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Ultrastructure of gum-resin secreting cells in the pith of *Ailanthus excelsa* Roxb.

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SUMMARY

The pith ducts of *Ailanthus excelsa* Roxb. (Simaroubaceae) secrete gum-resin. The synthesis of resin in the epithelial cells of the duct is associated with plastids and endoplasmic reticulum (ER) while dictyosomes are involved in the synthesis of the gum. In the secreting cells, large numbers of osmiophilic globules are scattered in the cytoplasm. Dictyosomes and ER produce abundant vesicles. The osmiophilic globules and vesicles fuse with the plasmalemma facing the duct lumen and are entrapped in plasmalemma invaginations. The walls of some of the epithelial cells have a few ingrowths which presumably enhance the efficiency of the cell in the transportation of materials from the cytoplasm. Some of the epithelial cells in a duct undergo autolysis and degeneration after secretion. The appearance of autophagic vacuoles containing portions of cytoplasm, the loss of tonoplast, the distortion of ER, mitochondria, dictyosomes and the darkening of cytoplasm are some of the autolytic features in the degenerating cells. The lysed cells with 'dark' cytoplasm, osmiophilic droplets and free ribosomes detach and disintegrate in the duct lumen, becoming part of the gum-resin content in the duct.

Key-words: Ailanthus excelsa, epithelial cell, gum-resin, secretion, ultrastructure.

INTRODUCTION

Ailanthus excelsa secretes a gum-resin known as 'Bossara' or 'Hog gum' which is used in the treatment of dysentery and in the preparation of incense sticks (Santapau 1966). The gum-resin is exuded mainly from the traumatic cavities induced in the secondary xylem by injury and infection, and the exudate is a mixture of lipids, proteins and carbohydrates (Shah & Babu 1986). The lysing axial parenchyma cells of the secondary xylem contribute to the gum-resin formation in the traumatic cavities (Babu *et al.* 1987). Normal gum-resin ducts are present in the pith of the stem which develop schizogenously from a group of initials below the shoot apex (Venkaiah 1982).

Using light microscopy methods, Venkaiah (1982) investigated the structure and development of gum-resin ducts, but an ultrastructural investigation has so far not been carried out to elucidate the process of secretion in the gum-resin ducts of *A. excelsa*.

