Persea americana, Mesocarp Cell Structure, Light and Electron Microscope Study
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the rest of the quiescent area, as delineated by CLOWES, only one division has been observed, whereas in the epidermal portion, a much smaller area, seven distinct divisions are recorded. While approximately 1800 divisions have been plotted, it would be desirable to study many more sections in order to clearly define the status of the epidermal region.

In the area immediately surrounding the quiescent center there is a gradual shading off of quiescence, divisions becoming gradually more frequent, so that the boundaries of the quiescent zone on the side away from the root cap cannot be defined with precision. In the remainder of the apex behind this region, there appears to be a fairly uniform distribution of divisions, with perhaps a rather higher density of division in the central, or "columella" area, than in the cortical and epidermal regions.

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PERSEA AMERICANA, MESOCARP CELL STRUCTURE, LIGHT AND ELECTRON MICROSCOPE STUDY[1]

FLORA MURRAY SCOTT, BARBARA G. BYSTROM, AND E. BOWLER

Introduction

The mesocarp of the avocado fruit consists primarily of parenchyma with abundant oil and a network of vascular strands (**1, 19**). The oil occurs as droplets in the cytoplasm of unspecialized cells and in oil sacs in scattered idioblasts (**3, 4**).

The structure of the oil-sac idioblast has been described in detail in the leaf of avocado, but the interconnection of idioblasts with surrounding cells is not indicated in the original diagrams (**4**). The present paper deals with cell-wall structure, intercellular connections, and the protoplasts in the tissue of the mesocarp of the fruit of *Persea americana*, var. Haas, as seen under light and electron microscopes.

Material and methods

Fresh material of young and mature[2] fruits was sectioned and examined under the light microscope, before and after staining with aqueous stains, also during and after treatment with standard microchemical reagents.

The distribution of cellulose, pectic substances, lignin, and suberin was determined by I_2KI–H_2SO_4, ruthenium red, phloroglucinol-HCl, and Sudan III. Ergastic substances, in particular oil and starch, were tested by staining with Sudan III, Sudan black, osmic acid, and I_2KI. Aqueous stains including neutral red, Janus green, anilin blue, and thionin were useful in staining differentially but not definitively the oils in parenchymatous cells and oil-sac idioblasts.

For electron microscope study of cell walls, young and mature tissues were cleared of the abundant oil and proteinaceous materials. Existing techniques were modified as required. The most successful methods involved the use of acid maceration: 1:1 10% chromic and 10% nitric acids; or acetylization: 1:4 glacial and concentrated sulfuric acids. These treatments were followed, when necessary, by removal of fatty components and by washing in 50% alcohol followed by distilled water.

For cell-wall study cleared material in distilled water was fragmented ultrasonically for 25 seconds or less in ultrasonic cell fragmentizer SF-50 (McKenna Laboratories, Santa Monica, California) and thereafter mounted on Formvar-coated grids and shadowed with palladium. Uncleared material was similarly processed for comparison.

For the study of fatty substances *in situ*, ultrathin sectioning was effective. Segments of fruit were fixed in buffered OsO_4 (**7**) or in $KMnO_4$ (**5**) and were imbedded in Epon 812. Material was sectioned at between 600 Å and 1000 Å on a Porter-Blum microtome. All sections were stained with Protargol.

[1] The authors thank the National Science Foundation (NSF G23387) for support of this work.

[2] The term "mature" is used of fruits at the "harvesting ripe" stage, full grown and firm in texture. Softening of the fruit occurs after picking.

Observations were made on an RCA–EMU-2 microscope.

Observations

LIGHT MICROSCOPE.—In the youngest mesocarp examined the parenchymatous cells are polyhedral and approximately equal in size. The future idioblasts are already marked by the refringence of their contents. Small air-filled intercellular spaces are ubiquitous. Mitosis occurs during the growth of the fruit, and recent cell division is indicated by variation in cell outline, diameter, and alignment, and by the presence of new thin cell walls between daughter nuclei. Idioblasts grow more rapidly than unspecialized parenchyma and are distinguished by their thicker walls and the presence of young stalked oil sacs.

