1/72

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A Study of Disinfection of the Skin: A Comparison of Povidone-Iodine with Other Agents used for Surgical Scrubs

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[This reprint of the original article has been edited slightly to eliminate certain opinions in the introduction which may be interpreted as claims for the preparation under study. This editing has been done by the author and publisher.]

A Study of Disinfection of the Skin: * A Comparison of Povidone-Iodine with Other Agents used for Surgical Scrubs

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THE HISTORICAL origin of antisepsis antedates by a few hundred years the work of Lister. Its discovery came about in a tangential manner when it was discovered that meats could be preserved through smoking.[1] It was not known at the time, of course, that smoke contains creosote and other phenols which inhibit the growth or destroy the bacterial agents of putrefaction, and it required the brilliant insight of Lister to adapt the principal of antisepsis to medicine. This was accomplished in 1867 when he employed phenol, the prototype of all antiseptics, on hands, surgical instruments and patients' skin, and thereby decreased the rate of postoperative infection.

It is almost 100 years since Lister made his great discovery, and since that epochal event, there has been a constant and diligent search for more effective antiseptics. The principal characteristics that are sought in an ideal agent are:[3-5]

1. It should be effective against both resident and transient flora.
2. It should be effective against all microorganisms.
3. It should be capable of being applied quickly and the effect should be sustained at least through the operation.
4. It should be capable of being used on any part of the body of any human, including infants, without irritating or sensitizing.
5. It should not be rendered ineffective by the common materials found in hospitals, such as alcohol, organic matter, soap, etc.

These characteristics are often claimed for antiseptics, but too frequently the claims are not borne out by clinical evidence.

The first specific reference made to the use of iodine in wounds[4] was in 1839. Since then, numerous investigators have praised the distinctive activity of iodine as an antiseptic. As late as 1956, Zintel[15] compared Iodine Tincture with hexachlorophene, Merthiolate, Zephiran, and alcohol, and considered iodine the most effective agent used, but noted that Iodine Tincture sensitized 15 per cent of the patients treated.

Hexachlorophene is one of the more popular antiseptics. Price,[10] after extensive research, however, found "Hexachlorophene does not disinfect the skin quickly"; and, "Even in those persons whose cutaneous bacterial flora has been shown by appropriate tests to be sensitive to hexachlorophene, an occasional brief wash with hexachlorophene soap cannot be relied upon to keep the hands relatively free from infectious germs (as has been alleged), either under rubber gloves or in ordinary

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conditions of life." Zintel[15] stated "An alcohol dip should never be used following hexachlorophene preparations because the alcohol inactivates the hexachlorophene deposited on the skin"; and, "The detergent-hexachlorophene preparation is not a superior agent for preparation of the skin of the operative area." Thus, the chief weaknesses of hexachlorophene would seem to be that it requires time (which may not be available in an emergency) before it is truly antibacterial, and it is removed by solvents such as alcohol. The former is a potential danger, especially when elective surgery is planned since it is recommended that the patient apply hexachlorophene daily for five days before coming to the hospital. Obviously, any contact during the prescribed five-day period with alcohol, as in after-shave lotion, etc., can remove the hexachlorophene film. The same hazard is faced by surgeons and operating room personnel. It is necessary that the soap used by them at home, in the office, hospital or club, contain hexachlorophene. The hexachlorophene-bacteriostatic film is removed by regular soap and by any lotions containing alcohol.

Quaternary ammonium compounds, such as benzalkonium chloride are also popular as bactericidal or bacteriostatic agents. Reddish,[12] however, found "They (quaternary ammonium compounds) are effective for the purpose in proper concentration, but are counteracted to an appreciable extent by organic matter, especially by blood serum." Malizia *et al.*,[9] in a study made at Walter Reed General Hospital, record two instances in which benzalkonium chloride was incriminated as a source of hospital contamination. One of the incidents involved serious clinical infections as a result of the inability of this disinfectant (in aqueous solution) to maintain germicidal activity in the presence of gauze sponges. Ellison,[5] in a report on hand scrubs and skin preps, restated the widely-known fact that quaternary ammonium compounds are neutralized by even the most minute residues of soap.

A striking new addition to the field of antiseptics is Povidone-iodine* (polyvinyl-pyrrolidone-iodine complex, or PVP-I). This topical antiseptic agent possesses the germicidal effect of iodine. Povidone-Iodine Antiseptic Solution,** when applied to the skin, delineates the operative area but has the advantage of washing off easily. Povidone is a polymer which has attained wide use because of its ability to detoxify and prolong the activity of many drugs. When it is combined with iodine, the resulting complex has many highly desirable advantages over elemental iodine: 1) It retains the germicidal activity of elemental iodine;[11] 2) it prolongs the activity of the iodine by liberating it slowly;[8] 3) it has a lower toxicity than iodine.[14] In addition, it maintains its germicidal activity in the presence of blood, pus, serum, and necrotic tissue.[11]

A substantial body of literature indicates the superiority of Povidone-Iodine as a germicidal agent in destroying bacteria, viruses, fungi, protozoa, and yeasts. Shelanski and Shelanski[13] observed that it is of low toxicity but of high germicidal action, valuable in the treatment of a variety of skin and mucosal infections caused by many different types of bacteria. Bogash[2] reported on the treatment with PVP-Iodine, in the form of a topical antiseptic, on 5,900 patients over a three-year period with only two reactions or idiosyncrasies, both of which responded to supportive therapy. Gershenfeld[3] found that the bactericidal

* Povidone-Iodine NND.

** Betadine® Antiseptic, Tailby-Nason Company, Inc., Dover, Delaware. (Currently sold under the trade name Betadine® solution by The Purdue Frederick Co., Yonkers, New York.)

efficiency of PVP-I, even in dilutions up to and including the 1:10 dilution without and with whole human blood (10%) or blood serum (5.0%), was adequate to kill separately on contact, within 15 seconds, the bacteria in a 4.0 mm. loopful inoculum of the following tests organisms: *M. pyogenes* var. *aureus*, F. D. A. 209; *Sal. typhosa*, Hopkins strain; and the following organisms isolated from pathologic processes: *M. pyogenes* var. *aureus* (antibiotic resistant); two different strains, each of *Str. pyogenes* and *Ps. aeruginosa* and another strain of *Sal. typhosa*. This includes both gram-negative and gram-positive bacteria.[14] Georgiade *et al.*,[7] in determining the effects of various skin sterilizing technics on the viability of skin, found no agent more effective than Povidone-Iodine. Garnes *et al.*[6] conducted a clinical evaluation of Povidone-Iodine Aerosol Spray in surgical practice and reported that, in 200 emergency suture procedures and 100 major operations, Povidone-Iodine proved to be unsurpassed as a germicidal agent for surgery, burns, and stasis ulcers. Rabinowitz[11] reported an unusually low incidence (0.8%) of postoperative infection in 125 consecutive orthopedic operations using Povidone-Iodine solution and spray as the antiseptic, and found no evidence of skin irritation, injury or sensitizing reaction.

Like hospitals throughout the country, The New England Center Hospital has been concerned with the problem of antibiotic resistant strains of microorganisms and the rising rate of postoperative infections. A re-evaluation of the present aseptic program and new technics reported in literature showed that Povidone-Iodine Antiseptic Solution was the most effective topical antiseptic available. When, therefore, we were presented with a surgical scrub procedure incorporating this potent germicide, Povidone-Iodine Surgical Scrub,*** a study *in vitro* and *in vivo* to determine whether the preoperative scrub could be improved and standardized was carried out. The investigations were designed to make a clinical evaluation of Betadine Surgical Scrub to determine its efficiency in preoperative scrubbing by the operating personnel; a comparison of Betadine Surgical Scrub with various skin degerming agents; and an evaluation of Betadine Surgical Scrub for preoperative preparations of the skin.

*** Betadine Surgical Scrub, Tailby-Nason Company, Inc., Dover, Delaware.

Methods

I. Hand Scrub. Fifty persons, nurses and technicians, were instructed to scrub their hands with Betadine Surgical Scrub in the following manner: washing twice for five minutes, using 5.0 ml. of the preparation each time. The subjects employed surgeon's hand brushes and used the standard technic of anatomical scrub; the initial scrub consisted of 50 strokes to nails and 30 strokes to hands. After the first five-minute scrub, a copious lather was developed by adding water, then the hands were rinsed and the scrub repeated for another five minutes; the secondary scrub consisted of 25 strokes to nails and 15 strokes to hands. After the final rinsing, the hands were patted dry with a sterile towel.

Cultures were taken before and after the scrub. In 30 subjects, cultures were taken after the operative procedures, when the hands had been inside rubber surgical gloves for a period of time ranging between one to five hours. Cultures were taken by a method that seemed most practical for this study. The fingertips of one hand were pressed gently against the surface of a freshly made nutrient blood agar plate for ten seconds. The plates were then incubated for 48 hours at 37° C., and the colonies counted with the aid of a Quebec colony counter. A qualitative examination of the colonies was made according to standard bacteriological technics. There was no residual Povidone-Iodine to give

falsely negative results, since it is water soluble and was washed off the hands.

II. Comparison of Various Skin Degerming Agents. Twenty-five volunteers participated in this study, which consisted of a control and six experimental series. In all tests the anterior surface of the forearm was employed as the *operative* site. Cultures were taken by swabbing one square inch of the skin vigorously for one minute with a cotton-tipped applicator, moistened with 1.0 ml. of buffer (pH 7.5). The applicator was then immersed in a tube of melted nutrient agar cooled to 45° C., and shaken vigorously ten times. The applicator was then removed and a poured plate was made from the melted agar. The plates were incubated at 37° C. for 48 hours. The colonies were counted with the aid of a Quebec colony counter. The cultured organisms were identified by standard bacteriological technics. In the control series, cultures were taken from the untreated skin of the subjects at the same time, but from the opposite arm (Table 2A).

In the first experiment, the skin was rubbed vigorously for one minute with a gauze sponge soaked in liquid soap, then washed off with sterile water, patted dry with a sterile towel, and cultures made (Table 2B). In the second experiment, the skin was washed with liquid soap for one minute, rinsed with sterile water and patted dry with a sterile towel. It was then wiped for one minute with a gauze sponge soaked in 70 per cent ethyl alcohol. The alcohol was allowed to dry and cultures were taken (Table 2C). A similar test was also done using 1:1,000 aqueous Zephiran as the germicide. The skin was wiped for one minute with a gauze sponge soaked in Zephiran. Cultures were taken after this was patted dry with a sterile towel (Table 2D). The fourth experiment employed pHisoHex as the germicidal agent. The skin was washed with the preparation for five minutes, rinsed with sterile water and

TABLE 1.

Bacterial Count Culture of Fingertips

Subject No.	Before Scrub	After* Scrub	After Operation
1	15 C	0	0
2	70 A	0	0
3	170 A, B	4 A	0
4	300 D	5 A	5 A
5	100 A	0	0
6	55 A	0	0
7	25 B	0	0
8	40 A	0	0
9	110 A	2 B	0
10	85 A	0	0
11	50 A	0	0
12	350 D	16 A	10 A
13	40 A	0	0
14	185 D	3 A	0
15	70 A	0	0
16	45 C	0	0
17	60 A	0	0
18	65 B	0	0
19	125 A, B	2 C	0
20	70 A	0	0
21	45 A	0	0
22	60 A	0	0
23	20 A	0	0
24	45 A	0	0
25	55 A	0	0
26	35 A	0	0
27	60 A	0	0
28	85 A	0	0
29	40 A	0	0
30	30 C	0	0
31	45 A	0	0
32	120 A, B	1 A	0
33	175 A, B	2 C	0
34	80 A	0	0
35	25 C	0	0
36	30 B	0	0
37	60 A	0	0
38	45 A	0	0
39	55 A	0	0
40	70 A	0	0
41	15 C	0	0
42	30 A	0	0
43	40 A	0	0
44	35 C	0	0
45	75 B, C	0	0
46	150 A, B	1 B	0
47	85 A	0	0
48	70 A	0	0
49	50 A	0	0
50	105 A	1 A	0

* Betadine Surgical Scrub.
A = *Staphylococcus albus*, coagulase negative.
B = Diphtheroids.
C = *Gaffkya tetragena*.
D = *Staphylococcus aureus*, coagulase positive.

TABLE 2. *Number Bacteria/Inch² of Skin*

Case	A Untreated Skin	B After Soap and Water	C After Liquid Soap and 70% Alcohol	D After Aqueous Zephiran 1:1,000	E After pHisoHex	F After Betadine Antiseptic	G After Betadine Surgical Scrub
1	250	200	25 A	25 A	34 A	0	0
2	280	150	30 A	65 A, B	12 A	1 C	0
3	400	165	35 A, B	85 A, B, D	21 A, B	0	0
4	450	85	32 A	75 A, B	18 A	0	0
5	600	105	41 A, D	55 A, C	2 B	0	3 A
6	480	250	28 A	30 A	14 B, C	0	0
7	500	200	22 A	95 A, B	31 A	2 A	0
8	320	275	35 A	80 A	18 A	0	1 B
9	380	160	39 A, C	50 A, B	40 A, D	0	0
10	210	130	32 A	65 A	8 A	0	0
11	330	180	23 A	70 A, D	36 A, B	0	4 A
12	450	250	27 A	55 A	40 A	0	0
13	340	315	30 A	75 C	32 A	1 C	0
14	370	300	31 A	60 A, C	28 A	0	0
15	350	185	30 A, B	80 B	21 A	0	5 A
16	420	210	22 A	85 A, B, D	16 C	0	0
17	580	240	37 A	60 A	20 A	0	0
18	400	160	20 A	45 A, B	34 A, B	0	0
19	350	140	36 A, D	60 A	27 A	0	0
20	320	110	40 A	75 B, D	18 A	0	3 A
21	420	130	38 A, C	60 A, C	10 B	0	0
22	500	145	28 A	55 A, B	22 A	1 C	0
23	480	285	26 A	70 A	34 A	0	0
24	360	175	22 A	45 A	29 A, C	0	2 B
25	500	240	31 A	55 A, D	31 A	0	0

A = *Staphylococcus albus*, coagulase negative.
B = Diphtheroids.
C = *Gaffkya tetragena.*
D = *Staphylococcus aureus*, coagulase positive.

patted dry with a sterile towel, after which cultures were taken. These cultures were grown in media containing 0.5 per cent Tween 80, in order to inactivate any hexachlorophene that might still be present (Table 2E). The fifth experiment was carried out to determine the effectiveness of Betadine Antiseptic. The skin was cleaned with soap and water, rinsed with sterile water, and then wiped for one minute with a gauze sponge soaked in Betadine Antiseptic. This was allowed to dry, and cultures were taken (Table 2F). In the sixth experiment the skin was washed for five minutes with gauze soaked in Betadine Surgical Scrub. The usual rinsing with sterile water and drying with a sterile towel followed before the cultures were taken. The sterile water rinse was considered sufficient to remove any traces of water soluble Betadine (Table 2G).

III. Preoperative Preparation of Patients Undergoing Surgery. A group of 40 patients undergoing general surgery were employed for the test of Betadine Surgical Scrub. Another group of 25 patients were used for testing pHisoHex. The skin preparation consisted of a five-minute scrubbing with two gauze sponges soaked with Betadine Surgical Scrub, and used in an anatomical scrub, i.e., from the center to the periphery of the area. This was followed by rinsing with sterile water and a gentle drying with sterile towels. The sterile water rinse was considered sufficient to remove any traces of water-soluble Betadine. In each case, the skin was cul-

tured before and immediately after the skin preparation, and at the end of the operations which were of 15 minutes to three hours duration.

To make a culture, a cotton-tipped applicator moistened with 1.0 ml. of pH 7.5 buffer was rubbed vigorously over one square inch of the skin for one minute. The applicator was then placed in a tube of melted blood agar cooled to 45° C., after which the agar was mixed thoroughly by shaking the tube vigorously ten times. Pour plates were made and incubated at 37° C. for 48 hours. The bacterial counts were made with the aid of a Quebec colony counter. Standard bacteriologic technics were employed to identify the cultured organisms.

Results

Hand Scrub. The results are summarized in Table 1. Of the 50 persons tested, 40 showed no bacteria after scrubbing with Betadine Surgical Scrub. In eight subjects the bacterial count was reduced by 98 per cent, and in two, the bacterial count was reduced by over 95 per cent. Of the 30 persons who wore gloves during the operative procedures, only two showed a positive culture. In one, the count was the same as immediately after scrubbing. In the other it was slightly decreased.

None of the organisms isolated after scrubbing or after the operation were pathogenic. Diphtheroids, *Staphylococcus albus*, coagulase negative, and *Gaffkya tetragena* were the ubiquitous skin saprophytes isolated, although three of the 50 subjects had *Staphylococcus aureus*, coagulase positive, on their fingertips when studied initially.

Comparison of Various Skin Degerming Agents. The results are presented in

TABLE 3. *Preoperative Skin Preparation with pHisoHex® Number Bacteria/Inch² of Skin*

Subject No.	Before Scrub	After Scrub	After Operation
1	0	0	0
2	2,240 D, E	332 D, E	2,880 E
3	352 A, F	928 D, F	0
4	14,400 F	3,520 D, F	0
5	287 B	105 B	416 B
6	2,200 A, E	330 A, E	2,900 E
7	352 B, E	930 B, E	0
8	14,000 E	3,500 A, B, E	0
9	36 E	5,600 B, E	0
10	>10,000 E	3,800 E	0
11	0	23 B	58 E
12	3,400 E	0	172 B, E
13	0	0	0
14	0	0	0
15	10,000 B, E	120 B	5,000 B, E
16	36 E	5,600 B, E	>10,000 E
17	1,600 B	46 E	0
18	>10,000 E	>10,000 E	>10,000 E
19	4 B	23 E	0
20	0	0	13 B
21	90 B	0	0
22	64 E	90 B, E	29 B
23	0	176 B	0
24	544 E	6,300 E	0
25	0	25 E	0

A = *Staphylococcus albus*, coagulase negative. B = Diphtheroids. C = *Gaffkya tetragena*.
D = *Staphylococcus aureus*, coagulase positive. E = Coliform bacilli.

Table 2. The bacterial counts in the untreated control series (Table 2A) ranged from 200 to 600 organisms per square inch of untreated skin. Following soap and water wash (Table 2B), the range was from 85 to 315. Washing with soap and water, followed by wiping with 70 per cent alcohol (Table 2C), reduced the bacterial count approximately tenfold. They ranged from 20 to 40 organisms per square inch of skin. After wiping with Zephiran (Table 2D), the count ranged from 25 to 95 organisms per square inch of skin. After five minute washing with pHisoHex (Table 2E), the count ranged from two to 40 organisms per square inch of skin. The bacterial counts after wiping with Betadine Antiseptic (Table 2F), ranged from 0 to two organisms per square inch of skin, while the Betadine Surgical Scrub series (Table 2G) ranged from 0 to five organisms per square inch of skin.

TABLE 4. *Preoperative Skin Preparation with Betadine Surgical Scrub*
Number Bacteria/Inch² of Skin

Subject No.	Before Scrub	After Scrub	After Operation
1	0	0	0
2	5 D	0	0
3	10 A, D	0	0
4	10 A	0	0
5	0	0	0
6	10 D	0	0
7	10 A	0	0
8	0	0	0
9	2,240 A	0	0
10	0	0	0
11	41 D	0	0
12	0	0	0
13	6 A	0	0
14	0	0	0
15	1,000 A	0	0
16	59 A	0	0
17	65 A	0	0
18	36,000 D	3 D	0
19	480 A	0	0
20	12 A	0	0
21	28,000 A	0	0
22	27 A	0	0
23	11 A	0	0
24	10 A	0	0
25	0	0	0
26	100 A	0	0
27	65 D	0	0
28	0	0	0
29	0	0	0
30	0	0	0
31	46 A	0	0
32	0	0	0
33	6 A	0	0
34	0	0	0
35	1,100 A	0	0
36	0	0	0
37	112 A	0	0
38	56 A	0	0
39	3 A	0	0
40	0	0	0

A = *Staphylococcus albus*, coagulase negative.
B = Diphtheroids.
C = *Gaffkya tetragena.*
D = *Staphylococcus aureus*, coagulase positive.

Preoperative Preparation of Patients Undergoing Surgery. The results are summarized in Tables 3 and 4. Of the 25 patients prepared with pHisoHex (Table 3), 19 had positive cultures immediately after the skin preparation. Ten had a positive culture after the operation. None of the 40 patients prepared with Betadine Surgical Scrub (Table 4) had any bacteria demonstrable on the scrubbed skin immediately after the preparation, or after the operation. There was one exception, however; in this case an initial count of 36,000 organisms was reduced to three by the scrub.

Discussion

A series of studies was performed to determine the most effective method of degerming the skin prior to doing surgical procedures. In order to prevent skin bacteria from being introduced into the deeper tissues or even into the circulation, three steps are considered necessary: 1) Removal of dirt and organic matter from the skin; 2) removal of fatty or greasy film from the skin; and 3) degerming of the cleaned skin. In order to remove dirt, vigorous rubbing with a gauze sponge soaked with liquid soap is very effective. However, it is necessary to rub off not only the surface dirt, but the dead layer of epithelial cells that harbor many of the skin bacteria.

In the present study, Betadine Surgical Scrub was submitted to intensive testing under conditions simulating preoperative hand scrubbing and preoperative skin preparation of the operative site, as well as in actual hospital use. Control studies using standard bacteriological technics established the types and numbers of microorganisms present on the skin of the subjects prior to application of the agent.

Both qualitative and quantitative bacteriological counts were made after scrubbing or skin preparation, and after operations, so that a continuous record was kept of the ability of the agent to degerm the skin. One study determined the efficacy of Betadine Surgical Scrub as a preoperative hand scrub, in nurses and surgical technicians highly proficient in operating room procedure; a second study compared the degerming ability of Betadine Antiseptic Solution, Betadine Surgical Scrub, pHisoHex, Zephiran, liquid soap and water, and liquid soap plus 70 per cent ethyl alcohol on a one inch *operative* site; the third test compared the skin degerming efficacy of Betadine Surgical Scrub and pHisoHex in the preoperative preparation of patients undergoing surgery.

Summary

It was demonstrated that 40 of 50 nurses and surgical technicians, after scrubbing with Betadine Surgical Scrub, showed no demonstrable bacteria on their skin. Of the other ten, eight showed reduction of the bacterial count by 98 per cent, and the other two by 95 per cent. Thirty subjects who wore gloves during operations were tested postoperatively after removal of the gloves. Only two of these subjects had positive skin cultures (*Staphylococcus albus*, coagulase negative). Neither after the preoperative scrubbing nor after the operation were any pathogenic organisms found, although the control culture indicated that *Staphylococcus aureus*, coagulase positive, was on the hands of three subjects (Table 1).

A control series established bacterial counts ranging from 200 to 600 organisms per square inch of skin on 25 volunteers. A soap and water wash showed a bacterial count ranging from 85 to 315; soap and water and ethyl alcohol reduced the bacterial count to a range of 20 to 40 organisms; after wiping with Zephiran the range was from 25 to 95 organisms; a five-minute washing with pHisoHex gave a bacterial count ranging from two to 40 organisms; Betadine Antiseptic gave a bacterial count ranging from 0 to two organisms; and a five-minute washing with Betadine Surgical Scrub reduced the bacterial count to a range of 0 to five organisms (Table 2).

Forty patients were prepared with Betadine Surgical Scrub and 25 patients were prepared with pHisoHex. Of the 40 patients in the group treated with Betadine Surgical Scrub, only one showed a bacterial count (of three), and no patient showed a count postoperatively. In contrast, of the 25 patients treated with pHisoHex, 19 showed a bacterial count, seven of them above 3,000; and after the operation 11 patients had a bacterial count, two of them above 10,000 organisms per square inch (Table 3).

Conclusion

1. Betadine Surgical Scrub was tested in comparison with liquid soap, liquid soap followed by 70 per cent ethyl alcohol, Zephiran 1:1,000, pHisoHex, and Betadine Antiseptic to determine its effectiveness for preoperative scrubbing by the operating personnel and for preparation of the patients' skin. Cultures were taken before and after preparing the skin, and after operations.

2. Betadine Surgical Scrub is a powerful degerming agent, superior to pHisoHex, Zephiran 1:1,000, liquid soap, and liquid soap followed by 70 per cent ethyl alcohol.

3. Betadine Surgical Scrub produced no irritation, side effects, or dryness of skin.

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13

Do Antiseptic Agents Reduce Surgical Wound Infections : Preoperative Bathing, Operative Site Preparation, Surgical Hand Scrub?

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Introduction

Skin antisepsis practices are based on sound theoretical principles of infection prevention, but are also fraught with ritual. A review of the biomedical literature through February 1994 was conducted to examine evidence of a causal association between risk of infection and three aspects of preoperative skin preparation: preoperative whole body patient bathing, preoperative patient skin preparation of the operative site, and the hand scrub of the surgical team. The purpose of this chapter is to summarize and evaluate the research relating preoperative skin preparation practices with infection rates.

Methods

The full computerized Medline System data base was searched using the following terms: nosocomial, cross infections, skin, anti-infective agents, bacterial infections (prevention and control of) and infection control. Articles were limited to those in English only, with human subjects, and covered the years of 1960 to February, 1994. Those articles available through the National Library of Medicine, Bethesda, MD, were incorporated into this literature review.

Results

Preoperative Whole Body Patient Bathing

Thirteen articles were found in which the effect of preoperative patient bathing regimens on surgical wound infection rates or levels of bacterial colonization was evaluated (Table I).

Several blinded, randomized trials were reported. In one (10), two groups of patients received three preoperative showers with either a 4% chlorhexidine detergent solution (n=57) or a placebo soap (n=58). The chlorhexidine group had

Table I. Studies of Effects of Preoperative Patient Bathing and Skin Preparation on Colonization and Infection Rates

Author/Year	Prep Type Bath/Scrub	Agents * A/I/C/O	Outcome: Col/Inf	Results
Brandberg (1981)	Bath	C	Inf	Significant decrease in inf in C Group
Aylliffe (1983)	Bath	C	Inf	No significant differences
Rotter (1988)	Bath	C	Inf	No significant differences
Hayek (1988)	Bath	C	Inf	Significant decrease in inf
Leclair (1988)	Bath	C, I	Col	Decrease with C
Kaiser (1988)	Bath	C, I	Col	Greater decrease with C - Repeated applications superior
Garibaldi (1988)	Bath	C, I, O	Col	Greater decrease with C
Mannion (1989)	Bath	C, I	Col	Decrease with C
Earnshaw (1989)	Bath	C	Inf	No significant differences between C and plain soap
Byrne (1990)	Bath	C	Col	Significant decrease with 2 showers
Byrne (1991)	Bath	C	Col	20-fold decrease in skin colonization
Lynch (1992)	Bath	C	Col/Inf	Col significantly reduced; no difference in inf
Ichida (1993)	Bath	C	Col/Inf	Lower, but no significant differences in col or inf in C Group

* A=alcohol, I=povidone-iodine or iodophor, C=chlorhexidine, O=other
Col=bacterial colonization, Inf=infection rates

a 20-fold reduction in skin flora as compared to no change in skin flora in the placebo group ($p<0.01$). There was a 5-day median time to recolonization in the chlorhexidine group and a 2-day median time in the placebo group. The sample size was too small to allow a meaningful comparison of infection rates.

In a second trial (19) of 575 surgical patients, chlorhexidine, povidone-iodine and medicated soap were compared. Two preoperative showers using four applications of chlorhexidine were significantly more effective in reducing levels of bacterial colonization than showering with either of the other treatments in like manner. Postshower cultures from patients using chlorhexidine yielded no growth on 43% of the surgical sites preoperatively as compared to 16% with the povidone-iodine group and 6% in the soap group ($p<0.001$).

In a prospective, randomized observer-blinded study of 39 patients, chlorhexidine, povidone-iodine and lotion soap were evaluated in one-stage (evening only) versus two-stage (both evening and morning) showers (26). Chlorhexidine was associated with a significant reduction in staphylococcal colonization, while the results obtained from povidone-iodine were inconsistent. Showering with soap actually increased staphylococcal colony counts. Repeated applications of chlorhexidine were superior to a single application.

Mannion, Thom, Reynolds and Strachan (37) studied the bacterial flora of 12 cardiac patients preparing for aortic grafts and three patients undergoing abdominal herniorrhaphies. The patients undergoing vascular grafts were divided into Group A, who received 2% aqueous povidone-iodine soaks to the groin at least one hour prior to surgery, and Group B, who received two baths with a 4% chlorhexidine gluconate product, one the day before and one the morning of surgery. The three patients undergoing repair of abdominal hernias served as controls. All groups received the same preoperative skin site preparation. Groups A and B also received antibiotic prophylaxis. A total of 1,106 isolates (892 staphylococci) were obtained from the 15 patients studied. Bacterial counts from all patients were twenty-fold higher from perianal swabs than from groin swabs and increased with hospital stay, except in Group B. Perianal bacterial counts from patients in Group B one week and two weeks postsurgically were lower than on admission. No statistical analyses were reported, probably because of the small number of patients studied.

Byrne et al. (9) evaluated chlorhexidine showering or bathing in 10 volunteers. A significant reduction ($p<0.005$) in skin flora was evident after two preoperative showers with 4% chlorhexidine. The authors recommended three showers using 4% chlorhexidine for optimum antiseptic activity.

Leclair *et al.* (29) randomized 151 patients undergoing neurosurgery into four groups to compare the efficacy of shampoo and/or site preparations using chlorhexidine in groups A and B and iodophor in groups C and D. All groups received surgical site preparation with their respective antiseptics while groups A and C also received shampoos with chlorhexidine or iodophor, respectively. Patients receiving both the chlorhexidine shampoo and site preparation had significantly fewer positive scalp cultures at the end of surgery. There were no reported postoperative infections from any group.

Hayek and Emerson (22) studied 2,915 patients assigned to one of three preoperative bathing regimens, each taking two showers: (1) chlorhexidine (n=689), (2) placebo (n=700), and (3) plain soap (n=626). Infection rates were: chlorhexidine, 9%; placebo, 11.7%; and plain soap, 12.9% ($p<0.05$). The initial placebo product was found to have some antibacterial effect, which might have explained the lower infection rate with placebo rather than soap.

Two randomized groups of patients undergoing prosthetic vascular grafts (16) received two baths: one group used chlorhexidine (n=31) and the other group used a non-medicated soap (n=35). All patients received chlorhexidine for skin site preparation just prior to surgery. Wound infection rate in the chlorhexidine group was 26%, as compared to 11% in the soap group ($p=0.12$). The small sample size in the study increased the risk of a Type II error.

A randomized, double-blind, placebo-controlled study of 3,482 general surgery patients (34) was conducted to measure the efficacy of whole body disinfection with chlorhexidine as compared with plain soap. After three showers, the patients using chlorhexidine had significantly reduced skin colony counts when compared with those showering with the detergent. Wound infection rates were similar in both groups (5.79% and 5.75%, respectively) and there was no economic benefit obtained from using the chlorhexidine suppression of skin flora.

Ichida (24) compared routine bathing of 71 burn patients (historical control group) to a total body chlorhexidine bath for 84 patients. The chlorhexidine group had overall lower colonization counts, rate of infection was 55% higher and median length of hospital stay was longer for the historical control group. However, no differences were statistically significant. A larger sample size would have been needed to increase the statistical power to 80%.

A non-randomized, prospective study was conducted in Sweden (8) to examine the effects of whole body bathing with chlorhexidine on wound infection rates. Half of the patients (n=171) received the standard preoperative site preparation and the others (n=170), in addition to the standard preparation, took 3-8 showers with chlorhexidine. In the standard group, 17.5% of the patients developed wound infections as compared to 8% of the patients in the shower-bath group ($p<0.05$).

Rotter *et al.* (46) compared two groups of patients in a prospective, randomized, double-blind, placebo-controlled whole body bathing study. In one group, 1,413 patients bathed twice using a chlorhexidine detergent and the other group of 1,400 patients used a non-chlorhexidine detergent. There was no significant difference in infection rates between the groups (2.6% and 2.4%, respectively).

In a similar study, chlorhexidine preoperative bathing was compared with non-medicated soap bathing (4) in 5,536 patients. The majority of patients (92.6%) had one bath, while 7.4% had either two or three baths. There was no significant difference ($p=.44$) in infection rates among those patients using chlorhexidine (48.8%) as compared with those patients using the non-medicated soap (51.2%).

Preoperative Patient Skin Preparation

Most of the studies assessing preoperative patient skin preparation have compared the effects of various products on bacterial colonization rates (Table II). Agents used in the past have included mercury-containing and quaternary ammonium compounds, iodine, and hexachlorophene. For various reasons, including low bactericidal activity, narrow spectrum of activity, or toxic side effects, these agents are rarely used today. Primary alternatives currently include alcohols, chlorhexidine gluconate, and iodophors. These agents have been studied using a variety of protocols (35). This section contains a review of recent studies of these products for preparation of patient skin prior to surgery.

Geelhoed *et al.* (20) randomly assigned 178 thoracic and general surgery patients to receive one of three skin preparations: a traditional 5-minute scrub with iodophor soap followed by painting and cloth draping; the same 5-minute scrub followed by alcohol cleansing and application of an antimicrobial drape; a 1-minute alcohol cleansing followed by application of the antimicrobial drape. While all three protocols resulted in a significant reduction of skin flora, the 1-minute alcohol cleansing was associated with a significantly better reduction than the 5-minute iodophor scrub.

Thirty subjects undergoing total joint surgery had a traditional 5-minute iodophor scrub followed by application of iodophor paint. The skin of 30 comparison subjects was painted with an iodophor tincture solution. Both protocols were comparably effective in reducing bacterial counts at the operative site (21).

Similarly, three skin preparation methods were compared on the faces of 14 volunteers: a 10-second alcohol wipe, and 60-second applications of either alcohol, povidone-iodine, or tincture of chlorhexidine. There were no significant differences in reductions in bacterial counts between these four groups five minutes after application. One hour after application, the povidone-iodine and chlorhexidine-treated sites maintained lower bacterial counts than the alcohol sites. None of the products had much impact on anaerobic flora (15).

One British group simultaneously tested five different preoperative protocols in 15 volunteers: 3-minute paint with chlorhexidine in alcohol; 3-minute paint with povidone-iodine in alcohol; 1-minute paint with 70% isopropyl alcohol followed by application of a plastic adhesive drape; 1-minute paint with alcohol followed by application of a plastic adhesive drape containing slow-release iodophor; and a control. At all time periods tested (5, 30, 60, 180 minutes), the site prepared with the alcohol and iodophor drape had the lowest bacterial counts (25).

Levy *et al.* (30) also studied the effect of an iodophor-impregnated adhesive drape on the contamination of intravascular catheters and the glove tips of anesthesiologists. Among the 30 patients for whom iodophor alone was used, 83% of glove tips and 13% of catheters were contaminated; none were contaminated in the group prepared with the impregnated film. Kutarski and Grundy (27) concluded that there were no significant differences in bacterial reductions up to 2 hours after application of povidone-iodine whether the antiseptic was wiped off after 30 seconds or allowed to dry.

Table II. Studies of Preoperative Patient Skin Preparation and Infection Rates

Author/Year	Prep Type Bath/Scrub	Agents* A/I/C/O	Outcome Col/Inf	Results
Levy (1980)	Scrub	I	Col	Higher levels of contamination of IV catheters and glove tips without iodophor-impregnated film
Geelhoed (1963)	Scrub	A, I	Col	1-min A significantly better than 5 min I
Alexander (1985)	Scrub	A,I	Col, Inf	No significant difference in bacterial counts.
Johnston (1987)	Scrub	A, I, C	Col	A with iodophor drape had lowest bacterial count up to 3 hrs after application
Dzubow (1988)	Scrub	A, I, C	Col	No significant differences in 10-sec A wipe and 60 sec application of A, I or C tincture immediately after application. Lower counts maintained after 1 hr with I and C
Payne (1989)	Scrub	I	Inf	Iodophor paint applied 12 hours prior to surgery associated with significant reduction in amputation site infections
Gilliam (1990)	Scrub	I	Col	Iodophor scrub plus tincture comparable to iodophor tincture alone
Longombe (1991)	Scrub	A	Inf	No infectious complication in 1,027 injections and spinal taps or 127 surgical procedures
Robson (1992)	Scrub	I	Inf	No significant difference in infection rates with two or one step regimen of iodophor scrub and paint.

*A=alcohol, I=povidone-iodine or iodophor, C=chlorhexidine, O=other

Col=bacterial colonization, Inf=Infection rates

Evidence is clear that preoperative shaving of the surgical site, particularly when done the day before surgery, increases the risk of wound infection by creating nicks which are more readily colonized. Depilatories or, if necessary, clipping or shaving of the site in the operating room immediately prior to surgery, are safer alternatives (2,11,12,22,43,47).

With the exception of shaving, very little work has been done to assess the impact of other aspects of preoperative patient skin preparation on surgical wound infection rates. Such studies present difficult logistical and practical problems and would require large sample sizes at prohibitive costs. A few relevant studies are reviewed below.

One group (1) conducted three randomized clinical trials including 1,324 patients to compare two skin preparation protocols: a 1-minute scrub with 70% alcohol and application of an iodophor-impregnated drape or a 1-minute scrub with 2% iodine in 90% alcohol followed by application of the drape. The investigators found that when drapes were lifted from the operative site during surgery, there was a significant increase in infection rates. Consistent with other studies, the infection rate was higher when hair was shaved and in clean-contaminated or contaminated cases. There were no infections in the 133 patients whose operations were shorter than 2 hours. However, there were no significant differences in bacterial counts at the site or in infection rates among patients receiving the alcohol or the iodophor preparation. In this study, the use of the drape rather than the antiseptic was a more important factor with regard to risk of wound infection.

Several investigators in Kenya tested the use of alcohol as an application to surgeons' hands before donning gloves and in a 2% iodine tincture for cleaning injection and operative sites. They reported no infectious complications associated with 1,027 injections and spinal taps and 127 surgical procedures, primarily herniorrhaphies and laparotomies. They suggested that alcohol, which is extremely inexpensive and readily available in developing countries, can be safely used for preoperative skin preparation (31).

In a group of 19 patients, Payne *et al.* (39) applied povidone-iodine paint 12 hours prior to amputation and covered the extremity with a sterile towel. These patients had significantly lower infection rates and shorter hospital stays than a comparison group of patients who received the same paint preparation just prio to surgery. However, nursing time to do the preparation was greatly increased an the investigators had difficulty with accrual to the study protocol because of a nursing shortage.

Two Canadian investigators compared a two-step regimen with povidone iodine scrub followed by povidone-iodine paint to a one-step paint alone. In 75 clean and clean-contaminated cases, there were no significant differences i surgical wound infection rates among those randomly assigned to either treatmer group (45).

Two studies comparing products used for cleansing of intravascular cath eter sites have also examined infection rates. In one study, 150 patients receivin brachial vein cutdowns were randomly assigned to daily site care with one three products: 70% ethyl alcohol, 1% chlorhexidine cream, or 5% iodoph

cream. The chlorhexidine group had more infectious complications and significantly higher incidence of positive catheter tip cultures (13). However, this was associated with daily application rather than initial site preparation and therefore has limited relevance to preoperative patient skin preparation.

In a prospective randomized trial, Maki *et al.* (36) compared local site infection and bacteremia rates associated with central line site preparation using either povidone-iodine, alcohol, or chlorhexidine gluconate. They reported a significantly lower rate of local infection and bacteremia in the aqueous chlorhexidine group. However, there were confounding variables which may have had an impact on the results (50). Additionally, aqueous chlorhexidine is not available in the United States.

Surgical Hand Scrub

The rationale for surgical hand scrub is to afford maximum reduction in total microbial populations on the hands of the surgical team member in order to minimize the possibility of patient wound contamination. To that end, a variety of techniques and products have been tested for their relative efficacy in reducing skin colonization. Elaborate efforts to reduce the quantity of skin flora on surgeons' hands were of paramount importance before the onset of universal use of gloves by surgical personnel. The wearing of sterile gloves has reduced by many fold the exchange of microorganisms between patient and surgical staff. However, the presence of undetected pinholes and leaks which occur during glove wearing has served as the rationale for a continued requirement for the preparation of the surgical team's hands with a lengthy application of an antimicrobial agent. The Association of Operating Room Nurses, for example, publishes prescriptive standards for the surgical hand scrub (3).

Studies of surgical hand antisepsis are limited primarily to evaluations of bacterial colonization. Examples of such studies, in chronologic order, which compare products or variations in technique are found in the reference list (5,6,17,28,32,38,42,48) and (14,33,40,41,44,49) respectively (Table III). Murie and Macpherson (38) reported no significant differences in infection rates when chlorhexidine was used in a detergent or a methanol base, although their sample size was insufficient for adequate statistical power.

Discussion

Preoperative Patient Bathing

Preparation of patients' skin prior to surgery has been a common practice since the late nineteenth century. It has two purposes: physical cleansing and removal of dirt, mircoorganisms, and residue; and antisepsis to minimize the total microbial load. Protocols for preoperative patient skin preparation are based on our understanding of the physiology of the skin and on the fact that the majority of surgical wound infections are associated with skin flora, often that of the

Table III. Studies of Skin Preparation of Hands of Surgical Team

Author/Year	Agents* A/I/C/O	Outcome Col/Inf	Results
Peterson (1978)	I,C,O	Col	Initial reductions:C > I > O (Hexachlorophene). Significant regrowth under gloves with I, but not with C or hexachlorophene
Eitzen (1979)	I, O	Col	Significant post-scrub reductions with I, triclosan, and hexachlorophene
Murie (1980)	C	Col, Inf	C in 95% methanol > aqueous detergent of C. No significant difference in wound infection rates
Ayliffe (1984)	I,C,O	Col	Alcoholic C > C in detergent > I
Soulsby (1986)	C,O	Col	C comparable to PCMX
Larson (1990)	A,I,C,O	Col	A > C > I > triclosan
Babb (1991)	A,I,C,O	Col	A > I > triclosan C products varied considerably by brand

*A=alcohol, I=povidone-iodine, C=chlorhexidine, O=other
Col=bacterial colonization, Inf=infection rates

patient. Hence, it is reasonable to expect that minimizing numbers of microorganisms on the skin immediately prior to surgery would reduce the risk of wound contamination and subsequent infection.

A series of clinical trials evaluating the effects of preoperative chlorhexidine showers on subsequent postoperative wound infection rates has been published over the past two decades, and yet results remain equivocal. Initial evidence of a reduction in risk of wound infection emanated from Sweden with the work reported by Brandberg (8). Subsequent European controlled trials with large sample sizes by Rotter *et al.* (2,813 patients) and Ayliffe and colleagues (5,536 patients) failed to demonstrate any protective effect. Hayek and Emerson (2,015 patients), on the other hand, reported a significantly lower infection rate among the chlorhexidine-bathed group. None of these three studies used similar definitions or surveillance systems. Additionally, the intervention varied from one to three applications and there was little attempt to monitor patient compliance with the prescribed regimens.

Since chlorhexidine requires several applications before its substantive effect is evident, it is not surprising that there would be little or no effect with one or perhaps even two applications. Even if two or three applications were effective, practical issues such as compliance and the fact that many of the highest risk surgical procedures are not elective (and therefore no preoperative showers could be planned) would limit the value of the practice of antimicrobial preoperative patient bathing. One component that needs to be added to the evaluation of this practice is a cost/benefit analysis.

Preoperative Patient Skin Preparation

The recent work assessing preoperative patient skin preparation has focused on incremental changes that could potentially result in time or cost savings (15,20,21,25,27,30). In general, shorter scrubs with agents containing alcohols or using antimicrobial-impregnated films are reported to have equivalent, and sometimes better, effectiveness as compared to longer procedures. Protocols that increase the need for either staff time or patient adherence, such as the one described by Payne (5), are unlikely to gain wide acceptance.

Variations in preoperative skin preparation, including type of product used, drape, and duration and method of product application do have an impact on levels of bacterial colonization and wound infection rates. However, too few studies with comparable protocols are available to assess the ideal components of the preparation. This is an area of patient care practice that is fraught with ritual and deserves thoughtful reconsideration to combine maximum efficacy with cost effectiveness and patient comfort.

Surgical Hand Scrub

Boyce and colleagues (7) recently reported a common-source outbreak of *Staphylococcus epidermidis* infections among patients undergoing cardiac surgery. The epidemic strain was traced to the hands of a surgeon, and the outbreak ceased when the surgeon changed his hand scrub practices. Such case studies, anecdotally common but rarely published in the 1950s and 1960s, are clear evidence of a causal link between the hand preparation of the surgical team and preoperative wound infection rates. Nevertheless, less than 2% of wound infections are associated with exogenous sources (18). Ayliffe (5) has suggested that although preoperative surgical hand scrub protocols may be rational, it is also reasonable to reconsider their impact on preventing infections. There well may be ways to streamline and improve the regimens prescribed.

Summary

Despite over a century of clinical experience, research relating antiseptic skin practices in surgery to postoperative wound infection rates continues. Evidence of the value of preoperative whole body patient bathing with an agent such as chlorhexidine is weak and this practice is likely to be cost effective only in

situations of high patient compliance and high risk. Preoperative preparation of patient skin and surgeon's hands seems important, but procedures should be streamlined for cost and time efficiency and for patient comfort as products and protocols which are fast acting and/or slow release are tested and improved.

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CHEMICAL GERMICIDES IN HEALTH CARE

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The Role of Microorganisms in Atopic Dermatitis

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Introduction

It is a well-known fact that atopics react abnormally to a variety of common environmental antigens. Such antigens may include products of the skin flora. Since the skin flora of patients with atopic dermatitis (AD) is qualitatively and quantitatively different from nonatopics, its interaction with the patient's defense mechanisms as well as the abnormal skin flora by itself may contribute to the inflammatory process clinically manifesting as eczema. We will therefore review here the clinical, microbiologic, and immunologic findings in atopics related to their abnormal skin flora, in particular *Staphylococcus aureus (S. aureus)*. This is also done because appropriate antimicrobial treatment can significantly contribute to the successful care of AD patients. Common viral diseases may present in AD patients a particular clinical course that requires prompt recognition and adequate treatment. Therefore, clinical aspects of selected viral skin diseases in AD are reviewed. Finally, because of the prevailing clinical notion that AD patients are more prone to infection, this point is critically evaluated for each microorganism presented in this article.

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Bacterial Microorganisms

The Normal Human Skin Flora

Of the many microorganisms isolated from human skin, only a few are regularly identified, and these constitute the normal skin flora *(1)*. Therefore, the skin microflora can be divided into two groups *(2)*. The first group, the resident flora, was considered to include organisms that:

1. Are consistently associated with the skin.
2. Possess physical attachment factors for human skin surface.
3. Are relatively stable in number.
4. Can multiply on the skin.

The second group, the transient flora, comprises a much wider variety of microorganisms that are derived from exogenous sources and found mainly on exposed skin. They vary greatly in both number and type. Other authors have distinguished a third group of organisms, the associate flora or the temporary residents, that may occasionally colonize the skin of a minority of persons because of environmental influences *(3,4)*.

The resident microorganisms live within the outermost layers of the epidermis and as small colonies on the skin surface. To assess the composition of the resident flora quantitatively and qualitatively, it is necessary to take samples from multiple sites, since a sample from a single area cannot be taken as representative of the entire flora *(5)*. Various methods of sampling and culture were used, yielding many quantitatively different results, until most investigators adopted the method of Williamson and Klingman *(6)*. This method consisted of sampling a limited skin surface area by scrubbing with a buffer containing a detergent. Nevertheless, the qualitative pattern of microorganisms recovered in the various studies was very similar, and it is now generally accepted that the resident bacterial flora is composed of the following: *Micrococci* (e.g., *M. luteus, M. varians*), *Staphylococci* (e.g., *S. epidermidis, S. capitis*), *Corynebacteria* (e.g., *C. minutissimum*), *Brevibacteria* (e.g., *B. epidermidis*), *Propionibacteria* (e.g., *P. acnes, P. granulosum*), and *Acinetobacter* (e.g., *A. calcoaceticus*).

Although it was reported in 1950 that the anaerobic bacteria outnumber aerobes by a ratio of more than a log (Evans et al.,

1950), the aerobic flora, which contains most of the pathogenic organisms, has been most extensively investigated. Its ecology is influenced by a large variety of factors, such as sex, age, and body location, as well as skin pH and humidity. Environmental factors include climate, seasonal variations, occupation, and the use of soaps and disinfectants as well as disease, hospitalization, and medications (reviewed in ref. *1*). As an example of the influence of the body location, the number of aerobic bacteria may vary quantitatively: from 2300/cm^2 on the forearm to 80,000/cm^2 in the axilla *(7)*, and qualitatively—*Corynebacterium* species reside particularly in moist intertriginous areas whereas *Propionibacterium* species are normal inhabitants of hair follicles and sebaceous glands. Skin flora maps may thus be helpful for the study of cutaneous microbial dynamics *(8)*.

The Bacterial Resident Flora in Atopic Patients

The skin of atopic patients is characterized by higher levels of colonization by aerobes. A significant increase of colony-forming units (CFU/cm^2) was found on the unaffected skin of 14 AD patients compared to 12 healthy subjects *(9)*. In another study, normal appearing skin of the forearm of 15 AD patients showed a mean of 1122 total CFU/cm^2 as opposed to 52 total CFU/cm^2 in 20 healthy controls ($p < 0.0005$) *(10)*. This increase in the density of total aerobe counts was also confirmed by an analysis of samples taken from more than 150 sites of a patient with severe AD and from a healthy volunteer *(8)*. Moreover, in the first quantitative study on 70 AD patients, the increased density of total aerobes was found to correlate with the severity of atopic skin manifestations. The mean bacterial density reached 18×10^6 bacteria/cm^2 in acute lesions, 6×10^5 in chronic plaques, and 2×10^5 in unaffected skin *(11)*. The skin of atopic patients is thus considered as a favorable milieu for aerobic bacterial colonization and multiplication, and is able to maintain a denser aerobic flora than skin of normal individuals.

In addition, the cutaneous flora of AD patients shows striking qualitative alterations. Coagulase-positive staphylococci *(S. aureus)*, which are usually not found on normal skin, account for the majority of the increased total aerobe counts in AD. Colonization by coagulase-negative staphylococci is also consistently

increased on atopic skin *(9)*. For example, *S. epidermidis* was the predominant organism (constituting 63% of the total flora) on the unaffected skin of 39 AD patients and was the second most predominant (after *S. aureus*) on lesional skin *(12)*. However, the clinical significance of extensive colonization by coagulase-negative staphylococcal strains in AD remains unknown.

The Role of S. aureus

Characteristics of S. aureus

Staphylococci are gram-positive, catalase-positive cocci of the Micrococcaceae family, and distinguishable from other micrococci by their ability to produce acid anaerobically from glycerol in the presence of erythromycin and their susceptibility to lysis by lysostaphin and nitrofuran *(13)*. They can be further subdivided into the coagulase-positive *S. aureus* and the coagulase-negative species. *S. aureus* is a nonmotile, nonspore-forming aerobic and facultative anaerobic gram-positive organism that is highly resistant to environmental changes. Its growth is optimum when cultured in a pH range of 7.0–7.5 and a temperature of 30–37°C. The differentiation of staphylococcal strains by bacteriophage typing allows a division into five subgroups (I–V). Strains reacting with only one group of phages are classified into groups I–IV. Untypable strains or strains reacting with more than one group of phages are classified into group V *(14)*. Cellular antigenic components include several capsular antigens, lipoteichoic acid, a membrane constituent and the cell-wall peptidoglycan, ribitol teichoic acids, and protein A. Finally, *S. aureus* can produce several exotoxins: coagulase, lipase, DNase, Hyaluronidase, and so forth; enterotoxins: Staphylococcal Enterotoxin (SE) A, B, C, D; and toxins specially associated with clinical virulence: Toxic Shock Syndrome Toxin (TSST)-1 and Exfoliative Toxins (ET) A and B.

Ecology of S. aureus

S. aureus may colonize the skin of healthy individuals *(8,15)*, but is not considered as a member of the normal resident flora *(1,15)*. In a large series of 382 normal children and 378 normal adults, *S. aureus* was found in <5% of the isolates *(16)*. Low colonization

rates were also found by several other investigators *(9,10,17,18)*. When present, *S. aureus* was found in small numbers and mainly in intertriginous areas *(15,19,20)*. Nasal carriage, up to 40% of the population, is more frequent than cutaneous colonization. Recurrent nasal carriers have a higher risk of localized infection (impetigo, furunculosis) and postoperative wound infections *(20,21)*.

In AD patients, *S. aureus* colonization is much more frequent, both in carriage and in quantity. The relationship between staphylococci and AD has been studied for decades. Already in 1900, it was reported that staphylococci induced eczematiform lesions in normal skin *(22)*. Later, Miescher *(23)* and Storck *(24)* challenged patients epicutaneously and intracutaneously with bacterial microorganisms. It was however, only with the advent of new methods that the increased colonization by staphylococci in AD was more clearly defined. In 1965, Selwyn *(25)* found *S. aureus* in 36 of 113 patients with eczema; only 24 were judged to be clinically infected. In 1974, Leyden et al. *(11)* examined *S. aureus* skin colonization in AD patients and found it to be the predominant skin organism. Three years later, this predominance was confirmed by Aly et al. *(12)*, who found that *S. aureus* constituted 91% of the total aerobic flora on AD patients. The carriage rate was 79% for the nares and 93% for the skin lesions. The same results (93% carriage rate) were found in children *(26)*. It has also been clearly shown that the *S. aureus* colonization rate depended on the type of skin involvement. *S. aureus* was found in 55–75% of AD patients on apparently normal skin *(9,12,18,27)*, in 85–91% on chronic lichenified lesions *(11,12,27)*, and in 80–100% on acute, exudative lesions *(10,11,18)*.

Quantification of colonization by *S. aureus* was studied by several authors *(7,10–12)* who reported a 100–1000 times higher density of *S. aureus* in lesional skin compared to normal appearing skin of AD patients. As an example, in one study, the following mean *S. aureus* counts were made: 1.9×10^3 CFU/cm^2 on normal appearing skin, 1.5×10^5 CFU/cm^2 on acute lesions, 2×10^5 CFU/cm^2 on lichenified skin, and 1.5×10^6 CFU/cm^2 on clinically superinfected skin. These results accounted for 72, 92, 70, and 95%, respectively, of the total aerobic flora, and represented a carriage state of 79, 100, 89, and 100%, respectively *(10)*.

It should be noted, however, that AD is not the only condition that shows a higher prevalence of *S. aureus* colonization. Diabetic patients, psoriatic patients, intravenous drug abusers, patients undergoing hemodialysis, and hospital workers are all susceptible to *S. aureus* colonization *(1)*, although to a much lower extent.

Specific Interactions of S. aureus *with Atopic Skin*

Since *S. aureus* was shown to be highly prevalent on atopic skin, several groups looked for a cutaneous abnormality that could explain this phenomenon. It is reasonable to assume that a skin disease with wet lesions, such as severe acute dermatitis, provides a favorable milieu for bacterial colonization and infection *(28)*. How can the increased *S. aureus* colonization found on unaffected skin of AD patients be explained? Carry-over from skin lesions may be explained by scratching caused by pruritus especially prominent in AD. Furthermore, microscopic lesions of apparently unaffected skin may provide the same favorable factors as visible lesions *(29,30)*. However, these explanations did not seem to satisfy all authors and, therefore, additional reasons were sought.

One of the first investigations providing evidence of a favorable milieu for *S. aureus* colonization was performed in the late 1960s. When treated with ether, the apparently unaffected skin of patients with eczema supported a higher multiplication of *S. aureus* than skin from controls without eczema *(31)*. Later, studies were performed to investigate the initial step toward bacterial colonization and infection, i.e., the bacterial adherence of *S. aureus* to corneocytes. Such experiments *(32–34)* led to the following conclusions: Larger numbers of *S. aureus* adhere to corneocytes and nasal mucosal cells of AD patients than to cells from normal controls, psoriatic patients, or patients with other dermatological diseases. The reported increased adherence was *S. aureus*-specific and not found with other gram-positive bacteria. Cole and Silverberg *(34)* favored protein A as the microbial adhesin responsible for the adherence of *S. aureus*, since free protein A inhibited the binding of *S. aureus* in vitro. However, another group suggested that the *S. aureus* adhesin was probably teichoic acid and not protein A *(32,35)*. Recent progress has been made in this field with the discovery of fibronectin as a receptor for staphylococci *(36)*. Fibronectin is a high-molecular-

weight glycoprotein found in association with basement membranes, in interstitial connective tissue, and in soluble form in plasma and other body fluids. Fibronectin was subsequently identified as one of the receptors involved in adhesion of *S. aureus (37)* by binding to teichoic acid *(37–39)*. Fibronectin has at least two potential sites capable of interacting with complementary structures on staphylococcal (and streptococcal) cell surfaces (38). The binding of *S. aureus* to fibronectin is time-dependent, functionally irreversible, and occurs both with live and heat-killed cells *(39)*. Other cutaneous factors, such as laminin *(40)*, may function as *S. aureus* receptors. Staphylococcal lipoteichoic acid *(41,42)* may also play a role in the adhesion to host cells.

The abovementioned studies provided the rational basis for a new treatment strategy, i.e., to block the adhesion of *S. aureus* to corneocytes in order to prevent and/or treat staphylococcal cutaneous infections. However, to date, this strategy has not found general acceptance probably because no specific biochemical abnormality of atopic corneocytes and/or their surrounding matrix has been identified, and also because it is unknown which of the several adhesion mechanisms is mainly responsible for the increased adherence of *S. aureus* to the skin of AD patients. Nevertheless, clindamycine and erythromycine may, in addition to their growth-inhibitory action on *S. aureus*, be successful antistaphylococcal agents, since they have been reported to decrease the adherence of *S. aureus* to fibronectin at concentrations that do not inhibit bacterial growth *(43,44)*.

S. aureus *and the Immune Response in AD*

Multiple immunological abnormalities have been described in AD (*see* article by Leung in this volume). Since there is a clear association between increased *S. aureus* colonization and AD, the question arises whether there is an interaction between *S. aureus* and the immune system of patients with atopic dermatitis. Therefore, a number of investigators have measured parameters of the adaptive and nonadaptive immune response to *S. aureus* in AD. Here, we will review these findings.

Granulocyte and monocyte functions were initially found to be abnormal *(45–47)* in patients with severe AD. Later, however, it was shown that there was no intrinsic granulocyte or mono-

cyte abnormality in these patients *(48)*. Likewise, there are no reports of specific and intrinsic granulocyte and monocyte abnormalities with regard to *S. aureus* in AD.

IgEs directed to many antigens present in the environment of AD patients have been identified. Similarly, several investigators have been searching for IgE that binds *S. aureus* antigens *(49–55)*. In their reports, a varying percentage of AD patients were identified to have increased *S. aureus* IgE in the serum. This is in contrast to the staphylococcal hyper-IgE syndrome (Job syndrome, Buckley syndrome) where increased IgE levels to *S. aureus* cell walls are a hallmark *(56)*. In one study, a method was used similar to that used to measure IgE directed to *S. aureus* cell walls in the hyper-IgE syndrome, but with increased sensitivity *(52)*. With this technique, several AD patients were found to have increased levels of IgE that bound to *S. aureus* cell walls. The values found in these patients were much lower than those reported in the hyper-IgE syndrome. These patients, however, also had much lower total serum IgE levels. In addition, several patients in this study were found to have IgE that bound to soluble *S. aureus* antigens. Interestingly, in this study, the presence of *S. aureus* IgE was correlated with regional lymphadenopathy. Therefore, *S. aureus* IgE might identify a subset of patients with a particular response to *S. aureus*. Infection did not seem to be the critical factor because there was no association with clinically recognizable superinfection. This was not confirmed in other studies in which increased *S. aureus* IgE was associated with severe extensive skin disease, superinfection, or high serum IgE *(55)*. In conclusion, it is not clear in how many AD patients *S. aureus*-specific IgE might contribute to their dermatitis by triggering IgE-dependent mechanisms. This has to be separated from other possible proinflammatory mechanisms triggered by *S. aureus*. That such mechanisms exist is supported by the fact that topic antibiotics together with corticosteroids clear AD more efficiently and more rapidly than corticosteroids alone *(57)*. It has also become clear that the immune response to *S. aureus* cannot be considered as the principal factor in the pathogenesis of AD. Staphylococcal products, such as protein A, teichoic acid, and other toxins, have been reported to induce the release of histamine from

mast cells and basophils *(58,59)*. Mast cell and basophil mediators may significantly modulate the response of other cells. For example, it is well known that histamine can decrease chemotaxis of neutrophils. Therefore, the release of mediators by mast cells or basophils may explain some of the granulocyte and macrophage abnormalities that were only detectable during infection and/or severe relapses of AD.

There are only a few studies that have investigated the lymphocyte response to *S. aureus* in vitro. A mitogen/superantigen-free *S. aureus* preparation induced higher proliferation in lymphocytes from AD patients than from healthy normal controls, suggesting that AD patients have a higher circulating number of T-cells that respond to *S. aureus* antigens *(60)*. Although the effect of adding *S. aureus* antigens to lymphocyte cultures from patients and controls had multiple effects on such parameters as immunoglobulin production and CD23 expression, no clear immunopathologic conclusions could be derived from these studies *(61,62)*.

The reason why the use of whole *S. aureus* in in vitro and in vivo studies has yielded unclear and even conflicting results may be because the pre-eminent biologic significance of some *S. aureus* toxins has only recently been recognized. Staphylococcal enterotoxins A, B, C1, C2, C3, D, E, toxic shock syndrome toxin-1, exfoliative toxin, and perhaps other *S. aureus* products still to be identified belong to a family of proteins with similar high biologic activity *(63)*. These proteins, collectively named superantigens, bind to major histocompatibiliy complex (MHC) molecules. As a consequence of this binding, they can activate, in an MHC-unrestricted manner, T-cells expressing distinct variable β chain families constituting their T-cell receptors. T-cells activated in this manner may represent a significant part of the T-cell repertoire and, thus, of the total number of T-cells. Immune modulation by these superantigens is presently a matter of intensive research *(64)*. If we are able to correlate the number or function of T-cells expressing these toxin-reactive receptor families with bacteriologic and other immunologic findings in AD, we may significantly increase our knowledge of immune modulation by *S. aureus*-derived toxins.

Possibly also because of the abovementioned new findings with *S. aureus* superantigens, the most controversial area of the *S. aureus* immune response in AD has been skin testing. In most studies that examined the immediate response to intracutaneously applied *S. aureus* or fractions thereof, reactions were found in most cases, but not more often than in normal controls *(28,65,66)*, or were even found to be less frequent *(52)*. In most reports, late readings (24 h and more) in AD patients were less frequently positive than in controls *(52,53,67)*. However, the skin test results are difficult to interpret because:

1. Test protocols and reading methods were not standardized.
2. Various and nonpurified materials were used in most studies.
3. In most studies, no care was taken to test during remission of AD (DTH-like responses seem to diminish during flare-ups *[68]*).
4. The influence of treatment is difficult to exclude.
5. Very few studies employed epicutaneous testing that might more closely imitate normal exposure to *S. aureus*.

With refined and better-defined skin test methods more meaningful information might be generated, similar to the field of aeroallergens, particularly dermatophagoides, where it is only recently that the results of skin test studies have supported a role for these microorganisms, despite the fact that the high prevalence of dermatophagoides-specific IgE in AD has been known for years *(69)*.

Clinical Implications

Signs of Bacterial Infection

It is difficult to draw a line between *S. aureus* colonization and infection in AD patients *(70,71)*. Large numbers of *S. aureus* can colonize the skin without overt signs of infection *(10,11)*. However, several physical signs are suggestive of infections, such as the presence of pustules, severe crusting, and a weeping discharge on acute eczematous lesions *(72)*. Occasionally, patients may develop regional lymphadenitis without systemic involvement *(52,72,73)*. It should be noted that the presence of bright erythema, lateral spread of pustules, tendency to cellulitis, and fever rarely occur with *S. aureus*; rather, they are suggestive of streptococcal impetiginization *(71)*.

Specific signs of primary *S. aureus* infection are more subtle and may frequently be overlooked. Small, superficial pustules appearing on unbroken skin and independent of hair follicles can be seen on patients with moderate to severe AD *(73)*. These pustules tend to occur on the extremities and occasionally on palmo-plantar skin, where they may be misdiagnosed as a dyshidrosiform condition. Pustulation may also be found in association with hair follicles *(65)*. It should be remembered that furuncles and "cold abscesses" are exceptional in AD. The latter sign rather evokes the diagnosis of a hyperimmunoglobulinemia E syndrome.

Frequency of S. aureus *Infection*

Staphylococcal infection in AD usually remains superficial and rarely becomes invasive *(74)*. Since specific signs of *S. aureus* might easily have been overlooked, until it was clearly demonstrated that *S. aureus* was associated with AD, bacterial infection was not considered to be a serious problem in AD. To describe the attitude that prevailed at that time, authors frequently quote Rajka *(75)*, who stated that in a disease characterized by scratching and excoriation, bacterial infections were uncommon and less frequent than might be expected. In later years, however, it was realized that staphylococcal infections might be of greater significance. This was mainly because of the observation that pustules containing *S. aureus* were commonly found during exacerbations of AD (73) and that acute eczematous lesions frequently harbor more than 10^6 *S. aureus*/cm^2. Such a density closely approaches that found in impetigo lesions. The significance of *S. aureus* was further confirmed by a prospective study in 190 children with a mean observation period of 13 mo. Seventy-six children (40%) had 164 episodes of exacerbation of eczema associated with bacterial infection; 15% of the episodes were severe enough to require hospitalization *(76)*. It is therefore now widely accepted that AD patients are prone to cutaneous staphylococcal infection during phases of exacerbation *(26,76,77)*. In addition, a distinct form of invasive *S. aureus* infection has been recently recognized in children with AD. Three children with osteomyelitis of the distal phalanges have been reported *(74)*. These lesions were probably the result of intense scratching of infected skin coupled with minor trauma to the finger tips. I

should be noted that emphasis was placed on the possibility of recognizing the insidious onset, which is characterized by subungual black macules followed by local edema, erythema, and pain. Since this form of invasive *S. aureus* infection has been only recently recognized and because its clinical signs may easily be missed, further studies are required to determine its frequency in AD. To our knowledge, staphylococcal scalded skin syndrome in AD has not been reported despite the fact that isolation of exfoliative toxin-producing strains from the skin of patients with AD is not rare.

Finally, the colonization of the skin of AD patients by *S. aureus* also represents an epidemiological problem. Crosscontamination between atopic children and their mothers or relatives has been documented *(26)*. Moreover, the dispersal of skin bacteria can cause infections: Several limited *S. aureus* epidemics were traced back to *S. aureus* carriers with AD *(11,26,78)*. It is therefore important to take preventive measures when persons with AD come into contact with individuals who are susceptible to bacterial infections. This is particularly important in a hospital setting.

Treatment

Heavily superinfected AD patients are often treated with systemic antibiotics *(11,76,79)*. The effect of such a treatment, however, has not been studied in a controlled manner. Erythromycin is probably the most common antibiotic used to treat AD patients. It seems to be effective and less frequently provokes allergic reactions than penicillin derivatives. Resistance of *S. aureus* to erythromycin can be as frequent as 30% of isolates from AD patients *(10,28,65,76)*. Caution is necessary when giving erythromycin at the same time as treatment with drugs that are metabolized in the liver, especially theophylline and histamine H1 receptor antagonists, such as terfenadine and astemizole *(80)*. Penicillin derivatives are also effective, but sensitization may be frequent. Methicillin-resistant strains are increasing and were recently found in 7% of 157 patients with infected eczema *(72)*. The antibiotic spectrum should cover also streptococci, since these have been frequently shown to be concomitant with *S. aureus* in acute infectious episodes of AD *(76)*. There is little experience

with cephalosporins, although several show a good activity against gram-positive bacteria. Resistant *S. aureus* strains are not usually found *(81)*. The risk of sensitization is very similar to that of penicillins, and crossreactivity is frequent. The effect of agents, such as rifampicin, used to treat chronic *S. aureus* carriers has not been studied in AD.

In contrast to systemic antibiotics, the use of topical antibiotics in AD has been assessed in several controlled trials. First, topical antibiotics have been demonstrated to be as effective as oral antibiotics. Leyden et al. *(11)* compared the effect of topical neomycin vs oral erythromycin, and found a similar decrease of *S. aureus* counts and total aerobic flora for both modes of application. Clinical improvement was also similar, although predominantly seen in patients with more than 10^6 *S. aureus*/cm^2. Topical mupirocin was also shown to be as effective as oral erythromycin in experimental infections *(81)* and in impetigo *(82,83)*. In addition, the beneficial effect of topical mupirocin was recently confirmed in a double-blind placebo controlled crossover study *(77)*. A reduction of both the *S. aureus* counts and the clinical severity was observed after treatment. It should be noted, however, that recolonization occurs quickly after cessation of treatment.

Second, there is substantial evidence that combined therapy with a topical antibiotic and corticosteroids is more effective than corticosteroids alone for the treatment of AD. In 1977, Leyden and Kligman reported the results of two studies on atopic dermatitis, comparing the use of 0.025% flucinolone acetonide cream vs the same cream with 0.5% neomycin sulfate, and a combination of polymyxin B, neomycin sulfate, and gramicidin cream vs the same cream with 0.5% hydrocortisone *(57)*. Combined treatment was significantly superior with regard to clinical improvement. Reduction of *S. aureus* was significantly better with the combined treatment in the first study and was similar for both creams in the second. Clinical improvement of impetinigized AD was also more marked with a combination of gentamycine/betamethasone compared to either component alone *(84)*.

Several topical antibiotics have been used over the years. Gentamycin is active against a broad spectrum of both gram-positive and gram-negative bacteria. However, its clinical use as a topical agent has been restricted because of possible resistance

and contact sensitization. This agent is therefore preferred for systemic use. Neomycin is an aminoglycoside that is very effective against staphylococci and gram-negative organisms (except *P. aeruginosa*), but that has little action against streptococci. It is not used for parenteral administration. Since neomycin is not absorbed by the gastrointestinal tract, oral preparations are available only for enteric decontamination. Its topical use is by far the most common mode of administration, but its use is limited by the risk of absorption from inflamed skin and subsequent development of allergic contact dermatitis, as seen in chronic leg ulcer patients in whom this local antibiotic was frequently used. Cross-sensitization with other members of the aminoglycoside group (framycetine, kanamycin, gentamycin) is common *(85)*. Bacitracin is highly effective against streptococci and *Staphylococcus pyogenes*. It is widely used for the topical treatment of streptococcal impetigo. Sensitization to bacitracin seems to be low. Fusidic acid has a strong activity against both staphylococci and streptococci. It has been used for nearly two decades in impetigo and AD. Despite increasing consumption, resistance rates still seem low *(86)*. However, increasing use of this topical antibiotic may lead to more frequent sensitization *(87)*. The last topical antibiotic to come on the market was mupirocin. It is derived from *Pseudomonas fluorescens*, which was found to produce a bacteriostatic substance nearly a century ago. Mupirocin is active against staphylococci, streptococci, and several gram-negative bacteria. Numerous recent publications have shown that it is a useful tool for the eradication of nasal *S. aureus*, as well as for the treatment of AD and impetigo *(77,81–83)*. It has little potential for sensitization. Systemic absorption of fusidic acid and mupirocin is negligible, and they are both equally efficient in the treatment of superficial skin infections *(88,89)*. However, complaints regarding the price and the greasiness of mupirocin ointment have been formulated *(79,88)*.

It should be noted that both sensitization *(90)* and development of *S. aureus* resistance to all these topical antibiotics after prolonged treatment have been described. Therefore, their use should be restricted to a short course of treatment. Furthermore chronic use of topical antibiotics in the management of AD has

not yet been assessed in controlled studies and is still a controversial issue *(72,91)*.

Finally, UVB phototherapy has also been proposed as being effective against *S. aureus* colonization/infection in AD. UVB was found to inhibit the growth of *S. aureus* in vitro *(92)*. Furthermore, in a clinical trial with AD patients, UVB significantly reduced *S. aureus* counts and yielded a significant clinical improvement *(93)*. However, since UV light has a strong anti-inflammatory action, and since the anti-inflammatory action of topical corticosteroid treatment given alone has also been shown to decrease *S. aureus* counts in AD significantly *(94)*, it is not known whether the clinical improvement was the result of the bactericidal or the anti-inflammatory action of UVB.

Viral Infections

Kaposi's varicelliform eruption *(95)* is a well-known complication of atopic dermatitis *(75, 96, 97)* that has been the subject of numerous publications *(98–118)* and emphasized the increased susceptibility of AD patients to unusually severe cutaneous infections of viral origin (at least vaccinia virus and herpes simplex virus). However, it is not clear whether AD patients just suffer from more severe forms or more frequently from viral infections. We will therefore review the data on cutaneous, and then extracutaneous, viral infections.

Cutaneous Viral Infections

Eczema Vaccinatum

Although Kaposi is generally credited with the first description of the varicelliform eruption *(95)*, it has been suggested *(103)* that priority should go to Martin, who reported three cases in 1882 and correctly attributed the eruption to smallpox vaccination. The isolation of vaccinia virus from a Kaposi's varicelliform eruption was then demonstrated in the mid 1930s, and such cases were subsequently reported as eczema vaccinatum in order to differentiate them from the clinically similar eruption caused by herpes simplex virus (HSV) *(119)*. Today, eczema vaccinatum (EV) is a problem of the past thanks to the worldwide eradication of smallpox.

Before the introduction of acyclovir, EV was estimated to occur in about one in 20,000 individuals after primary vaccination and to be fatal in 6% *(103)*. Although the eruption could be observed in patients with Darier's disease, EV was observed most frequently in AD patients regardless of whether they were in clinical remission, or had mild or severe disease *(103)*. In another report, it was shown that after the vaccination of 309 atopic patients, 36 cases had exacerbation of their disease, and 27 had secondary vaccinial lesions *(120)*. Besides inoculation by vaccination, heteroinoculation may represent a route of transmission. After an incubation of 5–12 d, the course of the disease varies from localized disease to generalized, potentially life-threatening conditions. The duration of the eruption lasts about 10 d, after which lesions may heal with scarring. Males were affected twice as frequently as females *(103)*.

Eczema Herpeticum

Characteristics of Herpes Simplex Viruses (HSV)

HSV, together with cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, and Herpes virus 6 are members of the family of Herpesviridae, which infects humans and is characterized by a similar viral structure. These double-stranded DNA viruses are characterized by persistence for life after primary infection, and endogenous reactivation and infection *(121,122)*. HSV-1 and HSV-2 can be distinguished by minor differences in biological behavior in vitro, restriction endonuclease analysis, and type-specific monoclonal antibodies. During natural infections, HSV-1 has a predilection for the oral mucosa, and HSV-2 for the genital mucosa. Both are transmitted by direct contact with infected secretions and are thought to enter sensory nerves that innervate the site of entry. After ascension through retrograde axonal transport, they become latent predominantly in the cell bodies of peripheral sensory neurons of the trigeminal (HSV-1) or sacral (HSV-2) ganglia. Detailed descriptions of replication and latency stages can be obtained elsewhere *(122,123)*.

HSV-1 and HSV-2 infections are found worldwide. Primary HSV-1 infection has a peak of incidence in the 2–4 yr old and late teen groups. Primary HSV-2 infection is unusual before puberty, correlates with sexual activity, and has therefore its highest incidence during the third decade of life. Mucocutaneous HSV infec-

tions follow a typical course in most individuals. After a prodromal period including increasing pain, tingling and/or numbness, grouped lesions progress from erythema, to the stages of vesicles, pustules, erosions, and sometimes ulcerations before healing. Primary infections usually last 14–21 d, and recurrent infections 7–10 d. Local lymphadenopathy is commonly found in primary HSV infection. Clinical criteria are insufficient to distinguish between primary and recurrent, as well as between HSV-1 and HSV-2 infections *(124)*.

Incidence of HSV Infections in AD Patients

In a retrospective study of almost 1000 patients with past or present AD *(125)*, it was found that a history of recurrent (>5 episodes/yr) cold sores was significantly more common in AD patients than in nonatopic controls. Furthermore, the incidence correlated positively with the severity of the atopic condition. Similarly, in a study of 2514 children attending nursery schools in Bari (Italy), atopic dermatitis was found in 8.3% of cases with recurrent cutaneous herpes simplex infection, but in only 2.6% in the control group (99). However, such data were not confirmed in a prospective study of 179 children *(104)*. Moreover, serological evidence of herpetic infection in AD patients (23/113 = 20%) was not found to be significantly different from that of matched controls (34/13 = 30%) *(105)*. Therefore, the question of whether AD patients are more often infected with HSV than normal individuals *(97,108,126,127)* remains unanswered.

Epidemiology of Eczema Herpeticum

The term eczema herpeticum (EH) refers to Kaposi's varicelliform eruption caused by HSV. Most cases are caused by HSV-1, but EH with HSV-2 has also been reported *(110)*. EH occurs mainly in AD patients, but has also been described in Darier's disease *(128–130)*, familial benign pemphigus *(131)*, pemphigus foliaceous *(132,133)*, autosomal dominant ichtyosis vulgaris (134), congenital ichtyosiform erythroderma *(135)*, mycosis fungoides *(136–138)*, Sezary's syndrome *(139)*, and extensive burns *(140)*. Common to all these diseases is a defective skin barrier. However, the role of cutaneous immunological abnormalities remains unclear.

In AD patients, the occurrence of EH is thought to coincide with natural HSV infection, with most cases occurring in small children (2–4 yr old) and, less frequently, in the late teens. Indeed, most reports described EH in children. However, in a more recent

series of 75 patients in Germany *(100)*, age at presentation ranged from 5 mo to 69 yr. In most patients, the disease developed during the second or third decade, indicating a possible shift of the disease into adulthood. In this study, a striking increase of cases in recent years was also reported. Such a trend needs confirmation, since the age-related prevalence of HSV-1 antibodies has a tendency to decrease in Western countries *(141)*.

EH is in most cases acquired by heteroinoculation from a close contact person, in children usually the parents, and in later life "best friends" or relatives, with cold sores *(114,142)*. In children, EH is usually the manifestation of a primary infection with HSV. However, adults may develop EH during a recurrent HSV infection. The latter was the case in at least 20% of the 63 patients reported by Bork and Brauninger *(100)*. However, in this report it was also stressed that numerous AD patients previously had recurrent cold sores without developing EH.

AD patients may have recurrent EH. In the report just mentioned, five of 63 patients had one to four episodes of EH in intervals ranging between several months to 6 yr. In another report *(142)*, seven out of 10 children had recurrent EH. Recurrent EH did not necessarily locate in the same areas as the first episode, and coincided with recurrent herpes labialis in other family members or close friends, thus suggesting exogenous reinfection. Finally, in a prospective study of 179 children followed for nearly 3 yr, five of 10 children who developed EH had a total of 11 recurrences. In these, recurrent episodes ranged from mild to severe, were trivial or as severe as the initial attack, and recurred in three patients at the same sites. A different distribution of the recurrences was also reported in the two other patients *(104)*.

Whether the presence of extensive lesions or recent use of corticosteroids prediposes AD patients to EH is controversial. Although such speculations have been put forward in isolated cases, they were not confirmed in larger series *(99,100,104,142)*.

Clinical Course of Eczema Herpeticum and Atypical HSV Infections

The incubation period of EH is estimated to be between 2 and 7 d. Classical EH is characterized by the appearance of discrete, localized clusters of tiny pruritic vesicles. They often erupt

in crops on erythematous skin and develop into pustules often with central umbilication and crusting. The presence of hemorrhagic crusts and erosions is frequent in severe cases. Lesions nearly always involve the face with dissemination to the neck, shoulders, upper trunk, and abdomen, usually in a symmetrical manner. Untreated, the disease lasts an average of 16 d. Constitutional symptoms, such as fever, malaise, headache, and lymphadenopathy, are frequent, as well as exacerbation of the AD.

Although the classical clinical presentation is hard to miss, less typical involvement may easily be overlooked. In most cases, the lesions may remain localized to areas of AD lesions. Round punched-out ulcerations may be the only specific sign *(142)*. On the other hand, EH may also run a very serious or even lethal course, particularly as a primary herpetic infection. Causes of death include viral dissemination to internal organs, such as the brain, lungs, and less frequently, to the liver or even muscles *(100)*, and secondary bacterial infections.

The more frequent complications include dehydration and hypokaliemia *(115)*, mostly in children owing to extensive lesions, and secondary infections with *S. aureus*, streptococci, or *Pseudomonas (97,104,114)*. Surprisingly, ocular involvement rarely leads to severe keratitis *(107)*.

Diagnosis of Cutaneous HSV Infection in AD

The classical picture of EH is hard to miss. However, since herpetic eruptions in AD may have a more silent course, it is wise to consider any appearance of clustered vesicles as herpetic infection. It has also been stressed that diagnosis of HSV infection has been delayed when herpetic lesions have been restricted to areas of eczematous lesions *(142)*. Therefore, this diagnosis should be suspected if acute exacerbations of eczema do not respond to conventional treatment *(104)*.

When suspected, the diagnosis can be confirmed by a Tsanck smear, which may show ballooning degeneration, multinucleate giant cells, and intranuclear inclusion bodies. Rapid diagnosis by electron microscopy (EM) was reported to take only minutes when available *(104)*. Routine diagnostic tests include viral culture and immunolabeling of lesional scrapings. The diagnostic value of tests that are based on genetic technology, such as *in*

situ hybridization and polymerase chain reaction (PCR), has to be assessed. Some authors strongly suggest that confirmation of the diagnosis should be routinely attempted to determine the type of HSV and to avoid misdiagnosis, since clinically similar eruptions may in some rare cases be caused by Coxsackie virus *(99)*.

Treatment

Established treatment against HSV includes vidarabine and acyclovir. Although the former has been used with success in EH *(101)*, the latter is now considered the treatment of choice *(102,112,113,117,129,143,144)* because of fewer side effects and better activity. Acyclovir shortens the course of the disease when taken at the beginning of the eruption *(124)* and has proven to be life-saving in neonates, immunosuppressed patients, and EH cases, although not in all cases *(111)*.

In all other than localized cases, acyclovir is best given intravenously at a dose of 5 mg/kg every 8 h. Oral treatment is also effective, but has the disadvantage of being subject to individually variable intestinal absorption *(144)*. The duration of treatment ranges from 5 *(104)* to 10 d. Minor relapses may occur in the 1 wk following discontinuation of therapy; they do not require a second course of therapy *(116)*.

In order to prevent complications, many advocate the administration of oral/systemic antistaphylococcal antibiotics as well as topical ophthalmic antiviral therapy. Concomitant general measures include cessation of corticosteroids, correction of fluid losses, and adequate local treatment.

As far as prevention is concerned, AD patients should avoid contact with people presenting active herpetic lesions. Lastly, parents with recurrent herpes labialis should be advised of the risks for children with AD and receive appropriate treatment.

Molluscum Contagiosum

The Molluscum contagiosum (MC) virus is a strongly epidermotropic DNA poxvirus that mainly infects children by direct person-to-person contact or indirectly by fomites. The typical skin lesion is a shining, whitish to pink, hemispherical, umbilicated small papule. Initial lesions often lack the central porus. Dissemination of lesions is commonly the result of autoinocula-

tion by scratching. After a period of months to years, MC shows spontaneous resolution. However, removal of the lesions is advocated by several authors.

MC infections are commonly reported to be increased in AD patients *(64,97,127)*. However, objective data are scant. To our knowledge, four case reports have been published: a young child with recurrent eruptive lesions *(145)*; a man with a diffuse eruption on the arms, neck, and face *(146)*; two children with extensive lesions *(118)*; and a 7-yr-old girl with pedunculate giant lesions *(147)*. We found only one report on the incidence of MC in a children's population *(108)*. In this study, various forms of atopy occurred in 9.5% of 57 children with MC, and MC was found in 4.2% of 1824 AD patients.

Even if there is underreporting resulting from low interest in publishing on such a condition, present data do not support the claim that MC is more frequent in AD. Similarly, the notion that MC is more severe in AD patients is not supported by published data.

Other Viral Diseases

Warts

On the one hand, it has been proposed that common clinical experience suggests that viral warts are found more frequently and in higher numbers in individuals with AD; on the other hand, atopy is usually not mentioned as a predisposing factor for warts. Currie et al. reported a higher frequency of warts in atopic patients *(148)*, and two recent Italian series argued for a very slight increase *(99,108)*. Extensive dissemination has been described *(118)*, but it is not known whether AD patients are definitely prone to more disseminated lesions. A patient with AD and bowenoid papulosis of the genitalia associated with HPV type 16 *(149)* has been reported.

Varicella Zoster Virus

Varicella occurs in nearly all children before adulthood. A higher frequency among atopic children has not been reported. It has been suggested that children with AD run a more severe course. However, there is no published data that unequivocally support this notion. An increased incidence of herpes zoster was

recently reported in a retrospective study of almost 1000 adults with past or present AD *(125)*. This needs confirmation, since a second report did not reach the same conclusion *(145)*.

Extracutaneous Viral Diseases

Reports on the frequency of extracutaneous viral infection in atopic patients are rare. One group *(103,125–127,150)* reported an increase of recurrent upper respiratory tract infection in children with past or present history of AD, particularly in those with severe disease. The same authors showed more frequent and higher titers of Epstein-Barr virus antibodies in children with AD *(150)*. However, another study did not confirm these results *(99)*. Only cutaneous viral infections were increased in children with AD. Upper respiratory infections correlated with children having asthma/rhinitis, but not AD.

The effect of viral infections on the course of AD does not seem to be uniform. Epstein-Barr virus, parainfluenza, respiratory syncytial virus, and cytomegalovirus have all been reported to trigger atopic manifestations, such as AD *(127)*. This has, however, not been researched in a controlled study. In contrast, measles may lead to temporary resolution of the disease *(151)*.

Fungal Microorganisms

Pityrosporum *Species*

Taxonomy and Ecology in Nonatopic Individuals

The genus *Pityrosporum* includes *P. orbiculare*, *P. ovale*, and *P. pachydermatitis*, the latter being only rarely found in humans. *Pityrosporum* species organisms are yeasts that require lipids for growth. Therefore, normal growth media have to be supplemented with olive oil *(152)*.

P. orbiculare was shown to have the capacity to adhere to human stratum corneum cells in vitro *(153)*. In vivo, *P. orbiculare* resides as a saprophytic yeast in a blastospore stage and can change to a pathogenic hyphal stage under the effect of various factors. These factors include increased heat and humidity, occlusive clothing, topical application of oils, corticosteroid treatment, as well as Cushing disease, malnutrition, depressed cellular immunity, and diabetes. The hyphal stage of *P. orbiculare* was origi-

nally described under the name of *Melassezia furfur*. It was later shown that *P. orbiculare* and *M. furfur* represent two stages of the same microorganism. *P. ovale* shows small morphological differences compared to *P. orbiculare* and was thought to represent a different species *(152)*. However, separation of these organisms on the basis of their lipid requirements is impracticable. In addition, they can both change to an nearly undistinguishable hyphal stage *(152,154)*. Moreover, a strong antigenic similarity between the two species has been demonstrated *(155)*. Therefore, *P. ovale* and *P. orbiculare / M. furfur* are now considered by many authors to be merely different stages of the same species. We will thus use only the term *Pityrosporum* when referring to studies on both *P. ovale (156–160)* and *P. orbiculare (153,154,161–165)*.

Pityrosporum microorganisms are part of the human resident flora *(1)*. Although usually found as resident in the blastospore stage, *Pityrosporum* may become pathogenic and is responsible for the development of tinea versicolor and *Pityrosporum* folliculitis. Except in association with seborrheic dermatitis, this microorganism is rarely found in small children *(157,160,166)*. *Pityrosporum* is increasingly detectable with age, reaching a maximum after puberty. This increase is probably related to its need of lipids provided by sebum *(166)*. With highest counts on the back and chest compared to the upper arm, the lower leg, or the back of the hand, the distribution of *Pityrosporum* in adults parallels both the regional variations in sebum excretion and the distribution of tinea versicolor *(162)*.

The Role of Pityrosporum in AD

Although it has been shown that AD does not lead to increased *Pityrosporum* colonization *(157,160)*, clinical and therapeutic experience has shown that *Pityrosporum* may play a role in AD as an environmental allergen. Indeed, in a large retrospective study of 741 patients, positive skin prick test (SPT) to *Pityrosporum* correlated with active AD, especially in patients with eczematous involvement in the seborrheic area, the so-called head and neck dermatitis *(165)*. Furthermore, ketoconazole treatment improved such patients *(165,167)*. Several investigators later confirmed that type I sensitivity to *Pityrosporum* occurred in a subgroup of AD patients: Specific IgE antibodies to *Pityrosporum* were

found in 14–79% of AD patients *(158,160,164,168,169)*, and immediate positive reaction to SPT could also be elicited in 23–79% of AD patients *(158–160,164,168,169)*. In contrast, only a few patients with atopic disorders other than AD, and no healthy controls had either IgE to *Pityrosporum* or positive SPT. The marked quantitative differences found in these studies are likely to be related to different methodology. It should be noted, however, that a significant correlation between positive SPT and the presence of IgE to *Pityrosporum* was not found in all studies. Furthermore, positive IgE to *Pityrosporum* and/or positive SPT did not consistently correlate with disease activity *(156)*.

Delayed hypersensitivity to *Pityrosporum* was also assessed in two studies and showed quantitatively different results. Seven of 55 (13%) AD patients were found to have a delayed reaction after epicutaneous testing *(159)*, whereas 75 of 118 (64%) AD patients were positive using the chamber scarification patch test *(158)*. Again, no reaction was described in nonatopic individuals.

Together, these results indicate that at least a subgroup of AD patients are capable of raising an allergic reaction to *Pityrosporum*. It remains to be determined to what extent the scratching behavior of AD patients facilitates the penetration of recently recognized allergenic components of *Pityrosporum (163,170)*, thus favoring sensitization. In any event, it has been proposed that allergy to *Pityrosporum* plays a role in the formation of eczematous lesions *(156,165)*. However, the present evidence is still too weak to support such a claim.

Candida albicans

Candida albicans (C. albicans) is a nonlipophilic yeast, considered to be a member of the normal flora of the alimentary tract in humans. It is not a resident member of the cutaneous microflora. However, it can be occasionally found on the skin of normal individuals, probably because of constant carry-over from the mouth. On the skin, *C. albicans* is mainly found as a nonpathogenic host, except under certain conditions that alter the host microenvironment. These include premature births, diabetes, prolonged antibiotic treatment, and immune deficiencies, such as in chronic mucocutaneous candidiasis.

C. albicans has been shown to play a role in atopic conditions, such as asthma and rhinitis, where a correlation was found between positive RAST, positive provocation test, and successful hyposensitization *(171)*. However, its role in AD is not well defined. It has been discussed as a flare factor *(172)*, but there are no reports on increased colonization/infection in AD.

Since recent interest has focused on the existence of IgE antibodies to *Pityrosporum*, similar studies were done to evaluate the prevalence of IgE antibodies to *C. albicans*. The presence of such antibodies was demonstrated in AD patients and correlated with IgE to *Pityrosporum (156,164)*. It is, however, not known whether this is mainly the result of the existence of crossreacting antigens. Indeed, sera of selected asthmatic children were found to react with several common proteins and the polysaccharide mannan *(170)*. Better characterization of these antigens, and of the allergenic variations between strains of *C. albicans*, is currently under investigation *(170,173,174)*.

Dermatophytes

Dermatophytes are filamentous fungi known to cause various infections of the skin, hair and nails. These infections are common, and are influenced by sex (males > females), age (rare in children), and a variety of local and environmental factors, such as maceration, humidity, footwear, and crowding conditions (as in the army). Two different open studies well established that dermatophytosis is more frequent among atopic patients. An incidence of atopic diseases, mainly hay fever or asthma, was reported in nearly 50% of patients with dermatophytosis *(175,176)*. More recently, it was shown that children with an atopic background had a relative risk of 3 of acquiring tinea pedis *(169)*. A tendency to chronic dermatophytic infections was also described *(169,177)*. Furthermore, a pathogenic role of *Trichophyton* antigens was demonstrated in a group of patients with asthma/rhinitis *(171)*.

Atopic patients have been shown to lack delayed-type hypersensitivity to intradermally injected *Trichophyton* antigens (especially from *T. rubrum*), despite frequent immediate reactions *(175,176,178)*. Since these reactions were found in atopic patients with and without dermatophytic infection, and associated with

positive reactions to molds, crossreactivity was thought to be responsible for reactions to *Trichophyton* antigens *(178)*.

