ELECTRON MICROSCOPY OF HUMAN AXILLARY SEBACEOUS GLANDS*

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Sebaceous glands have received considerable attention for many years because of their unusual mode of secretion. They are holocrine glands, secreting by extrusion of entire cells; as secretion occurs, new cells are forming at the periphery of the acini (1, 2, 3).

The factors which normally control secretion of sebaceous glands are not yet well understood, although it is known that the steroids, testosterone (4) and estrogen (5) influence both the growth and secretory activity of sebaceous glands.

The nature of sebum has been widely studied by chemical methods. Such studies involve the collection of secreted material from cut hair (6, 7, 8) or from the skin surface (9, 10). One study (11) made use of material obtained from individual orifices. These studies show that sebum is chemically different from other body lipid. It contains approximately 30% free fatty acids, 35% esterified fatty acids, and about 30% unsaponfiable matter. Such studies, of course, are concerned only with the composition of the end product and not with its synthesis.

Several recent histochemical studies have been made on both human (1, 12) and animal (13, 14) sebaceous glands. These studies have shown that the peripherally located cells contain only small amounts of lipid, and that these newly formed droplets are chemically different from the numerous, large lipid droplets in mature sebaceous cells. Montagna (1) has shown that there is a progressive centripetal loss of basophilic substance (ribonucleoprotein) in the sebaceous acini, so that the amount of ribonucleoprotein in these cells is usually inversely proportional to the amount of lipid.

The electron microscope, with its high resolving power, offers another approach to the study of the morphology of sebaceous glands, and of secretory processes in which chemical changes are reflected in changes in the morphology of cellular components. Electron microscopic studies have been made on sebaceous glands of newborn mice (15) and on those of the human adults (16). The present study is an attempt to describe, as seen with the electron microscope, the changes which the acinar cells with their secretory products undergo in the process of maturation.

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MATERIALS AND METHODS

Four-millimeter punch biopsy specimens of axillary skin were obtained from five Negro females. The tissue was cut into cubes of less than 1 mm. and fixed in 1% veronal buffered osmic acid, pH 7.4, for a period of two hours. The tissue blocks were then dehydrated in graded methanols and embedded in a prepolymerized mixture of 1 part methyl methacrylate, 4 parts n-butyl methacrylate. Sections were cut with either a Porter-Blum microtome or an LKB ultratome and observed in an RCA type EMU3C or Siemens Elmiscop 1.

OBSERVATIONS

The fundus of the sebaceous acinus is made up of several layers of cells, bounded peripherally by a basement membrane and sometimes, but not always, containing a central lumen. Most of the cells, except those nearest the basement membrane, contain dense granules whose size, density and number become greater toward the center of the acinus. The more centrally located cells are made up of closely packed granules with thin strands of cytoplasm separating them from each other. In the neck of the acinus there is a gradual transition from glandular cells to duct cells and then to cells of the outer root sheath of the hair follicle, hence it is difficult in electron microscopic sections to tell precisely where one of these structures ends and another begins.

In the following description, for purposes of convenience, the acinar cells are divided into basally located and superficial cells. This is not to imply that these are two distinct cell types, but only that they are the two morphologic extremes. Cells intermediate in structure between these two forms are numerous.

Basement membrane

The basement membrane varies greatly from one region of the gland to another. In some areas, especially in the most distal part of the acinus, it consists of a simple feltwork of small filaments. Numerous capillaries and flattened fibroblasts are frequently encountered against its external surface. In other areas the basement membrane is a more complicated structure, consisting of several layers of fine parallel filaments, those in adjacent layers running perpendicular to each other (fig. 3). In such regions closely packed collagenous and elastic fibers are usually present just under the deepest layer.

Basal cells

The acinar cells adjacent to and near the basement membrane are polygonal in shape and contain large, rather light staining nuclei. Nucleoli with a cord-like internal structure are frequently encountered. Small, rather dense mitochondria are scattered throughout the cytoplasm (fig. 1).

