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The Microbiology of the Human Axilla and Its Relationship to Axillary Odor

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The axillary microflora of 229 subjects was characterized quantitatively and the results correlated with whether the odor was pungent body odor or instead a faint "acid odor." The axillary flora was found to be a stable mixture of *Micrococcaceae*, aerobic diphtheroids and *Propionibacteria*. Significantly higher numbers of bacteria were recovered from the axillae of those with pungent axillary odor than in those with acid odor. Aerobic diphtheroids in high numbers were recovered in all subjects having typical body odor. These included lipophilic as well as large-colony diphtheroids.

When droplets of apocrine sweat placed on the forearm were inoculated with various bacteria which reside in the axilla, only diphtheroids generated typical body odor. Cocci produced a sweaty odor attributable to isovaleric acid.

Axillary odor is a distinctive malodorous scent of adults, popularly called "body odor." It is the dominant note among the odors originating in various body regions. The ultimate source of axillary odor is apocrine sweat which when it appears on the surface is both sterile and odorless [1]. The pungent odor is generated when resident microorganisms interact with apocrine sweat [1]. There is dispute regarding the types of bacteria capable of liberating the odiferous substances. At first it was thought that a wide range of gram positive and gram negative bacteria could produce the characterisic malodor [2]. Shehadeh and Kligman, however, demonstrated that only gram positive organisms had this capability [3].

It is not known whether there are quantitative or qualitative differences in the axillary microflora of individuals with different degrees of body odor. The object of this work was to compare the microflora of individuals without the acrid odor to those who were intensely odiferous. We found that the ability to produce the typical malodor is limited to aerobic diphtheroids.

MATERIALS AND METHODS

Subjects

Healthy, young-adult males and females aged 18 to 26 yr served as volunteers. Informed written consent was obtained. Deodorants and antiperspirants were prohibited for 1 week prior to, and throughout, each study. Each subject was given a nonmedicated soap (Ivory) for washing.

Microbiological Methods

Quantitative cultures were obtained by the detergent scrub technique of Williamson and Kligman [4]. This method employs 1 ml of 0.1% Triton-X-100 which is pipetted into a sterile glass cylinder placed in the axilla; the fluid is stirred vigorously for 1 min with a Teflon rod and removed. This procedure is repeated and the 2 samples pooled. Tenfold dilutions were made in 0.05% buffered Triton and drop-plated in duplicate on the following media: (1) Trypticase Soy Agar (TSA); (2) TSA with lecithin and Tween-80; (3) MacConkey's selective agar for gram negative organisms; (4) Sabouraud's media for yeast and fungi; (5) Brain Heart Infusion agar with 1% Dextrose and 0.1% Tween 80 for anaerobic diphtheroids. The first 4 media were incubated aerobically at 37° for 48 hr while the Brain Heart Infusion was incubated anaerobically in a Gas-Pak jar system at 37° for 7 days.

Identification of *Micrococcaceae* into *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Micrococcus* species was accomplished on 200 strains from 40 subjects using the scheme of Baird-Parker [5]. Subsequently, we went no further than recognition of *Micrococcaceae*. The classification of diphtheroids is still far from satisfactory. It has been our practice, for convenience, to distribute these into one of 2 broad groupings: (1) large colony and (2) lipophilic when the addition of Tween 80 enhanced growth. The classification of diphtheroids is currently being strengthened by application of sophisticated techniques such as cell wall analysis [6]. This work and that of Sharpe et al [7] makes it clear tht aerobic diphtheroids belong to 2 genera—*Corynebacterium* and *Brevibacterium* [6,7]. The genus *Corynebacterium* is characterized by meso-diamino-pimelic acid, arabinose, mannose, galactose and corynemycolic acid [6]. *Brevibacterium* can be distinguished by the production of methanethiol, DNAase activity and by the presence of proteolytic activity in gelatin and milk agar [7]. We examined axillary isolates of 10 "lipophilic" and 10 large colony diphtheroids as follows: Corynemycolic acid presence was determined by thin-layer chromatography as described by Goodfellow, Collins, and Minnikin [8]. The chain length of mycolic acid was confirmed by gas chromatography. Production of methanethiol was assessed by incubation in TSA supplemented with 0.1% L-methionine and 0.1% Tween 80 and assessment of the culture headspace by direct sampling using a Perkin-Elmer 3920 gas chromatograph equipped with a flame photometric detector. DNAase activity was determined on DNAase agar (Difco).

