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ULTRASTRUCTURE OF HUMAN SEBACEOUS FOLLICLES AND COMEDONES FOLLOWING TREATMENT WITH VITAMIN A ACID[1]

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Abstract. Ultrastructural findings in normal human sebaceous follicles, of comedones from acne patients, and from comedones during topical treatment with vitamin A acid (VAA) are described. In normal human sebaceous follicles, the two segments of the infundibulum—the acroinfundibulum and the infrainfundibulum—show differing ultramorphological patterns of keratinization. The incomplete horny layer of the infrainfundibulum is physiological and seems to be necessary for its normal function. On the other hand, no effective barrier function is established in this region. During comedo formation, excessive keratinization takes place in the infrainfundibulum, leading to a compact and coherent horny core. VAA-treatment leads to profound alterations of keratin production, with the appearance of small, irregular, non-coherent and foamy horny cells. The comedonal wall resumes almost physiological conditions. These changes are in agreement with experimental work using VAA in vivo and in vitro and may explain the therapeutic mechanism of action of this compound in the treatment of acne.

The ultrastructural characteristics of keratinization in normal human surface epidermis are well known (1, 3, 15), as are those of a variety of abnormal keratinization patterns (8, 15). On the other hand, the human sebaceous follicle has been neglected for a long time by electron microscopists. Information about normal and abnormal ultramorphology of this special structure of human skin is necessary for our understanding of the pathogenesis of frequently occurring, clinically important, follicular-bound diseases and their treatment. Only Knudson (7) recently paid attention to this problem in his extensive ultrastructural study of normal sebaceous follicles and acne lesions.

In this paper, we describe ultrastructural findings in normal human sebaceous follicles, comedones in acne vulgaris patients, and changes in comedones during topical vitamin A acid (VAA, tretinoin) application.

The latter part of the investigation was performed because of the increasing therapeutical use of VAA, especially in acne patients (6, 9, 10). It is shown that ultrastructural analysis can contribute to our understanding of the mechanism of action of this compound in the treatment of acne.

MATERIALS AND METHODS

A total of 24 biopsies were taken for this investigation. We used small excision biopsies either from the face (upper cheek and forehead) or the upper back or chest of normal volunteers or acne patients aged 14 to 46 years. In 10 cases, consecutive biopsies were taken from the same patient before, and after 2 to 14 weeks of VAA therapy. Routine VAA therapy consisted in one to three applications per day of the commercial topicals (0.05% cream, gel, or solution).[2]

The tissue was dissected into small pieces immediately after excision and fixed for 3 h in 1% osmic acid buffered in phosphate at pH 7.2. After microdissection and proper orientation, they were embedded in epon. Semithin sections of large areas were made with glass knives and stained with methylene blue and azur II. In these sections, special areas were selected for further trimming of the block and for ultrathin sectioning with diamond knives on the Reichert ultramicrotome OmU2 of OmU3. The sections were stained with uranyl acetate and lead citrate, and photographed with a Philips EM 300 electron microscope.

RESULTS

Normal sebaceous follicles

In normal human sebaceous follicles, different segments show distinctive characteristics of keratinization. In the following descriptions we shall restrict ourselves to the infundibulum which is limited above

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[2] Eudyna®, Nordmark, Uetersen, Germany; Vitamin-A-Säure Hoffmann-La-Roche, Grenzach, Germany.

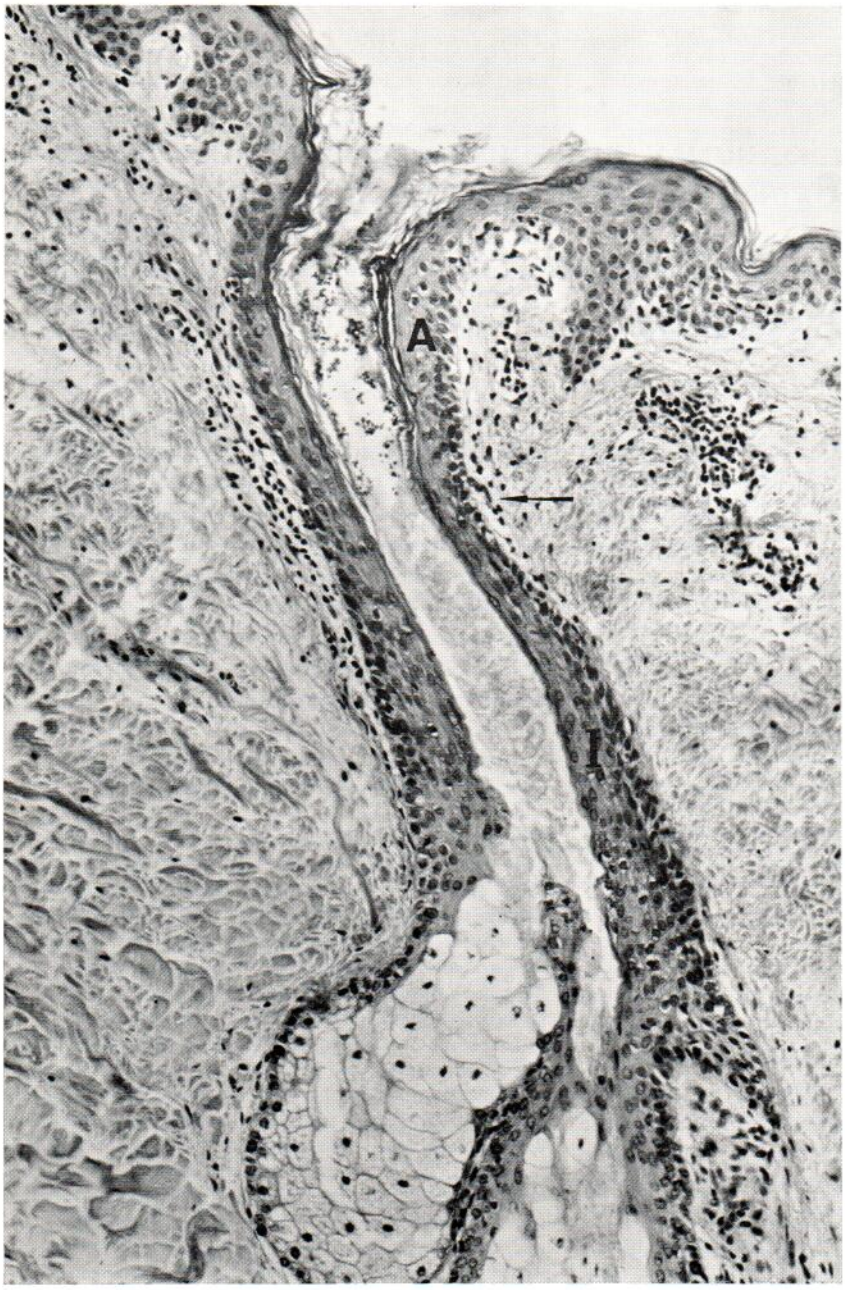

Fig. 1. Normal human sebaceous follicle, light microscopy. The infundibulum is divided into the acroinfundibulum (*A*) and the infrainfundibulum (*I*) by an arrow. Lipophilic yeasts are above, *C.* acnes below. H & E, 200:1.

