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Application of a Gloved-Hand Model for Multiparameter Measurements of Skin-Degerming Activity

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The application of an established gloved-hand model to multiparameter measurements of skin-degerming activity is described. In particular, appropriate experimental designs are illustrated which allow characterization of the performance of topical skin-cleansing preparations in terms of rapid, sustained, cumulative, and persistent skin-degerming effects on the hand. Single-contact studies were used to define the degerming activity profiles of selected commercial surgical scrub preparations, and to establish the optimal post-treatment sampling interval for individual preparations. Rapid and sustained skin-degerming effects were measured and contrasted. Rapid skin-degerming activity, namely, that occurring during actual hand contact, was demonstrated for an iodophor preparation. Sustained skin-degerming activity, namely, that occurring on the gloved hand during a postcontact interval, was shown and characterized for two hexachlorophene preparations. Multiple-contact studies with a 3% hexachlorophene preparation were used to illustrate cumulative and persistent skin-degerming effects. Cumulative skin-degerming activity was demonstrated in terms of progressive bacterial reductions after repeated contacts within a single day. Persistent skin-degerming activity was shown in terms of the profile of daily pretreatment bacterial counts after multiple contacts over successive days. Uniformity of treatment response was established for a broad range of pretreatment bacterial counts extending from approximately log 4 to log 7 per hand. The importance of the pretreatment bacterial count measurement and of adequate neutralization of hand extract samples is stressed. A randomized-hand experimental design is discussed relative to its versatility and amenability to statistical analysis.

The fundamental features of a model for measuring skin-degerming activity on the hand have been previously reported from this laboratory (4). The basic element of the model described in the earlier report was an improved gloved-hand sampling technique for the quantitative and reproducible determination of bacterial levels on the hand. The gloved-hand procedure involves the use of a sterile rubber surgical glove as a direct sampling device for the controlled extraction of skin bacteria from the hand. This direct sampling technique was shown to be a significantly more efficient microbiological extraction method (4) than the indirect glove-washing technique used by some investigators in the past (3, 7, 8). Recently, gloved-hand sampling methods have been used in several reported studies related to the degerming performance of various surgical scrub products (2, 5, 6).

The present report deals with the utility and versatility of the gloved-hand model in its application to multiparameter measurements of skin-degerming activity. Appropriate experimental designs are illustrated that allow the measurement of (i) rapid and sustained degerming activity after single-contact scrubbing, (ii) degerming activity profiles, (iii) cumulative activity after multiple-contact scrubbing and, (iv) persistent activity after extended multiple-contact scrubbing. A factor of basic importance is that data obtained from these designed experiments are readily amenable to statistical analysis for the evaluation of uniformity of treatment response and/or specific treatment comparisons with a high degree of confidence.

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MATERIALS AND METHODS

Skin-cleansing agents. pHisoHex (Winthrop Laboratories, New York, N.Y.), Betadine Surgical Scrub (The Purdue Frederick Co., Yonkers, N.Y.), Septisol (Vestal Laboratories, St. Louis, Mo.), and Maxine nonmedicated bar soap (Swift Chemical Co., Hammon, Ind.) were obtained from commercial sources. pHisoHex is an anionic liquid detergent containing 3% hexachlorophene (HCP). Betadine Surgical Scrub is an iodophor liquid cleanser containing povidone-iodine at an unspecified concentration. Septisol is a soap-based liquid preparation containing 0.75% HCP (0.375% HCP at use dilution). The undiluted products have pH values of approximately 5.5, 5.0, and 9.0, respectively.

Test panels. Test panels of 12 to 36 volunteer subjects were randomly selected from laboratory and secretarial personnel employed at this institution. The mixed male and female subjects were requested to avoid contact of the hands with all medicated and antiseptic materials beginning 3 days prior to, and for the duration of, the test period. During this interval, each subject was provided with nonmedicated bar soap (Maxine) to be used exclusively for all handwashing exposures except those prescribed experimentally. No other restrictions were imposed on the subjects, who were allowed to carry on their normal activities.

