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Regional Variations in Density of Cutaneous Propionibacteria: Correlation of *Propionibacterium acnes* Populations with Sebaceous Secretion

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Cutaneous *Propionibacterium acnes* populations were quantitatively measured in 33 young adults and compared with the rate and composition of sebum secretion in nine skin regions. Bacteriological and lipid analyses were performed on the forehead, cheek, anterior chest, abdomen, lower back, volar forearm, upper inner arm, thigh, and calf. *P. acnes* populations in these sites correlated significantly with the total amount of lipid produced ($r = 0.77$) as well as with di- and triglycerides ($r = 0.68$), squalene and wax esters ($r = 0.72$), cholesterol and cholesterol esters ($r = 0.67$), and free fatty acids ($r = 0.67$).

Propionibacteria are the only anaerobic bacteria which have been shown to be resident members of the cutaneous microflora in humans (4, 9). Three species may be found: *Propionibacterium acnes*, *Propionibacterium granulosum*, and *Propionibacterium avidum*. *P. acnes* is the species most commonly found and is present in nearly 100% of adults (4, 9).

Previously, we have shown that there are significant quantitative differences in *P. acnes* populations in different body sites. The highest levels were found in the face and scalp, where the mean count ranged from 10^5 to 10^6 organisms per cm^2. In contrast, very low levels (10^2 per cm^2) were found in the arms and legs (9). Since these results tend to parallel the density distribution of sebaceous glands, the possibility that sebum may play an important role in the ecology of propionibacteria must be considered. Indirect evidence that sebum may be important for *P. acnes* is the finding that *P. acnes* populations increase dramatically with the onset of puberty (5). At this time, sebaceous glands are enlarging due to androgenic stimulation, and sebum production is markedly increased. Further indirect evidence arises from the finding that in acne vulgaris, a disease characterized by elevated sebum production, *P. acnes* populations are also increased (6).

In this study, we have determined the number of *P. acnes*, the amount of sebum produced, and the composition of skin surface lipids for various body regions to determine whether any direct relationship exists between sebum and *P. acnes* in vivo.

MATERIALS AND METHODS

Subjects. A total of 33 young adults (20 males and 13 females; 24 white, 9 black), aged 18 to 24, who were free of skin disease and had no history of skin disorders, were studed. None of the subjects was taking any medications, and none had received antibiotic therapy within 1 month of this study.

Sites sampled. The sites sampled were selected on the basis of significant differences in the number of sebaceous glands and included the forehead, cheek, anterior chest (mid-sternum), abdomen, lower back, volar forearm, upper inner arm, thigh, and calf. Each site was cultured, and a 3-h sebum production measurement was performed.

Culture technique. A quantitative culture of the skin surface, using the detergent scrub technique of Williamson and Kligman (12), was made. This method involved placing a sterile glass cylinder with an internal area of 3.8 cm^2 over the area to be sampled, adding 1 ml of 0.1% Triton X-100 in 0.075% M phosphate buffer (pH 7.9), scrubbing with a blunted Teflon spatula for 1 min, and then withdrawing the sample fluid. This procedure was repeated, and the samples were pooled. Samples were processed by making 10-fold dilutions in half-strength scrub fluid (0.05% Triton X-100) to maintain the dispersion of bacteria. A 0.025-ml volume of each dilution was placed on brain heart infusion agar (BBL Microbiology Systems) supplemented with 0.8% dextrose, 0.1% Tween 80, 0.5% yeast extract, 1% sodium lactate, and 0.5% of a salt solution containing 4 g of $MgSO_4 \cdot 7H_2O$, 0.4 g of $MnSO_4 \cdot 4H_2O$, and 0.4 g of $FeSO_4 \cdot 7H_2O$, acidified with 2 drops of 10 N H_2SO_4. Plates were incubated for 7 days at 37°C in a GasPak anaerobic system (BBL). After incubation, the numbers of colonies of the various species were counted, and the number of bacteria recovered per square centimeter was calculated. Colony counts were performed on plates containing 10 to 30 colonies per dilution.

Marples and McGinley (8) have previously shown that the cutaneous propionibacteria can be presump-

tively identified on the basis of colonial morphology on primary subcultivation. These criteria were employed for initial *P. acnes* identification, and results were confirmed by bacteriophage susceptibility and biochemical tests as described by McGinley et al. (9).

