

The Accumulation and Persistence of Antibacterial Agents in Human Skin

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Synopsis—With the use of daily applications of a mixture of labeled HEXACHLOROPHENE and TRICLOCARBAN (3,4,4'-trichlorocarbanilide) in a soap vehicle, the accumulation and persistence of radioactivity on the dorsal surface of the hand were measured for five days. After the fourth application the amount remaining on the skin after rinsing seemed to approach equilibrium. During any one day the amount of antibacterial ingredients decreased by 50%. The accumulation of antibacterial agents on the SKIN may explain the observation that the effect of ANTIBACTERIAL SOAP on the microflora of the skin is related to the number of exposures.

INTRODUCTION

It is a peculiarity of washing with antibacterial soaps or detergents that the antibacterial agents are deposited onto skin from vehicles whose function it is to remove substances from the skin surfaces. Although the mechanism whereby these agents are deposited is poorly understood (1, 2), the molecular structure of the deposited material (3-6) and the nature of the vehicle (7-9) are involved. Deposition of particles on skin is stated (10, 11) to be related to the psi and zeta potentials of the skin and the particles.

Several techniques have been used to measure the quantity of chemicals deposited on viable skin from different types of vehicles. Compeau

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(2) and Manowitz and Johnston (12) used organic solvents to extract antibacterial substances deposited on skin and analyzed the extracts spectrophotometrically. Fahlberg *et al.* (9) assayed the extracted agent microbiologically. Investigating material deposited in particles large enough to be observed directly, Parran (13) stripped the skin with sticky tape and under magnification observed the amount of particles adhering to the tape. Shemano and Nickerson (14) and Stoughton (15) used radioactive hexachlorophene and measured the quantity sorbed onto the skin by surface counting. Using radioactive soaps, Hopf and Burmeister (16) extracted the skin and examined the extracts for the amount of soap retained after washing.

In the work reported here, which extrapolates the method of Shemano and Nickerson (14) to humans, the accumulation and persistence of a mixture of antibacterial substances deposited from soap onto human skin were studied. It is well known that antibacterial cleansing products such as hexachlorophene soaps or detergents exert their effect in a cumulative manner, the bacterial population decreasing with frequency of use until it is more or less stabilized at a level which is characteristic for each person (12, 17–20). Our work indicates that the amount of antibacterial agents on the skin approaches equilibrium.

The composition employed in this study consisted of a soap solution containing a 1:1 mixture of hexachlorophene (2,2'-dihydroxy-3,3',5,5',6,6'-hexachlorodiphenylmethane) and triclocarban (TCC, 3,4,4'-trichlorocarbaniide),* the former compound being C-14 labeled in the methylene group and the latter C-14 labeled in the carbonyl group. A 1:1 combination of hexachlorophene and triclocarban is found in a widely used antibacterial-deodorant soap.

METHODS

The experimental work included both animal and human studies. Albino rats were used to establish a relationship between skin surface counts and absolute radioactivity present in and on the skin. It was assumed that this relationship also would apply to the human studies.

Two male and two female albino rats weighing 250 to 300 g were employed. An area on the back (approximately 6 in.²) was closely clipped and depilated† to effect complete removal of hair.

Chemicals were weighed to the nearest 0.1 mg and transferred quantitatively to a 2% soap solution; the formulations were homogenized in a

* Active ingredients in Dial® soap, Armour-Dial, Inc., Chicago, Ill.

† Nair, Carter Products, Inc., New York, N. Y.

tissue homogenizer equipped with a tight-fitting pestle until successive aliquots of the suspension gave counts reproducible to within 2% or less. Before use, the formulation was warmed to 30°C and agitated continuously with a magnetic stirrer.

The composition of the test solution is given in Table I. The activity of the preparation was determined by scintillation counting of aliquots after homogenization.

Table I
Composition of Test Solution

Ingredients	g/100 ml
Hexachlorophene	
Labeled	0.400 \equiv 311.5 μ c
Unlabeled	0.350
TCC	
Labeled	0.092 \equiv 311.5 μ c
Unlabeled	0.658
Ivory Soap ^a	2.0
Total μ c/100 ml	623.0

^a Procter & Gamble Co., Cincinnati, Ohio.

