Disinfection of Hands : Removal of Transient Organisms

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Antiseptic detergent mixtures containing hexachlorophane or iodine have been found to cause a large reduction in the " resident " skin flora when used repeatedly for washing the hands—an effect which was not obtained by the use of ordinary soap and water (Traub, Newhall, and Fuller, 1944 ; Seastone, 1947 ; Lowbury, Lilly, and Bull, 1963). Hexachlorophane has little immediate effect after a single application, but repeated use of the antiseptic causes a progressive reduction in the number of resident organisms. The poor activity of hexachlorophane against Gram-negative bacilli is another limitation which does not affect its value in reducing the resident flora, among which Staphylococcus aureus is the only common pathogen.

Suppressing or removing " transient " organisms from the skin presents a different problem. The transient flora include any bacteria that are deposited on the skin, such as Pseudomonas pyocyanea and strains of salmonella, shigella, and Escherichia coli which may be carried on the hands of nurses in wards where infection with these pathogens occurs ; Staph. aureus may also be carried as a transient organism. Antiseptics and ablutions are required to remove these organisms rapidly rather than to suppress them by a progressive or cumulative action ; residual antiseptic on the skin may, nevertheless, be useful in preventing contamination or in destroying contaminants which are deposited after antiseptic ablutions or treatment with antiseptic creams.

In this paper we report a comparative study on alternative methods for the removal of transient flora ; we also describe experiments on the survival of bacteria deposited on the skin after treatment with antiseptics.

Comparison of Methods for Removal of Ps. pyocyanea and Staph. aureus

Materials and Methods

Overnight nutrient-broth cultures of Staph. aureus (62/2904) and Ps. pyocyanea (62/1274) were washed three times and resuspended in distilled water, the staphylococcus in the same volume and the pseudomonas, because of its poor survival on drying, in one-tenth of the volume of the original culture. One drop (0.02 ml.) of the bacterial suspension was inoculated on a marked area of the palm of each hand in volunteers, the hands

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having previously been rinsed for two minutes in 70% ethyl alcohol to reduce the numbers of resident bacteria and allowed to dry ; the bacterial suspensions were spread over the marked area with a wire loop and allowed to dry.

The hands were then washed or treated with antiseptic in the manner described below, or left untreated for use as controls. Bacteriological samples were taken from the marked areas by rubbing in a standard way, under a measured volume (5 ml.) of sterile Ringer's solution containing neutralizers (see below) which was retained by a truncated glass cylinder—a method originally described by Story (1952). With a calibrated pipette, 0.5-ml. amounts of tenfold dilutions of these washings were inoculated on plates of nutrient agar and spread with a glass rod. Viable counts were made with the aid of a hand lens after 48 hours' incubation at 37° C. Neutralizers were included in sampling fluid, and culture media and tests for carry-over of antiseptic were made as described elsewhere (Lowbury et al., 1963).

Nine methods of cleansing or disinfecting the hands were studied and compared with untreated controls ; in each case the period of treatment was 30 seconds, and a standard number of strokes palm to palm, palm over dorsum, and with fingers interlaced was used, either for rinsing in a bowl containing 100 ml. of antiseptic solution, or for washing under a running tap. The methods were:

(1) Washing with bar soap and running water.

(2) Rinsing with an aqueous solution of chlorhexidine (Hibitane) diacetate (0.5%).

(3) Rinsing with an aqueous solution of chloroxylenol (Dettol), diluted 1 in 40.

(4) Rinsing with Savlon (an aqueous solution of chlorhexidine gluconate 1.5% and cetrimide 15%) diluted 1 in 200 with water as recommended by the manufacturers for disinfection of the hands.

(5) Washing with 3% hexachlorophane detergent cream (Phisohex) under running water.

(6) Washing with Phisohex containing 1% orthophenyl phenol under running water.

(7) Washing with a liquid soap containing a mixture of five halogenated alkyl/aryl phenols (Hycolin liquid soap) under running water.