by the surface epidermis and below by the sebaceous gland (Fig. 1). The infundibulum itself can be separated into two parts (5, 10): an acroinfindubulum forming the upper portion of the follicle which is followed below by the infrainfundibulum. The two segments can easily be distinguished in the light microscope. The border is marked by an arrow in Fig. 1. Light microscopically, the acroinfundibulum has melanin pigmentation, granular and horny layers similar to the surface epidermis. These characteristics are absent in the infrainfundibulum.

In the electron microscope, the ultrastructure of the acroinfundibulum widely resembles the well-known surface epidermis: shape, arrangement, and con-

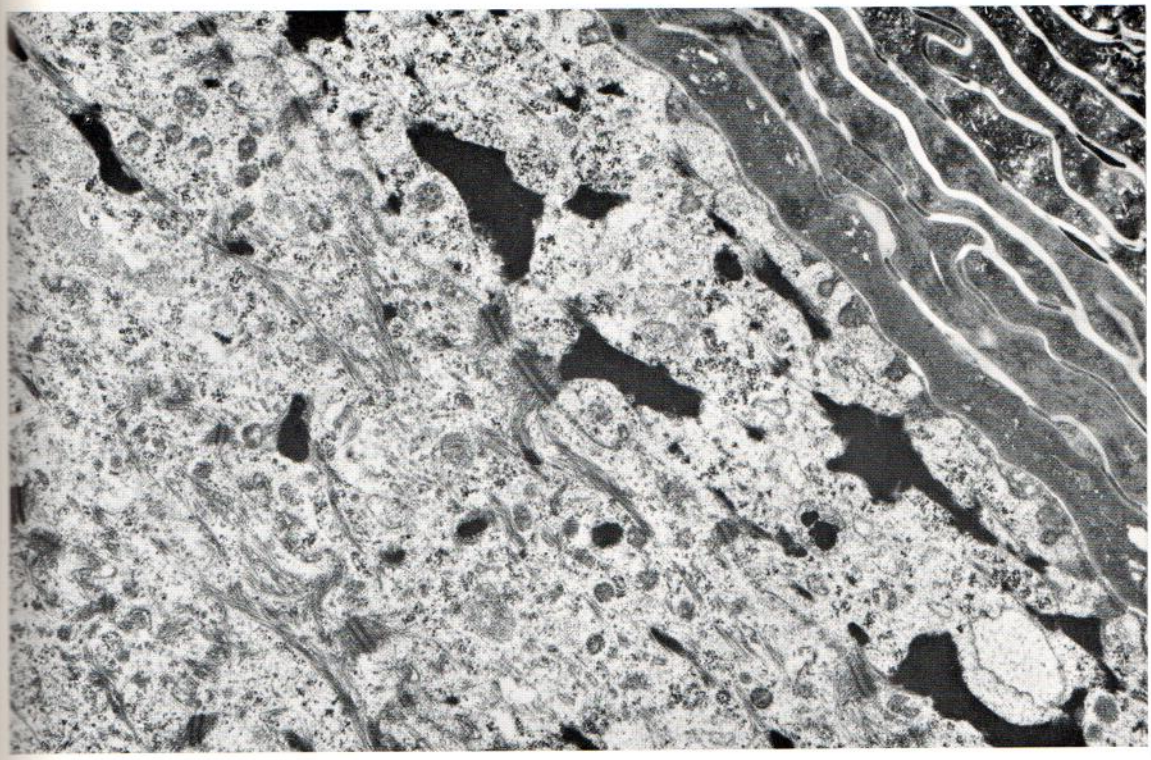

Fig. 2. Acroinfundibulum from a normal sebaceous follicle, upper cheek. The ultrastructural picture widely resembles normal epidermis. 22 000 : 1.

tents of basal, spinous, and granular cells do not differ markedly. Especially all structures specific to the keratinization process are present in a normal manner (Fig. 2). These are tonofilament bundles, desmosomes, keratinosomes, and keratohyalin granules. The horny layer is composed of normal, flat horny cells of regular arrangement showing the well-known A and B type of keratin appearance (8). In the infrainfundibular part of the follicle (Fig. 3), melanocytes and melanosomes are not present. Tonofilament formation is markedly reduced. The number of tonofilament bundles is diminished, and the individual bundles are very short and thin. Only a few, and very tiny desmosomes are formed. The keratohyalin granules—which are practically absent in the light microscopical picture of this region—are small and scarce. On the other hand, many keratinosomes can be found although it is difficult to give quantitative data without statistical analysis. The horny cells produced by this epithelium are small and flimsy (Fig. 3). They do not form a continuous, coherent layer as they are easily shed into the follicular canal, which is filled with a stream of sebum, carrying these detached horny cells to the skin surface through the follicular mouth. In the lowest part of the infrainfundibulum, incompletely keratinized and even living eopithelial cells of irregular shape are sloughed into the lumen (Fig. 4). Thus, in this region, no true horny cell barrier exists between the living epithelium and the follicular contents. These are composed of the already mentioned masses of sebum, horny cells, and of lacunae containing large colonies of *Corynebacterium acnes* (Fig. 5). These microorganisms, as well as the lipophilic yeasts and the micrococci in the upper part of the follicle, are always found in the intercellular spaces, and never within the horny cells.

Comedones

In initial comedones—"microcomedones" (5, 10)—the normal ultrastructural picture of the infrainfundibulum is no longer detectable. All segments of the follicular epithelium reveal signs of very active keratinization (Fig. 6). The granular layer is prominent.

