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Ultrastructural Changes in the Developing Ovaries of Citrus

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Additional index words: Orange, pericarp, plastid development, plasted degeneration.

Abstract

The ultrastructural changes in the ovary of *Citrus sinensis* (L.) Osbeck, cv. 'Navelate', during the transformation of the ovary into young developing fruit were studied. Epicarp and inner mesocarp cells have a large central vacuole surrounded by a peripheral cytoplasm. Outer mesocarp cells vary in their degree of vaculation containing abundant cytoplasm. Prominent organelles within cytoplasm of all these cells are the nucleus, mitochondria and plastids. Small amounts of rough endoplasmic reticulum, dyctyosomes and free ribosomes are also present. At petal fall, polysomal groups of ribosomes in outer mesocarp cells are observed. The main ultrastructural changes were alterations of plastid structure. Few days before anthesis, plastids of epicarp and outer and inner mesocarp, contain single or multiple starch granules. Their number decrease gradually until petal fall. Concomitantly an increase in the internal chloroplasts are visible, containing a number of small plastoglobuli. At the end of the fruit set period thylakoid breakdown in inner mesocarp chloroplasts is evident.

Abbreviations: CW = cell wall, EP = epidermis, G = Golgi area, Gr = grana, HY = hypodermis, IM = inner mesocarp, IS = intercellular space, M = mitochondrion, Mb = multivesicular body, OM = outer mesocarp, P = plastid, Pd = plasmodesmata, Pg = plastoglobule, R = ribosomes, RER = rough endoplasmic reticulum, St = starch grain, V = vacuole

Introduction

The growth and development of a citrus ovary into a mature fruit consists of a sequence of phases: cell division, cell enlargement and maturation, described by Bain (1958). Ultrastructural changes during fruit maturation have been the subject of investigation with the purpose of providing a basis for better understanding the process (2,5,7,8,9). However, only slight attention has been placed on the early phases of the growth and development process (1,10).

We are describing the ultrastructure of different cell strata making up an important portion of the pericarp from citrus ovaries and developing fruits. These strata are

epidermis, hypodermis and outer and inner mesocarp. The aim of the study was to understand the qualitative changes in cell organelles during the late stage of flowering and fruit set.

