ULTRASTRUCTURAL OBSERVATIONS IN ACNE VULGARIS: THE NORMAL SEBACEOUS FOLLICLE AND ACNE LESIONS*

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When under androgen influence the sebaceous glands and pilosebaceous canal enlarge, some vel­lus hair follicles of the face and upper trunk become sebaceous follicles (Strauss and Pochi, 1966). In some of these follicles in patients with acne, keratinized cells accumulate within the infundibulum of the pilosebaceous canal, that part of the follicle above the sebaceous ducts. As this impactation increases, either the follicles remain intact and develop into mature comedones or the epithelial wall disrupts; the resulting tissue reactions produce the inflammatory lesions of acne. The disrupted comedones can re-form by epithelial repair or heal by fibrosis (Strauss and Kligman, 1960).

Although hyperkeratinization is important in the pathogenesis of acne (Van Scott and MacCardle, 1956; Strauss and Kligman, 1960), its precise role is unclear. Since follicular impactation occurs most prominently in the infundibulum, its normal ultrastructure must be assessed before there can be any real understanding of the fine structural changes that occur in acne. Although the fine structure below the level of the sebaceous ducts has been described (Birbeck and Mercer, 1957a, b, c; Puccinelli et al., 1967), comparable ultrastructural descriptions of the follicle above this level are lacking. Histologic studies have dealt with acne (Lynch, 1940; Van Scott and MacCardle, 1956; Strauss and Kligman, 1960; Vasarirnsh, 1969) and the factors that are thought to induce comedo formation (Kligman and Katz, 1968; Kligman et al., 1970; Weary, 1970), but the fine structure of intact comedones and of the early follicular changes leading to their formation has not been reported. Furthermore, even though the inflammatory lesions of acne arise from a disruption of the follicular wall after comedo formation has begun (Strauss and Kligman, 1960), the ultrastructural alterations that precede this dissolution of the wall are not known.

The present report is based on a light and electron microscopic study of acne lesions and pilosebaceous units in normal and acne-afflicted skin; this study was undertaken (1) to determine whether there are any ultrastructural differences in the keratinized layer of the infundibula of normal and comedogenous follicles, and (2) to define the early morphologic changes within and around the epithelial lining of the comedo that precede its disruption.

MATERIALS AND METHODS

Subjects

Twenty-six female and 44 male patients with acne vulgaris† 14-35 years of age (mean: 18) were studied in a special acne clinic. These patients represented the full severity spectrum of the disease. They ate the same basic diet, but their total caloric intake differed. Acne treatment varied: 44 patients had received no treatment for at least 3 months before our initial examination and biopsy, and the others had been given simple topical treatment alone or in combination with systemic antibiotic therapy. When biopsy specimens were removed, the types and distribution of acne lesions were recorded for each subject.

Specimens

Under local anesthesia (1% lidocaine without epinephrine), 2.4 mm punch biopsy specimens were taken from normal skin and acne lesions of acne-affected parts of the body and from normal skin of acne-free areas. Noninflammatory open and closed comedones and inflammatory papules and pustules less than 3 mm in diameter were selected for these biopsies; no cysts or lesions larger than 3 mm were included. Specimens were immediately placed in 2.5% glutaraldehyde + 2% paraformaldehyde in 0.03 M phosphate buffer† overnight and then washed in 0.16 M phosphate buffer (Millonig, 1962). To facilitate orientation, the fixed specimens were examined under a dissecting microscope and bisected vertically through the central axis of lesions or of follicular openings. The small blocks (1-2 mm²) were then postfixed for 2-3 hr in 1% OsO₄ in 0.08 M phosphate buffer. After osmication all tissues were dehydrated through a graded series of ethanols to propylene oxide and subsequently embedded according to Spurr (1969). For electron microscopy, sections were cut on a Porter-Blum MT-2B microtome, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and viewed on uncoated grids in a Philips 200 electron microscope operating at 60 KV. Adjacent 1-μ sections were cut, mounted on slides, and stained with 1% toluidine blue + 1% borax for light microscope comparisons.