Gram negative rods were identified using the API Enteric system. *Propionibacteria* were tested for bacteriophage susceptibility (ATCC 29399B), indole and nitrate production and clearing of gelatin and milk as previously described by McGinley, Webster, and Leyden [9].

Study Designs

I. Quantitative bacteriology of the axilla: In the first study, we cultured 24 subjects, 12 males and 12 females on 5 consecutive days. Each sex was equally divided into left and right handedness.

In 6 subjects, 5 hairs from each axilla were individually cultured by vortexing each hair vigorously in 0.1% Triton X-100, followed by 10-fold dilutions in 0.5% buffered Triton.

Subsequently, 205 young adults (128 males, 77 females), were cultured on a single occasion (both axillae in 43, one in the other 162).

II: Correlation of quantitative bacteriology and axillary odor: Three observers independently examined 23 males and 20 females dividing these into 2 polar categories of odiferousness.

1. Axillary odor. The offensive pungent smell so typical of body odor and (2) no odor or a faint "acidy" odor. Isovaleric acid has been shown to produce a "sweaty" odor [12]. Henceforth, acid odor is the term we shall use for our second category. Twenty males had pungent axillary odor while 3 were classified as acid odor. Seven females had axillary odor and 13 were acid.

Quantitative cultures were obtained from each axilla 24 hr after washing with a nonmedicated soap.

III: Relationship of odor to skin surface lipids: Surface skin lipids were obtained from the above 23 males and 20 females. The axillae

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Abbreviations:
TSA: Trypticase Soy Agar

were thoroughly washed with 0.5% Triton-X-100, followed by wiping with gauze saturated with hexane to remove surface lipids and debris. Skin surface lipids were collected three hours later as follows.

One ml of hexane was pipetted into a glass cylinder with an area of 3.8 sq cm in the center of the axillary vault, agitated for 30 seconds and removed. The procedure was repeated with a second ml of hexane. The pooled sample was immediately passed through an 0.22 μ millipore filter to remove bacteria and debris, evaporated under nitrogen and stored at −20°C. Samples were reconstituted with 0.05 ml. of hexane and processed by the thin-layer chromatography method of Downing [10]. An internal standard of methyl nervonate was used to enable determination of the amounts of the various classes of lipids [11].

IV. In vivo *inoculation of bacteria and apocrine Sweat:* The volar forearms of 10 subjects were sterilized by compresses of 70% ethanol for 2 min. Sterilization was verified by culture. After evaporation, 3 μl of sterile apocrine sweat was spread over a small area which was then inoculated with 0.1 ml of a suspension of *S. saprophyticus* (4 sites), *S. epidermidis* (5 sites), lipophilic diphtheroids (10 sites), large colony diphtheroids (9 sites), and *E. coli* (2 sites). The apocrine sweat was collected by micropipette from droplets which appeared after the intradermal injection of 1:2000 adrenalin into the degermed axillary vault. All strains were isolated from human axillae; the inocula were standardized to 2×10^5 organisms per ml. An additional site was inoculated with 3 μl of apocrine sweat and 0.1 ml of 0.025% aqueous magnesium omadine (a broad spectrum antimicrobial agent). Each site was covered with 5 × 5 cm squares of impermeable plastic film (Saran) and secured by adhesive tape. Twenty-four hours later, the sites were evaluated organoleptically by 3 observers and then cultured quantitatively. An aliquot of the pooled apocrine sweat was cultured to assure bacterial sterility.

