Ecological effects of a deodorant and a plain soap upon human skin bacteria

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(Received 3 May 1976)

SUMMARY

The effects of a commercial trichlorocarbanilide-containing deodorant soap and a commercial plain soap upon the cutaneous flora of individuals were compared. Using a cross-over design, 21 volunteers (10 women and 11 men) washed their forearms at least once a day with one soap for 3 weeks and then switched soaps for another 4 weeks use. By analysis of variance no significant difference in total colony counts was noted among individuals in their use of the two soaps. With the exception of individual variation, neither sequence of use, sex, nor any combination was influential. However, in 20 of 21 subjects an alteration in the composition of skin flora was observed. The deodorant soap, which in six cases increased total flora, tended to reduce or eliminate diphtheroids in 12 of 17 carriers (71%). Fewer kinds of bacteria were also noted. More *Staphylococcus epidermidis* was seen with the plain soap, but washing with the deodorant soap seemed to favour *Acinetobacter calcoaceticus* and *Micrococcus luteus*. The impact of this alteration and the use of total counts to measure effectiveness of deodorant soaps were brought into question.

INTRODUCTION

Since the role of skin flora in cutaneous infectious disease and immunity is poorly understood, the purposeful alteration of the microbial habitat by deodorant soaps should be viewed with caution. The rationale for these antimicrobial soaps is the reduction of Gram-positive bacteria whose metabolic activities are responsible for the formation of pungent body odour in the several anatomically restricted areas with apocrine sweat glands (Shehadeh & Kligman, 1963). Although these soaps are touted as surgical scrubs or deodorants, an additional effect, the decline of infectious skin diseases, has been reported (Duncan, Dodge & Knox, 1969; MacKenzie, 1970). What impact these soaps have on normal flora and their interactions is as yet unknown, for only a few studies have examined skin ecology (Ehrenkranz, Taplin & Butt, 1967; Evans, Rendtorff, Robinson & Rosenberg, 1973; Voss, 1975). None have thoroughly focused on the differing ecosystems of individuals, and only two (Evans et al. 1973; Voss, 1975) have quantitatively investigated alterations in the composition of cutaneous flora. Furthermore, with the recent ban in the United States of hexachlorophene in non-prescription drugs ну**с** 78 I

and cosmetics, some of the previous studies are not directly applicable to today's products.

In this report the effects of a commercial trichlorocarbanilide-containing deodorant soap and a commercial plain soap upon the cutaneous flora of individuals are compared. Although changes in total colony counts were not significant, the qualitative alteration of resident flora was both common and profound.

MATERIALS AND METHODS

Volunteers

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Eleven males and 10 females of various races, all members or family of the laboratory staff, freely consented to this study. Subjects were in good health and had washed consistently with either a deodorant or a plain soap of their choice for at least 3 months.

Climate

The investigation took place in San Francisco in autumn, which was dry and cool, with temperature range of $7-20^{\circ}$ C.

Soaps

We compared the effects of two commercially available soaps: deodorant bars (Dial^R, Armour-Dial) containing 1.5% 3,4,4'-trichlorocarbanilide as the active antimicrobial agent and a plain compound soap (Ivory^R, Procter and Gamble).

Experimental design

A cross-over design was used. Volunteers were first assigned either the deodorant or the plain soap, depending on the type of soap then in use by the subject. Thus the 12 (5 males and 7 females) deodorant-soap and 9 (6 males and 3 females) plain-soap users would have a common beginning and avoid a possible and unnecessary ecological shock to their flora before the experiment. Both forearms were washed at least once a day during the study, under supervision whenever possible; additional washing was *ad lib*. Contact with any other soap or antimicrobial agent was not permitted. Test soaps were also placed at laboratory wash basins and bathrooms, and subjects were daily reminded of the experimental requirements. Every other day during the third week the mid-volar forearm opposite the dominant hand was sampled for a total of 3 times. Subjects then switched soaps. Since little change in flora was detected after 2 weeks, soap use was extended for an additional 2 weeks. Three samples from the forearm were obtained during the fourth week.