(8) Washing with detergent solution containing a complex of iodine with polyvinylpyrrolidone (povidone-iodine, or Betadine, surgical scrub) under running water.

(9) Washing for 30 seconds with soap and water followed by a rinse (30 seconds) in 0.5% aqueous chlorhexidine diacetate solution.

(10) No treatment (control).

TABLE I.—Logarithms of Viable Counts of Staph. aureus from 0.1 ml. of Samples from Test Areas

Experiment	Code Number of Method of Hand Treatment															
	1		2		3		4		5		7		8		10 (Control)	
1	(A.J.)	3·20	(R.B.)	2·43	(J.D.)	2·91	(A.K.)	4·01	(M.S.)	3·61	(V.B.)	3·47	(S.F.)	2·70	(B.D.)	5·53
2	(B.D.)	3·30	(A.K.)	1·54	(A.J.)	3·67	(R.B.)	3·77	(V.B.)	3·83	(S.F.)	2·58	(M.S.)	1·48	(J.B.)	5·87
3	(V.B.)	3·26	(A.J.)	2·30	(R.B.)	2·86	(B.D.)	1·60	(S.F.)	3·45	(A.K.)	2·04	(J.D.)	1·48	(M.S.)	5·47
4	(A.K.)	3·38	(S.F.)	2·90	(V.B.)	3·57	(J.D.)	3·54	(R.B.)	3·35	(M.S.)	3·61	(B.D.)	0·70	(A.J.)	5·46
5	(J.D.)	2·86	(M.S.)	2·56	(B.D.)	3·36	(A.J.)	4·57	(A.K.)	4·12	(R.B.)	3·39	(V.B.)	1·89	(S.F.)	5·85
6	(M.S.)	2·59	(J.D.)	3·31	(S.F.)	2·35	(V.B.)	3·32	(B.D.)	3·80	(A.J.)	2·36	(R.B.)	0·70	(A.K.)	5·18
7	(S.F.)	1·70	(V.B.)	1·94	(A.K.)	3·79	(M.S.)	3·70	(J.D.)	2·98	(B.D.)	3·26	(A.J.)	1·89	(R.B.)	5·18
8	(R.B.)	3·34	(B.D.)	2·28	(M.S.)	2·35	(S.F.)	3·42	(A.J.)	3·68	(J.D.)	2·77	(A.K.)	2·04	(V.B.)	5·38
Mean log count	2·95		2·41		3·11		3·49		3·60		2·93		1·61		5·49	
Mean count (not logs)	1,402		499		2,348		8,320		5,111		1,573		104		363,359	

Initials of subjects are given in parentheses.

Separate experiments were made with the strains of *Staph. aureus* and *Ps. pyocyanea* ; a Latin-square design was used, each volunteer being treated once by each of the methods. In the experiment with *Staph. aureus*, methods 1, 2, 3, 4, 5, 7, 8, and 10 were tested ; in the experiment with *Ps. pyocyanea* the methods tested were 1, 2, 5, 6, 7, 8, 9, and 10.

Results

Staph. aureus.—The counts of *Staph. aureus* from samples taken after the various treatments are shown in Table I. As in previous studies, logarithms of the counts were used for statistical analysis, since this makes the distribution closer to normal. Arithmetical means are also given for comparison. Analysis of variance (Table II) confirms that the effect of treat-

TABLE II.—*Analysis of Variance of Log Counts of Staph. aureus After Different Treatments*

	Degrees of Freedom	Mean Squares
Persons	7	0·1370 N.S.
Experiments	7	0·5430 N.S.
Treatments	7	10·0417 S. (P<0·001)
Residual	42	0·3332

ment was highly significant. Differences due to individuals and to experimental days were not significant. All the treatments were significantly more effective than the control (No. 10). The most effective was povidone-iodine (No. 8), and this was also significantly better than the next best, aqueous chlorhexidine (No. 2), which itself was significantly more effective than treatments Nos. 4 and 5. Soap and water (No. 1) and treatments Nos. 3 and 7 had effects intermediate between these two groups.