RESULTS

The initial study showed the axillary flora to be stable and reproducible. The geometric mean count for the 24 subjects was 420,000/cm^2 on day one, 380,000 on day two, 300,000 on day three, 590,000 on day four and 470,000 on day five. The coefficient of variation was 13%. Comparison of the axillary flora in terms of right and left handedness did not reveal any significant differences. The geometric mean for the left axilla in the 12 left-handed subjects was 308, 390/cm^2 compared to 433,111 for the right-handed subjects. Also, there was no difference between the right and left axilla of 43 subjects; the geometric mean count for the left was 361,275 compared to 328,473 for the right. Subsequently, cultures were obtained from only one axilla.

Individual hairs contained trivial numbers of bacteria, averaging only 327 aerobic organisms and four anerobic organisms. Because of these low counts, subsequent cultures were done without prior shaving of the axilla.

The results of the quantitative bacteriological survey of 205 subjects are found in Table I. *Micrococcaceae* were found in all subjects, lipophilic diphtheroids were recovered in 85% of males but only 66% of females ($p < 0.05$). Large colony diphtheroids were found in 26% and 25% respectively. Gram negative organisms were found in 20% of males and 19% of females. *Propionibacteria* were recovered from 70% of males but only 47% of females ($p < 0.05$). *P.acnes*, *P. granulosum* and *P. avidum* occurred in approximately equal numbers.

The strains of *Micrococcaceae* were identified as follows: 10% were *Staphylococcus aureus*, 51% were *Staphylococcus epidermidis*, 29% were *Staphylococcus saprophyticus* and 10% were *Micrococcus* species. Of the 41 gram negatives, 22 were *Escherichia* species, 15 *Klebsiella*, 11 *Proteus*, 8 *Enterobacter* and 5 *Acinetobacter*.

All 20 diphtheroids were classified as *Corynebacterium* on the basis of the presence of corynemycolic acid, and the absence of proteolytic activity and methanethiol production.

Figure 1 displays the bacteriological differences in the 2 odor groups. The persons with typical axillary odor had a significantly higher number of organisms, 1,300,000/cm^2 vs. 480/000 cm^2 ($p < 0.05$). More striking, however, were the marked differences in composition of the flora. Every subject with axillary odor had lipophilic diphtheroids (geometric mean of 810,000/cm^2 compared to only 55% prevalence with a mean of only 53,000 in the "acid" odor group. Large colony diphtheroids were found in 52% of axillary odor subjects (mean 250,000/cm^2) while only 9% of "acid" odor subjects had these organisms in small numbers (1,600/cm^2). *Propionibacteria* were found in 70% of those with axillary odor (mean of 36,000/cm^2) and in 49% of acid odor subjects (mean 36,000/cm^2).

The results of incubating apocrine sweat and bacteria on the forearms are shown in Fig 2. The typical, pungent axillary odor

