Disinfection of the Skin: An Assessment of Some New Preparations

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Summary

Repeated hand washing with a detergent solution containing 0.75% chlorhexidine digluconate was found to cause a large reduction in the resident skin flora which was slightly though significantly smaller than that caused by the use of 3% hexachlorophane liquid soap containing a phenolic preservative, chlorocresol 0.3%. Both agents caused a greater immediate reduction of bacteria after a single hand washing than the hexachlorophane liquid soap without a phenolic additive had shown in earlier experiments; the soap base containing chlorocresol 0.3% but no hexachlorophane was also found to cause a large reduction in skin flora. The chlorhexidine detergent solution had no residual disinfectant action on the skin after rinsing and drying the hands.

Disinfection of an operation site for two minutes with povidone-iodine containing 1% available iodine in 70% ethyl alcohol caused about as great a reduction in resident flora as a similar treatment with alcoholic 0.5% chlorhexidine. Both treatments were more effective than disinfection with aqueous 1% cetrimide or 0.1% benzalkonium chloride solutions.

Introduction

Studies in this unit on alternative methods for disinfection of the skin showed that liquid iodophor or hexachlorophane preparations gave the best results for repeated use on the surgeons' or nurses' hands and that alcoholic chlorhexidine or iodine gave the best results for single use on the operation site (Lowbury, Lilly, and Bull, 1960, 1963, 1964a, 1964b). Since the publication of these findings some new or modified preparations have been introduced. In this paper we report an assessment of the comparative effectiveness of a few recent preparations, and also of two older preparations which had not been studied for their effects on resident skin flora in the earlier series.

Materials and Methods

Preparations for Disinfection of Hands.—Such preparations are required to cause a progressive reduction of skin bacteria when the treatment is used repeatedly and a good detergent action to facilitate removal of dirt and desquamating epithelium. The following preparations were studied. (1) A chlorhexidine detergent solution (Dermofax) containing chlorhexidine digluconate B.P. (0.75% w/v), pendecamine (Betaine L7) (1.5% w/v), urea B.P.C. (10% w/v), with propylene glycol, hydroxy-ethyl cellulose as thickener, perfume, and colouring agent. (2) Hexachlorophane (Ster-Zac) liquid soap, containing 3% hexachlorophane and chlorocresol* 0.3% added to the original

*Patent applied for.

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product in order to suppress the survival or growth of contaminants; this addition was made by the manufacturers in response to reports of Gram-negative bacilli contaminating some stocks of the original product (Ayliffe, Barrowcliff, and Lowbury, 1969), and it has been shown to be highly effective in preventing such contamination (Collins and Deverill, 1971). (3) A 2.5% hexachlorophane soap gel (Steridermis) which has been reported to be more rapid in action than a 3% detergent product and to have a "broad-spectrum" activity against Gram-negative as well as Gram-positive organisms (Gibson, 1969). (4) Control application: unmedicated bar soap.

Preparations for Disinfection of Operation Site.—Such preparations are required to cause maximum reduction of bacteria on the skin at a single application. The preparations studied were: (1) povidone-iodine with 1% available iodine, in 70% alcohol; (2) benzalkonium chloride (0.1%) aqueous solution; (3) cetrimide (1%) aqueous solution; (4) chlorhexidine (0.5%) in 70% ethyl alcohol; and (5) control application, sterile distilled water.

TESTS FOR DISINFECTION OF HANDS

Separate experiments were made for the comparative assessment of (1) Dermofax, improved Ster-Zac liquid soap, and unmedicated bar soap; and (2) Steridermis, Ster-Zac liquid soap (original formulation), and unmedicated bar soap, each of the disinfectant soaps being tested separately for its effect when rubbed into the dry skin before rinsing with water, and for its effect on application to the hands moistened with water from the start. As in previous studies (Lowbury *et al.*, 1963, 1964b) the disinfectant action of different preparations was tested against the resident flora on members of the laboratory staff, and a Latin square design was used.

In the first experiment six members of the laboratory staff were chosen, and each of the three preparations was tested on each subject. The chlorhexidine detergent preparation (Dermofax) was applied to the dry skin (about 7 ml) and rubbed over all surfaces of the hands and forearms for one and a half minutes, after which a little water was added to produce a lather, and washing continued for half a minute; the lather was then rinsed off under running warm water. The other preparations were applied to the moistened skin and rubbed over all surfaces, while warm water was added at intervals to keep the detergent wet and finally to rinse off the residue; the wash was completed in two minutes. No brush was used.