Ps. pyocyanea.—A similar analysis of the counts of *Ps. pyocyanea* from sampling after the various treatments is summarized in Table III. Analysis of variance of the log counts shows a highly significant effect of treatment. Differences between individuals were also significant, but there was no significant difference between experimental days. All the treatments tested were more effective than the control (No. 10). The most effective was aqueous chlorhexidine (No. 2), and this was also significantly better than the next best treatment (No. 7), which itself was significantly more effective than soap and water (No. 1). The effects of the other treatments, however (Nos. 5, 6, 8, and 9), were not significantly different from that of soap and water (No. 1).

TABLE III.—*Summary of counts of Ps. pyocyanea after Different Treatments*

	Code Number of Method of Treatment							
	1	2	5	6	7	8	9	10 (Control)
Mean log count ..	3·29	1·50	3·49	3·22	2·48	2·88	2·97	6·09
Mean count (not logs)	4,016	167	3,515	27,322	1,405	1,726	3,498	2,956,037

Analysis of Variance

	Degrees of Freedom	Mean Squares
Persons	7	2·3924 S. (P<0·05)
Experiments	7	1·2108 N.S.
Treatment	7	13·7252 S. (P<0·001)
Residual	42	0·5963

Suppression of Contaminant Organisms by Residues of Antiseptic Left on Skin

The residues of antiseptic left on the skin after washing with an antiseptic soap or detergent, or after application of an antiseptic cream, might be expected to kill a proportion of the

bacteria that subsequently contaminate the skin. To test this hypothesis we made the following experiments.

Materials and Methods.—The methods of treatment were: (1) washing with Phisohex under a running tap for two minutes, followed by drying with a sterile towel ; (2) washing in a similar manner with povidone-iodine surgical scrub ; (3) application of chlorhexidine (1%) hand cream after washing with ordinary bar soap and water and drying with a sterile towel ; (4) washing with bar soap under a running tap for two minutes and drying with a sterile towel (control). Before these treatments the hands were rinsed for two minutes in a 70% aqueous solution of ethyl alcohol to reduce the resident flora. Immediately after the special treatment one drop (0.02 ml.) of a suspension of *Staph. aureus* (62/2904) was inoculated with a calibrated dropping-pipette on a marked circular area of the palm of each hand and spread over the whole area with a wire loop. The films of bacterial suspensions were allowed to dry and then left untouched for one hour. At the end of that time the area was sampled with sterile Ringer's solution containing neutralizers by the method of Story (1952), and viable counts were obtained in plates containing neutralizers ; tests for carry-over of antiseptic to sampling fluid and culture medium were made as described previously (Lowbury et al., 1963). A Latin-square design was used, each method being tested once on each subject.

Results.—The mean counts of *Staph. aureus* and the analysis of variance of the log counts are shown in Table IV. Treatment had a highly significant effect ; differences between individuals and between experimental days were not significant. All the antiseptic treatments had a significantly greater residual effect than soap and water. Phisohex had the greatest effect, but on the numbers studied this was not significantly greater than that of the other two antiseptic treatments.

TABLE IV.—*Residual Effect of Antiseptics. Summary of Counts of Staph. aureus from Hand-washing*

	Prior Antiseptic Treatment			
	Phisohex	Povidone-iodine	Chlorhexidine Cream	Soap and Water
Mean log count ..	2·94	3·71	3·93	5·57
Mean count (not logs)	4,246	8,190	43,284	435,500

Analysis of Variance

	Degrees of Freedom	Mean Squares
Persons	3	0·4449 N.S.
Experiments	3	0·9501 N.S.
Treatment	3	4·8820 S. (P<0·01)
Residual	6	0·5673

Comparison of Hypochlorite and Chlorhexidine Solutions and of Water in Removing Transient Flora

In 1847 Semmelweis showed that death from puerperal fever was greatly reduced after the introduction of hand-rinsing with chlorinated lime for those attending parturient women (see Sinclair, 1909). This result is commonly regarded as evidence that hypochlorite solutions have a valuable destructive effect upon *Streptococcus pyogenes* carried on the skin ; this organism, which does not multiply on normal skin, must be regarded as one of the transient flora.