Washing and scrubbing procedures. After removing all jewelry, subjects scrubbed or washed their hands and wrists under supervision for 2 to 6 min using tap water and the appropriate assigned product. Each product was used essentially in the manner recommended by the manufacturer. Hands and wrists were first wet, and each hand was scrubbed or washed separately for the specified time period using either 2 or 3 ml of the 3% HCP preparation, 5 ml of the iodophor preparation, or 2 ml of the diluted 0.75% HCP preparation (diluted 1:2 with an equal volume of water to give 0.375% HCP). In one study, the opposing hand to the one being scrubbed or washed was isolated and protected with a sterile surgical glove, and the procedure was then reversed. A nylon-bristle surgical brush was used for scrub contacts, with special attention given to scrubbing the fingernails and interdigital surfaces. After scrubbing or washing for the required time interval, subjects rinsed their hands thoroughly under running tap water, dried them with a sterile cotton towel, and donned sterile rubber surgical gloves. Pertinent details relative to the scrubbing or washing procedures for each study are noted in the legends of the tables and figures.

Hand-sampling procedures. Microbiological samples were obtained by a gloved-hand sampling technique previously described (4). Sterile 0.85% saline (pH 6.8) was used as the sampling fluid in each case except for the post-treatment samples after iodophor contacts, where saline containing 0.1% sodium thiosulfate was used as a neutralizer for residual iodine. In all cases, 10-ml samples of the resulting hand extracts were immediately added to an equal volume of chilled lecithin-phosphate buffer (LPB) (4), which served as a neutralizer for residual HCP. For studies of crossover design, a pooled pretreatment sample was prepared using 5-ml aliquots from the right and left hands of each subject. Decimal dilutions of the neutralized samples were prepared in chilled lecithin-phosphate buffer and aliquots were plated in triplicate with Letheen agar (BBL) using the pour-plate technique.

Gross et al. (1) reported that 0.5% sodium thiosulfate incorporated into solid agar media markedly inhibits the growth of cutaneous staphylococci. In the present studies, 0.1% sodium thiosulfate was used, as necessary, in the saline sampling fluid, but was not incorporated directly into the buffer diluent or solid culture media. Under these conditions, the maximal concentration of sodium thiosulfate transferred to the culture media was 25 μg/ml, a level that was established in control studies to be noninhibitory to normal skin bacteria. Moreover, Kundsin and Walter (2) have demonstrated that the viability of *Staphylococcus epidermidis* is unaffected after suspension of this predominant skin inhabitant for up to 1 h in saline containing 0.1% sodium thiosulfate.

Experimental designs and statistical analysis. Depending on the specific objective(s) of a particular study, either a crossover experimental design or a randomized-hand experimental design was used. In crossover studies, each subject was randomly assigned to one of two or more subgroups for the first treatment interval. After a minimal 3-day rest period to permit reestablishment of normal levels of skin bacteria, the experimental variable was crossed-over with respect to subjects. In this way, comparative measurements were obtained for each subject, thereby providing a within-subject contrast.

In comparative treatment studies, both hands of each subject were sampled for the enumeration of bacteria immediately before each scrub (Pre). Post-treatment samples were obtained from one hand of each subject immediately after each scrub (T_0) and from the opposite hand after a glove-wearing period of either 60 min (T_{60}) or 180 min (T_{180}).

A crossover design was also used for studies in which the degerming activity profile of single products was examined. After a 30-s prewash with nonmedicated bar soap to remove transient bacteria (4), both hands of each subject were sampled for the enumeration of resident skin bacteria immediately before scrubbing (Pre). Each subject then conducted a single-contact scrub with the preparation under study. Post-treatment samples were obtained from one hand of each subject immediately after the scrub (T_0) and from the opposite hand after a glove-wearing period of either 1, 2, 3, or 4 h. The procedure was repeated on each of 4 separate scrub days, allowing a 3-day rest period before each scrub day. Following a crossover design, each subject was sampled once for each glove-wearing interval over the course of the study.

In the randomized-hand study, the hands of each subject were randomly and separately assigned to one of six treatment regimens involving the same product. The design involved the isolation and protection of one hand with a surgical glove while the opposing hand was subjected to the specified treat-

ment regimen. For each treatment regimen, four contacts (either wash or scrub) were conducted on the assigned hands within a 6-h period, allowing a minimum of 1 h between contacts. The study was conducted over 3 successive days, with subjects and treatment regimens balanced with respect to days. Extract samples were obtained from each hand for the enumeration of resident skin bacteria immediately before (Pre) and at 60 min after (T_{60}) the first and fourth contacts. Sterile surgical gloves were worn after each of these contacts before T_{60} sampling.