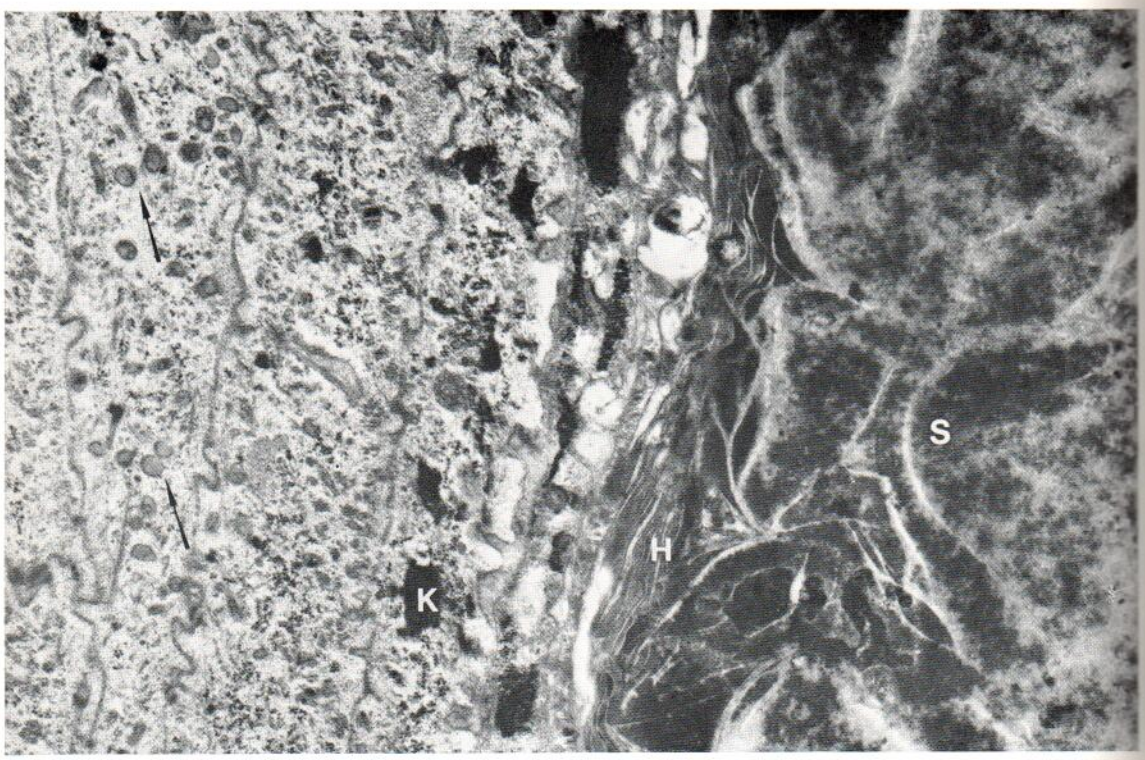

Fig. 3. Infrainfundibulum from a normal sebaceous follicle, from the forehead of a 17-year-old man. Few tonofilaments and desmosomes, little keratohyalin (*K*), small and flimsy horny cells (*H*) are produced. Keratinosomes (↗) are numerous. The content in the follicular canal, mainly sebum (*S*) is shown (*right*). 21 000 : 1.

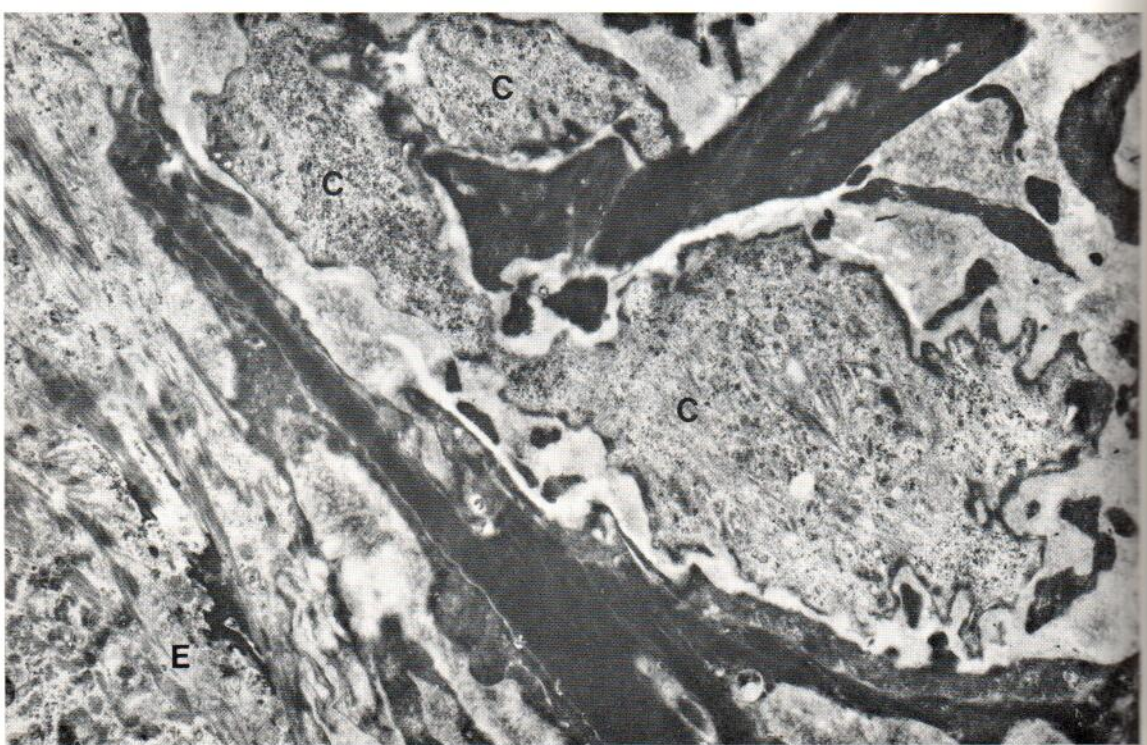

Fig. 4. Lower infrainfundibulum from a normal sebaceous follicle from the forehead of a 17-year-old man. Living epithelium (*E*) (*left*). No horny layer, sloughing of living cells (*C*) into the lumen. The follicular canal is seen (*upper right*). 13 000 : 1.

