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Ultrastructural Studies on the Secretory Cavities of *Citrus deliciosa* Ten. II. Development of the Essential Oil-Accumulating Central Space of the Gland and Process of Active Secretion

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Summary

Oil glands of *Citrus deliciosa* are multicellular secretory structures, globular to oval in shape, in the centre of which an essential oil-accumulating space is formed. Opening of this space begins from a single cell. It undergoes lysis which later extends to the neighbouring gland cells.

Secretory material in form of droplets is produced in plastids, from where it is transported to the parietal cytoplasm of the secretory cells via numerous ER-elements. After fusion of the ER-membranes with the plasmalemma, the exudate reaches the apoplast, through which it is driven to the central cavity of the gland.

Peripheral cells of the secretory complex are modified into a protective sheath with thick walls and large vacuoles, while their plastids are differentiated from leucoplasts into typical amyloplasts.

Keywords: Central space development; *Citrus deliciosa*; Secretion; Secretory cavities.

1. Introduction

In a previous work (Bosabalidis and Tsekos 1982) it was reported that secretory cavities of *Citrus deliciosa* originate from a pair of initial cells giving rise to a globular/oval glandular structure. In the centre of the gland a space is formed, into which the essential oil is accumulated. With respect to the manner of opening of this space in various representatives of *Rutaceae*, a difference in opinion exists among investigators. Thus, according to Thomson *et al.* (1976) secretory cavities develop schizogenously, while other authors (Martinet 1871, Fohn 1935, Amelunxen and Arbeiter 1967, Heinrich 1966, 1969) support the view of a lysigenous process. Sieck (1895), Biermann (1896), Brandt (1924), Sprecher (1956) and Peterson *et al.* (1978) consider that the central space of the secretory cavities is formed schizolysigenously.

In the present paper the development of the central cavity in the *Citrus deliciosa* oil gland, as well as the cellular origin and the manner of elimination of the essential oil into the lumen of the cavity, are investigated.

2. Materials and Methods

Material for transmission electron microscopy was treated according to the method previously published by Bosabalidis and Tsekos (1982).

3. Results

After divisions of gland cells have been completed, a cell located in the centre of the gland demonstrates an increased electron density of the cytoplasm, while at the same time degenerated organelles appear as "foamy" structures (Fig. 1). These features are typical for a lysing cell and are not observed in the neighbouring gland cells. Disintegration of the biomembranes results in a progressive reduction of the osmotic pressure of the lysing cell, so that the surrounding turgid cells make protrusions into its lumen. Protrusions later become

