976) and the ones observed are composed of curved or straight cisternae with few vesicles (Molenhauer and Morre, 1966; Bowes and Torrey, 1976; Yeoman and Street, 1973). The appearance of the plastids depends on the origin of the tissue; in the cortical cells of P. sativum root explants amylopasts containing several starch grains have been observed (Bowes and Torrey, 1976). In the tuber cells of H. tuberosus, plastids contain a granular stroma with some unbranched tubules; although chloroplasts of parenchymatous cells of photosynthetic tissue (N. tabacum, Tran Thanh Van and Chlyah, 1976) usually possess well developed grana and occasionally starch grains. Generally the plastids are scattered within the cells, but in some occasions they are found in tight clusters as in tuber cells of H. tuberosus (Tulett et al., 1969). The plastids of the quiescent cells from D. carota root explants are without internal lamellar structure but contain starch grains and sometimes carotene crystals (Israel and Steward, 1967). Frederick et al. (1968) reported the presence of crystal containing bodies (CCB's), bounded by a single membrane, in quiescent cells of Avena sativa and Nicotiana tabacum and suggested that these represent a specialised type of microbody characteristic of metabolically less active cells and that may be functional equivalent of lysosomes or microbodies in animal cells. CCB's were also observed by Fowke and Setterfield (1968), Bagshaw (1969) in quiescent cells of H. tuberosus and in N. tabacum by Tran Thanh Van and Chlyah (1976). In the parenchymatous explants the plasmalemma is generally closely adherent to the

cell wall but sometimes lomasomes may occur (Fowke and Setterfield, 1968). A well developed intercellular space system is usually present in quiescent tissues (Bowes and Torrey, 1976).

The tissues of the freshly hydrated cotyledon explant of P. vulgaris investigated in the present study differs considerably from the explant tissues described above since the storage cells of a cotyledon are non-vacuolated and packed with food storing organelles. In the air dried seed neither dictyosomes nor endoplasmic reticulum are apparently present, although, these begin to be discernible by Day 1 when the cotyledon is explanted. The food reserves become progressively digested during in vitro culture, leading to the early appearence of highly vacuolated, thin-walled cells in the adaxial storage tissue. At this stage this tissue generally resembles the parenchymatous explants derived from the other sources detailed previously.

The process of callus formation from quiescent cells of an explant may be divided into three stages, according to Yeoman, (1970): The lag phase (activation phase); the division phase, in which also an organized wound cambium is formed at the damaged surface of the tissue; and the differentiation phase.

Most of the microscopical observations on the first stage of development have been made on parenchyma cells of D. carota root explant (Israel and Steward, 1966, 1967; Jordan and Chapman, 1973), H. tuberosus tuber (Fowke and Setterfield, 1968; Jordan and Chapman, 1971; Bagshaw, 1969; Tulett et al., 1969; Yeoman et al., 1970), N. tabacum (Nitsch and Lance-Nougarède, 1967; Ross, Thorpe and Costerton, 1973), Lathyrous odoratus (Vasil, 1971, 1973), P. sativum (Bowes and Torrey, 1976; Feldman and Torrey, 1977). The changes occurring at this stage are summarised by

Yeoman and Street (1973, 1977) and Davidson *et al.* (1976). The structural differences largely reflect changes in the metabolism of the cells and normally lead to cell division. Within one hour of excision of *H. tuberosus* an increase in the number of polyribosomes, forming either spirals of helices, is detectable; the spirals are associated with the surface of the endoplasmic reticulum but the helices are scattered in the ground cytoplasm. Parenchyma cells usually contain storage products and their mobilisation starts during the preparation for division. *D. carota* root cells lose their starch grains and *H. tuberosus* tuber cells start forming starch in their plastids and lose their phytoferritin; the plastid population increases. The number of mitochondria also increases and is accompanied by an increase in the respiration rate. The excision also affects the shape of mitochondria and a large variety of shapes and sizes occurs in *H. tuberosus* (Bagshaw *et al.*, 1969). Crystal containing bodies are found to decrease in size during culturing (Bagshaw, 1969). Another indication of the cell activation is the increase in number of the dictyosomes which now have many vesicles of assorted sizes and flat cisternae in contrast to the curved ones with very few vesicles in the quiescent cells (Yeoman and Street, 1973). The changes occurring to the nucleus start with its change in shape and contents; soon after the excision it appears rounder but with an irregular outline (a 65% increse in surface area has been estimated by Jordan and Chapman, 1973, for the nucleus of *D. carota* root cells after 24 hours). There is an increase in the number of the nuclear pores and the nucleus migrates towards the centre of the cell. The nucleolus also undergoes changes during this activation period; nucleolar vacuoles appear and they contain

granular particles similar in size to ribosomes; the fibrillar and granular zones become intermingled. In addition electron transparent regions, smaller than the nucleolar vacuoles, form in the fibrillar zone surrounding the central granular area. The changes occuring in the nucleus are accompanied by changes in the surrounding cytoplasm. With the excision of the explant the volume of the cytoplasm increases and starts extending through the vacuole forming the phragmosome; the nucleus then moves into the phragmosome and settles somewhere away from the cell wall, but not necessarily in the centre of the cell (see Fig. 6.7. of Yeoman and Street, 1973). After the formation of the phragmosome during prophase, nuclear extensions appear in the cultured H. tuberosus cells (Yeoman et al., 1970); these structures differ from the nuclear envelope in not having nuclear pores and microtubules are often in association with these nuclear extensions. These microtubules may be involved in the movement of the nucleus as well as determining the point at which the phragmosome is formed (Davidson et al., 1976). Once the nucleus has reached its final location within the phragmosome, it can begin to divide mitotically. The changes in the metabolism of explant cells and the mechanical conditions under which they develop affects the appearance and the fine structure of their organelles. The range of forms of the various mitochondrial profiles which are found in clusters in H. tuberosus tuber tissue (Bagshaw et al., 1969) tend to increase during culture and they are divided into those appearing as long cylindrical rods, branched structures or plates (which all contain matrix and cristae and are probably derived from simple spheres and rod-shaped mitochondria) and another group consisting of plates and

bell-shaped forms which show the usual mitochondrial structure around the rim, but contain no cristae in the centre.

The development and fine structure of plastids has been studied in callus tissue of H. tuberosus (Tulett et al., 1969), D. carota root (Israel and Steward, 1967), Ranunculus sceleratus (Thomas et al., 1972) Streptanthus tortuosus (Sjolund and Weier, 1971). The clusters of plastids observed by Tulett et al. (1969) become fewer during culturing and starch is formed. Both Tulett et al. (1969) and Israel and Steward (1967) observed the "prethylakoid body", the corpo opaco, as it was described by Gerola and Dassu (1960), in H. tuberosus tubers.

In the P. vulgaris cotyledon explant cells an increase in the number of organelles is observed accompanying reserve food mobilisation and dedifferentiation. Endoplasmic reticulum elements are numerous (especially near the nuclei), amyloplasts are found either scattered in the cytoplasm or in clusters located near the activated nuclei and a variety of mitochondrial profiles is also observed in these cells similar to the previously described ones by Bagshaw et al. (1969) for the H. tuberosus explant. Dictyosomes are often found scattered in the cytoplasm. The fine structure of the cytoplasm of the storage cells in the in vivo germinating cotyledon appears very different from that of the explant cells; mitochondria and dictyosomes are rare and after mobilisation of their food reserves most cells senesce. However, the sub-epidermal cells are longer lived and their plastids develop very extensive grana and are photosynthetic. In the dedifferentiating and dividing storage cells of P. vulgaris explant it seems that replication of organelles occurs but (apart from nuclear divisions) this aspect has not been investigated in detail in the present study.

The first part of this study has examined a system (*P. vulgaris* cotyledon explant cultured *in vitro*) where cell proliferation is induced by exogenous growth substances in an organ which would in normal development undergo rapid senescence. The end product of this dedifferentiation is an amorphous mass of cells (the callus) which is potentially capable of proliferating indefinately (but without organogenesis) if routinely subcultured under aseptic conditions onto fresh nutrient medium. In the second part, of this thesis however, the dedifferentiation, which occurs spontaneously *in vivo* when young *Linum usitatissimum* seedlings are decapitated beneath their cotyledons, has been investigated. Following localised dedifferentiation of the epidermal cells *de novo* production of adventitious buds occurs; this capacity of the hypocotyl for organogenesis is apparently controlled both by endogenous and environmental factors (Link and Eggers, 1938, 1946a and b, and Gulline, 1960) and it is a short lived phenomenon which disappears after the hypocotyl is about 20-30 days old (Link and Eggers, 1946).

A description of the morphological stages of germination of *L. usitatissimum* and the histology of adventitious bud formation on the decapitated hypocotyl is given by Crooks (1933); some additional information is provided by Link and Eggers (1938, 1946a and b) and Gulline (1960). According to Crooks (1933) the *L. usitatissimum* cotyledons, still enclosed within the seed coat, show above the ground by about Day 4-5; the initially hook-shaped hypocotyl then straightens and at the same time the cotyledons are pulled free from the seed coat. The major zone of elongation of the hypocotyl is initially at ground level and this zone then progresses acropetally; the hypocotyl ceases elongation by Day

15-20 (Crooks, 1933) but higher temperatures give more rapid germination. The lower part of the hypocotyl is root-like in vascular arrangement and there is a gradual reorientation of the primary vascular tissue at successive higher levels throughout the hypocotyl.

In the present study of L. usitatissimum the normal differentiation of the epidermis and cortex of the hypocotyl during germination has been followed and is contrasted with the dedifferentiation changes in these tissues after decapitation of this organ. When examining the air dried hypocotyl (and cotyledons in preliminary experiments) the same technical problems in the processing of such material for microscopy, as previously listed for the P. vulgaris cotyledonary tissue, were faced. The cells of all tissues are packed with food reserves (protein 23%, lipid 34% and sugar 23%, Mayer, 1978, Table 2.2.). In the protein bodies four regions (a-d) are observed, and regions (b) and (d) seem to correspond to the globoid and crystalloid inclusions described by Poux (1965) for the storage cells of L. usitatissimum cotyledons. However, in the present investigation an additional region (a), which possibly represents an air space, is visible in the protein bodies of the air dried seed whereas Poux (1965) only examined hydrated seeds in which this region was not evident, presumably due to the swelling of the surrounding region (b). When all protein is digested, the protein bodies progressively join together and expand to form one central large vacuole; this digestion was also observed at the light microscopic level by Dhar and Vijayaraghavan (1979) in the protein bodies of the cotyledons of the same species.

As currently observed, protein body vacuolation, and

accompanying elongation of the cortical and epidermal cells is initially uneven. The hypocotyl (which at Day 0 is straight) becomes hook-shaped, with short cells (which are still packed with food reserves) on the concave side and relatively elongated and vacuolated cells on the convex side. Later the hypocotyl straightens as the food reserves are depleted on the concave side. As vacuolation proceeds, the nuclei of the cortical cells migrate from their original central position towards the peripheral cytoplasm lining the cell walls. By Day 2-4 the several types of epidermal cells have differentiated from the uniform cells present at Day 0. The epidermal nuclei migrate to the cytoplasm lining the inner (periclinal) walls and at the same time elongate and develop crystalline inclusions. The plastids in the cortical cells develop grana and some starch grains which are, however, digested by Day 8-10. The plastids in the normal and "swollen" epidermal cells do not develop grana but only some scattered thylakoids, and no starch is normally observed in the plastids. The stomatal guard and accessory cells do, however, develop mature chloroplasts and starch grains.

Crooks (1933) in his studies of the L. usitatissimum hypocotyl does not specify the presence of the "swollen" epidermal cells nor describe the nuclear inclusions in the epidermal cells. The "swollen" epidermal cells have been observed in all hypocotyls currently investigated and they develop at about Day 2-4; however, at considerably later stages of development, in both the intact (Day 40) and the decapitated (Day 10+30) hypocotyl, such are no longer present. No special functions are known for these cells but their arrangement in longitudinal ribs and their shape suggest that they might add some mechanical strength to the hypocotyl

until the xylem is well developed and also the fibres from the inner cortex.

Nuclear inclusions are observed in all types of epidermal cells of the hypocotyl of L. usitatissimum; they are absent in the embryo and first become evident after several days germination. It seems that a single inclusion occurs per nucleus and this is characteristically orientated with its long axis parallel to that of the hypocotyl. It is generally considered that such inclusions in other species are proteinaceous (Wergin et al., 1970) and in L. usitatissimum these may originate from the protein bodies present in the embryo. However, nuclear inclusions are absent from the cortex although this tissue also contains abundant protein in the embryo. According to Wergin et al. (1970) nuclear inclusions are commonly tissue specific as in L. usitatissimum. Although in L. usitatissimum the nuclear inclusions are clearly visible with the light microscope in semi-thin sections (1 or 2μm) these could easily be confused with the nucleolus if only transverse sections were investigated. Also the older and cruder fixation and embedding techniques employed by Crooks (1933), Gulline (1960) and Link and Eggers (1946a), for light microscopic observations on this specimen may possibly not preserve these structures; at any rate these authors failed to identify them. Nuclear inclusions very often occur in plant and animal cells and Wergin et al. (1970) in their report on nuclear inclusions in plants classified them as amorphous, crystalline, paracrystalline, fibrous and spherosome-like according to their structure as observed with the electron microscope; the nuclear inclusions observed in the L. usitatissimum hypocotyledonary epidermal cells could be described as fibrous although in a TS they appear as

hexagonal crystals. In the nuclei of the epidermal cells of L. usitatissimum the nucleolus and the nuclear inclusions occur in proximity to each other. This is also the case in many other species and in some there appears to be an inverse relationship during development between the size of the nuclear inclusions and the nucleoli (Meyer, 1920; Weber, 1926; Barton, 1967; Pickett-Heaps, 1967; Villiers, 1968; Weintraub et al., 1968). However, from these studies no definate conclusions can be made on the relation between these two types of nuclear bodies. The close association between nuclear inclusions and nucleoli in L. usitatissimum might be due to the limited nuclear space available but this does not exclude a physiological relationship between them. It is interesting to note that after the hypocotyl is decapitated, the nuclear inclusion may become re-orientated in a plane transverse to the longitudinal axes of this organ. This marks the orientation of the cell plate forming after the first mitosis in a bud primordium; the nuclear inclusions are absent from the daughter nuclei of this first division.

At about Day 4-5 the development of scattered fibres is visible in the inner cortex and further fibres develop in the older hypocotyl. According to Esau (1943) these fibres grow by apical intrusive growth in the young stem of the same species but their development has not currently been studied in detail in the hypocotyl. Occasionally fibre cells, up to 2-3mm long are observed in longitudinal sections of the Day 10 (intact) hypocotyl and such cells may contain up to 6 nuclei. The presence of fibres is more apparent in older hypocotyls and decapitation of the latter results in the development of more fibres (at Day 10+30) than in the intact (Day 40) organs. None of the previous workers

who examined the L. usitatissimum hypocotyl mentions the presence of fibres in the latter.

In the present study some dedifferentiation changes are observed in the young hypocotyl after decapitation which are not related to adventitious bud development. The upper end of the hypocotyl swells (but without forming wound cambium), in the cortex the intercellular spaces increase, the cell walls of the cortical cells become thicker, the cytoplasmic layer lining these cells becomes denser and wider whilst the chloroplasts increase in size and develop large starch grains. However, these changes are only temporary and when the new shoot is established from the outgrowth of the dominant adventitious bud most cortical cells revert to their original appearance. In the epidermal cells some accumulation of starch is observed but only in the stomatal guard and accessory cells and in some normal epidermal cells and these are possibly the initiative cells of adventitious buds. In the older(Day 40) intact hypocotyls several changes are evident compared to Day 10: the epidermis becomes replaced due to the activity of the cork cambium arising in the outer cortex whilst the hypocotyl becomes considerably thickened due to the development of a central mass of secondary vascular tissue (mainly xylem). However, in the Day 10+30 (experimental) hypocotyl the epidermis remains active (and shows some divisions) and the development of secondary vascular tissue is much sparser.

Careful examination of epidermal strips at both light and electron microscopic levels of control (Day 10) indicate that all normal epidermal cells of the hypocotyl possess similar cytological features and no indication exists as to which cells are going to give rise to adventitious buds. According to Crooks

(1933) all L. usitatissimum (var. Bison) seedlings produce 5-20 adventitious buds on the lower part of the hypocotyl even when only a few millimeters of stump is left above the ground level. However, Link and Eggers (1946a and b) reported bud initiations on both decapitated and non-decapitated hypocotyls of the same variety of this species and stated that buds develop from the upper half of the hypocotyl only if the latter is decapitated. Gulline (1960) using the variety Ventnor, noted bud development only on decapitated hypocotyls and this occurs from the upper regions only. Likewise, no bud development is observed on the intact hypocotyls of the variety of this species currently investigated and on the decapitated ones the sites of development (upper or lower half of the organ) seem to depend upon the time of the year; in decapitations carried out during Spring-Summer, the buds develop mainly on the lower half whilst in these experiments (the majority) carried out in Winter buds develop mainly on the upper half of the hypocotyl. In any event, one bud of the many initiated usually outgrows all the others,but this is not necessarily the first to have been initiated on the hypocotyl.

The development of an adventitious bud begins with a division in one of the normal epidermal cells. According to Crooks (1933) this division is a longitudinal, although according to Link and Eggers (1946a) several transverse divisions first occur and this is confined in the present study. It seems that Crooks (1933) only examined transverse sections of the hypocotyl and therefore he in fact only studied what was a somewhat later stage of development. In the current study no increase in the density or amount of cytoplasm in the epidermal cells was observed before the initial cell division and this agrees with the results obtained by

both Crooks (1933) and Link and Eggers (1946a). The present investigation shows that division in the hypocotyledonary epidermal cells occurs several days after decapitation of the fully elongated hypocotyl and the only prior changes which seem to indicate which epidermal cells are going to divide concern their plastids and nuclei. The plastids develop small starch grains and the elongated or tailed profiles sometimes visible suggest that plastid replication may be occurring. Some epidermal nuclei are wider and shorter than in the control and on a few occasions the nuclear inclusions are seen to be much shorter than in other epidermal cells and lying at right angles to their usual position parallel to the long axis of the epidermal cells. In such an epidermal cell the nucleus lies suspended in the central vacuole surrounded by plastids of the type described above. Following the first mitosis the two daughter cells quickly divide again transversely and further transverse divisions then occur; at this stage the cytoplasm becomes more prominent, vacuoles become smaller and the nuclei contain a single large nucleolus, sometimes vacuolated, with granular and fibrillar zones. No inclusions are observed in the daughter nuclei.

In Euphorbia esula L. both decapitated and non-decapitated hypocotyls develop adventitious buds from the dedifferentiation of the sub-epidermal cortical cells and also, infrequently, endogenously from the more peripheral stelar tissue. (Raju, 1975) Adventitious bud formation has also been reported in excised explants of leaves and hypocotyls of Torenia furnieri (Bajaj, 1972) and Anagalis arvense (Bajaj and Mäder, 1974); these buds arise from the epidermal as well as the sub-epidermal layers and the cells which initiate bud development are distinguishable from

the others by being smaller in size, highly cytoplasmic and with prominent, densely staining nuclei. By contrast in L.usitatissimum it appears that bud initials may form in any normal epidermal cell and there are apparently no cytological indications as to which cells will form bud primordia prior to decapitation of the hypocotyl. Tran Thanh Van and Trinh, (1978), make the generalisation (in respect to de novo development of adventitious organs that "buds arise from epidermal cells of stems or leaves or in some cases from parenchyma or cortical cells, i.e. mainly from superficial cells of the aerial parts of the plant. On the contrary, roots originate from inner tissues such as perivascular cells near cambial or pericyclic zones". In the L. usitatissimum hypocotyls the buds are exogenous in origin since the first divisions are always restricted to the epidermal cells.

In the present study, usually the young buds observed on the hypocotyl of L. usitatissimum consisted of several leaf primordia closely surrounding an apex; however, occasionally some more complicated, elongated structures, were observed; it is possible that these represented several buds (situated very close to each other) which had started to develop simultaneously so producing teratomatous organs (Bloch, 1965; Brown, 1969).

Link and Eggers (1938, 1946b) suggested that the plumular apex has an inhibitory effect on the development of adventitious buds on the hypocotyl of L. usitatissimum and showed that application of IAA on the decapitated surface of the hypocotyl suppresses bud development. The role of the apex in the development of lateral buds has been examined extensively by many workers and has been reviewed by Phillips (1975) and Rubinstein and Nagao (1976). In the non-decapitated hypocotyls of L.

usitatissimum currently examined no adventitious buds were ever observed. Such buds were, however, observed by Link and Eggers (1946) but these were restricted to the lower half of the non-decapitated hypocotyl whilst decapitation results in bud development on the entire length of this organ. In Euphorbia esula hypocotyls (Raju, 1975) the location of adventitious bud formation is not affected by decapitation, although, it is affected by the age of the seedling.

In preliminary experiments, in the course of this study, it was found that when hypocotyls of L.usitatissimum are inoculated in liquid (Murashige and Skoog, 1962) nutrient medium, containing only the basal constituents (i.e.no growth substances) only a small amount of callus tissue is produced at the excised ends and no organogenesis occurs even after several weeks of inoculation. Gamborg and Shyluk (1976) showed that hypocotyl explants of this species can be induced to proliferate in vitro to form both whole plants as well as callus tissue. However, it is likely that the bud initiating process had already started in the explants before the inoculation, since the hypocotyl used had been decapitated five days before the inoculation,and therefore the growth substances in the medium in this case only stimulated the development of buds which had already been initiated. Rybczynski (1975) has shown that depending on the ratio kinetin : NAA in the medium, cotyledon explants of L. usitatissimum when cultured in vitro may be induced to produce roots, shoots and whole plants as well as callus tissue of varying texture.

Crooks (1933) stated that in L. usitatissimum hypocotyls the "derivatives of the original epidermal cells by subsequent divisions eventually constitute the axis of the adventitious bud",

however, he does not provide evidence in the illustrations of the bud development for the exclusion of cortical involvment in this process. In the present study of the hypocotyl in this species, an attempt has been made to distinguish between dedifferentiated cells of epidermal or cortical origin based on fine-structural differences in the plastids. Thus in some cells (possibly epidermal in origin) these appear small and agranal whilst in other neighbouring cells (possibly of cortical origin) they appear as mature chloroplasts; however, it is also possible that the former type may also be of cortical origin but represent a more advanced stage of dedifferentiation. The dedifferentiation of the outer cortical and epidermal cells leads to the formation of the primary (apical-type) meristematic cells (as termed by Gautheret, 1966) which can then redifferentiate in sub-apical regions to produce the various tissues (e.g. epidermis, pith, cortex, vascular tissue). The fact that both epidermal and outer cortical cells can revert to a primary meristematic state suggests that these two types of cells are totipotent; no evidence has been found indicating that the outer cortical cells do not participate in the formation of the bud primordium, although their contribution to the multicellular mass of the bud apex is possibly less than that of the epidermis. Gamborg and Shyluk (1976) cultured protoplasts from the hypocotyl of L. usitatissimum and the majority of these contained chloroplasts and hence were of cortical origin. Although these protoplasts could be cultured and give rise to callus tissue no shoots could be induced. It would be interesting to attempt the in vitro culture of the isolated epidermal and isolated cortical tissues (as employed by Tran Thanh Van and Trinh, (1978), to determine whether both have an equal capacity

for organogenesis. It seems, however, that during dedifferentiation not all cells involved revert to the primary meristematic stage; for instance at least some of these in the mid-inner cortex (which form the procambial strands linking the bud to the axial vascular tissue of the hypocotyl) appear to undergo only partial dedifferentiation and still contain chloroplasts, and one or more relatively large vacuoles when their redifferentiation into mature vascular elements occurs.

In contrast to the L. usitatissimum hypocotyl, no organogenetic process is ever observed in the in vitro cultured P. vulgaris cotyledons even in the presence of strong stimulants such as coconut milk, 2,4-D, kinetin and IAA and this was also found by previous workers (Frame et al., 1976: Liau and Boll, 1970; Mante and Boll, 1978) in their attempts to induce organogenesis in P. vulgaris cotyledons. The absence of organogenetic capacity might be related to the high ploidy levels of storage cells in the mature cotyledons and also to the anomalous(amitotic) nuclear division occuring in vitro.

According to Crooks (1933) vascular bundles are differentiated in the first two or three leaves of the new bud by the time the growing point (shoot apex) is well established and before cell division has begun in the inner cortex of the hypocotyl; then the cells of the inner cortex become meristematic and vascular tissue differentiates progressively inward from the new bud through the cortex to the endodermis. Crooks (1933) does not distinguish between non-dormant and dormant adventitious buds, but the results currently obtained suggest that two such categories of buds exist and that they differ in their vascular linkages to the axial vascular tissue of the hypocotyl. For technical reasons

it is very difficult to distinguish the dominant from the dormant buds by only examining sectioned material of a hypocotyl; only one or two buds per hypocotyl can be examined by serial sectioning and usually only one (out of about 20 adventitious buds initiated on the hypocotyl) will subsequent grow out into a lateral replacement shoot. The external morphology or size of a bud cannot, unfortunately, be used to identify this bud at an early stage, but in cleared hypocotyls the presence or absence of protoxylem in these provascular strands can be investigated. Observations made on a number of both cleared and sectioned (transverse, longitudinal and paradermal serial sections) specimens indicate that (in certain buds only) dedifferentiation occurs throughout the cortex immediately after the first divisions have occurred in the epidermis. Such early changes in the cortex are thought to be associated with a non-dormant bud whereas in relation to potential dormant buds the provascular strands only begin to differentiate in the cortex much later when leaf primordia are already visible on the bud. In the non-dormant bud, by the time the first leaf primordia are visible, numerous tracheary elements have already differentiated within the cortical provascular strands and these sometimes seem to form long protoxylem strands.

Studies by Raju and Marchuk (1977) on the inhibited hypocotyledonary adventitious buds of Euphorbia esula (which occur on intact hypocotyls) using cleared specimens, indicate that at their bases the xylem strands are uninterrupted by partly differentiated tracheary elements; this contrasts with the buds which have been released from inhibition by decapitation, where uninterrupted xylem strands (containing vessels) connect with the hypocotyledonary stele.

Crooks (1933) describes the development of the protoxylem elements as progressing inwards from the hypocotyl towards the main (axial) vascular tissue. However, no such evidence is found in the hypocotyls currently observed by using serial sections and cleared specimens. On the contrary dedifferentiating cortical cells appear to occur simultaneously throughout the cortex along the paths of provascular strands and within these the protoxylem elements differentiate and then, in the case of a non-dormant bud these form continuous vessels whilst in the case of a dormant bud it seems likely that the xylem strands are discontinuous. It seems that Crooks (1933) examined only a small number of random sections which probably led him to draw his incorrect conclusions on this matter.

Phillips (1975) in reviewing the literature on the development of vascular tissue in relation to correlative inhibition of buds states that although suggestions have been made repeatedly that apical diminance involves regulation of the development of vascular connections between buds and stem, no generalisations can be made since the degree of vascularisation varies from one species to another, from one bud to another in a single plant, and also environmental conditions such as those of light and nutrient status influence bud trace development.

Transfer cells in plants possess an intricate system of convoluted, tubular inwardly projecting wall ingrowths from the main wall into the cytoplasm; the plasma membrane follows the labyrinthine contours of this wall and therefore the surface area is greatly increased; within the cytoplasm of such cells mitochondria are generally abundant (Gunning and Steer, 1975;

Pate and Gunning, 1972). Transfer cells are thought to be concerned with retrieval of solutes and their transfer to the sieve elements or exchange of solutes with the external environment and apparently both apoplastic and symplastic movements are possible (Pate and Gunning, 1972). These cells have been observed in a wide variety of species and at various anatomical positions in the plant, like leaf traces, root nodules, secretory glands (Bowes, 1973: Briarty, 1978; Czaninski, 1977; Gunning and Pate, 1969; Gunning *et al.*, 1970; Pate and Gunning, 1972; Gunning and Steer, 1975). Pate and Gunning (1969) reported that transfer cells are found in 22 of 200 families of dicotyledons and in one (*Zosteraceae*) of the 42 families of the monocotyledons studied by them.

A stereological analysis of micrographs has been made by Briarty (1978) in order to examine the development of root nodule xylem transfer cells in *Trfolium repens*. In the early stages of development, transfer cells and xylem vessels are rather similar. Briarty (1978) suggests that endoplasmic reticulum and dictyosomes may be involved in the deposition of wall material. As these transfer cells develop, an increase in vacuolation is observed and their nuclei become lobed. In the senescent stage of the nodule, transfer cells breakdown; their cytoplasm becomes electron-lucent and organelles become filled with electron dense droplets. In the provascular tissue of the germinating cotyledon of *Vicia faba* (Briarty *et al.*, 1970) wall ingrowths are found in the transfer cells surrounding both xylem and phloem and sometimes develop before the differentiation of these tissues; as in other species these transfer cells are characterised by numerous plastids and mitochondria, extensive endoplasmic reticulum and cytoplasm

densely populated with ribosomes. In the case of Linum usitatissimum transfer cells occur in the young shoot (Bowes, 1973) in proximity to the leaf trace protoxylem; the transfer cells are found to develop most ingrowths on the walls adjacent to protoxylem elements. In the present study of the hypocotyls of this species transfer cells occur in two locations following decapitation but are not observed in the intact organ. The first site is adjacent to the main (axial) vascular tissue of the hypocotyl where wall ingrowths develop only on the walls in contact with xylem elements but are uniformly distributed on all walls of transfer cells adjacent to phloem elements. The other position in which transfer cells develop is in relation to the ploem elements of de novo formed vascular tissue connecting the adventitious bud with the main vascular tissue; the wall ingrowths are uniformly distributed on all walls of these transfer cells. The transfer cells occurring in the decapitated hypocotyl seem to lose their wall ingrowths during the later development of the adventitious bud since no such cells can be identified in older hypocotyls (Day 10+30). The fine-structure of the transfer cells in L. usitatissimum appears similar to that generally encountered in other species (Gunning and Steer, 1975).

Following decapitation of the hypocotyl in L. usitatissimum the cortical cells in the vicinity of the bud primordia undergo varying degrees of dedifferentiation; the characteristics of the plastid populatios of such cells are apparently related to the extent of cellular dedifferentiation. Thus in cortical cells which have only divided once or twice the plastids are uniformly chloroplasts whereas in the more fully dedifferentiated cells

composing the provascular strands only proplastids occur. In between these two extremes daughter cells containing both chloroplast-like and proplastid populations are present. In such cells plastid replication is especially evident, it appears to occur in some cases by constrictive division into two (or sometimes more) equal portions or by the production of smaller "buds" from the main body of the plastid.

Leech (1976) in a recent review concluded that there is now well established evidence that young chloroplasts can divide and indeed this has been observed in vitro (in a medium lacking growth substances) after isolation from growing leaves (Ridley and Leech, 1970; Kameya and Takahashi, 1971). In mature chloroplasts Leech (1976) states that there is no clear evidence that their division can be induced in illuminated leaf discs cultured in a nutrient medium supplemented with kinetin (Boasson et al., 1972; Laetch and Boasson, 1971). In L. usitatissimum it seems possible that an accumulation of endogenous substances occurs in the upper hypocotyl in relation to the initiation and development of the adventitious buds (exogenous application of cytokinins to the in vitro grown hypocotyl stimulates bud initiation, Gamborg and Shyluk, 1976).

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FINE STRUCTURAL STUDIES ON DIFFERENTIATION AND DEDIFFERENTIATION IN THE HIGHER PLANT

BY

CONSTANTINOS FASSEAS B.Sc. Hons.

VOLUME II

A Thesis submitted for the Degree of

Doctor of Philosophy

in the

Botany Department

University of Glasgow

January 1980

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VOLUME II

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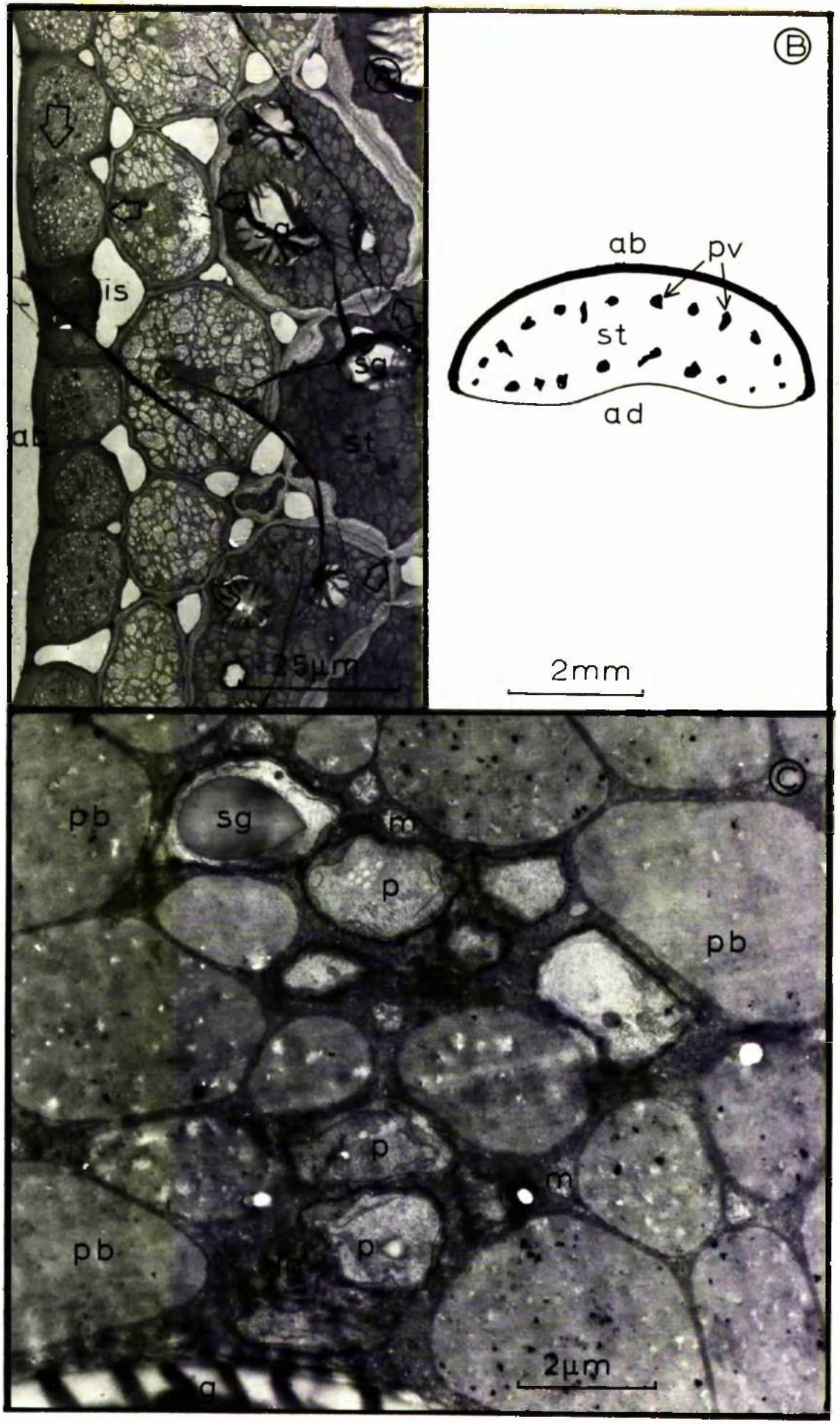
P. vulgaris

Plate 1A: TEM (Day 0) illustrating a TS of the abaxial epidermis, together with the unilayered sub-epidermis and the outer layer of the storage tissue. Note the relative size of the three types of component cells, the absence of large starch grains in both epidermis and sub-epidermis but the occurrence of protein bodies in all tissues, differences in cell walls of the various cell types and occurrence of intercellular spaces. The large arrow indicates a discontinuity in the anticlinal wall partly separating what appear to be two epidermal cells each containing a separate nucleus. The small arrows indicate pit areas. M: 1200x

Plate 1B: Diagramatic representation of a TS from the middle of a Day 0-1 cotyledon; 20-30 provascular strands (which are mainly longitudinally orientated) are visible. The adaxial surface is slightly concave and the abaxial surface is markedly convex. The position of the sub-epidermal layer is indicated by the thick black line on the abaxial surface. M: 12x

Plate 1C: TEM (Day 0) illustrating the cytoplasm of a storage cell; note the density of the ground cytoplasm (packed with free ribosomes) and crowding of the numerous organelles, which mainly here consist of angular protein bodies. Several proplastids are also present; these contain some membranes and sometimes electron translucent vesicles (arrow), whilst a prominent starch grain is visible in one plastid. Small lipid-like vesicles frequently closely surround the plastids. M: 12500x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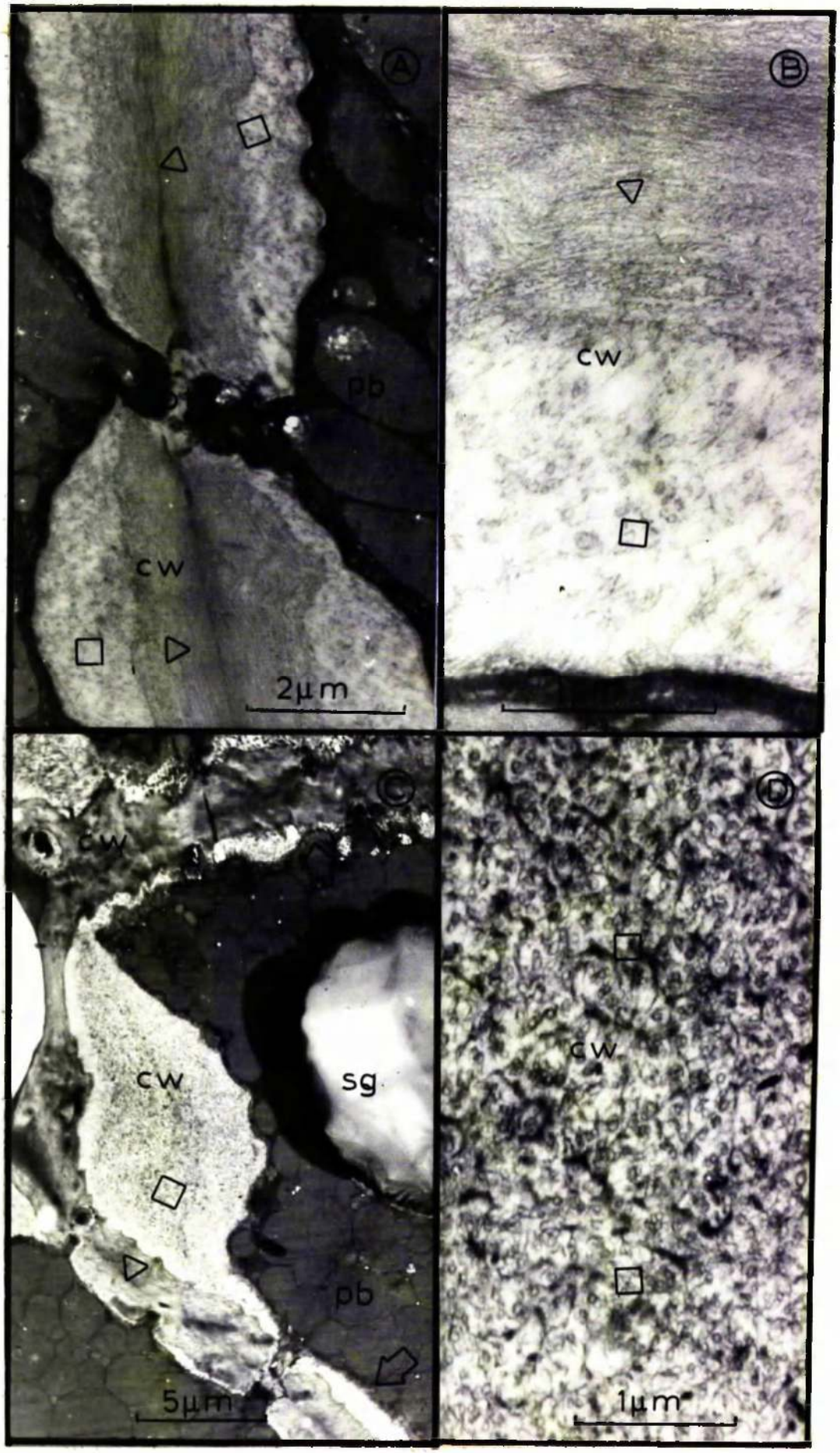
P. vulgaris

Plate 2A: TEM (Day 0) showing the thickened cell wall of a storage cell (interrupted by a pit) and consisting of two layers (△ and □, see detail in Pl. 2B) of wall material.
M: 13000x

Plate 2B: TEM (Day 0), detail of Pl. 2A, showing the fine-structure of the bilayered cell wall; outer fibrillar, cellulosic layer (△); inner non-fibrillar layer (□).
M: 36000x

Plate 2C: TEM (Day 1) showing part of a storage cell; the inner non-cellulosic layer (□) of the cell wall has swollen considerably (especially on the site near the intercellular space) compared to Day 0; (△) shows the outer cell wall in which there is no apparent change. The arrows indicate the layer of cytoplasmic lipid globules lining the cell wall.
M: 4400x

Plate 2D: TEM (Day 1), detail of Pl. 2C, showing the fine - structure of the inner (non-cellulosic) region of the cell wall of a storage cell. Note the great increase in fibrillar and vesicular-like structures. M: 26400x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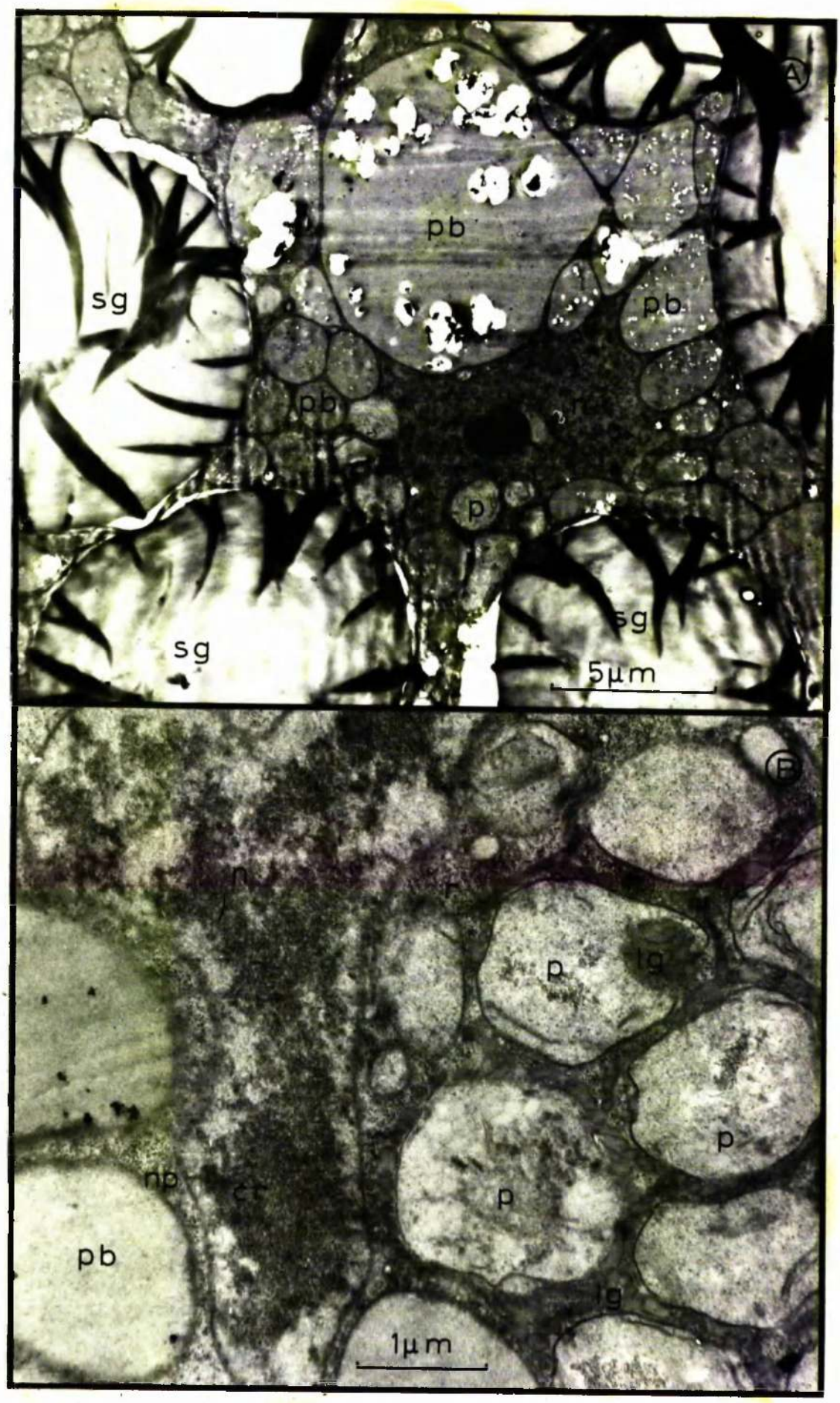
P. vulgaris

Plate 3A: TEM (Day 0) illustrating a typical storage cell with the multilobed nucleus occupying a central location surrounded by protein bodies, very large starch grains and various organelles. Note the radially arranged denser regions in the starch grains which probably represent artifacts. M: 6000x

