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INCIDENCE AND LIPOLYTIC ACTIVITY OF *PROPIONIBACTERIUM ACNES* (*CORYNEBACTERIUM ACNES* GROUP I) AND *P. GRANULOSUM* (*C. ACNES* GROUP II) IN ACNE AND IN NORMAL SKIN*

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ABSTRACT

To compare incidence and lipolytic activity of *Propionibacterium acnes* and *P. granulosum* (previously distinguished as *Corynebacterium acnes* groups I and II, see J Bacteriol 101:392, 1970) from acne lesions and from normal follicles, isolates were classified by biochemical and immunologic reactions, and their lipolytic activity on tributyrin was determined by potentiometric assay.

P. acnes occurs in normal sebaceous follicles of the nose of adults and adolescents 2 to 3 times as frequently as does *P. granulosum*. In pustules and comedones, *P. acnes* is about 8 times as frequent as is *P. granulosum;* in the follicles of the noses of these patients, the frequency of isolation of *P. acnes* is also higher than in normal follicles. These data suggest that *P. acnes* is more probably involved in pathogenesis in acne than is *P. granulosum*.

On the other hand, *P. granulosum* is more actively lipolytic than is *P. acnes*. Examination of isolates from acne and non-acne sources did not support the hypothesis that isolates from acne lesions are more actively lipolytic than are those from other sources.

It is widely held that early changes occurring in the pathogenesis of the lesions of acne vulgaris are due to irritating concentrations of free fatty acids, which are released from sebum triglycerides by the action of bacterial lipases [1–3]. Several workers have shown that *Corynebacterium acnes* is actively lipolytic [4–6]; Kellum et al [6] found evidence that isolates of *C. acnes* from acne patients are generally more lipolytic than are those from subjects without acne. Recently, Marples et al [7] showed that the numbers of *C. acnes* on the scalp are directly related to the free fatty acid concentration in the scalp lipids, and that reduction of *C. acnes* by treatment with demethylchlortetracycline is associated with a fall in the level of free fatty acids.

In investigations into the role of *C. acnes* in the etiology of acne, we have shown that two similar but distinct anaerobic corynebacteria can be isolated from normal skin and from acne lesions. For convenience, these organisms were designated as *C. acnes* groups I and II [8]. Recently, Johnson and Cummins [9] have shown the identity of *C. acnes* I with their *Propionibacterium acnes*, and of *C. acnes* II with their *P. granulosum*, principally on the basis of DNA homology. As it seems probable that the Johnson and Cummins nomenclature will be adopted, the organisms previously described as *C. acnes* I and II are here designated *P. acnes* and *P. granulosum*, respectively.

We report studies on the incidence of these two organisms in acne lesions and in normal follicles, and a comparison of the lipolytic activities of the two species, for the purpose of assessing their probable relative importance in the pathogenesis of acne.

MATERIALS AND METHODS

Sampling of bacterial flora. The subject wiped the surface of the nose with 70% (w/w) ethanol to remove the surface flora, and then squeezed the nose vigorously with the fingertips to express the contents of several sebaceous follicles. The contents were either streaked directly onto Brain Heart Infusion (BHI) agar (Difco) with 1% added glucose, or homogenized in a tissue homogenizer with a Teflon pestle in 2 ml of BHI broth before streaking. Plates were incubated under 95% N_2 and 5% CO_2 for five days at 35° C.

Comedones were expressed from the faces of acne patients with a comedo extractor, and homogenized and streaked as above. Pustules were opened and streaked directly on agar.

Subjects classed as adults (34 males, 9 females) ranged in age from about 25 to 50 years. Those classed as adolescents and as acne patients ranged from 15 to 22 years. Those adolescents considered "normal," with at most minimal evidence of acne, comprised 23 males and 14 females. Of the acne patients, 27 were males and 18 females; 35 were currently under treatment with one of the tetracyclines.

Indentification of bacterial flora. Several colonies (usually 10) of anaerobic diphtheroids from each subject were transferred to tubes of BHI broth with 1% added glucose; efforts were made to obtain a representative sampling of the various sizes and colors of colonies on the plates. Color ranged from white through tan to pink. After five days' anaerobic incubation, cultures were examined by Gram stain for purity and identity as

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diphtheroids. They were then tested for antigenic specificity by immunodiffusion precipitin tests, using a *P. granulosum* antiserum, and for proteolysis on gelatin agar [8]. Cultures which hydrolyzed gelatin and were antigenically similar to *P. acnes* were so classified. Gelatin-negative isolates which were antigenically similar to *P. granulosum* were identified as that organism. In a few cases where these two tests were in disagreement, the tests were repeated and the cultures further examined for indole production, fermentation of maltose and sucrose, and susceptibility to bacteriophage [8]. More than 90 percent of the isolates could be clearly identified as *P. acnes* or *P. granulosum*. The remainder were either intermediate in character, or were relatively more aerobic and probably represented part of the aerobic diphtheroid flora.

