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REVIEW

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Pathogenesis of acne

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Abstract Acne vulgaris is a skin disorder of the sebaceous follicles that commonly occurs in adolescence and in young adulthood. The major pathogenic factors involved are hyperkeratinization, obstruction of sebaceous follicles resulting from abnormal keratinization of the infundibular epithelium, stimulation of sebaceous gland secretion by androgens, and microbial colonization of pilosebaceous units by *Propionibacterium acnes*, which promotes perifollicular inflammation. The clinical presentation of acne can range from a mild comedonal form to severe inflammatory cystic acne of the face, chest, and back. At the ultrastructural level, follicular keratinocytes in comedones can be seen to possess increased numbers of desmosomes and tonofilaments, which result in ductal hypercornification. The increased activity of sebaceous glands elicited by androgen causes proliferation of *P. acnes*, an anaerobe present within the retained sebum in the pilosebaceous ducts. The organism possesses a ribosome-rich cytoplasm and a relatively thick cell wall, and produces several biologically active mediators that may contribute to inflammation, for instance, by promoting leukocyte migration and follicular rupture. In inflamed lesions, numerous neutrophils and macrophages infiltrate around hair follicles and sometimes phagocytose *P. acnes*. To examine the participation of neurogenic factors in the pathogenesis of acne, we quantitatively assessed the effects of neuropeptides on the morphology of sebaceous glands in vitro using electron microscopy. Substance P, which can be elicited by stress, promoted the development of cytoplasmic organelles in sebaceous cells, stimulated sebaceous germinative cells, and induced significant increases in the area of sebaceous glands. It also increased the size of individual sebaceous cells and the number of sebum vacuoles for each differentiated sebaceous cell, all of which suggests that substance P promotes both the proliferation and the differentiation of sebaceous glands. In this review, we introduce the general concept of pathogenic factors involved in acne, including typical electron microscopic findings and recent evidence of stress-induced exacerbation of acne from a neurological point of view. An improved understanding of the pathogenesis of acne should lead to a rational therapy to successfully treat this skin disease.

Key words Acne vulgaris · Sebaceous follicle · *Propionibacterium acnes* · Inflammation · Neuropeptides

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Introduction

Acne vulgaris is a complex, chronic, and common skin disorder of pilosebaceous units that occurs predominantly in the skin of the face, the upper back, and the upper chest. This disease usually begins at the time of the sharp increase in androgen production that occurs during adolescence. In recent years, the multifactorial nature of acne has been elucidated but much remains to be learned. Briefly, acne begins when the pilosebaceous ducts become plugged with keratinocytes to form comedones, sebum builds up and distends the follicles, and the anaerobe *Propionibacterium acnes* (*P. acnes*) proliferates in the sebum. If the comedo ruptures into the dermis, inflammation results and a pustule or papule forms.[1] This review article summarizes the pathogenesis of acne, including the clinicopathological relationship of acne lesions, the fine structural features of *P. acnes*, and the pathophysiology of sebaceous follicles. In particular, we focus on ultrastructural findings that help clarify why and how each therapeutic agent is rationally used for acne treatment. To clarify the participation of neurogenic factors in the etiology of acne, the effects of neuropeptides on the morphology of sebaceous glands were also studied.

Clinical aspects of acne

From the clinical point of view, acne is divided into noninflammatory or inflammatory lesions.[2] Noninflammatory acne is characterized by the presence of open or closed comedones, which begin as invisible microcomedones that proceed all other acne lesions. Microcomedo formation is caused by the abnormal keratinization of the infundibular epithelium of hair follicles. Further retention of a dense material composed of sebum and keratinous debris dilates the follicles of microcomedones, which leads to the formation of comedones. Two types of comedones can be distinguished morphologically, one being a closed comedo, or whitehead, and the other being a open comedo, or blackhead. In contrast, inflammatory lesions consist of papules, which are raised erythematous lesions measuring less than 0.5 cm, and pustules, which are papules with a visible collection of white pus at the surface. These lesions often enlarge, becoming firm or indurated, and are termed nodules. Cysts are a similar type of lesion with noted fluctuations. Scarring may be associated with any form of severe inflammatory acne. Clinical manifestations of acne vulgaris range from noninflammatory comedones to inflammatory papules, pustules, and cyst. In most patients with acne, these lesions are usually intermingled to various extent. The goal of treatment is to reduce the numbers of both types of lesions, with minimal or no side effects.[3]

Histology

Acne is a disease of the sebaceous follicles, which are equipped with large sebaceous glands and produce only fine vellus hairs. Sebaceous follicles are most common in the acne-prone areas such as the cheeks, the nose, and the forehead, as well as on the midline chest and the back. The follicular canal is considerably wider than that of a normal hair follicle and is lined with stratified squamous epithelium. At the proximal end of the canal, deep in the dermis, are sebaceous glands connected to the canal by short ducts. The outer part of the follicular canal comprises two histologically distinct regions. The distal region is termed the acroinfundibulum and the proximal region is the infrainfundibulum, the latter portion being important to the pathogenesis of acne. The acroinfundibulum is essentially a continuation of the usual surface epithelium. There is a similar granular layer and a stratum corneum, and desquamation of cornified cells proceeds normally. In the infrainfundibulum, the epithelial cells have fewer desmosomes and tonofilaments,[4,5] the granular layer is diminished, and the horny layer is much thinner. The cornified cells do not form a coherent layer, and they slough readily into the follicular canal. The resulting keratinous squamae are carried to the skin surface with the secreted sebum. In addition to sebum and shed cells, the canal normally contains a mixed microbial population. In the infrainfundibulum, the most common organism is *Propionibacterium acnes*, an anaerobic diphtheroid. The yeasts *Pityrosporum ovale* and *Pityrosporum orbiculare* colonize the upper acroinfundibulum. Aerobic cocci, usually *Staphylococcus epidermidis*, reside on the skin surface around the follicles.[6]

Pathogenesis of acne

There is general agreement that acne is of multifactorial origin. The four most significant pathogenic factors of acne have been identified as previously stated: (1) an androgen-stimulated increase in the production of sebum, (2) hyperkeratinization and obstruction of sebaceous follicles resulting from abnormal desquamation of follicular epithelium, (3) proliferation of *Propionibacterium acnes*, and (4) inflammation.[2]

Sebaceous secretion

The first factor in the genesis of acne is the androgen-induced hypertrophy of sebaceous glands and the overproduction of sebum, which is the by-product of the holocrine rupture of mature sebocytes. Androgens are the major sebotropic hormones; sebaceous glands are very sensitive to androgens and are less sensitive to estrogens.[7,8] Acne generally begins at puberty, when androgen levels increase significantly and stimulate excess sebum secretion.[9] In a microcomedo, the sebum is trapped behind a keratin plug. The follicle then becomes enlarged and contains a mixture of sebum and keratinous squamae, which leads to the obliteration of the normal architecture of the follicle and to the formation of a thin-walled cystic lesion, the comedo. The hormonal control of sebaceous gland function is complex. The pituitary is the main driver, and as a result of its influence on the adrenal and gonads, there is an interplay of hormones that control the pilosebaceous units.[10,11] The most important androgen is testosterone, which is converted to dihydrotestosterone by the iso-enzyme 5α-reductase type 1[12] (Fig. 1). On the other hand, it has been recognized that the adrenal androgen dehydroepiandrostenedione correlates well with early features of acne, in particular, the presence of comedones.[13] There are increased levels of 5α-reductase in the sebaceous glands of acne patients.[12] An increased number of androgen receptors is also found in sebaceous glands.[14] Acne patients are not endocrine misfits, and an end-organ hyperresponse of the glands to androgens is probably the most likely explanation for the seborrhea.[8] Sebum, when first secreted, consists principally of triglycerides, with significant quantities of wax esters and squalene and very small amounts of cholesterol and cholesterol esters.[1] However, as the secretion moves up the follicular canal, its composition is modified by microbial lipase hydrolysis of the triglycerides to yield free fatty acids and glycerol.[15] *P. acnes* produces a lipase, and it has been reported that 95% of the free fatty acids at the surface of the skin results from *P. acnes* activity.[16,17] Free fatty acids produced by *P. acnes* metabolism contribute to microcomedo formation as well as to inflammatory reactions in acne.

