

STUDY OF ELASTOLYTIC ACTIVITY OF PROPIONIBACTERIUM ACNES AND STAPHYLOCOCCUS EPIDERMIS IN ACNE VULGARIS AND IN NORMAL SKIN

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Abstract. Histopathological sections of anetoderma-like scars from 10 patients with acne vulgaris showed a selective absence of elastic fibers around pilosebaceous follicles. This finding is similar to the histologic changes of "perifollicular elastolysis" reported by Varadi. Bacteria isolated by anaerobic and aerobic cultures of swabs of the skin surface and pus of these 10 patients, 12 others with active acne vulgaris and 8 normal subjects were studied with particular attention to *Staphylococcus epidermis* and *Propionibacterium acnes*. These organisms were analysed for production of an elastolytic enzyme which might play a role in the observed selective loss of elastic fibers. No elastolytic activity was produced by *S. epidermidis* or *P. acnes* isolated from any of these individuals. Thus, we cannot attribute the perifollicular loss of elastic fibers in acne scarring to an elastase produced by organisms. The observed absence of elastic fibers might result from tissue necrosis produced by leukocytes during the inflammatory phase, followed by collagenous scar formation without regeneration of elastic fibers.

Key words: Acne; *Propionibacterium acnes*; *Staphylococcus epidermis*; Elastolytic activity

It is generally accepted that two classes of bacteria, *Propionibacterium acnes* and non-hemolytic *Staphylococcus epidermidis*, are the predominant micro-organisms in lesions of acne vulgaris (3, 5). The significance of either organism as primary pathogen or secondary invader is still not clearly established (2). Most research on acne has focused on early lesions—comedones, papules and pustules, while little attention has been paid to later stages of healing and scar formation. Acne scars are of several types: (i) depressed scars of varying depth, apparently resulting from destruction of a zone of dermal connective tissue around the acne lesion, (ii) hypertrophic, keloidal scars, composed of dense fibrous connective tissue—these usually follow large and deep lesions of acne, and (iii) a third type

of scar which is protuberant, lax, and resembles localized anetoderma (7) (Fig. 1). These scars usually contain a follicular orifice near the center. They thus resemble the lesions described by Varadi under the title "perifollicular elastolysis". Varadi isolated from normal skin (6) and from hair follicles located within the lesions of "perifollicular elastolysis" a variant of *S. epidermidis* which secreted an elastolytic enzyme. He postulated that this enzyme was the cause of the dissolution of elastin around the follicle. Rippon reported the isolation of elastases from *Trichophyton schoenleinii*, *verrucosum*, and the plus mating type of *Nannizzia fulva* (4). We have found no report of a search for elastolytic enzymes in organisms thought to be important in acne. Because of the clinical and histological similarity between the lesions of perifollicular elastolysis and the anetodermic type of acne scars, we studied the production of elastolytic enzymes by colonies of *S. epidermidis* and *P. acnes* cultured from the skin of normal individuals, and from that of treated and untreated acne patients, with or without the anetodermic type of scarring.

MATERIALS AND METHODS

A. Histological studies. Biopsy specimens of anetoderma-like scars with a central follicle were taken from the upper trunk of 10 patients with acne vulgaris. Sections were stained with hematoxylin and eosin, and Verhoeff or orcein stain.

B. Bacteriological studies. Bacteriologic cultures were made from pustules, cysts and closed comedones of 22 patients with acne vulgaris including the 10 with anetodermic scarring. Fourteen of these patients had received tetracycline at some time in the past, but none had had antibiotic therapy for at least one month prior to sampling. The other 8 patients were taking tetracycline at the time of



Fig. 1. Anetoderma-like scar on upper trunk of patient with acne vulgaris (in exact center of photo). The lesions are white, non-inflammatory, finely wrinkled and have a central dell corresponding to a hair follicle.

sampling. There were 8 males, 14 females, ranging in age from 14 to 40 (average age 23.6). The skin surface of acne patients was thoroughly cleansed with 70% ethanol. Pustules were opened with a sterile lancet. Samples were collected with a syringe and needle and placed immediately into a prereduced Anaport vial (Scott Laboratories) for transport to the laboratory. Material from the vial was streaked on growth media agar plates. For growth of aerobes the samples were streaked either direct onto Trypticase Soy Agar (BBL) plates with 1% added particulate elastin (Sigma) (TSAE) or onto 5% Sheep blood in Trypticase Soy Agar plates (GIBCO) (SBA), and incubated at 35°C. *S. epidermidis* was differentiated from *S. aureus* by the EDTA-Rabbit Plasma tube test for coagulase activity. A colony of *S. epidermidis* from SBA was then streaked aerobically on TSAE plates and incubated at 35°C. Examination for zones of clearing

around colonies of *S. epidermidis* growing on TSAE plates was made after 2, 3, and 7 days of incubation. If no zone of clearing was found after 3 days, the colony of *S. epidermidis* was considered negative for elastolytic activity.