Other strains. Fifty-five strains examined by Kellum et al [6] were obtained from Leon F. Ray, and classified as above. These strains were identified as coming from acne or non-acne sources.

Lipase determination. The optical density (OD) of 1:10 dilutions of 5-day cultures in BHI broth plus 1% glucose was determined at 660 nm, with a Bausch and Lomb Spectronic 20 spectrophotometer. Lipase activity on tributyrin substrate was determined as described by Hassing [10]; rate of hydrolysis was followed by potentiometric titration of the butyric acid released during the first 2–5 minutes at 25° C. Aliquots of the cultures taken for assay ranged from 0.5–2 ml, depending on activity of the enzyme. Results were corrected to rate of hydrolysis per ml of culture, and expressed as μeq of butyric acid released/min/0.1 OD unit, in order to compensate for differences in the amount of growth, as suggested by Freinkel and Shen [5].

RESULTS

Results of the evaluation of the incidence of *P. acnes* and *P. granulosum* in sebaceous follicles of subjects with and without acne, and in acne lesions, are shown in Table I. *P. acnes* was found in the normal follicles of the nose of both adolescents and adults 2 to 3 times as frequently as *P. granulosum*. In acne lesions, the ratio of *P. acnes* to *P. granulosum* increased to 8:1; i.e., *P. acnes* was found relatively much more frequently in lesions than in normal follicles. When incidence of the two organisms was compared in the follicles and lesions of 24 of these patients, *P. acnes* was more prevalent in the lesions than in the uninvolved follicles of the same individuals. No significant influence of tetracycline treatment on relative incidence of the two species in acne lesions could be seen.

Lipolytic activity of isolates of both species, taken from acne lesions, was determined. Results are shown in Table II. Although there is slight overlap between the groups, isolates of *P. granulosum* were significantly more actively lipolytic than were isolates of *P. acnes*. Many of the latter species did not show lipolysis under the conditions employed, although at least some of these strains will cause detectable lipolysis on incubation for a week in the presence of triglyceride.

The isolates of *P. acnes* from patients receiving tetracycline were slightly but significantly more actively lipolytic than were those from patients without tetracycline. A similar difference between isolates of *P. granulosum* was not significant.

Because Kellum et al [6] had reported that comparison of lipolysis during growth of their strains had shown more extensive lipolysis by strains from acne than from non-acne sources, 55 of their cultures were classified according to previously established criteria [8], and the rates of lipolysis by broth cultures were determined potentiometrically [10]. The results in Table III show little difference between rates of lipolysis by *P.*

TABLE I

Incidence of P. acnes *and* P. granulosum *in sebaceous follicles and in acne lesions*

Source	No. of subjects	No. of isolates		Ratio *P. acnes*/*P. granulosum*
		P. acnes	*P. granulosum*	
Normal follicles				
Adults	43	384	182	2.1
Adolescents	37	122	40	3.0
Acne lesions	45	360	45	8.0
Follicles in acne*	24	193	40	4.8
Lesions in same patients†	24	124	18	6.9
Patients with tetracycline	33	263	34	7.7
Patients without tetracycline	12	97	11	8.8

* Contents of uninvolved sebaceous follicles in acne patients

† Acne lesions compared with uninvolved follicles in the same patients

TABLE II

Lipolytic activity on tributyrin of P. acnes *and* P. granulosum *from acne lesions*

Organism	Isolates	Lipase, μeq/min/0.1 OD	
		Mean	Range
P. acnes	348	0.19*	0–1.64
From patients with tetracycline	257	0.20†	0–1.64
From patients without tetracycline	91	0.15†	0–0.52
P. granulosum	45	0.86*	0.16–3.15
From patients with tetracycline	40	0.90	0.16–3.15
From patients without tetracycline	5	0.55	0.36–1.16

* Difference between the means is statistically significant ($p < 0.01$), using the t-test.

† Difference between the means is statistically significant ($p < 0.025$), using the t-test.