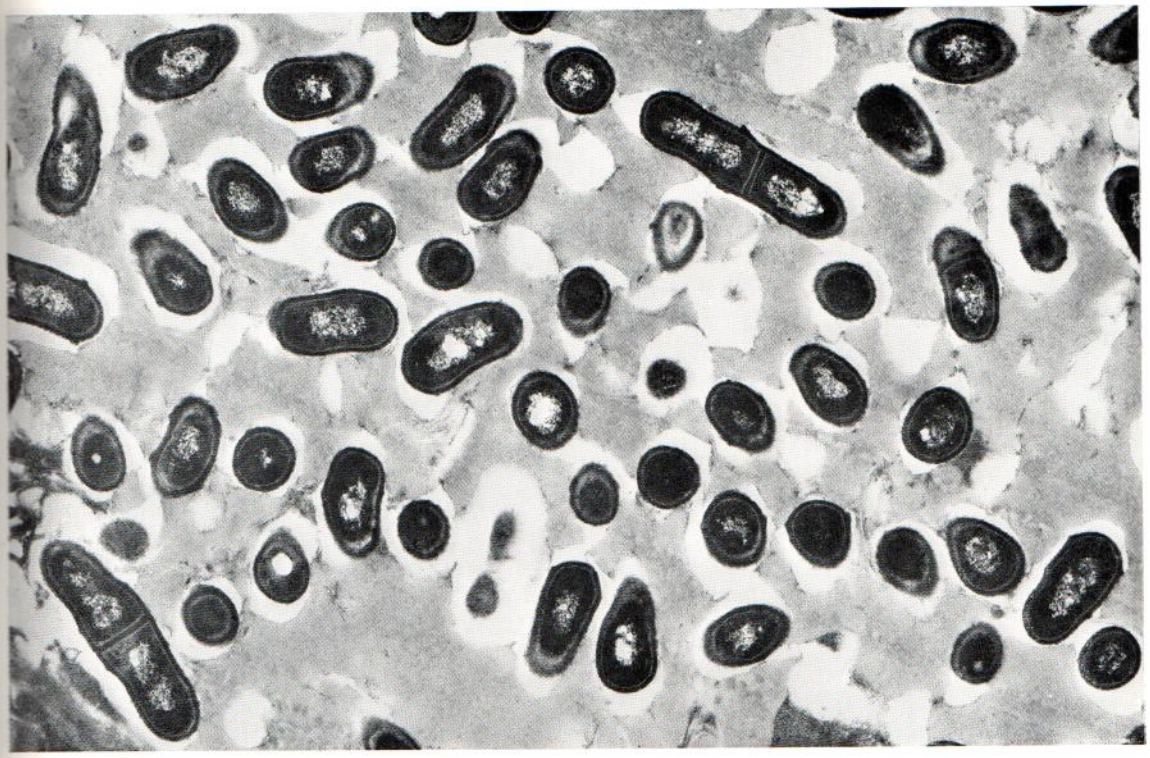

Fig. 5. Numerous *C.* acnes, embedded in sebum. Sebaceous follicle of a 21-year-old man. 21 000 : 1.

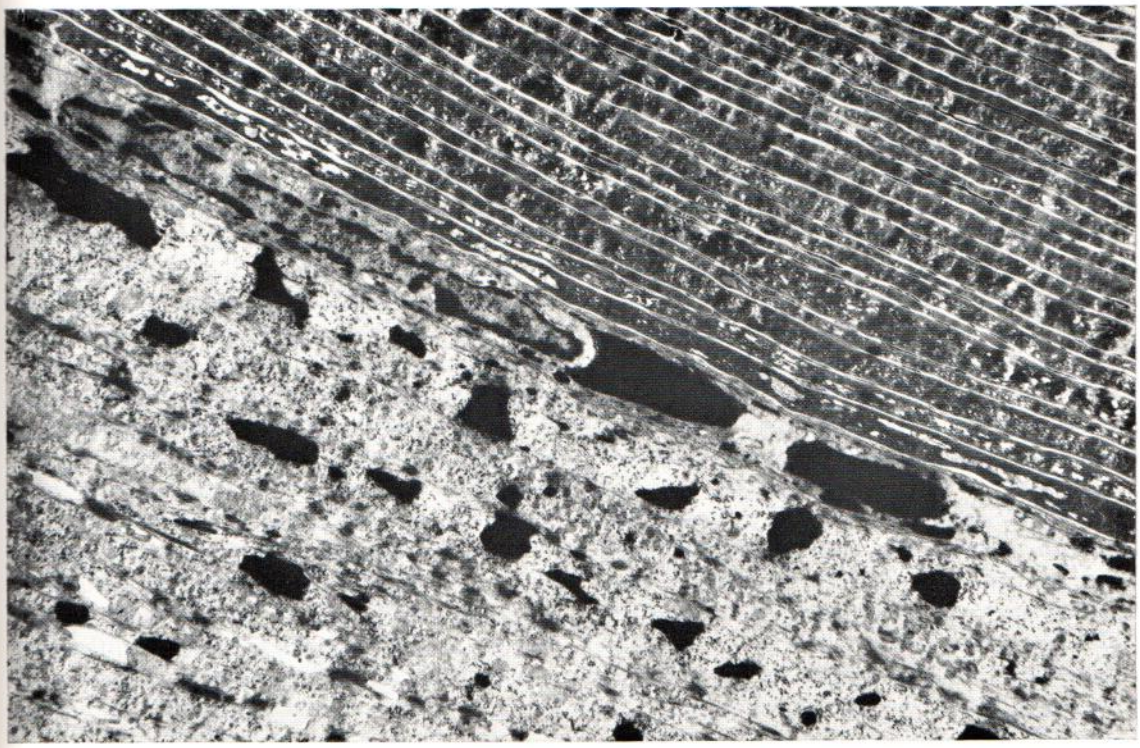

Fig. 6. Comedo from upper chest of a 21-year-old man with severe acne vulgaris, without treatment. The ultramorphological characteristics of excessive keratinization are evident, especially the thick granular and horny layers. 8 000 : 1.