For *P. acnes*, specimens were also streaked directly onto peptone-yeast extract-glucose agar (DIFCO) plates with 1% added particulate elastin (PYGAE) and incubated anaerobically in a BBL Gas Pak jar at 35°C, or streaked direct on pre-reduced Brain Heart Infusion Agar Supplement roll tubes (Scott Laboratories) (BHIA) (1) and incubated for 5 days at 35°C. A colony of *P. acnes* from the BHIA roll tube was then streaked onto PYGAE plates and incubated anaerobically in a BBL Gas Pak jar at 35°C. Examination for zones of clearing around colonies of *P. acnes* growing on PYGAE plates was made at 5, 7, and 14 days. If no zone of clearing was found after 14 days, the

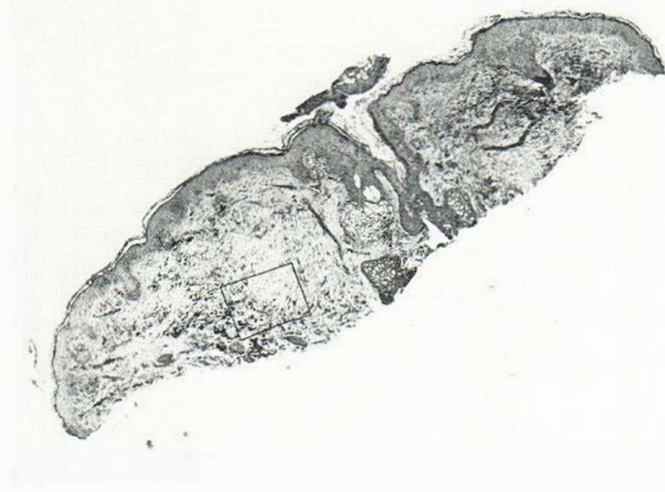


Fig. 2. Biopsy specimen of an anetoderma-like scar from a patient with acne vulgaris. Orcein stain for elastic fibers. No elastic fibers are present around the hair follicle and the accompanying sebaceous gland. There are no signs of inflammation or destruction of the follicle. (Orcein, $\times 12$.)

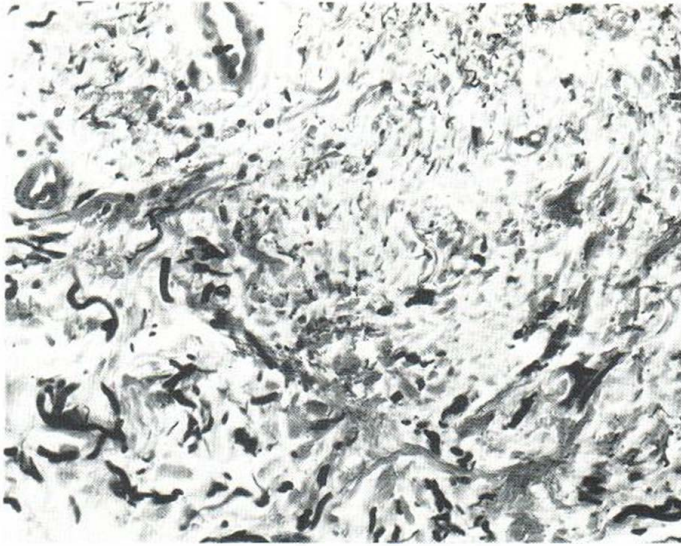


Fig. 3. Higher magnification from Fig. 1 (corresponding to boxed area) of a border region between an area with fewer elastic fibers (upper right corner) and one with intact elastic fibers (lower left). (Orcein, $\times 155$.)

colony of *P. acnes* was considered negative for elastolytic activity. *P. acnes* was identified with gas-liquid chromatography by demonstrating production of propionic acid from Peptone-Yeast extract-glucose broth cultures (PYG) of anaerobic Gram-positive bacilli, and by biochemical tests (esculin, glucose, lactose, maltose, mannitol, mannose, starch, sucrose, trehalose, xylose, indol, nitrate and catalase) (1).