Plate 3B: TEM (Day 0) showing greater detail of a part of a nucleus, protein bodies (and their uniformly staining amorphous contents), proplastids and lipid globules surrounding the plastids. Note the densely crowded ribosomes in the ground cytoplasm. M: 23200x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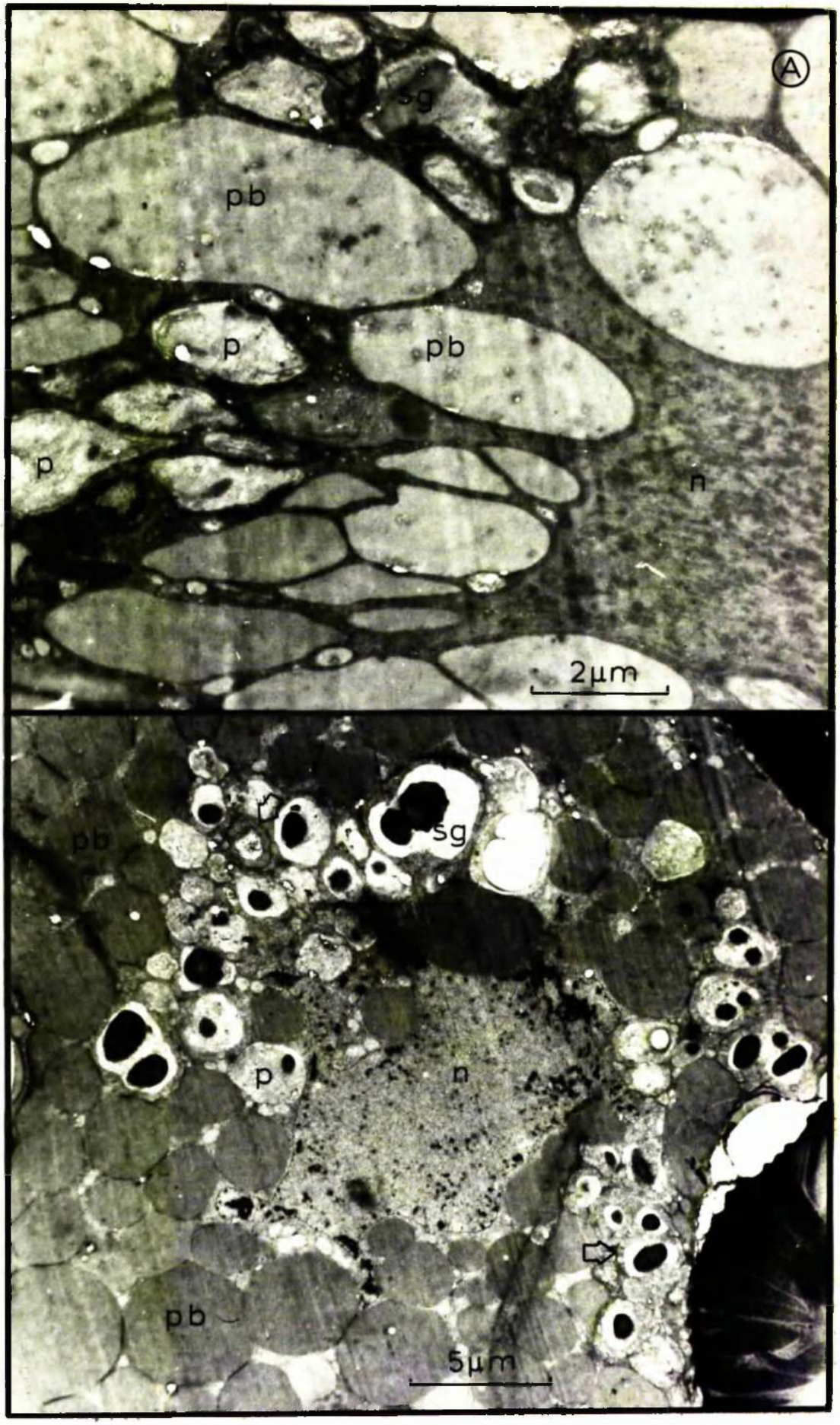
P. vulgaris

Plate 4A: TEM (Day 0) showing part of the multilobed nucleus, penetrating in between the surrounding organelles of a storage cell (c.f. Pl. 4B). M: 11200x

Plate 4B: TEM (Day 1) illustrating the nucleus and the surrounding organelles of a storage cell; note the abundant starch accumulation inside the proplastids, the more rounded and more crowded protein bodies and the changes which have occured in the distribution of the chromatin compared to Day 0 (c.f. Pl. 3A and 4A); the arrows indicate the lipid globules surrounding the small plastids . M: 5000x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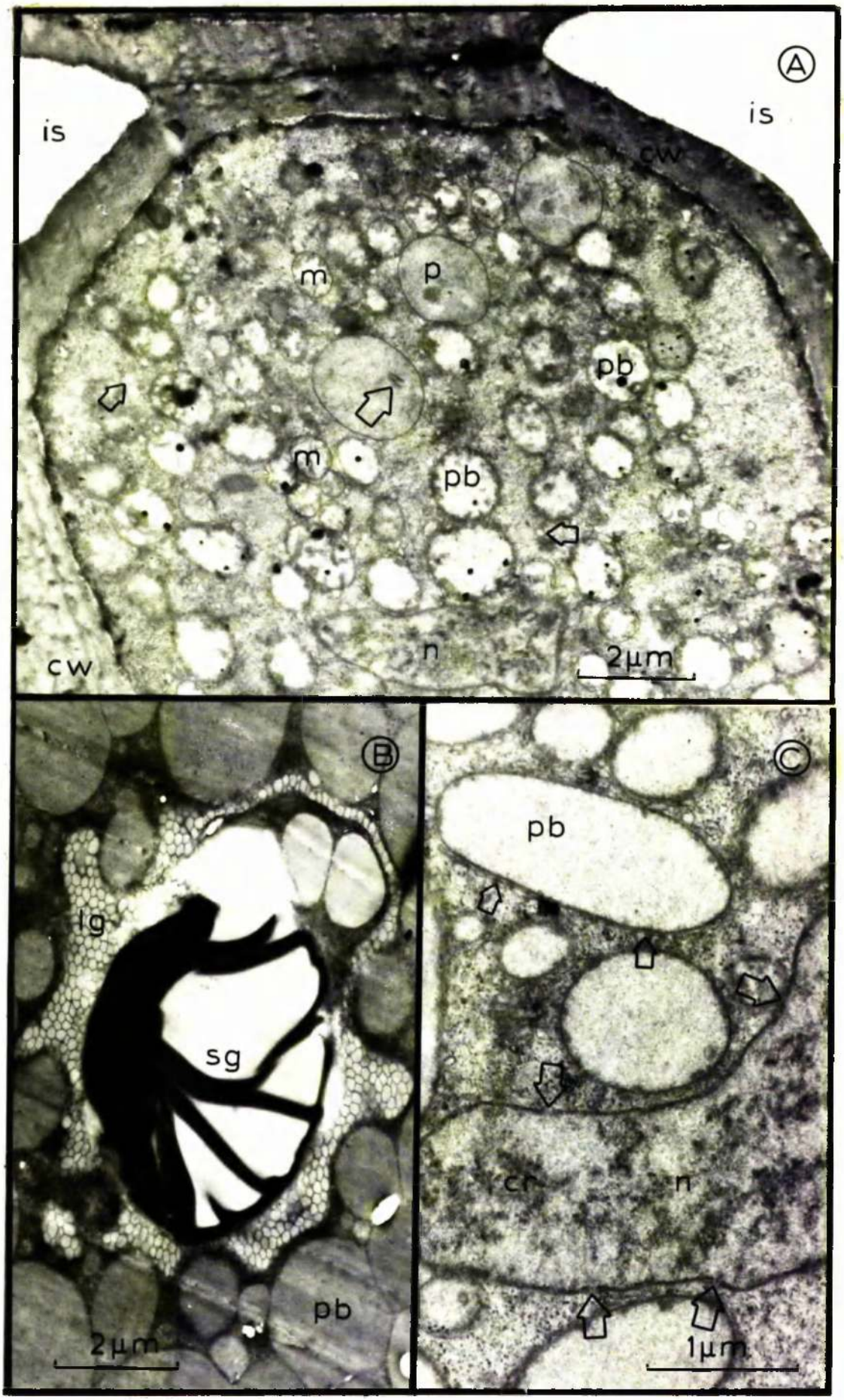
P. vulgaris

Plate 5A: TEM (Day 0) of an abaxial epidermal cell; the protein bodies are smaller than in the storage cells and their contents are not of uniform density. Note that starch grains do not apparently occur in the proplastids. The small arrows indicate some cytoplasmic vesicles which it is thought may give rise to the endoplasmic reticulum (Öpik, 1966) and the large arrow indicates some thylakoid membranes in one of the proplastids. M: 10700x

Plate 5B: TEM (Day 1) storage cell showing a small starch grain surrounded by numerous "honeycomb-like" vesicles which are thought to be lipid globules being digested. M: 10000x

Plate 5C: TEM (Day 0) showing part of a sub-epidermal cell; note the presence of nuclear pores (large arrows) and endoplasmic reticulum (small arrows) close to the protein bodies. M: 25700x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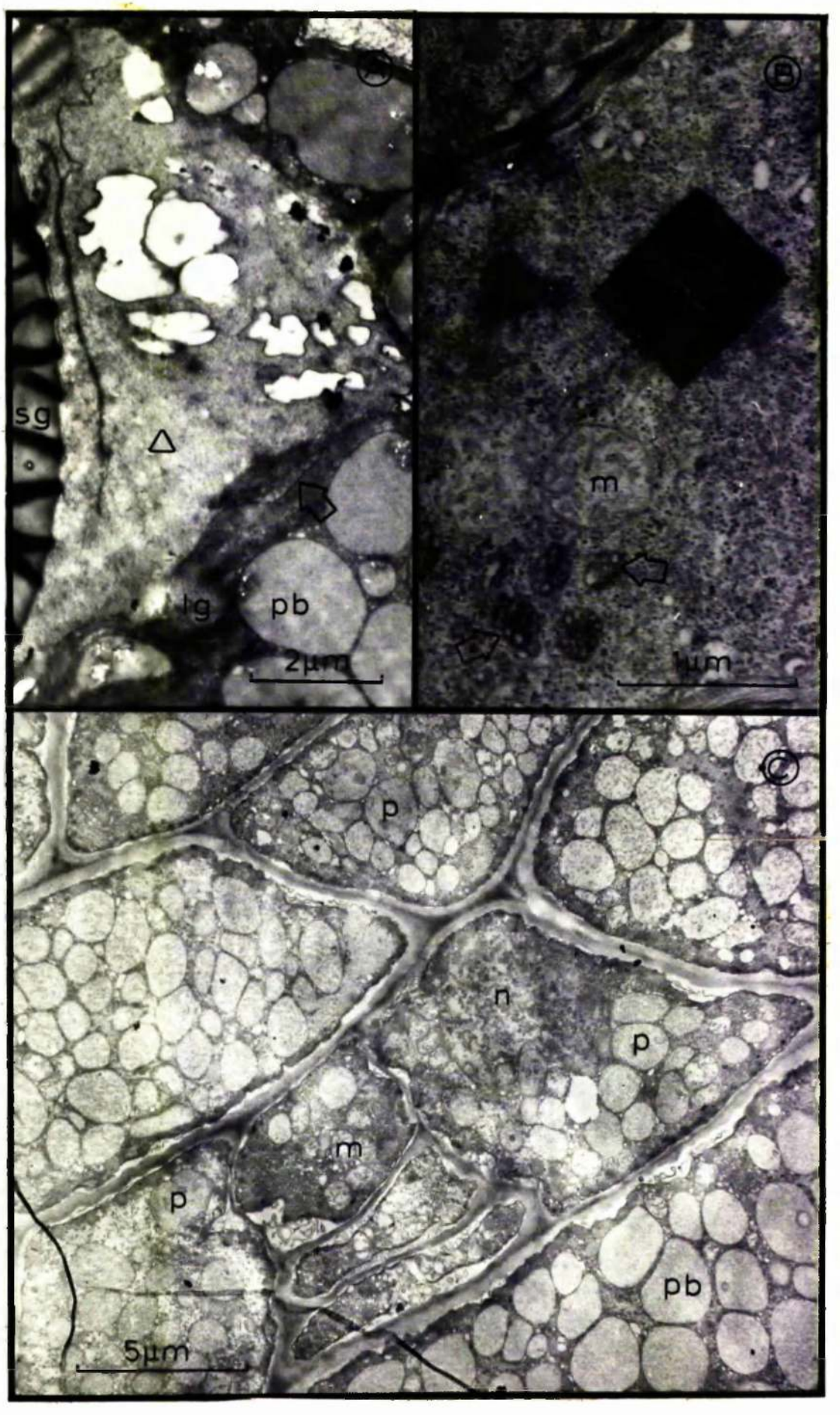
P. vulgaris

Plate 6A: TEM (Day 0) of a storage cell showing the margin of a large, densely staining starch grain which is partly surrounded by an irregular area (△) of material (differing in density from the ground cytoplasm) which may represent the remains of the amyloplast stroma. The arrow indicates what appears to be part of a membrane bounding the amyloplast. M: 12000x

Plate 6B: TEM (Day 1) illustrating part of a provascular cell. Note the presence of what appear to be p-protein crystals and also the multivesicular structures (arrows) possibly representing pro-dictyosomes. M: 31000x

Plate 6C: TEM (Day 0) illustrating a TS of a provascular strand; note the absence of starch grains and the difference in density of the protein bodies in comparison to the storage cells (c.f. Pl. 1C). M: 6000x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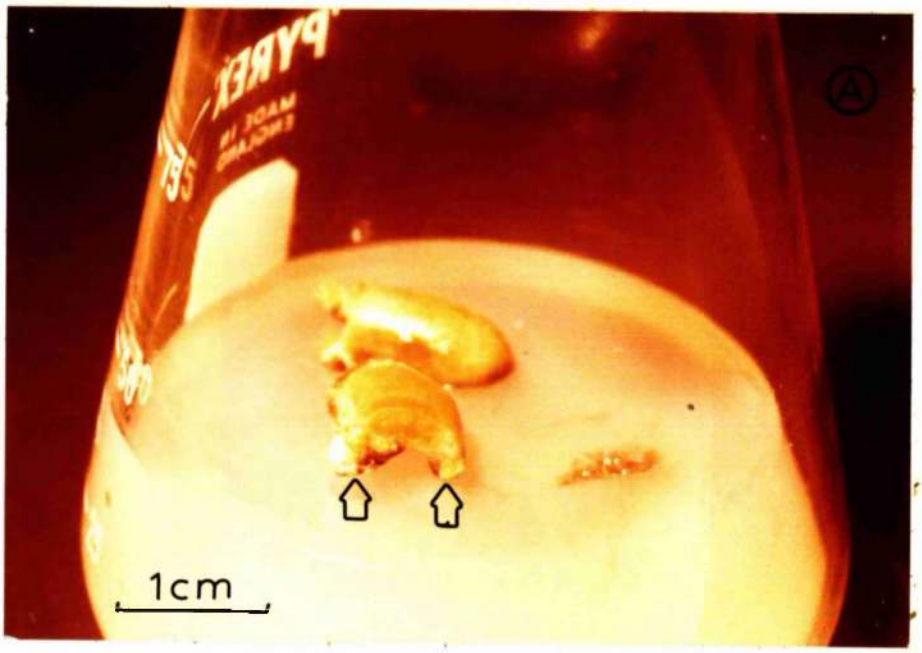
P. vulgaris

Plate 7A: Day 3 *in vitro* cultured cotyledons; note that the adaxial sides of the nearest cotyledon (arrows) are beginning to callus. M: 2x

Plate 7B: Day 7 *in vitro* cultured cotyledons; note the very pronounced adaxial callus development (cotyledon a, small arrows) and the small swelling on the transverse excised surface of cotyledon b (large arrow) corresponding to the provascular strands, which are transversely cut. M: 2x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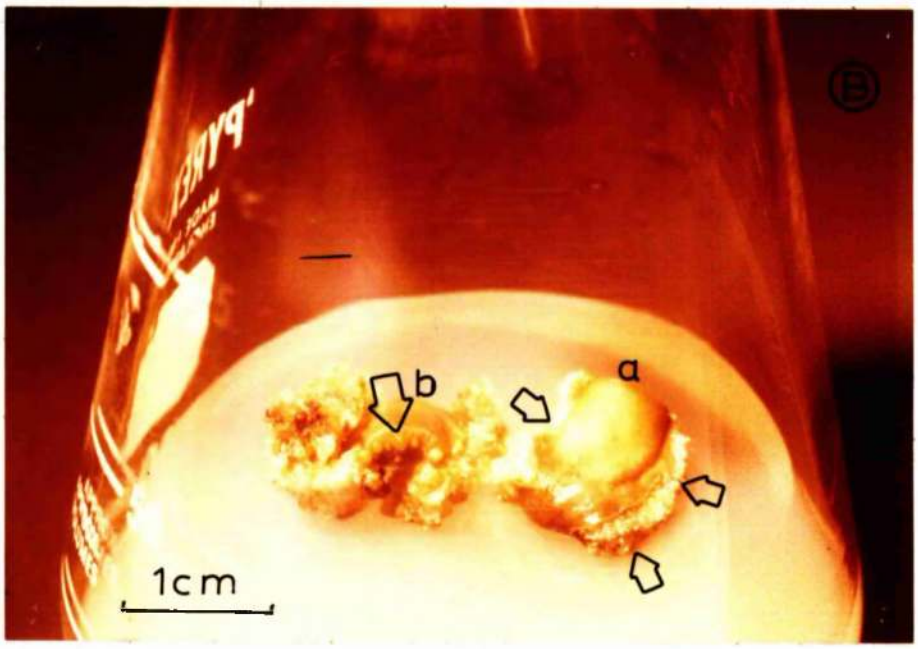
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P. vulgaris

Plate 8A: Day 10 in vitro cultured cotyledons; here the plastids in the abaxial sub-epidermal cells have developed green pigmentation but the newly-formed callus lacks chloroplasts.
M: 2x

Plate 8B: Day 30 in vitro cultured cotyledons; the callus tissue has occupied most surface of the nutrient medium (which is now somewhat dried-out) and the callus tissue needs to be subcultured. The arrows indicate the abaxial surfaces of the cotyledons; in this case no green pigmentation has been developed.
M: 1.5x


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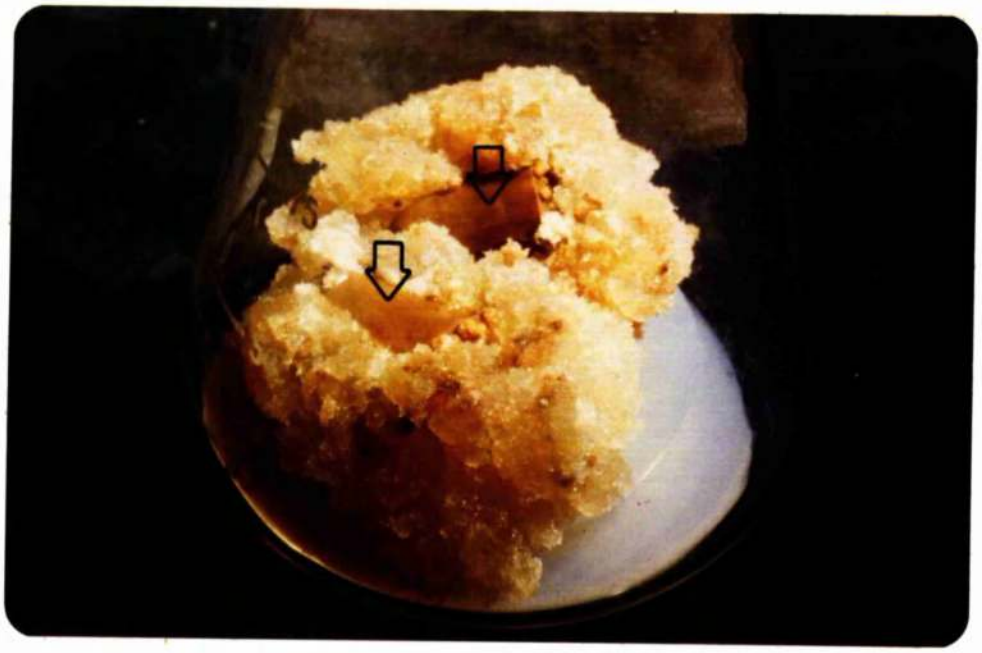

Plate 9A: LM (Day 1); TS through the margins of a cotyledon showing the adaxial surface at the base; note the existence of a unilayered sub-epidermis on the abaxial side (small arrows). Several small provascular strands (large arrows) are scattered between the large storage cells which contain conspicuous starch grains (these are the white "holes" in the micrograph). M: 100x

Plate 9B: LM (Day 3) of *in vitro* cultured, dedifferentiating storage cells. These have been sub-divided by freely-growing walls and several of these walls (small arrows) remain incomplete. Note also the large nuclei with prominent nucleoli; the darkly stained deposits inside the vacuoles (large arrows) are considered to be remains of the protein bodies. M: 650x

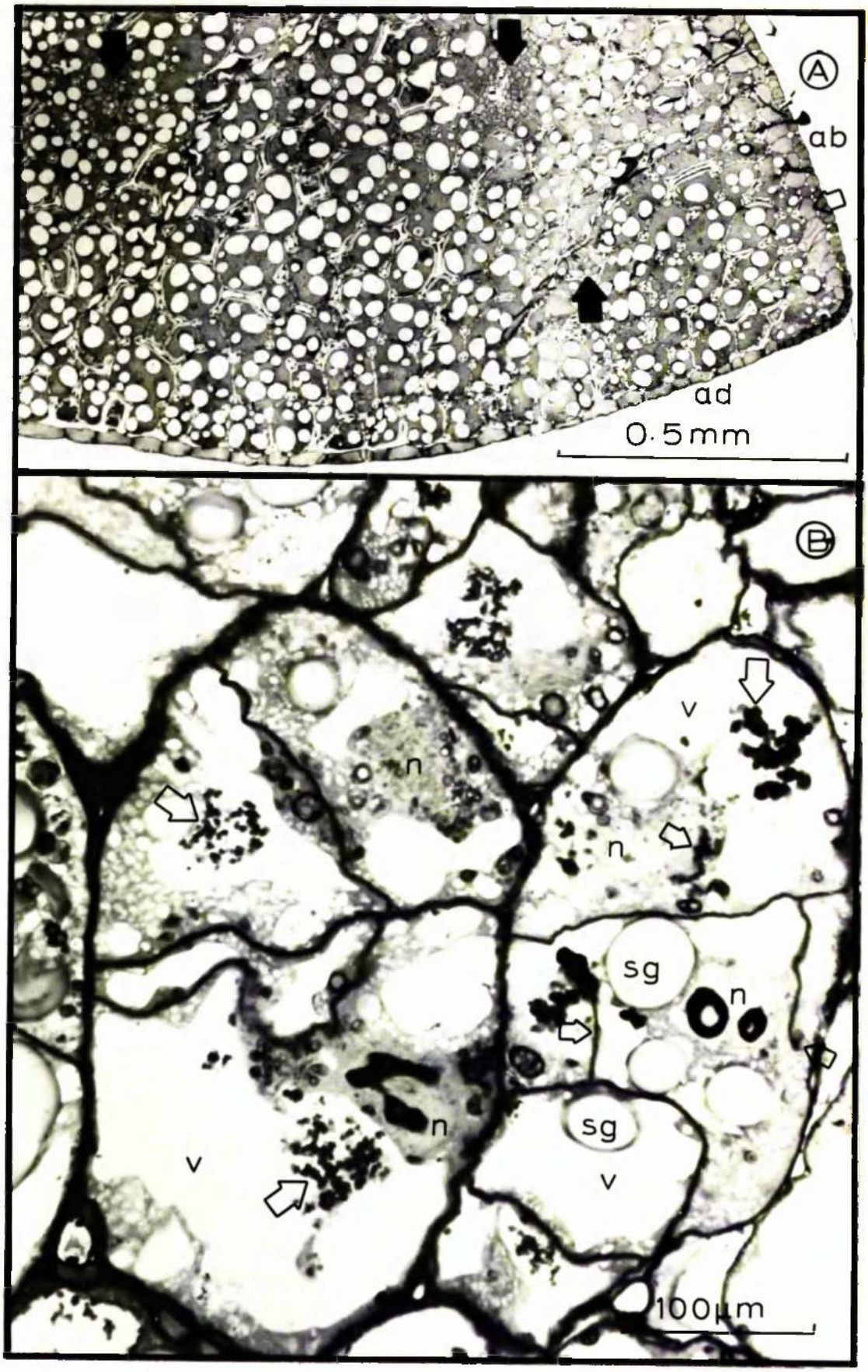
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Plate 10: LM (Day 3); TS through the adaxial margins of an *in vitro* cultured cotyledon. Note that the adaxial storage cells (apart from the sub-epidermal cells) have undergone considerable elongation and some have been sub-divided by freely-forming walls. The adaxial epidermis has collapsed but the adjacent layer of storage cells is still intact, although showing little enlargment from Day 1. Some of these cells (arrows) contain crystalline deposits in the protein bodies. The solid arrows indicate vascular bundles. M: 100x

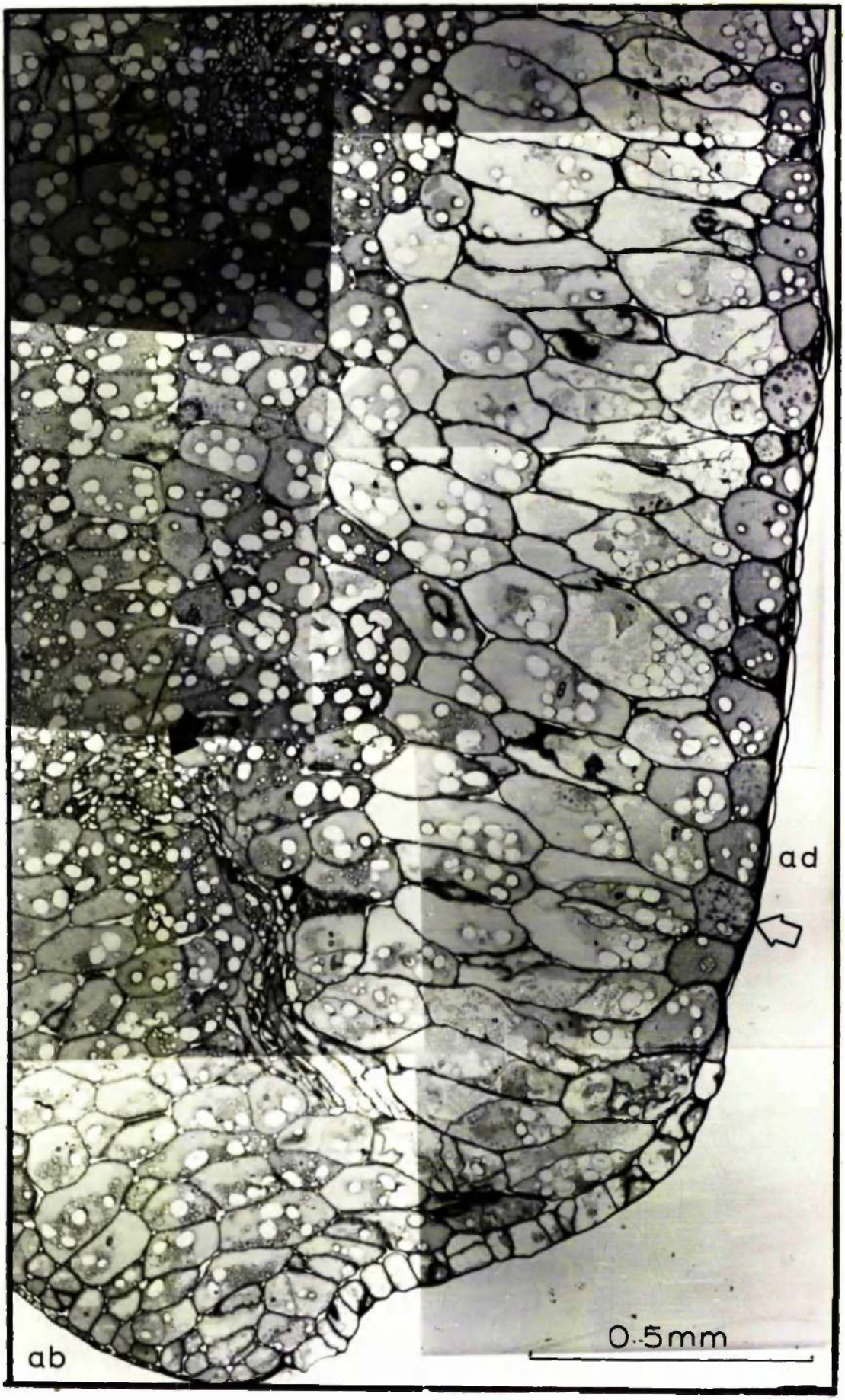
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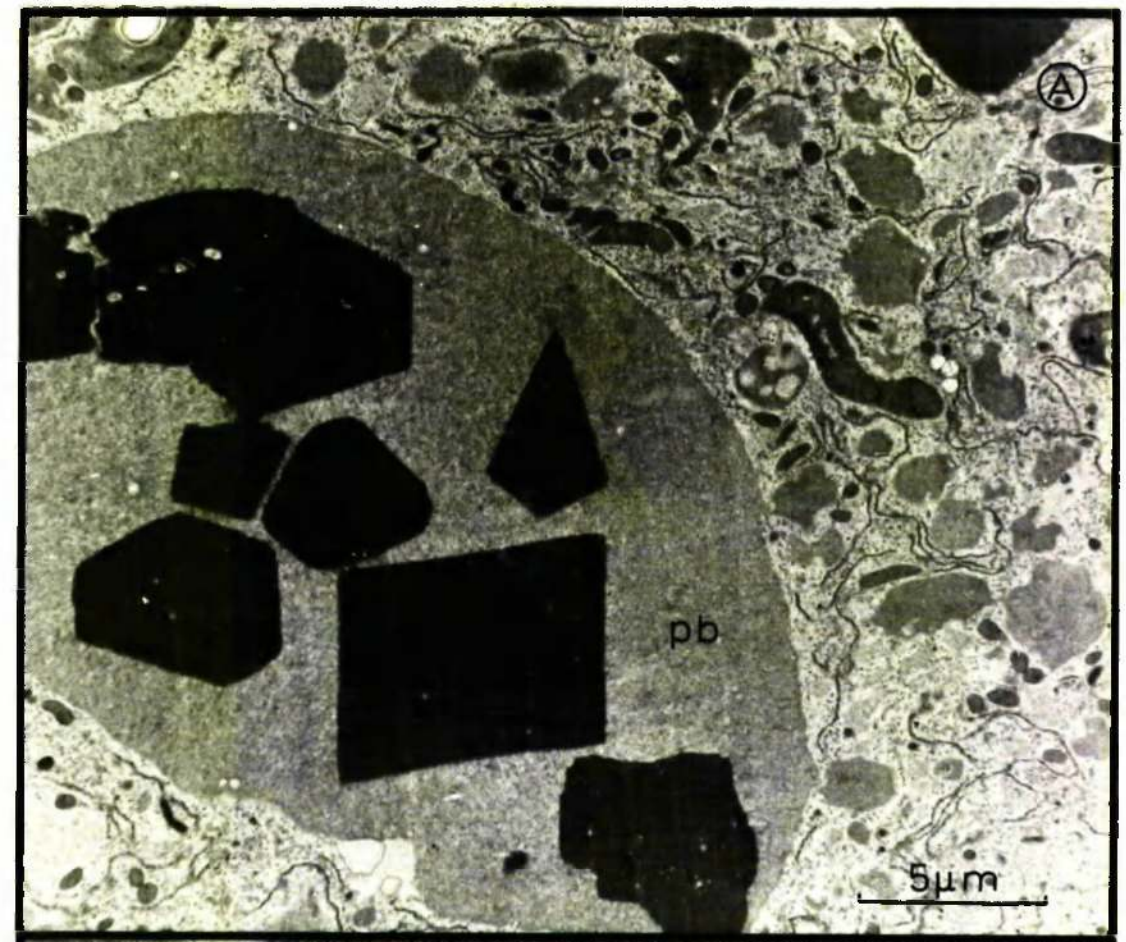
Plate 11A: TEM (Day 3) showing several protein crystals (△) in a protein body in a sub-epidermal storage cell of an *in vitro* cultured cotyledon. M: 5160x

Plate 11B: TEM (Day 3) of a protein crystal, such as in Plate 11A, illustrating the fine structure of the crystal lattice, the arrows indicate the margins of the protein body. M: 44000x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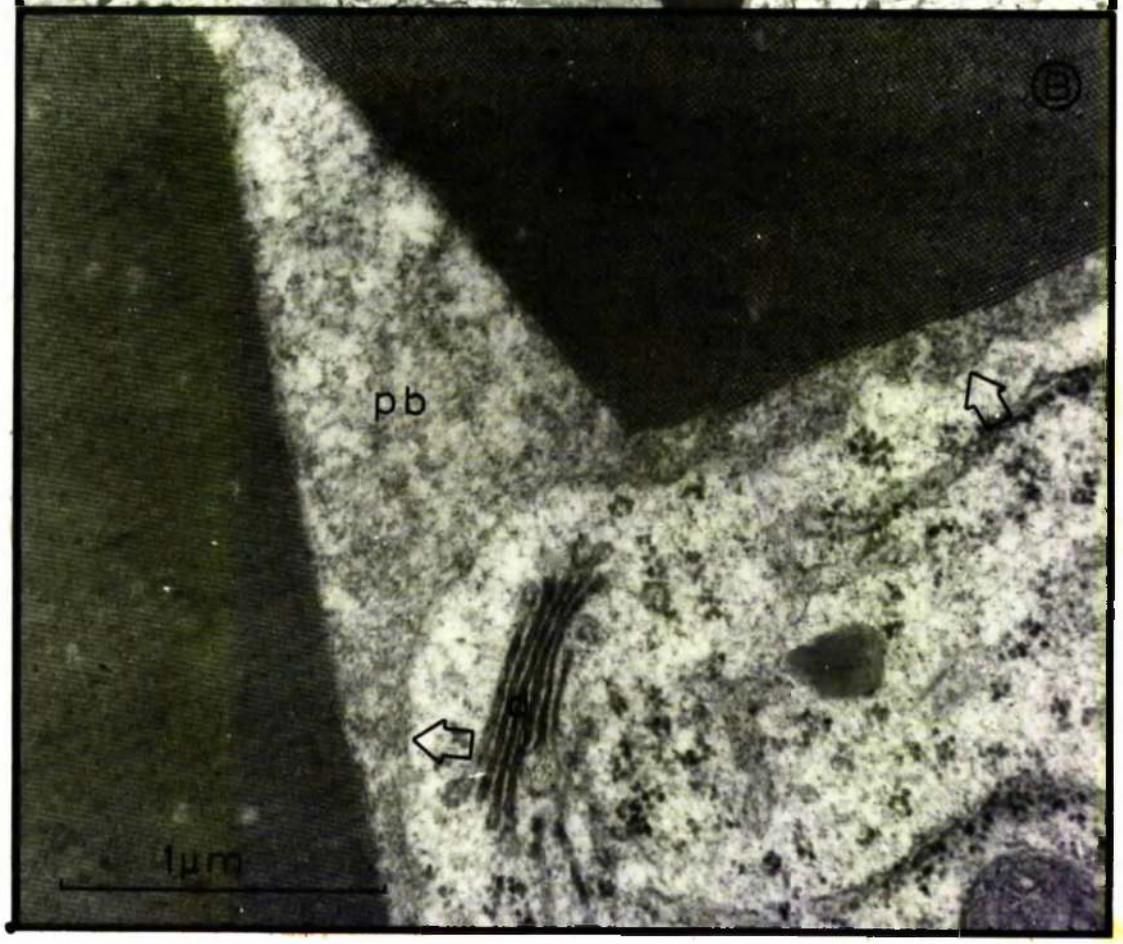
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Plate 12A: LM (Day 5) of a dedifferentiating in vitro cultured storage cell; this cell has already divided into six daughter cells and the new cell walls meet in a central, massive wall body; also note the isolated freely growing cell walls (arrows). M: 800x

Plate 12B: TEM (Day 5) showing freely-forming cell walls in a dedifferentiating in vitro cultured storage cell (similar to the one shown in Pl. 12A); note the presence of numerous profiles of endoplasmic reticulum which at places seem to be associated with the cell walls (small arrows); the large arrows indicate the very thin areas of the developing wall (see also Pl. 12C). M: 5160x

Plate 12C: TEM (Day 5) of a part of a freely-growing cell wall in an in vitro cultured storage cell; note its vesicular structure; the arrows indicate the sites where the endoplasmic reticulum is closely associated with the developing cell wall. M: 10750x

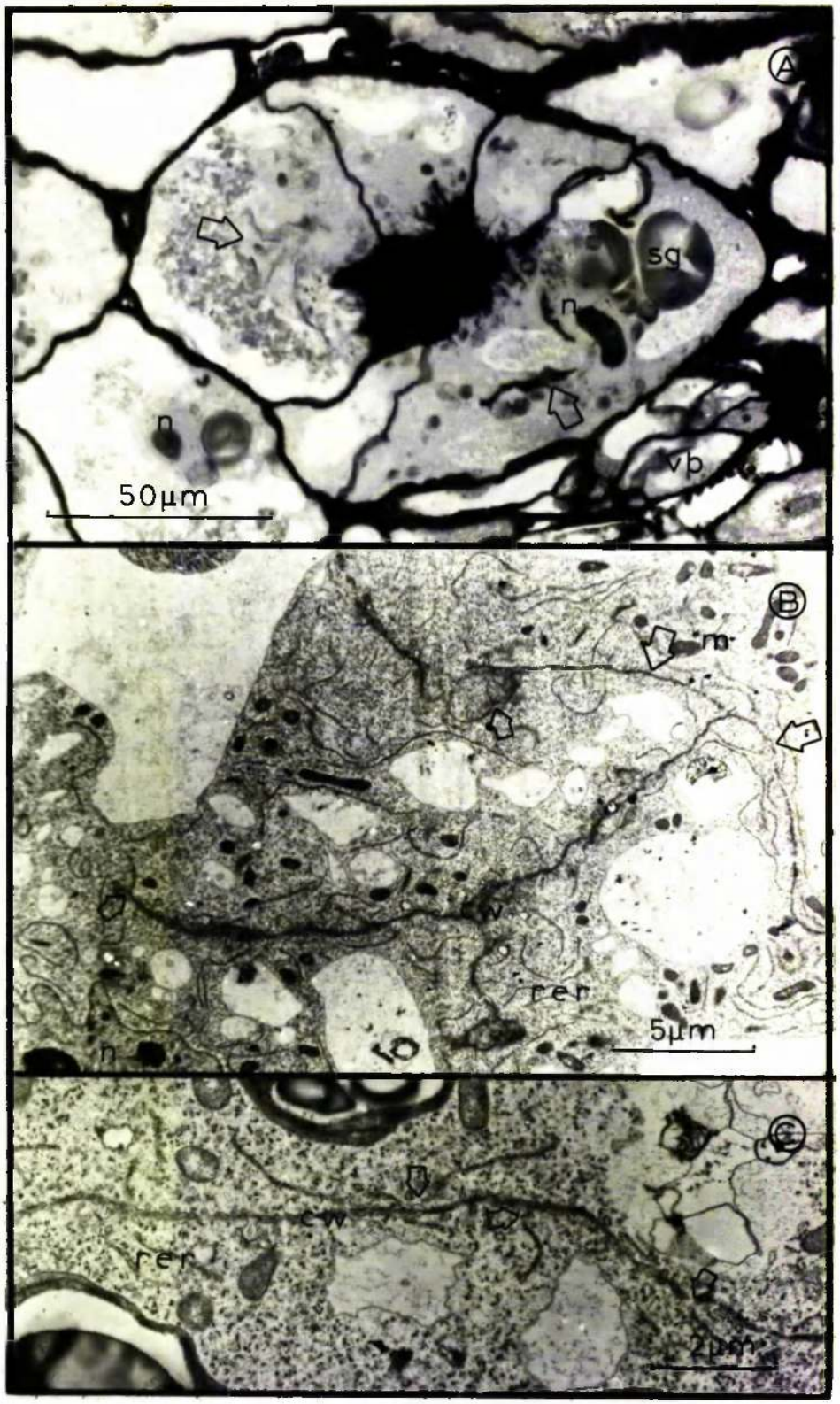
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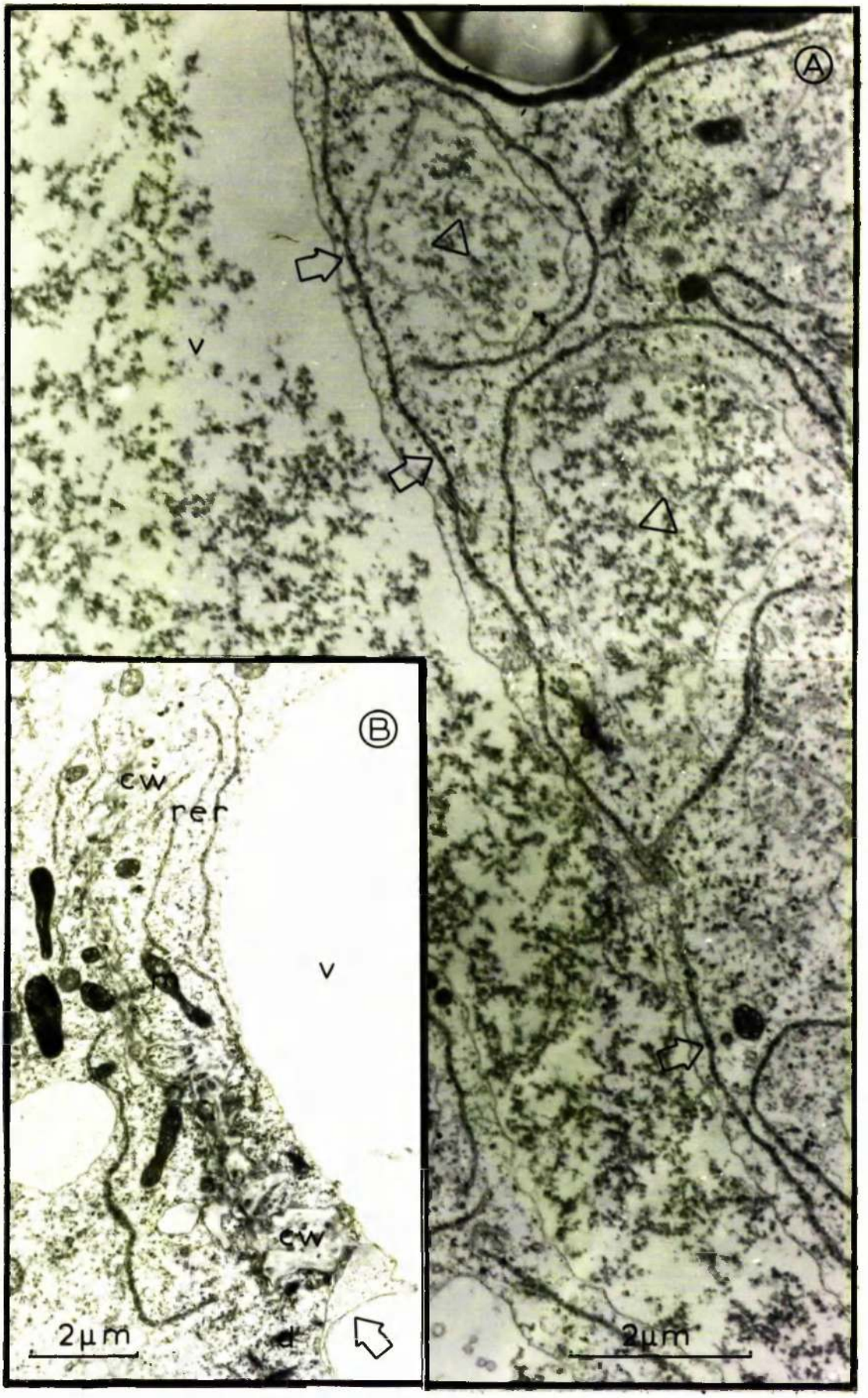
Plate 13A: TEM of an in vitro cultured dedifferentiating storage cell, showing rough endoplasmic reticulum (large arrows) possibly demarcating sites of new cell walls; also note the cisternae (△) formed by the endoplasmic reticulum elements which seem to represent either early stages of lysosomes or protein body vacuoles. M: 15800x

Plate 13B: TEM of an in vitro cultured storage cell showing the freely-growing end (arrow) of a cell wall which is protruding into a vacuole; this may indicate the location of a future cytoplasmic strand in which the freely-growing wall would traverse the vacuole. M: 10000x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cw
2µm

Plate 14A: TEM showing higher resolution detail of Pl. 13B; note the lesser density of the growing end of the cell wall (△) and the labyrinthine outline of this cell wall. M: 28500x

Plate 14B: TEM showing free wall formation associated with an apparently fragmenting nucleus in an *in vitro* cultured storage cell; note the tip of the growing walls (arrows) ending in the vicinity of the fragmenting nucleus. Note also the cytoplasmic enclave which is considered to be a lysosome. The solid arrow indicates what appear to be some organelle remains after digestion has occurred. M: 12500x