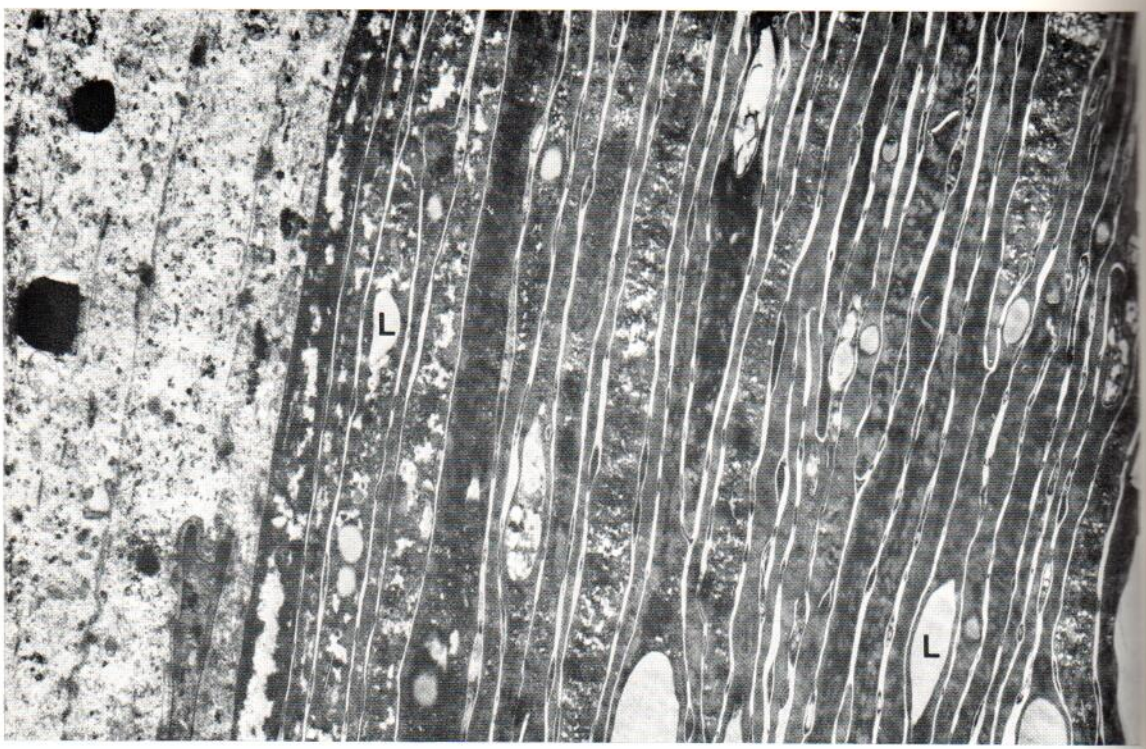

Fig. 7. Lower part of the comedo from the same patient as in Fig. 6, without treatment. Some lipid inclusions (*L*) are shown in the compact horny layer. 15 000:1.

It has increased several times in thickness, and contains many large keratohyalin granules. The numbers of tonofilaments and desmosomes have also increased, and sometimes long nexus are found between the granular cells. Keratinosomes seem to be diminished in number. The most obvious change is observed in the horny layer. It shows an enormous increase in thickness. It is composed of flat horny cells of fairly regular shape connected by many desmosomal discs. The contents of the horny cells are more inhomogeneous. A rather coarse, reticular keratin pattern and lipid droplets of different size and density may be present (Fig. 7). Inspite of these irregularities, the horny cells within the follicle stick together extremely well. This leads to the development of a core composed of the horny cells. In the center of comedones, the horny cells are often compacted in a meandering way (Fig. 8). Discharge of the newly formed horny cells is impaired, and the result is a distension of the follicle, and finally the development of a comedo.

Comedones during VAA-treatment

During topical VAA therapy, the ultramorphological characteristics of slight dermatitis can be observed in the lower layers of the comedonal wall. They correspond to the well-known clinical symptoms of dermatitis, namely erythema and scaling (6, 11). The changes consist mainly in slight spongiosis (Fig. 9*a*) and exocytosis (Fig. 9*b*), which we consider as nonspecific side effects in this treatment.

Much more important are the changes to be observed in the upper layers of the epithelium. Here remarkable alterations of the ultrastructural pattern of keratinocytic differentiation can be seen (Fig. 10): Keratohyalin production is widely reduced, only small and scarce keratohyalin granules are found in the thinned granular layer, especially at the onset of the typical reaction. Whereas the specific differentiation products of the keratinosomes—tonofilaments and desmosomes—are clearly diminished, the nonspecific morphological indicators of metabolic activity are prominent in the cytoplasm. We observe

Fig. 8. Central part of the horny core of a comedo from the upper chest of a 21-year-old man with acne vulgaris, without treatment. The large horny cells are compacted in a meandering way; they do not slough. 8 000 : 1.

many large mitochondria of irregular shape, well equipped with cristae, as well as many ribosomes, and even rough endoplasmic reticulum, which is otherwise a rare structure in these cells (Fig. 10). Keratinosomes are present in large numbers in the apical parts of the cells.

A highly altered horny layer is formed by this abnormal epithelium. As compared with untreated comedones, the horny cells differ considerably with respect to their size, shape, contents, arrangement, and coherence (Figs. 11–14). The horny cells produced during VAA-treatment are much thinner, smaller, and show irregular forms and arrangement. Within the horny cells, a normal keratin pattern cannot be detected. They contain lipid droplets, sometimes in large numbers and of huge size, as well as masses of foamy-looking material (Figs. 11, 14), which we have never seen in any other type of abnormal keratinization. They seem to disintegrate rather easily. In addition, very few desmosomal discs are present. The lipid and foamy material appears already in the granular layer where it may be detected in large amounts within the cells, together with keratohyalin granules (Fig. 12). All these changes lead to a marked decrease in horny layer coherence. As a result, only an incoherent horny layer may be built up (Fig. 14). Comedonal contents fail to adhere to its anchoring position and is extruded through the comedonal opening.

DISCUSSION

Normal sebaceous follicles

Electron microscopy reveals two distinctive types of keratinization within the infundibular part of human sebaceous follicle. In agreement with Kligman, we call these segments acroinfundibulum and infrainfundibulum (5, 10). Most ultrastructural characteristics described above have already been elaborated by Knudson (7) in his electron microscopical study.

For proper function, that is the lack of horny impactions in the follicular canal, the production of an incoherent horny layer by the infrainfundibulum is

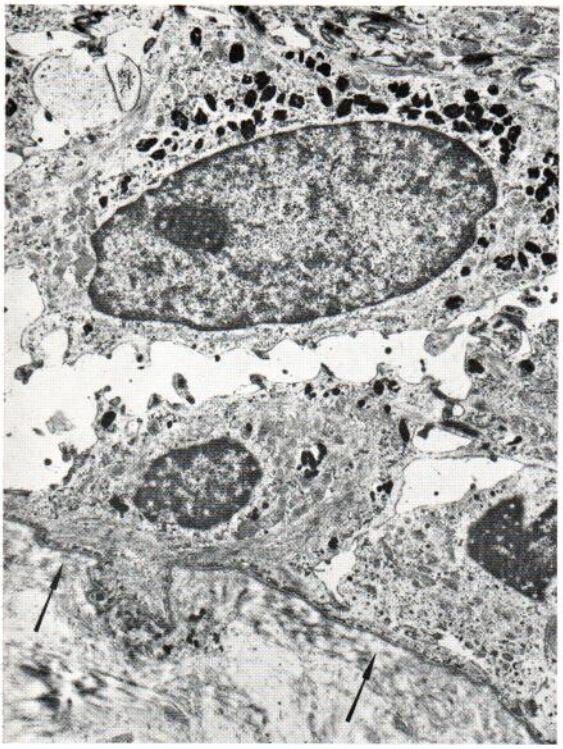

Fig. 9a. Epidermis of a 21-year-old man with acne vulgaris, upper back, treated with VAA for 4 weeks. Scaling was present at time of biopsy. Spongiosis (microacantholysis) is still visible in the basal layers of the epidermis. Arrows point to the basement membrane. 6 000:1.