Some specimens of acne lesions and normal skin were frozen in liquid nitrogen, cut on a cryostat at 20 μ, mounted on slides, and stained with Oil red O to determine sites of lipid accumulation.

OBSERVATIONS

Normal sebaceous follicles occurred in all of the

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† All of these patients were residents of The Fairview Hospital and Training Center, Salem, Oregon. Informed consent for their inclusion in this study was obtained via the Research Committee of this institution.

specimens examined from the acne-free areas and in several specimens of normal skin from the acne-affected areas. Some follicles from clinically normal skin from the acne-affected areas, but none from the acne-free areas, showed changes in the infundibulum that indicated early comedo formation. Despite variation in overall size and architecture, the ultrastructure of sebaceous glands from normal follicles and acne lesions did not differ significantly and looked like that previously described in normal human skin by others (Kurosami, 1961; Hibbs, 1962; Ellis and Henrikson, 1963; Ellis, 1967, 1968; Bell, this issue).

Normal Sebaceous Follicle

Light microscopy: Sebaceous follicles were identified by the many large multilobulated sebaceous glands connected by ducts to a wide pilary canal. The sebum in the lumina of these units consisted of lipid and loose floccular cell debris (Fig. 1). The pilary canal contained a single vellus hair, but no accumulations of keratinized cells. Although bacteria and yeast cells were fairly common at the orifices of normal follicles, large masses of bacteria were seen less often within the intradermal portion of the infundibulum or the sebaceous ducts; the few microorganisms that were present here occurred as single isolated bacterial cells (Fig. 1).

The epithelium lining the infundibulum was continuous with the epidermis. An irregular shelf of stratum corneum surrounding the follicular orifice decreased in thickness as it extended into the follicle and tapered significantly toward the deeper portions of the infundibulum (Fig. 1). Near the junction with the sebaceous duct, where it merged with the fine wispy remnants of disintegrating sebaceous cells and duct epithelium, this keratinized layer was almost absent. The granular layer became less prominent in the epithelium of the lower infundibulum and sebaceous ducts; the few keratohyaline granules were small and discrete.

Electron microscopy: Changes in the wall of the infundibulum occurred along a gradient from the distal intraepidermal portion at the orifice to the proximal intradermal portion at the junction with the sebaceous ducts. The structure of the epithelium lining the follicular orifice and intraepidermal infundibulum (Fig. 2) was similar to that of normal epidermis. (Since epidermal ultrastructure has been reviewed in detail by others [Odland and Reed, 1967; Odland, 1971], it will be described here only in comparison with the structure of the intradermal infundibulum.)

The epithelial lining became somewhat different in the deeper intradermal portions of the infundibulum; most strikingly, the keratinized layer became much thinner, wispy, and attenuated. Like the epidermis, however, distinct basal, intermediate, granular, and keratinized layers were present. The basal cells were columnar and a basal lamina separated them from the underlying dermis (Fig. 3). Intercellular spaces decreased in width from the basal to the granular layer (Figs. 2, 3).

Unlike the comparable layer of the intraepidermal infundibulum, the cells in the intermediate layer became flat and elongated nearer the basal layer and often had accumulations of glycogen particles in their cytoplasm (Fig. 3). They had fewer desmosomes and contained fewer tonofilaments, which were less uniformly arranged than in their usual distinctive peripheral radial orientations (Figs. 2, 3).

The cells of the granular layer lining the proximal intradermal infundibulum had fewer tonofilaments and desmosomes as well as smaller, more discrete keratohyaline granules than those of the intraepidermal infundibulum (Fig. 4). Lamellar granules (membrane-coating granules, Odland bodies, keratinosomes) were prominent in the cells of the upper intermediate layer and appeared in large clusters in the intercellular space at the junction of the granular and cornified layers (Figs. 4, 5).