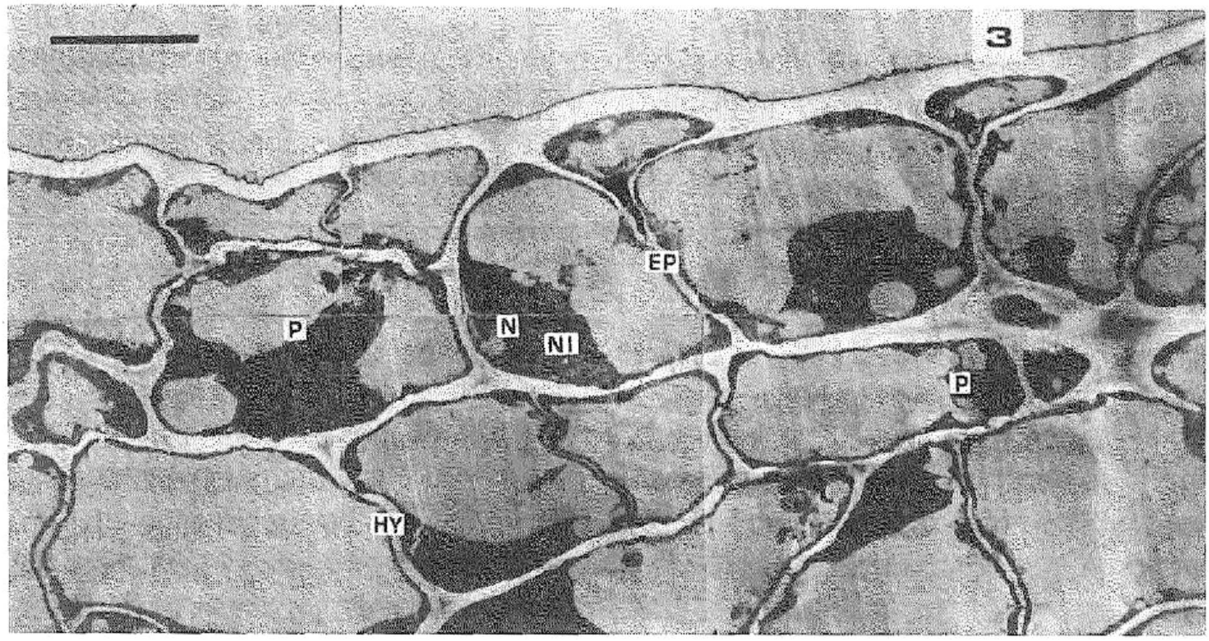
Material and Methods

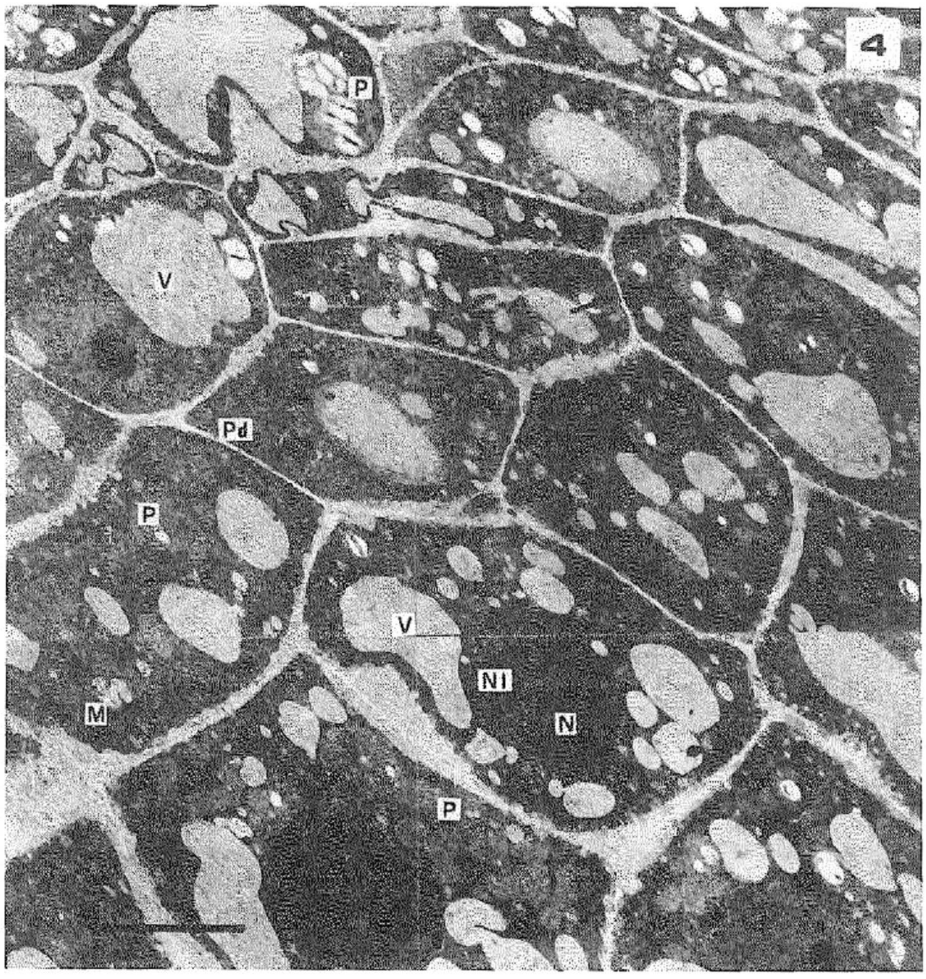
Ovaries and developing fruits were obtained from adult 'Navelate' orange (*Citrus sinensis* (L.) Osbeck) trees. The following stages of development were sampled: (I) closed flower ovaries 2.7 mm 0; (II) open flower ovaries 3.0 mm 0; (III) petal fall flower ovaries 4.0 mm 0; (IV) developing fruits 5.0 mm 0; (V) developing fruits 8.0 mm 0; (VI) developing fruits 15.0 mm 0. Sampling was made between April 15 and June 30, 1986.

Hand sections of pericarp at different stages of development were fixed overnight in a solution containing 2% glutaraldehyde and 0.1M cacodylate buffer (pH 7.4). The tissue was postfixed in 1.25 % Os04 in 0.1M Veronal-acetate buffer (pH 7.4) for 4 h and dehydrated in an ethanol series followed by propylene oxide. An intermediate step in dehydration was the *en bloc* staining with 2% uranyl acetate in 70% ethanol overnight. Dehydrated tissue was embedded in Durcupan. Gold-silver sections were cut with glass knives on a 8800 III LKB ultramicrotome and stained with lead citrate (3). Sections were viewed in a Jeol 100 S electron microscope at 80 kV.