In the extended multiple-contact study, each subject scrubbed both hands 20 times with a single product over a 5-day period, but only one hand (either right or left) was randomly assigned for microbiological monitoring over days. This design was similar to the randomized-hand design, except that a single-treatment regimen was used. Microbiological samples were obtained from the assigned hand immediately before (Pre) the first contact and at 60 min after (T_{60}) the first and fourth contacts on each day. A sterile surgical glove was worn after the latter contacts before T_{60} sampling. The same hand of each subject was also sampled once on days 3, 4, and 5 after discontinuation of the scrubbing regimen (days 8, 9, 10).

The model for studies using crossover (or split-hand) designs has been described previously (4). The randomized-hand design is described by the fixed effects covariance model (9):

$$Y_{ij} = \mu + \beta(X_{ij} - \bar{X}) + \gamma_j + e_{ij}$$

$$i = 1, \ldots, n \text{ hands}$$

$$j = 1, \ldots, m \text{ treatments}$$

That is, the post-treatment bacterial density on the hand (Y_{ij}) can be represented as a function of the population mean (μ), the influence of the pretreatment bacterial count [$\beta(X_{ij} - \bar{X})$], the treatment effect (γ_j), and the residual error (e_{ij}). The covariance component, $\beta(X_{ij} - \bar{X})$, is derived from the regression estimate of the influence (β) of the individual pretreatment count (X_{ij}) and the overall mean pretreatment count ($\bar{X}$) on the post-treatment bacterial level on the hand. The inclusion of the covariance component in this model provides a more precise estimate of residual error than could be obtained with a simple fixed effects model of the form $Y_{ij} = \mu + \gamma_j + e_{ij}$.

Bacterial count data were routinely transformed to logarithms before statistical analysis (4). The primary means for testing hypotheses and for determining statistical significance of associated contrasts was through the use of analysis of variance and analysis of covariance techniques. Standard errors based on the residual error term, and probability values associated with the calculated F ratios have been included to summarize the salient points of each analysis. Data from subjects who did not complete a particular study were not included in the statistical analyses.

RESULTS

Rapid versus sustained degerming activity. One of the parameters that may be used to evaluate a topical antimicrobial cleansing preparation is the rate of skin-degerming activity. To examine this variable, a preliminary study was conducted to directly compare the skin-degerming activity of the 3% HCP preparation with that of the iodophor preparation. A treatment crossover design was utilized with a test panel of 12 subjects. Each subject used each product once during the study.

As anticipated, there was no significant difference between the mean numbers of bacteria recovered from the hands immediately prior to scrubbing (Pre) for the two treatments (Table 1, study 1). This important contrast established a reliable reference for subsequent treatment comparisons. The mean number of skin bacteria recovered immediately after scrubbing (T_0)

TABLE 1. *Comparison of rapid versus sustained skin-degerming activity*

Measurement	Mean log no. of bacteria recovered/hand[a]					
	Study 1			Study 2		
	3% HCP	Iodophor	Standard error[b]	3% HCP	Iodophor	Standard error[b]
Sampling time[c]						
Pre	6.02	5.76	±0.21 ($P > 0.05$)	5.29	5.02	±0.15 ($P > 0.05$)
T_0	5.86	4.75	±0.20 ($P = 0.01$)	4.95	3.92	±0.22 ($P = 0.01$)
T_{60}	4.66	4.96	±0.35 ($P > 0.05$)			
T_{180}				4.26	4.74	±0.40 ($P > 0.05$)
Mean differences						
(Pre − T_0)	0.16	1.01	±0.23 ($P = 0.01$)	0.34	1.10	±0.20 ($P = 0.01$)
(Pre − T_{60})	1.35	0.79	±0.32 ($P > 0.05$)			
(Pre − T_{180})				1.03	0.28	±0.40 ($P > 0.05$)

[a] Crossover design; 12-subject panels; 3% HCP, 4-min scrub with 2 ml; iodophore, 6-min scrub with 5 ml.

[b] Standard error of the difference between means based on a within-subject comparison of each treatment, and the probability associated with this linear contrast in the corresponding analysis of variance.