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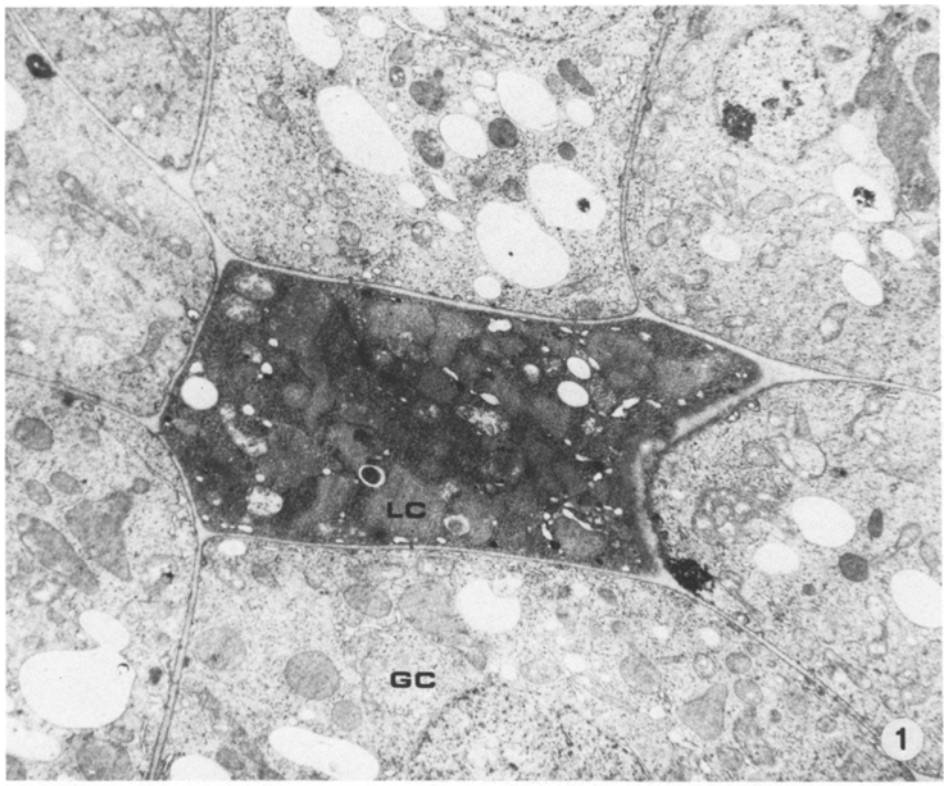
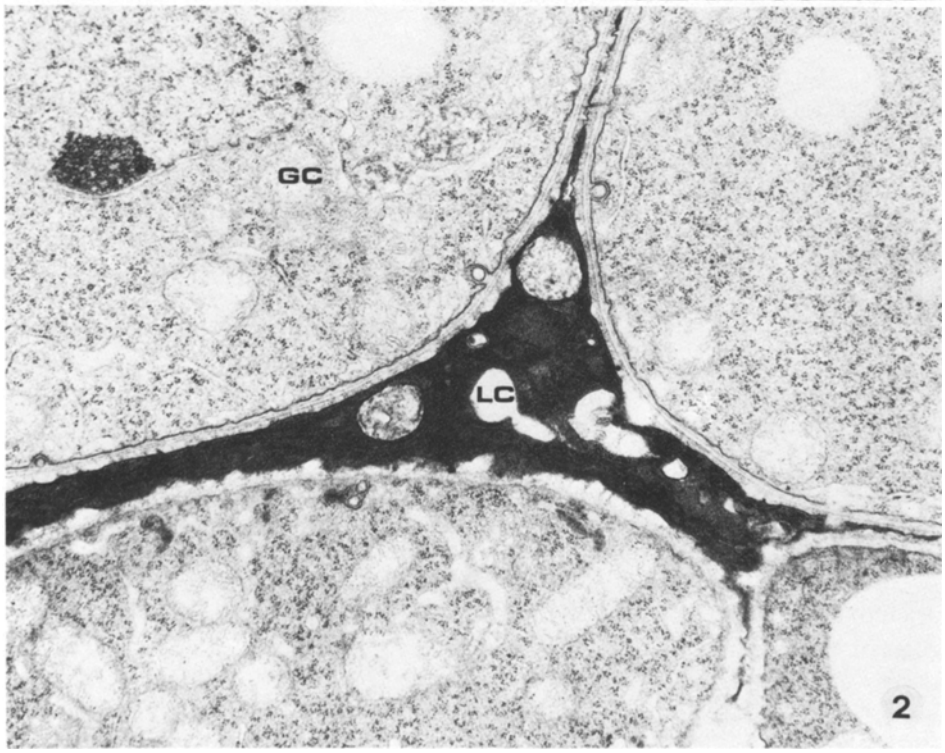




Abbreviations

CA Cavity, *CW* Cell wall, *ER* Endoplasmic reticulum, *GC* Gland cell, *LC* Lysing cell, *LP* Leucoplast, *ML* Multivesicular lomasoma, *N* Nucleus, *NE* Nuclear envelope, *OD* Oil droplet, *P* Plastid, *PC* Peripheral cell, *RER* Rough endoplasmic reticulum, *SC* Secretory cell, *SER* Smooth endoplasmic reticulum, *SG* Starch grain, *SS* Secretory substance, *V* Vacuole.

Fig. 1. Section of an oil gland, with a single lysing cell in the centre. × 8,000

Fig. 2. Advanced cytolysis in the central cell of the gland. Protrusions of boundary cells become so deep, that in low magnifications one gets the wrong impression that the opening of the inner cavity of the gland is performed schizogenously. × 20,000

very deep (Fig. 2) creating in low magnifications the wrong impression that the opening of the central cavity of the gland is performed schizogenously. After a central cell has been degenerated, a second one next to it begins to present features of cytolysis (Fig. 3). Thus, besides a significant increase in the electron density of the cytoplasm, a dilation of the nuclear envelope and the ER-elements as well as formation of numerous vacuoles are observed.