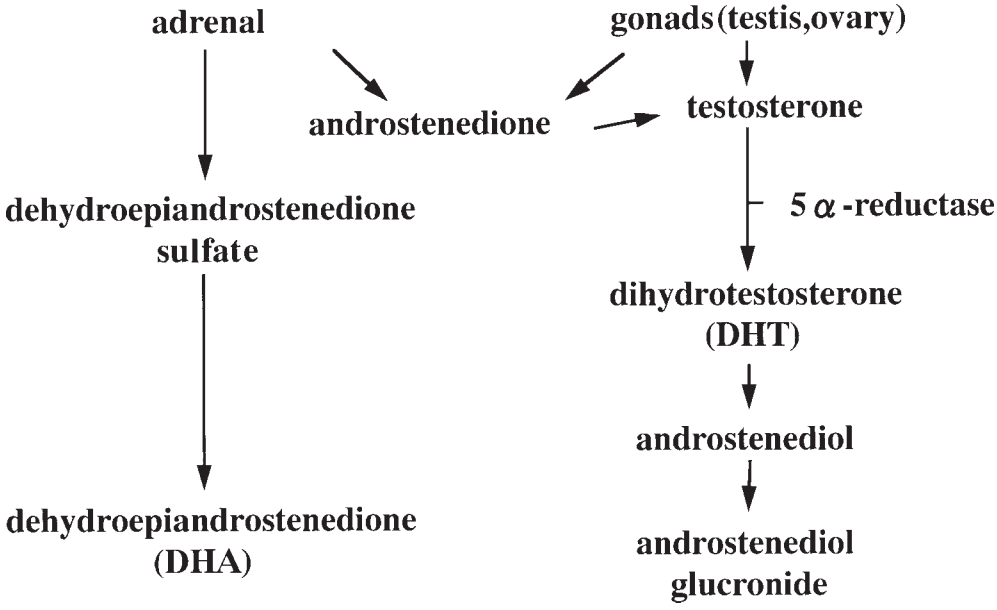

Fig. 1. Metabolic pathways of androgen and 5α-reductase

Microcomedones

The second factor in the genesis of acne is hyperkeratinization and the subsequent obstruction of sebaceous follicles. The ductal hypercornification can result from hyperproliferation of ductal keratinocytes or a reduced separation of ductal corneocytes. In follicles affected with acne, the process of desquamation of the epithelium is altered. The cells have more desmosomes and tonofilaments, and as a result the stratum corneum becomes thicker and more cohesive. The horny cells in the infundibular epithelium are thick and many layers accumulate. Many lipid droplets are present in the horny cells, and keratohyaline granules are large and are increased in number. Many lamellar granules are exocytosed into the intercellular spaces within the granular cell layer, resulting in a marked reduction in the number of lamellar granules (Fig. 2). Instead of sloughing off in the normal fashion, they become cohesive and are not shed into the lumen, and eventually occlude the follicular canal with a dense keratinous plug.

Although what initiates follicular hyperkeratosis is not known precisely, several factors may explain ductal hypercornification. The most significant comedogens are the free fatty acids produced by *P. acnes* metabolism, as first reported by Kligman and Katz in 1968.[18] Additional studies have revealed that exogenous free fatty acids are capable of inducing comedo formation in animal models.[19] Sebaceous linoleic acid has been shown to be reduced in comedones. Linoleic acid is an essential fatty acid, and animals deficient in linoleic acid become scaly. A comedo is caused by the accumulation of scales in the pilosebaceous duct. It is suggested that sebaceous follicles prone to acne have a relatively low concentration of linoleic acid, which bathes the ductal keratinocytes and triggers ductal hypercornification.[20,21] Squalene itself is only slightly comedogenic, but squalene peroxides are highly comedogenic. Squalene peroxides produced by *P. acnes* in sebum may induce hyperkeratosis of the epithelium in the follicular infundibulum.[18,22,23]

It has been shown that lower amounts of sphingolipids are observed in the stratum corneum of acne patients, which correlate with a diminished epidermal barrier function. An impaired water barrier function caused by decreased ceramides may be responsible for comedo formation.[24] As it has been demonstrated that follicular hypercornification can be induced in rats by exogenous administration of androgens, androgens may have an important role in controlling ductal hypercornification.[7] An important controlling effect of cytokines in comedogenesis has recently been pointed out. Comedones can be produced in an experimental system under the influence of interleukin-1α, and this process can be inhibited by adding an IL-1 receptor antagonist.[25,26] It has also been demonstrated immunohistochemically using a monoclonal antibody to Ki-67, a nuclear marker expressed by actively cycling cells, that increased numbers of basal keratinocytes in the follicle walls of comedones are labeled compared with normal follicles. Similarly, suprabasal immunolabeling of keratin 16, a phenotypic marker of hyperproliferating and abnormally differentiating keratinocytes, is found in ducal keratinocytes of acne lesions.[27]

Experimental comedones, which are visually similar to those in humans, can be produced in rabbit ears by applying certain comedogenic agents such as oleic acid.[22] The mechanisms of comedogenesis and comedolysis after the application of oleic acid, with or without vitamin A acid, to rabbit ears has been reported at the ultrastructural level.[28] The oleic acid-treated follicles showed multilayered, compact horny cells, suggesting increased cohesion of keratinized cells. The horny cells were thick and electron dense and included many lipid droplets. Keratohyaline granules were increased in number and size, and the horny cells were held

