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PROPIONIBACTERIUM LEVELS IN PATIENTS WITH AND WITHOUT ACNE VULGARIS

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Propionibacterium species were quantified on the foreheads and cheeks of persons with and without acne in three age groups: 11 to 15, 16 to 20, and 21 to 25. Propionibacteria were virtually absent in the pubertal non-acne group compared to a geometric mean density of 114,800 per sq cm in the acne group. A similar sharp difference existed between the acne subjects and normals in the age range of 16 to 20 years: 85,800 organisms per sq cm compared to 588 per sq cm. Patients with acne and normal subjects over age 21 showed no difference in Propionibacterium levels. In acne patients, while there was a trend for lower levels, no significant difference was seen as the severity of inflammation increased.

Researchers have long been concerned with determining what is different about the patient with acne vulgaris. Very little has been brought to light despite intensive search. While no unique feature has been found, there is one quantitative difference which has been firmly established; the intensity of the disease varies proportionately with the output of sebum [1,2]. The clinical observation that acne patients are "oilier" has been verified by measurement. The correlation is statistical and not strictly applicable to every case since factors other than seborrhea are involved. The larger sebaceous glands of acne patients, especially notable in acne conglobata, largely account for the enhanced production of sebum [3].

While sebum is an absolute prerequisite for the development of the disease, the anaerobe *Propriobacterium acnes* almost certainly figures significantly in pathogenesis. The suppression of *P. acnes* by antibiotics is one of the most dependable ways of modifying the disease [4,5]. The organism luxuriates in follicles which are about to transform into comedones and its products contribute to their eventual rupture. One would theoretically expect higher densities of *P. acnes* in acne patients. Curiously, such data have not been procured nor to our knowledge even seriously sought. It is our purpose in this communication to show that *P. acnes* levels are indeed strikingly higher in acne patients.

MATERIALS AND METHODS

Subjects. Subjects with and without acne were compared in each of the following age spans: 11 to 15, 16 to 20, and 21 to 25 years. There were 152 (100 males and 52 females) in the acne group and 135 normals (70 females and 65 males). The acne patients were further divided on the basis of severity of inflammation on a I to IV scale. There were 5 in grade I (no inflammatory lesions at the time of the study), 38 in grade II (small papules and pustules), 87 in grade III (numerous large papules and/or pustules), and 22 in grade IV (nodulocystic lesions in addition to numerous large papules and pustules).

Microbiologic methods. Propionibacteria were quantitatively determined using the detergent scrub technique of Williamson and Kligman [6]. Samples were obtained from the foreheads of all subjects and the cheeks in 73 nonacne and 88 acne subjects. Serial 10-fold dilutions were made in half-strength buffered detergent and drop plates prepared on modified Marshall and Kelsey media incubated anaerobically. The colonies were classified as either *P. acnes* or *P. granulosum* on the basis of colonial morphology and susceptibility to *P. acnes* bacteriophage [7]. Formerly such organisms were classified as *Corynebacterium acnes* Group I and Group II. The official genus is now Propionibacterium [8]. *P. acnes* corresponds to the previous *C. acnes* Group I, and *P. granulosum* represents the previous *C. acnes* Group II.

Statistics. Since microorganisms are log normally distributed, all values were transformed into logarithms per sq cm and significance then assessed according to the Student's *t*-test. Means are expressed in the Table as the geometric mean (anti-log of the logarithmic mean), while the standard deviation is expressed in logarithms. Although expressed in more familiar arithmetic terms, the geometric mean represents a logarithmic function.

RESULTS

Anaerobic Subgroups

P. acnes was isolated from all subjects and was the dominant species in both normals and acne patients. *P. granulosum* was recovered in only 2.4% of normal subjects and in 42.8% of those with acne. When present, *P. granulosum* represented 16% of the total anaerobic flora in acne subjects and less than 1% in normals. No statistical difference in either the prevalence or density of *P. granulosum* was detected with respect to the severity of acne. The subsequent quantitative data represent both Proprionibacterium subgroups.

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TABLE. *Forehead anaerobes in relation to disease*

Acne (*grade*)	Subjects	Geometric mean per sq cm[a]	Standard deviation[b]
I	5	250,000	1.60
II	38	96,000	1.40
III	87	99,000	1.24
IV	22	45,400	1.51

[a] The anti-log of the logarithmic mean count.
[b] Expressed as logarithms.

Quantitative Measurements

Propionibacterium counts were strikingly higher in acne patients during the period that the disease commences and flourishes. On both the forehead and cheek, acne patients in the 11 to 15 and 16 to 20 age range carried significantly greater numbers (Fig.). For example, normal subjects aged 11 to 15 carried a geometric mean count of 16.6 organisms per sq cm on the forehead and those in the 16 to 20 age range had a geometric mean count of 588 per sq cm. In the corresponding age group of acne patients, the levels were 114,800 and 85,800 per sq cm. At the onset of young adult maturity, this highly significant difference between acne and nonacne subjects no longer existed. Nonacne subjects, for example, had a geometric mean count of 124,000 per sq cm compared to 97,700 per sq cm in those with acne (a statistically nonsignificant difference). The same striking increase in Propionibacterium levels was found in the cheek. In normals, the geometric mean count was 27 organisms per sq cm in the 11 to 15 age group, rose to 447 per sq cm in the 16 to 20 age range, and then rose abruptly to 142,000 per sq cm in those age 20 (Fig.). In the acne patients, significantly higher levels were found on the cheek in the 11 to 15 and 16 to 20 age groups while no difference existed in those over age 20 (Fig.). In both acne subjects and normals, the counts on the cheeks were consistently higher than on the forehead in individual subjects. While this difference was uniform in individual subjects it was not necessarily reflected in the mean counts of every age group (Fig.). No significant difference existed in Propionibacterium levels on the forehead or cheeks of either normals or those with acne. In 68 paired samples from the acne group, the geometric mean count on the forehead was 69,000 per sq cm compared to 160,768 per sq cm on the cheek. ($r = 0.89$; $p < 0.01$) In a large series of normals we have also shown that the cheek carries a higher density of these anaerobes but significance is not achieved [9].