The lytic process beginning from the centre of the oil gland later extends centrifugally until an inner cavity is formed (Fig. 4), into which the essential oil will be accumulated. The opening of this cavity is followed by an activation of the gland cells, which enter the phase of intense secretion. Nuclei of secretory cells are centrally located and in most cases irregular in shape. Plastids are numerous and their matrix is often occupied by oil droplets of various electron density (Fig. 5). These droplets appear in contact with tubular elements, the membranes of which occasionally seem fused with the plastid envelope (Fig. 6, arrow).

The endoplasmic reticulum is well-developed and occurs either in form of elongated rough cisternae (Fig. 8) or in form of smooth tubular elements (Fig. 9). The former are found in the inner cytoplasm usually in close connection with the plastids. Periplastidal ER-cisternae are often dilated in the regions where they contact the plastids and are filled with an osmiophilic substance identical in appearance with the oil droplets of the plastid matrix (Fig. 7). Smooth ER is represented by numerous tubular elements, which at the stage of oil secretion are concentrated in the peripheral cytoplasm of the secretory cells (Fig. 9).

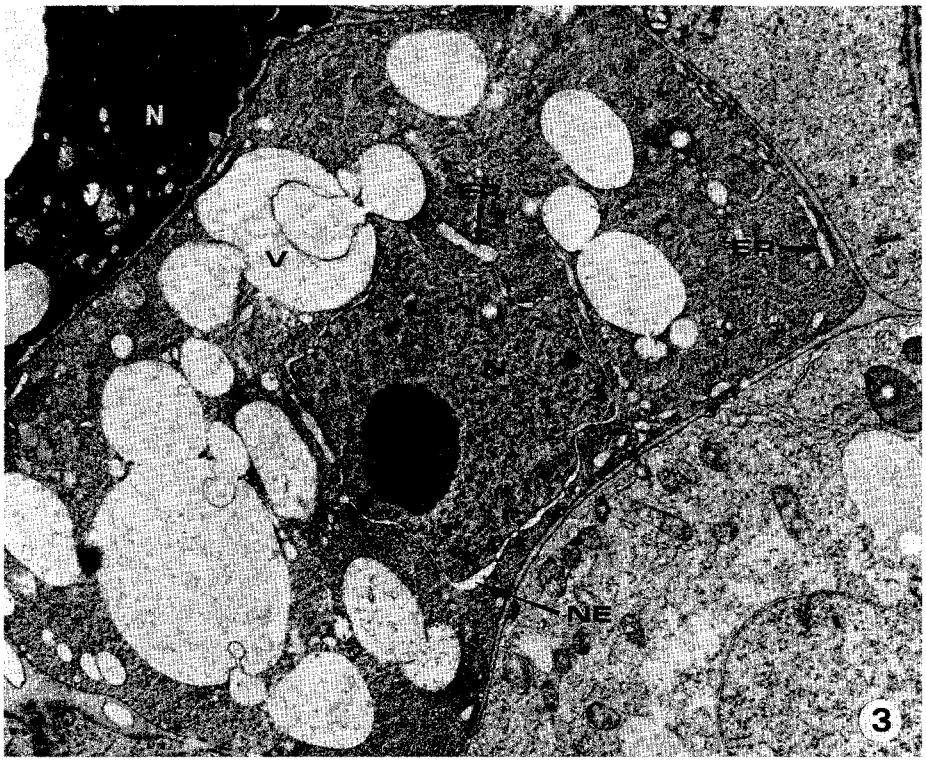
Mitochondria with a spherical or spindle-like shape are scattered in great number in the cytoplasm of the secretory cells during this stage. Multivesicular lomasomes (Fig. 10) are occasionally observed, some of which appear separated from the plasmalemma and released into adjacent vacuoles (Fig. 11). The walls of the secretory cells contain small or large deposits of an osmiophilic material in form of concentrically arranged lamellae (Fig. 12). In positions where a wall ends at the central cavity of the oil gland, a substance is often accumulated, which seems to come from the interior of the wall (Fig. 13). During the late differentiation of the secretory cavities (postsecretory period), small vacuoles with a "foamy" content reminding that of autophagic vacuoles, are found in the secretory cells (Fig. 14).