The formulation was applied 24 hours after depilation and after inspection of the animals to insure absence of abrasions or irritation. Prior to application of test material, the rats were anesthetized and circles having a diameter of 3.5 cm were circumscribed on each prepared skin site with waterfast ink. A 0.1-ml sample of the test solution was spread evenly over the test site by rubbing briskly with the tip of the delivery pipet for 3 min. At various intervals after application, ranging from 0 to 8 min in order to permit deposition of variable quantities of material on the skin, the test sites were thoroughly rinsed with tap water to remove excess test material.

Immediately after rinsing, each animal was sacrificed and the skin at each application site was excised, spread on aluminum foil, and counted using a Nuclear Radiation Detector Model D-34* (mica window mass of 1.4 mg/cm² with an effective diameter of 2.778 cm) connected to a Nuclear-Chicago Analyzer/Scaler 8725.*

Triplicate skin samples, each having an area of 0.363 cm², were taken from each counting area using a cork borer. These samples were dried and burned using the Schoeniger combustion method. The radioactive

* Nuclear Chicago, Des Plaines, Ill. 60018.

CO₂ formed was trapped in phenethylamine solution* and counted using standard procedures. The averages for each set of samples (in microcuries) were plotted against the observed surface counts. The relationship between surface counts and total radioactivity in and on the skin is shown in Fig. 1.

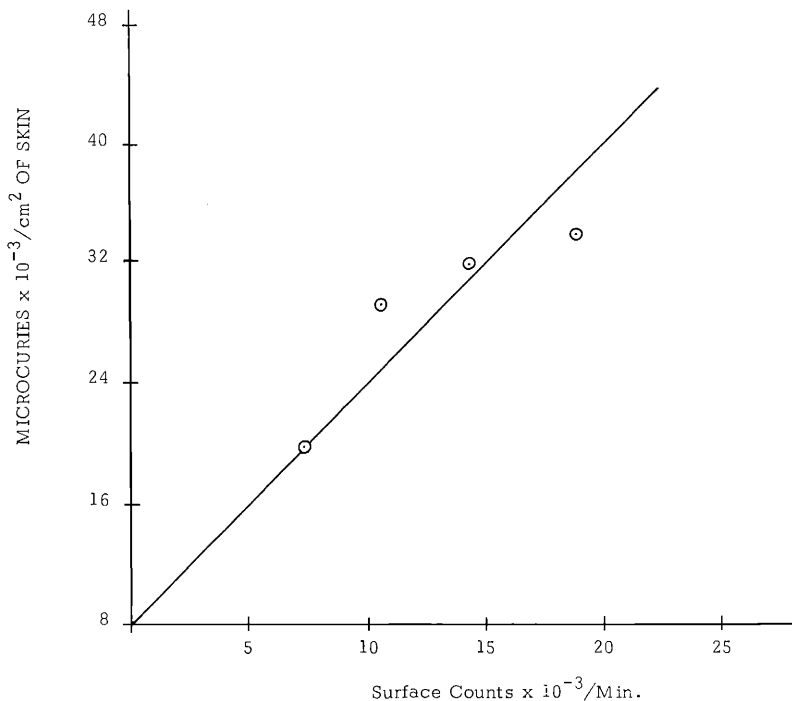


Figure 1. Relationship between surface count and radioactive content of skin

The average counting efficiency for the recovery of radioactivity applied to skin using the Schoeniger combustion technique was 56.2%. This efficiency was determined by burning samples of skin to which known quantities of radioactivity had been applied.

For the human study, five adult male and five adult female subjects were employed. The subjects were instructed to adhere to their usual habits in washing their hands using a control bar soap identical with that which was supplemented with bacteriostats when the antibacterial soap solutions were prepared. The use of hand creams, lotions, or other preparations was not permitted during the experimental periods.

* This solution consists of 27% v/v absolute methanol, 27% v/v 2-phenethylamine, 0.5% w/v PPO (2,5-diphenyloxazole), and 0.91% w/v POPOP [*p*-bis-2-(5-phenyloxazolyl)benzene] and toluene, *q.s.* to 100%.