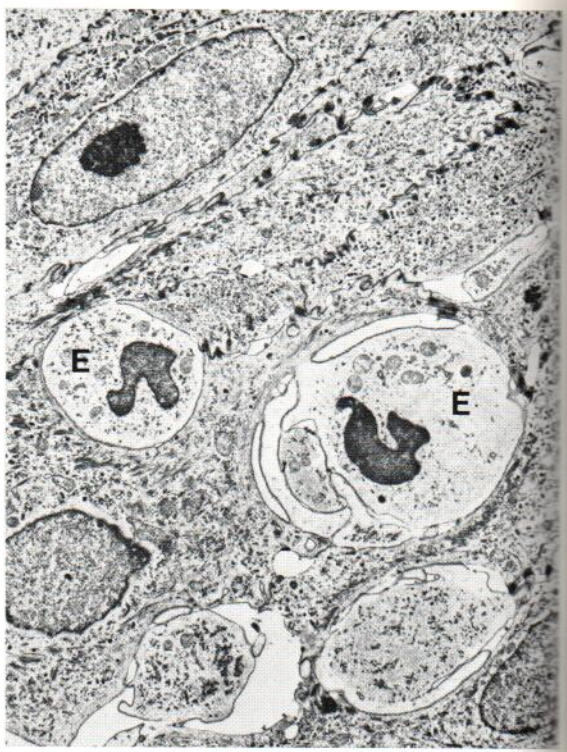

Fig. 9b. Epidermis of a 17-year-old man with acne vulgaris, forehead, treated with VAA for 6 weeks. Clinically, there was scaling and erythema. Slight spongiosis and exocytosis (*E*). 5 000:1.

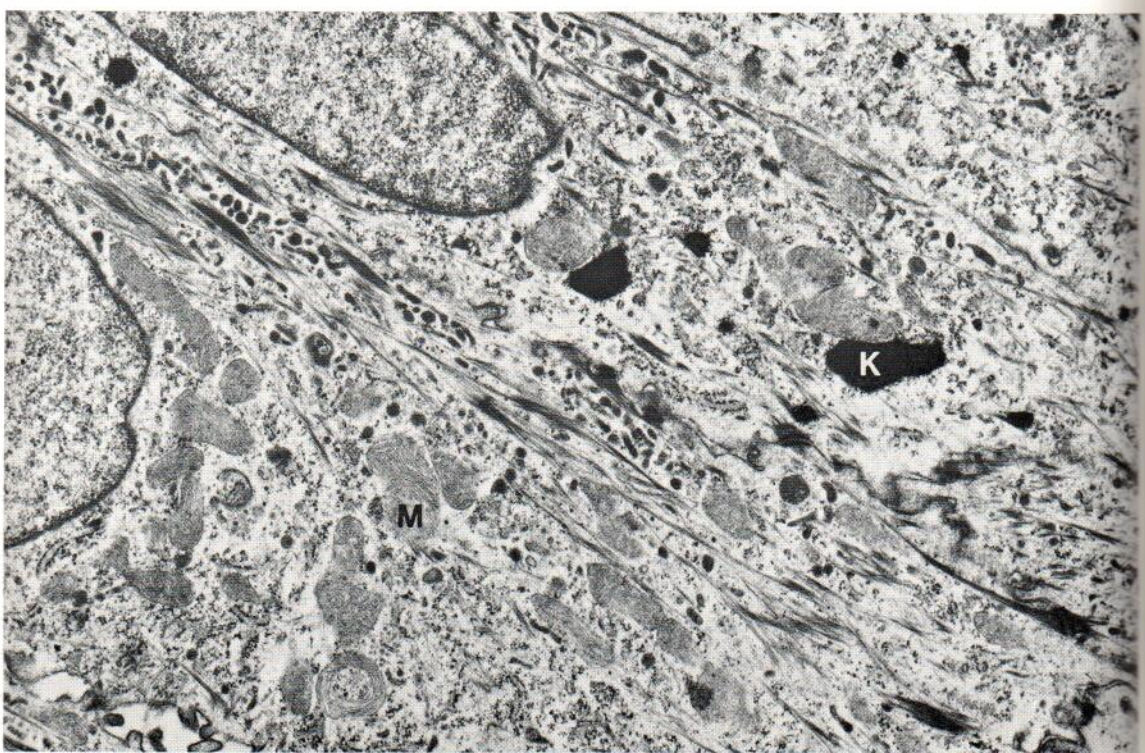

Fig. 10. Follicular wall (infrainfundibular part), upper back, from a 21-year-old patient, treated with VAA for 4 weeks. There are ultramorphological signs of high metabolic activity, e.g. mitochondria (*M*), but a decrease in the specific indicators of keratinization, e.g. keratohyalin (*K*). 14 000:1.