The peripheral cells of the oil gland possess large vacuoles and their walls are remarkably thicker (600 nm) than those of the inner secretory cells (120 nm) (Fig. 15). The ER-elements do not contain any osmiophilic substance, neither do they surround the plastids. The latter are a few and their matrix appears occupied by numerous starch grains, so that they are finally modified from leucoplasts into typical amyloplasts (Fig. 16).

4. Discussion

Secretory cavities of *Rutaceae* belong to the holocrine type of glands (SCHNEPF 1969). The opening of the central space in *C. deliciosa* oil glands begins from a single cell demonstrating a high electron density. A similar feature was also observed during the lysis of embryo suspensors (NAGL 1976), synergids (VIJAYARAGHAVAN *et al.* 1972) etc. Besides an alteration in electron density of cytoplasm, the central lysing cells of the gland exhibit a dilation of the ER-elements and the nuclear envelope. Swollen ER is frequently observed in degenerating plant cells (MOGENSEN 1978, TAN and UEDA 1978) as well as in senescent animal cells (PILAR and LANDMESSER 1976). The dilation of the nuclear envelope is not an artefact (WITHERS 1978); it is found in degenerating nuclei of sieve elements and nucellus cells (ESAU 1972, NORSTOG 1974), as well as during the effect of various external factors, such as low temperatures (PLATT-ALOIA and THOMSON 1976), cessation in supplying of tissues with nutrients (RAGETLI *et al.* 1970), attack by mycoplasmic diseases (LOMBARDO *et al.* 1970) etc. These data and our observations indicate that the opening of the *C. deliciosa* secretory cavities begins from a single central cell, which undergoes lysis. Features of lysis progressively extend to the neighbouring gland cells, so that finally an inner space is formed containing remnants of the dissolved cells (this space will be later filled with the essential oil). Based on these observations we can characterize the secretory cavities of *C. deliciosa* as truly lysigenous (about this subject cf. the views of various authors mentioned in Introduction).

As soon as a small cavity is formed in the centre of the gland, the secretory cells enter the stage of intense secretion. In their plastids the oil droplets strongly increase in number and size; some tubular elements of the plastid matrix, with which the droplets are in contact, often appear fused with the plastid envelope. These features are probably connected with an intraplastidal movement of the droplets towards the periphery of plastids. With respect to the removal of the exudate from the plastids, HAMPP and SCHMIDT (1976)