Sampling and identification

Skin sites were sampled with our linear-friction device using phosphate-buffered Triton X-100 (Bibel & Lebrun, 1975a), and all aerobic bacterial isolates on trypticase soy agar were enumerated and identified by a replica-plating procedure

Source	s.s.	D. F.	M.S.	F ratio
Between	10.21	20		_
Sequence of use	0.02	1	0.02	0.04
Sex	0.42	1	0.42	0.74
Sequence \times sex	0.00	1	0.00	0.00
Individuals	9.77	17	0.57	2.71*
Within	4 ·14	21		—
Soap	0.14	1	0.14	0.67
$Soap \times sequence$	0.02	1	0.02	0.10
Soap \times sex	0.03	1	0.03	0.14
$Soap \times sex \times sequence$	0.37	1	0.37	1.76
Error	3.58	17	0.21	
	* P < 0	-05.		

Table 1. Analysis of variance: comparison of total counts of skin bacteria after use of a deodorant and a plain soap

(Bibel, Smiljanic & Lebrun, 1975). *Micrococcaceae* were classified to the biotype level according to *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974), but owing to the lack of a suitable taxonomic scheme (Bibel & Lebrun, 1975), cutaneous diphtheroids were grouped. Anaerobic propionibacteria were initially examined, but were found both infrequently and in extremely low numbers (1-2 colonies/plate). With the additional burden of technical difficulties, the study was confined to the aerobic flora only.

Statistical analysis

Tallies of total flora in the three-way factorial design were transformed into logarithms for analysis of variance. The interactions of soap, sex, sequence of use, and their combinations were examined. We interpreted and compared qualitative data primarily with regard to the individual, but certain pooled data, when enumerated, were amenable to analysis by Student's t test.

RESULTS

When compared with the results obtained with a plain soap, the use of the antimicrobial deodorant soap, according to the analysis of variance (Table 1), did not significantly reduce the total colony count of aerobic bacteria of volunteers. Neither the sex of the subjects, the sequence of soap use, nor any combination was statistically influential. Only individual differences were important (P < 0.05).

An examination of individual data did show that in 13 cases the geometric mean of the flora was lower after use of the deodorant soap, but rarely was the count decreased to less than 10% and the reduction was generally to between 50 and 30%. The differences in kinds and proportions of cutaneous flora were clearly apparent. Indeed, we detected alteration of bacterial carriage in 20 of 21 subjects. Table 2 lists the types of changes and their incidence rate. The alteration of diphtheroid colonization was the most significant effect of the deodorant soap. Over 70% of the individuals who consistently harboured diphtheroids had

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	Total pop	ulation	Carrier pop	ulation†
Ecological effect of deodorant soap	Ratio	%	Ratio	%
Geometric means of total flora				
Deodorant so a < plain so a pla	13/21	62		
Deodorant soap > plain soap	6/21	29		<u> </u>
Deodorant soap = plain soap	2/21	10		
Changes in total kinds of flora	11/21	52		
Simplification	7/21	33		
Diversification	4/21	19		
Diptheroids				
Reduction	12/21	57	12/17	71
Partial decrease	7/21	33	7/17	41
Elimination	5/21	24	5/17	29
No change	4/21	19	4/17	24
Increase	1/21	5	1/17	6
Acinetobacter calcoaceticus				
Decrease	1/21	5	1/4	25
Increase	3/21	14	3/4	75
Micrococcus luteus				
Decrease	2/21	10	2/13	15
No change	2/21	10	2/13	15
Increase	9/21	43	9/13	69
Staphylococcus saprophyticus				
Decrease	3/21	14	3/15	20
No change	4/21	19	4/15	27
Increase	8/21	38	8/15	53
Staphylococcus epidermidis				
Decrease	7/21	33	7/19	37
No change	8/21	38	8/19	42
Increase	4/21	19	4/19	21
Recovery of deodorant soap flora profile	0/6	0		_
Recovery of plain soap flora profile	5/6	83	_	—