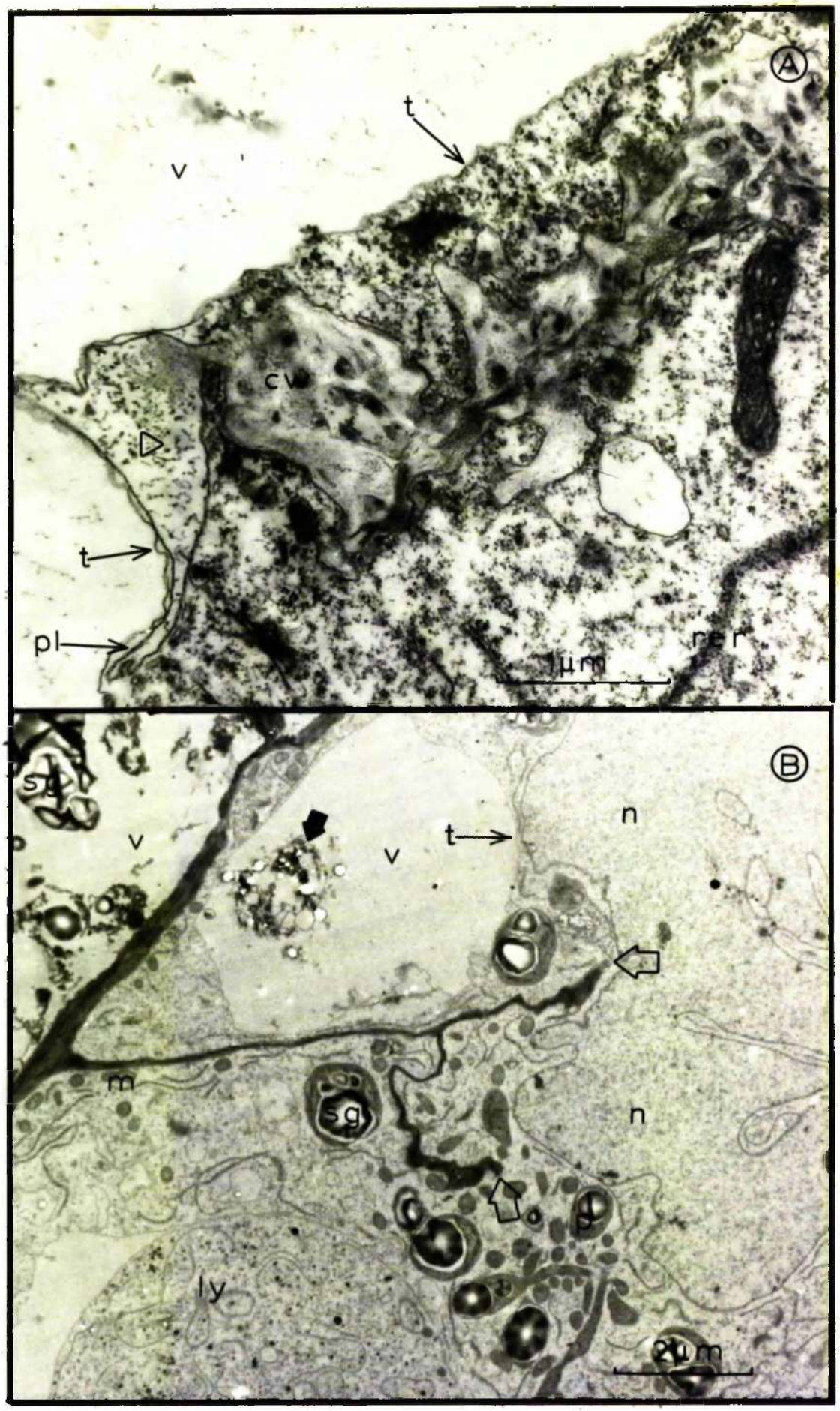

A
v
t
cv
t
pl
rer
1µm
B
st
v
v
t
n
m
sg
n
ly
2µm

Plate 15: TEM showing free cell wall formation associated with the nuclear fragmentation in an *in vitro* cultured storage cell. On either side of the isthmus (small arrows) connecting the larger and smaller lobe of the nucleus, tracts of freely-forming wall are visible (large arrows). Note also the numerous plastids in the cytoplasm; some of these are amyloplasts with multiple starch grains whilst clusters (△) of proplestids also occur. The solid arrows indicate what appears to be bell-shaped mitochondria cut at various angles. M: 5160x

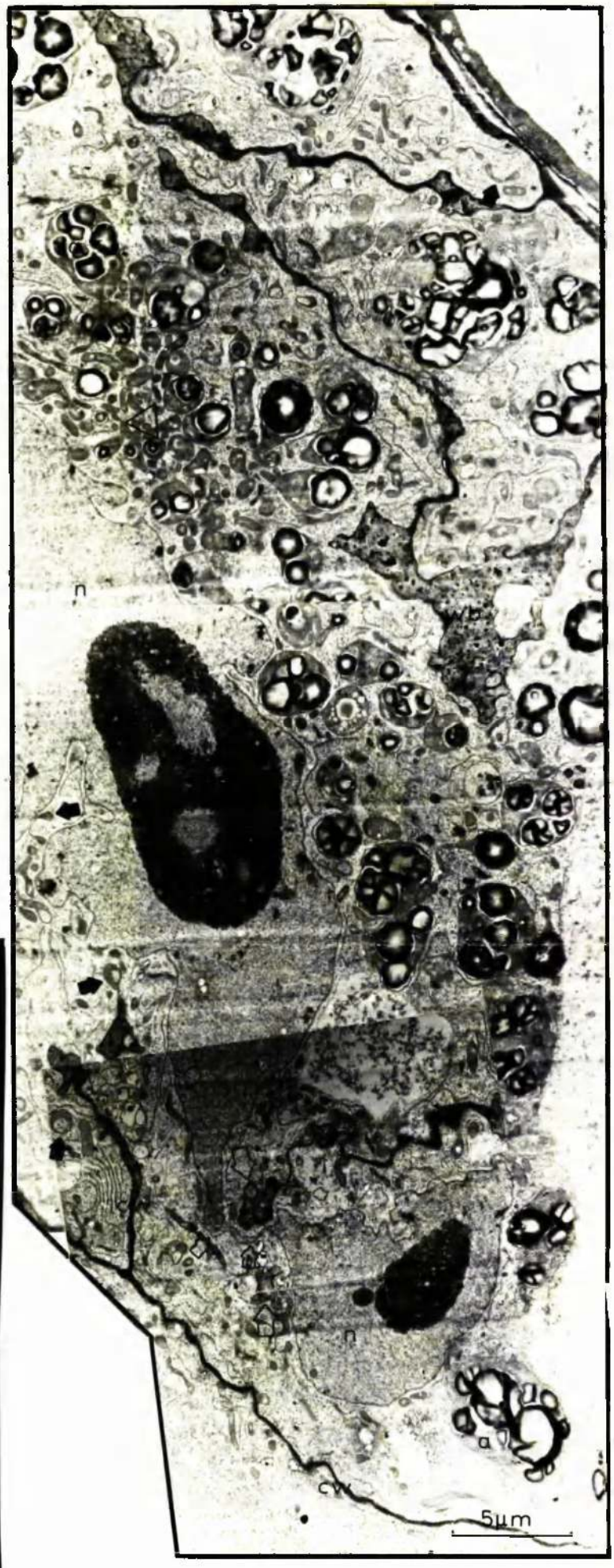

n
Wb
n
CW
5µm

Plate 16A: LM (Day 5); TS of an *in vitro* developing cotyledon showing a number of sub-divided storage cells, wall bodies (large arrows), freely-growing cell walls (small arrows) and a mitotic division (solid arrow). M: 220x

Plate 16B: LM from an *in vitro* cultured storage cell illustrating the labyrinthine structure of a wall body; the arrows show projections into the cytoplasm. M: 1000x

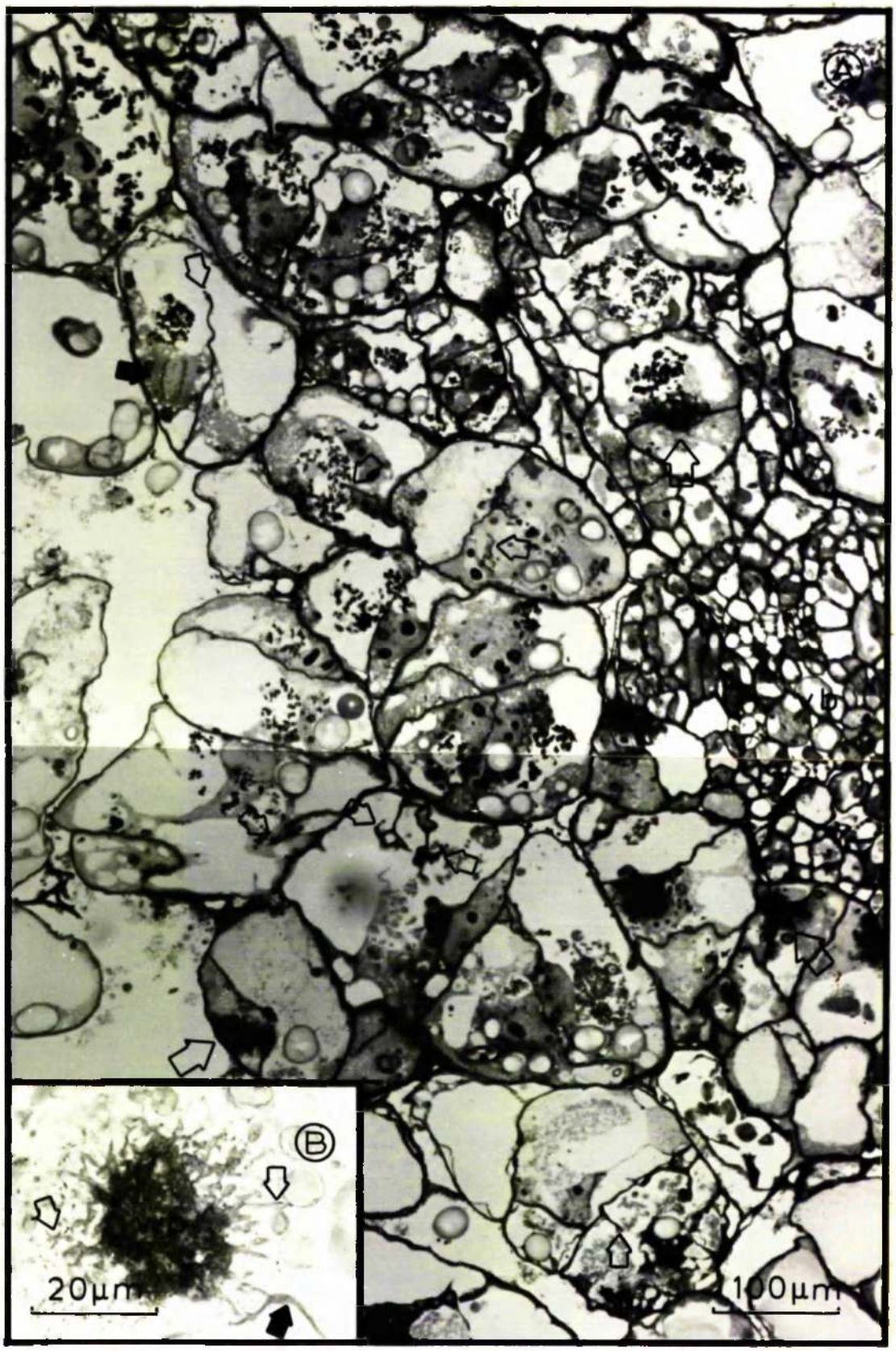

A
B
20µm
100µm

Plate 17: TEM (Day 3) of an *in vitro* storage cell showing part of a developing wall body. The arrows indicate parts of the cytoplasm entrapped inside the wall body and within these areas membranous structures can be seen. Note the presence of endoplasmic reticulum elements, mitochondria and dictyosomes with associated vesicles. M: 16500x


wb
rer
d
wb
m
d
1µm

P. vulgaris

Plate 18A-B: TEM's showing parts of the margins of a developing wall body from a Day 3 *in vitro* cultured storage cell. Dictyosomes have straight or slightly curved cisternae with vesicles attached to them whilst numerous vesicles can also be seen free in the cytoplasm (small arrows) or attached to the wall body (large arrows). The solid arrows show some connections between endoplasmic reticulum and wall bodies. Note also the presence of polyribosomes either in clusters or helices. M: 27500x

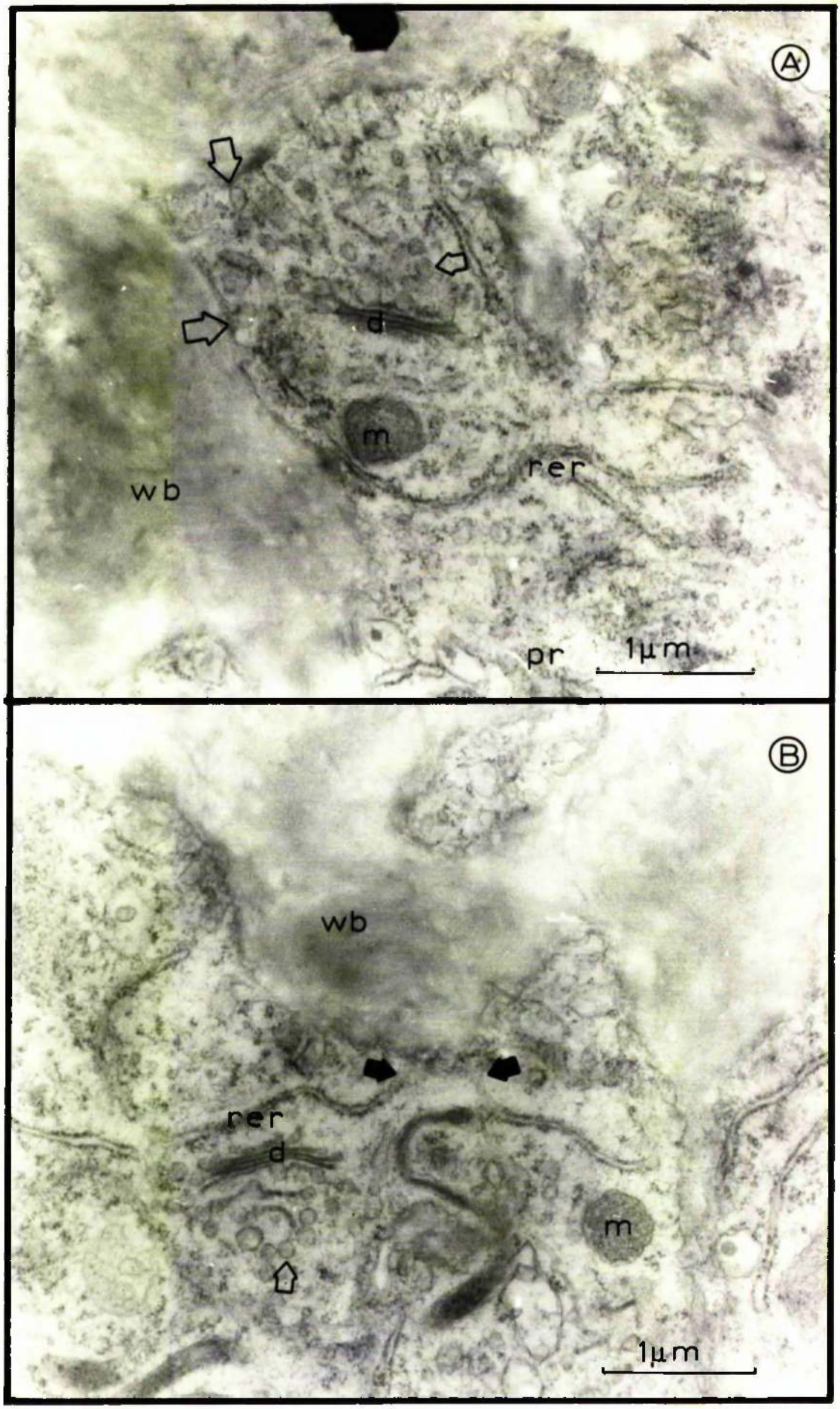
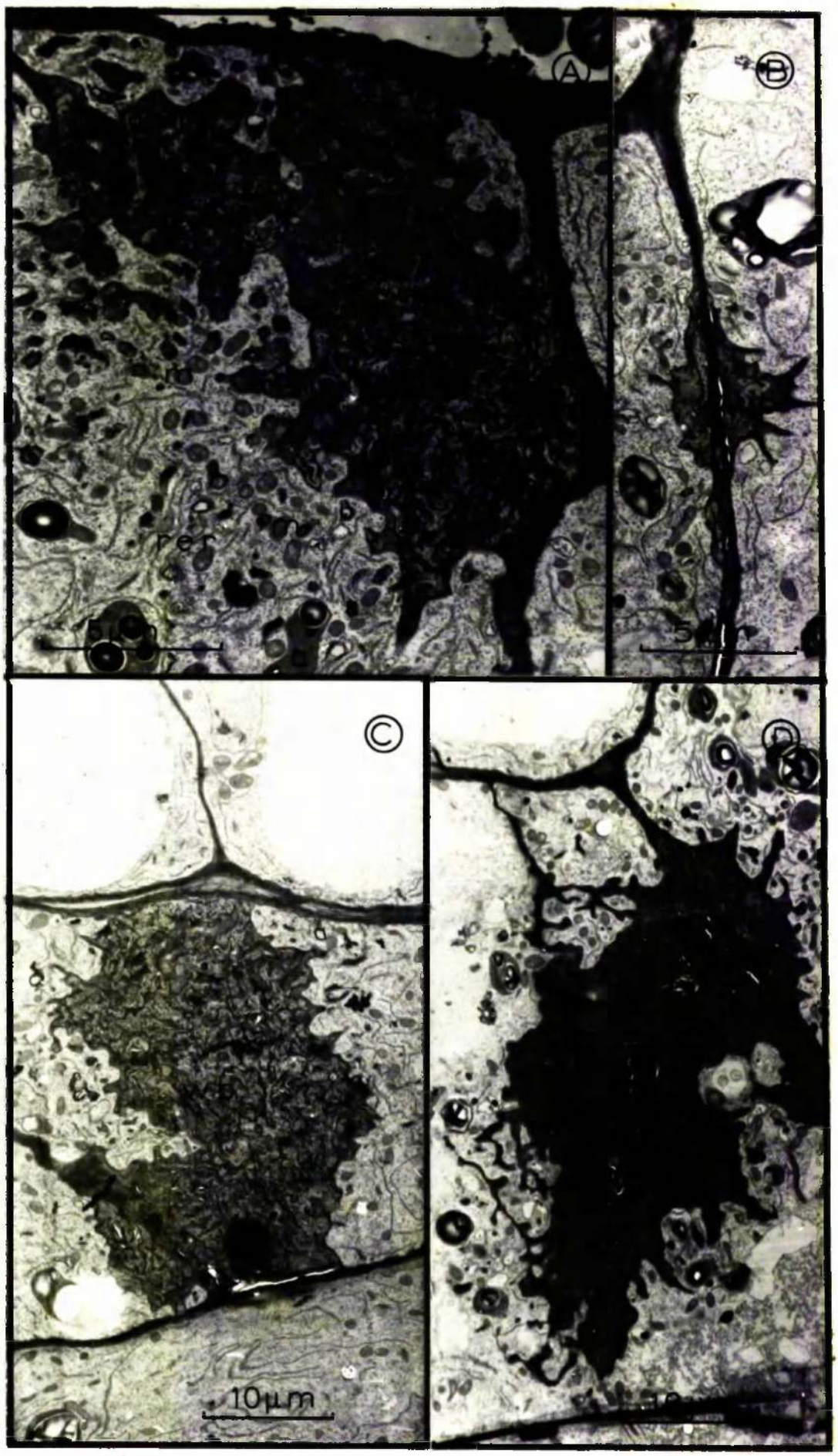

A
d
m
wb
rer
pr
1µm
B
wb
rer
d
m
1µm

Plate 19: TEM's (Day 3-7) showing a selection of labyrinthine wall bodies observed in the dense cytoplasm of dedifferentiating storage cells of *in vitro* cultured cotyledons.. M: A: 6500x

B: 5000x

C: 2750x

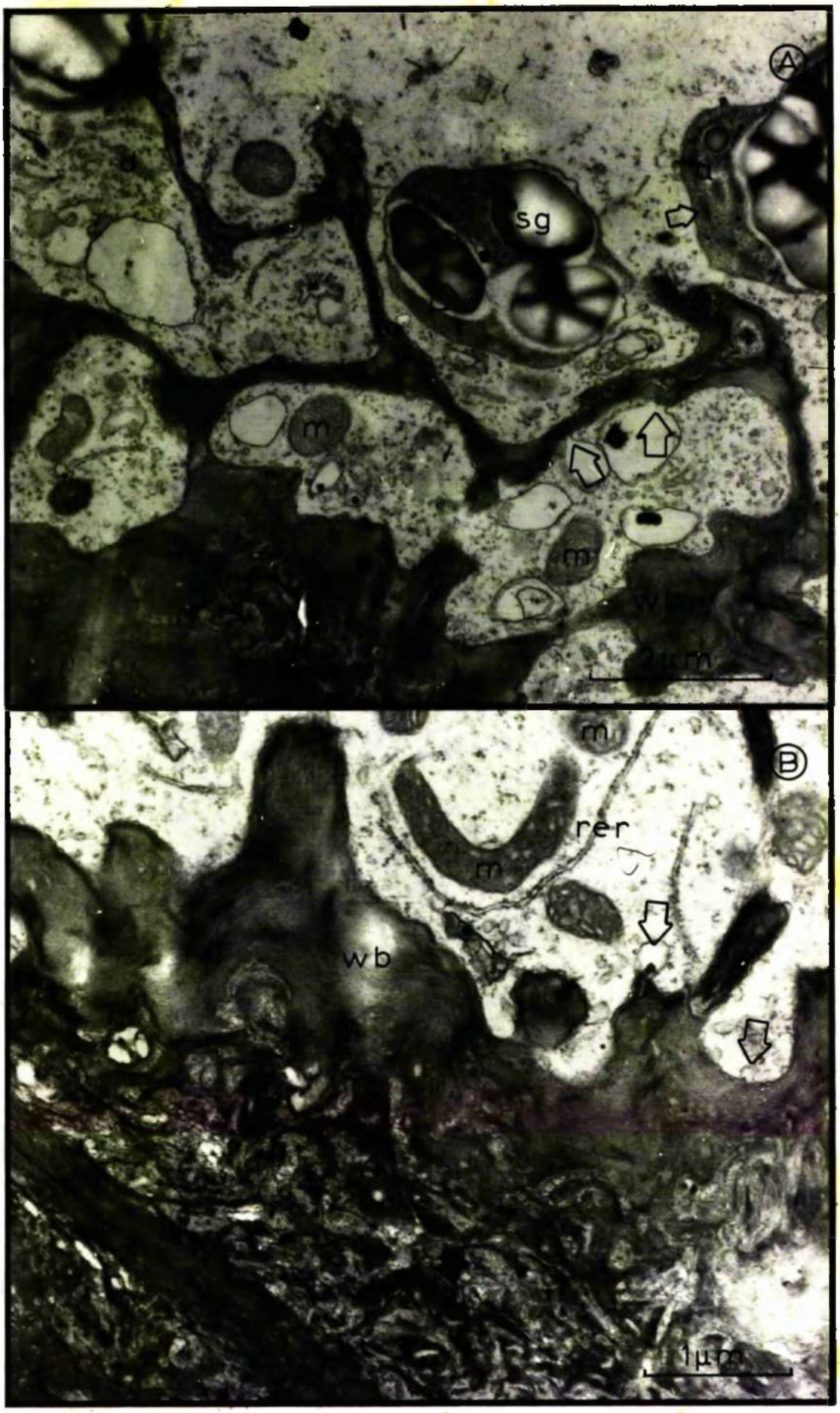
D: 2750x


A
B
C
D
10µm

P. vulgaris

Plate 20A: TEM showing part of the periphery of a wall body from an *in vitro* cultured storage cell; note the vesicles (large arrows), some containing electron dense inclusions, attached to its margins. The small arrows indicate thylakoids in the amyloplasts. M: 15600x

Plate 20B: TEM showing further detail of a wall body from an *in vitro* cultured storage cell. Note the variability of its appearance (c.f. Pl. 19A and C), ranging from a uniform fibrillar structure to a denser flecked region, and the vesicles (large arrows) attached to the margins. M: 27300x


A
sg
m
m
1µm
B
m
rer
m
wb
1µm

P. vulgaris

Plate 21A: TEM showing detail of the projecting margin of a labyrinthine wall body from an in vitro cultured storage cell. Note the rough endoplasmic reticulum elements associated with the wall body (arrows), and the presence of electron translucent vesicles (△), sometimes containing electron dense particles, which are found either attached to the wall body or free in the cytoplasm. M: 23200x

Plate 21B: TEM of a wall body from an in vitro cultured storage cell. The margins of the wall body show some attached vesicles (arrows); note the presence of apparently smooth endoplasmic reticulum in the vicinity of this wall body. M: 21000x

Plate 21C: TEM from an in vitro cultured storage cell showing a cluster of plastids. Such clusters are observed only in the in vitro dedifferentiating storage cells. M: 6450x

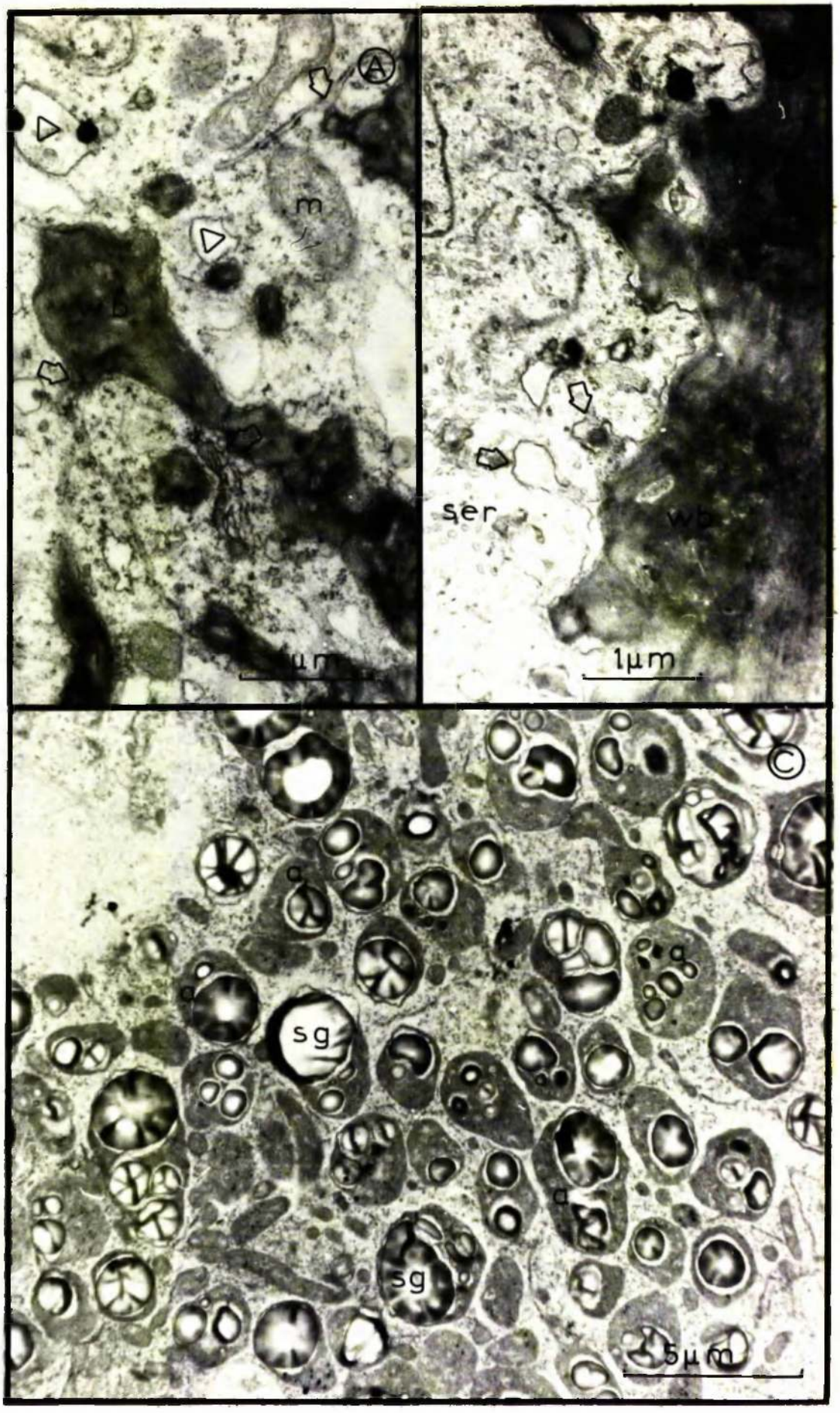
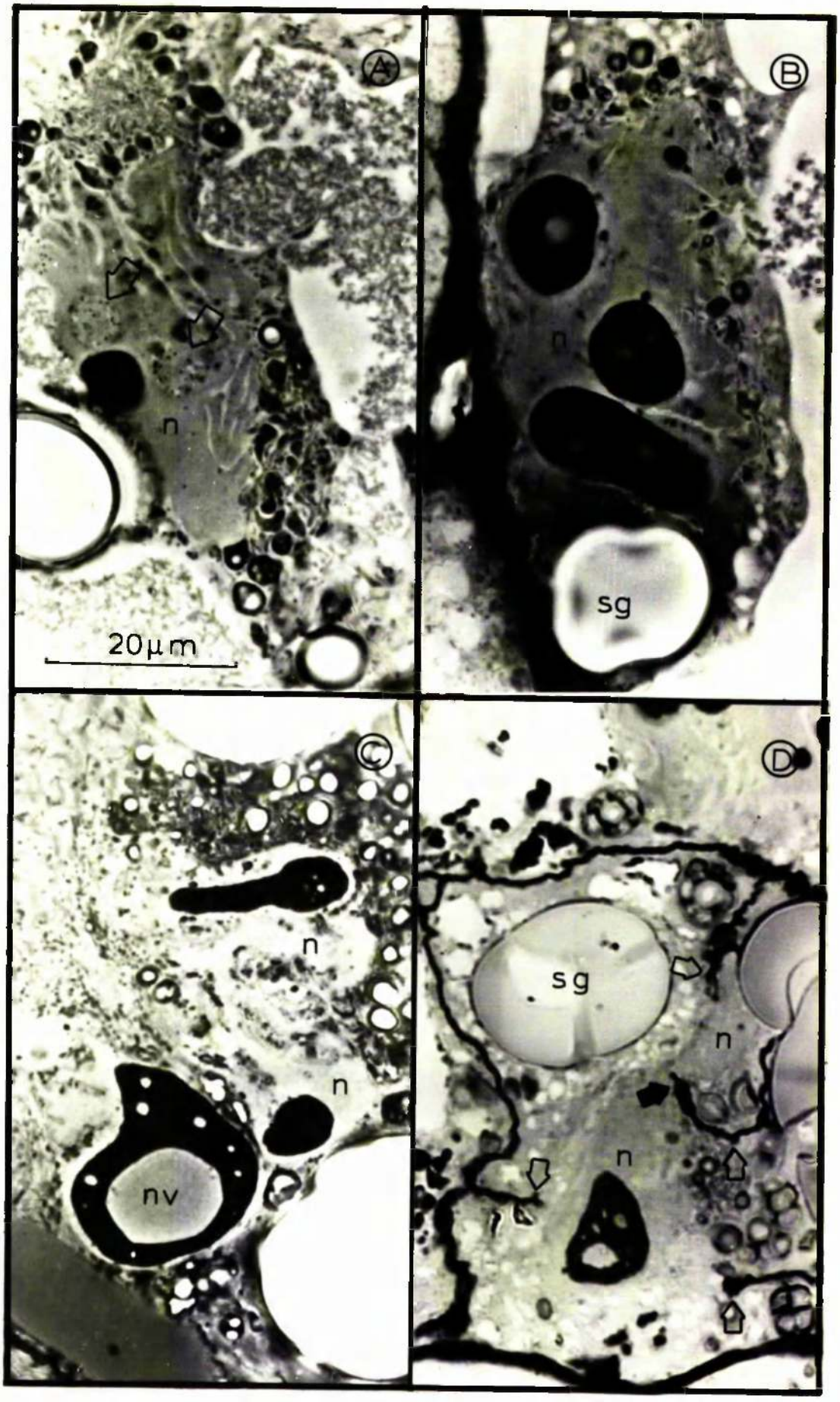

A
m
wb
1µm
ser
wb
1µm
C
a
a
a
a
sg
sg
5µm

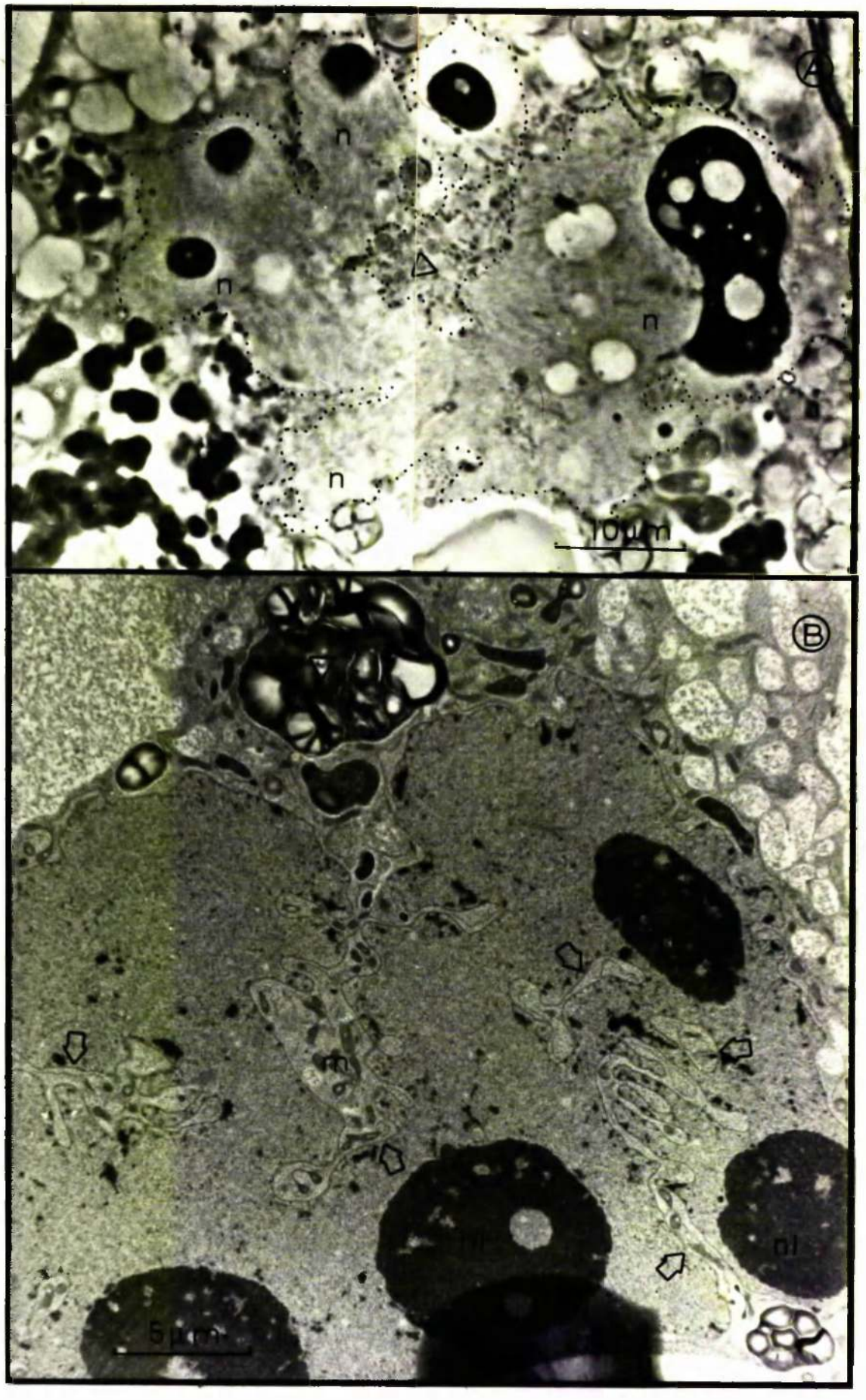
Plate 22A-D: LM's (Day 5) illustrating different stages of nuclear and nucleolar fragmentation in storage cells of the *in vitro* cultured cotyledon. Note the cytoplasmic enclaves (Pl. A, arrows) and complexity of the nuclear margins; the size and shape of the multiple nucleoli, which often contain vacuoles (Pl. B-D); and the freely-growing cell walls (Pl. D, arrows) in relation to the nuclear fragmentation. The solid arrow (Pl. D) shows a freely-growing cell wall with its growing end against a nuclear constriction. In all illustrations clusters of small plastids occur adjacent to the nuclei. M: All 1600x


A
n
20µm
B
n
sg
C
n
n
nv
D
sg
n
n

P. vulgaris

Plate 23A: LM (Day 5) illustrating fragmentation of a multinucleolate nucleus (the nuclear envelope is indicated by the dotted outline) occuring in in vitro cultured storage cell. Within the nucleus a cytoplasmic enclave (△) is visible. M: 2200x

Plate 23B: TEM (Day 5) from an in vitro cultured storage cell illustrating fragmentation of a multinucleolate nucleus (c.f. Pl. 23A). Note the amyloplast (△) with multiple starch grains, whilst there is an accumulation of mitochondria inside the cytoplasmic channels penetrating the nucleus. The arrows indicate the nuclear envelope. M: 5160x


A
n
n
n
10μm
B
nl
nl
5μm

P. vulgaris

Plate 24A: TEM from an *in vitro* cultured storage cell showing the fine-structure of the nuclear lobes (detail of Pl. 31A); note the numerous pores in the nuclear envelope (large arrows) and the elements of the rough endoplasmic reticulum in which localised swelling within their lumens (small arrows) are observed. M: 13000x

Plate 24B: TEM of a late stage of nuclear fragmentation from an *in vitro* cultured storage cell; the two nuclei are only connected (in this plane of section) by an isthmus (arrow). Note also the presence of numerous mitochondria in the cytoplasmic invaginations. M: 7200x

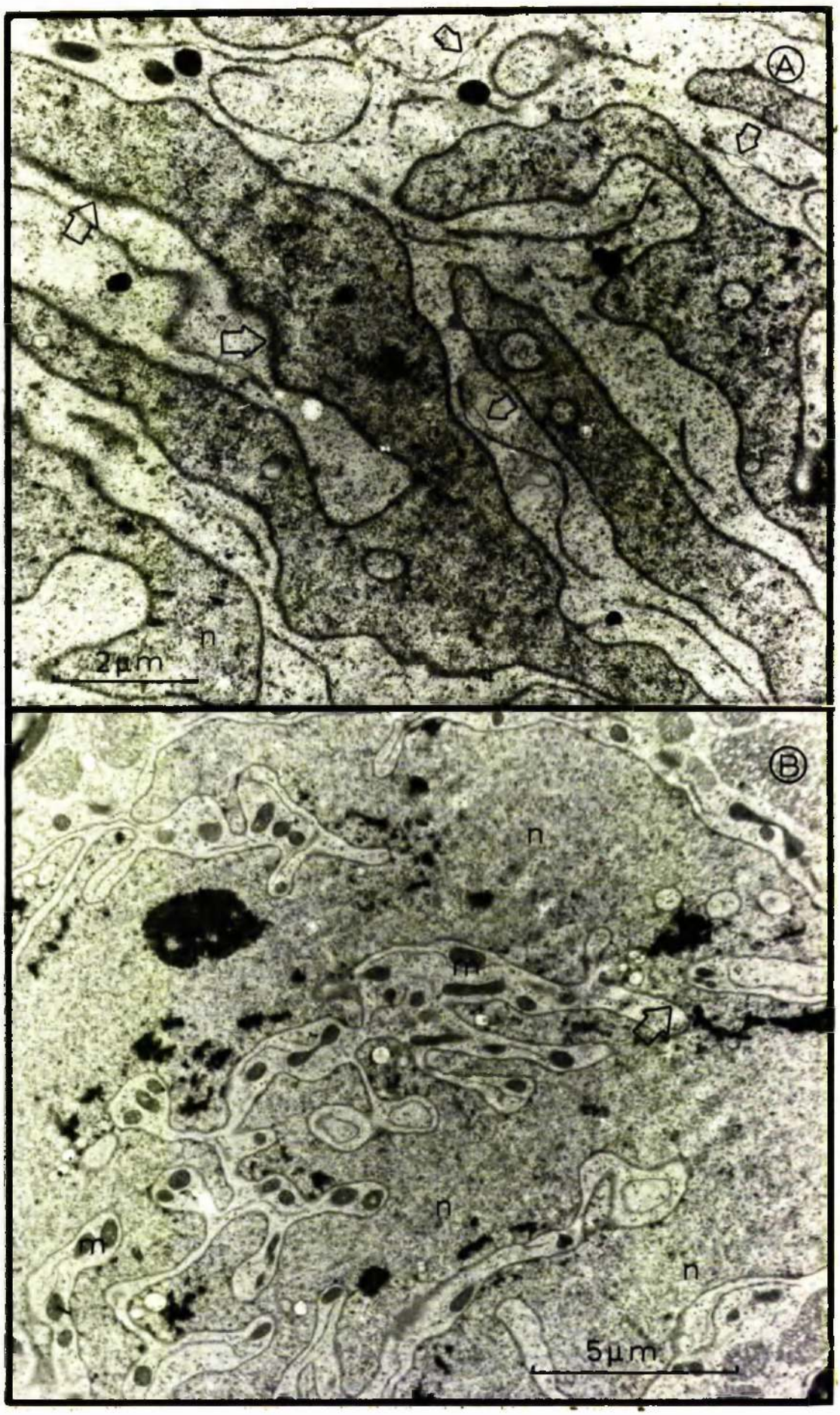
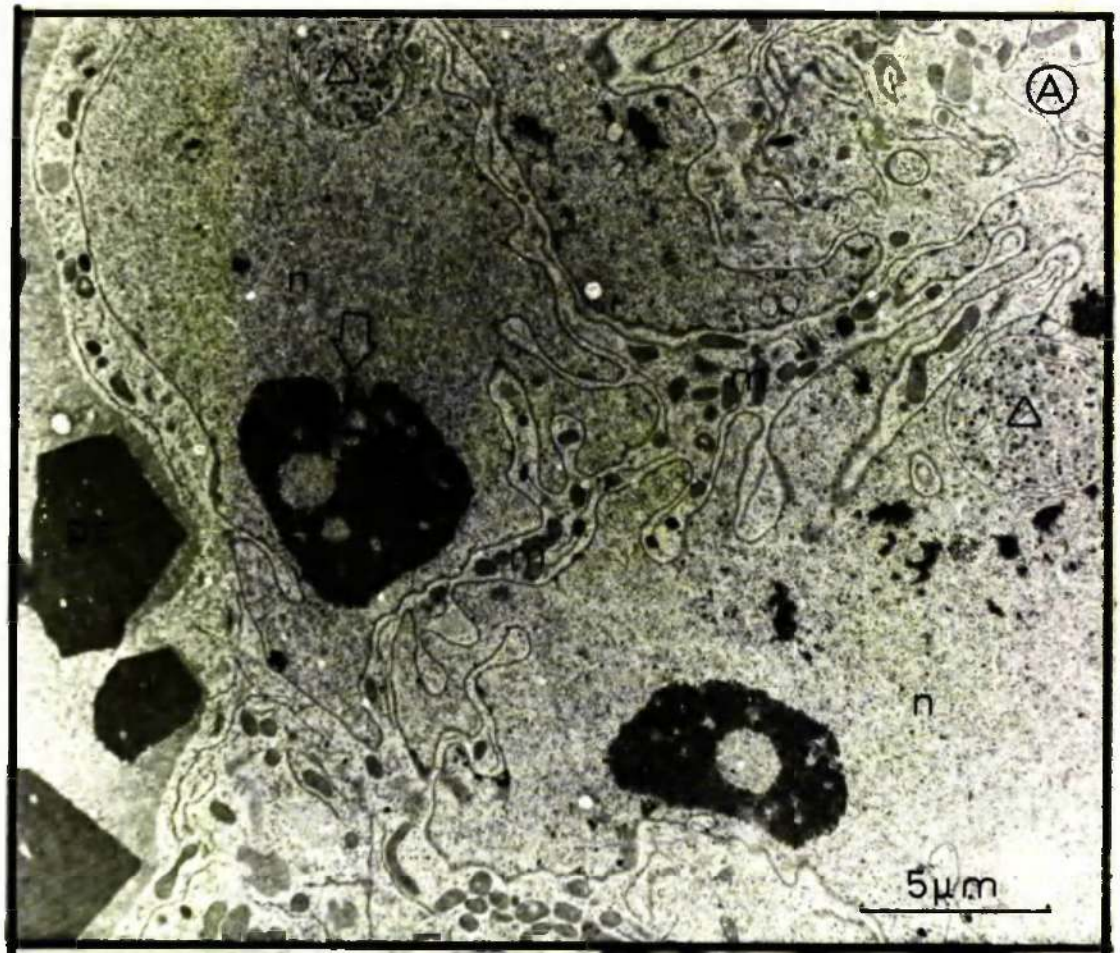
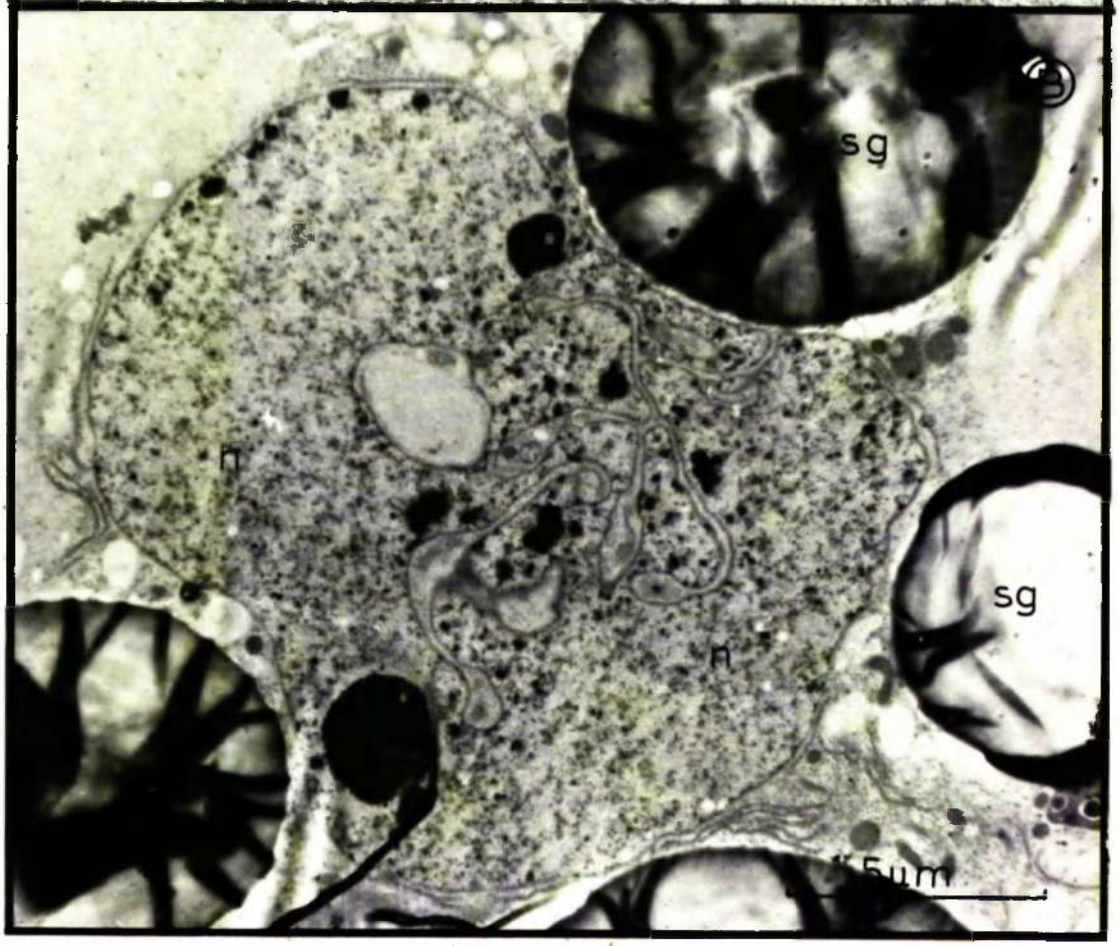