The most striking differences between the epithelium of the proximal infundibulum and that of the distal intraepidermal portion were in the layer of cornified cells around the lumen (Figs. 4, 5). Instead of forming a thick lamellar zone of discrete, intact keratinized cells as at the orifice, this layer was thin and appeared to disintegrate and slough into the pilosebaceous canal where it contributed to loose fragments of cell debris, the floccular material seen by light microscopy. This mate-
Fig. 2: The epidermis at the hair follicle orifice. This specimen was a normal sebaceous follicle from the upper back of a 15-year-old boy with facial acne. Similar morphology is seen in both normal follicles and early comedones. Prominent perinuclear tonofilament bundles (TF) of the spinous cells are attached to desmosomes at the cell boundaries. Keratohyalin (K) is abundant in the granular layer and distinct keratinized cells form a thick stratum corneum (SC). (× 3990)
Fig. 3: The intradermal infundibular epithelium in a normal sebaceous follicle. This specimen was taken from the back of a 21-year-old man with acne on the face and back. Tonomofilaments and keratohyaline granules are less prominent than at the orifice, and the cornified layer (C) is attenuated. Cytoplasmic glycogen accumulations (GL) are common. (× 3040)

The intradermal infundibular epithelium consisted of electron-opaque fragments of keratinized cells, round and elliptical masses of concentric keratinous lamellae, and granular or amorphous material (Fig. 4). The cornified cells, unlike those lining the orifice, were extremely thin and wispy, with indistinct cell membranes which were often indented by large masses of remnants of lamellar granules. Intercellular spaces within the cornified layer were frequently engorged with lamellar granules, and these granules or their remnants formed a greater part of this layer than the extremely thin cornified cells (Figs. 4, 5).

The epithelial wall of the sebaceous duct had the same basic structure in its intermediate and basal portions as the pilosebaceous canal. It was different only near the luminal surface where the lamellar granules and prominent bundles of tonofilaments were more numerous (Fig. 6). The superficial cells projected irregularly into the lumen, and aggregates of tonofilaments, some associated with small keratohyaline granules, were aligned in a plane parallel to the long axis of the cells. Many lamellar granules filled the apical portions of cells beneath the keratinizing layer. In some transitional cells that were partially keratinized, the organelles looked swollen and fragmented and the cytoplasm and nucleus were more electron-opaque; a few cells contained lipid droplets (Fig. 7). The horny layer consisted of irregular strands of attenuated keratinized material. In some areas of the duct, especially in the intralobular portion, lipid droplets (~1.3 μ) filled the cytoplasm of the epithelial cells, and the lumen contained lipid and discontinuous thin strands of keratinized material derived from sebaceous and duct cells (Fig. 7).

Noninflammatory Lesions

The term comedo is used here to indicate those follicles with apparent hyperkeratosis of the infundibulum. Mature open comedones (blackheads) and closed comedones (whiteheads) were grossly visible; however, early comedones were detected only by histologic examination of specimens of clinically normal skin from acne areas.

Early comedo. Light microscopy: Follicles in the early stages of comedo formation could be distinguished from normal follicles by changes that were usually restricted to areas of the intradermal
Epithelium and lumen (L) of the proximal infundibulum of a normal sebaceous follicle. This specimen was taken from the upper back of a 15-year-old boy with facial acne. Numerous lamellar granules are present within the cells beneath the granular layer (single arrows) and between the cornified cells (double arrows) (see Fig. 5 for greater detail). The keratinized layer is thin and discontinuous; its shedding adds to the loose fragments of keratinized cells within the lumen. (x 5980)
infundibulum; observation of some sections indicated that the changes first occurred at the junction of the sebaceous duct and the pilosebaceous canal. In early comedones, intraepidermal infundibulum and orifice and the sebaceous glands and intralobular sebaceous ducts were entirely like those of normal follicles.

Unlike the tapering cornified layer in normal follicles, the wall of the infundibulum in early comedones had a thicker horny layer and the lumen was filled with loose lamellae of vacuolated keratinized cells and masses of bacteria (Fig. 8). The vacuoles within the keratinized cells stained lightly with toluidine blue and with Oil red O. These changes were seen not only in the large patulous sebaceous follicles with wide pilosebaceous canals, small vellus hairs, and numerous multilobulated sebaceous glands, but also in some smaller follicles with fewer, small sebaceous glands and narrower pilosebaceous canals.