In atopic dermatitis, trichophytic infections are rarely severe, do not necessarily become chronic, and have not been mentioned as a possible flare factor. Furthermore, AD is a less important susceptibility factor than respiratory atopy *(169)*.

Conclusion

The skin of AD patients is particularly susceptible to colonization and infection with *S. aureus*. As a consequence, signs of a specific immune response can be identified in a subgroup of AD patients. A combination of an antibacterial with a topical corticosteroid treatment seems—even in cases without frank signs of superinfection—to be superior to corticosteroids alone, suggesting that abnormal bacterial skin colonization in AD patients aggravates their skin disorder. The aggravating role of other microorganisms, such as fungi, remains to be better defined.

In atopic dermatitis, some viral diseases, such as HSV infections, have particular clinical presentations that require prompt diagnosis in order to establish early, appropriate treatment. Finally, the notion that AD patients have an increased susceptibility to viral infections is not substantiated when published data are critically reviewed.

Notes Added in Proof

1. The role of *S. aureus* enterotoxin (SE), exfoliative toxin (ET), or toxic shock syndrome toxin (TSST-1) as immune mediators in AD has been further investigated recently *(179,180)*. It was shown that the (high) proportion of toxigenic strains of *S. aureus* recovered from AD skin was similar to that of psoriatic and healthy controls. However, subsets of AD patients are able to develop IgE antitoxins that may contribute to AD lesions.
2. A decreased risk of warts was found in children (at ages 11 and 16 yr) with either eczema or asthma/hay fever as compared to matched controls *(181)*. This report further confirms that the concept that all cutaneous viral infections occur more commonly in atopic subjects because of a generalized dysfunction in cell-mediated immunity is probably too simplistic a view.

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Comparative Study of Three Handwash Preparations for Efficacy Against Experimental Bacterial Contamination of Human Skin

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Abstract

The antimicrobial efficacy of three handwash preparations was measured by artificial contamination of the hands with *Serratia marcescens*, followed by treatment with the test preparation and recovery and enumeration of viable *Serratia*. A parachlorometaxylenol preparation (Acute-Kare®), a chlorhexidine gluconate preparation (Hibiclens®), and a nonmedicated liquid soap (Safe'N Sure®) were evaluated using 47 subjects randomly assigned to one of three groups. All subjects avoided antimicrobial substances for 14 days pretest. On the test day, the hands and forearms of each subject were contaminated with 6 ml of a stock *Serratia marcescens* culture containing at least 10^8 organisms/ml. After baseline recovery counts were determined with a glove-juice procedure, subjects' hands were recontaminated and treated with the assigned preparation for 15 seconds. Ten consecutive contamination/treatments were conducted in two hours. Viable *Serratia* were enumerated after the first, fifth, and tenth treatments (washes) by using the glove-juice procedure. The Hibiclens® group had significantly greater mean $\log_{10}$ reductions than Acute-Kare® and the nonmedicated liquid soap for

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Materials and Methods

Selection of Subjects

Forty-seven healthy adult male and female subjects, 21 years of age or older, were studied. All subjects were free of lesions or dermatoses of the hands and forearms, and none were taking topical or systemic antibiotics, oral contraceptives, or other drugs known to affect the bacterial flora of the skin. No subject was admitted to trial with a history of recent surgery, disease, burns, or any treatment which may have damaged the body's ability to defend itself against infections.

Experimental Procedure

PretestPeriod: For 14 days before testing, the subjects avoided the use of any medicated soaps, lotions, shampoos, deodorants, etc., as well as skin contact with any solvents, detergents, acids, or bases to prevent any possible build-up of antimicrobial agents contained in these products.

Test Period: Subjects were randomly assigned to one of three treatment groups. Each subject washed both hands and forearms with bland soap one hour before the test began. For baseline determinations 6 ml of a pigmented *Serratia marcescens* culture containing at least 10^8 organisms/ml was pipetted into the subject's cupped hands. The subject distributed the culture by gentle rubbing for ten seconds over the dorsal and ventral aspect of the hands, the fingers, and four inches above the wrist-line. After allowing the hands to air-dry for 60 seconds, baseline glove-juice samples of both hands were taken. This was followed by thorough washing for one minute with bland soap, thorough rinsing for one minute with tap water, and drying with a paper towel. The subject's hands were then contaminated with the *Serratia* culture as before and treated with the assigned handwash preparation for 15 seconds (first contamination/treatment). For treatment, the subject's hands were wet with warm tap water and 5 ml of Hibiclens®, Acute-Kare®, or nonmedicated liquid soap were dispensed into the subject's cupped hands. The subject washed his/her hands and arms to four inches above the wrist-line vigorously for 15 seconds and then rinsed with warm tap water for 15 seconds. Over a two-hour period, the hands were contaminated and treated nine additional times for a total of ten contamination/treatments. Glove-juice samples of both hands were taken after the first, fifth, and tenth contamination/treatments.

Glove-Juice Sampling and Plating Procedure

Sterile surgical gloves were placed on the subject and 75 ml of sterile 0.1% Triton X-100 in phosphate-buffered (pH 7.8) aqueous solution was immediately added to both gloves. The gloves were secured about both wrists and the fluid

nonmedicated liquid soap in mean $\log_{10}$ reductions. The comparisons were done for each hand and for the average of both hands after the first, fifth, and tenth washes (Table 1).

Table 1 — *Efficacy of three handwash preparations on experimentally contaminated human hands*

	Mean $\log_{10}$ reduction of *Serratia marcescens*								
	Right Hand			Left Hand			Average of Both Hands		
	Wash			Wash			Wash		
Treatment	1st	5th	10th	1st	5th	10th	1st	5th	10th
Hibiclens®	3.24*	4.88**	5.31**	3.21*	4.61*	5.29*	3.24**	4.76**	5.31**
Acute-Kare®	2.47	2.91	3.05††	2.34	2.86	3.12†	2.39	2.87	3.06†
Nonmedicated Liquid Soap	2.07	2.45	2.31	2.03	2.35	2.42	2.05	2.40	2.37

* Statistically significantly greater than Acute-Kare® ($p<0.01$) and nonmedicated liquid soap. ($p<0.001$)
** Statistically significantly ($p<0.001$) greater than Acute-Kare® and nonmedicated liquid soap
† Statistically significantly ($p<0.05$) greater than nonmedicated liquid soap
†† Statistically significantly ($p<0.01$) greater than nonmedicated liquid soap

Hibiclens® produced statistically significant greater mean $\log_{10}$ reductions than the Acute-Kare® and the nonmedicated liquid soap for both hands after the first, fifth, and tenth washes, with progressively greater reductions at each sampling time. The Acute-Kare® group had significantly greater mean $\log_{10}$ reductions than the nonmedicated liquid soap group only after the tenth wash.

Urinary tract infections due to gram-negative bacteria such as *Escherichia, Klebsiella,* and *Serratia* account for about 40% of hospital-acquired infections.[18] An effective health-care personnel handwash preparation should, therefore, demonstrate significant in-use efficacy against these bacteria and provide residual action against future contaminants. Significant reductions in numbers of *Serratia* bacteria were found on the hands of human subjects after first, fifth, and tenth contamination/handwashes with Hibiclens® and residual activity was observed; however, Acute-Kare® did not show any significantly greater activity than nonmedicated liquid soap until the tenth contamination/handwash. The conclusion from this study is that Hibiclens® (4% chlorhexidine gluconate) is a significantly more effective antimicrobial agent than Acute-Kare® (1% PCMX) and that Acute-Kare® is more effective than nonmedicated liquid soap only after multiple uses.

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Guidelines Committee

The Guidelines Committee is pleased to announce completion of the "Draft APIC Guideline for Handwashing and Hand Antisepsis," by Elaine Larson, RN, PhD, FAAN, CIC, and the Association for Professionals in Infection Control and Epidemiology, Inc. It is a working draft that has been subject to general comment after having been reviewed by the Guidelines Committee, the APIC Board of Directors, and a number of national organizations. At this time, we would like to invite all interested parties to comment on the proposed guideline before its final approval and adoption by APIC. To be considered, comments must be received in writing no later than January 2, 1995.

Please send your comments to:

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Draft APIC guideline for handwashing and hand antisepsis in health care settings*

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1992, 1993, 1994 APIC Guidelines Committee
Association for Professionals in Infection Control and Epidemiology, Inc.

The success of United States efforts in infection control has been due in large part to attention paid to the individual person as a primary source of the spread and thus the prevention of nosocomial infections. It is known, for example, that handwashing causes a significant reduction in the carriage of potential pathogens on the hands.[1,2] It is also known that handwashing can result in reductions in patient morbidity and mortality from nosocomial infection.[3-6] Although a definitive, double-blind, clinical trial of the effects of handwashing with an antiseptic product on nosocomial infection rates may be infeasible, it appears

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that, at least in certain high-risk situations, such antimicrobial products are beneficial.[7-10] Two major dilemmas facing ICPs in health care settings today, however, are when to use antiseptic agents and which agents to use.

In addition to the Association for Professionals in Infection Control and Epidemiology, Inc. (APIC),[11] several agencies and organizations have published guidelines, regulations, and standards regarding the topical use of antimicrobials for skin hygiene.[2, 12-14] This particular guideline will supplement those published by the Association of Operating Room Nurses (AORN),[12] the Centers for Disease Control and Prevention (CDC),[2] and the Food and Drug Administration (FDA)[13, 14] by describing specific characteristics of antimicrobial products available for topical use, summarizing the literature regarding their efficacy, and providing recommendations for their use by surgical personnel for hand scrubbing and by health care personnel for handwashing and hand antisepsis.

This guideline therefore provides information on skin flora of hands, characteristics of selected antimicrobial agents used on hands, handwashing and surgical scrub techniques, and related aspects of hand care and protection. In addition, recommendations are made regarding (1) health care personnel handwashing, (2) personnel hand preparation for operative procedures, and (3) other aspects of hand care and protection.

CHANGES SINCE 1988

This guideline focuses on handwashing, surgical personnel hand scrub, and related topics. Changes in this guideline from the 1988 publication[11] include a general updating of the research base for handwashing and surgical hand scrub products and the addition of sections on use of gloves and lotions; wearing of artificial nails, nail polish, and hand jewelry; and the behavioral and technical aspects of hand hygiene. Information relative to preparation of the patient's skin has been deleted. The myriad of products and practices related to preparation of the patient's skin before surgery or other invasive procedures is beyond the scope of this guideline. This guideline supersedes the document previously published as "APIC Guideline for Use of Topical Antimicrobial Agents."[11]

For the purposes of this Guideline, the following definitions will be used:

Transient flora (also termed "contaminating or noncolonizing flora"): microorganisms isolated from the skin but not demonstrated to be consistently present in the majority of persons. Such flora generally are considered to be transient but are of concern because of ready transmission by hands unless removed by mechanical friction and soap and water washing or destroyed by the application of an antiseptic handrub. Some microorganisms, particularly some gram-negative bacteria such as *Escherichia coli*, survive very poorly on the skin and are considered noncolonizing flora.[1, 2, 15-17]

Resident flora (also termed "colonizing flora"): microorganisms persistently isolated from the skin of most persons. These microorganisms are considered permanent residents of the skin and are not readily removed by mechanical friction. Colonizing flora include the coagulase- negative staphylococci, members of the genus *Corynebacterium* (commonly called diphtheroids or coryneforms) and *Propionibacterium*, *Acinetobacter* species, and probably certain members of the Enterobacteriaceae family.[1, 2, 15-18]

Plain or nonantimicrobial soap: detergent-based cleansers in any form (bar, liquid, leaflet, or powder) used for the primary purpose of physical removal of dirt and contaminating microorganisms. Such soaps work principally by mechanical action and have no bactericidal activity. Although some soaps contain low concentrations of antimicrobial ingredients, theseare used as preservatives and have minimal effect on colonizing flora.

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Handwash(ing): a process for the removal of soil and transient microorganisms from the hands.

Hand antisepsis: a process for the removal or destruction of transient microorganisms.

Surgical hand scrub: a process to remove or destroy transient microorganisms and reduce resident flora.

Antimicrobial soaps are considered drugs because they are intended to kill or inhibit microorganisms on skin when present in certain concentrations. They are included under the regulatory authority of the FDA. In 1978, the FDA published a tentative final regulation regarding the testing and classification of various topical antimicrobial ingredients.[13] Seven product categories were defined, three of which are used as handwash or hand scrub products:

Antimicrobial soap: a soap containing an ingredient with in vitro and in vivo activity against skin flora.

Health care personnel handwash: a broad-spectrum, antimicrobial preparation that is fast-acting, nonirritating, and designed for frequent use.

Surgical hand scrub: a broad-spectrum, fast-acting, persistent, and nonirritating preparation containing an antimicrobial ingredient designed to significantly reduce the number of microorganisms on intact skin.

The history of the role of the FDA in the regulation of topical antimicrobial products has been summarized,[19, 20] as has the need for standardized methods for testing the efficacy of such products by means of clinically relevant techniques.[21] A tentative final monograph for first-aid antiseptic products was published by the FDA in 1991,[14] and one for surgical scrub and health care personnel handwashing products is under development. Issues regarding efficacy criteria and testing methodology continue to be debated.

BACKGROUND RATIONALE

The indications for when handwashing should occur are well delineated in the "CDC Guideline for Handwashing and Hospital Environmental Control, 1985"[2] and the *APIC Curriculum for Infection Control Practice*.[22] The decision regarding when handwashing should occur depends on (1) the intensity of contact with patients or fomites, (2) the degree of contamination that is likely to occur with that contact, (3) the susceptibility of patients to infection, and (4) the procedure to be performed. Ranking schemes to identify health care activities that are likely to cause contamination of the hands have been developed and may be helpful for defining when handwashing is indicated.[23-25]

The 1985 CDC guideline states, "Plain soap should be used for handwashing unless otherwise indicated,"[2] and points out that the absence of randomized, controlled clinical trials regarding relative benefits of antimicrobial soap over plain soap preclude any Category I (strongly supported) recommendations for use of antiseptic agents for handwashing. Until such research studies are performed (if indeed such data ever are forthcoming, because the difficulties of conducting these trials may be prohibitive), some parameters for use of antiseptic agents are necessary. If an antimicrobial product is selected, it should be chosen for its inherent characteristics, its type and spectrum of activity, and the application for which it will be used.

Detergent (plain soap) with water can physically remove a certain level of microbes, but antiseptic agents are necessary to kill or inhibit microorganisms and reduce the level still further.[2, 9, 26, 27] For example, in a study by Lilly and Lowbury,[28] soap and water did not effectively reduce counts of artificially applied bacteria when the microorganisms were rubbed in. On the other hand, the application of 70% ethanol to contaminated hands resulted in a 99.7% reduction in counts.

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[redacted] demonstrated that even [redacted] level of reduction that can be reached [redacted] or integrity of h[redacted] preparations required less time to effect a maximum reduction than did a product containing chlorhexidine gluconate. These researchers also reported, however, that if the counts of hand flora were brought to a low equilibrium level with antiseptic washing and hands were then washed with plain soap, there was a sharp increase in bacterial yield.

Other studies confirm that use of soap and water for frequent daily handwashing results in minimal reduction and sometimes an increase in bacterial yield over baseline counts of clean hands.[30, 31] This increase is probably caused by increased shedding of viable bacteria in desquamating epithelium (resident flora) as a result of the trauma of frequent washing.[31, 32] That is, plain soap simply removes transient bacteria from skin but does not kill the bacteria released by shedding of skin squames and promotes their dispersal. Studies indicate that antimicrobials increase the likelihood of killing potentially pathogenic bacteria.[30, 31, 33-35]

In addition to their bactericidal and bacteriostatic effects on microorganisms, a second characteristic of certain antiseptic agents that sets them apart from plain soap is the ability to bind to the stratum corneum,[36] resulting in a persistent activity on skin. Microorganisms proliferate on the hands within the moist environment of rubber or plastic gloves,[36, 37] and gloves frequently become damaged during surgical procedures,[38, 39] increasing the risk of operative wound contamination.[40, 41] This characteristic of persistence (also called *substantivity* or *residual activity*) may be desirable to enhance continued antimicrobial activity when it is not possible to wash the hands during prolonged surgical procedures or when continued chemical activity on the skin is advantageous in other settings.

The primary action of plain soap is the mechanical removal of viable transient microorganisms, whereas the primary action of antimicrobial soap includes both mechanical removal and killing or inhibition of both transient and resident flora. The effect of antiseptic handrubs is only to inhibit flora, without any effect on soil.

The value of relative reductions (e.g., 85% vs 90% vs 99%) in total microbial counts on hands with regard to risk of subsequently transmitting infection-causing microorganisms is not known.[16, 21] High-risk situations in which patients are considered compromised and a maximum reduction in bacterial counts is thought to be desirable are of two major types: (1) during the performance of invasive procedures such as surgery or the placement and care of intravascular catheters, indwelling urinary catheters, or other invasive devices and (2) before contact with patients who have immune defects resulting from alterations in humoral or cellular immunity, damage to the integumentary system (burns, pressure ulcers, or wounds), and extremes of age.[1, 2, 10, 22] Handwashing with plain soap may fail to remove all transient microorganisms when contamination is heavy.[10, 42-45] The choice of plain soap, antiseptic soap, or antiseptic handrub should therefore be based on the degree of hand contamination and whether it is important to reduce and maintain minimal counts of resident flora, as well as to mechanically remove the transient flora on the hands of health care personnel.

Characteristics of selected antiseptic ingredients

This section discusses six antimicrobial ingredients commercially available in the United States that are designed for handwashing, surgical hand scrubbing, or hand antisepsis. Each is different, and none is ideal for all uses. In addition, many antimicrobial ingredients are quite sensitive to changes in formulation (e.g., pH, type of detergent base, and presence of certain emollients). Thus the selection of an appropriate antimicrobial agent for

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handwashing or surgical hand scrub should be made in three stages.[46] First, one must determine what characteristics of a topical antimicrobial agent are desired (e.g., absence of absorption across skin or mucous membranes, persistence, rapid reduction in flora, spectrum of activity) and then choose an ingredient that has these characteristics. Second, one must review and evaluate the evidence of safety and efficacy in reducing microbial counts. All antiseptic products should be tested as marketed.[27, 47] Compliance with use recommendations will depend on subjective reactions to features such as packaging, odor, and harshness, as well as safety and efficacy. Therefore a third step in the selection of a product is consideration of personnel acceptance of the product and the costs. In each subsequent section the following information is included: mode of action, spectrum of activity, safety and toxicity, rapidity of action, persistence, inactivation by organic matter, and available preparations.

Alcohols. Alcohols probably derive their antimicrobial effects by denaturation of proteins. They have excellent bactericidal activity against most vegetative gram-positive and gram-negative microorganisms and good activity against the tubercle bacillus. Although they are not sporicidal, they act against many fungi and viruses, including respiratory syncytial virus, hepatitis B virus, and HIV.[48] The data that demonstrate the virucidal activity of alcohols are derived, however, from in vitro studies. The significance of this activity in preventing transmission of viruses to health care workers is unknown. Alcohol may, rarely, be toxic; toxic reactions have been reported in children after sponging with isopropyl alcohol for fevers.[49] Nevertheless, alcohols applied to the skin are among the safest known antiseptics.[27, 41, 48]

In appropriate concentrations, alcohols provide the most rapid and greatest reduction in microbial counts on skin.[50-52] Alcohol applications as short as 15 seconds in duration have been effective in preventing hand transmission of gram-negative bacteria.[10] A vigorous, 1-minute rubbing with enough alcohol to wet the hands completely has been shown to be the most effective method for hand antisepsis.[27, 41, 53] Indeed, a 1-minute immersion or scrub with alcohol is as effective as a 4- to 7-minute skin preparation with other antiseptics in reducing the number of bacteria on skin.[41, 48, 54] Rubbing with alcohol for 3 minutes is as effective as 20 minutes of scrubbing.[41] Alcohols are effective as a surgical hand scrub[41, 55] and also as a health care personnel hand rinse.[45, 48]

It is necessary, however, to use a sufficient quantity of alcohol; that is, enough to thoroughly wet all surfaces of the hands. When used as a brief skin wipe in impregnated pads, alcohol's antimicrobial effects are less than those of liquid soaps with antiseptic ingredients.[56] The activity of alcohol does not appear to be significantly affected by small amounts of blood.[57] [redacted] cleaning agents, however, and are therefore not recommended in the presence of physical dirt. Further study is needed to determine the activity of alcohol in the presence of other types and larger amounts of organic material.

Although the alcohols do not leave a persistent chemical effect on the skin, the bacterial count on alcohol-scrubbed hands continues to drop for several hours after gloving, probably as a result of the continued deaths of damaged organisms.[58]

Three alcohols are most appropriate for use on the skin: ethyl (ethanol), normal-propyl (*n*-propyl), and isopropyl, although there are slight differences in their antimicrobial effects. For example, isopropyl alcohol may be less active against enteroviruses, more active against lipid-enveloped viruses, and slightly more bactericidal than ethanol.[48] Rotter[53] reported that the antibacterial effect of *n*-propanol was slightly superior to that of ethanol or isopropyl alcohol. The concentration of alcohol is of much more importance than the type, however, in determining its effectiveness. Alcohols must be diluted with water to denature protein. Alcohol concentrations between 60% to

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90% by weight are most effective. Generally, a concentration of no more than 70% by weight is used because it causes less skin drying and chemical dermatitis and is less costly than higher concentrations.[41, 48]

The major disadvantage of alcohol for skin antisepsis is its drying effect. Isopropyl alcohol, a more efficient fat solvent, may cause slightly more skin roughness than the other alcohols. However, some recently marketed preparations contain 60% to 70% ethanol or isopropyl alcohol with the addition of emollients to minimize skin drying.[59] These have been shown to be quite acceptable to users as well as having excellent antibacterial activity. The added emollient also may enhance antibacterial activity by slowing the drying time and thus increasing contact time of the alcohol with the skin.[34] Alcohol should be allowed to thoroughly evaporate from the skin to be fully effective and decrease irritation. A second disadvantage of the alcohols is that they are volatile and flammable and consequently must be stored carefully.

Chlorhexidine gluconate. Chlorhexidine gluconate (CHG) was used as a degerming agent in Europe and Canada for several decades before its approval for use in the United States in the 1970s. Chlorhexidine is a cationic bisbiguanide that derives its antimicrobial action by causing disruption of microbial cell membranes and precipitation of cell contents. Although it has a broad spectrum of activity, CHG is more effective against gram-positive than gram-negative bacteria. Action against the tubercle bacillus is minimal. CHG is only a fair inhibitor of fungi but in vitro is active against enveloped viruses including HIV, herpes simplex virus, cytomegalovirus, and influenza.[60-63] The significance of this activity in preventing transmission of these viruses to health care workers is unknown.

Numerous animal studies, as well as data from several decades of studies with human beings, indicate that CHG is nontoxic,[64, 65] even when used on the skin of newborn infants.[66, 67] Skin absorption is minimal.[36, 41, 66, 67] Ototoxicity can result, however, if chlorhexidine is instilled directly into the middle ear,[68, 69] and corneal damage can result from instillation of CHG into the eye.[70, 71] Contact urticaria syndrome leading to anaphylaxis and other allergic manifestations including respiratory symptoms and contact dermatitis have also been reported.[72-74] It has a relatively low skin-irritation potential.[61, 65]

Although the antibacterial activity of CHG is not as rapid as that of the alcohols, several clinical studies report good reductions in flora after a [illegible] classified as intermediate. In a recent study, CHG and a povidone-iodine product were compared in a surgical scrub protocol. The duration of scrub (3-minute initial and 30-second consecutive, as compared with 5-minute initial and 3-minute consecutive scrub) was not a significant variable when the iodophor was used, but the longer scrub was better when a CHG product was used. Additionally, CHG at both times resulted in a lower reduction of bacterial counts than did the povidone-iodine product.[76] One of the most important attributes of CHG is its persistence. It has strong affinity for the skin, remaining chemically active for at least 6 hours. Indeed, it probably has the best persistent effect of any agent currently available for handwashing.[36, 52, 77] After a few days of daily use of products that contain CHG, bacterial yield from hands is as low as that after use of alcohol-based products.[34, 52] The sequential use of CHG followed by a product containing 70% isopropyl alcohol and 0.5% CHG has been shown to be highly effective as a surgical scrub.[8]

The activity of CHG is not significantly affected by blood or other organic material.[57, 78, 79] Its activity is pH-dependent (5.5 to 7.0), however, and is reduced or neutralized in the presence of nonionic surfactants, inorganic anions (e.g., phosphate, nitrate, chloride), and other substances

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present in hard tap water and in many pharmaceutical preparations and hand creams and organic anions such as natural soaps.[60, 61, 65] For this reason, the activity of CHG is particularly formula dependent[80] and may be influenced by individual differences in skin pH, secretions, and moisture level.[81] Although efficacy data are difficult to interpret in terms of clinical impact, users may wish to compare data on reduction of flora when deciding which formulation to use. The potential for the development of resistance to CHG seems low[82] but has been reported.[83, 84]

CHG currently is offered in several formulations, the most common being 4% in a detergent base. Newer 2% aqueous formulations and foams appear to have antimicrobial activity slightly but not significantly less effective than that of the 4% liquid preparations.[85-87] CHG is also available in some countries as an alcohol-based hand rinse (0.5% CHG). A combination of the rapid effect of alcohol and the persistence of CHG would seem to offer a desirable antiseptic combination.[88-90]

Hexachlorophene. Hexachlorophene (HCP) is a chlorinated bisphenol that acts in high concentrations by disruption of microbial cell walls and precipitation of cell proteins. In low concentrations, it probably acts by inactivating essential enzyme systems within microorganisms.[91] At typical use concentrations (3%), it is bacteriostatic for gram-positive cocci but has little activity against gram-negative bacteria, the tubercle bacillus, fungi, or viruses.[60, 92-95]

As early as the 1940s, tests demonstrated potential toxicity of HCP, but for many years such evidence went unnoted. In the late 1960s and early 1970s, neurologic effects were reported in patients with burns who were bathed with HCP.[96] These neurotoxic effects were verified in animal studies and in premature infants.[97-99] As a result of these findings regarding the lack of safety of a product that was then in widespread use, the FDA instituted stringent regulations for testing and approval of antiseptic agents for over-the-counter (OTC) sale.[19, 36]

HCP is not fast acting, and one wash with HCP does not reduce cutaneous flora. Its rate of killing is classified as slow to intermediate. The major advantage of HCP is its persistence.[92] Unfortunately, long-term use of HCP followed by nonuse results in a temporary rebound increase in growth of skin flora.[41] Soaps and other organic materials have little effect on the activity of HCP.[41] HCP is available as an antiseptic, by prescription only, in a 3% formulation. Use on broken skin or mucous membranes or for routine total-body bathing is contraindicated.[98]

Iodine and iodophors. Tincture of iodine (the most effective formulation being 1% to 2% iodine and potassium iodine in 70% alcohol) has been used as a preoperative skin preparation for years. It is relatively safe and fast acting[50] but is not commonly used for handwashing. It must be removed from the skin after drying because of its potential to cause skin irritation.[13, 50]

The iodine-containing products that are used for handwashing and surgical hand scrub are the iodophors. Iodophors are complexes that consist of iodine and a carrier such as polyvinylpyrrolidone (PVP or povidone). The combination increases the solubility of iodine and provides a reservoir of iodine. The term "available iodine" indicates the extent of the reservoir, whereas "free iodine" is the amount of iodine in solution. The concentration of free iodine is the major chemical and microbicidal factor in the activity of iodophors and changes with the degree of dilution. A 10% povidone-iodine solution containing 1% available iodine will release free iodine to provide an equilibrium level of approximately 1 ppm.[100-102] Solutions with lower concentrations of iodophor may have higher relative activity because conditions favoring dissociation of iodine into solution are present.[103] Recommended levels of free iodine for antiseptics are 1 to 2 mg/L. Levels of free iodine

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Table 1. Types of hand care

	Purpose	Method
Handwash	To remove soil and transient microorganisms	Soap or detergent for at least 10-15 seconds
Hand antisepsis	To remove or destroy transient microorganisms	Antimicrobial soap or detergent or alcohol-based handrub for at least 10-15 seconds
Surgical hand scrub	To remove or destroy transient microorganisms and reduce resident flora	Antimicrobial soap or detergent preparation with brush to achieve friction for at least 120 seconds, or alcohol-based preparation for at least 20 seconds

below 1 ppm have been associated with contamination during manufacture.[101, 104] The antimicrobial effects of iodophors are similar to those of iodine and are the result of cell wall penetration, oxidation, and substitution of microbial contents with free iodine. Iodine and iodophors have a wide range of activity against gram-positive and gram-negative bacteria, the tubercle bacillus, fungi, and viruses. They also have some activity against bacterial spores.[41, 101, 105, 106] Iodophors are rapidly neutralized in the presence of organic materials such as blood or sputum.[79, 101, 107]

Iodine and, to a lesser extent, the iodophors are characterized by a propensity toward skin irritation and damage, as well as allergic or toxic effects in sensitive persons. Percutaneous and mucous membrane absorption occur, with the possible induction of hypothyroidism in newborn infants.[106, 108, 109]

The iodophor most commonly used is povidone-iodine. A formulation containing 7.5% is used as a surgical hand scrub. Lower concentration iodophors (0.05%) have been shown to have good antimicrobial activity[103, 110] because the amount of free iodine increases to some extent as the solution is diluted.[103] Other forms available for antiseptic use include 10% solution in applicators and various 2% solutions.

***Para*-chloro-*meta*-xylenol.** *Para*-chloro-*meta*-xylenol (PCMX or chloroxylenol) is a halogen-substituted xylenol that acts by microbial cell wall disruption and enzyme inactivation. It is less active than CHG and has good activity against gram-positive organisms, but it is less active against gram-negative bacteria. Its activity, especially against *Pseudomonas* species, is potentiated by the addition of ethylenediaminetetraacetic acid (EDTA) because of the binding of EDTA to metal ions in the cell wall of *Pseudomonas* species.[111, 112] It has fair activity against the tubercle bacillus, some fungi, and viruses.[47] In several in-use studies, various concentrations of PCMX have been shown to be less effective than either CHG or iodophors in reducing skin flora.[33, 113-115]

Even though PCMX penetrates the skin, the reported incidence of skin sensitization from PCMX is low.[116] Rapidity of activity of PCMX is intermediate, and it has a persistent effect over a few hours. It is active in alkaline pH but is neutralized by nonionic surfactants. For this reason, the efficacy of PCMX, like that of CHG, is highly formula dependent. Its activity is only minimally affected by organic matter.[117] PCMX is currently available in a number of handwashing products, usually in concentrations of 0.5% to 3.75%.

Triclosan. Triclosan (5-chloro-2-[2,4-dichlorophenoxyl] phenol, or Irgasan DP-300) is a diphenyl ether. Its antimicrobial activity is thought to derive from disruption of the microbial cell wall. It has good activity against gram-positive and most gram-negative bacteria; little information is available regarding its activity against viruses, and it appears to be a

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poor fungicide.[114, 118] Triclosan can be absorbed through intact skin but appears to be nonallergenic and nonmutagenic with short-term use. Its speed of antibacterial effect is intermediate, it has excellent persistent activity on skin, and its activity is only minimally affected by organic matter.[36, 117-120] It has been tested in concentrations from 0.3% to 2.0%. In one study, 0.3% triclosan was less effective than 2% CHG in reducing skin flora.[33] Another study however, found 1% triclosan superior to 4% CHG in reducing methicillin-resistant *Staphylococcus aureus* colonization in neonates.[121] One study in 20 healthy volunteers reported less prolonged effects and greater skin irritation with a 2% triclosan detergent than with a 4% CHG product.[122] Although it is commonly used in commercial soaps in concentrations of up to 1% to reduce body odor by inhibiting the growth of skin bacteria over time, additional safety and efficacy data are needed to determine the usefulness of higher concentrations in health care.

Technique

Handwashing. The purpose of handwashing is to remove dirt, organic material, and transient microorganisms (Table 1).[25, 123] Efficacy of handwashing is influenced by a number of factors. Although the amount of plain soap used does not appear to influence the result, antimicrobial products have a dose response, [illegible] is recommended. Hands should be thoroughly covered with the handwashing product used and vigorously rubbed together for 10 to 15 seconds, generating friction on all surfaces of the hands and fingers.[1, 2, 22-24] A technique to ensure coverage of all surfaces has been described[25] because parts of the thumbs, backs of the fingers, backs of the hands, and beneath the fingernails are often missed.[124, 125] Debris should be removed from under the fingernails because the subungual area has higher microbial counts and contamination of the hands can increase when gloves provide a warm, moist environment.[126] Duration of washing is important, not only for mechanical action but also to allow antimicrobial products sufficient contact time to achieve the desired effect when they are used.[127] Hands should be thoroughly rinsed to remove residual soap and then dried. When the sink does not have foot controls or an automatic shutoff, a paper towel should be used to shut off the faucet to avoid recontaminating the hands.

A variety of methods are available for drying hands.[128] Cloth towels are rarely used in health care settings because of concerns regarding contamination.[27, 129, 130] Although warm-air dryers are used in many public rest rooms, they are rarely found in patient care areas of health care facilities. Research is scant, but one study comparing cloth towels, paper towels, and warm-air drying found that all three methods resulted in a further reduction of flora, with warm-air drying producing the greatest reduction and cloth drying the least.[131] Another study found no difference in the numbers of bacteria remaining after paper towel and air drying.[132] However, it usually takes longer to dry hands with an air dryer, and many have standard 30-second cycles that may be inadequate.[27, 132] In addition, hand dryers can serve only one person at a time, and paper towels will not be available to use when shutting off faucets without foot or automatic controls. The noise associated with air dryers may also pose problems in patient care areas. Paper towels should be dispensed from holders that require the user to remove them one at a time. The hand-drying materials should be placed near the sink in an area that will not become contaminated by splash.

Hand antisepsis. The purpose of hand antisepsis is to destroy or remove transient microorganisms from hands (Table 1).[25, 123] Hand antisepsis can occur simultaneously with handwashing when soaps or detergents that contain antiseptics are used. Hand antisepsis can also be accomplished by use of alcohol-containing antiseptic handrubs

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Table 2. Intervention studies to improve handwashing practices in critical care areas

Reference	Setting	Type of intervention	Results
Doebbling et al.[7]	3 ICUs, 46 beds	A. Chlorhexidine gluconate vs soap-alcohol crossover trial	Handwashing compliance was significantly better during chlorhexidine use; infection rate was reduced with chlorhexidine but not significantly
		B. Videotaped and written instruction with refresher at midpoint	
		C. Visual observations; monthly summary of compliance posted	
Simmons et al.[210]	Two ICUs, 24 beds, community teaching hospital	Threefold:	No significant changes in handwashing rates
		A. Handwashing questionnaire; physician-provided compulsory in-service; supportive literature distributed	
		B. Button compaign	
		C. Observation of handwashing with critique of method and staff feedback	
Dubbert et al.[221]	12-bed ICU	A. Three series of classes by ICNs	A. Immediate increase in handwashing, followed by decline to baseline over 4 weeks
		B. Observation of handwashing with next-day feedback to staff	B. Improvement to 95% compliance, with feedback sustained to end of study
Graham[223]	18-bed ICU, Australia	Introduction of handrub solution after instruction	Significant increase (13%) in frequency of hand decontamination
Conly et al.[220]	16-bed ICU, Canada	A. Feedback on handwashing practices; memoranda regarding handwashing to attending staff and departments; posters	Significant increase in handwashing compliance; decrease in nosocomial infection rates
		B. Infection control staff "emphasized the importance of handwashing"; results of two previous surveys reviewed; ICU directors actively encouraged handwashing	
Mayer et al.[224]	ICU, VA medical center	Two phases:	
		A. Introduction of new emollient soap	A. No increase in handwashing after introduction of new soap
		B. Feedback on handwashing compliance by daily memo	B. Immediate increase in handwashing frequency when feedback phase began
Larson et al.[222]	6-bed postanesthesia recovery room and 15-bed neonatal ICU	Automated sink	Handwashing quality significantly improved but frequency declined significantly

ICU, Intensive care unit; *ICN*, infection control nurse; *VA*, Veterans Affairs.

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when hands are already clean; that is, not soiled with dirt or organic material.

A number of studies indicate that plain handwashing does not always remove transient microorganisms.[10, 42-45, 133] However, the concerns regarding adequacy of handwashing technique also apply to the use of alcohol handrubs. Failure to cover all surfaces of the hands because of poor technique or use of insufficient amounts of alcohol handrub solution can leave contaminated surfaces.[134] Although alcohol is often recommended for use in areas where handwashing facilities are not readily available, it is important to reiterate that alcohols are not good cleaning agents and are not recommended in the presence of physical dirt. In addition, although small amounts of blood do not appear to adversely affect the activity of alcohol,[57] it is unclear that the same is true for larger amounts of organic material. Health care personnel working in settings where handwashing facilities are not readily available and heavy hand contamination with organic material occurs may wish to use detergent-containing towelettes for physical cleansing of the hands before use of alcohol-containing handrubs for antisepsis.

Surgical scrub. Surgical hand scrub is performed to remove transient flora and reduce resident flora for the duration of surgery in case of glove tears (Table 1).[12] The process must begin with washing the hands and forearms thoroughly to remove dirt and transient bacteria. A nail cleaner should be used to clean under the nails.

The traditional surgical scrub in the United States has been 5 minutes in duration for both the initial and subsequent scrubs, with chlorhexidine or iodophor products,[12, 40, 135-137] agents that demonstrate persistence and help maintain lower microbial counts under gloves.[138] In Europe, alcohol-based preparations are often considered the agent of choice. These preparations are applied by rubbing on 3 to 5 ml until dry and repeating applications for approximately 5 minutes.[50] With alcohol preparations, persistence may be less important because bacterial counts are so low that it takes several hours for regrowth to occur to prescrub levels.[55] AORN has recommended that persons sensitive to antimicrobial agents with residual activity should scrub with a nonmedicated soap, followed by application of an alcohol-based preparation.[12]

The optimum duration of surgical scrub is unclear,[12] although research indicates that it may be agent dependent.* Also unclear is whether scrubs for subsequent consecutive cases may be shorter than for the first case.[40] O'Shaughnessy and associates suggest that scrub time can be reduced for subsequent cases when CHG is used because of its persistent and cumulative effect,[138] whereas Rehork and Ruden suggest that the time between initial and subsequent scrubs must also be considered.[139] The American College of Surgeons suggests that a surgical scrub of 120 seconds, which includes brushing of the nail and fingertip areas, is adequate.[141]

As with personnel handwashing, the clinical impact of relative reductions in microbial counts after surgical scrubs is unknown. Although Cruse and Foord[142] initially reported that glove punctures were associated with an increased clean surgery infection rate, their later work[143] and another study[144] showed no relationship between the incidence of surgical infections and glove tears during surgery.

Other aspects of hand care and protection

Glove use. The wearing of gloves to provide a protective barrier to microbial transmission has increased dramatically since the inception of universal precautions[145-147] and has been recommended to prevent heavy contamination of hands.[148, 149] Extreme variability in the quality of gloves has been reported, however, with leakage in 4% to 63% of vinyl gloves and 3% to 52% of latex gloves.[150-154] A recent study found little benefit in

*References 41, 76, 80, 113, 136, and 138-140.

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double gloving when latex gloves were worn.[155] Additionally, microbial contamination of hands and possible transmission of infection have been reported even when gloves were worn.[156-159] Soap and water handwash or an antiseptic handrub should therefore be used after glove removal.

Gloved hands should not be washed to reuse gloves. Studies have indicated that microorganisms are not always removed from gloves despite friction, a cleansing agent, and drying,[156] and washing may decrease the integrity of the glove.[160] The Occupational Safety and Health Administration Bloodborne Pathogens Standard prohibits washing or decontaminating disposable (single-use) gloves for reuse.[161]

Inappropriate glove use has been recognized as a problem.[162-164] Failure to change gloves between patients and contaminated body sites was identified as the cause of an *Acinetobacter* outbreak.[164]

Nails, nail polish and artificial nails. One report suggests that artificial nails may increase the microbial load on hands, particularly of gram-negative bacteria.[165] Another report showed no significant difference in colony counts between operating room nurses wearing artificial nails and those with natural nails. In two instances when the prosthetic nails were broken or had separated from the natural nails, however, high colony counts were found despite a 30-second wash with povidone-iodine.[166] Dermatologists report secondary infections with *Pseudomonas* and *Candida* when reactions to nail lacquers and hardeners cause onycholysis.[167, 168]

Nail polish applied to natural nails seems to have no detrimental influence on microbial load, as long as nails are short.[169] Short nails are probably important because the majority of flora on the hand is found under and around the fingernails.[126] Clear polish is preferable because dark colors may obscure the subungual space, reducing the likelihood of careful cleaning. In addition, long nails can make donning gloves more difficult and may cause gloves to tear more readily.

AORN recommends[12] that artificial nails not be worn by operating room personnel, citing reports of fungal and bacterial infections. Concerns have also been raised by others that use of artificial fingernails and nail polish may discourage vigorous handwashing.[124]

Jewelry. Total bacterial counts are higher when rings are worn,[170, 171] although Jacobson and coworkers did not find that rings interfered with removal of bacteria by handwashing.[170] Rings and nail jewelry can make donning gloves more difficult and may cause gloves to tear more readily.[124]

Lotion. Lotions are often recommended to ease the dryness resulting from frequent handwashing,[1, 124, 172] and more recently to prevent dermatitis resulting from glove use.[173] A study has demonstrated that the application of a lotion can reduce the dispersal of bacteria.[174] Hand lotion can become contaminated,[175-177] however, and should be considered as a possible reservoir in the event of an outbreak.

Petroleum jelly under gloves has been shown to be acceptable from a microbiologic point of view,[178] but concerns have been raised about the potential for oil-based lotion formulations to weaken latex gloves and cause increased permeability.[179] For that reason, lotions that contain petroleum or other oil emollients may affect the integrity of gloves. Lotions designed to protect against latex sensitivity are now on the market. Because latex allergy and anaphylactic reactions to latex products are being reported with increased frequency,[180-186] such products would be highly desirable if indicated. One study of such a lotion found no interference with effectiveness of the surgical scrub nor any increase in leakage in gloves worn for 2 hours after application of the lotion.[187] Anionic moisturizing products and surfactants, however, have been shown to interfere

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with the residual activity of CHG.[188, 189] Interaction between lotions and CHG antimicrobial products used must therefore be considered at the time of product selection.

Storage and dispensing of hand care products. Handwash products, including both plain soap and antiseptic products, can become contaminated or support the growth of microorganisms.[100, 104, 190-198] Bar soap should be provided in small bars that can be changed frequently, with soap racks that promote drainage.[2, 199-202] Liquid products should be stored in closed containers and dispensed from either disposable containers or containers that are washed and dried thoroughly before refilling.[1, 2] Some have suggested that dispensers should be foot- or elbow-operated to decrease the potential for contamination.[27, 203] Lotions can also become contaminated and support bacterial growth and should be dispensed in small, individual-use containers or from pump dispensers that are not opened or refilled.[175, 176]

Behavior and compliance. The primary problem with hand hygiene is not a paucity of good products, but rather the laxity of practice. Variations in handwashing practice have been reported by type of unit (pediatric personnel have been shown to have higher frequency of handwashing) and profession (in general, nurses wash more often than physicians, although physicians have been shown to wash more thoroughly).[129, 204-208] Overall, however, handwashing associated with general patient care occurs in approximately half of the instances in which it is indicated and usually is of shorter duration than recommended. Additionally, staff overestimate the frequency and quality of their handwashing behavior.[17, 125, 209-211]

A number of studies have examined the influence of various factors on handwashing behavior. Staffing,[212, 213] placement of sinks,[214] and the effect of handwashing on skin condition[215-218] have been identified as obstacles, whereas the main motivating factor has been awareness of the importance of handwashing in preventing infection.[215, 216] One study found senior British nurses were better handwashers than more junior nurses.[219]

Several studies, summarized in Table 2, have evaluated the influence of behavioral and educational interventions on handwashing practices in critical care units.[7, 210, 220-224] These interventions met with varying success, but even when handwashing increased the change in behavior was not sustained beyond the period of the study intervention. Clearly, simple educational efforts to influence handwashing practices are of minimal benefit. Studies indicate that health care personnel are aware of the reasons handwashing should be done.[215, 225] A number of studies also suggest, however, that sustained feedback on handwashing behavior or feedback about patient infections influences performance.[210, 220, 221, 224, 226] The use of role models or mentors to influence behavior has also been suggested.[215, 225-227]

A committed and thoughtful overall approach that includes staff involvement is important. In developing such approaches, the issue of which product to use must *not* take precedence over improving the quantity and quality of handwashing.[228]

Complications of handwashing and gloving

Handwashing can cause detrimental effects on the skin.[16, 59, 172] Some of these effects occur regardless of the products used; others involve reactions to the ingredients in various handwashing agents.[72, 73, 116, 229, 230] Contrary to popular opinion, antiseptics do not necessarily cause greater damage to skin than plain soap; often it is the detergent base that is harsh.[30, 31] Recently, as glove use has increased, reports of reactions to latex gloves have also increased.[183-186, 230-233] Dermatitis in health care personnel may place patients at risk because handwashing will not decrease bacterial counts on dermatitic skin,[1, 234] and dermatitic skin contains high numbers of microorganisms. Health care personnel with dermatitis may be at increased risk of exposure to

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blood-borne pathogens during skin contact with blood or body fluids because the integrity of the skin is not present.

A variety of solutions have been proposed to remedy these problems. Use of moisturizers to alleviate skin dryness has long been recommended.[1, 124, 172] Emollients have been added to soaps. Emulsions and antiseptic "no-wash" products have been suggested as substitutes for soap and water washes.[59, 235-237] Nonlatex, powder- or chemical-free gloves are available.[184, 230, 232, 233] Use of vinyl or cotton gloves under latex gloves or barrier lotions for latex-sensitive persons has also been suggested.[173, 230, 233] Unfortunately, none of these solutions has been studied under long-term, in-use conditions to determine either efficacy in alleviating the identified problem or the impact on the microbiologic condition of the skin.

New technologies

A variety of new devices have been proposed to improve handwashing compliance and technique. In one trial, automated sinks with water flow and soap dispensing controlled by electronic sensor improved the quality of handwashing when used but were avoided by staff during busy times.[222] Handwashing machines are also available[238] but are largely untested in clinical use. Recently, gloves containing a chlorhexidine coating on the inner surface were tested.[239] Undoubtedly, other items will be developed and promoted as solutions to the poor compliance with handwashing recommendations. All will require evaluation in clinical settings to determine their effectiveness.

Areas for future investigation

Definitive studies are needed to determine whether antimicrobial soaps or antiseptic handrubs are better than plain soap in preventing infection transmission. Circumstances under which use of an antiseptic agent will consistently reduce the occurrence of nosocomial infection need to be identified. Increased glove use in recent years may require a redefinition of the resident and transient flora of gloved and ungloved hands and the effect of handwashing, with or without antiseptic agents, on this flora. The use of nail polish, artificial nails, and hand jewelry by health care providers will remain controversial until further information is available. The optimum durations for surgical personnel hand scrub for both initial and subsequent cases need to be clearly delineated for each antimicrobial agent used. As new products and new technologies emerge to combat skin irritation and sensitivity to glove material, as well as to improve handwashing technique, they will need to be carefully evaluated for efficacy, compatibility, and adverse effects. Standardization of test methods is needed to allow consumers to evaluate studies conducted in different settings with different agents. Studies should be conducted under long-term, in-use clinical conditions.

Many handwashing studies focus on products and technology. Much less attention is given to investigating methods for improving compliance of health care personnel with recommended handwashing practices. Such studies are sorely needed. ICPs need to collaborate with other disciplines to determine how to maintain lasting improvement in handwashing, a behavior considered essential for infection prevention and control.

RECOMMENDATIONS

A. Health care personnel handwashing and hand antisepsis
   1. Hands must be washed thoroughly with soap and water when visibly soiled.
   2. Hands must be cared for by handwashing with soap and water or by hand antisepsis with alcohol-based handrubs (if hands are not visibly soiled):
      a. Before and after patient contact.
      b. After contact with a source of microorganisms (body fluids and substances,

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mucous membranes, nonintact skin, inanimate objects that are likely to be contaminated).
   c. After removing gloves.
3. Hands should be thoroughly covered with the handwashing product used and vigorously rubbed together for 10 to 15 seconds, covering all surfaces of the hands and fingers.
4. For general patient care, a plain, nonantimicrobial soap is recommended in any convenient form (bar, leaflets, liquid, powder). Such detergent-based products may contain very low concentrations of antimicrobial agents that are used as preservatives to prevent microbial contamination. If bar soap is used, small bars that can be changed frequently and soap racks that promote drainage should be used.
5. Hand antisepsis, achieved by handwashing or surgical scrub with antimicrobial-containing soaps or detergents or by use of alcohol-based antiseptic handrubs, is recommended in the following instances:
   a. Before the performance of invasive procedures such as surgery or the placement of intravascular catheters, indwelling urinary catheters, or other invasive devices.
   b. When persistent antimicrobial activity on the hands is desired.
   c. When it is important to reduce numbers of resident skin flora in addition to transient microorganisms.
6. Alcohol-based handrubs are recommended for use in settings where handwashing facilities are inadequate or inaccessible and hands are not soiled with dirt or heavily contaminated with blood or other organic material. In situations where such soilage occurs, detergent-containing towelettes should be used to cleanse the hands; alcohol-based handrubs can then be used to achieve hand antisepsis.
7. Alternative agents such as detergent-containing towelettes and alcohol-based handrubs should be available in the event of interruption of water supply.
8. Products used for handwashing, surgical scrubs, and hand care should be chosen by persons knowledgeable about the purpose of use, the advantages and disadvantages, cost, and acceptance of the product by users.
9. Routine use of HCP is not recommended.

B. Personnel hand preparation for operative procedures
   1. The procedure for surgical hand scrub should include the following steps:
      a. Wash hands and forearms thoroughly.
      b. Clean under nails with a nail cleaner.
      c. Rinse thoroughly.
      d. Apply antimicrobial agent to wet hands and forearm with friction for at least 120 seconds.
   2. If an alcohol-based preparation is selected for use, wash hands and arms, clean fingernails thoroughly, dry completely, and follow manufacturer's recommendations for application. Generally, application should last for at least 20 seconds.
   3. Personnel with allergic reactions to antiseptic agents other than alcohol may apply ethanol or isopropanol.

C. Other aspects of hand care and protection
   1. Glove use
      a. Gloves should be used as an adjunct to, not a substitute for, handwashing.
      b. Gloves should be used for hand-contaminating activities. Gloves should be removed and hands washed when such activity is completed, when the integrity of the gloves is in doubt, and between patients. Gloves may need to be changed during the care of a single patient, for

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example when moving from one procedure to another.
c. Disposable gloves should be used only once and should not be washed for reuse.
d. Gloves made of other materials should be available for personnel with sensitivity to usual glove material (such as latex).

2. Condition of nails and hands
   a. Nails should be short enough to allow the individual to thoroughly clean underneath them and not cause glove tears.
   b. The hands, including the nails and surrounding tissue, should be inflammation free.
3. Lotion
   a. Lotions may be used to prevent skin dryness associated with handwashing.
   b. If used, lotion should be supplied in small, individual- use or pump dispenser containers that are not refilled.
   c. Compatibility between lotion and antiseptic products and the effect of petroleum or other oil emollients on the integrity of gloves should be considered at the time of product selection.
4. Storage and dispensing of hand care products
   a. Liquid products should be stored in closed containers.
   b. Disposable containers should be used. If disposable containers cannot be used, routine maintenance schedules for cleaning and refilling should be followed. Reusable containers should be thoroughly washed and dried before refilling.
   c. There should be a routine mechanism to ensure that soap and towel dispensers function properly and are adequately supplied.
   d. Containers of alcohol should be stored in cabinets or areas approved for flammables.
5. Drying of hands
   a. Cloth towels, hanging or roll type, are not recommended for use in health care facilities.
   b. Paper towels or hand blowers should be within easy reach of the sink but beyond splash contamination.
   c. Lever-operated towel dispensers should be activated before beginning handwashing. Hand blowers should be activated with the elbow.
6. Behavior and compliance. Efforts to improve handwashing practice should be multifaceted and should include continuing education and feedback to staff on behavior or infection surveillance data. Unit clinical and administrative staff should be involved in the planning and implementation of strategies to improve compliance and handwashing.

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APIC GUIDELINES FOR INFECTION CONTROL PRACTICE

The Association for Professionals in Infection Control and Epidemiology, Inc. (APIC) Board of Directors and Guidelines Committee are pleased to present the "APIC Guideline for Handwashing and Hand Antisepsis in Health Care Settings."

Elaine Larson, RN, PhD, FAAN, CIC, was selected to revise the previously published "APIC Guideline for Use of Topical Antimicrobial Agents" because of her recognized expertise in infection control and extensive research in handwashing and hand disinfection. Initial drafts received review by the APIC Guidelines Committee, key individuals, and professional organizations before publication of the Draft in the October 1994 issue of the JOURNAL, soliciting further comment. All written comments were reviewed by the APIC Guidelines Committee and revisions were made. The Guideline was finalized by the Committee in February 1995 and approved by the APIC Board of Directors in March 1995.

The APIC Board of Directors and the APIC Guidelines Committee express our sincere gratitude to the author and to all who provided assistance in the guideline development and review process.

APIC guideline for handwashing and hand antisepsis in health care settings*

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1992, 1993, and 1994 APIC Guidelines Committee
Association for Professionals in Infection Control and Epidemiology, Inc.

The success of United States efforts in infection control has been due in large part to attention paid to the individual person as a primary source of the spread and thus the prevention of nosocomial infections. It is known, for example, that handwashing causes a significant reduction in the carriage of potential pathogens on the hands.[1, 2] It is also known that handwashing can result in reductions in patient morbidity and mortality from nosocomial infection.[3-6] Although a definitive, double-blind, clinical trial of the effects of handwashing with an antiseptic product on nosocomial infection rates may be infeasible, it appears that, at least in certain high-risk situations, such antimicrobial products are beneficial.[7-10] Two major dilemmas facing ICPs in health care settings today, however, are when to use antiseptic agents and which agents to use.

In addition to the Association for Professionals in Infection Control and Epidemiology, Inc. (APIC),[11] several agencies and organizations have published guidelines, regulations, and standards regarding the topical use of antimicrobials for skin hygiene.[2, 12-14] This particular guideline will supplement those published by the Association of Operating Room Nurses (AORN),[12] the Centers for Disease Control and Prevention (CDC),[2] and the Food and Drug Administration (FDA)[13, 14] by describing specific characteristics of antimicrobial products available for topical use, summarizing the literature regarding their efficacy, and providing recommendations for their use by surgical personnel for hand scrubbing and by health

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patients who have immune defects resulting from alterations in humoral or cellular immunity, damage to the integumentary system (burns, pressure ulcers, or wounds), and extremes of age.[1, 2, 10, 22] Handwashing with plain soap may fail to remove all transient microorganisms when contamination is heavy.[10, 42-45] The choice of plain soap, antiseptic soap, or antiseptic handrub should therefore be based on the degree of hand contamination and whether it is important to reduce and maintain minimal counts of resident flora, as well as to mechanically remove the transient flora on the hands of health care personnel.

Characteristics of selected antiseptic ingredients

This section discusses six antimicrobial ingredients commercially available in the United States that are designed for handwashing, surgical hand scrubbing, or hand antisepsis. Each is different, and none is ideal for all uses. In addition, many antimicrobial ingredients are quite sensitive to changes in formulation (e.g., pH, type of detergent base, and presence of certain emollients). Thus the selection of an appropriate antimicrobial agent for handwashing or surgical hand scrub should be made in three stages.[46] First, one must determine what characteristics of a topical antimicrobial agent are desired (e.g., absence of absorption across skin or mucous membranes, persistence, rapid reduction in flora, spectrum of activity) and then choose an ingredient that has these characteristics. Second, one must review and evaluate the evidence of safety and efficacy in reducing microbial counts. All antiseptic products should be tested as marketed.[27, 47] Compliance with use recommendations will depend on subjective reactions to features such as packaging, odor, and harshness, as well as safety and efficacy. Therefore a third step in the selection of a product is consideration of personnel acceptance of the product and the costs. In each subsequent section the following information is included: mode of action, spectrum of activity, safety and toxicity, rapidity of action, persistence, inactivation by organic matter, and available preparations.

Alcohols. Alcohols probably derive their antimicrobial effects by denaturation of proteins. They have excellent bactericidal activity against most vegetative gram-positive and gram-negative microorganisms and good activity against the tubercle bacillus. Although they are not sporicidal, they act against many fungi and viruses, including respiratory syncytial virus, hepatitis B virus, and HIV.[48] The data that demonstrate the virucidal activity of alcohols are derived, however, from in vitro studies. The significance of this activity in preventing transmission of viruses to health care workers is unknown. Alcohol may, rarely, be toxic; toxic reactions have been reported in children after sponging with isopropyl alcohol for fevers.[49] Nevertheless, alcohols applied to the skin are among the safest known antiseptics.[27, 41, 48]

In appropriate concentrations, alcohols provide the most rapid and greatest reduction in microbial counts on skin.[50-52] Alcohol applications as short as 15 seconds in duration have been effective in preventing hand transmission of gram-negative bacteria.[10] A vigorous, 1-minute rubbing with enough alcohol to wet the hands completely has been shown to be the most effective method for hand antisepsis.[27, 41, 53] Indeed, a 1-minute immersion or scrub with alcohol is as effective as a 4- to 7-minute skin preparation with other antiseptics in reducing the number of bacteria on skin.[41, 48, 54] Rubbing with alcohol for 3 minutes is as effective as 20 minutes of scrubbing.[41] Alcohols are effective as a surgical hand scrub[41, 55] and also as a health care personnel hand rinse.[45, 48]

It is necessary, however, to use a sufficient quantity of alcohol; that is, enough to thoroughly wet all surfaces of the hands. When used as a brief skin wipe in impregnated pads, alcohol's antimicrobial effects are less than those of liquid soaps with antiseptic ingredients.[56] The activity of alcohol does not appear to be significantly affected by small amounts of blood.[57] Alcohols are not good cleaning agents, however, and are therefore not recommended in the presence of physical dirt. Further study is needed to determine the activity of alcohol in the presence of other types and larger amounts of organic material.

Although the alcohols do not leave a persistent chemical effect on the skin, the bacterial count on alcohol-scrubbed hands continues to drop for several hours after gloving, probably as a result of the continued deaths of damaged organisms.[58]

Three alcohols are most appropriate for use on the skin: ethyl (ethanol), normal-propyl (*n*-propyl), and isopropyl, although there are slight differences in their antimicrobial effects. For example, isopropyl alcohol may be less active against enteroviruses, more active against lipid-enveloped viruses, and slightly more bactericidal than ethanol.[48] Rotter[53] reported that the antibacterial effect of *n*-propanol was slightly superior to that of ethanol or isopropyl alcohol. The concentration of alcohol is of much more importance than the type,

however, in determining its effectiveness. Alcohols must be diluted with water to denature protein. Alcohol concentrations between 60% to 90% by weight are most effective. Generally, a concentration of no more than 70% by weight is used because it causes less skin drying and chemical dermatitis and is less costly than higher concentrations.[41, 48]

The major disadvantage of alcohol for skin antisepsis is its drying effect. Isopropyl alcohol, a more efficient fat solvent, may cause slightly more skin roughness than the other alcohols. However, some recently marketed preparations contain 60% to 70% ethanol or isopropyl alcohol with the addition of emollients to minimize skin drying.[59] These have been shown to be quite acceptable to users as well as having excellent antibacterial activity. The added emollient also may enhance antibacterial activity by slowing the drying time and thus increasing contact time of the alcohol with the skin.[34] Alcohol should be allowed to thoroughly evaporate from the skin to be fully effective and decrease irritation. A second disadvantage of the alcohols is that they are volatile and flammable and consequently must be stored carefully.

Chlorhexidine gluconate. Chlorhexidine gluconate (CHG) was used as a degerming agent in Europe and Canada for several decades before its approval for use in the United States in the 1970s. Chlorhexidine is a cationic bisbiguanide that derives its antimicrobial action by causing disruption of microbial cell membranes and precipitation of cell contents. Although it has a broad spectrum of activity, CHG is more effective against gram-positive than gram-negative bacteria. Action against the tubercle bacillus is minimal. CHG is only a fair inhibitor of fungi but in vitro is active against enveloped viruses including HIV, herpes simplex virus, cytomegalovirus, and influenza.[60-63] The significance of this activity in preventing transmission of these viruses to health care workers is unknown.

Numerous animal studies, as well as data from several decades of studies with human beings, indicate that CHG is nontoxic,[64, 65] even when used on the skin of newborn infants.[66, 67] Skin absorption is minimal.[36, 41, 66, 67] Ototoxicity can result, however, if chlorhexidine is instilled directly into the middle ear,[68, 69] and corneal damage can result from instillation of CHG into the eye.[70, 71] Contact urticaria syndrome leading to anaphylaxis and other allergic manifestations including respiratory symptoms and contact dermatitis have also been reported.[72-74] It has a relatively low skin-irritation potential.[61, 65]

Although the antibacterial activity of CHG is not as rapid as that of the alcohols, several clinical studies report good reductions in flora after a 15-second handwash.[30, 75] Its speed of antibacterial effect is classified as intermediate. In a recent study, CHG and a povidone-iodine product were compared in a surgical scrub protocol. The duration of scrub (3-minute initial and 30-second consecutive, as compared with 5-minute initial and 3-minute consecutive scrub) was not a significant variable when the iodophor was used, but the longer scrub was better when a CHG product was used. Additionally, CHG at both times resulted in a lower reduction of bacterial counts than did the povidone-iodine product.[76] One of the most important attributes of CHG is its persistence. It has strong affinity for the skin, remaining chemically active for at least 6 hours. Indeed, it probably has the best persistent effect of any agent currently available for handwashing.[36, 52, 77] After a few days of daily use of products that contain CHG, bacterial yield from hands is as low as that after use of alcohol-based products.[34, 52] The sequential use of CHG followed by a product containing 70% isopropyl alcohol and 0.5% CHG has been shown to be highly effective as a surgical scrub.[8]

The activity of CHG is not significantly affected by blood or other organic material.[57, 78, 79] Its activity is pH-dependent (5.5 to 7.0), however, and is reduced or neutralized in the presence of nonionic surfactants, inorganic anions (e.g., phosphate, nitrate, chloride), and other substances present in hard tap water and in many pharmaceutical preparations and hand creams and organic anions such as natural soaps.[60, 61, 63] For this reason, the activity of CHG is particularly formula dependent[80] and may be influenced by individual differences in skin pH, secretions, and moisture level.[81] Although efficacy data are difficult to interpret in terms of clinical impact, users may wish to compare data on reduction of flora when deciding which formulation to use. The potential for the development of bacterial resistance to CHG seems low[82] but has been reported.[83, 84]

CHG currently is offered in several formulations, the most common being 4% in a detergent base. Newer 2% aqueous formulations and foams appear to have antimicrobial activity slightly but not significantly less effective than that of the 4% liquid preparations.[85-87] CHG is also available in some countries as an alcohol-based hand rinse (0.5% CHG). A combination of the rapid effect of

alcohol and the persistence of CHG would seem to offer a desirable antiseptic combination.[88-90]

Hexachlorophene. Hexachlorophene (HCP) is a chlorinated bisphenol that acts in high concentrations by disruption of microbial cell walls and precipitation of cell proteins. In low concentrations, it probably acts by inactivating essential enzyme systems within microorganisms.[91] At typical use concentrations (3%), it is bacteriostatic for gram-positive cocci but has little activity against gram-negative bacteria, the tubercle bacillus, fungi, or viruses.[60, 92-95]

As early as the 1940s, tests demonstrated potential toxicity of HCP, but for many years such evidence went unnoted. In the late 1960s and early 1970s, neurologic effects were reported in patients with burns who were bathed with HCP.[96] These neurotoxic effects were verified in animal studies and in premature infants.[97-99] As a result of these findings regarding the lack of safety of a product that was then in widespread use, the FDA instituted stringent regulations for testing and approval of antiseptic agents for over-the-counter (OTC) sale.[19, 36]

HCP is not fast acting, and one wash with HCP does not reduce cutaneous flora. Its rate of killing is classified as slow to intermediate. The major advantage of HCP is its persistence.[92] Unfortunately, long-term use of HCP followed by nonuse results in a temporary rebound increase in growth of skin flora.[41] Soaps and other organic materials have little effect on the activity of HCP.[41] HCP is available as an antiseptic, by prescription only, in a 3% formulation. Use on broken skin or mucous membranes or for routine total-body bathing is contraindicated.[98]

Iodine and iodophors. Tincture of iodine has been used as a preoperative skin preparation for years. It is relatively safe and fast acting[50] but is not commonly used for handwashing. It must be removed from the skin after drying because of its potential to cause skin irritation.[13, 50]

The iodine-containing products that are used for handwashing and surgical hand scrub are the iodophors. Iodophors are complexes that consist of iodine and a carrier such as polyvinylpyrrolidone (PVP or povidone). The combination increases the solubility of iodine and provides a reservoir of iodine. The term "available iodine" indicates the extent of the reservoir, whereas "free iodine" is the amount of iodine in solution. The concentration of free iodine is the major chemical and microbicidal factor in the activity of iodophors and changes with the degree of dilution. A 10% povidone-iodine solution containing 1% available iodine will release free iodine to provide an equilibrium level of approximately 1 ppm.[100-102] Solutions with lower concentrations of iodophor may have higher relative activity because conditions favoring dissociation of iodine into solution are present.[103] Recommended levels of free iodine for antiseptics are 1 to 2 mg/L. Levels of free iodine below 1 ppm have been associated with contamination during manufacture.[101, 104] The antimicrobial effects of iodophors are similar to those of iodine and are the result of cell wall penetration, oxidation, and substitution of microbial contents with free iodine. Iodine and iodophors have a wide range of activity against gram-positive and gram-negative bacteria, the tubercle bacillus, fungi, and viruses. They also have some activity against bacterial spores.[41, 101, 105, 106] Iodophors are rapidly neutralized in the presence of organic materials such as blood or sputum.[79, 101, 107]

Iodine and, to a lesser extent, the iodophors are characterized by a propensity toward skin irritation and damage, as well as allergic or toxic effects in sensitive persons. Percutaneous and mucous membrane absorption occur, with the possible induction of hypothyroidism in newborn infants.[106, 108, 109]

The iodophor most commonly used is povidone-iodine. A formulation containing 7.5% is used as a surgical hand scrub. Lower concentration iodophors (0.05%) have been shown to have good antimicrobial activity[103, 110] because the amount of free iodine increases to some extent as the solution is diluted.[103] Other forms available for antiseptic use include 10% solution in applicators and various 2% solutions.

***Para*-chloro-*meta*-xylenol.** *Para*-chloro-*meta*-xylenol (PCMX or chloroxylenol) is a halogen-substituted xylenol that acts by microbial cell wall disruption and enzyme inactivation. It is less active than CHG and has good activity against gram-positive organisms, but it is less active against gram-negative bacteria. Its activity, especially against *Pseudomonas* species, is potentiated by the addition of ethylenediaminetetraacetic acid (EDTA) because of the binding of EDTA to metal ions in the cell wall of *Pseudomonas* species.[111, 112] It has fair activity against the tubercle bacillus, some fungi, and viruses.[47] In several in-use studies, various concentrations of PCMX have been shown to be less effective than either CHG or iodophors in reducing skin flora.[33, 113-115]

Even though PCMX penetrates the skin, the reported incidence of skin sensitization from

Table 1. Types of hand care

	Purpose	Method
Handwash	To remove soil and transient microorganisms	Soap or detergent for at least 10–15 seconds
Hand antisepsis	To remove or destroy transient microorganisms	Antimicrobial soap or detergent or alcohol-based hand-rub for at least 10–15 seconds
Surgical hand scrub	To remove or destroy transient microorganisms and reduce resident flora	Antimicrobial soap or detergent preparation with brush to achieve friction for at least 120 seconds, or alcohol-based preparation for at least 20 seconds

PCMX is low.[116] Rapidity of activity of PCMX is intermediate, and it has a persistent effect over a few hours. It is active in alkaline pH but is neutralized by nonionic surfactants. For this reason, the efficacy of PCMX, like that of CHG, is highly formula dependent. Its activity is only minimally affected by organic matter.[117] PCMX is currently available in a number of handwashing products, usually in concentrations of 0.5% to 3.75%.

Triclosan. Triclosan (5 - chloro - 2 - [2,4 - dichlorophenoxyl] phenol is a diphenyl ether. Its antimicrobial activity is thought to derive from disruption of the microbial cell wall. It is broad spectrum, with good activity against gram-positive and most gram-negative bacteria; little information is available regarding its activity against viruses, and it appears to be a poor fungicide.[114, 118] Triclosan can be absorbed through intact skin but appears to be nonallergenic and nonmutagenic with short-term use. Its speed of antibacterial effect is intermediate, it has excellent persistent activity on skin, and its activity is only minimally affected by organic matter.[36, 117,120] It has been tested in concentrations from 0.3% to 2.0%. In one study, 0.3% triclosan was less effective than 2% CHG in reducing skin flora.[33] Another study however, found 1% triclosan superior to 4% CHG in reducing methicillin-resistant *Staphylococcus aureus* colonization in neonates.[121] One study in 20 healthy volunteers reported less prolonged effects and greater skin irritation with a 2% triclosan detergent than with a 4% CHG product.[122] Although it is commonly used in commercial soaps in concentrations of up to 1% to reduce body odor by inhibiting the growth of skin bacteria over time, additional safety and efficacy data are needed to determine the usefulness of higher concentrations in health care.

Technique

Handwashing. The purpose of handwashing is to remove dirt, organic material, and transient microorganisms (Table 1).[9, 25, 123] Efficacy of handwashing is influenced by a number of factors. Although the amount of plain soap used does not appear to influence the result, antimicrobial products have a dose response, and 3 to 5 ml is recommended.[35] Wet hands with running water. Apply handwashing agent and thoroughly distribute over hands. Vigorously rub hands together for 10 to 15 seconds, generating friction on all surfaces of the hands and fingers.[1, 2, 22-24] A technique to ensure coverage of all surfaces has been described[25] because parts of the thumbs, backs of the fingers, backs of the hands, and beneath the fingernails are often missed.[124, 125] Debris may be removed from under the fingernails because the subungual area has higher microbial counts and contamination of the hands can increase when gloves provide a warm, moist environment.[126] Duration of washing is important, not only for mechanical action but also to allow antimicrobial products sufficient contact time to achieve the desired effect when they are used.[127] Hands should be thoroughly rinsed to remove residual soap and then dried. When the sink does not have foot controls or an automatic shutoff, a paper towel may be used to shut off the faucet to avoid recontaminating the hands.

A variety of methods are available for drying hands.[128] Cloth towels are rarely used in health care settings because of concerns regarding contamination.[27, 129, 130] Although warm-air dryers are used in many public rest rooms, they are rarely found in patient care areas of health care facilities. Research is scant, but one study comparing cloth towels, paper towels, and warm-air drying found that all three methods resulted in a further reduction of flora, with warm-air drying producing the greatest reduction and cloth drying the least.[131] Another study found no difference in the numbers of bacteria remaining after paper towel and air drying.[132] However, it usually takes longer to dry hands with an air dryer, and many have standard 30-second cycles that may be inad-

equate.[27,132] In addition, hand dryers can serve only one person at a time, and paper towels will not be available to use when shutting off faucets without foot or automatic controls. The noise associated with air dryers may also pose problems in patient care areas. Paper towels should be dispensed from holders that require the user to remove them one at a time. The hand-drying materials should be placed near the sink in an area that will not become contaminated by splash.

Hand antisepsis. The purpose of hand antisepsis is to destroy or remove transient microorganisms from hands (Table 1).[9, 25, 123] Hand antisepsis can occur simultaneously with handwashing when soaps or detergents that contain antiseptics are used. Hand antisepsis can also be accomplished by use of alcohol-containing antiseptic handrubs when hands are already clean; that is, not soiled with dirt or organic material.

A number of studies indicate that plain handwashing does not always remove transient microorganisms.[10, 42-45, 133] However, the concerns regarding adequacy of handwashing technique also apply to the use of alcohol handrubs. Failure to cover all surfaces of the hands because of poor technique or use of insufficient amounts of alcohol handrub solution can leave contaminated surfaces.[134] Although alcohol is often recommended for use in areas where handwashing facilities are not readily available, it is important to reiterate that they are not good cleaning agents and are not recommended in the presence of physical dirt. In addition, although small amounts of blood do not appear to adversely affect the activity of alcohol,[57] it is unclear that the same is true for larger amounts of organic material. Health care personnel working in settings where handwashing facilities are not readily available and heavy hand contamination with organic material occurs may wish to use detergent-containing towelettes for physical cleansing of the hands before use of alcohol-containing handrubs for antisepsis.

Surgical scrub. Surgical hand scrub is performed to remove transient flora and reduce resident flora for the duration of surgery in case of glove tears (Table 1).[12] The process must begin with washing the hands and forearms thoroughly to remove dirt and transient bacteria. A nail cleaner should be used to clean under the nails.

The traditional surgical scrub in the United States has been 5 minutes in duration for both the initial and subsequent scrubs, with chlorhexidine or iodophor products,[12, 40, 135-137] agents that demonstrate persistence and help maintain lower microbial counts under gloves.[138] In Europe, alcohol-based preparations are often considered the agent of choice. These preparations are applied by rubbing on 3 to 5 ml until dry and repeating applications for approximately 5 minutes.[50] With alcohol preparations, persistence may be less important because bacterial counts are so low that it takes several hours for regrowth to occur to prescrub levels.[55] AORN has recommended that persons sensitive to antimicrobial agents with residual activity should scrub with a nonmedicated soap, followed by application of an alcohol-based preparation.[12]

The optimum duration of surgical scrub is unclear,[12] although research indicates that it may be agent dependent.* Also unclear is whether scrubs for subsequent consecutive cases may be shorter than for the first case.[40] O'Shaughnessy and associates suggest that scrub time can be reduced for subsequent cases when CHG is used because of its persistent and cumulative effect,[138] whereas Rehork and Ruden suggest that the time between initial and subsequent scrubs must also be considered.[139] The American College of Surgeons suggests that a surgical scrub of 120 seconds, which includes brushing of the nail and fingertip areas, is adequate.[141]

As with personnel handwashing, the clinical impact of relative reductions in microbial counts after surgical scrubs is unknown. Although Cruse and Foord[142] initially reported that glove punctures were associated with an increased clean surgery infection rate, their later work[143] and another study[144] showed no relationship between the incidence of surgical infections and glove tears during surgery.

Other aspects of hand care and protection

Glove use. The wearing of gloves to provide a protective barrier to microbial transmission has increased dramatically since the inception of universal precautions[145-147] and has been recommended to prevent heavy contamination of hands.[148, 149] Extreme variability in the quality of gloves has been reported, however, with leakage in 4% to 63% of vinyl gloves and 3% to 52% of latex gloves.[150-154] A recent study found little benefit in double gloving when latex gloves were worn.[155] Additionally, microbial contamination of hands and possible transmission of infection have been reported even when gloves were worn.[156-159] Soap and water handwash or an antiseptic handrub should therefore be used after glove removal.

Disposable single-use gloves should not be

*References 41, 76, 80, 113, 136, and 138-140.

reused. Studies have indicated that microorganisms are not always removed from gloves despite friction, a cleansing agent, and drying,[156] and washing may decrease the integrity of the glove.[160] The Occupational Safety and Health Administration Bloodborne Pathogens Standard prohibits washing or decontaminating disposable (single-use) gloves for reuse.[161]

Inappropriate glove use has been recognized as a problem.[162-164] Failure to change gloves between patients and contaminated body sites was identified as the cause of an *Acinetobacter* outbreak.[164]

Nails, nail polish, and artificial nails. One report suggests that artificial nails may increase the microbial load on hands, particularly of gram-negative bacteria.[165] Another report showed no significant difference in colony counts between operating room nurses wearing artificial nails and those with natural nails. In two instances when the prosthetic nails were broken or had separated from the natural nails, however, high colony counts were found despite a 30-second wash with povidone-iodine.[166] Dermatologists report secondary infections with *Pseudomonas* and *Candida* when reactions to nail lacquers and hardeners cause onycholysis.[167, 168]

Nail polish applied to natural nails seems to have no detrimental influence on microbial load, as long as nails are short.[169] Short nails are probably important because the majority of flora on the hand is found under and around the fingernails.[126] Clear polish is preferable because dark colors may obscure the subungual space, reducing the likelihood of careful cleaning. In addition, long nails can make donning gloves more difficult and may cause gloves to tear more readily.

AORN recommends[12] that artificial nails not be worn by operating room personnel, citing reports of fungal and bacterial infections. Concerns have also been raised by others that use of artificial fingernails and nail polish may discourage vigorous handwashing.[124]

Jewelry. Total bacterial counts are higher when rings are worn,[170, 171] although Jacobson and coworkers did not find that rings interfered with removal of bacteria by handwashing.[170] Rings and nail jewelry can make donning gloves more difficult and may cause gloves to tear more readily.[124]

Lotion. Lotions are often recommended to ease the dryness resulting from frequent handwashing,[1, 124, 172] and more recently to prevent dermatitis resulting from glove use.[173] A study has demonstrated that the application of a lotion can reduce the dispersal of bacteria.[174] Hand lotion can become contaminated,[175-177] however, and should be considered as a possible reservoir in the event of an outbreak.

Petroleum jelly under gloves has been shown to be acceptable from a microbiologic point of view,[178] but concerns have been raised about the potential for petroleum-based lotion formulations to weaken latex gloves and cause increased permeability.[179] For that reason, lotions that contain petroleum or other oil emollients may affect the integrity of gloves. Lotions designed to protect against latex sensitivity are now on the market. Because latex allergy and anaphylactic reactions to latex products are being reported with increased frequency,[180-186] such products would be highly desirable if indicated. One study of such a lotion found no interference with effectiveness of the surgical scrub nor any increase in leakage in gloves worn for 2 hours after application of the lotion.[187] Anionic moisturizing products and surfactants, however, have been shown to interfere with the residual activity of CHG.[188, 189] Interaction between lotions and CHG antimicrobial products used must therefore be considered at the time of product selection.

Storage and dispensing of hand care products. Handwash products, including both plain soap and antiseptic products, can become contaminated or support the growth of microorganisms.[100, 104, 190-198] Bar soap should be provided in small bars that can be changed frequently, with soap racks that promote drainage.[2, 199-202] Liquid products should be stored in closed containers and dispensed from either disposable containers or containers that are washed and dried thoroughly before refilling.[1, 2] Some have suggested that dispensers should be foot- or elbow-operated to decrease the potential for contamination.[27, 203] Lotions can also become contaminated and support bacterial growth and should be dispensed in small, individual-use containers or from pump dispensers that are not opened or refilled.[175, 176]

Behavior and compliance. The primary problem with hand hygiene is not a paucity of good products, but rather the laxity of practice. Variations in handwashing practice have been reported by type of unit (pediatric personnel have been shown to have higher frequency of handwashing) and profession (in general, nurses wash more often than physicians, although physicians have been shown to wash more thoroughly).[129, 204-208] Overall, however, handwashing associated with general patient care occurs in approximately half of the instances in which it is indicated and usually

Table 2. Intervention studies to improve handwashing practices in critical care areas

Reference	Setting	Type of intervention	Results
Doebbling et al.[7]	3 ICUs, 46 beds	A. Chlorhexidine gluconate vs soap-alcohol crossover trial B. Videotaped and written instruction with refresher at midpoint C. Visual observations; monthly summary of compliance posted	Handwashing compliance was significantly better during chlorhexidine use; infection rate was reduced with chlorhexidine but not significantly
Simmons et al.[210]	Two ICUs, 24 beds, community teaching hospital	Threefold: A. Handwashing questionnaire; physician-provided compulsory in-service; supportive literature distributed B. Button campaign C. Observation of handwashing with critique of method and staff feedback	No significant changes in handwashing rates
Dubbert et al.[221]	12-bed ICU	A. Three series of classes by ICNs B. Observation of handwashing with next-day feedback to staff	A. Immediate increase in handwashing, followed by decline to baseline over 4 weeks B. Improvement to 95% compliance, with feedback sustained to end of study
Graham[223]	18-bed ICU, Australia	Introduction of handrub solution after instruction	Significant increase (13%) in frequency in hand decontamination
Conly et al.[20]	16-bed ICU, Canada	A. Feedback on handwashing practices; memoranda regarding handwashing to attending staff and departments; posters B. Infection control staff "emphasized the importance of handwashing"; results of two previous surveys reviewed; ICU directors actively encouraged handwashing	Significant increase in handwashing compliance; decrease in nosocomial infection rates
Mayer et al.[224]	ICU, VA medical center	Two phases: A. Introduction of new emollient soap B. Feedback on handwashing compliance by daily memo	A. No increase in handwashing after introduction of new soap B. Immediate increase in handwashing frequency when feedback phase began
Larson et al.[222]	6-bed postanesthesia recovery room and 15-bed neonatal ICU	Automated sink	Handwashing quality significantly improved but frequency declined significantly

ICU, Intensive care unit; *ICN*, infection control nurse; *VA*, Veterans Affairs.

is of shorter duration than recommended. Additionally, staff overestimate the frequency and quality of their handwashing behavior.[17, 125, 209-211]

A number of studies have examined the influence of various factors on handwashing behavior. Staffing,[212, 213] placement of sinks,[214] and the effect of handwashing on skin condition[215-218] have been identified as obstacles, whereas the main motivating factor has been awareness of the importance of handwashing in preventing infection.[215, 216] One study found senior British nurses were better handwashers than more junior nurses.[219]

Several studies, summarized in Table 2, have evaluated the influence of behavioral and educational interventions on handwashing practices in critical care units.[7, 210, 220-224] These interventions met with varying success, but even when handwashing increased the change in behavior was not sustained beyond the period of the study intervention. Clearly, simple educational efforts to influence handwashing practices are of minimal benefit. Studies indicate that health care personnel are aware of the reasons handwashing should be done.[215, 225] A number of studies also suggest, however, that sustained feedback on handwashing behavior or feedback about patient infections

influences performance.[210, 220, 221, 224, 226] The use of role models or mentors to influence behavior has also been suggested.[215, 225-227]

A committed and thoughtful overall approach that includes staff involvement is important. In developing such approaches, the issue of which product to use must *not* take precedence over improving the quantity and quality of handwashing.[228]

Complications of handwashing and gloving

Handwashing can cause detrimental effects on the skin.[16, 59, 172] Some of these effects occur regardless of the products used; others involve reactions to the ingredients in various handwashing agents.[72, 73, 116, 229, 230] Contrary to popular opinion, antiseptics do not necessarily cause greater damage to skin than plain soap; often it is the detergent base that is harsh.[30, 31] Recently, as glove use has increased, reports of reactions to latex gloves have also increased.[183-186, 230-233] Dermatitis in healthcare personnel may place patients at risk because handwashing will not decrease bacterial counts on dermatitic skin,[1, 234] and dermatitic skin contains high numbers of microorganisms. Health care personnel with dermatitis may be at increased risk of exposure to bloodborne pathogens during skin contact with blood or body fluids because the integrity of the skin is not present.

A variety of solutions have been proposed to remedy these problems. Use of moisturizers to alleviate skin dryness has long been recommended.[1, 124, 172] Emollients have been added to soaps. Emulsions and antiseptic "no-wash" products have been suggested as substitutes for soap and water washes.[59, 235-237] Nonlatex, powder- or chemical-free gloves are available.[184, 230, 232, 233] Use of vinyl or cotton gloves under latex gloves or barrier lotions for latex-sensitive persons has also been suggested.[173, 230, 233] Unfortunately, none of these solutions has been studied under long-term, in-use conditions to determine either efficacy in alleviating the identified problem or the impact on the microbiologic condition of the skin.

New technologies

A variety of new devices have been proposed to improve handwashing compliance and technique. In one trial, automated sinks with water flow and soap dispensing controlled by electronic sensor improved the quality of handwashing when used but were avoided by staff during busy times.[222] Handwashing machines are also available[238] but are largely untested in clinical use. Recently, gloves containing a chlorhexidine coating on the inner surface were tested.[239] Undoubtedly, other items will be developed and promoted as solutions to the poor compliance with handwashing recommendations. All will require evaluation in clinical settings to determine their effectiveness.

Areas for future investigation

Definitive studies are needed to determine whether antimicrobial soaps or antiseptic handrubs are better than plain soap in preventing infection transmission. Circumstances under which use of an antiseptic agent will consistently reduce the occurrence of nosocomial infection need to be identified. Increased glove use in recent years may require a redefinition of the resident and transient flora of gloved and ungloved hands and the effect of handwashing, with or without antiseptic agents, on this flora. The use of nail polish, artificial nails, and hand jewelry by health care providers will remain controversial until further information is available. The optimum durations for surgical personnel hand scrub for both initial and subsequent cases need to be clearly delineated for each antimicrobial agent used. As new products and new technologies emerge to combat skin irritation and sensitivity to glove material, as well as to improve handwashing technique, they will need to be carefully evaluated for efficacy, compatibility, and adverse effects. Standardization of test methods is needed to allow consumers to evaluate studies conducted in different settings with different agents. Studies should be conducted under long-term, in-use clinical conditions.

Many handwashing studies focus on products and technology. Much less attention is given to investigating methods for improving compliance of health care personnel with recommended handwashing practices. Such studies are sorely needed. ICPs need to collaborate with other disciplines to determine how to maintain lasting improvement in handwashing, a behavior considered essential for infection prevention and control.

RECOMMENDATIONS

A. Health care personnel handwashing and hand antisepsis
   1. Hands must be washed thoroughly with soap and water when visibly soiled.
   2. Hands must be cared for by handwashing with soap and water or by hand antisepsis

with alcohol-based handrubs (if hands are not visibly soiled):
   a. Before and after patient contact.
   b. After contact with a source of microorganisms (body fluids and substances, mucous membranes, nonintact skin, inanimate objects that are likely to be contaminated).
   c. After removing gloves.
3. Wet hands with running water. Apply handwashing agent and thoroughly distribute over hands. Vigorously rub hands together for 10 to 15 seconds, covering all surfaces of the hands and fingers.
4. For general patient care, a plain, nonantimicrobial soap is recommended in any convenient form (bar, leaflets, liquid, powder). Such detergent-based products may contain very low concentrations of antimicrobial agents that are used as preservatives to prevent microbial contamination. If bar soap is used, small bars that can be changed frequently and soap racks that promote drainage should be used.
5. Hand antisepsis, achieved by handwashing or surgical scrub with antimicrobial-containing soaps or detergents or by use of alcohol-based antiseptic handrubs, is recommended in the following instances:
   a. Before the performance of invasive procedures such as surgery or the placement of intravascular catheters, indwelling urinary catheters, or other invasive devices.
   b. When persistent antimicrobial activity on the hands is desired.
   c. When it is important to reduce numbers of resident skin flora in addition to transient microorganisms.
6. In settings where handwashing facilities are inadequate or inaccessible and hands are not soiled with dirt or heavily contaminated with blood or other organic material, alcohol-based handrubs are recommended for use. In situations where soilage occurs, detergent-containing towelettes should be used to cleanse the hands; alcohol-based handrubs can then be used to achieve hand antisepsis.
7. In the event of interruption of water supply, alternative agents such as detergent-containing towelettes and alcohol-based handrubs should be available.
8. Products used for handwashing, surgical scrubs, and hand care should be chosen by persons knowledgeable about the purpose of use, the advantages and disadvantages, cost, and acceptance of the product by users.
9. Routine use of hexachlorophene is not recommended.

B. Personnel hand preparation for operative procedures
   1. The procedure for surgical hand scrub should include the following steps:
      a. Wash hands and forearms thoroughly.
      b. Clean under nails with a nail cleaner.
      c. Rinse thoroughly.
      d. Apply antimicrobial agent to wet hands and forearm with friction for at least 120 seconds.
   2. If an alcohol-based preparation is selected for use, wash hands and arms, clean fingernails thoroughly, dry completely, and follow manufacturer's recommendations for application. Generally, application should last for at least 20 seconds.
   3. Personnel with allergic reactions to antiseptic agents other than alcohol may apply ethanol or isopropanol.

C. Other aspects of hand care and protection
   1. Glove use
      a. Gloves should be used as an adjunct to, not a substitute for, handwashing.
      b. Gloves should be used for hand-contaminating activities. Gloves should be removed and hands washed when such activity is completed, when the integrity of the gloves is in doubt, and between patients. Gloves may need to be changed during the care of a single patient, for example when moving from one procedure to another.
      c. Disposable gloves should be used only once and should not be washed for reuse.
      d. Gloves made of other materials should be available for personnel with sensitivity to usual glove material (such as latex).
   2. Condition of nails and hands
      a. Nails should be short enough to allow the individual to thoroughly clean underneath them and not cause glove tears.
      b. The hands, including the nails and surrounding tissue, should be inflammation free.
   3. Lotion
      a. Lotions may be used to prevent skin dryness associated with handwashing.
      b. If used, lotion should be supplied in

small, individual-use or pump dispenser containers that are not refilled.

c. Compatibility between lotion and antiseptic products and the effect of petroleum or other oil emollients on the integrity of gloves should be considered at the time of product selection.

4. Storage and dispensing of hand care products
   a. Liquid products should be stored in closed containers.
   b. Disposable containers should be used. If disposable containers cannot be used, routine maintenance schedules for cleaning and refilling should be followed. Reusable containers should be thoroughly washed and dried before refilling.
   c. There should be a routine mechanism to ensure that soap and towel dispensers function properly and are adequately supplied.
   d. Containers of alcohol should be stored in cabinets or areas approved for flammables.
5. Drying of hands
   a. Cloth towels, hanging or roll type, are not recommended for use in health care facilities.
   b. Paper towels or hand blowers should be within easy reach of the sink but beyond splash contamination.
   c. Lever-operated towel dispensers should be activated before beginning handwashing. Hand blowers should be activated with the elbow.
6. Behavior and compliance. Efforts to improve handwashing practice should be multifaceted and should include continuing education and feedback to staff on behavior or infection surveillance data. Unit clinical and administrative staff should be involved in the planning and implementation of strategies to improve compliance and handwashing.

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Original Contributions

EFFECTIVE HAND DEGERMING IN THE PRESENCE OF BLOOD

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☐ **Abstract — This study evaluated the effect of blood on the antimicrobial activity of several agents used for handwashing. Seventy-one healthy adult volunteers used 1 of 6 products (70% isopropyl alcohol [IPA]; liquid hand rinse containing 70% ethyl alcohol and 0.5% chlorhexidine gluconate [EA]; detergent containing 7.5% povidone-iodine [PI]; detergent containing 4% chlorhexidine gluconate [CHG]; a nonantimicrobial soap; and a control group that used no product) in two tests: with and without 1.2 mL of dried sterile sheeps' blood on the hands. In the presence of blood, the two alcohol products (IPA and EA) resulted in significantly greater reductions in numbers of colony-forming units than other products ($P < 0.001$). When no blood was present, IPA was associated with significantly greater reductions, whereas soap and control groups had significantly lower reductions ($P = 0.008$). We conclude that hands are effectively degermed with a variety of products in the presence of blood, and that alcohols give greater initial reductions in colonizing flora. This is of particular relevance in emergency situations during which contamination with blood is likely and handwashing facilities are inaccessible.**

☐ **Keywords — handwashing; antisepsis**

INTRODUCTION

Attention to the effectiveness of topical antimicrobial products in the presence of organic material such as blood is important for several reasons. While there is increasing awareness of the importance of blood and body fluids in the transmission of important pathogens such as the human immunodeficiency virus and hepatitis B, there is at the same time increasing evidence of the limitations of gloves as barriers to both viruses and bacteria (1–5). Based on such evidence, the Occupational Safety and Health Administration recently published proposed rules addressing universal precautions and barrier techniques to minimize occupational exposure to bloodborne pathogens (6). In addition, bare-skin contact with blood or fluids by health care providers is most likely to occur outside the acute care setting, when both gloves and adequate handwashing facilities are unavailable. A recommended alternative to traditional handwashing in settings such as ambulances, emergency medical services, fire stations, day care centers, blood banks, and clinics has been the use of alcohol-based products in the form of foams, rinses, or impregnated papers, since these products eliminate the necessity for running water and towels. On the other hand, alcohols act by the denaturation of protein, and concerns about their activity in the presence of organic material are legitimate. The purpose of this study was to characterize the effect of blood on the antimicrobial activity of several products commonly used for hand degerming.