A
n
2µm
B
n
m
n
m
n
5µm

Plate 25A: TEM (Day 5) showing nuclear fragmentation in a sub-epidermal storage cell of an *in vitro* cultured cotyledon. Note the presence of cytoplasmic enclaves (△) within the nuclei and the protein crystals in the protein bodies. The arrow indicates a nucleoplasmic channel penetrating the nucleolus. M: 5160x

Plate 25B: TEM (Day 5) showing a nucleus from an *in vivo* (germinating) cotyledon storage cell. Note that the nuclear outline is less complex than in the comparable nucleus from an *in vitro* grown storage cell and that the nucleolus is relatively small. Few organelles (except for large starch grains) occur in the surrounding cytoplasm. M: 7000x


A
n
n
5µm


B
sg
n
sg
n
5µm

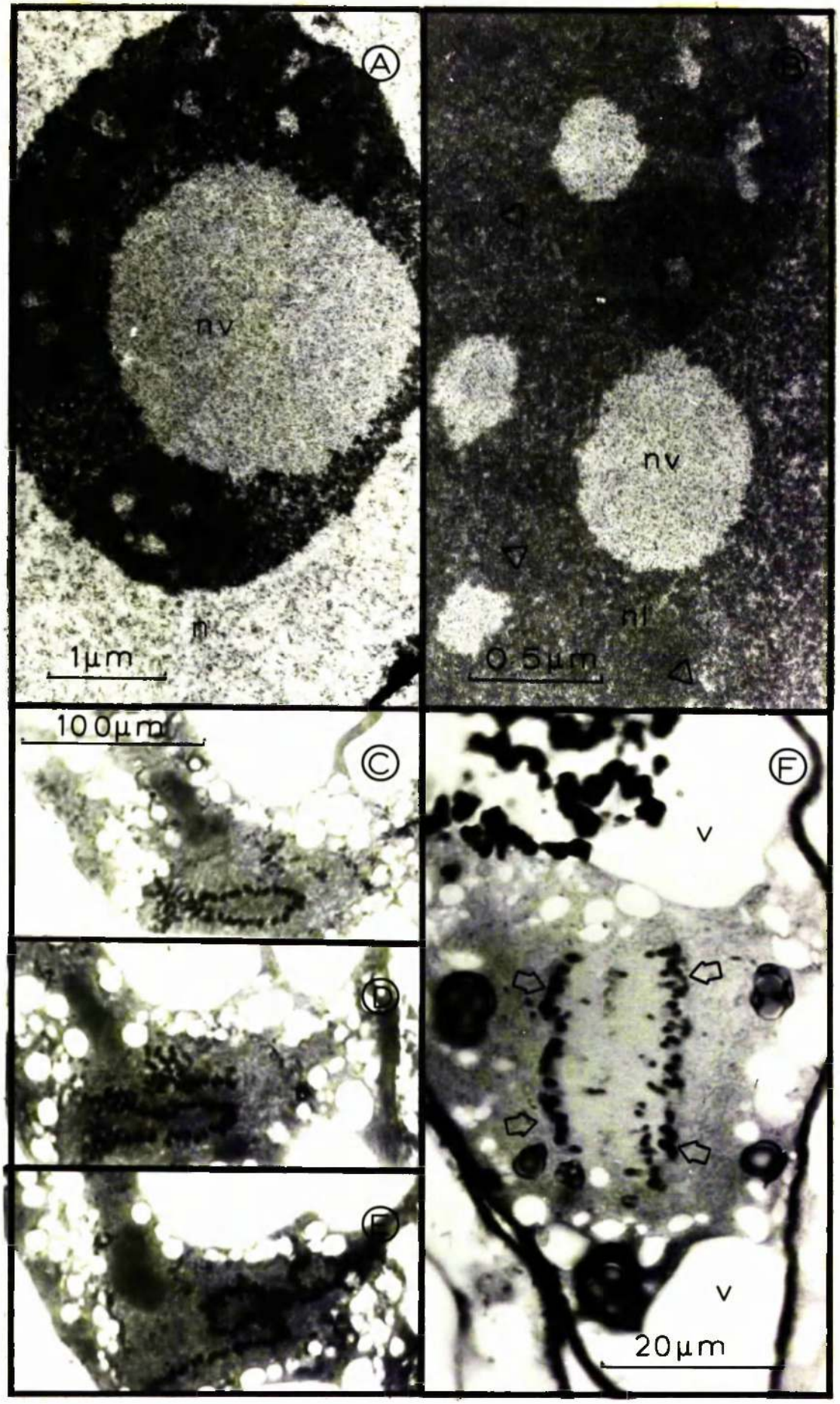
P. vulgaris

Plate 26A: TEM illustrating a highly vacuolated nucleolus from the fragmenting nucleus of a storage cell cultured in vitro. This shows the occurence of the granular (△) and so-called fibrillar zone (□). Note also the difference in density between the large nucleolar vacuole and the nucleoplasm. M: 21000x

Plate 26B: TEM showing higher resolution of the nucleolus from a fragmenting nucleus of an in vitro cultured storage cell. Note the differentiation into granular (△) and the so-called fibrillar zones (□); the nucleolar vacuoles also contain granular material. M: 50000x

Plate 26C-E: LM's (Day 5); serial sections of a metaphase stage in a mitotically dividing nucleus from an in vitro grown storage cell. Note the large number of chromatids present. M: 320x

Plate 26F: LM (detail of Pl. 16A) from an in vitro cultured storage cell illustrating a telophase in which a large number of chromosomes (arrows) are visible. M: 1600x


A
nv
n
1µm
B
nv
nl
0·5µm
100µm
C
D
E
F
v
v
20µm

P. vulgaris

Plate 27A: TEM from an in vitro cultured storage cell showing a metaphase plate in LS; note the kinetochore microtubules (large arrow) attached to one of the paired chromatids (see also Pl. 28A). Note also the newly-formed cell wall which shows secondary wall deposition in connection with the developing tracheary element on the right. M: 6000x

Plate 27B: LM from an in vitro cultured storage cell showing two newly-formed daughter nuclei with a cell plate (arrows) developing between them; each nucleus contains approximately 20 nucleoli. M: 1600x

Plate 27C: TEM from an in vitro cultured storage cell (c.f. Pl. 27B) showing two newly-formed daughter nuclei showing multiple nucleoli. Note the association of rough endoplasmic reticulum with the developing cell plate (arrows). M: 5040x

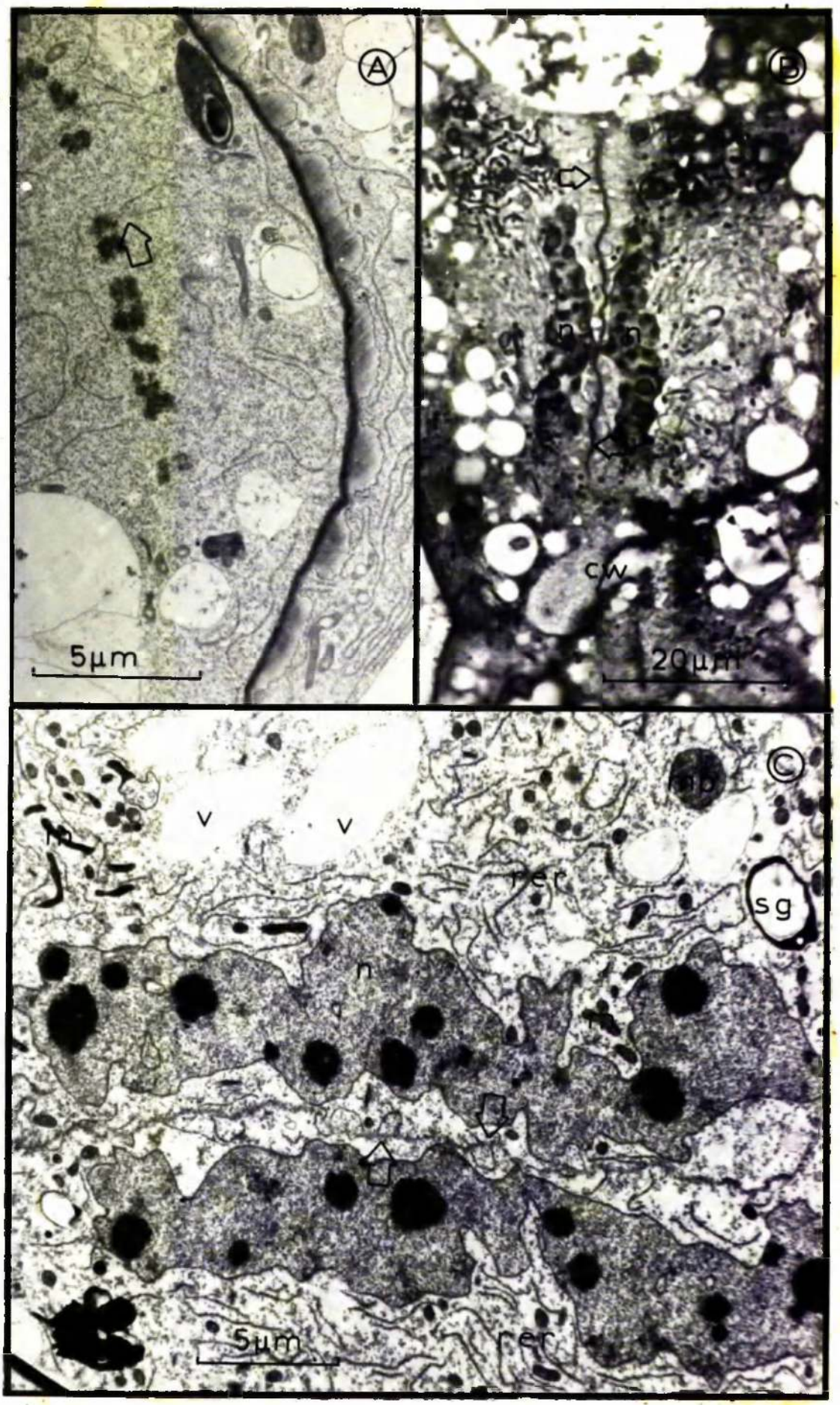
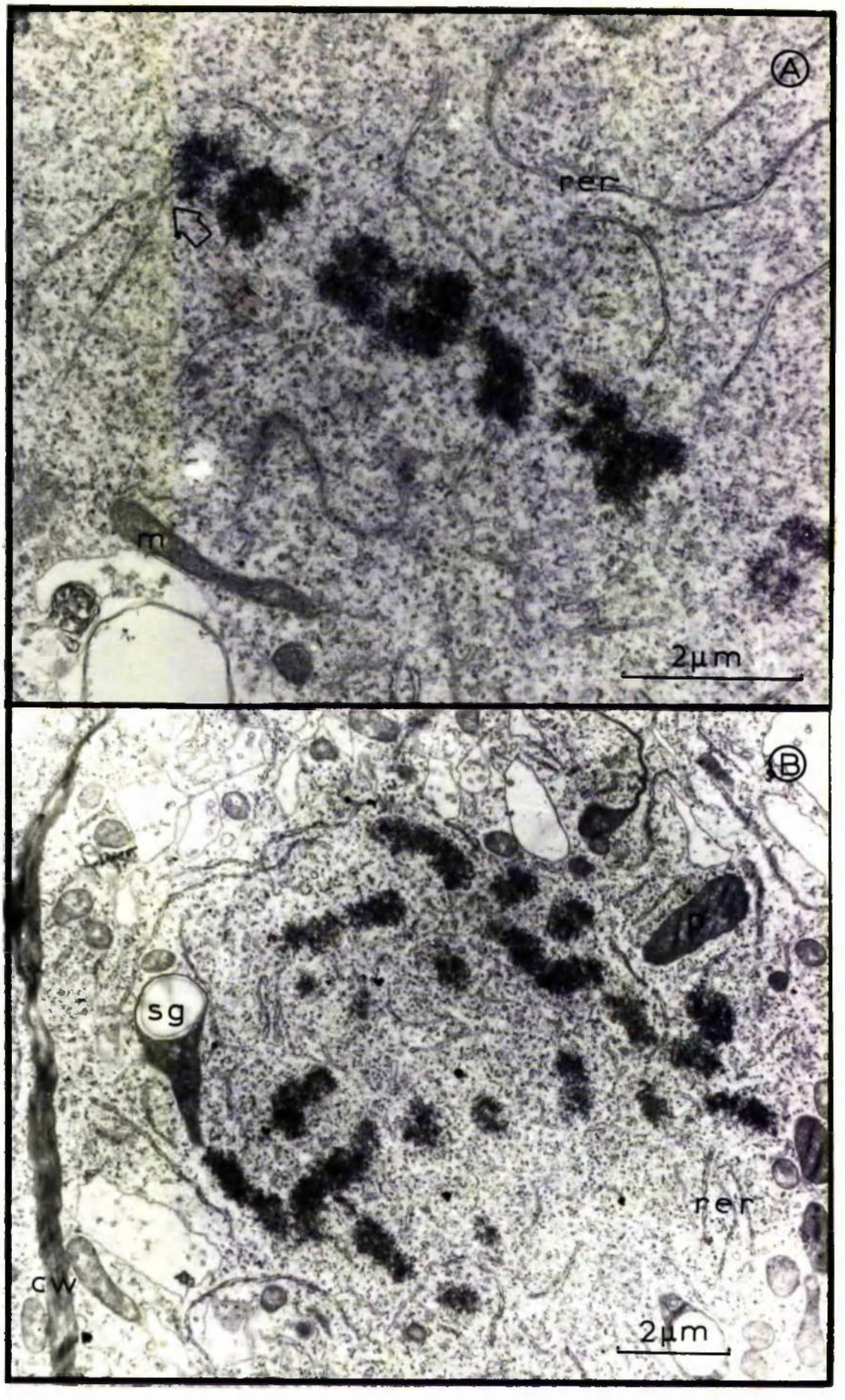

A
5µm
B
n
n
cw
20µm
C
v
v
mb
er
sg
n
5µm
rer

Plate 28A: TEM (detail of Pl. 27A) from an *in vitro* cultured storage cell showing the metaphase plate and two kinetochore microtubules (arrow) attached to a chromatide. Strands of rough endoplasmic reticulum and numerous ribosomes are also evident. M: 16200x

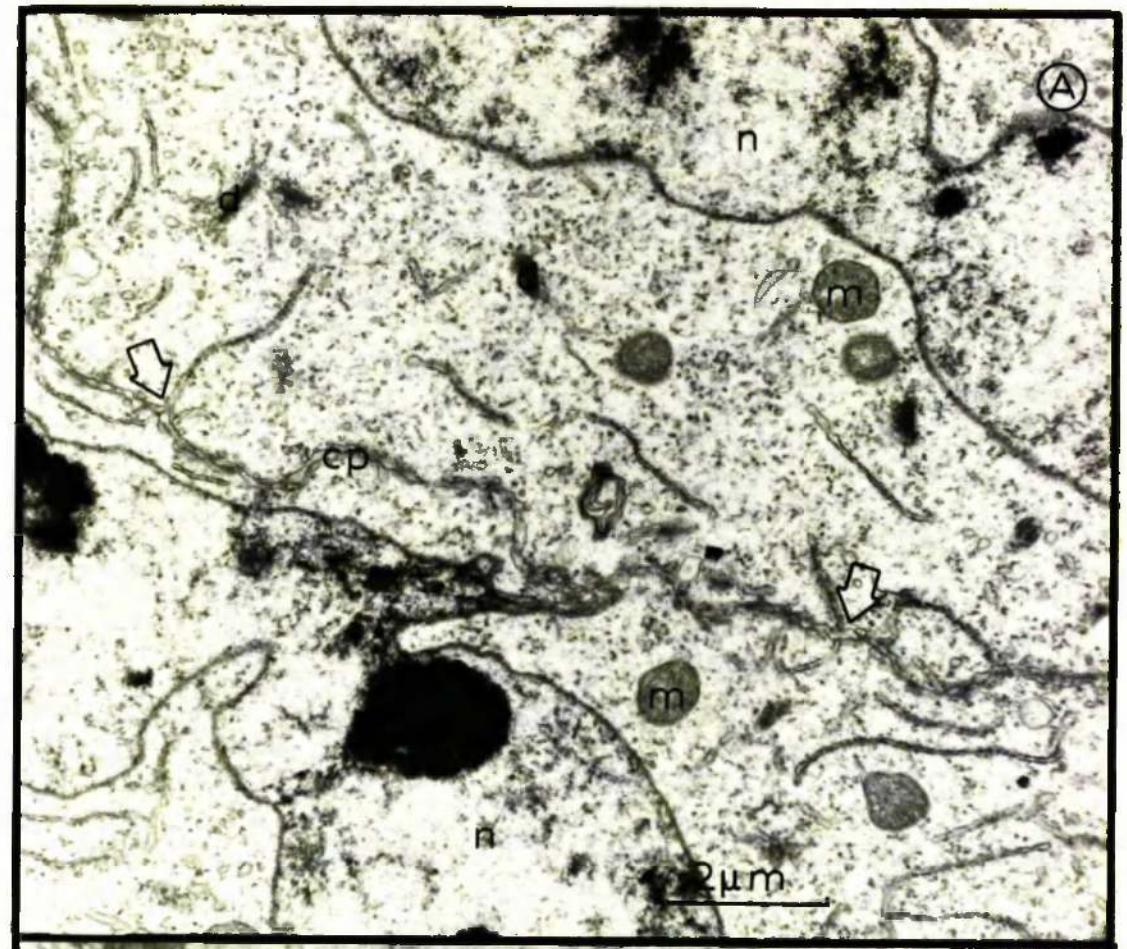
Plate 28B: TEM showing a face view of the metaphase plate of a mitotically dividing storage cell cultured *in vitro*; note the rough endoplasmic reticulum and free ribosomes interspersed between the chromatids. Numerous mitochondria and plastids surround the metaphase plate. M: 9600x

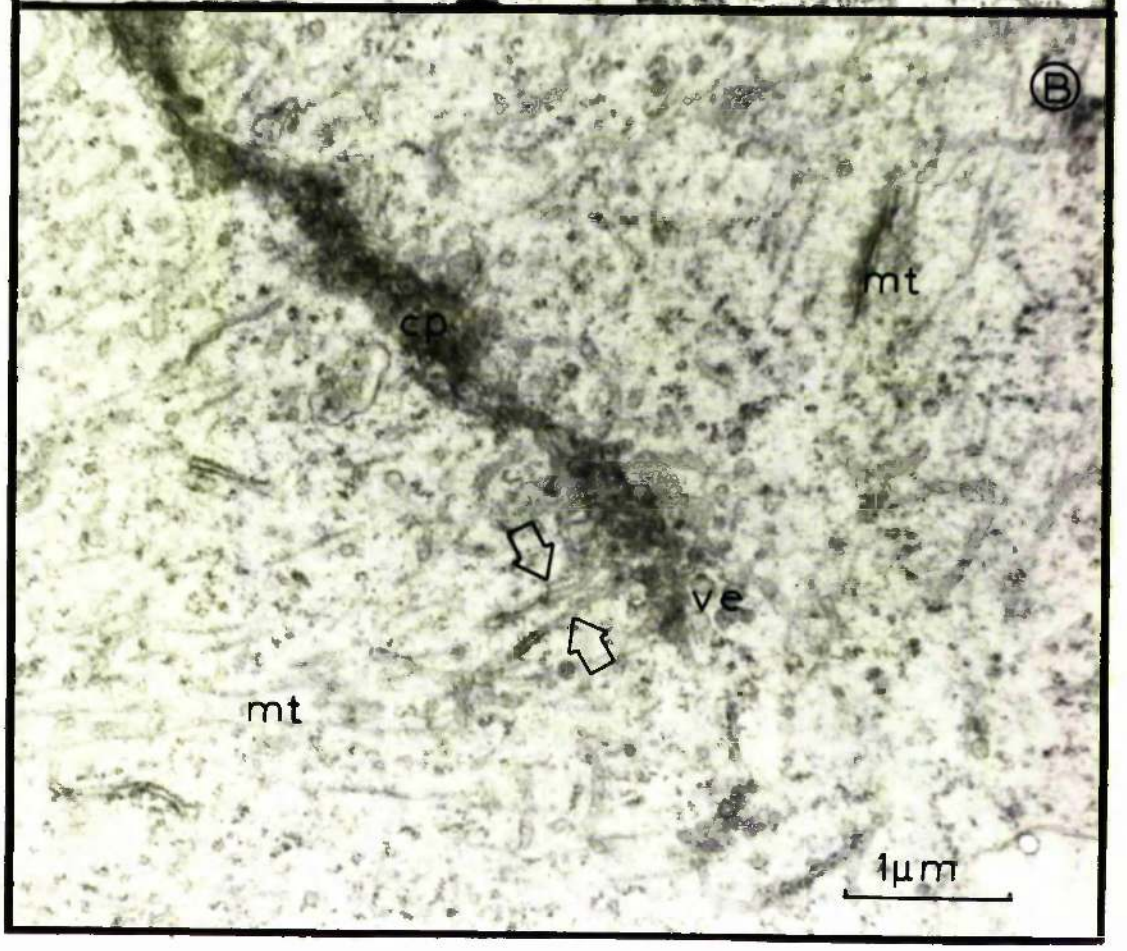

A
rer
m
2µm
B
sg
rer
cw
2µm

P. vulgaris

Plate 29A: TEM from an in vitro cultured storage cell showing part of the central region of the cell plate formed between two nuclei; note its vesicular structure with elements of the rough endoplasmic reticulum in close association with it, sometimes apparently fusing and, possibly, demarcating the sites of plasmodesmata (arrows). M: 11650x

Plate 29B: TEM from an in vitro cultured storage cell showing the margin of the centrifugally developing cell plate; note the rough endoplasmic reticulum elements in close association with it (arrows) together with a large number of cytoplasmic vesicles and microtubules. M: 23200x


A
n
m
cp
m
n
2µm

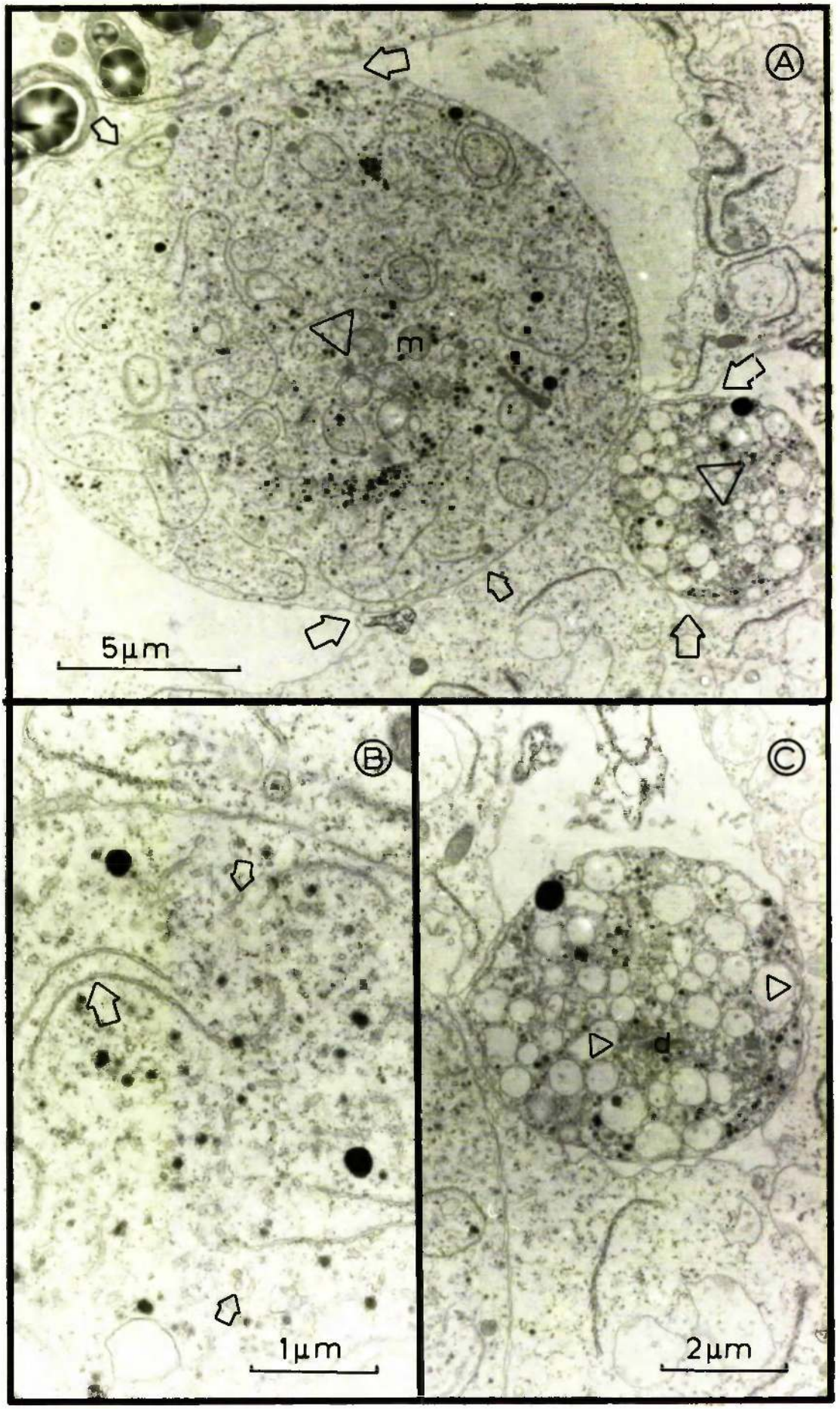

B
mt
cp
ve
mt
1µm

P. vulgaris

Plate 30A: TEM from an in vitro cultured storage cell showing two circular enclaves (△) of the cytoplasm, delimited by double membranes (small arrows) from the main cytoplasm. In places (large arrows) the two membranes are widely separated. Within the regions demarcated by the double membranes osmiophilic deposits, vesicles, elements of endoplasmic reticulum and profiles resembling mitochondria are abundant (see detail in Pl. 30B and C). M: 6500x

Plate 30B: TEM showing greater detail of part of the larger enclave of Pl. 30A; note the endoplasmic reticulum (large arrow) with few attached ribosomes, vesicles (small arrows) which are possibly transverse profiles of endoplasmic reticulum and osmiophilic deposits. M: 21000x

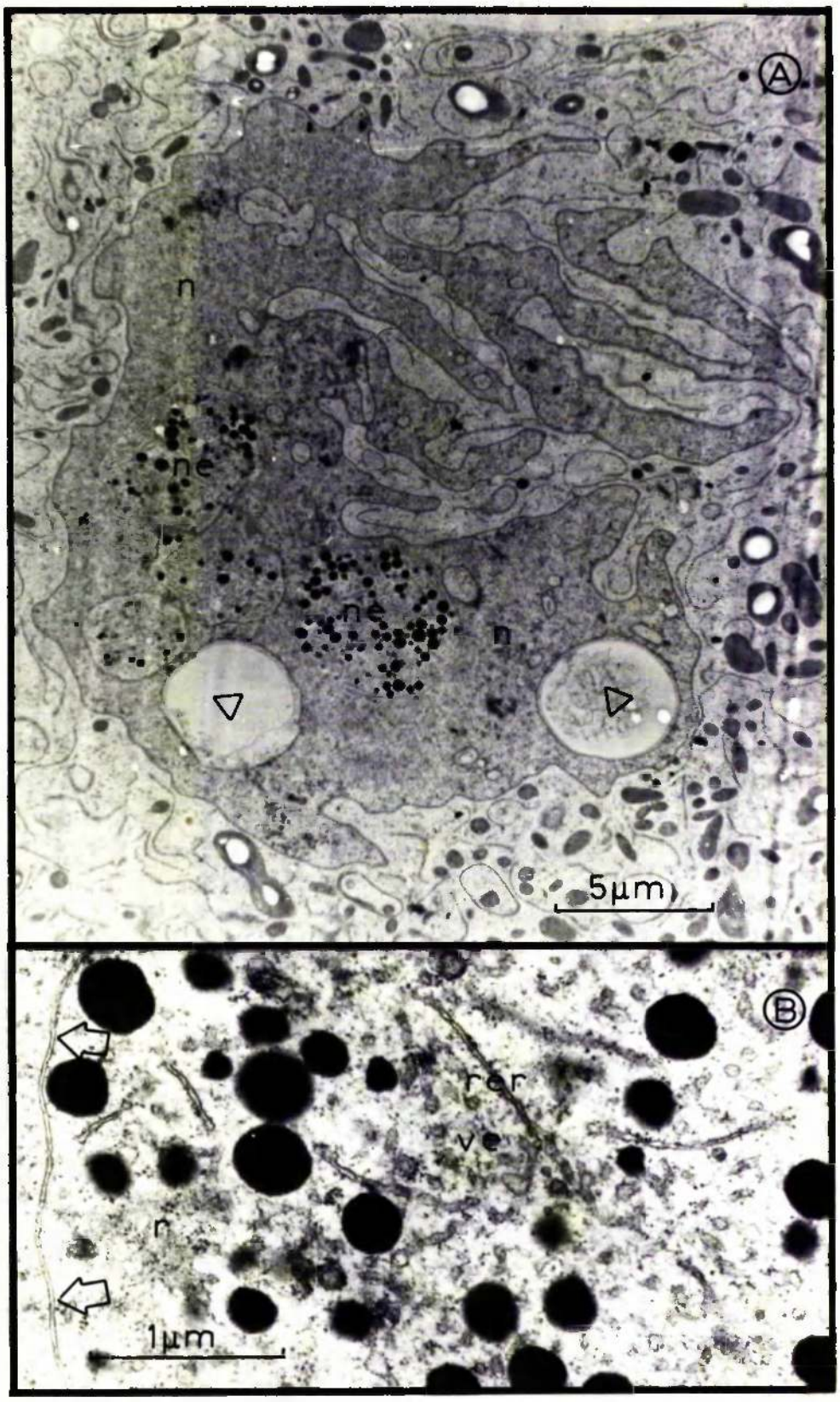
Plate 30C: TEM showing detail of the smaller enclave in Pl. 30A; note the large translucent vesicles (△), osmiophilic globules, and what appears to be a dictyosome. M: 10000x


A
m
5µm
B
1µm
C
d
2µm

P. vulgaris

Plate 31A: TEM of a highly lobed nucleus, from a storage cell cultured in vitro, with cytoplasmic enclaves containing osmiophilic globules, vesicles, rough endoplasmic reticulum segments, and some free ribosomes (see also Pl. 22A). (△) indicate translucent enclaves in the nucleus. M: 5160x

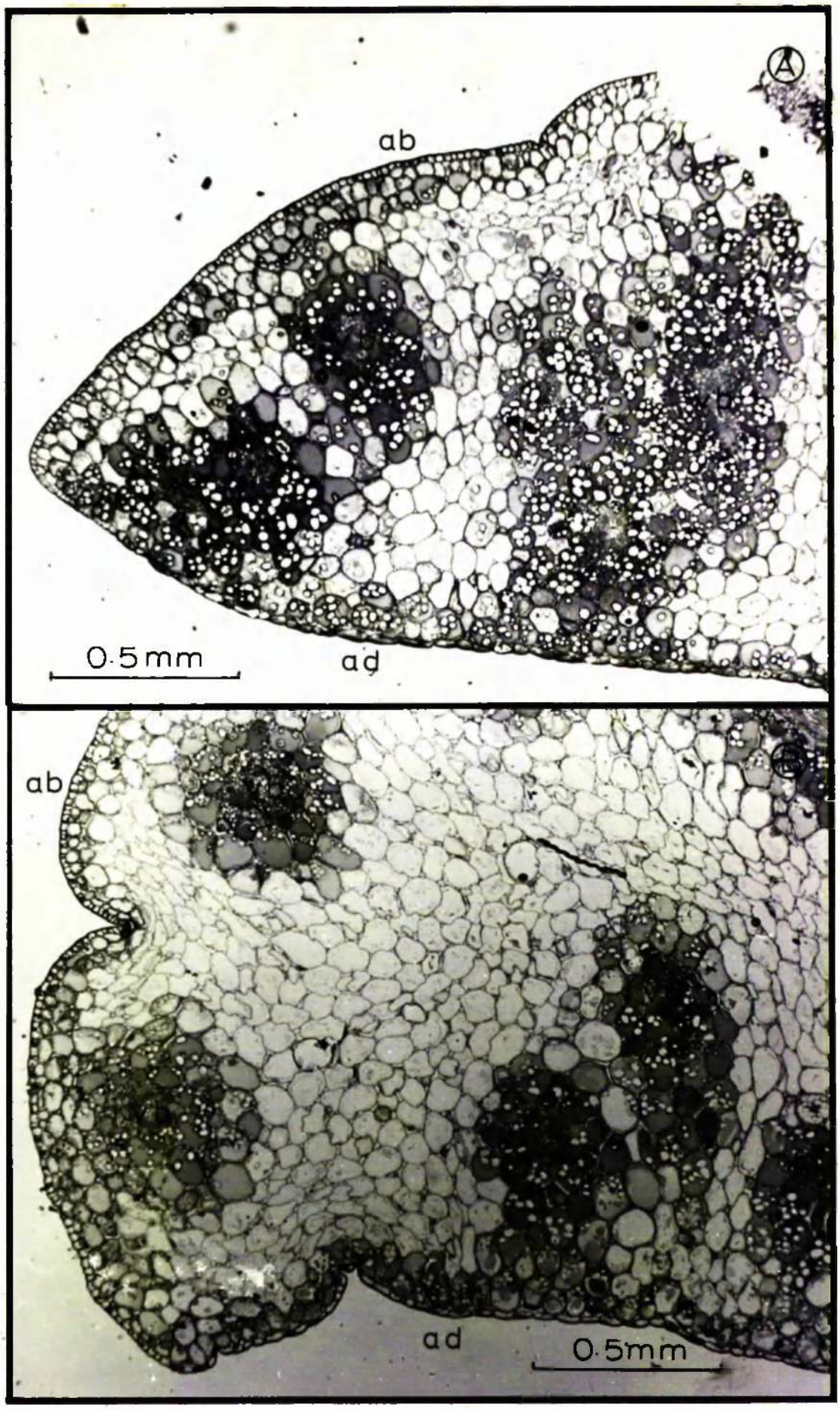
Plate 31B: TEM showing detail of a cytoplasmic enclave from the nucleus of Pl. 31A. Note the osmiophilic globules, endoplasmic reticulum segments, vesicles and free ribosomes. The arrows show the double membrane bounding the enclave. M: 31000x


A
n
ne
ne
n
5µm
B
rer
ve
n
1µm

P. vulgaris

Plate 32A: LM (Day 3) showing a TS of an *in vivo* germinating cotyledon; many of the storage cells appear empty since a large part of their reserve food has already been digested. M: 65x

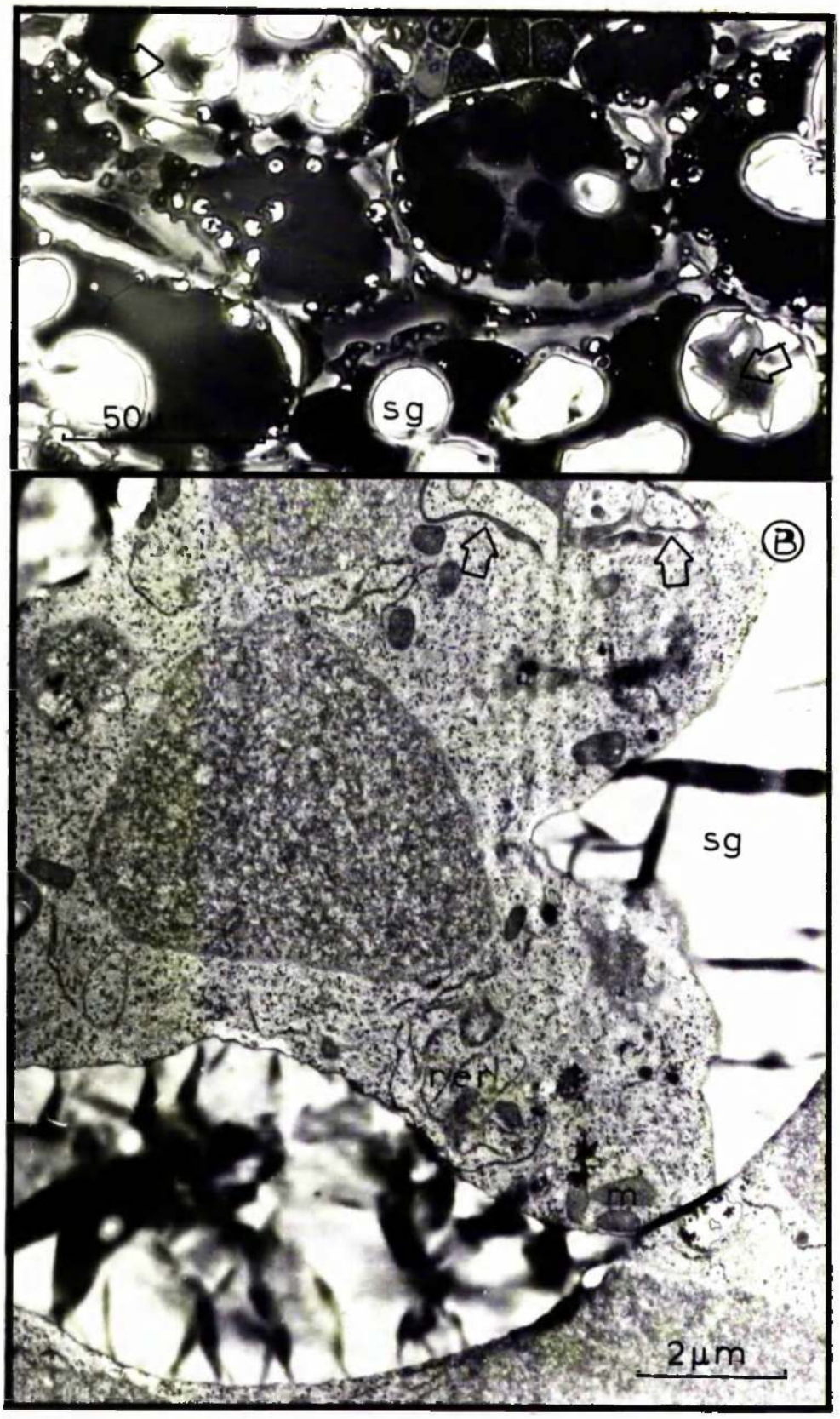
Plate 32B: LM (Day 5) showing a TS of an *in vivo* germinating cotyledon; the cotyledon has swollen considerably, most of the reserve food has been digested (however, around the vascular strands the storage cells still contain some deposits of protein and starch) and the empty storage cells are senescing. Some storage cells situated near the surface develop chloroplasts and become briefly photosynthetic. M: 65x


A
ab
0·5mm
ad
B
ab
ad
0·5mm

P. vulgaris

Plate 33A: LM of a TS of an in vivo germinating cotyledon showing part of a vascular bundle together with some storage cells. Note that some of the starch grains (ndicated by arrows) have developed cavities containing cytoplasm. M: 640x

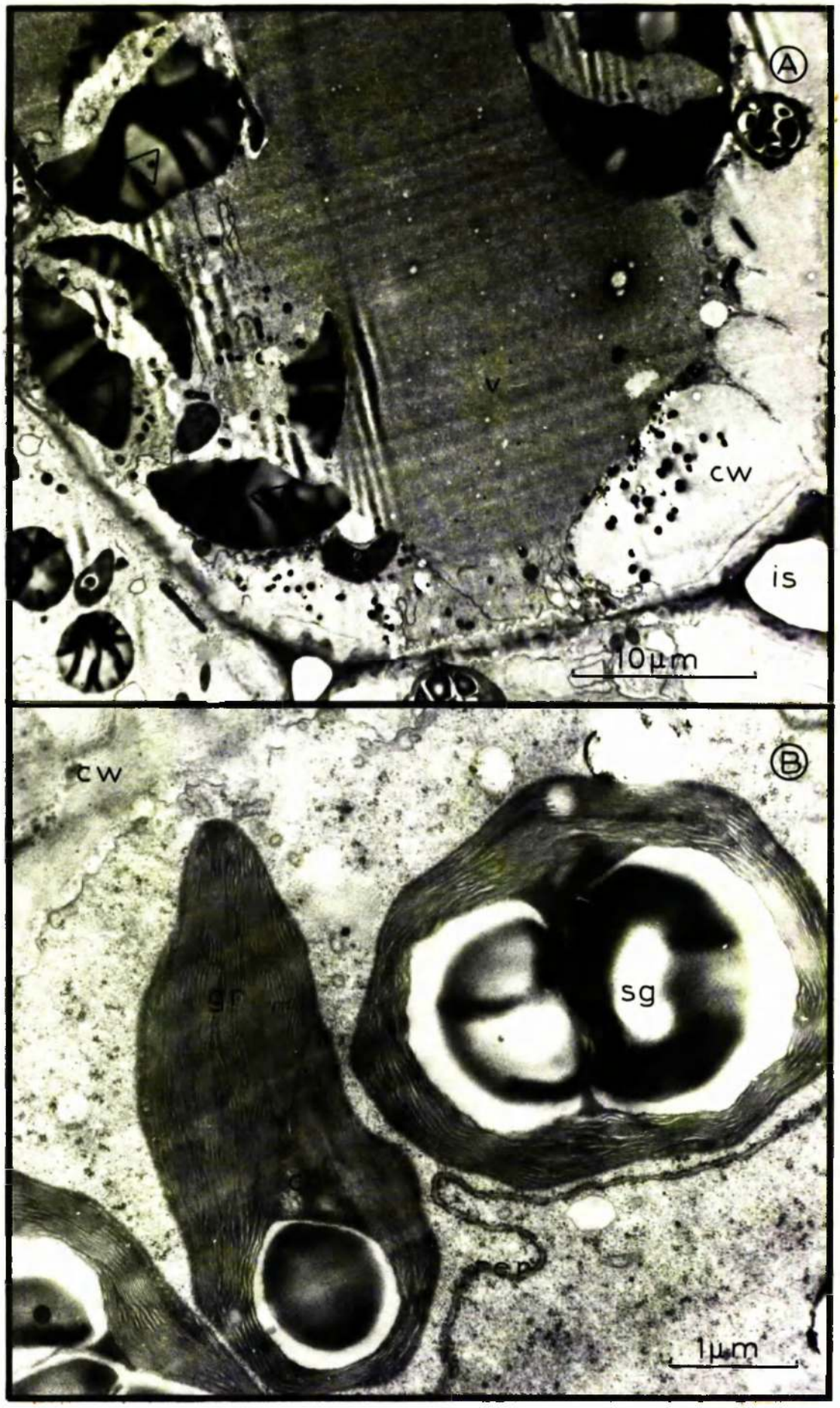
Plate 33B: TEM of a starch grain from an in vitro developing storage cell being digested; note the occurrence of cytoplasm within the digested cavity of the starch grain together with endoplasmic reticulum elements, mitochondria and filamentous plastids (arrows). M: 11600x


50
sg
B
sg
rer
m
2µm

P. vulgaris

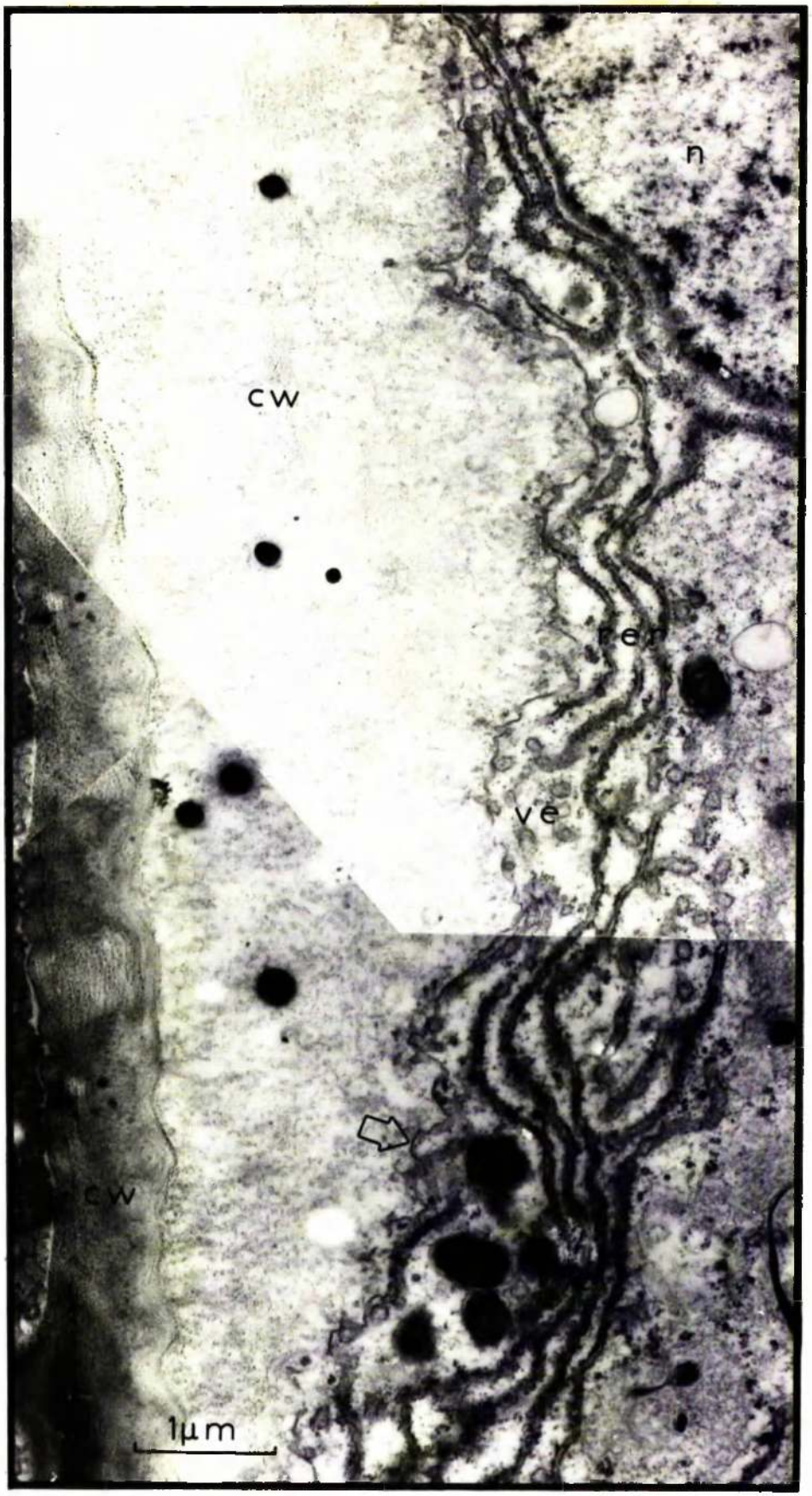
Plate 34A: TEM (Day 5) of an in vivo storage cell; note the expansion of the inner, non-cellulosic, part of the cell wall and also the fragmentation and digestion of the starch grains. M: 3300x.