Endoplasmic reticulum is difficult to recognize in most cells. Small smooth-walled vesicles are encountered (fig. 2) but the large, flattened, granule-covered vesicles characterizing most other glandular cells are not present. Many Palade particles, however, are found free in the cytoplasm. They are often grouped into small clumps, and appear to have no affinity for the membranous components of the cytoplasm (figs. 2, 5). The scarcity of the membraneous component and the lack of affinity of the particles for these membranes make it impossible to discern whether the membranes enclosing the newly-formed lipid droplets were originally part of the endoplasmic reticulum. However, formation of the granules within the particle-covered vesicles of endoplasmic reticulum has been demonstrated in several other types of glands.

Golgi material, which has been described in these cells by several workers using light microscopy, has been surprisingly difficult to locate in electron microscopic preparations. In the peripherally located cells it is present in small, widely separated islands (fig. 2), but in cells containing more lipid, Golgi material seldom can be recognized.

Droplets are present in some of the basal cells in the form of light-staining, membrane-enclosed globules. These globules consist of a finely granular substance with occasional mottled areas (figs. 1, 4). Electron density varies considerably from one droplet to another, but it is seldom as great as that of lipid inclusions encountered in other types of cells.

The limiting membranes of the basal cells are closely applied to each other so that intercellular spaces are seldom seen (figs. 1, 2). Convolutions of the cytoplasmic membranes are not uncommon, but are never as complicated as those in other epidermal glands. Desmosomes are also occasionally encountered, but are small and widely spaced.

Cells of the superficial layers

Cells of the more superficial layers of the sebaceous acini are extremely variable. The cells nearest the centers of the acini are degenerate in appearance, consisting of thin strands of cytoplasm separating numerous, large lipid droplets (fig. 6). Mitochondria are scarce and the nuclei are deep staining and compressed by the numerous lipid globules. Neither Golgi material nor endoplasmic reticulum has been encountered in these cells and the free Palade particles, so numerous in the basal cells, are absent.

Other cells of the sebaceous acini are transitional in form between the degenerate cells just described and the basal cells. In most instances there is a gradual increase in the size and number of lipid droplets as the center of the acinus is approached. These droplets increase in density but retain their granular consistency. The limiting membranes around the droplets remain intact during the maturation process, although it is difficult to visualize those of the more mature droplets because of their greater internal density. Most of the droplets are 2μ or greater in diameter and are reticulated or hollow, probably because of extraction during tissue preparation. Mitochondria are present in the cytoplasm of all cells, showing little change in form from the deepest to the most superficial layers, although there is a gradual decrease in number. Cytoplasmic vacuoles are numerous in some cells, but absent in others. When present, they are spherical, evenly distributed throughout the cytoplasm, and range from 0.05μ to 0.5μ in diameter (fig. 4). The number of such vacuoles in a cell is apparently unrelated to size or number of lipid droplets.

The membraneous component of the endoplasmic reticulum has not been recognized in the more superficial cells, but the Palade particles described in the basal cells are usually present, decreasing in number as the center of the acinus