METHODS

Seventy-two healthy adult volunteers without history of allergies or sensitivity to soaps or detergents, eczema, psoriasis, or other skin diseases were recruited primarily from among staff and students at the study institution. Subjects were required to have intact skin and not be receiving systemic antibiotic therapy within 30 days prior to testing. All subjects signed written consent

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forms and were paid for their participation. Rings were not worn during testing.

Subjects were assigned by block randomization to 1 of 6 products: (a) 70% isopropyl alcohol (IPA); (b) a liquid hand rinse containing 70% ethyl alcohol and 0.5% chlorhexidine gluconate (EA); (c) a liquid detergent containing 7.5% povidone iodine (PI); (d) a liquid detergent-based handwashing product containing 4% chlorhexidine gluconate (CHG); (e) a nonantimicrobial liquid soap (soap); and (f) a control group that used no degerming product. These were selected because they are typical of products commercially available. The alcohol-based products (IPA and EA) do not require a water rinse, whereas detergent-based products (PI, CHG, soap) must be removed with water.

Two separate tests were conducted with each subject. On day one, 1.2 mL of sterile sheep blood was dispensed by sterile syringe into the cupped hands of each subject. Sheeps' blood was used because it was readily available commercially. It was then rubbed over the entire surface of both hands and air-dried for 2 min. Following the application of blood, subjects performed a standardized 15-second hand cleansing using their assigned test product. Three days later, the same protocol was followed, except that blood was not applied to the hands. Since colonizing flora are considerably more difficult to remove from hands than are contaminating or artificially inoculated organisms, this protocol was designed to maximize the potential effect, if there was any, of the presence of blood by assessing the impact on colonizing flora.

The handwashing procedure was as follows. For the alcohol-based products, 5 mL of the test product, measured in a syringe, was dispensed into the palms of the subjects' hands and rubbed thoroughly over their entire surface for 15 seconds by the subject; the hands were then air dried. No rinsing or towel drying was used. For the detergent-based products, subjects first rinsed their hands in warm (approximately 40 °C) running tap water. Five milliliters of the test product, measured in a syringe, was dispensed into the palms, and hands were massaged thoroughly by the subject, with special attention to all surfaces, for 15 seconds. Subjects then rinsed their hands thoroughly with warm running water and dried with a clean paper towel. The control group applied no test product between baseline and pretreatment samplings.

Hands were cultured at 4 intervals: before and after application of the test product, with or without blood on the hands. Prior to baseline sampling, subjects performed a brief handwash with nonantimicrobial liquid soap to remove dirt and transient contaminants. To obtain samples, the subject inserted the dominant hand into a sterile polyethylene bag containing 50 mL of sampling solution (sterile distilled water containing [per liter] lecithin, 20 g; sodium thiosulfate, 6 g; sodium oleate, 6 g; protease peptone, 1 g; tryptone, 1 g; and Tween 80, 50 mL, pH 7.2 to 7.4) (7,8). The surface of the hand was rubbed vigorously through the wall of the bag for 1 min. All specimens were plated within 1 h of sampling. A 0.2-mL volume of each serial dilution up to 10^{-3} was plated on trypticase soy agar (Becton Dickinson, Cockeysville, MD) containing yeast extract (5 g/L) and Tween 80 (1 mL/L), and incubated aerobically at 37 °C for 48 h. Colony-forming units (CFU) were counted and reported as total counts per hand in log base$_{10}$ CFU.

Student's t test was used to assess the significance of differences between $\log_{10}$ reductions in CFU on each subject's hands in the presence and absence of blood. Analysis of variance was used to evaluate differences in mean $\log_{10}$ counts between product group at each of the 4 sampling times (baselines on both days, and following handwashing with and without blood on hands). A probability of $P < 0.05$ was considered statistically significant.

RESULTS

The final sample size was 12 subjects/product group, except IPA, which had 11 subjects; one subject failed to return for the second day of testing. Fifty-four subjects (76%) were female, ages ranged from 17 to 50 years, and 57 subjects (80.2%) were Caucasian. The overall mean $\log_{10}$ CFU count at baseline on day 1 of testing was 5.68 (standard deviation ± 0.57). Log_{10} reductions in counts following handwashing with blood on the hands ranged from −.17 for the control group to −.90 for the group using EA. Reductions in counts following handwashing without blood on the hands ranged from +.07 log (an increase in CFU after washing) for soap to −.87 for IPA (Table 1). Differences in $\log_{10}$ reductions were not significantly different with or without blood on the hands for the following groups: IPA ($P = 0.98$); PI ($P = 0.81$); CHG ($P = 0.81$); and control ($P = 0.41$). For subjects using EA and soap, there were significantly greater reductions in CFU with blood on hands, as compared to without ($P = 0.001$ and .007, respectively). Mean $\log_{10}$ counts among subjects in the 6 product groups were not significantly different at baseline on either test day ($P = 0.21$ and .08, respectively), but posttreatment differences were significant. In the presence of blood, the two alcohol products (IPA and EA) resulted in significantly greater reductions than did the other products ($P < 0.001$). When no blood was present, IPA was associated with significantly greater reductions, whereas the

Table 1. Mean Log_{10} Counts on Hands by Handwashing Product, with and without Blood on the Hands (n = 12 subjects per product)

	Mean Log Counts (± standard deviation)					
	With Blood			Without Blood		
Handwashing product	Before wash	After wash	Before–After Log change	Before wash	After wash	Before-After Log change
70% isopropyl alcohol	5.83 (.44)	4.97 (.66)	−.86*	5.91 (.58)	5.04 (.72)	−.87
70% ethyl alcohol/0.5% chlorhexidine gluconate	5.52 (.66)	4.62 (.85)	−.90*	5.44 (.43)	5.06 (.45)	−.38†
Detergent with 7.5% povidone-iodine	5.76 (.59)	5.50 (.72)	−.26	5.52 (.46)	5.31 (.44)	−.21
Detergent with 4% chlorhexidine gluconate	5.54 (.76)	4.89 (.57)	−.65	5.32 (.49)	4.75 (.63)	−.57
Non-antimicrobial liquid soap	5.97 (.36)	5.65 (.34)	−.32	5.68 (.50)	5.75 (.48)	+.07†
No soap (control)	5.49 (.43)	5.32 (.46)	−.17	5.44 (.49)	5.34 (.42)	−.10

*Significantly greater reductions than with other products ($P < 0.001$).
†Significantly greater reductions with blood on hands than without blood ($P < 0.01$).

soap and control groups had significantly lower reductions in colonizing flora than other groups ($P = 0.008$).

Although the alcohols were microbiologically effective, blood was not physically removed from the hands of subjects using IPA or EA. Small visible "clumps" formed on hands and it was necessary for all subjects in alcohol groups to wash their hands with soap following the testing protocol to remove visible blood.

DISCUSSION

Ulrich (9) reviewed the literature on in vitro testing of topical antiseptics in the presence of blood, serum, pus, sebum, triglycerides, surface active agents, and carbohydrates. He reported that antimicrobial activity of iodophors was most affected by organic matter, with blood being associated with the greatest reduction in activity. Quaternary ammonium compounds, CHG, and phenols were considerably less affected, but alcohols were not reviewed. Lowbury and Lilly (10) examined the effect of blood on hand hygiene in 6 volunteers by applying 2 mL of blood after a 2-min hand scrub, followed by gloving for 1 hour. The povidone-iodine product resulted in a significantly less effective microbicidal activity in the presence of blood as compared to control, whereas CHG and soap products were unaffected by the blood.

Results from in vitro studies of the activity of alcohols in the presence of organic material are inconsistent. Some researchers have reported the presence of continued activity of alcohol in the presence of organic matter (11,12), while others have noted reduced effectiveness (13,14). Wallbank (15) stated that 80% ethanol was neutralized by rabbit blood in his laboratory, but study methods were not described. Molinari and colleagues (16) reported that IPA was ineffective in removing saliva coating and blood/bacterial suspensions from several hard surfaces, whereas both were effectively removed by an iodophor. These studies have varied in design and with regard to important variables such as contact time and method of product application. For example, it has been demonstrated that bacteria in a dry environment are more resistant to the killing action of alcohols than in a moist environment (17,18), and drying time varied across studies. Thus, it may not be appropriate to draw conclusions from in vitro testing with regard to antimicrobial activity on the skin surface.

In contrast to results from in vitro studies, results from clinical studies of skin degerming are very consistent. Levels of reductions in flora and the rank ordering of product activity in this study in the absence of blood on the hands were in agreement with results from other studies of the same active ingredients. A group of British investigators demonstrated that alcohol-based products generally performed better initially, but that detergent formulations containing CHG and PI were equivalent to the alcohols after several applications (19–22). These results have been confirmed by investigators in Europe (23–26) and the United States (27–30). However, none of these studies evaluated antimicrobial activity in the presence of blood.

It is not surprising that topical antimicrobial agents would act differently on skin than on hard surfaces. Ethyl alcohol has the capability of altering the stratum

corneum to enhance its permeability and facilitate penetration of the skin (31). This may explain why scrubbed hands immersed for 1 min in 77% (v/v) ethyl alcohol have resulted in microbial reductions equivalent to those associated with 6.5 min of scrubbing with water (32). Brown and Hendley (33) recently reported evidence that the reservoir for normal skin flora is located below the stratum corneum, presumably in the sebaceous gland ducts. They further demonstrated that 70% ethyl alcohol eliminated bacteria not only from the surface of the skin, but from 15 layers of stratum corneum. Further, Mahl (34) reported that IPA and ethanol, unlike other products containing CHG or an iodophor, were effective in decreasing numbers of bacteria in areas around and under the fingernails which are laden with organic materials and difficult to reach.

The above evidence is consistent with our findings that a number of topical antimicrobial products continue to show activity even in the presence of blood. This is particularly reassuring for the alcohol products, since they are most likely to be used in emergency situations outside a hospital setting (for example, at an accident scene) when blood cannot be washed off the hands prior to degerming. Even in the emergency room where protective apparel to prevent blood contact is usually readily available, inadvertent contact with blood or other potentially contaminated organic matter such as feces and sputum is a frequent occurrence, through glove leaks, blood spills, unexpected hemorrhage, or when working with a combative patient. For this reason, it is vital that skin degerming agents remain active in the presence of proteinaceous body substances. Several caveats deserve mention. First, alcohol-based products cannot substitute for detergent solutions, since alcohols do not physically remove the blood, despite the fact that the blood does not seem to interfere with degerming action. Second, further research is indicated in which the antimicrobial activity of these products is measured against transient flora, particularly viruses, in a mixture of blood.

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Alcohol for Surgical Scrubbing?

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ABSTRACT

To test the effects of four surgical scrub products on colonizing hand flora, 60 healthy adult volunteers were assigned by block randomization (12 subjects per group) to use one of the following formulations: 70% ethyl alcohol with 0.5% chlorhexidine gluconate (ALC); a liquid detergent base containing 1% triclosan (TRI); a liquid detergent base containing 4% chlorhexidine gluconate (CHG); a liquid detergent base containing 7.5% povidone-iodine (PI); or a nonantimicrobial liquid soap (control). Using standard protocol, subjects performed a surgical scrub daily for five consecutive days. Hand cultures were obtained at baseline and on test days 1 and 5 immediately after the scrub and following four hours of gloving. After the first and last scrubs, ALC, CHG and PI resulted in significant reductions in colonizing flora when compared to the control. Additionally, by day 5 ALC was associated with an almost 3-log reduction as compared to an approximate 1.5-log reduction for CHG and PI and less than a 1-log reduction of TRI and the control ($p = .009$). After four hours of gloving on both days 1 and 5, microbial counts on hands of subjects using ALC, TRI and CHG were significantly lower than counts for the control ($p < .001$), whereas there was no significant difference in counts between the PI and control groups ($p = .41$). Skin assessment by study subjects [illegible] from least to most harsh as f[illegible] TRI, CHG, ALC and PI $p =$ [illegible] concluded that ALC could be [illegible] and acceptable alternative for surgical scrubbing. (*Infect Control Hosp Epidemiol.* 1990;11:139-143.)

INTRODUCTION

Healthcare personnel use three types of hand cleansing products: plain soap for removal of transient contaminants; healthcare personnel hand wash containing an antimicrobial product; and the antiseptic surgical scrub. Prior to operating, scrub team members attempt to remove not only extraneous organisms (transient flora) but also to minimize numbers of their own skin organisms (resident flora)[1] with a standardized scrub regimen, usually five minutes in duration. Repeated use of a surgical scrub product should result in a 2-log reduction in resident flora on the hands.[2]

Several antimicrobial ingredients for surgical scrub are available. Two important characteristics of such ingredients are the reduction of microbial counts on the hands and the binding of antiseptic to the stratum corneum, resulting in its sustained release. Alcohols and iodophors such as povidone-iodine (PI) rapidly reduce transient and colonizing flora but have little or no residual effect on the skin.[2-10] Chlorhexidine gluconate (CHG) and triclosan (TRI) are slower acting ingredients as compared to alcohols, yet bind to the stratum corneum.[3,5,11-14] Over time, use of these antiseptics can result in a sustained reduction in both transient and resident organisms on the skin. This characteristic may be important in clinical settings such as neonatal or adult critical care units where patients are at high risk for infection or when gloves are worn for sustained periods of time, such as in surgery.

Evaluating the efficacy of a topical antimicrobial product usually includes four stages.[15,16] First, in

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Table 1
Scrub Procedure

Area of Hand	Portion of Sponge/Brush	Timing
1. Fingernails	Sterile nail stick	Not timed
2. Subungual spaces and nail	Bristles	30 sec. each hand
3. Interdigital spaces	Sponge	30 sec. each hand
4. Palms	Sponge	30 sec. each hand
5. Dorsa	Sponge	30 sec. each hand
6. ⅔ of forearm	Sponge	30 sec. each hand

vitro and animal studies are conducted to examine the safety and antimicrobial spectrum. Second, the effect of a product on removal of transient flora is estimated by artificial inoculation of organisms onto skin and application of the antiseptic test agent. Third, controlled studies of the efficacy of the product in effecting a sustained reduction in normal colonizing skin flora are conducted. Finally, tests in the clinical setting should examine the efficacy of the agent in lowering nosocomial infection rates. Unfortunately, this final stage is often impossible or impractical because of the necessity of huge sample sizes, the presence of numerous confounding factors and the prohibitive cost. The purpose of this study (stage three above) was to compare the immediate and sustained antimicrobial effectiveness and user acceptability of surgical scrub preparations containing either alcohol, TRI, CHG or PI.

METHODS

Subject Selection

A convenience sample of 60 healthy adult volunteers was selected from a pool of volunteers, aged 18 to 65 years, who were not receiving systemic or topical antibiotics and who reported no history of skin disease or sensitivity to soaps. Using block randomization to assure equal size groups, subjects were assigned to one of five products: 70% ethyl alcohol with 0.5% CHG; a liquid detergent base containing 1% TRI; a liquid detergent base containing 4% CHG; a liquid detergent base containing 7.5% PI (Betadine, Purdue-Frederick, Norwalk, Connecticut); and nonantimicrobial liquid control soap.

Subject Training

Three days prior to the testing period all subjects were provided with a liquid nonantimicrobial soap (ProCare, Sani-Fresh International, San Antonio, Texas) and instructed to use this soap for general bathing and handwashing during a three-day weaning period and throughout the entire five-day test period. Vinyl disposable gloves were provided to the volunteers for use during shampooing, dishwashing or hand contact with other soaps, lotions or cleaning products during the weaning and test periods. Nail polish, rings and use of hand lotions were prohibited during the study, and subjects were requested to clip their fingernails to a length no longer than approximately 2 mm. After each volunteer was assigned to one of the five treatment groups, he or she was instructed in a standardized surgical scrub procedure[15] and practiced the technique until competence was demonstrated in the presence of an investigator.

Surgical Scrub Technique

The standard surgical scrub technique as defined by the American Society for Testing and Materials was followed. All jewelry and fingernail polish was removed for the entire study, subjects cleaned under their fingernails with a sterile nail stick, subjects applied the specified amount of the test formulation into the palm and kept their hands higher than their elbows during the procedure, and using a prepackaged sterile scrub sponge (Pre-op II Surgical Scrub Sponge/Brush, Davis & Geck, Danbury, Connecticut), hands were scrubbed in a systematic fashion (Table I) for five minutes.

Subjects using any of the four liquid detergent-based products rinsed their hands and arms in warm running water (38°C to 42°C) at the beginning and at the end of the five-minute procedure, but hands and arms were kept out of the running water during the scrub to avoid rinsing off the test product. Five milliliters was applied over the entire surface of the hands and two thirds of the forearms prior to cleaning the interdigital spaces, and a second 5 ml was applied prior to cleaning the palms. For the alcohol group, no water was used during the scrub procedure, and hands and forearms were kept wet with alcohol by applying 5 ml over their entire surface before each of the six steps listed in Table 1 (a total of 30 ml was used for the entire procedure).

Subjects performed the scrub protocol once daily for five consecutive days. All scrubs were performed in the laboratory under the supervision of an investigator. All steps of the procedure were timed with a stop watch and amounts of test product were premeasured in a syringe.

Microbiologic Sampling

Microbiologic sampling of flora from the hands was conducted five times: immediately prior to the study after a brief wash with control soap (baseline); immediately after the scrubs on days 1 and 5; and four hours after the scrubs on days 1 and 5 to measure residual activity. To test for residual activity on days 1 and 5, a sterile glove was placed on one hand of each subject immediately after the surgical scrub and worn for four hours. The subject then returned to the laboratory for final sampling.

Microorganisms on the hands were assayed using a modification of the glove juice tech-

nique.[16,17] Subjects inserted their dominant hand into a sterile polyethylene bag (Steri-Lok, 3M, St. Paul, Minnesota) containing 50 ml of sterile sampling solution (distilled water containing, per liter: lecithin, 20 g; sodium thiosulfate, 6 g; sodium oleate, 6 g; protease peptone, 1 g; tryptone, 1 g; and Tween 80, 50 ml, pH: 7.2 to 7.4).

The entire hand surface was rubbed vigorously through the wall of the bag for one minute by an investigator. After the rubbing, a 0.2 ml volume of sampling fluid was aseptically removed and plated within two hours of sampling. Serial dilutions up to 10^{-3} were placed on trypticase soy agar (BBL Microbiology Systems, Cockeysville, Maryland) containing yeast extract, 5 g/l and Tween 80, 1 ml/l. Plates were incubated at 37°C aerobically for 48 hours and at room temperature for 24 hours. Colony forming units (CFU) were then counted.

Product Acceptability

Two measures were used to assess user acceptability of the test products. First, a subjective instrument was completed by each subject to assess the skin on his or her hands at baseline and again at the end of the study (day 5). This instrument was composed of an ordinal, seven-point Likert scale, with a range of scores from 4 (lowest) to 28 (highest) rating the skin for appearance, sensation, intactness and moisture content. In previous studies, scores have been highly correlated with physiological measures of skin damage. Additionally, subjects were able to identify changes in their skin much earlier than dermatologists.[4,18]

Second, a commercially available sampling disc (D-SQUAME Skin Surface Sampling Discs, CuDerm Corporation, Dallas, Texas) was used to quantitate the extent of desquamation of the stratum corneum. The clear adhesive disc was placed on the dorsum of the hand between the second and third metacarpal space, removed and then fixed to a storage card with a black background. Shedding skin cells were removed by the disc and could be visualized as varying shades of white against the black background. Each storage card was imprinted with a four-level dry skin reference pattern. Subjects' hands were sampled at baseline and at the end of the five-day test period, and discs were read independently by three reviewers who were blinded to subject group assignment. A score of 1 to 4 (with 4 being the most skin shedding) was assigned, and changes in scores for each subject from baseline to post-scrub were calculated.

Data Analysis

Bacterial counts were calculated to reflect the total number harvested from the hand and then converted to log $base_{10}$. Analysis of variance (ANOVA) was used to compare log_{10} counts between the five treatment groups at baseline, after scrubbing and after four hours of gloving on days 1 and 5. To test the significance of differences in subject assessment of their skin, the day 5 scores were subtracted from baseline scores to calculate the pre-to-post change in score. A negative change score indicated that subjects felt their skin to be worse after the testing period than at the beginning; a positive score indicated that the subjective rating of their skin improved after the 5-day scrub period. The Kruskal Wallis ANOVA was used to compare mean pre-to-post changes in subject assessment scores between the five groups.

Interobserver agreement between the three independent reviewers of the desquamation discs was calculated using the intraclass coefficient formula. Two types of generalizability coefficients were calculated: generalizability of a single observation and generalizability of the average of K observations. The single generalizability coefficient represented an estimate of the average agreement between one observer and another. For K observations, an estimate of agreement was calculated for, the single random sample of reviewers and a theoretical set of other random samples drawn from the same universe.[19]

RESULTS

Sixty subjects, 53 (88%) females and seven (12%) males, completed the study. Ages of subjects ranged from 18 to 59 years, and the majority were employees of the study institution. There were 45 (75%) Caucasian and 15 black subjects. The mean log CFU counts from the hands of the 12 subjects in each treatment group at baseline ranged from 5.80 to 6.18 ($p = .39$). After the first scrub, day 1 mean log reductions for each treatment group were: alcohol, 1.98 (standard deviation ±1.20); TRI, 0.56 (±0.58); CHG, 0.86 (±0.95); PI, 1.08 (±0.47); and control, 0.39 (±.42). Reductions among subjects using alcohol, CHG and PI were significantly greater than among subjects using control soap (all $p<01$). Reductions among subjects using TRI were not significantly different from controls ($p = .07$). After the final scrub on day 5, mean log reductions were: alcohol, 2.85 (±0.72); TRI, 0.56 (±0.54); CHG, 1.56 (±.63); PI, 1.57 (±0.49); and control, 0.42 (±0.46). Again, all the antimicrobial-containing products except TRI resulted in significantly greater reductions when compared with control soap (alcohol, $p<.0001$; TRI, $p = .08$; CHG, $p = .0004$; PI, $p = .0003$). Additionally, alcohol was associated with significantly greater reductions than the other products ($p = .009$).

After four hours of gloving on day 1, mean log CFU counts were increased slightly from those immediately following the scrub in all groups. None, however, reached or exceeded the baseline (prescrub) levels. Log increases (i.e., bacterial regrowth) four hours after scrubbing an day 1 were: alcohol, +.44; TRI, +.11; CHG, +.27; PI, −.81; and control, −.38. Four hours after scrubbing,

Table 2
Mean Log Count (± Standard Deviation) From Hands of 60 Subjects Using Surgical Scrub Protocol

Active Ingredient	Baseline	After Scrub 1	After 4 Hrs. Gloving, Day 1	After Scrub 5	After 4 Hrs. Gloving, Day 5
Alcohol	6.04 (0.49)	4.06 (1.20)	4.83 (1.10)	3.19 (0.72)	3.62 (1.34)
Triclosan	5.84 (0.67)	5.28 (0.58)	5.39 (0.96)	5.28 (0.54)	5.69 (0.44)
Chlorhexidine gluconate	5.80 (0.58)	4.94 (0.95)	5.21 (1.10)	4.24 (0.63)	4.04 (1.11)
Povidone-iodine	6.18 (0.39)	5.10 (0.47)	5.91 (0.46)	4.61 (0.49)	5.68 (0.31)
Control	6.07 (0.57)	5.68 (0.42)	6.06 (0.47)	5.65 (0.46)	6.29 (0.51)
ANOVA comparing the five treatment groups at each testing interval	F:104 p .39	6.89 <.001	4.13 <.001	32.9 <.0001	23.3 <.0001

mean CFU counts were significantly lower for each antiseptic when compared to controls (all $p<.03$) except for the PI group, which did not differ significantly from control soap ($p=.41$). On day 5, log changes four hours after scrubbing as compared to immediately post-scrub were: alcohol, +.43; TRI, +.41; CHG, −.20; PI, +1.07; and control, +.64. After four hours of gloving on day 5, mean log CFU counts were significantly lower for the four antiseptic groups when compared with controls ($p<.0001$) (Table 2).

Subjective skin assessments, rating the condition of skin from baseline to post-scrub, were: alcohol, −.92; TRI, +.42; CHG, +.17; PI, −1.7; and control, +1.3 ($p=.00001$). Estimates of interobserver agreement among the three raters with the desquamation discs was .69 on postscrub samples. Pre-to-post changes in ratings made by these raters and the subjects themselves did not differ significantly (chi-square, 3.83; $p=.07$).

DISCUSSION

Until the early 1970s, hexachlorophene-based detergent was widely used for surgical hand scrubbing and for preoperative patient skin preparation in the United States. Hexachlorophene, however, has a narrow spectrum of antimicrobial activity, is very slow acting and is associated with rare but serious toxicity.[20] Therefore, it has been essentially replaced by iodophors and, to a lesser extent, by products containing ingredients such as CHG, TRI or para-chloro-meta-xylenol (PCMX).

Iodophors in detergent base have been one of the primary agents used for surgical hand scrub because of their rapid activity. However, they have several disadvantages. The chemistry of iodine complexes is not fully understood.[21] Iodophors are known to be harsh on the skin[18,22] and have slightly more risk of toxicity[10,23,24] when compared with other antiseptics, and they rapidly lose activity in the presence of organic matter such as serum.[25,26] Additionally, the aqueous solutions that are most effective because of higher levels of free iodine are associated with higher levels of skin sensitivity.[27]

CHG is slower acting than iodophors, but has been recommended as an alternative for surgical skin preparation because of its broad antimicrobial spectrum, residual activity, comparative mildness to skin and the fact that its activity is not significantly affected for organic matter.[28-30] TRI is also slow acting and has been shown to have less antimicrobial effect, but is recommended by some investigators primarily because of its marked residual activity on the skin.[12,31] All three of these active ingredients (iodophors, CHG and TRI) are currently available in this country in a number of commercial formulations as surgical hand scrubs.

In some European countries, state-of-the-art practice in regard to preoperative skin preparation differs from that in the United States. Germany and Austria, for example, require that an alcoholic preparation be used for surgical hand scrub. Indeed, 60% to 90% n-propanol, isopropanol and ethanol have been repeatedly demonstrated in European and American studies to have superior antimicrobial activity when compared to detergent-based preparations.[3,4,7,25,31-33] Despite the demonstrated superiority of alcoholic solutions, they are not widely used for scrubbing in the United States, perhaps because of problems such as flammability and skin defatting, resulting in drying.

This study is one of the first conducted in the United States for several decades that has compared an alcohol solution with other agents for surgical hand scrub. Results are quite consistent with European findings. The alcohol-based product

was superior in regard to antimicrobial efficacy after a single application, and that superiority remained after five days of use. The residual activity of TRI and CHG was demonstrated four hours after scrubbing on days 1 and 5 of testing. Despite this, the mean log counts four hours after scrubbing were still lower among subjects using the alcohol product. Residual effect, therefore, would seem to be of less importance than the initial reduction in bacterial counts from the scrub procedure.[34] Daily scrub with nonantimicrobial soap had minimal effect on the numbers of colonizing flora harvested from the skin surface. This is consistent with findings of others[35,36] and can be explained by increased shedding of skin squames containing viable bacteria associated with mechanical scrubbing.[37]

As expected, the nonantimicrobial control soap was rated by subjects as the mildest. Indeed, subjects using that product actually rated the condition of their hands as significantly better after five days of scrubbing. Subjects using TRI and CHG also had better post-scrub than baseline ratings. Consistent with what others have demonstrated, the iodophor was rated as the harshest. The alcohol was rated as less harsh than the iodophor but not as mild as the other test products.

The fact that subject assessments of changes in their skin condition after use of the five test products were consistent with similar assessments from previous studies[4,11,18,38] lends credence to this subjective rating scale as a reliable indicator of product acceptability. This was not the case, however, for the desquamation disc. Clearly, additional reliability and validity data are needed to evaluate the desquamation disc as a test methodology.

Alcohols are used rarely in the United States for surgical scrub. Deterrents to more widespread use seem to relate primarily to their flammability and their drying effect on skin. Enclosed dispensing systems (rather than open bowls) and complete air drying after application can minimize the problem of flammability. The addition of emollients to newer alcoholic formulations seems to minimize the drying effects. Therefore, we recommend the consideration of alcoholic preparations as an efficacious alternative for surgical hand preparation.

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CHG, (OH)

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24

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Efficacy of Alcohol-Based Hand Rinses under Frequent-Use Conditions

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Fifty volunteers randomly assigned to one of five hand washing agents (10 subjects per agent)—a nonantiseptic liquid soap (control), an antiseptic hand rinse containing 60% isopropyl alcohol (IPA) with emollients (Alc A), an antiseptic hand rinse containing 70% IPA and 0.5% chlorhexidine gluconate with emollients (Alc B), an antiseptic containing 4% chlorhexidine gluconate and 4% IPA (CHG), and 70% IPA—washed their hands 15 times per day for 5 days under supervision by using a standardized technique and measured amounts of test agent. Microbiologic samples of hand flora were obtained at base line and after hand washes 1 and 15 on test days 1 and 5. After the initial hand wash there were significant reductions over base line in aerobic and anaerobic log CFU among those using Alc A, CHG, and IPA. By the end of the first day of hand washing (15 washes), there were 2-log or greater reductions in aerobic counts among subjects using all antiseptics, but no significant reductions in controls. By the end of day 5, all agents produced signif reductions in aerobic ($P = 0.0002$) and anaerobic ($P = 0.002$) counts over control soap. Subj effects of hand washing on the skin and overall satisfaction varied significantly b respectively). We conclude that alcohol-based hand rinses are highly effica recommended as a health care personnel hand wash, particularly when inaccessible.

The testing of health care personnel hand washing products occurs in several stages. In the laboratory, initial safety and efficacy data are obtained in vitro and then in vivo with volunteers. In vivo testing should include an evaluation of the effect of the product on selected stains of gram-positive and -negative bacteria after artificial inoculation onto the skin and an evaluation of the initial effects of the product on colonizing flora after a few hand washes, as well as an assessment of the sustained effect after frequent use of the product over an extended period. Recently, several alcohol-based hand washing products designed for use without water have become commercially available and are recommended by some as being at least as, if not more, effective than the more traditional agents used with sink and running water (3; B. Nystrom, Letter, Infect. Control [Thorofare] 5:211, 1984). Use of such agents has been shown to result in significant log reductions in normal flora of the hands when compared with other antiseptics in single-use trials (A. Morrison, J. Gratz, I. Cabezudo, and R. P. Wenzel, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 930, 1985). They have not been evaluated, however, under frequent-use conditions such as those practiced in the health care setting. In this paper, we report the results of in vivo tests of the antimicrobial efficacy of five hand washing products after extended use, 15 hand washes per day for 5 consecutive days.

MATERIALS AND METHODS

Subject selection and training. A convenience sample of subjects was selected from a pool of healthy adults, primarily employees and students in the study institution, who had no history of allergies, psoriasis, eczema, other skin diseases, or sensitivity to soap. All subjects gave informed consent and were remunerated for their participation.

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Three days before the beginning of the testing period, subjects were provided with a mild, nonmedicated liquid soap (Safe 'n Sure; Calgon Corp., St. Louis, Mo.) for general bathing and hand washing and with disposable plastic gloves for use during shampooing, dishwashing, or hand contact with other soaps or lotions. They were instructed to use only this soap and to refrain from use of lotions or moisturizers for the 3-day weaning period and the entire test period (5 days). Subjects were instructed in a standardized 15-s wash technique. Each subject practiced this technique in the presence of an investigator until competence was demonstrated. Rings and nail polish were not worn during the study.

Hand washing regimen. Subjects were randomly assigned to one of five treatment groups: (i) liquid, nonmedicated soap (Safe 'n Sure; Calgon), (control), (ii) an antibacterial hand rinse containing 60% isopropyl alcohol in emollients (Cal Stat; Calgon), (Alc A), (iii) an antibacterial hand rinse containing 70% isopropyl alcohol and 0.3% (wt/wt) chlorhexidine gluconate in emollients (Hibistat; Stuart Pharmaceuticals, Wilmington, Del.) (Alc B), (iv) an antibacterial detergent containing 4% (wt/vol) chlorhexidine gluconate and 4% isopropyl alcohol (Hibiclens; Stuart) (CHG), and (v) 70% (wt/vol) isopropyl alcohol (IPA).

Samples from the skin of the hands were obtained for bacterial culture at five intervals: before the first hand washing (base line), after the first and last hand wash on the first testing day, and after the first and last hand wash on the last testing day. The hands were rinsed thoroughly in running water before sampling. For 5 days, subjects washed their hands according to a standard protocol (15 sec each wash) 15 times per day by using a syringe to measure 5 ml per hand wash (2 ml per hand wash for IPA). A lesser amount of IPA was used to reduce the risk of skin damage as a result of exposure to alcohol without emollients. All washings were performed with the prescribed agent according to the directions of the manufacturer under supervision

TABLE 1. CFUs from hands in five treatment groups

Type of bacteria	Treatment group	Base line	$\log_{10}$ CFU (% reduction)*			
			Day 1		Day 5	
			After hand wash 1	After last hand wash	After hand wash 1	After last hand wash
Aerobes	Control	7.00	6.41 (8.4)	6.19 (11.6)	6.67 (4.7)	6.31 (9.9)
	Alc A	6.25	4.91 (21.4)	2.63 (57.9)	3.04 (51.4)	3.24 (48.2)
	Alc B	6.91	6.11 (11.6)	3.47 (49.8)	3.89 (43.7)	3.03 (156.2)
	CHG	7.02	5.93 (15.5)	4.29 (38.9)	3.89 (44.6)	2.68 (61.8)
	IPA	7.09	5.89 (16.9)	4.26 (39.9)	5.11 (27.9)	3.92 (44.7)
Anaerobes	Control	6.88	6.38 (7.3)	5.96 (13.4)	6.26 (9.0)	6.18 (10.2)
	Alc A	6.70	5.44 (18.8)	3.77 (43.7)	4.14 (38.2)	3.26 (51.3)
	Alc B	6.24	6.13 (1.8)	3.43 (45.0)	4.97 (20.4)	2.79 (55.3)
	CHG	6.66	5.64 (15.3)	4.49 (32.6)	5.17 (22.4)	3.83 (42.5)
	IPA	7.07	6.05 (14.4)	5.62 (20.5)	5.31 (24.9)	4.49 (36.5)

* Percent reductions from base line calculated as [(base line count − test count)/base line count] × 100.

in a laboratory. For the control soap and CHG, hands were moistened with running water, washed, rinsed, and dried with a paper towel. For the alcohol-based products, no running water was used and hands were air dried.

In addition, subjects assessed the appearance, intactness, moisture content, and sensation on the skin of the hands at base line and again after the 5 days of hand washing. An ordinal scale with a maximum of 28 points (7 points on a Likert-type scale for each of the four factors assessed) was used; the lower the score, the worse the condition of the skin. At the end of the test week, subjects also rated the acceptability of their assigned hand washing product on a similar 7-point scale from "I hate this product; I would never choose it" (1 point) to "I love this product; I would always choose it" (7 points).

Microbiology. The sterile bag technique of Larson et al. (8) was used to assay microorganisms on the hand. The subject inserted the dominant hand into a sterile polyethylene bag containing 50 ml of sampling solution (sterile distilled water containing, per liter: lecithin, 20 g; sodium thiosulfate, 6 g; sodium oleate, 6 g; protease peptone, 1 g; tryptone, 1 g; and Tween 80, 50 ml; pH 7.2 to 7.4). In preliminary studies, we determined that there was no significant increase or decrease in CFUs in the solution within the first 2 h after sampling.

The entire hand surface was rubbed vigorously through the wall of the bag for 3 min. All specimens were plated within 2 h of sampling. A 0.1-ml volume of serial dilutions up to 10^{-3} was placed on three media. For aerobic incubation, Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing yeast extract, 5 g/liter; Tween 80, 1 ml/liter; and MacConkey agar (BBL) were used. For anaerobic incubation, prereduced brain heart infusion agar (BBL) containing 7% sheep blood and yeast extract, 5 g/liter, was used. Plates were incubated at 37°C aerobically for 48 h and anaerobically for 5 days. CFUs were counted.

Statistical analysis. Analysis of covariance, controlling for base line counts, was used to test the significance of differences in bacterial counts (counted as $\log_{10}$ CFU) between the five treatment groups. The nonparametric Kruskal-Wallis analysis of variance was used to test the significance of differences between subject assessment of their skin and of product acceptability among the treatment groups. In cases where multiple comparisons between groups were done, the Tukey technique was employed (13). A probability of $P < 0.05$ was considered to be statistically significant. A sample size of 10 subjects per treatment group would allow one to detect a statistically significant difference in bacterial counts of 0.70 log or greater with 80% power (14).

RESULTS

Fifty individuals were recruited for the study, and all completed the entire testing period. There were 15 (30%) men and 35 (70%) women ranging in age from 19 to 50 years (mean, 29.5 ± 6.8). Ages and sexes were evenly distributed throughout the five treatment groups.

Microbiology. There were minimal reductions in log CFU over the test period among subjects using the control soap (Table 1). After the first wash, there were significant reductions over base line in counts among those using Alc A, CHG, and IPA ($P = 0.002$). By the end of day 1 (15 hand washes) all antiseptics resulted in a 2-log or greater reduction ($P < 0.001$), whereas a less than 1-log reduction in counts was apparent in the control group ($P = 0.18$). This was the case for both aerobic and anaerobic counts. There were significant reductions in aerobic counts after the first hand wash on day 5 as compared with base line counts for Alc A, Alc B, and CHG ($P = 0.0001$). By the end of the test period, all agents produced significant reductions in both aerobic and anaerobic counts when compared with control soap ($P = 0.0002$ and 0.002, respectively) but were not significantly different from one another. Some subjects using control soap or IPA became colonized with gram-negative bacteria, whereas gram-negative bacteria were rarely detected after a week of washing with Alc A, Alc B, or CHG (Table 2).

Subject assessment. In four of the five treatment groups, mean subject ratings of the condition of the skin of their hands worsened. That is, subjects felt that their skin was adversely affected by the week of frequent hand washing.

TABLE 2. Effect of hand washing products on isolation of gram-negative bacteria

Treatment group	No. of subjects with gram-negative bacteria isolated from hands		
	Base line	After 1 wk	Difference
Control	0	4	+4
Alc A	3	0	−3
Alc B	2	0	−2
CHG	4	1	−3
IPA	1	4	+3

The mean changes in ratings from days 1 to 5 were controls, −1.9; Alc A, −4.5; Alc B, −2.5; CHG, +0.7; and IPA, −1.3 ($P = 0.04$ for differences between groups). Ratings by the subjects of overall satisfaction with the products (total possible = 7) were controls, 4.6; Alc A, 3.6; Alc B, 3.9; CHG, 5.9; IPA, 3.7 ($P = 0.05$).

DISCUSSION

To our knowledge, this is the first frequent-use trial of several alcohol-based health care personnel hand rinses designed for use without running water and the first trial in which these products are compared during frequent-use conditions with a product containing CHG. Previous comparisons have been primarily between CHG-containing products and products containing povidone-iodine (2, 4, 7, 12). It should be noted that this protocol did not mimic the situation on wards in that the hand washing practices of subjects were controlled, and subjects were not working in clinical settings in which they would have frequent or prolonged physical contact with patients. The two alcohol-based products designed to be applied without the use of water and air dried resulted in the most rapid immediate reduction in flora. The reductions in bacterial counts among those using CHG were initially less dramatic, but more consistent than those seen with the alcohol-based products. This was probably a result of the substantivity of CHG, a characteristic which results in the continuous release on the skin of chemical activity for several hours after application of the product. Substantivity is a desirable characteristic when prolonged antibacterial activity is important, such as during procedures during which gloves are worn.

At all test times for reducing counts of aerobes, anaerobes, and gram-negative bacteria, Alc A, Alc B, and CHG appeared to be superior to IPA. This finding is consistent with what others have also described (1). This superiority may be due in part to the substantivity which is known to occur with CHG, to the presence of emollients in the hand rinses which increase the time in which the active ingredient contacts the skin by delaying drying time, and to the fact that a larger amount of Alc A and B than IPA was used (5 versus 2 ml).

The efficacy of hand washing products is generally tested against aerobes only, since anaerobes likely to be transmitted from the hands seem to be of little clinical importance. It is reassuring to note, however, that the trends and patterns of the test agents noted against aerobes were similar for anaerobes. Hence, the testing of products against aerobes alone seems to be an accurate reflection of their effectiveness.

In this study, we did not identify species of organisms. In a previous study (7) we found that types of organisms isolated after frequent hand washing did not change substantially. However, an increased risk of harboring gram-negative bacteria on the hands has been associated with skin trauma (6), and this probably explains why gram-negative bacteria were isolated more frequently from individuals using the nonmedicated control soap, or alcohol, which contained no emollients to protect the skin.

The utility of a hand washing product is related not only to its antimicrobial effectiveness, but also to its user acceptability (5, 10). An effective product is of no benefit if it is not used. Users in this study judged the two alcohol-based rinses as having little more acceptability than plain IPA. As a matter of fact, Alc A was judged to cause significantly more skin irritation than any of the other products. This was probably because subjects were required to use 5 ml per hand wash, whereas the manufacturer recommends 1 to 2 ml per hand wash. Although only subjective measures of product acceptability were used, the results were consistent with previously reported findings based on objective measurements of skin trauma (7). That is, CHG was evaluated as the mildest and most preferred preparation, even when compared with control soap.

For some time, alcohol-based hand washing products have been successfully used in Europe (9, 11; Nystrom, Letter, Infect. Control [Thorofare] 5:211, 1985), but have come into some disfavor and disuse in the United States. Based on our data, we conclude that such disuse is unjustified. Alcohol hand rinses are highly efficacious, and their use is recommended in situations where an antiseptic health care personnel hand wash is indicated, particularly when traditional hand washing facilities (sink and running water) may be inaccessible or inconvenient.

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Influence of two handwashing frequencies on reduction in colonizing flora with three handwashing products used by health care personnel

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Four handwashing products (containing either 2% chlorhexidine gluconate, 0.6% parachlorometaxylenol, 0.3% triclosan, or a nonantimicrobial control) at two handwashing frequencies (6 or 18 times/day) were compared with regard to their effectiveness in reducing colonizing hand flora. Eighty adult volunteers were assigned by block randomization to one of the four products and one of the two frequency schedules (n = 10/group) and washed their hands under supervision for 5 consecutive days. There were no significant differences between products in mean $\log_{10}$ colony-forming units after the initial wash ($p = 0.61$), nor were there significant differences in products after 5 days among subjects washing six times per day. For those who washed 18 times per day, however, the effectiveness of all three antimicrobial soaps was significantly better than that of the control soap ($p < 0.05$). Chlorhexidine gluconate produced significantly greater reductions than triclosan or parachlorometaxylenol ($p < 0.05$), which were not significantly different from each other. On the basis of these findings an antimicrobial soap is recommended when handwashing frequency is high and a long-term reduction in colonizing flora is desirable. When handwashing frequency is low (6 times/day), there seems to be less advantage of one product over another, although the use of chlorhexidine gluconate resulted in greater reductions at both high and low handwashing frequencies. (Am J Infect Control 1988;17:83-8)

Three distinct types of hand-wash products are used by health care professionals. Each product type has its own specific requirements and applications. The first is plain soap, which is used for removal of dirt and many transient contaminants. The second is the antiseptic-containing health care personnel hand wash (HCPHW). Repeated 15- to 30-second hand washes with such a product should produce significant reductions in transient flora after artificial contamination of the hands and forearms. The repeated use of a HCPHW also will have some reductive effect on the resident or colonizing flora of the hands. The third type of handwash product is the antiseptic-containing surgical scrub. Scrubbing duration usually is approximately 5 minutes. Repeated use of a surgical scrub should produce at least a 2-log reduction in resident flora.

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Table 1. Mean $\log_{10}$ counts (± standard deviation) by product and washing frequency

	Control	Tricoslan	Parachlorometaxylenol	Chlorhexidine gluconate
Baseline, total group	5.73 (0.56)	6.14 (0.76)	6.31 (0.84)	6.09 (0.61)
Baseline, 6 wash/day	5.71 (0.68)	6.17 (0.61)	6.13 (0.83)	6.16 (0.71)
Baseline, 18 wash/day	5.75 (0.45)	6.11 (0.91)	6.48 (0.86)	6.01 (0.52)
After first wash, day 1, total group	5.81 (0.59)	6.13 (0.51)	6.01 (0.59)	5.81 (0.81)
After final wash, day 5				
6 wash/day	5.25 (0.65)	5.42 (0.62)	5.48 (0.44)	5.03 (1.12)
18 wash/day	5.45 (0.56)	4.56 (0.50)	4.74 (0.72)	3.62 (0.74)
Log change				
After first wash, day 1, total group	+ 0.08	− 0.01	− 0.30	− 0.28
After final wash, day 5				
6 wash/day	− 0.46	− 0.75	− 0.65	− 1.13
18 wash/day	− 0.30	− 1.55	− 1.74	− 2.39
Percentage change*				
After first wash	÷ 20.2	− 2.3	− 49.9	− 47.5
After final wash				
6 wash/day	− 65.3	− 82.2	− 77.6	− 92.6
18 wash/day	− 49.9	− 97.2	− 98.2	− 99.6

*$\frac{\text{Baseline} - \text{Test count}}{\text{Baseline}} \times 100$ (actual counts rather than log counts used).

ethylene bag (Steri-Lok; 3M Co., St. Paul, Minn.) containing 50 ml sterile sampling solution (distilled water containing, per liter, lecithin, 20 gm; sodium thiosulfate, 6 gm; sodium oleate, 6 gm; protease peptone, 1 gm; tryptone, 1 gm; and Tween 80, 50 ml, pH: 7.2-7.4). The sampling solution contained neutralizers for the three antiseptic products,[7,8] and in preliminary studies there was no significant increase nor decrease in CFUs in the solution within the first 2 hours after sampling. The entire hand surface was rubbed vigorously through the wall of the bag for 1 minute. All specimens were plated within 2 hours of sampling. A 0.2 ml volume of serial dilutions up to 10^{-3} was placed on trypticase soy agar, which contained yeast extract, 3 gm/L, and Tween 80, 1 ml/L (TYT), and on MacConkey agar (BBL Microbiology Systems, Cockeysville, Md.). The TYT plates were incubated at 35° C for 48 hours followed by incubation at room temperature for an additional 48 hours. The MacConkey agar plates were incubated at 35° C for 24 hours. Colony-forming units were counted and calculated to provide a total CFU count per hand. Gram-negative bacteria were not identified to species level. Microbiologic sampling of the hands was performed at three times: immediately before the study after a brief wash with control soap (baseline), after the first wash on day 1, and after the last wash on day 5.

Statistical analysis

Analysis of covariance, that is, controlling for baseline counts, was used to test the significance of differences in microbial counts (counted as $\log_{10}$ CFU) among the four product groups and between the two frequencies within each product group. When multiple comparisons between groups were completed, the Tukey technique was used.[9] A probability of $p < 0.05$ was considered to be statistically significant. With a sample size of 20 per product group a 75% difference in changes in $\log_{10}$ CFU could be detected between groups with a power of 80%.[10]

RESULTS

All 80 subjects completed the entire 5 days of testing. There were 60 women (75%) and 20 men, with a mean age of 31.9 years (range: 18-55 years). There were no significant differences

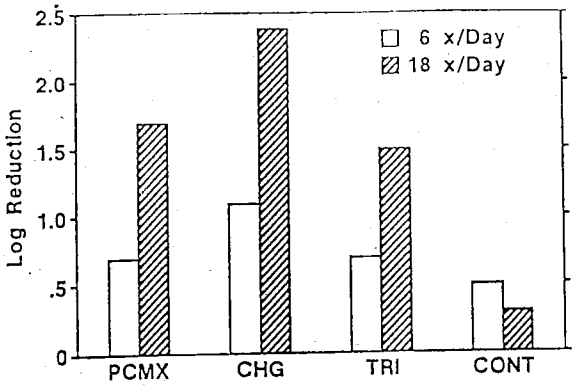


Fig. 1. Log reductions in colonizing flora by handwashing product and frequency, day 5.

in gender or age distribution between the product or frequency groups.

Baseline mean log counts for each group ranged from 5.71 (±0.68 standard deviation) to 6.48 (±0.86) (Table 1). After the initial 30-second wash, mean counts in the control group increased 0.08 logs and mean counts for subjects using one of the three antiseptics (TRI, PMCX, or CHG) decreased 0.01, 0.30, and 0.28 logs, respectively. There were no significant differences among the four test products ($p = 0.61$). Again, after six washes per day for 5 days there were no significant differences among products ($p > 0.50$), although subjects using CHG had the greatest reductions.

After 18 washes per day for 5 days, the control group mean count decreased 0.30 logs, and mean counts for TRI, PCMX, and CHG decreased 1.55, 1.74, and 2.39 logs, respectively (Fig. 1). All three antiseptics performed significantly better than control soap ($p < 0.05$). In addition, CHG produced significantly greater reductions than TRI or PCMX ($p < 0.05$); PCMX and TRI were not significantly different from each other ($p > 0.50$).

Many subjects (51/80, 64%) had gram-negative bacteria isolated from their hands at baseline, and subjects in all four product groups had substantial reductions in carriage of gram-negative bacteria by the end of the test period (Table 2). When gram-negative bacteria were present, they were also in greater numbers at baseline (baseline: mean log CFU = 3.95, range 2.9-4.3; end of test period: mean log CFU = 3.26, range 2.4-3.9).

DISCUSSION

Although plain soap performs well for removing dirt and some transient contaminants from hands, it has little effect on colonizing flora.[11, 12] It is not surprising, then, that in this study subjects using control soap had little change in log counts of normal flora after 5 days of handwashing. Health care personnel hand wash products, on the other hand, are chosen to minimize the numbers of transient contaminating flora and to aid in reducing the colonizing flora by means of their chemical bactericidal activity after a 15- to 30-second handwash. A high prevalence of gram-negative bacteria on hands of nonmedical personnel is not surprising and has been previously reported.[13, 14] The dramatic reductions in gram-negative bacteria among all test subjects indicated that the hands were clean and relatively free of transient flora.

Surgical scrub products containing 4% CHG frequently are used in the United States for health care personnel handwashing, surgical hand scrub, and occasionally for preoperative bathing or preparation of patient skin. A 2% concentration of CHG has been shown to be slightly lower in antimicrobial effectiveness than 4% concentrations of CHG.[15, 16] Several possible reasons explain the improved performance of CHG over that of the other test products. First, its concentration (2%) was greater than the HCPHW concentrations of either PCMX (0.6%) or TRI (0.3%). Perhaps the two latter agents at higher surgical scrub concentrations would have more antimicrobial activity. Indeed, others have used higher concentrations of each of these ingredients in comparative tests.[3, 17] PCMX, for example, is approved for topical use by the Food and Drug Administration for concentrations up to 3.75% and TRI up to 1.0%. Second, CHG has excellent substantivity, binding to the stratum corneum to effect prolonged release of chemical activity on the skin. TRI also has been shown to have this same characteristic, whereas the persistence on skin of PCMX may be less.[5, 6, 8] Third, CHG may give greater antimicrobial activity than the other two antiseptic ingredients tested. A recent comparison of 1.5% TRI and 4% CHG with the use of an HCPHW protocol showed that the two products were equally effective in removing artificially inoculated *Serratia marcescens*. How-

Table 2. Percentage (number) of subjects with gram-negative bacteria recovered from hands

Product	Baseline percentage	Final percentage	Percent reduction*
Parachlorometaxylenol	55 (11/20)	10 (2/20)	81.8
Chlorhexidine gluconate	60 (12/20)	15 (3/20)	75.0
Triclosan	80 (16/20)	15 (3/20)	81.3
Control	60 (12/20)	10 (2/20)	83.3
Total	63.8 (51/80)	12.5 (10/80)	

Chi-square to test differences in reductions between groups, $p = 0.86$.

*Percent reduction $= \frac{\text{Baseline} - \text{final}}{\text{Baseline}} \times 100$.

ever, the effectiveness of CHG was consistently, although not statistically, significantly better after 4, 7, and 10 washes.[18] The immediate effect (after a single 30-second wash) of products studied was not much different from that of plain, nonantimicrobial soap. It is clear that a 30-second HCPHW with such products does not meet surgical hand scrub criteria. Others have reported a similar result for removal of transient as well as colonizing flora.[18] When the most rapid reductions in flora are needed, a surgical scrub product and a surgical scrub procedure are required.

Products containing CHG, PCMX, or TRI at HCPHW levels are most effective when used over a sustained period of time. For this reason such products are appropriate for health care personnel handwashing when a reduction in colonizing flora is desirable and when handwashing frequency is high, certainly more than six times per work period. It also would appear that in situations when frequency of handwashing is very low, there is less advantage of one product over another and probably little advantage of an antiseptic over a plain soap. In most clinical situations, however, frequent handwashing is common and the antimicrobial-containing products, particularly CHG, result in a significantly greater reduction in colonizing flora as compared with plain soap.

This study examined the influence of two variables on the quality of handwashing: frequency of washing and product used. Both of these variables were clearly important. Other variables not examined in this protocol, inasmuch as the procedure was standardized to prevent confounding, include such factors as the duration of handwashing and the amount of soap used. Many of these factors probably have independent and combined influences on the effectiveness of handwashing.[19]

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The Microbiology of the Human Axilla and Its Relationship to Axillary Odor

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The axillary microflora of 229 subjects was characterized quantitatively and the results correlated with whether the odor was pungent body odor or instead a faint "acid odor." The axillary flora was found to be a stable mixture of *Micrococcaceae*, aerobic diphtheroids and *Propionibacteria*. Significantly higher numbers of bacteria were recovered from the axillae of those with pungent axillary odor than in those with acid odor. Aerobic diphtheroids in high numbers were recovered in all subjects having typical body odor. These included lipophilic as well as large-colony diphtheroids.

When droplets of apocrine sweat placed on the forearm were inoculated with various bacteria which reside in the axilla, only diphtheroids generated typical body odor. Cocci produced a sweaty odor attributable to isovaleric acid.

Axillary odor is a distinctive malodorous scent of adults, popularly called "body odor." It is the dominant note among the odors originating in various body regions. The ultimate source of axillary odor is apocrine sweat which when it appears on the surface is both sterile and odorless [1]. The pungent odor is generated when resident microorganisms interact with apocrine sweat [1]. There is dispute regarding the types of bacteria capable of liberating the odiferous substances. At first it was thought that a wide range of gram positive and gram negative bacteria could produce the characterisic malodor [2]. Shehadeh and Kligman, however, demonstrated that only gram positive organisms had this capability [3].

It is not known whether there are quantitative or qualitative differences in the axillary microflora of individuals with different degrees of body odor. The object of this work was to compare the microflora of individuals without the acrid odor to those who were intensely odiferous. We found that the ability to produce the typical malodor is limited to aerobic diphtheroids.

MATERIALS AND METHODS

Subjects

Healthy, young-adult males and females aged 18 to 26 yr served as volunteers. Informed written consent was obtained. Deodorants and antiperspirants were prohibited for 1 week prior to, and throughout, each study. Each subject was given a nonmedicated soap (Ivory) for washing.

Microbiological Methods

Quantitative cultures were obtained by the detergent scrub technique of Williamson and Kligman [4]. This method employs 1 ml of 0.1% Triton-X-100 which is pipetted into a sterile glass cylinder placed in the axilla; the fluid is stirred vigorously for 1 min with a Teflon rod and removed. This procedure is repeated and the 2 samples pooled. Tenfold dilutions were made in 0.05% buffered Triton and drop-plated in duplicate on the following media: (1) Trypticase Soy Agar (TSA); (2) TSA with lecithin and Tween-80; (3) MacConkey's selective agar for gram negative organisms; (4) Sabouraud's media for yeast and fungi; (5) Brain Heart Infusion agar with 1% Dextrose and 0.1% Tween 80 for anaerobic diphtheroids. The first 4 media were incubated aerobically at 37° for 48 hr while the Brain Heart Infusion was incubated anaerobically in a Gas-Pak jar system at 37° for 7 days.

Identification of *Micrococcaceae* into *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Micrococcus* species was accomplished on 200 strains from 40 subjects using the scheme of Baird-Parker [5]. Subsequently, we went no further than recognition of *Micrococcaceae*. The classification of diphtheroids is still far from satisfactory. It has been our practice, for convenience, to distribute these into one of 2 broad groupings: (1) large colony and (2) lipophilic when the addition of Tween 80 enhanced growth. The classification of diphtheroids is currently being strengthened by application of sophisticated techniques such as cell wall analysis [6]. This work and that of Sharpe et al [7] makes it clear tht aerobic diphtheroids belong to 2 genera—*Corynebacterium* and *Brevibacterium* [6,7]. The genus *Corynebacterium* is characterized by meso-diamino-pimelic acid, arabinose, mannose, galactose and corynemycolic acid [6]. *Brevibacterium* can be distinguished by the production of methanethiol, DNAase activity and by the presence of proteolytic activity in gelatin and milk agar [7]. We examined axillary isolates of 10 "lipophilic" and 10 large colony diphtheroids as follows: Corynemycolic acid presence was determined by thin-layer chromatography as described by Goodfellow, Collins, and Minnikin [8]. The chain length of mycolic acid was confirmed by gas chromatography. Production of methanethiol was assessed by incubation in TSA supplemented with 0.1% L-methionine and 0.1% Tween 80 and assessment of the culture headspace by direct sampling using a Perkin-Elmer 3920 gas chromatograph equipped with a flame photometric detector. DNAase activity was determined on DNAase agar (Difco).

Gram negative rods were identified using the API Enteric system. *Propionibacteria* were tested for bacteriophage susceptibility (ATCC 29399B), indole and nitrate production and clearing of gelatin and milk as previously described by McGinley, Webster, and Leyden [9].

Study Designs

I. Quantitative bacteriology of the axilla: In the first study, we cultured 24 subjects, 12 males and 12 females on 5 consecutive days. Each sex was equally divided into left and right handedness.

In 6 subjects, 5 hairs from each axilla were individually cultured by vortexing each hair vigorously in 0.1% Triton X-100, followed by 10-fold dilutions in 0.5% buffered Triton.

Subsequently, 205 young adults (128 males, 77 females), were cultured on a single occasion (both axillae in 43, one in the other 162).

II: Correlation of quantitative bacteriology and axillary odor: Three observers independently examined 23 males and 20 females dividing these into 2 polar categories of odiferousness.

1. Axillary odor. The offensive pungent smell so typical of body odor and (2) no odor or a faint "acidy" odor. Isovaleric acid has been shown to produce a "sweaty" odor [12]. Henceforth, acid odor is the term we shall use for our second category. Twenty males had pungent axillary odor while 3 were classified as acid odor. Seven females had axillary odor and 13 were acid.

Quantitative cultures were obtained from each axilla 24 hr after washing with a nonmedicated soap.

III: Relationship of odor to skin surface lipids: Surface skin lipids were obtained from the above 23 males and 20 females. The axillae

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Abbreviations:
TSA: Trypticase Soy Agar

were thoroughly washed with 0.5% Triton-X-100, followed by wiping with gauze saturated with hexane to remove surface lipids and debris. Skin surface lipids were collected three hours later as follows.

One ml of hexane was pipetted into a glass cylinder with an area of 3.8 sq cm in the center of the axillary vault, agitated for 30 seconds and removed. The procedure was repeated with a second ml of hexane. The pooled sample was immediately passed through an 0.22 μ millipore filter to remove bacteria and debris, evaporated under nitrogen and stored at −20°C. Samples were reconstituted with 0.05 ml. of hexane and processed by the thin-layer chromatography method of Downing [10]. An internal standard of methyl nervonate was used to enable determination of the amounts of the various classes of lipids [11].

IV. In vivo *inoculation of bacteria and apocrine Sweat:* The volar forearms of 10 subjects were sterilized by compresses of 70% ethanol for 2 min. Sterilization was verified by culture. After evaporation, 3 μl of sterile apocrine sweat was spread over a small area which was then inoculated with 0.1 ml of a suspension of *S. saprophyticus* (4 sites), *S. epidermidis* (5 sites), lipophilic diphtheroids (10 sites), large colony diphtheroids (9 sites), and *E. coli* (2 sites). The apocrine sweat was collected by micropipette from droplets which appeared after the intradermal injection of 1:2000 adrenalin into the degermed axillary vault. All strains were isolated from human axillae; the inocula were standardized to 2×10^5 organisms per ml. An additional site was inoculated with 3 μl of apocrine sweat and 0.1 ml of 0.025% aqueous magnesium omadine (a broad spectrum antimicrobial agent). Each site was covered with 5 × 5 cm squares of impermeable plastic film (Saran) and secured by adhesive tape. Twenty-four hours later, the sites were evaluated organoleptically by 3 observers and then cultured quantitatively. An aliquot of the pooled apocrine sweat was cultured to assure bacterial sterility.

RESULTS

The initial study showed the axillary flora to be stable and reproducible. The geometric mean count for the 24 subjects was 420,000/cm^2 on day one, 380,000 on day two, 300,000 on day three, 590,000 on day four and 470,000 on day five. The coefficient of variation was 13%. Comparison of the axillary flora in terms of right and left handedness did not reveal any significant differences. The geometric mean for the left axilla in the 12 left-handed subjects was 308, 390/cm^2 compared to 433,111 for the right-handed subjects. Also, there was no difference between the right and left axilla of 43 subjects; the geometric mean count for the left was 361,275 compared to 328,473 for the right. Subsequently, cultures were obtained from only one axilla.

Individual hairs contained trivial numbers of bacteria, averaging only 327 aerobic organisms and four anerobic organisms. Because of these low counts, subsequent cultures were done without prior shaving of the axilla.

The results of the quantitative bacteriological survey of 205 subjects are found in Table I. *Micrococcaceae* were found in all subjects, lipophilic diphtheroids were recovered in 85% of males but only 66% of females ($p < 0.05$). Large colony diphtheroids were found in 26% and 25% respectively. Gram negative organisms were found in 20% of males and 19% of females. *Propionibacteria* were recovered from 70% of males but only 47% of females ($p < 0.05$). *P.acnes*, *P. granulosum* and *P. avidum* occurred in approximately equal numbers.

The strains of *Micrococcaceae* were identified as follows: 10% were *Staphylococcus aureus*, 51% were *Staphylococcus epidermidis*, 29% were *Staphylococcus saprophyticus* and 10% were *Micrococcus* species. Of the 41 gram negatives, 22 were *Escherichia* species, 15 *Klebsiella*, 11 *Proteus*, 8 *Enterobacter* and 5 *Acinetobacter*.

All 20 diphtheroids were classified as *Corynebacterium* on the basis of the presence of corynemycolic acid, and the absence of proteolytic activity and methanethiol production.

Figure 1 displays the bacteriological differences in the 2 odor groups. The persons with typical axillary odor had a significantly higher number of organisms, 1,300,000/cm^2 vs. 480/000 cm^2 ($p < 0.05$). More striking, however, were the marked differences in composition of the flora. Every subject with axillary odor had lipophilic diphtheroids (geometric mean of 810,000/cm^2 compared to only 55% prevalence with a mean of only 53,000 in the "acid" odor group. Large colony diphtheroids were found in 52% of axillary odor subjects (mean 250,000/cm^2) while only 9% of "acid" odor subjects had these organisms in small numbers (1,600/cm^2). *Propionibacteria* were found in 70% of those with axillary odor (mean of 36,000/cm^2) and in 49% of acid odor subjects (mean 36,000/cm^2).

The results of incubating apocrine sweat and bacteria on the forearms are shown in Fig 2. The typical, pungent axillary odor

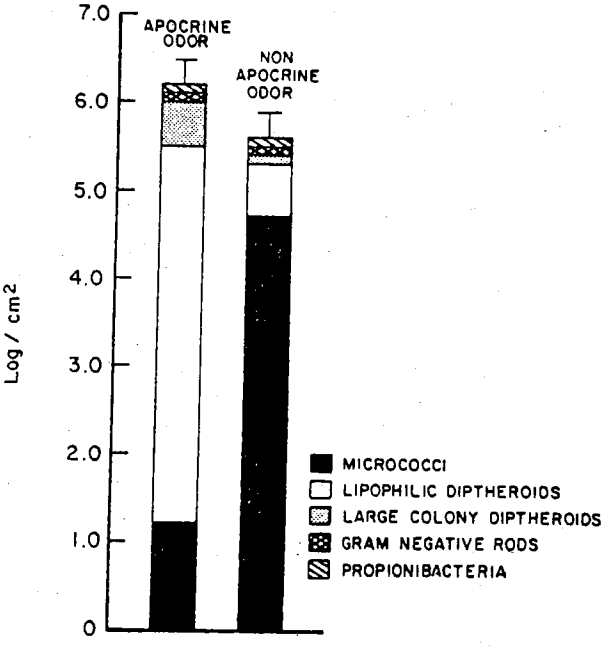


FIG 1. Quantitative bacteriology of subjects with intense axillary odor and those with absent or faint odor. Bacteriology is expressed in terms of the logarithm (base 10) per sq. cm.

TABLE I. *Prevalence and density of axillary resident bacterial flora*

	Males (N = 128)			Females (N = 77)		
	Prevalence	Density[a]	SEM[b]	%	Density	SEM
Aerobic flora	100	6.9×10^5	0.06	100	8.9×10^5	0.09
Micrococcaceae	100	1.2×10^5	0.07	100	3.6×10^5	0.11
Lipophilic diphtheroids	85	2.5×10^5	0.09	66	2.3×10^5	0.14
Large colony diphtheroids	26	2.7×10^4	0.21	25	3.7×10^4	0.24
Gram negative rods	20	2.3×10^3	0.25	19	2.1×10^3	0.31
Propionibacteria (total)	70	5.1×10^3	0.21	47	1.7×10^4	0.30
P. acnes	47	7.2×10^3	0.29	30	1.8×10^4	0.38
P. avidum	34	4.2×10^3	0.22	21	1.5×10^4	0.43
P. granulosum	8	4.1×10^3	0.32	5	4.5×10^3	0.28

[a] Geometric mean per square centimeter.
[b] Standard error of the mean, expressed in logarithms.

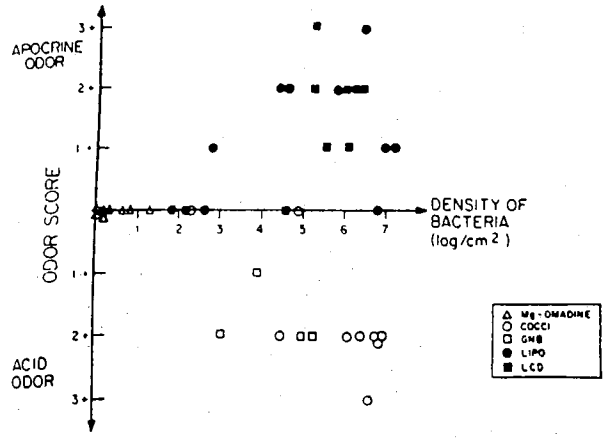


FIG 2. Results of *in vivo* inoculation of apocrine sweat and various organisms; cocci included *S. saprophyticus* and *S. epidermidis*, lipo refers to lipophilic diphtheroids; *LCD* refers to large colony diphtheroids; *GNB* refers to gram negative bacteria; mg-omadine is a broad spectrum anti-microbial agent. Odor production was evaluated organoleptically.

TABLE II. *Skin surface lipids in axilla*

	Strong odor	Faint or absent odor
Total amount of Lipid μg/cm²/3 hr	61.4	55.0
Cholesterol	9.7%	7.1%
Sterol esters	8.8%	7.2%
Triglycerides	27.3%	23.7%
Diglycerides	2.7%	3.4%
Fatty acids	17.4%	20.3%
Wax esters	21.4%	21.7%
Squalene	12.7%	16.4%

was found only when either lipophilic or large colony diphtheroids were inoculated with apocrine sweat and with 2 exceptions, only when their density was in excess of 10^4/cm². *S. epidermidis*, *S. saprophyticus*, and gram negative organisms produced odors of a quite different quality, easily distinguishable from classic body odor. Micrococci uniformly generated a distinctive "sweaty, acid odor."

The results of the skin surface lipid analysis are presented in Table II. No significant differences either in the amount of lipid or in composition was detected in the 2 groups. However, in the surface lipid the percentage of cholesterol was significantly higher than that in sebum rich areas such as the face. Stimulated by this finding, we processed 5 μl of sterile apocrine sweat by thin-layer chromatogrphy; [10] 76.2% of the lipid present was cholesterol, 0.9% was cholesterol esters, 3.6% was wax esters, 0.2% was squalene, 19.2% ws triglyceride and fatty acids.

DISCUSSION

The axillary flora comprises a stable population of aerobic and anerobic organisms, at densities generally between 500,000/cm² and 1,000,000/cm². Day-to-day variations were insignificant. The microflora was quantitatively and qualitatively the same in right and left axillae and was not affected by handedness or sex. In an extensive quantitative survey of the axillary flora, Prince and Rodger also found a very stable day to day flora [13]. Their results differ in two particulars: They found diphtheroids (not classified further) in all subjects. Moreover, our 10% prevalence of *S. aureus* is far lower than their 38% for the winter months and 73% in the summer months. Aly and Maibach surveyed multiple intertriginous areas of male prisoners. They found a higher level of diphtheroids than we did in the axilla (mean counts 1.3×10^7/cm² for nonlipophilic and 3.0×10^6/cm² for lipophilic strains [14]. These higher densities may reflect environmental and hygienic differences. Like us, they found *S. aureus* in 12% quantities. While the presence of hair contributes to the intensity of axillary odor [1], this does not appear to involve bacteria. The number of bacteria in hair was very low. Hairs are not a good substrate for bacterial growth. They probably serve to trap malodorous volatiles generated on the skin surface and also act as a dispersal device [1].

Our results point to diphtheroids as the organisms responsible for axillary odor. Both lipophilic and large-colony diphtheroids were more frequent and more numerous in persons with typical axillary odor. No subject with body odor lacked diphtheroids. Final proof of the odor-liberating capacity of the diphtheroids was forthcoming when both types of diphtheroids were incubated with apocrine sweat on the forearm. None of the other test organisms produced typical axillary odor. The sweaty odor of isovaleric acid was present when micrococci were incubated with apocrine sweat. We did not test *Propionibacteria* because these anaerobic organisms reside in the deep recesses of sebaceous follicles. If *Propionibacteria* were involved, apocrine sweat should be odorous by the time it reaches the surface and it clearly is not [1].

We strengthened the case for aerobic diphtheroids by trying to create body odor in 2 subjects whose axillae completely lacked these organisms. We occluded their axillae for 48 hr with wet gauze sealed under tape. The result was only an intensification of the original "acid" odor, presumably owing to expansion of cocci. Both subjects produced droplets of apocrine sweat after subcutaneous injection of epinephrine.

A current focus of our research is the identification of the odorous chemicals and their biosynthesis. We have already reported the presence of 2 steroids, dehydroepiandrosterone sulfate and androsterone sulfate while others have found 5x-androst-16-en-3-one and 5x-androst-16-en-3x-ol which have odor characteristics very similar to the natural axillary odor [15–18]. In this regard, the finding in apocrine sweat of large amounts of cholesterol, which can serve as the building block for steroid synthesis [19] is of particular interest.

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Announcement

The International Advanced Hair Replacement Symposium will be held in Birmingham, Alabama, U.S.A., February 3–4, 1982. It is sponsored by the American Academy of Facial Plastic & Reconstructive Surgery, Inc. Multidiscipline faculty composed of dermatologists, otolaryngologists & plastic surgeons. Registration fee: $420. Inquiries can be made to: D. B. Stough, III, M. D., Program Director, The Stough Dermatology & Cutaneous Surgery Clinic, P. A., Doctors Park, Hot Springs, Arkansas 71901, U.S.A.

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Updated *in vivo* Methods for Evaluating Topical Antimicrobial Agents on Human Skin

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Updated and expanded *in vivo* quantitative testing procedures to determine the efficacy of topical antimicrobial agents are presented. The occlusion test measures the ability of an agent to prevent the expansion of the resident microflora which occurs when an impermeable dressing is applied to the forearm. Measurements are made at 24 and 48 hr. The expanded flora test measures the ability of an agent to suppress a dense population of micro-organisms produced by expansion of the resident flora of the forearm by prior application of an impermeable occlusive dressing. Measurements are made at 6, 24 and 48 hr or after 10 min in the case for agents designed for immediate degerming. The persistence test measures the ability of an agent to establish a reservoir in skin and exert an antimicrobial effect up to 3 days after the last application of the test material. The ecological shift test determines any major alteration in cutaneous microbial ecology following several applications of the material under occlusive dressings. The serum inactivation test determines whether the presence of serum proteins interferes with antimicrobial activity.

Antimicrobial substances are applied to human skin for many different purposes, viz., treatment of superficial infections, degerming of operative sites, hand disinfection, prophylaxis of infection in cuts and abrasions, axillary deodorants, detergent cleansers to counter dissemination of virulent organisms in hospitals, nurseries, etc.

Of the many thousands of available antimicrobial chemicals, only a few are nontoxic enough to merit serious study in humans. It is a formidable and virtually impossible task to assess effectiveness of a new, untested agent in the clinical setting. For example, a properly controlled study for assessing prophylaxis of infection of cuts or abrasions, for several formulations of a new agent in a population practicing good hygiene, would require observations on thousands of individuals because only a small number of skin injuries become clinically infected. Moreover, adequate control of subjects and standardization of other variables, such as the type of wound, size of the innoculum of the pathogen and environmental factors are practically impossible in clinical studies. In fact, it is a current view of regulatory bodies and their advisory panels that proof of efficacy does not exist for most widely used antimicrobials, including antibiotics. The numerous clinical trials, offered as proof of efficacy, have been criticized for lack of adequate controls.

Given these complexities, one turns naturally to models. Animals are ruled out for the most part because their skins react differently and human bacterial ecology is unique. With the exception of experimental streptococcal lesions in hamster skin [1], it is not possible to induce infections which resemble those of the human. One is therefore obliged to develop human models.

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Marples and Kligman have described a human model for inducing localized, controlled infections with *Staphylococcus aureus* which permit analysis of an agent's ability to prevent or treat *S.aureus* pyodermas [2]. No *in vivo* streptococcal infection model in humans has been developed despite intensive study by Duncan, McBride, and Knox [3]. With respect to *in vitro* "degerming" models, the only established test is the so-called "glove juice test" [4]. This is a relatively complex but useful method for measuring an agent's ability to degerm hands and is primarily designed for evaluating pre-operative scrubbing agents.

In a previous publication, our laboratory described procedures for *in vivo* quantitative appraisal of certain attributes which effective antimicrobials might possess, for example, the capability of preventing the explosive expansion of the resident microflora when the surface is hydrated by covering it with an impermeable plastic film [5–7]. Since then we have had considerably more experience and have both modified and added to the original methods. We shall present our updated procedures in this paper and provide some illustrations of the results that can be obtained.

SUBJECTS

The subjects were healthy, young adult college students. Each volunteer was screened as follows: two 5-cm squares of impermeable plastic film were placed on the volar surface of each forearm and sealed occlusively for 24 hr by encircling the limb with adhesive tape. Unless the density of aerobic bacteria reached at least 1×10^5 organisms per sq cm, the subject was rejected. The reason for pretesting is that occasional subjects will fail to show substantial increase in the resident microflora. This problem is noted mainly in females and appears to be related to the use of antibacterial toiletries, especially perfumes. Subjects who fail to pass the initial screening are usually acceptable after a 1 to 2-week period of discontinuation of all cosmetics. Antibacterial soaps were not used for 2 weeks prior to testing.

MICROBIOLOGICAL METHODS

These have been previously described [8]. Sampling was by the detergent scrub method in which the surface within a glass cup 3.8 cm^2 is scrubbed with a Teflon policeman using 0.1% Triton X-100 (a nonionic fluid detergent) as the wash fluid. After serial dilutions in half-strength, buffered Triton X-100, single drops (0.025 ml) were allowed to fall on plates of Trypticase soy agar (BBL) with and without lecithin and polysorbate 80 (Tween 80) as detoxicants. MacConkey's Agar Medium (BBL) for gram negative organisms and Sabouraud's medium for yeast and fungi. Bacterial plates were incubated aerobically at 35°C for 2 days, plates for yeast and fungi at room temperature.

A potential source for error is the transfer of the antimicrobial substances from the skin to the culture system. This is monitored by assaying for residual antimicrobial activity in the sampling fluid. One drop (0.025 ml) of the original scrub sample is placed on lawnplate inoculated with a susceptible strain of *Staphylococcus epidermidis*. Inhibition of bacterial growth at the drop sites indicates "carry over." To evaluate the extent to which this invalidates results, the survival of a sensitive strain of *S. epidermidis* in the undiluted scrub sample is determined as follows: 0.9 ml of the undiluted culture fluid and 0.1 ml of a fresh saline suspension of *S. epidermidis* are mixed and allowed to stand for 4 hr. Quantitative subcultures are done at 30 min, 1 hr and 4 hr and compared with similar subcultures from a mixture of 0.1 ml of inoculum of the *S. epidermidis* suspension in 0.9 ml of Triton X-100. If the counts decline in relation to the control, the test is invalidated. In [illegible] either a neutralizer must be

found or sampling must be done at a time when the residue of antimicrobial agent on the skin is insignificant. "Carry over" is a rare event, but should be tested for with each new agent. The few occasions that we detected a carry-over occurred when the skin was cultured shortly after the antimicrobial agent was applied. This was seen, for example, in cultures taken 10 min after application of hexachlorophene, chlorhexidine, polymyxin, and Hyamine-10X. Tween-80 was found to neutralize the carryover of hexachlorophene and chlorhexidine. No suitable neutralizer could be found for polymyxin or Hyamine-10X and we are unable to assess immediate antibacterial effect of these agents.

When an antibiotic is tested, one must also take into account the possibility of false negative results through the selection and overgrowth of organisms which are resistant to the test agent. This can be assessed by simultaneously culturing samples on media containing appropriate concentrations of the test antibiotic. Growth of organisms on plates containing the antibiotic demonstrates the presence of resistant strains.

TESTING PROCEDURES

Occlusion Test

The principle of this test is that the microflora of the forearm skin is sparse (10^1 to 10^2 organisms per square cm [6]. An impermeable dressing will increase surface moisture by preventing diffusional water loss and thus enhance bacterial growth. The density of resident organisms increases significantly, with counts frequently reaching millions per sq cm by 48 hr [6,7]. The organisms involved in this expansion are primarily gram positive cocci and diphtheroids [6]. In earlier studies, Marples and Kligman compared antimicrobial agents for 48 hr time periods [7]. This measures not only antibacterial activity but persistence and stability as well. An unstable or rapidly cleared substance would falsely be judged as ineffective. Hence sampling is now done at 24 and 48 hr.

The procedure is as follows: on each arm 0.1 ml of the test material is delivered to each of two 5-cm squares (25 sq cm), by a plastic tuberculin syringe (0.1 ml). Each site is immediately covered with a 5-cm square of impermeable plastic film (Saran Wrap). The site is occlusively sealed by encircling the limb with plastic tape (Dermiclear). A strip of ½" wide white-backed adhesive tape (Zonas, Johnson & Johnson) is placed between each test site to prevent the possibility of translocation of test agents and organisms from one site to another. A third site on each arm is treated with 0.1 ml of the vehicle. The control site is always prepared first to prevent its potential contamination by the test substances. After 24 hr of occlusion, the 3 sites on one arm are quantitatively sampled. The opposite arm is sampled after 48 hr.

Expanded Flora Test

This test measures the ability of an agent to suppress a dense and flourishing population of organisms. The forearm is wrapped with several layers of impermeable plastic film and then sealed at the wrist and below the elbow for 24 hr to expand the resident aerobic flora. After expansion of the resident flora, the procedures are identical to those described in the occlusion test. Two 25 sq cm sites on each arm are treated with 0.1 ml of the test agent while a third is treated with the vehicle control. Sites are cultured at 6, 24 and 48 hr depending on the stability of the test agent. For agents designed to produce immediate bactericidal action, e.g., preoperative sterilization, cultures are performed 10 min after application. In such cases, extreme care must be taken to rule out carryover of the test material into the culture fluid, as mentioned above.

Persistence Test

The property that is evaluated in this procedure is the ability of the agent to establish a reservoir in the horny layer. Substantive agents which diffuse into the horny layer or bind chemically to it will not be readily removed either by loss from the surface or by absorption. The antibacterial effect will therefore last several days.

0.5 ml of the test agent is applied with a pippette twice daily for 4 days to one entire volar forearm. Twenty-four hours after the last application, occlusive dressings (as above) are applied to 3 sites on the chemically treated arm as well as to the other arm treated only with the vehicle. If an antimicrobial effect is demonstrable, the test is repeated, and the post-treatment interval is extended to 72 hr.

Ecological Shift Test

Many agents, particularly antibiotics, have restricted *in vitro* anti- [illegible] replacement of the resident microflora by potential pathogens which do not normally inhabit the site, except in very low numbers, viz., gram negative organisms and yeasts. Ecologic displacements of this kind can be assessed as follows: three 5 cm squares are marked on each forearm, one site serving as a control. The other 2 are treated every 48 hr with the test agent for a total of 3 applications (1 week of continuous occlusion). The dressings are replaced after each application. Quantitative cultures are obtained after the third 48-hour period of occlusion.

Serum Inactivation Test

Certain antimicrobial agents may be inactivated if their use entails application to exudative lesions or to superficial injuries that bring serum to the surface. The effect of serum in antimicrobial activity is assessed as follows: volunteers, who had given informed consent, have 50% ammonium hydroxide applied to their forearm within an 8 mm diameter glass cylinder according to the method of Frosch and Kligman [7]. Within 15 minutes, intraepidermal blisters are produced; these are immediately unroofed with sterile scissors exposing a serous base. This injury mimicks an abrasion. The blister is then inoculated with a marker stain of *S. epidermidis*, a nonpathogenic resident coccus which grows readily and can be recognized by its red pigment [9]. This assures that the microbial population will be roughly the same at each site and also counteracts invasion of virulent organisms such as *S. aureus* [2]. The wounds are covered for 6 hr with impermeable plastic film to allow growth of the implanted coccus. 0.02 ml of the test material is then applied occlusively for 24 hr.

MATERIALS

The following categories of materials were studied: (1) vehicles, (2) preservatives, (3) antibiotics, and (4) antimicrobial agents. The vehicles included 70% ethanol, U.S.P.; petrolatum; 100% acetone; 100% chloroform; hydrophilic ointment, U.S.P.; 60% propylene glycol; polyethylene glycol-400. Antibiotics included 1% erythromycin free base 1% clindamycin phosphate, and 1% neomycin sulfate. Antimicrobials included 5% benzoyl peroxide, 1% hexachlorophene, 1% chlorhexidine acetate, a commercially available ointment preparation of povidone iodine, the quaternary ammonium compound Hyamine-10X and 0.1% magnesium pyridinethione. Agents were evaluated in the occlusion test first and if they failed, no further testing was performed. Those that were active in the occlusion test were evaluated in the expanded flora test for activity at 6 and 24 hr. Those showing activity at 6 hr were evaluated at 10 min for immediate bactericidal activity and those effective at 24 hr were evaluated at 48 hr for persistence of bactericidal activity. Agents with activity in either the occlusion test or expanded flora test are evaluated in the persistence test.

For the ecological shift test the following agents were selected: a bacitracin-polymixin combination ointment, and a neomycin-bacitracin-polymixin combination ointment as examples of broad spectrum antibacterial agents with no activity against yeasts or fungi; 3% hexachlorophene as an agent with predominant activity against gram positive bacteria; and 1% magnesium pyridinethione as an example of an agent with activity against bacteria, yeast and fungi.

For the serum inactivation test the agents povidone-iodine and Hyamine-10X, both known to be inhibited by the presence of serum proteins, were contrasted with neomycin sulfate which is not affected by serum proteins.

RESULTS

Occlusion Test

Among the commonly employed vehicles, 70% ethanol, 60% propylene glycol, 100% polyethylene glycol and hydrophilic ointment exerted a significant effect over a 24-hr period while acetone, chloroform, petrolatum and lanolin failed. The results for hydrophilic ointment were surprising until we discovered that this agent contained sodium lauryl sulfate. Ethanol, propylene glycol and polyethylene glycol produced a significant reduction at 48 hr. Antimicrobial agents, such as benzoyl peroxide, povidone-iodine, chlorhexidine, hexachlorophene, Hyamine 10-X and topical antibiotics, such as clindamycin, erythromycin and neomycin were highly effective (Table I).

Expanded Flora Test

Results with representative agents are displayed in Table II to V. Povidone-iodine ointment and 70% ethanol demonstrated

TABLE I. *Occlusion test*

Agent	%	N	24-hr sampling		SEM	p	48-hr sampling		SEM	p
			Geometric mean*	Logarithmic mean*			Geometric mean	Logarithmic mean		
Control		9	932,000	5.970	0.149		1,960,000	6.292	0.12	
Ethanol	70	10	142	2.152	0.525	***	2,600	3.419	0.63	***
Petrolatum	100	10	860,000	5.935	0.137	NS				
Lanolin	100	8	183,000	5.263	0.362	NS				
Control		10	1,284,000	6.109	0.149		337,000	5.528	0.161	NS
Acetone	100	10	1,164,000	6.066	0.164	NS	2,000,000	6.301	0.254	
Chloroform	100	10	880,000	5.945	0.278	NS				
Hydrophilic ointment	100	8	4,500	3.653	0.411	***				
Propylene glycol	60	11	74	1.869	0.437	***	292,000	5.465	0.423	NS
Polyethylene glycol 400	100	12	504	2.702	0.652	***	31	1.491	0.717	***
Control		10	281,000	5.449	0.196		76,400	4.881	0.415	*
Benzoyl peroxide	2.5	10	7	0.845	0.378	***	2,219,000	6.346	0.054	
Benzoyl peroxide	5.0	10	3	0.477	0.306	***	31,000	4.491	0.151	***
Control		5	235,000	5.371	0.203		52	1.716	0.455	***
Povidone-iodine ointment (Betadine®)		10	181	2.258	0.489	***	1,078,000	6.033	0.241	
							990	2.996	0.563	***
Control		5	932,000	5.969	0.514					
Magnesium pyridinethione	1.0	10	7	0.845	0.378	***	6,501,000	6.813	0.143	
Control		8	692,000	5.840	0.155		34	1.531	0.155	***
Hexachlorophene	1.0	8	22	1.350	0.320	***	1,528,000	6.184	0.170	
Chlorhexidine	1.0	8	136	2.135	0.170	***	178	2.250	0.444	***
Control		8	169,000	5.228	0.231		860	2.935	0.197	***
Hyamine 10X	1.0	8	28	1.447	0.533	***	1,788,000	6.252	0.155	
Control		8	295,000	5.470	0.287		52	1.716	0.155	***
Erythromycin	1.0	8	1,200	3.095	0.292	***	1,857,000	6.269	0.130	
Clindamycin phosphate	1.0	6	525	2.720	0.814	***	122,000	5.086	0.241	**
Control		10	650,000	5.813	0.112		634	2.802	0.749	***
Neomycin sulfate	1.0	10	35	1.544	0.251		1,612,000	6.207	0.152	
							640	2.806	0.320	***

* Geometric mean density with standard error of the mean (logarithm): Student's *t*-test * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
Key to abbreviations: % = concentration; S.E.M. = standard error of the mean; p = significance (see note above); NS = not significant; and N = number of subjects.

TABLE II. *Expanded Flora test (10 min)*

Agent	%	N	Geometric Mean*	Logarithmic Mean*	SEM	p
Control		10	601,700	5.779	0.303	
Povidone-iodine ointment (Betadine)	100	10	620	2.793	0.600	***
Ethanol	70	10	80	1.904	0.415	***
Control		8	261,000	5.416	0.157	
Propylene glycol	60	8	2,051	3.312	0.561	**
Polyethylene glycol 400	100	8	34,900	4.544	0.276	*
Control		10	1,309,000	6.116	0.090	
Benzoyl peroxide	5	10	363,000	5.560	0.220	*
Control		9	56,500	4.752	0.142	
Chlorhexidine	1.0	9	4	0.560	0.314	***
Hexachlorophene	1.0	8	43,000	4.634	0.272	NS
Control		9	355,000	5.550	0.193	
Neomycin sulfate	1.0	9	77,500	4.889	0.003	*

* Geometric mean density with standard error of the mean (logarithm): Student's *t*-test * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
Key to abbreviations: % = concentration; N = number of subjects; SEM = standard error of the mean; p = significance; and NS = not significant.

highly significant immediate bactericidal properties. In the case of ethanol no effect was seen at 24 hr indicating that antibacterial properties were no longer present. Povidone-iodine ointment continued to exert a significant effect after 24 hr. While the results at 48 hr were still statistically significant, the effect was clearly diminishing since the geometric mean had risen to 10^4 organisms per sq cm. In the case of benzoyl peroxide, there was an immediate antibacterial effect (10 min) but not as substantial as the effect of ethanol or povidone-iodine ointment. However, the results at 48 hr showed benzoyl peroxide to be more effective than povidone-iodine. Of the various vehicles found effective in the occlusion test, 60% propylene glycol and 100% polyethylene glycol-400 produced a significant reduction [illegible] an established dense bacterial population even though it was effective in preventing expansion of the normal forearm flora.

Antibiotics, such as erythromycin and clindamycin which are bacteriostatic, were capable of suppressing an expanded flora over a 24-hr to 48-hr period, but did not have immediate (10 min or 6 hr) activity. Neomycin sulfate, however, proved to have both a significant immediate and sustained bactericidal effect. Agents, such as 70% ethanol, povidone-iodine ointment, benzoyl peroxide, hexachlorophene and chlorhexidine have immediate bactericidal action.

Persistence Test

Table VI summarizes the results of this test. Polyethylene glycol (100%), 1.0% hexachlorophene, 1.0% chlorhexidine, 1.0%

Hyamine 10-X and 1.0% neomycin sulfate were able to prevent expansion of the resident microflora 24 hr after the last application of the test material. Only 3 agents were capable of forming a sufficient reservoir in skin to prevent expansion of the microflora 72 hr after the last application, viz., Hyamine 10-X, neomycin sulfate and magnesium pyridinethione.

Ecological Shift Test

In this test, the repeated application of an ointment containing a mixture of bacitracin and polymyxin (active against gram positives and gram negatives), or an ointment containing a mixture of neomycin, polymyxin and bacitracin (also active against gram positive and gram negative organisms) resulted in the implantation of yeast—like fungi in 15 of 16 subjects. Hexachlorophene, effective against gram positive organisms, led to an increase in gram negatives in 5 of 8 subjects. No ecologic shifts were observed with magnesium pyridinethione, the microflora being uniformly and completely suppressed (Table VII).

Serum Inactivation Test

Table VIII compares 3 agents which can potentially be inactivated by serum (a quaternary ammonium compound, Hyamine 10-X and the iodine releasing compound povidone-iodine) with neomycin sulfate, whose antimicrobial effect is not influenced by serum proteins. The iodine compound was, in

TABLE III. *Expanded flora test (6 hr)*

Agent	%	N	Geometric Mean*	Logarithmic Mean*	SEM	p
Control		5	7,874,000	6.896	0.090	
Ethanol	70	6	14,000	4.149	0.344	***
Control		6	621,000	5.794	0.277	
Hydrophilic ointment		10	38,600	4.587	0.471	NS
Control		10	1,023,000	6.010	0.143	
Propylene glycol	60	10	274	2.438	0.499	***
Polyethylene glycol 400	100	8	415	2.618	0.336	***
Control		7	24,300	4.387	0.228	
Magnesium pyridinethione	1.0	7	65,110	4.814	0.282	NS
Control		10	1,320,000	6.121	0.212	
Povidone iodine ointment (Betadine)	100	10	1,000	3.000	0.142	***
Control		10	1,309,000	6.116	0.090	
Benzoyl peroxide	5.0	10	477	2.679	0.454	***
Control		8	144,000	5.158	0.267	
Hexachlorophene	1.0	8	1,400	3.147	0.423	**
Chlorhexidine	1.0	8	74	1.872	0.591	***
Control		5	1,552,000	6.191	0.087	
Hyamine 10X	1.0	10	16	1.204	0.454	***
Control		4	960,000	5.982	0.235	
Neomycin sulfate	1.0	7	44,000	4.643	0.312	*
Control		7	127,000	5.105	0.190	
Erythromycin	1.0	7	111,500	5.047	0.313	NS
Clindamycin phosphate	1.0	7	77,600	4.890	0.193	NS

* Geometric mean density with standard error of the mean (logarithm): * = $p < 0.05$; ** = $p < 0.01$; and *** = $p < 0.001$.
Key to abbreviations: % = concentration; SEM = standard error of the mean; p = significance (see note above).