In each experiment the subject first washed his hands briefly with bar soap and dried them on a sterile towel. A bacteriological sample of hand washings was then taken by a standard technique in 100 ml of Ringer's solution with neutralizers (see Lowbury *et al.*, 1963). After rinsing the hands under a tap, they were treated for two minutes with the selected agent, as described above, rinsed, and dried with a sterile towel. A second bacteriological sample was then taken by the hand-washing technique. Two further two-minute hand washings with the selected preparation were carried out in the manner described above on the same day and three such washings were done on the following day, allowing an interval of an hour or more between washings. After the last washing a third standard bacteriological sampling of the hands was carried out.

The same technique was used in the second experiment to assess the value of the hexachlorophane soap gel (Steridermis);

the experiment was, however, not completed because volunteers complained of irritation and reddening of the skin on repeated use of the soap gel, which was strongly alkaline (pH 9.6).

Tests for Residual Action of Chlorhexidine Detergent Solution on Skin.—Tests for residue of the antiseptic left on the skin after washing with this preparation were made in the manner described by Lowbury *et al.* (1964a). Measured volumes (0.05 ml) of suspensions of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were deposited, spread, and allowed to dry on the palms of the hands of two volunteers who had previously washed for two minutes, rinsed, and dried their hands, using either Dermofax or (controls) soap and water. After one hour the inoculated area was sampled with Ringer's solution by a standard technique (Story, 1952) and viable counts of bacteria were obtained. Tests with soap and with Dermofax were made on the same two volunteers.

TESTS FOR DISINFECTION OF OPERATION SITE

The method of assessment was that used in previous studies (Lowbury *et al.*, 1960, 1964b). Each of the five preparations was tested on each of five subjects by a Latin square design, a different preparation being tested on each subject on one day. Replicate tests on preparations were made, with change of subject in alternate weeks to allow the skin flora to return to its normal equilibrium level. Viable counts were obtained from standard hand washings taken immediately before and after the application of the disinfectant or the control fluid (sterile distilled water), which was rubbed on to the whole surface of both hands and wrists with a gauze swab and reapplied, when necessary, to ensure that the skin was kept visibly wet for two minutes. The hands were then briefly rinsed under running warm water and dried on a sterile towel. Sampling fluid and culture medium (pour plates of nutrient agar) contained Lubrol W (1%), lecithin (0.5%), Tween 80 (1%), and sodium thiosulphate (1%) as neutralizers, and tests for carry-over of inhibitory concentrations of disinfectant were made in each experiment.

Pour plates were incubated for 48 hours at 37%, and viable counts were then made.

Results

DISINFECTION OF HANDS

Chlorhexidine Detergent Solution and Improved Hexachlorophane Liquid Soap.—Table I shows the percentage reduction in bacterial counts of samplings from hands after a single treatment and after six treatments with improved hexachlorophane liquid soap (Ster-Zac), chlorhexidine detergent solution (Dermofax), and

TABLE I—Disinfection of Hands: Reduction in Bacterial Counts from Hand Washings Expressed as Percentage of Initial Count after First and Sixth Application

Replicate Experiments, Each on One Day	Hands Washed with					
	Improved 3% Hexachlorophane Liquid Soap (Ster-Zac)		Chlorhexidine Detergent Solution (Dermofax)		Control (Bar Soap)	
	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications
1	B.D. 44.2	99.6	S.D. 56.6	86.7	A.K. -37.0	67.2
2	A.K. 50.9	99.9	B.D. 67.2	84.5	S.D. 20.2	37.2
3	S.D. 30.4	98.3	A.K. 41.9	94.6	B.D. 14.4	-22.3
4	B.G. 53.0	99.6	E.R. 52.3	89.2	J.O. 11.5	7.8
5	J.O. 32.7	92.0	B.G. 72.5	97.0	E.R. 8.9	-6.0
6	E.R. 69.0	99.5	J.O. 68.8	96.6	B.G. 12.1	-9.2
Mean % reduction ..	46.7*	98.2†	59.9*	91.4†	5.0	12.4

Initials indicate experimental subjects.
**t* = 1.75, 0.2 > *P* > 0.1 (not significant).
†*t* = 2.70, 0.05 > *P* > 0.02.