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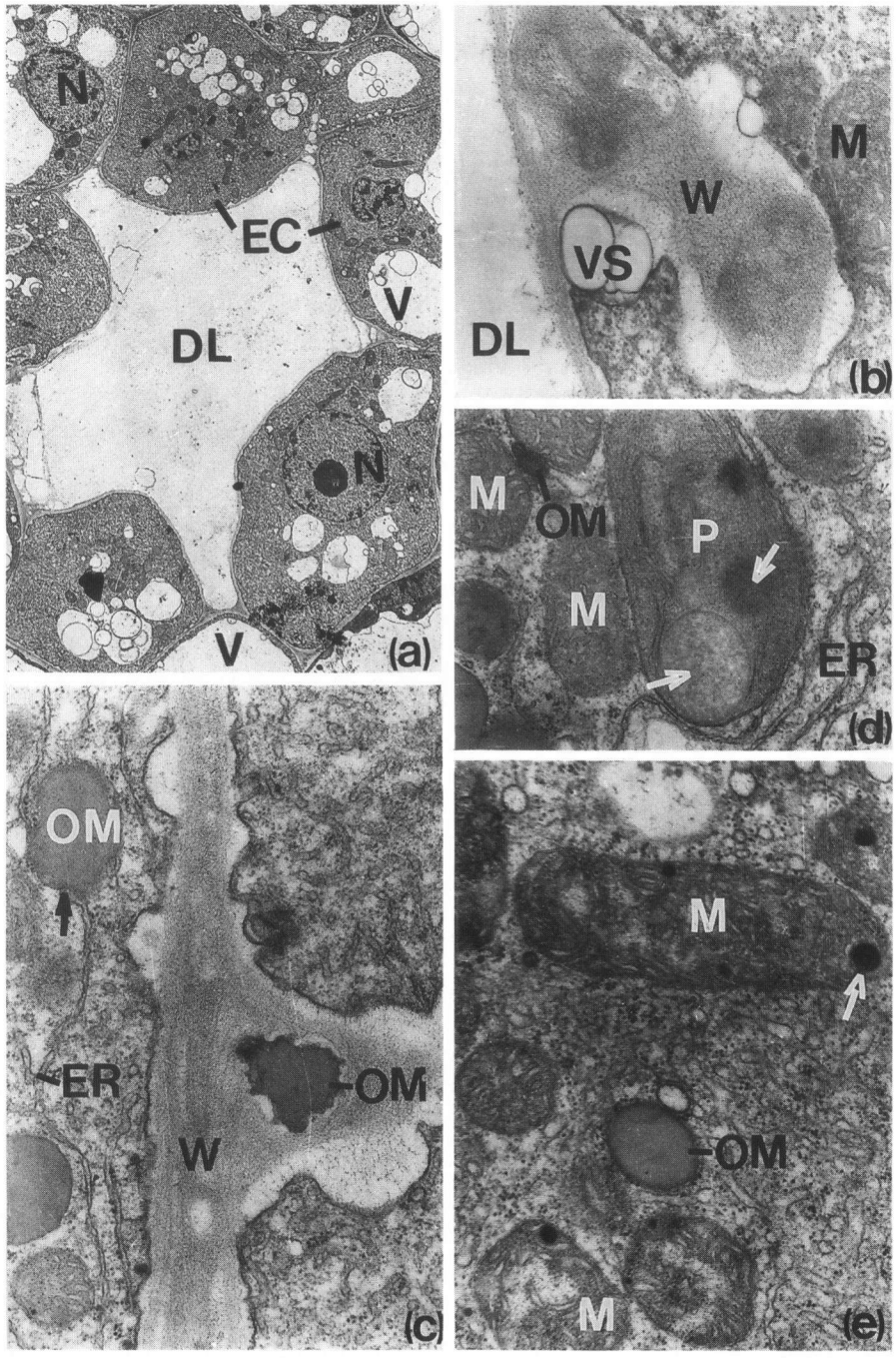
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Therefore, the present paper describes the ultrastructure of the ducts in the pith with particular reference to the site of synthesis and secretion of the gum and resin components of the exudate.

MATERIALS AND METHODS

Small pieces of second and third visible internodal regions from the shoot of *Ailanthus excelsa* Roxb. (Simaroubaceae) were collected and fixed in cold paraformaldehyde–gluteraldehyde in 0·05 M sodium cacodylate buffer at pH 7·1 (Karnovsky 1965). After a thorough washing with cacodylate buffer, the material was fixed overnight with 2% OsO_4 in cacodylate buffer at $4 \pm 1°C$. After washing in the same buffer and immersion in 2% aqueous uranyl acetate for 30 min, the material was dehydrated in a graded acetone series, infiltrated and embedded in a low viscosity epoxy resin (Spurr 1969). Silver–grey sections, cut on a Reichert OMU3 ultramicrotome, were stained with uranyl acetate followed by lead citrate (Dawes 1971) and viewed with a Philips 400 electron microscope.

