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THE ORIGIN OF FREE FATTY ACIDS IN SEBUM. I. ROLE OF COAGULASE NEGATIVE STAPHYLOCOCCI*

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In recent years the constituents of sebum, especially the free fatty acids, have become accepted as a primary factor in the pathogenesis of acne vulgaris (1). Lipolysis of preformed lipids by the lipase and esterase activity of the microbial flora of the skin has been shown to constitute one of the major sources of the free fatty acids of surface lipids (2, 3) although lipolytic activity of non-bacterial origin has not been exlcuded as an additional source of free fatty acids.

While both coagulase negative and positive staphylococci are known to produce lipases (4), lipolytic activity of the normal cutaneous flora has not been systematically assayed. Nor have the organisms been tested in model systems which are relevant to conditions that obtain to their natural environment in the skin. Inquiries into the precise role of the cutaneous flora with respect to the production of free fatty acids have been initiated. The present studies focus on the lipolytic activity of coagulase negative staphylococcus which together with *Corynebacterium acnes* and *Pityrosporum ovale* constitute the major resident microorganisms of the human skin.

METHODS

a. *Isolation of staphylococci.* Swabs were obtained from the unprepared skin of the forehead of 7 males and 5 females, between the ages of 20 and 25, and 3 prepubertal children. Comedones and follicular contents were expressed from the forehead or nose after the skin was cleansed with 70% alcohol in 4 subjects. Primary cultures were made on Staphylococcus Medium No. 110 (Difco). Nonpigmented colonies of staphylococci were propagated in beef heart infusion broth (BHI) and were stored at 4° as stock cultures. Organisms were identified as non-pathogenic staphylococci by appearance on solid agar, gram stain, and negative coagulase tests.

b. *Lipase assay.* For routine assay of lipase activity stock cultures were subcultured on solid medium containing lipid and spirt blue indicator (Difco). The color change produced by generation of free fatty acids afforded a visual test of lipase activity. Duplicate colonies were selected at random and each was inoculated into 11.0 ml BHI broth with 0.2% dextrose containing 1.0 ml emulsified olive oil. The emulsion (olive oil: 2% aqueous solution of gum acacia:1:4) was freshly prepared for each batch of assays, and the concentration of esterified fatty acid ranged from 22 to 60 μE/ml of culture medium. The free fatty acids accounted for less than 0.5% of the total fatty acids in these media. Cultures were incubated at 37° for 48 hours and aliquots were withdrawn at 24 hour intervals. Control incubations were performed with every assay; no liberation of FFA occurred in the absence of microorganisms.

In some experiments quantitative inoculations of organisms were employed. In these studies stock cultures were subcultured in BHI broth and aliquots innoculated directly into the liquid assay medium.

Following incubation, lipids were extracted from the aliquots of media by the method of Dole (5). Free fatty acids (FFA) were determined by direct titration of the lipid extract; total fatty acids (esterified and free) were estimated by titration after saponification of the lipid extract. Methods have been described previously (1).

c. *Bacterial counts.* Quantitative estimates of the bacterial population were obtained in some experiments from pour plates of serially diluted media. In the routine assay the bacterial population of a single culture reached its maximum at 24 hours (10^7 to 10^8 organisms per ml) and remained constant or declined at 48 hours. Twenty-four hour counts were the same in BHI broth in the absence or presence of olive oil suggesting that the products of lipolysis did not exert significant inhibition of growth.

RESULTS

All isolates tested not only gave a positive reaction on the solid lipid media but also produced free fatty acids (FFA) from the olive oil substrate in liquid media (Table I). Differences in lipolytic activity were evident between the various isolates. Although these may conceivably represent different lipase activities of various resident strains no quantitative significance can be assigned since no attempt was made to quantify and identify strains.

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Although lipase activity can be readily demonstrated *in vitro* it cannot be assumed that such activity is relevant in the natural environment on the human skin. Thus it was of interest to determine whether lipolytic activity could occur in the microaerophilic environment of the pilosebaceous follicle which is the area of greatest concentration of these organisms (6). Accordingly organisms were grown in a lipid medium under an atmosphere of pure nitrogen. As shown in Table II, lipase activity was present under anaerobic conditions.

In view of the preferential colonization of the sebaceous areas of the skin of adults (6), some relationship between lipolytic activity and the lipid environment might be anticipated. It was noted however that the few organisms cultured from prepubertal children (Table 1) displayed abundant lipase activity. The finding suggested that a heavy concentration of surface lipids is not a prerequisite for lipase activity in the staphylococci.

This observation prompted further inquiries into the interactions between microbial lipase and lipid substrate. Total fatty acids were assessed at the end of incubation in all 19 isolates and compared to the initial concentration. After 48 hours, total FA averaged 104 ± 5% of the control values, suggesting there was little if any discernible catabolism of fatty acids by the organisms to subserve energy requirements.