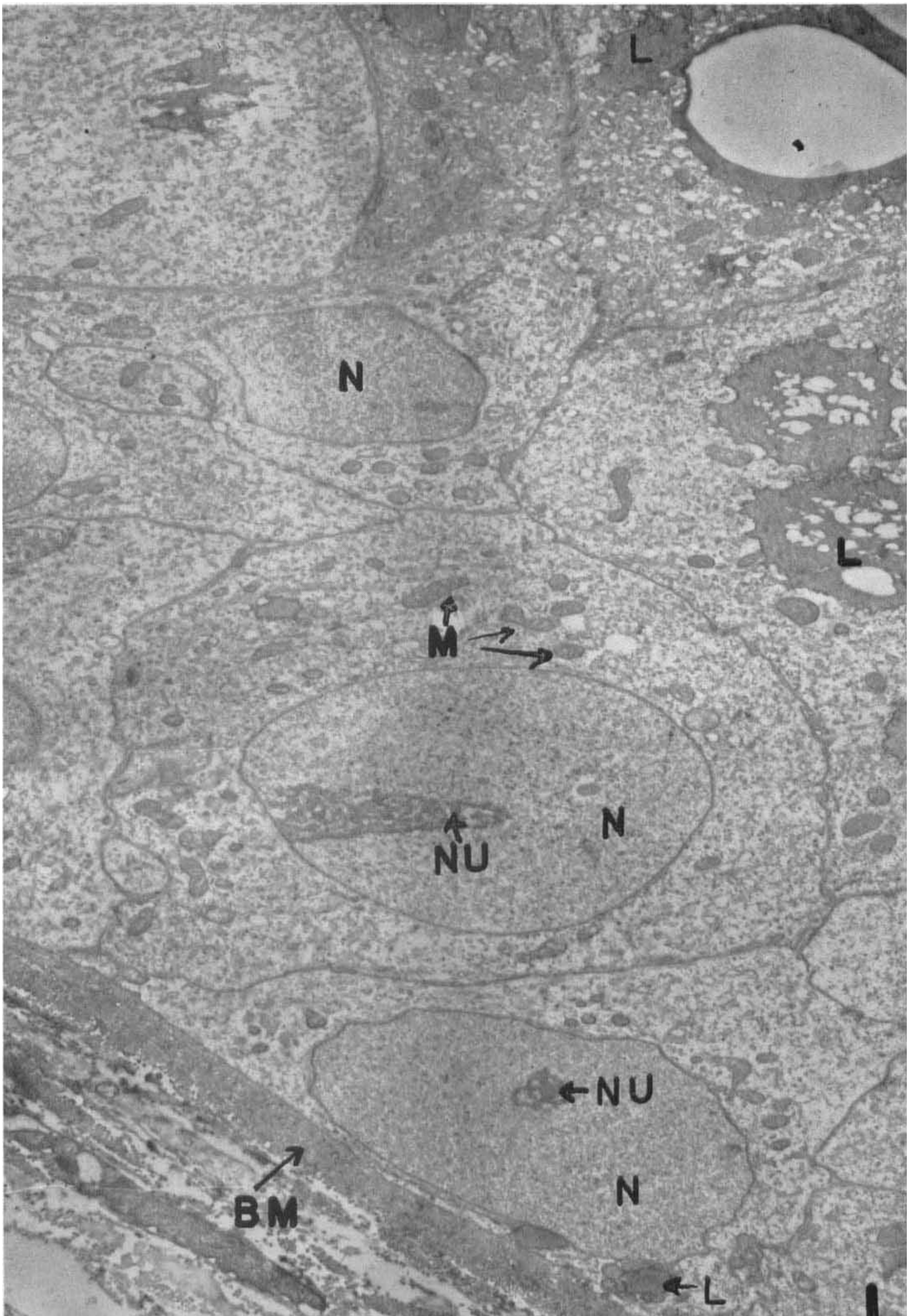


FIG. 1. Low magnification of a segment of a sebaceous acinus, extending from the basement membrane through two or three layers of cells. Note the extraction of lipid from the larger droplets. × 7,500. BM, basement membrane; M, mitochondria; N, nucleus; NU, nucleolus; L, lipid droplet.