TABLE IV. *Expanded Flora test (24 hr)*

Agent	%	N	Geometric mean*	Logarithmic mean*	SEM	p
Control		5	6,904,000	6.839	0.140	
Ethanol	70	6	4,694,000	6.672	0.011	NS
Control		6	1,319,000	6.120	0.191	
Hydrophilic ointment		10	165,000	5.219	0.162	NS
Control		10	1,198,000	6.079	0.153	
Propylene glycol	60	10	34	1.537	0.378	***
Polyethylene glycol 400	100	8	78	1.892	0.521	***
Control		10	2,603,000	6.415	0.082	
Benzoyl peroxide	5.0	10	10,400	4.019	0.275	***
Control		10	2,486,000	6.392	0.103	
Povidone-iodine ointment (Betadine)	100	10	8,700	3.940	0.200	***
Control		7	443,000	5.646	0.120	
Magnesium pyridinethione	1.0	7	19	1.282	0.529	***
Control		8	442,600	5.871	0.251	
Hexachlorophene	1.0	8	10	0.980	0.290	**
Chlorhexidine	1.0	8	14	1.134	0.455	
Control		5	2,342,000	6.370	0.130	
Hyamine 10X	1.0	10	4	0.602	0.331	**
Control		10	892,000	5.950	0.138	
Erythromycin	1.0	10	47,900	4.680	0.322	**
Clindamycin phosphate	1.0	10	121,000	5.083	0.225	**
Neomycin sulfate	1.0	10	2,050	3.312	0.451	

* Geometric mean density with standard error of the mean (logarithm): * = $p < 0.05$; ** = $p < 0.01$; and *** = $p < 0.001$.
Key to abbreviations: % = concentration; SEM = standard error of the mean; p = significance (see note above); NS = not significant; and N = number of subjects.

TABLE V. *Expanded flora test (48 hr)*

Agent	%	N	Geometric Mean*	Logarithmic Mean†	SEM	p
Control		10	1,819,000	6.250	0.078	
Propylene glycol	60	10	4,300	3.638	0.310	***
Polyethylene glycol 400	100	8	9,100	3.959	0.320	***
Control		10	2,877,000	6.459	0.094	
Benzoyl peroxide	5.0	10	62,400	4.795	0.131	***
Control		10	3,069,000	6.487	0.106	
Povidone-iodine ointment (Betadine)		10	101,000	5.004	0.245	***
Control		7	2,133,000	6.329	0.243	
Magnesium pyridinethione	1.0	7	0	0		***
Control		8	1,151,000	6.061	0.144	
Hexachlorophene	1.0	8	1,260	3.721	0.229	***
Chlorhexidine	1.0	8	76	1.883	0.381	***
Control		10	1,552,000	6.1909	0.087	
Hyamine 10X	1.0	10	7	0.8451	0.514	***
Control		10	487,500	5.688	0.170	
Erythromycin	1.0	10	129,000	5.109	0.170	NS
Clindamycin phosphate	1.0	10	63,400	4.802	0.113	NS
Control		10	1,819,000	6.260	0.167	
Neomycin sulfate	1.0	10	4,300	3.633	0.326	**

* (1) Geometric mean density with standard error of the mean (logarithm): Student's *t*-test * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
Key to abbreviations: % = concentration; N = number of subjects; SEM = standard error of the mean; p = significance (see note above); NS not significant

TABLE VI. *Persistence test*

Agent	%	N	Geometric mean*	24-hour sampling† Logarithmic Mean*	SEM	p	Geometric mean*	72-hour sampling† Logarithmic mean*	SEM	p
ontrol		8	723,000	5.859	0.125					
olyethylene glycol 400	100	8	18,400	4.265	0.419	**	717,000	5.855	0.111	NS
ontrol		6	117,800	5.071	0.378					
ropylene glycol	60.0	6	29,800	4.475	0.449	NS				
ontrol		8	206,500	5.315	0.305					
enzoyl peroxide		8	114,600	5.059	0.157	NS				
ontrol		6	117,000	5.068	0.190					
ovidone iodine ointment (Betadine)		6	41,800	4.621	0.195	NS				
ontrol		8	167,000	5.223	0.275					
exachlorophene	1.0	8	34	1.531	0.37	***	99,800	4.999	0.293	NS
hlorhexidine	1.0	8	12	1.08	0.54	***	87,000	4.939	0.292	NS
ontrol		6	381,000	5.581	0.08					
yamine 10X	1.0	6	26	1.409	0.471	***	27,100	4.433	0.275	**
ontrol		8	804,000	5.905	0.31					
rythromycin	1.0	8	634,000	5.802	0.19	NS				
lindamycin	1.0	8	405,000	5.617	0.396	NS				
ontrol		8	483,000	5.684	0.08					
eomycin sulfate	1.0	8	17	1.23	0.58	***	11,100	4.045	0.242	***
agnesium pyridinethione	1.0	8	14	1.146	0.53	***	120	2.079	0.42	***

† Occlusive dressings are applied 24 and 72 hr after the last of the 4 twice-daily applications of the test material. Quantitacultures are obtained per 24 hr of occlusion.
* Geometric Mean density with standard error of the mean (logarithm): Student's *t*-test * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
Key to abbreviations: % = concentration; N = number of subjects; SEM = standard error of the mean; p = significance; NS = not significant.

TABLE VII. *Ecological shift test*

Agent	N	Total Aerobic Flora*	Gram Negative Organisms*	Yeast*
ntrol	8	1,369,000	183,000 (37.5%)	42,000 (12.5%)
citracin-Polymixin	8	90,000	1,200,000 (12.5%)	80,000 (100%)
omycin-Bacitracin-Polymixin	8	5,500	90,000 (12.5%)	4,800 (87.5%)
% Hexachlorophene	8	2,000	13,600 (62.5%)	(0.0%)
% Magnesium pyridinethione	8	7	(0.0%)	(0.0%)

* Geometric mean average of all sites.
† Geometric mean of positive sites with % of positive sites in parentheses.

fact, inactivated. The quaternary compound was still capable of suppressing a dense population of micro-organisms but was not as effective as in the expanded flora test. This suggests that there was partial inactivation of this agent. Neomycin was just as effective in suppressing a dense flora in the presence of serum as in the expanded flora test.

COMMENT

These assays permit systematic, *in vivo* evaluation of antimicrobial activity. The sequence of study is as follows:

The 24-hr and 48-hr occlusion test is done first, thus establishing whether the agent is active and stable enough to prevent multiplication of resident micro-organisms. This is in reality a measure of bacteriostatic activity. If the antibacterial activity

TABLE VIII. *Serum inactivation test*

Agent	%	N	Geometric mean*	Logarithmic mean*	SEM	p
Control		8	5,122,000	6.709	0.09	
Povidone-iodine surgical scrub	100	8	1,448,000	6.161	0.31	NS
Hyamine 10X	0.5	8	528,000	5.723	0.32	**
Neomycin sulfate	1.0	8	1,700	3.236	0.41	***

* Geometric mean density with standard error of the mean (logarithm): Students' *t*-test * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Key to abbreviations: % = concentration; N = number of subjects; SEM = standard error of the mean; p = significance; NS = not significant.

is too low, no further tests are indicated, unless it is considered feasible to increase the concentration or to improve the vehicle. The expanded flora test is performed next. This provides a measurement of how quickly the test agent can suppress a dense, proliferating population, actually a test of bactericidal activity. If desired, immediate killing ability can be assessed within 10 to 30 min after application. One then decides whether it will be worthwhile to test for long-acting antimicrobial activity (reservoir effect). For example, if the agent is effective at 48 hr both in the occlusion and expanded flora tests, it should be assayed for persistance. Very few agents exert significant antimicrobial activity beyond 48 hr. Broad spectrum agents that are substantive would be useful in situation where exposure to fungi and pathogenic bacteria is high and frequent applications unfeasible. Broad spectrum antimicrobials with persistent activity would be useful to protect skin which is kept occluded for long periods such as around indwelling catheters.

If the materials are intended for use on cuts and abrasions or secondarily infected, exudative inflammatory processes, it should then be tested for antimicrobial activity in the presence of serum. Inactivation can often be overcome by increasing the concentration.

Except for raising ammonium hydroxide blisters in the serum inactivation test, these procedures are non-invasive and can easily be performed in volunteers without discomfort and provide quantitative measurements of several parameters.

With a new untested agent we can rapidly assess whether the material will have any potential benefit by measuring the effect in the occlusion test. If a material fails this test then it can be discarded unless there is the possibility of it being highly unstable or too dilute. The first possibility can be ruled out by measuring the effect of the agent in a 10-min expanded flora test. Newer more stable or more concentrated compounds are reassessed in the occlusion test. An agent which demonstrates activity in the occlusion test should then be evaluated in the expanded flora test to determine whether it is capable of suppressing a dense, thriving flora. Some agents will be effective in the occlusion test but fail to exert bactericidal activity. For agents which are already formulated and on the market, these tests can be used to substantiate or invalidate claims. For example, agents reported to be effective, rapid degerming agents with no danger of inducing adverse changes in the ecology of the resident flora, e.g., properties desirable in agents to be used frequently, such as disinfectant handwashers, can be tested in the expanded flora and ecological shift tests. Likewise, agents designed for sterilization around catheter sites should be capable of preventing expansion of the resident flora for at least 48 hr and should not lead to selection of resistant organisms or overgrowth of gram-negative organisms or yeasts if they are intended for prolonged usage. Agents designed for use in cuts should not be inactivated by serum proteins; this can be easily determined by the serum inactivation test. Agents reported to exert "long-lasting" antimicrobial effects can be tested in the persistance test.

In summary, these models provide noninvasive testing procedures which provide reproducible quantitative measurements in a variety of *in vivo* antimicrobial parameters.

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with 70% and 95% ethyl alcohol a similar though smaller effect was obtained. Previous studies had shown that cursory swabbing of the hands with 70% ethyl alcohol had relatively poor effects (Lowbury and Lilly, 1960). The incorporation of 1% glycerol in alcoholic solutions makes them acceptable to individuals who complain of excessive dryness of the skin after repeated use of alcohol. Aqueous solutions of chlorhexidine applied in the same way as the alcoholic solutions but followed by rinsing and drying of the hands were effective but less so than the alcoholic solutions or alcohol.

Alcoholic solutions rubbed into the skin have been widely used in Germany for disinfecting surgeons' hands (Molitor and Godry, 1972), and there are strong arguments for a more general adoption of this method. An incidental convenience in using alcoholic chlorhexidine for the surgeon's hands is that the same concentrate is used for preparing the solutions commonly used for disinfection of the operation site. There remains a need for detergent cleansing of the hands to remove dirt, blood, and other physical contaminants and probably for the first operation in a list. A "social" wash with soap and water should be adequate for this, but it is probably more convenient to combine the functions of cleansing and disinfection by the use of an antiseptic detergent preparation, such as 4% chlorhexidine detergent solution when a detergent is required. One of our experiments showed chlorhexidine to have greater skin disinfectant activity in isoproyl alcohol than in ethanol, and we found consistently lower viable counts, relative to pretreatment counts, when gloves were worn for three hours before sampling than when samples were taken immediately after disinfection of the hands. This effect could be attributed to residues of antiseptic left on the skin after the use of chlorhexidine, hexachlorophane, or Irgasan DP 300, but some other factor must be responsible in the case of the volatile alcohols, which also showed this effect. The alcohol might marginally damage bacteria, allowing them to recover if inoculated immediately on to culture media but not if left on the skin for three hours before inoculation of the medium. Another possibility is the destruction by drying of some bacteria which survived exposure to alcohol; this effect would be present only in the experiment in which gloves were worn. The self-disinfecting properties of the skin do not seem to be involved as there was no evidence of any reduction in the numbers of bacteria on the skin when gloves were worn after the use of non-antiseptic soap. Clinically, the reduction rather than increase in the bacterial flora of the skin during a three-hour period of wearing surgical gloves was a welcome finding. As such low levels of bacterial flora are maintained during the course of operations it seems unnecessary to disinfect the hands thoroughly before every clean operation in a long list, and three or four treatments on one day with an antiseptic should be quite sufficient for the maintenance of a low equilibrium level of skin flora.

Cleansing the hands with 5-10 ml of 70% ethyl alcohol solution containing 1% glycerol has been considered effective for nurses before they carry out aseptic ward procedures. A study on contamination of nurses' fingers when dressing operation wounds (Noy *et al.*, 1974) showed, by contact plate samplings, that three out of 32 hands (9·4%) carrying pathogens (*Staph. aureus* or coliform bacilli) lost less than 90% of these organisms on washing with soap and water, compared with a similar loss in three out of 47 (6·4%) hands disinfected with alcohol. Ethyl alcohol (70%) was therefore judged to have at least as great an effect in removing these organisms as soap and water. From our studies on the resident flora and from the evidence that *Staph. aureus* is often carried as a resident (Lowbury and Lilly, 1960) ethyl alcohol treatment of nurses' hands may be assumed to have a larger effect than soap and water in reducing the risks of contamination of wounds with staphylococci.

In a preliminary test for acceptance of alcoholic chlorhexidine handwashing by surgeons in operating theatres at this hospital the method was found to be comfortable and convenient. Further trials over a longer period will be required.

We thank Mr. M. D. Wilkins for help with statistical assessments and members of our staff for their co-operation in tests of skin disinfection.

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Disinfection of the Skin with Detergent Preparations of Irgasan DP 300 and Other Antiseptics

Triclosan

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Summary

An evaluation of the relative effectiveness of 2% hexachlorophane and 0·75% Irgasan DP 300 bar soaps in disinfection of the hands showed that the former caused a significantly larger reduction in natural skin bacteria than the latter after one handwash and after six handwashings, three on each of two successive days. Repeated use of Irgasan DP 300 bar soap caused a significantly greater reduction in skin flora than repeated handwashings with unmedicated bar soap, but a single handwash gave no significant reduction in skin flora compared with a single use of the unmedicated soap.

In a comparison of a 4% chlorhexidine detergent solution a 3% hexachlorophane detergent cream and a 2% Irgasan DP 300 detergent solution the 4% chlorhexidine detergent gave the largest mean reduction in skin bacteria after one handwash and after six handwashings and 2% Irgasan DP 300 a poor and erratic reduction after a single handwash. After six handwashings all three preparations gave large

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showed some residual activity on the skin after handwashing though less than that with chlorhexidine and with hexachlorophane-chlorocresol detergent preparations.

Introduction

Because of the possible toxic absorption of hexachlorophane from the skin of neonates (Curley *et al.*, 1971) the use of hexachlorophane preparations has been severely restricted and controlled by an order of the Department of Health and Social Security (Statutory Instruments, 1973). Alternative antiseptic soaps and detergent preparations containing 2,4,4'-trichloro-2'-hydroxydiphenyl ether (Irgasan DP 300, Geigy), a compound active against Gram-positive cocci and Gram-negative bacilli, have been introduced in place of hexachlorophane, sometimes bearing the trade names—for example, Derl and Cidal—previously used for the hexachlorophane preparations. Evidence of the value of repeated bathing with a bar soap containing 1% of 2,4,4'-trichloro-2'-hyroxydiphenyl ether has been reported by Bodey and Rosenbaum (1973).

We report here an assessment of two Irgasan DP 300 preparations compared with other antiseptic detergent preparations used for disinfection of the skin.

Methods

Preparations included in the study were: (*a*) 0·75% Irgasan DP 300 bar soap (New Derl Hospital Soap); (*b*) 2% hexachlorophane bar soap (Medisoap); (*c*) 2% Irgasan DP 300 bactericidal washing cream (Zalclense): the detergent base contains a potassium neutralized vegetable soap, emollients, "pearlising" agents, sequestering and viscosity modifying agents, perfume, and colour; (*d*) 4% chlorhexidine detergent solution (Hibiscrub); (*e*) 3% hexachlorophane detergent cream (Disfex); (*f*) Unmedicated bar soap (control application).

A standard two-minute handwash under running warm water with each of the preparations except 2% Irgasan DP 300 was carried out as described elsewhere (Lowbury and Lilly, 1973). The 2% Irgasan DP 300 cream was rubbed for 30 seconds on to the dry hands, after which rubbing continued with additions of warm water from the tap for one-and-a-half minutes in accordance with the manufacturer's instructions. In a separate study on three subjects 2% Irgasan DP 300 was applied in the same way as the other preparations. To assess disinfecting properties standard bowl sampling tests for viable counts of natural bacteria were made, as described elsewhere (Lowbury and Lilly, 1960), before the first use of the preparation, immediately after the first use, and again after the sixth of a series of handwashes with the preparation, three on one day and three on the next.

Two experiments were made in each of which a 0·5% lecithin, 1% Tween 80, and 1% sodium thiosulphate were used in the sampling fluid (Ringer's solution) and in the nutrient agar pour plates to prevent the effects of "carry-over" of antiseptic, and tests for carry-over were made on plates showing no bacterial growth or very scanty growth. The methods were identical with those used in earlier studies (Lowbury and Lilly, 1973).

Two experiments were made, one being a comparison of 0·75% Irgasan DP 300 bar soap, 2% hexachlorophane soap, and unmedicated soap and the other a comparison of 2% Irgasan washing cream, 4% chlorhexidine detergent solution, 3% hexachloraphane detergent cream, and unmedicated soap.

Experiments to determine whether active residues of Irgasan DP 300 were left on the skin one hour after washing with the 2% compound were made with inocula of *Staphylococcus aureus* and *Escherichia coli* on the skin (Lowbury and Lilly, 1973).

Results

The comparison of bar soaps containing 2% hexachlorophane, 0·75% Irgasan DP 300, and no antiseptic additive is shown in table I. The hexachlorophane soap gave consistently larger reductions in bacterial counts from skin samplings than Irgasan soap both after a single application and after six applications. Irgasan soap gave a consistently greater reduction in skin samplings than unmedicated soap after six applications. The reduction was greater with Irgasan than with unmedicated soap after one application in all but one of the experimental subjects, but there was a wide variation in the results of a single application and the mean reduction was very small.

A comparison of 2% Irgasan DP 300 cream, 4% chlorhexidine detergent, and 3% hexachlorophane detergent with unmedicated bar soap is shown in table II. The 4% chlorhexidine detergent gave the greatest reduction in bacterial counts from skin samplings both after one application and after six applications. After one application 2% Irgasan cream gave erratic results, and the reductions were consistently smaller than those which were given by either 4% chlorhexidine or 3% hexachlorophane. The cumulative effect of 2% Irgasan cream after six applications was good, the mean percentage reduction being intermediate between that of 4% chlorhexidine (98·1%) and that of 3% hexachlorophane (91·9%).

When 2% Irgasan DP 300 was applied in the same way as chlorhexidine and hexachlorophane preparations a larger and more consistent reduction was obtained after a single hand wash (mean percentage reduction (± S.D.) 57·2 ± 5·5%) than when it was applied as suggested by the manufacturer. After six applications a similar reduction was obtained with either method of application.

TABLE I—*Disinfection of Hands by Various Preparations: Reduction in Bacterial Counts from Hand Washings, expressed as Percentage of Initial Count*

Subjects	2% Hexachlorophane Bar Soap		0·75% Irgasan DP 300 Bar Soap		Unmedicated Bar Soap (Control)	
	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications
1	44·9	97·3	−2·9	54·2	17·2	50·5
2	88·1	97·1	38·3	91·3	22·4	8·7
3	49·1	94·1	19·2	61·7	6·8	37·1
4	42·0	88·9	19·7	21·1	6·0	16·9
5	61·0	78·7	34·2	68·0	13·8	31·0
6	41·7	70·0	16·9	40·7	9·2	14·2
Mean (± S.D.) % reduction	54·5 ± 7·3 (A)	87·7 ± 4·5 (B)	20·9 ± 5·9 (C)	56·2 ± 9·8 (D)	12·6 ± 2·6 (E)	26·4 ± 6·5 (F)

Comparison of treatments:
(A) *v.* (C): $t = 3{\cdot}56$; $P < 0{\cdot}01$.
(A) *v.* (E): $t = 5{\cdot}39$; $P < 0{\cdot}001$. (B) *v.* (D): $t = 2{\cdot}92$; $P < 0{\cdot}02$.
(B) *v.* (F): $t = 7{\cdot}74$; $P < 0{\cdot}001$. (D) *v.* (F): $t = 2{\cdot}53$; $P < 0{\cdot}05$.
(C) *v.* (E): N.S.

TABLE II—*Disinfection of Hands by Antiseptic Detergent Solutions and Cream: Reduction in Bacterial Counts from Hand Washings, expressed as Percentage of Initial Count*

Subject	2% Irgasan DP 300		4% Chlorhexidine Gluconate		3% Hexachlorophane		Bar Soap (Control)	
	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications
1	51·4	98·5	85·8	99·8	26·7	99·2	24·7	19·2
2	9·0	91·5	81·1	93·5	66·2	91·8	8·2	21·7
3	−42·7	99·1	84·3	99·6	59·5	95·2	22·3	31·5
6	27·0	93·9	97·2	99·7	32·9	81·3	15·4	17·1
Mean (± S.D.) % Reduction	11·2 ± 19·9 (A)	95·8 ± 1·8 (B)	87·1 ± 3·5 (C)	98·2 ± 1·6 (D)	46·3 ± 9·7 (E)	91·9 ± 3·8 (F)	17·7 ± 3·7 (G)	22·4 ± 3·2 (H)

Comparison of treatments:
(A) *v.* (C): $t = 3{\cdot}75$; $P < 0{\cdot}01$.
(A) *v.* (E): $t = 1{\cdot}58$ (N.S.).
(A) *v.* (G): $t = 0{\cdot}32$ (N.S.).
(C) *v.* (E): $t = 3{\cdot}95$; $P < 0{\cdot}01$.
(C) *v.* (G): $t = 13{\cdot}58$; $P < 0{\cdot}001$.
(E) *v.* (G): $t = 2{\cdot}74$; $P < 0{\cdot}05$.
(B) *v.* (D): $t = 1{\cdot}0$; (N.S.).
(B) *v.* (F): $t = 0{\cdot}91$; (N.S.)
(B) *v.* (H): $t = 19{\cdot}98$; $P < 0{\cdot}001$.
(D) *v.* (F): $t = 5{\cdot}27$; $P < 0{\cdot}01$.
(D) *v.* (H): $t = 21{\cdot}4$; $P < 0{\cdot}001$.
(F) *v.* (H): $t = 13{\cdot}94$; $P < 0{\cdot}001$.

RESIDUAL ACTION

The viable counts of *Staph. aureus* and *E. coli* in samplings from areas of skin previously washed with soap and water (controls) were 54,000/ml and 4,300/ml respectively. From areas previously washed with 2% Irgasan DP 300 *Staph. aureus* counts of 4,250/ml and 2,950/ml and *E. coli* counts of 965/ml and 1,300/ml were obtained. By comparison, areas washed with 4% chlorhexidine detergent before inoculation of bacterial cultures showed mean viable counts of 12·5 and 18·5 *Staph. aureus*/ml compared with 13,500/ml and 19,500/ml from control areas and 0 and 0·25 *E. coli*/ml compared with 900/ml and 1,150/ml on the control areas. A 3% hexachlorophane liquid soap with 0·3% chlorocresol (Ster-Zac) showed a reduction, by these criteria, from 24,250 to 25 *Staph. aureus*/ml washings and from 15,500 to 15 *E. coli*/ml washings (Lowbury and Lilly, 1973). From these results (see also Lowbury *et al.*, 1974) the 2% Irgasan preparation seemed to leave some residual activity after handwashing but less than that obtained with chlorhexidine and hexachlorophane detergent preparations.

Discussion

Both Irgasan DP 300 preparations showed significant skin disinfectant action compared with the inert control, but the effects of a single application were poor and erratic. The bar soap containing 2% hexachlorophane gave considerably better and more consistent skin disinfection than 0·75% Irgasan bar soap after one application or six applications. The 2% Irgasan soap compared favourably with 3% hexachlorophane and 4% chlorhexidine in its cumulative action on repeated use. Probably a bar soap containing 2%, rather than the existing 0·75%, of Irgasan DP 300 would have been more effective than the product which we tested and might have been as effective as the hexachlorophane bar soap.

The disinfectant action of 2% hexachlorophane bar soap after both single and repeated application was better than expected and apparently greater than that of a similar product tested previously (Lowbury *et al.*, 1963). The soap used in this study had been in store for over 12 years, but it seems unlikely that its activity would improve on storage. Hexachlorophane and, to a smaller extent, Irgasan bar soap used in wards might have some value in controlling cross infection. In high risk areas where hand disinfection, including removal of resident flora, is potentially more important, washing with 4% chlorhexidine detergent or a one- to two-minute wash with 10 ml 0·5% chlorhexidine in 95% ethyl alcohol would probably be more appropriate. The 0·5% chlorhexidine in 95% ethyl alcohol has been shown to have greater immediate disinfectant action against skin flora than any antiseptic detergent preparation (Lowbury *et al.*, 1964, 1974). Irgasan DP 300 detergent preparations may have a useful role in place of hexachlorophane for the prophylaxis of the newborn against staphylococcal infection, though its residual activity seems to be smaller than that of hexachlorophane and chlorhexidine detergent preparations.

We thank Mr. M. D. Wilkins for help with statistics, members of the staff of this unit for their co-operation in trials of skin disinfection, and Messrs. Izal Ltd., for supplies of Zalclense.

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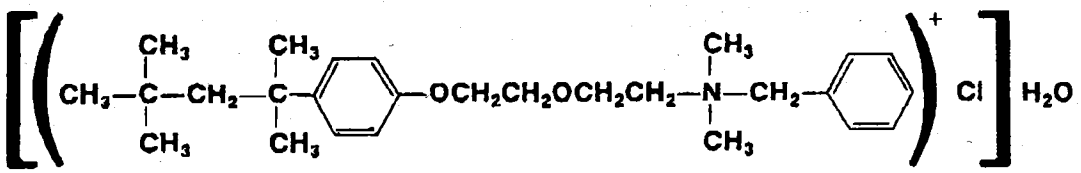
PRODUCT INFORMATION

HYAMINE® 1622

Hyamine 1622 microbiocide is a crystalline quaternary ammonium compound. Hyamine 1622-50 percent is a 50 percent active aqueous concentrate. These compounds are effective in low concentrations against a wide variety of microorganisms.

In suitable formulations the Hyamine products provide bactericidal and deodorant action. The crystalline form of Hyamine 1622 is readily soluble in water and other solvents. It can be formulated into powdered or liquid products for industrial or household sanitizers and disinfectants. Hyamine 1622 may also be useful in veterinary and proprietary pharmaceutical products as preservatives or antimicrobial agents for topical applications. Hyamine germicides are not corrosive to environmental surfaces when used according to directions.

Chemical and Physical Properties



Composition: Hyamine 1622 is diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride monohydrate; molecular weight 466.1

Appearance: Crystalline Hyamine 1622 is a white free-flowing powder. Hyamine 1622-50 percent is a moderately viscous light amber liquid.

Density: Hyamine 1622 Crystals: 27.5 lbs./ft.^{3}
Hyamine 1622-50%: 8.56 lbs./gallon.

Solubility: Hyamine 1622 is soluble in water, the lower alcohols, glycols, ethoxyethanol, ethyleneglycol monomethyl ether, tetrachlorethane, and miscible with ethylene dichloride and carbon tetrachloride. It is insoluble in aliphatic hydrocarbons.

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Compatibility: The compatibility of Hyamine 1622 was determined by mixing dilute solutions of the germicide with solutions of substances with which it might be combined in formulations. The presence or absence of turbidity indicates physical compatibility, but final formulations should be carefully evaluated for both physical and bactericidal stability.

Compatible	Partially Compatible	Incompatible
Acetic acid Aluminum chloride Borax Calcium chloride Citric acid Nonionic wetting agents Polyoxyalkylene lanolin Potassium hydroxide Sodium acetate Sodium bicarbonate Sodium carbonate Sodium hydroxide Sodium nitrate Sodium sulfate Tetrasodium pyrophosphate Trisodium phosphate	Potassium iodide Sodium hexametaphosphate* Sodium metasilicate Sodium tetraphosphate* Sodium tripoly-phosphate*	Anionic wetting agents Potassium chromate Potassium dichromate Soaps Sodium heptaphosphate

*Only in dilute solutions or in the presence of sufficient nonionic wetting agents or other suitable solubilizer.

Note: Hyamine biocides should not be used or compounded with oxidizing agents such as calcium hypochlorite, nitric acid or solid perchlorates, since mixtures of most organic materials with oxidants may be explosive.

AOAC Dilution

The AOAC Use Dilution Confirmation Procedure is used to determine the effective disinfectant concentration for hard surfaces using the two basic index bacteria, *Staphylococcus aureus* and *Salmonella choleraesuis*. If the product is to be used in hospitals, the effective disinfectant use dilution for *Pseudomonas aeruginosa* PRD-10 must be also determined.

The effective disinfectant concentrations of Hyamine 1622 (Crystals or 50 percent solution) for the microorganisms are:

Organism	Disinfectant Concentration of Active Ingredients, ppm	Disinfectant Dilution of Active Ingredient
Staphylococcus aureus	500	1:2000
Salmonella choleraesuis	500	1:2000
Pseudomonas aeruginosa PRD-10	1000	1:1000

Microbistatic and Microbicidal Titers

The maximum microbistatic and microbicidal dilutions of Hyamine 1622 are determined by means of serial dilutions in broth tubes inoculated with a fresh culture of the organism to be tested. After 24 hours of incubation, transfers are made from the tubes showing no growth to fresh broth containing a quaternary inactivator. After an additional 24 hours of incubation (total incubation time 48 hours) the initial set of tubes is reexamined for growth. The highest dilution of active ingredient preventing growth in the subculture tubes (quaternary inactivator present) after a total incubation time of 48 hours is the microbicidal titer.

Note: These values indicate the effect of Hyamine 1622 on these microorganisms under constant contact conditions as in preservative applications. They should not be interpreted as indicating the effective concentration for disinfecting or sanitizing applications.

Microbistatic and Microbicidal Titers of Hyamine 1622

Organism	Dilution of Active Ingredient	
	Bactericidal	Bacteriostatic
Streptococcus pyogenes C-203	1: 50,000	1: 50,000
Streptococcus viridans	1:200,000	1: 400,000
Escherichia coli	1: 16,000	1: 16,000
Salmonella gallinarum	1: 32,000	1: 32,000
Salmonella choleraesuis	1: 32,000	1: 32,000
Salmonella typhimurium	1: 16,000	1: 16,000
Salmonella schottmuelleri	1: 8,000	1: 32,000
Pseudomonas aeruginosa PRD-10	1: 8,000	1: 8,000
Lactobacillus casei	1: 50,000	1: 100,000
Shigella sonnei	1: 16,000	1: 32,000
Klebsiella pneumoniae	1: 16,000	1: 16,000
Saccharomyces cerevisiae	1:100,000	1: 100,000
Pityrosporum ovale	1:800,000	1: 800,000
Trichophyton interdigitale	1: 20,000	1: 40,000
Monilia albicans	1:400,000	1: 800,000
Aspergillus niger	1: 400	1: 800
Aspergillus oryzae	1:800,000	1:1,600,000
Penicillium notatum	1:800,000	1: 800,000
Penicillium luteum	1:400,000	1: 800,000

Effect of Hard Water on Antimicrobial Activity

Hyamine 1622 retains its effectiveness as a hard-surface disinfectant and preservative in the presence of hard water, but, like other quaternaries, its speed of action is slowed by the presence of calcium and magnesium salts. The effectiveness of hard-surface sanitizers is determined by the AOAC Germicidal and Detergent Sanitizer Test. Since this procedure requires that a 200-ppm active quaternary solution kills 99.999% of an *E. coli* culture in 30 seconds, the speed of kill is a determining factor in selecting a sanitizing agent.

The hard-water ceiling of Hyamine 1622 is relatively low, and it should be formulated with inorganic or organic sequestering agents to improve sanitizing performance in hard water. For 200 ppm active solution of Hyamine 1622, the limiting water hardness of $CaCO_3$ is 25 ppm.

Surface Tension

Hyamine 1622 is a surface-active compound and displays typical surfactant characteristics, such as the reduction of surface tension, foaming and penetration.

Surface Tension and Interfacial Tension				
Dynes Per Centimeter				
Hyamine 1622 Active Concentration	**1.0%**	**0.1%**	**0.01%**	**0.001%**
Surface Tension	33	30	45	55
Interfacial Tension*	4.9	2.6	15.8	25.8
*Interfacial tension vs. Arcoprime 90 mineral oil. All data via duNouy tensiometer at 25°C.				

Applications

Hyamine 1622 Crystals are powdered crystalline quaternary germicides and are especially suited for formulating dry powdered products. By mixing Hyamine crystals with inert compatible diluents, powdered disinfectant or sanitizer formulations can be prepared.

Convenience and exactness of dosage contribute to the popularity of tablets. Hyamine 1622 Crystals can be made into sanitizing tablets containing up to 50-percent active quaternary. As noted above, this product has a relatively low hard-water ceiling, and sequestering agents should be employed to improve the efficiency of the product in hard water. The tablet manufacturer should evaluate his final composition by the AOAC Germicidal and Detergent Sanitizer Test to assure proper sanitizing performance in his marketing area. Other excipients, such as lactose, ammonium sulfate, sodium bicarbonate, citric acid and other highly soluble substances, may be used to optimize the physical properties of the finished tablet.

Specialty Applications

Disinfectant formulations for surgical instruments, barber's tools, and dentures can be prepared in the form of aqueous concentrates, powders, or tablets. A quaternary concentration of 1000 ppm is required for instrument disinfection.

Hyamine 1622 finds wide use in the veterinary field as a disinfectant and a deodorant. Tinctures and lotions may be considered for topical antiseptics and for storage of sterile instruments.

Pharmaceutical formulations are subject to the New Drug Regulations of the Federal Food, Drug, and Cosmetic Act, as amended, pertaining to the safety and effectiveness of such products.

General Deodorization

Hyamine 1622 is a true deodorant and destroys rather than masks obnoxious odors. Odors due to microbial decomposition are also eliminated by the destruction of the microorganism.

A 1:1000 dilution of active ingredient of Hyamine 1622 products, applied by mopping, spraying, or sponging, quickly controls cooking odors, odors of putrefaction or personal illness, and odors in public rooms without contributing a chemical or disinfectant odor.

Algae Control

The growth of algae in swimming pools can be controlled by very low concentrations of Hyamine germicides. Concentrations of only 10 ppm active Hyamine 1622 kill most strains of algae, and levels of only 5 ppm can maintain algaestatic conditions. Premeasured quantities of Hyamine 1622 Crystals can be packed in water-soluble plastic and added to the water system as required. At the algaecidal concentration of 10 ppm active ingredient, Hyamine 1622 cannot replace chlorine as a disinfectant, but its algaecidal activity helps maintain the chlorine level.

Algae, like most organisms, may develop resistance to toxicants and necessitate a change in treatment. Should algae show resistance to Hyamine 1622, a shock treatment with a formulation of Hyamine 3500 at 5 ppm active ingredient is recommended.

Safety and Health Information

Danger: Keep out of the reach of children. Corrosive. Causes severe eye and skin damage. Do not get in eyes, on skin or on clothing. Wear goggles or face shield and rubber gloves when handling. Harmful or fatal if swallowed. Avoid contamination of food. When handling crystals, use an exhaust fan to keep dusts away from operators.

First Aid: In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. For eyes, call a physician. Remove and wash contaminated clothing before reuse.

If swallowed, drink promptly a large quantity of milk, egg whites, gelatin solution, or, if these are not available, drink *large* quantities of water. Avoid alcohol. Call a physician *immediately*.

Note To Physician: Probable mucosal damage may contraindicate the use of gastric lavage. Measures against circulatory shock as well as oxygen and measures to support breathing manually or mechanically may be needed. If persistent, convulsions may be controlled by the cautious intravenous injection of a short-acting barbituate drug.

Toxicity

Acute Toxicity — The acute oral toxidity (LD_{50}) of Hyamine 1622 in rats is 420 ± 25 mg/kg. Deaths occurred between 24 and 60 hours. Signs of toxicity are associated with severe depression.

The intraperitoneal and intravenous toxicities (LD_{50}) of Hyamine 1622 in rats are 33.1 ± 2.5 mg/kg and 19.1 ± 0.8 mg/kg, respectively

Eye irritation studies indicated that Hyamine 1622 in concentrations of 1 percent, 5 percent, and 10 percent produced progressively greater irritation manifested by varying degrees of erythema and conjuctivitis. A 0.01 percent solution instilled into the eye produced no irritation. The higher concentrations produced no irritation when the eyes were washed immediately after instillation.

On mucosal tissue 0.1 percent solutions produced no irritation, but some irritation resulted from the application of a 0.2 percent solution.

On the skin of rabbits and dogs, solutions of 1.0 percent caused erythema, blanching, and edema. A single application of a 0.6 percent solution was non irritating to the skin of dogs.

Repeated Dermal Application — Hyamine 1622 was applied to the skin of rabbits daily, 5 days per week, for 4 weeks at a concentration of 0.1 percent in water. No dermal or systemic effects were observed at this dilution, but a 1 percent solution produced erythema after 7 daily applications to the skin.

Chronic Toxicity — Hyamine 1622 was administered to rats in their diets for 2 years at levels of 0, 50, 200, 1000, 2500, and 5000 ppm. At the 5000 ppm level, growth was retarded and mortality rate increased. Histopathological examination showed that the testes of male rats were atrophied at the 2500 and 5000 ppm levels. No other pathology was found and no change in appearance, behavior, hematology, or tissues was observed.

Chronic dietary administration for one year to dogs at levels of 0, 5, 100, and 500 ppm produced no adverse effects on growth or survival. No hematologic or pathologic changes were noted.

Federal Registration

Microbicidal formulations containing Hyamine 1622 must be registered with the Environmental Protection Agency (EPA). Manufacturers of microbicidal formulations should consult with EPA for information on the procedures to obtain registration. Lonza Inc. will be pleased to advise applicants on the status of our own materials with respect to registration procedures.

The seller makes no warranty, expressed or implied, concerning the accuracy or any results to be obtained from the use of any information and no warranty expressed or implied concerning the use of the products other than indicated above. The buyer assumes all risks of use and/or handling. No statement is intended or should be construed as a recommendation to infringe any patent.

Skin Bacteria and Their Role In Infection
Maibach & Hildick-Smith ed.

237

F58

19

Removal of Bacteria from the Operation Site

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Almost all of the equipment used in modern surgery can be sterilized by heat, ionizing radiations, or ethylene oxide. The transition from antiseptic to aseptic surgery would be virtually complete were it not for the skin of operation sites which, today as in Lister's time, require antisepsis. The skin, moreover, is a medium in which bacteria, sometimes including pathogenic staphylococci, can multiply and may be very numerous. A residue of bacteria is likely to persist after disinfection of the skin, so it is important for the surgeon to minimize the risks of transferring bacteria to the tissues by using the most effective antiseptics.

An antiseptic for use on an operation site must act rapidly against a wide range of microorganisms; it must kill the organisms, not merely inhibit their growth, and it must combine these properties with an absence of toxic or sensitizing action on the skin or underlying tissues. The requirements for disinfection of an operation site are in some respects different from those of disinfecting the surgeons' or nurses' hands. Repeated washing with antiseptic soaps or detergents can cause a progressive reduction and maintenance of the hand flora at a low level; this is commonly achieved by the use of a slow-acting antiseptic, hexachlorophene, which causes little immediate reduction in the skin flora on first application. For the operation site, on the other hand, it is often necessary to achieve maximum disinfection in a single application, and quick-acting antiseptics are essential. When repeated applications are possible (e.g., in "cold" orthopedic operations) the cumulative action of antiseptics can be used, but the situation is still rather dif-

ferent from that of disinfecting the surgeon's hands; e.g., the removal of desquamated epithelium by washing with a detergent under running water is usually not practicable on an operation site. Disinfection is directed against both resident and transient organisms; but while in disinfection of the nurse's hands it is necessary to consider such transient organisms as the food poisoning and dysentery bacilli, the organisms against which antiseptics are directed in preparation of the operation site are wound pathogens and especially *Staphylococcus aureus* colonizing the skin (resident staphylococci). Transient organisms which may be important in operation sites are the clostridia of tetanus and gas gangrene, and these present special difficulties when it is necessary to operate on hands (e.g., of a garage mechanic or gardener) with thick horny skin and ingrained dirt.

Although nearly 100 years have passed since Lister published his first report on antiseptic surgery, there are still no accepted standard procedures for disinfection of the skin. The variety of practice is not surprising, for there have been many new antiseptics and few critical and comparative studies of their value in disinfecting the operation site. There has been confusion, too, because some authors studied the effect of antiseptics on transient organisms, which are readily removed by antiseptics (or by ablution), while others studied their effect on the resident organisms which are much more resistant to antiseptics and virtually unaffected by ablution without antiseptic.

Lister's [*1*] practice was to apply a 5 per cent solution of phenol for a few minutes—longer exposure he considered to be unnecessary and potentially harmful; he also deprecated the use of soap and water because, in his view, the full antiseptic action of phenol depended on its attachment to skin fats. Halsted [*2*] preferred mercuric chloride for preoperative treatment on the skin. This and other mercurial antiseptics became very popular, but were later shown to have little bactericidal activity [*3, 4*]; their apparent value in laboratory tests, which had deceived Robert Koch and many subsequent workers, was due to the transfer of strongly bacteriostatic traces of the antiseptics to culture media used in the tests.

At the end of the nineteenth century a number of other antiseptics gained favor, including zinc chloride, tincture of benzoin, and iodoform [*5*], but their value was probably even less than that of the mercurials. Two agents, ethyl alcohol [*6*] and iodine [*7*], which were introduced at about that time, are still counted among the most effective skin antiseptics available. Both are rapidly bactericidal toward a wide range of vegetative organisms, and in combination they are found to

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be even more effective than when used alone. Price [8] showed that ethyl alcohol has optimal activity in aqueous solutions of 70 per cent to 90 per cent; its freedom from irritant effects and its rapid evaporation make it a convenient and valuable skin antiseptic (except inside the mouth, where it appears to have little antiseptic action [9]). Iodine in alcohol or in aqueous solution with potassium iodide has been widely accepted, but it has the disadvantage of causing severe sensitivity reactions in some patients [*10, 11*].

Many other antiseptics have been used for the operation site in the past 50 years, and a few compounds or groups have been widely adopted. The quaternary ammonium compounds [*12, 13*] (e.g., cetrimide, benzalkonium chloride) became popular because they appeared to have good activity and combined antiseptic properties with detergent action; with alcohol they were found (unlike the mercurials) to have an additive effect [*14*]. Like the mercurials, however, they are apt to give misleading results in laboratory tests through transfer to culture media of bacteriostatic traces of the antiseptic [*15*]. They also appear to form a film on the skin over viable organisms [*16*], and they are deficient in activity against some Gram-negative bacilli (especially *Pseudomonas pyocyanea*). The acridines [*17*] (e.g., proflavine, 5 amino-acridine) are relatively non-toxic and retain their activity in the presence of organic matter. Their action, however, is slow and their effects are bacteriostatic. For these reasons they are not well suited for disinfection of the operation site. An alcoholic solution of one of the acridines (acrizane), however, was claimed by Murphy and his colleagues [*10*] to be more active than a number of other skin antiseptics, including 2 per cent iodine in alcohol. Complexes of iodine with organic solubilizers (the iodophors) have been developed and found to have skin disinfecting properties comparable with those of aqueous or alcoholic iodine, but to be free (or at least relatively free) from irritant and possibly from sensitizing properties [*18, 19*]. Two other antiseptics which have been found to have a particular value in skin disinfection are chlorhexidine [*20, 21*] and laurolinium [*22, 23*]. Comparative studies of their antiseptic properties are described below.

METHODS OF TESTING

The main criteria which have been used in assessing the value of the antiseptics are (1) removal of transient flora, represented by cultures of bacteria deposited on the skin; (2) removal of resident and transient flora as shown by viable counts of washings from the skin; (3)

removal of resident and transient organisms, as shown by cultures of skin biopsy; and (4) reduction of postoperative wound contamination and sepsis.

Tests showing the removal of bacterial cultures allowed to dry on the skin have some practical advantages. Pure cultures of staphylococci and other pathogens can be used, and a quantitative assessment of reduction in their numbers can easily be made. The method has been used in various ways [*24, 25, 26*]. In a comparison of different antiseptics used for the removal of applied bacteria, Gardner [*25*] showed that at least 99.9 per cent of the applied organisms were removed from skin treated for 20 seconds with 2 per cent iodine in 70 per cent alcohol, and this form of disinfection was the most effective of those tested.

The use of applied indicator organisms, however, provides a limited and artificial criterion. By showing the effect of antiseptics only on the superficial flora it exaggerates their practical effectiveness, and even as a test for removal of superficial organisms it has the flaw of artificiality, the level of contamination being different from that which occurs in nature. Tests for the removal of naturally occuring organisms (resident and transient) on the skin were developed by Price and others [*27, 28, 29*] and these have shown a much less complete removal of bacteria by antiseptics. For example, two minutes' application of 2 per cent iodine in 70 per cent alcohol caused a reduction of 95 per cent in the resident flora as shown by Price's serial basin test.

Culture of skin biopsy [*10, 20, 25, 30*] has been used less than other methods. It has the theoretical advantage of allowing assessment of the removal of bacteria from the full thickness of the skin. Paradoxically, though, this method has provided a flattering and improbable account of the value of certain skin antiseptics. Myers, Mackenzie and Ward [*20*] found that 23 out of 24 biopsies from skin previously disinfected with 0.5 per cent aqueous chlorhexidine were free from detectable bacteria. But the biopsies used in this method consisted of small fragments of skin, some of which yielded no bacteria even when the skin had not been disinfected. Tests in which samples from wider areas of the surface are used probably give a fairer assessment of antiseptics.

Assessment of the effect of skin antiseptics on the incidence of wound contamination and postoperative sepsis should give the most convincing evidence of their value. On the other hand, the difficulties of obtaining consistent estimates of sepsis acquired at operation and its generally low incidence have limited the practicability of this

method. Reports of the virtual elimination of sepsis following the introduction of a particular antiseptic [*31*] conflict with the regular finding of a proportion of patients (often about five per cent) who develop sepsis, whatever form of skin disinfection is used [*32, 33, 34*]. Some of the sepsis occurring after operations is undoubtedly acquired after the patient returns to the ward [*35, 36*] and even the most perfect preoperative skin antiseptics cannot prevent this. When the sepsis rate is low, an improvement in skin antiseptics cannot be expected to cause more than a marginal reduction in sepsis.

COMPARATIVE ASSESSMENTS OF ANTISEPSIS FOR THE OPERATION SITE

We have studied the value of a number of alternative agents, including some of the newer antiseptics, in the removal of bacteria from the skin of operation sites. Most of this work has been concerned with the natural, including resident, flora, but we also made some experiments on cultures of *S. aureus* applied to the skin to simulate transient flora. We examined the proportions of bacteria removed by different antiseptics, the value of repeated applications, the influence of time and manner of application of antiseptics, and the removal of bacterial spores from the skin with an iodophor preparation.

Removal of Superficial Flora. Our method was based on one described by Story [*26*]. Three circular areas were marked out on the front of each forearm. One drop (0.02 ml) of washed suspension of a penicillin-resistant *S. aureus* and (in some experiments) *P. pyocyanea* was spread over each area and allowed to dry. When dry, four out of the six sites were treated with antiseptic, which was spread over the whole area and kept moist for approximately two minutes. To assess the number of bacteria remaining in these areas and in the control areas which were not treated with antiseptic, the skin of the marked areas was rubbed in a standard way with a glass spreader for 15 seconds under 5 ml of Ringer's solution which was held in position by a truncated glass cylinder. Viable counts of the bacteria in the sampling fluid were made in pour plates containing appropriate neutralizers and 0.3 units of penicillin per ml to inhibit the growth of normal skin flora. Tests for carry-over of antiseptic to culture media were made, and when these were positive multiple pour plates of diluted sampling fluid were prepared.

The results (Table 19-1) showed no detectable indicator organisms after two minutes' treatment with 70 per cent ethyl alcohol, with

Table 19-1

Survival of "Transient" S. aureus on Skin After Two Minutes' Treatment of Skin with Antiseptics

Antiseptic	Viable counts per ml washings from untreated areas	Per cent organisms surviving after treatment*	Tests
Ethyl alcohol (70%)	236,000	<0.0004	3
Lugol's iodine (1%)	282,000	<0.0003	2
"Virac"	282,000	<0.0003	2
Chlorhexidine (0.5%) in water	282,000	<0.0003	2
Cetrimide (2%) in water	209,000	>0.09–>0.14	2
Penotrane (0.1%) in water	1,600–81,000	6.4–12.2	4

* Viable counts from treated areas expressed as percentage of viable counts from untreated areas.

aqueous solutions of chlorhexidine (0.5%), with Lugol's iodine (1%) or with an iodophor (Virac); after treatment with a mercurial antiseptic (0.1 per cent aqueous phenyl mercuric dinaphthyl methane sulphonate, or "penotrane") and with 1 or 2 per cent aqueous cetrimide, appreciable numbers of the test organisms survived.

These results illustrate the considerable effect which certain antiseptics can be expected to have on the superficial vegetative flora of the skin. It has been shown, however, in studies on disinfection of the surgeon's hands, that superficial organisms can be removed almost as effectively by ablution with soap and water as with antiseptics [37]; and while it is important for the surgeon to remove such transient contaminants from the operation site, the more important and more difficult objective is to remove the natural flora, including resident organisms.

Removal of Natural Flora [21, 23]. The hands were first washed with ordinary soap under a running tap for 30 seconds and dried. A sample was then taken by a standard washing of the hands in a bowl containing 100 ml of Ringer's solution with neutralizers. The hands were then rinsed in running water, dried, and treated with the antiseptic by applying it with gauze over the whole surface of both hands and keeping the whole surface visibly moist for about two minutes. A second sample of handwashings was then taken by the same method as the

Minutes' Treatment

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first sample. In control experiments the hands were rinsed briefly under a cold tap instead of being treated with antiseptic. To assess the comparative value of antiseptics we tested each of the agents and a control treatment on each of the experimental subjects, using a Latin-square design [38]. Two experiments were made, the results of which are summarized in Table 19-2. In the first experiment alcoholic solutions

Table 19-2

Mean Reduction in Bacterial Count from Handwashing after Skin Disinfection, Expressed as Percentage of Initial Count at 10^{-3} Dilution (Latin Square Design)

Treatment	First experiment: 7 replicates on 7 subjects; S.E. of percentage = 4.5	Second experiment: 6 replicates on 6 subjects; S.E. of percentage = 4.4
Ethyl alcohol (70%)	68.6	—
Alcoholic iodine (1%)	80.7	—
Lugol's iodine	67.4	—
Alcoholic chlorhexidine (0.5%)	81.3	84.0
Aqueous chlorhexidine (0.5%)	60.7	—
"Virac"	58.3	—
Povidone iodine	—	62.3
Alcoholic laurolinium (5%)	—	84.4
Aqueous laurolinium	—	81.2
Laurolinium spray	—	58.2
Control	14.7	8.5

of iodine (1%) and chlorhexidine digluconate (0.5%) had approximately the same effect, and were significantly more effective than the other agents tested (70% ethyl alcohol, Lugol's iodine, 0.5% aqueous chlorhexidine, and Virac). The largest mean estimated reduction in skin flora was 81.3 per cent with alcoholic chlorhexidine. In two separate tests by this method (not shown in Table 19-2) the mercurial antiseptic "penotrane" caused reductions of 28 and 38 per cent respectively, and therefore appeared to be considerably less effective than any of the agents tested in the first Latin-square experiment. In the second Latin-square experiment alcoholic chlorhexidine, alcoholic laurolinium, and aqueous laurolinium caused a similar reduction in the skin flora, but an iodophor (povidone iodine) and the application of laurolinium as a spray were significantly less effective.

These experiments suggested that equally good results could be ob-

tained by the use of alcoholic iodine or chlorhexidine, or by laurolinium either in alcohol or in aqueous solution. Application of antiseptics on gauze with friction appeared to give better results than spraying the surface with antiseptic from a pressure pack. Since iodine may cause sensitization and laurolinium has toxic action on skin tissue culture [39], while chlorhexidine has neither of these adverse effects, our experiments favored the use of alcoholic chlorhexidine.

Repeated Application of Antiseptics. It is common practice in orthopedic surgery to prepare the skin by repeated cleansing and disinfection for two or three days before an operation; it is assumed that the skin can be made bacteriologically cleaner by this procedure than by a single preparation on the day of operation. We have conducted some experiments to assess the validity of this concept.

Table 19-3

Effects of Repeated Disinfection with 70% Ethyl Alcohol (2 minutes) on Six Subjects*

Experiment	Reduction in bacterial counts from handwashings expressed as per cent initial count after		
	1st treatment	2nd treatment	3rd treatment
1	34	90.7	88
2	63	94.6	92.4
3	77	69	70
4	82	87	90
5	57	08	81
6	74	90	91

* Courtesy, *British Medical Journal*

We tested first the value of repeated two-minute treatments of the hands with 70 per cent ethyl alcohol on six subjects; the second and third treatments were given immediately after the samplings that followed the first and second treatments. In all but one of these subjects (see Table 19-3) the proportional reduction in skin flora after the first treatment was greater than after the second or third treatment, and in all but one there was no further reduction in bacterial count after two treatments. It appeared that more than two treatments, each of two minutes, would add nothing to what was achieved by two successive treatments with alcohol. But the data obtained in this study showed, in effect, that an application of antiseptic for six minutes is not more effective than one of four minutes. When some time is allowed to pass between antiseptic applications quite a different result

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is obtained, as we found in the following experiment [23] (see Table 19-4).

Table 19-4

Effect of Repeated Disinfection of Hands with Hexachlorophene Detergent Cream Followed by Treatment with Alcoholic Chlorhexidine Solution*

Subject	Percentage survival of skin flora after		
	One washing with pHisoHex®	Nine washings with pHisoHex®	Nine pHisoHex® washings followed by 2 minutes' treatment with alcoholic chlorhexidine
A.J.	92.4	0.29	0.063
M.W.	47.6	0.05	0
H.L.	65.0	0.81	0.030
J.O.	79.2	0.40	0.013
M.S.	70.4	0.03	0
A.K.	45.1	0.85	0.005
V.B.	21.2	0.18	0
G.B.	36.3	0.69	0
M.F.	86.1	0.31	0.015
Mean	60.4	0.40	0.014
Sd. mean	8.9	0.101	0.007

* Courtesy, *British Medical Journal*

A hexachlorophene detergent cream (pHisoHex®) was used four times a day by nine subjects for washing their hands. Before and after the first washing and again after the ninth washing the hand flora was estimated by a standard rinsing technique. This showed the progressive reduction in skin flora which is well known to follow repeated applications of hexachlorophene. The hands were then treated as in a preoperative preparation with two-minutes' application of 0.5 per cent chlorhexidine in alcohol. This caused a further reduction of skin flora, which was no less than the reduction found on similar treatment of skin which had not been prepared with hexachlorophene. The mean estimated reduction in skin flora after repeated treatments with hexachlorophene and final treatment with alcoholic chlorhexidine was 99.98 per cent; four out of nine subjects showed no detectable bacteria in skin washings after this treatment.

Repeated use of chlorhexidine at intervals, as of hexachlorophene detergent preparations, was shown to cause a progressive reduction in skin flora [23]. Other antiseptics can obviously be used to achieve a virtual elimination of the natural flora, but there may be some advan-

tages in the use of hexachlorophene preparations, which leave an active residue on the skin and prevent the growth of staphylococci with which the skin may become contaminated between treatments [23]. Since hexachlorophene preparations have little immediate action, however, it is important to use an agent with quick activity, such as chlorhexidine in alcohol, for the final treatment.

Removal of Clostridial Spores from Operation Sites

The spores of *Clostridium tetani* and of gas gangrene bacilli present a special problem in skin disinfection. Ethyl alcohol is totally inactive against them and, of the other antiseptics, only iodine has a potentially useful activity. In the conventional two minutes' skin preparation, however, iodine cannot destroy more than a small proportion of the spores exposed to it, and longer application of alcoholic or aqueous iodine solutions causes irritation and deep staining of the skin. When it is necessary to operate on hands with thick skin and ingrained dirt it is commonly thought that the most one can do is to remove the desquamated epithelial cells that carry the spores through repeated washing with detergent solutions or soap over a period of days.

Table 19-5

Removal of *B. subtilis* var. *globigii* Spores from Skin by Povidone Iodine (PVI)*

Minutes of exposure to PVI or control compresses	Viable counts of *B. subtilis* in washings from skin as per cent counts of initial washings taken when no compresses had been applied					
	Experiment 1		Experiment 2		Experiment 3	Experiment 4
	PVI	control	PVI	control	PVI	PVI
15	0.20	30	1.13	68	0.00	1.56
30	0.15	40	0.47	76	0.47	2.17
60	0.05	—	0.32	—	0.33	1.17
90	0.06	—	0.21	—	0.15	0.42
120	0.05	—	0.02	—	0.13	0.14

* Courtesy, *British Medical Journal*

With the introduction of the iodophors, preparations of iodine became available which could be applied to the skin for longer periods without causing irritation or staining.

We studied the value of an iodophor (povidone iodine, a complex of iodine with polyvinyl pyrrolidone) in the removal of spores of *Bacillus subtilis* var. *globigii* [23]; povidone iodine was found to have

about the same sporicidal activity in spore suspensions of these organisms and of *Clostridium welchii*. On measured circular areas of skin of the forearm, 0.02 ml amounts of a suspension of *B. subtilis* var. *globigii* were inoculated, spread and allowed to dry, after which they were covered with compresses of lint soaked with povidone iodine; control areas were treated with compresses of distilled water. Viable counts of the indicator organism in washings taken from areas treated with the iodophor showed a mean reduction of 99.1 per cent in 15 minutes (Table 19-5). A similar effect on *C. welchii* and on other clostridial spores seems likely.

EFFECT OF SKIN ANTISEPSIS ON COLONIZATION AND INFECTION OF OPERATION WOUNDS

For some years in our hospital we have kept a wound book and also taken swabs from the depth of clean operation wounds after incision and again before insertion of sutures. During this period we introduced improved methods of skin disinfection—for the operation site alcoholic chlorhexidine in place of aqueous cetrimide, and for the surgeons' hands scrubbing up with a hexachlorophene detergent cream or liquid soap instead of ordinary bar soap.

Preliminary assessments of this study, which will be reported later, show that the frequency of wound contamination and, to a smaller extent, sepsis has been reduced in the period following these improvements.

SUMMARY

From the experimental data we may conclude that the skin of operation sites can lose a large part (99.9% or more) of its natural flora, including the resident organisms, when prepared by repeated application of hexachlorophene detergent cream followed by a final preparation with 0.5 per cent chlorhexidine in alcohol; a single treatment with chlorhexidine in alcohol will cause a smaller reduction (between 80 and 90%) in the natural skin flora. The effect of these measures on resident *S. aureus* is likely to be no less than it is on the total organisms, to judge from tests in which the reduction of total organisms and *S. aureus* after disinfection were both measured. Spores of *B. subtilis* var. *globigii* (and, by inference, probably also of *C. tetani* and *C. welchii*) can generally be reduced to less than one per cent by application for 15 minutes of a compress soaked in povidone-iodine surgical scrub.

Postoperative infection may be derived from various sources. Even

if it were possible to eliminate the skin flora completely at the time of operation, this could not be expected to cause more than a partial reduction in the incidence of sepsis, because there would still be opportunities for the wound to become contaminated by airborne bacteria or by the returning skin flora after the patient has gone back to the ward. The evidence that such a reduction has probably occurred after improvements in the routine skin disinfection of one hospital is encouraging. The more strenuous techniques of repeated disinfection and of spore destruction can reasonably be reserved for patients who are exposed to special risks; but in all patients preparation of the operation site should be of a type which will ensure the removal of a large proportion at least of the resident skin flora.

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hypercapnia there is an upper blood pressure limit beyond which autoregulation fails and cerebral blood flow increases (Ekström-Jodal *et al.*, 1971). Such an upper limit of autoregulation was found in four of our patients but seemed unrelated to their $Paco_2$. No evidence of arteriolar spasm at high pressure was found in any of the patients studied. The 5 to 10 minutes of steady state maximum blood pressure should be ample time for autoregulation to operate, since this mechanism is known to be fully effective within 1 minute after blood pressure changes (Symon *et al.*, 1971). The fact that a similar increase in cerebral blood flow was found in one normotensive patient with a mean arterial blood pressure above 120 mm Hg suggests that the phenomenon is not caused by hypertensive vascular disease per se; it seems more likely that an upper limit of autoregulation is present in all persons and that even this upper limit is raised in hypertension.

None of our patients had symptoms during the short period of increased blood pressure. A sustained increase in blood pressure with hyperperfusion of the brain would be expected to cause exudation of plasma through the walls of arterioles and capillaries, this in turn giving rise to focal cerebral oedema, compression of capillaries, a decrease in cerebral blood flow, and the clinical picture of acute hypertensive encephalopathy (Byrom, 1969; Lassen and Agnoli, 1972). The initial event in this pathogenetic chain has been termed a "break-through of autoregulation" (Lassen and Agnoli, 1972). On a smaller scale similar phenomena may occur during sleep hypercapnia, causing the well known morning headache of the hypertensive patient.

Further investigations on the pathogenesis of hypertensive encephalopathy probably must rely on animal experiments with repeated measurements of cerebral blood flow with direct methods at very high blood pressure.

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Use of 4% Chlorhexidine Detergent Solution (Hibiscrub) and Other Methods of Skin Disinfection

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Summary

In a comparison of three antiseptic detergent preparations for hand washing, Hibiscrub, a 4% chlorhexidine detergent solution, caused a significantly greater estimated immediate reduction of skin flora (86·7% ± 3·0) than was obtained with Dermofax, a 0·75% chlorhexidine detergent solution (55·5% ± 5·1), or with Disadine scrub, a povidone iodine detergent preparation (68% ± 6·8). After six applications the mean estimated reductions of skin flora were 99·2% ± 0·2 for Hibiscrub, 97·7% ± 0·7 for povidone iodine, and 91·8% ± 1·6 for Dermofax.

After a series of hand washings with Hibiscrub, as with a hexachlorophane detergent preparation, a further large reduction of skin flora, shown by bacterial counts of hand sampling, was obtained by a second phase of disinfection consisting of two minutes' application on gauze swabs of 0·5% chlorhexidine digluconate in 70% ethanol; a further wash with Hibiscrub, in place of alcoholic chlorhexidine, for the second phase of disinfection caused an increase rather than a reduction in the yield of bacteria on skin sampling. Unlike this "two-phase" disinfection, the application for 30 minutes of compresses soaked in 10% aqueous povidone iodine or in 0·5% aqueous chlorhexidine digluconate did not cause a greater reduction in skin flora than that obtained by the conventional two minutes' application on gauze of 0·5% chlorhexidine in 70% ethanol.

Chlorocresol (0·3%) liquid soap (the base used for Ster-Zac liquid hexachlorophane soap) caused a mean reduction of skin flora when used for hand washing of 29% after one application and 72% after six applications spread over two days. This formulation, though less active and more variable as a detergent skin antiseptic than chlorhexidine, hexachlorophane, or povidone iodine detergent preparations, is an inexpensive disinfectant soap which could be useful in catering establishments. Alcoholic cetrimide applied as for disinfection of an operation site caused a reduction of skin flora greater than that shown by aqueous cetrimide but comparable to that shown by 70% ethyl alcohol in previous experiments.

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Introduction

Several developments in the technique of skin disinfection, including the use of a 0·75% chlorhexidine detergent solution for the hands, were recently reported from this unit (Lilly and Lowbury, 1971). Since then a more concentrated chlorhexidine preparation, Hibiscrub, has been introduced. In this paper we

TABLE I—*Disinfection of Hands: Reduction in Bacterial Counts from Hand Washings after Disinfection, Expressed as Percentage of Initial Count*

Replicate Experiments	(A) 4% Chlorhexidine Detergent Preparation (Hibiscrub)		(B) 0·75% Chlorhexidine Detergent Preparation (Dermofax)		(C) Povidone Iodine "Scrub" (Disadine)		(D) Bar Soap (Control)	
	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications	After 1 Application	After 6 Applications
1	G.S. 71·3	99·8	A.K. 40·5	87·0	B.D. 64·8	97·5	S.D. 12·7	20·8
2	S.D. 89·2	99·1	B.D. 76·1	96·1	G.S. 83·5	99·1	A.K. −40·7*	4·6
3	A.K. 89·3	99·4	G.S. 56·0	92·1	S.D. 29·9	99·7	B.D. −23·4*	28·1
4	B.D. 94·1	98·5	S.D. 45·8	86·1	A.K. 81·1	98·4	G.S. 14·2	28·3
5	E.R. 76·9	99·2	B.G. 72·1	94·5	S.F. 77·2	99·2	A.B. 9·0	29·2
6	B.G. 93·1	98·1	S.F. 64·3	98·1	A.B. 48·2	93·8	E.R. 8·8	21·8
7	S.F. 94·2	99·9	A.B. 36·3	92·7	E.R. 81·8	98·2	B.G. 13·0	18·7
8	A.B. 85·8	99·8	E.R. 52·6	87·7	B.G. 77·3	95·5	S.F. 32·5	56·0
Mean % reduction	86·7 (±3·6)	99·2 (±0·2)	55·5 (±5·1)	91·8 (±1·6)	68·0 (±6·8)	97·7 (±0·7)	3·3 (±8·3)	25·9 (±5·1)

Initials indicate experimental subjects.
* Values indicate increase expressed as percentage of initial count.

Comparison of treatments (*t* tests):
(A) and (B), 1 application: $t = 5{\cdot}27$, $P < 0{\cdot}001$.
(A) and (B), 6 applications: $t = 4{\cdot}65$, $P < 0{\cdot}001$.
(A) and (C), 1 application: $t = 2{\cdot}51$, $P < 0{\cdot}05$.
(A) and (C), 6 applications: $t = 1{\cdot}98$, $P > 0{\cdot}05$ (not significant).
(B) and (C), 1 application: $t = 1{\cdot}46$, $P > 0{\cdot}05$ (not significant).
(B) and (C), 6 applications: $t = 3{\cdot}11$, $P < 0{\cdot}01$.
(B) and (D), 1 application: $t = 5{\cdot}3$, $P < 0{\cdot}001$.
(B) and (D), 6 applications: $t = 12{\cdot}3$, $P < 0{\cdot}001$.

report comparisons of Hibiscrub with hexachlorophane and iodophor detergent preparations, and assessments of some other methods, including two special techniques for obtaining more intensive disinfection.

Assessment of 4% Chlorhexidine Detergent Solution (Hibiscrub) for Disinfection of Hands

PREPARATIONS STUDIED

The following preparations were compared. (*a*) Hibiscrub, a 4% solution of chlorhexidine (Hibitane) digluconate (*B.P.*) in a detergent base containing 25% w/v Pluronic F87 (a non-ionic surfactant described as polyoxyethylene-polyoxy propylene block polymer) and 3·7% w/v Ammonyx LO (an amphoteric surfactant, dimethyl lauryl amine oxide). (*b*) Dermofax, a 0·75% solution of chlorhexidine digluconate in a detergent base previously described (Lilly and Lowbury, 1971). (*c*) Disadine scrub, a povidone iodine detergent solution containing 0·75% available iodine in a base containing an anionic detergent (ammonium salt of sulphated alkyl phenoxy-polyethyleneoxyethanol). (*d*) Control application—unmedicated bar soap.

METHOD OF ASSESSING DISINFECTION OF HANDS

As in previous studies (Lowbury *et al.*, 1960, 1963; Lilly and Lowbury, 1971) each preparation was tested on each of a series of volunteers, and a Latin square design was used. Replicate experiments were made, the four subjects in the second experiment being different from those in the first.

The following methods were used for washing hands with Hibiscrub and with povidone iodine surgical scrub. After moistening the hands with water about 7 ml of the solution was poured on to the hands and rubbed systematically over all surfaces of both hands and wrists for two minutes; the hands were then rinsed under running water. With Dermofax, the preparation was rubbed over the dry skin of both hands for one and a half minutes after which water was added to produce a lather for a further half minute, before rinsing. In the control treatment with bar soap and water the hands and wrists were systematically washed for two minutes and rinsed under a tap, as in the treatment with disinfectant-detergent preparations.

Bacterial counts on pour plates were obtained from standard hand samplings taken before and immediately after the first disinfectant and control hand washings and again after the last of five similar hand washings with the same preparations, two on the first and three on the second day. The technique of sampling was the same as that described by Lowbury *et al.* (1963). The sampling solutions and nutrient agar contained neutralizers for the antiseptics studied (1% Lubrol W, 0·5% lecithin, 1% Tween 80, and 1% sodium thiosulphate). Tests were made for carry-over of disinfectant to plates showing no growth or scanty growth (Lowbury *et al.*, 1960). An interval of at least seven days was allowed between experiments on subjects to allow a return to the normal level of skin flora.

RESULTS

The results of the trial are given in table I. Washing with Hibiscrub caused a significantly greater mean immediate reduction (86·7%) in bacterial counts from standard skin samplings than was found with Dermofax* (55·5%) or with Disadine (povidone iodine) scrub (68·0%); the reduction found after six disinfectant washes spread over two days was greater with Hibiscrub (99·2%) than with Dermofax (91·8%) ($P < 0{\cdot}01$) or with Disadine Scrub (97·7%) (not significant); each of the disinfectant treatments caused a highly significant reduction in bacterial counts from skin samplings compared with the control treatment (soap and water). There was no evidence of carry-over of disinfectant to the culture plates. An analysis of variance of one of the Latin squares is shown in table II.

TABLE II—*Disinfection of Hands (see table I): Analysis of Variance (Experiments 1–4)*

No. of Applications	Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares	Significance
1	Experiments	920·3	3	306·7	>0·2
	Treatment	20191·2	3	6730·0	<0·01
	Persons	522·1	3	174·0	>0·05
	Residual	3654·7	6	609·1	
	Total	25288·3	15		
6	Experiments	57·0	3	19·0	>0·2
	Treatment	17351·9	3	5783·9	<0·001
	Persons	156·4	3	52·1	>0·2
	Residual	226·6	6	37·7	
	Total	17791·9	15		

*The manufacturers now supply Dermofax containing a higher concentration of chlorhexidine.

TABLE III—*Disinfection of Operation Sites by Compresses, etc.: Reduction in Bacterial Counts from Hand Washings Expressed as Percentage of Initial Count*

Method of Disinfection	Replicate Experiments				Mean % Reduction
	1	2	3	4	
(*a*) Povidone iodine compress (30 min.)	B.D. 97·7	B.G. 90·9	S.D. 90	E.V. 74·4	88·3 (±4·9)
(*b*) 0·5% Aqueous chlorhexidine compress (30 min.)	E.V. 30	S.D. 63	B.G. 81·3	B.D. 76·5	62·7 (±11·6)
(*c*) 0·5% Alcoholic chlorhexidine swabbing (2 min.)	S.D. 82·2	B.D. 69·6	E.V 82·4	B.G. 89·1	80·8 (±4·1)
(*d*) Hibiscrub swabbing (2 min.)	B.G. 42·2	E.V. 84·3	B.D. 85·3	S.D. 72·4	71·1 (±10·1)

Comparison of methods:
(*a*) and (*b*) $t = 2{\cdot}04$, $0{\cdot}05 < P < 0{\cdot}1$
(*a*) and (*d*) $t = 1{\cdot}50$, $0{\cdot}1 < P < 0{\cdot}2$
(*a*) and (*c*) $t = 1{\cdot}17$, $0{\cdot}2 < P < 0{\cdot}3$
(*b*) and (*d*) $t = 0{\cdot}57$, $0{\cdot}5 < P < 0{\cdot}6$
(*b*) and (*c*) $t = 1{\cdot}48$, $0{\cdot}1 < P < 0{\cdot}2$
(*c*) and (*d*) $t = 0{\cdot}66$, $0{\cdot}4 < P < 0{\cdot}5$

Initials indicate experimental subjects.

Special Methods Designed for More Intensive Disinfection of Operation Sites

The following studies were made in order to assess methods by which the skin flora might be reduced to a greater degree than it is by the standard methods used for the operation site. Such methods would be indicated before operations on patients with enhanced susceptibility to infection—for example, in immunodeficient subjects or when immunosuppressive drugs are used.

Application of Compresses.—Compresses of povidone iodine applied for 30 minutes have been found highly effective in destroying bacterial spores on the skin (Lowbury *et al.*, 1964b; Drewett *et al.*, 1972). In this study we compared the effectiveness of (*a*) compresses containing an aqueous solution of 10% povidone iodine (Disadine) with 1% available iodine, (*b*) compresses containing 0·5% aqueous chlorhexidine digluconate solution, (*c*) 0·5% chlorhexidine gluconate in 70% ethyl alcohol, rubbed on to the skin for two minutes with gauze swabs, and (*d*) 4% chlorhexidine detergent solution (Hibiscrub) applied on a swab as with (*c*).

METHODS

After a quick "social" wash with bar soap and water, the hands were rinsed, dried on a sterile towel, and sampled for viable counts by a standard hand washing technique, with neutralizers in sampling fluids and culture media (Lowbury *et al.*, 1963). The hands were then rinsed and dried on a sterile towel. In the experiments with compresses, strips of gauze soaked in the antiseptic solutions (*a*) and (*b*) were applied over the whole surface of both hands and wrists. After 30 minutes the compresses were removed; the hands were rinsed under running warm water, dried, and sampled again for viable counts. In the experiments with alcoholic chlorhexidine (*c*), the application of this preparation to the hands was carried out as in routine preoperative disinfection. The 4% chlorhexidine detergent preparation (*d*) was applied in the same way for two minutes. Bacteriological sampling of hands before and after application of (*c*) and (*d*) was carried out as in treatment groups (*a*) and (*b*).

A Latin square design was used, all four methods being used by each of the four experimental subjects. Experiments on subjects were separated by a week or more to allow the normal levels of skin colonization to be restored.

RESULTS

These are shown in table III. Though the largest mean percentage reduction was 88% (with povidone iodine compresses) and the smallest (with aqueous chlorhexidine compresses) was 63%, the differences between them and between other pairs did not reach significance levels. Hibiscrub applied on a swab gave a lower mean estimated reduction in bacterial flora of the skin (71%) than the same preparation used in washing the hands, as shown in table I (87%). Thirty minutes' application of compresses did not offer any apparent advantage over a two minutes' application of the same compound rubbed on with gauze swabs—for example, aqueous chlorhexidine compresses in this experiment caused a mean reduction of 63%, compared with a mean reduction of 60% obtained by rubbing on of 0·5% aqueous chlorhexidine diacetate with gauze in a previous experiment (Lowbury *et al.*, 1960). The standard method of operating site disinfection with 0·5% chlorhexidine in alcohol gave a mean reduction of 80%, which was slightly (though not significantly) lower than that given by povidone iodine compresses.

Repeated Use of Detergent Antiseptic Followed by Alcoholic Antiseptic Preparation ("Two-phase" Disinfection).—Lowbury *et al.* (1964b) showed that 0·5% alcoholic chlorhexidine applied with friction, as in preoperative preparation of the skin, caused as large a reduction in bacterial flora if the skin had previously been disinfected by repeated washing with a hexachlorophane detergent cream as when the skin had not been thus disinfected; the mean estimated reduction in viable counts from skin after such "two-phase" disinfection was 99·98% and about half of the subjects on whom such treatment had been carried out showed no detectable bacteria in standard hand wash samplings. It seemed likely that the large reduction in skin bacteria caused by this treatment was due to the use of different methods of application, and possibly also to the use of different antiseptics in the two phases of treatment, the second phase causing a further reduction after an equilibrium had been established on repeated use of the first phase treatment (detergent antiseptic hand washes). The following experiments were made in an attempt to confirm and clarify this mechanism.

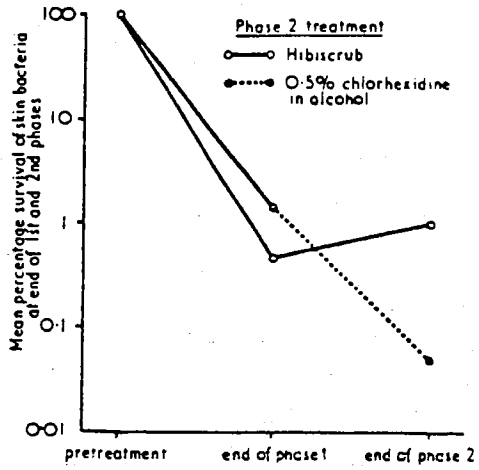
METHODS

Experiment 1.—Subjects who were using disinfectant-detergent preparations three times a day were sampled for viable bacterial counts of hand washings before the first use of the preparation and after the first, third, sixth, ninth, and twelfth hand wash. Experiments were made on four subjects, two each with Hibiscrub and with Disfex (3% hexachlorophane cream containing a *p*-hydroxybenzoic acid ester as preservative).

Experiment 2.—In this experiment we tested the hypothesis that "two-phase" disinfection depends on a further reduction in the numbers of bacteria on the skin below the equilibrium level, caused by the use of a different vehicle for the antiseptic and a different form of application in the second phase of disinfection. Eight subjects carried out the standard two minutes' hand washing with Hibiscrub on nine occasions, three each on three successive days. Standard samplings for viable counts were made before the first hand wash and again after the ninth hand wash. Four of the subjects were then given, for the second phase, a standard two minutes' skin disinfection with 0·5% alcoholic chlorhexidine rubbed on with gauze, (Lowbury *et al.*, 1964b). In the four other subjects the second phase of disinfection consisted of a further two minutes' hand wash with Hibiscrub immediately after the sampling which followed the ninth Hibiscrub hand wash. Standard samplings for viable counts were

made on the eight volunteers immediately after the second-phase disinfection.

Experiment 3.—Further confirmatory studies were made with three different preparations (Hibiscrub, Disfex, and 3% hexachlorophane liquid soap containing 0·3% chlorocresol (Ster-Zac)) in the first phase of disinfection, and with 0·5% alcoholic chlorhexidine, applied for two minutes on gauze swabs, in the second phase. The first phase consisted of three standard hand washes on each of three successive days. Samplings for counts of skin bacteria were obtained by the standard technique before and immediately after the first disinfectant hand wash, after the ninth disinfectant hand wash, and after disinfection with alcoholic chlorhexidine.



"Two-phase" disinfection of hands: mean percentage survival in hand sample counts after nine standard handwashes with Hibiscrub (the first phase), followed immediately by "second phase" disinfection with alcoholic chlorhexidine or with Hibiscrub; eight subjects were studied, four treated with one and four treated with the other "second phase" preparation.

RESULTS

These are shown in table IV. In experiment 1 the estimated mean reduction in skin flora after the first application was greater with Hibiscrub than with Disfex, but the eventual reduction achieved by these two preparations was similar. Though an equilibrium level seemed to be reached after six treatments, the pour plate counts from 1 ml of undiluted washings taken at this stage were very low (between 1 and 3). Similar very large reductions to very low counts were obtained with Hibiscrub and with Ster-Zac liquid soap in the first phase of experiment 3, so that the further reduction or elimination of bacteria which were found in phase 2 in all but one of the subjects who were treated with these agents in the second phase had little or no significance; by contrast, after the use of Disfex in phase 1 reductions from 97 to 1, 27 to 3, and 23 to 1 were found in colony-counts of pour plates from undiluted washings taken after phase 2 disinfection.

In experiment 2 (see chart), phase 1 of disinfection (nine washings with Hibiscrub) caused a large estimated mean reduction in skin bacteria (about 99%), which was followed by a large further mean reduction (about 95%) in the second phase of disinfection when the agent used for this was 0·5% chlorhexidine in 70% ethanol; counts per ml undiluted washings fell from 271 to 1, 52 to 9, 7 to 0, and 3 to 0; by contrast, when the second phase of disinfection consisted of an immediate further standard hand wash with Hibiscrub there was no further reduction in any subject, and in three there was an increase in estimated skin flora after the "second phase" treatment (from 51 to 138, from 8 to 87, and from 58 to 91 bacteria per ml undiluted washings).

Disinfection of Hands by Chlorocresol (0·3%) in Liquid Soap

The inclusion by a manufacturer of 0·3% chlorocresol in liquid hexachlorophane soap was effective in preventing contamination of the soap by Gram-negative bacilli (Collins and Deverill, 1971). This additive appeared to enhance the skin disinfecting properties of the hexachlorophane soap, and was shown to have an independent skin-disinfectant action in a liquid soap containing 0·3% chlorocresol but no hexachlorophane (Lilly and Lowbury, 1971).

In this study we compared the skin disinfectant action of the soap base containing 0·3% chlorocresol with that of a 3% hexachlorophane detergent cream (Disfex). The techniques of hand washing and hand sampling were the same as in the experiments with Hibiscrub described above, sampling times being immediately before and immediately after the first standard two minutes' hand wash, and the last sampling being after the sixth hand wash, the hands being washed three times on each of two successive days. Five volunteers were used, each using each of the two preparations.

RESULTS

These are shown in table V. The chlorocresol soap gave smaller reductions in bacterial skin flora after one and after six applications than those obtained with Disfex, and there was more variation between results on different subjects. The mean reduction after six washes with chlorocresol soap (72%), however, shows that it might be useful in situations where some disinfection is desirable but the most intensive disinfection is not essential—for example, for catering staff.

TABLE IV—*"Two-phase" Disinfection of Skin*

Experiment	No. of Subjects	Mean % Survival, with range, of Skin Flora Estimated from Viable Counts of Hand Washings							
		After Phase 1					After Phase 2		
		Treatment	Result, Expressed as % Pretreatment Counts, After Treatments					Treatment	Result Expressed as % Count at End of Phase 1
			1	3	6	9	12		
1	2	Hibiscrub	5·9 (5·9, 5·9)	0·35 (0·3, 0·4)	0·09 (0·16, 0·03)	0·06 (0·12, 0)	0·1 (0·2, 0)	—	—
	2	Disfex	52·5 (39, 66)	2·3 (0·6, 4·1)	0·13 (0·08, 0·18)	0·1 (0·19, 0·02)	0·06 (0·07, 0·04)	—	—
2	4	Hibiscrub	—	—	—	1·5 (0·3–2·0)	—	Alcoholic chlorhexidine	5·2 (0–10)
	4	Hibiscrub	—	—	—	0·4 (0·05–1·0)	—	Hibiscrub	371 (83–1,087)
3	3	Hibiscrub	19 (8–31)	—	—	0·01 (0·008–0·01)	—	Alcoholic chlorhexidine	[17 (0–50)]*
	3	Ster-Zac liquid soap	38 (24–45)	—	—	0·01 (0·005–0·02)		"	[44 (0–100)]*
	3	Disfex	61 (65–68)	—	—	0·5 (0·2–0·95)		"	5 (1–11)

*Numbers of bacteria in undiluted washings at end of phase 1 were very low, so that further meaningful reduction in phase 2 could not be shown.

TABLE V—*Comparison of 0·3% Chlorocresol Liquid Soap and 3% Hexachlorophane Detergent Cream (Disfex): Reduction of Bacterial Counts on Skin Sampling Expressed as Percentage of Initial Count*

Experiments	Methods of Disinfection			
	0·3% Chlorocresol Soap		3% Hexachlorophane Detergent Cream (Disfex)	
	1 Application	6 Applications	1 Application	6 Applications
1	B.G. 79·8	90·4	A.K. 58·7	97·5
2	A.K. 14·3	27	J.B.O. 44·1	99·8
3	A.B. 18·1	86·1	B.G. 44·3	99·4
4	B.D. 19·9	82·2	A.B. 66·4	99·8
5	J.B.O. 17·3	75·5	B.D. 42·2	95·5
Mean % reduction	29·9 (±14·9)	72·2 (±11·6)	51·1 (±4·8)	98·4 (±0·84)

Initials indicate experimental subjects

Disinfection of Operation Site with Alcoholic Cetrimide

In a recent study (Lilly and Lowbury, 1971), aqueous cetrimide (1%) used for disinfection of an operation site caused a mean reduction of 55% in bacterial counts from skin samplings, compared with a 79·6% reduction after a similar use of 0·5% chlorhexidine in 70% ethyl alcohol. The following experiment was made to assess the value of alcoholic cetrimide solution against the natural skin flora. This fills a gap left in our earlier studies, which omitted cetrimide from the series of antiseptics examined for their ability to remove resident flora because of its failure to eliminate transient flora (Lowbury *et al.*, 1960).

EXPERIMENT

Disinfection of the operation site with 1% cetrimide in 70% ethyl alcohol was assessed in comparison with a control treatment (distilled water). Eight experiments, each on a different subject, were made with cetrimide, and similar experiments were made with distilled water on the same eight subjects. The technique of assessment was as in previous studies (Lowbury *et al.*, 1960, 1964b; Lilly and Lowbury, 1971), but slightly modified to prevent effects of carry-over.

The whole surface of both hands was disinfected by systematic and vigorous application of the alcoholic cetrimide solution with a gauze swab soaked in the solution; the application was continued for two minutes, after which the skin was allowed to dry. Bacterial counts were obtained from washings taken immediately before and immediately after the disinfection. The sampling technique was similar to that used in our previous studies, but modified to prevent the effects of carry-over of cetrimide which occurred when the usual technique was used. After the standard sampling in 100 ml of neutralizer solution, 10 ml of the washings were mixed with 10 ml of sterile milk; 1 ml of the mixture was transferred, in 0·1-ml amounts, to 10 pour plates of nutrient broth with neutralizers. Viable counts were made after overnight incubation at 37°C, and tests for carry-over were made (Lowbury *et al.*, 1960) on all the pour plates and on a sample of the washings mixed with sterile milk.

RESULTS

The result of this experiment is shown in table VI. The mean percentage reduction was 69·5% (range 64·4 to 74·1%). This mean reduction was greater than that obtained with aqueous cetrimide in our previous study (Lilly and Lowbury, 1971), but in the same range as that of 70% ethyl alcohol (Lowbury *et al.*, 1960), and lower than that of 0·5% chlorhexidine in 70% ethyl alcohol, which varied between 79·6% and 84·9% in three previous studies (Lowbury *et al.*, 1960; 1964b; Lilly and Lowbury, 1971).

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

Treatment	Replicate Experiments				Mean % Reduction
	1	2	3	4	
1% Cetrimide in 70% alcohol	L.M. 66·7 V.R. 70·6	J.B.O. 70·3 E.R. 64·4	J.R.B. 66·3 A.K. 69·4	B.D. 73·8 S.D. 74·1	69·5
Distilled water (Control)	A.K. 7·8 E.R. 3·1	V.R. 11·1 B.D. 6·4	J.B.O. 11·9 S.D. 7·7	J.R.B. 9·5 L.M. 7·6	8·1

Initials indicate experimental subjects.

Residual Action of Hibiscrub

METHODS

The following tests (see Lowbury *et al.*, 1964a) were made for residues of antiseptic deposited on the skin after the use of Hibiscrub and Ster-Zac liquid soap. Measured volumes (0·05 ml) of suspensions of *Staphylococcus aureus* and *Escherichia coli* in Ringer's solution were spread and allowed to dry on the palms of the hands of volunteers who had previously washed their hands for two minutes with Hibiscrub or Ster-Zac, and then rinsed the hands thoroughly and dried them on a sterile towel. After one hour the inoculated area was sampled by a standard sampling technique for superficial "transient" organisms (Story, 1952), and viable counts were obtained from the washings.

RESULTS

It is shown in table VII that washing with Hibiscrub left a large antibacterial residue on the skin, and this was active against *E. coli* as well as against *Staph. aureus*. Washing with Ster-Zac liquid soap had a similar residual action against both organisms; the residual action of Ster-Zac against *E. coli* may have been due to the chlorocresol in this preparation, since hexachlorophane has relatively poor activity against Gram-negative bacilli.

TABLE VII—*Residual Effects of Hibiscrub and Ster-Zac Liquid Soap*

Preparation Used for Hand Wash before applying Bacterial Suspensions	Mean Viable Counts per ml Washings from Areas Inoculated with Bacterial Suspensions			
	Staph. aureus		*E. coli*	
	1	2	1	2
Hibiscrub	12·5	18·5	0	0·25
Control (bar soap) ..	13,500	19,500	900	1,150
Ster-Zac	25	19	15	21
Control (bar soap) ..	24, 250	19,400	15,500	11,000

Discussion

The new 4% chlorhexidine detergent preparation, Hibiscrub, was shown in this study to give a significantly greater immediate reduction in resident skin flora after a single two-minutes' hand wash than was obtained after similar use of povidone iodine surgical scrub or of a 0·75% chlorhexidine detergent preparation (Dermofax). After six successive hand washes spread over two days, however, there was little difference in the reduction of skin flora obtained with these agents and with liquid hexachlorophane detergent preparations, though Hibiscrub had a significantly larger effect than Dermofax. The curve of disinfection on repeated hand washes with any of these preparations shows a steep fall on the first day (steeper with Hibiscrub or povidone iodine than with hexachlorophane preparations), flattening

after two to three days to an equilibrium level at 1% or less of the initial levels.

Our earlier studies (Lowbury *et al.*, 1964b) showed that the low counts of skin bacteria reached after six successive treatments with a hexachlorophane detergent cream could be further reduced by disinfection of the same area with alcoholic chlorhexidine rubbed on with gauze ("two-phase" disinfection); this method could be useful in the disinfection of operation sites before elective operations in high-risk patients. These findings were confirmed in the present study, which also showed that two-phase disinfection could be obtained with Hibiscrub as the first-phase detergent preparation. When, however, the hands were washed with Hibiscrub immediately after the ninth of a series of hand washes with the same agent the second-phase treatment failed to cause any further reduction of skin bacteria. This finding parallels that obtained in an earlier study, when hands which had shown a large reduction in bacteria on two-minutes' disinfection with ethyl alcohol failed to show a further reduction on receiving a similar alcohol disinfection immediately afterwards (Lowbury and Lilly, 1960). In some of the experiments few bacteria were found on sampling the skin after the first-phase treatment so that the effect of the second phase could not be shown. The hypothesis that "two-phase" disinfection of the skin depends for its effect on the distinct action of two different forms of disinfection was, however, supported by some of these experiments, in which there were differences in the chemistry of the disinfectant used, in the nature of the solvent, and in the mode of application. It is possible that alcohol used in the second phase of disinfection was active against bacteria persisting on the skin through the first phase because they were relatively resistant to chlorhexidine or hexachlorophane.

The other methods which seemed potentially useful for skin disinfection of the operation site of highly susceptible patients gave a somewhat disappointing result. Application for thirty minutes of povidone iodine compresses caused a reduction in the resident flora which was only marginally better than that obtained in a standard two minutes' disinfection with alcoholic iodine or chlorhexidine, and similar treatment with compresses of 0·5% chlorhexidine gave less satisfactory results. Compresses of povidone iodine provide the most effective method of eliminating bacterial spores on the skin (Lowbury *et al.*, 1964), and when this procedure has been deliberately adopted to reduce hazards of gas gangrene—for example, before amputation of a leg in a patient with arterial insufficiency—the reduction in numbers of vegetative organisms is about the same as that which would be obtained by a standard skin disinfection of the operation site.

A comparison between repeated hand washing with 0·3% chlorocresol liquid soap and similar treatment with a 3% hexachlorophane detergent cream showed a larger and more consistent disinfectant action from the hexachlorophane preparation. A large cumulative reduction of skin bacteria was, however, usually caused by the use of chlorocresol soap. It is clearly a much more effective agent for cleaning the skin than ordinary soap, and probably more effective than hexachlorophane bar soap (Lowbury and Lilly, 1960).