Electron microscopy: During early comedo formation, the wall of the follicular infundibulum, like that of the normal follicle, was composed of stratified squamous epithelium. In the cornified and granular layers, some striking differences occurred and frequently began at the junction of the sebaceous duct with the pilosebaceous canal (Fig. 9).

The cornified layer of the early comedo consisted of numerous lamellae of compacted keratinized cells with distinct, thickened cell membranes. Instead of disintegrating and sloughing, the keratinized cells maintained their integrity and usually contained variable numbers of prominent intracellular round or ovoid inclusions (Figs. 9, 10). These inclusions varied from 0.2 μ to 14 μ in size, contained a homogenous material of low electron opacity, and corresponded to those sites which stain with Oil red O in light microscope preparations. Near the center of the lumen, they seemed to increase in size by the flattening of the cornified cells and the coalescence of adjacent inclusions (Figs. 9, 10). Nucleated cornified cells were rare.

Interspersed among the cells of the cornified layer and along the junction of cornified and granular layers were a few lamellar granules or their remnants (Fig. 10) similar in quantity to those at the orifice (epidermis) but unlike the large accumulations in the intradermal infundibulum of normal follicles (Figs. 4, 5).

Several of the most superficial granular cells adjacent to cornified cells contained lipid inclusions (Fig. 9), which were not seen in this region in normal follicles. Usually the number and size of discrete keratohyaline granules increased and the number of lamellar granules and tonofilaments was decreased; desmosomes appeared normal (Figs. 9, 10).

**Mature comedo.** Light microscopy: Open and closed comedones appeared to be histopathologi-
Fig. 6: A portion of a normal sebaceous duct from the face of a 21-year-old man with acne. Many lamellar granules fill the apices of differentiating cells (single arrows) and are grouped in extracellular lacunae near the keratinized layer (double arrows). Cornified cells are shed as fragments into the lumen. Note a few intracellular lipid inclusions (L). (× 5600)

cally similar to those described by others (Lynch, 1940; Strauss and Kligman, 1960), i.e., epithelium-lined follicular cysts of different sizes and shapes and containing masses of concentric lamellae of keratinized cells, lipid, hair, and focal masses of bacteria. The often-thin walls of the comedones occasionally had small sebaceous glands attached to them. In closed comedones, the epithelium-lined sac opened to the surface by a narrow ostium, but in open comedones the orifice was widely distended by a horny impaction continuous at the surface with keratinized lamellae in the deeper portion. When frozen sections of comedones were stained with Oil red O, tiny droplets within the keratinized cells absorbed the material and therefore were composed of lipid.

Electron microscopy: Parts of the horny mass of mature comedones resembled the keratinized layers in early comedones except that the cells were more compact, flatter, and more compressed (Fig. 11). The keratinized cells had distinct margins with little visible intercellular material and usually contained several large intracellular lipid inclusions. Parakeratotic cells were rare. The cells of the granular layer resembled those in early comedones with few tonofilaments and lamellar granules, large keratohyaline granules, and intracytoplasmic lipid inclusions.

In some parts of the comedo, lipid inclusions were less prominent in the keratinized cells and cells of the granular layer. The cornified cells closely resembled those of the epidermal stratum
corneum, but the granular cells did not (Fig. 12). Occasionally, layers of keratinized cells containing many large lipid inclusions alternated periodically with layers containing only a few small lipid droplets (Fig. 13).

**Inflammatory Lesions**

We examined multiple sections of clinically normal skin and uninflamed comedones to identify the early changes that immediately precede disruption of the comedo wall and the formation of inflammatory lesions. The walls of a few mature comedones became so thin that the keratinized lamellae were separated by only one cell from the dermis, but there was no sign of an inflammatory reaction (Fig. 14a). In some early comedones, the follicular impaction seemed to protrude like a wedge through the follicular wall although the orifice was patent and inflammatory cells were absent (Fig. 14b).