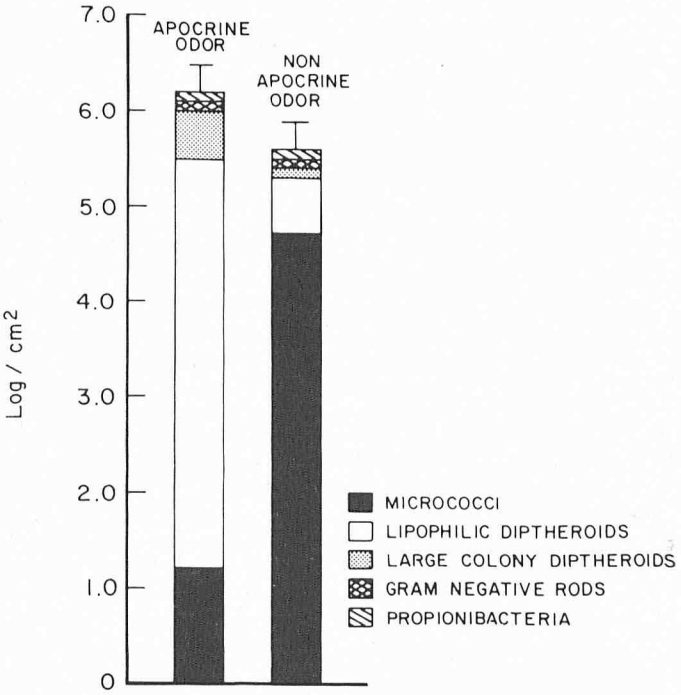

FIG 1. Quantitative bacteriology of subjects with intense axillary odor and those with absent or faint odor. Bacteriology is expressed in terms of the logarithm (base 10) per sq. cm.

TABLE I. *Prevalence and density of axillary resident bacterial flora*

	Males (N = 128)			Females (N = 77)		
	Prevalence	Density[a]	SEM[b]	%	Density	SEM
Aerobic flora	100	6.9×10^5	0.06	100	8.9×10^5	0.09
Micrococcaceae	100	1.2×10^5	0.07	100	3.6×10^5	0.11
Lipophilic diphtheroids	85	2.5×10^5	0.09	66	2.3×10^5	0.14
Large colony diphtheroids	26	2.7×10^4	0.21	25	3.7×10^4	0.24
Gram negative rods	20	2.3×10^3	0.25	19	2.1×10^3	0.31
Propionibacteria (total)	70	5.1×10^3	0.21	47	1.7×10^4	0.30
P. acnes	47	7.2×10^3	0.29	30	1.8×10^4	0.38
P. avidum	34	4.2×10^3	0.22	21	1.5×10^4	0.43
P. granualosum	8	4.1×10^3	0.32	5	4.5×10^3	0.28

[a] Geometric mean per square centimeter.
[b] Standard error of the mean, expressed in logarithms.

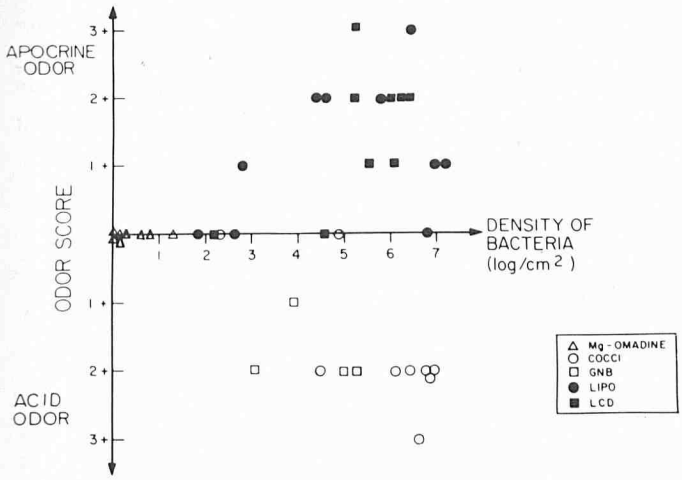

FIG 2. Results of *in vivo* inoculation of apocrine sweat and various organisms; cocci included *S. saprophyticus* and *S. epidermidis*, lipo refers to lipophilic diphtheroids; *LCD* refers to large colony diphtheroids; *GNB* refers to gram negative bacteria; mg-omadine is a broad spectrum anti-microbial agent. Odor production was evaluated organoleptically.

TABLE II. *Skin surface lipids in axilla*

	Strong odor	Faint or absent odor
Total amount of Lipid μg/cm²/3 hr	61.4	55.0
Cholesterol	9.7%	7.1%
Sterol esters	8.8%	7.2%
Triglycerides	27.3%	23.7%
Diglycerides	2.7%	3.4%
Fatty acids	17.4%	20.3%
Wax esters	21.4%	21.7%
Squalene	12.7%	16.4%

was found only when either lipophilic or large colony diphtheroids were inoculated with apocrine sweat and with 2 exceptions, only when their density was in excess of $10^4/cm^2$. *S. epidermidis*, *S. saprophyticus*, and gram negative organisms produced odors of a quite different quality, easily distinguishable from classic body odor. Micrococci uniformly generated a distinctive "sweaty, acid odor."