These studies have shown that a 4% chlorhexidine detergent solution is a potentially outstanding addition to the small list of detergent-disinfectant preparations for the hands of surgeons and nurses, with a greater immediate effect than that obtained with the hexachlorophane or even the iodophor preparations. Like Ster-Zac liquid soap and unlike the 0·75% chlorhexidine detergent preparation previously studied, the 4% chlorhexidine preparation showed a residual action against bacteria, both *Staph. aureus* and *E. coli*, deposited on the skin after washing, rinsing, and drying the hands. These properties make Hibiscrub a potentially useful alternative to hexachlorophane detergent preparations for the protection of neonates against staphylococcal infection. Such alternatives are clearly required in the light of possible toxic absorption of hexachlorophane in some infants (Curley *et al.*, 1971), but there appears to be none of proved effectiveness. Hibiscrub, if found safe and effective in infants, would probably have the additional advantage of giving no selective encouragement to Gram-negative infections—a side effect attributed to hexachlorophane (Forfar *et al.*, 1968).

We are grateful to Dr. J. P. Bull for advice and to Mr. M. D. Wilkin for help with statistical assessments; to I.C.I. (Pharmaceuticals) Ltd. for supplies of Hibiscrub and Disadine; to Messrs. Hough-Hoseason for supplies of 0·3% chlorocresol liquid soap (Ozac), and of Dermofax; and to members of the staff of this unit for their collaboration in tests of skin disinfection.

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Forfar, J. O., Gould, J. C., and MacCabe, A. F. (1968). *Lancet*, 2, 177.
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Lowbury, E. J. L., Lilly, H. A., and Bull, J. P. (1964a). *British Medical Journal*, 2, 230.
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Postoperative Deep Vein Thrombosis in Sudanese Patients

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British Medical Journal, 1973, 1, 515–517

Summary

The incidence of postoperative deep vein thrombosis diagnosed by radioisotope scanning in 100 Sudanese patients aged 40 or over was 12%. This compares with an incidence of nearly 30% in 542 patients reported from British hospitals using the same diagnostic technique. The reason for the difference is obscure and needs further investigation.

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Introduction

The incidence of postoperative deep vein thrombosis in patients aged over 40 in British hospitals is from 25-30%. Its incidence in the Sudan and, indeed, throughout Africa is generally believed to be lower than in Western society, but this has not, to our knowledge, been substantiated by proper clinical studies.

CHAPTER 13

QUATERNARY AMMONIUM ANTIMICROBIAL COMPOUNDS

John J. Merianos

The quaternary nitrogen moiety is an essential component for many biologically active compounds. Quaternary ammonium compounds play an important role in the living process. From vitamins (vitamin B complex and thiamine) to enzymes carboxylase, which participates in the carbohydrate metabolism, to choline, which is involved in transmethylation reaction of the fat metabolism, and to acetylcholine, a mediator in the transmission of nerve impulses (Burger, 1960), all play a fundamental function.

There are at least four types of physiologic actions (Burger, 1960) associated with quaternary ammonium compounds: (1) Curare-like (curaremimetic or curareform) action, a muscular paralysis with no involvement of central nervous system or heart, produced by d-tubocurarine chloride used to induce muscular relaxation during surgery; (2) muscarinic-nicotinic action, which is a direct stimulation of smooth muscles, and is a primary transient stimulation and secondary persistent depression of sympathetic and parasympathetic ganglia; (3) ganglia-blocking action; and (4) neuromuscular blockade. Table 13–1 illustrates the chemical structures of representative compounds responsible for these physiologic actions.

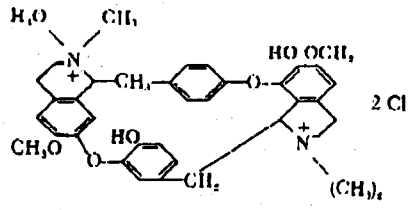
Medicinal chemists using the principle of structure activity relationships (Goldstein, 1974) have synthesized many quaternary ammonium compounds that will mimic certain biologic effects. Thus a very complex structure of d-tubocurarine chloride can be reduced to a much simpler decamethonium structure, a neuromuscular blocking agent. Hexamethonium acts as a ganglionic blocker by preventing the receptor from responding to acetylcholine. The decamethonium is too long to fit the ganglionic receptor but would act as a neuromuscular blocker by preventing the combination of acetylcholine with muscle end plate receptors. When the number of carbon atoms separating the quaternary nitrogens is increased above 12 carbons, the autonomic nervous activity disappears and the compounds become surface active and antimicrobial. However, there are exceptions to this rule, as we will discuss later on in the case of bis-quaternary and polymeric quaternary ammonium compounds, where two and four carbon atoms separate the quaternary nitrogen, and the products have antimicrobial properties. This chapter is concerned exclusively with antimicrobial quaternary ammonium compounds.

DISCUSSION

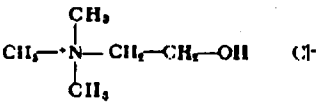
Although there are many stages in the historical development of quaternary ammonium germicides, there is general agreement on at least two truly historical milestones. The first is the work of Jacobs et al., which examined structure, preparation, and antimicrobial activity. Jacobs and Heidelberger, in 1915, published a number of papers describing the preparation of various different series of the quaternary ammonium salts of hexamethylenetetramine. In 1916 Jacobs and his coworkers published three papers describing the antimicrobial activity of many of the quaternary ammonium compounds they had previously synthesized and of additional derivatives prepared therefrom. In these publications, they related structure to antimicrobial activity. Although some reviews have challenged the work of Jacobs et al. as the earliest investigations of quaternary ammonium germicides, their preeminence is assured by their quality, quantity, and treatment, which included antimicrobial activity and correlation between structure and antimicrobial activity. The only valid criticism of the work of Jacobs may be the fact that some of the antimicrobial activity of his products may be due to the release of formaldehyde from hexamethylenetetramine. Jacobs reported that the antimicrobial activity is due to the presence of hexamethylenetetramine nucleus. Methenamine mandelate USP is used as a urinary tract anti-infective

Table 13–1. *Quaternary Structures with Physiological Actions*

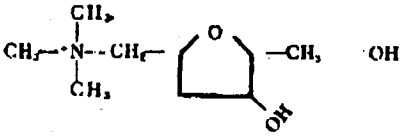
[1] d-Tubocurarine Chloride USP



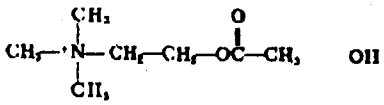
[2] Choline Chloride



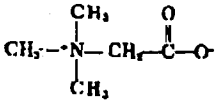
[3] Muscarine Hydroxide



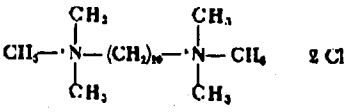
[4] Acetylcholine Hydroxide



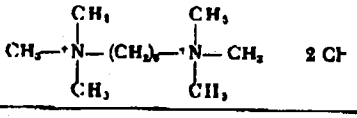
[5] Betaine



[6] Decamethonium Chloride



[7] Hexamethonium Chloride



even today, acting by releasing formaldehyde in an acid medium (Gennaro, 1985).

In any consideration of earlier investigations, the reader is reminded that our interests are solely with the various aspects of the antimicrobial activity of the quaternary ammonium compounds. Consequently, we may exclude those early publications dealing completely with synthesis of these compounds and in addition refute the role attributed by other reviews to the nineteenth century research on curare (Cucci, 1949).

Not until the 1920s did any additional information appear, at which time Browning et al. (1922, 1926) and Browning (1926) described the bacterial activity of quaternary derivatives of pyridine, quinoline, and other ring structures. Hartmann and Kagi, in 1928, reported on the antibacterial activity of quaternary ammonium compounds of acylated alkylene diamines. It was not until 1935, when Domagk disclosed the antibacterial activity of the long-chain quaternary ammonium salts, that the second and most important milestone in the development of antimicrobial quaternary ammonium compounds took place. The improved germicidal activity that occurred when a large aliphatic residue was attached to the quaternary nitrogen atom established the practicability and utility of these compounds, first in medicine and later in many other applications. This important disclosure stimulated research in synthesis and antimicrobial testing of quaternary ammonium compounds, with the consequent frequent publications and patents issued from 1935 to the present. After Domagk's discovery of the biocidal properties of cationic surface active agents several generations of structurally variable quaternary ammonium antimicrobials of commercial importance were developed.

The first generation is the standard benzalkonium chloride of specific alkyl distribution, namely C_{12}—40%, C_{14}—50%, C_{16}—10%. Another version of equally commercially successful alkyl distribution in the benzalkonium series is C_{12}—5%, C_{14}—60%, C_{16}—30%, C_{18}—5% as shown in Table 13–2. The official United States Pharmacopeia recognizes the benzalkonium chloride as a pharmaceutical aid (antimicrobial preservative). The USP specification for the C_{12}/C_{14} homologs components is 70% minimum of the total alkylbenzyldimethyl ammonium chloride content. This broad specification does not always give the most efficacious product. The major determining factor for biocidal efficacy is the hydrophilic-lipophilic balance of the products. The peak for biocidal activity of the homolog series is illustrated on Table 13–3, with a carbon chain of 14 offering the best activity (Cutler et al., 1966; Daoud et al., 1983; Hansch et al., 1973, 1964; Lien et al., 1976, 1968).

Modifications in the first-generation quaternaries by substitution of the aromatic ring hydrogen with chlorine, methyl, and ethyl groups resulted in the second generation of the substituted benzalkonium compounds. Out

Table 13–2. *Commercial Antimicrobial Quaternary Ammonium Compounds*

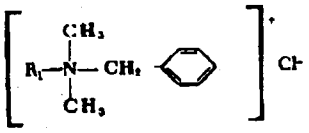
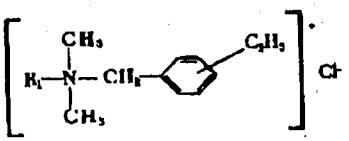
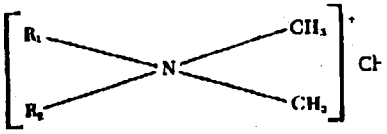
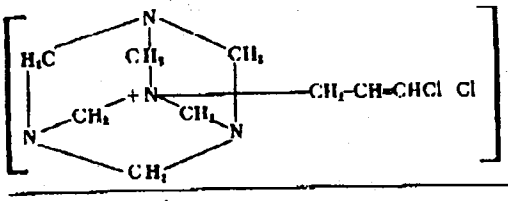
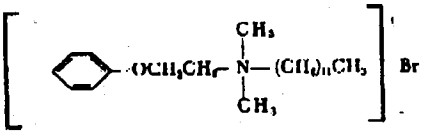
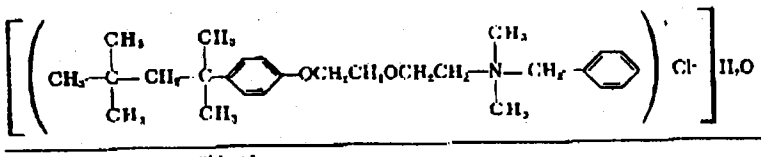
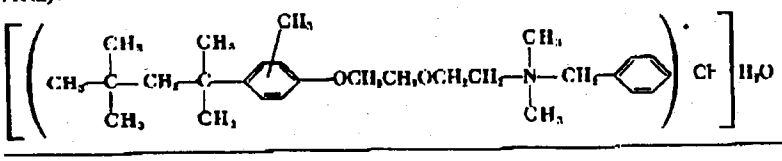
Chemical Structure	*Trade Names*	*Manufacturers*
Benzalkonium Chlorides: 	BTC 835, BTC 824	Stepan (Onyx)
	BTC 50 USP	"
	Barquat MB-50	Lonza
	Variquat 50 MC	Sherex
	Zephiran Chloride	Winthrop
	Hyamine 3500	Lonza
	Maquat MC 1412	Mason
	Maquat MC 1416	Mason
R_1 = C_{12}—40%, C_{14}—50%, C_{16}—10%		
R_1 = C_{12}—5%, C_{14}—60%, C_{16}—30%, C_{18}—5%		
Substituted Benzalkonium Chlorides:* 	BTC 471	Stepan (Onyx)
	BTC 2125M	"
	Barquat 4250	Lonza
	Maquat MQ-2525M	Mason
R_2 = C_{12}—50%, C_{14}—30%, C_{16}—17%, C_{18}—3%		
R_2 = C_{12}—68%, C_{16}—32%		
*See Text and Brochures		
Twin Chain Quaternaries: 	BTC 818, BTC 812	Stepan (Onyx)
	BTC 1010	"
	Bardac 2050	Lonza
	Bardac 205M	"
	Bardac 2250	"
Dioctyl 25%, Didecyl 25%, Octyldecyl 50%		
R_1 = Octyl, R_2 = Dodecyl		
Cetylpyridinium Chloride: 	Cepacol Chloride	Merrell Labs.
	Cetapryn Chloride	"
N-(3-Chloroallyl)Hexaminium Chloride: 	Dowicide Q	Dow
	Dowicil 200	Dow
	Dowicil 75	Dow
Domiphen Bromide: 	Bradosol	Procter & Gamble
	Oradol	Ciba
	Modicare	"
Benzethonium Chloride: 	Phemerol Chloride	Parke-Davis
	Hyamine 1622	Rohm & Haas
		Lonza
Methylbenzethonium Chloride: 	Diaparene Chloride	Rohm & Haas
	Hyamine 10X	Rohm & Haas

Table 13–3. *Effect of Length of Carbon Chain on Bactericidal Activity of Benzalkonium Chloride*

Long-Chain Length	Bactericidal Test (Minimum Concentration That Kills in 10 Minutes but Not in 5 Minutes in ppm)		
	Staphylococcus aureus #6538	*Salmonella typhosa* #6539	*Pseudomonas aeruginosa* #15,442
8	3000	4500	6000
9	800	1400	2500
10	450	300	1200
11	160	130	400
12	45	40	120
13	25	20	50
14	15	12	40
15	25	20	70
16	30	25	200
17	170	15	360
18	450	60	1000
19	330	90	1300

of this group the product with commercial significance is the alkyldimethylethylbenzyl ammonium chloride under the trade name BTC 471 with alkyl distribution C_{12}—50%, C_{14}—30%, C_{16}—17%, C_{18}—3%. Another product with high biocidal activity in this group is alkyldimethyl-3,4-dichlorobenzyl ammonium chloride under the trade names of Tetrosan 3,4D, and Riseptin with same alkyl distribution as above.

By far the product of greatest commercial significance today is of the third generation of quaternary ammonium compounds, the *dual quats*, developed in 1955 under the trade name BTC 2125M. This product is a mixture of equal proportions of alkyldimethylbenzyl ammonium and alkyldimethylethylbenzyl ammonium chlorides of specific alkyl distribution, as shown in Table 13–2.

This combination of benzalkonium chloride with alkyl distribution (C_{12}—5%, C_{14}—60%, C_{16}—30%, C_{18}—5%) and alkyldimethylethylbenzyl ammonium chloride with alkyl distribution (C_{12}—68%, C_{14}—32%) is BTC 2125M, with superior microbiologic performance (Onyx or Stepan brochures). This synergistic combination of the third generation of quaternaries not only had an increased biocidal activity, it reduced the acute oral LD_{50} from 0.3 g/kg of benzalkonium chloride to an acute oral LD_{50} of 0.750 g/kg for BTC 2125M.

In summary, the third generation—the dual quats—offer improved biocidal activity, stronger detergency, and a relatively lower level of toxicity. In the early 1950s the nonionic detergents were being developed with far greater cleaning power than natural soaps. The compatibility of quaternary ammonium compounds with nonionic detergents resulted in superior formulations that helped overcome the environmental factors, such as hard water, anionic residues of soap, and proteinaceous soils, which were found to weaken their effectiveness (Greene and Petrocci, 1980).

A continual change and improvement in advancing and broadening the spectrum of biocidal activity enabled disinfectants to work under the most adverse conditions and produced safer, more economical products. In 1965 another technological development, catalytic amination of long-chain alcohols, made commercially feasible the production of dialkylmethyl amines, which in turn can be quaternized with methyl chloride to give us the *twin chain quats*, the fourth generation of quaternaries antimicrobials with high performance, unusual properties, and tolerances (Ditoro, 1969; Petrocci et al., 1974). The twin chain quats, like dioctyl dimethyl ammonium bromide and didecyldimethyl ammonium bromide were first introduced by the British Hydrological Corporation (BHC, 1947. "DECIQUAM 222") for the British food industry.

These products displayed outstanding germicidal performance; unusual tolerance for anionic surfactants, protein loads, and hard water; and even low-foaming characteristics. Table 13–4 illustrates the cidal activities and pseudomonicidal, fungicidal, and hard-water tolerance of five of the most active twin chain quats, out of 20 as measured by the official AOAC procedure reported by Petrocci et al. (1974). The product of choice in this series is C_8/C_{10}DMAC because of its superior water solubility and germicidal activity. However, the odd number chain C_9/C_{11}DMAC is an equally active product, although its commercial feasibility has not been explored because of high cost of the odd carbon chain alcohols. Recently several odd carbon chain alcohols have been offered in semicommercial quantities. The concept of synergistic

Table 13–4. *Antimicrobial Activity of Twin-Chain Quaternary Ammonium Compounds*

Compounds	Pseudomonicidal	Fungicidal	HWT
1. C_8/C_{10}DMAC	500 PPM	210 PPM	1100 PPM
2. C_8/C_{12}DMAC	500 PPM	200 PPM	1200 PPM
3. C_9/C_{11}DMAC	500 PPM	190 PPM	1400 PPM
4. C_9/C_{13}DMAC	550 PPM	235 PPM	1300 PPM
5. C_{10}/C_{12}DMAC	550 PPM	210 PPM	1300 PPM

HWT = Hard Water Tolerance; DMAC = Dimethylammonium Chloride

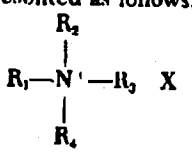
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combination in the dual quats has been applied to twin chain quats dialkyldimethyl ammonium chlorides (dioctyl—25%, didecyl—25%, octyldecyl—50%) was combined with benzalkonium chlorides (R=C_{12}—40%, C_{14}—50%, C_{16}—10%). According to the work of Schaeufele (1984), a 60/40 blend of the above quaternaries proved to be superior to the individual components tested via the AOAC Use-Dilution Test (Official Methods of Analysis of the AOAC, 1984). This newest blend of quaternaries represents the fifth generation of quaternary ammonium compounds. The blend remained active under the most hostile conditions, was less toxic and less costly, and provided more convenient disinfectants.

In the 1980s the toxicity of quaternaries underwent scrutiny by the EPA and other U.S. regulatory agencies. The safety of the biocides in general received top priority over their efficacy. A new class of biocides, the polymeric quaternaries, has emerged, which are less toxic than the standard benzalkonium chlorides and less powerful than the dual quats or twin chain quats. The polymeric quaternaries are milder and have found applications in pharmaceuticals as preservatives (Stark, 1985; Merianos, 1988). The polymeric quaternary ammoniums are polyelectrolytes, representing the sixth generation of quaternary ammonium compounds. They are milder and safer than all other classes based on LD_{50} and cytotoxicity (Stark, 1985). More recently another synergistic combination, which may be considered the seventh generation of quaternary blends of bis-quats and polymeric quaternary (Polylonenes), offer excellent antimicrobial activity against oral flora bacteria, namely *Bacteroids gingivalis* 318, A7A128, *Actinomyces viscosus*, and *Streptococcus mutans* in the level of 1 to 5 ppm in a pharmaceutically accepted polymer formulation (MerPan Chemical Consultants Brochures, MerPanocide L. and MerPanocide LHD) (Merianos, 1988). Reduction of toxicity in the bis-quats without compromising efficacy is not an easy task. These preliminary results required further testing to confirm safety and true synergism in this series of polymeric quaternaries with bis-quats blends (Merianos, 1986).

CHEMISTRY

The quaternary ammonium compounds are the products of a nucleophilic substitution reaction of alkyl halides with tertiary amines. Chemically, they have four carbon atoms linked directly to the nitrogen atom through covalent bonds, while the anion in the original alkylating agent becomes linked to the nitrogen by an electrovalent bond. The general formula for the quaternary ammonium compounds is represented as follows:



R_1, R_2, R_3, and R_4 are alkyl groups that may be alike or different, substituted or unsubstituted, saturated or unsaturated, branched or unbranched, and cyclic or acyclic, and that may contain ether or ester or amide linkages; they may be aromatic or substituted aromatic groups. The nitrogen atom plus the attached alkyl groups forms the positively-charged cation portion, which is the functional part of the molecule. The portion attached to the nitrogen by an electrovalent bond may be any anion, but is usually chloride or bromide to form the salt.

Depending on the nature of the R groups, the anion, and the number of quaternary nitrogen atoms present, the antimicrobial quaternary ammonium compounds may be classified as follows.

Monoalkyltrimethyl Ammonium Salts

In this instance, one R group is a long-chain alkyl group, and the remaining R groups are short-chain alkyl groups, such as methyl or ethyl groups. All of the quaternary compounds in this group are prepared from the reaction of a tertiary amine with an alkyl halide (Domagk, 1935; Shelton et al., 1946; Hartmann, 1929). The tertiary amine may be the long-chain alkyldimethylamine or the short-chain trimethylamine, which react with methyl halide or with the long-chain alkyl halide, respectively. Examples of commercially available products in this group are cetyltrimethylammonium bromide as CTAB, alkyltrimethyl ammonium chloride as Arquad 16, alkylaryltrimethyl ammonium chloride as Cloquat C, cetyldimethyl, ethylammonium bromide as Cycloton D256B, Ammonyx DME, and Bretol.

Monoalkyldimethylbenzyl Ammonium Salts

In this group, one R is a long-chain alkyl group, a second R is a benzyl radical, and the two remaining R groups are short-chain alkyl groups, such as methyl or ethyl groups. These compounds are prepared by the reaction of a long-chain alkyldimethylamine with the benzyl halide (Wakeman and Tesoro, 1954; Domagk, 1938; Dunn, 1936).

Examples of commercially available products in this group are alkyldimethylbenzyl ammonium chlorides, as BTC 824, Hyamine 3500, Cyncal Type 14, and Catigene.

In addition, there are substituted benzyl quaternary ammonium compounds such as dodecyldimethyl-3,4-dichlorobenzyl ammonium chloride, sold under the trade name of Riseptin. There are also mixtures of alkyldimethylbenzyl and alkyldimethyl substituted benzyl (ethylbenzyl) ammonium chlorides, such as BTC 2125M, Barquat 4250 (Stepan; Lonza Brochures).

Dialkyldimethyl Ammonium Salts

In this instance, two R groups are long-chain alkyl groups, and the remaining R groups are short-chain alkyl groups, such as methyl groups. These compounds are prepared by the reaction of the long-chain alkyldimethylamine with a long-chain alkyl halide or dialkylmethylamine with methyl halide (Kirby and Frick, 1963; Tonaka, 1944; Kuhn et al., 1940).

Examples of commercially available products in this

group are didecyldimethyl ammonium halides, such as Dequam 222 and Bardac 22, and octyldodecyldimethyl ammonium chloride, such as BTC 812 (Stepan; Lonza Brochures).

Heteroaromatic Ammonium Salts.

In this group, one R chain is a long alkyl group, and the remaining three R groups are provided by some aromatic system. Thus, the quaternary nitrogen to which these three R groups are attached is part of an aromatic system such as pyridine, quinoline, or isoquinoline.

These compounds are prepared by reaction of the aromatic amine with a long-chain alkyl halide (Browning et al., 1922; Shelton et al., 1946; Mosher and Howard, 1948). Examples of commercially available products in this group are cetylpyridinium halide (CPC and Ceepryn), reaction product of hexamethylenetetramine with 1,3-dichloropropene to give cis-isomer 1-[3-chloroallyl]-3,5,7-triaza-1-azoniaadamantane (Dowicil 200), alkylisoquinolinium bromide (Isothan Q), and alkyldimethylnaphthylmethyl ammonium chloride (BTC 1100).

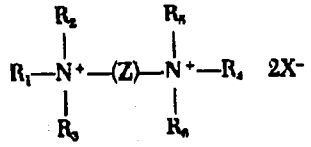
Polysubstituted Quaternary Ammonium Salts

In this group, the cation portion of the molecule is the same as that described for any of the aforementioned groups. However, the anion portion is not a small inorganic ion as previously described, but a large, high-molecular-weight organic ion. These compounds are prepared by reaction of the quaternary ammonium halides with the sodium, potassium, or calcium salt of a high-molecular-weight organic moiety, so that an exchange of the anions is effected (Wakeman et al., 1971; Shibe et al., 1955).

Examples of commercially available products in this group are alkyldimethylbenzyl ammonium saccharinate (Onyxide 3300 and Lonquat QA 100), and alkyldimethylethylbenzyl ammonium cyclohexylsulfamate (Onyxide 172).

Bis-Quaternary Ammonium Salts

In this group of compounds, there are two symmetric quaternary ammonium moieties arranged in the general formula:



Here the R groups are as described for any of the aforementioned groups, Z is a carbon-hydrogen chain, and an anion is attached to each quaternary nitrogen via an electrovalent bond. These compounds are prepared by reaction of a bis-tertiary amine with alkyl halide or of a di-halo compound with a tertiary amine (Hwa, 1963; DeBenneville and Bock, 1950; Babbs et al., 1956).

An example of a commercially available product in this group is 1,10-bis(2-methyl-4-aminoquinolinium chloride)-decane, sold under the trade name of Dequadin or Sorot. Another example of bis-quat is 1,6-Bis [1-methyl-3-(2,2,6-trimethyl cyclohexyl)-propyldimethylammonium chloride] hexane or triclobisonium chloride sold by trade name Triburon. Another commercially available bis-quat is CDQ (Buckman Brochures), used for industrial water treatment for controlling sulfate-reducing bacteria (*Desulfovibrio* sp.). The CDQ is prepared by reaction of alkyl[C_{12}—40%, C_{14}—50%, C_{16}—10%] dimethylamine with dichloroethyl ether. Also, reaction of 1,4-dichloro-2-butene with two moles of alkyldimethylamines or hexamethylenetetramine offer another example of bis-quats with broad spectrums of biocidal activity.

Polymeric Quaternary Ammonium Salts

Many different types of polymeric quaternary ammonium salts have been reported to have antimicrobial activity (Ghosh, 1988, 1986; Ikeda and Tazuke, 1983, 1984, 1985; Samour, 1978; Rembaum, 1973, 1975, 1977). The methods of preparation of these polymers are also many, from free radical polymerization of monomers containing quaternized nitrogen, to cationic, anionic polymerization, polycondensation of diamines with dihalides, or polycondensation of haloamines. The last method was used by Rembaum for the preparation of ionenes, which are polyelectrolytes with positively-charged nitrogen atoms located in the backbone of polymeric chain. This type of polycation was first reported in 1941 and is formed by the Menchutkin reaction from ditertiary amines and dihalides (Rembaum, 1973). Although a number of patents were published since 1941 concerning the applications of ionene polymers (Fush and Stamberger, 1965; Boyer and Santiago, 1974; Walker and Cambre, 1970), little information was available on the mechanism and kinetics of their formation, or their solution properties. The scope of this polycondensation reaction, the effect of concentration and solvent on rates and molecular weight of ionenes, and a proposed mechanism were first reported by Rembaum in 1968, when it was realized that under well defined conditions relatively high molecular weight (3,3 ionene chloride 65,000) ionenes were obtainable. The polymerization conditions for the formation of ionenes involves a total concentration of 3.0 mol [1.5 mol of each monomer] in a mixture of DMF/MEOH solvent [1:1 or 4:1] and 5 to 7 days of reaction time at room temperature to give polyionenes with molecular weight of about 65,000. In condensation polymerization the purity of the condensing species and stoichiometry are critical in obtaining high-molecular-weight polymer. Rembaum (1973) reported that polyionenes exhibit the following biologic effects: (1) *Bactericidal action*, (2) *Formation of insoluble complexes with DNA and heparin*, (3) *Neuromuscular blocking action*, (4) *Cell lysis and aggregation*, and (5) *Cell adhesion*. The antimicrobial and antifungal properties of ionenes were studied by the zone of inhibition method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida sporogenes*, *S. typhimurium*, *M. smegmatis*, *Pseudomonas mirabilis*, *Pseudomonas vulgaris*,

Table 13–5. *Minimum Inhibitory Concentration of Polyionenes*

Polyionene	MIC (ppm)	
	S. aureus	*E. coli*
1. 3, 3-ionene bromide	>128	>128
2. 6, 6-ionene bromide	16	16
3. 6, 10-ionene bromide	4	4
4. 2, 10-ionene bromide	4	8
5. 6, 16-ionene bromide	4	32

Candida globosum, *M. verrucaris*, *F. oxysporum* and *Alternaria sp.* The MIC for five of the most active ionenes bromides against *S. aureus* and *E. coli* are listed in Table 13–5.

The 6,10 ionene bromide is the most active in these series, and at concentrations up to 4 ppm stimulated the growth of normal cells while this range showed inhibition and death of the transformed human cells W138, possibly by electrostatic cytotoxic interaction (Rembaum et al., 1975). It has been suggested that malignant cells are more electronegative than normal cells. If so, malignant cells should demonstrate a greater affinity for the electropositive ionene polymer. This hypothesis has been supported by experimental work. However, this preliminary study appears to warrant more extensive studies to evaluate this class of polymers as chemotherapeutic agents. Rembaum concluded that polyionenes and their low-molecular-weight analogues constitute a unique model system for a molecular probe of the living cell machinery, because their structure, their positive charge densities, their counterions, and their molecular weights can be varied systematically. These considerations apply not only to the study of toxicity or antimicrobial activity, but also to the understanding of the interaction of the polyionenes with DNA or as molecular probes to elucidate the properties of cell membranes.

The high charge density of polyionenes is responsible for their bactericidal and fungicidal activities, for the prolonged duration of the curarizing action, and for the formation of complexes with DNA and heparin. The differences in the biologic properties or stability of the complexes may be explained on the basis of electrostatic association between the negative and the positive moieties of the interacting molecules.

Because the normal and neoplastic cells show an array of different surface properties, including an increase in anodic mobility after transformation, some polyionenes (e.g., 6,10 ionene bromide) may preferentially bind to cancer cells and inactivate them. This differential binding and toxicity to transformed cells conforms with topologic changes of membrane binding sites in the transformed cells. All four products in Table 13–6 are ionenes containing the quaternary nitrogen atom on the backbone of the polymer chain. The main difference is the molecular weight, which is due to reactivity of the alkylating monomer, reaction time, solvent, temperature, and method of preparation. The ionene bromides (MW 65,000) reported by Rembaum, however, are not commercially available, despite their many biologic effects and potential applications, perhaps due to poor yields, incomplete reaction (65 to 75% conversion), long reaction time, and toxicity. The other three products listed in Table 13–6 are items of commerce. The WSCP or Busan 77 from Buckman Laboratories, which is chemically identified as poly[oxyethylene(dimethyliminio)-ethylene (dimethyliminio)-ethylenedichloride] is made by condensation of N,N′ tetramethylethylenediamine with dichloroethyl ether in water to give molecular weight 2000 to 3000. The molecular weight was measured by Gel Permeation Chromatography (GPC) using polyethyleneglycol standard according to Levy and Dubin's reported method (Merianos, 1986).

The manufacturer has provided brochures and data sheets describing minimum inhibitory concentration (MIC of 10 to 50 ppm) levels indicating a broad spectrum of antimicrobial activity, with dual functionality as microbicide and polymer clarifier for swimming pools. The material is registered with the Environmental Protection Agency as a swimming-pool algicide, with 2 ppm of active product used as a maintenance dose, and 5 to 8 ppm of active product used to rid pools of heavy, objectionable algae growth. The presence of WSCP at only 2 ppm can reduce by 80% or more the chlorine needed to kill the bacteria. In general, WSCP enhances the activity of all oxidizers used in the swimming pools. In addition, the material is registered with the Environmental Protection Agency as a cooling water treatment biocide at 20 to 40 ppm of active product. WSCP microbicide formulations can be used to control the growth of algae, bacteria, and fungi on cooling towers and other parts of commercial and industrial recirculating cooling water systems.

Another polymeric quaternary ammonium compound is the product Mirapol A-15 from the Miranol Chemical Company, which is chemically identified as poly[N-3-dimethylammonio)propyl]N-[3-ethyleneoxyethylenedimethylammonio)propyl]urea dichloride] or by the CTFA name Polyquaternium 2. This product is made by reacting a symmetrically substituted urea ditertiary amine with dichloroethylether in water to give MW 2000 to 3000.

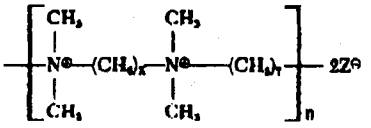
The manufacturer, who is primarily concerned with hair care products, reports a static level of 80 ppm and a destruction level of 100 ppm against *Pityrosporum ovale*. Additional examination of this structure demonstrates inhibitory levels at 100 ppm against both *Staphylococcus aureus* and *Escherichia coli*. By a time/kill water treatment screening procedure, the product demonstrates a high-percentage kill of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* at 10, 15, and 20 ppm of active product following a 30-minute contact period. Although unpublished reports by Petrocci have demonstrated antimicrobial activity for this product, attempts to repeat these results with current production of this product failed to confirm biocidal activity. This product

is not sold as an antimicrobial but as a hair conditioning agent for shampoos.

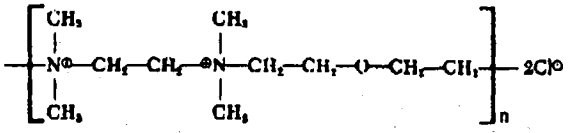
The last structure on Table 13–6 of the polymeric quaternary ammonium compound, from Onyx (Stepan) Chemical Company, is chemically identified as α-4-[1-tris(2-hydroxyethyl)ammonium chloride-2-butenyl] poly[1-dimethyl ammonium chloride-2-butenyl]-W-tris(2-hydroxyethyl)ammonium chloride, or Onamer M or Polyquat or with the CTFA name Polyquaternium 1. The Onamer M is made by reacting 1,4-bis[dimethylamino]-2-butene (0.9 mol), triethanolamine (0.2 mol), and 1,4-dichloro-2-butene (1.0 mol) in water to give an average molecular weight of 5000 to 10,000 (Green et al., 1975, 1977, 1978, 1982; Good et al., 1987). The purpose of the triethanolamine in the preparation was to randomly terminate the polymeric chain, so that we obtained a low-molecular-weight product by design. The 1,4-dichloro-2-butene alkylating agent is very reactive and gave us a 98% conversion of organic chloride to ionic within 6 to 8 hours in water. Polyquaternium 1 was originally intended for hair conditioning applications (Merianos et al., 1977); however, with the good antimicrobial activity combined with excellent toxicologic data, it emerges as an excellent preservative candidate for ophthalmic preparations (Stark, 1985). Today this product is registered with FDA as a preservative for contact lens solutions (Opti-Tears, Opti-Clean, Opti-Soft, Opti-Frec, Polyflex Tears-Naturale II—all are trade names of Alcon Labs, Fort Worth, TX 76134). Table 13–7 lists the comparative cytotoxic response of Onamer M at various concentrations and other solutions by in-vitro testing of Mouse L929 cells (Stark, 1985). The most common ophthalmic preservatives are thimerosal, benzalkonium chloride, and chlorhexidine. These compounds are toxic to the eye and may cause corneal erosion and corneal ulceration resulting in pain. This problem is particularly severe with quaternary ammonium compounds that are concentrated more than 400 times by hydrophilic lenses. Chlorhexidine is concentrated as much as 100-fold by hydrophilic contact lenses, which results in the potential for injury to the eye. The comparative cellular toxicity of soft contact lenses soaked in Onamer M at various concentrations and other solutions was determined by in-vitro testing. Mouse L929 cells were grown

Table 13–6. *Polymeric Polyquaternary Ammonium Compounds*

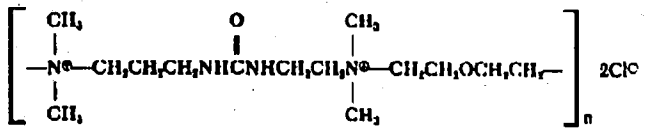
A. Ionenes A. Rembaum Applied Polymer Symposium No. 22 299-317 (1973)



B. Poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylene dichloride] (WSCP or Busan 77)

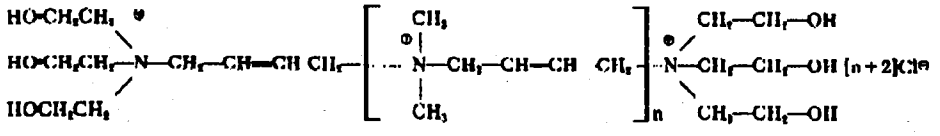


C. Polyquaternium 2 CTFA-adapted name (Mirapol-A15)



n = 6 (average)

D. Polyquaternium 1 CTFA-adapted name (Onamer M)



plus triethanolamine hydrochloride

Table 13–7. *Comparative Cytotoxicity of Soft Contact Lenses Soaked in Preserved Solutions on Mouse L929 Cells*

Lens Soaked	Cytotoxic Response: Cell Lysis	Cytotoxic Response: Zone of Cell Death (mm)	Cytotoxicity Conclusion†
Saline	–	0	None
0.001% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.01% Onamer M		0	None
0.01% Onamer M	–	0	None
0.1% Onamer M	–	0	None
0.3% Onamer M	–	0	None
1.0% Onamer M	+	31	Moderate
Alkyltriethanol ammonium*			
Chloride [0.03%] + thimerosal [0.002%]*	+	16	Minimal
Chlorhexidine [0.005%] + thimerosal [0.001%]*	+	25	Moderate
Thimerosal [0.001%]*	+	10	Minimal
Sorbic acid [0.1%]*	+	16	Minimal
0.01% Benzalkonium chloride	+	48	Severe
0.01% Benzalkonium chloride	+	64	Severe

*Commercially available marketed solutions.

†Cytotoxicity was rated as follows: Minimal zone of decoloration was 20 mm; Moderate zone of decoloration was 20–40 mm; Severe zone of decoloration was 40 mm.

on a basal salts medium using standard tissue culture techniques. The cells were grown until confluent growth was obtained. HEMA soft contact lenses were cycled through seven 8-hour cycles of fresh solution prior to exposure to the mouse cells. After soaking, the lenses were rinsed in water. The mouse cells were then exposed to each respective lens for 24 hours, whereupon the cell growth was examined microscopically and with staining procedures. The cytotoxic response cell lysis and zone of cell death was reported as in Table 13–7. The results from Table 13–7 conclusively show that Onamer M is not cytotoxic to Mouse L929 cells. This cytotoxic response may be related to the acute oral LD_{50} of these compounds listed on Table 13–8. Additional work is needed to prove if there is a correlation of cytotoxicity and acute oral LD_{50} of the biocides within the same class or different ones. This seems to be the case, if one compares Onamer M with the benzalkonium chloride and the other products. All are more toxic than Onamer M, as can be seen from LD_{50} reported in the literature. Petrocci et al. in 1979 examined this compound and determined that the product was bacteriostatic against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus faecalis* at 50 ppm of active product.

Table 13–8. *Acute Oral LD_{50}: Commercial Antimicrobial Quaternary Ammonium Compounds*

Compounds	LD_{50}
1. Cetylpyridinium chloride	0.20 g/kg
2. Benzalkonium chloride	0.30 g/kg
3. Domiphen bromide	0.32 g/kg
4. Methylbenzethonium chloride	0.35 g/kg
5. Benzethonium chloride	0.42 g/kg
6. Didecyldimethylammonium chloride	0.53 g/kg
7. Octyldodecyldimethylammonium chloride	0.72 g/kg
8. BTC 2125M	0.75 g/kg
9. Dowicil 200	2.20 g/kg
10. 6,10-tonene bromide	1.00 g/kg
11. WSCP or Bussan 77	2.77 g/kg
12. Mirapol A-15 (not sold as antimicrobial)	2.85 g/kg
13. Onamer M or Polyquat or Polyquaternium-1	4.47 g/kg

Chem. Pharm. Bull., 34, 4215–4224.

In addition, Onamer-M, when tested by the EPA-required Use-Dilution method, demonstrated efficacy as a hard surface disinfectant at 300 ppm of active product against *Salmonella choleraesuis*, at 350 ppm of active product against *Staphylococcus aureus*, and at 800 ppm of active product against *Pseudomonas aeruginosa*. The product demonstrated excellent activity by a time/test at 10 and 15 ppm of active product against *Pseudomonas aeruginosa* and *Enterobacter aerogenes* following a 30-minute contact period.

At least two characteristics are common to the WSCP and Onamer-M polymeric quaternaries, making them uniquely different from the ordinary biocidal quaternary ammonium compound. One is the absence of foaming, even at high aqueous concentrations. The other is the remarkably low toxicity shared by these polymeric quaternary products. As an example, for Onamer-M at 30% active material, primary abraded and intact skin irritation scores were zero for all observation periods. Draize eye irritation studies recorded a mild conjunctival irritation with scores of 2 or 4 in each rabbit, which cleared on the second day of observation. The acute oral LD_{50} in rats was determined to be 4470 mg/kg. The product was described as nonmutagenic by the mouse lymphoma forward mutation assay and by the sex-linked recessive lethal test in *Drosphila melanogaster*. In addition, it was not considered carcinogenic by the in-vitro transformation of Balb/3T3 cells assay.

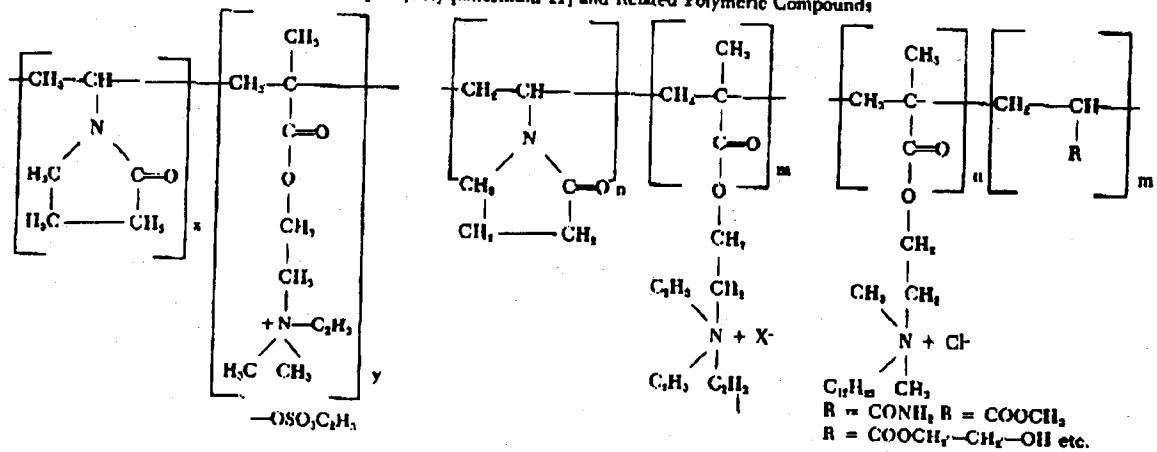
For reasons of structure, size, foaming, and toxicity, those polymeric quaternary compounds must be consid-

ered uniquely different from the biocidal quaternary ammonium compounds heretofore utilized. The results obtained to date should encourage synthesis within this structure group to produce additional compounds for consideration. Besides the polyionenes quaternary polymers there are other classes of antimicrobial polymers in which the quaternary nitrogen is pendant away from the backbone chain of the polymer (Table 13–9). Samour and coworkers (1978) reported a number of quaternary monomers, homopolymers, and copolymers of N-vinylpyrrolidone and 2-methacryloxyethyl-N,N,N-triethyl ammonium bromide and iodide with antimicrobial activity. This activity increases as the content of quaternary ammonium moiety increases. The quaternary nitrogen is pendant away from backbone chain and requires the proper lipophilic groups and proper charge density of the quaternary nitrogen for biocidal activity. In Table 13–9 are listed the most common polymers made by free radical polymerization. None of these polymers is used as an antimicrobial, but they are commercially available as hair conditioning agents for shampoos and other cosmetic products. Examples are Cafquats (Polyquaternium 11) and Merquats (Polyquaternium 7). These products are made by the free radical polymerization of diallyl quaternary monomer, and their molecular weight is over 100,000 (Butler et al., 1966—U.S. Patent No. 3,288,770).

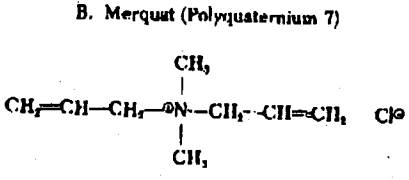
However, more recently Andrews et al. (1981—U.S. Patent No. 4,304,894) reported related Terpolymer, with molecular weight 10,000 to 20,000, to have surprisingly effective sterilizing activity against *Candida albicans* at 0.1%, when it was used as a preservative for soft contact lenses without accumulation into the lenses.

Table 13–9. *Free Radical Polymeric Quaternary Ammonium Compounds*

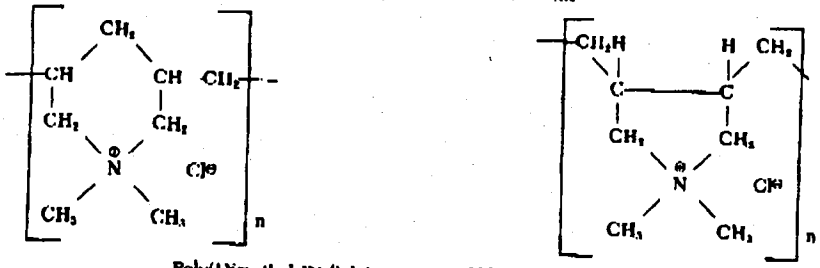
A. Cafquat [Polyquaternium 11] and Related Polymeric Compounds



B. Merquat (Polyquaternium 7)



Diallyl Dimethyl Ammonium Chloride



Poly(Dimethyl Diallyl Ammonium Chloride) (DMDAAC)

Ikeda and Tazuke (1983) reported that antimicrobial activities (MIC) of poly[trialkyl(vinylbenzyl)ammonium chloride] type of polycations against bacteria and fungi were more active than the corresponding monomers; however, the absolute activity is low compared to commercially used quaternaries. The antibacterial assessment was based only on the conventional spread plate method, which has been widely used to evaluate the antibacterial activity of ordinary antibiotics and disinfectants. However, the antibacterial activity of the polycations can not be determined precisely by this method on account of the adsorption of the polycations by some constituents in the culture media. The activity must be evaluated in the absence of any interfering material. More recently, Ikeda and Tazuke (1984, 1985) obtained peculiar results for poly[dodecyldimethyl(vinylbenzyl)ammonium chloride] and its monomer using the conventional spread plate method for evaluation. The MIC of the monomer was 10 to 100 ppm against *Escherichia coli*, *Aerobacter aerogenes* and *Pseudomonas aeruginosa*, whereas the MIC of the polymer product was greater than 1000 ppm against the same organisms.

The poly[dodecyldimethyl(vinylbenzyl)ammonium chloride interacted with the negatively charged species (such as sodium caseinate of the agar plate) and produced an insoluble complex, leading to inactivation of the polymer. In order to evaluate the antibacterial activity of such disinfectants correctly, it is preferred that the products come in contact with bacterial cells in sterile water or saline, and then that the surviving cells be counted after being cultivated on agar plates. Using this viable counting method the antibacterial activity of poly-[dodecyldimethyl(vinylbenzyl)ammonium chloride] was determined against *Staphylococcus aureus*, and at a concentration of 0.5 ppm all the bacterial cells were killed within 30 minutes of contact, whereas the corresponding monomer was inactive. Compounds with the longest alkyl chain studied (dodecyl) were found to exhibit high activity, and this was ascribed to the contribution of the increased hydrophobicity of the compounds to the activity.

In conclusion, the most significant finding was that the polymers are more active than the corresponding monomers, particularly against gram-positive bacteria. The higher activity of the polymers was interpreted as due to favored adsorption onto the bacterial cell surface and the cytoplasmic membrane with subsequent disruption of its integrity, although they have the disadvantage of diffusing through the cell wall, especially with the gram-negative bacteria.

Sheldon et al. (1985—U.S. Patent No. 4,532,128) reported that polymeric quaternary ammonium compounds having repeat vinylbenzylammonium units were antimicrobial and particularly useful for preserving ophthalmic solutions.

Ikeda and Tazuke in 1985 reported an optimal MW region of 14,300 for the 6,6-ionene bromide with antibacterial activity minimum bactericidal concentrations (MBC) of 6.6 ppm to 10 ppm against *Staphylococcus aureus*. The same workers reported that polymeric biguanides of MW 11,900 and poly{alkyl[C_8–C_{18}]-dimethyl[vinylbenzyl]ammonium chloride} of similar MW exhibit better bactericidal action against gram-positive than gram-negative bacteria. The poly{dodecyldimethyl[vinylbenzyl]ammonium chloride} was the most active with 0.5 ppm against *Staphylococcus aureus*, which suggests that hydrophobicity plays an important role in bactericidal action.

In conclusion, in all three types of polymers reported by Ikeda and Tazuke (1985), there is a clear MW dependence of the biocidal activity—that is, polymers with low MW as well as high MW exhibit lower bactericidal activity—and there exists an optimal MW region for the biocidal action. This MW dependence was confirmed in my work with Onamer M analogues (Merianos, 1986). It is my belief that a fine balance exists in the polymeric biocides between MW, biocidal activity (cytotoxicity), and (toxicity) acute oral LD_{50} as seen in Tables 13–8 and 13–7.

ANALYSIS

The chemical structure of quaternary ammonium compounds permits a variety of quantitative procedures to determine their concentration. Recently various analytical methods have been reported for the determination of benzalkonium chlorides by high-performance liquid chromatography (HPLC) (Marsh et al., 1983; Sato et al., 1984) and gas chromatography (GC) (Suzuki et al., 1989; Cybulski, 1984; Ng et al., 1986). Chemical ionization mass spectroscopy (MS) has been used to identify and determine the proportions of various alkyl chain lengths in commercial mixtures of benzalkonium chlorides (Dauod et al., 1983). The diphasic or the dye transfer method or the Epton method (1947) cannot be used for the determination of polymeric quaternary ammonium compounds. Recently, two methods for determination of low concentration of Onamer-M or polyquaternium 1 antimicrobial preservative in ophthalmic solutions (Good et al., 1987; Stevens and Eckardt, 1987) were reported. A direct titration technique for the accurate, quantitative determination of cationic and anionic polyelectrolytes has been reported by Wang and Shuster (1975). The technique is based on a direct neutralization reaction between cationic and anionic forms of the polymers. Modification of poly[vinylsulfuric acid] potassium salt (PVSAK) method has been used for determination of high concentration of Onamer-M (Merianos et al., 1977). The cationic polyelectrolytes show a light blue color in the presence of toluidine blue O dye, and the blue color turns to bluish purple when the titration end point is reached. With the many methods available, the method of choice is usually dictated by the level of quaternary ammonium compound anticipated in the sample. For solutions estimated to contain 0.5% or more quaternary ammonium compound, the chemist may employ a diphasic or direct titration procedure.

STRUCTURE-ACTIVITY RELATIONSHIPS

Jacobs and Heidelberger in 1916 were the first to report correlation between chemical structure and antimicrobial activity of benzyl and substituted benzyl hexamethylenetetrammonium salts. The results obtained with some 40 substances upon *Bacillus typhosus* demonstrated the existence of direct relationships between chemical constitution and bactericidal action within the series. The degree of the bactericidal action, however, is determined by the position, character, and number of groups substituted in the benzene nucleus. By introduction of the methyl, chlorine, bromine, iodine, cyano, and nitro groups into the benzene nucleus of the parent benzylhexamethylenetetrammonium salt, the bactericidal power of this compound was notably enhanced. The substitution of these groups in the ortho position almost invariably resulted in substances that were more active than their meta or para isomers; the introduction of the methoxy group was without marked effect.

Several substances in which two hexamethylenetetrammonium groups on the same aromatic nucleus (namely bis-quats 1,2-xylylenebis[hexamethylenetetrammonium chloride and mesitylenebis[hexamethylenetetrammonium chloride]) were found to be the most active of the substances of this series when tested against *Bacillus typhosus*. Comparative tests with other bacterial types demonstrated that these compounds possessed a marked degree of specificity for *Bacillus typhosus*. The bactericidal character is directly attributable to the presence of the hexamethylenetetramine nucleus.

The same general principle of increased antimicrobial activity was observed with benzalkonium and substituted benzalkonium chlorides, as illustrated in Table 13–10. The AOAC germicidal and detergent sanitizers test was used, and the hard water tolerance in ppm as $CaCO_3$ was measured. The test organism was *Escherichia coli* ATCC No. 11229; a 99.999% reduction in 30 seconds is required with quaternary level of 200 ppm to pass.

A synergistic blend of alkyl[C_{12}/C_{14} 70/30] 2,4,6-trimethylbenzyldimethyl ammonium chloride [C] has the highest hard water tolerance (HWT) of 1300 ppm, with the 2,4,5 isomer [D] having the next best HWT of 1200 ppm, which is twice as high as the corresponding benzalkonium chloride [A]. The mesitylenyl and pseudocuminyl quaternary compounds are highly crystalline nonhygroscopic materials with excellent microbiocidal properties; however, economics favors alkyl[C_{12}/C_{14} 70/30] ethyl[15% ortho, 85% para]benzyldimethylammonium chloride[B]. The HWT of the alkyl[C_{12} and C_{14}] ethylbenzyldimethylammonium chloride is reported in U.S. Patent Nos. 3,472,939 and 3,525,793.

Synergistic blends of microbiocidal quaternary ammonium compounds with dodecyl to tetradecyl proportion 85/15 to 55/45 are listed in Table 13–11.

Kourai and coworkers (1985) have reported on quantitative structure-activity relationships of antimicrobial N-laurylpyridinium iodides (Table 13–12). The antimicrobial activities of N-laurylpyridinium iodides (19 compounds) having various substituents (methyl, ethyl, propyl, amino, carboxyl, and carbomoyl groups) on the pyridine nuclei were studied against *Escherichia coli* K12, *Bacillus subtilis* var. *niger*, *Aspergillus niger*, and *Candida utilis* with respect to their chemical structures. The N-lauryl-2,4,6-trimethylpyridinium iodide had the highest antimicrobial activity, whereas N-lauryl-2-carboxylpyridinium iodide had the lowest activity.

In general it can be seen from Table 13–12 that electron-releasing groups on the pyridine nucleus such as amino or methyl group markedly enhanced the activities, and the electron-attracting groups such as carboxyl or carbamoyl group highly reduced them. There was a clearly linear relationship between the antimicrobial activities and the acidic dissociation constant (pK_a) for the corresponding pyridines, and the same relationships exist between the pK_a and both bacteriolytic and bactericidal activities.

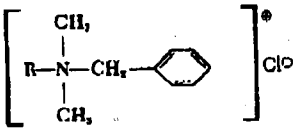
In conclusion the findings obtained suggest that the antimicrobial activities of the N-laurylpyridinium iodides are linearly dependent on the electron density of the ammonium moiety. It needs to be mentioned here, however, that the authors did not report on the stability of the iodide salts. It is well known that iodides under acidic conditions in the presence of oxygen and light can be oxidized to iodine, which is a well known biocide (Merianos, 1988). The only commercially used pyridinium quaternary is cetylpyridinium chloride, as the active ingredient in mouthwash (Cepacol), and in preservatives in pharmaceutical preparations. Economic considerations have prevented the commercialization of the substituted N-laurylpyridinium iodide quaternaries.

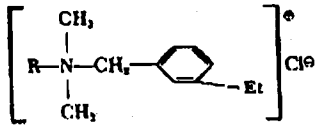
Kourai and coworkers (1989) reported on the relationship between hydrophobicity of bacterial cell surface and drug susceptibility to alkylpyridinium iodides. The value of antimicrobial activity (log 1/MIC) of quaternary iodides having long alkyl chains (hexyl, octyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl) against each bacterium was regarded as the drug susceptibility. The partition coefficient of bacterial cells between n-hexadecane and physiologic saline was regarded as representing their hydrophobicity of the cell surface.

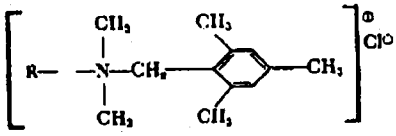
The logarithm of the coefficient was employed as a measure of the hydrophobicity at 37°C. The hydrophobicity of the gram-negative bacteria was always higher than that of the gram-positive bacteria. The susceptibility of the bacteria to each of the alkylpyridinium iodides was analyzed by use of the hydrophobicity, and a quantitative relationship between the susceptibility and their hydrophobicity was found. These findings eventually lead to a conclusion that the first step of the antimicrobial action of the iodides is a hydrophobic interaction between the cell surface and the iodide, and that the magnitude of the drug-susceptibility of the bacteria depends upon the hydrophobicity of the cell surface. Daoud et al. (1983) investigated the relationships between physicochemical properties and the antimicrobial activity of a homologous series of alkyldimethylbenzyl ammonium chlorides

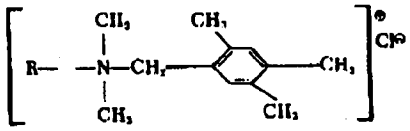
Table 13–10. *AOAC Germicidal and Detergent Sanitizers Test Method* Escherichia Coli *ATCC No. 11229 200 ppm Active Quat to Give 99.999% Reduction of* E. Coli *in 30 Seconds*

	Hard Water Tolerance (ppm) $CaCO_3$			
Alkyl	A	B	C	D
R_{12}	100	400	900	1250
R_{14}	700	700	1000	700
R_{16}	400	400	600	500
R_{18}	100	300	550	350
R_{12} (50) R_{14} (50)	650	1000	1300	1200

(A) 

(B) 

(C) 

(D) 

U.S. Patents 3,525,793, 3,472,939

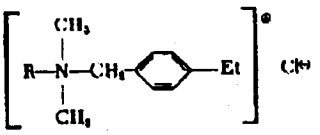
against a variety of microorganisms, and considered whether benzalkonium chloride mixtures could be improved as antimicrobial agents through a more rational choice of alkyl chain length composition. The minimum growth inhibitory concentrations (MIC) of various members of the homologous series of alkyldimethylbenzyl ammonium chlorides were determined against 12 strains of microorganisms, representative of gram-positive and gram-negative bacteria, yeast, and fungi.

The critical micelle concentrations and octanol-water partition coefficient (P) were measured for alkyldimethylbenzyl ammonium chlorides. The partition coefficients were calculated as the ratio of the concentration in the aqueous and oil (alcohol) phases expressed as the means of the replicate determination. Physicochemical data such as log P and log 1/MIC were subjected to multiple regression analysis to produce quadratic equations of the general form:

$$\text{Log } 1/\text{MIC} = a + b \log P + C [\log P]^2$$

The log P values were plotted against biologic activity for a series of compounds, and a parabolic relationship was obtained. Log P_o would in this instance be the value of log P for compounds with optimal biologic activity. This value of log P_o will vary between different types of organisms. Many such parabolic relationships have been demonstrated, including several for quaternary ammonium compounds (Hansch and Clayton, 1973; Hansch and Fujita, 1964). The levels of antimicrobial activity were observed to be parabolically related to the alkyl chain length, and thereby to log P. The chain lengths with optimal activity varied from organism to organism, reflecting differences in their cell-wall structures. The lower chain lengths [C_{10} to C_{12}] were more active against yeast and fungi, whereas gram-negative organisms were most susceptible towards the more lipophilic C_{16} compounds. This was probably a consequence of the lipophilic nature of the gram-negative cell wall and of the difficulties often encountered by hydrophilic molecules to traversing it. As result gram-negative microorganisms were the least sensitive towards all of the homologues, MIC values being approximately 10 times greater than those towards the gram-positive organisms and the fungi. The possibility of dual binding sites has experimental support from the observations of Salt and Wiseman (1968) working with cetyltrimethyl ammonium bromide, which binds in two stages, representing high- and low-affinity binding sites for the drug. Such a mechanism implies potential synergy in combination of benzalkonium chlorides and might influence the choice of alkyl chain components for preservative mixture. Hansch and Fujita (1964) and Lien et al. (1968) reported that the differences in the biologic activity of chemically related antimicrobials, acting at similar sites within the cell, would reflect their relative ease of penetration through the various liquid barriers of the cells and their ability to react at that site. The outer components of the cells were considered as a series of aqueous and lipophilic layers. Sub-

Table 13–11. *AOAC Germicidal and Detergent Sanitizers Test* Escherichia Coli *ATCC No. 11229 200 PPM Active Quat to Give 99.999% Reduction of* E. Coli *in 30 Seconds*



U.S. Patent 3,525,793

R		Hard Water Tolerance PPM	
C_{12}	C_{14}	Calculated	Determined
100	0	400	400
95	5	415	500
90	10	430	750
85	15	445	1000
82.5	17.5	453	1050
80	20	460	1110
77.5	22.5	468	1000
75	25	475	1050
70	30	490	1000
65	35	505	1000
60	40	520	900
55	45	535	1000
50	50	550	900
45	55	565	1000
40	60	580	900
35	65	595	800
30	70	610	750
25	75	625	800
20	80	640	800
10	90	670	800
5	95	685	600
0	100	700	700

stances of low water solubility would be unable to penetrate these aqueous layers and would accumulate within the lipid regions; similarly, those with low oil solubility would be unable to cross the lipophilic barriers. Compounds between these two extremes must exist that possess the optimum balance between hydrophilicity and lipophilicity for traversing the cell barriers.

In summary, antimicrobial activity of the compounds was found to be a parabolic function of their lipophilicity and maximized with n-alkyl chain length of between C_{12} and C_{16}. Generally yeast and fungi were most sensitive towards C_{12}, gram-positive bacteria towards C_{14}, and gram-negative bacteria towards C_{16}. The gram-negative cells were the most resistant towards all the compounds, and gram-positive cells the least.

Tomlinson and coworkers in 1977 reported on the effect of colloidal association on the measured activity of alkyldimethyl benzylammonium chlorides (ADBAC) homologous series C_8 to C_{18} against *Pseudomonas aeruginosa* (ATCC 9027). The critical micelle concentrations (CMC) is an important factor in the study of antimicrobial activity of cationics; at high concentrations of ADBAC, formation of a micellar state of the salt is interfering with the cationics' antibacterial action. Tomlinson et al. (1977) questioned the normally accepted parabolic relationship reported to exist between the lipophilicity of many quaternary ammonium salts and their antibacterial activities. The high concentration of nutrient salts, which is used in screening, would alter the concentration of monomeric quaternary ammonium salt. This may be responsible for some of the parabolic relationships, rather than the intrinsic antibacterial activity of the compounds. As early as 1953 it was reported that for the homologous series of cationic surface active agents, a parabolic relationship could exist between their antibacterial properties and their hydrophobic character. Thus, there is a linear relationship between activity and alkyl chain length with increased carbon number up to a maximum of between C_{12} and C_{14}, at which region there is observed a decrease in activity. In Table 13–3, the work of Cutler et al. (1967) showed that with broth dilution tests a peak activity against various microorganisms could be observed at an alkyl chain length of 14 carbon atoms. The turndown in activity is probably related to more than one physical property of the compounds: (a) The longer the chain the greater the tendency for the molecule to be adsorbed at the surface of a bacterium; (b) There is a reduction of aqueous solubility of the molecule as the carbon number is increased. These workers failed to discuss the relevance of micellization to the thermodynamic state of the ADBAC molecule in the test system. Salt additives are known to increase the tendency of ionic surface active agents to micellize in water. In some instances the micellar molecular weights can increase, indicating a change in micellar type.

In conclusion, Tomlinson et al. (1977) have measured the antibacterial activities of ADBAC homologous by the minimum inhibitory concentration procedure and by a sterilization kinetics test carried out in deionized water. There was a log-linear relationship between activity measured by kinetics test and carbon number. With the MIC method there was a log-linear relationship up to C_{14}, then there was a turndown in activity. So consideration of the colloidal association of ADBAC in deionized water and in a simple salts growth media led these authors to suggest that use of high concentrations of nutrient salts in MIC tests will lower the effective concentration of the surface active agents. This change may be responsible for the turndown in activity observed in MIC tests, and that in such circumstances the MIC test does not give a true reflection of the intrinsic activity of the compounds.

Jono and coworkers (1986) investigated the effect of alkyl chain length of benzalkonium chloride (BAC) on the bactericidal activity and binding to organic materials during a short contact time (10 s, 30 s, 1 min, 10 min) by counting survivors. Table 13–13 lists the results. The effect of human serum and dried yeast on the bactericidal activity of ADBAC standard blend OSN Alkyl[C_{12}—59

Table 13–12. *Minimum Inhibitory Concentration of N-Laurylpyridinium Iodides Against* Escherichia Coli *K12*, Bacillus Subtilis *Var.* Niger, Candida Utilis, *and* Aspergillus Niger

No.	2	3	4	5	6	Abbreviation	MICs(M)*			
							E. coli K12	*B. subtilis* var. *niger*	*C. utilis*	*A. niger*
1.	H	H	H	H	H	Py	53	5.6	61	85
2.	CH_3	H	H	H	H	2-Me-Py	21	3.6	26	26
3.	H	CH_3	H	H	H	3-Me-Py	51	4.9	59	82
4.	H	H	CH_3	H	H	4-Me-Py	31	4.6	45	72
5.	CH_3	H	CH_3	H	H	2,4-Me-Py	20	4.0	32	35
6.	CH_3	H	H	H	CH_3	2,6-Me-Py	20	5.0	30	40
7.	H	CH_3	CH_3	H	H	3,4-Me-Py	40	2.7	20	35
8.	H	CH_3	H	CH_3	H	3,5-Me-Py	74	5.5	17	69
9.	CH_3	H	CH_3	H	CH_3	2,4,6-Me-Py	2.6	0.39	0.49	29
10.	H	$CONH_2$	H	H	H	3-$CONH_2$-Py	71	20	210	67
11.	H	H	$CONH_2$	H	H	4-$CONH_2$-Py	84	24	220	77
12.	COOH	H	H	H	H	2-COOH-Py	1100	380	640	460
13.	H	COOH	H	H	H	3-COOH-Py	780	280	430	400
14.	H	H	COOH	H	H	4-COOH-Py	1000	350	700	460
15.	NH_2	H	H	H	H	2 NH_2-PY	44	10	13	23
16.	H	NH_2	H	H	H	3-NH_2-PY	29	1.8	3.8	35
17.	H	H	NH_2	H	H	4-NH_2-PY	10	1.6	7.0	23
18.	C_2H_5	H	H	H	H	2-C_2H_5-Py	45	12	160	130
19.	C_3H_7	H	H	H	H	2-C_3H_7-Py	24	6.3	53	120

*MICs against bacteria and yeast were measured by a Hitachi Recording Incubator, and MICs against mold by the test-tube dilution method.

to 63%, C_{14}—29 to 34%, C_{16}—6.8 to 7.2%]dimethylbenzyl ammonium chloride and pure C_{12}BAC, C_{14}BAC, C_{16}BAC was compared against 13 strains of bacteria.

It was concluded that from the practical point of view C_{12} benzalkonium chloride was the most effective component of the homologues series in the presence of organic materials. The bactericidal activity of BAC was inhibited by both dried yeast and human serum. The inhibition by 2.5% dried yeast was stronger than that by 10% human serum. As the carbon chain was increased, the inhibition increased. The C_{12}BAC was bactericidal against *Pseudomonas cepacia, Pseudomonas aeruginosa, Achromobacter guttatis, Alcaligenes faecalis,* and *Serratia marcescens* at 5000 ppm in a suspension of 2.5% dried yeast and at 2500 ppm in a 10% solution human serum at 1 min of contact, but C_{14}BAC and C_{16}BAC could not kill them at 10,000 ppm at 10 min. Many factors might be affected by the alkyl carbon length of BAC, producing changes in the bactericidal activity; for example, (1) aqueous solubility; (2) aqueous critical micelle concentration; (3) lipophilicity; and (4) the cell surface characteristics of the microorganisms used. The C_{12}BAC and C_{14}BAC were effectively bactericidal with all bacteria tested, even at a short contact time (30 s to 1 min). The inhibition of bactericidal activity by organic materials was least for the C_{12}BAC (Table 13–13). To clarify the relationship between inhibition of bactericidal activity of BAC by organic materials and binding of BAC to them, the authors assayed unbound C_{12}BAC and C_{14}BAC in a solution of bovine serum albumin by HPLC. As the carbon chain was lengthened, aqueous solubility decreased.

When the carbon chain is longer than C_{14}, the solubility and the critical micelle concentration of BAC are extremely low. The binding to bovine serum albumin is increased with increase in the carbon chain; the C_{14}BAC bound to bovine serum albumin 2.5 to 3.7 times more than C_{12}BAC did. The bactericidal activity of BAC in a solution of bovine serum albumin depended on the amount of unbound BAC. The results in Table 13–13 show that other factors in human serum also inhibited the activity. Human serum tended to stimulate bactericidal activity against *Staphylococcus aureus* and *Pseudomonas cepacia* when the contact time was short. Human serum may make the surface of some bacteria more sensitive to BAC. These results suggest that C_{12}BAC is the most effective component of the homologous series of BAC in the presence of organic materials. The greater the content of C_{12}BAC in the blends, the more effective the mixture will be as a sanitizer from the practical point of view.

ANTIMICROBIAL ACTIVITY

The assessment of the antimicrobial activity of the quaternary ammonium compounds has, in some recent instances, demonstrated a lack of scientific objectivity. Those statements about antimicrobial quaternary ammonium compound testing that, by the selection of the

Table 13–13. *Effect of Human Serum and Dried Yeast on Bactericidal Activity of Homologues of the Long-Chain Alkyl Benzalkonium Chlorides*

Organism		Bactericidal Concentration ($\times 10^3$ μg/ml)[a]															
		OSN				C_{12}-BAC				C_{14}-BAC				C_{16}-BAC			
		10s	30s	1 min	10 min	10s	30s	1 min	10 min	10s	30s	1 min	10 min	10s	30s	1 min	10 min
S. aureus	DW	0.5	0.5	0.2	0.05	5	1	0.5	0.1	1	0.4	0.1	0.025	0.4	0.2	0.025	0.01
FDA 209P	HS	1	0.5	0.5	0.5	1	1	0.5	0.5	5	1	0.5	0.5	0.5	0.5	0.5	0.5
	DY	5	5	2.5	2.5	5	5	2.5	2.5	5	5	2.5	2.5	>10	2.5	2.5	2.5
S. epidermidis	DW	0.2	0.1	0.0125	0.006	1	0.4	0.4	0.025	0.8	0.025	0.025	0.006	0.05	0.006	< 0.006	< 0.006
IFO 3762	HS	0.5	0.5	0.5	0.5	2.5	1	0.5	0.5	0.5	0.5	0.5	0.5	2.5	0.5	0.5	0.5
	DY	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	1
P. cepacia	DW	5	1	1	0.4	1	0.8	0.5	0.5	>10	0.5	0.2	0.1	>10	>10	0.2	0.05
ATCC 17774	HS	2.5	0.5	0.5	0.5	2.5	1	0.5	0.5	>10	5	2.5	0.5	>10	>10	>10	2.5
	DY	10	5	5	2.5	5	5	5	2.5	>10	>10	10	5	>10	>10	>10	>10
P. cepacia	DW	5	5	0.2	0.1	5	5	1	0.5	10	5	5	0.2	>10	>10	>10	2.5
H130	HS	5	1	0.5	0.5	5	1	1	0.5	5	5	2.5	1	>10	>10	>10	5
	DY	>10	5	5	2.5	10	5	5	5	>10	>10	>10	10.5	>10	>10	>10	>10
P. aeruginosa	DW	0.05	0.05	0.025	0.01	0.1	0.1	0.05	0.025	0.05	0.5	0.025	0.001	0.1	0.025	0.025	0.01
IFO 13736	HS	2.5	1	0.5	0.5	10	5	0.5	0.5	10	5	2.5	1	>10	>10	5	1
	DY	10	10	5	5	10	5	5	5	>10	>10	10	5	>10	>10	10	5
P. aeruginosa	DW	0.1	0.1	0.025	0.01	0.2	0.1	0.1	0.025	0.2	0.2	0.025	0.01	10	0.1	0.05	0.01
8L-2-32	HS	2.5	2.5	0.5	0.5	5	5	2.5	1	10	5	2.5	1	5	5	0.5	0.5
	DY	>10	10	10	10	10	5	5	5	>10	>10	10	10	>10	>10	>10	>10
P. mirabilis	DW	0.2	0.2	0.05	0.05	0.4	0.2	0.01	0.01	0.2	0.05	0.05	0.025	0.1	0.05	0.01	0.01
ATCC 21100	HS	5	2.5	0.5	0.5	5	1	1	0.5	10	2.5	2.5	1	>10	5	5	1
	DY	>10	5	5	2.5	10	5	5	2.5	>10	10	5	5	>10	>10	>10	10
P. mirabilis	DW	5	0.2	0.1	0.01	1	0.4	0.2	0.05	1	0.1	0.05	0.05	>10	0.2	0.05	0.025
82-1-4	HS	5	1	0.5	0.5	5	2.5	1	1	10	2.5	2.5	0.1	>10	>10	2.5	0.5
	DY	>10	5	5	5	10	10	5	5	>10	>10	5	5	>10	>10	>10	10
P. morganii	DW	0.5	0.2	0.1	0.05	0.2	0.2	0.05	0.05	1	0.05	0.01	0.01	1	0.05	0.05	0.025
82-2-11	HS	2.5	1	0.5	0.5	10	5	0.5	0.5	10	5	1	1	>10	>10	5	1
	DY	5	5	5	5	10	5	5	5	10	10	5	5	>10	>10	>10	10
S. marcescens	DW	5	0.4	0.2	0.05	>10	0.4	0.4	0.025	>10	0.4	0.2	0.025	>10	>10	0.025	0.025
82-2-52	HS	5	2.5	1	0.5	10	5	1	0.5	>10	>10	5	2.5	>10	>10	>10	2.5
	DY	>10	>10	5	5	>10	5	5	5	>10	>10	10	5	>10	>10	>10	10
Flavobacterium	DW	0.1	0.05	0.05	0.025	0.2	0.05	0.05	0.025	0.2	0.05	0.025	0.025	0.2	0.1	0.025	0.01
sp. 82-1-98	HS	2.5	0.5	0.5	0.5	5	1	0.5	0.5	5	5	1	0.5	10	5	2.5	0.1
	DY	2.5	2.5	2.5	2.5	5	2.5	2.5	1	10	10	5	1	10	5	5	2.5
A. guttatis	DW	5	5	1	0.2	>10	>10	1	1	>10	>10	10	1	>10	>10	>10	0.5
A-39	HS	5	5	1	0.5	5	5	1	1	>10	>10	>10	>10	>10	>10	>10	>10
	DY	>10	>10	>10	2.5	>10	>10	5	5	>10	>10	>10	>10	>10	>10	>10	>10
A. faecalis	DW	1	1	0.2	0.1	1	0.4	0.2	0.2	>10	0.2	0.2	0.2	>10	>10	>10	0.2
572	HS	10	2.5	1	0.5	10	5	2.5	0.5	>10	10	10	1	>10	>10	>10	10
	DY	>10	>10	10	5	>10	10	5	5	>10	>10	>10	5	>10	>10	>10	>10

[a]Killed from 10^8 to <10 cfu/ml at 25 C. DW, deionized water; HS, 10% human serum; DY, 2.5% dried yeast. OSN, alkyl(39–63% $C_{12}H_{25}$–, 29–34% $C_{14}H_{29}$–, 6.8–7.2% $C_{16}H_{33}$–) dimethylbenzylammonium chloride.

Joyo, K., et al. 1986. Chem. Pharm. Bull., 34, 4215–4224.

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proper test method, the compounds may appear as good disinfectants (Mallman et al., 1946), or that the phenol coefficient test cannot be used to test quaternary ammonium compounds (Sykes, 1965), or that disinfection end points obtained via inoculated carrier tests require much larger amounts of compound over those observed from phenol coefficient testing (Mallman and Hanes, 1945; Stuart et al., 1953), or that tests with quaternary ammonium compounds measure bacteriostatic activity instead of bactericidal activity (Klarmann and Wright, 1946) are grossly misleading unless some additional interpretive and qualifying information is provided. Unfortunately, a large amount of the experimental work of the middle 1930s, immediately following Domagk's publication (1935), did indeed indicate a widespread spectrum of antimicrobial activity, including sporicidal and tuberculocidal activity at extremely low concentrations of quaternary ammonium compounds. There is little doubt that this was a result of inadequate neutralization of the test material carried over to the subculture medium, so that a static condition produced in the subculture was mistaken for destructive activity.

However, it must be remembered that the entire area of neutralization to prevent carryover stasis was inadequately dealt with at the time. A firm distinction between the biostatic and biocidal activities of an antimicrobial was not always determined. The necessity to obviate stasis from the subculture medium was not always demanded as a requisite of antimicrobial testing, and the neutralization with specific chemical moieties used for individual classes of antimicrobials was not always insisted upon. Any neutralization that occurred utilized dilution of the carryover antimicrobial from the aliquot sample to the volume of subculture medium to obtain a less than static concentration of the antimicrobial in the subculture medium.

More recently, the successful identification of discrete chemicals as specific quaternary ammonium compound neutralizers, which may be incorporated into subculture media or into diluents, has made it mandatory that antimicrobial testing be performed only in the presence of such neutralizers (Quisno et al., 1946). Consequently, the more recent experimental results with quaternary ammonium compounds cannot be discredited as static instead of destructive. The statement that the quaternary ammonium compounds may be made to look good by the proper test selection (Mallman et al., 1946) may be applied to any antimicrobial chemical.

Certainly, a manipulation of the various test parameters such as type and amount of soil, selection of test organisms, and many others will bias the results, so that any chemical product will appear as a good or poor antimicrobial agent. Of the many inadequacies attributed to the phenol coefficient test method (Klarmann and Wright, 1946; McCulloch, 1947), the most pertinent objection was its use as an index of actual disinfection levels for quaternary ammonium compounds (Sykes, 1965). However, this same objection may be raised for the entire spectrum of modern disinfectant products, because extrapolation from the phenol coefficient to recommended use-dilution for a product by way of some arbitrary factor is invalid and untenable in any and every instance (Klarmann, 1959; Ortenzio et al., 1961). If the in-use characterization of a disinfectant is divorced from the phenol coefficient test, the method becomes entirely useful to describe the antimicrobial activity of a substance as the concentration that will destroy a bacterial suspension in 5-, 10-, or 15-minute contact periods. The method, which fixes many independent variables such as operation equipment, media, temperature, and volumes of test solution and inoculum, may be applied with some minor reservations to any species of microorganism. That carrier type test methods provide lower dilution end points than the phenol coefficient method has already been stated for quaternary ammonium products (Mallman and Hanes, 1945; Stuart et al., 1953) and, more importantly, for other kinds of chemical products (Klarmann, 1959; Ortenzio et al., 1961). The dynamics of testing carriers prepared with inocula as dried films of microorganisms provide a more severe test than the liquid inoculum system of the phenol coefficient method. This feature of carrier-type tests has led to the development and use of these tests by the United States regulatory agencies to measure bactericidal, sporicidal, tuberculocidal, and sanitizing activity (Official Methods, AOAC, 1984).

Of special note are the efforts to determine the validity and accuracy of product-label statements and claims made by manufacturers of disinfectant products for sale in the United States. This regulation of antimicrobials intended for treatment of inanimate surfaces was formerly exercised by the United States Department of Agriculture and currently by the United States Environmental Protection Agency. It includes deliberate scrutiny of a product for antimicrobial efficacy and toxicologic and chemical properties in compliance with the product-label claims and other attendant information—all prior to the initial sale of the product. Further, the agency is required to monitor future production obtained on a periodic inspection schedule. To facilitate the antimicrobial examination, a number of testing protocols are prescribed to examine specific antimicrobial activity (Greene and Petrocci, 1980).

For those catalogued as AOAC methods, the reader is referred to the AOAC publications (Official Methods of Analysis of the AOAC, 1984) for the purpose, scope, precise methods, and application areas served by these tests. In addition to the AOAC methods, the Environmental Protection Agency recommends methods to determine the sanitizing activity on inanimate, nonfood contact surfaces, the initial and residual bactericidal and bacteriostatic activity of treated laundry fabric, the sanitizing activity on carpeting, the virucidal activity on environmental surfaces, and the initial and residual fungicidal and fungistatic activity on inanimate surfaces. This requirement to test products by specified protocols pro-

vides a useful means to collect data that may be compared, and so to more accurately characterize the antimicrobial activity of a substance. The recent installations of prescribed test methods by other agencies and in other countries is a most salutary development to eliminate the confusing conclusions developed heretofore from many different kinds of experimental studies.

Quaternary ammonium compounds have been tested by a multitude of different procedures, which may be generally classified as (1) In vitro static or minimum inhibitory level tests, usually as broth dilution tests; (2) In vitro or killing dilution tests, with phenol coefficient as an example; (3) Simulated use-dilution tests, including inoculated carriers of representative surfaces such as stainless steel, unglazed porcelain, and fabric; and (4) In-use tests, which use the product in the actual environment (Greene and Petrocci, 1980). The results of such testing with quaternary ammonium compounds may be summarized as follows: the active quaternary ammonium compounds sold as items of commerce are algistatic, bacteriostatic, tuberculostatic, sporostatic, and fungistatic at low concentration levels of 0.5 to 5 ppm (Hueck et al., 1966; Freelander, 1940; Schneider, 1935); they are algicidal, bactericidal, fungicidal, and virucidal against lipophilic viruses at medium concentration levels of 10 to 50 ppm (Lawrence, 1950; Petrocci et al., 1974; Klein and Deforest, 1963); they are not tuberculocidal or sporicidal or virucidal against hydrophilic viruses at high concentration levels (Klein and Deforest, 1963; Smith et al., 1950; Davies, 1949). They are bactericidal to both gram-positive and gram-negative bacteria, with some evidence for greater activity against the gram-positive bacteria (Quisno and Foter, 1946). In this regard, it should be remembered that the gram-negative bacteria as a group, and especially the pseudomonas species, are more resistant to all the antimicrobial compounds currently available.

The quaternary ammonium compounds are bactericidal agents in acid and alkaline environments, with some evidence for greater activity in the alkaline range (Gershenfeld and Perlstein, 1941). The following descriptions of antimicrobial results are presented as representative data for quaternary ammonium compounds. They are compiled and prepared from the published scientific literature, from Onyx (Stepan) unpublished work, from brochures and data sheets supplied by the quaternary ammonium compound manufacturers, and from patents issued for quaternary ammonium compounds. It is important to recognize the absence of bias in information supplied by the manufacturers' literature that is carefully scrutinized by the regulatory agencies in advance of the sale of the chemical. The bacteriostatic, fungistatic, and algistatic activities of fatty nitrogen compounds, including quaternary ammonium compounds, were described

Table 13–14. *Inhibiting Concentrations (in PPM) of Fatty Nitrogen Compounds For Some Bacteria, Fungi, and Algae*

	Bacteria			
	C–N		*C–P*	
Compound	*Escherichia coli*	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*
Benzethonium chloride	1,000	300	3	3
Benzalkonium chloride	200	300	3	4
Dodecyltrimethyl ammonium chloride	500	500	5	5
Dodecylbenzyldimethyl ammonium chloride	750	750	2	2
Cocobenzyldimethyl ammonium chloride	225	225	2	2
Didecyldimethyl ammonium chloride	225	750	0.7	7

	Fungi			
Compound	*Aspergillus niger*	*C. globosum*	*M. verrucaria*	*T. viridae*
Benzethonium chloride	300	30	300	200
Benzalkonium chloride	60	10	40	60
Dodecyltrimethyl ammonium chloride	500	50	500	500
Dodecylbenzyldimethyl ammonium chloride	75	7	150	75
Cocobenzyldimethyl ammonium chloride	20	7	20	20
Didecyldimethyl ammonium chloride	75	7	20	20

	Algae			
Compound	*Ch. vulgaris*	*Stigeoclonium species*	*A. cylindrica*	*Os. tenuis*
Benzethonium chloride	3	1	1	1
Benzalkonium chloride	1	0.7	1	0.6
Dodecyltrimethyl ammonium chloride	50	5	5	0.5
Dodecylbenzyklimethyl ammonium chloride	0.2	0.2	0.2	0.2
Cocobenzyldimethyl ammonium chloride	2	0.5	2	0.7
Didecyldimethyl ammonium chloride	2	0.7	0.2	0.7

by Hueck et al. (1966); data selected therefrom for the quaternary ammonium compounds are presented in Table 13–14. The killing dilutions for some chemically different, commercially available quaternary ammonium compounds after a 10-minute contact period at 20°C by the phenol coefficient method against the recommended test organisms *Staphylococcus aureus* and *Salmonella typhosa* are compiled from the manufacturers' brochures (e.g., Brochure: Hyamine, Brochure: Roccal, BTC 2125M BTC, Brochure: Bardac) as Table 13–15.

These are at high dilutions against both organisms, and they are at significantly different dilutions depending on the specific chemical structure of the quaternary compound. The effect of the size of the quaternary molecule on the antimicrobial activity of a homologous series of alkyldimethylbenzyl ammonium chlorides is well documented by Cutler et al. (1966). They demonstrated a maximum activity as the highest 10-minute killing dilution against *Staphylococcus aureus* and *Salmonella typhosa* and *Pseudomonas aeruginosa* for the C_{14} member compound tested. Their results are shown in Table 13–3. Additional evidence for the structure and antimicrobial activity relationship is offered by the work of Petrocci et al. (1974), who evaluated the interference of hard water with the sanitizing activity against *Escherichia coli* of a group of dialkyldimethyl ammonium chloride compounds of varying long-chain lengths. This comparison of the hard-water tolerances, which are defined as the maximum concentrations of synthetic hard water (as $CaCO_3$), in which 200 ppm of active quaternary compound will continue to effectively sanitize (at 99.999% reduction) a suspension of *Escherichia coli* after a 30-second contact period, are displayed in Table 13–16.

Quisno's work with cetyl pyridinium chloride (Quisno and Foter, 1946) illustrates several characteristics of quaternary ammonium compounds, such as the greater activity for gram-positive bacteria over gram-negative bacteria (Table 13–17), and the positive effect of temperature and the negative effect of organic matter on antimicrobial activity (Table 13–18).

Lawrence (1950) described the effect of pH on the antibacterial activity of an alkyldimethylbenzyl ammonium chloride at 5-, 10-, and 15-minute contact periods against *Staphylococcus aureus*. A portion of these data is available as Table 13–19. Over many years, Petrocci has examined the fungicidal activity of many quaternary structures against *T. interdigitale* (Petrocci, unpublished). Some of these data are available as Table 13–20. This small section of antimicrobial activity is only a minute portion of the total information that has been published and is available for examination. The space limitations of this chapter do not permit a complete listing of this information; instead, I have attempted to select and present representative data that are compatible with the bulk of current opinions in the field.

TOXICOLOGY

On March 4, 1987, the EPA issued a Data Call-In Notice requiring all registrants of antimicrobial active ingredients to submit subchronic and chronic toxicologic data to support the continued registration of their products. The EPA will permit registrants to select among several options for ways to comply with the data requirements of this notice. The EPA has concluded that it should be possible to evaluate risk by acquiring exposure data and by acquiring toxicity data under a tiered approach. The three-tier studies may be summarized as follows:

A. *The first-tier studies:*
   1. 90-days dermal
   2. 90-days inhalation
   3. Teratogenicity 1st
   4. Mutagenicity

B. *The second-tier studies:*
   1. Subchronic feeding

Table 13–15. *Killing Dilutions of Commercially Available Quaternary Ammonium Germicides for 10-Minute Contact Periods at 20°C from Phenol Coefficients in Manufacturers' Brochures*

	Killing Dilutions vs.	
Chemical	*Staphylococcus aureus*	*Salmonella typhosa*
Di-isobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride	1/25,000	1/25,000
40% Methyldodecylbenzyl trimethyl ammonium chloride, 10% methyl-dodecylxylylene bis (trimethyl ammonium chloride)	1/24,000	1/22,500
N-alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethyl benzyl ammonium chlorides	1/45,000	1/45,000
Benzyldimethyltetradecyl ammonium chloride	1/42,800	1/67,500
N-alkyl (60%C_{12}, 30%C_{16}, 5%C_{14}, 5%C_{18}) dimethylbenzyl ammonium chlorides	1/44,000	1/39,600
25% *N*-alkyl (68%C_{12}, 32%C_{14}) dimethylethylbenzyl ammonium chlorides	1/55,500	1/54,300
N-alkyl (98%C_{16}, 20%C_{14}) dimethyl 1-naphthylmethyl ammonium chlorides	1/60,000	1/58,500
N-alkyl (50%C_{12}, 30%C_{14}, 17%C_{16}, 3%C_{18}) isoquinolinium bromides	1/38,000	1/40,000
Octyldodecyldimethyl ammonium chlorides	1/61,800	1/57,500
Alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethylbenzyl ammonium saccharinates	1/43,500	1/40,500
Didecyldimethyl ammonium chlorides	1/83,000	1/84,000

Table 13–16. *Hard Water Tolerances (PPM $CaCO_3$) of Dialkyldimethyl Quaternary Ammonium Compounds with Long-Carbon Chains (R_1 + R_2) that Total 19 to 22 Carbons*

Totals Carbons (R_1 + R_2)*	Hard Water Tolerance (ppm $CaCO_3$)						
19	700	800	500	550			
20	500	900	1200	1400	1100		
21	300	700	900	1300	1300		
22	400	550	750	900	800	650	
	6	7	8	9	10	11	R_1 carbon length

*R_2 carbon chain length obtained by subtracting R_1 carbon chain length from the R_1 + R_2 total carbon chain length.

Table 13–17. *Germicidal Activity of Cetyl Pyridinium Chloride Aqueous Solution*

Organism	No. Strains Tested	Average Critical Killing Dilution In Terms of Active Ingredients at 37°C, No serum
Staphylococcus aureus	5	1:83,000
Staphylococcus albus	1	1:73,000
Streptococcus viridans	1	1:42,500
Streptococcus hemolyticus	2	1:127,500
Neisseria catarrhalis	2	1:84,000
Diplococcus pneumoniae I	1	1:95,000
Pseudomonas aeruginosa	2	1:5,800
Klebsiella pneumoniae	2	1:49,000
Corynebacterium diphtheriae	1	1:64,000
Mycobacterium phlei	1	1:1,500
Eberthella typhosa	5	1:48,000
Escherichia coli	2	1:66,000
Proteus vulgaris	2	1:34,000
Shigella dysenteriae	1	1:60,000
Shigella paradysenteriae (Flexner)	2	1:52,000
Shigella paradysenteriae (Hiss)	1	1,49,000
Shigella sonne	2	1:68,000

2. Teratogenicity 2nd
3. Dermal absorption
4. 90-days neurotoxicity

C. *The third-tier studies:*
1. Chronic feeding, two species
2. Oncogenicity, two species
3. Reproduction
4. Metabolism to clarify issues concerning structure activity relationships

On February 26, 1988, the EPA clustered the quaternary ammonium compounds into four groups, so that the toxicity study would be facilitated by selecting one member from each group for testing.

Group I: The straight-chain alkyl or hydroxyalkyl quaternaries; the most common product in this group is the twin chain quats. The EPA will allow a full set of subchronic and chronic toxicity studies on didecyldimethyl ammonium chloride and teratology studies on alkyltrimethyl ammonium chloride.

Group II: The nonhalogenated substituted benzyl and alkyldimethylbenzyl ammonium compounds (ADBAC); namely benzalkonium chlorides. The EPA will allow a full set of subchronic and chronic studies on alkyl[C_{12}—40%, C_{14}—50%, C_{16}—10%] dimethylbenzyl ammonium chloride. The EPA notes that after evaluating the data it expects to receive on the Group I and II compounds, it may also require one or more studies on alkyl[C_{12}—68%, C_{14}—32%]dimethylethylbenzyl ammonium chloride, to determine whether the Group I and ADBAC data is representative of the toxicity of the ethylbenzyl quats.

Group III: The alkyl[di- and tri- chlorobenzyl]dimethyl ammonium compounds. The EPA will allow a full set of subchronic and chronic studies on alkyl[C_{12}—5%, C_{14}—90%, C_{16}—5%] dimethyl 3,4-dichlorobenzyl ammonium chloride.