[c] Pre, Immediately before scrub; T_0, immediately after scrub; T_{60}, 60 min postscrub; T_{180}, 180 min postscrub.

was signficantly lower ($P = 0.01$) for the iodophor treatment than that observed for the 3% HCP treatment. Correspondingly, the mean (Pre-T_0) reduction in bacterial levels was significantly greater ($P = 0.01$) for the iodophor treatment (90%) versus the 3% HCP treatment (31%). In contrast, the comparative mean bacterial levels on the hand at 60 min postscrub (T_{60}) were not significantly different for the two treatments. Likewise, no significant difference was observed between the (Pre-T_{60}) reductions in bacterial levels for the iodophor treatment (84%) compared to the 3% HCP treatment (96%). It will be noted, however, that the mean post-treatment bacterial levels decreased considerably between T_0 and T_{60} after scrubbing with the 3% HCP preparation, whereas the corresponding bacterial levels were essentially unchanged in the case of the iodophor treatment.

A second study of similar design was conducted with the 3% HCP preparation and the iodophor preparation to further examine comparative differences between the two products with respect to rates of skin-degerming activity. In this study the post-treatment sampling interval was extended to 180 min (T_{180}).

As was the case in the previous study, no significant differences were observed between the mean pretreatment bacterial levels on the hands for the two treatments (Table 1, study 2). The 3% HCP treatment and the iodophor treatment again differed significantly ($P = 0.01$) with respect to the comparative mean T_0 bacterial levels on the hand and also for the observed (Pre-T_0) reductions in bacterial levels. Results for the latter parameter were in good agreement with those noted in the previous study. The mean numbers of bacteria recovered from the hand at T_{180} were not significantly different for the two treatments. Comparative (Pre-T_{180}) reductions in mean levels of skin bacteria were indicative, but not significantly different for the 3% HCP treatment (91%) versus the iodophor treatment (48%). It will be noted, however, that mean bacterial levels after the iodophor treatment increased appreciably between T_0 and T_{180}, such that the T_{180} value approached the pretreatment value. In contrast, a further decrease in mean bacterial levels between T_0 and T_{180} was observed after the 3% HCP treatment.

Degerming activity profiles. It is clear from the above studies that the pretreatment bacterial count is an important reference from which skin-degerming activity can be measured. It is also obvious that a single comparison of pre- and post-treatment bacterial levels cannot adequately characterize the performance of skin-degerming preparations. Therefore, it was of interest to simultaneously examine a number of post-treatment sampling intervals to determine the point of maximal treatment effect and to gain insight into postscrub trends in the reestablishment of resident skin bacteria.

Separate studies were conducted to examine the degerming activity profiles of three antimicrobial cleansing preparations after single-contact use. The preparations studied contained, respectively, 3% HCP, an iodophor, and 0.375% HCP. For each study, a sampling crossover design was utilized with a test panel of 16 subjects.

For each of the three studies, there was no significant difference in mean pretreatment levels of skin bacteria over the four separate scrub days (Table 2). The absence of treatment carryover effects between scrub days allowed the calculation of overall mean Pre values. Likewise, in each study, the mean T_0 bacterial levels did not differ significantly over the 4 separate scrub days. Thus, the calculation of overall mean T_0 values and overall mean (Pre-T_0) reductions was also justified since each separate scrub day could be considered as a replicate experiment. Although the experimental design was not intended for direct comparisons among the three products studied, it is noteworthy that overall (Pre-T_0) reductions in bacterial levels were different for the 3% HCP preparation (60%), the iodophor preparation (84%), and the 0.375% HCP preparation (13%).