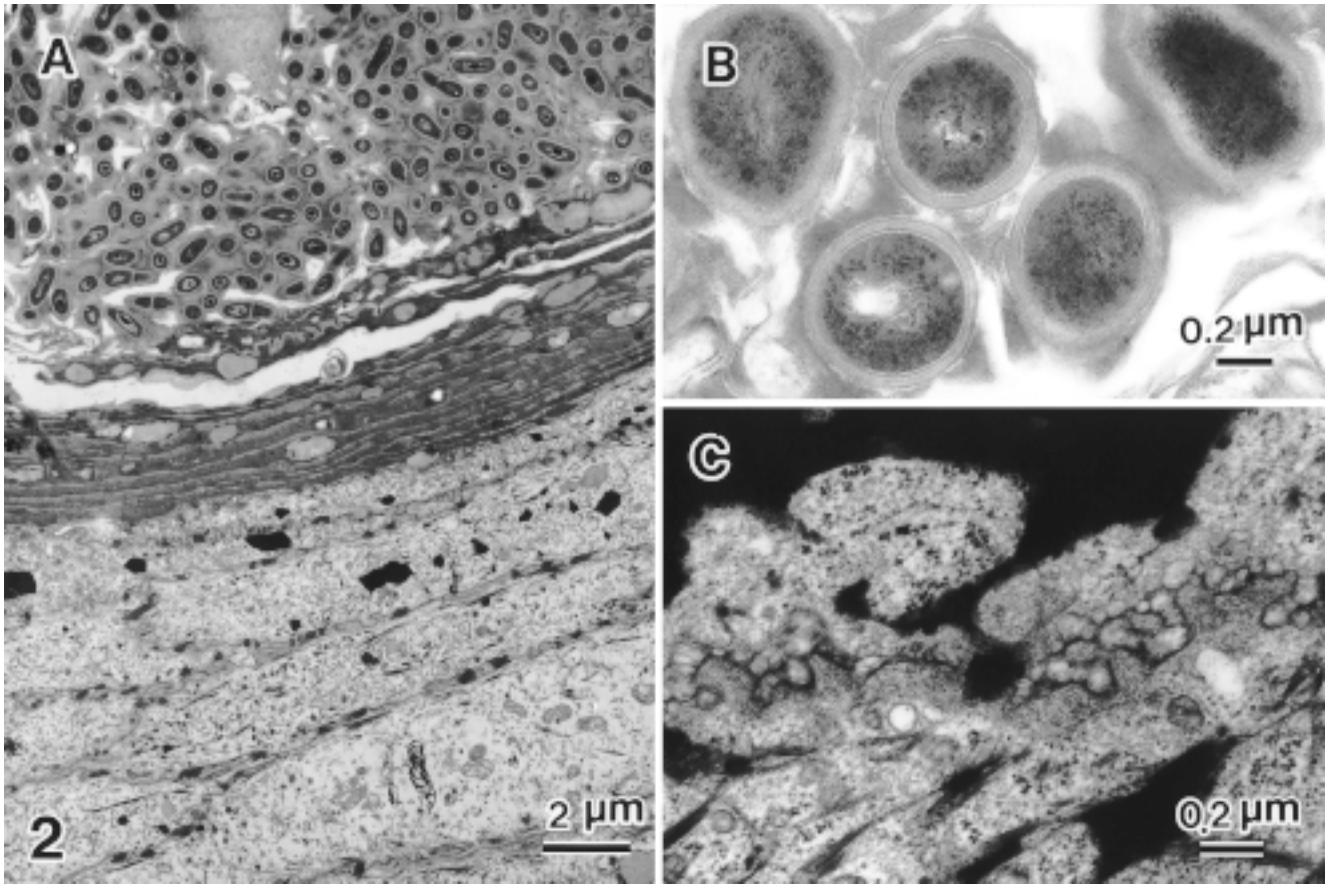

Fig. 2A–C. Follicular infundibulum of comedones. **A** The infundibular keratinocytes posses increased numbers of large keratohyaline granules. The horny cells with lipid droplets accumulate and appear to be cohesive. Note that numerous *Propionibacterium acnes* are present within the follicular canal. **B** Higher magnification of *P. acnes* in the transverse section. **C** In the granular cell layer, many lamellar granules have exocytosed into the intercellular spaces

together tightly by desmosomes and by desmosomal discs in the oleic acid-treated follicles. Thus, experimentally induced comedones in rabbit ears appeared to be induced both by the hyperkeratinization of the follicular epithelium and by the delayed desquamation of horny cells caused by the persistence of the intercellular binding apparatus. On the other hand, vitamin A acid strongly inhibited the formation of experimentally induced comedones. In vitamin A acid-treated follicles, several characteristic ultrastructural changes in the follicular infundibulum have been observed; the follicular epithelial cells were markedly edematous, and keratinized cells were not recognized. Further, keratohyaline granules and tonofilaments were scanty or undetectable, and desmosomes were reduced in number and size. Interestingly, vitamin A acid caused a characteristic increase in Odland bodies with lamellar structures, supporting the theory that Odland bodies may have a desquamating function as extracellular lysosomes.[29,30] Taken together, vitamin A acid disturbs follicular epithelial keratinization and reduces intercellular bindings between horny cells, preventing the cohesion and accumulation of horny cells and inhibiting comedo formation.

Microbial proliferation

The third factor in the pathogenesis of acne is the proliferation of the normal flora, typically *P. acnes*, in the retained sebum. *P. acnes* colonizes sebum-rich follicles in the skin of the face, the chest, and the back. *P. acnes* is a strictly anaerobic gram-positive fine rod and produces propionic and acetic acids. In the follicular infundibulum of comedones, a large number of *P. acnes* are observed (Fig. 3). Conditions for overgrowth of *P. acnes* are ideal in comedones in that they are anaerobic sites filled with a suitable lipid substrate as a nutrient source. Ultrastructural observation shows that *P. acnes* are 0.4 to 0.7 μm in width and 3 to 5 μm in length, possessing a ribosome-rich cytoplasm and a relatively thick cell wall composed of peptidoglycan (Fig. 4). As mentioned, free fatty acids produced by lipase secreted from *P. acnes* may act as comedogenic and acnegenic factors in sebaceous follicles and may irritate the follicular walls as well as the surrounding dermis following follicular rupture.

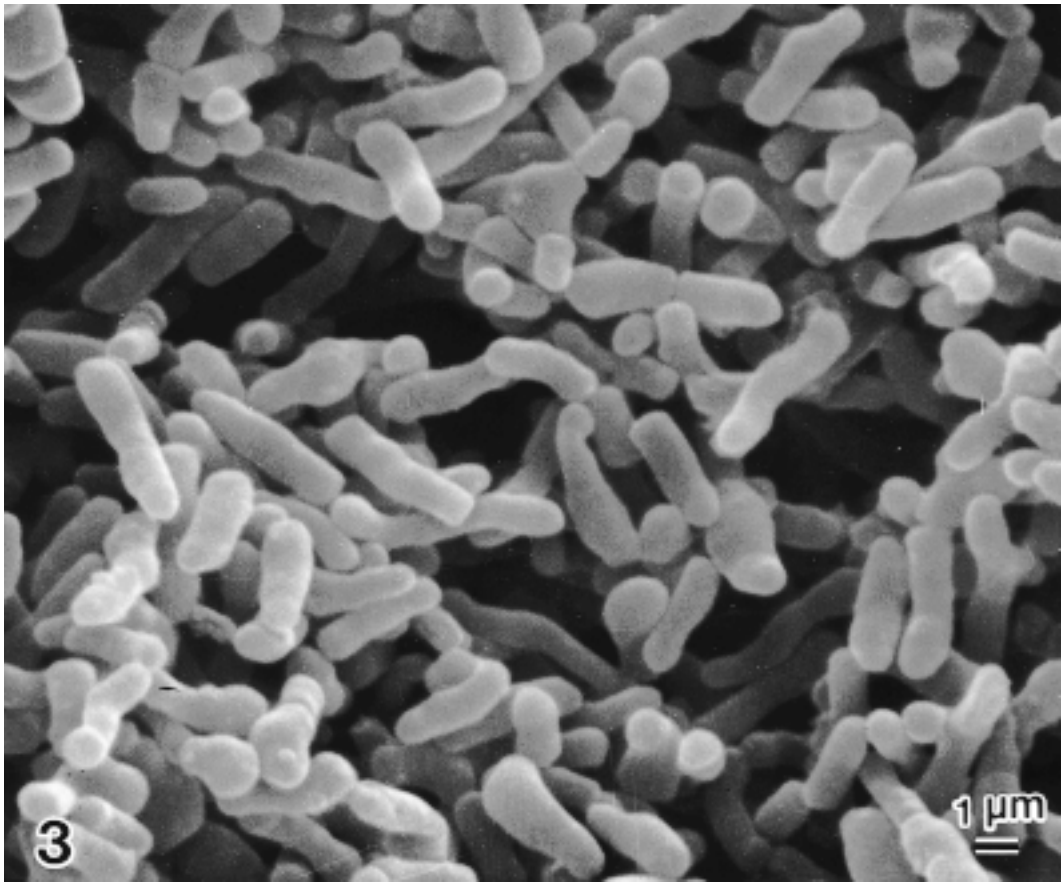

Fig. 3. Scanning electron photomicrograph of cultured *P. acnes* isolated from the follicular infundibulum of comedones