Plate 34B: TEM (Day 5) of chloroplasts from an in vivo storage cell located near the surface of the cotyledon; note the large number of grana and the densely stained stroma. M: 21500x


A
v
cw
is
10µm
B
cw
gr
sg
ep
1µm

P. vulgaris

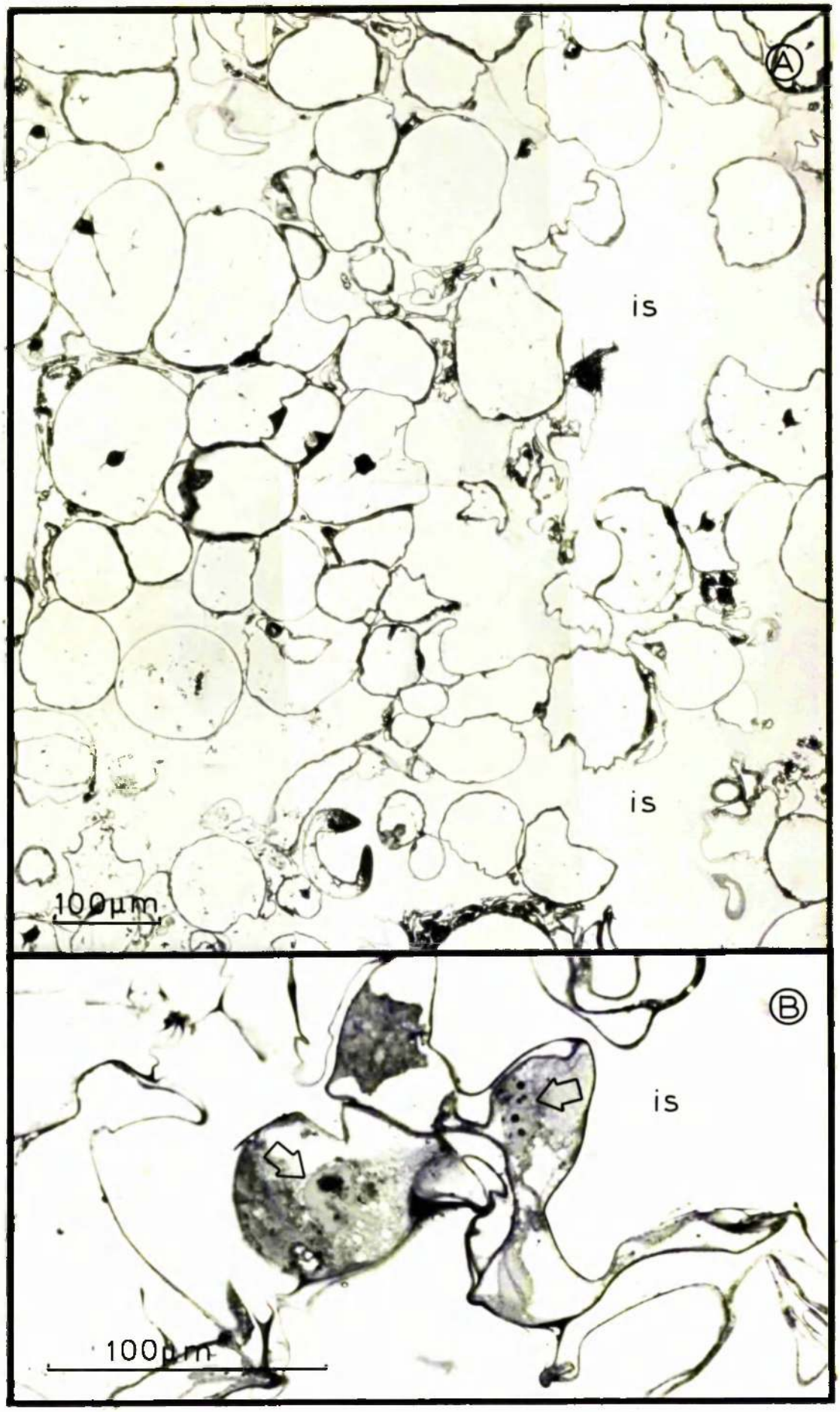
Plate 35: TEM (Day 5) showing cell wall digestion in a storage cell from an *in vivo* germinating cotyledon; note the rough endoplasmic reticulum elements lying parallel to the swollen inner cell wall and the numerous associated vesicles. The arrows indicate the plasmalemma. M: 21500x


n
cw
rer
ve
cw
1µm

P. vulgaris

Plate 36A: LM of a section through the established callus tissue derived from a cotyledon explant; note the highly irregular size and shape of the greatly vacuolated cells and the large intercellular spaces. M: 190x

Plate 36B: LM illustrating some meristematic-looking cells from the periphery of the established callus; note the irregular outline of the nucleus indicated by a large arrow and the multinucleolate nucleus on the right (small arrow). M: 500x


A
is
is
100µm
B
is
100µm

P. vulgaris

Plate 37A: LM showing nuclear fragmentation in an established callus cell; note the nuclear enclave (arrow) and the massive wall body (△). M: 2000x

Plate 37B: LM of an established callus section showing some cells from near the periphery of the callus; note their highly irregular shape, the large nuclei with prominent nucleoli and the mitotic division in one cell (arrow). M: 500x

Plate 37C: LM detail of the mitosis from Pl. 37B showing the anaphase plate in more detail; note its similarity to the one in Pl. 26C-E. Clusters of small amyloplasts are observed near the nucleus. M: 2000x

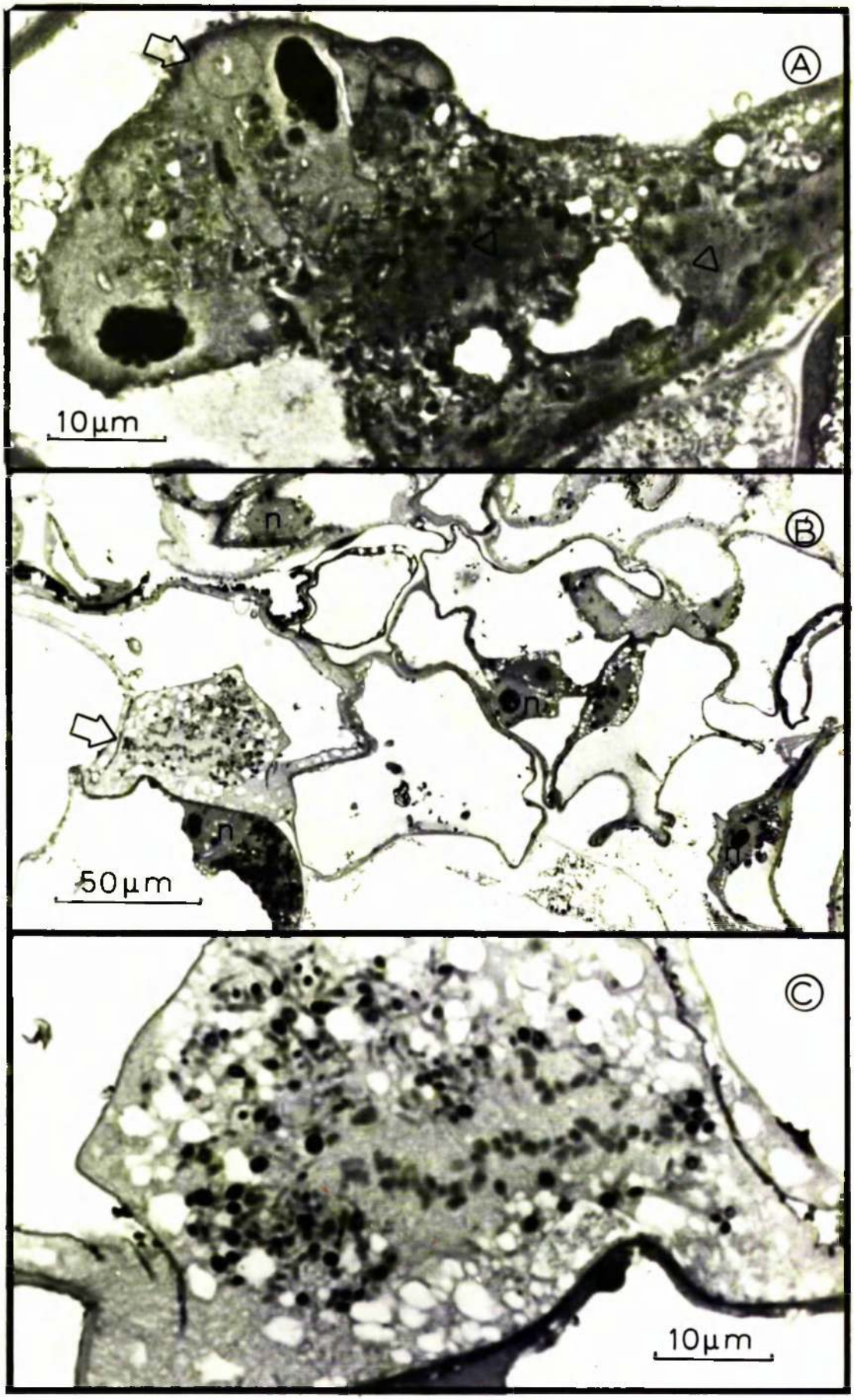
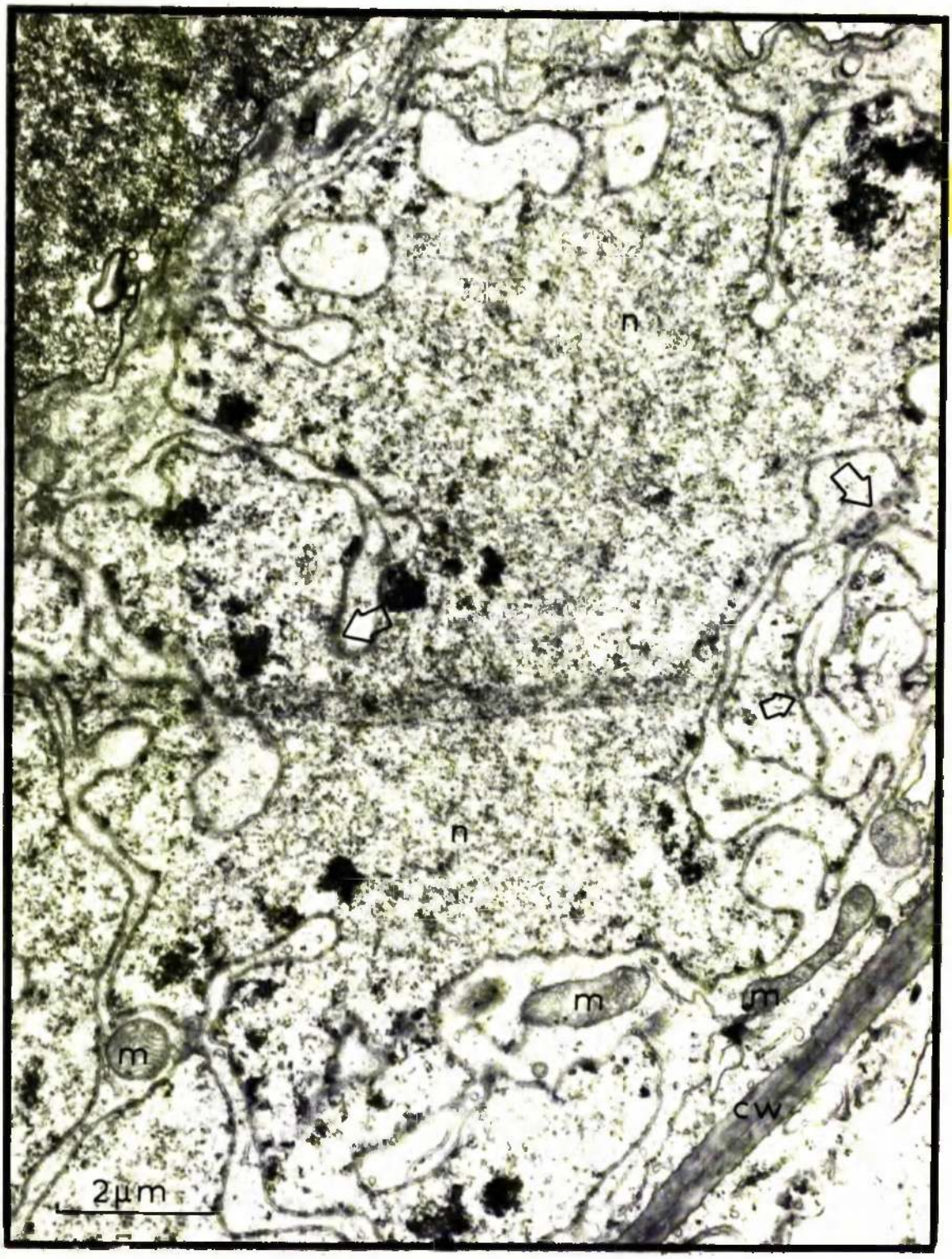

A
10μm
B
n
n
n
n
50μm
C
10μm

Plate 38: TEM of the nucleus of an established callus cell; note its highly irregular outline showing nuclear pores (large arrows) and the continuity of an element of the endoplasmic reticulum with the nuclear envelope (small arrow). M: 12500x

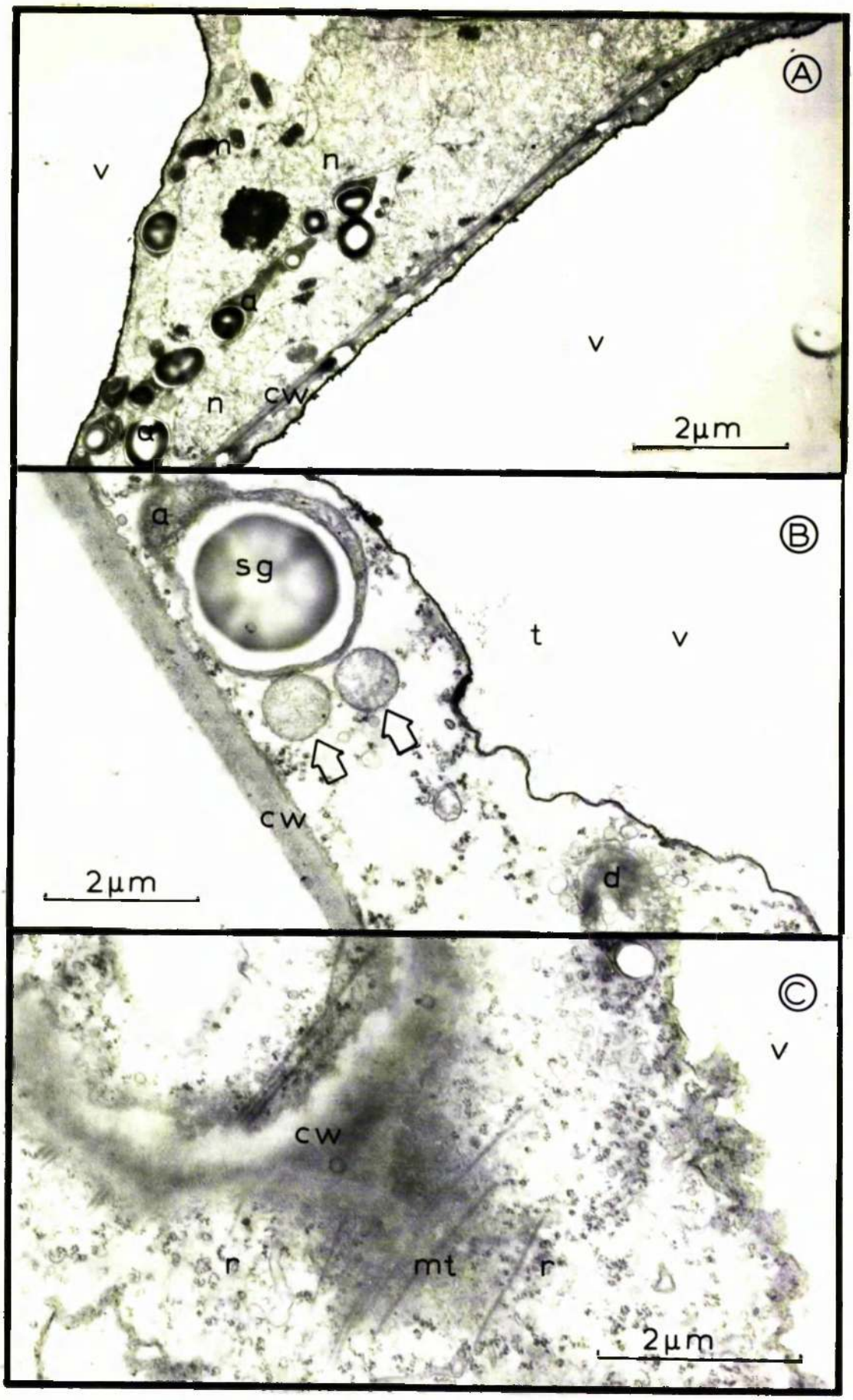

n
n
m
m
m
cw
2µm

P. vulgaris

Plate 39A: TEM showing the nucleus of a highly vacuolated cell from the established callus; note the presence of agranal amyloplasts with tails and the densely stained mitochondria in the thin layer of cytoplasm. M: 5400x

Plate 39B: TEM illustrating part of the cytoplasm of a highly vacuolated established callus cell; the swollen dictyosome, the very translucent cytoplasm with very few ribosomes and the organelles indicated by the arrows (possibly representing mitochondria) suggest that this cell has become senescent. M: 13500x

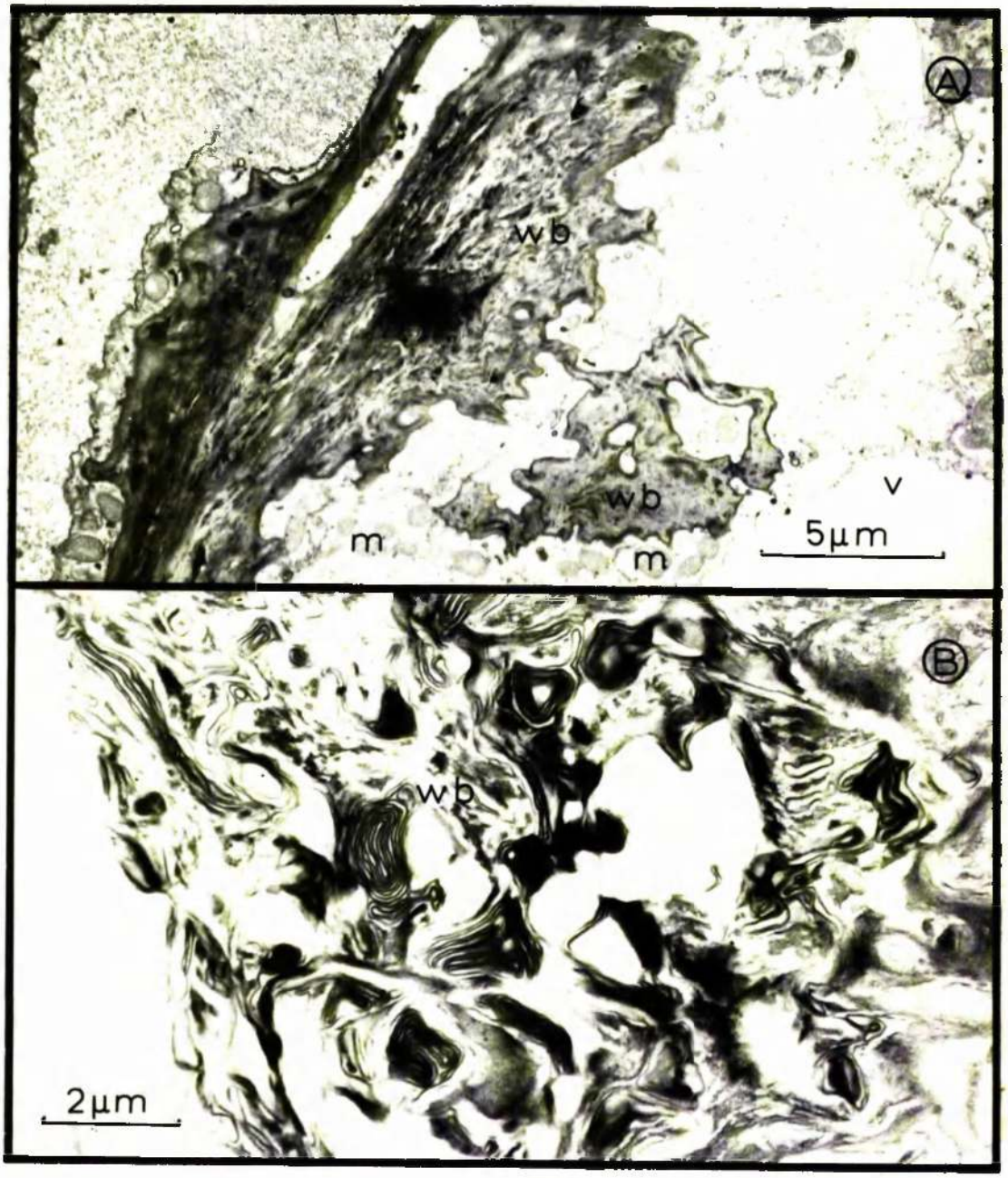
Plate 39C: TEM illustrating part of the cytoplasm of a highly vacuolated established callus cell in which numerous microtubules and coiled polyribosomes are present. M: 18000x


A
v
n
n
v
n
cw
2µm
B
a
sg
t
v
cw
2µm
d
C
v
cw
n
mt
r
2µm

P. vulgaris

Plate 40A: TEM showing a wall body in an established callus cell. M: 5400x

Plate 40B: TEM showing at higher resolution part of a wall body from an established callus cell; note the membranous structures and myelin-like figures enclosed in it; possibly representing remnants of organelles engulfed by the expansion of this body in the cytoplasm. M: 10000x


A
wb
wb
v
m
m
5µm
B
wb
2µm

L. usitatissimum

Plate 41A: Macrophotograph showing a Day 10+4 decapitated hypocotyl (on the left) and a Day 14 (control) seedling. Note the bud primordium, which has (exceptionally) developed at the base of the hypocotyl (arrow), and the swollen upper region. No adventitious bud primordia are evident on the intact hypocotyl (on the right). M: 2x

Plate 41B: Macrophotograph showing a Day 10+10 hypocotyl; the dominant adventitious bud has already grown considerably giving rise to a replacement (lateral) shoot; at the same time a number of other adventitious buds at different steges of development (ranging from leaf primordia to leafy buds) are present on the upper half of the hypocotyl; the arrow indicates the decapitated hypocotyl surface. M: 2x

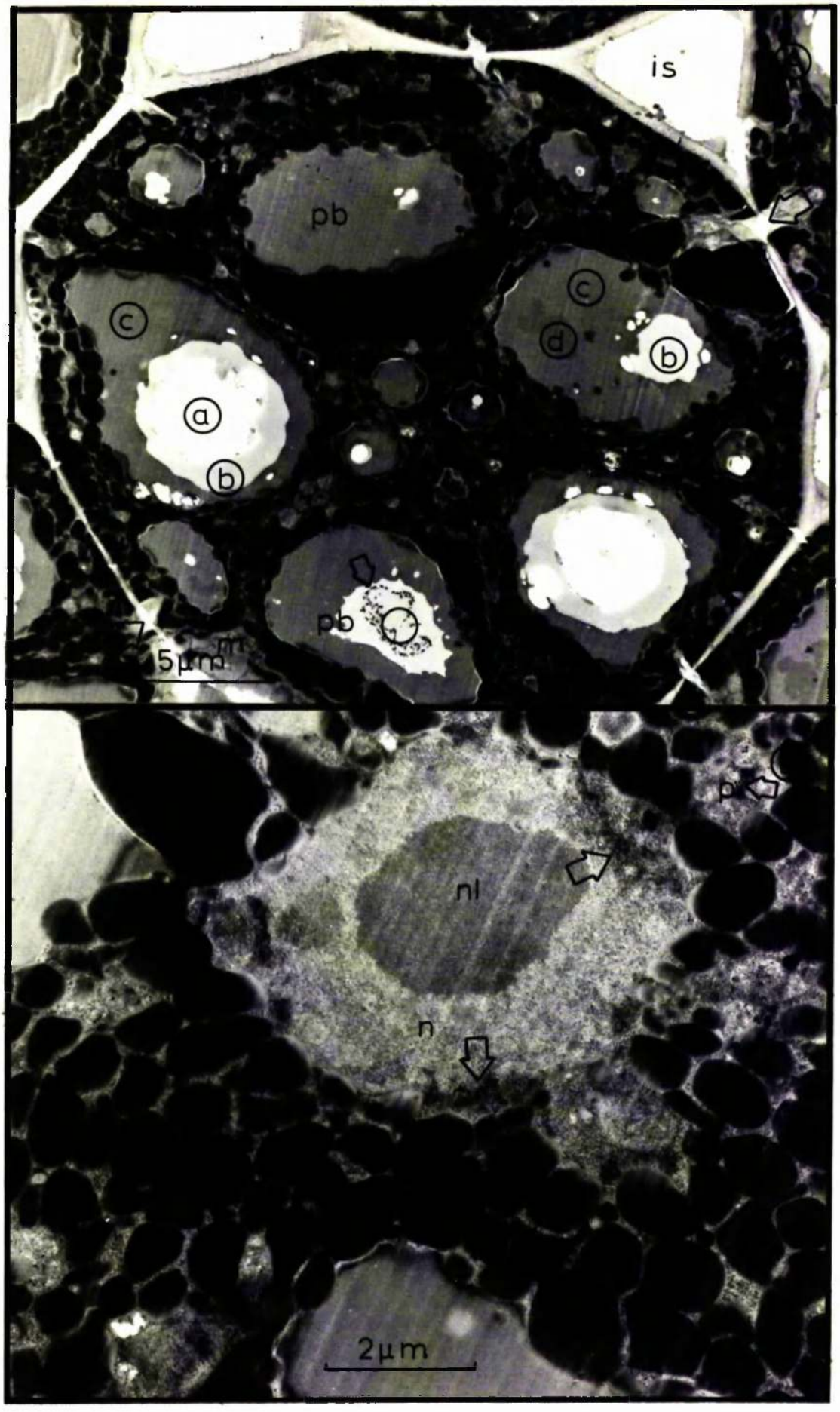
Plate 41C: Macrophotograph similar to Pl. 41B except for the location of the buds; this experiment was carried out during Spring and the majority of adventitious buds were developed on the lower half of the decapitated hypocotyl instead of the upper region as normally occurred in most experiments (c.f. Pl. 41B). M: 2x


C
A

L. usitatissimum

Plate 42A: TEM (TS) showing a Day 0 cortical cell from the upper part of the hypocotyl. The areas (a), (b), (c) and (d) are the parts of the protein bodies identified as air spaces, globoid inclusions, crystalloid inclusions and ground protein respectively. Note the variation in lipid globuli size and the presence of cytoplasmic organelles tentatively identified as plastids and mitochondria. The arrows indicate some "channels" which might be either artifacts or represent sites where plasmodesmata occur. The small arrow indicates some granular inclusions observed inside area (b) of a protein body. M: 5160x

Plate 42B: TEM (TS) of a Day 0 cortical cell from the upper part of the hypocotyl. Neither the nuclear envelope nor other membranes are clearly defined, but some organelles are identified as plastids because of the presence of plastoglobuli (small arrows). Note the angular outlines of the lipid globules. The large arrows indicate some peripheral darkly stained granular areas of the nucleus; however it is not certain whether this is a staining artifact (c.f. Pl. 45B) or a genuine densely stained area of the nucleus.
M: 13000x


is
pb
c
d
b
a
b
pb
5µm
p
nl
n
2µm

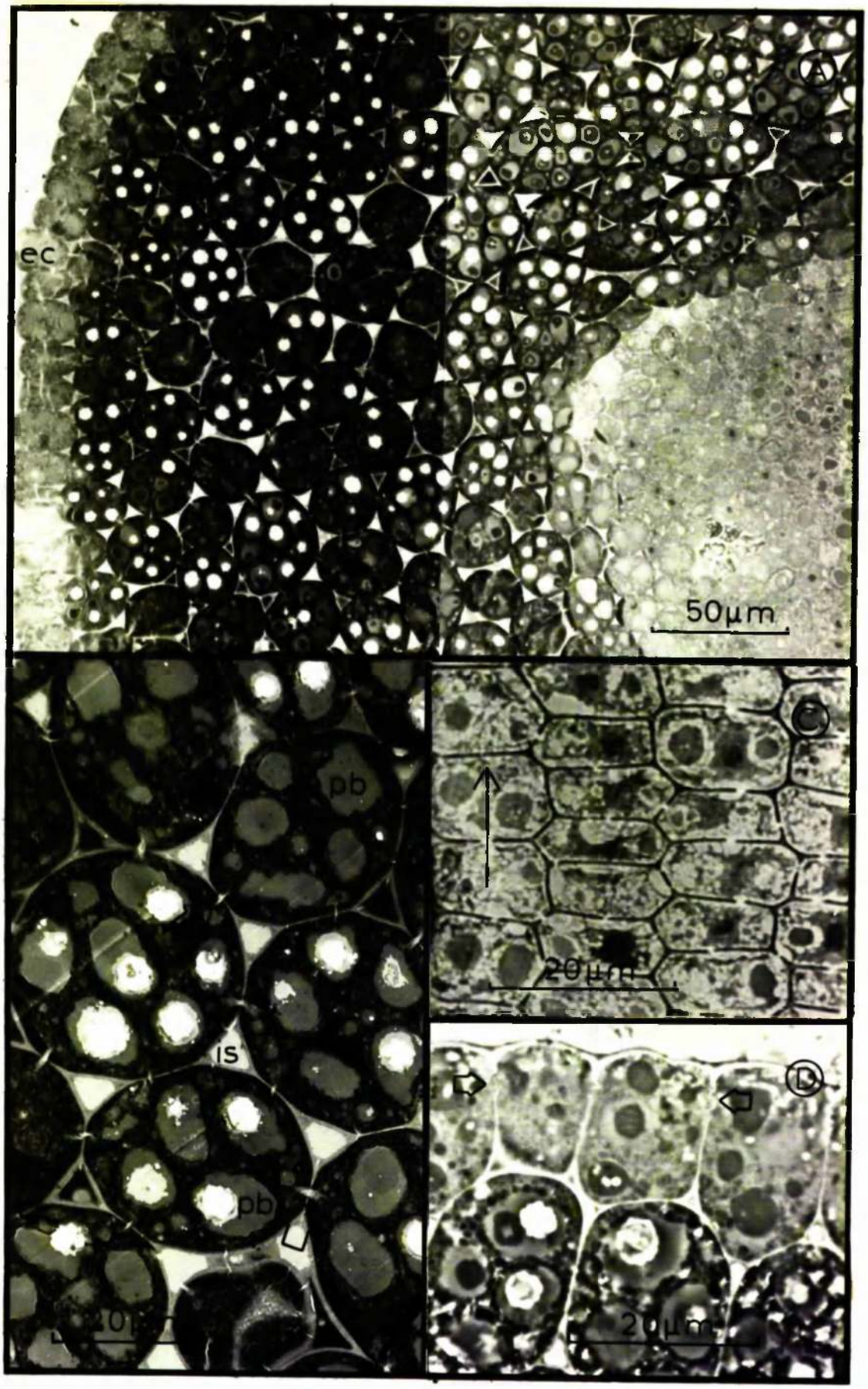
L. usitatissimum

Plate 43A: LM (TS) from a Day 0 hypocotyl showing the various tissues (epidermis, cortex, provascular tissue and pith). The difference in staining intensity with toluidine blue between cortex and all other tissues possibly reflect differences in the amount of food reserves. The cortical cells are more densely packed with food stores than other tissues. M: 450x

Plate 43B: TEM (TS) showing some cortical cells of the Day 0 hypocotyl. Note the occurrence of the "channels" which are frequently present on walls in contact with other cells but not on cell walls contacting intercellular spaces. M: 1200x

Plate 43C: LM (PS) of the Day 0 hypocotyledonary cells. The arrow indicates the long axis of the organ (acropetal direction). Note the presence of "channels" on the cell walls and the squat shape of the cells (c.f. the Day 10 epidermal cells in Pl. 50A, which are about 15-20 times longer). M: 1600x

Plate 43D: LM (TS) of the Day 0 hypocotyl showing part of the epidermis. The arrows indicate the anticlinal walls which appear corrugated separating the otherwise well preserved epidermal cells. Note the difference in protein deposits between epidermal and cortical cells; the former contain fewer and smaller protein bodies than the latter. M: 1600x


ec
A
50µm
pb
is
pb
B
20µm
C
20µm

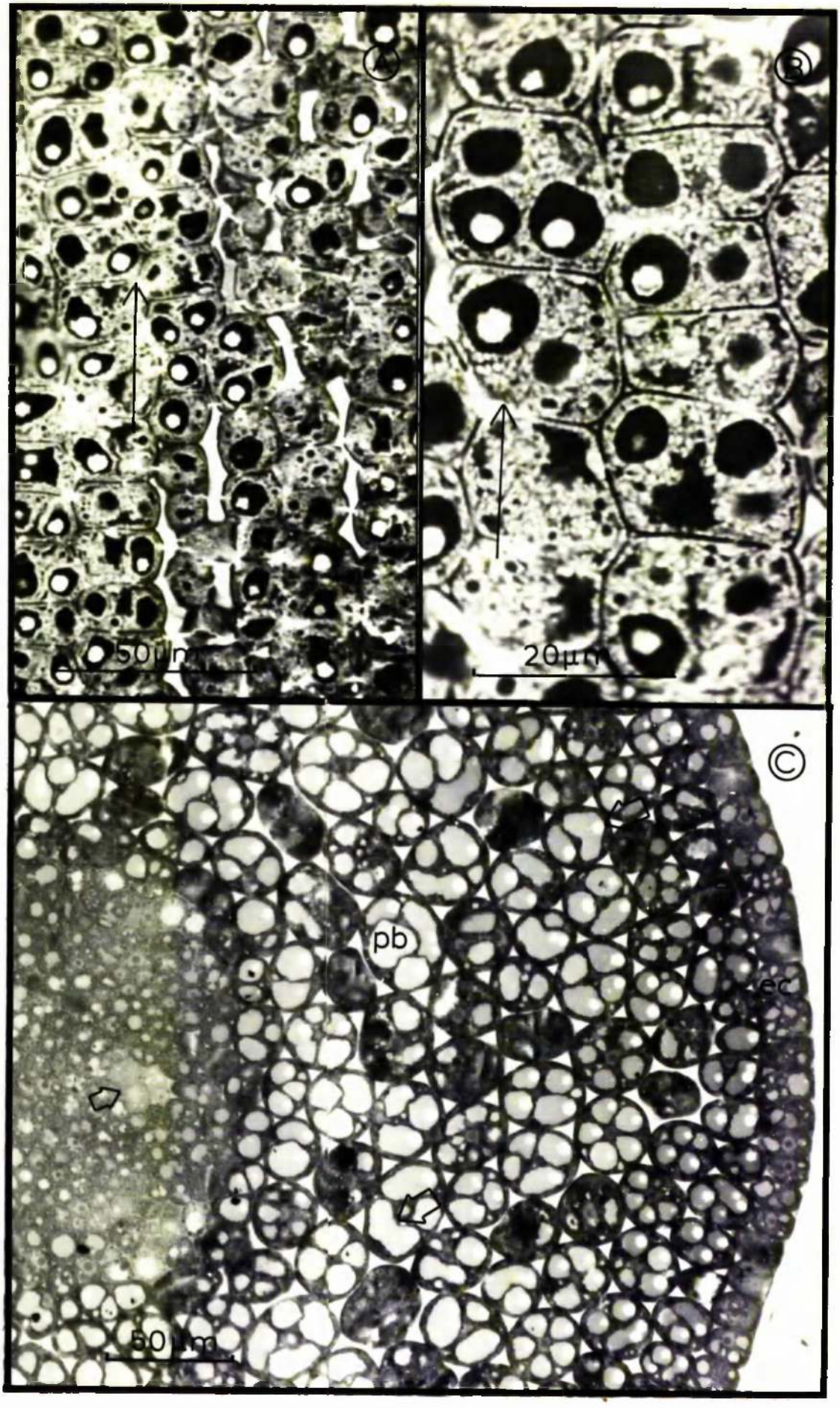
L. usitatissimum

Plate 44A-B: LM's (RLS) of the Day 0 hypocotyl showing part of the cortex. The thin arrow indicates the long axis of the hypocotyl. Note the presence of the long channels (Pl. A, arrows) formed by the intercellular spaces between cortical cells.

M: A: 750x

B: 1800x

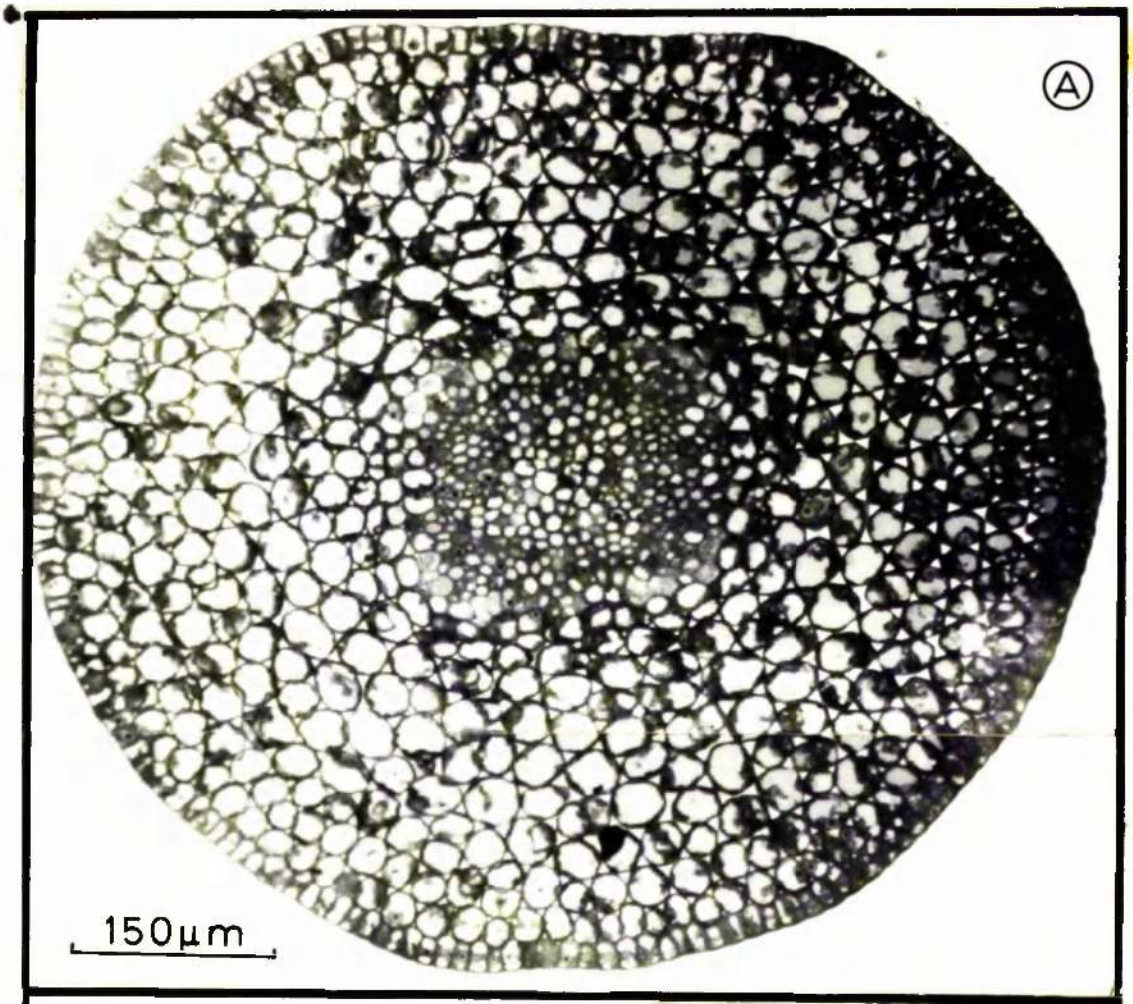
Plate 44C: LM (TS) of the Day 1 hypocotyl. The protein bodies are now bigger than in the Day 0 hypocotyl (c.f. Pl. 43A) and some of them have fused together (large arrows) giving rise to vacuoles. The small arrows indicate some provascular cells which appear less densely stained because of lack of food stores and these are possibly the cells which differentiate into vascular elements. M: 450x

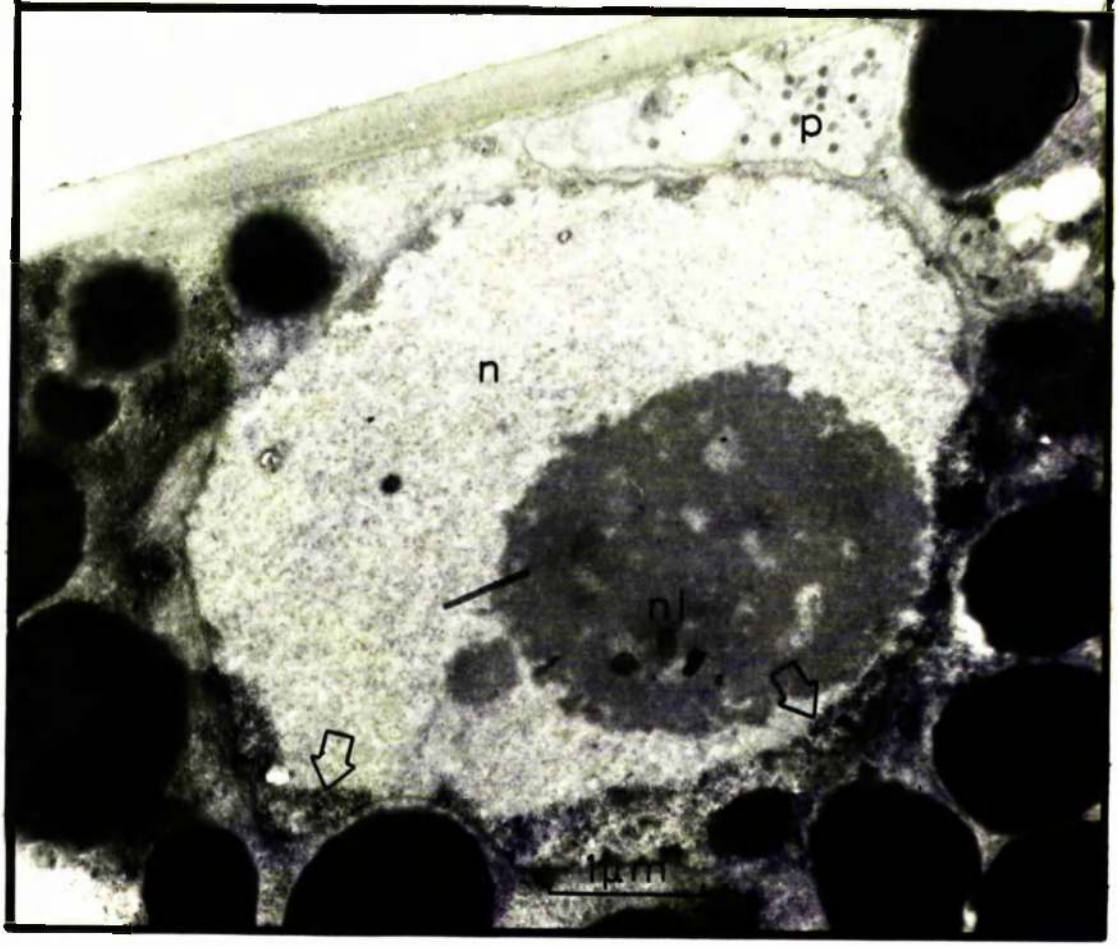

A
50µm
B
20µm
C
pb
ec
50µm

L. usitatissimum

Plate 45A: LM (TS) of the Day 3 hypocotyl (hook stage); note that food digestion is faster on the left side of the micrograph which corresponds to the convex side of the hypocotyl (c.f. Pl.46A) than on the right which corresponds to the concave side. The small arrows indicate cortical nuclei which on the concave side appear to occupy a more central position. On the convex side, nuclei occupy a more peripheral position and they are more rarely sectioned because cells on this side of the hypocotyl are more elongated. M: 180x

Plate 45B: TEM (TS) of a Day 1 hypocotyledonary epidermal cell. The lipid globules are rounder than in the Day 0 cells; the membranes bounding organelles such as plastids, dictyosomes and the nuclear envelope are now visible. Note the granular, densely stained area of the nucleus indicated by the arrows; however, this could represent an artifact introduced during staining or due to contamination of the specimen in the microscope. M: 20000x