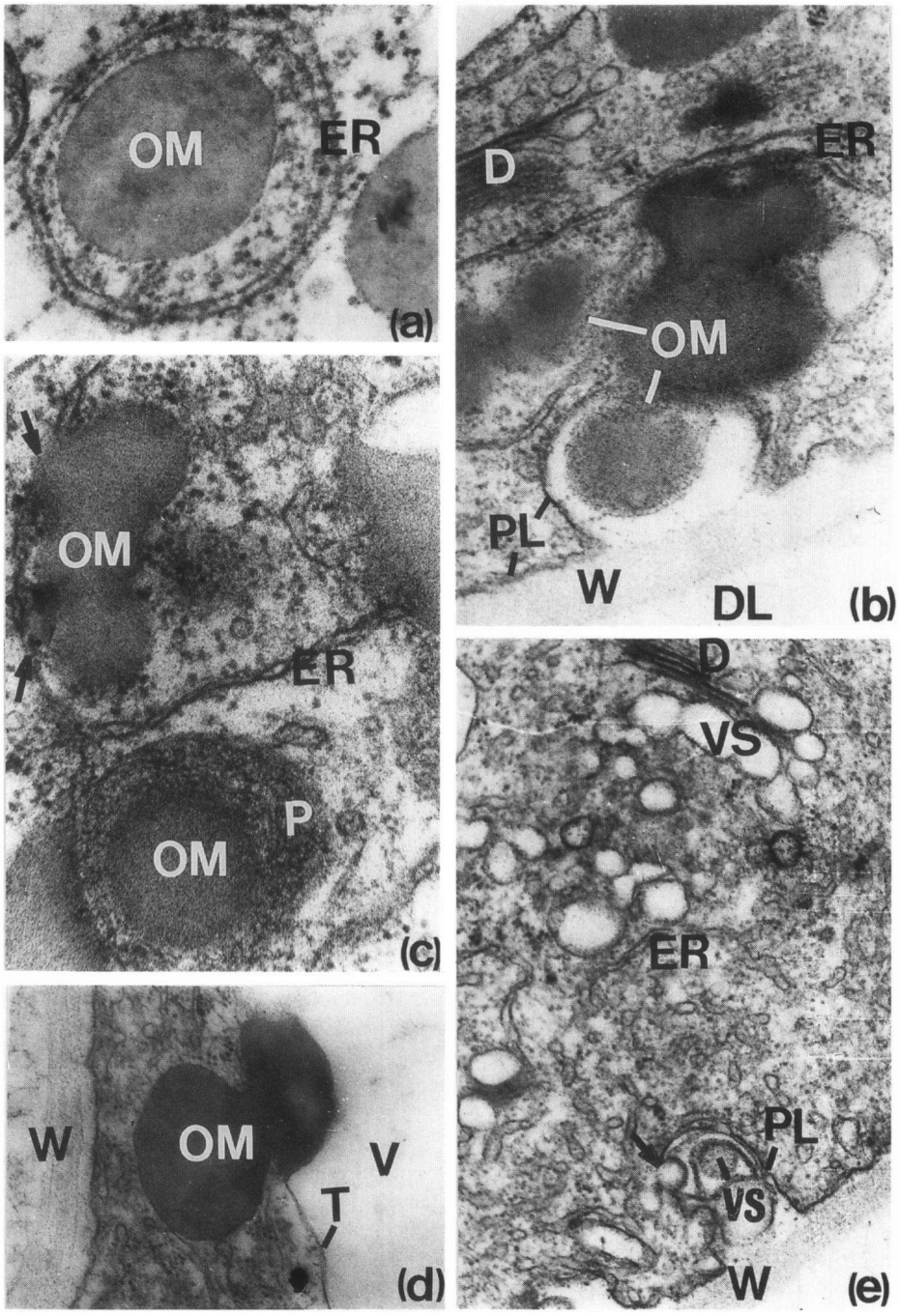
RESULTS

The epithelial cells surrounding young ducts are densely stained, moderately vacuolated and rich in organelles (Fig. 1a), whereas the adjacent pith cells are highly vacuolated with thin parietal cytoplasm. The radial and inner tangential walls (i.e. the wall facing the duct lumen) of many epithelial cells possess scattered papillose ingrowths (Fig. 1b and c). The wall facing the duct lumen usually has a loose microfibrillar texture and a sloughed-off appearance. Microtubules are frequently detected near the plasmalemma of the secreting cells. The cytoplasm contains abundant free ribosomes, polysomes, plastids, mitochondria, dictyosomes and rough endoplasmic reticulum (ER). Plastids are characterized by a dense matrix and poorly developed internal membranes (Fig. 1d). The numerous mitochondria present have well-developed cristae and small osmiophilic globules in their matrix (Fig. 1e). Coalescence of vacuoles is a consistent feature of epithelial cells (Fig. 1a).