Table 2. Changes in cutaneous flora by a trichlorocarbanilide deodorant soap

† Organism present in minimum of two of three test samples.

a reduction of these micro-organisms. Sometimes the magnitude of decrease was only of the order of $\frac{1}{3}-\frac{1}{2}$, yet 29% of carriers lost diphtheroids completely. Pooling the subjects' diphtheroid data, the difference in carriage was found to be statistically significant (P < 0.001, Fig. 1). Staphylococcus epidermidis, especially biotype 1, was another organism whose loss or diminution seemed to be associated with the variety of soap used.

A reduction of certain micro-organisms plus the continuance of total bacterial counts at about the same level requires that other micro-organisms are substituted, the composition of the flora is simplified to fewer types, or a combination of both phenomena occurs. Such events were observed. *Micrococcus luteus* and *Acinetobacter calcoaceticus* increased proportionally or even existed solely with use of the deodorant soap. However, when individual data were pooled, differences in



Fig. 1. Effect of different soaps on the presence of M. *luteus* and diphtheroids on human skin. Horizontal bar indicates geometric mean. Only differences in diphtheroid populations are significant (P < 0.001).



Fig. 2. Examples of soap-induced alterations of the skin flora of individuals. (A) Bars show the composition of bacterial flora of one volunteer at each of the six sampling times. Subject used the first soap for 3 weeks, and washed with the second soap for 4 weeks. (B) Results of a second volunteer. Flora indicated by key in previous diagram.

		Tabl	le 3. Compa	rison of studies us	sing deodo	rant soaps.		
Study	Deodorant Soap†	Subjects	Interval	Sequence‡	Sampling Method	Samples	Sites	Results
Hurst et al. (1960) (8)	TBS, TCS TCC, G-11	12, Latin square	1 week	$\begin{array}{c} C & \downarrow D \\ C & \downarrow C \\ C & \downarrow C \\ \end{array}$	Multiple basin	Daily, 2nd and 3rd week	Hand	Reduction of flora
Kooistra et al. (1966) (9)	TBS/TFC/TCC	500	4 days	C → D	Multiple basin	1, each soap	Hand	Reduction of flora
	TBS/TFC/TCC	150, D 150, C	2 weeks	C ↑ C C ↑ C	Multiple basin	1, each soap	Hand	Reduction, $P < 0.05$
	TBS/TFC/TCC, TCC/G-11	160, D ₁ 160, D ₃ 160, C	2, 4 weeks	$\begin{array}{c} \mathbf{C} \leftarrow \mathbf{D}_{\mathbf{I}}\\ \mathbf{C} \leftarrow \mathbf{D}_{\mathbf{S}}\\ \mathbf{C} \leftarrow \mathbf{C}\\ \mathbf{C} \end{array}$	Multiple basin	1, each soap	Hand	Reduction, $P < 0.05$
Ehrenkranz <i>et al.</i> (1967) (6)	TBS/TCC	1	Days, 2 weeks	C ↑ D C ↑ 1D		6/2 months	Toewebs, both feet	Loss of gram-positive cocci, gain of gram- negative bacilli
Dravnieks, et al. (1968) (3)	TCC/G-11	4	2 weeks, 2, 3 days	$\mathbf{C} \rightarrow \mathbf{D} \rightarrow \mathbf{C}$	Cup scrub	1, each soap	Axilla	Flora is reduced; recovery at 72 h
Duncan <i>et al.</i> (1969) (4)	TBS/TFC/TCC	2550, Crossover	9 months	$\begin{array}{c} \mathbf{C} \ \downarrow \mathbf{D} \\ \mathbf{C} \ \downarrow \mathbf{D} \ \downarrow \mathbf{C} \end{array}$	I	I	Ι	Skin infections reduced, $P < 0.01$
MacKenzie (1970) (10)	TCC/G-11	602, D 599, C	6 months	$\begin{array}{c} X \\ X \\ X \\ \downarrow C \end{array}$		I	ļ	Skin infections reduced, $P < 0.01$
Wilson (1970) (21)	TCC, G-11	21	4 weeks	$\mathbb{C} \to \mathbb{D}_1 \to \mathbb{C} \to \mathbb{D}_2$	Multiple basin	1, each soap	Hand	Reduction, $P < 0.01$
	TCC, G-11	13	4 weeks	$\mathbb{C} \to \mathbb{D}_1 \to \mathbb{C} \to \mathbb{D}_2$	Sellotape strip	1, each soap	Forearm	No change
	TCC, G-11	40	2 weeks	$C \to D_1 \to C \to D_2$	Contact plates	1, each soap	Forehead, thigh, forearm, chest, back	No change
	TCC, G-11	6		ļ	Slit-air sampler	1	Surrounding air	Significant reduction in 3
	TCC, G-11	Ward	I	1	Swabs	2/week	Nose, chest, groin	Reduction of <i>S. aureus</i> acquisition