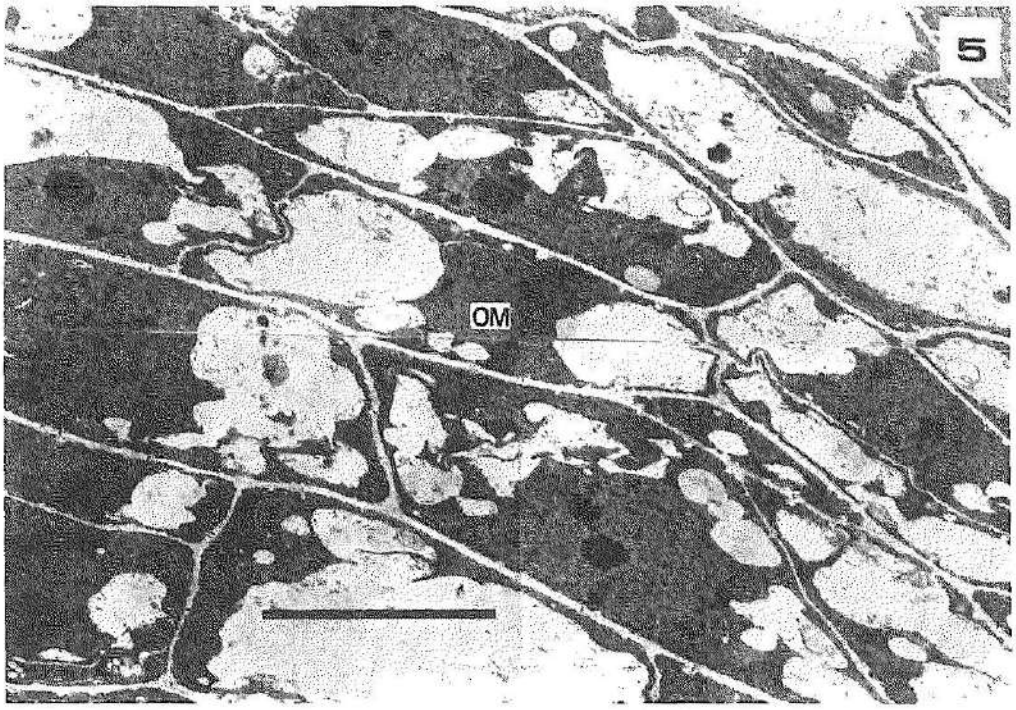
Results

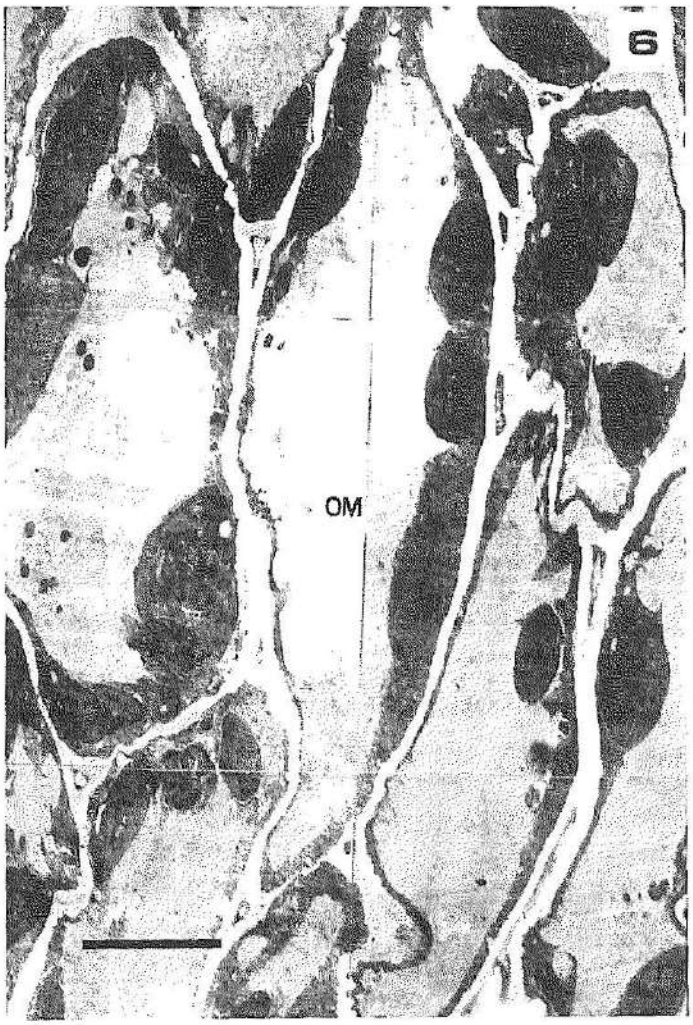
The single-layered epidermis was mainly composed by parenchymatous cells that appeared rectangular in cross section (Figs.1-3). Their cellular size did not vary during the late stage of flowering and the fruit set periods and were 10 to 12 µm long. A thin cuticle overlying the epidermis was observed. Immediately below the epidermis and containing the oil glands, two cellular strata called hypodermis (figs. 1-3) and outer mesocarp (Figs. 4-6) were found. They differed in cellular size, the outer mesocarp cells being larger than the hypodermal. The hyperdermis was the first stratum placed below the epidermis and was composed of a variable number of cell layers, 3 to 5. The hypodermis was composed of parenchymatous cells and were 10 to 14 µm diameter. The outer mesocarp was placed underneath the hypodermis and was composed of cells with an irregular structure, rather spherical during the initial stages of development adopting an elongated shape as fruits reached more advanced development stages. The number of cell layers was variable, 10 to 15, and were 15 to 22 µm long. The inner mesocarp was the largest zone of the pericarp, placed underneath the outer mesocarp (Figs. 7–9). As a result of ovary and fruit development process, the inner mesocarp seemed to contribute significantly to increase their size. The inner mesocarp was composed of parenchymatous cells having a diameter between 24 and 27 µm. The smaller diameter value was the most commonly found over the late stages of flowering, and the larger diameter value were seen during the fruit set period. The number of cell layers increased with ovary and fruit development.