Secretion

The secretory epithelial cells are characterized by the presence of numerous globules of osmiophilic material mostly representing the resin component of the gum-resin. The osmiophilic material is usually found in association with plastids (Fig. 1d) and ER (Figs 1c and 2a–c) of the secreting cells. Frequently the ER remains closely associated with the plastid-containing osmiophilic globules (Figs 1d and 2c). Such ER is also found in close association with the osmiophilic globules present in the cytoplasm and near the wall facing the duct lumen (Fig. 2b).

Fig. 1. (a) Transverse section of a young gum-resin duct in the pith. The epithelial cells have dense cytoplasm with prominent nuclei. Note the coalescing vacuoles in the cytoplasm of many of the epithelial cells (× 3420). (b) Ingrowth in the inner tangential wall of an epithelial cell. Vesicles are associated with the plasmalemma. (× 29 700). (c) Ingrowth in a radial wall of an epithelial cell. Note the close association of osmiophilic material with ER (arrow) and its presence within the wall ingrowth (× 29 880). (d) Plastid with osmiophilic globules in its matrix (arrows). Osmiophilic material is also seen associated between two mitochondria (× 21 600). (e) Mitochondria with osmiophilic droplets in their matrix (arrow) (× 21 600). D, dictyosome; DL, duct lumen; EC, epithelial cell; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; OM, osmiophilic material; P, plastid, PL, plasmalemma; T, tonoplast; V, vacuole; VS, vesicle; W, wall.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The formation of the gum component of gum-resin is apparently associated with the dictyosomes. They are very prominent and produce abundant vesicles lacking osmiophilic materials. These vesicles, as well as those produced from ER, appear to be fusing with each other (Fig. 2e) and also with the highly invaginated plasmalemma along the inner tangential wall (Fig. 2e).

In an actively secreting cell, a large number of osmiophilic globules are scattered in the cytoplasm (Fig. 3a). Several of them are often aggregated near the inner tangential wall and some globules are in contact with the plasmalemma and inside its invaginations (Fig. 2b). Osmiophilic globules of similar electron density are also found in the vacuoles and in the cytoplasm passing through the tonoplast (Fig. 2d). Degenerating membranes and myelin-like structures with their associated osmiophilic globules are sometimes present in the vacuoles. (Fig. 3b).

Lysis of epithelial cells

Some of the epithelial cells in a duct undergo lysis after a stage of secretion. The lysing cells show increased vacuolation, rupture of tonoplasts and loss of polysomes (Fig. 3e). The vacuoles contain portions of degenerating cytoplasm and the mitochondria show clear matrix with few disorganized cristae (Fig. 3e) and dictyosomes become distorted. The number of vesicles derived from ER and dictyosomes increases in the cytoplasm (Fig. 3c). The ER often forms concentric rings (Fig. 3d). The cytoplasm turns more electron dense and the organelles disappear (Fig. 4a). Subsequently, the cells fill with the darkened cytoplasm containing large osmiophilic droplets, distorted ER and free ribosomes, becomes detached and degenerates in the duct, mostly contributing to gum-resin formation (Fig. 4b and c). Once an epithelial cell disintegrates completely, the adjacent parenchyma cell exposed to the duct lumen differentiates into an epithelial cell.

DISCUSSION

In *A. excelsa*, the secreted gum-resin is a mixture of lipids, proteins and carbohydrates (Shah & Babu 1986). As chemically different compounds are produced, different systems must be operating in the secretory cells for their synthesis. The synthesis of resin is apparently associated with plastids and ER.

