

## CHAPTER 3

### **Ultrastructure of the Mesocarp of Mature Avocado Fruit and Changes Associated with Ripening**

Platt-Aloia, K. A. 1980. Ultrastructure of Mature and Ripening Avocado (*Persea americana* Mill.) Fruit Mesocarp; Scanning, Transmission and Freeze Fracture Electron Microscopy. PhD. Dissertation. University of California, Riverside. 113 pages.

Chapter 3. Ultrastructure of the Mesocarp of Mature Avocado Fruit and Changes Associated with Ripening

# Ultrastructure of the Mesocarp of Mature Avocado Fruit and Changes Associated with Ripening

**K. A. Platt-Aloia and W. W. Thomson**

Department of Botany and Plant Sciences

University of California, Riverside, California, 92521, U.S.A.

## ABSTRACT

The mesocarp tissue of ripening avocado fruits was studied by freeze fracture, thin section, and scanning electron microscopy. CO<sub>2</sub> and ethylene production by individual fruit were monitored, and samples were analyzed at several stages of the ripening process.

The tissue is composed primarily of large, isodiametric, lipid-containing parenchyma cells. At maturity these cells contain the normal complement of plant cell organelles, and all membranes appear intact. When ripening begins, several changes in the ultrastructure occur. The most obvious changes are a loosening and eventual breakdown of the cell wall, and swelling and vesiculation of the rough endoplasmic reticulum. In freeze fracture replicas a significant increase in the number of intra-membranous particles in the EF face of the plasmamembrane was observed at the climacteric peak.

In postclimacteric, soft fruit the particle density of the EF face of the plasmamembrane decreased to the density observed in the membrane of preclimacteric cells. All of the organelles and membranes appear whole and intact whether examined by thin section, freeze fracture, or scanning electron microscopy. However, the cell walls in postclimacteric fruit have almost completely disappeared.

These results indicate that the ripening process per se in avocados does not involve a complete loss of compartmentalization nor a breakdown of organelle and membrane integrity. It may, however, lead to these or similar senescence changes as a result of the loss of the cell walls. The variations in particle density of the plasmamembrane during ripening may reflect one or more of several structural, compositional, or functional membrane phenomena, and this aspect of ripening warrants further study.

*Key words: Freeze fracture, fruit ripening, scanning electron microscopy, senescence, ultrastructure*

## INTRODUCTION

For many years the ripening process of most fruits has been considered to be a senescence phenomenon (Sacher, 1967). It is often thought to involve a loss of membrane integrity resulting in a breakdown of compartmentalization, release of hydrolytic enzymes, organelle disintegration, and cellular death (Blackman and Parija,

1928; Bain and Mercer, 1964). These ideas have gained support from studies showing 1) increased cell membrane permeability in ripening tissues (Sacher, 1967; Brady, O'Connell, Smydzuk, and Wade, 1970); 2) loss of membrane lipids from ripening tomatoes (Kalra and Brooks, 1973) and from senescing cotyledons (McKersie, Lepock, Kruuv, and Thompson, 1978); and 3) structural breakdown as observed with the electron microscope (Bain and Mercer, 1964; Rhodes and Wooltorton, 1967; Butler and Simon, 1971; Knee, Sargent, and Osborne, 1977).

However, some questions concerning the validity of this interpretation have been raised. For example, several studies (Burg, Burg, and Marks, 1964; Simon, 1977; see Sacher, 1973) have shown that much of the observed leakage of solutes from fruit discs is due to osmotic bursting of the delicate cells of ripe fruit. The delicacy of the cells is supported by ultrastructural studies of ripening fruit which describe cell wall degradation (Pesis, Fuchs, and Zauberman, 1978; Ben-Aire, Kislev, and Frenkel, 1979; Platt-Aloia, Thomson, and Young, in press). Loss of the walls would result in cells which are easily broken or disrupted. Furthermore, Galliard (1968) showed that quantitative changes in lipids of pre- and postclimacteric apples involved a selective change, primarily in plastid membranes, and not from other organelles or the plasmamembrane. Additionally, there are numerous studies on ripening fruits which show large increases in enzyme activity (Dilley, 1970; Frenkel, Klein, and Oilley, 1968; Awad and Young, 1979), RNA synthesis (Frenkel et al., 1968; Looney and Patterson, 1967; Richmond and Biale, 1966) and increased protein synthesis (Dilley, 1970; Frenkel et al., 1968), all of which indicate that the cells of a ripening fruit are active in the synthesis of new materials. These biochemical and physiological studies are supported at the ultrastructural level (Thomson and Platt-Aloia, 1976). In a study of developing, ripening and senescing Navel oranges, they showed that cellular deterioration did not occur until well after ripening was complete. Thus, the results of these studies suggest that ripening apparently is not associated with a loss of compartmentalization or increasing cellular deterioration. Although there are, undoubtedly, changes during ripening, the maintenance of cellular integrity and function leads to the view that ripening is a determinant developmental process which is followed by senescence.

The ripening process in the avocado may provide information concerning this question of whether ripening should be considered a senescence phenomenon. The avocado is a climacteric fruit and therefore exhibits a characteristic rise in respiration and ethylene production during the ripening process, thus allowing a correlation of physiological and structural events. Furthermore, an avocado fruit will not ripen until it is harvested from the tree, and this provides a temporal separation of maturation and ripening, as well as control of the ripening process. Consequently, a study of the ultrastructural changes in cytoplasmic organelles correlated with the physiological process of ripening in avocado fruit may lead to a better understanding of the ripening process.

Because of the delicate nature of the cells of ripening fruit, we have studied this process in the avocado both by chemical fixation of tissues for thin sections and scanning electron microscopy, and by quick freezing for freeze fracture electron microscopy. By taking this approach of a comparison of the three methods using quite different preparative techniques, we can arrive at a more reliable conclusion as to changes in the ultrastructure of organelles and membranes during the ripening process of the avocado.

## MATERIALS AND METHODS

Avocado fruits (*Persea americana* Mill. var. Hass) were collected from trees at the South Coast Field Station of the University of California. Uniformly-sized fruit were placed in individual containers at 18 or 20°C under air passed at 100 ml/min CO<sub>2</sub> and ethylene released from each fruit were monitored every 4 h (Awad and Young, 1979). Fruit were removed from their containers at various stages of ripening and samples were prepared for microscopy. Some studies have shown that ripening in avocados may not be uniform throughout the fruit (Biale and Young, 1971). To avoid excess variability, we consistently took our samples from the area of largest diameter, on the side of the fruit where the distance from the stem to the blossom end is smallest. After sampling, the fruit was returned to its container for continued monitoring of CO<sub>2</sub> and ethylene production. Awad and Young (1979) have shown that removal of tissue samples from avocados does not affect the pattern of respiration or ethylene production.