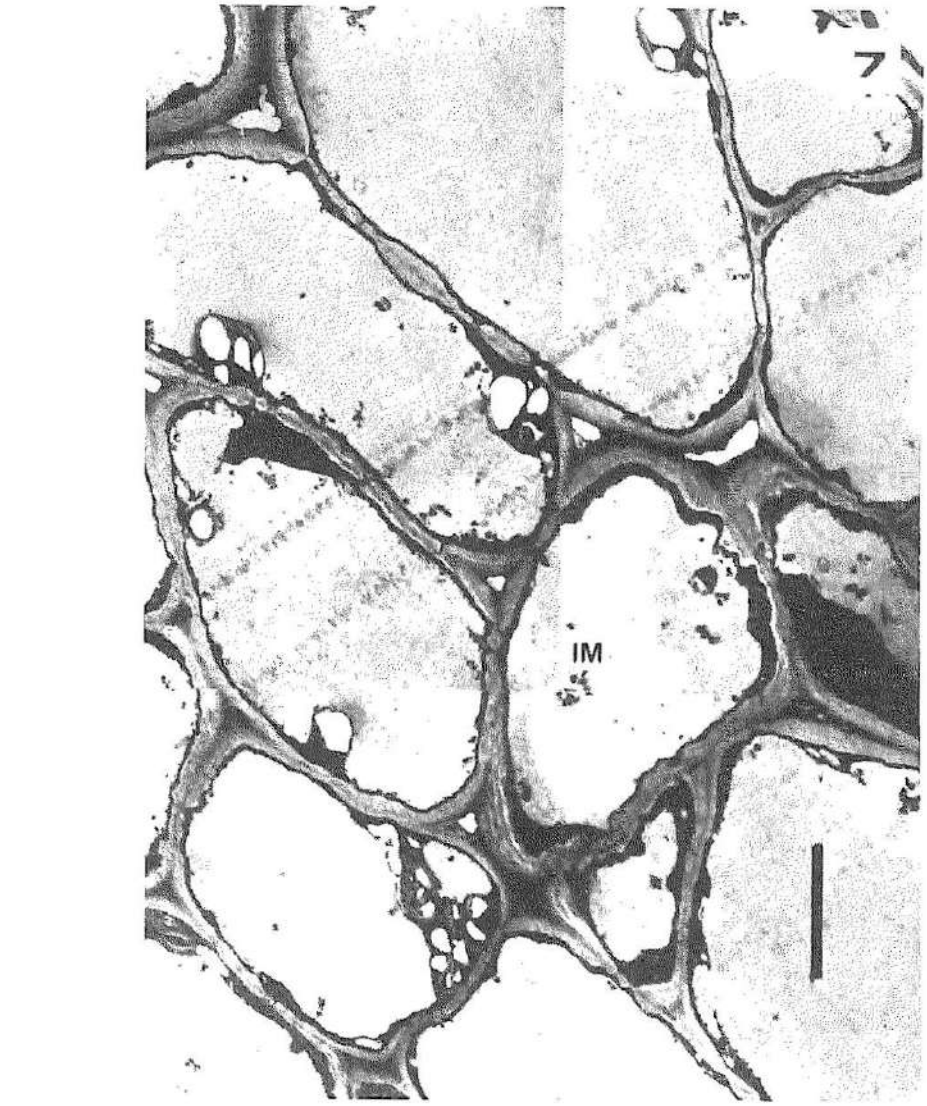


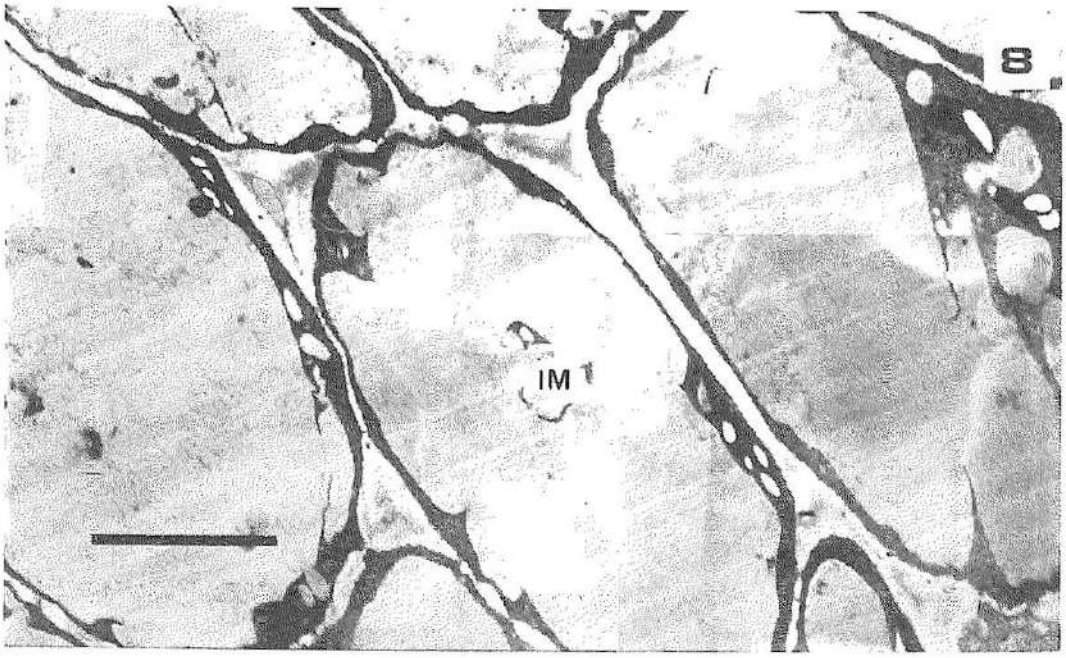
Figures 3 and 4: Thin cross-sections of epidermal and hypodermal cells from stage V (3) and outer mesocarp cells from stage III (4). Arrows indicate newly formed walls. Scale bar = 5 µm.



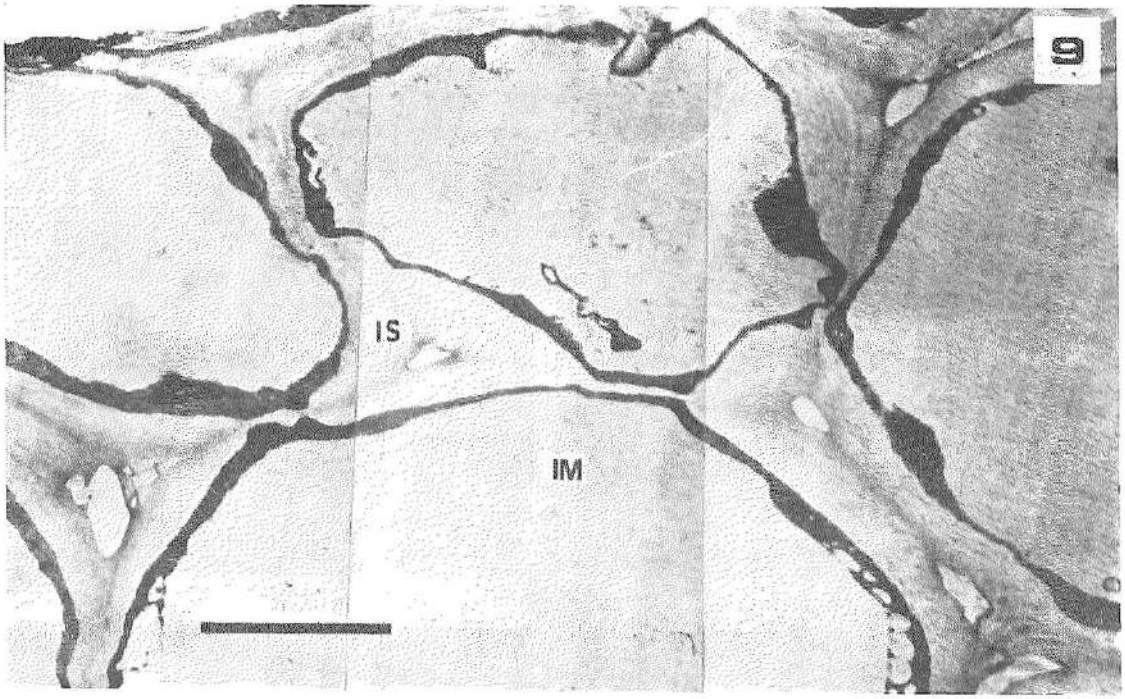


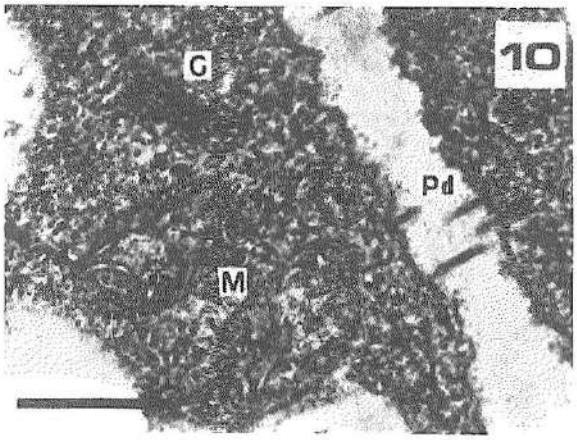
Figures 5 and 6: Thin cross-sections of outer mesocarp cells from stages V (5) and VI (6). Arrows indicate newly formed walls. Scale bar = 5 μm.