Group IV: The heterocyclic ammonium compounds with unusual substituents. In this group the EPA was unable to reach a conclusion in testing a small subset of quats. Each chemical in this category must be tested separately, unless registrants can develop a testing scheme using representative compounds that is acceptable to the EPA. Representative compounds in *Group IV* are (a) alkyl[C_{12}/C_{16}] substituted Picolinium compounds; (b) alkyl[C_{17}/C_{18}] imidazolinium compounds; (c) alkyl[C_{14}/C_{16}] N-ethyl-morpholinium compounds; and (d)

Table 13–18. *Effect of Temperature and Serum Upon Germicidal Activity of Cetyl Pyridinium Chloride*

Organisms	Critical Killing Dilutions Expressed in Terms of Active Ingredients			
	37°C		20°C	
	No Serum	10% Bovine Serum	No Serum	10% Bovine Serum
Staphylococcus aureus	1:110,000	1:11,500	1:67,500	1:6,750
Eberthella typhosa	1:83,500	1:4,000	1:62,500	1:1,300

Table 13–19. *Effects of pH on the Antiseptic Efficiency of a Quaternary Ammonium Germicide Against* Staphylococcus Aureus

Quaternary Ammonium Compound Conc.	Acid or Alkali per 100 ml	pH	Bacterial Growth after Minutes of Exposure		
			5	10	15
1:15,000	0.10 ml N/10 HCl	4.3	–	–	–
1:15,000	0.25 ml N/10 HCl	3.8	–	–	–
1:15,000	0.50 ml N/10 HCl	3.5	+	+	+
1:15,000	1.00 ml N/10 HCl	3.2	+	+	+
Control (no quaternary ammonium compound)	1.00 ml N/10 HCl	3.2	+	+	+
1:25,000	0	6.7	+	+	+
1:25,000	0.10 ml N/10 NaOH	9.4	+	+	+
1:25,000	0.25 ml N/10 NaOH	10.0	+	+	–
1:25,000	0.50 ml N/10 NaOH	10.2	+	+	–
1:25,000	1.00 ml N/10 NaOH	10.5	+	–	–
1:25,000	1.25 ml N/10 NaOH	10.6	+	–	–
Control (no quaternary ammonium compound)	1.25 ml N/10 NaOH	10.6	+	+	+

alkyl[C_{12}/C_{16}] isoquinolinium compounds and other heterogeneous structures.

A knowledge of the toxicologic properties of quaternary ammonium compounds is important so that the safety, health, and well-being of man and animals are not compromised by contact with these chemicals as they are manufactured, shipped, compounded, or used. Although these specific investigations are not germane, the studies to characterize the general toxicologic properties of antimicrobial quaternary ammonium compounds and the studies of the harmful consequences of these chemicals at concentrations simulating their use patterns are of primary importance. Toxicologic effect is measured at three levels: (1) at the amount necessary to bring about the response with 100% of the experimental animals, LD_{100}; (2) at the amount that will cause the response with 50% of the animals, LD_{50}; and (3) at the amount that does not produce the response with any of the animals, LD_0. The LD_{50} level is most frequently used to characterize toxicity. In addition, the methods of administering the chemical are also identified using descriptions such as oral, subcutaneous, intraperitoneal, intravenous, and dermal. Also, the toxicity is determined as an acute response following a single administration of the chemical, as a short-term or subacute response following a multiple, periodic administration of the chemical over a short period such as 30 or 90 days, and finally as a chronic response following a multiple, periodic administration over a long period, such as the animal's life span. The LD_{50} amounts of quaternary ammonium compound depend on the route of administration. A notable example of this is the work of Nelson and Lyster (1946), who examined myristal picolinium chloride and reported an LD_{50} of 250 mg/kg by oral administration, 200 mg/kg for subcutaneous injection, 7.5 mg/kg for intraperitoneal injection, and 30 mg/kg for intravenous injection. Using the acute oral LD_{50}, which is the toxicologic characteristic most frequently described, one may compare the toxicities of different quaternary compounds. When this is done with the work of Shelanski (1949) and Finnegan and Dienna (1954), we observe acute oral LD_{50} values in white rats of 445 mg/kg for alkyldimethylbenzyl ammonium chloride, 500 mg/kg for alkenyldimethylethyl ammonium bromide, 730 mg/kg for alkyldimethyl 3,4-dichlorobenzyl ammonium chloride, 420 mg/kg for diisobutylphenoxyethoxyethyldimethylbenzyl ammonium chloride, and 389 mg/kg for alkyltolymethyltrimethyl ammonium chloride. These and similar data obtained from studies with man and laboratory animals have led to the generalized conclusion that the antimicrobial quaternary ammonium compounds examined to date exhibit similar toxicologic and pharmacologic properties.

Dermal toxicity was also examined by Shelanski (1949) and Finnegan and Dienna (1954), who concluded from animal and human testing that 0.1% was the maximum concentration of the aforementioned compounds that would not produce primary irritation on intact skin or act as a sensitizer. The conjunctival mucous membrane obviously requires special consideration, which was undertaken by a number of investigators. Nelson and Lyster (1946) observed that a 1:3000 (333 ppm) solution of myr-

Table 13–20. *Critical Killing Dilution (10-Minute Contact Period) Required for Aqueous Dilutions of Quaternary Compounds to Kill Spores of* Trichophyton Interdigitalis; *Dilutions are Based on Anhydrous Germicide*

Chemical Structure	Avg. Killing Dil.
40% methyldodecylbenzyltrimethyl ammonium chloride, 10% methyldodecylxylylene bis (trimethyl ammonium chloride)	1/2000
25% n-alkyl (60%C_{14}, 30%C_{16}, 5%C_{12}, 5%C_{18}) dimethylbenzyl ammonium chloride, 25% n-alkyl (68%C_{16}, 32%C_{14}) dimethylethylbenzyl ammonium chlorides	1/800
Octyldodecyldimethyl ammonium bromide	1/5000
Didecyldimethyl ammonium chloride	1/4750

istal picolinium chloride produced a slight irritation of the conjunctival mucosa of rabbits. Walter (1938) reported that a 1:1000 dilution (1000 ppm) of alkyldimethylbenzyl ammonium chloride instilled into the eyes of human subjects produced burning and stinging reactions, whereas a 1:5000 dilution (200 ppm) produced no unpleasant sensations. Whitehill (1945) determined that the maximum nonirritating concentrations of alkyldimethylbenzyl ammonium chloride and cetylpyridimium chloride in the eyes of rabbits were 1:3000 (333 ppm) and 1:2000 (500 ppm), respectively. Finnegan and Dienna (1954) observed comparable irritation levels for diisobutylphenoxyethoxyethyldimethyl benzyl ammonium chloride and for alkyltolymethyltrimethyl ammonium chloride (Shelanski, unpublished); in Shelanski's investigation of laurylisoquinolinium bromide, he observed that the compound at 0.5% produced moderate to severe corneal involvement of the rabbit eye, which cleared up in 5 days. However, rabbit eyes similarly exposed and washed at 2 or 4 seconds after instillation produced only a slight conjunctival involvement, which cleared up after 2 days. A most important toxicologic consideration arising from the widely encountered application area of sanitizing food contact surfaces is the cumulative effect in animals and man of the ingestion of food contacted by the small residues of the quaternary sanitizer. The laboratory study simulating this situation is the daily oral administration of quaternary ammonium compound in the food or drinking water of test animals over a considerable period of time, including the normal life span of the animal. Such studies were made early by Nelson and Lyster (1946), who determined that myristal picolinium chloride at 0.1% in the diet of rats did not interfere with their normal growth rate, but this concentration produced death when administered as a sole source of fluid and required reduction to 0.05% to permit a normal growth rate of the test animal.

Fitzhugh and Nelson (1948) summarized the results of a 2-year chronic feeding study of rats on a diet containing 0.5, 0.25, 0.125, or 0.063% alkyldimethylbenzyl chloride; they found that all the quaternary compound concentrations were toxic to rats, as evidenced by lower growth rates over an untreated control group and as determined by the histopathologic lesions present in the treated animals. Alfredson et al. (1951), however, using a significantly larger number of experimental animals and a more randomized statistical design, demonstrated that alkyldimethylbenzyl ammonium chloride at 0.25% in the diet of rats over a 2-year feeding period did not demonstrably affect the growth, food consumption, blood picture, or histopathology of the treated animals. Dogs that were fed over a 15-week period a diet containing 0.12% or less quaternary compound demonstrated similar nontoxic effects. Shelanski (1949) noted a minimal toxicologic effect, evidenced by weight loss, a depressed growth rate, and an abnormal blood or histopathology, for dogs that were allowed a 1:5000 aqueous dilution of either alkyldimethylbenzyl ammonium chloride, alkenyldimethylethyl ammonium bromide, laurylisoquinolinium bromide, or alkyldimethyl 3,4-dichlorobenzyl ammonium chloride as their sole source of drinking water over a 6-month period, for guinea pigs administered 25, 12.5, or 5 mg/kg of the aforementioned quaternary ammonium compounds daily for a 1-year period, and for rats similarly treated daily over a 2-year period. Finnegan and Dienna (1954) demonstrated that a concentration of 2500 ppm of alkyltolylmethyltrimethyl ammonium chloride or diisobutylphenoxyethoxyethyldimethyl benzyl ammonium chloride in the diet of rats produced minimal deleterious toxicologic effects over a 2-year period, whereas a concentration of 1000 ppm produced no undesirable toxicologic effects over the same period.

The concern of the Food and Drug Administration over the unsafe adulteration of food, especially of milk, with residues from quaternary sanitizers led to label statements for these products requiring a potable water rinse following the quaternary compound sanitization and reuse of the food equipment. Recently, a careful scrutiny by the FDA of additional chronic feeding studies supplied by manufacturers of quaternary ammonium compounds has concluded that the food additive regulations should be amended to provide for the safe use of a sanitizing solution on food processing equipment and utensils and on food contact surfaces in bars and restaurants if the solution does not exceed 200 ppm of quaternary compound and if the quaternary compound solution contains equal amounts of n-alkyl [C_{12}–C_{16}] benzyldimethyl ammonium chloride and n-alkyl [C_{12}–C_{14}]dimethylethylbenzyl ammonium chloride having an average molecular weight of 384. In this instance, safe use does not require a potable water rinse of treated food contact surfaces and equipment prior to reuse (Federal Register, 1969).

A short time later, the same statement was made for an aqueous solution containing n-alkyl [$C_{12}C_{16}$] benzyldimethyl ammonium chloride having an average molecular weight of between 351 and 380 and consisting principally of alkyl groups with 12 to 16 carbon atoms with or without, but not over, 1% each of groups with 8 and 10 carbon atoms (Federal Register, 1969).

Most recently, the same statement was made for a detergent-sanitizer solution containing the first sanitizing product described as a mixture of benzyl and ethylbenzyl quaternary compounds, with the optional adjuvant substances tetrasodium ethylenediaminetetraacetate or a(p-nonylphenyl)-w-hydroxypoly(oxyethylene) (or both) having an average poly(oxyethylene) content of 11 moles (Federal Register, 1974). Gleason (1969) points out that at least 10 human fatalities implicating quaternary ammonium compounds are medically recorded as resulting from alkyldimethylbenzyl ammonium chloride solutions of 10 or 15% that were introduced into the victims via oral ingestion, intramuscular or intravenous administration, or intrauterine instillation. The reader is referred to Gleason's work for a complete description of the medical and clinical symptoms of quaternary ammonium

compound poisoning and also for the recommended treatment, including first aid, for the same. One symptom should be mentioned: namely, the ingestion of concentrated solution leads to immediate burning pain in the mouth, throat, and abdomen.

The toxicologic information presented is generalized as follows: quaternary ammonium compound germicides as concentrated solutions of 10% or more are toxic, causing death if taken internally and severe irritation to the skin and conjunctival mucosa when applied externally. With normal precautions and operating procedures, the probability of such contact is extremely remote, because the effective use-dilutions of these materials are well below the concentrated level causing death and severe injury. In addition, oral ingestion, the most likely route of accidental internal administration, is self-limiting for concentrated quaternary ammonium solutions, because the immediate burning in the mouth and throat acts as a signal. At the dilute solutions of the use levels of quaternary ammonium compound germicides, which range from several to about 1000 ppm, only the most deliberate distortions of normal operating procedures could offer acute toxicity problems. Finally, chronic toxicity, as from the cumulative effect of food adulterated with quaternary ammonium compound germicides via residues from dilute sanitizing solutions used on food processing equipment, is not a problem.

Aursnes (1982) studied the ototoxic effects of quaternary ammonium compounds (QAC), benzethonium chloride, and benzalkonium chloride, frequently used for skin disinfection. The disinfectant QACs, at concentrations of 0.1% in water solution or in 70% alcohol, were introduced into the tympanic cavity (middle ear) of guinea pigs for exposure times of 10, 30, or 60 minutes. The animals were sacrificed 2 or 9 weeks after the exposure, and the organ of Corti and vestibular neuroepithelia were studied as surface preparations with phase contrast microscopy. It was found that most of the ears exposed to the disinfectants had suffered damage, affecting both the vestibulum and the perilymphatic and endolymphatic spaces of the cochlea of the inner ear. The extent of the damage was related both to the duration of exposure and to the length of the animal's survival after the exposure. Furthermore it was found that the tympanic cavity and the perilymphatic spaces of vestibulum and cochlea were pathologically changed.

The final report on the safety assessment of benzethonium chloride and methylbenzethonium chloride has been issued by CTFA (1985) in the journal of the American College of Toxicology. It was concluded that both compounds are safe at concentrations of 0.5% in cosmetics applied to the skin. A maximum concentration of 0.02% is safe for cosmetics used in the eye area. Chronic and subchronic feeding studies indicated little or no toxic effects for both ingredients. In clinical studies, benzethonium chloride produced mild skin irritation at 5% but not at lower concentrations. Neither ingredient is considered to be a sensitizer.

APPLICATIONS

Following Domagk's publication in 1935, a large number of application areas were developed for the quaternary ammonium compounds. Initially, they were used as an adjunct to surgery, such as in preoperative patient skin treatment, degerming the hands of the surgical team preoperatively, and disinfection of surgical instruments. Wetzel, in 1935, described the superiority of 0.1% alkyldimethylbenzyl ammonium chloride over 60% alcohol for treatment of the surgeon's hands prior to surgery. Much additional evidence was provided for the treatment of the surgeon's hands with alkyldimethylbenzyl ammonium chloride by Caesar in 1935, Walter in 1938, Naegel in 1940, and Swan et al. in 1949. In 1938, both Walter and White et al. reported that the use of alkyldimethylbenzyl ammonium chloride as a skin preparation for a patient prior to surgery reduced the incidence of wound infection. Hagan et al., in 1946, compared the activity of cetylpyridinium chloride and alkydimethylbenzyl ammonium chloride to mercurials as skin preparation prior to surgery and found the quaternary compounds superior. However, Miller et al., in 1943, offered evidence that quaternary compounds applied to the skin formed a continuous residual sheet, beneath which bacteria survived.

Rahn (1946) proposed that the film is formed as an oriented adsorption of the quaternary compound on an organophilic surface, with the nontoxic ends directed toward the skin and the germicidally active ends directed toward the outside, which explained the favorable results obtained for quaternary compounds in many studies. Blank and Coolidge, in 1950, rejected the film concept and proposed instead that treatment with the quaternary compounds altered the charge of the skin to positive, which attracted and held the negatively charged bacteria, leading to the favorable, if inaccurate, results obtained in basin tests with quaternary compounds. In the same publication, they speculated that phospholipids present on the surface of the skin inhibited the antimicrobial activity of the quaternary compound.

These negative comments and the widespread popular use of hexachlorophene-based products should have seriously curtailed the use of quaternary compounds as skin degerming agents. Thus it is interesting to note that King and Zimmerman, in 1965, reporting on skin degerming practices in hospitals, did determine that 37% of hospitals were using quaternary compounds, compared to the 48% observed by Price in 1948. Walter, in 1965, described a hand degerming treatment for the surgeon as a mechanical soap hand scrubbing followed by application of a mixture of isopropyl alcohol, alkyldimethylbenzyl ammonium chloride, and cetyl alcohol. Verdon, in 1961, tested a number of skin degerming agents used in British hospitals and demonstrated the utility of quaternary compounds.

Caesar and Schmidt in 1935 and Walter in 1938 recommended the use of alkyldimethylbenzyl ammonium chloride for the disinfection of surgical instruments, Cae-

sar specifying a 1:100 concentration of quaternary compound for a 10-minute contact period and Walter a 1:1000 concentration over a 30-minute contact period. Hornung (1935) recommended the addition of sodium carbonate to the quaternary solution to prevent rusting of the instruments; Post (1946) later proposed sodium nitrite for this purpose. The early investigators, with some few exceptions, did indeed subscribe to the idea of a total spectrum of germicidal activity for the quaternary compounds and thus endorsed them as surgical instrument sterilizers. Early exceptions were Zeissler and Gunther (1939), who pointed out that Zephirol would not kill spores from the soil.

Sommermeyer and Frobisher, in 1952, using glass rods inoculated with sputum containing tubercle bacilli to simulate contaminated thermometers, demonstrated that aqueous solutions of alkyldimethylbenzyl ammonium chloride and cetylpyridinium chloride at 1:1000 dilution did not kill the organism after a 10-minute contact period, whereas tinctures of the same compounds (50% alcoholic) did in fact kill the organism. The subsequent use of specific quaternary compound neutralizers as a requisite for testing has prevented additional instances of residual bacteriostasis that were interpreted as bactericidal activity, as in the case of tuberculocidal and sporicidal testing.

The inability of quaternary compounds to kill the tubercle bacillus or bacterial spores should seriously limit their use as surgical instrument sterilizers in the future. However, in some few special cases, they are still recommended for the disinfection of comparable devices (Kasper and Kirk, 1972).

The widespread use of quaternary ammonium compounds for environmental disinfection of floors, walls, and equipment surfaces in hospitals, nursing homes, public places, and gymnasia required the development of a detergent compatible with the quaternary compounds that would permit the combination of the two operations of cleaning and disinfection. Rahn and Esseltine (1947) pointed out the utility of such a system and indicated work in progress as described by Guiteras and Shapiro (1946), who combined cetyldimethylethyl ammonium bromide with the nonionic wetting agent alkylated aryl polyether alcohol and inorganic salts. The formulation prepared by Guiteras and Shapiro diluted as 1 oz/gal aqueous solution cleaned glass slides contaminated with heavy artificial soil inoculated with bacteria. When the contact period of the slide and diluted formulation was extended to 10 minutes, all the bacteria on the soiled slides were killed, as demonstrated by negative broth subcultures of the slides and by zero-count agar plates prepared from the broth subcultures.

The concept of a one-step operation to produce cleaning and antimicrobial activity, which made the treatment of large surface areas practical, met with success and furthered research to the present time toward the development of quaternary nonionic formulations with optimum cleaning and antimicrobial properties (Greene and Petrocci, 1980). Publications (Kundsin and Walter, 1961; Lenhart et al., 1961) of both in-vitro laboratory tests and of field tests under actual conditions attest to the utility of these products. Treatment of large areas of environmental surface is accomplished by a mop-and-bucket operation, by mechanical flooding and vacuum pick-up, by sponge or cloth hand-wipe operation, and by liquid spray. As an additional treatment procedure for disinfection of surfaces, there is available liquefied gas, quaternary ammonium compound germicidal formulation discharges from pressurized containers as an aerosol spray that, according to Fulton et al. (1948), killed both *Escherichia coli* and *Staphylococcus aureus* as air-borne suspensions and surface contaminations. The patent issued to Taylor and Prindle (1966) is an instance of popular and widely used surface disinfectant and space deodorant aerosol spray composition.

Recently, treatment of surfaces is reported via a mist of quaternary ammonium compound germicide generated from commercial fogging devices that produce droplets of approximately 50 μm in diameter by the shearing action of air pressure on the liquid germicide solution. This procedure is suggested as an adjunct for terminal disinfection of hospital areas. The germicidal results obtained with 0.2% active aqueous solution of a 50:50 mixture of alkyldimethylbenzyl ammonium chloride and alkyldimethylethylbenzyl ammonium chloride in field tests and in simulated in-use tests are described by Friedman et al. (1968) and Hauser et al. (1963).

Treatment of food contact surfaces, i.e., those found on equipment in the food processing industries and on food and beverage utensils in eating and drinking establishments, in order to obtain sanitary conditions that will prevent transmission of disease through the food, is defined as a sanitizing treatment or as sanitation of such surfaces. Practicable sanitizing requires that any treatment residual will not in itself be harmful; it does not require a destruction of all the bacteria present on the treated surface. The utility of quaternary ammonium compounds in sanitizing such surfaces was suggested by Krog and Marshall (1940), who experimentally contaminated the rims of glass drinking tumblers, allowed them to air dry, and treated them with a 1:5000 aqueous dilution of alkyldimethylbenzyl ammonium chloride for various contact periods before swabbing and plating procedures were employed to enumerate the viable bacteria present; this demonstrated a reduction to less than 100 bacteria per rim after only a 1 minute contact period. Two years later they reported that the use of alkyldimethylbenzyl ammonium chloride at a 1:6000 dilution in a dairy pasteurizing plant caused a 60 to 98% reduction of the bacteria count on the milk handling and processing equipment (Krog and Marshall, 1942). Frayer (1943), Mueller et al. (1946), DuBois and Dibblee (1946), Puhle (1950), and Speck and Lucas (1957) are several of the many investigators who determined the utility of quaternary ammonium compounds as germicides for the dairy farm and dairy plant. Similar reports to demon-

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strate the advantage of quaternary compounds as germicides in other food processing industries have appeared, such as those of Penniston and Hedrick (1945) in the egg processing industry, Tressler (1947) in the fishing industry, Lehn and Vignolo (1946) and Resuggan (1951) in the brewing industry, and Casey (1972) in the sugar refining industry.

The treatment of large amounts of water to destroy and prevent the proliferation of disease-producing microorganisms, of industrial process-interfering microorganisms, and finally of noxious, esthetically undesirable microorganisms is an important application area of the quaternary ammonium compounds. The destruction of cysts of *Entamoeba histolytica* was reported by Fair et al. (1945) and by Kessel and Moore (1946). Kessel and Moore offered their opinion that quaternary ammonium compounds should be considered for the emergency sterilization of drinking water; this opinion was based on the high germicidal levels observed. Sotier and Ward (1947) observed that a product containing 0.5% of Hyamine 1622 was an effective disinfectant of new water mains and of hemp used to prepare joints on the mains. Quaternary ammonium compounds have been recommended for treating process water in paper mills (Erskine et al., 1958; Shema, 1966) to prevent the proliferation of microorganisms on the surface of equipment contacting the pulp solution. These proliferations form microbial slimes that become detached and are carried along to become spots in the paper, often causing paper breaks during the manufacturing process that result in expensive, time-delaying machine shutdowns. Quaternary compounds are used in cooling water systems (Darragh and Stayner, 1954; Berneschot et al., 1964) to prevent proliferation of bacteria in the circulating cooling water system that would form microbial slimes that may reach the heat exchange units and impair their operational efficiency. Prusick and Gregory (1956) recommended the use of dicocodimethyl ammonium chloride to prevent proliferation of bacteria in injection water or brine used in secondary oil recovery operations. The injection water is pumped under pressure into injection wells drilled around the producing well, so that as it spreads through the subterranean sand structures containing residual oil, it will displace the residual oil in the direction of the producing well and increase the productivity of the well. If there is a proliferation of microorganisms in the injection waters, the subterranean sand structures will become plugged, requiring greater pressures to be applied to the injection waters until the operation cannot function. Steinberger (1963) described the ability of N-alkyl crude tar base quaternary ammonium compounds at 5 to 20 ppm to inhibit the proliferation of *Desulfovibrio desulfuricans*, which with pseudomonas species are primarily involved in plugging the sand formation.

Outdoor swimming pools are regularly treated with quaternary ammonium compounds to prohibit growth of algae, an esthetic rather than a public health problem. Palmer and Maloney (1955) determined that 2 ppm of several different quaternary ammonium compounds did effectively inhibit the growth of various algae species. Antonides and Tanner (1961) recommended a formulation containing trimethylalkyl ammonium compound at 4.5 ppm as a swimming-pool algicide and bactericide. Shay et al. (1964) demonstrated that alkyldimethylisopropylbenzyl ammonium chloride at 7 ppm killed both *Escherichia coli* and *Streptococcus faecalis* at a 30-second contact time by the AOAC test method prescribed for swimming pool bactericides.

The positively charged functional portion of the quaternary molecule is attracted to and substantive to negatively charged fabric; it may be applied to the fabric from a quaternary solution by rinsing, padding, or spraying. Taking advantage of this phenomenon, Benson et al. (1947, 1949) proposed that diapers be treated with [methylbenzethonium chloride]p-diisobutylcresoxyethoxyethyldimethylbenzyl ammonium chloride monohydrate in the final rinse of the laundry cycle. Treated diapers would prevent the formation of ammonia from urine by the action of ammonia-producing bacteria found in the infant's feces; thus, ammonia dermatitis leading to diaper rash could be prevented. Lawrence (1950) pointed out the need to apply chemical treatment between the laundry loads of different customers using public automatic clothes-washing machines.

Although large commercial laundries could provide sufficient volumes of hot water at disinfecting temperatures, the small automatic installations could not. Lawrence used alkyldimethylbenzyl ammonium chloride to treat laundry in the final rinse cycle of automatic machines in a coin-operated self-service laundry. He determined that there were high bacteria counts in the first soak and wash cycle water, which following treatment with the quaternary compound at 1:5000 (200 ppm) or 1:10,000 (100 ppm) dilution, were reduced to zero counts in the deep rinse cycle.

McNeil and Choper (1962) treated naturally soiled wash loads at the warm or hot water setting and in the wash or rinse cycle with alkyldimethylbenzyl ammonium chloride to provide 200 ppm of active quaternary compounds in the presence of anionic or nonionic laundry detergent. Compared to untreated wash loads, the quaternary compound used with the warm or hot water setting consistently reduced the numbers of bacteria in the wash and rinse waters and on fabric swatches attached to laundry items. This occurred with the quaternary compound added to the rinse cycle, and to the wash cycle when a nonionic detergent was used. Utilizing the AOAC antimicrobial laundry additive test procedure (Petrocci and Clarke, 1969), a simulated use test, Shay and Petrocci (1968) demonstrated sanitization of fabric during the laundry cycle following addition of a 50:50 mixture of alkyldimethylbenzyl ammonium chloride and alkyldimethylethylbenzyl ammonium chloride at a 200 ppm level based on the weight of dry laundry fabric. In addition, fabric similarly treated and allowed to air dry demonstrated residual bacteriostatic activity against

Staphylococcus aureus, *Escherichia coli*, and *Brevibacterium ammoniagenes*, and also demonstrated residual self-sanitizing activity against *Staphylococcus aureus* and *Escherichia coli*. Some of the miscellaneous applications for quaternary ammonium compounds are as preservatives. Lawrence (1950) evaluated a large number of chemicals as preservatives for ophthalmic solution and selected benzalkonium chloride at 1:5000 and 1:10,000 as superior. More recently, Stark (1985) used Polymeric quaternary ammonium compounds, namely Polyquat, for disinfecting and preserving solution for contact lenses, ointments, and other ocular medicaments. The polyquaterium-1 (Onamer M) has been approved by the FDA as a preservative in several brands of contact lens soaking solutions at 0.01 to 0.001% [Opti-Soft, Opti-Clean, Opti-Tears, Opti-Free, and Tears-Natural II]. This product has replaced benzalkonium chloride and Thiomerosal in these preparations. Bernarducci and Harrison (1988) have discovered that water-soluble cationic polymers (Busan 77, WSCP, Onyxsperse 12S, Onamer M), when formulated in an aqueous medium with one or more nonionic surfactants, provide stable, isotropic liquid laundry detergent and sanitizer compositions having good detergency and bactericidal properties, and in addition are less irritating to the eye and practically nonirritating to the skin.

Like et al. (1975) examined a number of compounds, including a 50:50 mixture of alkyldimethylbenzyl ammonium and alkyldimethylethylbenzyl ammonium chlorides, as preservatives for cosmetic oil-in-water systems using amine oxides as emulsifiers. At 1000 or 2000 ppm, the quaternary ammonium compounds reduced the microbial count of the cosmetic system inoculated with bacteria and fungal spores from an initial count of several million per ml to less than 10 per ml over an 8-week incubation period, including a reinoculation after 4 weeks of incubation.

In another instance, as a fungistat for exterior latex paint films, Ramp et al. (1966) demonstrated that alkyldimethyl ethylbenzylammonium cyclohexylsulfamate at about a 1% level in exterior latex paints would produce a paint film resistant to attack by fungi. In another instance, as a moth-proofing agent for wool fabric, Tolygesi et al. (1971) concluded that tricaprylylmethyl ammonium chloride was superior to all other quaternary ammonium compounds tested for this purpose. Both compounds applied to wool fabric at 0.15 to 0.40% add-on levels protected the fabric from the black carpet beetle and the webbing clothes-moth larvae.

MODE OF ACTION

The earliest accounts of the quaternary ammonium compounds related antimicrobial activity to chemical structure by utilizing homologous series of quaternary compounds and noting the effect on antimicrobial activity offered by variations in structure (Jacobs, 1916). This method of collecting information on the antimicrobial attributes of a quaternary compound is constantly employed to determine the most effective structure that may be used as an item of commerce. Beyond this purpose, this method contributes little to our understanding of the basic mechanism of quaternary ammonium compounds as antimicrobial agents. Although the mode of action has not yet been completely or definitively described in detail, there are well defined and accepted steps explaining the mode of action of cationic disinfectants or antiseptics as reported by Ikeda and Tazuke (1985) and Franklin and Snow (1989). Many techniques have been used in elucidating the mode of action of antimicrobial agents. Once the primary site of action is established the overall effect of the drug on the metabolism of microbial cells can often be explained. The most important site of adsorption is the cytoplasmic membrane. Spheroplasts or protoplasts lacking the outer cell wall layers will bind the cationic antiseptic and may be lysed or damaged. Adsorption by isolated cell membranes can be demonstrated.

The extent of killing of the bacteria is governed by five principal factors: (a) Concentration of antiseptic or disinfectant; (b) nature of bacterial cells and density; (c) time of contact; (d) temperature of medium; (e) the pH; and (f) the presence of foreign matter.

The adsorption of a given amount of the compound per cell leads to the killing of a definite fraction of the bacterial population in the chosen time interval. The lowest concentration of the antiseptic that causes death of the bacteria also brings about leakage of cytoplasmic constituents of low molecular weight. The most immediately observed effect is loss of K^+ ions. The increased permeability is a sign of changes in the membrane that are initially reversible but become irreversible on prolonged treatment. The necessary characteristic of an antiseptic is its bactericidal action, but there is often a low and rather narrow concentration range in which its effect is bacteriostatic. At this low concentration, certain biochemical functions associated with the bacteria membrane may be inhibited. In the presence of a higher concentration of antiseptic and after prolonged treatment, the compound usually penetrates the cell and brings about extensive ill defined disruption of normal cellular functions. The primary effect of these antiseptics on the cytoplasmic membrane is thus established beyond doubt, but secondary actions on the cytoplasmic processes are less defined and may vary from one compound to another.

A review of the most reliable evidence on the mode of action of cationic antiseptics suggests the following generalizations:

1. Adsorption of compound on the bacterial cell surface
2. Diffusion through the cell wall
3. Binding to the cytoplasmic membrane
4. Disruption of the cytoplasmic membrane
5. Release of K^+ ions and other cytoplasmic constituents

6. Precipitation of cell contents and the death of the cells

It is well known that the bacterial cell surfaces are ually negatively charged, and then that adsorption of olycations on to the negatively charged cell surfaces rocess 1) is expected to be enhanced with increasing olecular weight of the polymer due to the increasing iarge density of the polycations. The binding of polyicrs to the cytoplasmic membranes and its disruption expected to be facilitated by increasing the molecular eight of the polymer and by increasing the amount of ie bound polymers to the bacteria cells. To examine is hypothesis Ikeda and Tazuke (1985) studied the effect MW of the polymers on lysis of protoplasts, which are acteria cells freed entirely of cell walls, so that the nteractions processes 1 (adsorption) and 2 (diffusion) of he polymer with the protoplasts can be left out of conideration. The amount of cytoplasmic constituents reeased from protoplasts and from intact cells of *Bacillus ubtilis* was measured at 260 nm after exposure to fracionated samples of polymers of various MW. Lysis of protoplasts was clearly enhanced with increase in MW of polymer[poly(n-butyldimethyl[vinylbenzyl]ammonium chloride)]. A bell-shaped curve relationship of the activity to the MW of fractionated polymer was observed in the case of intact cells of *B. subtilis*. Because separate experiments have shown that release of cytoplasmic constituents from intact cells correlates well with the death of the cells, these results support the concept that the mode of action of the polymeric biocides is disorganization of cytoplasmic membrane followed by rupture of membrane, leading to the death of the bacteria (Franklin and Snow, 1989).

Ikeda and Tazuke in 1985 reported an optimal MW region of 14,300 for the 6,6-ionene bromide with antibacterial activity with minimum bactericidal concentration of 6.6 ppm to 10 ppm against *Staphylococcus aureus*. The same workers reported that polymeric biquanides of MW 11,900 and poly{alkyl[C_4–C_{18}]dimethyl[vinylbenzyl]ammonium chloride} of similar MW exhibit better bactericidal action against gram-positive than gram-negative bacteria. The poly{dodecyldimethyl[vinylbenzyl]ammonium chloride} was the most active at 0.5 ppm against *Staphylococcus aureus*, which suggests that hydrophobicity plays an important role in bactericidal action.

A primary consideration in examining the mode of action is the characterization of quaternary compounds as surface-active agents, or surfactants. These are adequately defined by James (1965) as compounds with a structural balance between one or more water-attracting (hydrophilic) groups; depending on the nature of the charge or absence of ionization of the hydrophilic group, they may be classified as anionic, cationic, or nonionic. Quaternary ammonium compounds are cationic surface agents and possess such properties as a reduction of the surface tension at interfaces upon absorption; a ready attraction to an absorption on surfaces possessing a negative charge such as wool, glass, protein, and bacteria; the formation of ionic aggregates or micelles with attendant changes in electrical conductivity, surface tension, and solubility; a precipitation, complex formation, and denaturing effect on proteins; and an inhibiting or stimulating effect on enzyme activity (James, 1946).

With these demonstrable properties for cationic surfactants, it was natural for some investigators (Cowles, 1938) to attribute the antimicrobial activity of quaternary ammonium compounds entirely to the presence and amount of surface activity. However, this obvious explanation has been refuted (Gershenfeld and Milanick, 1941; Hotchkiss, 1946).

An excellent presentation on the mode of action of cationic surface-active agents on microbial cells was given by Hugo (1965). The concepts concerning the mode of action of surface-active agents on microorganisms may be divided into five broad categories.

1. *Effects on protein.* See Putnam (1948) for protein-denaturing agents, Khun and Dann (1940) for enzyme-disrupting agents. Quaternary ammonium compounds are surface-active agents and will denature protein or cause dissociation of an enzyme from its prosthetic group. This effect is usually caused at concentrations much higher than those which are lethal to the microbial cells, and it is unlikely that this effect is the primary cause of the antibacterial activity of surface-active disinfectants except at high concentration.

2. *Effects on metabolic reactions.* See Baker et al. (1941) on the aerobic and anaerobic respiration of glucose by a variety of bacteria and Ordal and Borg (1942) on the oxidation of lactate by *Escherichia coli* and *Staphylococcus aureus*. Attempts have been made to relate inhibition of metabolism with inhibition of growth. Such a correlation has been observed at high concentration, and almost any degree of agreement or disagreement may be demonstrated depending upon the enzyme system chosen and the test organism. It can be expected that those enzymes located in the cytoplasmic membrane will be the first to be affected. Penetration of the cell will follow, and cytoplasmic enzymes will then be inhibited. The enzyme inhibition is not the primary or main lesion caused by these compounds. A specific detergent-sensitive enzyme does not exist or has not been discovered.

3. *Effects on cell permeability.* See Armstrong (1957) on cytolytic damage and phosphorus loss and Scharff and Maupin (1960) on membrane damage and loss of potassium. The cytoplasmic membrane is probably the organelle most sensitive to surface-active agents within the cell of bacteria and yeasts, and the alteration in the semipermeable properties of this structure can lead to leakage of metabolites and coenzymes and disturbance in the delicate balance of metabolite concentrations within the cell. This lesion may be a major contribution to the death of a cell and cause an apparent loss in enzymic activity due to the loss or dilution of coenzymes or substrates. There is a well established relationship between cytolytic

action and surface tension. This lends support to the idea that cytolytic damage may in fact be the primary lesion caused by surface-active substances.

4. Stimulatory effect of the glycolysis reaction. This is suggested by the work of Strickland (1956) and Bihler et al. (1961). This reaction is of interest, but because the effect is elicited at concentrations well below those which are antibacterial, it cannot be considered of significance for the antibacterial action of surface-active agents.

5. Effect on an enzymatically-maintained dynamic membrane. This is offered as a speculative theory by Newton (1958). Results to support this interesting hypothesis are awaited. This concept of the dynamic cell membrane enzymatically maintained could well be the detergent-sensitive enzyme, which as it was stated before does not exist.

Hugo has selected the effect on the cytoplasmic membrane controlling the cell permeability as the mode of action for quaternary ammonium compound germicides. This, he feels, is most consistent with the data presented to date, and it is well accepted (Franklin and Snow, 1989; Ikeda and Tazuke, 1985).

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ND DISINFECTANTS

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Alcohols

Disinfection, Sterilization and Preservation S.S. Block 3rd Ed.

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As a chemical group, the alcohols possess many features desirable for a disinfectant. They have a bactericidal rather than a bacteriostatic action against vegetative forms: they are relatively inexpensive and usually easily obtainable. They have a cleansing action and evaporate readily. They are colorless, but if this property is undesirable, a coloring substance may be added. Like chemical disinfectants in general, their destructive action against spore forms is much less than that against vegetative forms. Although studied extensively as a group, their mode of action is far from being clearly understood. The greatest amount of work has been done with ethyl alcohol.

Wirgin (1904) pointed out that insofar as methyl, ethyl, propyl, butyl, and amyl alcohols were concerned, the bactericidal action of the aliphatic alcohols increased with the increase in the molecular weights of the alcohols, with the exception of the tertiary alcohols. Testing alcohols in a liquid environment against *Salmonella typhosa*, Tilley and Schaffer (1926) observed an increase in the phenol coefficients from 0.026 for methyl to 21.0 for octyl alcohol. For *Staphylococcus aureus*, the phenol coefficients varied from 0.03 for methyl to 0.63 for amyl alcohol.

Tanner and Wilson (1943) tested alcohols containing from 1 to 11 carbon atoms by the agar cup-plate method. Methyl alcohol had no noticeable effect on the test organisms. The size of the zones in which no growth of the test organisms took place increased in size from that for ethyl alcohol to that for amyl alcohol, then decreased in size for the primary-normal alcohol series. The size of the zone of inhibition for primary-normal undecyl alcohol, the last alcohol in the series to be tested, was comparable to that for ethyl alcohol. In the case of *Pseudomonas aeruginosa*, growth was stimulated rather than inhibited by the sample of undecyl alcohol. The bactericidal action varied from greatest to lesser in the following order: primary–normal > primary-iso->secondary-normal> and tertiary alcohols, as was observed by Tilley and Schaffer (1926). Nine months' contact with 100% primary-normal methyl, ethyl, propyl, butyl, amyl, hexyl, heptyl, octyl, nonyl, and undecyl alcohols did not kill *Bacillus subtilis* and *B. megatherium* spores.

The bactericidal action of alcohols is important because proprietary preparations are frequently marketed as alcoholic solutions. Molinas and Brewer (1949) studied 27 compounds for their ability to reduce the number of bacteria on rabbit, guinea pig, and human skins. Solutions of 50% ethyl alcohol and 10% acetone or 50% isopropyl alcohol and 10% acetone were used as controls. These investigators concluded that the action of proprietary preparations designated for disinfecting the skin depends largely upon the alcoholic solutions in which they are incorporated. The results of in

preferable to 75% concentration (Kulpers, 1947). An alcoholic solution of cetrimide ap-

peared clinically more effective than an aqueous solution of cetrimide for preoperative skin preparation (Noone, 1970). The length of time the alcohol is in contact with the skin is important (Lowbury, et al., 1960). In quantitative tests, Novak and Hall (1939) observed the 50% alcohol-10% acetone mixture to be highly germicidal. It killed 96% of the organisms on the skin, whereas no disinfectant tested was able to kill 100% of the organisms, and tincture of green soap was the poorest, killing only 53% of the organisms. The tests were performed on the skin over the abdomen where, on the average, 12 staphylococci and diphtheroids were found per square centimeter of skin.

MODE OF ACTION OF ALCOHOLS

Denaturing Action

The most plausible explanation for the antibacterial action of the alcohols is their denaturing of proteins. In the absence of water, proteins are not denatured as readily as when water is present. This affords an explanation of why absolute ethyl alcohol, a dehydrating agent, is less bactericidal than mixtures of alcohol and water.

Interference with Metabolism

Dagley et al. (1950) observed that a 0.41-molar concentration of ethyl alcohol increased the lag phase of *Enterobactor aerogenes*. This effect was decreased by the presence of the amino acids: DL-methionine, L-leucine, L-glutamic acid, L-histidine, and DL-tryptophane. The lag was increased by L-proline, glycine, DL-alanine, DL-serine, and DL-aspartic acid. The authors concluded that the bacteriostatic action was due to the inhibition of the production of metabolites essential for rapid cell division.

After being exposed to various chemical and physical agents, bacterial cells are often judged to be killed when they fail to multiply in suitable growth medium. When incubated in special media, however, these supposedly killed cells frequently can be rendered viable and capable of growth. Heinmets et al. (1954) reported that *Escherichia coli* B/r failed to grow after being exposed to 20% ethyl alcohol for 10 minutes, but grew after being treated with various metabolites. Best results in demonstrating viable cells in the "sterilized" suspensions of treated cells were obtained with *cis*-aconitic acid, α-ketoglutaric acid, and a mixture of 11 metabolites that included the two already mentioned. The concentration of 20% approaches the borderline of effectiveness of ethyl alcohol and represents a concentration much lower than that used for the purpose of disinfection. Such reactivation of "inactivated" cells needs to be studied under conditions that more nearly approach those in actual practice. The work indicates that it is possible to reverse the action of weak concentrations of ethyl alcohol.

The inhibition of spore germination by ethyl and other alcohols may be due to the inhibition of enzymes necessary for germination. The inhibition is reversible as only the removal of the alcohol from the environment is necessary for germination to take place (Trujillo and Laible, 1970).

Lytic Action

Pulvertaft and Lumb (1948) reported that lysis of microorganisms occurred with many antiseptics when used at a concentration approximately double that of the minimum concentration producing bacteriostasis. Lysis was observed with formalin, mercuric chloride, thimerosal, phenol, and sodium hypochlorite, as well as with the antibiotic penicillin. Lysis was most marked with staphylococci, pneumococci, *Bacillus subtilis*, and *Escherichia coli*, less marked with Flexner and Sonne strains of dysentery bacilli, and only slight with streptococci. The organisms were most susceptible to lysis when the antiseptic was added to the culture during its logarithmic phase, and lysis usually set in about the third hour thereafter. The lytic action was thought to be due possibly to using the antiseptic in a concentration that inhibited growth of the microorganisms without inhibiting the autolytic enzymes.

Lecce (1953) observed that a suspension of the Campo strain of mycoplasma isolated from man, estimated to contain 140 μg nitrogen per ml, was lysed within a few minutes by tertiary-butyl and iso-amyl alcohols in a concentration of 0.1%. The organisms were not lysed under similar conditions by methyl, ethyl, propyl, isopropyl, and normal butyl alcohols. *Escherichia coli* in a suspension estimated to contain 500 μg dry weight per ml was lysed in the

presence of 33% propyl and tertiary-butyl alcohols.

Razin and Argaman (1963) found that *Acholeplasma laidlawii* and *Mycoplasma mycoides* var. *capri* were lysed by ethyl alcohol in concentrations between 4.3 and 5.4 molar, by propyl alcohol in concentrations greater than 0.84 molar, and by normal butyl alcohol in concentrations greater than 0.24 molar.

METHYL ALCOHOL

Methyl alcohol has the weakest bactericidal action of the alcohols and thus is seldom considered for use as an antibacterial agent. Its slight action was noted by Wirgin (1904) and Tilley and Schaffer (1926), although Tanner and Wilson (1943) observed no noticeable effect. It apparently does not noticeably affect the bactericidal action of ethyl alcohol when used as a denaturant (Olivo, 1948). Kolb et al. (1952) reported that methyl alcohol in concentrations of 0.004 to 95% has no killing action on the spores of *Bacillus anthracis*. While strains of influenza virus A and B are completely precipitated by 31% methanol, continued contact for even 10 hours produced no loss of infectivity (Cox et al., 1947).

ETHYL ALCOHOL (ETHANOL)*

Alcohol* is widely used for the destruction of the vegetative forms of microorganisms preceding such procedures as venipunctures, hypodermic injections, finger pricks to obtain blood for blood counts and hemoglobin determinations, and other procedures that break the intact skin. During the latter part of the last century, it was common practice to test disinfectants against microorganisms dried on threads or beads. This brought out the fact that some water must be present for alcohol to exert its most effective bactericidal action. It also gave the erroneous impression that alcohol is a poor disinfectant because it failed to readily kill the dried organisms, and, like chemical disinfectants in general, it possessed poor killing action against bacterial spores. The erroneous statement was perpetuated in many textbooks that 95% alcohol was practically worthless as a disinfectant, possibly because 70% alcohol possessed a stronger killing action. In 1939, Price attempted to correct the erroneous impression that alcohol was a poor disinfectant.

Harrington and Walker (1903) tested organisms on moist and dry threads against various concentrations of alcohol. *Escherichia coli* on moist threads was killed in less than 5 minutes' exposure to alcohol of 60 to 99% concentrations, whereas only the concentrations of 50 and 60% killed within 5 minutes when the organisms were dried on threads. Concentrations of 94 and 99% did not kill *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*, and *Staphylococcus aureus* in an exposure of 24 hours when the organisms were dried on threads. *Pseudomonas aeruginosa* on moist threads was killed in 5 minutes by concentrations of 40 to 99%, but on dried threads only the concentrations of 40 to 70% killed in the same period of time. *Staphylococcus epidermidis* on moist threads was killed in less than 1 minute by 50% alcohol. Concentrations of 40 to 99% killed in 5 minutes, but when the organisms were dried on threads, only the concentrations of 40 to 70% killed in the same period of time. Practically the same results were obtained with *Staphylococcus aureus*. On moist threads, *Salmonella typhosa* was killed in 5 minutes by concentrations of 30 to 99%, but on dried threads only by concentrations of 30 to 80%. *Corynebacterium diphtheriae* on moist threads was killed in 5 minutes by concentrations of 40 to 99% and on dried threads by concentrations of 40 to 90%. These authors concluded more than three fourths of a century ago that against the commoner, nonsporing pathogenic bacteria in a moist condition, any strength of alcohol above 40% by volume is effective within 5 minutes, and certain preparations are effective within an exposure time of 1 minute. Against organisms in the dry state, concentrations of 60 to 70% are the most effective, and they are equally effective against the organisms in the moist state. Typical results are reproduced in Table 10–1. Ethyl alcohol in a concentration of 70% was observed by Hare et al. (1963) to kill in an exposure of 30 seconds microorganisms of ten species when dried on glass surfaces. This indicates it is possible for an aqueous alcoholic solution to exert its bactericidal action on even dried organisms before it evaporates. Bacterial spores were not killed during an exposure of 24 hours, as had been observed by Heuzenroeder and Johnson (1958).

*In this section, the word alcohol, when used alone, refers to ethyl alcohol.

TABLE 10–1. THE KILLING ACTION OF VARIOUS CONCENTRATIONS OF ALCOHOL AGAINST *STAPHYLOCOCCUS AUREUS* ON MOIST AND DRIED THREADS (HARRINGTON AND WALKER, 1903)

Alcohol, %	Exposure Time																		
	Moist Threads									Dried Threads									
	Minutes					Hours				Minutes					Hours				
	5	10	15	30	45	1	2	7	24	5	10	15	30	45	1	2	7	24
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
20	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+
25	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	–
30	+	+	+	+	+	+		–	–	+	+	+	+	+	+	+	–	–
40	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
75	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
80	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
85	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
90	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
94	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–
99	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	+	–	+

– growth + no growth

By adding a loopful of suspension of microorganisms to the solution of disinfectant, Post and Nicoll (1910) found that 70 and 50% concentration of alcohol killed *Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria gonorrhoeae,* and *Salmonella typhosa* in less than 1 minute. Even 30% alcohol killed *Neisseria gonorrhoeae* in less than 1 minute.

Morton (1950) tested various concentrations of alcohol against a variety of microorganisms in exposure periods beginning with 10 seconds. In order not to alter the concentrations of the alcohol, the test organisms in 0.5-ml amounts of broth culture were placed in sterile tubes and centrifuged. The supernatant was removed, and the alcoholic solution was thoroughly mixed with the moist cells. One drop of the culture-cell mixture was subcultured at stated intervals. *Pseudomonas aeruginosa* was killed in 10 seconds by all concentrations of alcohol from 100 to 30% by volume. The lowest concentration of alcohol tested, 20%, killed in 30 minutes. *Serratia marcescens, Escherichia coli,* and *Salmonella typhosa* were killed in 10 seconds by all concentrations of alcohol from 100 to 40%. Nathanson (1951) stated that 2% alcohol added to their products (sulfonated vegetables oils) prevented the growth of *Serratia marcescens* and *Pseudomonas aeruginosa* in most cases. Ethyl or isopropyl alcohol in concentrations of 4 to 7% have been recommended for preservatives of certain disinfectant solutions (see Chapter 20). The gram-positive vegetative organisms *Staphylococcus aureus* and *Streptococcus pyogenes* were a little more resistant, being killed in 10 seconds by concentrations of 60 to 95%. Exposures of 50 and 90 seconds, respectively, were required to kill the two organisms with absolute alcohol. Table 6 from Morton (1950) is reproduced as Table 10–2. The temperature of medication was room temperature. At higher temperatures, the killing action would be expected to be more rapid (Tilley, 1942). Price (1939) employed an original technique in which the alcohol-culture mixture was diluted with water to stop the germicidal action, aliquot portions seeded into plates, and colonies from surviving organisms counted. He observed *Escherichia coli* to be killed in less than 60 seconds by 60% alcohol (by weight), in less than 40 seconds by 70% and in less than 30 seconds by 80% alcohol. *Staphylococcus epidermidis* was killed in less than 60 seconds by either 50 or 70% alcohol. *Staphylococcus aureus* was killed in less than 15 seconds by 70% alcohol. However, by employing essentially the same technique, Price (1950) reported that *Escherichia coli* required an exposure of 5 minutes in order to be killed by 60% alcohol. *Staphylococcus aureus* required a similar exposure to be killed by either 70 or 80%, and *Staphylococcus epidermidis* was not killed in 10 minutes by any concentration of alcohol. Hayes (1949) reported that

TABLE 10–2. THE KILLING ACTION OF VARIOUS CONCENTRATIONS OF ALCOHOL AGAINST *STREPTOCOCCUS PYOGENES* (MORTON, 1950)

Alcohol		Exposure of Test Organism to Germicide																
		Seconds					Minutes											
% by vol.	% by wt.	10	20	30	40	50	1	1½	2	3	3½	4	5	10	15	30	45	60
100	100	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−		
95	92	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
90	85	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
80	73	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
70	62	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
60	52	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
50	42	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−		
40	33	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−		
30	24						+	+	+	+	+	+	+	+	−	+	−	−
25	20						+	+	+	+	+	+	+	+	−	+	+	−
20	16						+	+	+	+	+	+	+	+	−	+	+	−

\+ growth − no growth

Escherichia coli, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were killed in less than 5 minutes when exposed to concentrations of ethanol ranging from 50 to 100%. A concentration of 25% ethanol did not kill these organisms during an exposure of 30 minutes.

Seelig and Gould (1911) were annoyed at the earlier methods of testing germicides, as the microorganisms are usually found in the deeper layers of the skin and tissues or covered with blood or exudate. For practical purposes, a disinfectant must possess power of penetration, so to test the power of penetration, the authors made a pouch of celloidin or living animal tissue at the end of a glass tube, placed the culture under study inside of the pouch, and immersed it in the disinfectant solution. After varying periods of time, subcultures were made of the culture within the pouch. *Salmonella typhosa* within celloidin pouches was killed within 6 minutes by 95 and 99.8% alcohol concentrations. Concentrations of 50%, 70%, and 80% did not kill in 1 hour. In a pouch of living rabbit omentum immersed in 95% alcohol, the organisms were killed within 3 minutes. In a pouch of living rabbit skin immersed in 95% alcohol, the organisms were alive after 5 minutes but dead after 10 minutes. When placed in a pouch of dried rat intestine and immersed in 99 and 95% alcohols, the organisms were still alive after 18 hours. An important incidental observation was that when the organisms lost their motility they were no longer viable. By making microscopic observations of the organisms inside the pouches, they could determine if the organisms had been killed, and the results of subculturing verified this in every case. In celloidin pouches, *Escherichia coli* was killed in 20 but not in 10 minutes by 95% alcohol, in 60 but not in 30 minutes by 80%, in 18 hours but not in 2 hours by 70%; 50% alcohol did not kill in an exposure of 18 hours. Comparable results were obtained with a staphylococcus culture. When certain germicides were dissolved in alcohol, their germicidal action was increased. An alcoholic solution of iodine killed microorganisms within the pouches quicker than 95% alcohol or aqueous solution of iodine alone.

Alcohol in the body may suppress the normal clearing mechanism of the body for bacteria. The retention of *Staphylococcus aureus* by the lungs of mice was 3.6 times greater when the mice were given an intraperitoneal injection of alcohol (Laurenzi et al., 1963). When 8% alcohol was supplied in the drinking water and the mice were challenged by intravenous injection of the organisms, the survival time was shorter and the number of staphylococci in the liver greater than in the control group. When the mice were given 5% alcohol in their drink for 3 weeks prior to challenge, the number of staphylococci in the liver was greater, but there was no significant difference between the number of organisms in the spleens and kidneys of the mice in the ethanol and control groups, and the mortality rates were the same for all groups (Wasz-Höckert et al., 1959).

In testing the effect of ethyl alcohol against *Mycobacterium tuberculosis*, Smith (1947) observed that 95% alcohol killed the tubercle ba-

cilli in sputum within 15 seconds, absolute and 70% alcohol required 30 seconds, and 50% alcohol required 60 seconds. Tubercle bacilli in water suspensions were killed in 15 seconds by 95% alcohol, in 30 seconds by absolute alcohol, and in 60 seconds by 70% alcohol. When tuberculous sputum was allowed to dry on cover slips and then subjected to the action of the alcohols, 50% alcohol killed the tubercle bacilli in 1 or 2 minutes, depending on the thickness of the smears. In thin smears, 70% alcohol killed the organisms in 1 minute, but in thick smears the organisms were not killed in 5 minutes. In thick smears, 95% alcohol killed the organisms in 30 minutes, and absolute alcohol did not kill in an exposure of 60 minutes.

Smith concluded that 95% ethyl alcohol was best for wet surfaces, 50% for dry surfaces, and 70% for either wet or dry surfaces. The number of tubercle bacilli has an effect upon the killing action of 95% alcohol. When 0.0001 or 0.1 mg of bacilli were used, some of the subcultures were sterile after the organisms had been exposed for 2 minutes, and all subcultures from the longer exposures were sterile (Cohn, 1934). The size of the inoculum was important also in testing tetradecylamine (Smith, 1951).

In contrast to the results obtained with suspensions of tubercle bacilli and smears of suspensions and sputa, sputum, especially if it is viscous, presents a different problem. Frobisher and Sommermeyer (1953) observed that 80 to 100% concentrations of ethanol were effective in killing tubercle bacilli only if precautions were taken to ensure intimate contact, such as violent shaking, between organisms and alcohol before the sputum became coagulated.

Like most chemical disinfectants, alcohol appears to be more toxic for certain tissue cells than for bacterial cells. Welch and Brewer (1942) found absolute alcohol to be 7.5 times more toxic for the phagocytic cells of human and guinea pig blood than for *Staphylococcus aureus*.

Effect of Ethyl Alcohol on Spiroplasmas

Using a microtiter technique, it was found that various species of *Spiroplasma* (microorganisms lacking cell walls) varied in their susceptibility to ethanol as well as to other antibacterial agents, such as formalin, glutaraldehyde, and phenol. A strain of *Spiroplasma* of tick origin, designated SMCA (suckling mouse, cataract agent), and two strains of honey-bee origin were comparable to *S. aureus* and *E. coli* (cell-wall bacteria) in susceptibility to the antibacterial action of alcohol; the five cultures survived in 30% alcohol for as long as 40 minutes. *Spiroplasma citri* was the most susceptible of the strains tested, surviving 20% ethanol for only 5 minutes. Against phenol, one of the honey-bee strains was comparable to *S. aureus* and *E. coli* in susceptibility, while the remaining strains of spiroplasma were more susceptible (Stanek et al., 1981).

Effect of Ethyl Alcohol on Bacterial Spores

It is well known that ethyl alcohol has little effect against bacterial spores. The greater killing action of alcohol for vegetative cells as compared to that for spores has been employed to facilitate the isolation of *Clostridium botulinum* type E (Johnson et al., 1964). Coulthard and Sykes (1936) demonstrated that the bactericidal action of ethyl alcohol was greatly increased by the addition of 10% amyl-meta-cresol, or 1% hydrochloric, nitric, phosphoric, or sulfuric acid, or sodium or potassium hydroxide. They stated that the bactericidal action of methyl and isopropyl alcohols could be similarly enhanced. Samples of alcohols of various concentrations have been reported to occasionally contain bacterial spores. Gershenfeld (1938) tested 100 samples of 95% alcohol and 25 samples of absolute alcohol obtained on the open market from many different sources in the United States and found all to be free of bacteria and bacterial spores.

Alcohol may become contaminated with spores from having contaminated materials immersed in it. Kuhn and Dombrowsky (1932) reported half of the samples of alcohols tested to be contaminated. Fatal infections with *Clostridium* may result when depending upon alcohol for sterilization of surgical instruments contaminated with bacterial spores, as demonstrated by Nye and Mallory in 1923. Saegesser (1941) demonstrated gas gangrene bacilli in 70% alcohol in which syringes were stored and emphasized that alcohol was not an adequate method for sterilization.

Effect of Ethyl Alcohol on Fungal Spores

The spores of *Trichophyton gypseum* were not killed by 50% alcohol but were killed by

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85% alcohol in an exposure of 30 minutes (Emmons, 1933). Seventy-percent alcohol inhibited the growth of *Microsporum audouini* from naturally infected hairs in 40% of the hairs by an exposure of from 1 to 2 minutes, and the inhibition was increased to 100% by an exposure of 1 hour (Loewenthal, 1961). However, when 70% alcohol was used instead of water to cleanse the skin prior to collecting material to be cultured on Sabouraud's agar for filamentous dermatophytes, the percentage of positive isolations was increased and the percentage of contamination decreased. Cleansing with alcohol was not necessary if the material was cultured on cycloheximide agar (Loewenthal, 1964).

Seventy percent was the most effective concentration of ethyl alcohol for killing the tissue phase of *Cryptococcus neoformans*, *Blastomyces dermatidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* and the culture phase of the latter three organisms when aerosolized onto painted wood, glass, stainless steel, neoprene, and asphalt floor tile surfaces (Kruse et al., 1963, 1964). Against the conidia of *Penicillium tardum*, Lüthi (1954) observed that, within the range of 70 to 96%, the concentration of 90% ethanol exerted the greatest sporicidal effect.

Effect of Ethyl Alcohol on Viruses

Vaccinia virus was reported by Gordon (1925) to be inactivated by 50% ethyl alcohol in 1 hour but not by 20% alcohol in 24 hours. Methyl alcohol appeared to be slightly more effective in that a 20% concentration had a noticeable effect in 1 hour and inactivated the virus in 24 hours. Groupé et al. (1955) likewise have reported a strong virucidal action of ethanol on vaccinia virus by the action of 70% ethanol on chick embryo material during an exposure at 25° C for 10 minutes. The virus of Newcastle disease was observed by Cunningham (1948) to be inactivated by 70 and 95% alcohols in an exposure period of 3 minutes. Alcohols of 40 and 25% concentrations did not inactivate the virus under similar conditions. The results with 25% ethyl alcohol were comparable to those with isopropyl alcohol and the same virus as reported by Tilley and Anderson (1947).

When mixed with an equal volume of saline filtrate of the virus of hoof-and-mouth disease and held at room temperature, alcohol did not inactivate the virus in 3 days but did so in 6 days (Stockman and Minett, 1926).

The danger of transmitting hepatitis virus by transferring material from one individual to another has become apparent. The use of a syringe and needle on more than one individual without proper sterilization between individuals is now recognized as a hazardous procedure. The use of a blood lancet or knife blade for obtaining small amounts of blood must be recognized, likewise, as a procedure not without risk. Dipping a blood lancet into alcohol and allowing it to dry or wiping a knife blade with a pad moistened with alcohol should not be considered a safe procedure. The resistance of serum hepatitis virus to alcohol is illustrated by the observations of Caspó et al. (1962). The serum from persons with hepatitis still contained active virus, although it was attenuated, after treatment with 40% ethyl alcohol for 14 days at —5° C.

Ethyl alcohol in a dilution of 1 in 6, formalin 1 in 15, eosin (yellowish) 1 in 300, sodium oleate 1 in 50, and liquor cresolis compositus 1 in 200 were reported by Frobisher (1930) as inactivating the virus of yellow fever (Asibi strain) contained in monkey serum. Bichloride of mercury 1 in 7,500, phenol 1 in 150, hexylresorcinol 1 in 1,500, and sodium oleate 1 in 150 did not inactivate the virus of yellow fever.

The virus of eastern equine encephalomyelitis (1% aqueous suspension of chick embryo material) was reduced in activity four logs or more after 1 hour of exposure at 18 to 20° C by 50% ethyl alcohol (Bucca, 1956).

Ethyl alcohol in a final concentration of 20% brought about a loss in activity exceeding 95% of the virus of mouse encephalomyelitis, in mouse brain suspension, in 45 minutes' exposure in the icebox (Theiler and Gard, 1940), but ether had no apparent effect.

Two strains of the virus of rabbit fibroma (Shope) were inactivated in 1 hour at 0° C by 30 and 40% concentrations of ethyl alcohol (Pontier and Chaumont, 1954). Against the Lansing strain of poliomyelitis virus, ethyl alcohol in concentrations of 63 and 70% had little virucidal action (Faber and Dong, 1953).

Influenza virus, type A, in chick embryo material, was exposed to varying concentrations of ethyl alcohol for 10 minutes at 25° C. A concentration of 14% ethanol exerted no action, 35% exerted a weak action, and 70% com-

pletely inactivated the virus under the conditions of the test (Groupé et al., 1955).

Ethyl alcohol in the concentrations of 70 to 90% inactivated all of seven viruses tested that were representative of the general types of viruses (Klein and Deforest, 1963). The greatest virucidal action was against those viruses with a lipid envelope. For general virucidal activity, ethanol was stronger than isopropyl alcohol. A comparison of the alcohols is summarized in Table 10–3.

Ability of Ethyl Alcohol to Prevent Infection

The crucial test of any disinfectant is the ability of the substance to prevent infection in a susceptible animal with the test organism. Neufeld and Schiemann (1938) demonstrated by intraperitoneal injection in mice that a culture of *Streptococcus pneumoniae*, type 1, on the finger tips was killed by treating with 80% alcohol. Not all of the pneumococci were destroyed when 96% alcohol was used.

Nungester and Kempf (1942) devised a test in which the tail of a mouse was scrubbed with 4 or 5 strokes of a swab that had been moistened with a broth culture of the test organism. The tail was then dipped for 2 minutes into a tube containing the disinfectant. At the end of this time, a half-inch portion of the tail was cut off and implanted in the peritoneum of the same animal. In the case of *Streptococcus pneumoniae*, type 1, 100% of the mice died following treatment of their contaminated skin with either physiologic salt solution, which served as a control, or aqueous thimerosal (Merthiolate), 1:1000. Mortality was 69% among the mice when a mixture of 50% alcohol and 10% acetone was used; mortality was 63% when 70% alcohol (by volume) was employed. Employing 2% aqueous iodine solution resulted in a mortality of only 5%, but when a 2% iodine solution in 95% alcohol was employed, the mortality was zero. In the case of some mice, a portion of tail was also cultured. In general, more animals died of the infection than growth was obtained in the subcultures. When using a hemolytic streptococcus, none of the animals was protected by the use of the alcohol-acetone mixture.

Employing the technique of Nungester and Kempf and *Streptococcus pneumoniae*, Prombo and Tilden (1950) obtained a mortality of slightly more than 10% when using 70% alcohol by weight, and slightly less than 20% mortality when using 70% alcohol by volume. With *Streptococcus pyogenes* and 70% alcohol by weight, mortality was 20% in the mice.

Ethyl Alcohol in Skin Degermation

It is nearly impossible to sterilize the skin. The best one can hope to accomplish is to reduce the number of viable organisms on or in the skin and to destroy the pathogenic organisms that may be on the skin as transients. The term "degermation" is more accurate than "disinfection" (Price, 1939). Price pointed out that, in the surgical scrub, immersion of the hands and arms in a 65.5% solution of alcohol by weight for 1 minute was as effective as scrubbing for 4.2 minutes as far as reducing the number of bacteria on the skin is concerned. This effectiveness of alcohol appears to have been amply confirmed (Pohle and Stuart, 1940; Pillsbury et al., 1942; Hatfield and Lockwood, 1943; Gardner and Seddon, 1946; Gardner, 1948; Story, 1952).

Employing a testing procedure somewhat similar to that of Price, Hatfield and Lockwood (1943) concluded that ethyl alcohol in strengths

TABLE 10–3. THE VIRUCIDAL ACTION OF ETHYL AND ISOPROPYL ALCOHOLS ON SEVEN VIRUSES (KLEIN AND DEFOREST, 1963)

	Lowest Concentration Inactivating in 10 Minutes		*Lipid Envelope*	*Lipophilic*
	Ethyl	*Isopropyl*		
Poliovirus, type 1	70%	95% (Negative)		
Coxsackie B-1	60%	95% (Negative)		
ECHO 6	50%	90%		
Adenovirus, type 2	50%	50%		+
Herpes simplex	30%	20%	+	+
Vaccinia	40%	30%	+	+
Influenza, Asian	30%	30%	+	+

of 95 or 70% by weight was preferable to any of a group of commercially prepared agents specifically designed for skin sterilization at that time. The ideal concentration was found to be 95%, but for economy, the concentration could be reduced to 70%. Pillsbury et al. (1942) also observed that 80% or more by volume produced a satisfactory decrease in the number of viable bacteria remaining on or in the skin. They expressed doubt about the necessity of alcohol of an exact concentration for clinical use so long as the concentration exceeds 70% by volume.

Skin bacteria were found to be of two kinds, "transients" and "residents" (Price, 1938). The transient bacteria lie free on the skin or are loosely attached by lipoidal substances. Their removal is relatively easy compared to the resident bacteria, which are a relatively stable population in size and kinds. The terms *exposed* and *sheltered* flora, in place of *transient* and *resident* flora, were proposed by Evans et al. (1980). They observed that in 15 individuals with a sparce flora in the antecubital fossa, no surviving bacteria were detected after alcohol treatment (60 seconds), but in 10 individuals with a more abundant flora, viable bacteria remained, and their number bore no quantitative correlation with the surface flora. Following scrubbing of the skin after alcohol treatment, sheltered or resident bacteria could be demonstrated in each of the 5 individuals studied.

While alcohol has a bactericidal action on the organisms on skin, it may leave the skin with a decreased bactericidal activity. Lacey (1968) demonstrated this by cleaning the skin on the forearm with alcohol, applying *Staphylococcus aureus*, and covering with a self-adhesive label for 5 hours. The degree of decrease in bactericidal activity of the skin was about the same as for soap and was thought to be due to the removal of bactericidal substances from the skin.

Ethyl Alcohol for the Chemical Sterilization of Instruments

Some instruments, especially those with sharp cutting edges, do not lend themselves well to sterilization by heat, so "cold" sterilization by means of a chemical solution is often attempted. The ability of a chemical solution to sterilize instruments certainly can be no better than the germicidal action of the solution in vitro. Indeed, its ability to sterilize instruments may be lessened because of the rough surfaces or joint on some instruments that offer potential harboring sites for microorganisms. Contaminated instruments frequently are soiled with blood, pus, or other body fluids that make bacterial destruction more difficult. The inability of alcohol, like other chemical disinfectants, to destroy bacterial spores makes this method of sterilization hazardous. In the absence of bacterial spores, alcohol is as effective for sterilizing instruments as its germicidal action is indicated by in vitro bactericidal and infection-prevention tests; namely, it is better than many substances, but is not 100% reliable. In its ability to sterilize Bard-Parker knife blades contaminated with pus or blood, wet or dry, Spaulding (1939) observed that 70% alcohol killed *Candida albicans*, *Escherichia coli*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* in either ½ or 1 minute; rarely was an exposure of 2 minutes required. *Staphylococcus aureus* was slightly more resistant when the pus and blood were dried. Exposures of 10 minutes were required to kill the organisms in dried pus and 5 minutes in dried blood. *Clostridium tetani*, *Clostridium perfringens*, and *Bacillus anthracis* were not killed after an exposure of 18 hours, except *B. anthracis* in wet pus, which was killed in that period of time.

Next to moist heat, which was damaging to the anesthesia equipment, Ziegler and Jacoby (1956) found that soaking in 70% alcohol for 15 minutes was satisfactory for sterilizing airways, endotracheal tubes, and cuffs. However, since soaking in 70% alcohol did not always sterilize rubber tubing, Winge-Hedén (1962) recommended boiling water for 5 minutes.

Sterilization of Clinical Thermometers

Force and Kerr (1920) gave serious thought to the necessity for sterilization of clinical thermometers. Because of their construction, clinical thermometers are best sterilized by chemical disinfectants. Since alcohol is nonpoisonous, colorless, nearly tasteless, readily evaporates, and leaves no residue, it was selected for the sterilizing agent. These investigators reported that an exposure of 4 minutes to 50% alcohol would adequately disinfect oral thermometers, if they were wiped with a cotton sponge wet with water to free them of mucus before placing them in the alcohol. Results of

two rather extensive investigations have been published. Sommermeyer and Frobisher (1952) used glass rods or unfilled mouth thermometers and contaminated them with a thin film of tuberculous sputum that was strongly positive for acid-fast bacilli by direct smear examination. Cultures of *Corynebacterium diphtheriae* were added to the samples of sputum, which contained staphylococci, streptococci, and other bacteria, in addition to the acid-fast bacilli. The contaminated thermometers were allowed to dry for 30 minutes before applying the sterilizing procedures. The contamination probably exceeded that encountered in ordinary practice, so the results possibly err on the side of safety. These authors found that wiping the thermometers with cotton and a mixture of equal parts of 95% ethyl alcohol and tincture of green soap before placing in the disinfectant decreased the number of viable organisms in practically every case. After the cleaning procedure, immersion of the thermometers for 10 minutes in 70% ethyl or isopropyl alcohol containing 0.5 or 1% iodine reduced the number of viable organisms to a low number. Aqueous iodine solutions or 70% solutions of ethyl or isopropyl alcohol were nearly as effective as the alcoholic iodine solutions. The importance of wiping the thermometers was emphasized by Ryan and Miller (1932).

Gershenfeld et al. (1951) tested various disinfectants for their ability to sterilize 2-inch segments of clinical thermometers contaminated with bacterial cultures. Ethyl alcohol, 95%, 70%, and 50% concentrations, isopropyl alcohol, 70 and 50%, and 2% iodine, aqueous solution and tincture, were tested. *Streptococcus pneumoniae* was killed by all disinfectants within 20 seconds. *Streptococcus pyogenes* was killed by all disinfectants within 20 seconds except 95% ethyl alcohol, which required 60 seconds. Cultures of these two organisms were used to contaminate the thermometer segments and, without drying, the segments were transferred to the disinfectants. This may be one factor that accounts for the fast killing action in the experiments. An article by Ecker and Smith (1937), which appears to have been overlooked, reported that thermometers contaminated with sputum from cases of lobar pneumonia were not sterilized by exposures for as long as 30 minutes to 70 and 95% solutions of ethyl alcohol. This is difficult to interpret in light of the findings of Sommermeyer and Frobisher (1952) and of Gershenfeld et al. (1951) with pure cultures of *Streptococcus pneumoniae*. The sputum may have exerted a protective action, as Gershenfeld et al. observed for plasma. It must be borne in mind that the work of Ecker and Smith (1937) was carried out prior to the general practice of neutralizing the bacteriostatic action of mercury in the subculture medium. This might account for the apparent quick sterilization of the thermometers by the mercurial compounds. In the case of the other organisms tested by Gershenfeld et al. (1951), the contaminated segments were thoroughly dried in an incubator at 37° C, which might account for delayed killing action.

On the basis of his work with alcohols on *Mycobacterium tuberculosis*, Smith (1947) stated that if thermometers are kept immersed in 70% alcohol, either ethyl or isopropyl, they should remain noninfectious if the alcohol is changed often enough to keep its strength within the effective range.

Denatured Alcohol

Ethyl alcohol (96%) denatured by the addition of 10% methyl alcohol was found by Olivo (1948) to have practically the same bactericidal power as ethyl alcohol. His tests were made with *Staphylococcus aureus* and *Escherichia coli*.

PHENYLETHYL ALCOHOL

Phenylethyl alcohol (benzylcarbinol) was discovered by Lilley and Brewer (1953) to have a unique property among the alcohols in that it has a greater inhibitory effect against gram-negative than against gram-positive organisms. By incorporating 0.25% phenylethyl alcohol in the culture medium, it is possible to isolate gram-positive organisms from material containing troublesome contaminating gram-negative organisms such as *Proteus* species. It is better to add the compound aseptically to the sterilized medium to avoid evaporation during sterilization of the medium. Slow evaporation of the compound also takes place during storage of the medium. Although the gram-negative organisms are inhibited, some growth will take place, and the growth of some gram-positive organisms may be slowed.

The combination of colistin and nalidixic acid has been proposed by Ellner et al. (1966)

for inhibiting gram-negative organisms. The combination of the two antimicrobials is more stable than phenylethyl alcohol, and the inhibition of gram-negative organisms is more complete, while at the same time the growth of gram-positive organisms is less likely to be retarded. These three features of the two antimicrobials make them preferable to phenylethyl alcohol for use in the selective inhibition of gram-negative organisms.

Mycoplasmas have been observed to be totally inactivated within 20 minutes by exposure to 1% phenylethyl alcohol, but enveloped viruses resisted such treatment (Staal and Rowe, 1974). The stable L-form of *Streptococcus faecium*, but not the parent *S. faecium*, was lysed by 0.5 to 0.6% (v/v) phenylethyl alcohol (King, 1974). Phenylethyl alcohol as a preservative and in combination with other germicides is discussed in Chapter 20.

ISOPROPYL ALCOHOL

The propyl alcohols (normal-propyl and isopropyl) occupy an enviable position in that they are the alcohols of the highest molecular weight that are miscible with water in all proportions. Tanner and Wilson (1943) found normal-propyl alcohol to be the strongest bactericide of the water-soluble alcohols. Because ethyl alcohol is subject to restrictions and heavy taxation and is important in many manufacturing processes, a suitable substitute has been sought for it as an antiseptic. Isopropyl alcohol appears to fill this need. In a review of the literature on isopropyl alcohol, Grant (1923) pointed out that it had no noticeable harmful effect on the human skin although it is slightly more toxic than ethyl alcohol. However, isopropyl alcohol vapors may be absorbed through the lungs and produce narcosis. Senz and Goldfarb (1958) stated that isopropyl was twice as toxic as ethyl alcohol and produced greater and longer-lasting toxic effects. The toxic reactions have been reported in children who were given alcohol sponge baths to reduce fever (Garrison, 1953; Wise, 1969).

As might be expected, the bactericidal action of isopropyl alcohol is slightly greater than that of ethyl alcohol. By making counts of surviving organisms after 30 seconds of exposure to varying concentrations of alcohols, Coulthard and Sykes (1936) found isopropyl alcohol slightly more bactericidal than either ethyl or methyl alcohol for *Escherichia coli* and *Staphylococcus aureus*. The alcohols had little effect against bacterial spores.

Powell (1945) reported that *Staphylococcus aureus* was killed by a 1-minute exposure at 20° C to 50%, 60%, 70%, 80%, and 91% isopropyl alcohol solutions, but not by 20%, 30%, and 40% solutions. Other tests showed that the same organism was killed in 5 minutes by 40% and greater concentrations of isopropyl alcohol, but not by 10%, 20%, or 30% solutions. *Escherichia coli* was killed in 5 minutes at 20° C by 30% and greater concentrations of isopropyl alcohol but not by 10 or 20% solutions. Well-spored cultures of *Bacillus subtilis* and *Clostridium novyi* were not killed in 60 minutes at 20° C by any concentration of isopropyl alcohol ranging from 20 to 91%. In tests performed some 10 years earlier, Powell stated that the stocks of isopropyl alcohol were contaminated with a saprophytic spore-forming organism, which further substantiates its ineffectiveness against bacterial spores. The sporicidal activity of isopropyl alcohol can be greatly increased by the addition of 5% propylene oxide and maintaining a temperature of 30° C (Hart and Ng, 1975).

Tainter et al. (1944) reported that *Staphylococcus aureus* was killed in less than 10 seconds by a 50% aqueous solution of isopropyl alcohol. A 90% solution failed to kill the organisms in an exposure of 2 hours, thus emphasizing the importance of the presence of water for effective bactericidal action, as in the case of ethyl alcohol.

Against tubercle bacilli in dried sputum smears, Smith (1947) observed that the bactericidal activity of isopropyl alcohol paralleled that of ethyl alcohol in the upper and middle ranges of concentrations, but surpassed that of ethyl alcohol in the range of lower concentrations. Frobisher and Sommermeyer (1953) obtained results with 70% to 50% isopropyl alcohol that were comparable to those obtained with 80 to 100% ethanol against tubercle bacilli and other bacilli in sputum when precautions were taken to ensure intimate contact between the bacteria and alcohol before the sputum became coagulated. For a comparison of the ability of isopropyl alcohol to disinfect clinical thermometers with that of ethyl alcohol, the publications of Gershenfeld et al. (1951) and Sommermeyer and Frobisher (1952) should be consulted.

TABLE 10–4. THE FATE OF *PSEUDOMONAS AERUGINOSA* IN SALINE, SODIUM CHLORIDE AND IN SALINE CONTAINING 0.9% BENZYL ALCOHOL

	Saline, 0.9% NaCl	*Saline 0.9% NaCl, + 0.9% Benzyl Alcohol*
Time zero	62,000 per ml	55,000 per ml
24 hours	18,000,000 per ml	5,000 per ml (92.5% killed)
48 hours	Not determined	52 per ml (99.9% killed)
72 hours	Not determined	0 per ml (100% killed)
96 hours	Not determined	0

In preventing infection in mice with *Streptococcus pneumoniae*, Prombo and Tilden (1950) found isopropyl alcohol (99%) as effective as ethyl alcohol, 70% by weight, and nearly as effective as ethyl alcohol, 70% by volume. It was less effective than ethyl alcohol, 70% by weight, in preventing infection in mice with *Streptococcus pyogenes*. Isopropyl alcohol in concentrations of 48.5 and 99% showed a strong inactivating effect on vaccinia virus and influenza virus, type A (Groupé et al. 1955). In a concentration of 49.5%, it reduced the activity of eastern equine encephalomyelitis virus four logs or more after 1-hour exposure at 18 to 20° C (Bucca, 1956).

Tilley and Anderson (1947) mixed equal volumes of 50% isopropyl alcohol and suspension of the virus of Newcastle disease. After varying periods of exposure up to 60 minutes at 20° C, portions of the mixtures were diluted with distilled water and portions of this diluted mixture inoculated into chick embryos. All the embryos died, but it must be pointed out that a 25% solution is much weaker than that which gives maximum bactericidal action.

Isopropyl alcohol was less active than ethyl alcohol against the entero viruses, but slightly more active than ethanol against the viruses with a lipid envelope (Klein and Deforest, 1963). These investigators concluded that it cannot be considered equivalent to ethanol as a virucide. A comparison of the activities of the two alcohols against viruses is summarized in Table 10–3.

AROMATIC ALCOHOLS

Of the aromatic alcohols, benzyl alcohol appears to be the only one tested in recent years. It is soluble in water with difficulty, which perhaps explains its limited usage. Prombo and Tilden (1950) found 4% benzyl alcohol by weight less effective than 70% ethyl alcohol (either by weight or by volume) or isopropyl alcohol (99%) in preventing infection in mice with *Streptococcus pneumoniae*. Benzyl alcohol, 4% by weight, was slightly better than ethyl alcohol (70% by weight) or isopropyl alcohol (99%) in protecting mice against infection with *Streptococcus pyogenes*.

Sodium chloride, 0.9%, and sodium chloride, 0.9%, containing 0.9% benzyl alcohol, were tested for their bacteriostatic activity. Gram-negative microorganisms are more prevalent than gram-positive microorganisms in hospital environments and frequently cause serious nosocomial infections. Moreover, infections with some gram-negative microorganisms, such as *Pseudomonas*, are not readily amenable to successful treatment with antimicrobial drugs. The fate of *Pseudomonas aeruginosa* was determined in saline, 0.9%, and in saline, 0.9%, containing 0.9% benzyl alcohol. One ml of a 1:100 dilution of an overnight culture of *P. aeruginosa* in broth was added to 150-ml bottles of saline and saline plus 0.9% benzyl alcohol. The contents of the bottles were thoroughly mixed by shaking and the bacterial concentration determined immediately and at 24-hour intervals by making pour plate preparations of appropriate dilutions of the contents of each bottle. The results are summarized in Table 10–4. It cannot be overemphasized that "bacteriostatic" preparations are not necessarily sterile preparations.

LONG-CHAIN ALCOHOLS

Maximum inhibition of growth of *Mycoplasma gallisepticum* and *M. pneumoniae* was obtained by the saturated primary alcohols varying in chain length from 16 to 19 carbon atoms at 64-μM concentration. The unsaturated alcohols, oleyl, linoleyl, and linolenyl, were less effective as inhibitors. The inhibitory effect of 48-μM concentration of stearyl alcohol was not significantly relieved by a concentra-

tion of 128 μM cholesterol (Fletcher et al., 1981).

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A Comparative Evaluation of Different Hand Cleansers

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INTRODUCTION

The potential for foodhandlers to be a vector in transmitting foodborne disease continues to be a significant issue (*2,3,5,6,7*). Additionally, there continues to be an increasing number of situations where foodhandlers have been implicated as a primary vector in contributing to foodborne illness. Various kinds of foodborne epidemics may be transmitted to consumers via this vector, but gastrointestinal outbreaks are the most commonly reported (*4,7*).

The microorganisms which normally colonize the hand surfaces pose little threat of infectious disease transmission from foodhandlers to consumers. The threat, instead, comes from "transient" pathogenic microorganisms which contaminate the hand surfaces. In the food industry, contamination usually occurs from contact with one's own excretions or infected areas, or those of others, most commonly through hand to hand transmission. However, food may also become contaminated by food processing personnel via exposure to the feces or raw animal products being processed. The most significant bacteria in this area include species of *Salmonella*, *Shigella*, *Escherichia*, *Yersinia*, *Klebsiella*, *Proteus*, *Clostridium*, *Citrobacter*, *Staphylococcus* and *Streptococcus* (*2,4*).

Foodborne disease outbreaks result in staggering financial costs, as well as necessitating regulatory processes to assure food safety in the food processing and food service industries. The most effective way to break the contamination vector between foodhandling/processing personnel and consumers is for foodhandlers to perform an effective handwash using an effective hand cleanser.

There is some confusion as to the actual antimicrobial effectiveness of commercially available products. The purpose of this study is to aid food sanitarians in choosing the most appropriate hand cleansing products for their needs. Prior to evaluating any hand cleanser, it is critical that a valid experimental design, as well as valid testing and sampling methods be employed.

The hand sampling methods most commonly used in the food industry to evaluate hand cleanser effectiveness are the "swab" and "finger press" techniques. In brief, the swab sampling method consists of swabbing the palmar hand surfaces as well as between the finger digits with a pre-moistened cotton swab and culturing it on an agar plate. The other sampling method, the finger press method, is conducted by having the test subjects press their palmar surfaces and/or finger tip pads onto an agar plate. A third method (glove juice), although not widely used in the food industry, is the only method accepted by regulatory agencies for evaluating hand disinfectant products. This "glove juice" method is conducted by having the subject don latex surgical gloves, instilling a microbial stripping solution into the glove, and then massaging the hands and fingers through the glove for 1 min.

The major problem with the first two hand sampling methods is lack of reliability. They are neither accurate nor precise in measuring known quantities of marker microorganisms (*5*). Accuracy, in this context, refers to the ability of the sampling method to estimate true microbial population. Precision is the ability of the sampling method to repeatedly and closely estimate the true microbial population.

In a study conducted in our laboratory comparing these three hand sampling methods at hand contamination levels of 10^2 through 10^8, the glove juice method was the only technique which accurately and precisely measured all of the contamination levels (*5*). Both the swab and finger press methods grossly underestimated the actual contamination populations, and were also imprecise in repeated estimations of known microbial population numbers (*5*). Hence, the method of choice is the glove juice sampling procedure.

Additionally, there has been some argument concerning the need to sample the entire hand when mainly the fingers are involved, particularly in fecal contamination. This is a valid argument, but when trying to ascertain the reliability of measuring a known number of microorganisms on the finger tips, the sampling variability is unacceptable, due to the finger tips having such a small surface area. One-hundred-fold accuracy and precision errors are not uncommon (*1*). Additionally, once the fingers have been contaminated, the contaminating microorganisms are quickly disseminated over the entire palmar surfaces via finger and hand movement. Hence, for reliability, it is suggested that the entire hand surface be measured.

Another problem in evaluating hand disinfectants is that one must know the number of transient microorganisms existing on the hands and not confuse them with the normal or resident microbial populations in order to evaluate the

product's true degerming effects. Recall that the microorganisms encountered on the hand surfaces fall into two categories. The first consists of transient microorganisms "picked up" by foodhandlers. They are called transient for they reside on the hand surfaces only temporarily. The second category (resident bacteria) consists of those microbes which permanently reside on the hand surfaces, e.g., the normal skin flora.

Since it is nearly impossible to reliably quantify the microbial populations of either of these categories, it is simpler and more effective to use a marker microorganism as the transient microorganism. In this study, *Serratia marcescens* was used as a marker organism, because it produces a characteristic red colony that is easily distinguishable from the normal microbial population of the hands, as well as other contaminating microorganisms. The use of *S. marcescens* provides a clear indication of the effectiveness of the handwash without confounding or "mixing up" transient and normal microorganisms.

Additionally, the use of *S. marcescens* is valuable in several other ways. First, it is not normally a pathogenic or disease-producing microorganism so there is little risk of infection to human volunteer subjects. Second, it is as resistant to mechanical removal from the hands as potential pathogens, such as *Escherichia coli*, *Salmonella* sp. and *Shigella* sp. Third, because it is seeded onto the hands at a known population, the antimicrobial properties of the handwash products can be easily and directly compared because the baseline levels for all subjects will be equivalent.

In evaluating handwash products designed mainly to remove transient microorganisms, two parameters of interest are the immediate and persistent antimicrobial effects. The immediate antimicrobial effect is the measurement of both the mechanical removal (if a handwash is used) and the immediate inactivation of the microorganisms by the antimicrobial compound (e.g., alcohol, triclosan, parachlorometaxylenol), if one is used in the product. The persistent effect is the measurement of the product's antimicrobial ability to prevent microbial recolonization of the skin surfaces (either by inhibition or lethality) after having used the product.

However, even if a hand cleansing product is effective in removing transient microorganisms, it must also be mild to the skin after many consecutive applications if foodhandlers are to use it. Few people will conform to a handwashing program when the handwash product irritates their hands.

In view of what has been discussed, this study was designed to compare six common handwash product configurations (a non-antimicrobial lotion soap, an antimicrobial lotion soap, an E2[1] sanitizing soap, an alcohol [ethanol] gel sanitizer and combinations of a non-antimicrobial lotion soap used in conjunction with an alcohol gel sanitizer, and an antimicrobial lotion soap used with an alcohol gel sanitizer).

In brief, non-antimicrobial soaps are useful degerming agents because of their ability to assist mechanical removal of transient microorganisms during a warm water hand Antimicrobial soaps, including E2 soaps (usually contai. triclosan or parachlorometaxylenol [PCMX]), have imme ate antimicrobial effects mainly due to mechanical degermi during the wash but partly due to the antimicrobial compoun used. They also have persistent antimicrobial action due specifically to the antimicrobial compound they contain.

Alcohol and alcohol gels, where alcohol levels exceed 50%, have the most pronounced immediate antimicrobial effects. However, they lack persistent antimicrobial properties. Alcohol gels, which are used without a water wash, have excellent immediate antimicrobial effects. However, without the mechanical removal of the microorganisms via a handwash, there is tendency for microbial build-up upon repeated exposure to contaminating microorganisms. A number of manufacturers recommend a regimen involving the use of both a soap and water handwash to degerm the hands, as well as the use of an alcohol gel to augment the soap's immediate antimicrobial properties.

In this study, we were interested in the immediate and persistent antimicrobial properties as well as the product's irritation to the hands upon repeated use.

MATERIALS AND METHODS

The six product configurations were used for this study as follows:

Configuration	Regimen	Active Ingredient
1	Non-antimicrobial Lotion Soap	None
2	Antimicrobial Lotion Soap	0.6% PCMX
3	E2 Sanitizing Soap	2.5% PCMX
4	Alcohol Gel Sanitizer	62% Ethanol
5	Non-antimicrobial Lotion Soap plus Alcohol Gel Sanitizer	62% Ethanol
6	Antimicrobial Lotion Soap plus Alcohol Gel Sanitizer	0.6% PCMX plus 62% Ethanol

Subjects.

Thirty human subjects over the age of 18 but under the age of 70 were recruited for this study. Subjects were of both sexes; all were free of clinically evident dermatoses or injuries to the hands and/or forearms. No known immune comprised subjects were admitted into this study. Subjects were randomly assigned one of the six product configurations. Five subjects per product configuration were used.

Contaminating microorganism.

Serratia marcescens (ATCC No. 14756), red-pigmented strain was used in this evaluation. A 24-h culture of *S. marcescens* with a population of approximately 1.0×10^8 colony forming units (CFU) per ml was used. Since *S. marcescens* colonies appear red on the tryptic soy agar

[1] E2 denotes the United States Department of Agriculture (USDA) classification of handwashing and sanitizing compounds which are dispensed from adequate dispensers located a sufficient distance from the processing lines to prevent accidental product contamination. With E2 compounds, the hands need not be washed prior to their use but after use the hand must be thoroughly rinsed with potable water. Additionally, E2 compounds must be used at dilutions applicable as provided on the label instructions. E2 compounds are accepted handwash/hand sanitizing compounds based upon their equivalency of 50 ppm chlorine.

plates, they can be clearly identified as the marker (transient) microorganism. This prevents "mixing up" or confounding the normal and transient microorganisms found on the hands. Any non-red colonies appearing on the agar plates were not counted.

Pre-test period.

The first 7-day period of this study was designed the "pre-test period." During this period, subjects avoided using medicated soaps, lotions, detergents, acids and bases. Bathing in chlorinated pools and hot tubs as well as using ultraviolet (UV) light tanning beds were prohibited. This regimen allowed for stabilization of the normal microbial flora of the hands.

Experimental period.

The second 7-day period (following the "pre-test" period) constituted the experimental period. Each subject was utilized 1 day of that week for approximately 4 to 5 h. During this period, 5 ml portions of the *S. marcescens* culture were pipetted into each subject's cupped hands. The inoculum was then distributed evenly over both hands and the area comprising approximately one-third of the forearm, via gentle massage. After a 1 min air dry, the glove juice sampling procedure was performed.

The first microbial inoculation cycle constituted the baseline sample. It assured that all of the microorganisms inoculated onto the subjects' hands could be removed. It was followed by a reinoculation of the microorganisms and use of the assigned hand cleansing product configuration. The microbial inoculation/assigned product use procedure was repeated 10 consecutive times with a minimum of 5 min between cycles. A transient microorganism count of the hands was performed following washes 1, 3, 7 and 10, using the glove juice sampling procedure.

Wash procedure.