Inflammation

The fourth and final factor involved in the pathogenesis of acne is the inflammatory reaction, which is fueled by a variety of pathological processes. Inflamed papules, pustules, and nodules develop when comedones rupture and extrude their contents into the dermis rather than onto the surface of the skin. The keratin, hair, and lipids in the extruded sebum directly initiate inflammation by a nonimmune foreign-body reaction.[31] It is believed that *P. acnes* sets the stage for the rupture of a comedo by releasing low molecular weight chemotactic factors. These factors diffuse through the thinned follicular epithelium and attract neutrophils (Fig. 5), which are sometimes also observed within the cornified cell layer of the infundibulum of follicles. Macrophages phagocytose *P. acnes* in inflammatory acne lesions (Fig. 6). The neutrophils release inflammatory factors, such as lysosomal enzymes[32,33] and reactive oxygen,[34,35] which then damage the follicle wall and cause it to rupture. The free fatty acids released with the sebum are cytotoxic and probably also contribute to this process. *P. acnes* can also stimulate both the classic and the alternative pathways of complement activation, leading to the production of C5-derived neutrophil chemotactic factors, which attract more leukocytes and further exacerbate the inflammation.[36] The bacteria also release enzymes, such as hyaluronidase and various proteases, that may add significantly to the pathological conditions.[37–39] The host's immune response to *P. acnes* antigens may also perpetuate the local inflammation.

Neurogenic factors

It is well known that exacerbation of acne can occur as a result of emotional stress, and several lines of clinical evidence suggest that the nervous system, including psychological factors, can influence the course of acne.[40–42] To examine whether cutaneous neurogenic factors affect the morphology of sebaceous glands, we used electron microscopy to observe alterations of sebaceous glands in a skin organ culture system treated with several kinds of neuropeptides or nerve growth factor. Skin samples obtained from ICR mice were cut into circular pieces, then placed in 24-well flat-bottom tissue culture plates and floated in 1 ml Dulbecco's modified Eagle's medium (DMEM). The skin organ culture dishes were then supplemented with neuropeptides (calcitonin gene-related peptide, substance P: SP, vasoactive intestinal polypeptide, or neuropeptide Y) or nerve growth factor at a final concentration of 10^{-7} M. DMEM alone was added as a control in each experiment. Samples were observed after 72 h of treatment. The ultrastructure of sebaceous glands supplemented with DMEM only was identical to that of intact sebaceous glands in

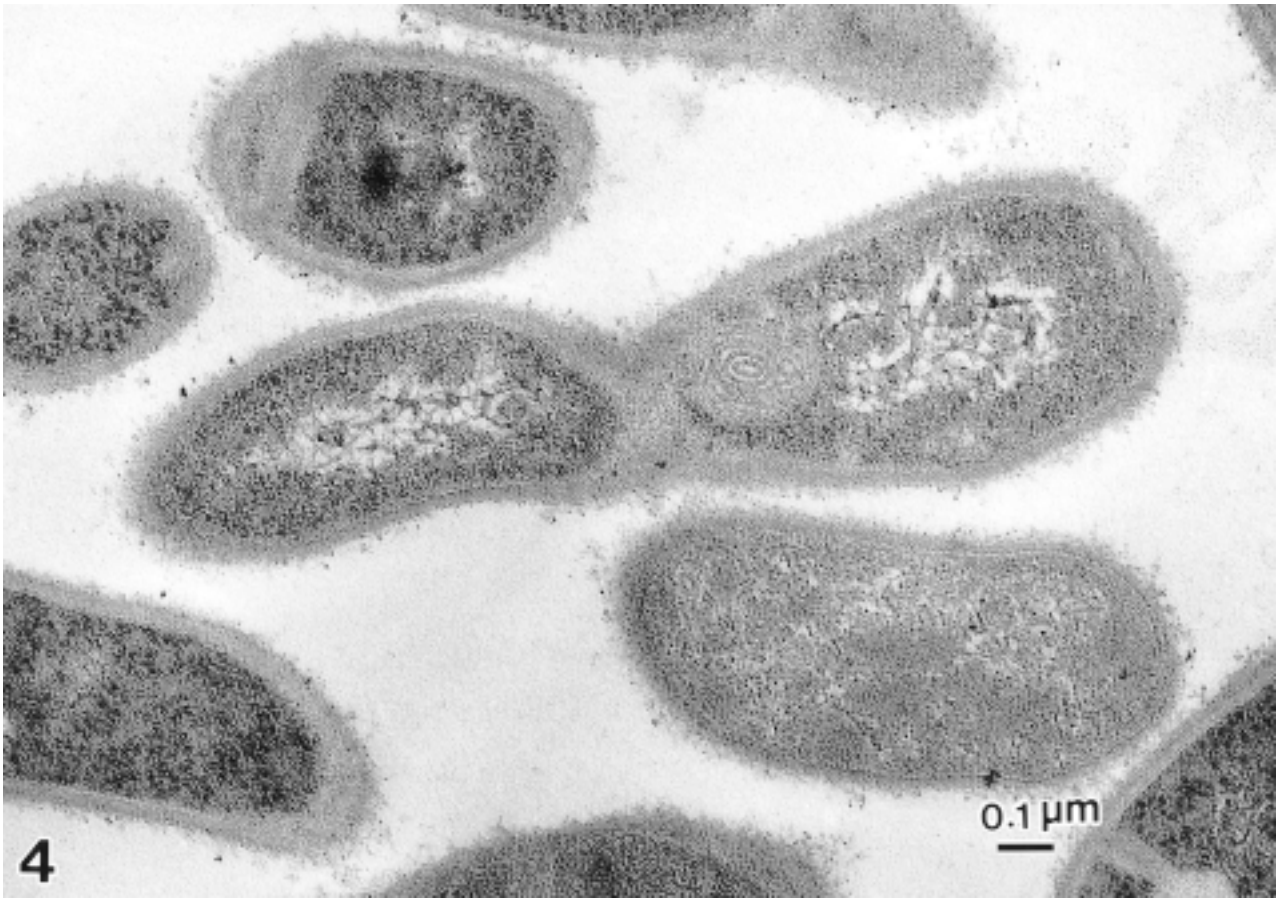

Fig. 4. Transmission electron photomicrograph of *P. acnes* in a longitudinally cut section. *P. acnes* is a fine rod in shape and possesses a ribosome-rich cytoplasm and a thick cell wall

mouse skin. From the exterior aspect to the interior, sebaceous glands consisted of the germinative, undifferentiated, and differentiated sebaceous cell layers (Fig. 7). In contrast, sebaceous glands stimulated with SP showed that most sebaceous cells contained numerous lipid droplets, which disintegrated to form an acellular sebum secretion, even in the peripheral area of the glands (Fig. 8). These observations indicate that SP can accelerate lipid synthesis. There were numerous free ribosomes and mitochondria, which often appeared to be elongated, within the cytoplasm 6 h after exposure to SP (Fig. 9). Twenty-four hours after exposure to SP, the cytoplasm of sebaceous cells was densely packed with smooth-surface membranes of the endoplasmic reticulum (Fig. 10), suggesting the active phase of lipid synthesis.

