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CONTROL OF FREE FATTY ACIDS IN HUMAN SURFACE LIPIDS BY *CORYNEBACTERIUM ACNES**

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ABSTRACT

Aerobic bacteria were excluded as a major source of lipases when it was found, in a previous study, that their virtual eradication by topical neomycin did not lower the free fatty acids (FFA) in scalp lipids. We now report the reciprocal experiment in which an oral tetracycline was used to suppress the anaerobic organisms.

Nine healthy adults received 600 mgm of demethylchlortetracycline (DMCT) daily for 6 weeks. During this time we followed the composition of scalp lipid by thin layer chromatography and the densities of *C. acnes*, aerobes and yeasts on scalp and forehead.

Six subjects had high initial levels of *C. acnes*. In these the FFA fell from 43% to 23% with a more than 99% reduction in the density of *C. acnes*. In three who initially had insignificant quantities of *C. acnes* the FFA were at the start only 25% and did not decline with treatment.

The density of the aerobic population was not affected by DMCT owing to development of resistance. Yeasts were also unaffected. The finding that the FFA were proportional to the level of *C. acnes* suggests that these organisms are the chief source of lipolytic enzymes. The FFA levels began to decline earlier than the density of *C. acnes*. This might be explained either by enzyme inhibition or by bacteriostatic effects.

In the passage of lipid from the sebaceous gland to the surface, free fatty acids (FFA) are liberated from triglycerides by lipases which are almost certainly derived from microorganisms (1, 2, 3). Three groups of microorganisms inhabit sebaceous follicles: coagulase negative cocci, the anaerobic diphtheroid *Corynebacterium acnes* and yeast-like fungi of the genus *Pityrosporum*. Production of lipases has been demonstrated in culture for each of these: cocci (4, 5), *C. acnes* (5, 6) and *P. ovale* (7). Studies *in vitro*, however cannot determine which of these are important in lipolysis in living human skin. In a previous study we found that practically complete elimination of the aerobic coccal scalp flora by topical neomycin did not change the proportion of FFA in the surface lipids (8). Since the populations of the other two members of the flora were not reduced, we concluded that aerobes do not normally participate in lipolysis. The purpose of this present study was to evaluate the role of *C. acnes* by selectively suppressing this component of the flora with an antibiotic. Systemic demethylchlortetracycline (DMCT) treatment was chosen because the cocci quickly develop resistance so that the population density is not altered (9), while *C. acnes* remains susceptible and its numbers gradually decline over a period of weeks (10). Yeasts are not susceptible to this antibiotic.

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MATERIALS AND METHODS

Subjects. Nine healthy adult male negro inmates of the Philadelphia County Prison at Holmesburg served as volunteers.

Treatment. Each subject received 600 mgm of (DMCT) daily in two doses for 6 weeks. Medicated soaps and cosmetics were prohibited for three weeks prior to starting the study. The scalp was washed at weekly intervals with a non-medicated shampoo.

Surface lipids. A) *Collection.* Ether washings of the scalp were made on two successive days following each bacterial sampling and shampoo day as previously described (8). The washings were fil-

tered through a Millipore HA47 to remove particulate matter, before the ether was driven off by gentle heat and finally in a nitrogen stream. The lipid sample was stored frozen in screw capped tubes with teflon cap liners until analysis.

B) *Lipid analysis.* The major components were resolved using a triple development quantitative thin layer chromatographic method as previously described (8, 11).

Bacteriological methods. A) Quantitative sampling. The detergent scrub technique of Williamson and Kligman was used (12). Two sites on the vertex of the scalp were clipped and a glass cylinder 3.8 sq. cm. in area held to the skin. Each site was sampled with two successive one minute scrubs in one ml of 0.1% Triton X-100 in phosphate buffer and the two suspensions pooled. Duplicate samples were taken twice before treatment and after 1, 2, 4 and 6 weeks of treatment from the scalp and the forehead.

B) *Cultural methods.* Four tenfold dilutions were made in half strength wash fluid and 0.25 ml quantities incorporated into the following media:

Trypticase Soy Agar (BBL) for aerobes.