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			Table 3 (c	ont.)				
Study	Deodorant Soap†	Subjects	Interval Sequence‡	Sampling Method	Samples	Sites	Results	E
Evans et al. (1973) (7)	G-11	15, D 15, C	1, 2 weeks $X \rightarrow D$ $X \rightarrow C$	Scrub	2, each	Toewebs, both feet	Reduction, $P < 0.01$ staphylococci and diphtheroids reduced, increase of Gram-negatives	Ecological e
Voss (1975) (18)	TCC/TFC	132, D 93, C	$2-7 \text{ months } \mathbf{X} \rightarrow \mathbf{D}$ $\mathbf{X} \rightarrow \mathbf{C}$	Cup scrub	-	Axilla, back, chest, forearm, calf, foot	Reduction except in axilla, $P < 0.05$ to P < 0.001; no increase in Gram- negatives, less S. <i>aureus</i>	ffects of soap
† G-11 = 2,2'-dil	hydroxy-3,5,6,3'	',5',6'-hexachlc	prodiphenylmethane (hexachle	orophene).	$\Gamma CC = 3,4,4'-t$	richlorocarbanilide.	TFC = 4,4'-dichloro-3-	

(trifluoromethyl) carbanilide. TBS = 3,4',5-tribromsalicylanilide. TCS = 3, 3', 4',5-tetrachlorsalicylanilide. $\ddagger C = Control soap; D = deodorant soap; X = soap unknown.$

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total numbers of *M. luteus* were not significant (P > 0.05, Fig. 1). Staphylococcus saprophyticus biotypes also increased. Although diversification of flora was occasionally seen, simplification of flora was noted in one-third of the subjects washing with the antimicrobial soap. Examples of such ecological alterations of one's cutaneous flora are presented in Fig. 2.

One month after the experiment 6 volunteers from each group who had resumed use of the initial soap were again sampled. Washing was *ad lib*. during the interim. The desire was to determine if the skin flora had returned to the earlier state. None of the subjects who came back to the deodorant soap had a return of their original flora, whereas most of those using the plain soap did recover their 'normal' flora.

As a clinical note it should be mentioned that 9 volunteers (43%) developed temporary pruritus upon switching soaps. Separating them by sequence of use, 6 of 9 subjects (67%) going from the plain to the deodorant bar and 3 of 12 persons (25%) substituting the neutral for the antimicrobial compound complained of itchiness. Inspection of their data showed that 5 of the first group and one of the latter category eventually lost their diphtheroid population. No further correlation could be found. There were no other abnormal manifestations, and all subjects remained otherwise healthy.