Morphometric analysis using a computer-assisted image analysis system (NIH) revealed that of all the factors tested, only SP induced significant increases in the area of sebaceous glands (Fig. 11) as well as in the size of individual sebaceous cells (Fig. 12). Furthermore, SP significantly increased the number of sebum vacuoles per each differentiated sebaceous cell at the electron microscopic level (Fig. 13). The number of sebum vacuoles induced by SP increased in a dose-dependent manner when various concentrations of SP were added to the culture medium (Fig. 14). These findings suggest that SP may stimulate the proliferation as well as the differentiation of sebaceous glands, and further, that it upregulates lipid synthesis in sebaceous cells. Taking into account that stress can elicit the release of SP from peripheral nerves,[43] it is tempting to speculate that these findings partially explain the participation of the cutaneous nervous system in the pathogenesis of acne.

Other factors

A large number of exogenous factors can trigger or exacerbate acne, either by stimulating follicular hyperkeratosis or by contributing to the inflammatory response.[1] Genetic influences may determine an individual's susceptibility to comedogens, as well as the severity of the course of disease.[44] Comedogenic substances, either in the environment or intentionally applied by the patient, can produce outbreaks of acne. Notable among these are halogenated hydrocarbons and certain greasy or occlusive cosmetics. Heat, humidity, and ultraviolet radiation may also induce follicular hyperkeratosis. Pressure, friction, and excessive scrubbing or washing can exacerbate existing acne by causing

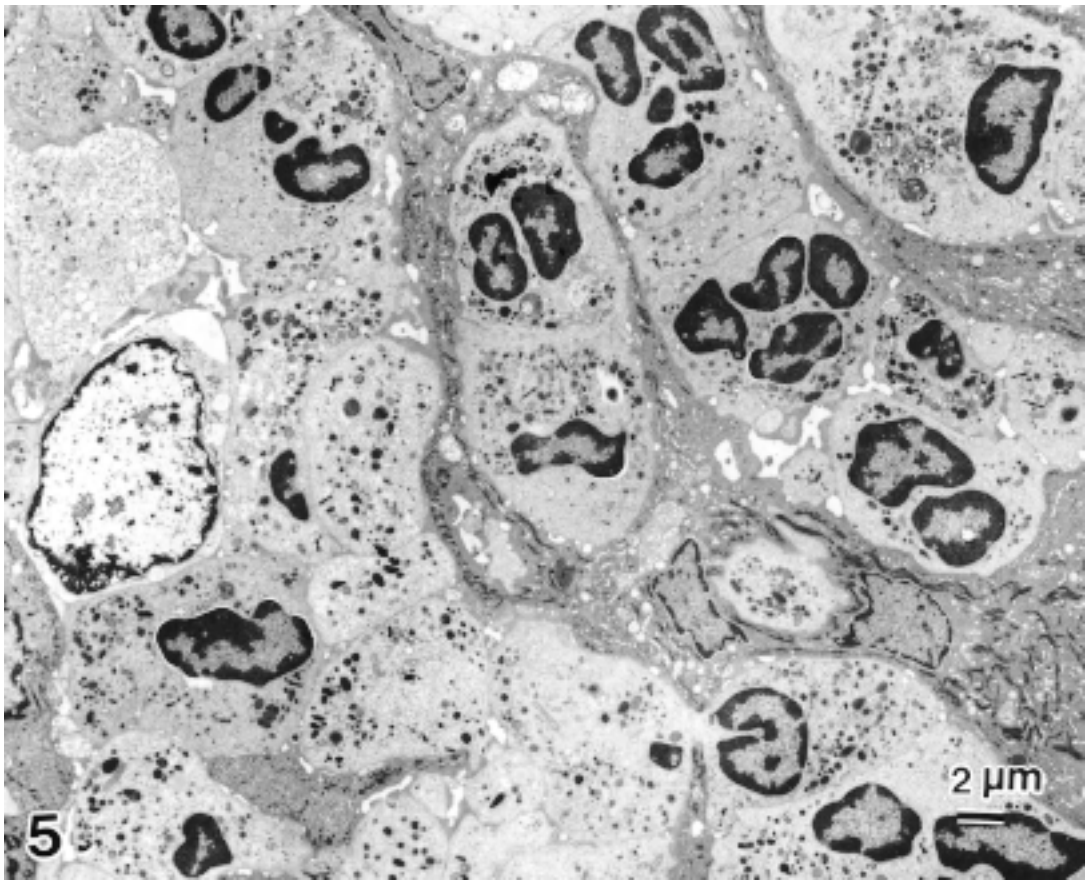

Fig. 5. Transmission electron photomicrograph of inflammatory acne lesions. Numerous neutrophils infiltrate and densely accumulate in perifollicular areas

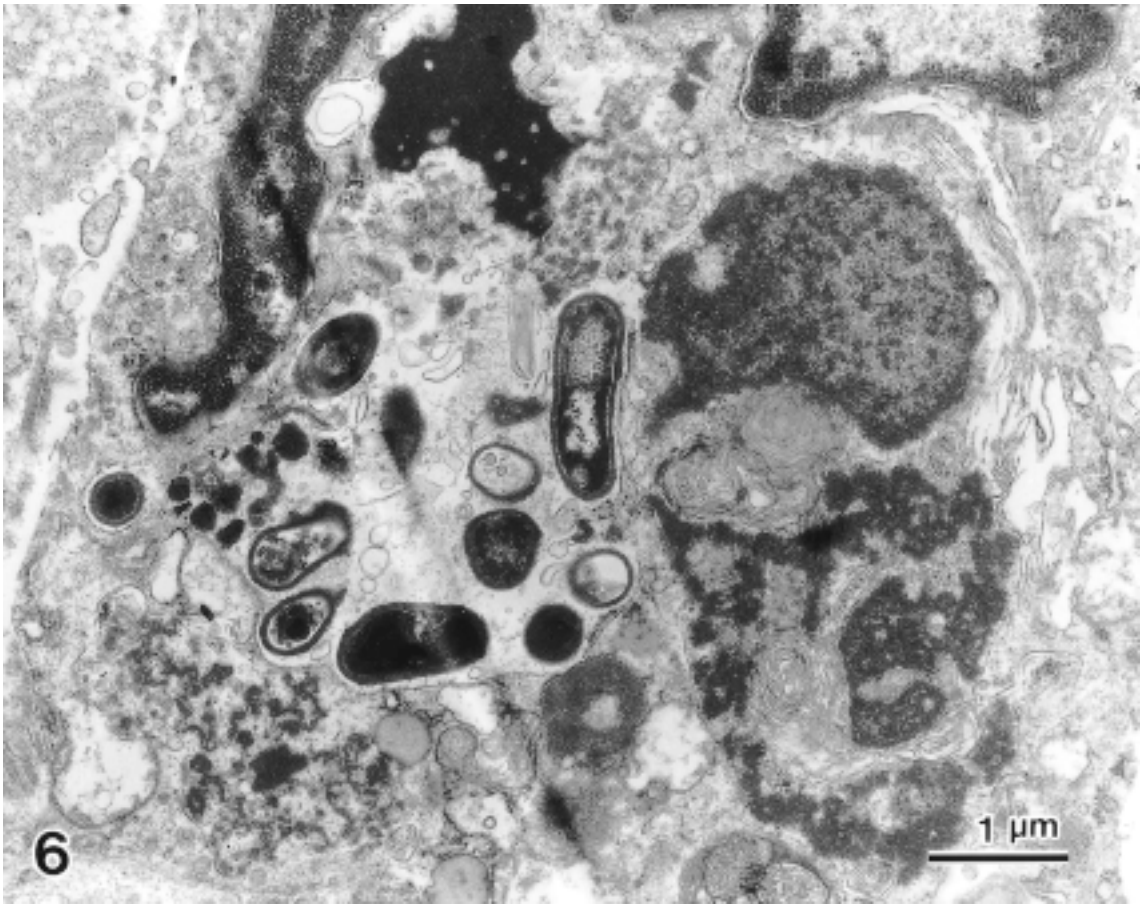

Fig. 6. Numerous *P. acnes* seen within the phagosomes of an infiltrating macrophage in an inflammatory lesion