The appropriate test formulation (0.1 ml) was applied once each morning for 5 consecutive days to the dorsal surface of the left hand of each subject on an area measuring 3.5 cm in diameter. The method of application was as described for the animal studies. Excess material was rinsed off with tap water 3 min after application.

The surface skin counts were determined immediately and 15 min, 4 hours, and 24 hours after each application of the test material. No washing of the test sites was allowed for 4 hours following application of the test material. Applications were made daily for 5 days.

Relationship of Surface Counts to Total Radioactivity in and on the Skin

The activity (in microcuries) in and on the skin of each subject treated with the labeled soap suspension was determined by counting the surface of the skin using a Nuclear Radiation Detector and converting surface counts to total activity using the graph (Fig. 1) constructed from data obtained in the animal studies, and assuming analogy between human skin and rat skin.

RESULTS

Efficiency of Surface Counting

From Fig. 1, a surface count of 30,000 cpm is equivalent to 0.0565 $\mu\text{C}/\text{cm}^2$ of skin. If all of the radioactivity were on the surface and if counting were 100% efficient, the detectable surface counts may be calculated using the following conversion formula:

$$\begin{aligned}\frac{\text{cpm}^a}{6.06^c} &= \frac{\mu\text{C} \times 2.22 \times 10^{10}^b}{1.33^d} \\ \text{cpm} &= \frac{0.0565 \times 2.22 \times 10^6 \times 6.06}{1.33} \\ \text{cpm} &= 5.72 \times 10^5\end{aligned}$$

$$\begin{aligned}\frac{\% \text{ of radioactivity in and on the skin}}{\text{detectable by surface counting}} &= \frac{30 \times 10^3}{5.72 \times 10^5} \times 100 = 5.25 \\ (\% \text{ efficiency})\end{aligned}$$

^a Does not include a background of 65 cpm.

^b 1 $\mu\text{C} \equiv 2.22 \times 10^6$ disintegrations per min.

^c The effective surface area of the Nuclear Radiation Detector used was 6.06 cm^2 .

^d 1.33 is an aliquot adjustment. After combustion of a skin sample, the carbon dioxide formed was collected in 20 ml of basic scintillator solution and 15 ml of the solution was counted in a liquid scintillation counter.

Table II
Counting Efficiency

$\mu\text{c}/\text{cm}^2$ in and on Skin	Surface Counts	% Efficiency
0.0202	7,500	3.65
0.0320	15,000	4.63
0.0565	30,000	5.25
	Mean	4.51

Use of this value and others calculated similarly for different levels of total radioactivity gave the results shown in Table II.

Persistence and Accumulation of Bacteriostats on the Skin

From Fig. 1, the surface counts for male and female subjects and for the composite groups taken four times each day for a 5-day period were converted to microcuries of total radioactivity in and on the skin. Since there were no significant differences between male and female subjects, only the composite data are presented (Table III). These data are shown graphically in Fig. 2. Analysis of variance shows the differences between days and time periods within any day to be significant ($p < 0.01$).

Table III
Accumulation and Persistence of Labeled Germicides on Human Skin After Repeated
Daily Applications^a
(Mean Values for Five Male and Five Female Subjects)

Time of Determination Post-Dosing	Day 1	Day 2	Day 3	Day 4	Day 5	Increase After 5 Days
Immediate	25.0 ^b	28.0	29.4	30.8	32.3	7.3
15 Minutes	25.0	28.4	31.3	33.3	32.3	7.3
4 Hours	22.2	24.1	26.2	27.0	27.3	5.1
24 Hours	11.7	14.3	15.4	15.4	...	3.7

^a Read across for accumulation and down for persistence.

^b All values are given in $\mu\text{c} \times 10^3/\text{cm}^2$ of skin.