Tissue samples for transmission (TEM) and scanning (SEM) electron microscopy were placed immediately into 1% glutaraldehyde in 50 mM cacodylate buffer, pH 7.2, cut into smaller (~1x4 mm) pieces, and then transferred to fresh 1% glutaraldehyde for 2-3 h. After a brief rinse in buffer, the tissue was postfixed 3 h or overnight in 1% OsO<sub>4</sub> in 50 mM cacodylate buffer.

The tissue for TEM was dehydrated in acetone and embedded in an epoxy resin (Spurr, 1969). Thin sections were cut with a diamond knife using a Porter Blum MT-2 ultramicrotome, stained with 1% aqueous uranyl acetate for 1 h and with lead citrate (Reynolds, 1963) for 1-2 min.

The tissue to be examined in the SEM was prepared by a modification of the osmium thiocarbohydrazide method (Malick and Wilson, 1975; Kelley, Dekkar, and Bluemink, 1973; see Platt-Aloia and Thomson, 1980). After post-fixation in 1% OsO<sub>4</sub> in buffer, tissue was washed in distilled water, then treated with freshly made, filtered, 1% thiocarbohydrazide (TCH) for 20-30 min with frequent agitation. The tissue was again washed with distilled water and treated with 1% OsO<sub>4</sub> in distilled water overnight. The above procedure (water rinses, 1% TCH for 20-30 min, rinsing, and treatment with OsO<sub>4</sub> overnight) was repeated the next day. The tissue was then dehydrated in acetone and critical point dried according to the method of Anderson (1951) with a Tuisimas CO<sub>2</sub> critical point dryer. After drying, pieces of tissue were broken in half and mounted on aluminum stubs with conductive paint containing colloidal silver. They were oriented so that the freshly broken surface, relatively free from contamination, was exposed. Samples were stored in a desiccator until viewing with a Joelco JSM-U3 scanning electron microscope at 10 or 20 KV.

Samples taken for freeze fracture electron microscopy were prepared in two different ways. Some were placed directly into buffer, without chemical fixation or cryoprotection, and cut into pieces 1-2 mm<sup>3</sup>. Others were fixed for 1.5 h in 2.5% glutaraldehyde, 50 mM cacodylate buffer, then infiltrated, stepwise, into 20% glycerol. All samples were then quickly placed into gold-nickel planchets, frozen in Freon 22 near its freezing point, and stored in liquid nitrogen. Fracturing and replication was according to Moor and Mühlethaler (1963) at -120°C and less than 2 x 10<sup>-6</sup> torr with a Balzers BAF 301 equipped with a quartz thin film monitor. The replicas were cleaned with chromic sulfuric acid and 12% KOH in 95% EtOH. The nomenclature for membrane faces follows that

of Branton et al. (1975). Particle densities were determined by counting the number of particles within a subdivided  $0.25 \mu\text{m}^2$  quadrant on micrographs enlarged to 100,000 X. Statistical analysis of these particle density counts was performed using one-way analysis of variance. Significant differences between the means of each group (pre, rise, peak, and post climacteric) was determined by use of the Student-Neumann-Kuels a posteriori test, as described in Scheffler (1979).

All thin sections and replicas were studied with a Philips EM 300 or a Philips EM 400 electron microscope. Serial section reconstructions were accomplished according to the method of Atkinson et al. (1974). Mitochondria enlarged to a magnification of 36,000, were traced from micrographs onto transparent sheets. The sheets were spaced with 3 mm thick plates of glass and photographed with background light.

## RESULTS

The mesocarp of a mature hard, unripe avocado fruit was found to be composed primarily of parenchyma cells, scattered oil cells (idioblasts) and a few vascular strands. The majority of cells were isodiametric, lipid containing parenchyma cells with an average diameter of  $50 \mu\text{m}$  (Plate 1A & B). The following description of the ultrastructure and subcellular organization of these cells is divided into two parts: 1) those organelles and cellular components which apparently do not change significantly in ultrastructure during the ripening process, and 2) those organelles which do undergo some degree of structural change or reorganization.

### Organelles which do not change during ripening

The most prominent component of the parenchyma cell cytoplasm was the numerous large lipid bodies (Plate 1B & C, L). These were generally circular in outline, but their smooth contour was frequently interrupted by indentations which were often occupied by various organelles such as plastids, mitochondria, and microbodies (Plate 1C, arrows). The indentations provided a landmark to identify the large spherical structures with numerous invaginations seen in the SEM as lipid bodies (Plate 1A and D, L). Neither a full unit membrane nor a half membrane was readily apparent surrounding the lipid bodies as seen in thin sections. In freeze fracture, although most of the lipid bodies cross-fractured, (Plate 2A), occasionally the fracture plane appeared to follow the contour of the lipid body, either as a convex or as a concave surface. In both these instances the surface appeared textured and devoid of particles (Plate 2B). As a result of the large quantity of lipid in these cells, the vacuoles were a relatively minor component of the cellular space. They were irregular in outline and the contents varied from electron transparent to a fine flocculent or granular material.

Plastids were another prominent component of these cells. When viewed in fresh cross section, the avocado mesocarp exhibits a gradient in color from dark green in the outer cells just under the skin, to a lighter green and finally yellow deeper into the fruit. The structure of the plastids reflected this gradient from the outer to the inner tissues by a decrease in the size and number of grana, and an increase in the size and number of prolamellar bodies. Thus, the chloroplasts in the outer dark green tissues (Plate 2C) had large grana stacks and a few small prolamellar bodies or tubular complexes. The chloroplasts from cells in the light green region (Plate 2D) contained smaller grana and

larger prolamellar bodies. Additionally, these light green chloroplasts often contained membrane bound bodies, the limiting membrane of which was often continuous with the granal and prolamellar body membranes (Plate 2D, 5C). The etioplasts of the inner, yellow tissue were devoid of grana or contained very small grana and large prolamellar bodies (Plates 1C & 3A). Membrane bound bodies were not observed in these innermost etioplasts. The plastids in all tissues of the avocado mesocarp were irregular in shape and all types contained starch and plastoglobuli.

Several other cellular organelles exhibited no apparent changes during ripening. The dictyosomes were simple in structure and usually composed of 4-6 cisternae and a few associated vesicles (Plate 3A). Microbodies were numerous and contained an electron dense, granular matrix. Nuclei were usually elongate, located in the peripheral regions of the cell, and had a prominent nucleolus (Plate 2D). Most of the ribosomes occurred as single, free constituents, rather than linked together as polysomes, and many were seen to be bound to the endoplasmic reticulum.