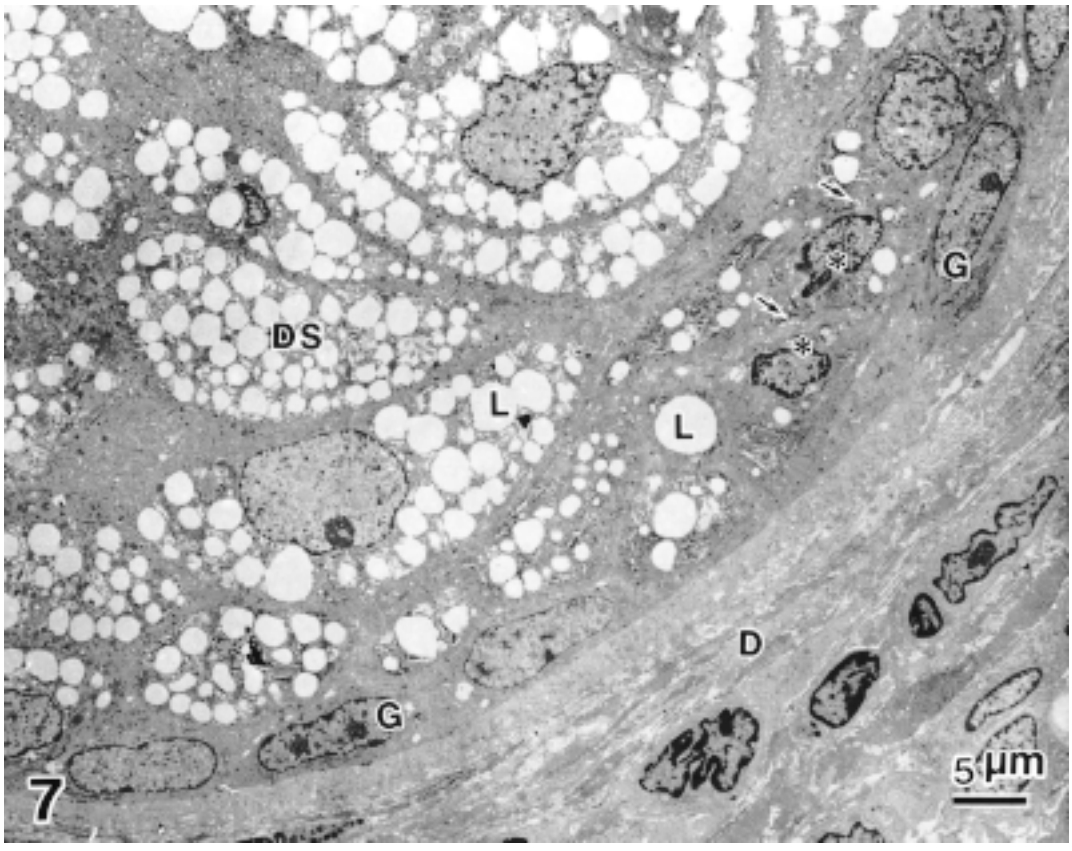

Fig. 7. A low magnification electron photomicrograph of a sebaceous gland 72 h after exposure to Dulbecco's modified Eagle's medium (DMEM) (control culture). The germinative cells (*G*) can be seen at the periphery of the bland. A layer (*asterisks*) internal to the germinative cell layer is composed of cells containing small lipid vesicles (*arrows*), suggesting an early phase of lipid synthesis. Differentiation of the sebaceous cells (*DS*) becomes evident toward the central portion of the gland, with the appearance of relatively large lipid vacuoles (*L*) within the cytoplasm. *D*, dermal connective tissue

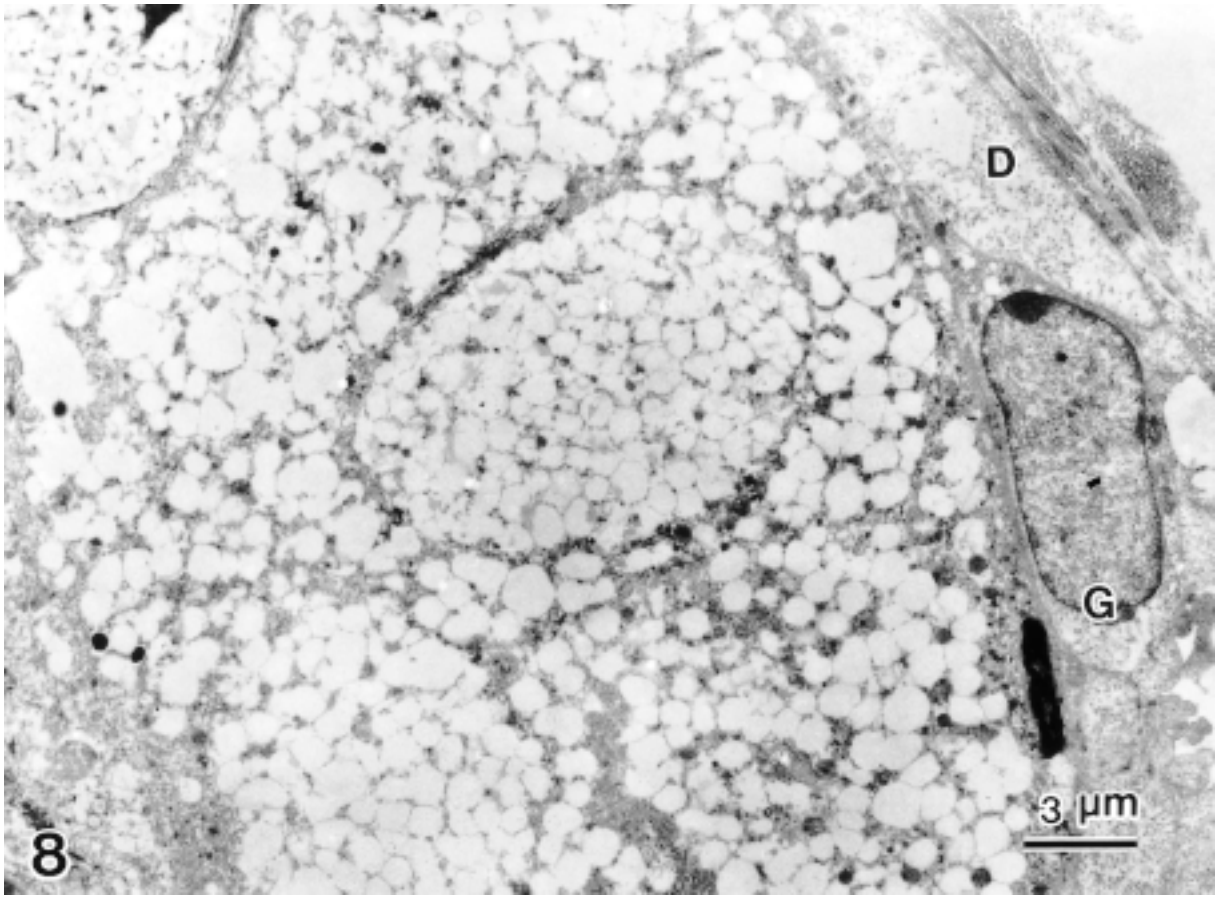

Fig. 8. A sebaceous gland 72 h after exposure to SP. Fully differentiated sebaceous cells containing numerous lipid vacuoles are seen. Most sebaceous cells are disintegrating to form an acellular secretion sebum, even in the peripheral area of the gland. *G*, germinative cells, *D*, dermal connective tissue