DISCUSSION

Inspection of Table III indicates an apparent gradual leveling out of the amount of sorbed material by day 4, the values for that day being about 25% greater than on day 1. It is likely that this represents equilibrium conditions related to the quantity of antibacterial agents applied per unit area of skin. Of interest is the decrease of activity during any

one day: 24 hours after application the amount of agent present decreases by 50%. Our results differ from those of Compeau (2), who found no difference between the amount of hexachlorophene extractable from skin immediately after scrubbing the hands with an antibacterial detergent or after using the product exclusively for five days, but are in agreement with those of Manowitz and Johnston (12), who measured the hexachlorophene extractable from the entire forearm. Using a detergent

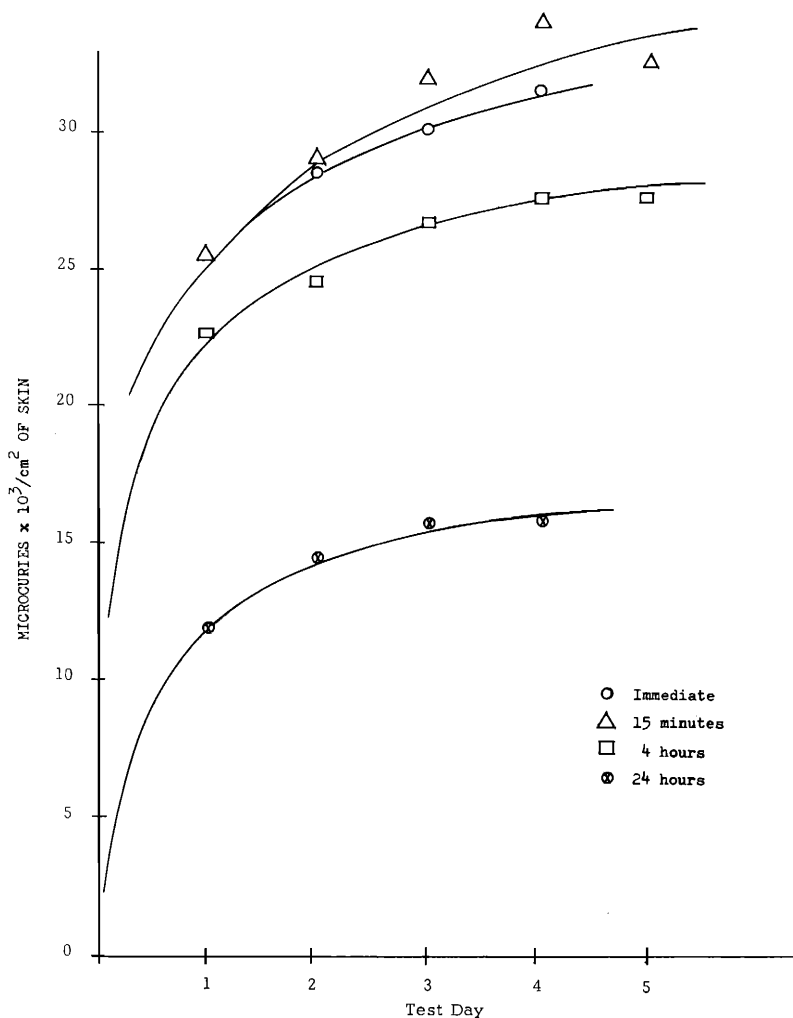


Figure 2. Relationship between number of applications to humans of soap solution containing labeled hexachlorophene and triclocarban and the radioactive content of skin

containing radioactive hexachlorophene, Stoughton (15) found that only 0.5% of the hexachlorophene applied to the skin remained one day after application, unlike the 50% of the mixture of agents found by us. A major difference between the two experiments, aside from the fact that we used soap, not detergent, as the vehicle, and a mixture of antibacterial agents rather than hexachlorophene, is that Stoughton washed the test site with plain soap and water immediately after applying the detergent, a procedure which can be assumed to remove a considerable portion of the deposited chemical.

Diminution of the amount of antibacterials with time probably is due to a great extent to normal shedding, turnover of the *stratum corneum*, and, to a lesser extent, penetration (21). At the end of our experiment, application sites were stripped with cellophane tape followed by two washings with soap and water. Two such treatments failed to remove all of the radioactivity from the skin, but about 64 hours later skin surface counts had reverted to background level.

The accumulation of bacteriostats in skin may explain the observation (12, 17) that the effect of antibacterial soap or detergent on the microflora of the skin is related to the number of exposures to the product. Our data suggest that an antibacterial bar soap may be most effective if used regularly to allow maximum buildup of bacteriostatic film on the skin.

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