The overall mean Pre and overall mean T_0 values, as well as the postscrub mean bacterial counts for each study, are summarized in Fig. 1. Referring to study 1 with the 3% HCP preparation, it will be noted that the postscrub bacterial level on the hand continued to decline from the T_0 level, reaching a low value at 1 h postscrub, corresponding to an overall reduction of 97%. Thereafter, reestablishment of resident skin bacteria ensued, but the mean bacterial level was significantly depressed ($P = 0.01$), relative to the pretreatment level, even at 4 h postscrub. In study 2, after a single contact with the iodophor preparation, the maximal decrease in bacterial levels was observed at T_0, and corresponded to a reduction of 84%. This decrease was immediately followed by reestablishment of resident bacteria, which returned to the pretreatment level by 3 h postscrub. In study 3, after a single scrub with the 0.375% HCP preparation, skin-degerming activity progressed very slowly to a maximal effect at 2 h postscrub, corresponding to an overall reduction of 72%. Rapid reestablishment of skin bac-

TABLE 2. *Measurements of rapid degerming activity for three skin-cleansing preparations*

Study	Product	Sampling time[a]	Mean log no. of bacteria recovered/hand[b]					Standard error[c]
			Week 1		Week 2		Overall mean	
			Scrub 1	Scrub 2	Scrub 3	Scrub 4		
1	3% HCP	Pre	5.25	5.10	5.20	5.14	5.17	±0.10 ($P > 0.05$)
		T_0	4.88	4.66	4.68	4.85	4.77	±0.11 ($P > 0.05$)
		(Pre-T_0)[d]	0.37	0.44	0.52	0.29	0.40	±0.09 ($P > 0.05$)
2	Iodophor	Pre	5.37	5.42	5.38	5.23	5.35	±0.06 ($P > 0.05$)
		T_0	4.44	4.59	4.49	4.62	4.54	±0.13 ($P > 0.05$)
		(Pre-T_0)[d]	0.93	0.83	0.89	0.61	0.81	±0.11 ($P > 0.05$)
3	0.375% HCP	Pre	5.20	5.42	5.32	5.48	5.35	±0.10 ($P > 0.05$)
		T_0	5.23	5.38	5.17	5.36	5.29	±0.13 ($P > 0.05$)
		(Pre-T_0)[d]	−0.03	0.04	0.15	0.12	0.06	±0.06 ($P > 0.05$)

[a] Pre, Immediately prior to scrub; T_0, immediately after scrub.

[b] Crossover design; 16-subject panels; 3% HCP, 4-min scrub with 2 ml; iodophor, 6-min scrub with 5 ml; 0.375% HCP, 4-min scrub with 2 ml; scrubs preceded by a 30-s prewash with nonmedicated bar soap.

[c] Standard error of an individual mean based on a within-subject comparison among the four scrubs, and the probability associated with this linear contrast in the corresponding analysis of variance.

[d] Mean difference.

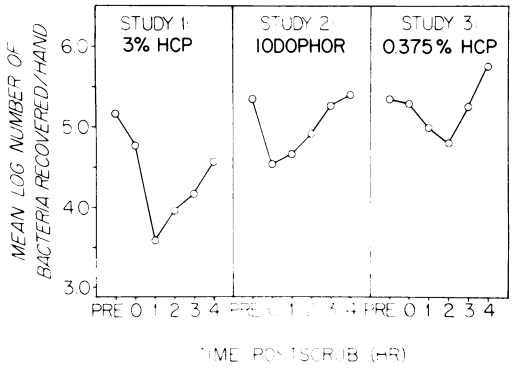

FIG. 1. *Degerming activity profiles of three skin-cleansing preparations. Sixteen-subject panels; crossover design with respect to postscrub samples; 3% HCP, 4-min scrub with 2 ml; iodophore, 6-min scrub with 5 ml; 0.375% HCP, 4-min scrub with 2 ml. Scrubs preceded by a 30-s prewash with nonmedicated bar soap. The Pre and T_0 values represent the overall mean of four separate measurements per subject. A single measurement per subject was obtained for each of the other postscrub intervals.*

teria followed immediately, such that the mean bacterial level had returned to the pretreatment level by 3 h postscrub and was even higher ($P = 0.05$) at 4 h postscrub.

Uniformity of treatment response. Because of the rather broad distribution of pretreatment bacterial counts normally observed among subjects, it was important to ascertain that each subject panel, as a group, responded uniformly to treatment. This was confirmed for each of the three preceding studies by linear regression analysis of individual subject responses. To illustrate this point, individual T_0 counts have been plotted as a function of the corresponding pretreatment counts for each study (Fig. 2). It can be seen that, in each case, response to treatment was uniform over a range of pretreatment bacterial counts extending from approximately log 4 to log 6.5. That is, the line of best fit for the data closely approximated the theoretical line of uniform response.