In the following experiment we compared the effects of rinsing with a hypochlorite solution, with a chlorhexidine solution, and with distilled water on a transient skin contaminant.

Materials and Methods.—The materials tested were: (1) aqueous hypochlorite solution (Milton 1 in 80) ; (2) aqueous chlorhexidine diacetate solution (0.5%) ; (3) distilled water ; (4) no treatment (controls). A suspension of *Staph. aureus* (62/2904) was applied to marked areas on the palms of both hands, and the effects of 30 seconds' rinse with each fluid was

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assessed on four subjects by the method described above ; a Latin-square design was used.

Results.—The mean counts of *Staph. aureus* and the analysis of variance of the log counts are shown in Table V.   Treatment

TABLE V.—*Comparison of Hypochlorite and Chlorhexidine Solution. Summary of Counts of Staph. aureus*

	Treatment			
	Hypo-chlorite	Chlor-hexidine	Distilled Water	Nil
Mean log count ..	4·18	2·15	4·61	5·60
Mean count (not logs)	20,870	984	45,031	485,250

Analysis of Variance		
	Degrees of Freedom	Mean Squares
Persons	3	0·2797 N.S.
Experiments	3	0·7467 N.S.
Treatment	3	8·4050 S. (P<0·001)
Residual	6	0·3231

had a highly significant effect: differences between individuals and between experimental days were not significant.   Aqueous chlorhexidine was significantly more effective than hypochlorite solution.   The observed difference between the latter and a rinse with distilled water was not significant on the numbers tested, but both were significantly better than no treatment.

Removal of Transient Organisms from Rubber Gloves and from Skin

Because of the imperfect removal of resident organisms from skin even by the best antiseptics, it is sometimes urged that rubber gloves should be used by nurses for certain ward duties. The following experiment was made to determine the value of various methods of ablution and disinfection in removing transient bacteria from the finger-tips of the gloved hand ; similar tests were made on hands without gloves.

Materials and Methods.—Overnight broth cultures of *Staph. aureus* (62/2904), *Ps. pyocyanea* (62/1274), and *E. coli* (N.C.T.C. 9002) were washed three times and resuspended in distilled water (*Ps. pyocyanea* and *E. coli* in one-tenth of the original volume of fluid).   In each experiment one drop (0.02 ml.) of a suspension was spread and allowed to dry on each of the fingers of both hands (gloved or ungloved).   The following methods of cleansing (in each case for 30 seconds) were used: (1) washing with soap and water ; (2) washing with soap and water, followed by a rinse in 0.5% aqueous chlorhexidine diacetate (30 seconds each) ; (3) washing with 2% hexachlorophane liquid soap (Ster-zac) and water ; (4) washing with Phisohex and water ; (5) washing with Phisohex containing 1% orthophenyl phenol ; (6) washing with Hycolin liquid soap and water ; (7) rinsing with 0.5% aqueous chlorhexidine diacetate solution ; (8) no treatment (controls).   Two experiments were made with each organism, a different agent being used on each individual in each experiment.   Surface viable counts were obtained on plates inoculated with 0.5-ml. amounts of Ringer's solution in which the finger-tips had been sampled by rubbing against glass beads in a standard manner, as described elsewhere (Lowbury and Lilly, 1960).   The usual precautions were taken to neutralize and detect antiseptic carried over to sampling fluids and media.

Results.—These are summarized in Table VI.   All treatments, including soap and water, caused a considerable reduction in the transient flora ; even the largest proportion of survivors (group + + +) is less than 1% of the numbers found on the untreated skin of the controls.   The degree of reduction was too variable for numerical comparisons, but there was a consistent absence of detectable *E. coli* from rubber gloves after every type of treatment ; no treatment caused such elimination

from the ungloved hands.   Another consistent feature was the absence of *Staph. aureus, Ps. pyocyanea,* and *E. coli* from the gloved (but not the ungloved) hand after treatment with chlorhexidine solution.