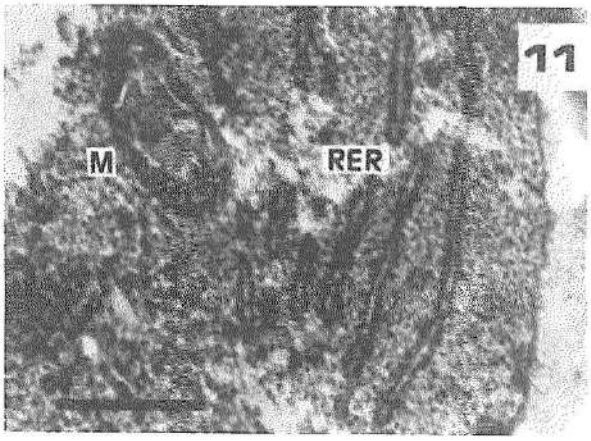


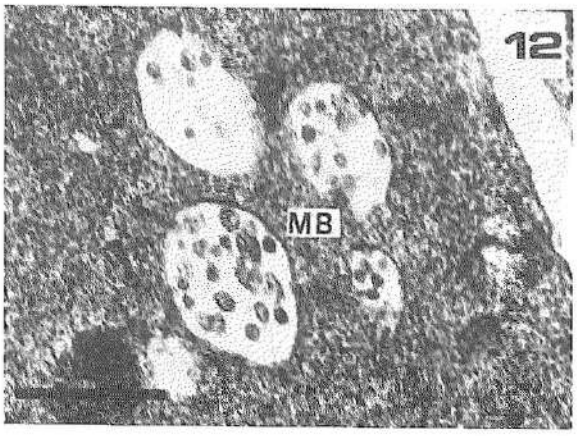


Figures 7 and 8: Thin cross-sections of inner mesocarp cells from stages I (7) and III (8). Scale bar = 5 μm.









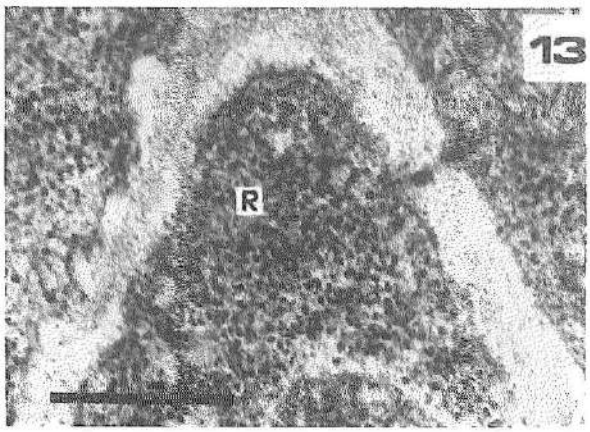
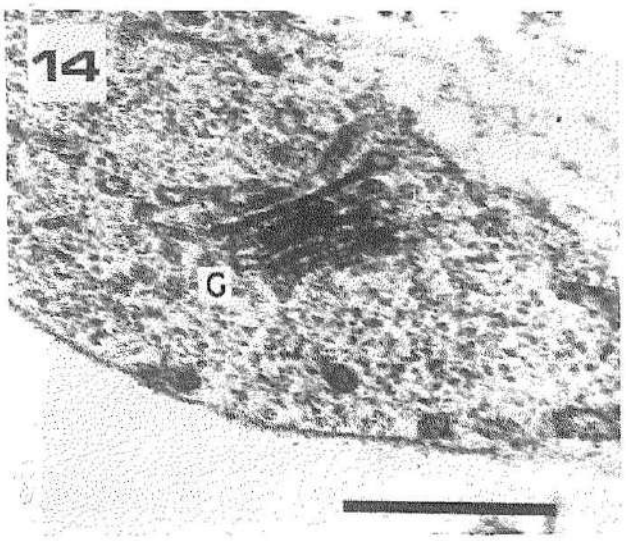
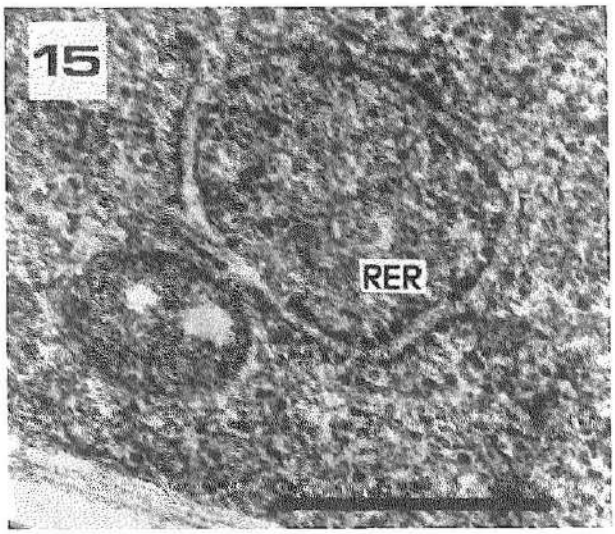
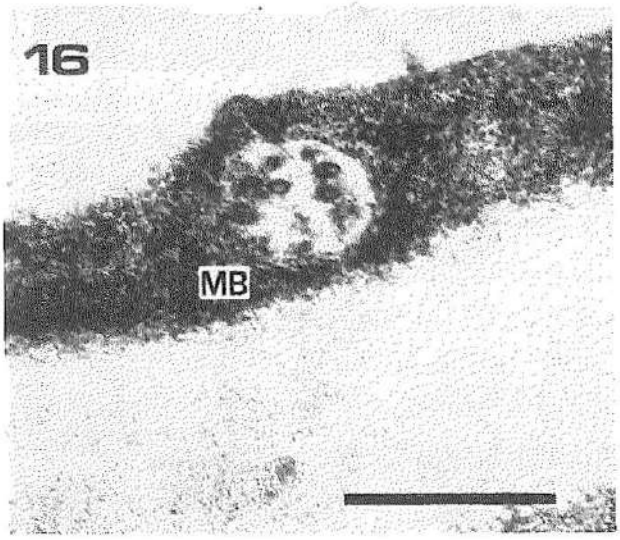
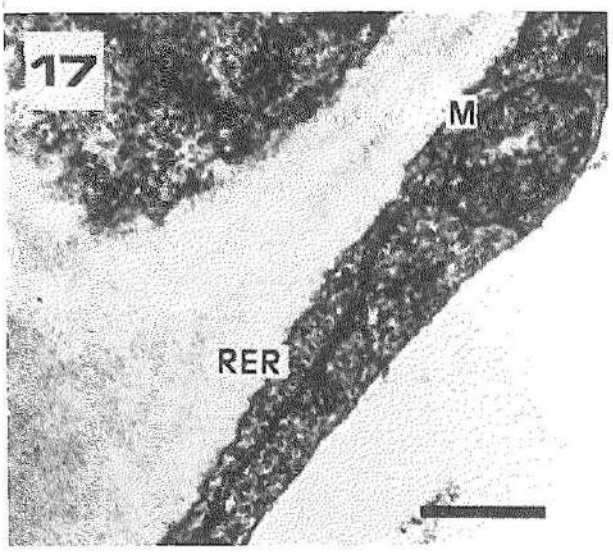