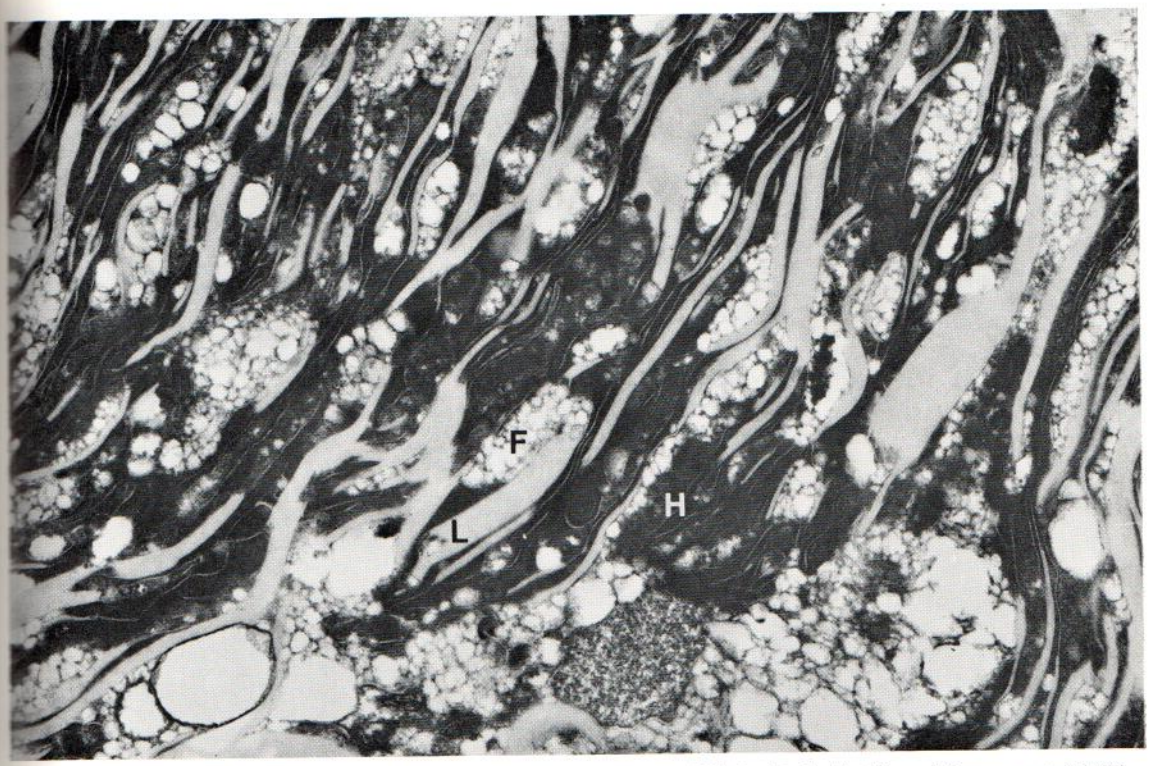

Fig. 11. Horny cells from a comedo from the upper chest of a 19-year-old man with acne vulgaris, after 6 weeks of treatment with VAA. The horny cells (*H*) are irregular, incoherent, and filled with lipids (*L*) and foamy material (*F*). 25 000 : 1.

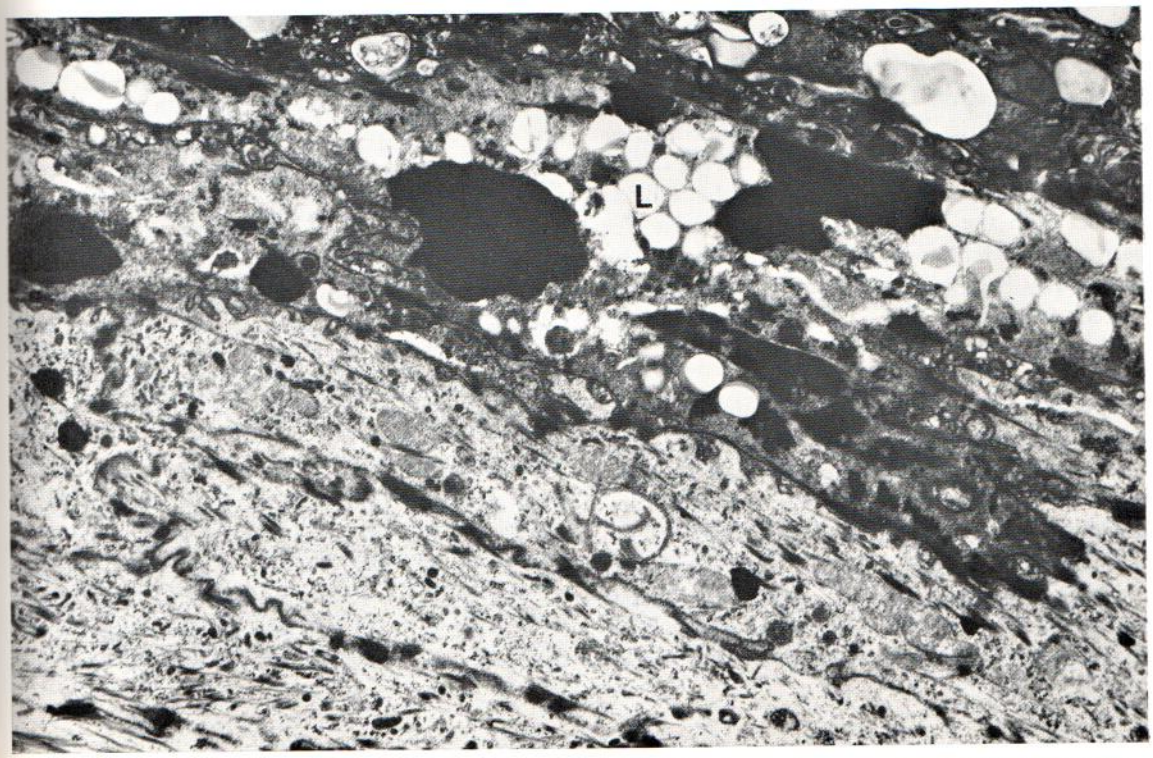

Fig. 12. Lipid inclusions (*L*) in granular cells of a comedonal

Fig. 10. Note changes in upper spinous and in horny layers.

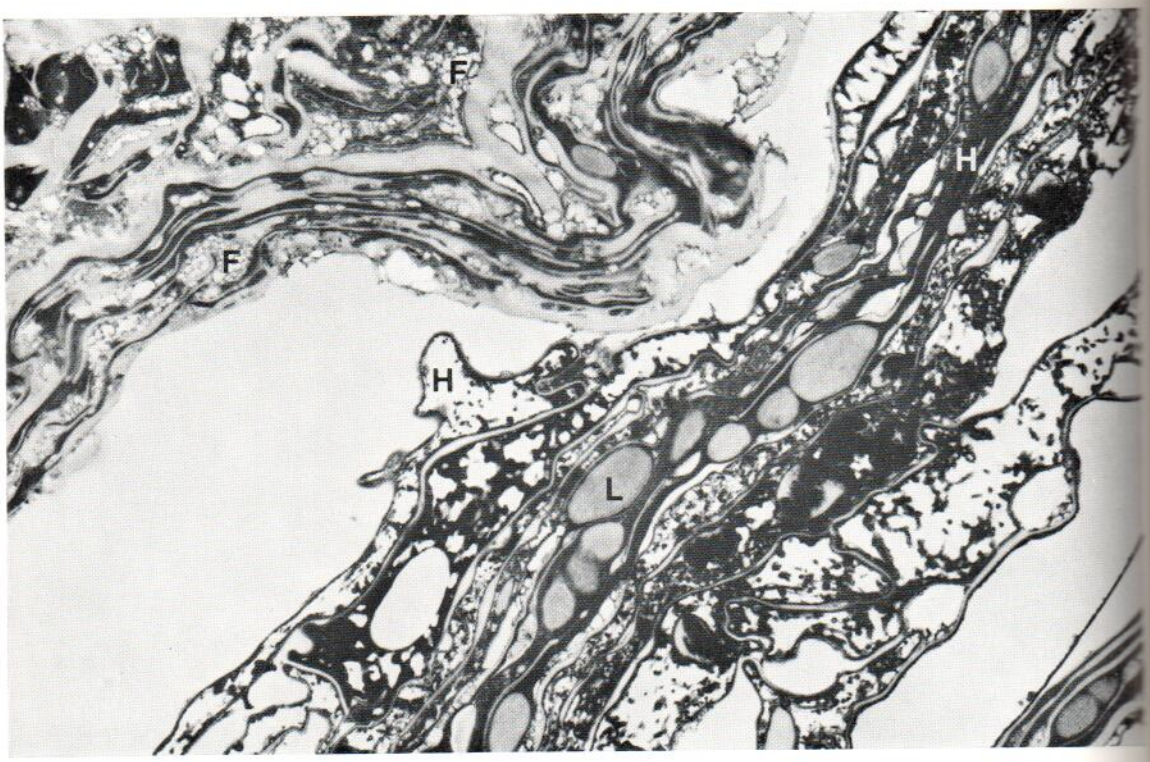

Fig. 13. Horny cells from a comedo of a 19-year-old man with acne vulgaris after 6 weeks of VAA-treatment, same patient as in Fig. 11. Incoherence of horny cells (*H*), lipid droplets (*L*), and foamy material (*F*) are obvious (cf. Figs. 6–8, before treatment). 13 000:1.

