THE PATHOLOGICAL AND ECOLOGICAL SIGNIFICANCE OF MICROORGANISMS COLONISING *ACNE VULGARIS* COMEDONES

J. P. LEEMING, K. T. HOLLAND AND W. J. CUNLIFFE*

*University Departments of Microbiology and *Dermatology, University of Leeds,
Leeds LS2 9JT*

SUMMARY. A microbiological survey has been undertaken of comedones isolated by micro-dissection from skin biopsies. Of closed comedones 10·7% and of open comedones 7·1% did not contain *Pityrosporum* spp., *Propionibacterium* spp. or *Staphylococcus* spp., the organisms most frequently associated with the pathogenesis of acne. Mature comedones were more frequently colonised than were young comedones. These results support the argument that the presence of microorganisms is not a prerequisite for comedo formation. Other pathological and ecological implications of these results are discussed.

INTRODUCTION

Acne vulgaris is a disorder of the pilosebaceous units (follicles) of human skin. For convenience, the morphogenesis of lesions can be divided into two phases. The first is a non-inflammatory phase during which keratin accumulates in affected follicles producing whiteheads (closed comedones), which have very small orifices, and blackheads (open comedones) which have distended orifices. The second is an inflammatory phase during which a variety of inflamed lesions may develop from a proportion of comedones. This study concentrated on the primary non-inflammatory stage.

The importance of microorganisms in the pathogenesis of acne has proved difficult to establish. All microbial groups implicated are also common commensals on healthy skin and, until recently, the available skin sampling methods were inadequate for the comparison of normal and acne-affected follicles. Calcium chloride-mediated micro-dissection of skin biopsies (Kellum, 1966) has recently been exploited for microbiological investigations of normal skin (Puhvel *et al.*, 1975). This technique isolates entire follicles and, therefore, offers great improvements in penetration, accuracy and resolution over traditional non-invasive sampling methods. A study extending the use of this procedure to acne comedones was undertaken to clarify the significance of micro-organisms in this disorder.

MATERIALS AND METHODS

Subjects. A total of 49 volunteer patients with *acne vulgaris* attending the dermatology clinic at Leeds General Infirmary was studied. Their average age was 21·5 years (range 13–39) and all had mild to moderate acne. None had been receiving antibiotic treatment for at least 6 weeks.

Received 18 Jun. 1984; accepted 29 Oct. 1984.

Biopsy processing. A 3-mm diameter punch biopsy with a comedo at the centre was taken from the upper back of each patient. Local anaesthetic (lignocaine hydrochloride) was administered intradermally but the skin was otherwise unprepared. Micro-dissection of the biopsy was facilitated by soaking in 1M CaCl₂ solution for 2 h at 4°C. Each comedo isolated was homogenised individually in phosphate-buffered Triton X-100 0.1% v/v solution (wash fluid; Williamson and Kligman, 1965).

These procedures have been shown to have no adverse effect on the viability of cutaneous bacteria and yeast (Puhvel *et al.*, 1975; Leeming *et al.*, 1984).

Microbiological assessment. Viable counts of cutaneous bacteria and yeasts, and a microscopical count of yeast cells, were performed on the homogenates as described previously (Leeming *et al.*, 1984).

RESULTS

Comedo isolation

The techniques employed proved successful for the isolation of open and closed comedones (see fig. 1). In all, 30 closed and 29 open comedones were obtained. Isolated lesions showed diverse morphologies. There was a great degree of overlap in the appearances of open and closed comedones, which often could not be differentiated

FIG. 1.—A closed comedo and closely associated small normal follicle attached to the epidermis after stripping from the rest of the biopsy ($\times 50$).

after micro-dissection but all were easily identified before biopsy. Open comedones exhibited a greater range of sizes, from slightly to greatly distended, than did closed comedones and a crude division of these lesions into 'early' and 'mature' groups could be made on this basis.

Microbiological findings

The microorganisms most frequently isolated from comedones were propionibacteria, staphylococci and *Pityrosporum* spp. Other organisms, primarily aerobic coryneform bacteria, were occasionally isolated, but when present were found either in low numbers or in the presence of greater numbers of staphylococci.

Fig. 2 compares the bacterial colonisation patterns of comedones with those obtained in a parallel study of normal follicles isolated from closely matched acne patients (for details, see Leeming *et al.*, 1984). The cubed-root transformation employed in fig. 2 clearly differentiates follicles containing few or no bacteria of a given type from those harbouring substantial populations. Bacteria present in low numbers were considered to be non-resident organisms derived from other areas of skin. Hence, in analysis of these results only those follicles containing substantial populations were regarded as colonised. The rationale behind this division has been discussed in detail previously (Leeming *et al.*, 1984).

Follicles were regarded as colonised by *Pityrosporum* spp. if either microscopical or

FIG. 2.—A comparison of bacterial densities occurring in normal pilosebaceous follicles and comedones isolated from the upper backs of acne patients. Closed circles (●) represent units considered to be colonised, open circles (○) represent units considered not to be colonised. N.F. = normal follicles, C.C. = closed comedones, O.C. = open comedones.