bar soap. A comparison of differences between paired observations on the use of improved Ster-Zac and Dermofax on the same individuals showed that Dermofax had a slightly (not significantly) larger immediate effect than improved Ster-Zac, and that there was a slightly but significantly larger effect by Ster-Zac after six applications. Both the immediate and the late effect of Ster-Zac were greater than had been found in similar tests with the original formulation of Ster-Zac liquid soap (Lowbury *et al.*, 1963); the immediate effects were greater than were to be expected from a hexachlorophane preparation, in view of the slow action of hexachlorophane. It seemed likely that the superiority of the new product was due to independent activity of chlorocresol 0.3% which was introduced to prevent the survival or growth of contaminants. A comparison was therefore made, in single tests on three subjects, of the skin-disinfecting properties of the Ster-Zac soap base containing chlorocresol 0.3% but no hexachlorophane; from the results (see Table II) it seemed that the Ster-Zac soap base containing chlorocresol 0.3% had a considerable independent skin-disinfecting activity.

TABLE II—Disinfection of Hands by Washing with Liquid Soap Base containing Chlorocresol 0.3%

Time of Sampling	Viable Counts per ml Washings (diluted 10 ⁻⁵), and % Reduction after Washing		
	Subject A.K.	Subject E.R.	Subject S.D.
Before hand washing ..	503	473	262
After single hand washing ..	259 (48.5)	149 (68.5)	131 (50)
After six hand washings ..	74 (85.3)	27 (81.8)	83 (68.3)

Tests for Residual Action of Chlorhexidine Detergent Solution.—Table III shows that the skin of hands washed with Dermofax had no greater bactericidal action against organisms inoculated after washing than the skin of hands washed with unmedicated soap and water.

TABLE III—Tests for Residual Action of Chlorhexidine Detergent Solution (Dermofax) on Skin after Washing and Drying the Hands

Bacterial Strains Applied to Skin	Subject	Viable Counts per ml Washings from Skin Washed before Bacteria were Inoculated	
		With Dermofax	With Unmedicated Soap
<i>E. coli</i> {	1 2	107 129	126 181
<i>Ps. aeruginosa</i> {	1 2	39 105	43 82
<i>Staph. aureus</i> {	1 2	214 139	252 151

Hexachlorophane Soap Gel.—The experiment with this agent was not completed because of its irritant properties, but the tests on three subjects (Table IV) show the type of effect which was obtained. The most striking feature was the large reduction in bacterial counts in two individuals after a single treatment when the gel was rubbed on to the skin without previous moistening; if the skin was first moistened the initial effect (in the one subject tested) was small. The effects of Ster-Zac

TABLE IV—Individual Experiments with Hexachlorophane Liquid Soap (Ster-Zac, Original Formulation) and with Hexachlorophane Soap Gel (Steridermis)

Method of Application		Percentage Reduction in Viable Counts of Hand Washings after Washing with			
Ster-Zac	Steridermis	Ster-Zac		Steridermis	
		After 1 Application	After 6 Applications	After 1 Application	After 6 Applications
On moist skin	On dry skin	B.D. 44	97.1	B.G. 99.5	98.9
On moist skin	On dry skin	B.G. 40	96.8	A.K. 75.5	98.6
On dry skin	On moist skin	B.G. 53	99.1	B.D. 24	91.2

Initials indicate experimental subjects.

TABLE V—Disinfection of Operation Site: Reduction in Bacterial Counts from Hand Washings Expressed as Percentage of Initial Count

Treatment	Replicate Experiments, Each on One Day					Totals	Mean % Reduction
	1	2	3	4	5		
(a) Benzalkonium chloride, aqueous, 0.1%	T.K. 45.6	J.O. 43.3	E.R. 51.4	J.B. 36.8	B.G. 54.1	231.2	46.2
(b) Cetrimide, aqueous, 1%	J.O. 61.5	T.K. 61.3	J.B. 52.2	B.G. 42.0	E.R. 58.0	275.0	55.0
(c) Chlorhexidine 0.5% in 70% ethyl alcohol ..	B.G. 68.3	J.B. 74.9	T.K. 82.8	E.R. 83.0	J.O. 88.8	397.8	79.6
(d) Povidone iodine (1% available iodine) in 70% ethyl alcohol	E.R. 82.9	B.G. 62.1	J.O. 63.5	T.K. 88.1	J.B. 75.4	372.0	74.4
Control	J.B. 12.7	E.R. 33.6	B.G. 27.4	J.O. 13.6	T.K. 3.6	23.7	4.7
Total	271.0	208.0	277.3	263.5	279.9	1299.7	