Three milliliters of each hand washing product was dispensed into each subject's hands. Each subject washed for 20 s, rinsed for 30 s with lukewarm water, and allowed their hands to dry thoroughly. The alcohol gel (3 ml) was applied to dry hands, rubbed to distribute evenly, and allowed to dry for 5 min before sampling. No water wash was used in conjunction with the alcohol gel product.

Glove juice sampling procedure.

Following the prescribed wash and rinse, non-powdered, sterile surgical gloves were donned. Seventy-five milliliters of a sterile aqueous phosphate buffer (pH 7.8) solution containing 0.1% Triton X-100 were instilled into the glove. The glove was secured at the wrist and the hand massaged through the glove for 60 s. Portions of the "glove juice" were removed and serially diluted in Trypticase Soy Broth (TSB) containing 1.0% Tween 80 and 0.3% Lecithin as neutralizing agents. Duplicate Trypticase Soy Agar (TSA) spread plates containing 1.0% Tween 80 and 0.3% Lecithin were prepared for each dilution. The plates were incubated at 30 to 35°C until a distinguishable red color developed. Those plates providing between 25 and 250 red pigmented colonies were utilized in this study. The number of viable red pigmented bacteria recovered was determined using the formula:

$$B = A\left[\frac{\Sigma x}{n}\right] 10^{D}$$

Where:

B = estimated number of microorganisms
A = portion volume = 75 ml
$\frac{\Sigma x}{n}$ = average colony forming unit count of the agar plates for each dilution level
D = dilution level

Since the data collected in this study were on the exponential scale, in order to linearize the data, each B value was transformed into the $\log_{10}$ scale. This linearization process was a requirement of the statistical models employed.

Irritation study.

After microbial inoculation/product use cycle ten, the subjects entered the irritation potential phase of the study. The subjects continued to wash their hands with their assigned product configuration for 15 more consecutive washes/product applications, for a total of 25 washes. There was a minimum of 5 min and a maximum of 15 min between washes/product applications. The subjects' hands were examined for irritation after washes 15, 20 and 25, or at the first sign of irritation. Irritation was scored according to Table 1. The entire study took about 6½ h to perform.

TABLE 1. *Hand irritation scoring system.*

Erythema: (redness)	0 =	No reaction
	1 =	Mild and/or transient redness limited to sensitive areas
	2 =	Moderate redness persisting over much of the product-exposed area
	3* =	Severe redness extending over most or all of the product-exposed area
Edema: (swelling)	0 =	No reaction
	1 =	Mild (just perceptible)/ transient
	2 =	Moderate - definitely palpable
	3* =	Severe
Rash:	0 =	No reaction
	1 =	Mild - few, small eruptions
	2 =	Moderate - scattered eruptions > 10/hand
	3* =	Severe
Dryness: (redness)	0 =	No reaction
	1 =	Mild - transient, generally limited to cuticles, knuckles
	2 =	Moderate - persistent, extending over much of the hand
	3* =	Severe - persistent, extending over most of the hand, characterized by cracking and roughness

* Represents significant irritation and requires subject's removal from study.

RESULTS

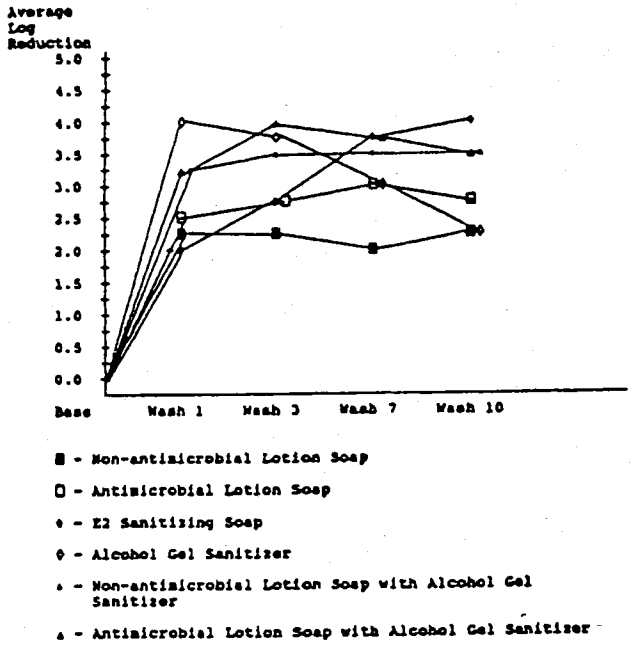
Figure 1 represents the results in graphic form and Table 2 presents the data in $\log_{10}$ reductions from the baseline as well as percent reduction from baseline form.

As can be seen in Fig. 1 and Table 2, each of the six product configurations was highly statistically significant in reducing the baseline population by at least two logs ($p<0.05$).

Non-antimicrobial lotion soap.

The non-antimicrobial lotion soap reduced the baseline microbial counts by about two logs (99.00%) after the first wash. Upon repeated microbial inoculations and washes, the product configuration was capable of keeping the contaminating microorganism counts down by nearly two logs (99.00%) from the baseline value over the course of the ten inoculation/wash cycles.

Figure 1. *Graph of the six product regimen efficacies over time.*



Antimicrobial lotion soap.

The antimicrobial lotion soap reduced the initial microbial counts after the first inoculation/wash cycle by approximately 2.5 logs (99.68%) and demonstrated antimicrobial consistency over the ten consecutive inoculation/wash cycles.

E2 sanitizing soap.

The E2 sanitizing soap reduced the baseline microbial population by nearly two logs (99.00%) after the first wash. After the third inoculation/wash, the product was able to reduce baseline microbial populations by 3 logs (99.90%), after the seventh inoculation/wash cycle by 3.75 logs (99.98%), and by wash ten, nearly 4 logs (99.99%). As the product was used repeatedly over time, it demonstrated increasing antimicrobial effectiveness.

Alcohol gel sanitizer.

The alcohol gel sanitizer achieved approximately a 4-log (99.99%) reduction in microorganism from baseline after the first inoculation/product application cycle. But, since it is applied without the use of a water wash, a slight but continuous build-up in microorganism populations was noted. Even so, after inoculation/application number ten, the microbial populations were still at least two logs (99.00%) below the baseline value ($p<0.05$).

Non-antimicrobial lotion soap with alcohol gel sanitizer.

The non-antimicrobial lotion soap and the alcohol gel sanitizer, when used in conjunction with each other, reduced the baseline counts about 3.25 logs (99.94%). After the first inoculation/product use cycle, and from inoculation/product use cycles three, seven, and ten, the microbial counts were kept down from baseline by greater than 3.5 logs (99.97%).

Antimicrobial lotion soap with alcohol gel sanitizer.

The antimicrobial lotion soap used in conjunction with the alcohol gel sanitizer, after the first inoculation/product use wash cycle, provided about a 3.25 log (99.94%) reduction from baseline and remained at about 3.5 logs (99.97%) for the remainder of the ten inoculation/product use cycles.

Irritation.

No significant skin irritation was observed over the 25 consecutive wash/product application cycles with the non-antimicrobial lotion soap, the antimicrobial lotion soap or the alcohol gel sanitizer. Neither of the soaps used in conjunction with the alcohol gel sanitizer produced any significant skin irritation over the course of the 25 handwashes.

Mild irritation consisting of the transient redness (score of 1 on the scoring system) and generalized dryness (score of 1 on the scoring system) was observed with the E2 Food Industry Sanitizing Soap.

DISCUSSION

Although each of the six product configurations was effective in reducing transient microbial population levels on the hands, the overall results illustrate the range and complexity involved in setting an effective hand hygiene regimen for the foodhandling environment. Each configuration has associated advantages and disadvantages.

The non-antimicrobial soap illustrates the generally sound principle of standard hand washing for hygiene purposes. This configuration showed that mechanical removal of transient skin bacteria is relatively effective and consistent, but perhaps not optimum. Importantly, no skin irritation was found from this regimen.

The antimicrobial lotion soap was statistically more effective than the plain soap in both immediate and persistent antimicrobial properties. A properly formulated general purpose antimicrobial soap can exhibit both effective removal/kill of transient bacteria and low skin irritation and, thus, can be considered as an alternative to plain non-antimicrobial soaps.

The E2 sanitizing soap was very effective in both immediate and persistent antimicrobial effects, providing sustained high log reductions in transient organisms. However, these types of soaps typically show more aggressive skin effects, as was found in this study. Skin irritation can increase resistance to handwash compliance, so the use scenario in total must be evaluated when considering an E2 product.

The alcohol gel sanitizer was, as expected, the most immediately effective product in reducing microorganism populations. It is the product of choice when transient organism contamination is possible, and water is not available for a thorough hand wash. It is also preferred in situations where

TABLE 2. *Log percent reduction values from baseline.*

Product Configuration	Mean Log Baseline Value	Standard Deviation	Mean Log Reduction From Baseline Wash 1	Standard Deviation	Percent Reduction From Baseline Wash 1	Mean Log Reduction From Baseline Wash 3	Standard Deviation	Percent Reduction From Baseline Wash 3	Mean Log Reduction From Baseline Wash 7	Standard Deviation	Percent Reduction From Baseline Wash 7	Mean Log Reduction From Baseline Wash 10	Standard Deviation	Percent Reduction From Baseline Wash 10
1	7.98	.27	2.29	.52	99.49%	2.68	.63	99.15%	1.95	.72	99.28%	2.89	.68	99.15%
2	7.98	.27	2.64	.22	99.68%	2.73	.41	99.81%	2.95	.63	99.88%	2.76	.27	99.83%
3	7.98	.27	1.99	.12	99.98%	2.83	.72	99.84%	2.72	.43	99.98%	2.99	.89	99.99%
4	7.98	.27	2.93	.84	99.99%	2.79	.18	99.98%	2.95	.17	99.99%	2.76	.13	99.99%
5	7.98	.27	2.27	.14	99.99%	2.79	.10	99.98%	2.64	.23	99.99%	2.92	.29	99.98%
6	7.98	.27	2.79	.13	99.99%	4.13	.23	99.99%	2.74	.27	99.98%	2.85	.17	99.98%

1 = Non-antimicrobial Lotion Soap

2 = Antimicrobial Lotion Soap

3 = E2 Sanitizing Soap

4 = Alcohol Gel Sanitizer

5 = Non-antimicrobial Lotion Soap with Alcohol Gel Sanitizer

6 = Antimicrobial Lotion Soap Alcohol Gel Sanitizer

one cannot continually wash his/her hands. Although the alcohol gel maintains significant log reductions over a series of hand washes, the data trend suggests that it be supplanted with an antimicrobial or non-antimicrobial hand wash product after three to five consecutive uses. Use of the alcohol gel showed low skin irritation, comparable to that of the plain soap.

LIMITATIONS

This study provides food service professionals with information and guidelines to help choose effective hand cleansing products and regimens that reduce the risk of foodhandler-vectored infections. However, it is the responsibility of the end user to assure the products are utilized appropriately and regimens are adhered to as required as appropriate to each situation.

A potential limitation of this study is that it was conducted in a laboratory setting, rather than under actual field conditions. However, correlation between the glove juice test results and actual field results has been established by extensive studies in the healthcare industry. The fact remains that glove juice testing is the only methodology accepted by regulatory agencies for demonstration of handwash efficacy. Further, any study, field or otherwise, must use accurate and valid techniques before correct conclusions can be drawn. The glove juice procedure has been shown to be accurate and precise while many other methods have not.

CONCLUSION

The most effective product regimens, from an overall microorganism reduction profile as well as a skin irritation standpoint, were the combinations of alcohol gel with antimicrobial or plain lotion soap. Both of these configurations showed tremendous immediate transient organism reduction due to mechanical degerming by the soap hand wash coupled with the alcohol's tremendous immediate antimicrobial properties. Both regimens also demonstrated persistent antimicrobial properties.

Effective hand washing is a cornerstone in preventing foodhandler-borne illness. A second key issue is selection of effective hand cleansing product. The interaction of the product regimen selected and a well-managed handwashing/sanitizing program, which includes compliance measurement, provides the key to assuring the safety of the American food supply.

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C59

COMPARATIVE EVALUATION OF SURGICAL SCRUB PREPARATIONS

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IN A RECENT PUBLICATION (6), the first series of studies carried out in the United States to establish the safety and efficacy of the skin antimicrobial chlorhexidine gluconate has been discussed. It was suggested that it offers an alternative to other antimicrobial agents currently available for use in surgical scrub preparations, specifically the most widely used, hexachlorophene and the iodophors. While much is known about the individual characteristics of each of these agents, few well controlled clinical studies have been conducted to compare directly the efficacy of scrub preparations. The Food and Drug Administration (1), with the guidance of a panel of experts, has determined several methodologies to be the most appropriate for demonstrating the efficacy of topical skin antimicrobials. This study was undertaken to compare directly the efficacy of three currently available surgical scrub preparations using these methods which are currently recognized as the most meaningful by the Food and Drug Administration and this panel.

MATERIALS AND METHODS

The three formulas tested in this study were Betadine surgical scrub, 0.75 per cent available povidone-iodine; Hibiclens, 4 per cent chlorhexidine gluconate, and pHisoHex, 3 per cent hexachlorophene.

Efficacy against resident flora. One hundred and eight persons were used to test the immediate and persistent effects of the three test formulations on the bacterial flora of the hands under conditions mimicking those encountered in the operating room. With the use of the glove fluid technique (5), samples from both hands of each person were taken three times during the base line week, and mean left and right hand base line levels were established. Only those individuals with mean counts of 1.5×10^6 to 4.0×10^6 organisms per hand were admitted to the trial. On admission, each person was randomly assigned to one of the three treatment groups. During the test week, each person performed six minute scrubs with 10 milliliters of the assigned test preparation for a total of 11 scrubs. Samples were taken after the first scrub, day 1; the second scrub, day 2, and the 11th scrub, day 5. On each of the three sampling days, the left hands were occluded by surgical gloves for one, two, three, four, five or six hours, with 18 different persons being sampled at the end of each hour.

Efficacy against transient flora. The three formulas were further studied for efficacy against transient flora in a 15 second hand wash study designed to mimic routine hand washing by health care personnel. One hundred and eight persons were randomly assigned to three treatment groups. Each individual contaminated his hands with a known concentration of the marker organism Serratia marcescens, and glove fluid samples were taken to determine the actual number of applied organisms. Then, the hands were recontaminated 25 times during the course of one day, each contamination being followed by a 15 second hand wash with 5 milliliters of the assigned test preparation. Glove fluid samples were subsequently taken of all hands after every fifth contamination hand wash.

Analysis. Analysis was based upon the base 10 logarithm transformations of the raw bacterial counts. Alternately, percentage reductions, computed from the raw counts, were used.

RESULTS

Resident flora study. In Tables I and II, the results of the resident flora glove fluid test are given in detail. As can be seen from the immediate postwash bacterial counts (Table I), all three test preparations reduced the bacterial flora of the hands but with significant difference in the magnitude of the reductions. When compared with base line, the level of bacteria remaining on the hands after one wash, day 1, was lowest with the chlorhexidine gluconate scrub, 2.74 $\log_{10}$ organisms, followed by 4.13 $\log_{10}$ organisms with the povidone-iodine scrub and 5.20 $\log_{10}$ organisms with the hexachlorophene scrub. During

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TABLE I.—MEAN LOG, BASE 10, BACTERIAL COUNTS

	Povidone-iodine			Chlorhexidine gluconate			Hexachlorophene		
	Day 1	*Day 2*	*Day 5*	*Day 1*	*Day 2*	*Day 5*	*Day 1*	*Day 2*	*Day 5*
Base line count—mean, days, 1, 3 and 7									
36 hands, right	6.37			6.38			6.41		
36 hands, left	6.37			6.41			6.42		
Immediate postwash counts, 36 hands, right	4.13	3.91	3.62	2.74	2.48	2.27	5.20	4.47	3.78
Delayed postwash counts, six hands each hour, left									
1 hr	4.60	4.77	3.76	2.41	2.09	1.92	4.16	3.96	3.31
2 hrs	4.32	4.51	3.51	2.69	2.51	2.46	4.75	4.16	3.30
3 hrs	4.20	4.16	4.09	2.42	2.13	1.89	4.12	3.47	3.42
4 hrs	5.34	4.87	4.32	3.09	2.70	2.53	4.89	4.08	3.35
5 hrs	4.86	4.84	4.38	3.03	2.92	2.59	4.72	4.94	4.34
6 hrs	5.23	5.22	5.05	2.36	2.32	2.19	5.34	4.86	3.65
Combined delayed postwash counts, 36 hands, left	4.76	4.73	4.19	2.67	2.44	2.26	4.66	4.25	3.56

TABLE II.—IMMEDIATE POSTWASH REDUCTIONS IN MEAN BACTERIAL COUNT

	Povidone-iodine		Chlorhexidine gluconate		Hexachlorophene	
Day No.	*Log_{10} reduction*	*Per cent reduction*	*Log_{10} reduction*	*Per cent reduction*	*Log_{10} reduction*	*Per cent reduction*
1	2.24	97.27	3.64	99.90	1.21	83.28
2	2.46	97.79	3.89	99.94	1.94	95.82
5	2.76	99.25	4.11	99.98	2.63	99.00

TABLE III.—MEAN LOG_{10} BACTERIAL COUNTS

Time	*Povidone-iodine*	*Chlorhexidine gluconate*	*Hexachlorophene*
Base line	7.83	8.07	8.05
After fifth wash	4.05	3.75	6.39
After tenth wash	3.94	2.58	6.36
After 15th wash	3.94	2.43	6.22
After 20th wash	3.03	2.03	6.11
After 25th wash	3.90	1.45	6.16
Combined wash counts	3.97	2.45	6.25

TABLE IV.—REDUCTIONS IN MEAN BACTERIAL COUNT

	Povidone-iodine		Chlorhexidine gluconate		Hexachlorophene	
Time	*Log_{10} reduction*	*Per cent reduction*	*Log_{10} reduction*	*Per cent reduction*	*Log_{10} reduction*	*Per cent reduction*
After fifth wash	3.78	99.98	4.32	99.99	1.66	96.97
After tenth wash	3.89	99.98	5.49	>99.99	1.69	97.03
After 15th wash	3.90	99.98	5.63	>99.99	1.83	97.61
After 20th wash	3.80	99.96	6.04	>99.99	1.94	98.18
After 25th wash	3.93	99.98	6.61	>99.99	1.89	98.13

the course of the test week, bacterial counts were further reduced in all three treatment groups, with significantly lower counts being noted in the chlorhexidine group each day than in either the povidone-iodine or hexachlorophene groups. The day 1 log_{10} reduction in the chlorhexidine gluconate group was increased from 3.64, 99.90 per cent, to 3.89 on day 2, 99.94 per cent, and 4.11, 99.98 per cent, on day 5 (Table II). The log_{10} reduction in the povidone-iodine group after the first wash was significantly lower, 2.24, 97.27 per cent, increasing slightly to 2.46, 97.79 per cent, on day 2 and 2.76, 99.25 per cent, on day 5. Of all the treatments, the smallest reduction on day 1 was in the hexachlorophene group, 1.21 log_{10} organisms, 83.28 per cent; 1.94 log_{10} reduction, 95.82 per cent, on day 2, and a 2.63 log_{10} reduction, 99.00 per cent on day 5. By comparing the delayed postwash counts with the immediate counts in the povidone-iodine group, a significant regrowth occurred during the six hour test period on each test day (Table I). In the chlorhexidine group, no bacterial regrowth occurred under the gloves on any test day; data from the hexachlorophene group indicate a slight, further decrease in bacterial counts on the gloved hands on any given test day.

Transient flora study. Data from the artificial contamination hand wash study are detailed in Tables III and IV. As can be seen from the mean log_{10} bacterial counts (Table III), 15 second hand washes with chlorhexidine gluconate resulted in the lowest over-all log_{10} bacterial count, 2.45, compared with 3.97 for the povidone-iodine and 6.25 for the hexachlorophene groups. Log_{10} reductions in the chlorhexidine group increased significantly from 4.32 after five washes to 6.61 after 25 washes; corresponding reductions for the povidone-iodine and hexachlorophene groups showed no similar trend (Table IV).

DISCUSSION

Several reports have appeared in the literature which describe the comparative efficacy of chlorhexidine gluconate, hexachlorophene and povidone-iodine in formulations similar to those tested herein. Sevens and co-authors (7) found the 4 per cent chlorhexidine gluconate detergent solution to give the best reductions with a one minute wash, 81.8 per cent, compared with 79.8 per cent for an iodine detergent solution and 64.5 per cent with a 3 per cent emulsion of hexachlorophene. Ojajarvi (4), using a standard wash method to sample hands, found a 98 per cent reduction in the natural flora following a hand wash with 4 per cent chlorhexidine gluconate detergent solution compared with a 55 per cent reduction after a 0.75 per cent available povidone-iodine wash and a 51 per cent reduction after a 3 per cent hexachlorophene emulsion treatment. Lowbury and Lilly (2), using the same sampling methodology, reported an 86.7 per cent reduction in resident hand flora following a 4 per cent chlorhexidine gluconate detergent wash versus 68 per cent following an identical wash with a 0.75 per cent available povidone-iodine solution. In this same study, hands were also tested following six hand washes with each test preparation. The chlorhexidine gluconate detergent solution gave slightly better reductions than did the povidone-iodine detergent solution, 99.2 versus 97.7 per cent, an indication that both preparations have cumulative effects. However, when Smylie and colleagues (8) tested hands which had been gloved for one, two or three hours, 0.75 per cent available povidone-iodine detergent showed no residual or cumulative effect, as was shown with chlorhexidine gluconate and hexachlorophene scrubs.

In a recent review of data available on skin antimicrobials, The Medical Letter (3) concluded that ". . . chlorhexidine, a new antiseptic, combines the broad spectrum antibacterial activity of alcohol and the iodophors with the prolonged action of hexachlorophene." These conclusions can be further elaborated on the basis of this report, which details the first comparative study of three major surgical scrub preparations following the more rigorous test methodologies, as recommended by the Food and Drug Administration in a proposed monograph on antimicrobial agents. Chlorhexidine gluconate in detergent solution has been shown to produce the best immediate and persistent reductions in normal hand flora when compared with a povidone-iodine detergent solution and a hexachlorophene emulsion. Further, the activity of chlorhexidine against repetitive transient contamination has been demonstrated to be superior to the other test preparations. It appears that the 4 per cent chlorhexidine gluconate detergent solution is indicated when strict skin antisepsis is required.

SUMMARY

The efficacy of 0.75 per cent available povidone-iodine scrub solution, 4 per cent chlorhexidine gluconate detergent solution and 3 per cent hexachlorophene emulsion against resident and transient flora of the hand has been compared using two currently accepted study designs. Chlorhexidine gluconate produced the greatest initial reductions against resident flora, followed by povidone-iodine and hexachlorophene, respectively. In subsequent washings, all three treatments further reduced the flora, chlorhexidine giving the greatest reduction on any test day. On gloved hands, there was significant regrowth following the use of povidone-iodine; there was no such significant regrowth on gloved hands which had been washed with chlorhexidine gluconate or hexachlorophene.

Against transient flora, chlorhexidine gluconate gave the lowest over-all counts, followed by povidone-iodine and hexachlorophene, respectively. The reductions in bacteria counts increased following the repetitive use of chlorhexidine; no such trend was noted with either povidone-iodine or hexachlorophene.

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NUMBER 22 SURGICAL BACTERIOLOGY—PRICE 1993

learn to select the treatment intelligently. For a very small tumor of a high grade of malignancy in the anterior commissure, I would recommend surgical removal by laryngofissure followed by irradiation.

Dr. Joseph C. Beck, Chicago: I am sorry Professor Coutard is not here to defend his side of the radiologic question. There should be in this discussion a "radiumologist" and a radiologist, because, as Dr. Jesberg said and as the Jacksons' paper would indicate, we have not yet reached the point where we can speak definitely. I have great hopes for the effect of proper irradiation carried out by experts on cancer, because I have seen excellent results in the treatment of certain kinds of tumors of the larynx. I would say that the results depended on whether the tumor was radiosensitive or radioresistant. However, in association with Dr. Cutler in one of the largest institutions for the care of carcinoma of the larynx, namely the Veterans Administration Facility at Hines, Ill., I have seen similar results in the case of radioresistant tumors that were really operative and yet were subjected to irradiation at the patient's request. Recently Dr. Schall painted an excellent picture of laryngectomized patients and said truly that they are not a great unhappy family, as they are so often described. Most of the people who are cured of cancer of the larynx are glad that they are permitted to live on with an educated or artificial voice. If they become dependents now, since so many people are dependents they don't have to care so much about that. Most laryngectomized patients do become dependents, according to my experience. They are not productive, especially if they are salesmen. I am definitely at variance with Professor Coutard about making the fixed cord which is immovable from inflammation move again. I have not seen this happen. The inflammation is chronic and is not, according to radiologists, affected by irradiation. I do not think it should be considered in the prognosis. Dr. Jesberg also was definitely wrong when he said that for a tumor which was very sensitive to irradiation he would operate. I have seen the worst kind of results from such a procedure, namely rapid spread not only into the glands of the neck but throughout the system. The surgeon should be much more alert to the pathologist's decision as to what type tumor he is treating.

Dr. Chevalier L. Jackson, Philadelphia: I appreciated very much hearing Dr. Cutler's discussion. Dr. Jesberg and Dr. Beck have both spoken about lesions of the anterior commissure. We feel that it is much more important to consider the histologic picture with such lesions than with lesions in the middle third of the cord. It is with lesions of the anterior commissure that the final decision will depend on the histologic picture. The highly malignant lesion will certainly not be suitable for operation, but the lesion of low grade malignancy will be operable, and very successfully so, even if it does involve the anterior commissure. One of our tables lists thirty-four cases of lesions of the anterior commissure in which laryngofissure was done by the Chevalier Jackson anterior commissure technic. In this operation introduced by Dr. Chevalier Jackson in 1922, we divide the thyroid cartilage from the outside with a saw instead of clipping through the lesion with shears. Of our series of thirty-four patients, twenty-seven, or 79 per cent, were free from recurrence for three years. The last thing I wish to say with regard to movement of the cord, a point which Dr. Beck mentioned. I have frequently seen irradiation restore the motion of a fixed cord whether the immovability was due to neoplastic infiltration or to inflammation.

Muscle Physiology.—Several observations by Bernard foreshadow the intensive work of the last decade in the field of muscle physiology. He found that blood leaving a contracted muscle is more venous than that leaving the same muscle at rest, i. e., although a living muscle always consumes oxygen, the consumption is increased during activity; and there is an accompanying increase in heat production. But if the motor nerve to the muscle is cut, there is very little oxygen consumed, often less than in a normal inactive muscle. This shows that, while the constant nerve impulses which keep the muscle in a state of semiactivity which we call tonus cause the muscle to assume a certain amount of oxygen, active contraction causes it to consume still more.—Olmsted, J. M. D.: Claude Bernard, Physiologist. New York, Harper & Bros., 1938.

NEW STUDIES IN SURGICAL BACTERIOLOGY AND SURGICAL TECHNIC

WITH SPECIAL REFERENCE TO DISINFECTION OF THE SKIN

PHILIP B. PRICE, M.D.

BALTIMORE

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Surgical technic has from the first suffered the lack of a quantitative test for degree of disinfection of the skin. It has been impossible to determine the precise effect of any hand disinfectant or stronger cutaneous germicide because the size of the bacterial flora could not be measured either before or after employment of the agent. In consequence there is a tremendous literature on the subject but little unanimity of opinion or uniformity in practice.

A NEW QUANTITATIVE METHOD OF STUDYING THE BACTERIA OF THE SKIN

If a large number of basins of sterile water are prepared and the hands and forearms are scrubbed with soap and brush in a standard manner for exactly one minute in each basin, one after another, the washings will be found to contain a slowly diminishing number of bacteria. The cumulative totals plotted against time produce a curve. This is found to be a regular, logarithmic curve that is constant. From a study of this curve the size of the flora of the scrubbed area can be determined accurately.

Furthermore, by employing two such series of basins and plotting the two curves independently, the germicidal effect of any disinfectant used in the interval may be studied quantitatively. This introduces a new scale of germicidal value—the measured action of germicides in reducing the bacterial flora of skin as compared with the effect of scrubbing in a standard manner. The experiment shown in the accompanying table and chart will serve as an example to illustrate more fully application of the test.

The method also permits a much more accurate study of the bacterial flora of the skin than has been possible heretofore, lending itself admirably to investigation of the size and location of the bacterial flora as well as qualitative studies of the flora at various stages of degermation, the mechanism of degermation and reestablishment of the usual flora after degermation.[1]

With generous help from colleagues and various groups of students, I have applied this new quantitative method to a reexamination of certain aspects of surgical bacteriology and surgical technic. The study has extended over a period of several years. Some of our results and conclusions are reported briefly herewith. A full description of the test and a more detailed account of the work on which the following conclusions are based will be published elsewhere.

A SUMMARY OF RESULTS AND CONCLUSIONS

The bacterial flora of normal skin is found to be composed of "transients" and "residents."

Transients may be present in enormous numbers at times, but as a rule relatively few are present on grossly clean hands or on clean protected (unexposed) skin.

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1. The word degermation is used here in preference to disinfection to designate reduction in the total number of bacteria present, irrespective of whether the organisms are or are not pathogenic.

Transients, which are collected from extraneous sources, are comparatively easy to remove or kill. That is not true of the basic flora. Hence tests of germicidal power, which utilize test bacteria placed on skin and exposed subsequently to some disinfectant, may give thoroughly misleading results.

The size of the basic (or resident) flora at any given time is the net result of factors constantly acting, some to increase, others to lessen, the bacterial "population." Increase results largely from multiplication of resident organisms already present. Decrease is brought about by washing, friction of clothing and influences deleterious to bacterial life. Certain transients change their status and become residents, but this process seems to be a slow one and how it takes place is not clear. But the fact that it does happen makes it important to disinfect contaminated hands without undue delay.

The resident flora of my hands and arms, under conditions of ordinary life, tends to strike an equilibrium at about eight million micro-organisms.

This flora is located not in the skin nor to any extent in glands or hair follicles but on the skin.

The basic flora includes some pathogenic germs. If hands are in frequent contact with contaminated objects a dangerously large proportion of the resident bacteria may be pathogens. In such a case it is almost impossible to disinfect the hands. In this hitherto unsuspected manner a person may become a carrier of virulent organisms.

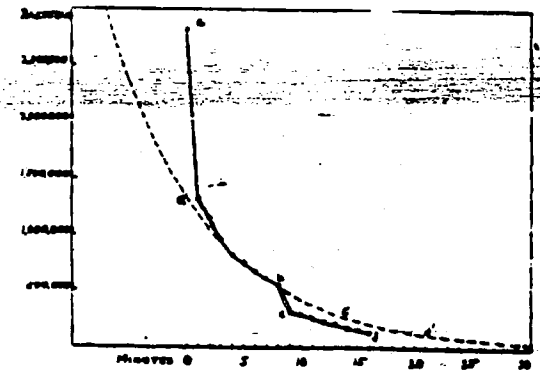
Results of an Experiment Done to Determine the Effect of Washing in 78.5 Per Cent (by Weight) Alcohol on the Bacterial Flora of Hands and Arms

Basin	Scrubbing Time, Minutes	Total Bacterial Count for Basin, Organisms	Cumulative Totals Washed Off, Organisms	Actual Totals or Sum of Flora Left, Organisms
				2,822,570 (a)
1	1	1,497,440	1,272,570	1,324,000
2	1	166,200	774,000	1,150,510
3	1	197,360	608,510	950,000
4	1	122,720	410,510	827,800
5	1	66,800	277,800	761,200
6	1	74,000	211,200	687,200
7	1	74,000	137,200	613,200
8	1	62,200	62,200	538,000 (b)
At this point hands and arms dried in the air and then washed without friction in 78.5 per cent (by weight) alcohol for exactly sixty seconds. Scrubbing resumed immediately. Temperature of alcohol 25 C.				
				318,200 (c)
9	1	30,000	178,200	288,200
10	1	31,200	148,200	258,000
11	1	22,000	116,000	226,800
12	1	28,000	94,000	204,800
13	1	24,000	66,000	181,200
14	1	17,200	41,200	168,200
15	1	23,200	23,200	140,000 (d)

(a) Total number of bacteria on the hands and arms at the beginning of the experiment; (b) after eight minutes of scrubbing; (c) after application of the germicide; (d) after the second period of scrubbing; b and d are arrived at by mathematical projection of the curves produced.

Scrubbing with brush, soap and warm water, as practiced by me, reduces this basic flora at a constant rate. Irrespective of the initial size of the flora, the number of bacteria is reduced by approximately one half with each six minutes of scrubbing. The kind of soap used makes no difference. Variations in the temperature of the water do not influence the rate appreciably. The amount of vigor used in brushing is a very important factor, however. Sterile water has been found to possess no advantage over ordinary tap water in reducing this flora.

Ethyl alcohol has a very narrow range of effective germicidal concentrations. The optimum germicidal strength, both in vitro and on the skin, is 70 per cent by weight (not by volume as ordinarily prepared).[2] At precisely this concentration, alcohol is more effective than any other hand disinfectant now in general use. Each minute spent in this particular solution (at 25 C.) has a degerming effect equivalent to about six and one-half minutes of scrubbing. This effect may be increased considerably by friction, i. e. by rubbing with gauze or a rough wash cloth. Even slight variations from that particular concentration of alcohol results in marked decrease of bactericidal power. Sixty per cent by weight (about 70 per cent by volume) is almost worthless.



Bacteriologic effect of washing the hands and arms in 78.5 per cent (by weight) alcohol for one minute. The broken line *a'd'* represents the rate at which the basic flora of the author's hands and arms always are degermed by scrubbing in a standard manner. The solid line *ab* indicates the effect of scrubbing eight minutes, while *cd* shows the result of scrubbing seven minutes in the second series of basins. The initial deviation, *aa'*, from the broken line is due to contaminating (transient) bacteria improperly placed on the hands at the outset. So, of the initial number of 2,822,000 organisms, only 1,330,000 were "residents." This relatively small flora was due to the hands and arms having been well degermed two days previously and the usual flora not yet having been reestablished. The second deviation, *bc*, was due to use of the alcohol. It is clear that, by scrubbing 4.2 minutes, *bc'*, the same effect (as *bc*) could have been obtained. *cd* is equivalent to *c'd'*. Hence each minute spent in this particular solution of alcohol may be said to be equivalent to 4.2 minutes of scrubbing.

Mercury bichloride solutions do not reduce the flora on the skin appreciably. Paradoxically, a sterile cutaneous surface may be produced. This phenomenon is due, we believe, to the formation of a transparent "film" on the skin under which the bacteria are imprisoned. There conditions are so suitable to life that multiplication takes place, the existing bacterial flora doubling every fifty minutes. The "film" may be broken up, either with an alkaline sulfide or by prolonged friction, whereupon the bacteria are released uninjured.

The same phenomenon is observed when potassium mercuric iodide (biniodide) or Harrington's solution is used. Neither of these is a true germicide when applied to the skin. When tested, these agents should always be followed by an alkaline sulfide.

Kelly's method of hand disinfection (with hot saturated solutions of potassium permanganate and oxalic acid) is very effective. The procedure requires from two and one-half to more than five minutes, depending

2. It is important to differentiate clearly in solutions of alcohol between per cent by volume and per cent by weight. Per cent by weight solutions are exact and invariable. Per cent by volume solutions, as ordinarily prepared, are inexact and undependable owing to three uncontrolled variables—temperature expansion, specific gravity and contraction on solution. Thus 70 per cent alcohol by weight always the equivalent of 76.8 per cent by volume if the latter is prepared at 15 C. but is the equivalent of 80.5 per cent by volume if the volumetric measurements are made at 25 C. Accurate weight-per-cent solutions can be prepared from commercial 95 per cent alcohol with the help of hydrometer, thermometer and a reprint tables. The following formula will give a solution approximately 70 per cent by weight: 95 per cent alcohol at 25 C. 815 cc., plus cold distilled water sufficient to make 1,000 cc.

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he temperature of the solutions. The total degerm- effects are equivalent to between twenty and thirty utes of scrubbing.

he lime and soda method is also very effective. en rubbing with the paste was continued for four utes the reduction of flora was as great as could be mplished by twenty minutes of scrubbing.

aponated solution of cresol, though a relatively ng germicide against test organisms in vitro (a n which we have verified), proved worthless as a nfectant of the skin; 1:400 and 1:200 solutions, l without friction at 25 C., do not reduce the flora reciably. In 1:100 solution, which causes some ning and anesthesia of skin, the flora is reduced ut as rapidly (or as slowly) as scrubbing.

search for a more nearly ideal hand disinfectant resulted in the production of a new germicidal mix- which seems to possess certain advantages over of the agents now in general use. It is powerfully micidal, each minute spent in it (at 25 C., without tion) being equivalent to more than eleven minutes scrubbing. It is simple and pleasant to use. It does irritate or injure the skin. It is more stable than ple ethyl alcohol solutions. This germicide consists ethyl alcohol 50 parts by weight, normal propyl hol 20 parts by weight and water 30 parts by weight. nay be prepared as follows: ethyl alcohol (95 per t) 675 cc., pure n-propyl alcohol 250 cc., distilled er 250 cc., all measurements being made at 25 C. y rational and conscientious use of the most effec- means, the bacterial flora of the hands can be uced to a relatively low figure but practically never zero. The remaining organisms may, and on sur- ns' hands often do, include pathogens. Hence the essity for wearing gloves when operating.

eneath rubber gloves, however, the bacteria remain- on the skin multiply rapidly, their number doubling ry forty minutes if the hands are dry or every fifty utes if the gloves have been put on wet. If gloves worn long enough, the cutaneous flora may increase il it exceeds by far the ordinary flora. I found that one occasion the bacterial count of my hands and is had increased to more than 31,000,000. If patho- s are present they participate proportionately in this ltiplication. No means has yet been found to prevent delay this increase. These extraordinary bacterial pulations," we find, cannot be reduced any more idly than the ordinary. This phenomenon of rapid ease of cutaneous flora does not occur under cotton ves or ordinary clothing.

he basic flora of protected skin per unit area is no ller than that of the hands.

ther, a representative of a group of the stronger micides thought to disinfect skin chiefly by means letergent properties, when subjected to our quanti- ve test proved utterly powerless to reduce the basic a of clean skin.

reshly prepared U. S. P. tincture of iodine (7 per t), applied to grease-free skin and followed by an idote, came nearer to full sterilization of the epider- than any other germicide tested. U. S. P. "half- ngth" tincture of iodine (3.5 per cent) proved far effective, reducing the flora irregularly, but on the rage by less than half. Two per cent iodine in 70 cent (by weight) alcohol was much more effective, ering the bacterial count to nearly 18 per cent of its ginal size. On drying, the germicidal action of iodine practically ceases. The 2 per cent iodine in 70 per cent alcohol solution has certain important advantages: even spread, slow evaporation, nonirritation, and contributory bactericidal effect of the 70 per cent alcohol.

Mercurochrome, a prominent representative of a large group of the newer germicides, the organic compounds of mercury or silver, was tested. From bacteriostatic and bacteriocidal standpoints, mercurochrome was found to be similar in many respects to the inorganic salts of mercury. If no antidote is used, an exaggerated idea of its germicidal power is obtained. Aqueous solutions of mercurochrome on the skin may produce a nearly germ-free surface, but subsequent use of 10 per cent ammonium sulfide will show that the bacterial flora has been reduced little if any. Scott's alcohol-acetone-2 per cent solution of mercurochrome, applied liberally and allowed to dry slowly, reduced the bacteria of the skin to about one-third their original number. This whole group of germicides, the organic compounds of heavy metals, needs to be reinvestigated, appropriate antidotes being used.

After the skin has been degermed, reestablishment of the usual flora proceeds slowly (except under rubber gloves), apparently at a rate represented by a sigmoid curve. When the hands and arms have been thoroughly "disinfected," the number of bacteria being reduced, say, to 200,000, full reestablishment of the ordinary flora may be expected in about a week.

Parallel studies of our quantitative test on the one hand and the commonly used scraping and rubbing tests for cutaneous contamination on the other show that both of those time-honored methods become increasingly untrustworthy as the flora of the skin diminishes.

PRACTICAL RECOMMENDATIONS

The following principles and procedures are based on our experimental studies. Before recommending them, however, we subjected them critically to a prolonged test of clinical application, with highly satisfactory results.

Preoperative Preparation of Hands.—1. The hands are scrubbed with soap, a good brush and warm water for at least seven minutes. This will usually suffice to remove gross dirt, transient bacteria and fats, and incidentally about half the basic flora. The nails should be short and clean at the start.

2. The resident flora is much more effectively attacked by germicides than by scrubbing. We recommend ethyl alcohol, 70 per cent by weight, or the mixture of alcohols described. These solutions should be freshly and accurately prepared. Before entering the alcohol basin, the hands and arms should be dried thoroughly with a sterile towel, for to carry water into the alcohol will weaken the solution and markedly lessen its germicidal power. In the alcohol basin the skin should be rubbed firmly with sterile gauze or a washcloth.

The time spent in these solutions is of the utmost importance. We suggest that ethyl alcohol be used with friction for three minutes by the clock or the mixture of alcohols for two. This may be expected to reduce the original flora from 50 per cent (result of scrubbing) to something less than 2 per cent.

The occasional operator will increase the margin of safety by washing also in a 1:1,000 bichloride or 1:500 biniodide solution for one minute. The mercuric salt should follow, never precede, the alcohol, for the film formed by the mercurial is impervious to alcohol as well

as to bacteria. Bichloride, biniodide and Harrington's solutions are not recommended, however, for hands that must be disinfected frequently.

3. Gloves and gown are put on. An ungloved hand inevitably increases the risk of wound infection. In operating rooms where expense is not a major consideration, where only perfect (unpatched) gloves are used and where preparation of gloves are in highly competent hands, we have shown dry gloves to be preferable, and even safer. Elsewhere wet gloves will probably have a larger margin of safety. In lieu of gloves, the operator should wash his hands in bichloride or biniodide of mercury.

4. Between operations the hands should be washed in a germicidal solution in order to counteract the increase of cutaneous bacteria which has taken place beneath the gloves. A useful rule is one minute in alcohol for every hour that the gloves have been worn.

Preparation of the Field of Operation.—Preparation of the patient's skin the day before operation by wrapping the part in sterile towels is psychologically harmful and bacteriologically useless. Before coming to the operating room the patient should receive a bath, however, the site of operation being especially well washed with soap and water to remove dirt, most of the fats and any transient bacteria. If, as in the presence of a wound, this is not possible, a chemical detergent should be used.

Immediately before operation the site of incision should be washed with gauze and 70 per cent (by weight) alcohol or the suggested mixture of alcohols. It should be allowed to dry slowly, for in disinfection time is a factor that cannot be ignored.

This is to be followed by one of the stronger germicides. U. S. P. tincture of iodine (7 per cent) is extremely effective but in many cases too strong a solution to be tolerated or used safely. We recommend weaker solutions of iodine dissolved in 70 per cent (by weight) alcohol. After application, iodine solutions should be permitted to dry slowly. Washing a dried coat of iodine off the skin with alcohol increases rather than diminishes the total germicidal effect.

As an alternative to the iodine technic, the field of operation may be painted with Scott's alcohol-acetone-2 per cent solution of mercurochrome, or an irregular area such as that of the hand or foot may be soaked for a minute in 1:500 biniodide solution. In either case an aseptic surface will be produced. But the line of incision must first be specially prepared (disinfected), else the knife will necessarily pass through germ-laden skin beneath the "film." One way to do this is to rub the site of incision for two or three minutes firmly with gauze and 70 per cent (by weight) alcohol or our mixture of alcohols.

Disinfection of Contaminated Hands.—Ordinary hands, contaminated by contact with infectious patients or materials, are not difficult to disinfect. Saponated solution of cresol, however, is almost useless for the purpose, and solutions of bichloride or biniodide of mercury may be actually dangerous. We recommend the following as a simple dependable method for routine use:

1. The hands should be washed as soon as possible with soap and running water for at least thirty seconds. This may be expected to remove about nine tenths of the contaminating organisms. If there is pus, blood, secretion from the wound, saliva, mucus or other infectious material on the hands, washing should be continued for a minute or more, perhaps with the use of a brush.

2. The hands should be well dried on an individual towel.

3. Every part of the hands should be wet with 70 per cent (by weight) alcohol. A few cubic centimeters dripped on the hands will suffice. The alcohol should not be wiped or shaken off but the skin should be allowed to dry by evaporation. It is the germicidal action that is required, and that takes time.

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PHOTOGRAPHY OF THE UTERINE CERVIX

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The present knowledge of medicine must be handed on to students, and teaching can be accomplished more advantageously with a combination of auditory and visual methods than by either method alone. Photographs are also of value to the practitioner as office records. The physician may wish to know what the exact appearance of the cervix, or any other lesion, was six months previously.

The patient also appreciates knowing why the cervix or other inaccessible part of the body needs repair or other treatment. She has no idea of the appearance of the cervix because she has never seen it and has never been aware of any sensation from it, since there are no sensory nerve endings in it. No other part of the body undergoes as much injury or is so neglected after being injured. The cervix is also the sharp junction of two distinctly different types of epithelium, which means that it is constantly subject to change due to the erosion and healing processes going on in such an area. Other parts of the body may lend themselves just as readily as the cervix to photographic records. Portrayal of operative procedures and photographs of pathologic tissue in situ are both valuable and should be preserved not only for records but for use in teaching.

In the field of gynecology and obstetrics, teaching material is somewhat more difficult to find than in other branches of medicine; therefore, every effort possible must be made to record by accurate drawings and photographs examples of pathologic lesions and normal tissue.

Not only must this material be recorded, but it must be done in such a way that it is easily available for teaching purposes. The records must be where they can be readily shown and they must be shown repeatedly.

Students spend long hours in clinics attempting to see examples of lesions about which they have read, but most find by the time their course is over that there are many important lesions which they have not had an opportunity to see. By making accurate colored photographs over a period of years of the many differ-

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294

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REVIEW ARTICLE

Handwashing and hand disinfection

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Introduction

One of the most efficient methods of restricting the transfer of infectious material from one patient to another is by the application of a handwashing and hand disinfection policy (Reybrouck, 1983). Since members of hospital staff are often confused about the object of different forms of cleansing and disinfection, it seems appropriate to define these general aims and terms before discussing systematically the individual methods and their efficacy.

Definitions and objectives

Disinfection of hands

The term hand-disinfection implies the application of a chemical substance possessing antimicrobial activity. In the USA (Simmons, Hooton & Mallison, 1981; Block, 1983), France (Association Française de Normalisation, 1981) and the UK (Lowbury, 1982) the terms 'antisepsis' and 'antiseptic' are used when referring to antimicrobial agents applied to living tissues. In this review I have followed the convention more prevalent in Europe and used the terms 'disinfection' and 'disinfectant' in this context (Schweizerische Mikrobiologische Gesellschaft, 1972; Deutsche Gesellschaft für Hygiene und Mikrobiologie, 1981; Österreichische Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin, 1981*a*, 1981*b*; Lowbury, 1982).

The forms of hand disinfection

Four distinct types of hand disinfection are recognized in Europe. The aim of the first of these, ***hygienic hand disinfection***, is to kill pathogenic organisms that have been deposited on the hands and are part of the transient flora. The agents used for this purpose are likely to have a secondary effect on the resident flora, but that is not the primary aim. Since the nurse on duty

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usually has little time for handwashing, preparations used for hygienic hand disinfection should act quickly, within a short period (i.e., 15–30 s).

A second type of hand treatment which is sometimes used in hospital wards is *hygienic hand disinfection with residual action*. The purpose of this form is to kill not only the pathogenic bacteria present in the transient flora at the time of washing, but also those which might contaminate the hands thereafter; the aim is to obtain a continuing residual antibacterial effect on the transient flora, and particularly on *Staphylococcus aureus*.

The third form of hand disinfection is *surgical hand disinfection*, i.e., the pre-operative disinfection of the surgeons' hands. This is effective against the resident as well as the transient flora. Since most surgical operations may be of some duration and since a large percentage of gloves appear to be perforated at the end of the operation (Devenish & Miles, 1939; Church & Sanderson, 1980), it is important that the surgeon's hands remain as germ-free as possible for some hours. For this purpose, not only an immediate antibacterial effect but also an activity lasting at least 2–6 h is considered desirable (Schweizerische Mikrobiologische Gesellschaft, 1972). As the surgeon is usually less pressed for time before he starts to operate than is the nurse on the ward, it is less important for his skin disinfection to reach its full effect immediately; indeed perforation of a glove will usually not occur immediately at the beginning of the operation.

There is a fourth kind of hand disinfection, referred to here as *basic disinfection*, the purpose of which is to cause a continuing reduction in the density of the resident flora, without necessarily affecting the transient flora (Schweizerische Mikrobiologische Gesellschaft, 1972), e.g., persons working in food manufacturing or pharmaceutical industry, or nurses in a protective isolation unit for immunocompromized patients, should have as low a resident flora as possible. But the form of skin disinfection used to achieve this does not have to destroy transient bacteria quickly, as when a nurse disinfects her hands before ward procedures or a surgeon before he operates. The method differs from surgical disinfection in that it depends upon the cumulative or progressive reduction in density of skin bacteria on repeated application of a disinfectant, although the surgeon who disinfects his hands repeatedly in each operating session does, in fact, achieve the same objective of basic disinfection. Basic disinfection can, therefore, be defined as the effects of repeated applications of a disinfectant, which cause a greater reduction in the hand flora than can be obtained from a single treatment with a surgical disinfectant. A summary of these requirements for the different forms of hand hygiene is given in Table I.

The laboratory evaluation

Different laboratory evaluation techniques are used for the different forms of disinfection. In the case of surgical and basic disinfection, the influence on the total (resident) hand flora is assessed, whereas with hygienic hand disinfection, the hands are artificially contaminated, and the effect of the

Table I. *The different forms of hand disinfection and handwashing*

Forms of treatment	Main clinical purpose	Influence on bacterial flora	Application time	Action period	Principle of laboratory evaluation
Handwashing	Cleansing after patient contact	Removes and reduces density of transient flora	A few seconds	Short	Influence on the transient flora represented by artificial contamination
Hygienic hand disinfection	Disinfection after contamination	Kills and eliminates most transient flora	<2 min (usually 15–30 s)	Short	Influence of the application of the disinfectant after artificial contamination
Surgical hand disinfection	Pre-operative disinfection	Kills and reduces resident and transient flora	<10 min (usually 2–5 min)	Short (with sustained effect)	Influence on total hand flora, immediately and after 1 or more hours
Hand disinfection with residual action	Prevention of contamination	Kills contaminating organisms on deposition		Long	Influence on artificial contamination 1 h after the application of the disinfectant
Basic hand disinfection	Routine disinfection in protected areas	Causes low level of resident flora	Repeated	Very long (cumulative and persistent effect)	Influence on total hand flora after repeated treatment for several days

disinfectant on these organisms is assessed. Tests for residual action are designed to show how many of the bacteria applied to the hands after disinfection survive or die. Many hand disinfection procedures aim to achieve more than one type of disinfection and many test techniques are not focused on the evaluation of a single type of disinfection. The scheme of testing is, therefore, not always as simple as that presented in Table I.

The most extended batteries of tests were those developed by Lowbury and collaborators in Birmingham (Lowbury, Lilly & Bull, 1963, 1964*a*, 1964*b*) and by the Austrian and German societies for microbiology and hygiene (Deutsche Gesellschaft für Hygiene und Mikrobiologie, 1981; Österreichische Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin, 1981*a*, 1981*b*). In evaluating a skin disinfectant two main difficulties are encountered. The first of these is common to all tests of antimicrobial activity of disinfectants, namely the possible carryover of inhibitory concentrations of disinfectant from the site of disinfection to the culture medium used for assessing the results of disinfection; because of this possibility it is necessary to inactivate unwanted residues with neutralizers (Reybrouck, 1979; Reverdy, Martra & Fleurette, 1984*a*). The other difficulty is that the different quantitative methods used to assess the number of bacteria surviving on the skin after disinfection do not necessarily give the same results (Reybrouck, 1978; Desvignes *et al.*, 1979). These difficulties explain to some extent the differences reported in the literature.

In the evaluation of the same formulations by different authors, not only may different experimental designs be used, but the method of disinfection procedure may also be different, e.g., different use-dilutions, duration of treatment, and quantity of disinfectant may be prescribed by different workers. All these tests are performed in the laboratory but, ideally, the true value of a disinfectant should be assessed by the effect on the infection rate. Today it is impossible to prove the effectiveness of skin disinfection as Semmelweis did in the last century, when a reduction in puerperal sepsis transmitted by hand provided the criterion of success.

Handwashing

Though handwashing has been described as the most important procedure in preventing nosocomial infections (Steere & Mallison, 1975), there has been a general lack of attention among hospital personnel to the ways in which the hands should be cleansed (Fox, Langner & Wells, 1974; Albert & Condie, 1981), and the quality of the handwashing is often inadequate; the procedure is usually unstandardized, and some parts of the hand (especially the thumb) (Taylor, 1978) often remain unwashed during routine handwashing. In practice, the duration of the handwash by health care personnel is below the standards recommended (Quoraishi, McGuckin & Blais, 1984), and the measured frequency is far less than the nurses' claimed

frequency (Broughall *et al.*, 1984). The objective is to remove superficial desquamated skin scales, sweat and sebaceous secretions by the action of a detergent (e.g., soap) and water, and with them a variable proportion of the transient bacteria are also removed.

Laboratory tests prove the antibacterial activity of the simple handwash, when the hands are inoculated by contaminants. Lowbury *et al.*, (1964*a*) found a log reduction factor of 2·54 in the case of *Staph. aureus* and 2·80 in the case of *Pseudomonas aeruginosa*, after a wash of only 30 s. Similar results were obtained by Ayliffe, Babb & Quoraishi (1978), namely log reduction factors between 2·2 and 2·5 for *Ps. aeruginosa, Staph. aureus, Staph. saprophyticus* and *Escherichia coli*. Mittermayer & Rotter (1975) found even more than 3 log reduction for *E. coli*. By a contact transfer model, Mackintosh & Hoffman (1984) demonstrated that the log reductions in transfer of bacteria from donor to recipient fabrics by handwashing were 3·0 for *Staph. saprophyticus* and more than 4 in the case of *Ps. aeruginosa*, *Klebsiella aerogenes* and *Serratia marcescens*. In general, less than 1% of the contaminating microbes persist after cleansing with soap and water, but the proportion varies with the mode of deposition (Lilly & Lowbury, 1978). A thorough wash with soap is nearly as effective as many aqueous disinfectants, but the application of ethyl alcohol is far more effective.

The micrococcacae of the resident flora, in contrast with transients, are not readily removed by washing with soap and water. *Staphylococcus aureus* is commonly a transient but may sometimes colonize the skin and is then more difficult to remove (Ayliffe *et al.*, 1975; Ojajärvi, 1980). In fact, when after a simple wash, counts of the total hand flora are made, the reduction in the number of bacteria is far less than in tests with artificial contamination; it does not reach 90%, i.e., 1 log reduction (Dewar & Gravens, 1973; Lowbury & Lilly, 1973; Lilly & Lowbury, 1974; Lowbury, Lilly & Ayliffe, 1974). In contrast, there is sometimes an apparent increase (Ojajärvi, 1976; Lilly, Lowbury & Wilkins, 1979), which is possibly due to the dispersion of micro-colonies. It must be remembered that the objective of social washing is not to remove the resident flora and that tests using the resident flora, as for the evaluation of pre-operative disinfectants, are not suitable for assessing the efficacy of handwashing.

The routine quick handwash, as usually employed by busy nurses, seems to be effective in removing many of the transient bacteria acquired from patients and the choice of the detergent is of no real significance (Sprunt, Redman & Leidy, 1973). The few salmonellae left on the fingers of symptomless excreters after defaecation are also transients and can be readily removed by washing (Pether & Scott, 1982). Washing with soap and water is also an effective method of removing viruses (Hendley, 1973). Ojajärvi (1980), however, found that after dry or moist contamination of the hands by handling an infected patient, colonies of *Staph. aureus* were often detected in fingerprint samples of hands washed, after such contamination, with water and soap only; Gram-negative bacilli were more easily removed

than staphylococci. On the other hand, pathogenic bacteria are rarely isolated from the hands after washing with soap and water, and this would suggest that there is no need for routine use of hand disinfection by staff in normal hospital wards (Ojajärvi, 1981).

The evidence that handwashing influences the infection rate is more difficult to produce. Most studies are confined to special wards, such as nurseries, and in many medicated soap containing hexachlorophane is used. Frappier-Davignon, Frappier & St-Pierre (1959) found that the staphylococcal infection rate in isolated infants was the same in hospitals where unmedicated soaps were used and those which used hexachlorophane preparations. Where the babies were not strictly isolated in the nursery from birth until discharge, the infection rate was 1·24% when the hands were washed and 2·65% when nurses did not wash their hands before handling the babies. Provided that all infected patients were strictly isolated, a simple handwashing appeared to be adequate. Isolation and gloving procedures are more effective in reducing the prevalence of pathogenic organisms on hands than washing and disinfecting. Black *et al.* (1981) evaluated the effect of a programme of frequent handwashing on the incidence of diarrhoea in two day-care centres; they found that the incidence of diarrhoea in these centres was about half that of the control centres where no special programme had been introduced.

It is to be concluded that by handwashing the greater part of the contaminants is removed, but that the resident flora is hardly influenced. The reduction required to reduce cross-infection is unknown. In practice, washing with soap is usually thought to be adequate in the UK, Belgium, the Netherlands, but in some other European countries, such as Austria and Germany, disinfection with an alcoholic solution is considered necessary for routine patient care (Rotter & Reybrouck, 1983).

Liquid soap is often preferred in hospital as it is more economical and consumption can be more precisely estimated. Bar soap lying in a pool of water is apt to become contaminated with skin bacteria and Gram-negative bacilli (Jarvis *et al.*, 1979; McBride, 1984). The same risk occurs with liquid soap if the dispenser is replenished without emptying and cleaning. If a non-irritant soap (liquid or bar) is not used, skin damage, dermatitis or allergy may occur after frequent applications, which may be as many as 50 a day. Personnel with dermatitis will tend to avoid handwashing and have higher bacterial counts on damaged skin; they are, therefore, a greater potential source of infection (Steere & Mallison, 1975). The same hazard was encountered during a prolonged field trial, in which an antimicrobial detergent proved to be highly irritant, especially in a neonatal unit where the nurses washed their hands very frequently (Ojajärvi, Mäkelä & Rantasalo, 1977). More emphasis should therefore be placed on the use of disposable gloves, the acceptability of the soap preparations, and on techniques which avoid the use of washing, e.g., alcohol with an emollient.

Agents for hand disinfection

The choice of disinfectants intended for hand disinfection of hospital personnel is rather limited. One of the most important requirements is the absence of toxicity, even after daily use for many years. Agents selected for skin disinfection (Steere & Mallison, 1975; Ayliffe, 1980; Kaul & Jewett, 1981) are those which have good bactericidal activity usually against a wide range of micro-organisms, and minimal toxicity to the skin and tissues. In this survey the disadvantages and side-effects will be mainly considered.

Hexachlorophane is a bis-phenol characterized by a narrow antimicrobial spectrum: it is principally active against Gram-positive cocci, and has little or no activity against Gram-negative bacilli. Hexachlorophane is rather slow in action, but residual activity is obtained when a liquid preparation (e.g., 'pHisoHex') is used repeatedly and exclusively. The continual use of hexachlorophane preparations changes the normal ecosystem of the skin by a selective reduction in carriage of staphylococci which allows an increased carriage of Gram-negative organisms, such as *Ps. aeruginosa* and coliform bacilli (Forfar, Gould & Maccabe, 1968; Evans *et al.*, 1973). Hexachlorophane may be absorbed by the skin (Butcher *et al.*, 1973). Toxic effects, however, are due to the use of too high concentrations or to application on burnt and non-intact skin surfaces. One report (Halling, 1979) refers to a suspected link between handwashing with hexachlorophane soap and congenital malformations in the offspring.

Chlorhexidine is a bisbiguanide effective against Gram-positive cocci and to a lesser extent Gram-negative bacteria. Its general and local toxicity is very low. Accidental contamination of aqueous solutions of low concentrations of chlorhexidine has been reported on several occasions (Marrie & Costerton, 1981; Rutala & Cole, 1984). In hand disinfection, chlorhexidine is mainly used as a 4% detergent solution ('Hibiscrub', 'Hibiclens') or a 0·5% alcoholic solution ('Hibisol').

Quaternary-ammonium compounds have a good bactericidal effect on Gram-positive organisms and they are well tolerated by skin, wounds and mucous membranes, but their role in hospital is restricted to that of a cleansing agent, since Gram-negative bacteria can survive and multiply in aqueous solutions of these compounds (Rutala & Cole, 1984).

Iodophors (Görtz & Häring, 1981) are complexes of iodine and a stabilizer, e.g., polyvinylpyrrolidone (PVP-I, povidone-iodine or 'Betadine'). They have the same broad spectrum of antimicrobial activity as iodine. Accidental contamination of an iodophor has been described (Berkelman *et al.*, 1981; Craven *et al.*, 1981). Allergy and local irritation may occur, but is less than with aqueous or alcoholic iodine solutions (Kunze, Kaiser & Petres, 1983). Percutaneous absorption occurs mainly in neonates and when applied to mucous membranes, leading to an increase in the serum iodine level and urinary output of iodine (Connolly & Shepherd, 1972) and possible induction of hypothyroidism in neonates.

The alcohols are very active and at the same time harmless formulations; 70% ethanol or 60% isopropanol, and to a lesser extent 50% n-propanol are mainly used. Washing the hands for 1 min with a quantity of alcohol that wets the hands completely and rubbing vigorously until the skin is dry will disinfect them more effectively than other methods of disinfection. Application of a smaller amount for a lesser time, e.g., 20–30 s, will usually be sufficient for routine hygienic hand disinfection provided all surfaces of the hands are covered. Although alcohols show no residual activity, a delayed antimicrobial effect was found when rubber gloves were worn for some hours after disinfection, a phenomenon explained by sublethal damage to some of the skin bacteria (Lilly *et al.*, 1979). A potential disadvantage of alcohol is that frequent use dries the skin. The addition of 1% glycerol will usually prevent this (Lowbury *et al.*, 1974). Another potential, though probably negligible, disadvantage of alcohol is that it removes the unsaturated fatty acids of the skin secretions which have antimicrobial action, so that the self-disinfecting activity of the skin may be impaired for several hours. Commercial alcoholic preparations usually contain skin-protecting agents and other germicidal substances. The antimicrobial activity of many of these preparations is for the greater part due to the alcohol, but some of these solutions are more active after a short application than alcohol alone.

Hygienic hand disinfection

The purpose of hygienic hand disinfection is to eliminate, as quickly as possible, bacteria that survive on the hands after contact with a contaminated object or infected patient, to ensure that the persons concerned do not develop an infection and that contamination will not be transmitted on their hands to other patients. Although the aim is merely to destroy the transient flora, many of the resident flora are also likely to be killed by the methods which are used. The main requirements of a disinfectant in hygienic hand disinfection are to be active within a short time and to reduce the numbers of contaminating organisms to a safe level. For the former, it is proposed that the application time should not be longer than 1 min and preferably as short as 15 or 30 s (Schweizerische Mikrobiologische Gesellschaft, 1972). To be acceptable, a preparation must give a similar or greater log reduction than that obtained by the action of a standard preparation, i.e., 60% isopropanol (Deutsche Gesellschaft für Hygiene und Mikrobiologie, 1981; Österreichische Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin, 1981*a*). Further, it should not irritate the skin if frequently applied. To test disinfectants proposed for hygienic hand disinfection, the hands are inoculated with marker organisms, preferably potential pathogens such as *Staph. aureus*, enterobacteriaceae or pseudomonas, and the activity of the preparation measured by a quantitative culture method. Although disinfection of the

skin site before puncture, injection, operation, etc., must fulfil the same conditions of rapid and effective action, we will not consider this form of skin disinfection here, as the tested skin area, usually the forearm, does not correspond to or have the same structure as the hands.

The preparations selected for hygienic hand disinfection can be classified into medicated soaps, germicidal creams and foams, aqueous disinfectants and tinctures. The so-called germicidal soaps containing chlorocresol, triclosan, trichlorocarbanilide or hexachlorophane, are usually insufficiently active for hygienic disinfection and too slow to kill pathogenic organisms within 2 min. This holds for antiseptic creams, lotions, gels or foams, which also appear to be ineffective within the short action time required. In fact, they are no more, or only a little more, active than an unmedicated soap (Lowbury *et al.*, 1964*a*; Mittermayer & Rotter, 1975; Sheena & Stiles, 1982).

In general, chlorhexidine-detergent or iodophor surgical scrubs are better than the above-mentioned preparations (Lowbury *et al.*, 1964*a*; Ayliffe *et al.*, 1978; Ojajärvi, 1980; Rotter, Koller & Wewalka, 1980; Sheena & Stiles, 1982). Of the aqueous solutions, some iodophors and 0·5% chlorhexidine seem to be more, and other iodophors, quaternary ammonium compounds and hypochlorite solutions less active as skin disinfectants (Lowbury *et al.*, 1964*a*; Rotter, Koller & Wewalka, 1981; Sheena & Stiles, 1982). Only the alcohols and tinctures, i.e., alcoholic solutions, which are rapid in action, satisfy the requirements of hygienic disinfection (Mittermayer & Rotter, 1975; Grün & Heyn, 1976; Rotter, Koller & Kundi, 1977; Wewalka *et al.*, 1977; Ayliffe *et al.*, 1978; Ojajärvi, 1980; Ulrich, 1982; Bartzokas *et al.*, 1983; Rotter, 1984), but the aqueous preparations may still be preferred in some circumstances.

If hands are washed with a disinfectant only in a quick and superficial way, the reduction in transient flora will not be sufficient to cause an effective reduction in the hazard and the hands may remain contagious (Sprunt *et al.*, 1973), although the clinical evidence for this is uncertain. The main hazards in the use of these disinfectants when frequently applied are skin dryness, irritation, cracking, dermatitis or allergic reactions. It is preferable to prevent the hands from becoming contaminated, e.g., by use of gloves, than to try and eliminate contaminants.

Surgical hand disinfection

The purpose of disinfection of the surgeons' hands is to prevent the transfer of bacteria from them to the operation site, and to ensure that even if the surgical gloves become perforated during the operation, few bacteria from the hands will be implanted in the operation wound. This means that the transient flora as well as the resident flora should be reduced, and that this reduction should last for several hours. Both the immediate (or rapid) and the sustained effect of disinfection on the total (resident and transient) hand

flora are important and must be considered when evaluating the effectiveness of products intended for disinfection of the surgeons' hands.

In the past it was usual to scrub the hands with soap and a brush for at least 5 min, followed by a rinse with alcohol; prolonged scrubbing, however, can cause excoriations of the skin, which become infected. This probably explains why more bacteria were found on the hands after wearing gloves for 1 h when they were scrubbed for 10 min rather than 5 (Dineen, 1969). Today disinfection rather than physical cleaning is the method recommended for reducing the hazard of contamination from the surgeon's hands; disinfection is quicker as well as being more effective. The brush is only helpful in removing dirt, especially under the nails, and should be used for the first operation of a list only, not for a prolonged scrub. The first disinfectant used was hexachlorophane, but preparations containing iodophors or chlorhexidine, which were introduced later, were more rapid in action and more successful.

It is very difficult to determine which preparations are the best for pre-operative hand disinfection. There is no general agreement on the testing technique to be followed; the results obtained are expressed in different ways and the base of comparison is not always the same. Table II summarizes these and other details of tests of surgical hand disinfection in papers published in the past 10 years. When so many different methods of testing have been used, the results are not comparable. Most of the publications examine only two or three products, and it is not certain that products with the same brand name have exactly the same composition in different countries. The quantity of product applied and the medication time can differ. Nevertheless, in spite of these problems the overall conclusions on the relative effectiveness of products are often similar (Ayliffe *et al.*, 1975).

With reference to the immediate activity of disinfectants, povidone-iodine (PVP-I) surgical scrub is more active than 'pHisoHex' (Crowder *et al.*, 1967; Gross *et al.*, 1973; Kundsin & Walter, 1973; Michaud, McGrath & Goss, 1976; Tucci *et al.*, 1977; Peterson, Rosenberg & Alatary, 1978); 4% chlorhexidine scrub is sometimes found to be marginally more effective than PVP-I detergent solution (Lowbury & Lilly, 1973; Peterson *et al.*, 1978; Aly & Maibach, 1983), and sometimes less effective (Rotter, 1981; Rotter *et al.*, 1981). The mean reduction in viable counts of skin bacteria is greater when the hands are disinfected with 0·5% chlorhexidine in alcohol applied with friction and rubbed to dryness than with 4% chlorhexidine detergent preparation (Rosenberg, Alatary & Peterson, 1976; Lilly *et al.*, 1979; Rotter, 1981). Results regarding the sustained action after 1 h are even less conclusive: e.g. 'pHisoHex' appears to be better than 'Betadine' according to some authors (Kundsin & Walter, 1973; Peterson *et al.*, 1978) but not others (Michaud *et al.*, 1976). Kundsin & Walter (1973) contend that the sustained effect should be given greater attention than the rapid action. However, most operations are not sufficiently long to allow much growth of bacteria from the low levels

reached after rapid and effective hand disinfection, so there is some doubt for the need to test sustained reduction in resident flora after a single treatment. Although laboratory tests show a difference in antimicrobial activity among the different preparations, clinical trials show no significant difference between different preparations (White & Duncan, 1972; Murie & Macpherson, 1980); this is explained by the small number of infections observed and also by the importance of other sources of infection, including air, and the patients internal flora.

Hand disinfection with residual action

When it is considered important to prevent the survival on skin of bacteria acquired after hand disinfection, in isolation wards or in intensive care units, where ungloved hands are likely to become contaminated with bacteria from infected patients, disinfectants that have residual activity and also rapid activity against transient flora are desirable (Lowbury *et al.*, 1964*a*). In laboratory experiments the residual activity of some disinfectants has been demonstrated. On skin previously treated with hexaclorophane preparations, iodophors, an alcoholic chlorhexidine solution or a chlorhexidine detergent preparation, *Staph. aureus* (Lowbury *et al.*, 1964*a*; Bruun, Bøe & Solberg, 1968), and in some cases also *E. coli*, did not survive as easily as on the untreated skin (Lowbury & Lilly, 1973; Lilly & Lowbury, 1974; Lowbury *et al.*, 1974). Although rational, the usefulness of residual activity in the hospital has not been demonstrated. The use of agents with a residual activity might cause a false sense of security. The killing of transients may be too slow to be of clinical value.

Basic hand disinfection

The cumulative effect of hexachlorophane has been known for a long time (Traub, Newhall & Fuller, 1944). Even recent articles, in which more modern testing techniques were used, mention the persistent and cumulative action of the basic hand disinfection properties of hexachlorophane (Gravens *et al.*, 1973; Lowbury & Lilly, 1973; Michaud *et al.*, 1976; Peterson *et al.*, 1978; Jarvis *et al.*, 1979). There are more active germicidal substances which also show a cumulative action. As in the case of hexachlorophane, the main requirement for cumulative action is frequent use of the antiseptic. A broader antimicrobial spectrum than that of hexachlorophane is desirable including activity against Gram-negative bacilli. Disinfectants with residual action are also used for basic disinfection, e.g., liquid hexachlorophane soaps, iodophors, a chlorhexidine detergent preparation, an alcoholic chlorhexidine solution, trichlorocarbanilide (Lowbury & Lilly, 1973; Lilly & Lowbury, 1974; Lowbury *et al.*, 1974; Rosenberg *et al.*, 1976; Peterson *et al.*, 1978; Aly & Maibach, 1979; Jarvis *et al.*, 1979; Sprössig & Sauerbrei, 1982). Some authors, however, regard tests for persistent and cumulative effect of

Table 11. *Different elements of the testing technique in the assessment of disinfectants for pre-operative disinfection of surgeons' hands*

Reference	Determination of the rapid (immediate) activity	Determination of the sustained activity	Technique for the determination of the no. of survivors	Basis of comparison of the products tested	Expression of results
Dewar & Gravens, 1973	+	1 h	Fingerprint impression	Same panel; pre-treatment values	Per cent reduction
Gravens *et al.*, 1973	+	Field test	Fingerprint impression	Same panel; pre-treatment values	Count distribution
Gross *et al.*, 1973	+	2 h	Fingertip impression; impression of the hand dorsum	Same subject; pre-treatment values	Per cent reduction
Kundsin & Walter 1973	+	1 h	Gloved-hand sampling	Same panel; pre-treatment values	Per cent reduction
Lowbury & Lilly, 1973	+	–	Counts from handwashings (bowl)	Same subject; pre-treatment values	Per cent reduction
Smylie, Logie & Smith, 1973	–	Field test	Finger-streak	Mutual comparison	Count distribution
Lowbury *et al.*, 1974	+	3 h	Counts from handwashings (bowl)	Same subject; pre-treatment values	Per cent reduction
Lowbury & Lilly, 1975	–	Field test	Glove washing	Mutual comparison	Counts; count distribution
Reber *et al.*, 1975	+	2, 4, 6 h	Counts from handwashings (plastic bag)	Same panel; pre-treatment values	Log reduction factor
Reybrouck & Van de Voorde, 1975	–	1 h	Fingertip impression; glove washing	Mutual comparison	Per cent reduction
Michaud *et al.*, 1976	+	1, 3 h	Gloved-hand sampling	Same panel; pre-treatment values	Log reduction factor

Rosenberg *et al.*, 1976; Peterson *et al.*, 1978	+	1, 2, 3, 4, 5, 6 h	Glove fluid	Same panel; base line counts	Log reduction factor; per cent reduction
Charrel *et al.*, 1977	+	1 h	Counts from handwashings	Same panel or subject?; pre-treatment values	Per cent reduction
Tucci *et al.*, 1977	+	–	Swab	Mutual comparison	Count distribution
Galle, Homesley & Rhyne, 1978	+	1 h	Fingertip impression	Same panel; pre-treatment values	Per cent reduction
Reybrouck, 1978	–	1, 3 h	Fingertip impression; glove washing; counts from handwashings (plastic bag)	Mutual comparison	Count distribution
Aly & Maibach, 1979	+	1, 2, 3, 4, 5, 6 h	Gloved-hand sampling	Same panel; base line counts (soap)	Log counts
Eitzen *et al.*, 1979	+	Field test	Hand palm and finger impression	Same panel?; pre-treatment values	Per cent reduction
Jarvis *et al.*, 1979	+	1·5 h	Counts from handwashings	Same subject; washing with soap (bar)	Per cent reduction
Rotter *et al.*, 1980; DGHM, 1981; ÖGHMP, 1981*b*	+	3 h	Fingertip washing	Same subject; pre-treatment values	Log reduction factor
Aly & Maibach, 1983	+	3, 6 h	Gloved-hand sampling	Same panel; base line counts (soap)	Log reduction factor
Crémieux *et al.*, 1984	–	Field test	Glove washing	Mutual comparison	Log counts
Reverdy, Martra & Fleurette, 1984*b*; Savage *et al.*, 1984	+	–	Counts from handwashings (plastic bag)	Same subject; pre-treatment values	Log reduction factor

preparations intended for surgical or basic hand disinfection as unnecessary (Rotter *et al.*, 1980). Whether the use of disinfectants with a cumulative action is of any value in intensive care or protective isolation wards is uncertain. Cumulative reduction of resident flora in these units would seem to be of little value and as with residual activity might give a false sense of security. In any case, agents with a greater immediate effect are preferred, e.g., an alcohol rub. In addition, the ecosystem of the hands may be disturbed (Sprössig & Sauerbrei, 1982; Larson, 1984). It would perhaps be a justifiable policy to introduce chlorhexidine or hexachlorophane as a routine if outbreaks of *Staph. aureus* occur. Other measures, such as the isolation of infected patients and barrier nursing, probably influence the infection rate as much as hand disinfection or the whole-body bathing of the patients; the use of disinfectant preparations with a persistent or cumulative effect does not release hospital staff from the obligation of wearing disposable gloves (Frappier-Davignon *et al.*, 1959; Gehlbach *et al.*, 1975; Ojajärvi, 1980).

Facilities and equipment for hand disinfection

It is important that facilities should be available so that hospital personnel can carry out the chosen policy of handwashing or disinfection. The money saved through a reduction of the infection rate justifies some extra costs for the equipment needed in providing these facilities.

Handbasins should be present not only in a sufficient number, but they should also be strategically placed. The construction of the soap and disinfectant dispensers is very important. In some soap or germicidal preparations, Gram-negative bacteria may survive after accidental contamination and can even multiply in the liquid. So the construction of elbow- or foot-operated dispensers is recommended. The same problem exists in the construction of taps and other sanitary equipment. If the equipment is malfunctioning or if poor techniques are followed, the equipment can become the source of an outbreak (Anagnostakis *et al.*, 1981). Alcohols and alcoholic solutions can and should be rubbed into the hands until the skin is dry, but after handwashing, or after disinfection with aqueous preparations, the wet hands must be dried with a towel or with hot air. On multiple-use cotton towels, pathogenic bacteria (*Staph. aureus* and enteric organisms) may accumulate in large numbers causing recontamination of the washed or disinfected hands (Robinton & Mood, 1968). For this reason, towels used in hospital should be disposable; these may be made of paper or of non-woven tissue. The quality of disposable towels is important; if they are made of material which is too rough the skin is likely to be irritated and abraded; dermatitis may follow and may be wrongly attributed to the soap or disinfectant. Personnel will then either avoid handwashing and disinfection, or they will return to the use of the linen towel. Some institutes have therefore introduced linen towels which

are used once only and laundered. Contradictory opinions are held about hand drying by means of hot air. Some people emphasize the possible risk that bacteria may be carried with the air current, but others consider the number of bacteria from this source, as well as infectivity of hands after drying, to be rather small. In wards, drying of hands by hot air may be used, but the method is not recommended in operating theatres. The main problem is the slowness of hot air drying, and staff are likely to move away before their hands are dry.

A policy for hand hygiene

As washing and disinfection of hands is probably the most important factor in the prevention of spread of hospital-associated infections, it is necessary to establish a policy that will be practiced rigorously by all members of staff. Such a policy cannot be laid down for use in all hospitals, since each hospital is different from others in regard to the patients treated, in equipment and facilities available and even in personnel. It would serve little purpose to introduce measures of handwashing if no washbasins are available, or to prescribe a germicidal preparation which is not accepted by the personnel because it is too irritant.

Directives for hand hygiene should, therefore, be laid down by the infection control committee and adapted to the particular needs of the hospital. Guidelines should be clear and distinct, and measures should be followed by everyone in the hospital, including doctors and domestic personnel. Only a restricted number of preparations should be available, but every product should be applied at the right dilution for the recommended time with standard methods of application. The policy should state the circumstances in which each disinfectant should be used. For staff members complaining of allergy or dermatitis, an alternative preparation should be offered. It must be stated too, in which cases disposable gloves must be worn.

Clear guidelines, standardization of technique and strict discipline are the main requirements of a successful policy for hand hygiene.

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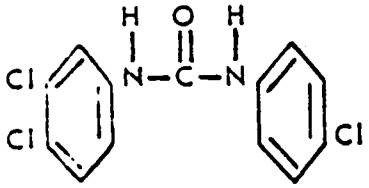
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Cutaneous Antiseptic Activity of 3,4,4′-Trichlorocarbanilide

DANIEL P. ROMAN, E. HARVEY BARNETT and ROBERT J. BALSKE*

With the discovery that hexachlorophene, 2,2′-methylenebis(3,4,6-trichlorophenol) maintained its activity in soap [illegible], a new era in cutaneous antisepsis began. Prior to this development no compound was available which could so successfully maintain the number of resident bacteria on the skin at such reduced numbers. Years later, a second compound was introduced. Shumard *et al.* (2) described the properties of a related compound, bithionol, 2,2′-thiobis (4,6-dichlorophenol) as a skin antiseptic. Vinson (3) in 1954 introduced tetramethylthiuramdisulfide as a third bacteriostatic agent compatible with soap. These products have gained acceptance and have been widely used in the soap and cosmetic industries for several years.

This paper describes an outstanding new compound, 3,4,4′-trichlorocarbanilide (TCC) as a skin antiseptic. The synthesis of this and related compounds along with preliminary reports of their *in vitro* activity was published by Beaver *et al.* (4) in early 1957. TCC is a chlorinated diphenyl urea having the following structure:



It is a fine white powder with a molecular weight of 315.6 and a melting point of 252° C. It is very sparingly soluble in water, in the order of 0.1 ppm at pH 7.0 at 25°C. This low solubility may in part explain its apparent low activity in zone-of-inhibition tests where diffusion in an aqueous medium is important. Solubility in organic solvents for the most part is also quite low as illustrated in Table I.

TABLE I

Solubility of 3,4,4′-Trichlorocarbanilide

Solvent	Solubility, %
Dimethyl phthalate	< 1
Cottonseed oil	< 0.5
Propylene glycol	ca. 1
Acetone	" 4
Dioxane	" 2
Methyl isobutyl ketone	" 4
Dimethyl formamide	" 35

The material *per se*, diluted in organic solvents, or at a 2 per cent concentration in a toilet soap does not discolor on prolonged exposure to light, nor does it form highly colored complexes in the presence of iron.

*[illegible]

Toxicity

Acute oral toxicity of 3,4,4′-trichlorocarbanilide as a water suspension was determined in rats. There was no mortality at the highest single dosage employed, 10 g/Kg. The dosage was increased to 25 g/Kg given in 4 equal feedings twice daily for two consecutive days, again there were no deaths. Rats autopsied after 1 week's observation appeared normal by microscopic examination.

Patch tests were done on 50 human subjects using powdered TCC. The primary application was of 48 hours duration. At the end of 48 hours the patches were removed and the reactions read. Twenty-four hours after removal of the patches, the patch sites were re-examined for delayed reactions. Two weeks later a challenge patch was applied to determine sensitization. After 24 hours, those patches were removed and the reactions read. Twenty-four hours later the patch sites were re-examined for delayed reactions. None of the subjects showed any immediate or delayed reactions following the primary or challenge applications.

This test was repeated using soap containing 2 per cent 3,4,4′-trichlorocarbanilide. The test soap was used in 10 per cent aqueous solutions in the patch test. Twelve of the subjects showed 1+ reactions following the primary applications. None of the subjects showed any immediate or delayed reactions following the challenge application. The 1+ reactions are characteristic of a 10 per cent soap solution.

The conclusion was reached that 3,4,4′-trichlorocarbanilide is neither a primary irritant nor a sensitizer.

Ophthalmic and abraded skin tests on rabbits confirmed that TCC has very low mammalian toxicity.

Bacteriostasis

TCC exhibits a high degree of *in vitro* activity against gram positive organisms normally found on the skin. Table II illustrates effective dilutions at which TCC may be expected to inhibit these organisms.

TABLE II

Bacteriostatic Activity of 3,4,4′-Trichlorocarbanilide

Organism	Dilution Inhibiting
Micrococcus pyogenes var. aureus ATCC 6538	1:10 million
Micrococcus pyogenes var. albus ATCC 4840	1:5 "
Micrococcus pyogenes (coagulase +)	1:10 "
Corynebacterium diphtheriae ATCC 296	1:10 "
Bacterium ammoniagenes ATCC 6871	1:10 "
Diplococcus pneumoniae	1:10 "
Streptococcus viridans	1:10 "
Lactobacillus casei	1:10 "
Bacillus subtilis	1:1 "

These are relative figures, and inhibition at a dilution of 1:30 million does not necessarily represent an end point. We have had difficulty in establishing a precise end point for TCC against *Micrococcus pyogenes* var. *aureus*. End points of from 1:30 million to 1:75 million have been obtained, with 1:30 million appearing to be the most likely figure.

Since the true effectiveness of a bacteriostat for cutaneous antisepsis depends on its *in vitro* activity and its substantivity to skin, handwashing tests must be the final measure of effectiveness. The Cade (5) handwashing test was the method of choice. Briefly this test requires that subjects be used who have not been using antiseptic soaps or cosmetics for two weeks prior to test. The initial count is then determined by using a control soap in a series of five washes. After 5, 11, and 12 days use of the test soap, additional counts are similarly made to follow the course of the reduction of the numbers of bacteria on the skin. It is important to note here that we felt it necessary to issue definite instructions to the subjects regarding their part in the test. Subjects were told to use the test soap a minimum of three times a day, they were also forbidden the use of any soap, solvent, cosmetic, or any other substance which would add to or subtract from the bacteriostatic film being built up on the hands. Rubber gloves are issued to each subject for use in such unavoidable tasks as dishwashing which would expose the hands to cleansing products other than the test soap. While we cannot be sure that our instructions are always carried out, it does impress the subjects with the fact that their cooperation is required.

Objections to the use of the per cent reduction as a measurement of activity have been expressed in the literature. Quinn *et al.* (6) observed that the normal flora of the hands fluctuates from day to day, and pointed out that this is the greatest error involved in measuring reductions of the bacterial population. Baer and Rosenthal (7) commented on the inadequacies of per cent reduction in the statistical handling of the data.

The purpose of incorporating a bacteriostat into toilet soaps is primarily to reduce the number of bacteria on the skin to the point where maximum cosmetic and deodorant benefits are obtained. Despite the disadvantages involved, expressing this reduction as a certain per cent of an original count has some merit. We have retained these units and at the same time we have eliminated the major objections to this method. Since the initial count of a subject is not a fixed characteristic of an individual, we have taken the mean of several hundred initial 5th basin counts as the proper base from which to obtain our per cent reduction figures. We have chosen the log of the count in thousands for convenience in handling the data. The mean initial 5th basin count is then 3.20 log units, equivalent to 1,580,000 bacteria. The true efficiency of a bacteriostat can then be stated in terms of the mean final count in log units, or transformed to per cent reduction from the initial standard count.

In Table III the result of adding TCC at various levels is tabulated. With this test we can expect the final count to be as low as log 2.0, or 100,000 organisms.

TABLE III

Effect of TCC on the Resident Bacteria

% TCC	Log of the 5th Basin Count in Thousands: Standard Initial	5th Day	11th Day	12th Day
0.5	3.2	2.3321	2.3926	2.2567
1.0	3.2	2.3650	2.0276	2.1168
2.0	3.2	1.7464	1.5119	1.5386

These data are presented in the more familiar form, as per cent reduction in Table IV. The per cent reduction has been obtained by transforming the logs back to counts and calculating from an initial count of 1,580,000.

TABLE IV

TCC Test Results as Per Cent Reduction

% TCC	Per Cent Reduction 5th Day	11th Day	12th Day
0.5	86.4	84.4	88.5
1.0	85.3	93.2	91.7
2.0	96.5	97.9	97.8

Although the count has been greatly lowered by all three concentrations of TCC, the reduction achieved by the 2 per cent concentration is significantly greater than that obtained by the use of the lower concentrations.

These results can have meaning only if related to present knowledge. To see where TCC stands in relation to other bacteriostats, compare the data presented in Table V.

TABLE V

Comparative Data on Bacteriostatic Soaps on 12th Day

Compound, %		No. of Subjects	Log Mean	Most Probable Reduction %	95% Confidence Limit of % Reduction
TCC	2	25	1.5386	97.8	96.6-98.6
Bithionol	2	18	1.8534	95.5	92.4-97.3
Hexachlorophene	2	17	1.9797	94.1	89.7-96.5
TCC	1	11	2.1168	91.7	83.9-95.8
Bithionol	1	18	2.1818	90.4	83.8-94.3
TCC	0.5	7	2.2567	88.5	73.4-95.1
Hexachlorophene	1	17	2.5858	75.6	58.4-85.8

Standard error = 0.48

For the number of subjects tested in each group, the most probable per cent reduction represents our best estimate for true reduction. The range of per cent reductions represents the 95 per cent confidence limits within which the true reduction lies. We have 96 per cent confidence that TCC at a 2 per cent level is superior to the next most effective material (bithionol) by our test, and, of course, somewhat more confidence that TCC is superior to the other alternatives tested.

Thus we have a bacteriostat which at the 2 per cent level is stable and effective. The antimicrobial activity of 3,4,4'-trichlorocarbanilide has been demonstrated to be high both *in vitro* and *in vivo* by accepted test procedures. Activity against gram positive cocci is in the range of 1:30 million. Skin degerming activity of 2 per cent TCC is significantly better than that of equal amounts of bithionol or hexachlorophene. The number of resident bacteria on the skin is decreased by 96.6-98.6 per cent as calculated by the method described.

The color stability of TCC enables it to be incorporated into skin care products without danger that discoloration will affect the products sales appeal.

TCC's almost complete lack of toxicity furnishes ready assurance of the safety of this product for incorporation into topically applied preparations.

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TABLE I.

Bacteriostatic Action of TCC

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Staphylococcus aureus (ATCC 6538)	37	NA	[illegible]	[illegible]
Staphylococcus aureus (ATCC 4840)	37	NA	236	1:5 million
Streptococcus pyogenes Group A Type I (ATCC 12344)	37	NA	276	1:10 million
Corynebacterium diphtheriae Park 8, ATCC 296	37	NA	277	1:10 million
Bacillus subtilis (ATCC 6633)	37	NA	313	1:5 million
Pseudomonas aeruginosa (PPL 500)	30	NA	277	1:10 million
Escherichia ammoniagenes (ATCC 6871)	30	NA	266	1:5 million
Streptococcus viridans Vectar	37	TSI	303	1:10 million
β-Hemolytic streptococcus (St. Louis U.)	37	TSI	443	1:1 million
γ-Hemolytic streptococcus (St. Louis U.)	37	TSI	447	1:1 million
Lactobacillus arabinosus (St. Louis U.)	30	TSI	524	1:10 million
Lactobacillus casei (St. Louis U.)	30	TSI	520	1:10 million

1. Lactobacilli were incubated 96 hrs., all others 24 hrs.
2. NA—nutrient agar. TSI—Tryptone beef infusion agar.
3. The degree of turbidity of the inoculum as measured in the Coleman Nephelometer.

TABLE II.

*Fungistatic Action of TCC**

FUNGI**	MODES. [illegible] TO INSURE GROWTH
Fomes annosus FPL 517	1:10 thousand
Trichophyton gypseum	1:5 thousand
Trichophyton inguinale	1:5 thousand
Microsporum globoseum (ATCC 10214)	1:10 thousand
Aspergillus niger (ATCC 9642)	1:5 thousand
Chaetomium globosum (ATCC 6205)	1:5 thousand

*The following fungi showed [illegible] of the order of 1:5 thousand: Aspergillus niger, Penicillium notatum, Candida albicans, [illegible] Saccharomyces cerevisiae, Saccharomyces sake, Rhizopus nigricans. The following [illegible] 1:1 thousand: [illegible]

**Fungi incubated for 10 days at 30°C. [illegible] on Sabouraud's dextrose agar.

Soap & Chem Spec. Vol 34 p 35 1958

New Germicide for Soap

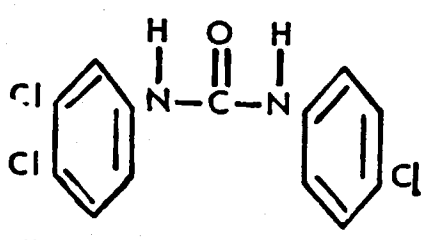
By Daniel P. Roman, E. Harvey Barnett and Robert J. Balske*

Monsanto Chemical Co.

St. Louis, Mo.

WITH the discovery that hexachlorophene, 2,2′-methylenebis (3,4,6-trichlorophenol) maintained its activity in soap (1), a new era in cutaneous antisepsis began. Prior to this development no compound was available which could so successfully maintain the number of resident bacteria on the skin at such reduced numbers. Years later, a second compound was introduced. Shumard *et al.* (2) described the properties of a related compound, bithionol, 2,2′-thiobis (4,6-dichlorophenol) as a skin antiseptic. Vinson (3) in 1954 introduced tetramethylthiuramdisulfide as a third bacteriostatic agent compatible with soap. These products have gained acceptance and have been widely used in the soap and cosmetic industries for several years.

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*Reprinted from the Proceedings of the Scientific Section of the Toilet Goods Association, Inc., No. 28:12 (1957)

It is a fine white powder with a molecular weight of 315.6 and a melting point of 252°C. It is very sparingly soluble in water, in the order of 0.1 ppm at pH 7.0 at 25°C. This low solubility may in part explain its apparent low activity in zone-of-inhibition tests where diffusion in an aqueous medium is important. Solubility in organic solvents for the most part is also quite low as illustrated in Table I.