TSA with 0.5% 'Tween 80' (Atlas Chemical) as a growth supplement for lipophilic diphtheroids.

Thioglycollate agar with 1% glucose for Corynebacterium acnes.

TSA containing 10 μgm/ml of DMCT to determine the number of resistant organisms.

Surface inocula and methods of identification were as previously described.

C) *Yeast Counts.* The quantity of yeasts was assessed by a modification (13) of the corneocyte count technique before and after treatment.

TABLE I

Percentage of coccal cells resistant to 10 μgm/ml DMCT

Pretreatment			Weeks of treatment			
	1	2	1	2	4	6
Scalp	8.1	9.1	22.2	79.1	84.6	92.0
Forehead	23.1	20.4	38.3	76.0	73.2	80.2

RESULTS

It soon became obvious that the nine subjects did not form a homogenous group bacteriologically. Three subjects (7, 8 and 9) had very low initial densities of *C. acnes*. Counts ranged from 10^3 to 10^5 organisms per sq. cm. on the scalp with 3 of 12 samples yielding no *C. acnes*. The densities were even lower on the forehead (10^2 to 10^3) with 9 of the 12 pretreatment samples yielding no *C. acnes*. These values are very much less than the 10^6 usually found, a level present in the remaining six subjects. In our experience less than 10% of randomly selected subjects display such low levels. These three subjects are thus quite unusual with regard to *C. acnes* populations, although they did have a normal density of aerobes. Their data were treated separately.

Pretreatment microflora. The aerobic flora was typical in all subjects, being dominated by coagulase negative cocci. On the scalp the Baird Parker type (14) principally found was *Micrococcus* type 3, but *Staphylococcus* type II (S II) was abundant in 8 of the nine subjects. The forehead flora was dominated by S II which was most numerous in 5 and second in the remaining 4 subjects. Small numbers of other Baird Parker types were also recovered.

Effect of treatment on the composition of the microflora. Essentially the aerobic flora of both scalp and forehead remained a coccal flora. Some increase in the proportion of S II was evident within the cocci.

The major effect of treatment was to increase the proportion of coccal cells resistant to 10 μgm/ml of DMCT from less than 10% on the scalp and from 25% on the forehead to 80–90% (Table I). This increase was detectable in one week and was practically complete by two weeks of treatment.

TABLE II

Effect of DMCT on the density of resident organisms

Pretreatment				Weeks of treatment			
		1	2	1	2	4	6
Aerobes	Scalp	1,570,000	6,488,000	3,648,000	1,581,000	2,335,000	2,373,000
	Forehead	6,430	5,610	9,430	10,320	21,880	29,330
C. acnes	Scalp	2,023,000	1,080,000	4,013,000	817,000	23,900	15,400
	Forehead	3,330,000	1,226,000	8,441,000	2,677,000	174,400	30,670
Yeasts	Scalp	340,000	—	—	—	—	361,000

TABLE III

C. acnes per sq cm on scalp

Subject	Pretreatment		Weeks of treatment			
	1	2	1	2	4	6
1	8,535,000	2,797,000	15,750,000	5,790,000	133,100	25,780
2	1,688,000	1,506,000	2,959,000	1,095,000	11,530	36,460
3	974,500	635,000	1,551,000	1,053,000	29,770	5,000
4	7,800,000	594,200	10,690,000	2,098,000	18,230	1,370
5	983,600	440,900	1,837,000	77,900	3,720	—
6	634,800	2,260,000	2,939,000	273,700	60,000	148,800
7	1,050	631	1,580	—	2,100	1,890
8	1,050	—	263,000	—	—	—
9	8,710	89,130	77,600	2,630	5,260	1,050

TABLE IV

Effect of DMCT on the percentage of free fatty Acids

Subjects*	Pretreatment		Weeks of treatment			
	1	2	1	2	4	6
1	41.25	39.4	38.8	28.95	25.6	20.4
2	42.5	35.1	39.85	22.4	24.85	22.8
3	37.7	42.4	35.25	31.25	38.25	23.0
4	44.15	35.85	24.2	25.9	27.15	22.9
5	49.75	56.15	49.3	33.6	38.3	—
6	—	44.75	24.7	23.1	34.0	28.15
Average	43.1	42.5	34.4	28.1	31.4	23.45
7	22.4	20.1	18.7	26.85	32.05	23.5
8	26.0	30.1	17.8	—	—	—
9	17.6	18.6	30.5	28.45	32.6	26.7
Average	22.0	24.7	20.5	27.65	32.63	25.1

* Subjects 7, 8 and 9 had unusually low anaerobic counts.