Plastids lacking well-defined membrane structure have been a general feature of resin-producing cells (Dell & McComb 1977). In several plants, the plastids are directly involved in the synthesis of lipophilic substances (Dell & McComb 1977; Werker & Fahn 1981; Bosabalidis & Tsekos 1982b; Pridgeon & Stern 1983). In *A. excelsa* they also seem to play a direct role in the synthesis of resin or its component, because the plastids in the epithelial cell contain abundant osmiophilic droplets, while similar inclusions are absent in the plastids of adjacent cells.

The conspicuous sheathing of plastids by ER is indicative of the co-ordination between these organelles in resin synthesis. Sheathing of plastids by ER is frequently observed in

Fig. 2. (a) Endoplasmic reticulum enclosing an osmiophilic globule (× 68 400). (b) Osmiophilic material in cytoplasm associated with the plasmalemma and inside its invagination. ER cisternae are associated with the osmiophilic material (× 39 900). (c) An ER cisterna associated with osmiophilic material and a plastid which contains a large osmiophilic droplet in its matrix (× 68 400). (d) Osmiophilic material in the cytoplasm traversing the tonoplast into a vacuole (× 39 900). (e) Vesicles near a dictyosome and ER. Note similar vesicle in contact with the plasmalemmal invaginations (arrow) (× 31 450). Abbreviations are given in Fig. 1.