TABLE VI.—*Transient Organisms Remaining After Various Cleansing Treatments of Gloved and Ungloved Fingers*

Treatment	Staph. aureus		Ps. pyocyanea		E. coli	
	Skin	Glove	Skin	Glove	Skin	Glove
Soap and water ..	+ + +*	+ +	+ + +	+	+ +	0
Soap and water, followed by aqueous chlorhexidine rinse	+ +	0	+	+	+	0
Ster-zac and water ..	+ +	+ + +	+	+	+	0
Phisohex and water ..	+ +	+	+ +	+	+ + +	0
Phisohex (with ortho-phenyl phenol) and water	+ +	+ +	+	+	+ +	0
Hycolin liquid soap and water	+ +	+ + +	+ +	+	+ +	0
Aqueous chlorhexidine rinse	+	0	+ +	0	+ + +	0

* Mean proportion of bacteria persisting on eight fingers as percentage of control counts : + + + =0·1%—<1% remaining.   + + =0·01%—<0·1% remaining. + =detectable—<0·01% remaining.   0=none detectable.

Discussion

It was shown in earlier studies—for example, Lowbury *et al.* (1963)—that the numbers of resident skin bacteria on the hands are considerably reduced by treatment with certain antiseptics but virtually unaffected by washing or scrubbing for the same time with soap and water.   In contrast, transient bacteria—represented in the studies described above by cultures of bacteria inoculated on the skin—were almost completely removed either by rinsing with antiseptics or by washing with soap and water ; there was little advantage in the use of antiseptics against these organisms, and neither rinsing with hypochlorite solution nor washing with hexachlorophane detergent cream had much advantage over physical ablution in removing them.   From the result with hypochlorite it seems probable that the historic success of Semmelweis in controlling puerperal fever by routine hand-rinsing with chlorinated lime was due more to physical effects of frequent and thorough rinsing than to the antiseptic used for the rinse.   Chlorhexidine solution and povidone-iodine surgical scrub were rather more effective than simple ablution, the former especially against *Ps. pyocyanea,* the latter against *Staph. aureus.*   Rinsing with chlorhexidine was found to have little effect if the hands were wet after a wash with soap and water, presumably because of inactivation of the antiseptic by soap ; drying the hands after washing might be expected to reduce or prevent such inactivation.   It is important, too, that chlorhexidine should not be diluted beyond the range in which it is effective ; this appears to have happened with Savlon diluted 1 in 200, which contained 0.0075% chlorhexidine gluconate and 0.075% cetrimide.

Although washing with soap and water—or even with water alone—removed the great majority of these inoculated transient organisms, some of them persisted even after treatment with antiseptic solutions.   More effective removal of these bacteria (especially of *E. coli* and, to a smaller extent, of *Ps. pyocyanea*) seemed possible, either by ablution or disinfection, from hands wearing gloves than from the bare skin—an argument for the more frequent use of gloves, which also protect the patient from the resident flora of the nurses' hands.

The application of an antiseptic which stays on the skin may have some value in preventing the survival or multiplication of bacteria that are subsequently deposited on the skin.   In our studies Phisohex and povidone-iodine surgical scrub were found to have such an effect.   With the former the result was not unexpected ; but in the case of the iodophor, which leaves no detectable iodine after the hands are rinsed with water, the persistent suppressive effect is presumably due to some other component of the preparation.   Chlorhexidine hand cream,

though quite ineffective in removing resident organisms (Lowbury et al., 1963) was found to have a considerable suppressive effect against bacteria inoculated later. It is, of course, uncertain how much of this suppressive effect occurs after treatment with these antiseptics when transient organisms are acquired naturally ; the result presumably depends on the amount of moisture present on the skin and in the inoculum, and is likely to vary with the individual and with environmental conditions.