The apparent failure of the organisms to utilize fatty acids does not exclude the pos-

TABLE I

Production of free fatty acids by isolates of coagulase negative staphylococci from human skin

Subject	Sex	Source	Initial conc. of esterified FA: μE/ml	Net production FFA per 24 hrs. μE/ml	
				Day 1	Day 2
1	Male	Surface	40.0	4.2	8.6
2	Male	Surface	36.0	6.3	5.7
3	Male	Surface	23.4	14.4	5.6
4	Male	Surface	33.2	13.4	2.8
5	Male	Surface	22.1	11.8	10.3
6	Male	Surface	60.4	0	2.6
7	Male	Surface	41.6	2.5	6.4
8	Female	Surface	57.5	0.3	2.6
9	Female	Surface	45.0	15.0	10.1
10	Female	Surface	32.0	12.2	10.2
11	Female	Surface	39.2	12.2	11.8
12	Female	Surface	44.8	15.4	10.1
13	Child	Surface	21.6	13.0	4.3
14	Child	Surface	40.0	16.5	9.2
15	Child	Surface	42.4	7.2	6.4
A	Female	Comedone	53.6	12.8	19.7
B	Male	Follicle	46.4	9.7	17.5
C	Male	Follicle	37.0	13.9	18.3
D	Female	Follicle	43.3	8.5	10.9

LEGEND: Single colonies inoculated into BHI broth containing emulsified olive oil (initial conc. of esterified fatty acids given as μE/ml). Production of free fatty acids (FFA) (μE/ml) estimated at 24 and 48 hrs. Values for net production on day 2 = FFA at 48 hrs. − FFA at 24 hrs.

TABLE II

Effect of anaerobic incubation

Organisms incubated in 100% N_2 with 43 μE/ml esterified fatty acid

Subject	μE FFA/ml at 48 hrs.
6	8.6
1	4.9
D	14.8

TABLE III

Effect of carbohydrate on lipolytic activity

Organisms incubated with 55 μE added esterified fatty acid in heart infusion broth ±0.2% dextrose

Dextrose	μE FA/ml at 48 hrs.
+	26.9
−	25.8

TABLE IV

Effect of triglyceride concentration of bacterial lipolysis

Subject	μE esterified fatty acid added	μE FFA/ml at 48 hours	# organisms per ml
1	21.0	4.4	Not Done
	45.0	8.9	Not Done
D	21.0	7.8	8.0×10^7
	45.0	19.0	8.2×10^7
	84.0	26.4	7.8×10^7

sibility that the glyceride-glycerol liberated by fat cleavage served nutritional requirements. If lipase activity were quantitatively geared to provide such glycolytic intermediates in the natural environment of the skin surface, the relative availability of alternative sources of carbohydrate might be expected to affect lipolytic activity *in vitro*. Thus, aliquots of stock cultures were grown in the presence of lipid with and without 0.2% dextrose. As shown in Table III lipolytic activity was not enhanced in the absence of added carbohydrate.

The effect of lipid concentration on enzyme activity was then assessed. Aliquots of organisms were grown with varying concentrations of lipid and FFA assayed after 48 hours (Table IV). Bacterial counts were the same at all concentrations of lipid, but the production of FFA increased with increasing concentrations of substrate. The finding suggests that there is a proportional relationship between substrate and enzyme activity which is independent of the size of the bacterial population.

DISCUSSION

The uniform presence of lipase activity in isolates of coagulase negative staphylococci from the normal skin suggests that this organism may contribute to the formation of the free fatty acids of sebum. Experimental models were devised to determine the relevance of this activity to conditions on the skin surface.

The studies have demonstrated that potential for lipolytic activity due to staphylococci exists in microaerophilic conditions of the follicle where high local concentrations of FFA may influence the development of acne.

Staphylococci are extremely hardy organisms with very indifferent growth requirements. It has been shown in *S. aureus* that the lipase is an extracellular enzyme which is elaborated in the absence of lipid (7). The present studies also suggest that lipolytic activity is not geared to the nutritional demands of the cutaneous coagulase negative staphylococci. For example, lipolysis was not affected by the availability of alternative sources of carbohydrate. Moreover, in a stable population of organisms, lipolysis was proportional to substrate concentration without evident catabolism of the liberated fatty acids. Thus it may be postulated that lipase activity exceeds the functional requirements of the organisms, so that FFA would tend to accumulate in concentrations that would depend both on the amount of substrate lipid and on the size and activity of the bacterial population. This formulation has obvious implications for the patient with acne who produces more sebum than the normal population (8).

Quantitation of the relative role of staphylococcal lipase with respect to similar activity by other organisms as well as with respect to the development of acne awaits further study.

SUMMARY

Lipase activity has been uniformly found in isolates of coagulase negative staphylococci from the surface and follicles of human skin. Potential for the production of excessive quantities of free fatty acids from preformed lipids of sebum in the natural medium of the skin has been demonstrated in model systems. The results suggest that the lipolytic activity of resident staphylococci may be an important source of the free fatty acids of cutaneous surface.

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