#### Organelles which change during ripening

Thin section electron microscopy revealed that in the mature, unripe fruit, mitochondria had numerous cristae and an electron dense matrix. They usually occurred in groups and were either round or elongated in outline (Plate 3B). However, serial section reconstructions of these groups of mitochondria showed most of them to be one or two long, branching mitochondria (Plate 3C), indicating their identification in the SEM to be the long worm-like structures (Plate 1D). During the period of ripening, there was no obvious structural change in the mitochondria as revealed by thin sections (compare Plates 3B and 3D). However, in SEM of avocado mesocarp cells, although no quantitative measurements were made, there was evidence of an increase in the length of the mitochondria during ripening (compare Plates 1D and 4A). The structural integrity of the mitochondria, as well as other organelles just described (plastids, microbodies, dictyosomes and nuclei) appeared to be retained through ripening and in the post-climacteric, soft fruit (Plates 3D, 4B).

Another cytoplasmic organelle which exhibited structural alteration during the ripening process was the rough endoplasmic reticulum (ER). In the SEM, the ER of the hard, unripe fruit exhibited a variety of forms, primarily tubular and sheet-like, with only a minor amount of vesiculation apparent (Plates 1D and 4C). In thin sections of fruit in the early stages of the climacteric rise, the ER appeared to swell (Plate 4D) as compared to recently picked fruit (Plates 2D, 3B). This swelling was apparently followed by vesiculation which predominates both at the climacteric peak (Plate 3D) and in the post-climacteric, soft fruit (Plates 4B, 5C). These vesicles of ER, with ribosomes attached, were frequently seen to be in close proximity to, and possibly in the process of fusion with, the plasmamembrane (Plates 3D, 4E).

The plasmalemma also exhibited changes in structure or organization during ripening and appeared to become increasingly irregular as seen in thin sections of ripening fruit (Plate 3D). Additionally, counts made from freeze fracture replicas of fruit at various stages of ripening showed a statistically significant change in the density of intramembraneous particles (IMPs) on the EF face of the plasmamembrane at the climacteric peak. Densities of 1500 IMPs/ $\mu\text{m}^2$  were found for pre-, rise and

postclimacteric membranes (Plate 5A, Table 1). However, the density of these particles at the peak of the climacteric (Plate 5B, Table 1) were 1900 IMPs/pm<sup>2</sup> which is significantly higher ( $P < 0.01$ ) than any other stage.

## DISCUSSION

It is apparent in the present study that the mature avocado fruit consists primarily of physiologically active and ultrastructurally intact parenchyma cells at all stages of ripening. Mature avocado fruits of the Hass variety have been reported to contain approximately 20% fat on a whole fruit, fresh weight basis (Biale and Young, 1971). Approximately 85% of these lipids are triglycerides, and occur as droplets or oil bodies within the cytoplasm of the parenchyma cells (Plate 1A, B, C). A limiting membrane is not evident either in thin sections, or in freeze fracture replicas. This is in contrast to the full unit membrane reported to surround spherosomes of barley aleurone layers (Buttrose, 1971), and the half-unit membrane as reported in peanut cotyledons (Yatsu and Jacks, 1972). At present, our conclusion is that the boundary of avocado lipid droplets is determined by an aqueous/nonaqueous interface with the cytoplasm rather than a membrane.

The vacuoles of some plant cells have been considered to have a lysosomal function (Matile, 1975; Thomson and Platt-Aloia, 1976; Matile, 1978) and have been shown to undergo considerable change in content during senescence processes (Thomson and Platt-Aloia, 1976). The small vacuoles of avocado mesocarp, on the other hand, appear to remain fairly consistent in their structure and content throughout the ripening process. This apparent lack of activity might be significant in reflecting a balance of turnover and sustained metabolic activity, rather than a loss of recycling ability, which would result in the accumulation of degradative products as has been observed in senescent cells of orange rind or leaves (Butler and Simon, 1971; Thomson and Platt-Aloia, 1976).

Plastids are probably the most frequently studied and described organelle of fruit tissue at the ultrastructural level (Bain and Mercer, 1964; Rosso, 1968; Thomson, 1969; Mohr and Stein, 1969; Phan, 1970; Ljubescic, 1977). This is primarily due to the fact that most fruits undergo a color change during ripening. Avocados, on the other hand, ripen without any apparent color change of the mesocarp. The structure of their plastids varied with their location in the fruit which is probably dependent on the degree of penetration of light into the tissue. This observation is in agreement with the study by Cran and Possingham (1973) on avocado fruit plastids. Our observation that no apparent change in the ultrastructure of plastids occurred during ripening of avocados is significant. Previous studies on fruit ripening have concluded that the loss of grana and an increase in pigments in the plastids are a sign of deterioration and thus of senescence (Bain and Mercer, 1964; Mohr and Stein, 1969; Knee et al., 1977). The retention of structural integrity of avocado plastids, including the chloroplasts of the outer green tissue (Plate 4B) is evidence of continued function and suggests the possibility that structural changes in plastids of other ripening fruits may be only a reflection of developmental transition of function, rather than one of a senescent deterioration. This opinion was also expressed by Spurr and Harris (1968) in a study of changes in the ultrastructure of tomato fruit plastids during ripening. They interpreted the transitions which occurred to be an indication of a dynamic synthetic activity rather

than breakdown.

The maintenance of structural integrity during ripening which is exhibited by the plastids can also be seen in the mitochondria. The mesocarp cells of avocado fruit are capable of a considerable increase in CO<sub>2</sub> production during the ripening process (Biale and Young, 1971). Thus, it is not surprising to find the mitochondria of these cells rather large and complex organelles with a compact matrix and numerous tubular cristae. The size and morphology of mitochondria may be a indication of their functional capacity or state (Heywood, 1977). An apparent extreme has been reported by Pellegrini (1978) in the giant mitochondrion of *Euglena*. It was shown, by serial section reconstruction, that *Euglena* contain a single, anamostosing mitochondrion, the morphology and size of which changed markedly, depending on the conditions of growth. It may, therefore, be a significant reflection of the functional status of the avocado fruit that its mitochondria appear to occur as large, branching structures. Additionally, the apparent increase in the size or length of these mitochondria during ripening as seen in SEM (compare Plates 1D and 4A) as well as the maintenance of structural integrity might be evidence of increased activity during this time.