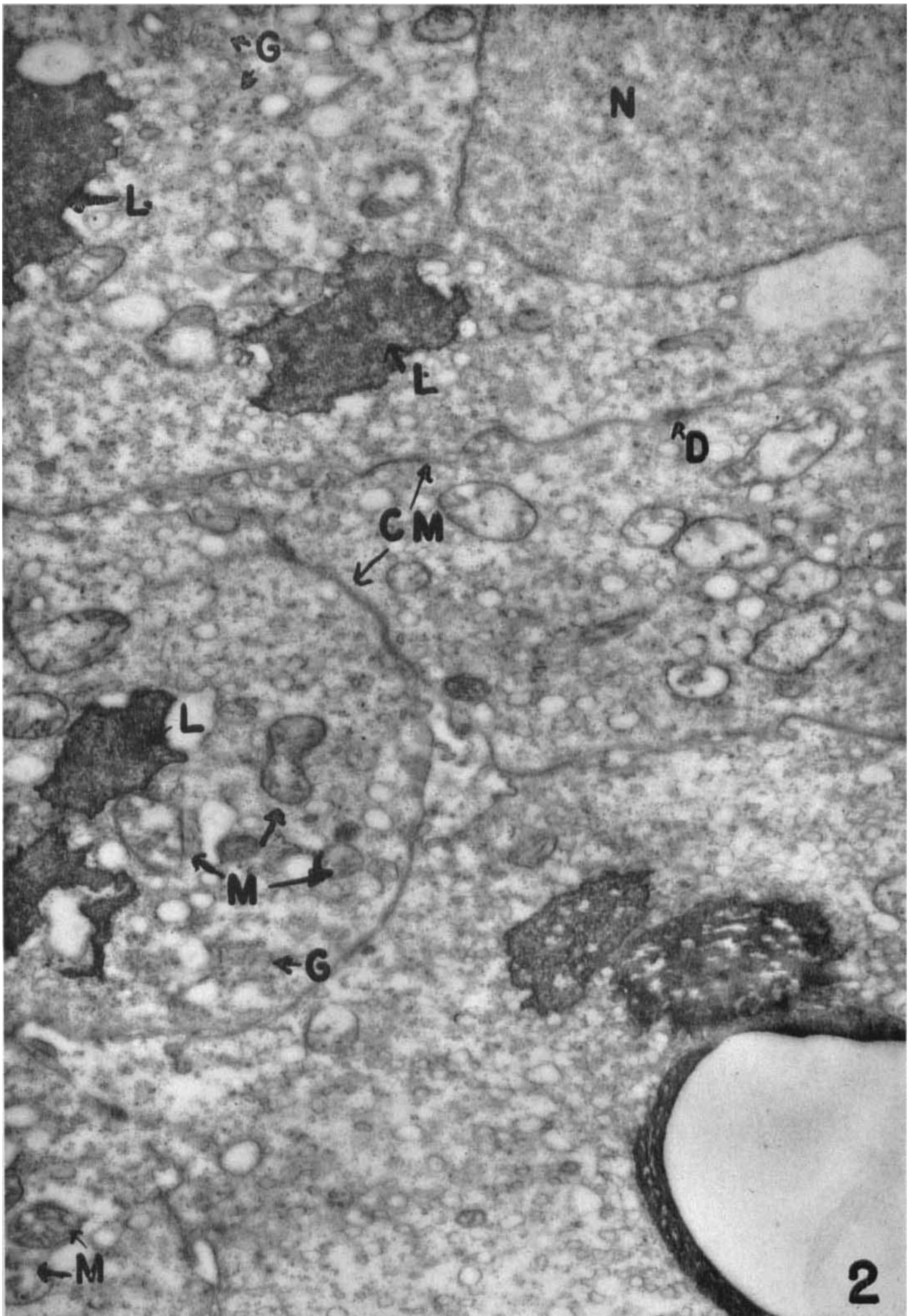