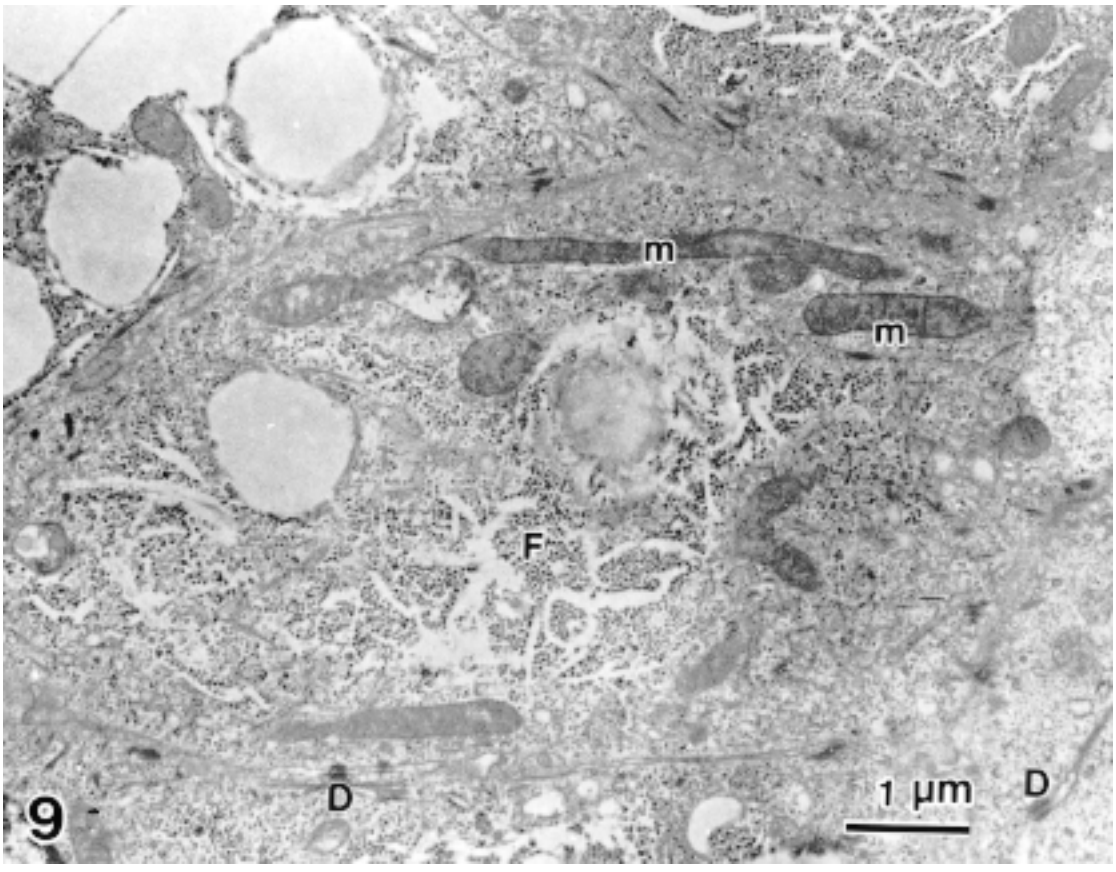

Fig. 9. A sebaceous gland 6 h after exposure to substance P (SP). There are numerous free ribosomes (*F*); mitochondria (*m*), which often appear to be elongated, are seen within the cytoplasm. *D*, desmosome

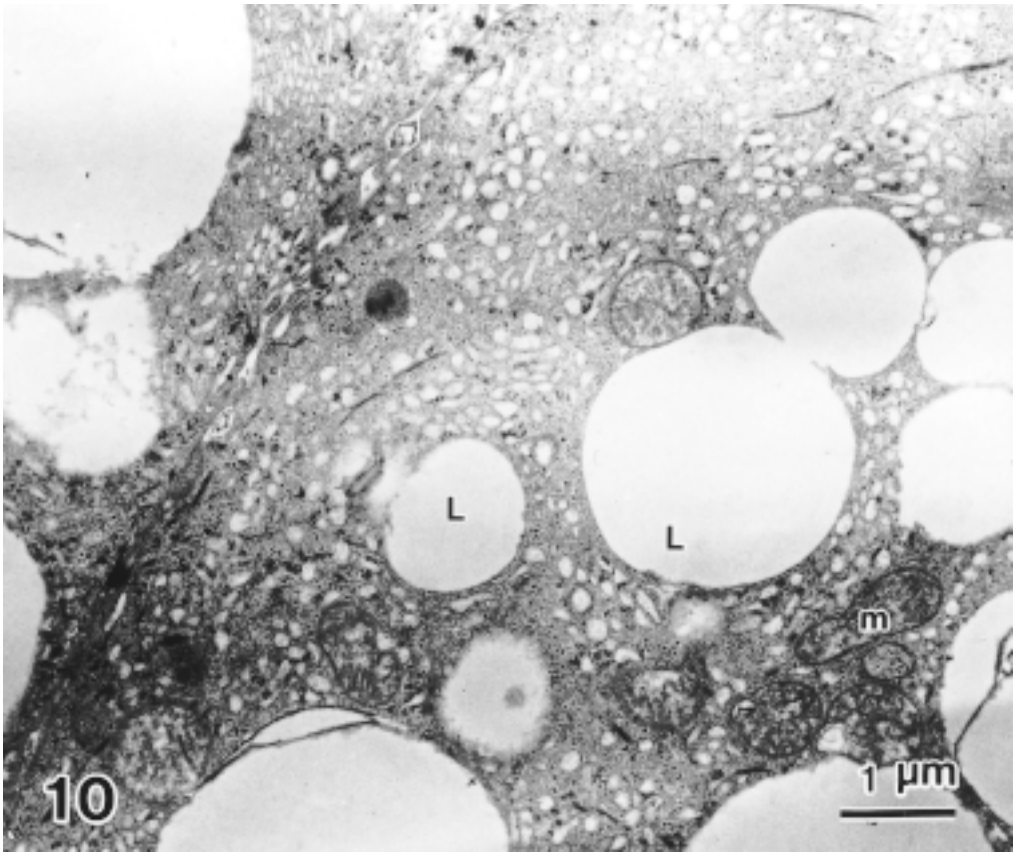

Fig. 10. A sebaceous gland 24 h after exposure to SP. The cytoplasm is densely packed with smooth-surface membranes of the endoplasmic reticulum. *L*, lipid vacuole

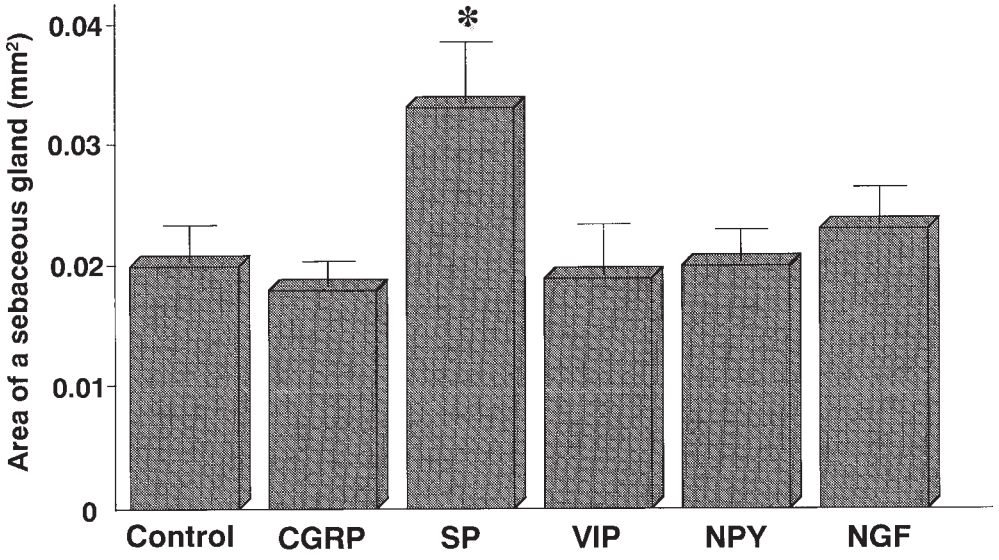

Fig. 11. Effect of neuropeptides and nerve growth factor on the area of sebaceous glands. Morphometric analysis was performed 72 h after exposure to 10^{-7} M of each factor. $^{*}P < 0.01$ compared with the control