Cumulative activity after multiple contacts. The foregoing studies allowed characterization of the skin-degerming activity of topical antimicrobial cleansing preparations after a single contact on the hands. Another important parameter relevant to the skin-degerming performance of these preparations is the effect of multiple contacts on the resident skin bacteria. Although the basic crossover experimental design may be adapted to multiple-contact studies, a randomized-hand experimental design of greater utility was developed for this purpose, where the hand, rather than the subject, was used as the test object.

Utilizing the randomized-hand design, the multiple-contact skin-degerming performance of the 3% HCP preparation was evaluated under several use conditions. Specifically, six treatment regimens were examined, corresponding to contact times of either 2, 4, or 6 min with or without the use of a surgical brush.

At each of the four sampling intervals, no significant difference was observed among the mean bacterial levels on the hand for the six treatment regimens (Table 3). Similarly, the mean (Pre-T_{60}) reductions in skin bacteria after a single contact were not significantly different

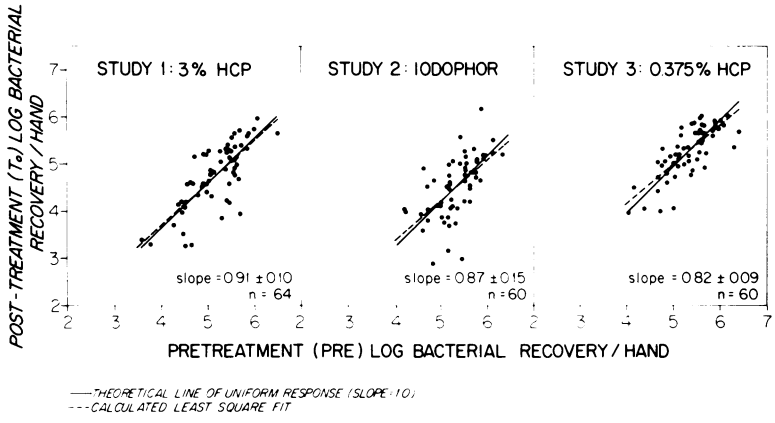

FIG. 2. *Uniformity of treatment response for subjects participating in three separate studies. Individual subject data for the mean Pre and T_0 values summarized in Table 2.*

TABLE 3. *Cumulative skin-degerming activity of a 3% HCP preparation*

Measurement	Mean log no. of bacteria recovered/hand[a]						Standard error[b]
	Wash			Scrub			
	2 min	4 min	6 min	2 min	4 min	6 min	
Sampling time[c]							
Contact 1 Pre	5.73	5.50	5.68	5.71	5.98	5.86	±0.17 ($P > 0.05$)
Contact 1 T_{60}[d]	4.80	4.69	4.63	4.61	4.85	4.71	±0.15 ($P > 0.05$)
Contact 4 Pre[d]	4.49	4.53	4.50	4.58	4.55	4.17	±0.16 ($P > 0.05$)
Contact 4 T_{60}[d]	3.64	3.20	3.78	3.77	3.97	3.98	±0.21 ($P > 0.05$)
Mean differences							
(C_1Pre-C_1T_{60})	0.93	0.90	1.08	1.11	1.03	1.11	±0.18 ($P > 0.05$)
(C_1Pre-C_4T_{60})	2.09	2.33	1.90	1.94	1.98	1.86	±0.25 ($P > 0.05$)

[a] Randomized-hand design; 36-subject panel ($n = 12$); four wash or scrub contacts with 3 ml as indicated within a 6-h period; contacts one and four preceded by a 30-s prewash with nonmedicated bar soap.

[b] Standard error of an individual mean based on either the pretreatment analysis of variance or the post-treatment analysis of covariance error estimate, and the probability associated with the corresponding linear contrast among means.

[c] Pre, Immediately prior to contact; T_{60}, 60 min postcontact; C_1, first contact; C_4, fourth contact.

[d] Mean values adjusted through covariate analysis based on the highly significant correlation with pretreatment values.

for the various treatment regimens (range 87 to 92%). Overall mean reductions in bacterial levels from contact 1 Pre through contact 4 T_{60}, likewise, did not differ significantly for the six treatment groups of hands (range 98.6 to 99.5%). It is clear, however, that each multiple-contact regimen resulted in progressive reductions in levels of skin bacteria. The degerming efficacy of this product, however, was not enhanced by washing regimens longer that 2 min, nor by the use of a surgical brush regardless of contact time.