Skin cultures from normal subjects were handled in a similar manner. There were 3 males and 5 females, ranging in age from 21 to 50 (average age 30.4). Swabs were obtained from cheek, chin and forehead areas, and cultures for *S. epidermidis* and *P. acnes* were observed for elastin clearing.

To measure the sensitivity of our systems for recognizing elastolytic activity we placed serial dilutions of porcine pancreatic elastase (Sigma, Type III chromatographically purified) in 3.5 mm diameter wells in TSAE plates and incubated as above. After one day 2×10^{-3} μ moles produced a concentric zone of clearing 1 cm in diameter.

RESULTS

Histologic studies (Figs. 2, 3)

Hematoxylin and eosin and elastin-stained sections of typical anetodermic acne scars showed a zone around the pilosebaceous follicle which was partially or totally devoid of elastic fibers. In these areas the collagen appeared normal, or perhaps slightly increased in density. In most specimens a well preserved follicle was found centrally, many of these containing a growing hair. While the amount of destruction of elastin in the surrounding zone may seem surprising, it should be remembered that these lesions were selected for biopsy precisely because they demonstrated clinically a protuberant, anetodermic scar around a well defined follicular orifice.

Table 1. Summary of culture results

Group	Number	Organisms	Elastin clearing
Normal: ages 21-50	8	2 <i>S. epidermidis</i> 6 <i>P. acnes</i> and <i>S. epidermidis</i> (mixed)	None
Treated acne: ages 16-36	8	1 No growth 1 <i>P. acnes</i> 6 <i>P. acnes</i> and <i>S. epidermidis</i>	None
Untreated acne: ages 14-40	14	1 No growth 3 <i>S. epidermidis</i> 1 <i>P. acnes</i> 8 <i>P. acnes</i> and <i>S. epidermidis</i> 1 <i>Bacillus</i> (contaminant)	None +

Bacteriological studies (Table I)

The tabulation of organisms cultured from patients and normals is shown in Table I. We were unable to demonstrate any elastolytic activity produced by colonies of *P. acnes*. However, one aerobic Gram-positive rod, identified as *Bacillus* species, recovered from an acne patient not receiving antibiotic therapy, did demonstrate elastolytic activity. This organism was considered a probable laboratory contaminant or saprophyte, but did confirm that our system for demonstrating elastolytic activity was appropriately sensitive. Similarly, no *S. epidermidis* colonies exhibited elastolytic activity within the prescribed 3-day time period. However, one strain of *S. epidermidis* did show elastin clearing after 13 days of anaerobic incubation. This was attributed to induced enzyme production, since an elastolytic enzyme was not isolated from the strain after sub-culture in liquid medium containing no elastin.

The finding that organisms from 30 consecutive subjects, with or without acne, failed to demonstrate elastolytic activity indicates that at the 95% confidence level, no more than 11% of the population may have a variant of *P. acnes* producing elastase, and that less than 10% of the population harbors an elastase-producing *S. epidermidis*.

DISCUSSION

Ten histopathological sections of anetoderma-like scars from 8 patients with active acne vulgaris, or a history of acne, showed a selective loss of elastic fibers surrounding hair follicles. This was similar to the histopathology of "perifollicular elastolysis" reported by Varadi.

Twenty-two patients with acne vulgaris and 8 normal subjects were studied in regard to the production of an elastolytic enzyme produced by a variant of *S. epidermidis* or *P. acnes*, because such an enzyme might cause selective loss of elastic fibers surrounding hair follicles in one type of scarring associated with acne. We were unable to demonstrate elastolytic activity by *S. epidermidis* or *P. acnes* cultured from acne patients and normal controls.

Because we could not demonstrate elastolytic

activity in variants of *S. epidermidis* or *P. acnes*, we cannot attribute the perifollicular loss of elastin fibers in acne scars to degradation by bacterial enzymes. An alternative explanation for the elastolysis could be the production of elastases by inflammatory cells in the active acne lesions, and subsequent replacement of collagen without elastin regeneration. This phenomenon has been noted in scar formation in other types of wound healing.

ACKNOWLEDGEMENTS

We wish to thank Donna Blazevec, Department of Laboratory Medicine, Anaerobic Section, University of Minnesota Hospitals, for her assistance with the isolation and identification of micro-organisms.

We also wish to thank Dr Bruce Bart of Minneapolis for assistance in obtaining cultures.

This work was supported by USPHS Training Grant No. 5-T01-AMO5560-08 and the Minnesota Medical Foundation.

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Received September 12, 1975

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