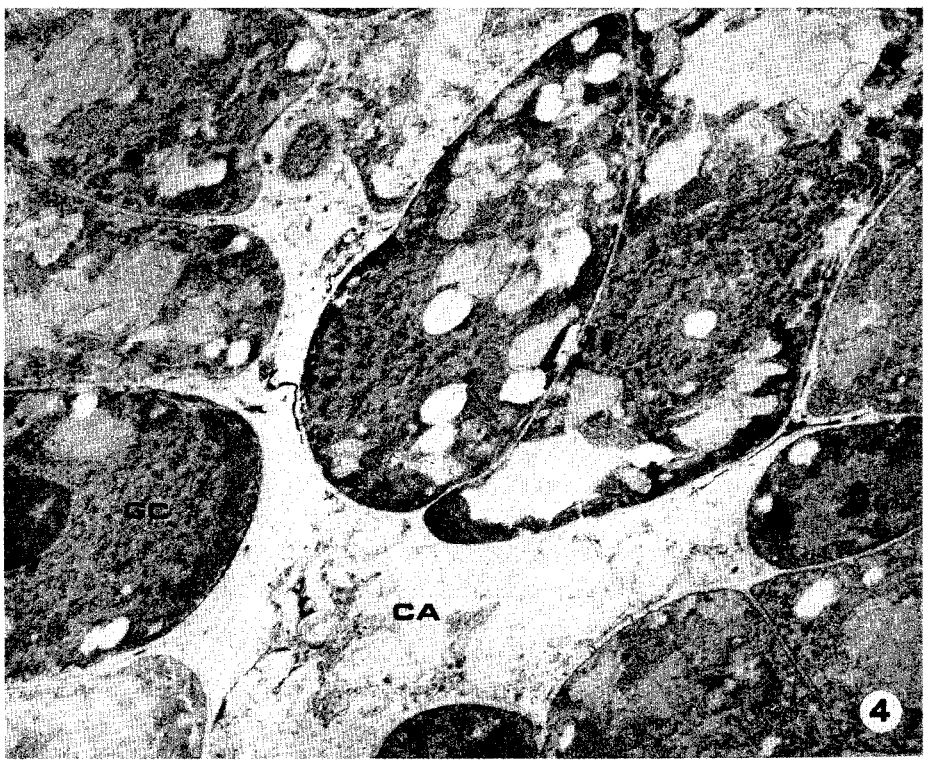
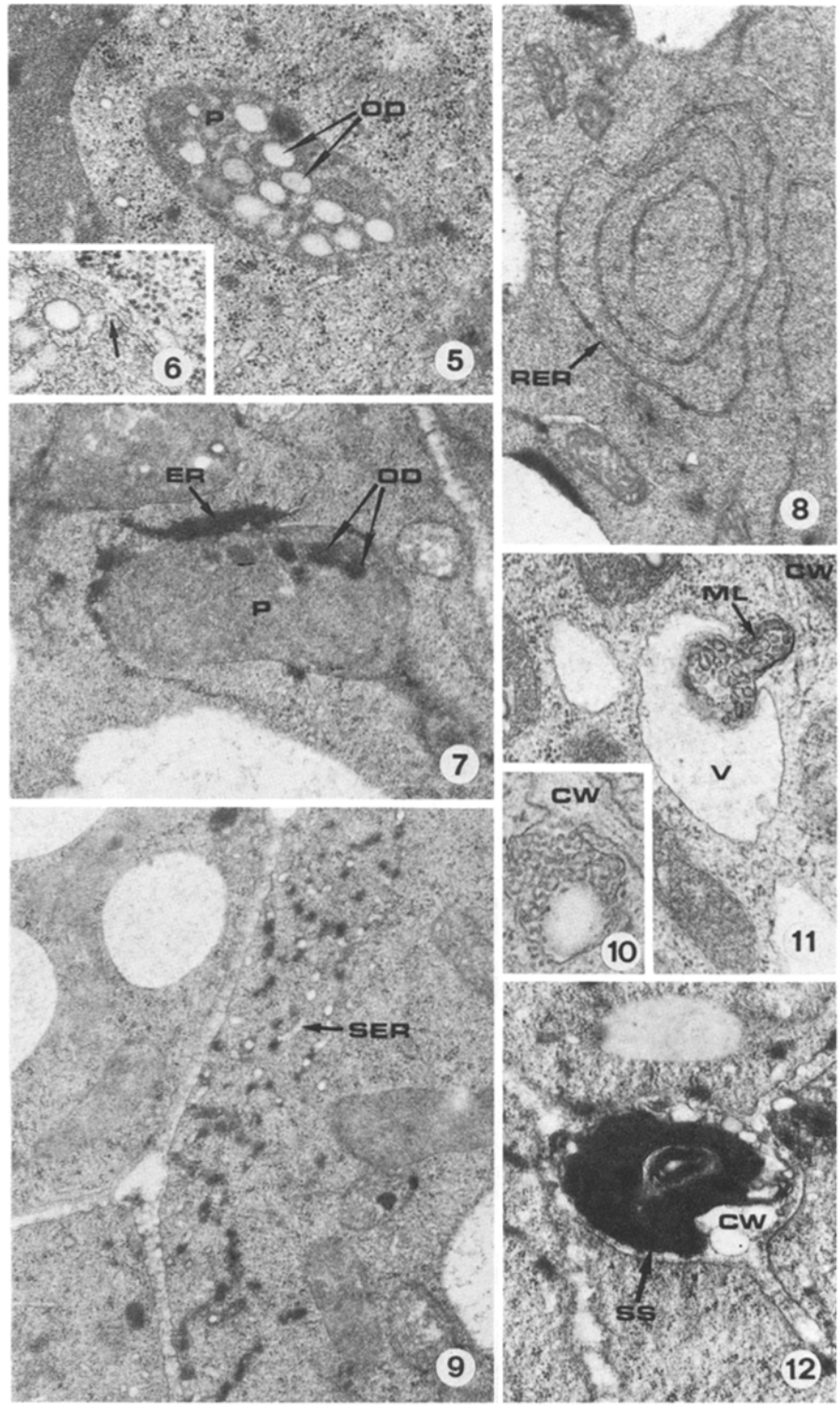