Another structural indication of the functionally dynamic nature of ripening in avocados is the rather extensive endoplasmic reticulum found in these cells. The variety of forms in the unripe fruit (sheet-like, tubular and vesiculating) as seen in the SEM (Platt-Aloia and Thomson, (1980) implies a dynamic and physiologically active system and may be indicative of active protein synthesis. In avocados, during the climacteric rise in respiration and increased ethylene production, there is also a significant increase in the activity of the wall hydrolytic enzymes polygalacturonase and cellulase (Pesis et al., 1978; Awad and Young, 1979). Although studies have not been done to determine whether this increase in activity is due to de novo synthesis or an activation of inactive forms of the enzymes, the apparent activity of the rough ER during ripening might be suggestive of the former. Additionally, since the site of action of these enzymes is presumably in the cell wall, transport across the plasmamembrane is necessary. The vesiculation of the rough ER and its apparent fusion with the plasmamembrane during ripening (Plates 3D, 4E) are suggestive of a possible mode of synthesis, compartmentalization, and secretion by exocytosis of these enzymes. In studies of the ultrastructure of leaf abscission zones, Sexton and colleagues (Sexton and Hall, 1974; Sexton, Jamieson, and Allan, 1977) found significant increases in rough ER and dictyosomes during abscission. They suggested this is probably related to the increased protein synthesis and is possibly involved in secretion of the wall hydrolytic enzymes which, as in ripening avocados, are active during abscission. Since few and small dictyosomes are apparent in ripening avocados, the vesicles of the ER may be involved in the exportation of wall hydrolytic enzymes.

Fusion of vesicles with the plasmalemma is apparent both in thin sections and freeze fracture replicas (Plates 2A, 3D and 4E). Additionally, the increasing irregularity of the plasmalemma during ripening suggests an increase in surface area due to insertion of vesicle membrane. Similar irregularities ("raised areas") in freeze fractured membranes were reported by Mullins (1979) in the fungus *Achlya* at sites of active secretion of cellulase. In other studies, distinctive patterns of IMPs have been reported to be associated with membrane fusion events (Satir, 1980). These rosette patterns or other



structural arrangements of IMPs were not found in the avocado membranes at any stage of the ripening process. There was, however, a significant increase in the particle density of the EF face of the plasmalemma which closely coincided with the respiratory climacteric, maximum ethylene evolution, and with changes in wall hydrolytic enzyme activities (Awad and Young, 1979). Parish (1974) reported increased particle density in the plasmalemma of *Salix* cambial-zone cells in the spring as compared to the winter, dormant cells. This increase was correlated with increased physiological activity as well as with new wall synthesis. The significance of the increase in particle density observed at the climacteric peak of ripening avocados is unknown and interpretation would be pure speculation. The nature of the particles is still questionable; whether they represent intrinsic membrane proteins (Fujimoto and Ogawa, 1980), lipopolysaccharides (Verregaert and Verkleij, 1978), or are a manifestation of a phase change of the membrane lipids themselves (DeKruiff et al., 1979; DeKruiff, Cullis, and Verkleij, 1980) is unknown at this time. However, the observation that the particle density returns to the preripening level in the post climacteric fruit indicates that this change reflects a transitory event and may be related to one or more of the processes of ripening, i.e. ethylene production or release, enzyme secretion, plasmalemma-vesicle fusion, or other unknown phenomena.

In conclusion, the ultrastructural changes which occur during the ripening of avocado fruits, are, with the exception of the cell wall, apparently not degradative or senescent. Further, the lack of change of some organelles (e.g. plastids and vacuoles) is additional evidence of the preservation of compartmentalization through membrane integrity. Although ripening may be the last anabolic period in the life of a fruit, it evidently does not include, in the avocado, a loss of organizational resistance or degradation of the cytoplasmic organelles.

## LITERATURE CITED

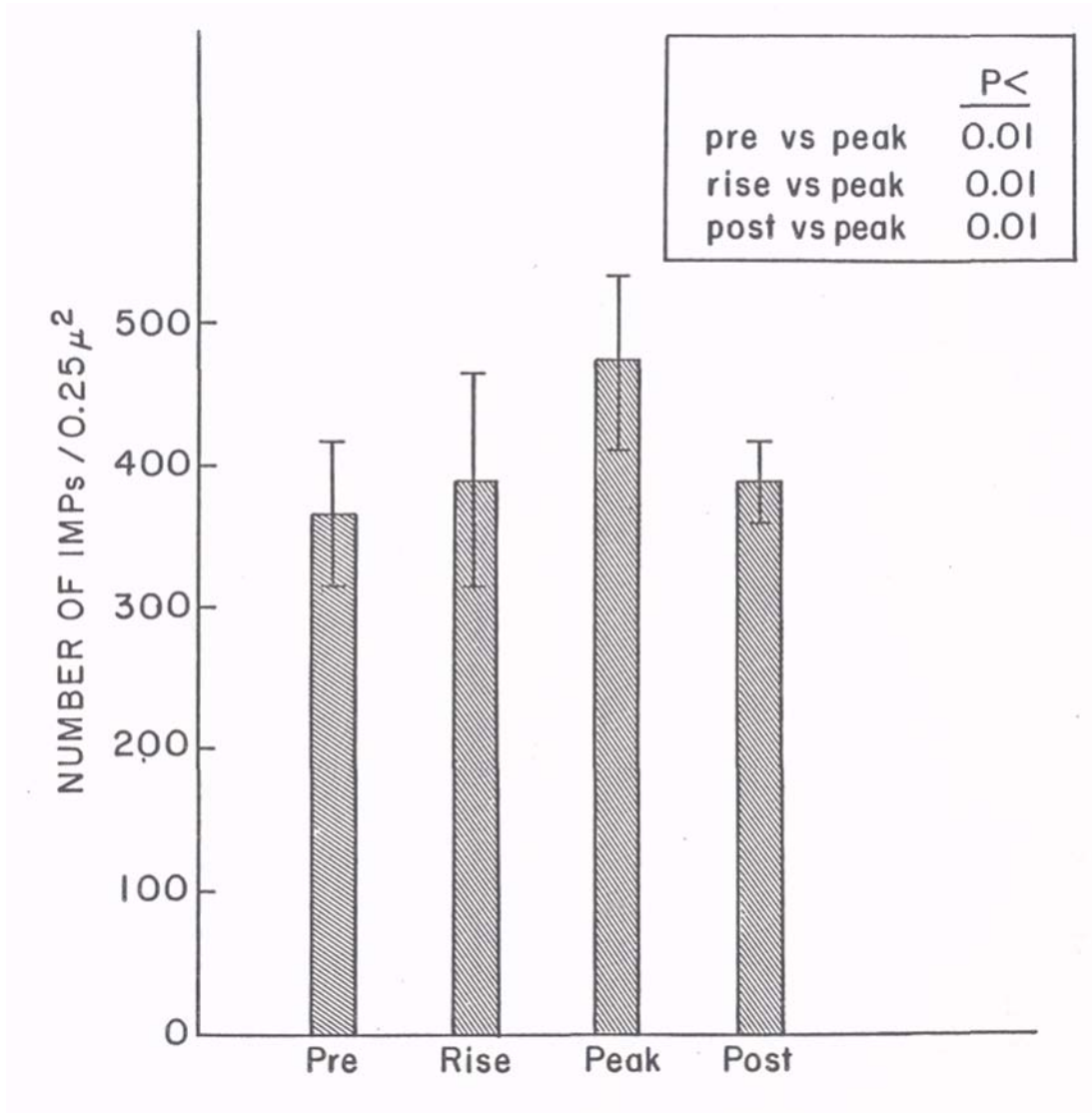
- Anderson, T. F., 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *Trans. N.Y. Acad. Sci.* 13, 130-4.
- Atkinson, Jr., A. W. , John, P. C. L. and Gunning, B. E. S., 1974. The growth and division of the single mitochondrion and other organelles during the cell cycle of *Chlorella*, studied by quantitative stereology and three dimensional reconstruction. *Protoplasma* 81, 77-109.
- Awad, M. and Young, R. E. 1979. Postharvest variation in cellulase, polygalacturonase, and pectinmethylesterase in avocado (*Persea americana* Mill. cv. Fuerte) fruits in relation to respiration and ethylene production. *Plant Physiol.* 64, 306-8.
- Bain, J. M. and Mercer, F. V., 1964. Organization resistance and the respiratory climacteric. *Aust. J. Biol. Sci.* 17, 78-85.
- Ben-Aire, R. , Kislev, N. and Frenkel, C. , 1979. Ultrastructural changes in the cell walls of ripening apple and pear fruit. *Plant Physiol.* 64, 197-202.
- Ben-Yehoshua, S., 1964. Respiration and ripening of discs of the avocado fruit. *Plant Plantarum.* 17, 71-80.
- Biale, J. B. and Young, R. E., 1971. The avocado pear. In *The Biochemistry of Fruits*