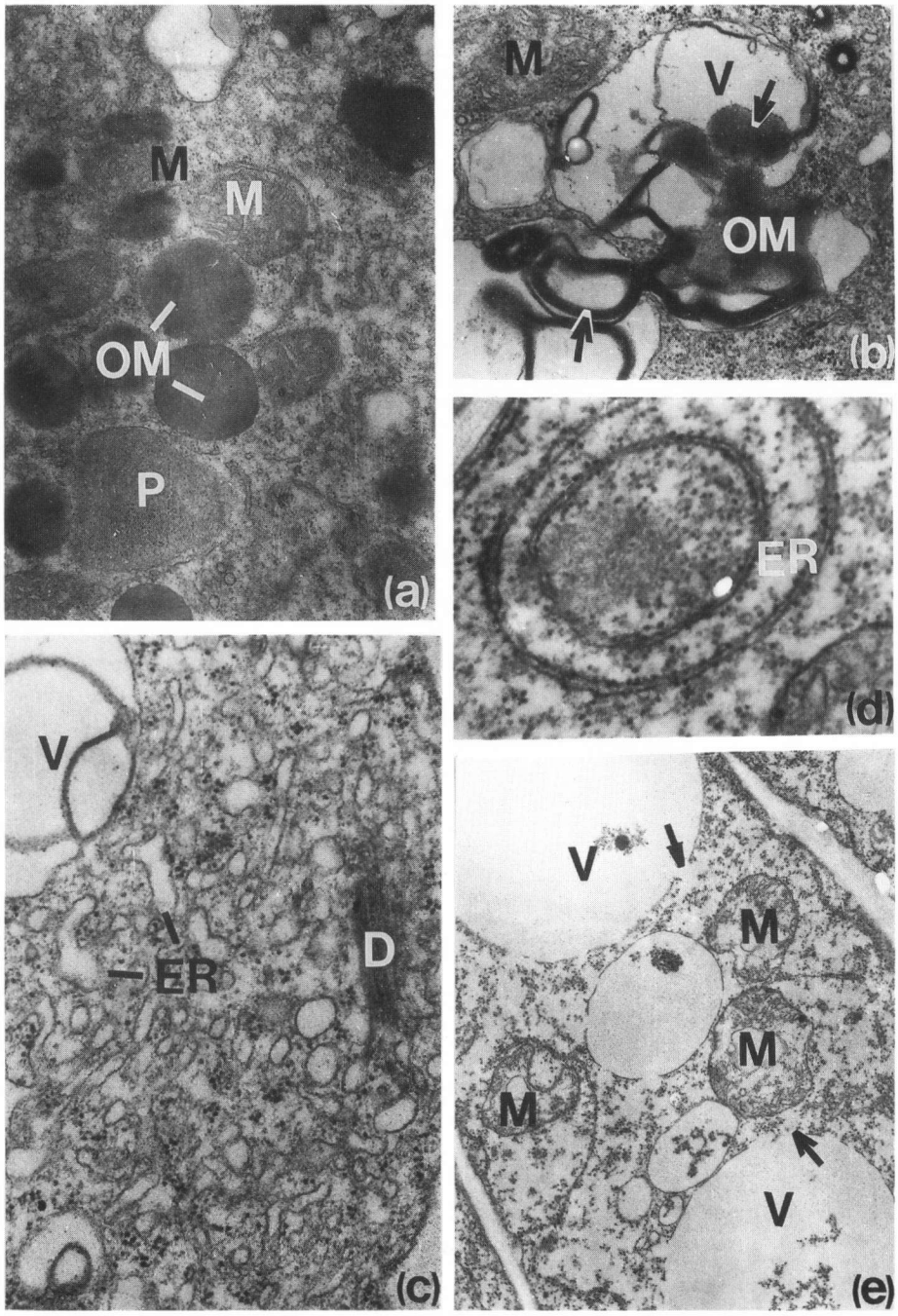

Fig. 3. (a) Cytoplasm of an actively secreting cell containing numerous droplets of osmiophilic material (× 34 200). (b) Coalescing vacuoles containing myelin-like structures (arrows) and osmiophilic material (× 22 800). (c) Extensive vesiculation of the ER (× 31 540). (d) Concentric rings of ER (× 39 900). (e) Senescing epithelial cell showing degenerating mitochondria and ruptured tonoplast (arrows) (× 15 200). Abbreviations are given in Fig. 1.