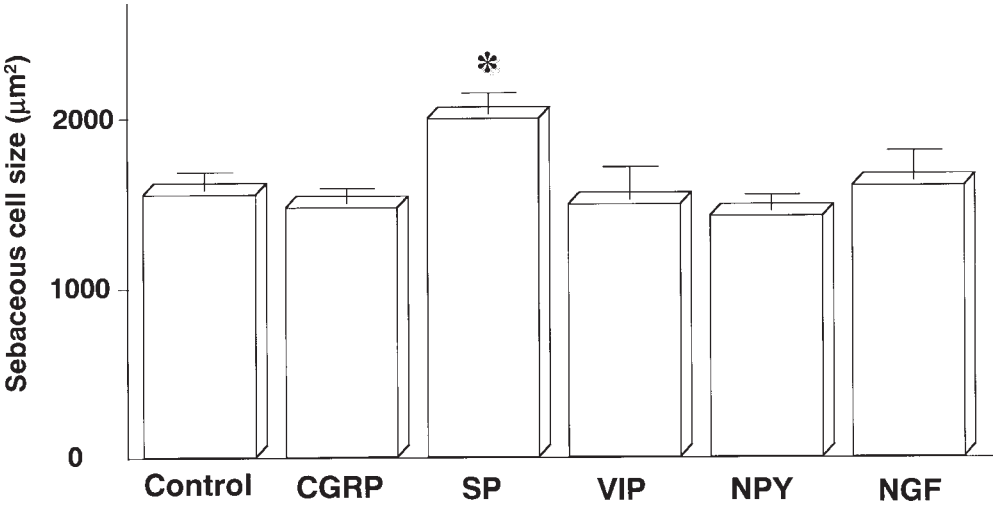

Fig. 12. Effect of neuropeptides and nerve growth factor on sebaceous cell size. Morphometric analysis was performed 72 h after exposure to 10^{-7} M of each factor. $^{*}P < 0.05$ compared with the control

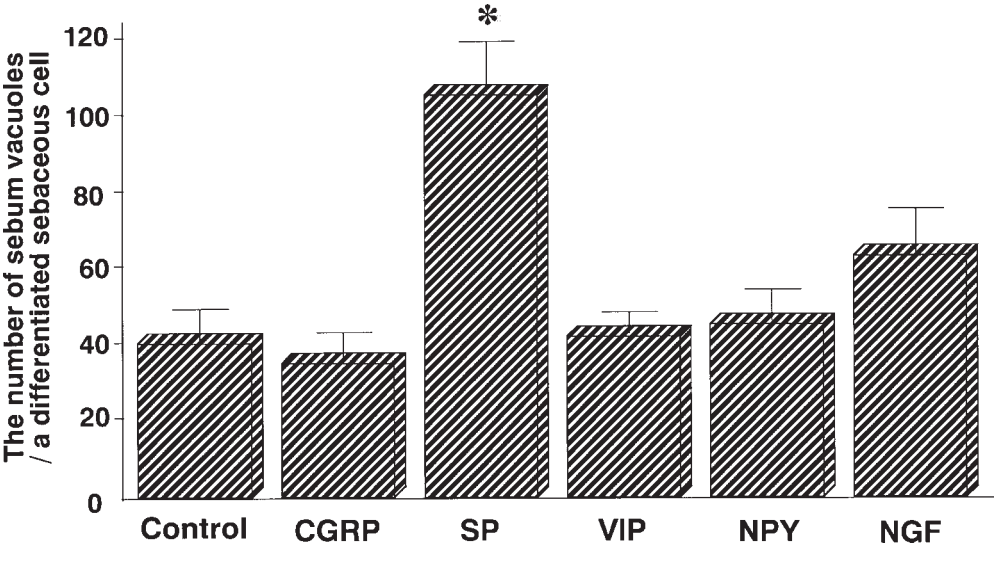

Fig. 13. Effect of neuropeptides and nerve growth factor on the number of sebum vacuoles per differentiated sebaceous cell. Morphometric analysis was performed 72 h after exposure to 10^{-7} M of each factor. $^{*}P < 0.001$ compared with the control

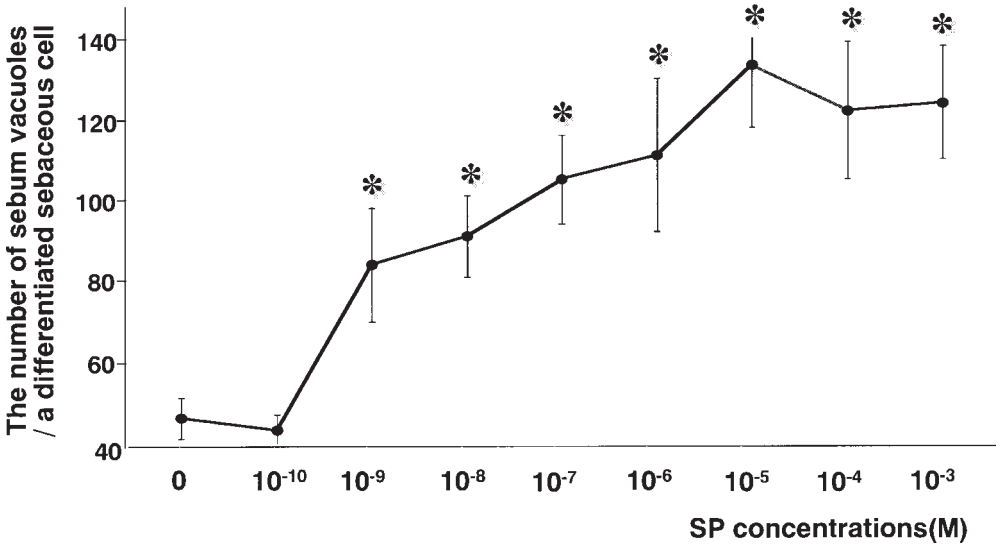

Fig. 14. Effect of various concentrations of SP on the number of sebum vacuoles per differentiated sebaceous cell. Morphometric analysis was performed 72 h after exposure to 10^{-7}M of each factor. $*P < 0.01$ compared with the control (0)

microcomedones to rupture and thus initiate an inflammatory reaction.[44]

Conclusions

The characteristic morphological features associated with the pathophysiology of acne have been discussed in this review. We also demonstrated in this report that at least one of the neuropeptides present in the skin induces morphological alterations in sebaceous glands, and that this might partially explain the pathological significance of the neurogenic aspect in the disease process. We believe that a rational approach to acne therapy must begin with a clear understanding of the pathological basis of the disease. Although the cause of acne is multiple and complex, electron microscopic observations have been beneficial to further understanding of the etiology of acne.

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