- and their Products, vol. 2, ed. A. C. Hulme, pp. 1-63. Academic Press, London.
- Blackman, F. F. and Parija, P., 1928. Analytical studies in plant respiration. I. The respiration of a population of senescent ripening apples. *Proc. Roy. Soc. JB.* 103, 412-45.
- Brady, C. J., O'connell, P. B. H. , Smydzuk, J. and Wade, N. L., 1970. Permeability, sugar accumulation, and respiration rate in ripening banana fruits. *Aust. J. Biol. Sci.* 23, 1143-52.
- Branton, D., Bullivant, S., Gilula, N. B., Karnovsky, M. J., Moor, H., Mühlethaler, K., Northcote, D. H., Packer, L., Satir, B., Satir, P., Speth, V., Staehelin, L. A., Steere, R. L. and Weinstein, R. S., 1975. Freeze-etching nomenclature. *Science* 190, 54-6.
- Burg, S. P., Burg, E. A. and Marks, R., 1964. Relationship of solute leakage to solution tonicity in fruits and other plant tissues. *Plant Physiol.* 39, 185-95.
- Butler, R. D. and Simon, E. W., 1971. Ultrastructural aspects of senescence in plants. *Adv. Geront. Res.* 3, 73-129.
- Buttrose, M. S., 1971. Ultrastructure of barley aleurone cells as shown by freeze-etching. *Planta* 96, 13-26.
- Cran, D. G. and Possingham, J. V., 1973. The fine structure of avocado plastids. *Ann. Bot.* 37, 993-7.
- DeKruiff, B., Cullis, P. R. and Verkleij, A. J., 1980. Non-bilayer lipid structures in model and biological membranes. *Trends Biochem. Sci.* 5, 79-81.
- DeKruiff, B., Verkley, A. J., Van Echteld, C. J. A., Gerritsen, W. J., Mommers, C., Noordam, P. C. and DeGier, J., 1979. The occurrence of lipidic particles in lipid bilayers as seen by <sup>31</sup>P NMR and freeze-fracture electron-microscopy. *Biochim. Biophys. Acta* 555, 200-9.
- Dilley, D. R., 1970. Enzymes. In *The Biochemistry of Fruits and Their Products*, vol. 1, ed. A. C. Hulme, pp. 179-207. Academic Press, London.
- Frenkel, C., Klein, I. and Dilley, D. R., 1968. Protein synthesis in relation to ripening of pome fruits. *Plant Physiol.* 43, 1146-53.
- Fujimoto, T. and Ogawa, K., 1980. Intramembranous particles on freeze-fractured membrane replica and sulfhydryl groups. *Histochemistry* 65, 217-22.
- Galliard, T., 1968. Aspects of lipid metabolism in higher plants. II. The identification and quantitative analysis of lipids from the pulp of pre-and post-climacteric apples. *Phytochemistry* 7, 1915-22.
- Heywood, P., 1977. Evidence from serial sections that some cells contain large numbers of mitochondria. *J. Cell Sci.* 26, 1-8.
- Kalra, S. K. and Brooks, J. L., 1973. Lipids of ripening tomato fruit and its mitochondrial fraction. *Phytochemistry* 12, 487-92.
- Kelley, R. O., Dekkar, R. A. F. and Bluemink, J. G., 1973. Ligand-mediated osmium binding: its application in coating biological specimens for scanning electron microscopy. *J. Ultrastr. Res.* 45, 254-8.

- Knee, M., Sargent, J. A. and Osborne, D. J., 1977. Cell wall metabolism in developing strawberry fruits. *J. Exp. Bot.* 28, 377-96.
- Ljubescic, N., 1977. The formation of chromoplasts in fruits of *Cucurbita maxima* Duch "Turbaniformis". *Bot. Gaz.* 138, 286-90.
- Looney, N. E. and Patterson, M. E., 1967. Changes in total ribonucleic acid during the climacteric phase in yellow transparent apples. *Phytochemistry* 16, 1517-20.
- Malick, L. E. and Wilson, R. B., 1975. Modified thiocarbohydrazide procedure for scanning electron microscopy: Routine use for normal, pathological, or experimental tissues. *Stain Tech.* 50, 265-9.
- Matile, P., 1975. *Cell Biology Monographs; The Lytic Compartment of Plant Cells*, Vol. 1, 183 pp. Springer-Verlag, Wien/New York.
- Matile, P., 1978. Biochemistry and function of vacuoles. *Ann. Rev. Pl. Physiol.* 29, 193-213.
- McKersie, B. D., Lepock, J. R., Kruuv, J. and Thompson, J. E., 1978. The effects of cotyledon senescence in the composition and physical properties of membrane lipid. *Biochim. Biophys. Acta* 508, 197-212.
- Mohr, W. P. and Stein, M., 1969. Fine structure of fruit development in tomato. *Can. J. Plant Sci.* 49, 549-53.
- Moor, H. and Mühlethaler, K., 1963. Fine structure in frozen-etched yeast cells. *J. Cell Biol.* 17, 609-28.
- Mullins, J. T., 1979. A freeze-fracture study of hormone-induced branching in the fungus *Achlya*. *Tissue and Cell* 11, 585-95.
- Parish, G. R., 1974. Seasonal variation in the membrane structure of differentiating shoot cambial-zone cells demonstrated by freeze-etching. *Cytobiologie* 9, 131-43.
- Pellegrini, M., 1978. The giant mitochondria of *Euglena gracilis* Z. Qualitative and quantitative variations in photoautotrophic, photoheterotrophic and heterotrophic synchronous cultures. In *Plant Mitochondria*, ed. G. Ducet and C. Lance, 454 pp. Elsevier/North Holland Biomedical Press, Amsterdam.
- Pesis, E., Fuchs, Y, and Zauberman, G., 1978. Cellulase and softening in avocado. *Plant Physiol.*, 61, 416-9.
- Phan, C. T., 1973. Chloroplasts of the peel and the internal tissues of apple-fruits. *Experientia* 29, 1555-7.
- Platt-Aloia, K. A. and Thomson, W. W., Aspects of the three-dimensional intracellular organization of mesocarp cells as revealed by scanning electron microscopy. *Protoplasma* (in press).
- Platt-Aloia, K. A., Thomson, W. W. and Young, R. E., Ultrastructural changes in the walls of ripening avocados: Transmission, scanning, and freeze-fracture microscopy. *Bot. Gaz.* (in press).
- Reynolds, E. S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17, 208-12.