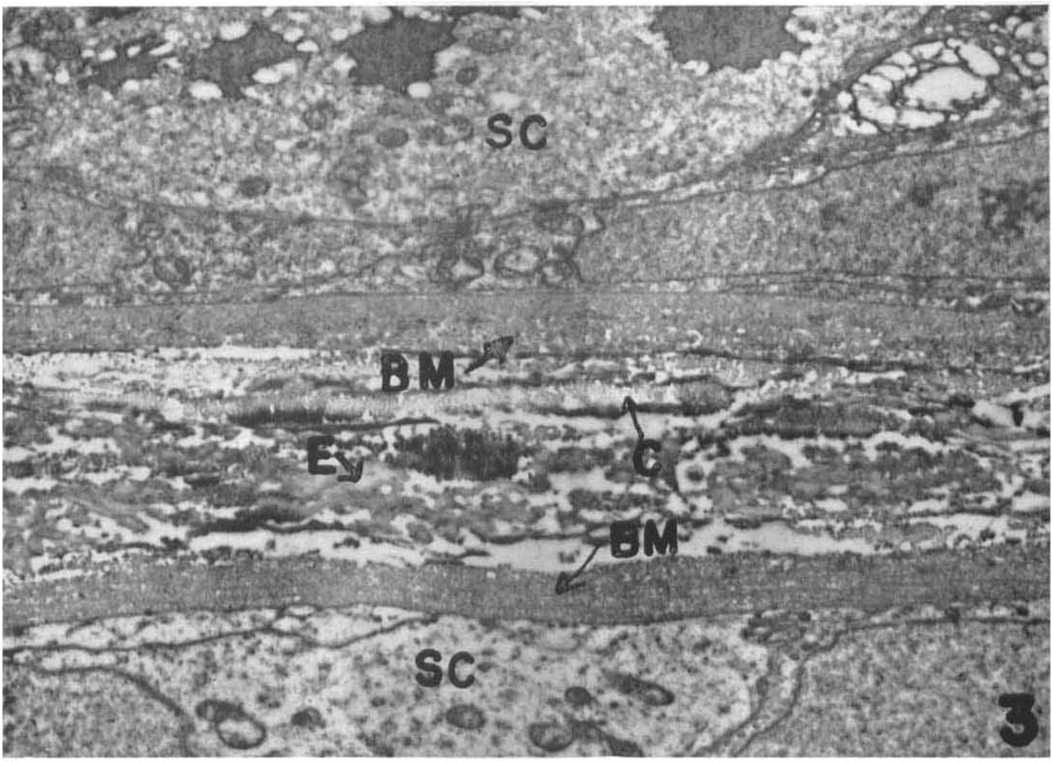