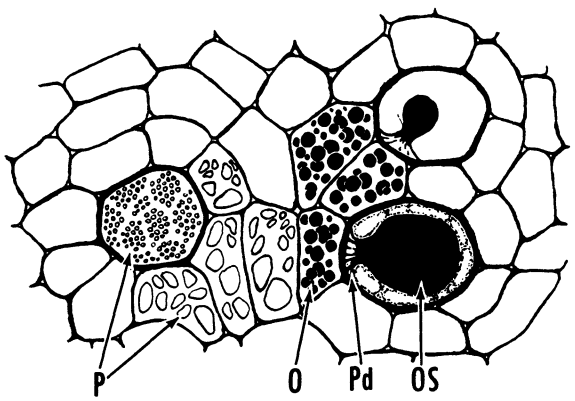


FIG. 1.—Diagram of avocado mesocarp cells showing pitted cell wall and oil drops in protoplasts of parenchyma cells and thickened cell wall and oil sacs of idioblasts. In latter note grouped pits, younger and older oil sacs, plasmodesmata at base, and radiating protoplasmic strands. *P*, pits; *O*, oil; *OS*, oil sac; *Pd*, plasmodesmata. Actual diameter of idioblasts is 25 μ.

In mature fruits the distribution of the polyhedral parenchyma cells and the scattered idioblasts is clearly seen. The cell faces of the parenchyma appear netlike because of the very numerous pits (fig. 1). The pits are elliptical, oval, or occasionally circular; the major axis or diameter is from 3 to 5 μ, except on cell faces contiguous with the idioblasts. On the latter, the pits, smaller, circular in outline, and more numerous, match those of the idioblast wall. All intercellular spaces throughout the tissue, including those around the idioblasts, are lined with a suberin film.

The vacuoles of mature parenchyma cells are filled with conspicuous oil drops of varying size in addition to chloroplasts, mitochondria, and numerous granules. All chloroplasts are encircled by a corona of minute lipid droplets visible in fresh material before and after staining with Sudan black (**13**).

The polyhedral idioblasts in the same fruits are distinguished from the parenchyma by larger size and thicker walls and by the presence of oil sacs. Traces of lignin occasionally occur in the cell wall. The pattern of pitting on all cell faces is evident in younger fruits before the wall is too heavily suberized. In older suberized walls pitting is observed in ultrasonically shattered fragments. The protoplast is vacuolated, and the stalked oil sac is clearly defined during various stages of growth. The sac membrane and stalk consist of cellulose but are impregnated from the earliest stages with a certain amount of lipid—a suberin-like material. The membrane appears very finely punctate or ultraporous. From the sheath that surrounds the sac, cytoplasmic strands radiate between vacuoles toward the peripheral cytoplasm, as in developing cystoliths and calcium oxalate idioblasts (**9, 10**). In the full-grown idioblast, the oil sac fills almost the entire cell cavity and masks the nucleus and other organelles.