Persistent activity after extended multiple contacts. To further define the skin-degerming effects of multiple-contact scrubbing, a study was undertaken that simulated extended use of the 3% HCP preparation. An adaptation of the randomized-hand experimental design was utilized, wherein the preparation was used four times daily for 5 consecutive days.

On each of the first 2 treatment days, cumulative skin-degerming activity was apparent during multiple scrubbing with the 3% HCP preparation (Fig. 3). The mean bacterial reductions on day 1 were 94% at scrub 1 T_{60} and 99% at scrub 4 T_{60}. By treatment day 3, the mean number of skin bacteria on the hand at scrub 1 T_{60} was reduced to a minimal level and showed no further decrease with additional scrub contacts on this day or on subsequent days. The overall mean bacterial reduction achieved by treatment day 3 was 99.9%.

Mean levels of bacteria immediately before scrubbing on each treatment day (scrub 1 Pre) showed a progressive and significant decrease ($P = 0.01$) over the 5 days (Fig. 3). It will be

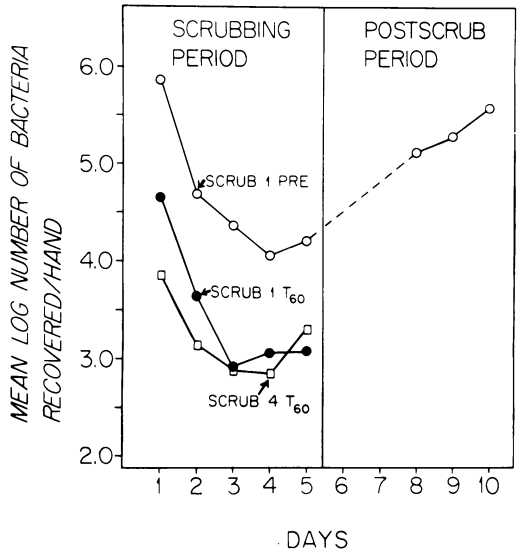

FIG. 3. *Cumulative and persistent skin-degerming activity after extended multiple-contact scrubbing with a 3% HCP preparation. Twelve-subject panel; one hand of each subject was randomly assigned for microbiological sampling; four 2-min scrub contacts with 2 ml of the preparation were conducted daily within a 6-h interval on days 1 through 5; scrub 1 Pre measurements on all days were preceded by a 30-s prewash with nomedicated bar soap.*

noted that reduced bacterial levels persisted overnight on successive treatment days, and did not return to pretreatment levels until day 5 postscrub (day 10).

DISCUSSION

The application of the gloved-hand model to multiparameter measurements of skin-degerming activity allows systematic characterization of the skin-degerming effects exerted by topical antimicrobial cleansing preparations. The key parameters which yield useful information are measurements of rapid, sustained, cumulative, and persistent skin-degerming effects. A schematic representation of these different parameters of activity is shown in Fig. 4.

Collectively, the studies reported herein show that a single comparison of pre- and posttreatment bacterial levels cannot adequately characterize the performance of a skin-degerming preparation. Ideally, a systematic evaluation should first focus on examination of the single-contact activity of the preparation under study.

Single-contact studies are useful to define the skin-degerming activity profile of a preparation over a number of post-treatment sampling intervals. The activity profile establishes the point of maximal bacterial reduction and allows discrimination between rapid and sustained skin-degerming activity. The (Pre-T_0) measurement provides an index of rapid skin-degerming activity, namely, that which occurs during actual hand contact. Conversely, a decrease in bacterial levels subsequent to T_0, denotes sustained skin-degerming activity, namely, that which occurs on the gloved hand during a postcontact interval. It is clear from the studies reported here that the iodophor preparation exerted rapid activity, but no detectable sustained activity after a single exposure. In contrast, the two HCP preparations produced a slower initial activity, but exhibited characteristic sustained activity after a single contact. With the appropriate selection of posttreatment sampling intervals, the activity profile defined in single-contact studies also provides useful insight into postscrub trends in the reestablishment of resident bacteria on skin. Single-contact studies may also be used to determine optimal dose and contact time for a particular preparation.