- Rhodes, M. J. C. and Woollorton, L. S. C., 1967. The respiration climacteric in apple fruits. The action of hydrolytic enzymes in peel tissue during the climacteric period in fruit detached from the tree. *Phytochemistry* 6, 1-12.
- Richmand, A. and Biale, J. B., 1966. Protein and nucleic acid metabolism in fruits: studies of amino acid incorporation during the climacteric rise in respiration of the avocado. *Plant Physiol.* 8, 1247-1253.
- Rosso, S. W., 1968. The ultrastructure of chromoplast development in red tomatoes. *J. Ultrastruct. Res.* 25, 307-22.
- Sacher, J. A., 1967. Studies of permeability, RNA and protein turnover during ageing of fruit and leaf tissues. *Symp. Soc. Exp. Biol.* 21, 269-304.
- Sacher, J. A., 1973. Senescence and post-harvest physiology. *Ann. Rev. Pl. Physiol.* 24, 197-224.
- Satir, B. H. 1980. The role of local design in membranes. In *Membrane-Membrane Interactions. Society of General Physiologists Series*, vol. 34, ed. N. B. Gilula, 218 pp. Raven Press, New York.
- Scheffler, W. C., 1979. *Statistics for the Biological Sciences*, 230 pp. Addison Wesley Publishing Co., Reading, Mass.
- Sexton, R., Jamieson, G. G. C., and Allan, M. H. I. L., 1977. An ultrastructural study of abscission zone cells with special reference to the mechanism of enzyme secretion. *Protoplasma* 91, 369-87.
- Sexton, R. and Hall, J. L., 1974. Fine structure and cytochemistry of the abscission zone cells of *Phaseolus* leaves. I. Ultrastructural changes occurring during abscission. *Ann. Bot.* 38, 849-54.
- Simon, E. W., 1977. Leakage from fruit cells in water. *J. Exp. Bot.* 28, 1147-52.
- Spurr, A. R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26, 31-43.
- Spurr, A. R. and Harris, W. M., 1968. Ultrastructure of chloroplasts and chromoplasts in *Capsicum annum*. I. Thylakoid membrane changes during fruit ripening. *Am. J. Bot.* 55, 1210-24.
- Thomson, W. W., 1969. Ultrastructural studies on the epicarp of ripening oranges. *Proc. First Int. Citrus Symp.* 3, 1163-69.
- Thomson, W. W. and Platt-Aloia, K., 1976. Ultrastructure of the epidermis of developing, ripening, and senescing navel oranges. *Hilgardia* 44: 61-82.
- Ververgaert, P. H. J. T. and Verkley, A. J., 1978. A view on intramembraneous particles. *Experientia* 34, 454-5.
- Wade, N. L. and Bishop, D. G., 1978. Changes in the lipid composition of ripening banana fruits and evidence for an associated increase in cell membrane permeability. *Biochim. Biophys. Acta.* 529: 454-64.
- Yatsu, L. Y. and Jacks, T. J., 1972. Spherosome membranes, half unit- membranes. *Plant Physiol.* 49, 937-43.

**TABLE 1.** Histogram of the intramembranous particle density of the EF face of the plasma membrane of avocado fruits at various stages of ripening. As determined by one-way analysis of variance, the density of particles at the climacteric peak was significantly higher, at the 0.01 level, than any of the other stages counted.



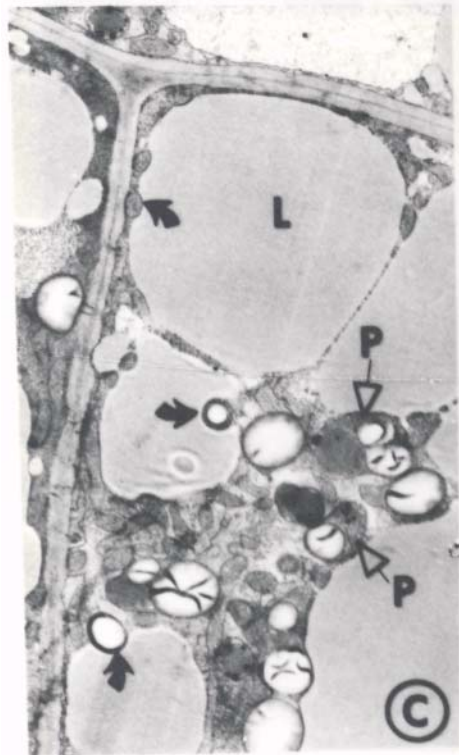
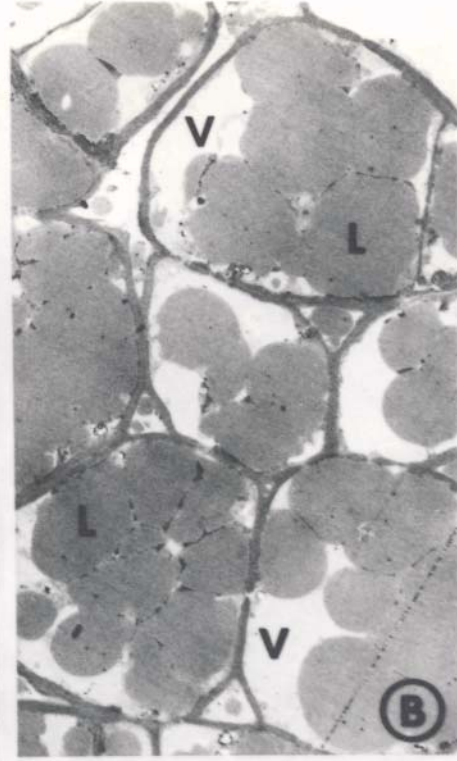
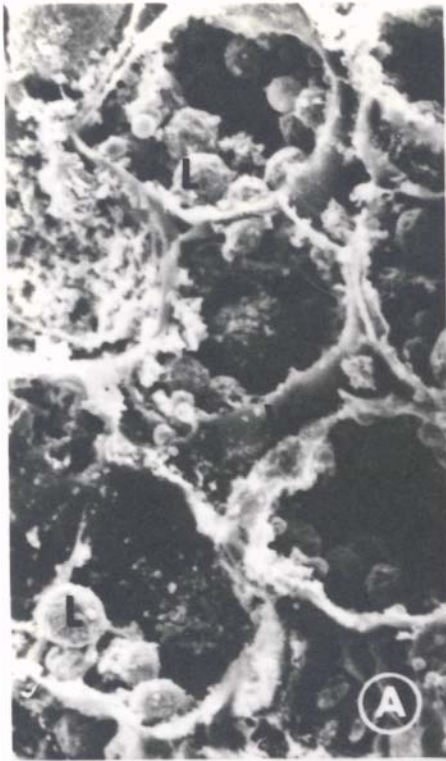
## FIGURES AND FIGURE LEGENDS