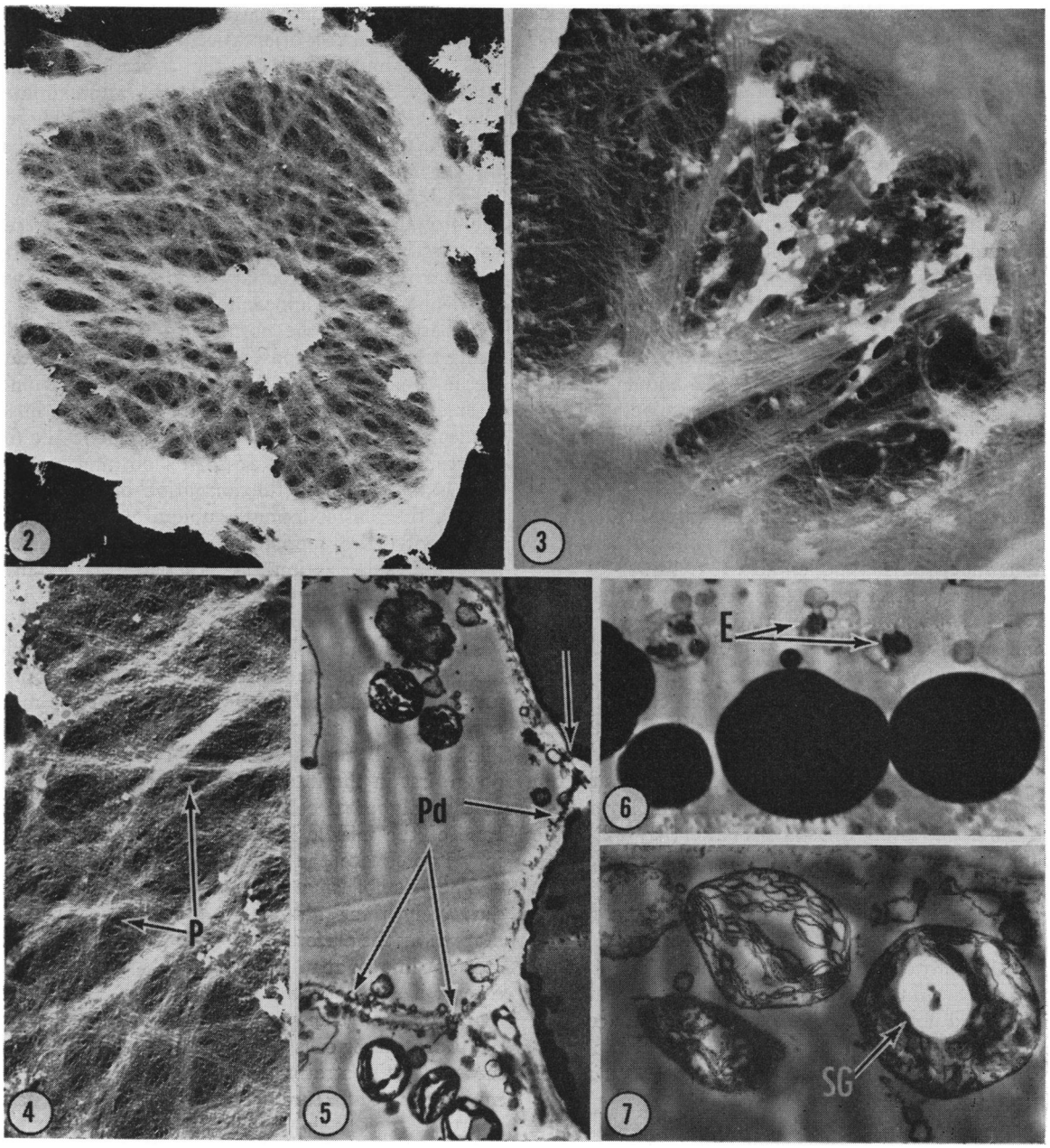
In the tissues of younger fruits the cell walls and the protoplasts of the idioblast react immediately by change in color to standard microchemical reagents and to aqueous stains. In mature fruits the idioblasts generally remain impervious indefinitely to the same reagents and stains. It is not known at what stage of development this impermeability becomes effective.

ELECTRON MICROSCOPE.—The cellulose microfibrillar pattern of the youngest cell walls examined is a reticulum with pores and groups of pores that mark the beginning of pits (**15**). Differentiation and wall thickening of unspecialized parenchymatous and idioblast cells is evident at an early stage of growth.

In the interparenchymatous cell faces, as already noted, the ubiquitous pits are large, elliptical or circular in outline, and become delimited by a macroreticulum of microfibrils (fig. 2). In maturing cells, while deposition of microfibrils thickens the wall as a whole, the pit membrane also thickens (fig. 3). Near the rim of a cell face, some smaller pits, possibly adjacent to an intercellular space, and others in random locations, are partially or completely occluded by microfibrils. Conspicuous pits remain unobstructed and functional, however.

On cell faces contiguous to idioblasts, the pit pattern is similar to that of the idioblast. The meshes of the macroreticulum surround numerous small circular pits (fig. 4).