Lipid collection and analysis. Skin surface lipids were obtained as follows: the skin surface was wiped for 30 s with a piece of gauze (2 by 2 in.; ca. 5 by 5 cm) saturated with 0.1% Triton X-100 to remove surface debris, desquamating epithelial cells, and bacteria, then rinsed three times with water, dried, and then washed for 30 s with hexane-soaked gauze. The site was then protected with a plastic weighing boat with several perforations to allow evaporation of sweat. Skin surface lipids were harvested 3 h later by pipetting 2 ml of nanograde hexane from a volumetric pipette into a glass cup with a 3.8 cm^2 area and scrubbing with a blunted Teflon policeman for 30 s. The hexane was withdrawn, filtered through a 0.22-μm membrane filter (Millipore Corp.) to remove bacteria, debris, and desquamating cells, evaporated to dryness with nitrogen, and stored at −20°C in Teflon-capped vials. The hexane scrub fluid contained 100 μl of methyl nervonate per 2 ml for lipid-rich areas (face and upper trunk) and 50 μl per 2 ml for the remaining sites. Methyl nervonate served as an internal standard, permitting quantification of the lipid during subsequent thin-layer chromatographic analysis. The internal standard also allowed for correction of any sample loss before spotting, since its loss would be proportional to the loss of extracted sebum.

Thin-layer chromatography. Before analysis, the scrub samples were brought to room temperature and redissolved in 0.2 ml of hexane. Suitable volumes of the lipid solutions (3 to 20 μl) were applied to 5-mm lanes ruled on commercially prepared 250-μm-thick silica gel G plates (20 by 20 cm; Analtech, Newark, Del.). The plates were sequentially developed in hexane (to 20 cm), in benzene (to 20 cm), and twice in a mixture of hexane-ether-acetic acid (75:25:1, vol/vol) to 9 cm. Lipid spots were then visualized by charring with 50% sulfuric acid, followed by heating to 220°C as described by Downing (2). The plates were scanned in the dual beam mode on a Schoeffel SD300 spectrodensitometer interfaced with a strip chart recorder. Peak areas on the recorder chart correspond to the intensity of spots on the chromatogram. These areas were determined by polar planimetry and corrected for the relative efficiency of charring of each lipid class (2).

The lipids detected were triglycerides, diglycerides, free fatty acids, wax esters, squalene, cholesterol, and cholesterol esters. Quantification of lipid was achieved by comparing the peak area for methyl nervonate with the peak areas of each lipid constituent as described by Greene et al. (3). For purposes of analysis, cholesterol and cholesterol esters, which are primarily of epidermal origin, and wax esters and squalene, which are of sebaceous gland origin, were considered separately.

RESULTS

The recovery of *P. acnes* and the quantity and composition of skin surface lipids are summarized in Table 1. *P. acnes* was recovered from more than 90% of subjects on the face and upper trunk (areas rich in sebaceous glands). On the abdomen, the recovery rate was only 60%, and the geometric mean count was low (138 colony-forming units per cm^2, compared to 64,565 in the upper trunk and 371,535 on the face). Likewise, on the upper extremities, *P. acnes* was recovered from only 61% of subjects, with a geometric mean count of 41 colony-forming units per cm^2, and from only 21% of lower extremities, with a mean count of 3 colony-forming units per cm^2.

The 3-h accumulation of lipid on the skin surface varied considerably, from 105 $\mu g/cm^2$ for the face to 52 $\mu g/cm^2$ for the upper trunk to 10.2 $\mu g/cm^2$ for the lower extremities. The number of *P. acnes* organisms for a given body area showed a significant correlation with the total amount of lipid production ($r = 0.77$; $P < 0.001$). Significant correlations were also found between *P. acnes* and the amount of di- and triglycerides ($r = 0.68$; $P < 0.001$), squalene and wax esters ($r = 0.72$; $P < 0.001$), cholesterol and cholesterol esters ($r = 0.67$; $P < 0.001$), and free fatty acids ($r = 0.67$; $P < 0.001$).

Significant differences in the amounts of lipids derived mainly from sebaceous glands (squalene, wax esters, and glycerides) were seen from one body area to another, as illustrated in Table 1.

P. acnes hydrolysis of glycerides to fatty acids previously has been shown to account for 95% of the free fatty acids recovered from the skin surface (7). The relationship of *P. acnes*, total lipid, and the hydrolysis of sebaceous gland triglycerides to free fatty acids is shown in Fig. 1. A significant correlation exists between *P. acnes* and free fatty acids ($r = 0.67$), and between *P. acnes* and glycerides plus free fatty acids ($r = 0.75$).

DISCUSSION

The intent of this study was to determine whether any correlation exists between the total number of *P. acnes* organisms and the amount of sebum produced at various skin sites. Our results demonstrate that such a correlation does exist. The highest density of *P. acnes* was found on the face, an area rich in sebaceous glands. On the upper trunk, an area with abundant sebaceous glands but fewer found than on the face, the geometric mean was approximately one order of magnitude lower. The amount of lipid delivered to the skin surface of the face in a 3-h period was twice as high as for the upper trunk. On areas with only scattered sebaceous glands, such as the lower trunk and the extremities, the prevalence of *P. acnes* was much lower than for the face and upper trunk, and the average num-

TABLE 1. *P. acnes populations, lipid secretion, and composition in five cutaneous environments*