### Abbreviations

D	Dictyosome	N	Nucleus
ER	Endoplasmic reticulum	Nu	Nucleolus
G	Granum	P	Plastid
L	Lipid body	PB	Prolamellar body
LS	Lipid body surface	PM	Plasma membrane
M	Mitochondrion	V	Vacuole
MB	Membrane bound body	W	Cell wall
Mb	Microbody		

### PLATE 1

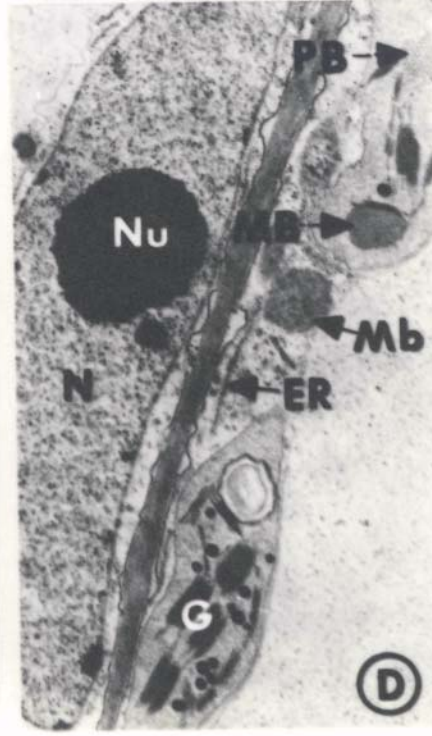
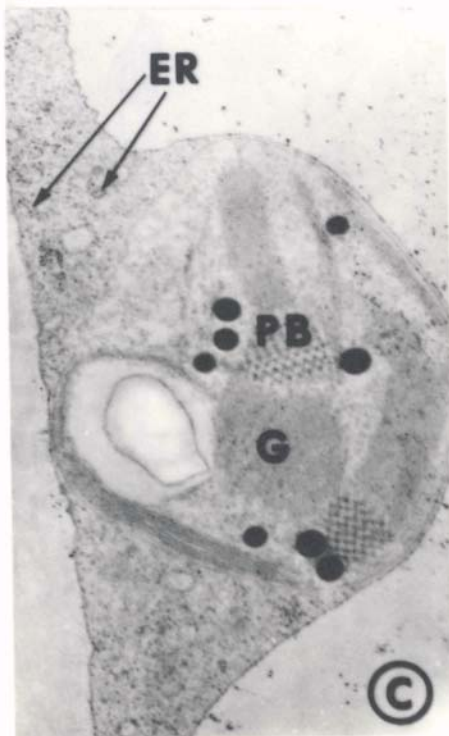
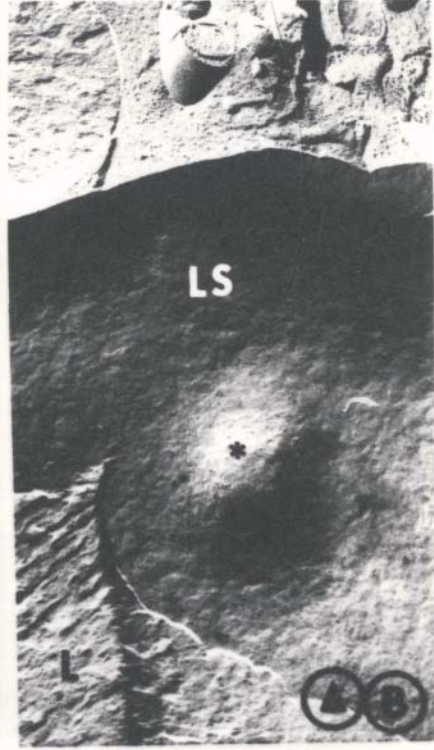
- A. Scanning electron micrograph of parenchyma cells of mature, unripe avocado mesocarp tissue. The cell walls are intact and the cells are filled with spherical lipid bodies which have numerous indentations. Small, smooth surfaced structures are vesicles and other organelles. X 700.
- B. Thin section of tissue similar to that shown in A. The major component of the cells are the lipid bodies. The vacuoles are irregular in shape. X 900.
- C. Portion of a parenchyma cell from the internal yellow portion of a mature unripe avocado. This illustrates invaginations of the lipid bodies (curved arrows) which correspond to the indentations seen in the SEM (A & D of this plate). These invaginations are frequently occupied by organelles such as mitochondria and plastids. Plastids are etioplasts with large prolamellar bodies. X 4,000.
- D. Scanning electron micrograph of a portion of an unripe preclimacteric avocado showing indentations in the lipid body (curved arrows), tubular endoplasmic reticulum, and elongate mitochondria. X 3,500.