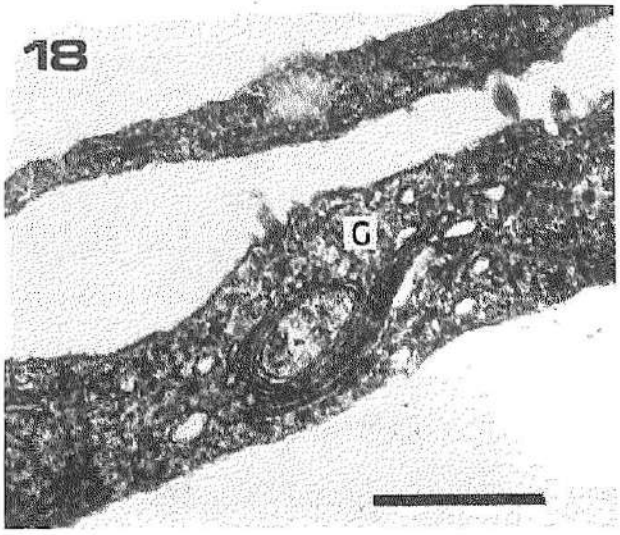
Figure 9: Thin cross-section of inner mesocarp cells from stage IV. Scale bar = 5 μm.
Figure 10–13: Portions of cytoplasm of epidermal cells from stages III (10) and IV (11) and outer mesocarp cells from stages II (12) and III (13) containing several cell organelles. Scale bar = 0.5 μm.