Light microscopy: Once inflammatory lesions became clinically apparent as papules or pustules, all of them exhibited a generally focal inflammatory reaction on one wall of the follicle. In papules, the inflammatory infiltrate, which consisted predominantly of polymorphonuclear leukocytes and macrophages, tended to localize intradermally (Figs. 15a, c), whereas in pustules it assumed a more superficial position within the epidermis at the follicular orifice (Figs. 15b, f). Inflammatory lesions were always associated with comedones but in some the comedones were not fully developed.
some, the cytoplasm and nucleus had increased electron opacity. Mitochondria appeared swollen and keratohyaline granules and lamellar granules were rare. The adjacent keratinized mass contained many lipid inclusions, and the cells were highly electron opaque and so compacted that barely perceptible intercellular spaces could be discerned.

In some follicles, the wedge-like protruding keratinized mass formed a convex front in direct contact with the dermis. On each side of the advancing edge, a gradient of changes occurred within the wall (Fig. 17). Individual cells showed some evidence of necrosis, including mitochondrial swelling, cytoplasmic opacity, and pyknotic nuclei. The basal lamina could be traced up to this necrotic cell mass but not beyond. Here, too, the keratinized cells were opaque and compressed, and the number of keratohyaline granules and lamellar granules decreased along a gradient toward the point of rupture. The only cells within the adjacent dermis were fibroblasts and a few macrophages; collagen fibers appeared normal.

In clinically apparent papules and pustules where follicular disruption was more advanced and inflammatory cells were present, the infiltrate consisted of polymorphonuclear neutrophils and macrophages containing multiple lipid inclusions and phagocytic vacuoles of electron-opaque materials (Fig. 18). In addition, focal accumulations of cornified cells contained lipid inclusions similar to those in the phagocytic cells; intact bacteria and yeasts rarely occurred.

**DISCUSSION**

**Comedo Formation**

Although considerable interest has been shown in the factors that are thought to initiate comedo formation, scarcely anything is known about the nature of comedo keratinization. Some observers have considered the process to be an exaggeration of normal follicular keratinization, and recent evidence indicates that the rate of formation of horny cells is greater in comedones than in normal sebaceous follicles (Plewig et al., 1971). Although increased cell cohesiveness has also been suggested as a basic factor in comedogenesis (Kligman et al., 1969; Plewig et al., 1971), very little corroborative data have been reported.

Aberrations in follicular keratinization have also been implicated in the formation of comedones (Puccinelli, 1969). Puccinelli and Califano (1965), who studied the ultrastructure of the extruded contents of follicles and of comedones from patients with acne, described two types of comedones. The easily expressed contents of the “normal” infundibulum were defined as being derived from a “false” comedo. This material, which keratinized differently from the epidermis, consisted of isolated, flat, cornified cells which contained numerous empty spaces. The cells here appeared to have lost their contents and to lack a
Fig. 9: An early comedone from the face of a 21-year-old man. At the junction of the sebaceous duct (SD) and the pilary canal (PC), the cornified layer increases in thickness and has many layers of discrete intact cells. Intracellular lipid inclusions are prominent within completely keratinized cells (L₁) and the cells of the granular layer (L₂). The granular layer of the pilary canal has larger keratohyaline granules (K) and fewer tonofilaments than normal (see Fig. 3). Numerous bacteria (B) occupy the lumen. (× 3110)
Fig. 10: A higher magnification of the infundibulum distal to that shown in Figure 9. The keratinized cells have thickened cell margins (single arrows) and many layers accumulate. In comparison with the normal follicle (Fig. 5), there are fewer small clusters of lamellar granules (double arrows) at the junction of the granular and cornified layers. Note intracellular lipid inclusions (L). (× 11,400)