Effects of treatment on microbial population density. The data are summarized in Table II which shows the geometric means for each sampling occasion. The averages of the duplicate results for *C. acnes* on the scalp for each subject are tabulated in Table III as an example of the data.

The density of aerobes showed no significant change through the treatment period although the proportion of resistant cells was rising. The density of *C. acnes*, however, fell dramatically on both the scalp and forehead. This decline was first detectable at 2 weeks on the scalp, and at 4 weeks on the forehead. By 6 weeks the count had been reduced by more than 99% at both sites. The density of yeasts was unaffected.

Effect of treatment on surface lipid. Changes were found only in the proportion of FFA and the reciprocal change in triglycerides. The values of FFA for each subject are listed in Table IV. In the 6 subjects with high initial densities of *C. acnes* the FFA averaged more than 40%. Treatment reduced this by almost 50% (average = 23.5%). The FFA in the three unusual subjects with low *C. acnes* counts were not reduced by the antibiotic. Indeed, instead of a decline a slight increase could be appreciated.

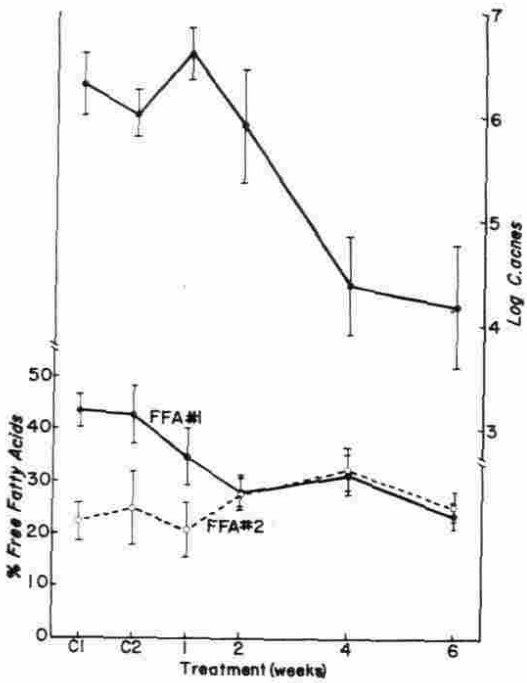

FIG. 1. Changes in *C. acnes* density and free fatty acids on the scalp. Upper curve: Density of *C. acnes* in 6 subjects with initially high *C. acnes* levels. Lower continuous curve: Free fatty acid levels in the same 6 subjects. Lower broken curve: Free fatty acid levels in the 3 subjects with low initial *C. acnes* levels. Bars represent 95% confidence limits of the mean. C_1 and C_2 indicate the two pretreatment controls.

DISCUSSION

The microbiological effects of treatment with DMCT were similar to our previous studies (9, 10). In the coccal dominated scalp and forehead, the most sensitive indicator of arrival of the antibiotic at the skin surface is the rise in the proportion of coccal cells resistant to the antibiotic. Because resistant cells are usually present initially, little change in aerobic density occurs as these proliferate to replace sensitive cocci when the latter are eliminated from the flora. *C. acnes* on the other hand remains sensitive to DMCT but the decline in density is delayed because the route of delivery of the antibiotics is by holocrine excretion (10). Yeasts should not be directly affected. In the previous study, when the aerobes were suppressed, yeasts appeared to increase in density. In this study no change was found, suggesting that *Pityrosporum* occupies a niche more like that of the cocci than that of *C. acnes*.