FIG. 2. Higher magnification of parts of several peripherally located cells of a sebaceous acinus. Note the numerous smooth-walled cytoplasmic vesicles. × 20,000.

M, mitochondria; N, nucleus; L, lipid droplet; CM, cytoplasmic membranes; G, Golgi material; D, desmosome.



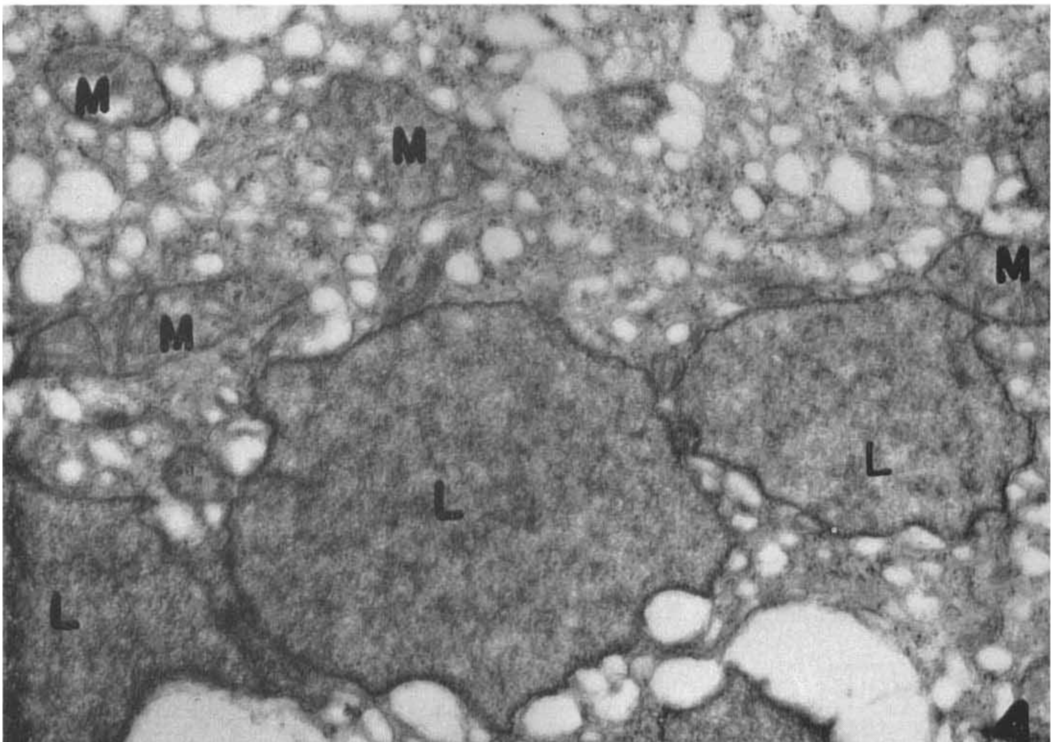
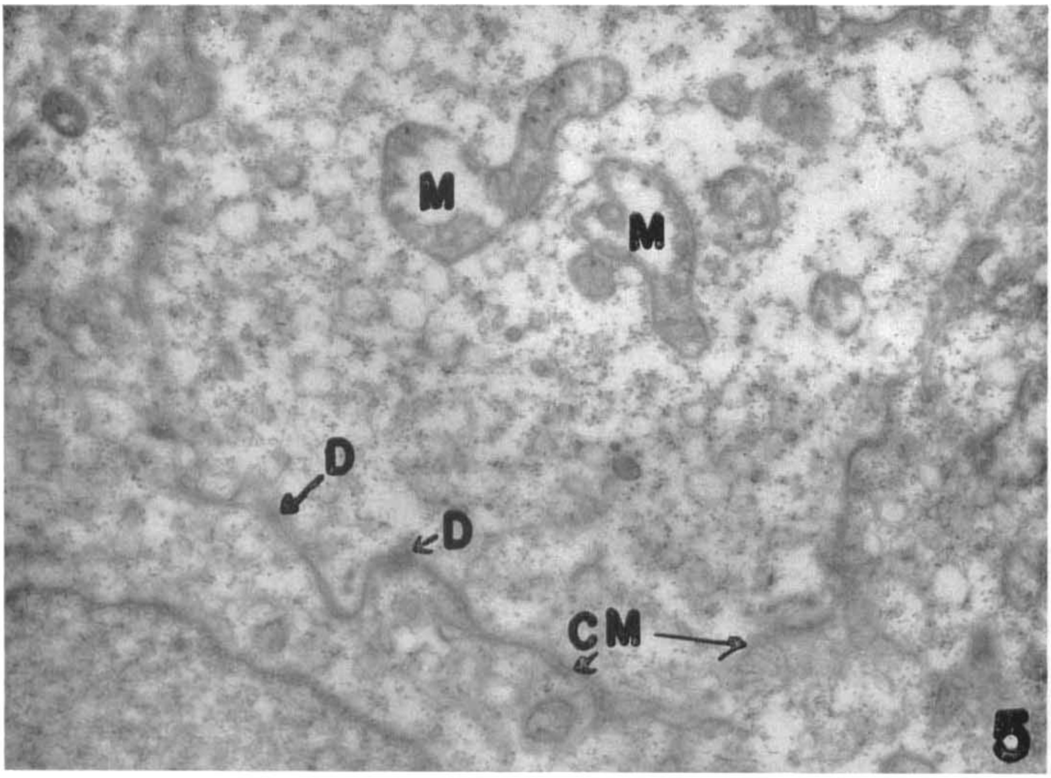