Fig. 11: Epithelial lining and the keratinized contents of a closed comedo from the back of an 18-year-old male patient with acne. Intracellular lipid inclusions (arrows) are present in both cornified and granular cells. Keratohyalin occurs in large discrete granules (K) and few tonofilaments are present. Cells of the keratinized layer are flattened and compressed. (× 3325)
Fig. 12: Wall of a closed comedo from the face of a 17-year-old boy. The keratinized cells contain only a few lipid inclusions (arrow). The keratohyaline granules (K) are large and discrete. Compare with Figure 11. (x 3610)

Fig. 13: Keratinized cells within a closed comedo from the back of a 16-year-old male patient with acne. Some zones of cornified cells contain many lipid inclusions and these alternate with others in which lipid is less apparent. (x 3640)
Fig. 14: (a) Noninflamed closed comedo from the face of a 17-year-old boy with acne. Note the attenuated portion of the epithelial lining where the keratinized contents are separated from dermal components by only one cell (arrow). There are no inflammatory cells adjacent to this region. (× 125)
(b) A cross-section of a noninflamed comedo from the back of an 18-year-old male patient with acne. A wedge-like mass of keratinized cells protrudes through the epithelial wall (arrow). Figure 17 shows the ultrastructural features of the area within the square. (× 120)

Fig. 15: (a) An inflammatory papule, from the back of a 17-year-old boy, arises from a closed comedo with a double orifice. The wall is disrupted, and some of the contents of the comedo are discharged into the dermis. An infiltrate of neutrophils and macrophages is invading the disrupted area. (× 30)
(b) A pustule from the face of a 17-year-old boy. A dense mass of neutrophils distends the orifice of the antecedent comedo. Several hair shafts are visible within the lesion, but the sebaceous glands have atrophied. (× 30)
(c) A papule from the back of a 17-year-old male with acne. The left wall of the hair follicle is disrupted and
infiltrated by an acute inflammatory infiltrate which also fills the lumen. Sebaceous glands are still prominent. The intraepidermal infundibulum contains an impaction of keratinized cells. (× 40)

(d) A higher magnification of the bottom of the field shown in Figure 15a. The contents of the comedo stream past the intact portion of the epithelial wall and are penetrated by neutrophils and macrophages. (× 90)

(e) A pustule from the face of an 18-year-old girl with acne. The epithelial wall of the antecedent comedo is completely collapsed, and the keratinized lamellae are surrounded by acute inflammatory cells. Centrally located masses of bacteria are enveloped by layers of cornified cells. (× 70)

(f) A pustule arising from a closed comedo on the face of a 19-year-old male patient with acne. Neutrophils penetrate the epithelial wall, dissect between the wall and keratinized cell mass, and aggregate most abundantly near the comedo orifice. (× 70)
filament-matrix pattern. On the other hand, the contents of the "true" comedo were firm, compact, and difficult to extrude. The keratinized cells here resembled those in the stratum corneum of the epidermis.

The present observations suggest that comedo formation does involve a significant change in the formation and desquamation of the keratinized cell layer inside the infundibulum. Although light microscopy descriptions of the normal follicle have stated that keratinization of the infundibulum is similar to that of the epidermis (Montagna and Van Scott, 1958; Pinkus, 1972), electron microscopy shows that normal keratinization of the upper follicle is not the same as that of the epidermis but varies along a gradient from the follicular orifice to the sebaceous gland. The cells do not normally accumulate and distend the infundibulum because of the increasing disintegration and sloughing of the horny layer, particularly in the proximal intradermal portion of this zone. Comedo formation, on the other hand, is characterized not only by quantitative (Plewig et al., 1971) but also by qualitative changes in follicular keratinization. In comedones, the keratinized layer of the intradermal infundibulum does not disintegrate but remains intact. Discrete cornified cells accumulate and differ from those in the normal follicle by having distinct thickened cell membranes. The granular cells that form this layer in the comedo have fewer tonofilaments and larger keratohyaline accumulations than normal cells. Although cornified cells of many normal keratinizing epithelia have thickened cell membranes (Farbman, 1966; Hashimoto, 1969, 1971a), this is unusual in comedo formation. If the disintegration of the horny layer of the normal infundibulum, as well as the shedding of the inner root sheath (Straille, 1965; Gemmell and Chapman, 1971) occurs by enzymatic degradation, then this layer might be more resistant to enzymatic action because of the differentiation of keratinized cells with resistant thickened cell membranes; horny cells could thereby accumulate. Alternatively, a mechanism (perhaps enzymatic) for their degradation could be lacking. Accumulation of horny cells could also result from a defect in a desquamating mechanism. Large and numerous clusters of lamellar granules occur in the intercellular spaces of the cornified layer in normal follicles but are relatively
Fig. 17: An electron micrograph of the area within the square in Figure 14b. A gradient of necrotic changes occurs in the epithelial cells from left to right as the leading edge of the wedge-like mass of keratinized cells is approached. Mitochondria (M) are swollen, nuclear and cytoplasmic electron opacity is increased, and keratohyaline granules are absent. A basal lamina underlies the basal cells at the left but can be traced only to the asterisk: farther to the right where most necrosis occurs, it is absent. Intercellular spaces (arrows) between the keratinized cells are almost imperceptible. The only cells in the adjacent dermis are fibroblasts (F). (x 3640)