Multiple-contact studies allow the measure-

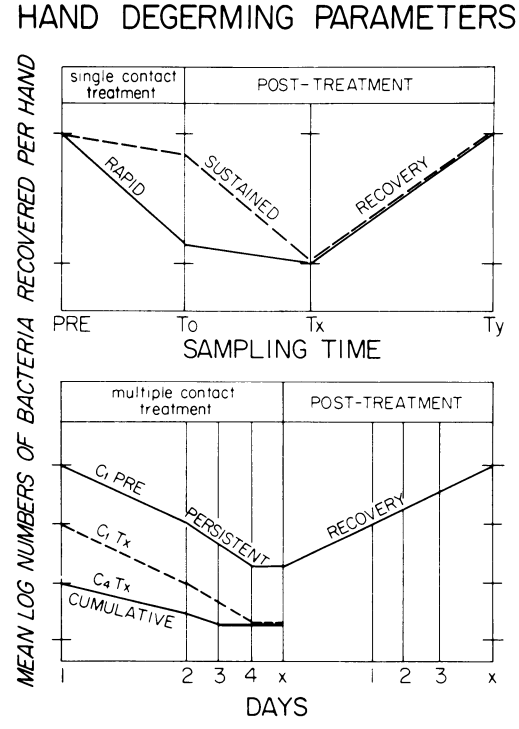

FIG. 4. *Schematic representation of rapid, sustained, cumulative, and presistent skin-degerming activity parameters. Pre, Immediately before treatment; T_x and T_y, x and y minutes post-treatment. C_1, Contact 1; C_4, contact 4; x, xth treatment day or post-treatment day.*

ment of cumulative skin-degerming activity on the hand after repeated contacts within a single day. This activity parameter is characterized by progressive bacterial reductions as a function of multiple contacts. When extended over a number of successive days, multiple-contact studies also provide a measurement of persistent skin-degerming activity over days. The latter parameter, which is characterized by the profile of daily pretreatment levels of skin bacteria, reflects the net difference between maximal daily bacterial reductions and subsequent regrowth of skin bacteria during overnight noncontact intervals. The studies presented here demonstrate that the 3% HCP preparation exerted both cumulative and persistent skin-degerming activity after multiple-contact exposure.

For both single-contact and multiple-contact studies, the pretreatment bacterial count is the critical reference from which activity measurements are made. It is important, therefore, that pretreatment samples be obtained immediately before treatment on each day to accurately measure the microbiological state of the hand at that specific point in time.

It is also essential that post-treatment extract samples obtained from the hand be appropriately neutralized with a suitable nontoxic agent and/or adequately diluted before culturing to avoid possible interference due to carryover of residual amounts of the active antimicrobial agent. In the case of multiple-contact studies, however, it is obvious that neutralizers should not be incorporated directly into the hand-sampling fluid.

In view of the rather broad range of pretreatment bacterial counts normally observed within the general population, it is equally important that subjects under study respond uniformly to treatment. Based on the results and analyses presented herein, candidate subjects with pretreatment bacterial counts in the range from approximately log 4 to log 7 per hand may be expected to respond uniformly to treatment, and, therefore, would be generally acceptable for these types of studies. Appropriate analyses of pre- and post-treatment data may be used to verify the satisfaction of this requirement for each study.

The selection of appropriate experimental designs affords considerable flexibility for studies of the types described. The major objectives of an appropriate experimental design are to minimize the risk of bias, maximize precision, and provide for corresonding hypothesis testing. These requirements were an integral feature of the skin-degerming studies reported here, and all data were readily amenable to statistical analysis.

The randomized-hand experimental design described herein is especially useful in that it permits the simultaneous examination of a number of experimental variables, or topical cleansing products, with the use of a minimal number of subjects. The increased versatility of this design, over traditional crossover and split-hand designs, is achieved at a relatively small and acceptable sacrifice in statistical discriminatory power. It has been the experience of this laboratory that a minimum of 12 independent measurements for each variable examined in hand-degerming studies will provide an adequate basis for direct comparisons. This number of observations will usually permit the statistical discrimination of differences in bacterial reductions as small as 0.5-log unit. Significant differences of this magnitude provide practical and meaningful information related to product comparisons and/or comparisons of variables such as dose and contact time.

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