The protoplast of unspecialized parenchymatous cells includes nucleus and cytoplasm, chloroplasts, elaioplasts (**2**), mitochondria, Golgi apparatus, and such ergastic substances as oil, starch, and undetermined granules. All cells are interconnected by plasmodesmata (fig. 5). Osmiophilic drops are conspicuous in the cytoplasm of cells of all ages. They



FIGS. 2–7.—Electron micrographs of various aspects of avocado mesocarp parenchyma; *P*, pits; *Pd*, plasmodesmata; *E*, elaioplast; *SG*, starch. Fig. 2, young; cell face with macroreticulum and pits, ×2900; fig. 3, older; pit with early stage of membrane thickening, ×15,500; fig. 4, wall contiguous to idioblast with characteristic smaller pits, ×5900; fig. 5, young; plasmodesmata, chloroplasts, fragment of nucleus, various granules, and part of two adjacent oil sacs; Luft fixation, ultrathin section, ×3150; fig. 6, young; oil drops (black) in cytoplasm, elaioplasts (see fig. 8); Palade fixation, ultrathin section, ×2800; fig. 7, young; chloroplasts with lamellation and starch grain; Luft fixation, ultrathin section, ×10,000.

vary in size and in older cells may mask nucleus, other organelles, and cytoplasm (fig. 6). Chloroplasts show typical lamellation and contain minute osmiophilic droplets and, frequently, starch grains (fig. 7). Elaioplasts, in contrast, are unstratified in the present preparations. They occasionally occur in small groups of two or three in random locations, and all possess a corona of osmiophilic droplets (fig. 8).

The walls of differentiating idioblasts, in contrast to the parenchymatous walls, are comparatively dense from the earliest visible stages of differentiation and remain so during later growth. A conspicuous macroreticulum with numerous small circular pits is laid down at an early stage (fig. 9*a*). Subsequent microfibrillar deposition may partially occlude some of these pits (fig. 9*b*). Deposition of suberin-like material in the idioblast wall begins about the time that the suberin-pellicled intercellular spaces become conspicuous. Isolated fragments of the suberized layer of the wall resemble the ultraporous or punctate suberin layer in the seed coat of *Cercidium* (**14**). Idioblast suberin, however, is distinguished by the ubiquitous perforations that mark the paths of the interconnecting plasmodesmata (fig. 10).

In the mature fruit ultrasonic treatment is effective in isolating complete idioblasts, in shattering their walls, and in releasing the enclosed suberized oil sacs frequently intact. The oil sac, attached to the wall, distinguishes the idioblast at an early stage of development (fig. 11). The sac develops in a typical vacuolated cell, and lipid material is laid down in its membrane. The latter is sheathed by cytoplasm from which strands radiate to the peripheral layer with its interconnecting plasmodesmata (fig. 12). The older, partially suberized sac is generally coated with projecting remnants of the radiating strands (fig. 13). In occasional small fragments of the membrane a microfibrillar pitted structure is indicated.

In the ultrathin sections of mature idioblasts the irregular outline of the sac membrane presumably indicates a normal localized variation in the consistency of the living protoplasm (fig. 14). The sac membrane here is indistinguishable from the surface of the oil drop.