Infrequent in comedones. Although the function of these granules is not known, Wolff and Holubar (1967) have reported that they contain hydrolytic enzymes and Weinstock and Wilgram (1970) suggested that they represent a specialized extracellular lysosome involved in desquamation. If this is so, then their apparent decrease in comedones would account for the decreased desquamation of the cells that form the impaction. On the other hand, if lamellar granules have a cementing function and account for increased cell cohesiveness as suggested by Hashimoto (1971b), they should be increased rather than decreased in comedones.

The most striking difference between comedones and normal follicles is the multiple intracellular lipid inclusions within keratinized and granular cells. Although the lipid nature of these is not proven, they have an electron opacity similar to that of lipids derived from adipocytes and from sebaceous cells, and droplets inside the keratinized cells of comedones stain with Oil red O.

According to light microscopic studies (Strauss and Pochi, 1966), the visible lipid in comedones may be derived from sebaceous glands before they atrophy during comedogenesis. Certainly some of it may arise this way, but the rapid turnover of comedo contents (Plewig et al., 1971), coupled with the often tiny or absent sebaceous glands associated with comedones, would make this an unlikely exclusive source of lipid. Furthermore, lipid droplets within the cytoplasm of granular cells in comedones suggest that the process of abnormal keratinization begins below the keratinized layer, not by an absorption of sebaceous lipid. This process could result from (1) production of an abnormal lipid, (2) lack of an enzyme(s) for degradation of normal lipid, or (3) production of an abnormal keratin which cannot complex normally with lipid. The appearance in comedones of alternating layers of keratinized cells with and without lipid inclusions suggests that this type of cell differentiation occurs intermittently.

Lipid droplets within keratinized cells are not apparent in normal mammalian epidermis but have been described in normal avian epidermis (Matoltsy, 1969). They have also been observed in parakeratotic psoriatic scales (Hanusova, 1960, 1961, 1965; Brody, 1962; Matoltsy and Matol-
sy, 1962; Swanbeck and Thyresson, 1962; Bonnevill et al., 1968), in parakeratotic layers in eczema and pityriasis rubra pilaris (Hanusova, 1961, 1965), and in small numbers in fungus-infected nails (Matoltsy and Matoltsy, 1962), but not in orthokeratotic cells in such large quantities as in comedones. Swanbeck and Thyresson (1962) proposed that the lipid droplets in psoriatic scales are a crystalline form unable to disperse in the horny layer, and Matoltsy and Matoltsy (1962) suggest they are a product of abnormal keratinization.

The events leading to the altered follicular keratinization in comedo formation are unknown. Abnormal keratinization could be a primary change coinciding at puberty with an increase in sebum production, or it may arise as a secondary cellular response to comedogenic substances within susceptible follicles. The conversion to a more epidermoid cornified layer which at the same time contains lipid in vacuoles may be a manifestation of the pleuripotential nature of follicular epithelium.