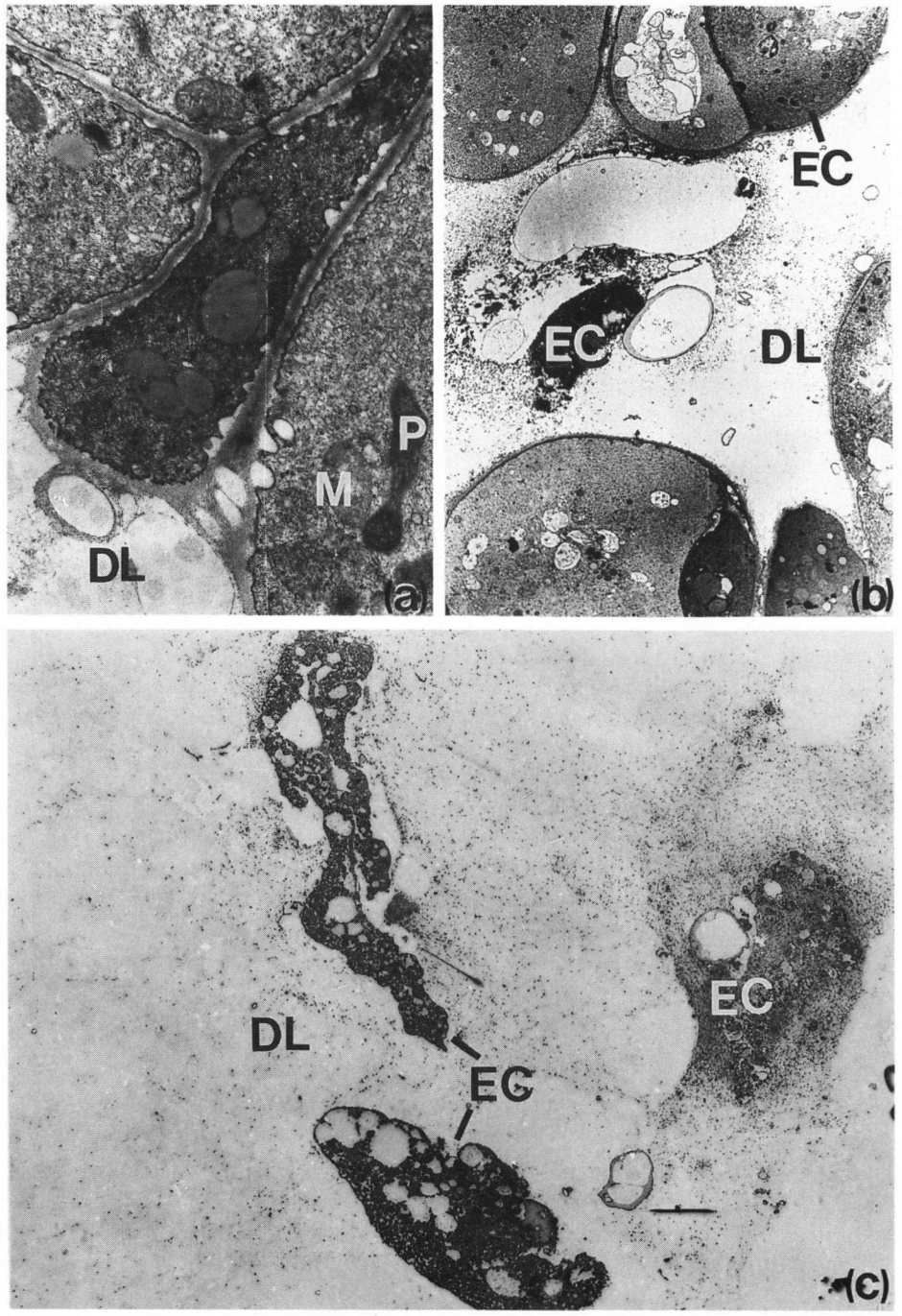

Fig. 4. (a) A degenerating epithelial cell with electron-dense cytoplasm containing abundant osmiophilic droplets. Note the sloughed-off appearance of the inner tangential wall (× 11 780). (b) A duct with gum-resin and distintegrating cells in its lumen. Some epithelial cells have very dense cytoplasm (× 9120). (c) The lumen of a duct showing detached and disintegrating cells (× 11 020). Abbreviations are given in Fig. 1.

many types of secretory cells synthesizing lipophilic substances (Dell & McComb 1977; Galatis *et al.* 1978; Nair *et al.* 1983; Pridgeon & Stern 1983). According to Bosabalidis & Tsekos (1982a) and Galatis & Apostolakos (1977), the significance of the plastid–ER association which becomes prominent in the secretory cells at the stage before secretion, lies either in the involvement of the ER in the transfer of soluble carbohydrates or other products to and from the plastids, or in that the ER cisternae are the site of synthesis of substances required for plastid differentiation. However, the conspicuous sheathing of ER around large numbers of plastids which lack a distinct normal membraneous system but contain osmiophilic material, indicates its role in resin synthesis rather than in plastid differentiation.

In many resin-secreting tissues, the ER plays an active role in the intracellular transport of resin (Dell & McComb 1977; Benayoun & Fahn 1979; Joel & Fahn 1980; Bosabalidis & Tsekos 1982b). In *A. excelsa*, ER does not show osmiophilic material in its cisternae and hence its role in the transport of resin is doubtful. However, the consistent association of ER with osmiophilic material in the cytoplasm is indicative of its participation in the synthesis rather than transport.

In *A. excelsa*, plastids and ER are consistently associated with the osmiophilic material. This kind of association of a secondary metabolite with different cell organelles may indicate that either both types of organelles are capable of resin synthesis or that different resin components are synthesized by different organelles (Joel & Fahn 1980). In *Rhus glabra* (Fahn & Evert 1974), plastids, ER, mitochondria and dictyosomes are involved in the synthesis and/or transport of the lipophilic material.