Discussion

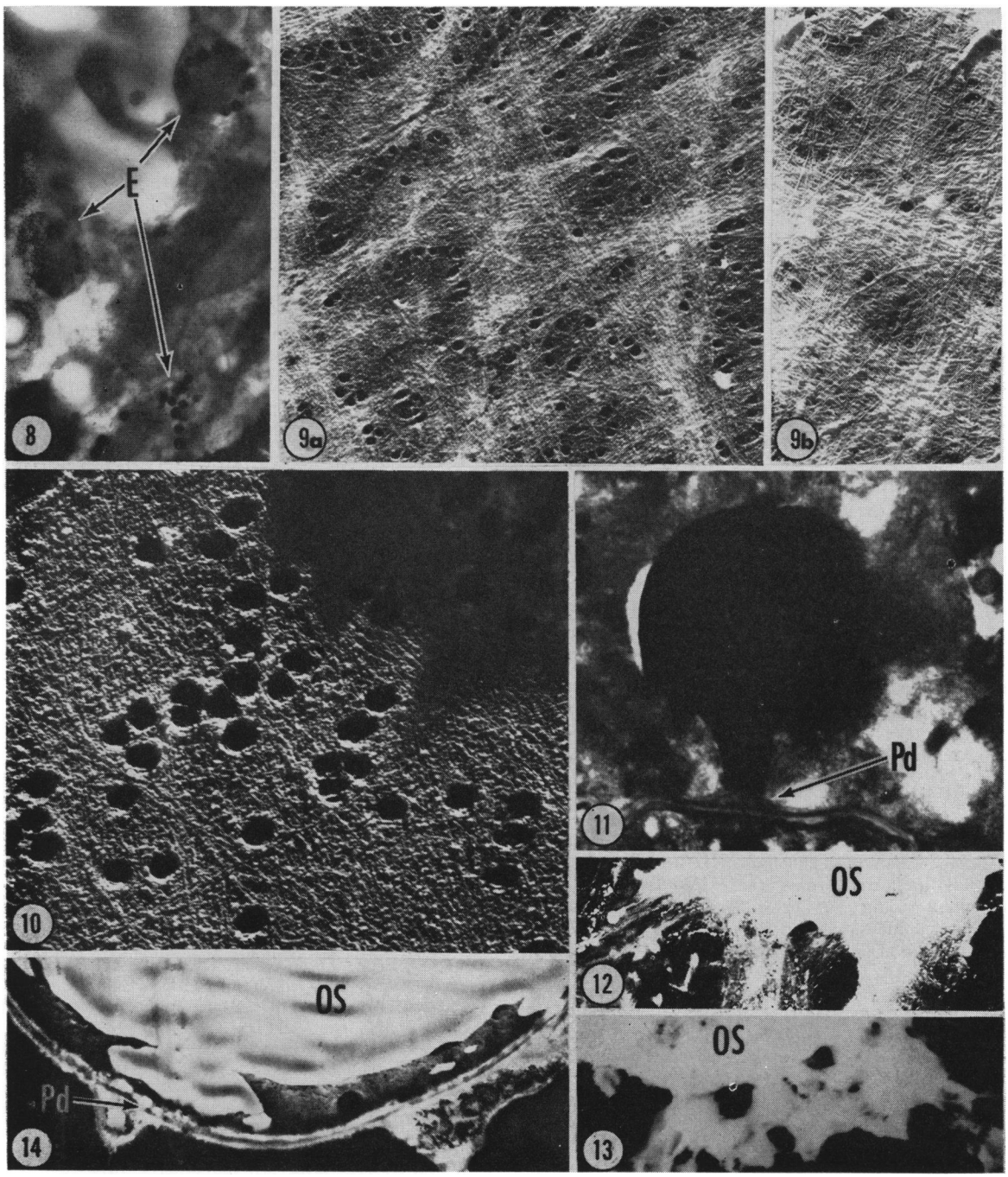
The content of the oil sacs in the leaf of *Persea* is stated to be terpene (**4**). The content of the sacs in the fruit is not necessarily identical. A recent analysis (**6**) of the oils in the mature avocado fruit, variety Haas, indicated a mixture of saturated and unsaturated fatty acids with slight traces of terpene: saturated acids (palmitic, stearic), 23% and unsaturated acids (palmitoleic, oleic, linoleic), 77%. It is considered probable that traces of terpene present in the oil sacs are responsible for differences in staining.

During the marked elongation of the parenchymatous cells in the onion root tip, the microfibrillar pattern changes from the characteristic meristematic network to parallel orientation (**15**). In the polyhedral isodiametric cells of the avocado mesocarp, where all cell faces increase in area approximately evenly, no such change in pattern occurs. The cell wall thickens by deposition of successive lamellae, but the microfibrillar pattern remains reticular. Since the cell wall begins to thicken in the early stages of the growth of the fruit, long before any of the cells have reached their maximum volume, the terms "primary" and "secondary," in relation to wall thickening, do not appear to be applicable.

Suberization is the normal reaction of wounded living tissue when exposed to air, as seen, for example, in the cut tuber of a potato (**8**). Suberization of intercellular spaces appears to be ubiquitous in the tissues of higher plants and was evident in the first intercellular spaces that arose in shoot and root tips (**11, 12, 17**). During the formation of intercellular spaces, the plasmodesmata, ubiquitous on all faces of the polyhedral meristematic cells, are broken. Intercellular suberization may, therefore, be regarded as a reaction of the protoplast to wounding. In the protoxylem of *Ricinus* the suberization of the spiral vessel wall began at the intercellular spaces, extended along the middle lamella, and eventually impregnated the cylindrical vessel wall (**18**). In the mesocarp of *Persea* the pattern of suberization of the idioblast wall may be similar. Suberin may be laid down first in the intercellular spaces at the corners of the polyhedral cells, may then extend along the zone of the middle lamella, and at the same time may impregnate the cellulose framework.