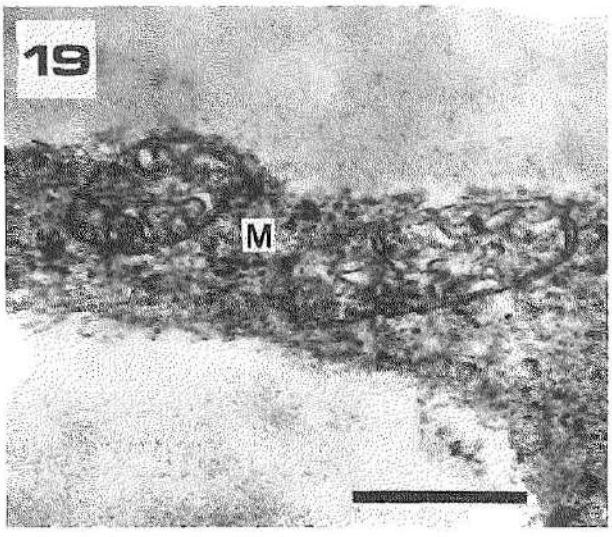




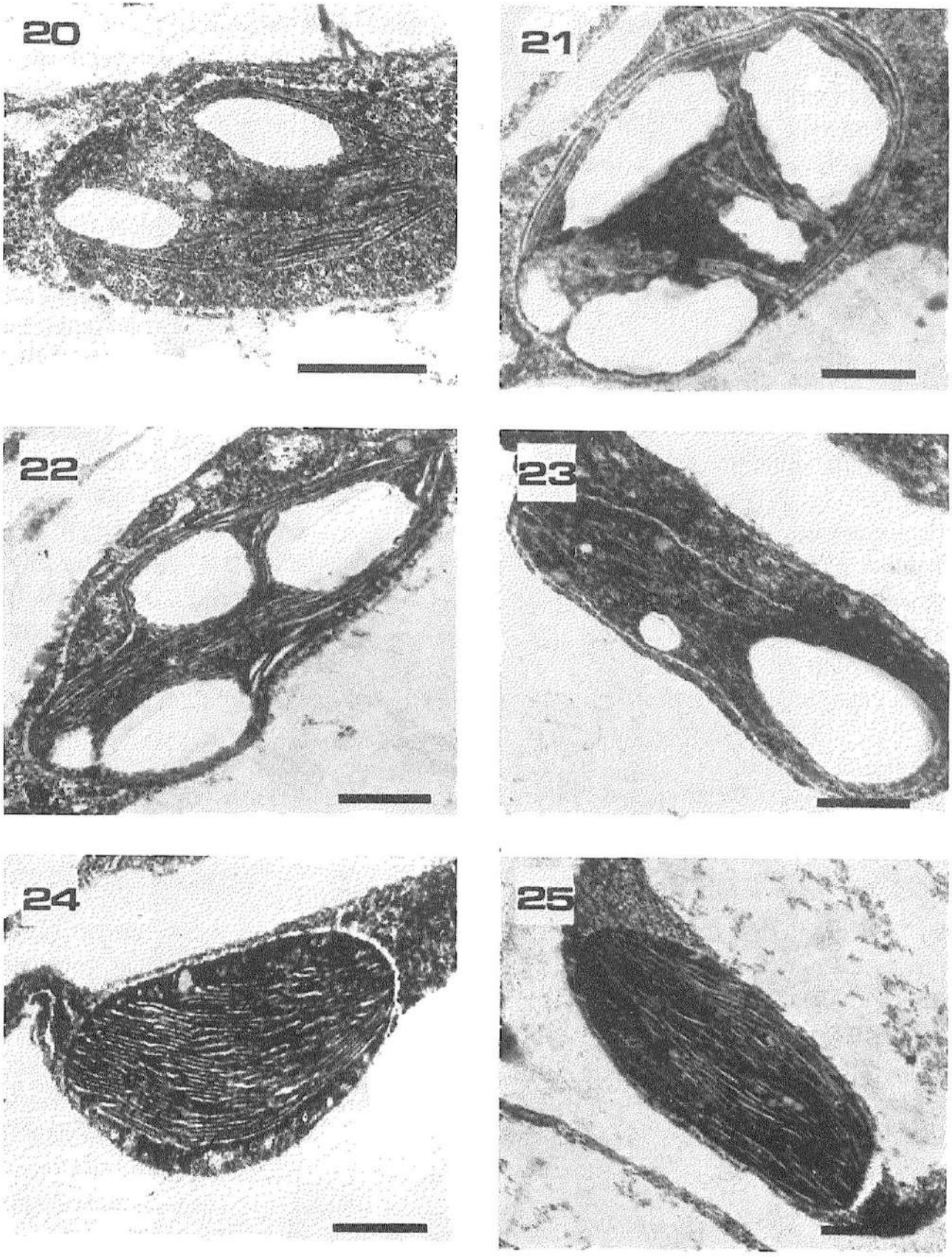




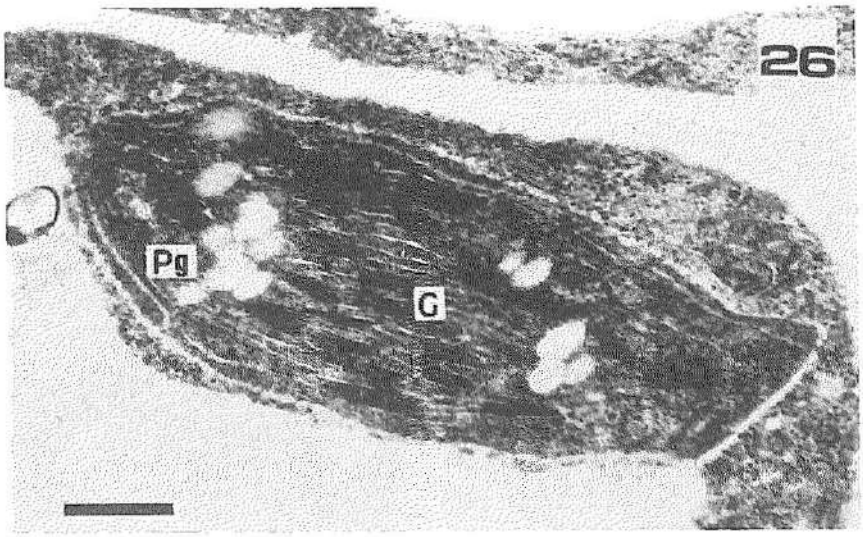


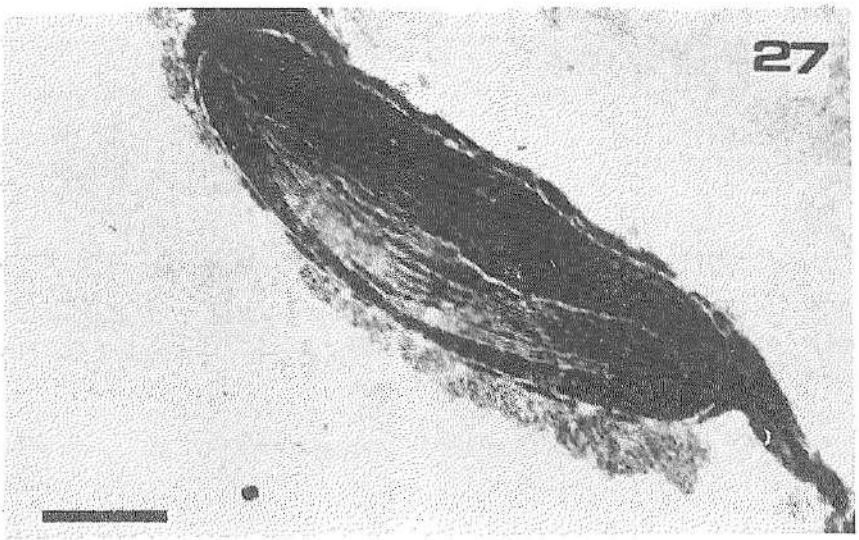


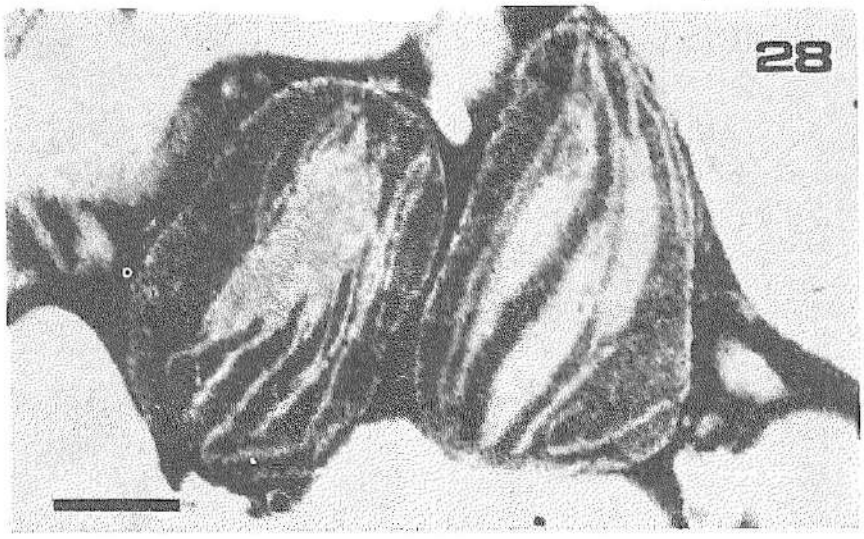
Figures 14–19: Portions of cytoplasm of outer mesocarp cells from stage IV (14 and 15) and inner mesocarp cells from stages I (16), II (17 and 18) and VI (19) containing several cell organelles. Scale bar = 0.5 μm.