Summary

Suspensions of *Staph. aureus* and *Ps. pyocyanea* were allowed to dry on marked areas of the palms of both hands. A number of alternative methods of disinfection and ablution were compared in respect of their activity in removing these "transient" contaminants. A Latin-square design and standard methods of washing, rinsing, and sampling were used.

All the methods tested had a large effect. In the experiments with *Staph. aureus* the most active methods (washing with povidone-iodine surgical scrub and rinsing with 0.5% aqueous chlorhexidine diacetate solution) caused reduction of 99.97% and 99.86% respectively in mean counts of samplings from the treated areas, as compared with untreated control areas ; these effects were slightly but significantly greater than that of bar soap and water, which caused a reduction of 99.62% in the mean count. Similar results were obtained in the experiment with *Ps. pyocyanea*, though with that organism an effect significantly better than that of soap and water was obtained only with chlorhexidine solution.

A chlorhexidine solution was significantly more active in removing *Staph. aureus* from the hands than a solution of hypochlorite (Milton 1 in 80) ; the latter did not cause a significantly greater effect than rinsing with distilled water.

Dried suspensions of *E. coli* were more effectively removed from hands wearing rubber gloves than from ungloved hands.

Suspensions of *Staph. aureus* allowed to dry on hands which were previously washed with a hexachlorophane or an iodophor detergent preparation or treated with chlorhexidine cream showed a significantly smaller proportion of survivors than similar inocula on hands which had not been treated with an antiseptic.

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Medical Memoranda

Insufficiency of Vertebral Artery Treated by Decompression of its Cervical Part

[WITH SPECIAL PLATE]

Vertigo, dizziness, weakness of the limbs, and episodes of falling to the ground without loss of consciousness, called "drop attacks" by Kremer (1958), have been attributed to insufficient blood supply in the territory of the vertebro-basilar system (Kubik and Adams, 1946 ; Silversides, 1954 ; Meyer, Sheehan, and Bauer, 1960). The symptoms are often provoked or exacerbated by movement of the neck. Obstruction of the vertebral artery caused by extension and rotation of the head was demonstrated in cadavers at the atlas and axis (de Kleyn and Nieuwenhuyse, 1927 ; Tissington Tatlow and Bammer, 1957) and in the foramina transversaria (Virtama and Kivalo, 1957). Hutchinson and Yates (1956) described distortion of the second part of the vertebral artery in cadavers affected by cervical spondylosis. Associated atheroma was found in 19 of the 48 cases examined.

Sheehan, Bauer, and Meyer (1960) demonstrated distortion of the vertebral artery by cervical osteophytes during life by transbrachial vertebral angiography. Extension and rotation of the head increased compression of the artery.

Surgical treatment of vertebro-basilar insufficiency has been devised previously. DeBakey, Crawford, Cooley, and Morris (1959) and DeBakey, Crawford, and Fields (1959) treated atheromatous obstruction at the origin of the vertebral artery by thromboendarterectomy or a by-pass operation. Fusion of the cervical spine was sometimes advocated but seldom performed.

No previous account could be found describing decompression of the second part of the vertebral artery for drop attacks and ischaemic symptoms precipitated by movements of the neck.

CASE REPORT

A man of 56 was admitted to hospital complaining of pain in the neck, weakness of the right limbs, dizziness while standing, and attacks of dropping to the ground.

When he was 10 years of age a mass of tuberculous glands were excised from the right side of his neck. In 1940, when aged 33, the roof caved in on him while he was engaged in fire-fighting during an air raid. He suffered from pain in the neck and shoulders for two months. When aged 41 the pain in his neck returned and he began to experience short attacks of dizziness and occasional loss of consciousness, associated with extension of the neck and turning of the head to either side. He was given a long cervical support to wear. He managed to drive a motorized invalid chair, but had to be careful, because even the slight degree of neck movement permitted by the cervical support could induce a feeling of impending loss of consciousness. About 10 months before his admission the periods of dizziness became so frequent that he could not get to work. His cervical spine was manipulated without anaesthesia at