## PLATE 2

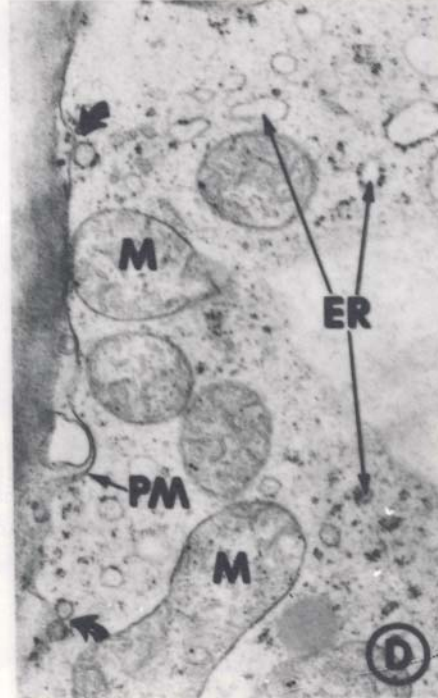
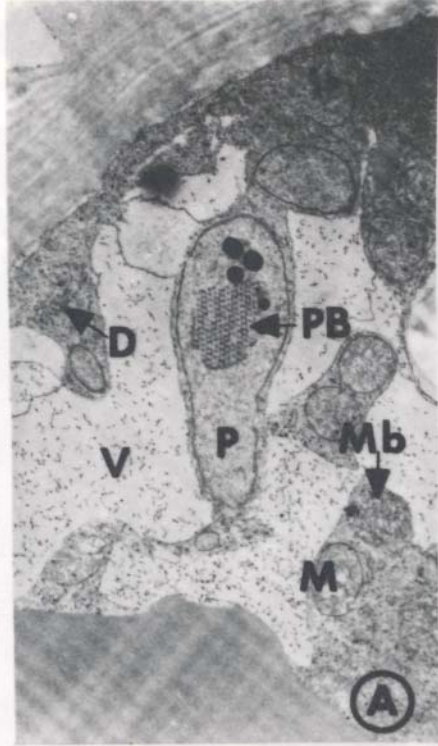
- A. Freeze fracture electron micrograph of a portion of a cell from a preclimacteric avocado. The lipid body has been cross-fractured. The irregular plasma membrane may indicate fusion of vesicles (curved arrows). X 36,000.
- B. Freeze fracture replica of a lipid body in an avocado at the climacteric peak. Part of the lipid body has cross-fractured (lower left) and part has apparently fractured along the concave surface. An indentation, typical of lipid bodies in avocados, is indicated by an \*. X 22,000.
- C. Chloroplast from the outer, dark green tissue of the mesocarp of an avocado during the climacteric rise. The grana stacks are large, Prolamellar bodies are present, but are minor components. The endoplasmic reticulum shows some swelling or vesiculation. X 20,000.
- D. Electron micrograph of portions of two cells from the light green portion of a preclimacteric avocado. The plastids have smaller grana than in the dark green tissue. Additionally, many of these plastids have membrane bound bodies, the membrane of which is continuous with the grana. The nucleus is elongate and has a prominent nucleolus. The rough endoplasmic reticulum is not swollen or vesiculated. The microbody has an electron dense, granular matrix. X 8,500.





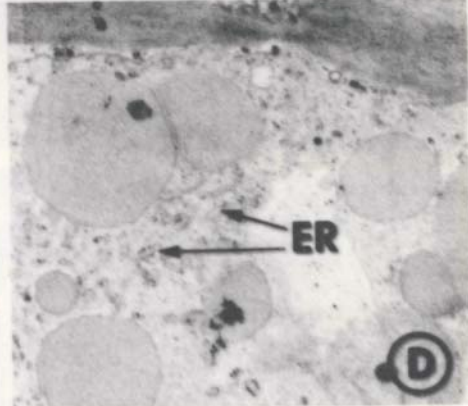
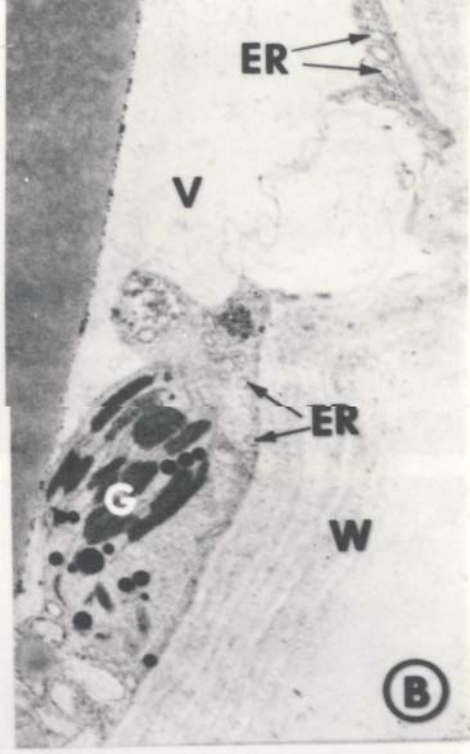
### PLATE 3

- A. Portion of a cell from the inner yellow tissue of a preclimacteric avocado. The plastid contains no grana or lamellae in this section. The prolamellar body is the major component. The vacuoles are irregular in shape. The dictyosome is simple with a few associated vesicles. X 12,000.
- B. Electron micrograph of a thin section through a portion of a cell from a preclimacteric avocado. The mitochondria occur in a group and the central one is elongate and branched. The matrix of these mitochondria is fairly compact and there are numerous tubular cristae. The endoplasmic reticulum is not swollen or vesicular. X 22,000.
- C. Serialsection reconstruction of the group of mitochondria shown in Plate 3B. Apparently, all the "separate" mitochondria shown in the thin section are part of one large, anamostosing mitochondrion. Additional transparencies were made which prove this, but photographing more than shown here was impractical for the sake of clarity. X approx. 18,000.
- D. Portion of a cell from an avocado at the climacteric peak. The endoplasmic reticulum is swollen and apparently vesiculated. In some cases these vesicles appear to be associated with or possibly fusing with the plasma membrane (curved arrows). The plasma membrane is quite irregular in outline. The mitochondria appear to have maintained their structural integrity during ripening. X 20,000.



#### **PLATE 4**

- A. Scanning electron micrograph of a portion of a post climacteric, soft fruit. The mitochondria are extremely elongate. The lipid bodies have the characteristic indentations. X 4,000.
- B. Electron micrograph of a portion of a cell from the outer, dark green mesocarp of a post climacteric avocado. The chloroplast contains numerous, large grana stacks and has retained its structural integrity. The cell wall has lost much of its structural components. X 10,400.
- C. Scanning electron micrograph from a preclimacteric avocado showing a sheet of endoplasmic reticulum over a lipid body. X 12,500.
- D. Thin section of a mesocarp cell from an avocado during the climacteric rise. The endoplasmic reticulum shows apparent incipient vesiculation. X 15,200.
- E. Electron micrograph from a climacteric peak avocado illustrating apparent fusion of endoplasmic reticulum vesicles with the plasma membrane (curved arrows). X 42,000.



## PLATE 5

- A. Freeze fracture replica of the EF face of a preclimacteric avocado. Impressions of the wall striations are visible. Arrow indicates the direction of shadow. X 50,000.
- B. Freeze fracture replica of the EF face of the plasma membrane of an avocado at the climacteric peak. This membrane is more irregular than that shown in A, and the increased particle density is apparent. Impressions of wall striations are not visible. Arrow indicates the direction of shadow. X 50,000.
- C. Thin section of a portion of a plastid from the light green tissue of avocado mesocarp. The curved arrow indicates possible continuity of the membrane bound body with that of the prolamellar body. The arrowheads indicate points of continuity of the membrane of the membrane bound body with the granal membranes. X 49,000.