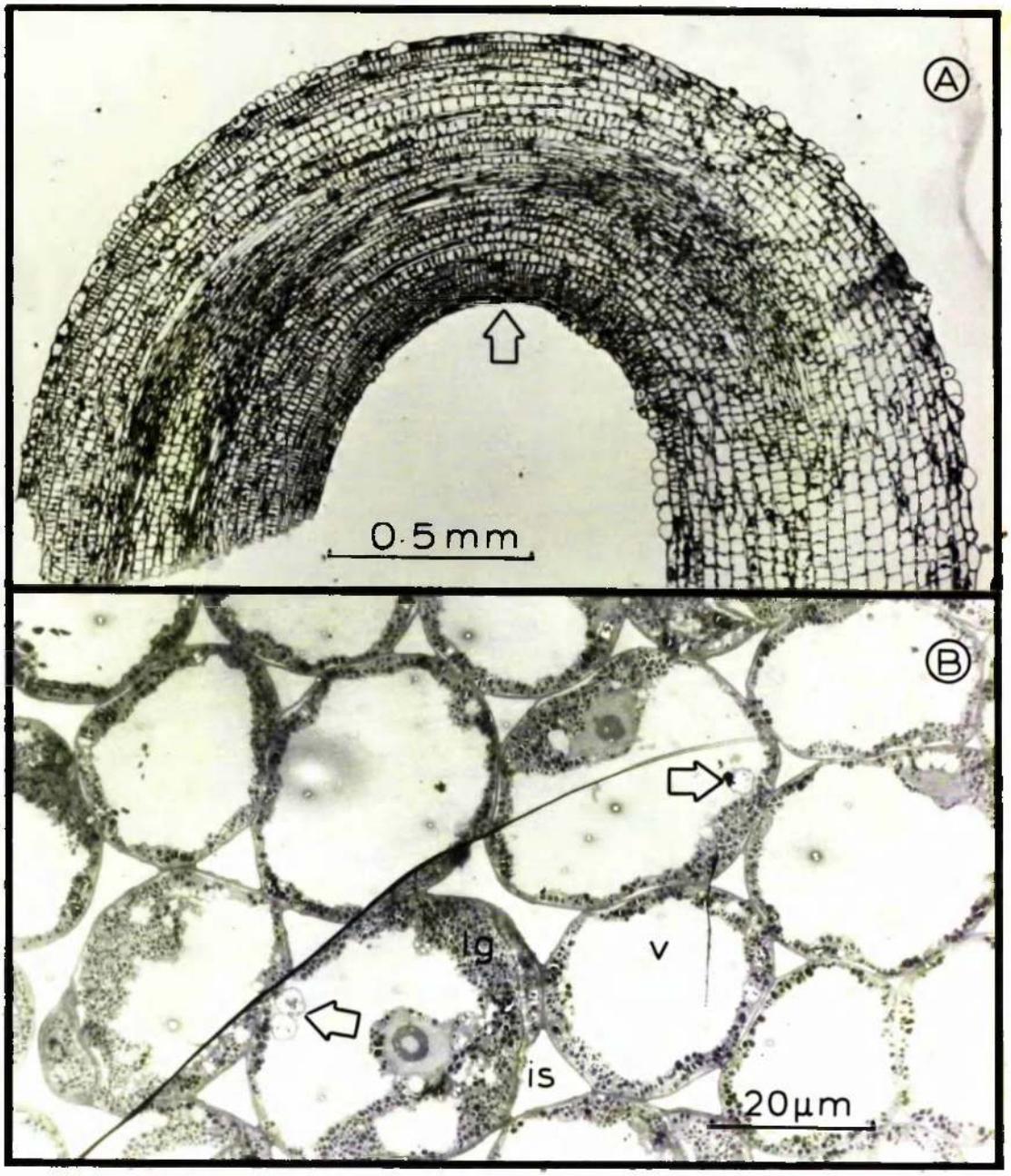

Ⓐ
150µm


p
n
nl
1µm

L. usitatissimum

Plate 46A: LM (RLS) of a Day 3 (hook stage) hypocotyl. The large arrow indicates the concave side with still short epidermal cells and the small arrows some epidermal cells protruding to the outside ("swollen cells") now visible in the convex surface. The distal end of the hypocotyl is on the left of the photograph. M: 60x

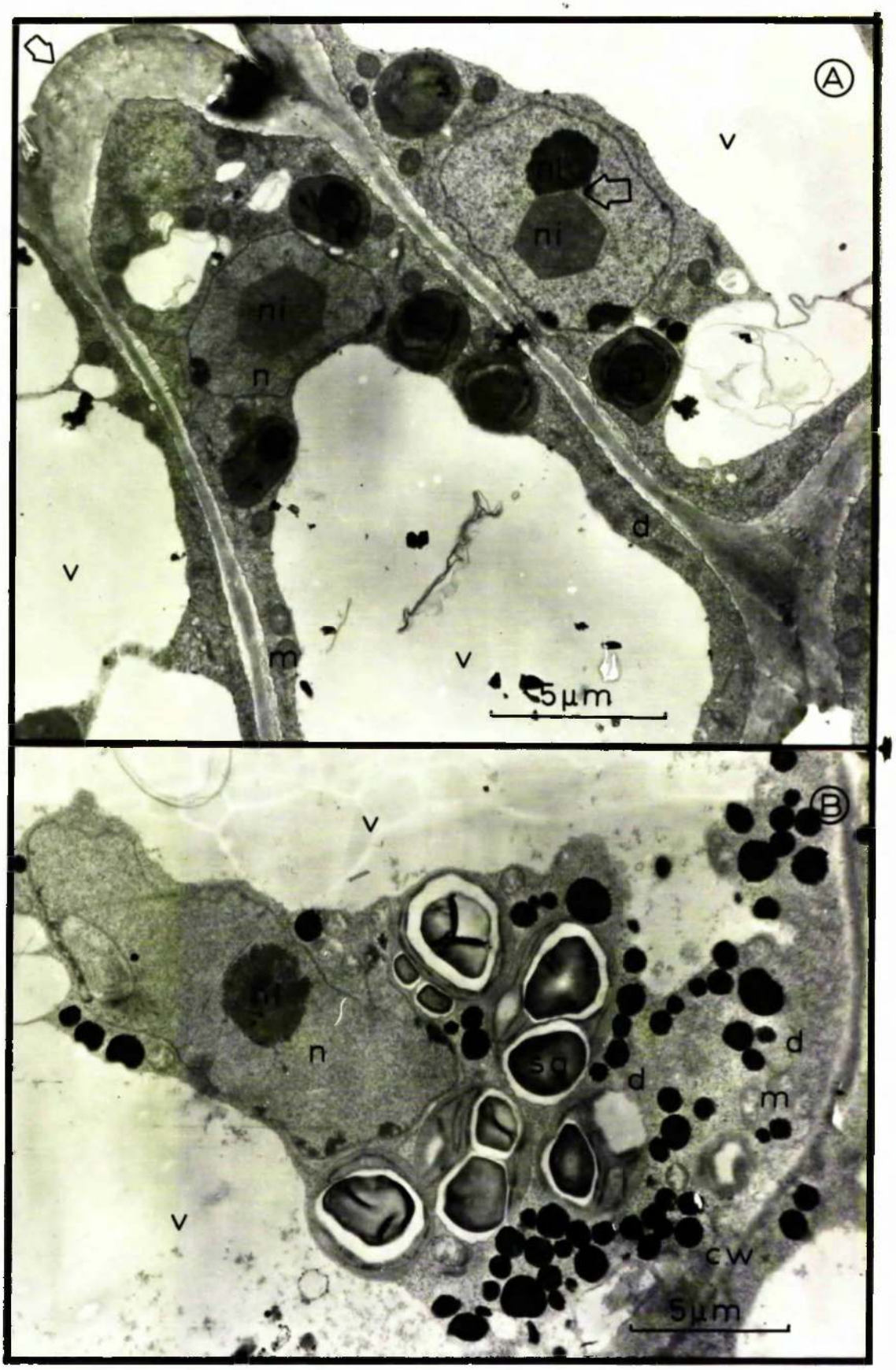
Plate 46B: TEM (TS) of a Day 3 hypocotyl showing several cortical cells which have already been considerably vacuolated; note the presence of membraneous or granular inclusions (large arrows) originally found in the protein bodies (globoid inclusions) of the Day 0 cortical cells. Numerous lipid globules are still present peripherally arranged. Also note the nuclei with large, sometimes vacuolated (small arrow) nucleoli. M: 450x


A
0·5mm
B
lg
v
is
20µm

L. usitatissimum

Plate 47A: TEM (TS) of Day 4 epidermal cells. The peripheral nuclei contain inclusions (with hexagonal profiles in a cross section) which are closely associated with the nucleolus (large arrow). The plastids contain some thylakoids and a large densely stained body. The small arrow indicates the external cell wall which is thickened and covered by a thin cuticle. M: 6000x

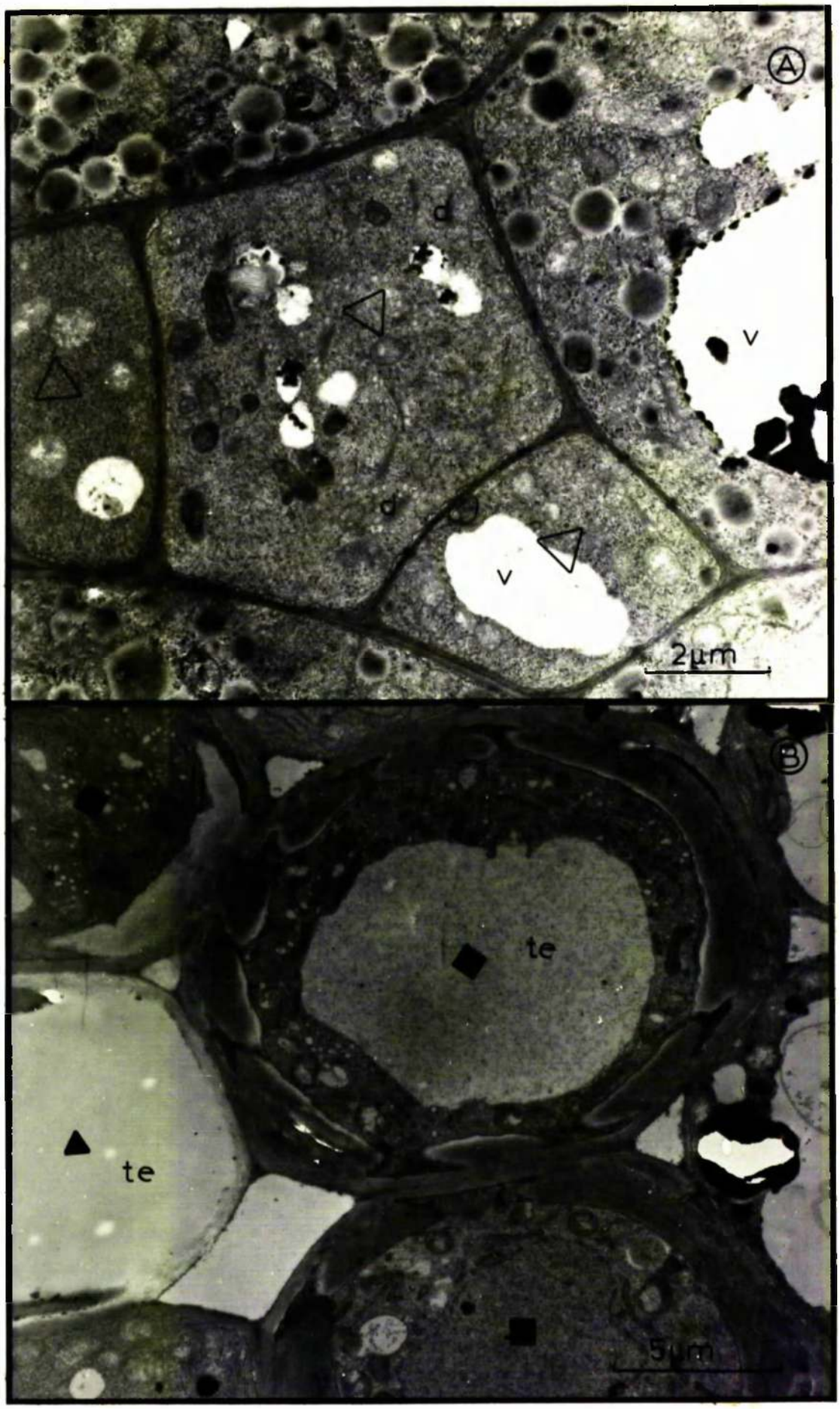
Plate 47B: TEM (TS) showing part of a Day 4 cortical cell. The nucleus is peripherally located and numerous small lipid globules are still present in the cytoplasm; note the presence of relatively large starch grains and some thylakoids in the plastids. M: 5400x


A
v
ni
ni
ni
n
d
v
m
v
.5µm
B
v
m
n
sg
d
d
m
v
cw
5µm

L. usitatissimum

Plate 48A: TEM (TS) from the provascular tissue of a Day 1 hypocotyl. Note the absence of food stores in the cells marked with a (△) and the presence of several dictyosomes, mitochondria and small vacuoles in their cytoplasm. These cells possibly are destined to differentiate into vascular elements. M: 10750x

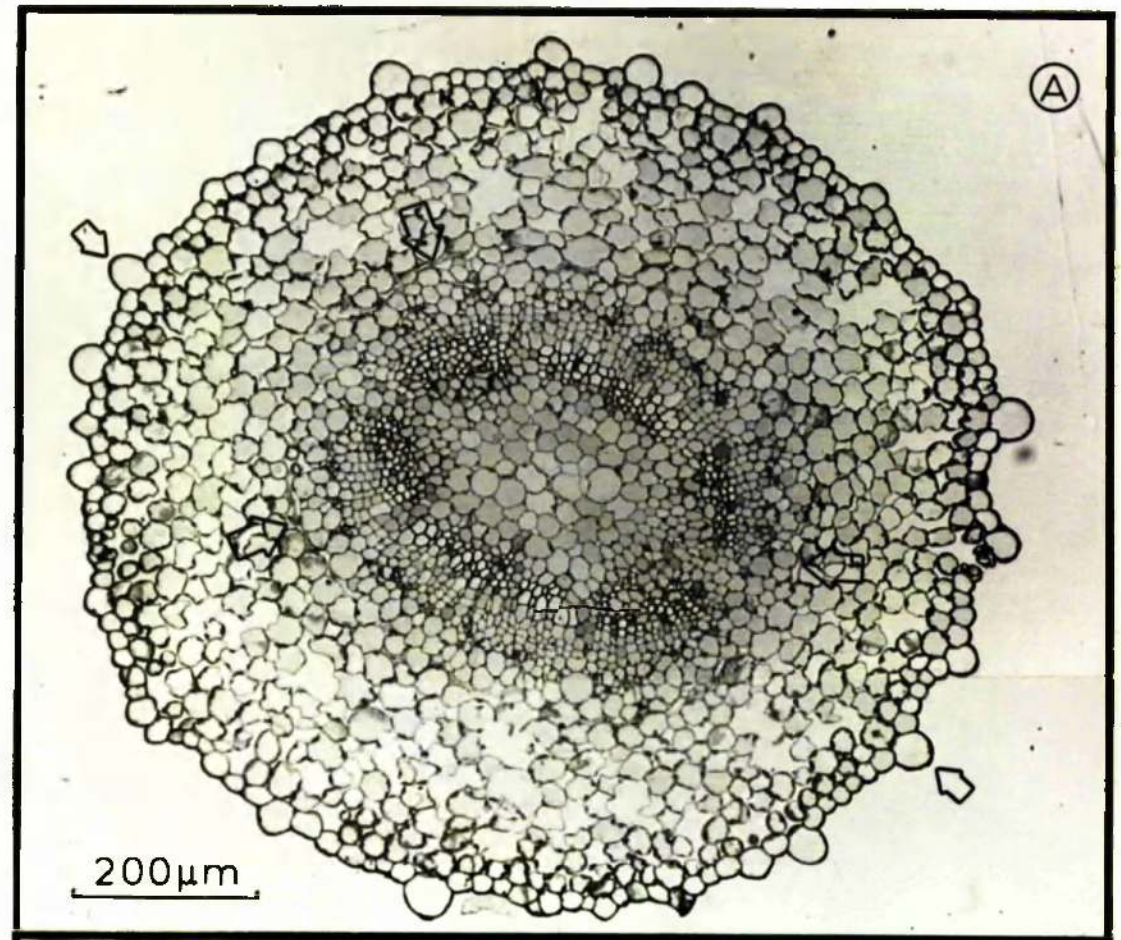
Plate 48B: TEM (TS) from a Day 4 vascular bundle; some tracheary elements are already mature (△) whilst others are still developing (□) and in their peripheral cytoplasm dictyosomes, endoplasmic reticulum, mitochondria and translucent vesicles are often observed. The arrows indicate the secondary thickened walls of the tracheary elements. M: 6000x


A
d
d
v
v
2µm
B
te
te
5µm

L. usitatissimum

Plate 49A: LM (TS) of a Day 10 hypocotyl (about 5mm beneath the cotyledons) illustrating the location of the various tissues. The small arrows indicate the "swollen" epidermal cells and the large arrows the fibres which appear as flattened cells with secondary thickened walls. M: 125x

Plate 49B: SEM showing part of the Day 10 upper hypocotyl; note the rows of "swollen" epidermal cells with the normal epidermal cells and stomata (arrows) in between them. M: 100x


A
200µm


B
sec

L. usitatissimum

Plate 50A: LM (PS) of an epidermal strip from a Day 10 hypocotyl. Note the presence of the normal epidermal cells, "swollen" epidermal cells and stomatal guard and subsidiary cells. The arrow indicates a nuclear inclusion in one of the subsidiary cells. M: 450x

Plate 50B: LM (PS) showing several Day 10 epidermal cells. Note the presence of nuclear inclusions (arrows) inside the uni- or binucleolate nuclei. M: 450x

Plate 50C: TEM (TS) of a Day 10 hypocotyl showing fibre cells. The two cells on the right appear as highly vacuolated and the secondary walls have pulled away from the primary walls whilst the two central cells still contain dense cytoplasm and the walls have not separated. M: 10800x

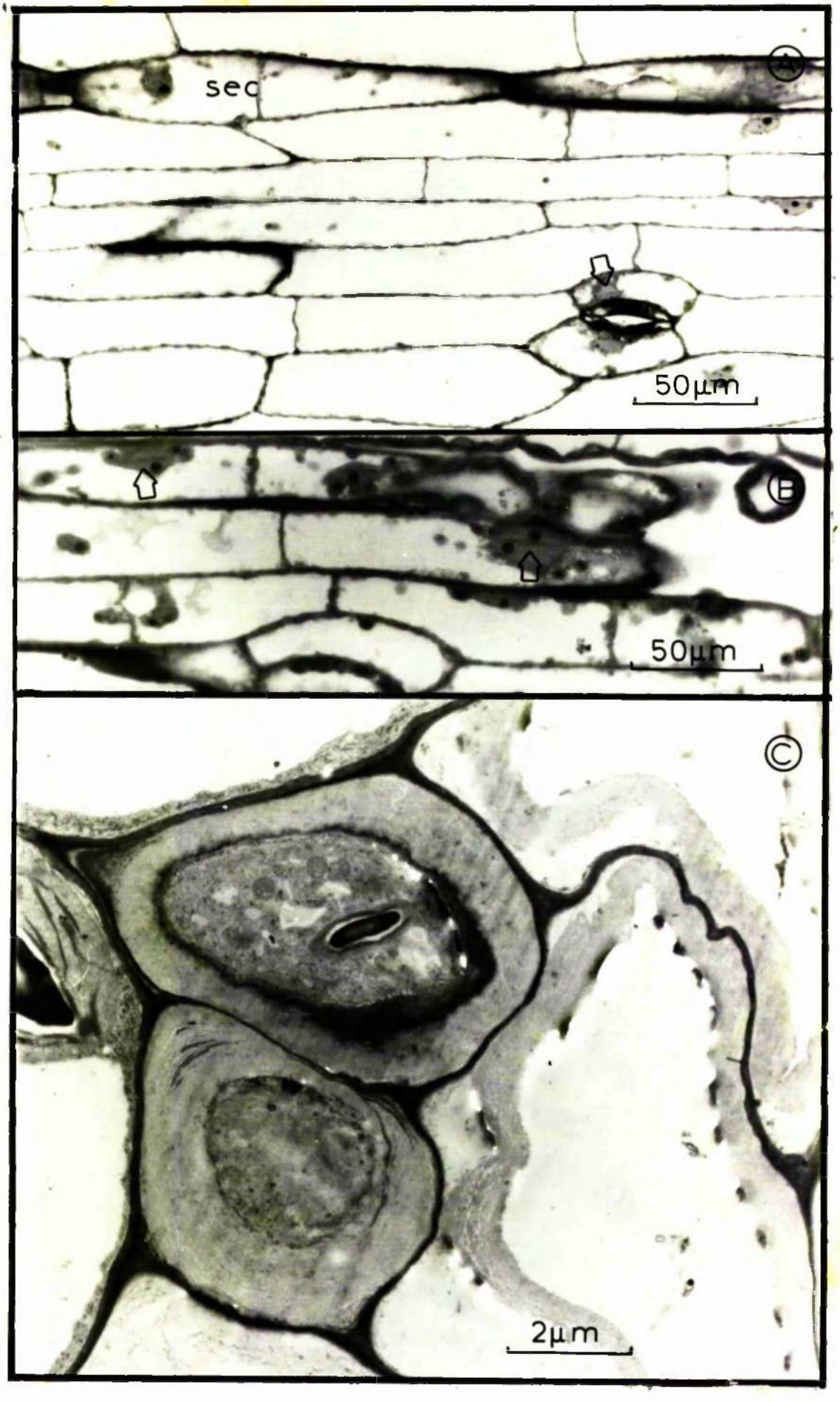
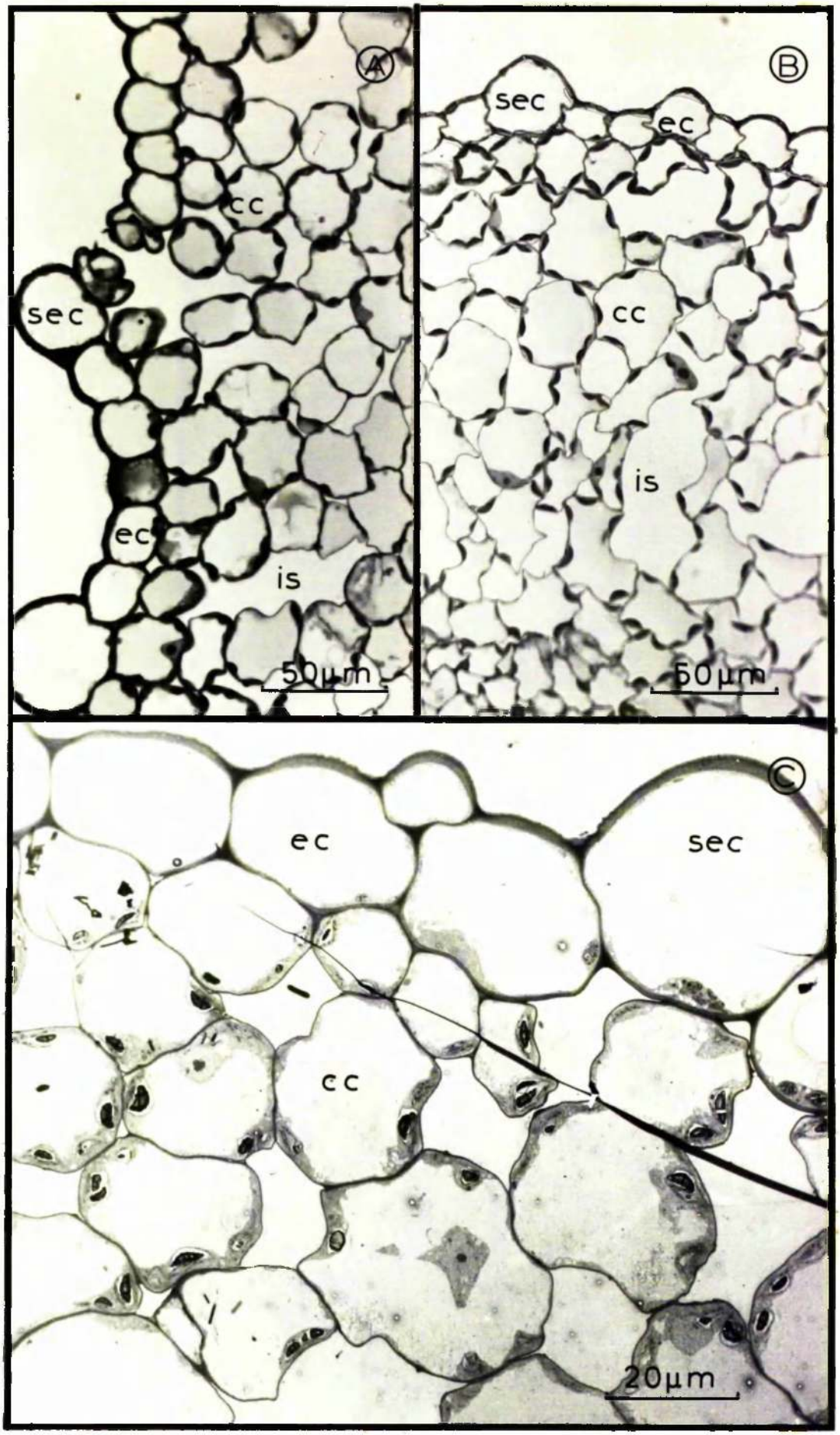

sec
A
50µm
B
50µm
C
2µm

Plate 51A: LM (TS) of a Day 10 hypocotyl showing the outer cortical and epidermal tissues. Note that the shape of the cortical cells is more-or-less round; although these cells contain relatively few chloroplasts they are nevertheless more abundant than in those in the mid and inner cortex (c.f. Pl. 49A). M: 450x

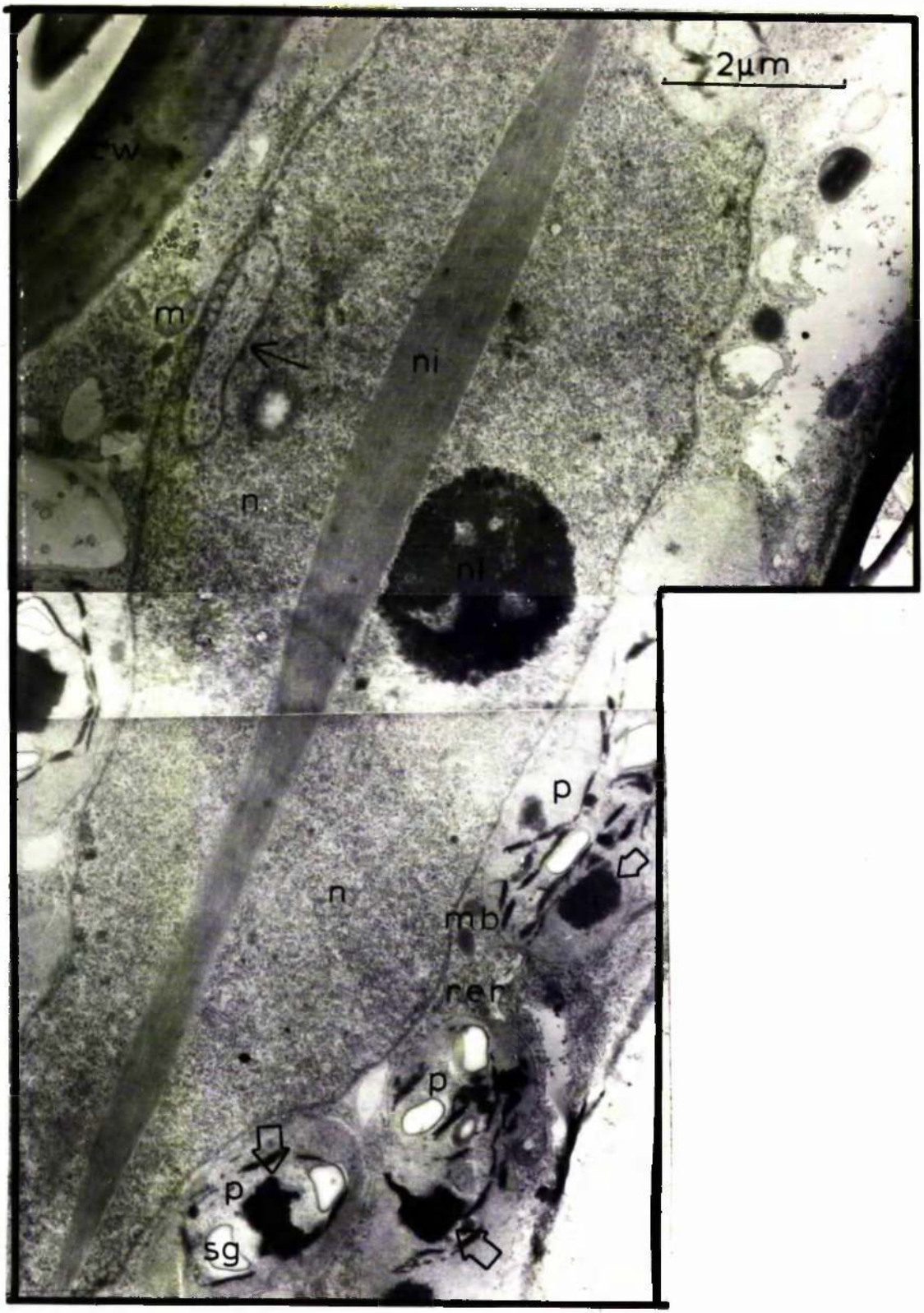
Plate 51B: LM (TS) of a Day 10+5 hypocotyl showing part of the cortical and epidermal tissues. Note that the shape of cortical cells is now irregular and somewhat reminiscent of spongy mesophyl, whilst more chloroplasts are now present, even in the inner cortical cells. M: 450x

Plate 51C: TEM (TS) of a Day 10+8 hypocotyl showing some epidermal and cortical cells. Note the presence of massive starch grains inside the chloroplasts of the cortical cells (large arrows). The presence of small starch grains (absent at Day 10, c.f. Pl. 54A) in the plastids of some epidermal cells (small arrows) may indicate cells begining to dedifferentiate. Note also the cortical nucleus (solid arrow) which lies in the centre of one cell and with cytoplasmic extensions from its margins which presumably link with the peripheral cytoplasm. M: 1200x


A
sec
cc
ec
is
50µm
B
sec
ec
cc
is
50µm
C
ec
sec
cc
20µm

L. usitatissimum

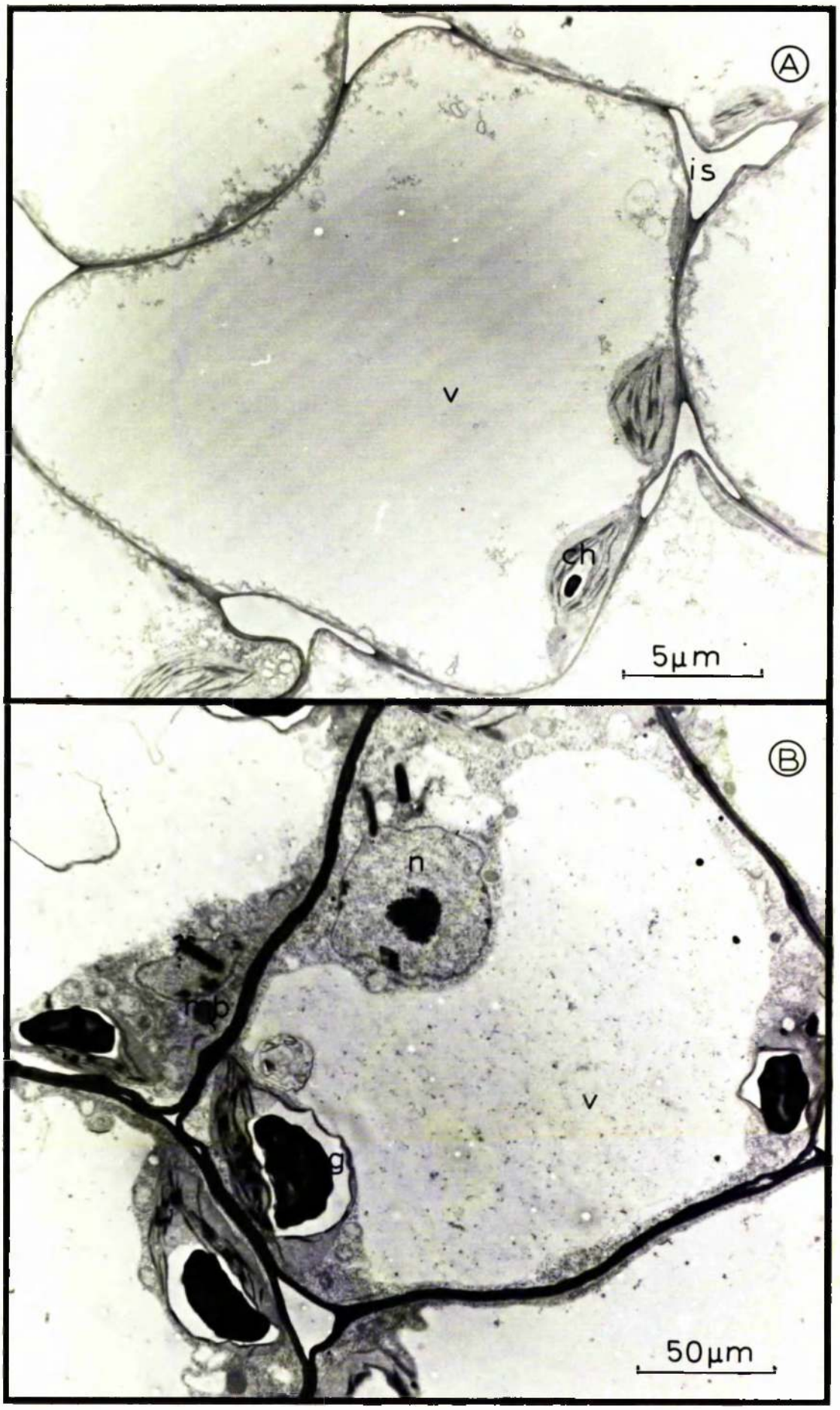
Plate 52: TEM (PS) from a Day 10+8 hypocotyl. The plastids have developed some starch grains which probably indicates the initiation of the dedifferentiation process leading to the development of an adventitious bud. The large arrows indicate some densely stained bodies in the plastids with irregular outlines, possibly representing lipids; the small arrows indicate densely stained, granular bodies of phytoferritin; whilst the solid arrows indicate some plastoglobuli. The outline of the nucleus appears somewhat irregular and a cytoplasmic invagination is visible (thin arrow). Note the presence shape, size and sub-structure of the nuclear inclusion and also its close association with the nucleolus; the latter appears to be vacuolated and with some dense and less dense (granular) zones. Note also the presence of microbodies in the cytoplasm.
M: 15000x


2µm
cw
m
ni
n
ni
p
n
mb
reh
p
p
sg

L. usitatissimum

Plate 53A: TEM (TS) of a Day 10 cortical cell of the hypocotyl. Note the very thin cytoplasm lining the thin cell walls and the occasional relatively small starch grains in the chloroplasts. M: 5160x

Plate 53B: TEM (TS) of a Day 10+8 cortical cell of the hypocotyl. Note the increased amount of cytoplasm lining the thickened cell walls and the larger chloroplasts with massive starch grains as compared to Pl. 53A. M: 5160x

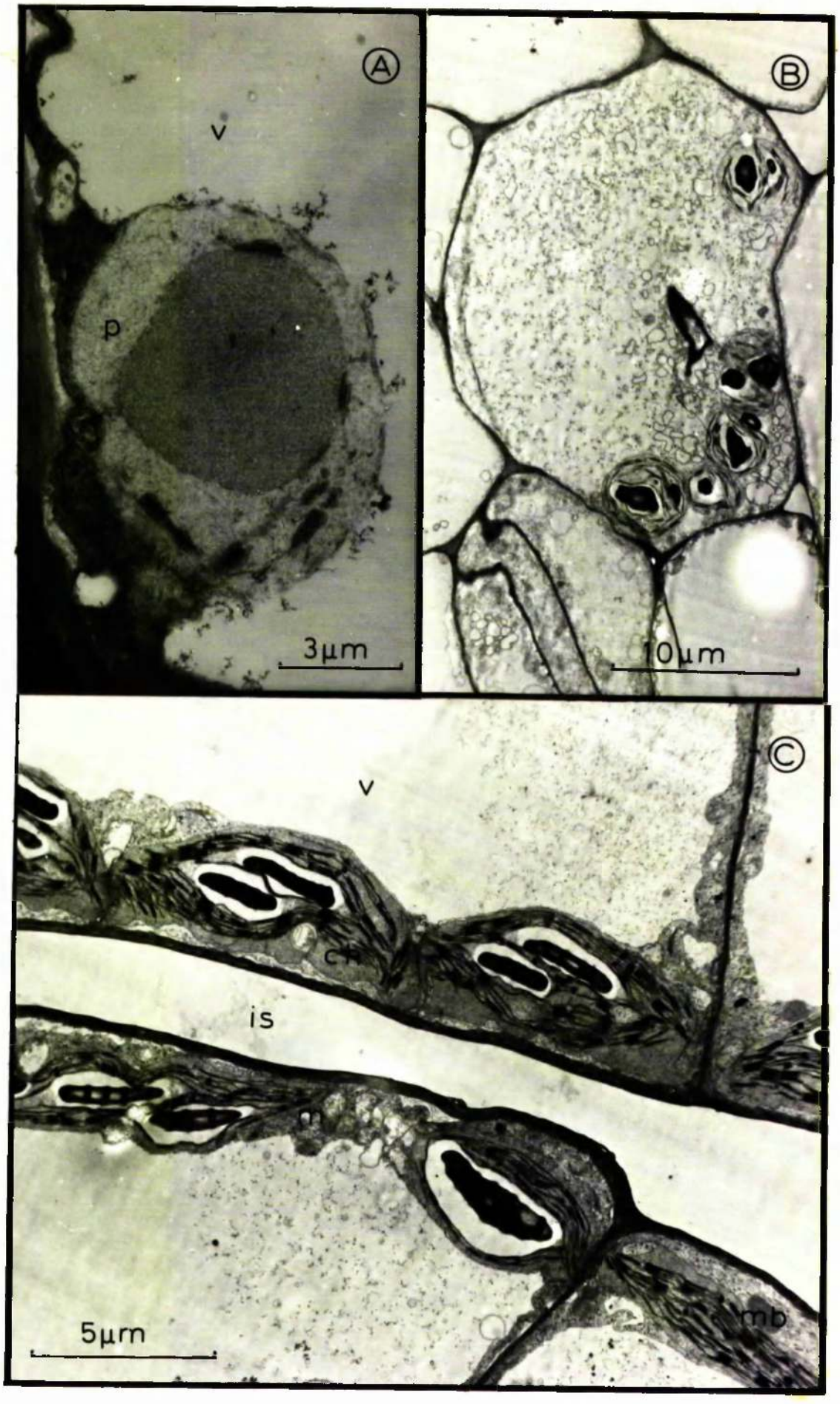

A
is
v
ch
5µm
B
n
rep
v
g
50µm

L. usitatissimum

Plate 54A: TEM of a typical plastid of an epidermal cell of a Day 10 hypocotyl. Note the few thylakoids and the amorphous central densely stained body. All epidermal plastids appear similar except for some decapitated hypocotyls, where in some cells small starch grains occur (c.f. Pl. 52) and such cells are considered to be sites of bud initiation. M: 7500x

Plate 54B: TEM (TS) of the inner cortex of a Day 10 hypocotyl. These cells are considered to be the initials of the hypocotyledonary fibres (c.f. Pl. 49, large arrows); note the presence of prominent starch grains in the chloroplasts and numerous vesicles in their protoplasts which are features that are not normally observed in the adjacent cortical cells. (c.f. Pl. 53A). M: 3300x

Plate 54C: TEM (RLS) from the cortex of a Day 10+8 hypocotyl; note the irregular outlines of the chloroplasts and their large starch grains showing an inner densely stained region and a translucent area surrounding it; also note the presence of microbodies in the cytoplasm. M: 6500x

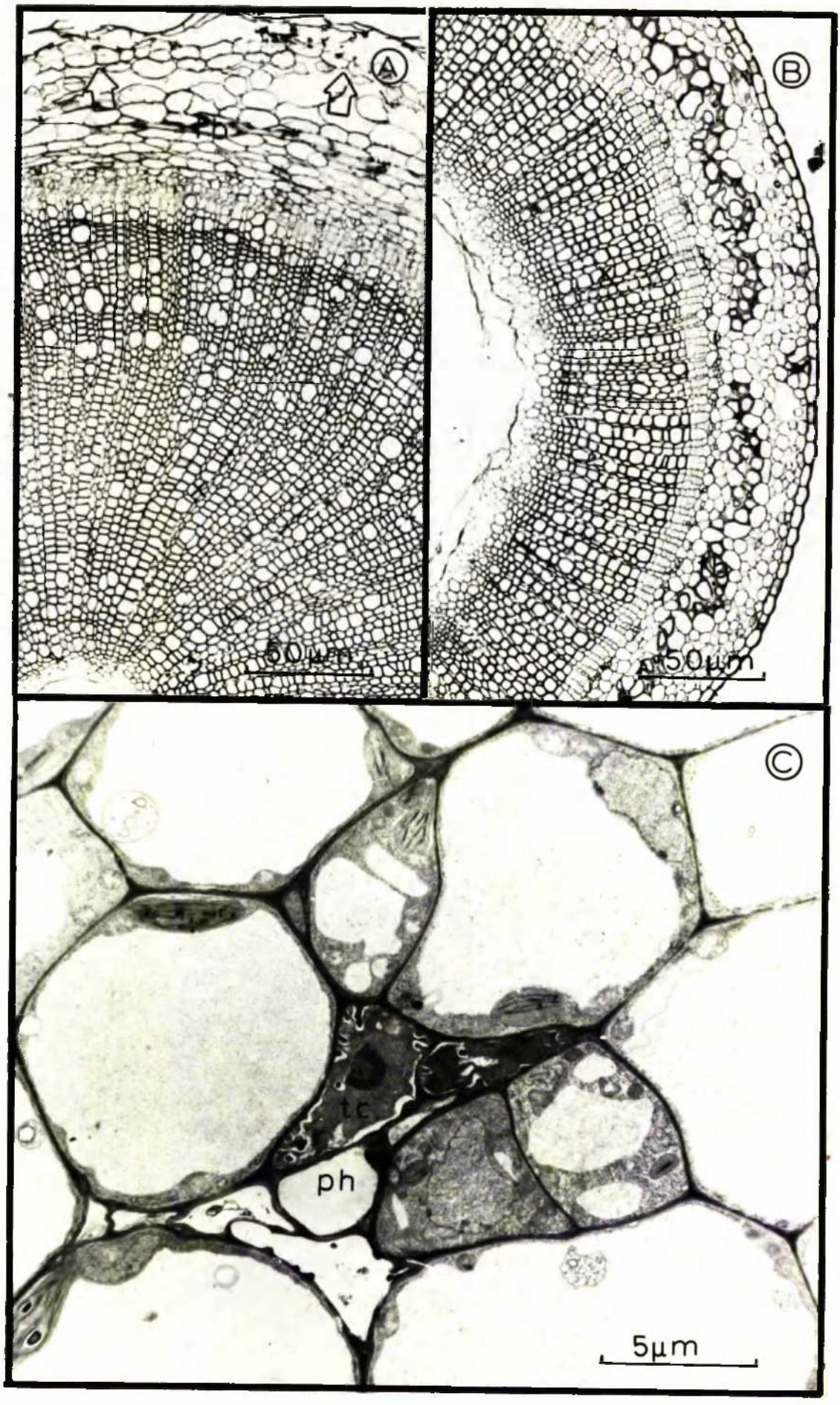

A
v
p
3µm
B
10µm
C
v
cw
is
pm
mb
5µm

L. usitatissimum

Plate 55A: LM (TS) of the Day 40 (control) hypocotyl. Note the extensively developed secondary xylem and the developing cork cambium (arrows) beneath the senescent epidermal cells. The fibres appear no further developed than these at Day 10 (c.f. Pl. 49A). M: 450x

Plate 55B: LM (TS) of the Day 10+30 (experimental) hypocotyl beneath a dominant bud. Note the limited development of secondary xylem, the large pith cavity, the extensive development of fibres and the intact epidermis in comparison to Day 40 (c.f. Pl. 55A) hypocotyl. M: 450x