Comparison of treatments (t tests, 12 degrees of freedom) (see Table VI):

- (a) and (d) $t = 3.62$, $0.01 > P > 0.001$
 (a) and (c) $t = 4.28$, $0.01 > P > 0.001$
 (a) and (b) $t = 1.12$, $P > 0.1$ (not significant)
 (b) and (d) $t = 2.49$, $0.05 > P > 0.02$
 (b) and (c) $t = 3.15$, $0.01 > P > 0.001$
 (c) and (d) $t = 0.67$, $P > 0.1$ (not significant)

when the soap was applied without initial moistening of the skin were only marginally better than those obtained when the skin was moistened at the beginning of the hand wash. Good effects were obtained from six applications with both preparations.

DISINFECTION OF OPERATION SITE

Tables V and VI show the results of a comparison of four disinfectant preparations and a control application. Alcoholic povidone-iodine and chlorhexidine caused comparable reductions in estimated skin flora as shown by viable counts of hand washings before and after treatment. Aqueous cetrimide

TABLE VI—Disinfection of Operation Site—Analysis of Variance

Sources of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	Significance
Experiments ..	1,018.07	4	254.52	> 0.2
Persons ..	513.31	4	128.33	> 0.2
Treatment ..	17,996.55	4	4,499.14	< 0.001
Residual ..	1,836.82	12	153.07	
Total ..	21,364.75	24		

and benzalkonium chloride caused significantly smaller reductions in skin flora than alcoholic chlorhexidine or povidone-iodine; the difference between the skin disinfecting activity of alcoholic and aqueous chlorhexidine shown in earlier studies (Lowbury *et al.*, 1960, 1964b) was only slightly smaller than the difference between the skin disinfecting action of alcoholic chlorhexidine and of the aqueous cetrimide solution in this study; aqueous cetrimide was slightly but not significantly more effective than aqueous benzalkonium chloride. Tests showed no "carry-over" effects of cetrimide or benzalkonium chloride in the washing and dilution fluids and culture media used for viable counts.

Discussion

A 3% hexachlorophane liquid soap, to which the manufacturers have added a phenolic compound, chlorocresol 0.3%, as a preservative against Gram-negative bacilli, was found to have greater effects, especially after a single application, than were obtained in earlier experiments on the same hexachlorophane soap without the phenolic additive. Tests with the soap base containing chlorocresol 0.3% alone showed that this had an independent skin disinfectant action, differing from that of hexachlorophane in its rapid action on a single application.

A 0.75% chlorhexidine detergent solution (Dermofax) showed comparable immediate effects and a cumulative skin disinfectant action only slightly (though significantly) smaller than that of the improved hexachlorophane liquid soap. An earlier detergent

formulation of chlorhexidine containing cetrimide (Savlon) contained too low a concentration of chlorhexidine at its usual dilution for skin disinfection (Lowbury *et al.*, 1963). The new chlorhexidine product, in which an effective concentration of the antiseptic is applied to the skin, must be regarded as a valuable addition to the small list of effective disinfectant detergent solutions for the surgeons' and nurses' hands.

In disinfecting the operation site, alcoholic povidone-iodine containing 1% available iodine was as effective as 0.5% chlorhexidine in alcohol, and the results with both of these agents approximated to those obtained in earlier studies with alcoholic 1% iodine and 0.5% chlorhexidine. The main advantage of chlorhexidine over iodine is an absence of irritant and sensitizing effects on the skin, but previous experience with aqueous povidone-iodine suggests that the alcoholic solution of this compound might also cause less irritation and sensitization than 1% alcoholic iodine. We had not previously studied the effect of aqueous quaternary ammonium compounds in removing the resident skin flora, because pilot studies had shown aqueous 1% cetrimide to be less effective than a selection of other agents in removing bacteria deposited on the skin to simulate transient flora (Lowbury *et al.*, 1960). In this study two quaternary ammonium compounds were tested in comparison with alcoholic chlorhexidine and povidone-iodine for their effects on the resident flora; though their activity was significantly smaller than that of the alcoholic solutions, it was not much smaller than the activity of aqueous chlorhexidine shown in earlier studies. A larger effect would be expected from alcoholic solutions of quaternary ammonium compounds, which were shown by Price (1957) to be more active against the skin flora than 70% alcohol.

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