important. This special type of keratinization allows easy sloughing of horny cells and an unimpaired stream of sebum towards the surface. On the other hand, this situation leads to a disadvantage concerning the barrier function of the epithelium. As is demonstrated in Figs. 3 and 4, in the infrainfundibular part the follicular contents is in almost direct contact with the living epithelial cells, as a true barrier of coherent horny cells is not produced. This discrepancy is important for our understanding of comedo formation.

Comedones

For reasons that are still under discussion, the keratinization pattern of the infrainfundibulum may change. According to Kligman, the lipophilic diphtheroids, *C.* acnes (Fig. 5), living deep in the follicular canal in the region of the infrainfundibulum may play an important role (5). These microorganisms split the triglycerides of the sebum, thus liberating highly irritating free fatty acids which could attack the unprotected epithelium. Also, in different types of acne, acne venenata, acneiform eruptions, the comedogenic substances may preferably penetrate through the follicular wall in this region.

Whatever may be the true ethiopathogenetic factor, electron microscopy reveals a change in keratinization in the infrainfundibulum during comedo formation. This epithelium may transform into a well keratinizing structure. A very compact and adherent horny layer is built up (7, 12), the follicle is distended, and discharge of horny cells, cellular debris, and bacteria is impaired. Once a microcomedo has formed, the functional conditions inevitably worsen, thus leading to an escalation of this mechanism. The well known result is a comedo.

VAA-treatment

Considering the structure and ultrastructure of comedones and the conditions under which they are formed, the best therapy would be to decrease horny material formation and to decrease horny cell adherence, especially in the infrainfundibular segment of the sebaceous follicle. The latter can be done by

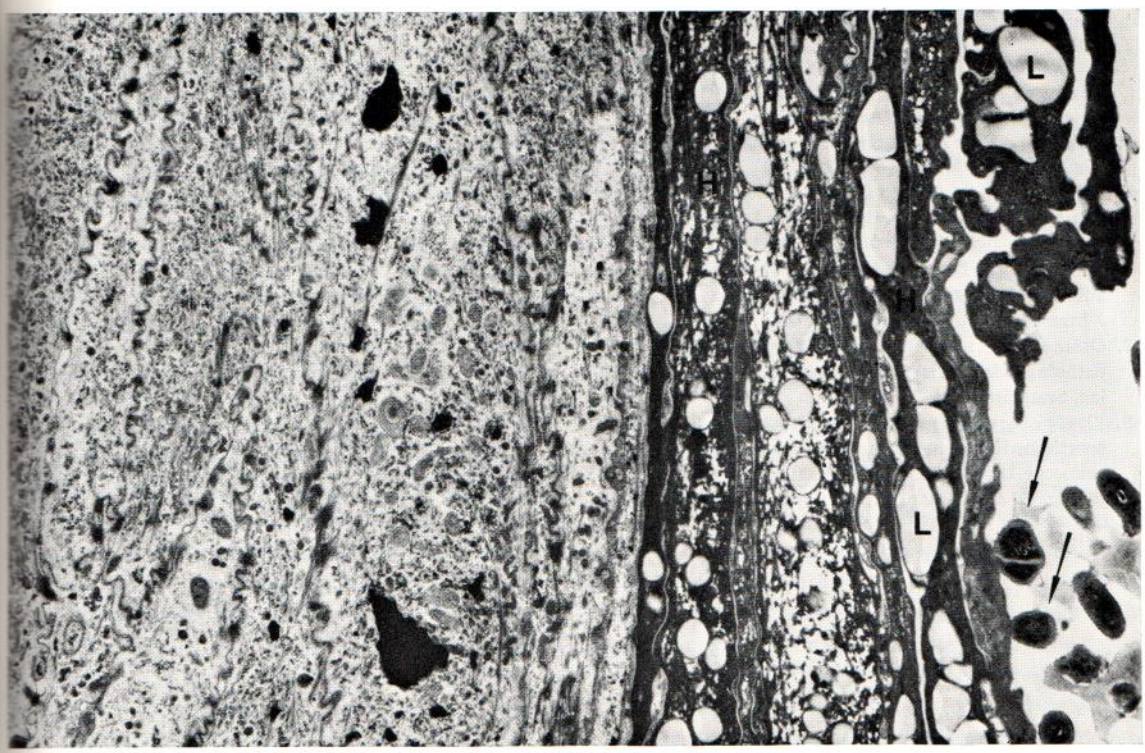

Fig. 14. This was a comedo on the forehead of a 17-year-old man with acne vulgaris. Six weeks of VAA treatment. Only a few horny cell layers (*H*) are present between the altered living epithelium (*left*) and the follicular lumen (*right*), which was the site of the original comedo. Lipid droplets (*L*) are conspicuous. *C. acnes* (↗) are now in close apposition to the epithelium. 10 000 : 1.

topical application of VAA (6, 9, 10). Experimental work has shown that VAA profoundly influences proliferation and differentiation of epidermal cells in vivo (2, 11, 13, 14) and in vitro (4). In short, an enormous increase in cell proliferation is observed (2, 13), whereas keratinization is inhibited or greatly altered. The obvious morphological changes of the keratinization process have been demonstrated in histological, histochemical, and electron microscopical studies of animal and human epidermis (2, 11, 13, 14).

In this paper, for the first time the effects of VAA on human comedones during acne therapy are investigated with the electron microscope. This study offers ways to explain the successful action of the compound in the treatment of acne lesions. It is shown that the thick, compact, and coherent horny layer produced by the wall of comedones is changed back into a more "physiological", thin, and incoherent horny layer. This consists of small horny cells of irregular shape and arrangement, almost completely lacking desmosomal discs, containing lipid droplets and foamy materials, and falling apart very easily. Once the horny core of the comedo is removed, the sebaceous follicles may retain their original size and function.

ACKNOWLEDGEMENT

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