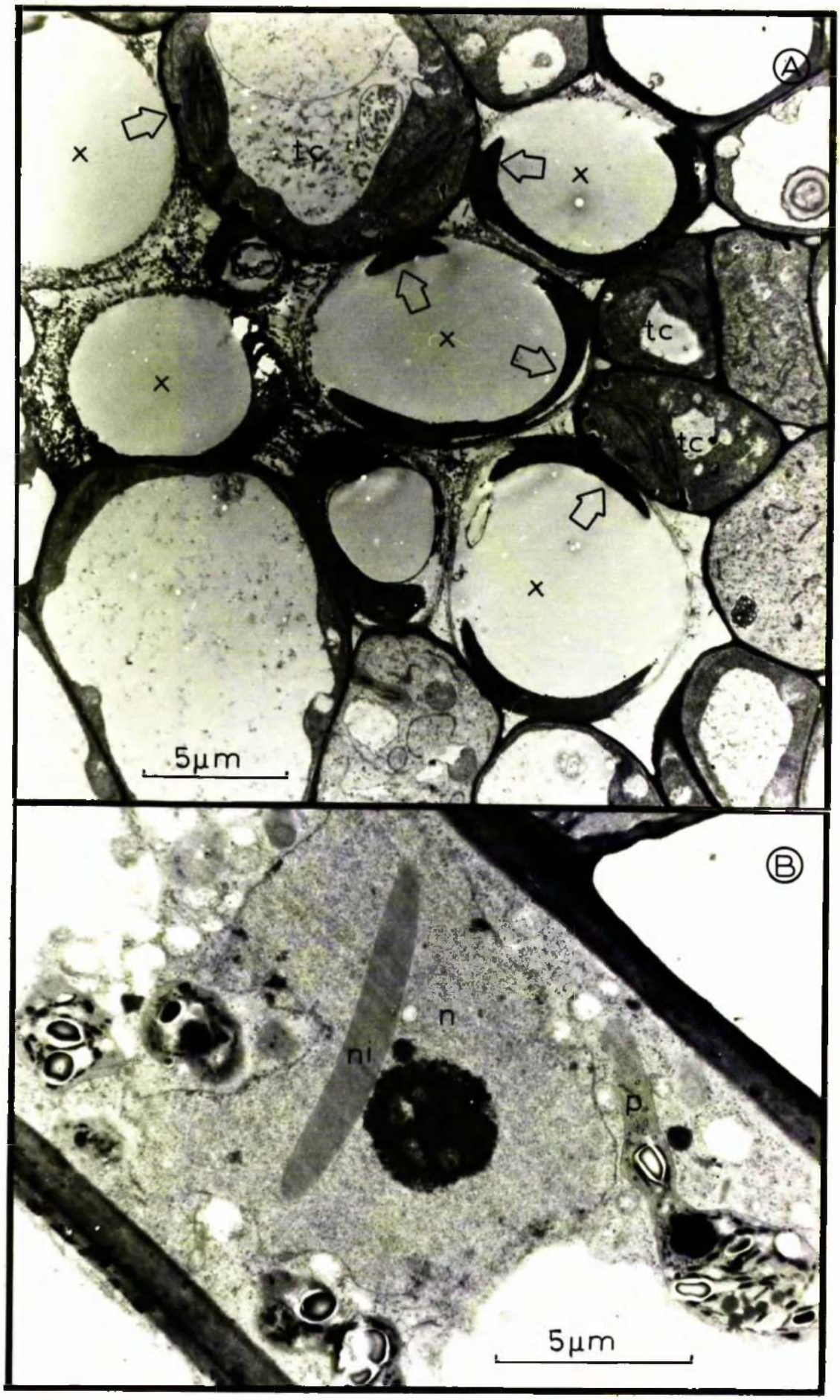
Plate 55C: TEM (TS) of a Day 10+10 hypocotyledonary main (axial) vascular strand. Note the presence of transfer cells in relation to phloem elements. The transfer cell wall ingrowths are uniformly distributed on all cell walls. M: 5600x


A
50µm
B
50µm
C
tc
ph
5µm

L. usitatissimum

Plate 56A: TEM (TS) of a Day 10+10 hypocotyledonary axial vascular strand. Note the presence of transfer cells which possess wall ingrowths (arrows) only on the walls in contact with xylem elements (in contrast to the uniform development of wall ingrowths in relation to phloem, c.f. Pl. 55C)
M: 5600x

Plate 56B: TEM (PS) of a Day 10+8 epidermal cell; the nucleus has lost its normal spindle shape and the nuclear inclusion, now lies across the long axis of the cell; also note the shape of the plastids which now contain some small starch grains. The absence of these features from the other epidermal cells of the same material (which are of similar cytological character to the control material, c.f. Pl. 54A) suggests that this epidermal cell might be beginning to dedifferentiate. M: 7200x

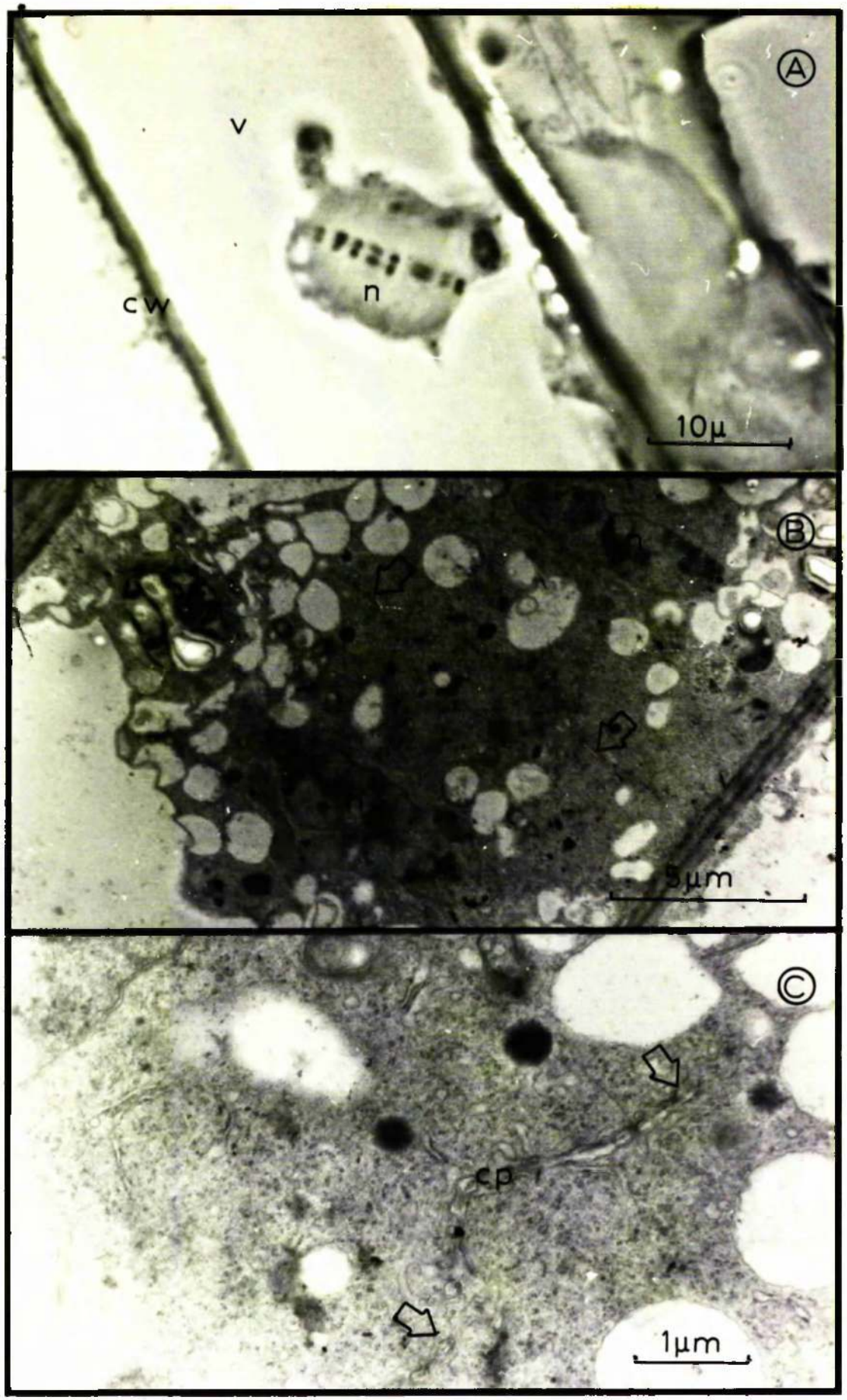

A
x
tc
x
x
tc
x
tc
x
5µm
B
n
ni
p
5µm

L. usitatissimum

Plate 57A: LM (PS) of a Day 10+8 epidermal cell illustrating the obliquely orientated anaphase plate of what it is believed to be the first division in the initiation of an adventitious bud. M: 2400x

Plate 57B: TEM (PS) of a Day 10+8 epidermal cell showing the formation of the cell plate (arrows) between the daughter nuclei resulting from the first division of an epidermal cell. M: 7200x

Plate 57C: TEM (PS) detail of Pl. 57B illustrating the formation of the cell plate (arrows). M: 20000x


A
v
n
cw
10µ
B
5µm
C
cp
1µm

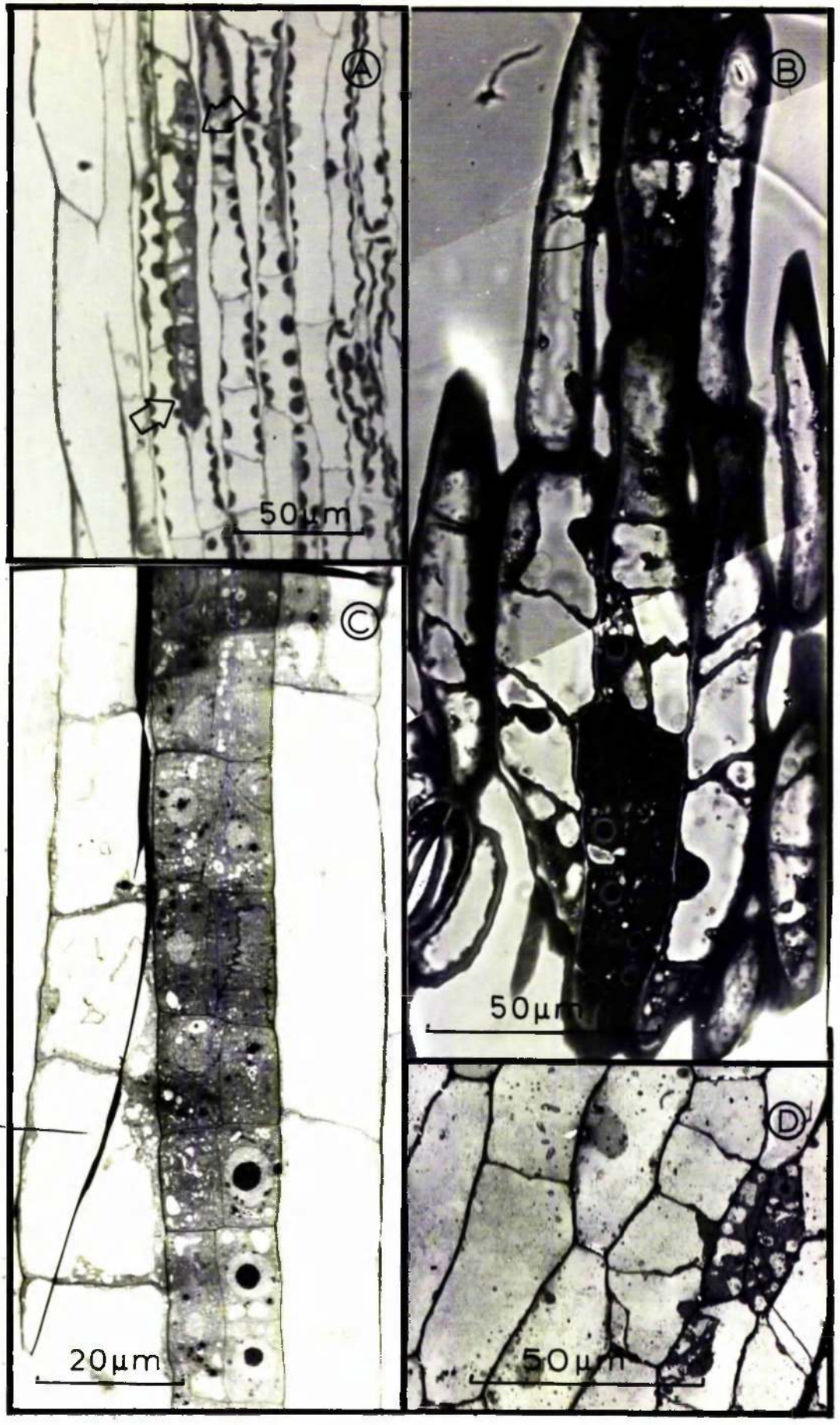
L. usitatissimum

Plate 58A: LM (PS) of a Day 10+8 hypocotyl showing a bud initiation; the arrow indicates the daughter cells which are derived from a single mother epidermal cell and these are still within the original limits of the cell walls of the mother cell. M: 450x

Plate 58B: LM (PS) of an adventitious bud initiation; several epidermal cells have undergone divisions and some of the daughter cells have reached the primary meristematic stage. M: 800x

Plate 58C: TEM (PS) of an adventitious bud initiation; several mother epidermal cells have undergone division and dedifferentiation and become meristematic whilst some others have divided less frequently and they are still highly vacuolated. M: 1200x

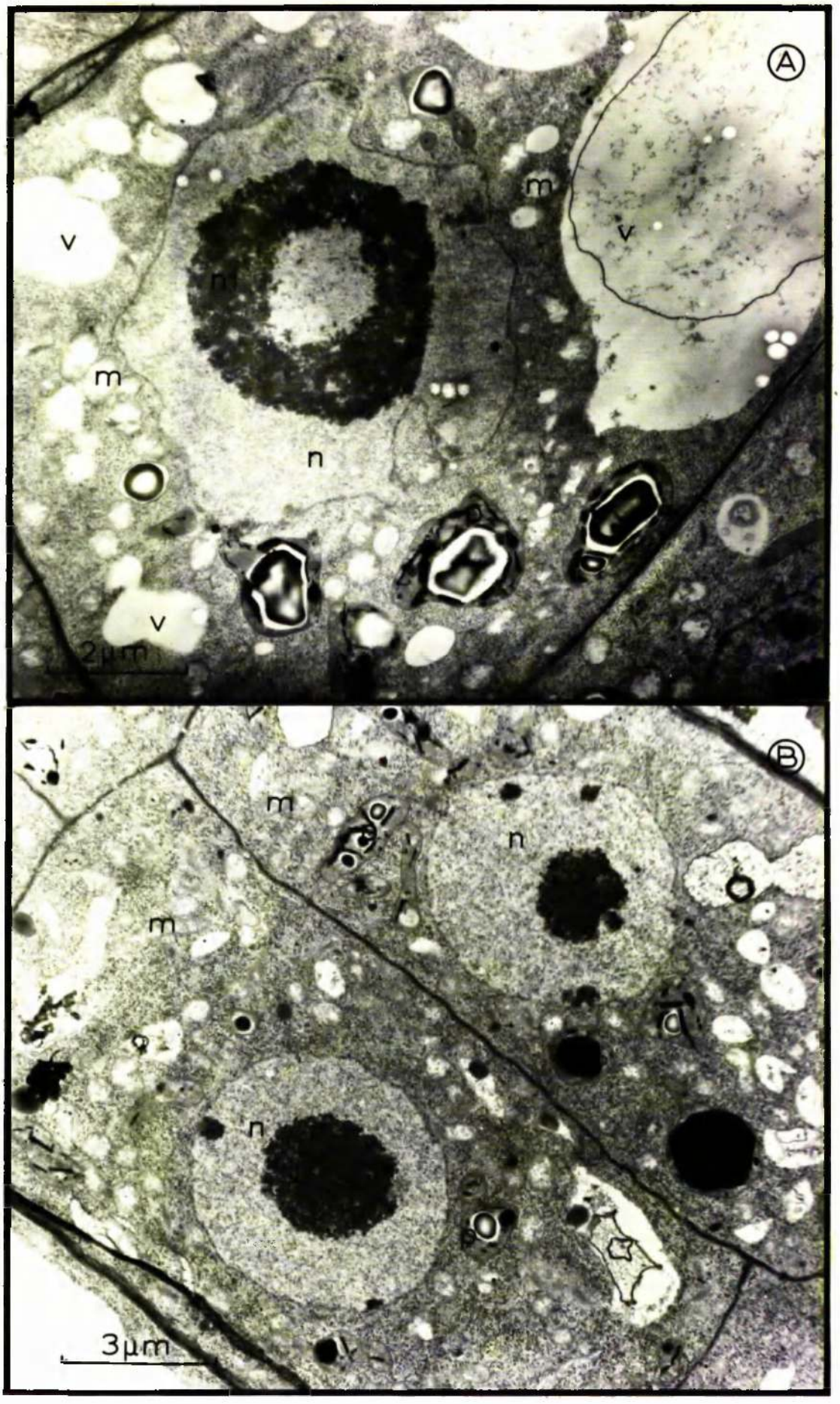
Plate 58D: TEM (PS) similar to Pl. 58C but here all epidermal cells surrounding the mother cell undergo dedifferentiation at the same time. M: 800x


A
50µm
B
50µm
C
20µm
D
50µm

L. usitatissimum

Plate 59A: TEM (PS) of a daughter epidermal cell during dedifferentiation leading to adventitious bud development; this cell has not reached yet the primary meristematic stage; some relatively large starch grains are present in the plastids, vacuoles are still prominent and the nucleus has an irregular outline with a vacuolated nucleolus showing granular and fibrillar zones. The arrows indicate some plasmodesmata penetrating the cell walls. M: 12500x

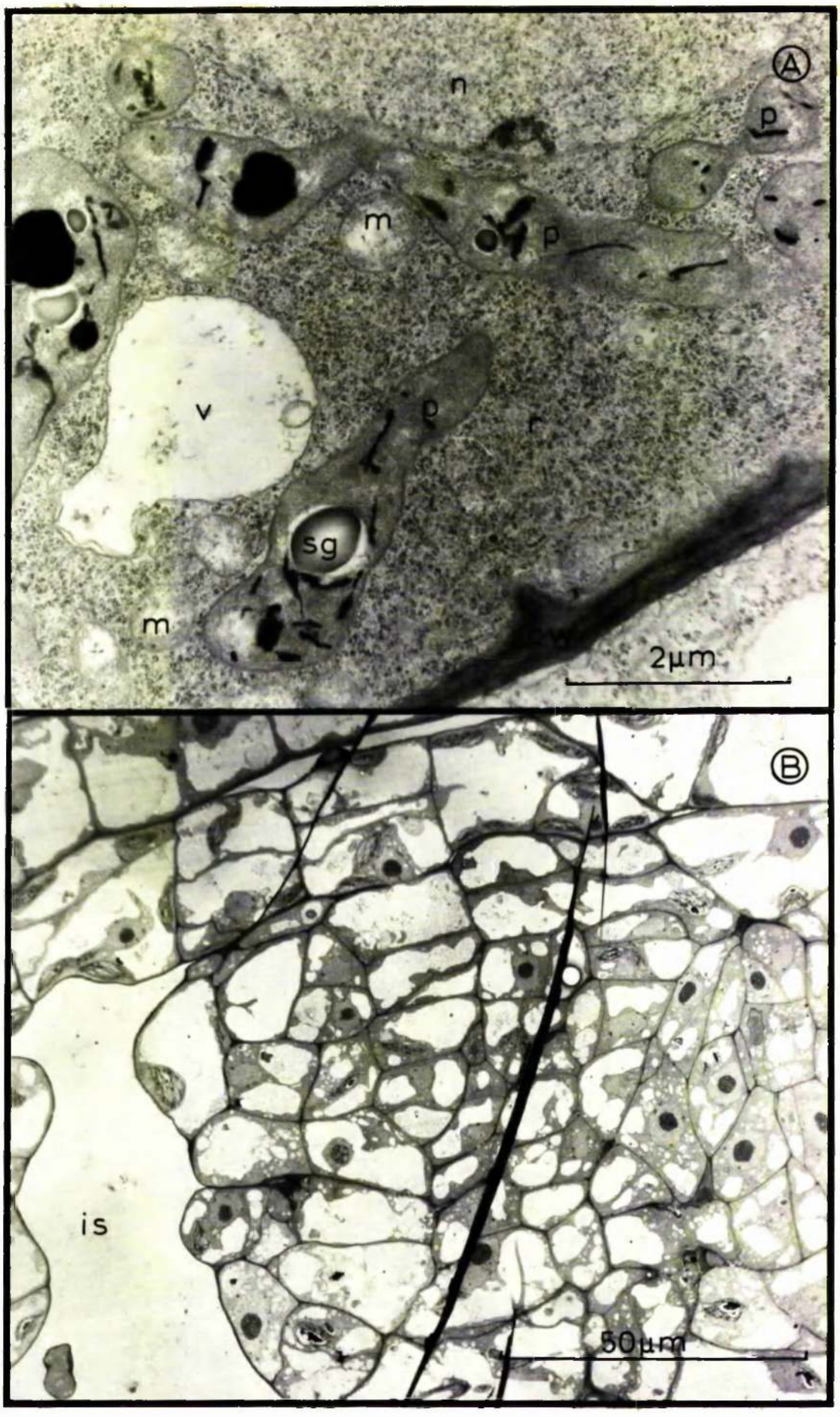
Plate 59B: TEM (PS) of daughter epidermal cells which seem to have reached the primary meristematic stage. Some starch grains are still present in the plastids which are much smaller in non-activated epidermal cells; only small vacuoles are found in the cytoplasm, the nuclear outlines are more regular (round) and nucleoli are non-vacuolated, although the fibrillar and granular zones are visible; the dense cytoplasm is crowded with free ribosomes. The small arrows indicate some plasmodesmata penetrating the daughter cell walls whilst the large arrows show the thicker mother epidermal cell walls. M: 9000x


A
v
m
v
n
m
n
v
2µm
B
m
n
m
n
3µm

L. usitatissimum

Plate 60A: TEM (PS) of a dedifferentiating daughter epidermal cell showing what appears to be dividing plastids. Note the packed cytoplasm with free ribosomes. The arrows show rough endoplasmic reticulum elements. M: 2000x

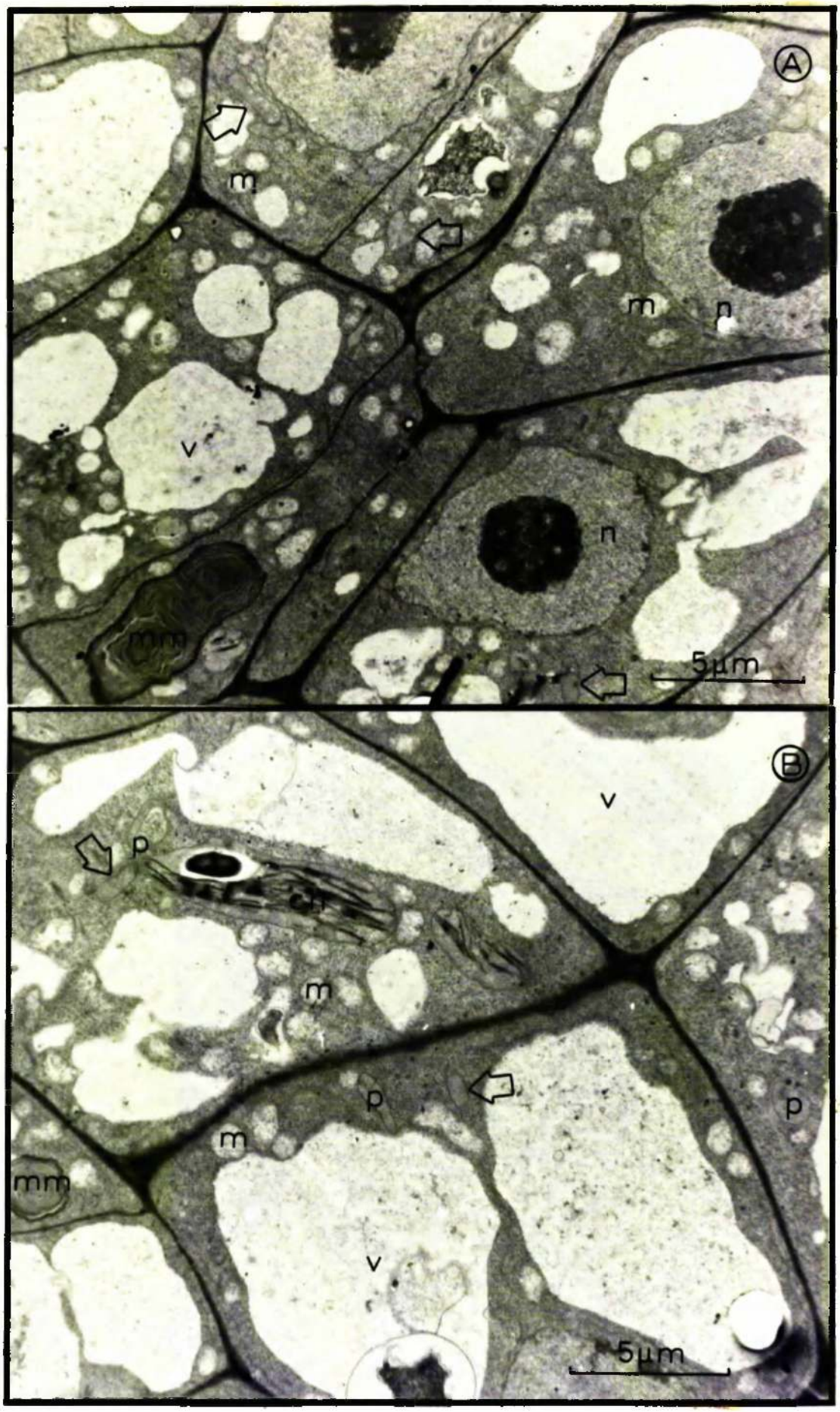
Plate 60B: TEM (PS to the hypocotyl and TS to the bud primordium) showing a group of cells at different stages of development during dedifferentiation. The cells at the top represent cortical cells which have undergone one or two divisions, they contain large chloroplasts (possibly dividing) and they are still highly vacuolated. The smaller, dedifferentiating cells (centre of micrograph) contain chloroplasts relatively small vacuoles and dense cytoplasm in comparison to the cells at the top. M: 1100x


A
n
p
m
p
v
p
r
sg
m
2µm
B
is
50µm

L. usitatissimum

Plate 61A: Detail of Pl. 60B (cells from right hand side of the micrograph). Note the presence of dense cytoplasm and numerous small vacuoles. The arrows indicate some small agranal plastids. Also note the presence of $^{a}_{v}$multi-membraneous body and lightly stained mitochondria. These cells may be of either epidermal or cortical origin and if the latter is the case, are more dedifferentiated than the ones shown in Pl. 61B. M: 5600x

Plate 61B: Detail of Pl. 60B (cells from left hand side of the micrograph) showing dedifferentiating cells which are considered to be of cortical origin since they contain small chloroplasts with grana as well as small agranal plastids (arrow). Also note the presence of a multi-membraneous body and lightly stained mitochondria. M: 5600x

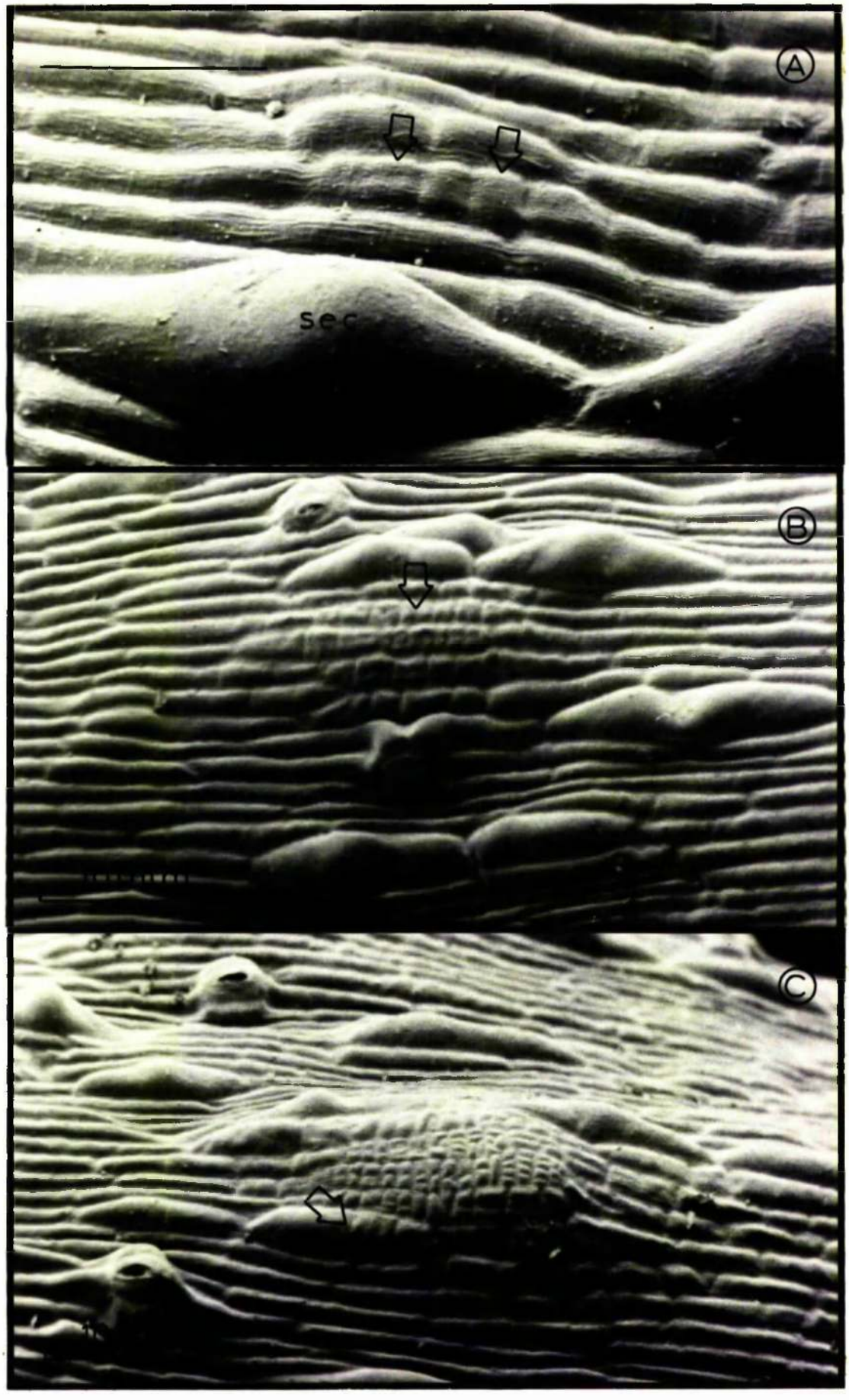

A
m
m
n
v
n
mm
5µm
B
v
p
m
p
m
p
mm
v
5µm

L. usitatissimum

Plate 62A-C: SEM's illustrating successive stages of adventitious bud development; (A) shows a very early stage where one epidermal cell has apparently undergone several transverse divisions. In (B-C) about 4 rows of normal epidermal cells are involved with numerous transverse divisions; in (B) a few radial longitudinal divisions appear to have also occurred whilst in (C), the latter divisions are abundant. Note that the "swollen" cells do not initiate bud formation but are involved later (C, arrow). No obvious relation between bud initiation and stomata is observed.

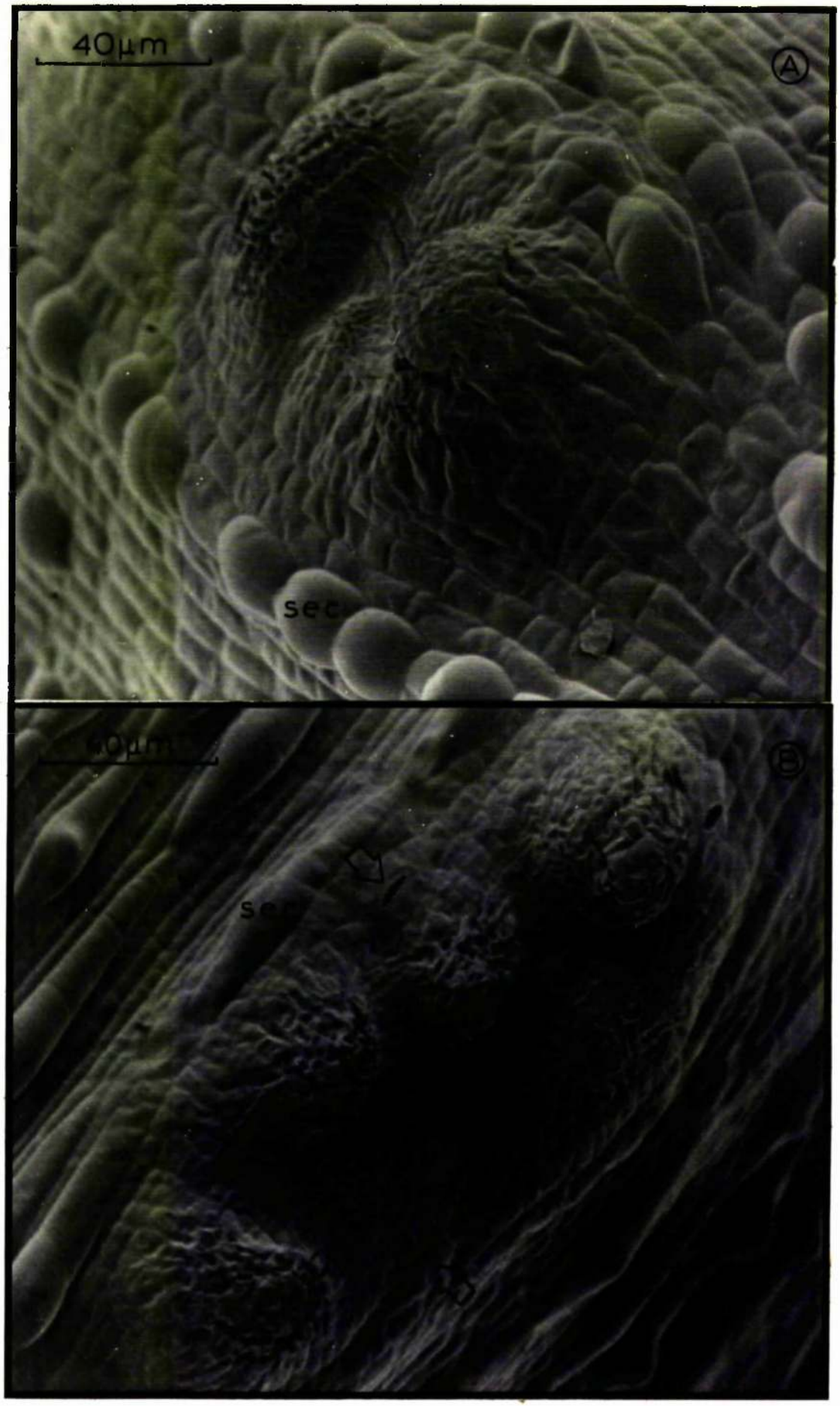
M: A: 800x

B-C: 300x


A
sec
B
C

L. usitatissimum

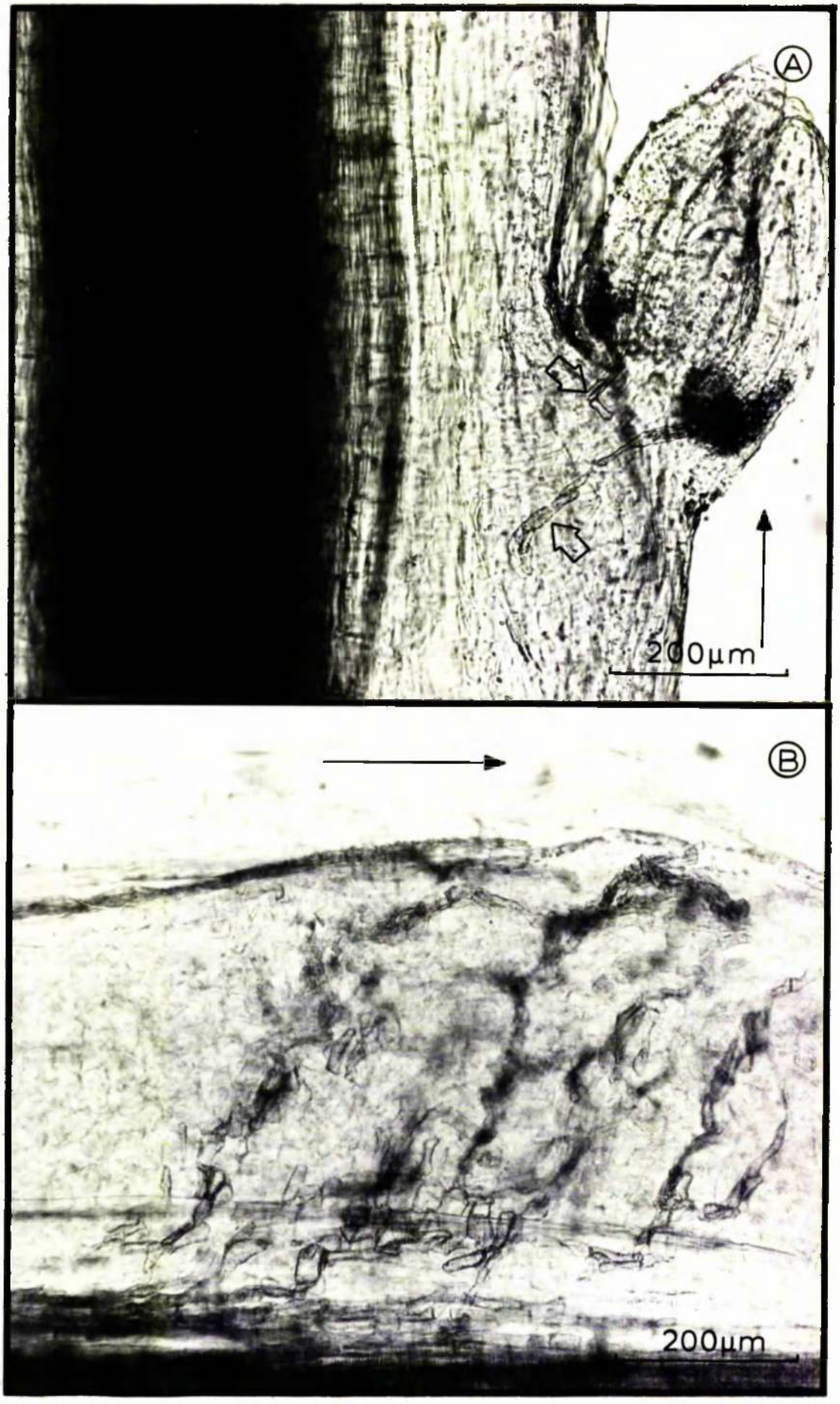
Plate 63A-B: SEM's of later stages of adventitious bud primordia; in A two leaf primordia are visible whilst in B these are more numerous, possibly due to the presence of two bud initials at the same site; note the guard cells (B, arrows) do not undergo dedifferentiation even at later stages of development. The shrivelled appearence of the leaf primordia is presumably an artifact, perhaps related to the thinner cuticle present in the compact cells compared to the mature epidermal cells of the hypocotyl. M: 750x


40µm
A
sec
B

L. usitatissimum

Plate 64A: LM of a cleared specimen showing a dormant adventitious bud primordium; note the absence of protoxylem connection to the main hypocotyledonary vascular cylinder; the arrows indicate some blindly ending tracheary strands and the long arrow indicates the direction of the long axis of the hypocotyl. M: 165x

Plate 64B: LM of a cleared specimen showing protoxylem elements differentiated in the cortex but (at varying levels of focus); no bud primordium has apparently developed in relation to them. The long arrow indicates the acropetal direction of the organ. M: 165x


A
200µm
B
200µm

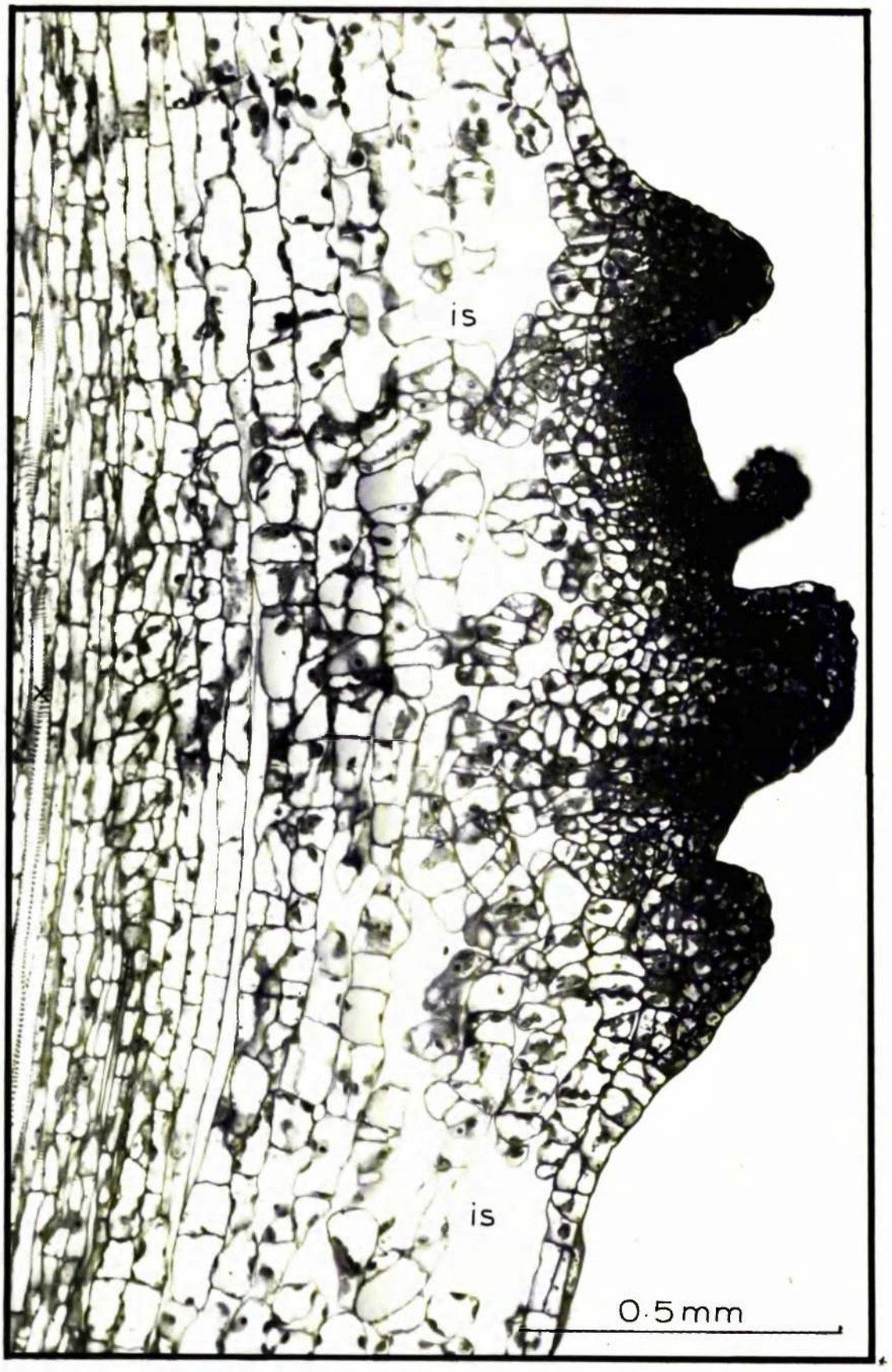
L. usitatissimum

Plate 65: LM of a cleared specimen of a Day 10+15 decapitated hypocotyl (c.f. Pl. 418); note that the two dormant buds (indicated by the arrows) have no protoxylem connections to the main cylinder, although the dominant bud (on the left) forms an extension of the main vascular cylinder. The part of the hypocotyl above the connection with the dominant bud ceases development and later abscinds. M: 50x


1mm

L. usitatissimum

Plate 66: LM (RLS) of an "oversized" adventitious bud (c.f. Pl. 63B). The morphology of this type of bud appears more complicated than the normal bud primordium (c.f. Pl. 63A) and possibly results from more than one initials. The absence of provascular tissue in the cortex underneath the bud suggests that this is a dormant bud. M: 110x


is
is
0·5mm

L. usitatissimum

Plate 67A-F: LM's (TS) serial sections (approx. 0.2mm apart) of the hypocotyl showing an adventitious bud. Plate A shows the upper end of the bud and the cortical cells show little dedifferentiation at this level whereas in B-E the cortical cells show considerable involvement. The predominantly radial longitudinal divisions in relation to the formation of provascular strands are particularly evident in the cortical cells at level E.
M: 80x