Although these changes have been described in follicles from selected body areas of young patients with acne vulgaris, it is not known whether similar changes occur in other conditions where comedones are found (e.g., chloracne, actinic or senile comedones). The observations do suggest that in acne vulgaris, some hair follicles of the face and upper trunk undergo a metaplasia so that the wall of the pilosebaceous canal produces an abnormal keratinized layer which contains large quantities of intracellular lipid inclusions and exhibits increased integrity and decreased desquamation of cells. The morphologic differences between the infundibulum of the normal and the comedogenic follicle are summarized in Figure 19.

**Inflammatory Lesions**

Our observations support the view that inflammatory lesions derive from antecedent comedones. Comedones are frequently seen without associated inflammatory infiltrates, but no inflammatory lesions are seen without comedo formation. Although lesions occurring in the absence of a comedo and composed predominantly of lymphocytic infiltrates have been described in acne by others (Strauss and Kligman, 1960), we have not seen similar lesions in this study. Instead, all inflammatory lesions have had cellular infiltrates of neutrophils and macrophages.

Whether inflammatory cells appear before or after disruption of the comedo wall cannot yet be completely answered. However, the fortuitous finding of comedones whose contents are in direct contact with the dermis but without visible inflam-

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**Fig. 18:** The inflammatory infiltrate from the lesion shown in Figures 15a and 15d. Keratinized cells (K), macrophages (M), and neutrophils (N) contain lipid inclusions (arrows). Some cells have phagocytic vacuoles (V). No bacteria can be identified. (× 2660)
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19: A diagram comparing the structure of a normal sebaceous follicle (Fig. 19a) and an early comedo (Fig. 19b). The sites enclosed in the small square (Fig. 19a) are shown in greater detail in the larger drawings. The orifice, sebaceous duct, and sebaceous gland are similar in both figures.

(a) The lumen of a normal follicle contains sebum and loose keratinized fragments (F). The cornified layer is attenuated and composed of noncompacted keratinized cells which shed into the pilary canal. Clusters of lamellar granules (C) fill interstices within the horny layer. Many individual lamellar granules (C) localize at the luminal surface of the cells of subjacent layers.

(b) Comedogenesis begins in the intradermal infundibulum (I). Many keratinized cells in an early comedo are maintained in a compact cornified layer and contain multiple nonsebaceous lipid inclusions (L). The number of lamellar granules is decreased. Large masses of bacteria (B) colonize the follicular lumen.

information suggests that disruption of the wall precedes the inflammatory reaction. In these areas, the loss of intercellular spaces creates a compressed mass of keratinized cells, and there are signs of cell injury within the adjacent wall. Loss of the basal lamina and decreases in tonofilaments, keratohyaline granules, and lamellar granules suggest that the synthetic functions of the affected cells are impaired. This loss could result from pressure necrosis from the expanding follicular impaction, although the fact that disruption occurs in immature comedones with apparently patent orifices casts some doubt on this mechanism.

Whether bacteria play a role in the genesis of inflammation in acne is not clear. Although large masses of bacteria are often seen in inflammatory lesions, they are usually surrounded by a horny mass and rarely occur free within the infiltrate. Seldom can bacterial cells be discerned in the multiple phagocytic vacuoles of neutrophils or macrophages. However, even though the microorganisms appear physically separated from the inflammatory infiltrate, the possible effects of toxic products (e.g., free fatty acids) or chemotactic factors generated by them cannot be ruled out.

Other substances associated with the genesis of the inflammatory reaction are the keratinized and lipid contents that are derived from the disrupted follicle and may act as foreign materials within the dermis (Strauss and Pochi, 1965). Although the lipids are mainly regarded as sebaceous in origin, the present observations demonstrate that lipid inclusions within keratinized cells are a prominent nonsebaceous source of lipid within the inflammatory infiltrate. The similarity between these cytoplasmic lipid droplets and those in adjacent neutrophils and macrophages suggests that some of the lipid within the inflammatory cells originated from the keratinized cells. The nature of this lipid and its significance in the pathogenesis of acne vulgaris should be considered in future studies of this perplexing disease.

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