Figures 20–25: Chloroamyloplasts and chloroplasts of epidermal cells from stages III (20) and V (24), outer mesocarp cells from stages I (21), III (22) and V (25), and an inner mesocarp cell from stage III (23). Scale bar = 0.5 μm.







Figures 26–28:Chloroplasts of an outer mesocarp cell from stage VI (26) and inner mesocarp cells from stages V (27) and VI (28). Note the disappearance of thylakoid system in degenerating chloroplasts of Figure 28. Scale bar = 0.5 µm.

During normal development of ovaries and fruits, numerous cell divisions of pericarp layers took place and this could be shown by the presence of walls, thinner than those usually surrounding the protoplast. Epidermal cells only divided anticlinally (Figs. 1-3, arrows), whereas hypodermical cells underwent both periclinal (Fig. 2, arrow) and anticlinal divisions (Fig. 3, arrow). Outer and inner mesocarp cells divided into anticlinal, periclinal and oblique planes (Figs. 4,5,7 arrows).

Cell walls of different layers of pericarp were thin (Figs. 1-8) even though thickness of inner mesocarp walls increased as development advanced (Fig. 9). On the other hand, periclinal walls of epidermal cells were usually thicker than the anticlinal ones (Figs. 1-3). A limited number of plasmodesmata was observed (Figs. 1-9) forming clusters or alone.

Intercellular spaces between pericarp cells were usually observed, even though in more advanced stages of development they were more conspicuous and larger (Figs. 1-9). There was no evidence of intercellular spaces between epidermal cells and the first layer of hypodermal cells (Figs. 1-3).

Degree of vacuolation of pericarp cells was variable. In general, outer mesocarp cells were slightly vacuolated containing abundant cytoplasm (Figs. 4-6), whereas epidermal and hypodermal cells had a higher degree of vacuolation. Inner mesocarp cells were highly vacuolated, the cytoplasm being limited to a narrow peripheral strip (Figs. 7-9). Pericarp cells contained a variable number of vacuoles, and a characteristic of the cells was the presence of a large central vacuole and many small vacuoles associated. Sometimes vacuoles contained small vesicles (Fig. 8).

The nuclei were characterized by a condensed nucleolus and by chromatin associated with the inner nuclear membrane (Figs. 1–6). The nuclei of epidermal and hypodermal cells tended to be located close to the internal periclinal walls (Figs. 1–3).

The plasmalemma of pericarp cells had no special features, the cytoplasm being fairly dense. The inner mesocarp cells sometimes contained invaginations of the tonoplast with cytoplasmic material. Number of risosomes was moderate, occurring free, in clusters, or as a constituent of rough endoplasmic reticulum (figs. 10, 15, 17). During petal fall (stage III) an increase in free ribosome content of epidermal and outer mesocarp cells was observed (Figs. 11, 13) resuming normal content afterwards. There was a small number of Golgi areas with a few vesicles associated (Figs. 10, 14, 18). They preferably located near the cell wall with associated plasmodesmata. Number of mitochomdria was moderate, showing a normal appearance (Figs. 10, 11, 17–19).

Outer mesocarp cells and, in small proportion, the inner mesocarp cells contained multivesicular bodies with an abundant small vesicles (Figs. 12, 16).

The more remarkable ultrastructural changes were related to plastids. There were a few plastids tending to be elongated. During the late stages of flowering, plastids (amyloplasts and chloroamyloplasts) stored starch granules, number and size being

variable (Figs. 20–23). An increasing gradient in plastid starch content from epidermis to inner mesocarp was observed. As development advanced a gradual disappearance in starch inclusions took place, initiating thylakoid membrane system formation. From petal fall (stage III) plastids without starch (chloroplasts) completely developed their endomembrane system with prominent grana and some plastoglobules (figs. 24–27). At the end of the fruit set period (stage VI) the degeneration of inner mesocarp plastids was observed. A breakdown of the thylakoid membrane system occurred, and hence the loss of their photosynthetic nature (Fig. 28).

Discussion

The ultrastructural study of the various layers of 'Navelate' pericarp has been carried out throughout stage I and the beginning of stage II which had been described by Bain (1958) in an anatomical and physiological study of development and ripening in 'Valencia' oranges. This expansion of pericarp by cell division was observed by Bain (1958) and later by Thompson and Platt-Aloia (1976) in epidermis layer. The main ultrastructural features of outer mesocarp cells such as little vacuolation, thin walls and abundant cytoplasm reinforced the view of this cellular strata as an active meristem (4) that originated cells towards the inner mesocarp.

The main ultrastructural changes were related to plastid development. Initially plastids contained an abundant amount of starch and a limited lamellar membrane system. Subsequently, there was an intermediate stage of development with little starch content and some membrane stacks resemble the grama of mature chloroplasts. Finally, plastids became real mature chloroplasts that had lost the starch and contained a more extensive endomembrane system and grana with photosynthetic ability. The ability of floral tissues to carry out photosynthesis, was previously reported (11). An additional photosynthetic supply would be the result of the conversion of the plastids with starch and a limited lamellar membrane system into mature chloroplasts. As fruit growth progressed a loss of internal membrane system of inner mesocarp plastids was observed in Stage VI of development. As a result of the increase of size and hence no light penetration into deeper layers of pericarp, a breakdown of the thylakoid system and chloroplast with systems of degeneration were observed. Plastid degeneration together with the presence of invaginations of the tonoplast, extensive vacuolation and large intercellular spaces in inner mesocarp might be the initial steps of subsequent transformation of this zone in a loose anastomosed network of parenchymatous cells with numerous large air spaces typical of citrus albedo (6).

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