Site	No. of sites sampled	*P. acnes*		Lipid concn[a] (μg/cm² per 3 h)				
		Prevalence	Density per cm²[b]	Total lipid	Free fatty acid	Diglycerides and triglycerides	Squalene and wax esters	Cholesterol and cholesterol esters
Face	65	99	5.57 (0.22)	105.5 (5.1)	17.1 (1.6)	32.4 (3.1)	48.5 (3.1)	6.6 (0.3)
Upper trunk	34	94	4.81 (0.34)	52.1 (5.1)	9.7 (1.7)	16.9 (3.0)	20.5 (1.8)	3.8 (0.3)
Lower trunk	43	60	2.13 (0.35)	23.5 (2.2)	2.4 (0.4)	10.9 (1.2)	7.9 (1.0)	2.5 (0.2)
Upper extremities	49	61	1.61 (0.24)	18.7 (2.6)	2.3 (0.3)	8.0 (1.1)	6.7 (1.3)	2.3 (0.2)
Lower extremities	38	21	0.47 (0.16)	10.2 (0.7)	1.0 (0.1)	5.0 (0.4)	2.1 (0.2)	1.9 (0.2)

[a] Mean quantity of excreted lipid (standard error of the mean).
[b] Log mean of all *P. acnes* per square centimeter (standard error of the mean).

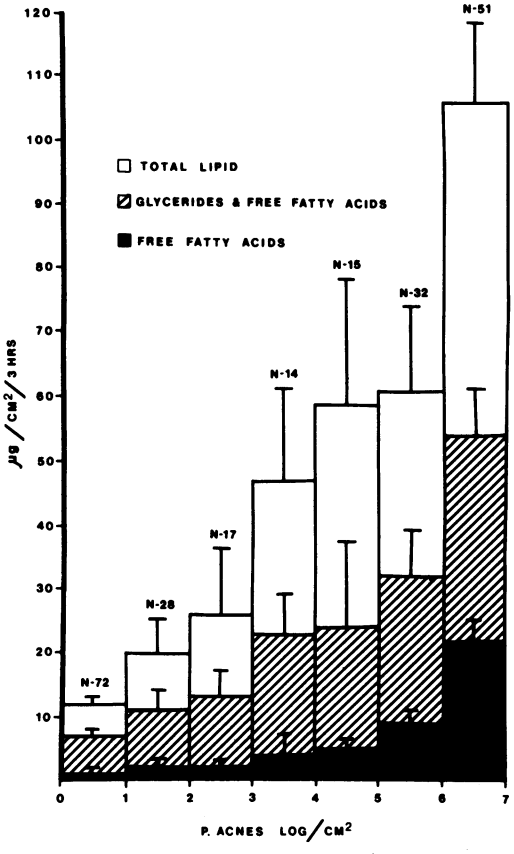

FIG. 1. *Correlation of mean P. acnes populations (±95% confidence limits) with total lipid, glycerides plus free fatty acids, and free fatty acids secretion.*

ber was significantly lower.

The total number of *P. acnes* organisms significantly correlated with all the fractions of skin surface lipids including diglycerides, triglycerides, squalene, wax esters, cholesterol, and cholesterol esters. Cholesterol and its esters are primarily derived from the epidermis (3) and as such would not likely be important substrates for *P. acnes*, which resides in the deeper recesses of sebaceous follicles. Wax esters and squalene are primarily derived from sebaceous gland secretions and possibly could be utilized by *P. acnes*. No in vitro data exist to clarify this question. The correlation of *P. acnes* with glycerides and with products of glyceride hydrolysis suggests that glycerol or fatty acids may be important substrates for *P. acnes*. The in vitro demonstration that *P. acnes* can utilize glycerol (10) helps support this possibility, as does the finding that acne patients, who have increased levels of *P. acnes* along with increased sebum production, have lowered levels of skin surface glycerol than do controls or acne patients treated with antibiotics (11). The findings of increased sebum production with lower levels of glycerol when associated with higher densities of *P. acnes*, coupled with increasing glycerol levels as a result of antibiotic suppression of *P. acnes*, strongly suggests that glycerol is an important substrate for *P. acnes* growth.

Many factors have been shown to contribute to the cutaneous microenvironment and affect the kinds and numbers of bacteria on human skin. The availability of water, for example, greatly influences the number of organisms. Areas rich in mosture-yielding eccrine sweat glands, such as the axilla, groin, and feet, support higher numbers of organisms than relatively dry areas, such as the extremities (1, 4, 9). The role of water as an environmental determinant has also been experimentally demonstrated. Artificial hydration of normally dry areas (forearms) produces an explosive growth of the resident organisms and an eventual shift to a flora similar to that of the axilla (4). In the past, skin lipids have been studied mainly from the point of view of their ability to inhibit growth of organisms, particularly streptococci (4). In this study we have shown that a strong correlation exists between the density of *P. acnes* and the amount of sebum produced in various body regions. Sebum

thus appears to act as a positive force determining *P. acnes* colonization and proliferation.

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