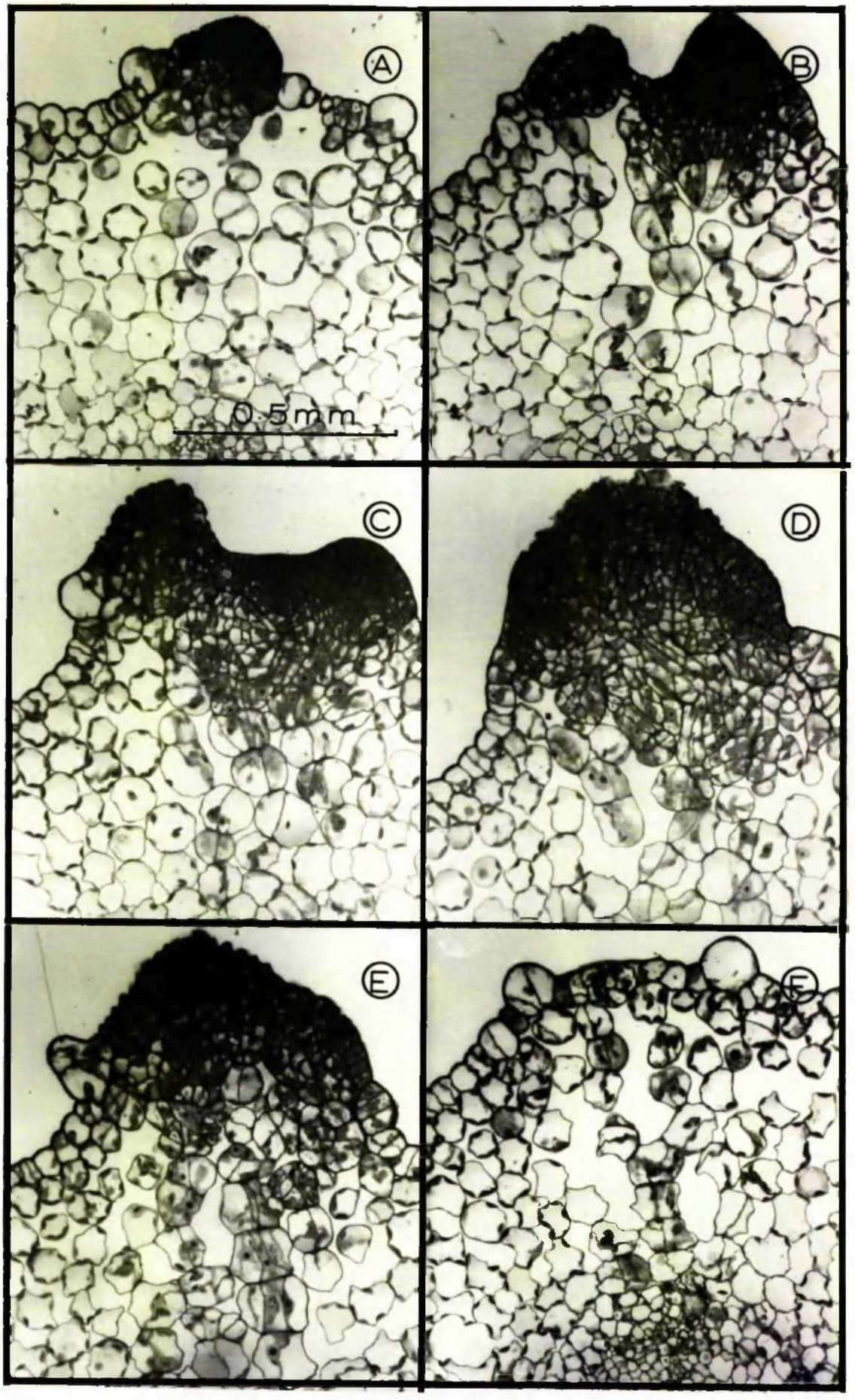
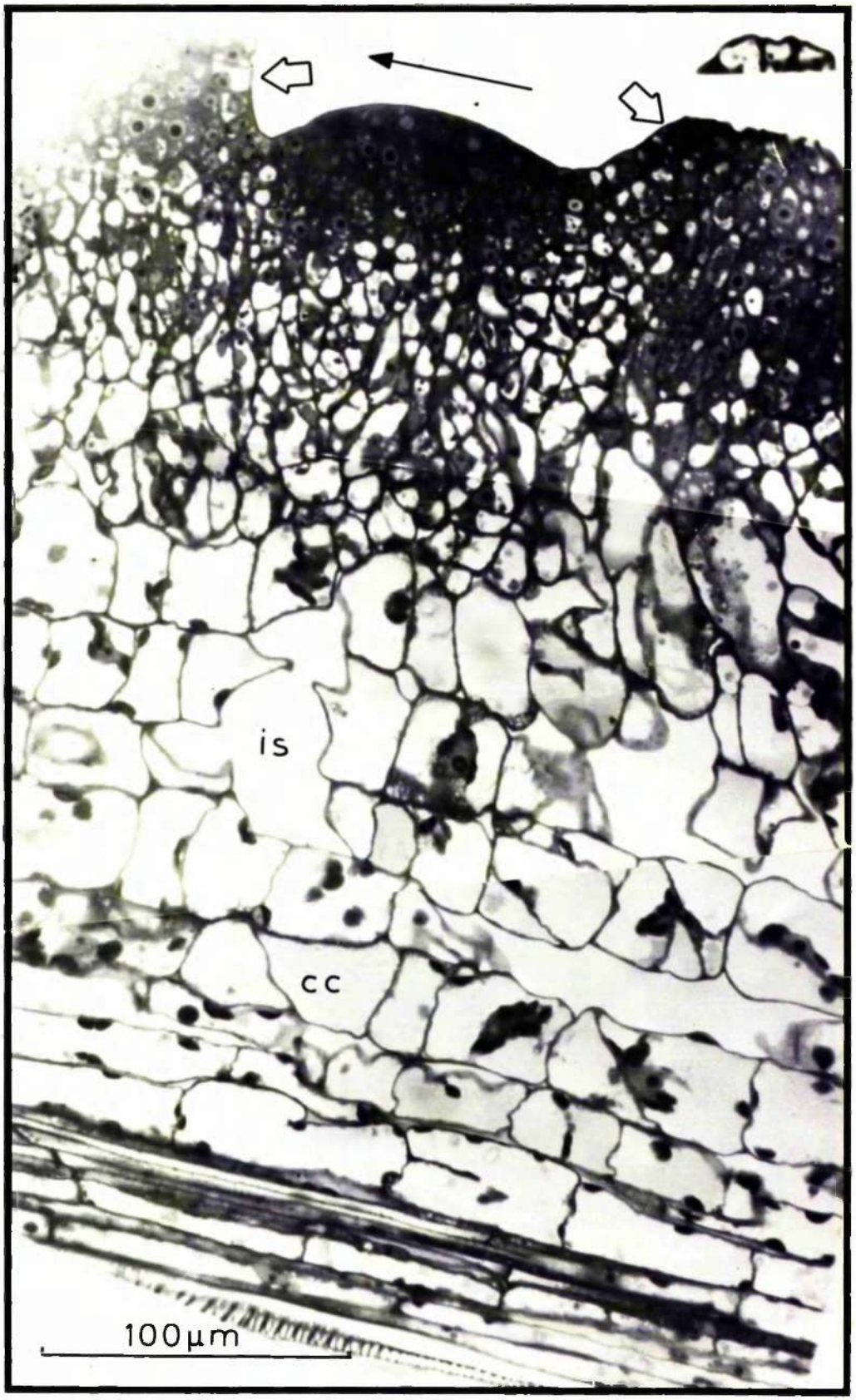

A
B
0.5mm
C
D
E
F

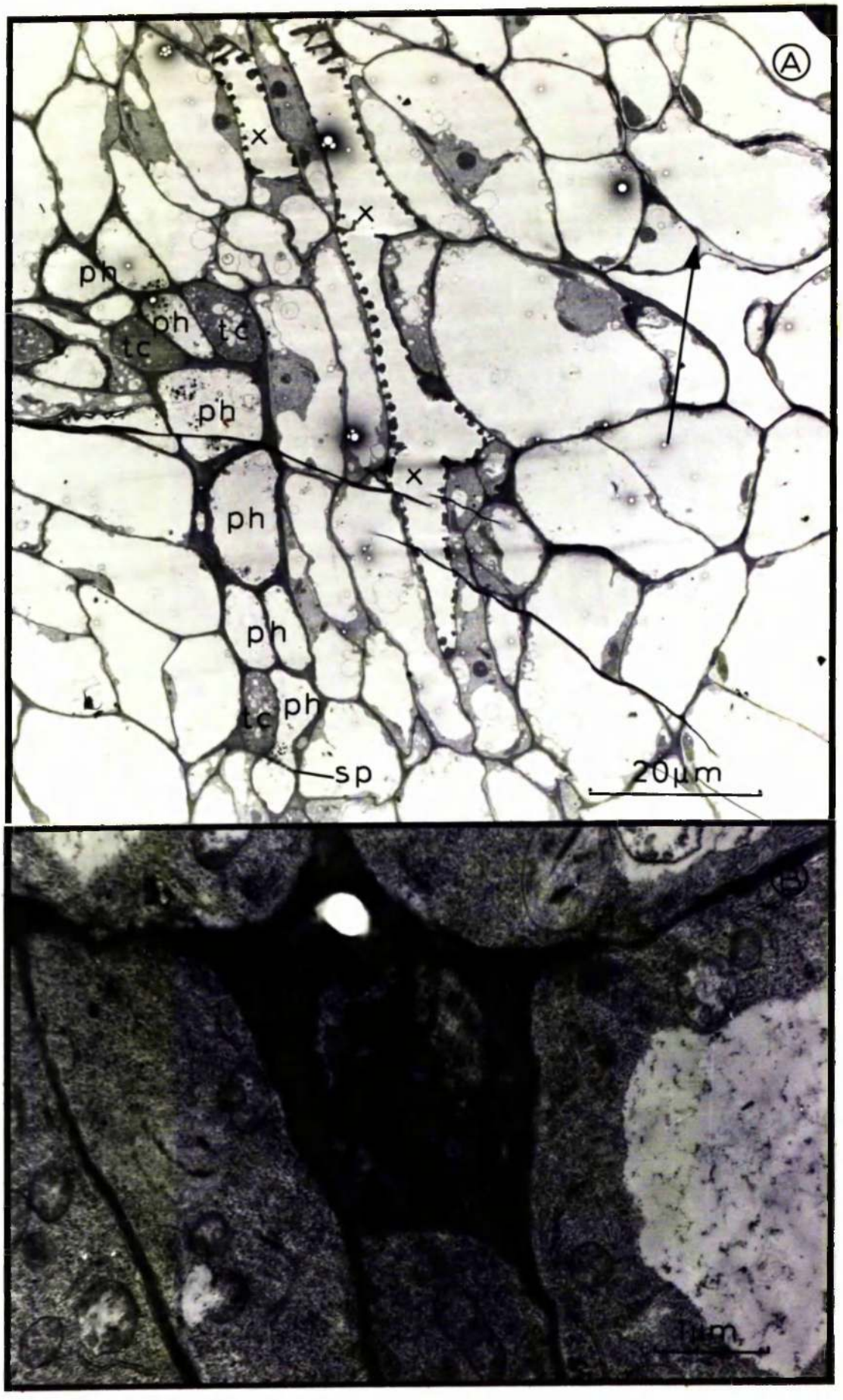
Plate 68: LM (RLS) of an adventitious bud where leaf primordia have developed (large arrows); note the divisions of the cortical cells indicating the oblique direction of the differentiating provascular strands. The cells of the bud apex are densely staining whereas beneath this region the cells are less dedifferentiated. The dotted line shows the approximate plane of the section shown in Pl. 60B and 61A-B. M: 500x


is
cc
100µm

L. usitatissimum

Plate 69A: EM (RLS) through the cortex showing the development of the tracheary and phloem elements connecting the adventitious bud to the main (axial) vascular tissue of the hypocotyl. Note the presence of transfer cells only in relation to the phloem elements. The thin arrow shows the acropetal direction. M: 1500x

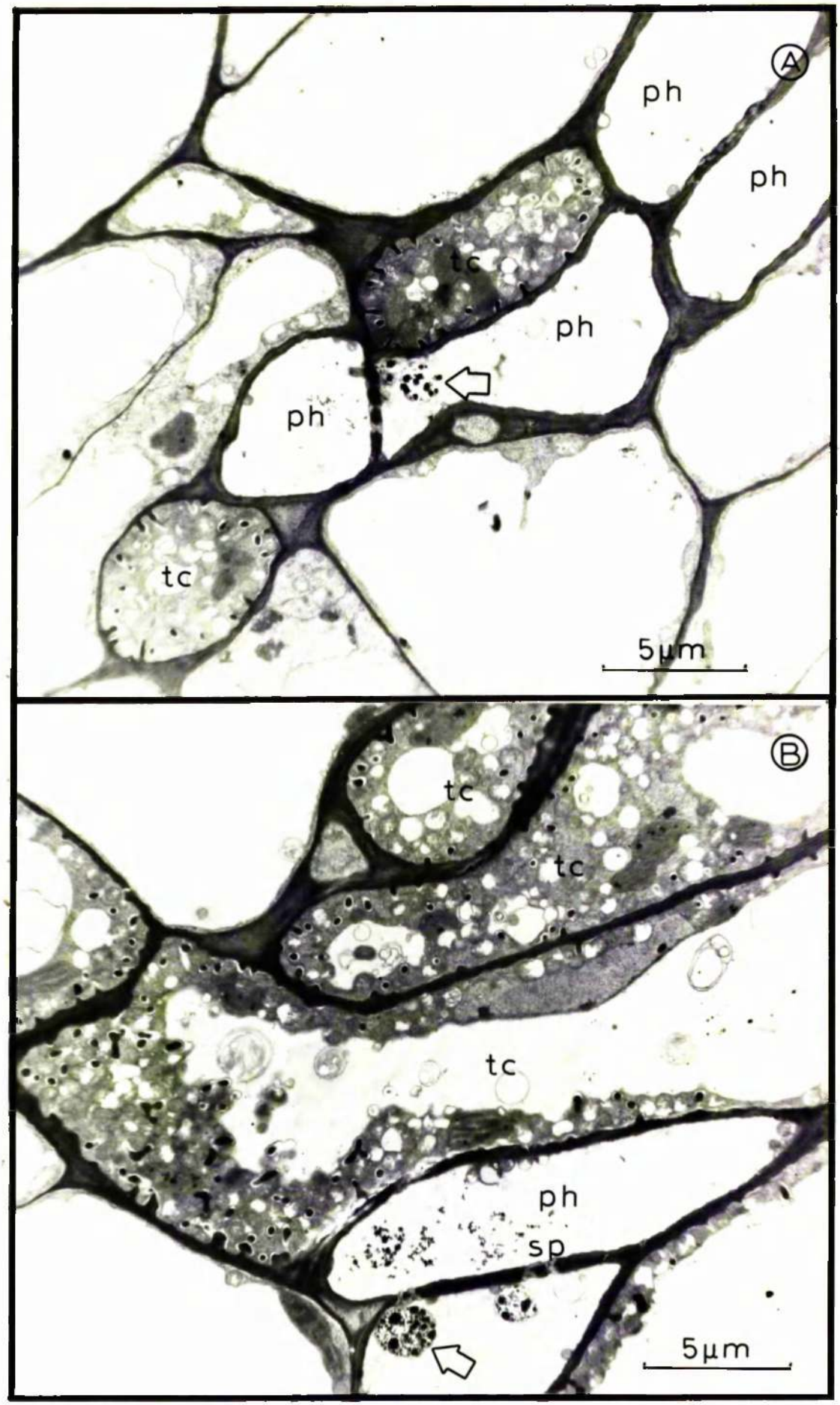
Plate 69B: EM (PS to the hypocotyl and TS to the bud primordium) showing a face view of a developing sieve plate. Note that the surrounding cells are still meristematic suggesting that phloem elements differentiate before transfer cells and protoxylem elements. M: 19800x


A
x
x
x
ph
ph
tc
tc
ph
ph
ph
ph
tc
ph
sp
20µm
B
1µm

L. usitatissimum

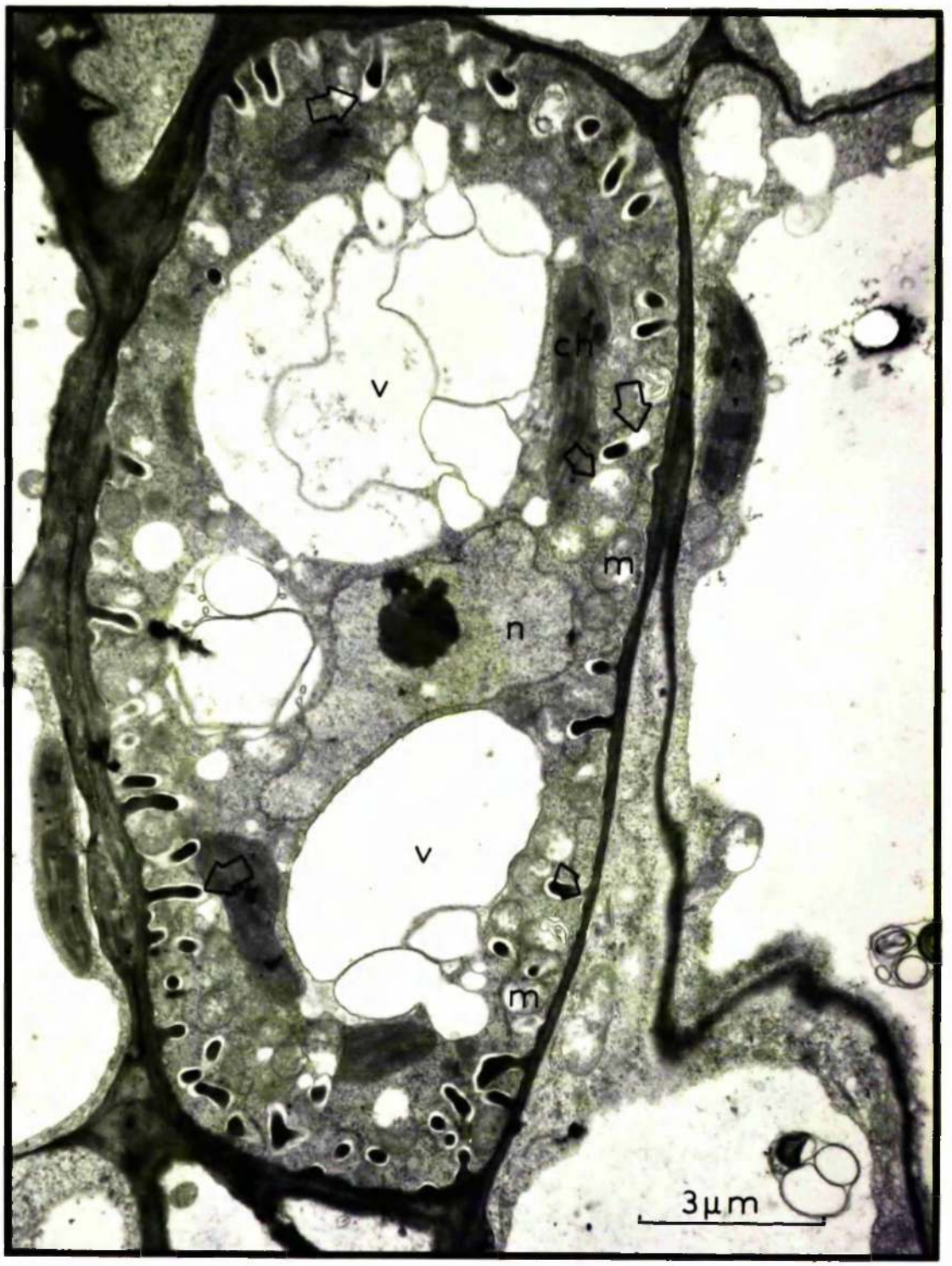
Plate 70A: Detail of Pl. 69A showing transfer cells in relation to phloem elements; note the dense cytoplasm in the former cells and the finger-like wall protrusions; the arrow indicates a highly modified plastid characteristic of sieve tubes. M: 5160x

Plate 70B: EM from an adjacent area to Pl. 69A showing further detail of the phloem transfer cells. The large central transfer cell appears vacuolated and wall ingrowths are mainly observed on the walls where the peripheral cytoplasm is more extensive. The arrow indicates a highly modified plastid characteristic of sieve tubes. M: 5160x


A
ph
ph
tc
ph
ph
tc
5µm
B
tc
tc
tc
ph
sp
5µm

L. usitatissimum

Plate 71: TEM showing a phloem transfer cell (as in Pl. 66A-B); note the dense cytoplasm containing free ribosomes, rough endoplasmic reticulum elements, and small chloroplasts. The numerous mitochondria possess some translucent areas (small arrows) and these possibly represent an artifact; also the development of the large vacuoles by amalgamation of smaller ones. The large arrows indicate translucent areas usually surrounding the densely stained regions of the wall ingrowths. M: 9000x


v
ch
m
n
v
m
3µm

L. usitatissimum

Plate 72A: LM (RLS) showing the upper hypocotyl at the point where, what is believed to be, a non-dormant adventitious bud has already grown out considerably (about 1cm). From a single section like this it is not possible to tell whether a continuity of vascular tissue is established between bud and main axis. However, more information can be obtained, if serial sections are cut transversely to the bud. M: 40x

Plate 73B-F: LM sections cut at various levels paradermal to the hypocotyl (TS to the bud axis) showing the ring of vascular bundles connecting the bud to the main cylinder of the hypocotyl; B is at the base of the bud whilst F lies near the axial vascular tissue of the hypocotyl. M: 40x

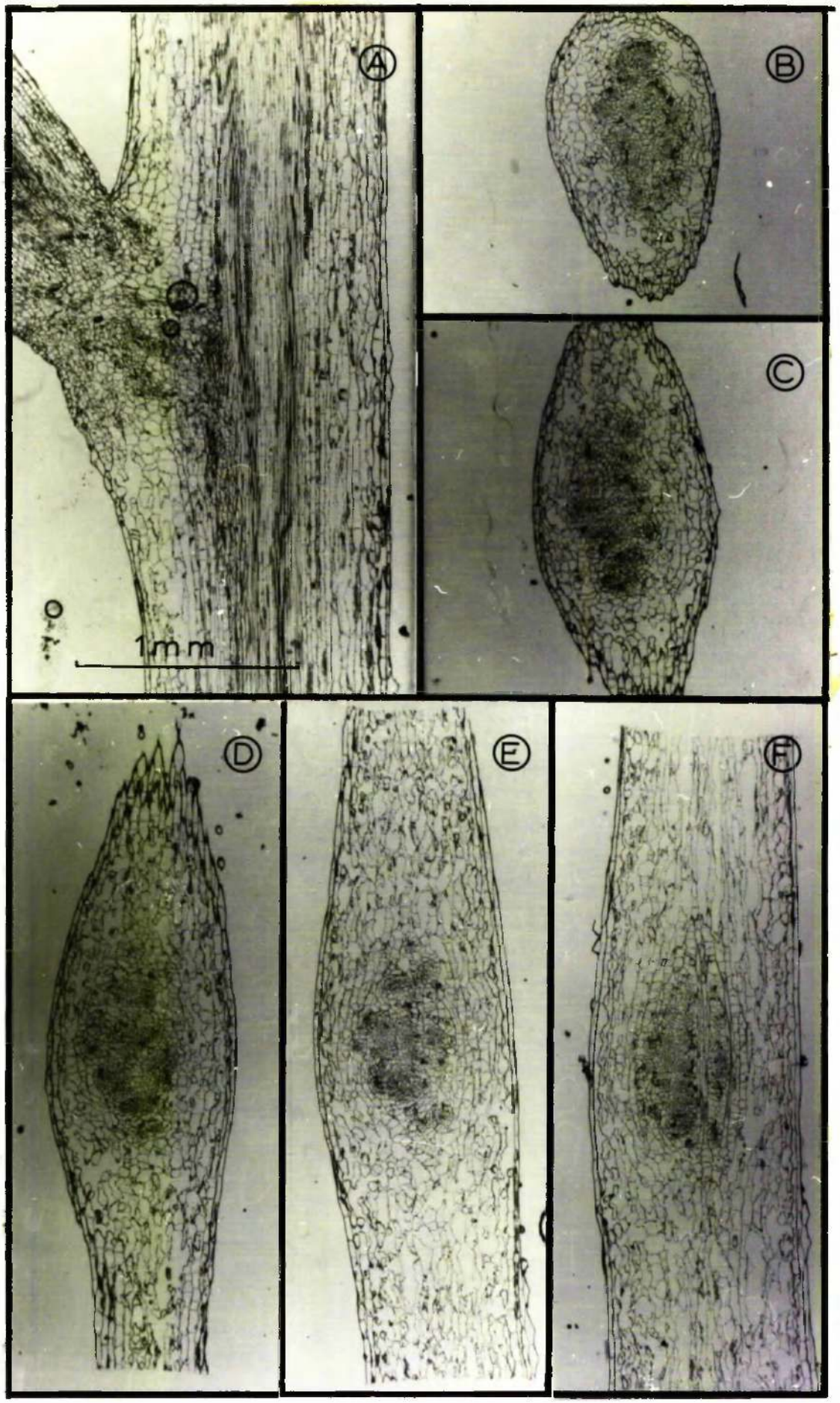

A
B
C
D
E
F
1mm

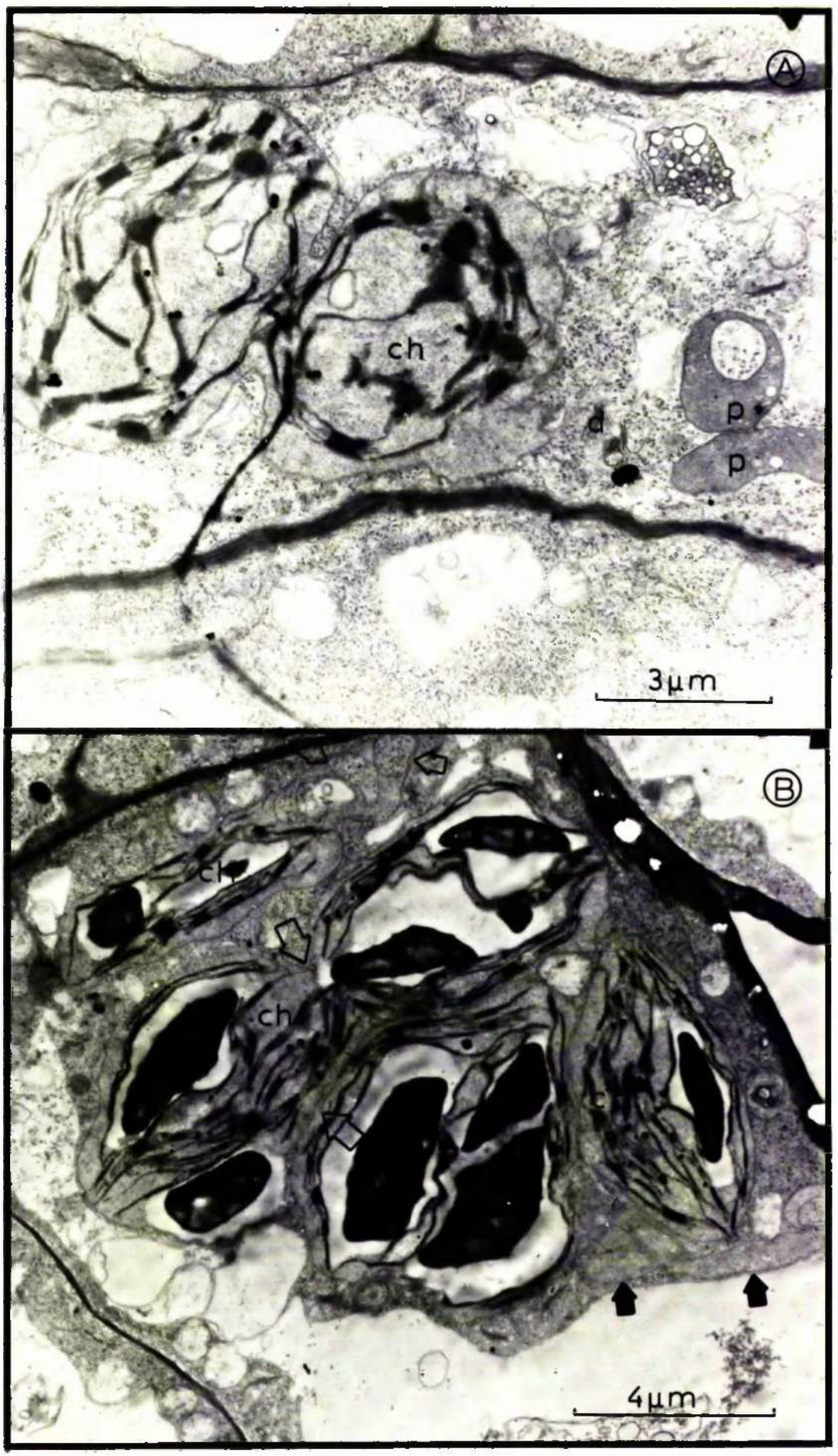
Plate 73: TEM showing a chloroplast of a Day 10+10 cortical cell (in LS) of the hypocotyl from an area remote from adventitious bud development; note the central densely stained regions of the rod shaped starch grains and the translucent areas around them, the well developed thylakoid system and also the presence of microbodies. M: 18000x


v
g
mb
v
2µm

L. usitatissimum

Plate 74A: TEM showing a possible late stage of chloroplast division in a dedifferentiating cortical cell (Day 10+10); note also the other type of plastids shown by the large arrows which are more densely stained and contain some translucent vesicles in their stroma. M: 10000x

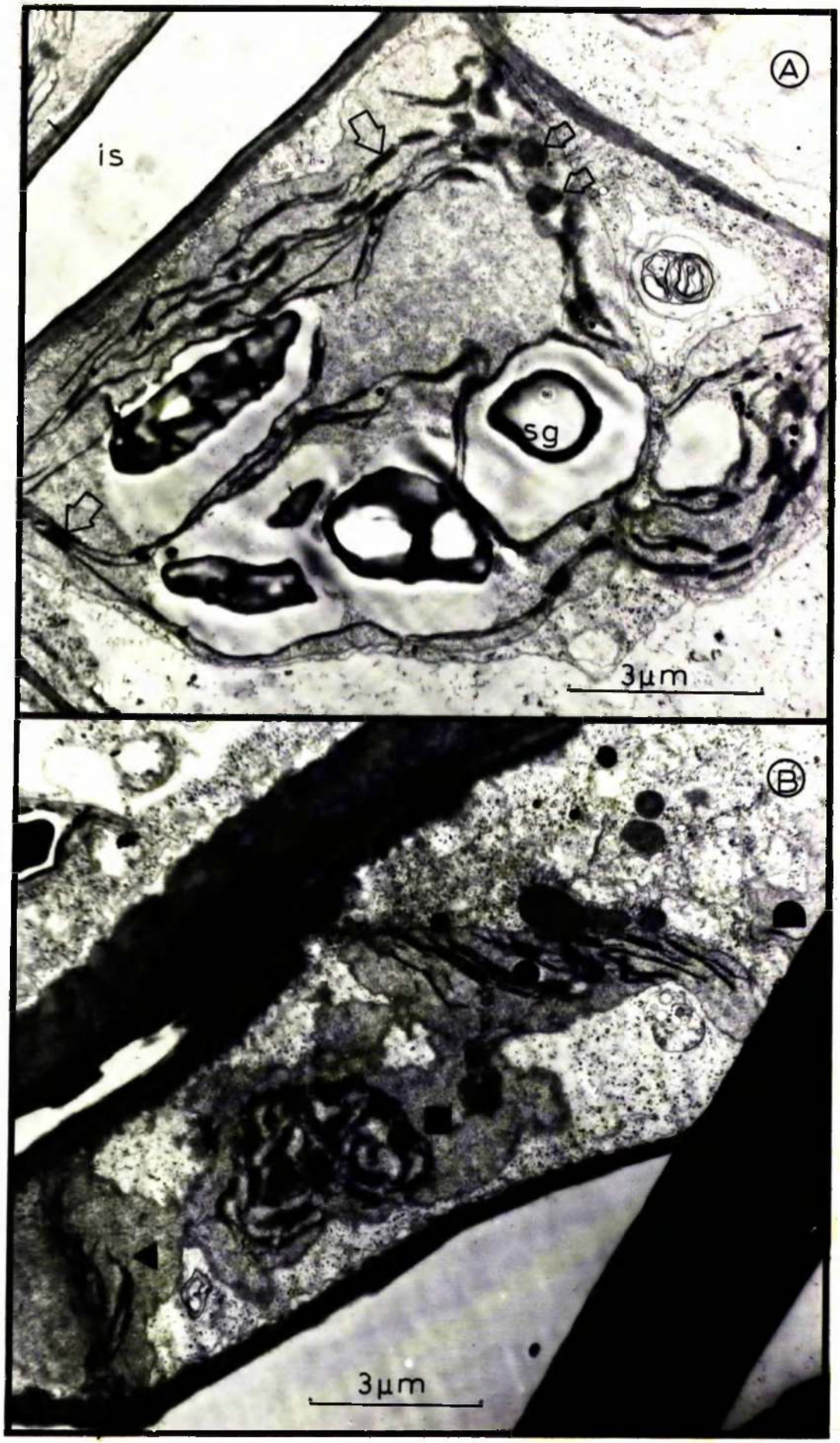
Plate 74B: TEM showing a group of chloroplasts which are apparently undergoing replication in a dedifferentiating cortical cell (Day 10+10). The three large chloroplasts in the centre are apparently connected through narrow constrictions (large arrows) whilst the chloroplast on the right appears isolated. The chloroplast at the top has developed enclaves occupied by cytoplasm (small arrows); also note the agranal plastids at the bottom right (solid arrows) which may be connected to the group of the large chloroplasts although this cannot be clearly visualised in this plane of section. M: 8800x


A
ch
d
p
p
3µm
B
ch
ch
4µm

L. usitatissimum

Plate 75A: TEM showing a possible late stage of chloroplast division in a dedifferentiating cortical cell (Day 10+10). Note the large translucent areas around and inside the denser areas of the starch grains; also note that although most grana are seen in a "side" view, some of them (large arrows) are seen from the "top" (small arrows). M: 11000x

Plate 75B: TEM showing a possible stage of a multiple chloroplast division in a dedifferentiating cortical cell. Note the highly irregular outline of the chloroplast and also the different angles the grana are seen in the three parts (▲ ■ ●) of the chloroplast (see also adjacent sections in Pl. 76A-B). M: 9600x

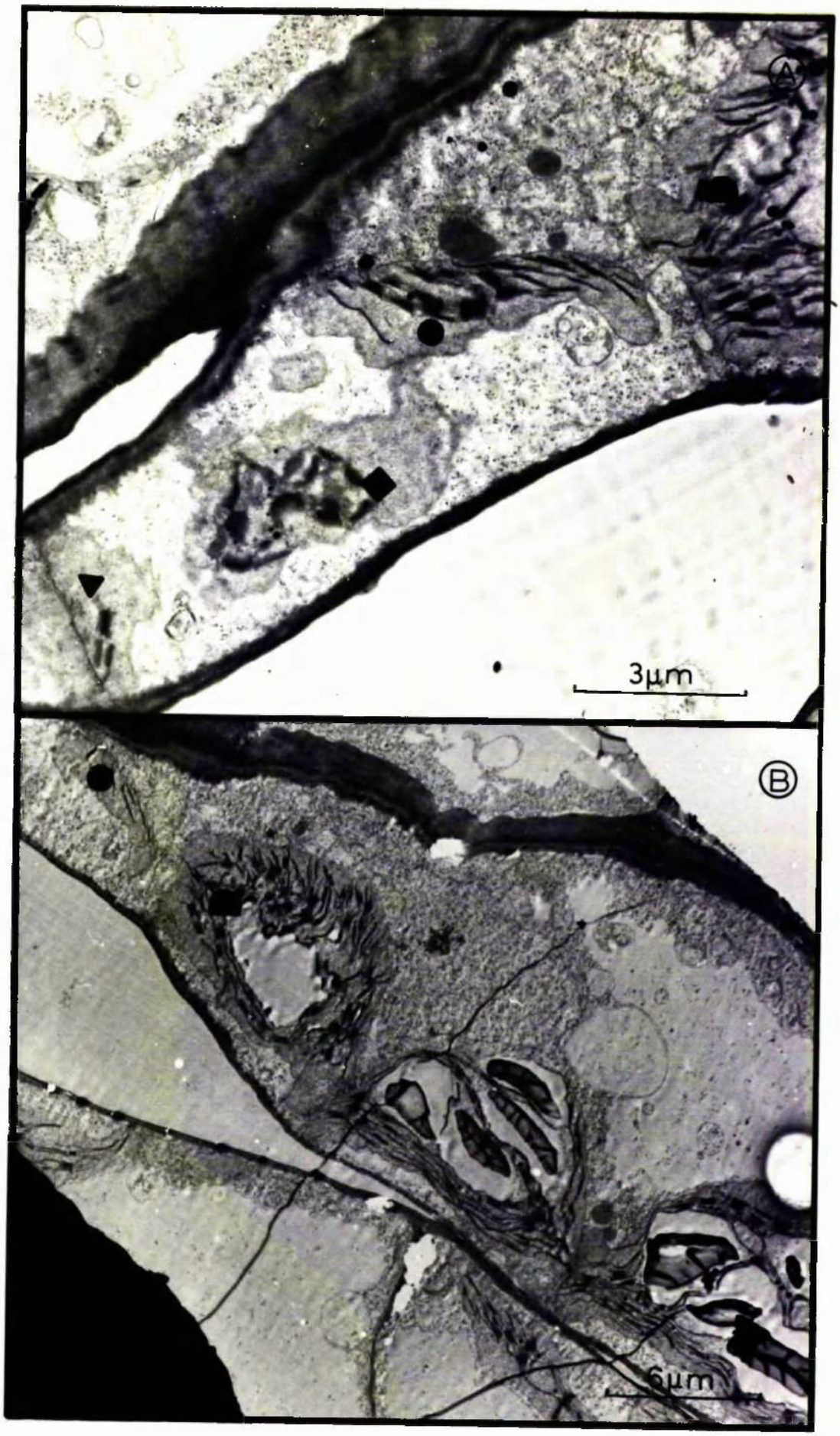

A
is
sg
3µm
B
3µm

L. usitatissimum

Plate 76A-B: TEM's from closely adjacent sections to Pl. 75B. The symbols (▲ ■ ● ⬬) indicate the same chloroplasts in the three plates (Pl. 75B and 76A-B). In A the unit (▲) appears as a separate chloroplast whilst units (■) and (●) are still connected through a narrow isthmus. Note that no envelope can be clearly seen delimiting chloroplast units (▲) and (■) but this is visible (arrows) in unit (●). In B some oversized chloroplasts are visible which are highly irregular in shape and content.

M: A: 9600x

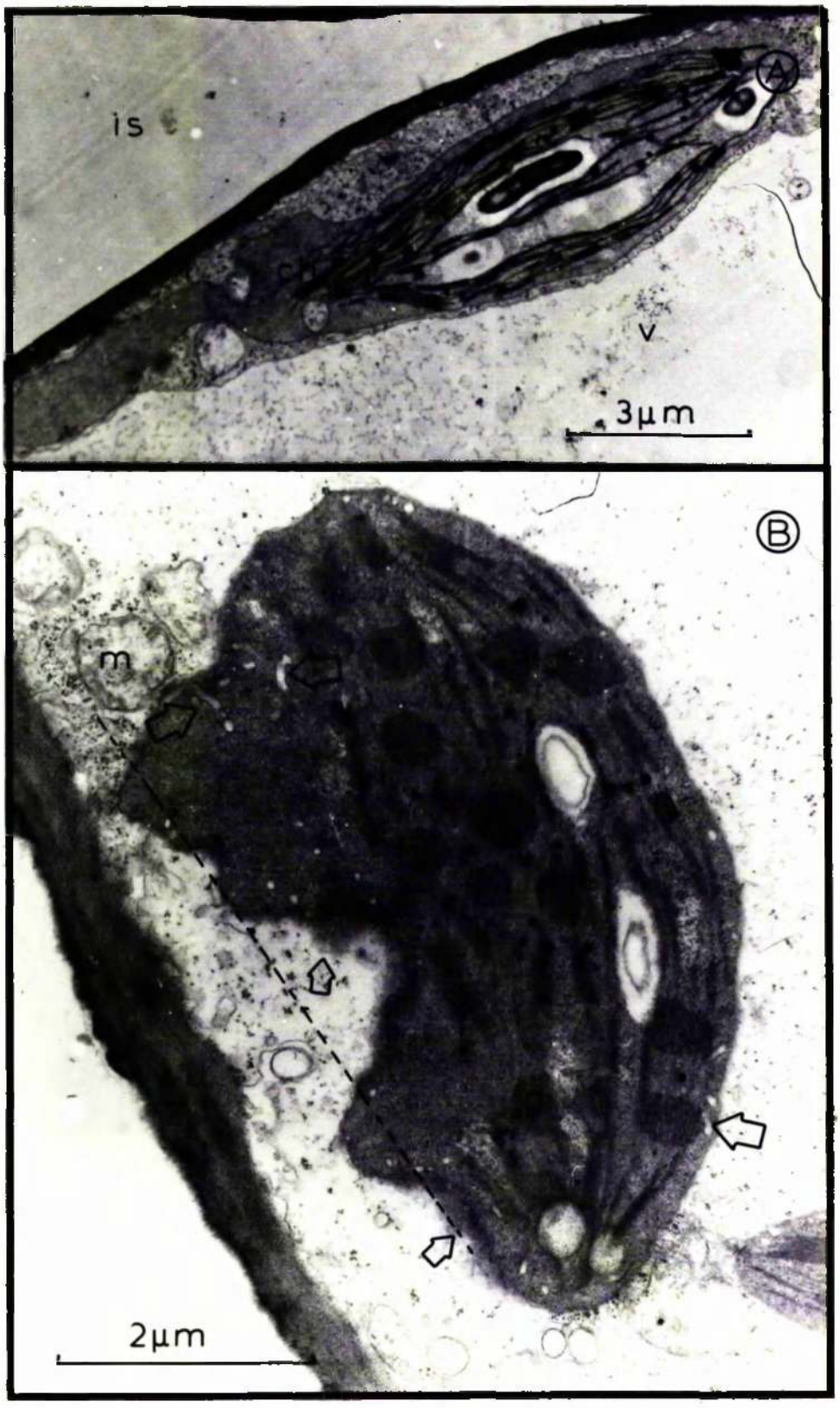
B: 5160x


A
3µm
B
6µm

L. usitatissimum

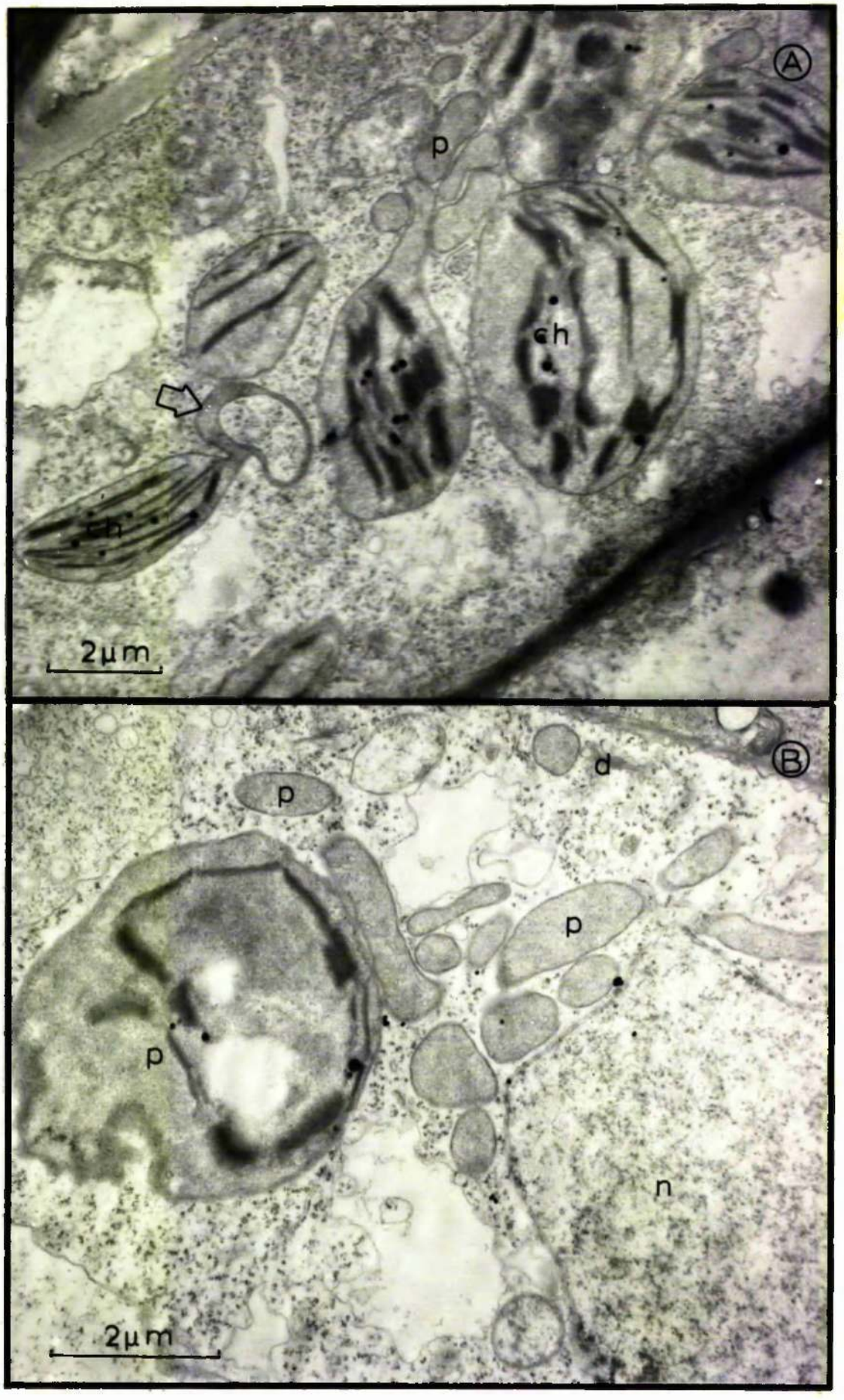
Plate 77A: TEM showing a chloroplast of a Day 10+10 dedifferentiating cortical cell of the hypocotyl possessing a long agranal tail. This might represent a division stage giving rise to a larger chloroplast component and a small agranal plastid. M: 9600x

Plate 77B: TEM showing a chloroplast of a Day 10+10 dedifferentiating cortical cell of the upper hypocotyl. The large arrows show some vesicles present in the stroma of the chloroplasts. Note that no envelope can be seen delimiting the chloroplast (especially on the irregular side) although membrane preservation seems reasonably good (see chloroplast at bottom right and mitochondria at top left of micrograph); on the left irregular side of the chloroplast (small arrows) it seems as if chloroplast stroma and cytoplasm are in continuity. The grana in this chloroplast are obliquely sectioned, therefore the latter cannot be clearly seen. The dotted line shows the plane of an imaginary section which might perhaps give a picture similar to the one shown in Pl. 78B. M: 20000x


A
is
v
3µm
B
m
2µm

L. usitatissimum

Plate 78A-B: TEM's showing groups of chloroplasts and agranal plastids from Day 10+10 dedifferentiating cortical cells of the hypocotyl. It is possible that all or most of the plastids shown here are derived from one large mother chloroplast from which they are budding. M: 10000x


A
p
ch
ch
2µm
B
d
p
p
p
n
2µm

ABBREVIATIONS

a : amyloplast

ab : abaxial

ad : adaxial

cc : cortical cell

ch : chloroplast

cr : chromatin

cp : cell plate

cw : cell wall

d : dictyosome

ec : epidermal cell

EM : electron microscope

fb : fibre

g : grana

is : intercellular space

lg : lipid globule

LM : light microscope

ly : lysosome

m : mitochondrion

mb : microbody

mm : multi-membranous body

mt : microtubule

n : nucleus

ne : nuclear enclave

ni : nuclear inclusion

nl : nucleolus

np : nuclear pore

nv : nuclear vacuole

p : plastid

pb : protein body

pc : protein crystal

ph : phloem

pp : p-protein

pr : polyribosome

pt : pit

pv : provascular

r : ribosome

rer: rough endoplasmic reticulum

sg : starch grain

sec: swollen epidermal cell

SEM: scanning electron microscope

ser: smooth endoplasmic reticulum

sp : sieve plate

st : storage cell

t : tonoplast

tc : transfer cell

te : tracheary element

TEM: transmission electron micr.

v : vacuole

ve : vesicle

vb : vascular bundle

wb : wall body

x : xylem