FIG. 3. Section through two closely approximated acini showing the thick laminated basement membranes. × 10,000.

BM, basement membrane; E, elastic fibers; C, collagenous fibers; SC, sebaceous cells.

FIG. 4. Portion of a peripherally located sebaceous cell showing the granular consistency and limiting membranes of the immature lipid droplets. Note the many vacuoles (no limiting membranes) dispersed among the smooth vesicles of endoplasmic reticulum. × 30,000.

M, mitochondria; L, lipid droplets.



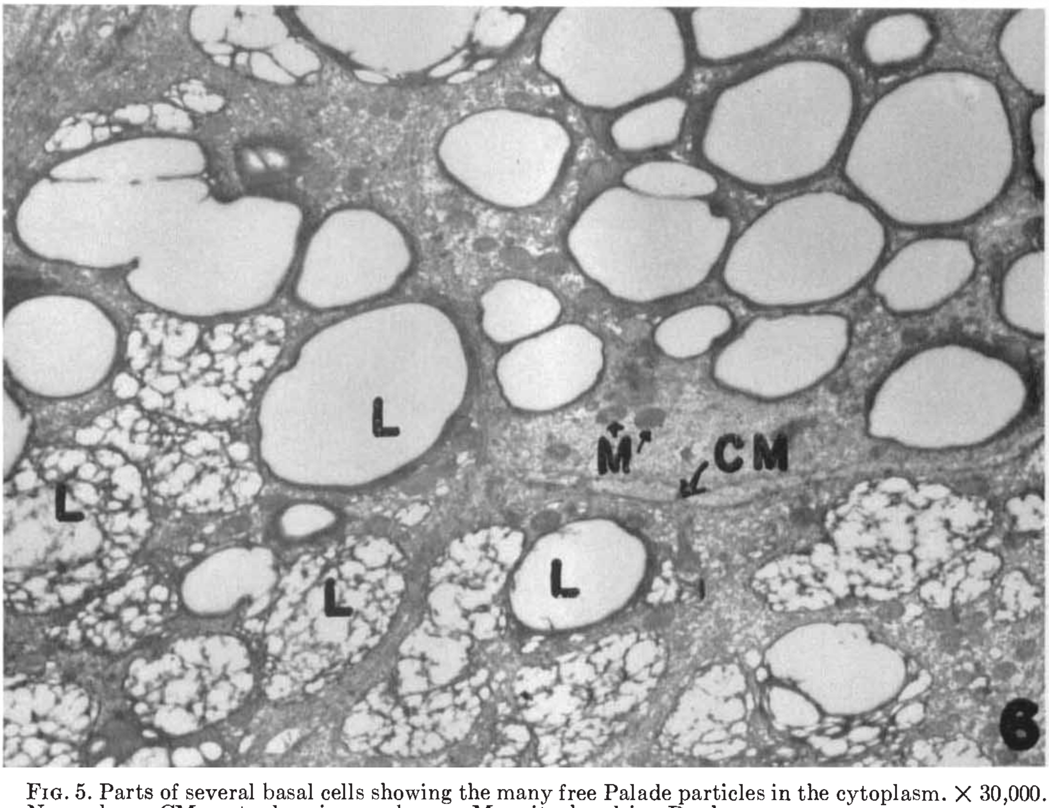


FIG. 5. Parts of several basal cells showing the many free Palade particles in the cytoplasm. × 30,000. N, nucleus; CM, cytoplasmic membrane; M, mitochondria; D, desmosomes.

FIG. 6. Low power view of parts of three centrally located sebaceous cells. Note the marked extraction of material from the lipid droplets. × 5,500.
M, mitochondria; L, lipid droplets; CM, cytoplasmic membrane.

is approached. Only in the degenerating central cells are the particles absent.

DISCUSSION

The observations reported here confirm many of the studies made on sebaceous glands with the light microscope, and clarify some of the fine details of morphology which cannot be resolved with that instrument.

The reason for the greater basophilia of the peripherally located cells than of the superficial cells is evident in the presence of many Palade particles (ribonucleoprotein) in the cells. These particles are most numerous in those cells in which lipid droplets are just beginning to appear, leading one to suspect that they might be involved in the synthesis of the sebaceous secretion.

The transition through which the lipid droplets pass before they reach maturity can be followed in electron microscopic preparations. The newly formed droplets are light staining and finely granular; each is enclosed in a membrane. There is commonly some shrinkage of the droplets during preparation, as is shown by their stellate shape (figs. 1, 2), but there is seldom any extraction or vacuolation. Droplets of larger size are of greater electron density; extraction of material from them often occurs, leaving the droplets vacuolated or reticulated. In the final stages, the droplets are even larger in size and often appear as rings of dense material, with hollow centers (fig. 6). No coalescence of granules has been seen, except in cells which are in the process of breaking up. Although several droplets may lie immediately adjacent to each other, the enclosing membranes apparently prevent coalescence.

The changes in osmiophilia and physical properties indicate a progressive change in the composition of the droplets. The newly formed droplets have little resemblance to lipid seen in other cells, but the mature droplets are easily recognized as lipid. This is in agreement with the findings of Montagna (1) who reported a difference in staining between the small, newly formed droplets in the peripheral part of the gland and those of mature cells. He also found a difference in composition between the material in the outer shell of the mature granules and that in the central regions. This could explain why, in preparation for electron microscopy, only the central regions of many granules are dissolved away.

The contention of Nicolas *et al.* (18) that lipid droplets form directly from mitochondria cannot be confirmed. Rogers (15) reported the presence of dense inclusions within mitochondria of the sebaceous cells of young mice. These, he believed, could be the beginning of such a transformation, but so far none of these bodies has been encountered in the present studies, although all of the specimens studied showed active lipid formation.

It seems more probable that the lipid droplets originate within Golgi vesicles. Even the smallest of the droplets encountered were enclosed by membranes. Although it is not certain that the enclosing membranes are Golgi material, previous studies (19, 20) have shown that when the lipid droplets are just large enough to be detected with the light microscope they are almost always closely associated with the Golgi substance.

SUMMARY

Axillary sebaceous glands of five Negro females were studied by electron microscopy.

The successive layers of cells in the sebaceous acini are described.

The formation of lipid droplets, from their first appearance in the basal cells to maturation is described, and their possible origin discussed.

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