DISCUSSION

The experiment demonstrated a significant alteration in the composition of cutaneous flora after at least 4 weeks use of a commercial deodorant soap containing trichlorocarbanilide. The effects were highly individual, but analysis showed the following trends. The loss of one species of micro-organism was compensated by an increase in the proportion or total of another. This was often coupled with a reduction in the total kinds of flora. Washing with the deodorant soap did cause a lowering of total aerobic flora in some subjects, but the effect was generally not significant. Although 4 weeks avoidance of the deodorant soap permitted a change of flora, a subsequent month's washing with the antimicrobial soap did not return the flora to the initial condition, indicating that long-term use might have caused a secondary alteration.

The finding of a statistically insignificant reduction of total aerobic flora is contrary to the results of almost every previous study (Table 3) and demands explanation. Comparisons, however, are not easy, for the field suffers from a lack of standardization. Variation in experimental design, antimicrobial agents, sampling sites, and assay procedure is extreme. Table 3 compares these points. Many factors within the experimental design need to be considered in any analysis, and these include the number of subjects, the manner and period of soap use, whether washing is *ad lib*. or regimented, the form of controls, and the sequence of soap use. Furthermore, any residual deposit of the antimicrobial agent (Stoughton, 1966) must be weighed against its removal by washing and by the constant replacement of the epidermis, which from basal layer to desquamation takes about 4 weeks (Weinstein & van Scott, 1965).

The investigation shares many features of those listed in Table 3, but certain

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aspects of experimental design were different. Most other studies have used the hands, in testing soaps as a surgical scrub, or the axilla or toe-web, when a deodorant function was considered. The forearm was selected for this study because of the arm's general anatomical similarity to about 85 % of the body surface, regions without apocrine sweat glands. The population of skin flora may vary widely on adjacent sites (Shaw, Smith, McBride & Duncan, 1970; Williamson & Kligman, 1965) and change daily in the same area (Williamson & Kligman, 1965). This variation may occur over 10%. Most investigators have taken only one sample before and after treatment. In this study three specimens were obtained for each soap used. No previous study had monitored the alteration of flora following a change from a deodorant to a neutral soap. Because after 4 weeks the epidermis should be replenished and free of the antimicrobial compound, the results should be equivalent to the reverse sequence. Present data agree with this hypothesis, but indicate the possibility of secondary, long-term effects. Only Voss's (1975) report was similar with regard to site and sampling method, but in the present case subjects were their own controls whereas in his design two independent groups were compared. The intent of my investigation was to examine alterations in the microbial ecology of individuals. In short, the study cannot be directly compared with others and the lack of reduction of total skin flora was probably due to a combination of mentioned factors. Until there are standardized trials the degree of flora reduction by deodorant soaps remains in doubt.

This report brings out a second point, the profound alteration of the composition of skin flora by deodorant soap. The phenomenon has also been mentioned by others (Ehrenkranz *et al.* 1967; Evans *et al.* 1973; Taplin, 1972) who, testing different soaps, have noted the increase in Gram-negative flora at the expense of the gram-positive cocci. Whereas Taplin (1972) observed no change in lipophilic diphtheroids, Evans *et al.* (1973), as I, found a decrease in diphtheroids. I further observed an increase in M. *luteus* and S. saprophyticus in the quantitative study.

Knowledge of the function of normal flora in host resistance to infectious skin disease is meagre. Is the whole-body alteration of resident flora to prevent localized natural body odour safe and worth while? Is there any real advantage of deodorant soaps over neutral soaps when one follows a course of proper hygiene? What are the risks when the use of deodorant soaps is inconsistent, causing frequent wild fluctuations of flora? Taplin found that even under strict supervision the use of a deodorant soap may actually be detrimental, for it seemed to be correlated with increased streptococcal pyoderma (personal communication); yet other workers indicated that skin infections may be diminished (Duncan *et al.* 1969; MacKenzie, 1970). More work is needed to answer these questions and to determine whether or not the long-term alteration of one's normal flora by deodorant soaps is universally safe or proper.