Since there were more males than females in the acne group (100 vs 52), Propionibacterium levels were analyzed with reference to sex. Males demonstrated a geometric mean count of 75,788 per sq cm compared to a geometric mean of 138,166 per sq cm in females, a nonsignificant difference. The highly significant increased levels found in acne patients is present in both sexes.

There was trend toward lower counts of Propionibacteria as the inflammatory expressions of the disease increased but significance was not achieved (Tab.).

DISCUSSION

These data portray another unequivocal quantitative difference between adolescents with and without acne. Both in the epoch after puberty, ages 11 to 15, and during the period when the disease peaks up to age 20, Propionibacterium counts are strikingly higher. Indeed, upon attainment of puberty, most normal subjects have virtually none, only about 16 organisms per sq cm. By contrast, acne subjects after puberty attain levels which are as high as seen in young normal adults, age 21 to 25. In the latter age range, the two groups become indistinquishable, the count being in the range of 100,00 organisms per sq cm.

Do these data prove a pathogenic role for the Propionibacterium? The answer must be in the negative; the high densities in acne patients certainly need not be a primary event in inciting the disease. Larger populations of organisms might simply reflect a more ample habitat and nutrients. Acne subjects are oilier and produce more sebum. The anatomic basis for this is larger glands, the implication of the latter is that the canals of the sebaceous follicles where Propionibacteria reside are also more spacious. With the "surface biopsy" method using cyanoacrylate resins, one can easily determine that the material removed from the depths of sebaceous follicles occupied canals of greatly increased diameter compared to those without acne (unpublished observations). *P. acnes* therefore, might simply expand in this increased space.

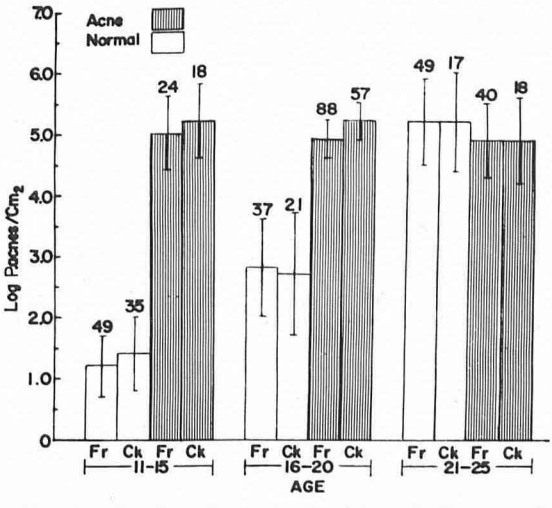

FIG. Forehead and cheek densities of *P. acnes* in acne and nonacne subjects. *Bars* represent the 95% confidence limits of the standard error of the mean.

We think it unlikely, however, that *P. acnes* and *P. granulosum* will be found to be bystanders in the acne process. There is considerable circumstantial evidence that they participate in pathogenesis to some extent even though their effects are indirect. The organisms do not proliferate in living tissue, healthy or diseased, and are certainly not primary pathogens. They are implicated on the following grounds:

1. Crowds of these diptheroids accumulate in follicles at the onset of comedo formation [10].

2. Lipids secreted by the organisms are strongly comedogenic; moreover, lipases produced by the organisms cleave triglycerides to form free fatty acids. The latter are both comedogenic and irritating [11].

3. The intense colonization of comedones by *P. acnes* is responsible for rupture and the incitation of inflammatory lesions. Injection of living *P. acnes* into the sterile cysts of steatocystoma multiplex results in inflammatory lesions [12]. Furthermore, comedones produced by bacteriostatic acnegens such as coal tar tend not to rupture. Likewise, comedones produced in the rabbit ear contain no bacteria and never break down despite large sizes attained by continual application of comedogenic chemicals. *P. acnes* elaborates a variety of enzymes including proteases which could attack the epithelial capsules of comedones.

4. Finally, antibiotics capable of suppressing *P. acnes* or reducing the free fatty acids are the very drugs which appreciably moderate the disease.

If *P. acnes* and *P. granulosum* are important in causation, one would argue that the disease should eventually develop in everyone in whom the populations match those of subjects with adolescent acne. That, in fact, occurs in young adulthood, yet acne is decidedly on the wane in the early twenties. Our hypothesis is that the follicle attains anatomic maturity by that time, and is no longer susceptible owing to a presence of a horny layer barrier lining the canal. Whether or not this is substantiated, *P. acnes* is only one of many factors which combine to produce the disease.

Our finding of a trend toward lower levels of Propionibacterium in more severely inflamed acne is in agreement with Cunliffe's observation of lowered free fatty acid levels in such patients [2]. At first glance, this is paradoxical since greater not lesser quantities of these organisms and free fatty acids might be expected in the worst forms of the disease. An explanation that tentatively appeals to us is that *P. acnes* being an anaerobic organism is antagonized by higher oxygen tension or some other aspect of inflamed skin such as temperature or pH changes. Whatever the mechanism, we have observed similar low levels of *P. acnes* in other inflammatory conditions such as seborrheic dermatitis. [13].

If we are to achieve a greater understanding of how the various causative factors combine to produce acne, it is essential to obtain quantitative data on sebum production, Propionibacterium levels, and free fatty acid on the same person. We may then begin to analyze how these factors are influenced by age, sex, race, and the presence of disease.

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