The results of the skin surface lipid analysis are presented in Table II. No significant differences either in the amount of lipid or in composition was detected in the 2 groups. However, in the surface lipid the percentage of cholesterol was significantly higher than that in sebum rich areas such as the face. Stimulated by this finding, we processed 5 μl of sterile apocrine sweat by thin-layer chromatogrphy; [10] 76.2% of the lipid present was cholesterol, 0.9% was cholesterol esters, 3.6% was wax esters, 0.2% was squalene, 19.2% ws triglyceride and fatty acids.

DISCUSSION

The axillary flora comprises a stable population of aerobic and anerobic organisms, at densities generally between 500,000/cm² and 1,000,000/cm². Day-to-day variations were insignificant. The microflora was quantitatively and qualitatively the same in right and left axillae and was not affected by handedness or sex. In an extensive quantitative survey of the axillary flora, Prince and Rodger also found a very stable day to day flora [13]. Their results differ in two particulars: They found diphtheroids (not classified further) in all subjects. Moreover, our 10% prevalence of *S. aureus* is far lower than their 38% for the winter months and 73% in the summer months. Aly and Maibach surveyed multiple intertriginous areas of male prisoners. They found a higher level of diphtheroids than we did in the axilla (mean counts $1.3 \times 10^7/cm^2$ for nonlipophilic and $3.0 \times 10^6/cm^2$ for lipophilic strains [14]. These higher densities may reflect environmental and hygienic differences. Like us, they found *S. aureus* in 12% quantities. While the presence of hair contributes to the intensity of axillary odor [1], this does not appear to involve bacteria. The number of bacteria in hair was very low. Hairs are not a good substrate for bacterial growth. They probably serve to trap malodorous volatiles generated on the skin surface and also act as a dispersal device [1].

Our results point to diphtheroids as the organisms responsible for axillary odor. Both lipophilic and large-colony diphtheroids were more frequent and more numerous in persons with typical axillary odor. No subject with body odor lacked diphtheroids. Final proof of the odor-liberating capacity of the diphtheroids was forthcoming when both types of diphtheroids were incubated with apocrine sweat on the forearm. None of the other test organisms produced typical axillary odor. The sweaty odor of isovaleric acid was present when micrococci were incubated with apocrine sweat. We did not test *Propionibacteria* because these anaerobic organisms reside in the deep recesses of sebaceous follicles. If *Propionibacteria* were involved, apocrine sweat should be odorous by the time it reaches the surface and it clearly is not [1].

We strengthened the case for aerobic diphtheroids by trying to create body odor in 2 subjects whose axillae completely lacked these organisms. We occluded their axillae for 48 hr with wet gauze sealed under tape. The result was only an intensification of the original "acid" odor, presumably owing to expansion of cocci. Both subjects produced droplets of apocrine sweat after subcutaneous injection of epinephrine.

A current focus of our research is the identification of the odorous chemicals and their biosynthesis. We have already reported the presence of 2 steroids, dehydroepiandrosterone sulfate and androsterone sulfate while others have found 5x-androst-16-en-3-one and 5x-androst-16-en-3x-ol which have odor characteristics very similar to the natural axillary odor [15–18]. In this regard, the finding in apocrine sweat of large amounts of cholesterol, which can serve as the building block for steroid synthesis [19] is of particular interest.

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Announcement

The International Advanced Hair Replacement Symposium will be held in Birmingham, Alabama, U.S.A., February 3–4, 1982. It is sponsored by the American Academy of Facial Plastic & Reconstructive Surgery, Inc. Multidiscipline faculty composed of dermatologists, otolaryngologists & plastic surgeons. Registration fee: $420. Inquiries can be made to: D. B. Stough, III, M. D., Program Director, The Stough Dermatology & Cutaneous Surgery Clinic, P. A., Doctors Park, Hot Springs, Arkansas 71901, U.S.A.