TABLE I
Statistical comparison of the colonisation patterns of normal follicles and comedones

Type of follicle	Proportion of follicles* colonised by			Geometric mean counts† in colonised follicles of		
	staphylococci	propionibacteria	<i>Pityrosporum</i> spp.	staphylococci	propionibacteria	
Normal follicles	3.6%	12.3%	12.7%	5.5×10^3	2.6×10^5	p = < 0.05 n.s. 3.8×10^5
Closed comedones	28.6%	39.3%	69.2%	1.9×10^4	3.0×10^5	
Open comedones	17.9%	71.4%	77.8%	7.2×10^4	3.8×10^5	

n.s. = No significant differences at the 5% level.
* Statistical analysis by the G-test.
† Statistical analysis by Analysis of Variance.

TABLE II

A comparison of the microbial colonisation of early and mature comedones isolated from the upper back and classified according to size

Stage of comedones (number)	Number of open comedones colonised by		
	staphylococci	propionibacteria	<i>Pityrosporum</i> spp.
Early comedones (10)	0	5	5
Mature comedones (14)	5	11*	13
	†p=0.048	p=0.099	p=0.028

* Out of 13 tests analysed.

† p=probability that mature comedones are no more likely to be colonised than early comedones (Fisher's Exact Test).

cultural counts showed this organism to be present. *Pityrosporum* spp. counts were not analysed quantitatively because they were not considered to be sufficiently accurate.

Table I summarises the colonisation patterns of normal follicles and comedones. The species of staphylococci and propionibacteria isolated did not differ significantly amongst normal follicles, closed comedones and open comedones. However, *Prop. granulosum* was isolated more frequently from comedones than from normal follicles and this difference might have achieved statistical significance with a larger sample size (Gehse *et al.*, 1983).

Table II analyses the microbial colonisation patterns of open comedones designated as "early" or "mature" according to the degree of ductal distension observed. Data from lesions of intermediate size were disregarded for the purpose of this analysis. For each microbial group the colonisation frequency of apparently mature lesions was greater than that for early lesions—this was statistically significant at the 5% level for staphylococci and *Pityrosporum* spp. but only at the 10% level for propionibacteria.

Host factors

The age, sex and acne severity of donor patients were analysed to determine if these factors affected the frequency of ductal colonisation or the resultant population sizes. There was no evidence of any such influence on either the normal follicles or the comedones isolated in this study.

DISCUSSION

Pityrosporum spp., staphylococci and propionibacteria all colonised comedones more frequently than they did normal follicles, but in no instance did the prevalence of a particular microbial group approach 100%. Even if organisms believed to be contaminants were included as colonists, 10.7% of closed comedones and 7.1% of open comedones were apparently sterile (compared with 34.4% of normal follicles). These data are consistent with previous reports of failure to demonstrate bacteria in expressed comedonal material from a proportion of comedones (Marples *et al.*, 1973; Puhvel and Amirian, 1979; Lavker *et al.*, 1981).

The observation that mature open comedones are more frequently colonised than early lesions has important implications. It is hard to reconcile this result with arguments suggesting that microorganisms once present in comedones have either lost viability before examination, in which case the opposite result would be expected, or have been rendered non-viable by the processing techniques. Indeed, this result suggests that many comedones which are colonised have become so during progressive enlargement of the comedones and that microorganisms were not present at the outset of comedogenesis. This observation supports the argument that microbial colonisation is not a prerequisite for comedo formation.

If microbial colonisation is the effect of, and not the cause of, comedo formation, microorganisms must be exploiting environmental changes brought about during comedonal morphogenesis. The qualitative similarity between the microflora of comedones and normal follicles suggests that the factors important in producing favourable conditions for microbial growth in comedones are probably very similar to those factors determining whether normal follicles are colonised (Leeming *et al.*, 1984). This is an important area for future research.

The observation that microorganisms are not present in all comedones also has an important implication in the study of lesion inflammation. Inflamed lesions are believed to be derived from comedones or micro-comedones (sub-clinical lesions showing histological comedonal changes) and, therefore, whilst it was assumed that all comedones contained microorganisms, it followed that all inflamed lesions contained microorganisms when inflammation was triggered. Recognition that microorganisms are not universally present in comedones negates this argument and, therefore, the presence of microorganisms at the outset of inflammation remains to be proven. Preliminary investigations of this problem are currently being undertaken.

We thank the West Riding Medical Research Trust and the Leeds Foundation for Dermatological Research for financial support of this investigation.

REFERENCES

- Gehse M, Hoffer U, Gloor M, Pulverer G 1983 Propionibacteria in patients with acne vulgaris and in healthy persons. *Archives of Dermatological Research* **275**:100-104.
- Kellum R E 1966 Isolation of human sebaceous glands. *Archives of Dermatology* **93**:610-612.
- Lavker R M, Leyden J J, McGinley K J 1981 The relationship between bacteria and the abnormal follicular keratinization of acne vulgaris. *Journal of Investigative Dermatology* **77**:325-330.
- Leeming J P, Holland K T, Cunliffe W J 1984 The microbial ecology of pilosebaceous units isolated from human skin. *Journal of General Microbiology* **130**:803-807.
- Marples R R, McGinley K J, Mills O H 1973 Microbiology of comedones in acne vulgaris. *Journal of Investigative Dermatology* **60**:80-83.
- Puhvel S M, Amirian D A 1979 Bacterial flora of comedones. *British Journal of Dermatology* **101**:543-548.
- Puhvel S M, Reisner R M, Amirian D A 1975 Quantitation of bacteria in isolated pilosebaceous follicles in normal skin. *Journal of Investigative Dermatology* **65**:525-531.
- Williamson P, Kligman A M 1965 A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology* **45**:498-503.