It was shown in a previous investigation (15) that the composition of the surface lipid of different individuals is remarkably constant over long periods, and that composition differs between individuals only in triglycerides and FFA. Free fatty acids in different subjects ranged from 8 to 39%. The factors controlling the generation and level of FFA are of great interest, not only to investigators, but also to clinicians since FFA are strongly implicated in the pathogenesis of acne. These substances probably contribute to the induction of comedones and to the inflammatory lesions as well.

FFA arise secondarily through the splitting of triglycerides synthesized by sebaceous glands (2). Microbial lipases are certainly involved in this process for there are no free fatty acids in sterile cysts such as steatocystomas (1), though these contain sebaceous secretions. Any of the three resident microorganisms of lipid rich areas, yeasts, cocci or *C. acnes* could theoretically participate in lipolysis. Our previous study excluded the aerobes. An important finding at that time

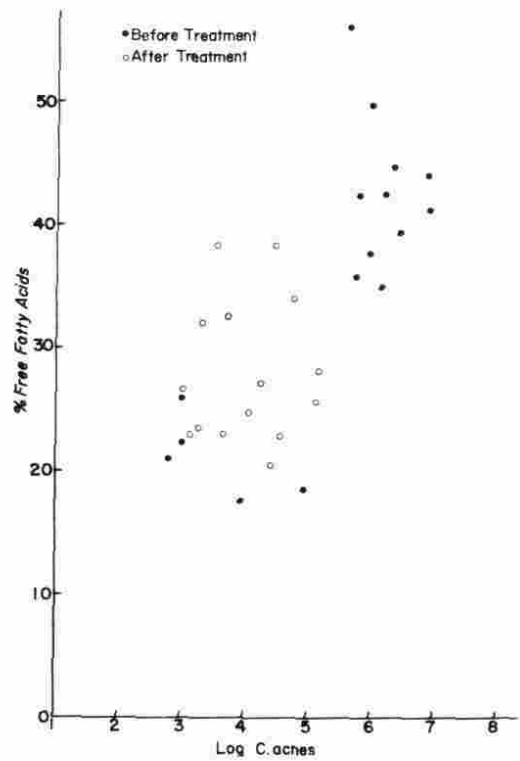

FIG. 2. Correlation between *C. acnes* density and the proportion of free fatty acids in scalp surface lipid.

was a significant relationship between the proportion of FFA and the *C. acnes* count. Such correlations, of course, do not prove a functional relationship. The results of the present study, Figure 1, provide more substantive evidence that it is the density of *C. acnes* which controls the amount of FFA in the surface lipids. When DMCT caused a great reduction in the anaerobic population, the FFA correspondingly declined. Moreover, the correlation between FFA and *C. acnes* was evident both before and after DMCT (Fig. 2). Important questions still remain. For example, the FFA began to decline before the *C. acnes* count (Fig. 1). Perhaps this reflects inhibition, but not actual death of the organisms. Prolonged bacteriostasis of course eventually kills. An alternative explanation is that tetracyclines directly inhibit bacterial lipases as Shalita *et al.* (16) and Kellum *et al.* (17) have demonstrated in their *in vitro* studies.

While we think that *C. acnes* plays a dominant role in lipolysis, it cannot be the sole agent regulating FFA in surface lipid. Despite the virtual elimination of this organism the reduction in FFA did not exceed 50%. This agrees with the earlier findings of Strauss and Pochi (18). In those subjects with initially high values of FFA and *C. acnes*, the percentage of FFA fell only to the levels normally obtaining for the three subjects with unusually low *C. acnes* counts. Tetracycline had no effect in these three. This limited effect can be interpreted in different ways. It has been adequately demonstrated that cocci and *Pityrosporum* can elaborate lipases *in vitro*. Though usually of slight account, these might become important when the normally dominant anaerobic flora is absent or is artificially suppressed by antibiotics. It is still possible that lipases could arise from the skin itself.

Finally, percentage measurements may be very misleading. Though more than 99% of the original anaerobic population was destroyed, this still left sizeable numbers of organisms.

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