The plasmodesmata of the idioblasts occur in comparatively thick strands, the paths of which are marked by the perforations of the suberin membrane. It is not known at what stage of growth, nor to what extent, if at all, suberization blocks the plasmodesmata and the transport of materials from chlorophyll-containing cells into the idioblast. In the young mesocarp, the strains and reagents used penetrate rapidly; in the mature mesocarp, on the other hand, many enter very slowly or possibly not at all.

The texture of the isolated suberin membrane, whether described as ultraporous or finely punctate, is strikingly similar in *Persea* and in *Cercidium* (**14**) except for the conspicuous perforations in the former. Under the electron microscope it is also indistinguishable from the cutin layer of the onion leaf and of the epidermis of the root (**16**). It is evident that the physical properties of suberin and cutin require reconsideration in relation to the functions of absorption in the root and of penetration of fertilizer sprays into the leaf.



FIGS. 8–14.—Electron micrographs of various aspects of avocado mesocarp parenchyma or oil-sac idioblasts; *OS*, oil sac; *Pd*, plasmodesmata; *E*, elaioplast. Fig. 8, young parenchyma; elaioplasts with coronae of osmiophilic drops; Palade fixation, ultrathin section, ×10,500; fig. 9*a*, young idioblast wall showing pit distribution, ×10,250; fig. 9*b*, older idioblast wall with partial occlusion of pits by microfibrils, ×18,500; fig. 10, idioblast; isolated fragment of suberin layer with perforations and underlying microfibrils, ×52,500; fig. 11, young idioblast with oil sac in cytoplasm, attached to wall; plasmodesmata evident; Palade fixation, ultrathin section, ×6250; fig. 12, young idioblast; fragment of membrane of isolated sac with microfibrillar remains of wall and stalk and radiating cytoplasmic strands, ×10,000; fig. 13, older idioblast; fragment of membrane of isolated sac with remaining stumps of projecting cytoplasmic strands, ×20,000; fig. 14, mature idioblast; oil sac with plasmodesmata at oil-sac base, peripheral cytoplasm, and uneven surface of oil sac; Luft fixation, ultrathin section, ×3150.

The anatomical development of the oil sac of *Persea* resembles that of the intracellular cystolith of *Beloperone* (**10**) and also that of the intracellular sheath of calcium oxalate druses (**9**). In the latter structures the intracellular envelope consists initially of cellulose and is sheathed by cytoplasm from which strands radiate to the peripheral cytoplasm with its plasmodesmata. In older cells a certain amount of suberin is deposited in the cystolith framework and in the calcium oxalate crystal envelope. To what extent this suberization blocks interconnection with the surrounding cells is undetermined. In the case of *Persea* it is evident that experimental work, possibly with radioactive tracers, is required to follow the path of oil precursors from adjacent chlorophyll-containing cells into the oil sacs of the idioblasts.

Summary

1. The bulk of the mesocarp of the fruit of the avocado (*Persea americana*) consists of polyhedral parenchymatous cells and scattered oil idioblasts. Oil is abundant in the cytoplasm of the parenchyma and also fills the oil sacs of the idioblasts.

2. In young fruits all cell walls consist principally of cellulose. The thick walls of idioblasts in the mature fruit are heavily suberized. Intercellular spaces are lined with a suberin pellicle.

3. Plasmodesmata are ubiquitous; the pits on the interparenchymatous walls are large, in contrast to those of the parenchyma-idioblast cell faces.

4. In fresh material of the mature fruit the suberized idioblasts are frequently impermeable to aqueous stains and to standard microchemical reagents that react immediately with parenchymatous elements. At what stage of growth this impermeability develops is not known, nor is it known to what extent wall suberization slows down or blocks the transport of materials from chlorophyll-containing parenchyma to idioblast.

5. The details of interparenchymatous and of parenchyma-idioblast microfibrillar pit patterns are evident under the electron microscope.

6. Chloroplasts with lamellae and starch and elaioplasts with coronae of oil droplets occur in the parenchymatous cytoplasm.

7. The oil sac is ensheathed by cytoplasm from which strands radiate to the peripheral cytoplasm and the cell wall with its plasmodesmata. Plasmodesmata also occur at the base of the stalk.

8. Pores in the suberized layer of the cell wall of the idioblast mark the path of plasmodesmata.

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