Fig. 3. Central region of a just opening secretory cavity. After a central cell of the gland has been degenerated, a second one next to it is undergoing lysis showing a dilation of the nuclear envelope and the ER-elements, as well as a remarkable increase in the electron density of the cytoplasm. Upper- left part of the picture illustrates the degenerated nucleus of the first lysing cell. ×8,000

Fig. 4. Oil gland with an open inner cavity containing remnants of the degenerated cells. This cavity will be later filled with the essential oil. ×7,000



Figs. 5-13. Secretory cells at the stage of active secretion.

Fig. 5. A plastid containing numerous oil droplets of various electron density. × 31,000

Fig. 6. A tubular element in continuity with the inner membrane of the plastid envelope (arrow). × 54,000

Fig. 7. Periplastidal ER-cisterna containing a substance identical in appearance with the oil droplets of the plastid matrix. × 19,000

Fig. 8. Elongated ER-cisternae occasionally arranged concentrically. × 22,000

Fig. 9. Accumulation of numerous smooth tubular ER-elements with an osmiophilic content, in the peripheral cytoplasm. × 23,000

Fig. 10. A multivesicular lomasoma. × 50,000

Fig. 11. Multivesicular structure entering a vacuole. × 32,000

Fig. 12. Osmiophilic substance within the walls. × 27,000

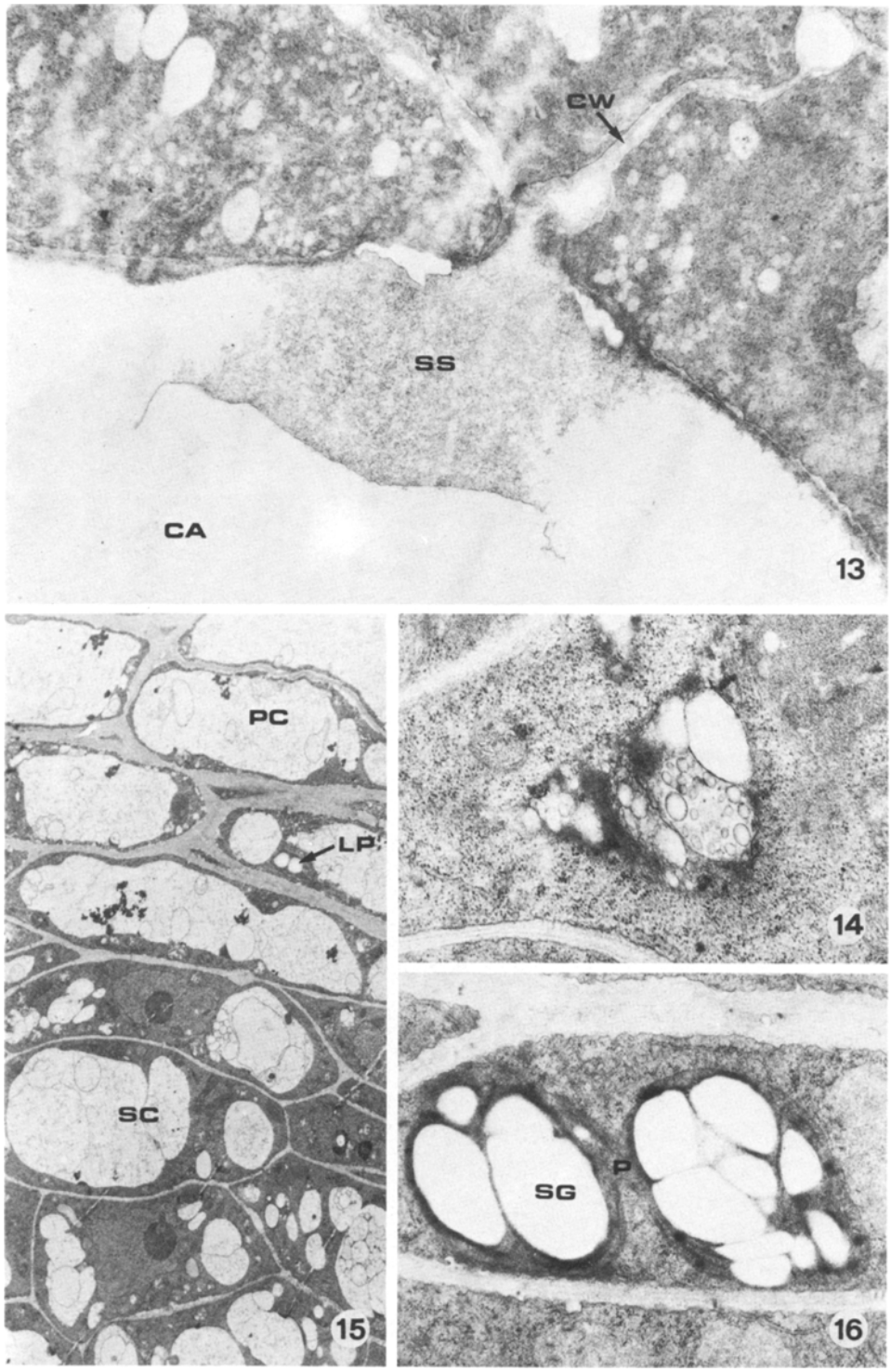


Fig. 13. Discharge of the secretory substance from the interior of the walls into the central cavity of the gland. × 17,000

Fig. 14. Autophagic vacuole-like structure in a secretory cell during the postsecretory period. × 26,000

Fig. 15. Peripheral cells of the gland possessing thick walls, large vacuoles and amyloplasts. × 4,000

Fig. 16. Amyloplast in a peripheral cell of the oil gland. × 17,000

consider that the permeability of the plastid envelope increases with the process of differentiation. COCKBURN and WELLBURN (1974), using labelled mevalonic acid, came to the conclusion that in the outer membrane of the plastid envelope an active mechanism controlls the transport of terpenoids from the interior to the exterior of plastids. The accumulation of the secretory material at the periphery of plastids is accompanied by a close arrangement of the ER-cisternae around the plastids. Connection of both organelles is believed to be related with a direct movement of the secretory product from the plastids into the ER. In this way, a possible poisoning of the cytoplasm by the essential oil (HEINRICH 1970) during its migration from the one compartment into the other, is avoided.