TABLE III

Lipolytic activity on tributyrin of P. acnes *and* P. granulosum *from acne and non-acne sources**

Organism	Source	No. of isolates	Avg. lipolytic activity μeq/min/0.1 OD
P. acnes	Acne	34	0.061
	Other	13	0.056
P. granulosum	Acne	4	0.32
	Other	4	0.065

* Strains of Kellum, Strangfeld, and Ray [6].

acnes strains from acne and from other sources. On the whole, the few *P. granulosum* cultures were again more actively lipolytic than were those of *P. acnes*.

DISCUSSION

In the course of this work, it was observed that the contents of the normal sebaceous follicles of the nose are often essentially pure cultures of *P. acnes* or *P. granulosum*. Most frequently, either organism occurs alone, although in some cases the two species occur together. Plate counts of the follicular contents usually show about 20 to 100 $\times$ 10^6 anaerobic diphtheroids per mg wet weight; thus, much of the mass of the contents consists of bacterial cells. Staphylococci are found only infrequently in the normal follicle. Mixed populations of *P. acnes* and *P. granulosum* may occur in pustules and comedones as well as in the follicles, although only one organism is usually isolated from a lesion. On the other hand, staphylococci are cultured from the lesions much more frequently than from the follicles, suggesting that they may appear as secondary invaders or that they may be directly involved in pathogenesis. As has been reported many times, the flora of the acne lesion may consist solely of cocci or of anaerobic diphtheroids, or both types of organism may occur together.

It must be admitted that the very large follicles of the nose, from which the contents were expressed for sampling, may not be representative of the follicle population as a whole. However, as we have pointed out earlier [11], much of the difficulty in interpretation of results of surface collections of bacteria or lipids may be due to marked inhomogeneity of the population of follicles with respect to lipolysis, bacterial population, and production of sebum.

Survey of the frequency of occurrence of *P. acnes* and *P. granulosum* in acne lesions and in normal sebaceous follicles showed that *P. acnes* occurs with greater frequency than *P. granulosum* in normal follicles of adolescents and adults, and that its relative frequency is increased two- to fourfold in acne lesions. Even in acne patients, the ratio of *P. acnes* to *P. granulosum* is higher in the lesions than in the uninvolved follicles. There is, therefore, reason to believe that *P. acnes* is more likely than *P. granulosum* to be significant in the pathogenesis of acne, if the organisms do indeed play a causative role in the disease. It is possible that *P. granulosum* frequently precedes *P. acnes* in the follicle, and that appearance of acne is associated with a shift in the flora to a greater proportion of the latter organism.

On the other hand, Tables II and III show that in the assay system we have used *P. granulosum* is much more actively lipolytic than is *P. acnes*. If the production of irritating concentrations of free fatty acids from sebum triglycerides is indeed significant in the early stages of formation of the lesion and if the in vitro assay reflects the in vivo state, one would expect *P. granulosum* to be the more important pathogen. However, we were unable to confirm the conclusion of Kellum et al [6] that isolates from acne are more actively lipolytic than those from non-acne sources. This may be due, at least in part, to differences in the methods used. The other workers incubated growing cultures for seven days in the presence of various triglycerides before estimating the extent of lipolysis, whereas we determined activity of the lipase by potentiometric titration of the rate of hydrolysis of tributyrin by 5-day cultures.

The data of Kellum et al [6] suggest that isolates from patients treated with tetracycline show depressed lipolytic activity. Our data (Table II), on the other hand, show more active lipolysis by *P. acnes* from patients receiving tetracycline than from those without the antibiotic. A similar difference was seen with *P. granulosum*, as well; lack of statistical significance of the difference may be attributable to the small number of isolates which had not been exposed to tetracycline.

It should be mentioned that use of tributyrin as substrate in these assays is based on the demonstration that hydrolysis of different triglycerides occurs at rates dependent on composition of the glycerides [12]; tributyrin is chosen as substrate because its greater rate of hydrolysis simplifies assay of the lipase.

Lower lipolytic activity of strains long maintained in culture (Table III), as compared with that of fresh isolates (Table II), may reflect a gradual diminution of lipase production by the organism on continued subculture.

Other results [11] show no relation of free fatty acid levels on the skin surface to the particular anaerobic diphtheroid which the subject carries; residence time in the follicle may be more important than concentration or activity of the lipase. Moreover, the relative frequency of occurrence of the two species in normal follicles and in acne lesions does not support an assumption of greater significance of *P. granulosum*.

Recent reports by Šalomon et al [13] and by Gloor and others [14] have shown reduced free

fatty acid levels in acne. Kanaar [15] has observed a similar reduction in male patients, but not in females. These findings raise questions concerning the importance of free fatty acids as a determining factor in acne. Therefore, the greater lipolytic activity of *P. granulosum* noted in our laboratory may not be of significance in the etiology of the disease.

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