I thank David J. Lovell and Roko J. Smiljanic for their excellent technical assistance and James B. Clarke for the statistical analysis of the data.

REFERENCES

- BIBEL, D. J. & LEBRUN, J. R. (1975). Changes in cutaneous flora after wet-occlusion. Canadian Journal of Microbiology 21, 496.
- BIBEL, D. J., SMILJANIC, R. N. & LEBRUN, J. R. (1975). A replica-plating method for the identification of Micrococcaceae. Canadian Journal of Microbiology 21, 1676.
- BUCHANAN, R. E. & GIBBONS, N. E. (ed.) (1974). Bergey's Manual of Determinative Bacteriology, 8th ed. Baltimore: Williams & Wilkins.
- DRAVNIEKS, A., KROTOSZYNSKI, B. K., LIEB, W. E. & JUNGERMANN, E. (1968). Influence of an antibacterial soap on various effluents from axillae. *Journal of the Society of Cosmetic Chemists* 19, 611.
- DUNCAN, W. C., DODGE, B. G. & KNOX, J. M. (1969). Prevention of superficial pyogenic skin infections. Archives of Dermatology 99, 465.
- EHRENKRANZ, N. J., TAPLIN, D. & BUTT, P. (1967). Antibiotic-resistant bacteria on the nose and skin: colonization and cross-infection. Antimicrobial Agents and Chemotherapy-1966, 255.
- EVANS, Z. A., RENDTORFF, R. C., ROBINSON, H. & ROSENBERG, W. (1973). Ecological influence of hexachlorophene on skin bacteria. *Journal of Investigative Dermatology* 60, 207.
- HURST, A., STUTTARD, L. W. & WOODROFFE, R. C. S. (1960). Disinfectants for use in bar soaps. Journal of Hygiene 58, 159.
- KOOISTRA, J. A., BANNAN, E. A. & CARTER, R. O. (1966). Use of human subjects for product evaluation: an evaluation of antibacterial soap bars. *Journal of the Society of Cosmetic Chemists* 17, 343.
- MACKENZIE, A. R. (1970). Effectiveness of antibacterial soaps in a healthy population. Journal of the American Medical Association 211, 973.
- SHAW, C. M., SMITH, J. A., MCBRIDE, M. E. & DUNCAN, W. C. (1970). An evaluation of techniques for sampling skin flora. *Journal of Investigative Dermatology* 54, 160.
- SHEHADEH, N. H. & KLIGMAN, A. M. (1963). The effect of topical antibacterial agents on the bacterial flora of the axilla. Journal of Investigative Dermatology 40, 61.
- STOUGHTON, R. B. (1966). Hexachlorophene deposition in human stratum corneum: Enhancement by dimethylacetamide, dimethylsulfoxide, and methylethylether. Archives of Dermatology 94, 646.
- TAPLIN, D. (1972). The use of antibiotics in dermatology. In *Pharmacology and the Skin* (ed. W. Montagu, R. B. Stoughton and E. J. van Scott), pp. 315-323. Advances in Biology of the Skin 12.
- Voss, J. G. (1975). Effects of an antibacterial soap on the ecology of aerobic bacterial flora of human skin. *Applied Microbiology* **30**, 551.
- WEINSTEIN, G. D. & VAN SCOTT, E. J. (1965). Autoradiographic analysis of turnover times of normal and psoriatic epidermis. *Journal of Investigative Dermatology* 45, 257.
- WILLIAMSON, P. & KLIGMAN, A. M. (1965). A new method for the quantitative investigation of cutaneous bacteria. Journal of Investigative Dermatology 45, 498.
- WILSON, P. (1970). A comparison of methods for assessing the value of antibacterial soaps. Journal of Applied Bacteriology 33, 574.