The dictyosome and its vesicles lack osmiophilic contents and hence its role in resin synthesis is doubtful. However, the abundance of vesicles near the dictyosomes and in the cytoplasm, and the presence of similar vesicles associated with the plasmalemma, suggest a probable role in the synthesis of the carbohydrate component of the exudate. The role of dictyosomes in the synthesis of carbohydrates has enormous support in the literature (Moore & McClelen 1983; Gedalovich & Fahn 1985; Morrison & Polito 1985; Tsekos 1985). In *A. excelsa*, the inner tangential wall of the epithelial cells has a loose microfibrillar structure and a sloughed-off appearance. This suggests that the wall material is continuously removed, which presumably becomes a component of the gum. A similar phenomenon is also reported in *R. glabra* (Fahn & Evert 1974), *Commiphora wightii* (Shah *et al.* 1982), *Anacardium occidentale* (Nair *et al.* 1983) and *Magnifera indica* (Bhatt & Shah 1985). Thus it seems that by replenishing the wall material, the dictyosomes are indirectly involved in the gum synthesis in *A. excelsa*.

The contents of the vesicles and the resin are apparently discharged from the cytoplasm by a process of exocytosis. The vesicles fuse with the plasmalemma and the contents are accumulated in the extra-cytoplasmic space and subsequently incorporated into the wall. However, once the osmiophilic (resin) materials reach the extra-cytoplasmic space, it is not clear how they are released into the duct lumen. The products may be released into the duct along with the cell contents when the epithelial cell undergoes lysis. It may also migrate through the microfibrillar layers of the cell wall into the duct lumen as suggested in several other plants (Nair *et al.* 1981, 1983; Bosabalidis & Tsekos 1982b; Pridgeon & Stern 1983; Bhatt & Shah 1985). By following special procedures for retaining resinous material in the cell during the processing for electron microscopy, Nair *et al.* (1981) and Bhatt & Shah (1985) could locate osmiophilic material in the wall matrix of epithelial cells in *C. wightii* and *M. indica* respectively.

Some of the epithelial cells are characterized by wall ingrowths similar to those in transfer cells. As the plasmalemma follows the contours of the wall ingrowths, this adaptation enhances the efficiency of the epithelial cell in transport of material from the cytoplasm. Microtubules are usually aligned parallel to the wall ingrowths of these cells. In many types of plant cells, microtubules are clustered in regions where the wall is thicker and they appear to control the thickening (Dustin 1978; Juniper *et al.* 1981). Thus the presence of wall ingrowths in the epithelial cells is indicative of its high efficiency of transport of materials into the duct lumen.

The epithelial cells undergo autolysis and disintegration after a stage of secretion. The autolysing cells become darker due to an increase in their osmiophilic contents. The 'darkening' of cytoplasm is reported as a developmental phenomenon preceeding the destruction or degeneration of the secretory cell in many other plants (Bosabalidis & Tsekos 1982a; Nair *et al.* 1983; Morrison & Polito 1985). The senescing cells in *A. excelsa* show heavy vacuolation, the presence of autophagic vacuoles, membrane degeneration, loss of tonoplast, distortion of mitochondria and dictyosomes and dilation of ER cisternae. These cytological alterations are indicative of the loss of integrity of the phytolysosomal system with loss of compartmentation (Gahan 1981). The dead cell filled with osmiophilic contents and free ribosomes is detached and it disintegrates in the lumen. The adjacent cell exposed to the duct lumen differentiates into an epithelial cell and thus the process of secretion in a duct is continued. The merging of the distintegrated product of the lysed epithelial cell with the contents of the duct lumen indicates this phenomenon as one of the possible mechanisms of secretion of gum and resin.

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