The exudate-containing ER-cisternae are modified into smooth tubular elements, which by fusion with the plasmalemma (cf. also FAHN 1979) release their content into the walls. The apoplastic movement of the exudate is probably controlled by the protoplasm (SCHNEPF and BENNER 1978) and is directed exclusively towards the cavity of the gland. This movement takes place through the wall capillaries, which are formed during the dissolution of the interfibrilar matrix by the myelin-like lomasomes (cf. BOSABALIDIS and TSEKOS 1982). The secretory substance appears within the walls in form of concentrically arranged lamellae. This observation supports the view that this substance is of lipophilic constitution (GEBICKI and HICKS 1973).

HEINRICH (1969, 1970), examining the ultrastructure of secretion in the oil glands of *Citrus* and *Poncirus* concluded that the secretory droplets of the plastids empty directly into the cavity of the gland by the lysis of the secretory cells (droplets are not transported via the ER and the apoplast, as found by us).

The multivesicular lomasomes observed in the secretory cells of *C. deliciosa* are likely related with the maintenance of constancy in plasmalemma surface, which probably increases during the granulocrine secretion (cf. HEINRICH 1973, SCHNEPF and BUSCH 1976).

The peripheral cells of the oil gland, which have at the beginning of the ontogeny the same morphology with the inner secretory cells, are progressively modified into a protective sheath with thick cell walls. The presence of large vacuoles in peripheral cells suggests that they may store assimilates, which are further transported to the secretory tissue (secretory cells lack chlorophyll). Plastids are gradually differentiated from leucoplasts into typical amyloplasts. In the oil glands of other representatives of *Rutaceae*, such as *Dictamnus albus* (AMELUNXEN and ARBEITER 1967), *Ruta graveolens* (HEINRICH 1969) and *Citrus sinensis* (THOMSON *et al.* 1976), plastids of peripheral cells are reported as chloroplasts with a well-developed grana system.

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