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Solvent	Solubility, %
Dimethyl phthalate	< 1
Cottonseed oil	< 0.5
Propylene glycol	ca. 1
Acetone	ca. 4
Dioxane	ca. 2
Methyl isobutyl ketone	ca. 4
Dimethyl formamide	ca. 35

The material *per se*, diluted in organic solvents, or at a 2 per cent concentration in a toilet soap does not discolor on prolonged exposure to light, nor does it form highly colored complexes in the presence of iron.

Toxicity

ACUTE oral toxicity of 3,4,4′-trichlorocarbanilide as a water suspension was determined in rats. There was no mortality at the highest single dosage employed, 10 g/Kg. The dosage was increased to 25 g/Kg given in 4 equal feedings twice daily for two consecutive days, again there were no deaths. Rats autopsied after 1 week's observation appeared normal by microscopic examination.

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This test was repeated using soap containing 2 per cent 3,4,4′-trichlorocarbanilide. The test soap was used in 10 per cent aqueous solutions in the patch test. Twelve of the subjects showed 1+ reactions following the primary applications. None of the subjects showed any immediate or delayed reactions following the challenge application. The 1+ reactions are characteristic of a 10 per cent soap solution.

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Organism	Dilution Inhibiting
	million
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Micrococcus pyogenes var. var. albus ATCC 4840	1:5
Micrococcus pyogenes (coagulase +)	1:10
Corynebacterium diphtheriae ATCC 296	1:10
Bacterium ammoniagenes ATCC 6871	1:10
Diplococcus pneumoniae	1:10
Streptococcus viridans	1:10
Lactobacillus casei	1:10
Bacillus subtilis	1:1

These are relative figures, and inhibition at a dilution of 1:10 million does not necessarily represent an end point. We have had difficulty in establishing a precise end point for TCC against *Micrococcus pyogenes* var. *aureus*. End points of from 1:10 million to 1:75 million have been obtained, with 1:30 million appearing to be the most likely figure.

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In Table III the result of adding TCC at various levels is tabulated. With this test we can as log 2.0, or 100,000 organisms.

Table III. Effect of TCC on the Resident Bacteria

% TCC	Log of the 5th Basin Count in Thousands: Standard Initial	5th Day	11th Day	12th Day
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Although the count has been greatly lowered by all three concentrations of TCC, the reduction achieved by the 2 per cent concentration is significantly greater than that obtained by the use of the lower concentrations.

These results can have meaning only if related to present knowledge. To see where TCC stands in relation to other bacteriostats, compare the data presented in Table V.

For the number of subjects tested in each group, the most probable per cent reduction represents our best estimate for true reduction. The range of per cent reductions represents the 95 per cent confidence limits within which the true reduction lies. We have 96 per cent confidence that TCC at a 2 per cent level is superior to the next most effective material (bithionol) by our test, and, of course, somewhat more confidence that TCC is superior to the other alternatives tested.

Thus we have a bacteriostat which at the 2 per cent level is stable and effective. The antimicrobial activity of 3,4,4'-trichlorocarbanilide has been demonstrated to

in other colleges of pharmacy.

The aerosol industry, for its own benefit should do all it can to stimulate study and research in pharmaceutical aerosols. This it may do by contacting pharmaceutical manufacturers. It is equally important that the aerosol industry should stimulate basic researches in colleges of pharmacy by personal contacts, equipment and supply contributions and financial support of research programs. In this way, basic data of importance to the pharmaceutical industry may be obtained and published. This will result in a stimulation of aerosol product development researches.

Summary

AN analysis of the types of pharmaceuticals which may be used effectively as aerosol dosage forms in the near future reveals a market of at least $190,000,000. With the aid of imaginative research and development programs and effective educational campaigns, there is every reason to believe that a good portion of this market can be represented by aerosol products within the next few years.

Among the types of preparations for which aerosol applications either have been found or will be found are the following: dermatologicals, such as analgesics, anesthetics, anti-pruritics, antibacterials, burn remedies, emollients, anti-inflammatories and, fungicides; spray bandages, ear, nose and throat preparations, dental preparations, rectal and vaginal preparations and inhalation products. The latter group of products are the ones which appear the most interesting to pharmaceutical manufacturers at present. Basic research on the localization site of various medicaments inhaled as aerosols and studies on this type of drugs which are absorbed into the blood stream when administered as inhalation aerosols will open many avenues for the development of pharmaceutical aerosol dosage forms.

Pressure packagers should take cognizance of the potential for

Analysis of ABS

(*From Page 44*)

CALCULATIONS:

$$\frac{\text{Weight of Sodium Tartrate (Step 1)} \times 0.1566}{\text{ml Karl Fischer Reagent (Step 3)}} = \text{Grams } H_2O \text{ equivalent to 1 ml of Karl Fischer Reagent}$$

$$\frac{(\text{ml Karl Fischer Reagent (Step 3)} - \text{ml Karl Fischer Reagent (Step 4)}) \times \text{Grams } H_2O \text{ equivalent to 1 ml of Karl Fischer Reagent} \times 100}{\text{Weight of Sample (Step 1)}} = \% H_2O$$

The intra laboratory precision (95% limits) for simultaneous duplicate determinations by one analyst is ±0.22% at a moisture level of 70%. Overall precision (intra laboratory) is statistically estimated (95% limits) to be ±0.17% at a moisture level of 70%.

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New Soap Germicide

(*From Page 36*)

be high both *in vitro* and *in vivo* by accepted test procedures. Activity against gram positive cocci is in the range of 1:30 million. Skin degerming activity of 2 per cent TCC is significantly better than that of equal amounts of bithionol or hexachlorophene. The number of resident bacteria on the skin is decreased by 96.6-98.6 per cent as calculated by the method described.

The color stability of TCC enables it to be incorporated into skin care products without danger that discoloration will affect the products sales appeal.

TCC's almost complete lack of toxicity furnishes ready assurance of the safety of this product for incorporation into topically applied preparations.

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—★—

New Liquid Cleaner

A new liquid cleaner for use on floors, walls, tile, woodwork, metals and upholstery was introduced recently by George Stearns Chemical Corp., Madison, Wis. Tradenamed "GS 77," the product does not contain soap. It is claimed to be effective for wax stripping. "GS-77," may be obtained in packages of four, one-gallon containers and five, 10, 30 and 55-gallon drums.

Table V. Comparative Data on Bacteriostatic Soaps on 12th Day

Compound, %		No. of Subjects	Log Mean	Most Probable Reduction %	95% Confidence Limit of % Reduction
TCC	2	25	1.5386	97.8	96.6-98.6
Bithionol	22	18	1.8534	95.5	92.4-97.3
Hexachlorophene	2	17	1.9797	94.1	89.7-96.5
TCC	1	11	2.1168	91.7	83.9-95.8
Bithionol	1	18	2.1818	90.4	83.8-94.3
TCC	0.5	7	2.2567	88.5	73.4-95.1
Hexachlorophene	1	17	2.5858	75.6	58.4-85.8

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Preface

The field of antiseptics and disinfectants had its beginnings with the discovery that germs were associated with infection and that the use of certain chemicals prevented the spread of germs, resulting in fewer ve infection. Lister's use of phenol around operating theate ressings and Semmelweis' use of handwashing were the begi ermicides as disinfectants and antiseptics. It is indeed a task t chemicals as germicides in all fields (e.g., medicine, agricu thers). It is the purpose of this book to look at those subject control, the use of germicides in preventing the spread of infe nate objects or from person to person. The use of disinfectant ts (disinfectants) or on human tissue (antiseptics) has come past decade because of unsubstantiated claims made by ma use of the importance of effectiveness necessitated by dis culosis, acquired immunodeficiency syndrome (AIDS), an

The number of chemicals in use, like the po lications, is too great to be covered in one book. I have attem chemicals that have the widest use for both disinfection of pment and antisepsis. Some obvious omissions may be no quaternary ammonium compounds are not reviewed, primaril routinely used on critical medical equipment, and also beca nents have been reported for some time. Also, several recent p se chemi-

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cals in depth, and there is no need to repeat that information. Likewise, with iodine, although covered somewhat in Chapter 8, an exhaustive review at this time would be repetitious and unwarranted, as little new information has been generated.

This book covers the chemicals that have the most widespread use in current disinfection and antisepsis practices, both in the United States and abroad. The chemistry, microbiology, and toxicology of each agent is presented. In order to better understand the microbiology of these chemical agents, a section has been included to describe the procedures, both in-use and laboratory, that are used to generate microbiological claims for disinfectants and antiseptics. Because of the similarity in chemicals used in contact lens disinfectant solutions and antiseptics and disinfectants, an additional chapter has been included to discuss the methods used to evaluate these chemicals in this particular application.

It is my hope that this book will provide readers in the medical profession, academia, and industry with pertinent information in the field of disinfection and antisepsis.

Joseph M. Ascenzi

Contents

Preface ..... *iii*

Contributors ..... *vi*

I. METHODS

1. Test Methodology for Evaluation of Germicides 1
   Eugene C. Cole and Richard Robison
2. A Broad-Based Approach to Evaluating Topical Antimicrobial Products 17
   Daryl S. Paulson
3. Neutralizer Evaluations as Control Experiments for Antimicrobial Efficacy Tests 43
   Scott V. W. Sutton

II. APPLICATION

4. Antiseptics and Their Role in Infection Control 63
   Joseph M. Ascenzi
5. Disinfectant Use in Dentistry 73
   James A. Cottone and John A. Molinari

NOV-06-1995 15:45 P&G SWITC CLIN RSCH & BIO. 513 626 3275 P.04

6. Contact Lens Disinfectants 83
Michael J. Miller

III. DISINFECTANTS AND ANTISEPTICS

7. Glutaraldehyde-Based Disinfectants 111
Joseph M. Ascenzi

8. Chlorine and Iodine Formulations 133
Sally F. Bloomfield

9. Antimicrobial Effects of Hydrogen Peroxide as an Antiseptic and Disinfectant 159
Andrea M. Lever and Scott V. W. Sutton

10. Alcohols for Antisepsis of Hands and Skin 177
Manfred L. Rotter

11. Chlorhexidine 235
N. S. Ranganathan

12. Chloroxylenol: An Old–New Antimicrobial 265
Mary K. Bruch

Index 295

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NOV-05-1995 16:45 P&G SWC CLIN RSCH & BIO. 513 626 3275 P.05

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10

Alcohols for Antisepsis of Hands and Skin

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I. DEFINITION

Alcohols are organic compounds, of which the general chemical formula is R–OH. (R stands for an alkyl or substituted alkyl radical.)

Alcohols, therefore, can be described as hydroxyl derivatives of paraffins (= hydrocarbons). Whereas the functional hydroxyl group determines the kind of possible chemical or physiological reaction, the speed and extent of these reactions are mainly controlled by properties of the alkyl radical.

II. NOMENCLATURE AND CHEMISTRY OF ALCOHOLS USED IN SKIN ANTISEPSIS

Alcohols (or "alcanols") are named by terminating the name of the hydrocarbon with the ending "-ol," for example methan*ol*.

The *number* of hydroxyl groups in a paraffin molecule determines the valency of an alcohol. Methanol, therefore, is a monovalent alcohol; 1,2-ethandiol

("glycol") is a divalent alcohol, and glycerol, which contains three OH-groups, is a trivalent alcohol.

According to the *position* of the hydroxyl groups, an alcohol is classified as primary, secondary, or tertiary:

Primary $R-CH_2-OH$

Secondary $R-\underset{\displaystyle R}{\overset{\displaystyle |}{CH}}-OH$

Tertiary $R-\underset{\displaystyle R}{\overset{\displaystyle R}{\overset{\displaystyle |}{\overset{\displaystyle |}{C}}}}-OH$

Aliphatic (chain) alcohols are distinguished from aromatic (ring) alcohols.

Only the short chain, aliphatic alcohols are used in antisepsis, mainly for practical reasons. The only aromatic alcohol of some importance is benzyl alcohol; it is used virtually exclusively as an additive to other alcoholic antiseptics to support the antimicrobial effect.

III. PHYSICOCHEMICAL PROPERTIES

An important feature for their usability in antisepsis is the miscibility of alcohols with water. As can be seen from Table 1, only short-chained alcohols, such as methanol, ethanol, and the propanols, are completely miscible. Higher alcohols, such as hexanol, heptanol, and so on, or benzyl alcohol, have little or no solubility in water. Alcanols with chain lengths above the undecyl alcohols are not liquid at 20°C, thereby rendering their use impractical.

The longer the alkyl chain, the more lipophilic (and less hydrophilic) alcohols become. This is somehow counteracted by branching of the chain. The extent of the lipophilic characteristics is expressed by the partition coefficient, *P*, of an alcohol in systems such as octanol/water or diethyl ether/water [1]. As an example, some values are given for the system diethyl ether/water in Table 1.

If concentrations are given in fractions of 100 ("percent"), they must be clearly defined as percentage by weight (g/g or w/w) or by volume (ml/ml or v/v). The transformation of percentage by volume into percentage by weight is not possible by merely multiplying volume percent by the specific weight of the alcohol, since, in mixtures with water, a volume contraction of the alcohol occurs that is greatest when mixing equal parts of alcohol and water. Thus, for example, adding 50 ml of water to 50 ml of absolute ethanol results in a total volume of 96.3 ml, rather than 100 ml. Therefore, to prepare 100 ml, one would have to top up 50 ml of absolute alcohol with (distilled) water right to the 100-ml mark of a measuring cylinder. Table 2 lists the corresponding percentages by volume and

weight for ethanol. As the corresponding values for propan-2-ol and propan-1-ol were lacking, they were generated especially for this chapter in laboratory experiments [H. Felsch, personal communication]. According to these measurements the corresponding curves are similar to, although not identical with, that of ethanol. In the area of greatest deviation, 60% by volume of propan-2-ol corresponds to 53.7% by weight, and of propan-1-ol to 54%. The best way to avoid difficulties in preparation of alcoholic solutions, however, is to work with concentrations by weight, rather than by volume, as early recommended by Beyer [2] in 1911 and, later, by Price [3]. The short-chain, aliphatic alcohols are inflammable liquids. Table 3 lists various safety data for these.

Because of their polar structure, low-weight alcohols possess considerably less surface tension than water. This is the reason for their better wetting characteristics, an important feature for antiseptics used on the skin surface. In this context, an old observation by Neufeld and Schütz [4] showed that ethanol absorbs six times more air than water, thus, it more easily enters crevices and blind-ending glandular ducts of the skin.

IV. HISTORY OF ALCOHOLS AS ANTISEPTICS

The name *alcohol* is taken from the Arabic *Al Kohl*, which was used for refined antimony sulfide used by ancient Egyptians as a remedy against eye infections and, in combination with a blue dye, as a cosmetic. In the early 16th century, the famous physician, scientists, and philosopher Paracelsus Theophrastus von Hohenheim gave the name "Al-Kool" to the distillate of wine for its antiseptic properties [5]. In antiquity and medieval times, alcohol was used in the form of wine or its distillate probably for wound antisepsis only. According to Beck [6] it was recommended for this purpose by Galen (131–201 AD) and Guy de Chauliac (1363), and Dittrich [7] reports that the surgeon Augustin Belloste (17th century) favored its use as did J.-F. Bathaile.

L. Buchholtz [8] was probably the first to investigate the antimicrobial activities of ethanol on a scientific basis in 1875 and to relate these to the possible application for antiseptic purposes. Robert Koch, however, concluding from the results of his experiments with anthrax spores, which he had dried onto silk threads, that ethanol was completely unsuitable for antiseptic and disinfection purposes [9]. Despite this erroneous belief, he was right in his observation that alcohols are not (or hardly) sporicidal. This was later often demonstrated [10–18].

Preoperative skin disinfection with alcohol was first carried out in the latter half of the last century [6] by Nelaton using alcohol soaks at the site of operation. P. Fürbringer [19] was the first to recommend alcohol in 1888 for hand disinfection of surgeons and medical staff.

However, it was a long time until the controversies over the necessary concentrations and times of action were resolved by the discovery that the pres-

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TABLE 1 Synopsis of Some Physical Properties of Short-Chain Alcohols

Alcohol	Mol wt	Specific gravity at 20°C	Boiling point (°C)	Melting point (°C)	Solubility in water at 20°C (g/100 g water)	Partition coefficient (log P)[a]	Viscosity (mPa·s)	at (°C)[b]
Methanol	32.04	0.791	64.7	−98	Unlimited		0.52	20
Ethanol	46.07	0.789	78.4	−117	Unlimited	−0.58	1.22	20
Propan-1-ol[c]	60.10	0.805	97.2	−127	Unlimited	0.28	2.75	20
Propan-2-ol[d]		0.785	82.0	−89	Unlimited	−0.19	2.27	20
Butan-1-ol[e]	74.12	0.810	117.0	−89	7.7	0.89	2.95	20
Butan-2-ol[f]		0.808	99.8	−114	12.5	0.84	4.21	20
2-Methylpropan-1-ol[g]		0.802	108	−108	9.5	0.65	6.68	20
2-Methylpropan-2-ol[h]		0.779	82.9	24	Unlimited	0.34	3.30	20
Pentan-1-ol[i]	88.15	0.815	138	−79	2.7		3.68	30
Pentan-2-ol		0.808	119	−50	13.5			20
3-Methylbutan-1-ol[k]		0.816	131	−117	2.5			
2-Methylbutan-1-ol[l]		0.816	128	70	3.6			
2-Methylbutan-2-ol[m]							5.10	20
Hexan-1-ol	102.20	0.800	102	−12	17.7 (30°C)			
Heptan-1-ol	116.21	0.814	157	−45	0.6		3.70	25
Octan-1-ol	130.23	0.824	175	−34	Slight		0.45	24
Benzyl alcohol	108.13	0.827	195	−16	Slight			
		1.042	204.7	−15.3	3.5		8.40	20

[a]System: Diethylether/water [54]
[b]Centipoise
[c]*n*-Propyl alcohol
[d]Isopropyl alcohol
[e]*n*-Butyl alcohol
[f]*sec*-Butyl alcohol
[g]Isobutyl alcohol
[h]*tert*-Butyl alcohol
[i]*n*-Amyl alcohol
[k]*sec*-Amyl alcohol
[l]Isoamyl alcohol
[l]*act*-Amyl alcohol
[m]*tert*-Amyl alcohol
Source: Courtesy of Merck Corp., Darmstadt; Refs. 250, 251.

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Table 2 Corresponding Concentrations as Percentages by Weight and Volume for Ethanol, Propan-2-ol and Propan-1-ol

Concentrations by			
Weight (% g/g) Alcohol	Volume at 20°C (% ml/ml) Ethanol	Propan-2-ol	Propan-1-ol
40	47.4	47.0	46.5
50	57.8	57.0	56.5
60	67.7	67.2	66.2
70	76.9	76.5	76.1
80	85.4	85.0	84.5
90	93.2	93.8	93.5
95	96.7	97.5	97.2
100	100.0	100.0	100.0
Volume at 20°C (% ml/ml)		Weight (% g/g)	
40	33.4	33.5	34.0
50	42.8	43.0	43.4
60	52.1	53.7	53.9
70	62.6	62.8	63.5
80	73.4	73.5	74.5
90	85.8	86.0	86.0
95	92.5	92.8	93.0
100	100.0	100.0	100.0

Source: Courtesy H. Felsch, 1992.

ence of water plays an important role in the antimicrobial activity of alcohols [20–24], the necessary amount of water depending mainly on the status—dry or moist—of the bacterial cell. The hypothesis is that a high percentage, or pure, alcohols deprive bacterial cell walls of water, thereby inducing the formation of impermeable protein layers which, in turn, prevent the alcohol from penetrating into the cell [25]. Hence, as a prerequisite for antimicrobial activity of alcohols on *dry* cells, the absorption of water by the cell wall is necessary [2,26,27]. But this was not true for moist cells, as Morton [16] has demonstrated in his experiments. Therefore, the statement, found in many textbooks, that high-percentage alcohols, such as 95% ethanol, are practically worthless as germicides needs to be qualified.

In contrast with European countries, in many of which the performance of

Table 3 Various Safety Data on Alcohols Used for Antiseptic Purposes

Alcohol	Vapor pressure at 20°C hPa	Vapor density at 20°C	Flash point[a] (°C)	Explosion limits in air (vol %)	Autoignition temp. (°C)	Odor threshold (ppm)
Methanol	128	1.1	11	6.0–36.0	385	5
Ethanol	59	1.6	13	3.3–19.0	363	10
Propan-1-ol	19	2.1	15	2.2–13.7	410	30
Propan-2-ol	43	2.1	12	2.0–12.7	399	40
Butan-1-ol	7	2.5	30	1.4–11.3	343	10
Butan-2-ol	17	2.6	23	1.7–9.8	405	0.6
Isobutanol	12	2.6	29	1.7–10.9	430	40
tert-Butanol	40	2.6	11	2.3–8.0	478	73
Pentan-1-ol	3	3.0	49	1.3–10.5	300	1
Pentan-2-ol	3	3.0	41	1.2–9.0	347	
Isoamyl alcohol	3	3.0	42	1.2–8.0	350	70
akt-Amyl alcohol	5[b]	?	50	1.9–9.3	340	
tert-Amyl alcohol	13	3.0	20	1.3–9.6	425	2.3
Hexan-1-ol	1	3.5	63	2.1–7.7	292	5.2
Heptan-1-ol	0.5	4.0	70		350	0.5
Octan-1-ol	0.3	4.5	81	0.8	270	
Benzyl alcohol	0.1	3.7	101		436	

[a]For concentrated solutions
[b]At 50°C
Source: Courtesy of Merck Corp., Darmstadt, and Refs. 250, 251.

alcohols has remained the standard of hand and skin antisepsis since the beginning of this century. In the United States the attitude toward the use of ethanol or propan-2-ol has changed completely during the last five decades. In the 1930s, ethanol was the favorite hand and skin antiseptic. In 1938, the surgeon P. B. Price added further evidence for the excellent performance of ethanol in hand and skin antisepsis, as demonstrated by the results of his intelligently designed tests [28–30]. Even in his later papers reporting on his findings with the then more modern preparations that were based on quaternary ammonium compounds, hexachlorophene, and iodophors [31,32], he mentions that "seventy per cent alcohol by weight is still believed to be the solution of choice for disinfection of the skin." In his survey of 1948 [33], 64% of the hospitals in the United States were using ethanol (although nearly 70% also used organic mercurials, and 48% used a quaternary ammonium compound). In 1965, however, only 18% of North American hospitals had alcohol on their list of hand and skin disinfectants [34] and, today (1992), alcohols are seldom used for this purpose, presumably for reasons of flammability and their allegedly drying effect on skin [35]. During the period discussed, alcohols were replaced on the North American continent mainly by hexachlorophene and iodophors, the former of which was withdrawn from the market in the 1970s because of its potential toxicity. To a lesser extent, preparations containing chlorhexidine gluconate, triclosan, or phenolic compounds such as *p*-chloro-*m*-xylenol (PCMX) are used.

V. ANTIMICROBIAL PROPERTIES

The antimicrobial activity of alcohols is highly dependent on their concentration and on the status—dry or moist—of the microorganism. The short-chain, aliphatic monovalent alcohols, ethanol and the propanols, seem to be the most suitable for antisepsis because they are miscible with water to an unlimited degree, hardly toxic, and are nonallergenic, fast-acting, and are microbicidal, rather than microbiostatic, if used in the correct range of active concentrations. The bi- or trivalent alcohols do not play a role in the field of antisepsis, nor do aromatic alcanols. Some of them are used as solvents and for preservation because of their bacteriostatic and fungistatic characteristics. They will not be discussed in this chapter.

A. Mechanisms of Antimicrobial Action

- The mechanism for the disinfection effect of alcohols is generally believed to originate from the coagulation of proteins [36–39]. Kamm [36] explained the biological effects of alcohols more precisely with both their capability to coagulate proteins and their solubility in lipids. Both features become more prominent with increasing molecular weight. For example, the relative efficiencies of molar

solutions to inactivate enzymes for ethanol, propan-1-ol, and butan-1-ol are 1, 2.6, and 8.0, respectively.

The coagulation of proteins occurs within certain concentration limits encompassing the concentration optimum. Coagulation of enzymatic protein results in the loss of specific cellular functions. For bacterial dehydrogenases, Sykes [40] has demonstrated an increasing effect in the following range of order:

ethanol < propan-2-ol < butan-1-ol < pentan-1-ol

An alcohol-induced prolongation of the growth lag phase of *Enterobacter aerogenes* could be counteracted by the addition of amino acids [41]. Ethanol- and, to a lesser extent, propan-1-ol–induced effects, however, were "curable" by only certain amino acids, such as D,L-methionine, L-leucine, L-glutamic acid, or L-histidine and D,L-tryptophan; the addition of some other amino acids such as D,L-alanine, D,L-serine, or D,L-aspartic acid caused the inverse effect (i.e., a further increase of the lag phase). Therefore, this bacteriostatic action may be caused by an alcohol-induced inability of the cell metabolism to produce some metabolites necessary for growth. Other workers [42] succeeded in resuscitating cells of *E. coli*, which were apparently killed by a 10-min exposure to 20% ethanol, by "treating" them with several metabolites of the citrate cycle, such as *cis*-aconitic acid, α-ketoglutarate, or a mixture of several metabolites, including both of these. This could also be demonstrated under conditions of hand disinfection [43]. The activity of cholinesterase was repressed, within certain concentration limits, by the normal C_3 to C_6 alcohols, such that, with increasing chain length, the activity decreased. Beyond certain concentration, the enzyme was completely inactivated. This critical concentration for each alcohol also decreased with increasing chain length [44].

Bacterial enzymes localized in the cell wall are easier to inactivate than those inside the cell [45]. The coagulation of plasma proteins is visually demonstrable in electron micrographs, in which mixing of cell plasma and nucleoid can be observed [46]. However, coagulation of nucleoproteins does not occur [27].

- Another target in the microbial cell is the cytoplasmic membrane, the destruction of which leads to an outflow of cytoplasm, amino acids, and nucleic acid constituents [47–49] at relatively low concentrations. As with other disinfectants, bacterial lysis may occur at relatively low concentrations (about twice the minimum inhibitory concentration; MIC) [50]. Also, lysis of human *Mycoplasma* strains has been reported [51].

There are associations between the structure and the biological effects of alcohols. Those of monovalent alcohols were summarized by Friedemann and Treuhoff [52]. An extensive account on the dependence of biological effects on the chemical and physical properties of alcohols was given by Franke and Kramer [53]. An important review on alcohols as disinfectants has been written by Morton [38]. A more recent survey was presented by Heeg et al. [54].

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The main facts are the following: The antibacterial effect of monovalent, aliphatic alcohols increases with the boiling point [55]. Elongation of the unbranched alkyl radical up to six carbon atoms improves the bactericidal effect, further elongation impairs it. Eggensperger [56], for example, demonstrated that *Staphylococcus aureus* was killed by a 2-min exposure in suspensions of alcohols with the following critical concentrations (by weight): methanol 60%, ethanol 50%, propan-1-ol 20%, butan-1-ol 10%, hexan-1-ol 5%, but dodecan-1-ol at 15% (see also Table 4).

The branching of the alkyl radical increases water solubility, but diminishes the disinfection effect. In the foregoing experiments, for instance, a concentration of 40% was necessary for propan-2-ol and tertiary butanol to achieve an effect comparable with that of the corresponding normal alcohols.

Among the isomers, the following range order of the antibacterial effect has long been known: *n*-primary > *iso*-primary > secondary > tertiary alcohols [22,57]. Of the alcohols that are completely miscible with water, propan-1-ol, therefore, is the most active [11,58,59].

As with all disinfectants, there is a clear association of temperature and disinfection efficacy [11,27,60,61].

Controversial reports exist on the impairment of disinfection efficacy by the presence of organic material, especially proteins. Whereas Friedemann [62] showed that bacteria suspended in serum or wound secretions are protected by coagulated protein from the killing effect of alcohols, and Schürmann and Eggers [61] found a concentration-dependent inhibition of the antiviral activity of an ethanol/propan-2-ol mixture, other authors [63,64] have shown that, under practical conditions, doubling the exposure time easily compensates for this factor. Rotter and colleagues [65] found, in practical experiments, that the mean log reductions of the release of test bacteria (*E. coli*) from the fingertips of artificially contaminated hands by rubbing in 60% (v/v) propan-2-ol for a total of 60 s were only slightly (and not at all significantly) different if the test organisms were suspended in broth, horse serum, or skim milk, and then dried on the hands for 3 min before disinfection. The respective log reductions were: 4.74, 4.69, and 4.48.

With some combinations of alcohols, synergistic effects have been observed. For example, with the combination of methanol/butan-1-ol, the killing times were reduced by 30–80%, compared with those of either alcohol alone [66].

Development of resistance against alcohols does not play a role, at least in concentration ranges suitable for disinfection. In 50 passages of *Staphylococcus aureus* and four gram-negative bacterial species on media containing 7% and 3.5% propan-2-ol, respectively, Wille [67] could not detect any indications of an increase in alcohol resistance. In experiments by Wigert et al. [68] using concentrations of various alcohols below the bacteriostatic concentrations, the sensitivity of *S. aureus* was decreased by 50% (i.e., one dilution step), and for gram-negative bacteria, the change was even less [69]. In addition, this behavior of the test strains

was not stable, but disappeared immediately after termination of the exposure to alcohol. Therefore, any genetic basis for it can be excluded [70].

B. Antimicrobial Spectrum

In this section, the current knowledge of the antimicrobial activity of alcohols as used in disinfection and antisepsis (against bacterial spores, vegetative forms of bacteria, fungi, and viruses) will be presented in the order that this knowledge was accumulated during the last century.

1. Bacterial Spores

As pointed out in Section IV, alcohols are generally considered to be completely inactive against bacterial spores. It would be imprecise, however, to state that they are never sporicidal. Even if it is true that spores of *Bacillus subtilis* stay alive in 95% ethanol for years [39], such that a high ethanol concentration was used as a suspension liquid to preserve them as standard inoculum for antibiotic assays, this is not true for all bacterial spores and not under all conditions. Regamay [71], for instance, has demonstrated that spores of anaerobic bacteria indeed survived in 10% ethanol for more than 10 months, but in concentrations of 40% and 80% they survived for only 7½ and 4 weeks, respectively. Morton [38] produced evidence that, in contrast with Koch's and other authors findings, spores of *B. anthracis* may well be sensitive to ethanol concentrations between 42 and 100%. Under his experimental conditions they were killed within 48 h. With propan-2-ol this was possible even with concentrations of 30–40% within 2–5 min [72], but methanol was reported to be completely inactive against spores of this species in concentrations of 0.004–95% [17].

Spores of other species, namely *B. subtilis* and *Clostridium novyi*, remained unaffected by exposure to 20–91% propan-2-ol over 1 h [73]. Although one cannot advocate the use of alcohols for indications that require sporicidal actions, one would often wish that the differences in activity were better defined in terms of an exact description of the bacterial strains, the condition of the spores—dry or moist—and accompanying factors. Some of these may well explain controversial findings, since sporicidal activity of alcohols can be augmented or induced by the addition of agents such as alkali, mineral acids [74], H_2O_2 (0.5%), or some tensides [75,76] and formaldehyde [66].

2. Vegetative Bacteria

When ethanol was recommended in 1888 by Fürbringer [19] for disinfection of hands, the idea was to use it as a cleansing agent [see also Ref. 77] for its alleged fat-dissolving properties, rather than as a bactericide. The actual disinfection with a mixture of 0.2% sublimate in 3% carbolic acid was the next step in Fürbringer's recommendation. In those days, others [78,79] explained the reduction of bacterial

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release from the hands, as observed after the application of alcohol, by the fixation of microbial cells to the skin or by trapping them in the depths of the alcohol-indurated skin surface. This hypothesis, however, was soon refuted (a) by the results of laboratory experiments proving that bacteria are readily killed by ethanol [22,55,80], (b) by the elegant experiment of Neufeld and Schiemann [12] demonstrating that an alcohol rub reduced the release of *E. coli*, but not that of *B. subtilis* spores from hands previously contaminated with a mixture of both organisms, and (c) by Bernstein [81], who demonstrated that a 2-min alcohol treatment of hands did not reduce the release of epithelial cells from the skin.

Table 4 summarizes the antimicrobial activity of various alcohols in terms of the concentrations necessary either to inhibit growth or to kill *S. aureus* and *E. coli*. As pointed out in Section V.A, it is evident that the antimicrobial activity increases with the chain length of the alcohols and that tertiary alcohols are less

TABLE 4 Comparison of Minimum Bacteriostatic and Microbicidal Concentrations of Various Alcohols in Suspension Tests

Effect	Minimum effective concentrations (% v/v)							
	Growth inhib.	Killing effect in suspension test						
Test Organism	*S. aureus*	*S. aureus*				*E. coli*		
Exposure (min):		1	2	2	10	1	2	12
Alcohol								
Methanol	9	65		67[c]		60–65		
Ethanol	7	50		58[c]		45–50		
Propan-1-ol	4	20		23[c]		17		
Propan-2-ol	4	45		48[c]		26		
Butan-1-ol	3	9		11[c]		5		
Isobutanol	3	18				6.5		
sec-Butanol		15				11		
tert-Butanol	5		28	46[a]			14	
Pentan-1-ol	1				3	2		
Isopentanol			4			2.75		
sec-Pentanol		7.5			4			
tert-Pentanol	3	15				9		
Hexan-1-ol		0.6[a]	5[a]		0.7			
Heptan-1-ol		0.12[b]						0.12
Octan-1-ol		0.08[b]				0.08		
Ref.	22	55	55	56	55	55	55	55

[a]In 30 min.
[b]In >30 min.
[c]Converted from % w/w.

active than primary or secondary ones. This is also true for exposure of the test organisms on carriers such as batiste patches or glass slides (Table 5).

From these tables and from reports in the literature, the following conclusions can be drawn: Methanol exerts a definite, although comparatively low, activity [22,55,56,58,66]. Concentrations of above 50% (v/v) are necessary for bactericidal effects. However, it was noted by some authors [83] that, in contrast with other alcohols, it is also active on dried bacteria (not spores) without the presence of water. The combination of ethanol (96%) with methanol (10%) for denaturation of the former does not impair its disinfection efficacy [86].

Ethanol exerts bacteriostatic effects at approximately 10% (v/v). At this concentration germination of bacterial spores is also inhibited [39]. Bactericidal concentrations exist from about 30% (v/v) upward, depending on the bacterial species, its water content, and the exposure time [16]. Table 6 gives examples of the times necessary to kill various bacterial species in ethanol at the optimum concentrations of 60–80% (v/v). Except for *Streptococcus pyogenes*, all species listed are killed within 1 min. This is also true for *Mycobacterium tuberculosis* in this experiment. A very high percentage (90% up to absolute ethanol) is less active than the less-concentrated solutions.

An informative picture of the influence of test organism status—dry or moist—and of the weak action of highly concentrated ethanol is given by the results of experiments carried out by Harrington and Walker [20] with *S. aureus* on moist and dried threads. Table 7 demonstrates that, in moist conditions, the test

TABLE 5 Comparison of Minimum Bactericidal Concentrations of Various Alcohols in Carrier Tests

Test Organism	Minimum effective concentrations (% v/v)								
	Staph. aureus			*E. coli*	*M. tuberculosis*				
Exposure (min):	2	30	30	0.25–15s	10[b]	10[b]	15	30	120
Alcohol									
Methanol		57[a]	70	95					90
Ethanol	80	43[a]	60	80	96	80		80	
Propan-2-ol	50		50	50	60			20	
Propan-1-ol		23[a]	30	20	50	50		20	
Butan-1-ol								5	
Isobutanol									10
Propen(1)-ol(3)								20	
Benzyl alcohol							~4		
Refs.	82	11	83	83	84	82	85	85	85

[a]Converted from %w/w.
[b]Dried in sputum on slide.

OCT-17-1995 17:04 FROM SMITH CLIN RSCH & BIO. 513 526 3275 P.08

Table 6 Bactericidal Efficacy of Ethanol at Various Concentrations (% [v/v]) in Suspension Tests

Bacterial species	Killing time (s)		
	60%	70%	80%
Staphylococcus aureus	15	15	10
S. epidermidis	30	30	
Streptococcus pyogenes			90
Escherichia coli	60	30	30
Serratia marcescens		10	
Salmonella typhi		10	
Pseudomonas aeruginosa		10	
Mycobacterium tuberculosis	60	30	30

Source: Ref. 39.

organism is killed in 5 min by the highest ethanol concentration of 99%, but on dried threads, this concentration is still inactive after 24 h. Later, Morton [16] produced results comparable with these, but with more bacterial species and with anthrax spores. He points out that the results of alcohol on dried organisms are unjustly taken as typical of the germicidal action of ethanol.

The effect of ethanol against *Mycobacterium tuberculosis* depends on the suspending medium—sputum or water—and on its moisture content—liquid or dried. Table 8 summarizes results of Smith [87] demonstrating the influence of these variables on the tuberculocidal effect of ethanol. From these results the author concluded that 95% ethanol was the most effective on wet surfaces, 50% on dry, and 70% on either wet or dry surfaces. The reports of other authors [82,84,85], as summarized in Table 8, show the same concentration effects. Ethanol is also active against other mycobacterial species, such as *M. phlei* [88].

A bactericidal effect of ethanol was also shown for *Mycoplasma* strains by Razin and Argaman [51], who found a concentration range of 4.3–5.4 mol effective, and for *Spiroplasma* strains by Stanek et al. [89], who described effective concentration/time relationships from 20%/5 min to 30%/40 min.

The two propanols, which are homologues with the highest molecular weight, are miscible with water in any proportion. Of the water-soluble alcohols, propan-1-ol (syn: *n*-propanol, *n*-propyl alcohol) was reported to be the most active [11,58,59,90–92]. For the more sensitive species, such as *E. coli*, propan-1-ol is bactericidal at 13% [55], the optimum concentration being 40–60% (v/v). The antibacterial spectrum is comparable with that of ethanol [25,39,54,83; see also Tables 4 and 5].

Table 7 Bactericidal Activity of Various Concentrations of Ethanol Against *Staphylococcus aureus* on Moist and Dried Threads

Conc. (%)	Exposure of test organism[a]																							
	Moist threads												Dried threads											
	min					h							min					h						
	5	10	15	30	45	1	2	7	24	5	10	15	30	45	1	2	7	24
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+
40	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−
50	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−
60	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
70	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
80	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−
85	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−
90	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−
94	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−
99	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−

[a] +, growth; −, no growth.
Source: Ref. 20.

OCT-17-1995 17:05 FROM SMTC CLIN RSCH & BIO. 513 626 3225 P.03

Table 8 Tuberculocidal Effect of Ethanol Under Various Conditions

Condition	Tuberculocidal exposure	
	Conc. (% v)	Time
In sputum (wet)	100	30 s
	95	15 s
	70	30 s
	50	60 s
In water	100	30 s
	95	15 s
	70	60 s
In sputum (dried)		
Thin layer	70	60 s
	50	60 s
Thick layer	100	>60 min
	95	30 min
	70	>5 min

Source: Ref. 87.

For its outstanding antimicrobial properties propan-1-ol is a common constituent of commercial preparations for skin and hand disinfectants in Europe. At a concentration of 60% (v/v), it is now the standard, the activity of which acts as a reference for every new surgical hand disinfectant to be approved in German-speaking countries [93,94]. Another reason for its frequent employment is its relatively high flash point of 23°C. In many European countries inflammable liquids with a flash point below 21°C are not permitted for general use in hospitals. Therefore, many alcoholic antiseptic solutions contain propan-1-ol to raise the flash point.

Propan-2-ol (syn: isopropanol, isopropyl alcohol), the antimicrobial activity of which ranges between those of ethanol and propan-1-ol (see Tables 4 and 5) also requires the presence of water around the microorganism or in mixture with the alcohol to attain a microbicidal effect [95]. The optimum bactericidal concentration ranges between 50 and 70% (v/v) [39,73,74,82,83,96,97]. Tuberculocidal activity is achieved in 10 min at 40% [85]. In sputum, however, 50–70% is necessary [82,84,98].

Butane- and pentane-derived alcohols are of little importance in this context. Except for the tertiary alcohols, their antimicrobial activity seems to be higher than that of the propanols (see Tables 4 and 5). Their low solubility in water and their offensive smell are obstacles for practical use. Nevertheless, some

commercial preparations contain one or the other as an additive in low concentration.

Allylalcohol and benzyl alcohol are active against *M. tuberculosis* at 20% and in saturated solution (ca. 3.5%), respectively, in 15 min [85].

3. Fungi

The fungicidal activity of short-chain, monovalent alcohols, mainly ethanol, is well documented. As with their activity against bacteria, with methanol higher concentrations (50%) are necessary to kill *Candida* or *Aspergillus* species within 1 min than with ethanol (35%) or with the propanols, which are active in the range of 10–30% [66].

Table 9 summarizes the fungicidal activity of ethanol as reported in the literature. Except for the results of Szerémi [66], which demonstrate an unusually high sensitivity of *Candida* and *Aspergillus* spp. (killing by 35% ethanol in 1 min), the most effective concentrations range is between 70 and 90%, acting in 5–60 min.

4. Viruses

Controversial opinions appear in the literature on the antiviral activity of alcohols. There is, however, general agreement that enveloped, lipophilic viruses are easier to inactivate by disinfectants than are "naked" viruses [104]. This is also true for alcohols.

Among the enveloped viruses the vaccinia virus has been studied extensively for sensitivity to alcohols. As shown in Table 10, methanol is reported to inactivate the virus at concentrations of 80% in 15 min [105], of 50% in 1 h, and of 20% in 24 h [106]: the latter was not possible with ethanol. However, at 50% [106] to 60% [105] ethanol inactivated the virus within 1 h, even under dried conditions [105]. In a suspension of chick embryo material it seems to be much easier to attain inactivation. Only 2, 3, or 10 min were necessary at the respective concentrations of 70% [107,108], 80% [109], 70 or 40% [110,111]. Compared with ethanol, the propanols seem to be more active against this virus, since lower concentrations are needed for the same effect within the same times or even shorter [105,108–111].

Togaviruses such as yellow fever or Eastern equine encephalitis are readily inactivated by otherwise suboptimal concentrations of ethanol, 17% and 50%, respectively, in 30–60 min [113,114]. The effect of propan-2-ol was comparable with that of ethanol.

At an optimum concentration of 70%, Kewitsch and Weuffen [108] found ethanol effective against influenza A in 2 min; within the same time, only 40% of propan-1-ol caused the same effect. With propan-2-ol the virus was inactivated within 10 min at concentrations of 30 [111] and 48.5% [110].

Against the mumps virus, 36% ethanol was effective in 30 min [115], and another paramyxovirus, Newcastle disease virus, was inactivated by 70% ethanol

Table 9 Fungicidal Effect of Ethanol Against Various Fungal Species

Fungus	Minimum effective conc. (%) Exposure time (min) 1	5	30	60	Not stated	Ref.
Yeasts		75				99
Candida albicans	35					66
C. krusei	35					66
Cryptococcus neoformans					70	100
Histoplasma capsulatum[a]					70	100
Blastomyces dermatitidis[a]					70	100
Coccidioides immitis[a]					70	100
Dermatophytes		50				99
Microsporum gypseum			80			39
(spores)			85			101
M. audouinii				70		102
(on naturally infected hairs)					70	109
Aspergillus niger	35					66
Penicillium tardum					90 (70–98)	103
(conidia)						

[a]Tissue and culture phase.

in 3 min [116] or by 25% propan-2-ol in 1 h [117]. Herpes simplex lost its infectivity after contact for 10 min with either 30% ethanol or 20% propan-2-ol [111].

These reports demonstrate that enveloped viruses are readily inactivated by short-chain aliphatic alcohols in due time, if these are used at their optimum concentrations. At least, for the viruses discussed, the same alcohol order range of virucidal capacity applies as that for effect against bacteria: methanol < ethanol < propan-2-ol < propan-1-ol.

An unusual behavior among the lipophilic viruses, however, was reported by Jaeger et al. [118] for the rabies virus which could not be inactivated by ethanol within 30 min. Although adenoviruses do not number among the enveloped viruses, they behave like them in their tenacity. Klein and Deforest [111] found them sensitive to ethanol and propan-2-ol at 50% in 10 min in a suspension test model. Propan-2-ol was reported active against the respiratory syncytial virus at 35% in 1 min [119].

In contrast to the foregoing viruses, naked viruses are much more difficult to inactivate with higher alcohols than with ethanol. Especially the picornaviruses pose considerable disinfection problems (Table 11). Against poliovirus type 1, 70% ethanol was found effective in 2 min [108]. Within the disinfection time of 10

Table 10 Inactivation of Enveloped Viruses by Short-Chain, Aliphatic Alcohols in Vitro

Virus	Alcohol[a]	Minimum effective conc. (%) Exposure times (min) 0.5	2	3	10	15	30	(h) 1	24	Ref.
Vaccina	M					80				105
Dried	E						70	50	20	106
								60		105
Suspension			70					50		106
				80						107,10
					70					108,11
					40					110
	P 1					30	15			111
		40								105
				60						108
	P-2				48.5					109
					30					110
Yellow fever	E						17			111
Eastern equine	E							50		113
Encephalitis	P-2							49.5		114
Influenza	E		70							114
					70					108
					30					110
	P-1		40							111
	P-2				48.5					108
					30					110
Mumps	E						36			111
Newcastle disease	E		70							115
	P-2							25		118
Herpes simplex	E				30					117
	P-2				20					111
										111

[a]M, methanol; P-1, propan-1-ol; P-2, propan-2-ol.

min, which Klein and Deforest [111] have chosen for their suspension test, this concentration was also effective; with only 25%, 240 min was needed for the same effect [120]. With a concentration of 80% (v/v) Steinmann et al. [196] reported increasing reductions of the poliovirus I (Sabin) of 2.5, 3.7, and 4.8 logs after 1, 2, and 5 min, respectively. In contrast, Tyler et al. [200] found, in their suspension tests, that the titer of poliovirus (Sabin 1 an) was reduced with ethanol 70% (v/v) by only 0.4 logs in 1 min. Propan-2-ol was inactive even at 95% [111]. Schürmann

OCT-17-1995 17:05 FROM SMITH CLIN RSCH & DEV 513 626 3275 P.11

Table 11 In Vitro Inactivation of Picornaviruses by Short-Chain, Aliphatic Alcohols

Virus	Alcohol	Minimum effective conc. (%) Exposure times (min) 1	2	4	6	8	10	240	(d) 6	Ref.
Poliovirus type 1	E		70							108
							70			111
								25		120
	P-2						(95[a])			111
	CP[b]				100					121
Coxsackie B	E						60			111
	P-2						(95[a])			111
	CP[b]		100							121
Echo 6	E						50			111
	P-2						90			111
Echo 9 (Hill)	CP[b]					100				121
Echo 11	M	76								122
	E	(76[a])								122
Hoof-and-mouth disease	E								25	123
Hepatitis A	E	(70[a])								124
	P-2	(45[a])								124

[a]Not effective at this concentration.
[b]Commercial preparation of ~90% ethanol, 10% propan-2-ol, and 0.1% tetrabromo-6-methylphenol.

and Eggers [121], who have extensively tested the antiviral properties of a commercial preparation containing 78.2% (w/w) of 95.3% (v/v) ethanol (about 90% v/v), 10% (v/v) propan-2-ol and 0.1% of a tetrabromo-6-methylphenol in a standard suspension test, demonstrated a 4-log reduction of polio type 1 in 6 min, but reported only a 2-log reduction of polio type 2 in 10 min. Coxsackievirus B was inactivated by 60% ethanol, but not by 95% propan-2-ol in 10 min [111]. With the aforementioned commercial product containing mainly ethanol, the titer of coxsackievirus B4 was reduced by 4 logs in 2 min, but with coxsackievirus B3, this reduction could not be achieved in 10 min [121]. To inactivate echovirus 6 in 10 min was possible with 50% ethanol; but not with 90% propan-2-ol [111]. A strain of echovirus 9 was reduced by 4 logs in 8 min by the commercial product mentioned earlier [121]. The authors noted, however, considerable differences between the five strains tested. A strain of echovirus 11 was sensitive to methanol, 76% in 1 min, but not to ethanol, propan-1-ol, propan-2-ol, or butan-2-ol, even

at 90% [122]. Hoof-and-mouth disease virus was not inactivated in 3, but was in 6 days by 25% ethanol [123].

Hepatitis A, now classified as an enterovirus, is known for its high resistance to chemical and physical influences. In a recent study, 20 disinfectants were tested for their capability of inactivating, within 1 min, the virus contained in a fecal suspension and dried on polished, stainless steel disks. Only 2% glutaraldehyde, sodium hypochlorite (dissolved to give 5000 ppm free chlorine), and a quaternary ammonium compound in 23% HCl were effective; however, 70% ethanol and 45% propan-2-ol were not effective.

Among other enteric viruses, rotaviruses behave as enveloped viruses, being increasingly sensitive to methanol upto butan-2-ol (Table 12) [122]. In contrast, astrovirus was sensitive only to high concentrations (90%) of methanol and ethanol [122].

In the past, the hepatitis B virus (HBV) was considered extremely resistant to the action of chemical disinfectants. However, since the experiments of Bond et al. [125] and Kobayashi et al. [126], who treated dried or liquid human plasma containing high-titer HBV with various chemicals or heat, and inoculated it into susceptible chimpanzees, this belief is no longer justified. Both groups found all treatments effective, including 70% propan-2-ol for 10 min and 80% ethanol for 2 min, respectively.

5. Protozoans

In a suspension test, trophozoites of *Toxoplasma gondii* were killed in 3 min by ethanol 30% and by propan-1-ol 20% [127]. But this information is of importance

Table 12 Activity of Short-Chain Alcohols on Enteric Viruses

Alcohol	Minimum effective conc. (%)[a] Virus tested: Rota	Astro	Echo 11
Methanol	>50	90	76
Ethanol		90	(76[b])
Propan-1-ol	40	No effect	No effect
Propan-2-ol	40	No effect	No effect
Butan-2-ol	30	No effect	No effect

[a]Minimum concentration reducing virus titer by at least 4 $\log_{10}$ in 1 min in a suspension test system.
[b]Only 3-log reduction.
Source: Ref. 122.

OCT-17-1995 17:07 P&G SWTC CLIN RSCH & BIO. 513 626 3275 P.12

only for laboratory workers handling these stages of *Toxoplasma*, for instance in Sabin–Feldmann tests. For the really relevant question about the activity of alcohols against the persistent stages of this protozoan, as excreted with cat feces, only little is known. The same applies to cysts of *Entamoeba histolytica* or *Cryptosporidium*, all of which can be transmitted with fecal material by hands. For oocysts of *Toxoplasma gondii*, Duby et al. [128,129] have reported an antiprotozoal activity for 95% ethanol in 1 h and of 20% ethanol in 7 days.

C. Antimicrobial Activity in Vivo

Most results of in vitro experiments on the efficacy of disinfectants and antiseptics offer only little information about the real time–concentration relationships of the antimicrobial action in vivo. Therefore, disinfectants and antiseptics must be tested under conditions simulating practical conditions as closely as possible. For disinfectants of hands and skin, for instance, real skin must be used as a carrier for test organisms if the efficacy against transient flora has to be tested, and for surgical hand disinfection, real hands with their natural resident microbial flora must be employed in tests.

1. Microbial Flora of Hands and Skin

All considerations on the microbial flora of hands and skin must take into account two groups of microorganisms: one group that can be found regularly and usually in great numbers because its members reside in and on the skin as autochthonous flora, and the other groups consisting of the flora that happens to be found there by chance. The first group is generally known as "resident flora," whereas the latter is called "transient flora" [28,29]. However, distinguishing between the two may be difficult [130,131].

The term *resident flora*, as specified very cautiously by Price [28,29], covers microorganisms that not only survive on the skin, but also multiply there. By mechanical means—with or without a brush—they are difficult to remove. To significantly reduce their viable counts, suitable disinfectants, antiseptics, or antibiotics are necessary [131–133]. Washing hands with soap and water only halves the release of skin bacteria every 6 min [28].

The composition of the microflora of the healthy and diseased skin has been described extensively in several reviews [134–141]. There are differences between different parts of the body [142–144], between individuals [143,145], between persons of different ages, and between the sexes [146,157]. Possibly, there also exists an association with the seasons [148].

The preponderant part of the normal, resident flora consists of only a few bacterial species, the pathogenic potential of which is usually considered to be low [149], unless they are introduced into body tissue by trauma or by adhering to foreign bodies such as implants [150,151].

Except for very greasy skin areas, most skin bacteria grow aerobically [142,149]. Most of them belong to the family *Micrococcaceae* or are members of the "diphtheroid" group [133,142,152]. The most prevalent species is *Staphylococcus epidermidis*. Fortunately, *S. aureus*, a well-recognized pathogen, only rarely colonizes the healthy skin, mainly in the perineal region [142]. But also hands, face, and the nape are preferential areas of temporary colonization. The skin of the hands of health care personnel, however, is considerably more often colonized with *S. aureus* [28,153,154].

Lipophilic diphtheroids are located mainly in the armpits [145], nonlipophilic ones usually at all visible skin areas. Gram negative rods, mainly *Acinetobacter*, *Enterobacter*, and *Proteus* spp. can be found in moist regions [133,155]. *Klebsiella* has been reported to colonize hands in a few instances [156].

Of the anaerobic bacteria *Propionibacterium acnes* is the most prevalent organism [134]. It multiplies in skin regions with strong sebum production, but can be found all over the body surface as a transient. Other anaerobic bacteria of the skin are peptococci and *Propionibacterium granulosum* and, in moist regions, *P. avidum* [142,157,158].

The population density of resident skin bacteria ranges between "hundreds and thousands," rather than within "billions" per square centimeter, as sometimes assumed [130,142]. One reason for differing reports in the literature may be that different methods of quantifying the skin flora are used. Nevertheless, from studies with comparable sampling techniques, it is known that intertriginous regions are the most densely populated, and forehead, face, nape, and soles are the next most densely colonized skin regions of the human body [143,144]. The individual population density at a site remains remarkably unchanged over a long time [28,143,159]. Long-term changes occur only with skin diseases and as a consequence of forceful interference with the biocenosis by antibiotics, disinfectants, or antiseptics [132,136,145,160]. The strongest short-term (1–2 h) fluctuations can be observed after intensive contact with water, as with bathing or showering [143,161].

As opposed to former assumptions, the deep layers of the epidermis are not colonized by a microbial flora. Except for the anaerobe propionibacteria, which reside in the infundibula of the sebaceous glands, most skin bacteria colonize only the uppermost layers of the stratum corneum [145,162]. There, they are embedded in the pars disjuncta of keratinized epithelial cells, a mass of lipids and cell detritus [143,157,163]. Probably, the fastest microbial multiplication takes place in the upper parts of the hair follicle, which was also demonstrated by electron microscopy [164]. In contrast, the ducts of eccrine and apocrine sweat glands of the healthy skin are usually sterile [142].

Members of the transient flora are all microorganisms that do not multiply in or on the skin, but are found there by chance after contamination. Therefore, pathogens may also be encountered. Usually, transient bacteria do not survive

OCT-17-1995 17:03 P&G SWTC CLIN RSCH & BIO. 513 626 3275 P.13

very long on healthy skin [165]. Although the reasons for this are not completely understood, it is generally accepted that possible mechanisms for the colonization resistance of skin could be explained not only by the inhospitable environment resulting from physicochemical conditions, but also by several active self-defense mechanisms [130,166]. The most important factors for colonization control by the skin are (a) free fatty acids liberated from skin lipids by bacterial metabolism—mainly by *P. acnes*; (b) the low water content of the stratum corneum; and (c) the production of bacteriocins and other antibioticlike inhibitors by the resident flora [130,149,167,168]. In contrast, pH and osmotic conditions are of little importance [148]. From the foregoing, the protective role of the autochthonous skin flora against infection becomes evident [169].

The transient flora is easily removable by mechanical means [179]. A simple handwash with soap and water for 1 min reduces the numbers released from the hands by two to three orders of magnitude [59,171,172]. Rubbing hands with water alone is nearly as effective [170,171].

2. Principles of Assessment

Various approaches are possible to assess the effectiveness of alcohols as hand and skin disinfectants. One may, for instance, try to directly assess the influence of using alcohol for hand disinfection on the ratio of nosocomial infections. This seems to be a difficult undertaking, as the frequency of nosocomial infections is influenced by many factors other than merely hand transmission of pathogens. In addition, it would also be a very uneconomical method of assessment: To demonstrate a statistically significant reduction of hand-transmitted infections by 50% from 2 to 1% (at $\alpha = 10\%$, directional, and a testing power of 90%) one would have to observe approximately 2500 patients in each of the treatment and control groups [173].

One could also assess the efficiency of alcoholic handrubs to prevent the transfer of pathogens by measuring their effect on the frequency of indicator bacteria. Again, this is tedious and prone to be influenced by other factors that are difficult to control.

Another in vivo test can be done by either in-use tests or by employing a model that simulates practical conditions in the laboratory. With both, the reduction of the release of organisms from the hands or skin is assessed. With the former, many variables are difficult to control, whereas with the latter, employing volunteers, the antimicrobial efficiency of alcohols can be measured in a standardized and well-controlled manner. Therefore, several models that make use of this possibility have been described [93,94,153,170,173–182].

3. Test Models

In test models of hand disinfection, hands are more realistic test objects than is animal skin. Therefore, laboratory tests with volunteers are preferred. Furthermore, when testing hand disinfectants, the purpose of the antiseptic measure has to be taken into consideration. If the elimination of transient organisms is important, as for example, with health care personnel trying to prevent transmission of pathogens from one patient to the next, a model with artificial contamination should be used to assess the effect of the disinfectant on the release of transient organisms. This type of handcare is known as "postcontamination treatment of hands" and can be carried out either as a "hygienic handrub" (in German-speaking countries—"hygienic hand disinfection") or as a "hygienic handwash" with disinfectant–detergents. An effect against the resident flora is usually not necessary, or even desired, nor is it a sustained effect. If, however, the suitability of a surgical handrub or scrub is to be tested, the reduction in the release of resident skin bacteria must be assessed. Because a sustained action might also be required under a surgeon's glove, this can simultaneously be tested with the split-hand model (one hand gloved for a specified time), as suggested by Michaud et al. [183].

Test models for either type of hand treatment should assess the number of viable bacteria released from the hands, both before and after treatment, to estimate the "degerming" efficacy of the treatment by using the ratio of both values as a measure. In addition, the test model must allow for individual attributes of the volunteers that may influence the test results (e.g., size of the hands and skin quality), as well as the way the treatment is performed. Therefore, it was suggested by Rotter and co-workers [59,174,179,182] that each volunteer acts as his or her own control by performing a standard disinfection procedure in the same test. If, at the same time, the efficacy of the standard disinfection has been chosen such that it may serve as a break-point for the efficacy of hand treatments under test, then the results of evaluation in different laboratories or with different groups of volunteers are rendered homogeneous [179]. At present, for testing hygienic hand disinfection procedures, only the Vienna model [59,173–175,179], which was adopted by the Austrian [93] and German [94] societies for hygiene and microbiology as an official test, and the Birmingham model [172] compare against a parallel standard disinfection procedure. For testing surgical handrubs or scrubs this principle is included in only the official test models of the Austrian [184] and German [94,181] societies and in a test proposed recently by the Birmingham group [181].

VI. ALCOHOLS USED IN SKIN ANTISEPSIS

Together with the information on the principles of assessing the efficacy of hand disinfectants and skin antiseptics, as outlined in the foregoing, the following explanations are intended to summarize the present knowledge on the efficiency of alcohols in hand and skin antisepsis.

OCT-17-1995 17:03 P&G SWTC CLIN RSCH & BIO. 513 626 3275 P.14

A. Hygienic Hand Disinfection

The objective of hygienic hand disinfection is to render hands safe after contact with dangerous pathogens. This may, possibly, be accomplished by a simple handwash with soap or with a disinfectant–detergent (hygienic handwash). To prevent dissemination of the pathogens during and by the wash process itself, inactivation of microorganisms should take place while they are still on the hands. Therefore, procedures that include rubbing a disinfectant on to the hands (hygienic handrub) are to be preferred. Furthermore, as time is usually scarce for postcontamination treatments, only fast-acting chemicals are suitable.

As can be seen from Table 13, alcohols are very fast-acting agents, at least against bacteria. When rubbed onto artificially contaminated hands they reduce the release of test bacteria within 30–60 s by 2.60–5.78 log, depending on the type of alcohol and the concentration used. Longer exposure times, such as 2 to 4 min, do not greatly improve effectiveness.

Although the results obtained by various authors are comparable when similar test methods are employed, the association of effectiveness with the type of alcohol, the concentration used, and the exposure time are better demonstrated by tests with a homogeneous technique. In Table 14, results obtained with the Vienna model are summarized. All data stem from the author's laboratory, ensuring the greatest technical homogeneity possible. In addition, comparisons can be made on the effectiveness of procedures for hygienic handwash. The table demonstrates that

1. There is a clear order range for the effectiveness of the compared alcohols: propan-1-ol > propan-2-ol > ethanol.
2. The efficacy is also associated with the concentration used.
3. A handrub with povidone–iodine aqueous solution is not significantly less effective than with propan-2-ol 60% (v/v), the performance standard for hygienic handrubs in several European countries.
4. Each of the hygienic handwash procedures tested is significantly less effective than the alcohol standard.
5. Of all hygienic handwash procedures tested, only that with povidone–iodine liquid soap meets the requirement of performing significantly better than soft soap, the control treatment [182].

These results clearly demonstrate that, in comparison with other methods of postcontamination hand treatment, alcoholic handrubs are now the most efficient measure to quickly reduce the release of transient flora from the hands. Consequently, it may be inferred that, after contamination, alcohol-treated hands are less likely to transfer bacteria than soap-washed hands. Indeed, this has been shown by Ehrenkranz and Alfonso [193], who demonstrated that, after contact with heavily colonized patients' groins, handwashing with soap failed to prevent the transfer of

TABLE 13 Efficiency of Short-Chain Alcohols to Reduce the Release of Various Test/Bacteria From Artificially Contaminated Hands at Various Concentrations and Exposure Times in Hygienic Handrubs[a]

Alcohol	Conc. (%v/v)	Test/Bacterium	Mean log reduction: Exposure time (min) 0.5	1.0	2.0	4.0	Ref.
Ethanol	60	*Escherichia coli*		3.8			92
	70	*E. coli*	2.6–2.9				186
			3.4	4.1			177
			3.6	3.8	4.5		82
				4.0			179
				4.3	4.9	5.4	171
				4.3	5.1		185
		Staph. saprophyticus	3.5				177
				4.0			187
		Staph. aureus	2.6				188
			3.7				177
	80	*E. coli*		4.5			92
Propan-2-ol	50	*E. coli*	3.4	3.9	4.4		92
	60	*E. coli*		4.0			182
				4.2			189
				4.3			190
				4.4			179
				4.7			185
		Serratia marcescens		4.1			191
	70	*E. coli*	3.4–3.6				186
				4.8			192
				4.9			92
Propan-1-ol	40	*E. coli*		4.3			92
	50	*E. coli*	3.7	4.7	4.9		92
				5.0			92,179
	60	*E. coli*		5.5			92
	100	*E. coli*		5.8			91

[a]Tested with comparable methods.

aerobic gram-negative bacilli by health care workers' hands to urinary catheters in 11 of 12 experiments, but when isopropanol (propan-2-ol) 70% (v/v) was used, transfer occurred in only 2 of 12 experiments. Furthermore, soap failed to prevent subsequent colonization in each of the 12 experiments, alcohol in 5. The risk ratio was calculated with 2.4 (95% confidence interval: 1.2–21.7). From this, the authors concluded that soap was generally ineffective in preventing hand transfer

TABLE 14 Comparison of the Effectiveness of Various Degerming Procedures to Reduce the Release of *E. coli* from the Artificially Contaminated Hands as Assessed by the Vienna Model

Procedures (1 min each)	Conc. %	Mean (N = 12–15) log reduction	Remarks
Hygienic handrub with			
Propan-1-ol	100	5.8	
	60	6.5	
	50	5.0	
	40	4.3	
Propan-2-ol	70	4.9	
	60	4.0–4.3	Standard
	50	3.9	
Ethanol	80	4.5	
	70	4.0	
	60	3.8[a]	
Povidone–iodine sol	1 w/w	4.0	
Hygienic handwash with			
Povidone–iodine liq. soap	0.75 w/w	3.5[a,b]	
Chlorhexidine gluconate liq. soap	4.0 w/w	3.1[a]	
Triclosan liq. soap	0.1 w/w	2.9[a]	
Phenol derivatives liq.soap	2.0 w/w	2.6[a]	
Soft soap Eur. Pharmacop.	20 w/v	2.7[a]	Comparative basis

Con., concentration in % w/v unless otherwise stated.
[a]Significantly less effective than standard for hygienic handrub.
[b]Significantly more effective than comparative basis for hygienic handwash.
Source: Refs. 59, 182.

of gram-negative bacilli to catheters following contact with a heavily contaminated source, whereas alcohol was generally effective.

Except for their tuberculocidal activity, the bactericidal effectiveness of alcohols on the skin does not greatly differ among the bacterial species [64,177]. Ayliffe et al. [177], for instance, found the following log reductions with 70% ethanol: *E. coli* 3.4; *S. epidermidis* 3.5; and *S. aureus* 3.7. Rotter and Koller [65], using propan-2-ol 60% (v/v) observed, in one experiment, log reductions for *S. aureus* of 4.9 and for *E. coli* of 4.8.

As reported previously, alcohols are tuberculocidal. The range of order describing their antibacterial activity is the same as that for other bacteria. Possibly, longer exposure times are required [84]. Unfortunately, exact information

on practical conditions is lacking as, to our knowledge, experiments with *Mycobacterium tuberculosis* or *M. bovis* on the hands have not yet been carried out. Models with other mycobacteria, such as *M. aquae* [194] and *M. avium*, *M. terrae*, or *M. smegmatis* [195], have been proposed. Present recommendations for specific methods of hand disinfection, however, are based on results of in vitro experiments. Usually, they include a disinfection time of 1–5 min with 70% ethanol, 60–70% propan-2-ol, or 50–70% propan-1-ol [65].

The antiviral activity of alcohols in vivo has been studied on the model of artificially contaminated hands or fingertips by several groups. One finding of these studies was that the effectiveness of alcohols against some "difficult" viruses, such as entero- and rotaviruses is significantly better than that of handwashing with soap and water [196,199]. Steinmann and co-workers [196] have shown, for instance, that disinfection with absolute ethanol or with propan-1-ol reduces the release of poliovirus from the contaminated hands approximately 100 times more efficiently than a social handwash with soap and water, a postcontamination procedure generally recommended (Table 15). It is notable, however, that the alcoholic handrubs tested take 10 min. Absolute ethanol, which produced a reduction of viral release of 3.2 log, was more effective than 80% (v/v) ethanol (2.2), absolute propan-1-ol (3.0) or absolute propan-2-ol (2.4). In contrast, with an individual handwash of 10–55 s, a reduction of only 1 log was observed.

Schürmann and Eggers [121], who have extensively investigated methods of testing procedures for virus disinfection of hands, found enveloped viruses, such as fowl plague, influenzae A, or the complex vaccinia virus completely inactivated

TABLE 15 Reduction of the Release of Poliovirus Type 1 (Sabin) from Artificially Contaminated Hands by Handrubs[a] with Short-Chain Aliphatic Alcohols and With Social Handwash[b]

Alcohol	Conc. (% v/v)	Mean log reduction[c]
Ethanol	100	3.2
	80	2.2
Propanol-2-ol	100	2.4
Propanol-1-ol	100	3.0
Handwash		1.1

[a]Five consecutive 2-min rubs with 1.4 ml each totaling 10 min with 7 ml.
[b]Free-style handwash with soap and tap water (10–55 s)
[c]log ID_{50} recovered from untreated hands minus log ID_{50} recovered from disinfected hands.
Source: Ref. 196.

OCT-17-1995 17:11 F&G SWTC CLIN PSCH & BIO. 513 626 3275 P.16

on the hands and fingertips when rubbing a commercial preparation of ethanol (90% w/w) plus propan-2-ol (8% w/w) and tetrabrome-6-methylphenol (0.1%) onto the hands (Table 16). Before this, the vaccinia virus had already been shown to be completely inactivated on the skin by 70% propan-2-ol alone, within 5 min [197]. In the foregoing tests, naked viruses were more slowly inactivated, and inactivation was usually incomplete after 10 min. From their results the authors concluded that the high-alcohol–content preparation tested is effective against naked viruses, such as enteroviruses, provided the surrounding conditions (high temperature, large disinfectant/virus volume ratio, low protein load) are favorable [121].

Hendley et al. [198], who investigated the effectiveness of handrubs—liquids or foams—in preventing the transmission of rhinovirus in nasal secretions by direct hand-to-hand contact, found that alcohols were only moderately active when used alone (Table 17). In combination with hexachlorophene or benzalkonium chloride and applied as a foam, ethanol proved more effective, even in a concentration as low as 50% (w/w). The most effective agent, however, was iodine in ethanol or water.

Data about the antiviral activity of alcohols on the skin are also available for the human rotavirus (Table 18) confirming the favorable results of tests in vitro (see Table 12). Propan-2-ol and ethanol, both at 70% (v/v), bring about a reduction

TABLE 16 Reduction of the Release of 11 Viruses from Artificially Contaminated Hands or Fingertips by Handrub[a] or Fingerrub[b] with an Alcoholic Commercial Preparation[c]

	Log reduction[d]			Log reduction[d]	
Virus	Hand	Fingertips	Virus	Hand	Fingertips
Fowl plague	>2.5		Coxsackie B3	1.1	2.9
Influenza A/WSN	>2.5		Coxsackie B4	1.3	3.0
Vaccinia MVA	>1.5		Echo 9 Hill	0.7	2.6
Adeno 5	2.1	>2.3	Echo 9 Barty	1.3	2.2
Polio 1	1.0	2.5	SV 40	0.9	1.8
Polio 2	0.2	0.7			

[a]Five consecutive 2-min handrubs with 1.4 ml each, totaling 10 min with 7 ml.
[b]Five consecutive 2-min fingerrubs with 30 µl/finger, each totaling 10 min with 150 µl/finger.
[c]Commercial preparation of ~90% ethanol, 10% propan-2-ol, and 0.1% tetrabromo-6-methylphenol.
[d]Log virus titer recovered from water-treated hands (fingers) minus log virus titer recovered from disinfected hands (fingers).
Source: Ref. 121.

TABLE 17 Reduction of the Release of Rhinovirus 29 and 39 from Artificially Contaminated Fingertips by Handrubs[a] with Alcoholic or Alcohol-Based Liquids or Foams

	Mean log reduction[b]	
	Rhinovirus	
Preparations and concentrations (%w/w)	29	39
Liquids		
Ethanol, 70%		1.5
Ethanol, 70% in towels		1.8
Ethanol, 79% + propan-2-ol, 10%		2.0
Ethanol, 62% + benzyl alcohol, 3%		2.0
Ethanol, 62% + benzyl alcohol, 6%	0.7	
Ethanol, 10% (placebo)	0.1	1.1
Water		0.8
Foams		
Ethanol, 50% + hexachlorophene, 0.23%		2.7
Ethanol, 50% + benzalc. chlor. 0.2%	1.9	2.5
Ethanol, 10% (placebo)	0.8	

[a]1 ml of liquid or 3-cm–diameter ball vigorously rubbed over the hand and allowed to dry completely.
[b]Geometric mean log ID_{50} recovered from fingertips of untreated hands minus geometric mean log ID_{50} recovered from fingertips of disinfected hands.
Source: Ref. 198.

of viral release from contaminated hands and fingers that is approximately 100 times that of tap water [199].

As opposed to their antiviral activity on skin, alcohols are not sufficiently effective against viruses, such as hepatitis A [124], polio [200], or rhinovirus [201] on hard surfaces, such as steel, glass, or tiles, respectively.

B. Surgical Hand Disinfection

The objective of surgical hand disinfection is the removal of all the readily detachable transient flora and as many of the resident flora as possible to reduce the release of skin bacteria from the surgical team's hands to a safe level for the duration of the surgical operation, should the glove be punctured or broken. Therefore, not only a strong immediate, but also a continuing, antibacterial effect is desirable. Some agents without residual activity, such as alcohols may, however, exert an immediate effect strong enough to cause a long delay in regrowth of the skin flora. Consequently, their activity is also long-lasting [202,203].

OCT-17-1995 17:11 FROM SMITH CLIN RSCH & BIO. 513 626 3275 P.17

TABLE 18 Reduction of Human Rotavirus from Artificially Contaminated Fingertips by Combined Handrub/Wash[a] or Fingertip Treatment[b] with Alcoholic or Alcohol-Based Products and Other Agents [199]

Preparations and concentrations (%)[c]	Mean log reduction[d]	
	Hand	Fingertips
Propan-1-ol, 70%	3.0	2.7
Ethanol, 70%		2.7
Ethanol, 70% + CHG, 0.05% + CETR, 0.5%	2.0	2.1
CHG, 0.008% + CETR, 0.075% in water	0.8	0.7
Liquid soap 10	1.2	0.9
Tap water	0.8	0.8

CHG, chlorhexidine gluconate; CETR, cetrimide.
[a]0.5 ml of preparation vigorously rubbed over the hand for 10 s, then washed with 500 ml of warm tap water, dried with paper towel.
[b]Vial containing 1 ml of preparation placed on fingertip and inverted ten times within 10 s followed by scraping fingertip on inside rim of vial and rinsing with 15 ml tap water for 5 s.
[c]Alcohol concentrations (% v/v), other concentrations (% w/v).
[d]Log virus titer recovered from untreated hands (fingers) minus log virus titer recovered from disinfected hands (fingers).
Source: Ref. 199.

Although evidence that surgical hand disinfection is really necessary is lacking from epidemiological data, it is a logical consequence of the concept of asepsis in surgery. Indirect evidence, however, has been produced by Furuhashi and Miyamae [204] demonstrating that 10^3–10^4 colony-forming units of skin bacteria escaped through the pinholes of experimentally punctured gloves during a period of 5 min when hands had been scrubbed earlier without a disinfectant. However, when the hands and forearms had been brushed with a disinfectant, bacterial counts were no more than 100, even 3–4 h after handwashing. As skin bacteria are known to cause infections, especially in operations involving prosthetic surgery, it is sensible to prevent them from entering the surgical wound.

There are three basic techniques for reducing the skin flora: surgical handwash, surgical handrub, and a combination of both, employing them consecutively. The effects of these techniques are markedly different (Tables 19–21).

Whereas, depending on the preparation and the time taken, surgical handwashes with unmedicated soap or disinfectant–detergents cause immediate 0.2–1.3 log reductions in the bacterial release (see Table 19), with handrubs, applying alcohols or a povidone–iodine aqueous solution, log reductions of 0.6–2.9 can be

TABLE 19 Efficacy of Surgical Handwash on Resident Skin Flora from the Hands

Preparation	Conc. (%)[b]	Time (min)	Mean log reduction after surgical handwash[a]		Ref.
			Immediate	3 h	
Unmedicated soap		5	0.4	−0.1	28,35,192
		4	0.2		204
		2	0.06	0.09	202
Povidone–iodine detergent	0.75	6	0.9		204
		5	0.9–1.1	0.2–0.3	35,190,192
		2	0.5–0.6		204,205
Chlorhexidine gluconate detergent	4.0	6	1.3		204
		5	0.9	0.9	35,192
		3	0.8–1.0	0.8–1.0	35,190,192
		2	0.9	1.5–1.6	202,206
Hexachlorophene detergent	3.0	4	0.3	1.0	183
Triclosan detergent	1.0	5	0.6	0.5	35
Benzethonium detergent	10.0	6	1.3		204
		3	1.0		204
		2	0.9		204
Cetrimide detergent	1.0	2	0.4		205

[a]Log pretreatment counts minus log posttreatment counts.
[b]All alcohol concentrations are % v/v, all other agents are % w/v.

achieved (see Table 20). Thus, the minimum and maximum effectiveness of both techniques differ by factors of 2.5–40, respectively. For the range of achievable sustained effects, reductions observed with surgical handwash techniques were −0.1–1.6 log, or by handrubs 0.6–2.7 log; therefore, handrubs were 5–13 times more effective.

With sequential techniques of surgical hand treatment both strong immediate (1.7–2.9 log) and sustained (1.1–3.0 log) effects were observed by several authors (see Table 21).

A more detailed analysis of Table 19 reveals that, in comparison with the poor effects (log reductions 0.06–0.4) of unmedicated soap [28,35,192,202,204] or cetrimide [295], a surgical handwash with a disinfectant–detergent containing povidone–iodine [35,190,192,204,205], chlorhexidine gluconate [35,190,192, 202,204,206], or benzethonium chloride [204] causes stronger immediate effects of 0.5–1.3 log. With hexachlorophene [183] and triclosan [35], as well as with chlorhexidine gluconate-containing detergents, sustained effects, which differ between 0.5 and 1.6 log from the baseline counts, are clearly demonstrated.

TABLE 20 Efficacy of Surgical Handrub on Resident Skin Flora from the Hands

Preparation	Conc. (%)[b]	Time (min)	Mean log reduction after surgical handrub[a] Immediate	3 h	Ref.
Propan-1-ol	60	5	2.5–2.9	1.6–2.4	192,207,215
Propan-2-ol	70	5	2.4	2.1	208
		3	1.8	1.2	209
		2	1.2	0.8	202
		<1	0.8		213
	60	5	1.7	1.0	190
	50	2	0.7		204
Ethanol	88	2	2.1		210
	60	5	2.5		211
		2	1.5		212
	70	5	2.0	1.2	35
		2	0.6–1.0	0.6	202, 204
Povidone–iodine sol.	1.0	5	1.9	0.8	189
		2	1.0	0.4	202
Ethanol + chlorhexidine gluconate	70 0.5	2	1.0–1.4	0.7	202,204
Ethanol + chlorhexidine gluconate	90 0.5	2	1.1		204
Propan-2-ol + chlorhexidine gluconate	70.5	5	2.5	2.7	208
		4	1.7	1.1	207
		2	1.5	1.0	202
		<1	0.8	1.2	213

[a]Log treatment counts minus log posttreatment counts.
[b]All alcohol concentrations are %v/v, all other agents are %w/v.

Of the alcohols tested for effectiveness in the surgical handrub, propan-1-ol has long been recognized as the most efficient [11,22,58,90]. This has also been demonstrated by more recent studies [192,207,215]. A 5-min rub with this alcohol at 60% (v/v) can be expected to reduce the release of skin bacteria by 2.5 to 2.9 log. Because of this strong immediate activity, the difference from pretreatment counts is still high (1.6–2.4 log) after 3 h. Propan-2-ol is also a powerful agent for handrubs, but less active than its isomeric alcohol. Ethanol is the least active of the three, but is still more effective than any of the disinfectant–detergents shown in Table 19.

The only nonalcoholic agent with similar efficacy and acceptable skin tolerance is an aqueous solution of povidone–iodine. Its effect is comparable with that of 60% (v/v) propan-2-ol, and similarly it has no residual effect [189,202].

TABLE 21 Efficacy of Sequential Surgical Hand Treatments on Resident Skin Flora

Preparation	Conc. (%)[b]	Time (min)	Mean log reduction after sequential treatment[a] Immediate	3 h	Ref.
Propan-1-ol[d] (comparison)	60	5	2.6	1.1	214
Liquid soap[c]		2	1.8	1.1	214
Propan-1-ol[d]	60	5			
Liquid soap[c]		3	1.7	1.1	207
CHG in propan-2-ol[d]	0.5 + 70	4			
CHG in detergent[c]	4.0	3	2.5	1.7	207
CHG in propan-2-ol[d]	0.5 + 70	4 (5)	(2.9)	(2.2)	
CHG in detergent[c]	4.0	3	2.5	3.0	204
CHG in ethanol[d]	0.5 + 90	1			
Benzethonium chloride in detergent[c]	10.0	3	2.5	3.0	204
Benzethonium chloride in ethanol[d]	0.5 + 90	1			
Benzethonium chloride in detergent[c]	10.0	3	2.1	3.0	204
CHG in water[d]	0.02	1			

[a]Log pretreatment counts minus log posttreatment counts.
[b]All alcohol concentrations are %v/v, all other agents are %w/v. CHG, chlorhexidine gluconate.
[c]1st phase: scrubbing with c.
[d]2nd phase: rubbing on d.

Mixtures of alcohols and agents with a real sustained action, such as chlorhexidine gluconate, provide the rapid effect of the former and the residual activity of the latter. Acceptance of some preparations, however, may be a limiting factor because of alleged skin damage.

The principle of combining the effects of both groups of agents can also be applied by using them consecutively in two phases. From the work of Lilly and associates [216], it is known that the alcohol must always follow the antiseptic handwash to obtain best results; but, from Table 21, it is evident that a handwash with unmedicated soap directly preceding the application of alcohol may impair the effectiveness of the latter [214]. Otherwise, two-phase surgical hand treatments ensure strong immediate and long-lasting activity if suitable preparations are chosen [204,207]. Disadvantages may be the longer duration of the total procedure and a higher risk of adverse skin reactions.

An indication for a combined surgical handwash–rub is probably the first

hand preparation for the day's surgery, and when a handwash is necessary or desired between and during surgical operations (e.g., when gloves are changed), a surgical handrub with alcohol is the method of choice for both its effectiveness and time-saving.

To summarize the knowledge on the suitability of alcohols for surgical hand disinfection, it seems evident from the foregoing that alcohols are now the most effective chemicals in reducing the release of skin flora from the hands of the surgical team.

C. Skin Disinfection

The aim of skin disinfection is the quick removal and killing of as many of the skin flora as possible at the site of a planned invasive procedure, to prevent their translocation into the underlying tissues by a knife blade or a needle. This measure should be distinguished from procedures intended to treat skin areas for colonization with potential or true pathogens, a topic that will not be covered in this chapter.

In terms of strategies, preoperative skin disinfection differs from hand disinfection in that the aim is to hit all microorganisms in and on the skin, regardless of how easily they are released.

Thus, their actual number is of interest, rather than the number of detachable microorganisms. This causes a technical problem for their representative enumeration, unless biopsy techniques are used. Selwyn and Ellis [149] have shown that the sensitivity of most other sampling procedures was 0.5% and less than that of their biopsy method. Unfortunately, taking skin biopsies cannot be considered a routine sampling technique in tests for skin disinfectants. Therefore, many authors employed variations of the cylinder scrub method [147,218,219,220]. Others, such as Lowbury and the Birmingham group [205,210,216,221,222] used the skin of the hands and tested for effectiveness against the resident flora there. Evans and Stevens [157], investigating the flora of normal skin and the efficacy of alcohol disinfection of the antecubital fossa, the palm, and the forehead, produced their results by the combined use of the cotton swab and scrub methods [137,159,223]. Also, when using a combination of two consecutively employed sampling techniques—a modification of the detergent scrub method of Williamson and Kligman [220] and the cyanoacrylate method of Holland et al. [224]—Hartmann found a way to assess the bacterial population density in two skin compartments of different depth [225,226] and states that his technique could replace skin biopsies. Leyden and co-workers [227] proposed to test topical antimicrobial agents on the skin of the forearm after stimulating bacterial multiplication by an occlusive dressing.

From Table 22 it can be determined that on the finger surface, including the nails and the subungual spaces, a 2-min rub with 70% (v/v) ethanol produces a slight, but definite, reduction of resident flora by 0.5 log, when compared with a

TABLE 22 Efficacy of Various Treatments on Skin in Reducing the Content of Skin Bacteria at Various Body Sites and Assessed with Different Methods

Site	Preparation	Conc. (%)a/ time (min)	Mean log reduction								Ref.
			Aerobic skin flora					Propionibacterium			
			Hand[b]	Biop	Swab	Scrub	CAM	Swab	Scrub	CAM	
Hand	Liquid soap	—/2	0.03								210
	Ethanol	70/2	0.5								153
	+ iodine	1	0.7								153
	+ CHG	0.5	0.7								153
	Ethanol	96/2									210
	+ CHG	0.5	1.8								
	Lugol	/2	0.5								153
	CHG aqueous	0.5/2	0.4								153
			0.2								221
Operation site	Ethanol	70/0.75									
	+ iodine	1.5		1.3		2.4					149
	+ CHG	0.5		0.9		2.2					149
Palm	Ethanol	70/4			2.3			3.0			223
Forehead	Ethanol	70/4			1.3	0.5		0.9	0.3		223
	Propanol-2	60/5				0.8	0.4		0.5	—0.1	226
	Propanol-1	60/5				2.1	1.4		0.8	0.4	226
	Povidone–iodine solution	10/5				1.8	0.4		1.3	0.3	226

[a]All alcohol concentrations are %v/v, all other agents are %w/v.
[b]Bowl-sampling of fingers [222].
CAM, cyanoacrylate-sampling; Biop, biopsy; CHG, chlorhexidine gluconate.

JUL-17-1995 11:13 P&G SWTC CLIN RSCH & BIO. 513 626 2242 P.22

handwash with liquid soap [153,210]. This result can be slightly improved, by the addition of iodine (1%) or chlorhexidine gluconate (0.5%), to 0.7 log [153]. However, only an increase in alcohol concentration to 95% led to an augmentation of effectiveness worth mentioning, namely, to 1.6 log [210]. With nonalcoholic antiseptic solutions, such as Lugol's or an aqueous chlorhexidine gluconate (0.5%) solution, only marginal antimicrobial effects were observed [153].

These results cover only the effects of antiseptics on the skin of a very small and sebum-free part of the human body. Therefore, they may not be representative for regions typical for injections or for surgical operations. Furthermore, in these studies, the effect on the bacterial counts released from the skin, rather than actually residing there, was assessed. In contrast, Selwyn and Ellis [149] have shown, at various surgical sites, that combinations of 70% ethanol with iodine (1.5%) or chlorhexidine gluconate (0.5%) both act equally well on the surface: as found by scrub-sampling, the respective reductions were 2.4 and 2.2 log. However, biopsy samples lead to the conclusion that ethanol plus iodine causes a more pronounced effect (log reductions: 1.3 vs. 0.9).

In distinguishing between the more superficial flora, detachable by swabbing, and the anaerobic propionibacteria residing in the infrainfundibula of sebaceous glands and detectable by scrubbing, Evans and Mattern [223] showed that 70% ethanol within 4 min may considerably reduce the aerobic flora and propionibacteria from the palm. As there are no sebaceous glands, propionibacteria do not multiply on the palms, but exist there as superficial transient flora stemming from other skin regions. On the forehead, however, the deep flora, staphylococci or propionibacteria, were scarcely reduced in number, as demonstrable by the scrub technique (log reductions 0.5 and 0.3, respectively). From the more superficial layers sampled by swabbing, they were reduced by 0.9 and 1.3 log, respectively.

Hartmann's results [226], finally, confirm these findings and what has been said about the antimicrobial effectiveness of various alcohols; namely, that propan-1-ol is more active than propan-2-ol. His investigations also clearly show that disinfection of skin in regions of strong sebum production is difficult and that to attain sterility of the skin is impossible. Furthermore, interestingly, the reduction of more superficial propionibacteria is more readily achieved with povidone–iodine solution than with alcohol. As found with the cyanoacrylate technique, propionibacteria in the depth can seldom, or never, be attacked successfully by a single disinfection attempt. On the contrary, as a consequence of alcohol treatment a high rate of bacterial multiplication has been observed [226]. This was interpreted as a rebound effect attributable to the lipolytic action of the alcohol, which provoked an increased sebum production. However, the presence of salicylic acid and phenol, as contained in the isopropanol base of Fabry's tincture, obviously prevented this phenomenon and led to an increasing reduction of the bacterial skin population with long-lasting effect [226].

To make the best use of alcohols in skin disinfection, they should be applied by rubbing them onto the skin, applying a minimum pressure, since friction increases their antibacterial effect [228]. Whereas, for instance, chlorhexidine gluconate in 70 or 95% ethanol applied by gauze led to bacterial reductions of 1.0 and 1.2 log, the respective effects with the same preparations using the gloved hand as applicator were 1.9 and 3.0 log. In contrast, alcoholic sprays have relatively little effect [229].

When long-term effects of antiseptic skin care are needed, alcohols may not be the best choice. This applies to both treatment for carrier status or for colonization with unwanted or pathogenic organisms and for prevention of these conditions. For the prevention of vascular catheter-related septicemias in an intensive care unit, Maki and co-workers [230] compared the effect of three antiseptics for disinfection of patients' central venous and arterial catheter sites before insertion and for site care every other day. The use of aqueous chlorhexidine gluconate (2% w/v) was associated with the lowest incidence of local infection (2.3%), whereas with 70% (v/v) propan-2-ol and povidone–iodine solution, infections occurred in 7.1 and 9.3%, respectively; catheter-related bacteremias were seen in 0.5 versus 2.3 and 2.6%, respectively. These findings indicate that for this indication, the strong residual action of chlorhexidine gluconate on the skin flora in the vicinity of the catheter insertion site may be more important than the undoubtedly stronger immediate effect of alcohol, which does not possess residual bacteriostatic activity.

Alcohols for preoperative skin disinfection and for use before injections must be free of bacterial spores. This is usually achieved by filtration. In some countries it is routine to add 0.5% hydrogen peroxide to ethanol. This is reported to inactivate bacterial spores in the alcohol within 24 h [75,231].

To kill spores on the skin surface, for instance, before amputation of a malperfused leg, skin disinfection has been proposed with ethanol or propan-1-ol, with added 0.2% peracetic acid. This was reported to augment considerably both virucidal and sporicidal activity of alcohols in vivo [232]. On cadaver skin, 0.2% peracetic acid in 33% propan-1-ol has reduced spores of *Clostridium perfringens* by 3.0 log within 2 min [233].

VII. TOXICOLOGICAL ASPECTS

If disinfectants are used according to their intended purpose, only contact and inhalatory exposure need be considered. Oral toxicity of alcoholic disinfectants or antiseptics may be a problem in countries where alcohol (ethanol) is prohibited by religion or law, or when it is used with suicidal intent.

For most alcohols used in disinfection, standards exist for occupational exposure limits. Some of these are listed in Table 23.

OCT-17-1995 17:14 F&G SWTC CLIN RSCH & BIO. 513 626 3275 P.21

Table 23 Occupational Exposure Limits of Some Standards for Various Alcohols

Alcohol	Type of limit	Concentration (ppm)	Concentration (mg/m³)		Standard OSHA	ACGIH	NIOSH	DFG
Methanol	TWA/MAK	200	262		s	s	s	s
	STEL	250	328		•	•	•	
	MAK-ST	400	524	(30 min, 4×)				•
Ethanol	TWA/MAK	1000	1880		•	•	•	•
	STEL	2000	3760	(60 min, 2×)				•
Propan-1-ol	TWA	200	492		•	s	s	
	STEL	250	614		•	•	•	
Propan-2-ol	MWA/MAK	400	983		•	•	•	•
	STEL	500	1230		•	•	•	
	MAK-ST	800	1966	(30 min, 4×)				•
Butan-1-ol	MAX	50	150	s	s	s		
	TWA/MAK	100	300					•
	STEL	200	600	(6 min, 8×)				•
Butan-2-ol	TWA/MAK	100	303		•	•	•	•
	STEL	150	455				•	
	MAK-ST	200	606	(5 min, 8×)				•
Butanol, tert.	TWA/MAK	100	303		•	•	•	•
	STEL	150	455		•	•	•	
	MAK-ST	200	606	(5 min, 8×)				•
Isobutanol	TWA	50	152		•	•	•	
Pentan-1-ol	No limits				Established			
Pentan-2-ol	No limits				Established			
Pentan-3-ol	TWA/MAK	100	361			•		
	STEL	125	462			•		
Isoamyl alcohol	TWA/MAK	100	361		•	•	•	•
	STEL	125	452		•	•	•	
	MAK-ST	200	722	(30 min, 4×)				•

OSHA, Occupational Safety and Health Administration
ACGIH, American Conference of Government Industrial Hygienists
NIOSH, National Institute for Occupational Safety and Health
DFG, Deutsche Forschungsgemeinschaft
TWA, Time-weighted average
STEL, short-term exposure limit
MAX, ceiling concentration
MAK, maximum working place concentration
MAK-ST, MAK short-term

A. Percutaneous Absorption

Percutaneous absorption of alcohols does occur, but it is very small. It increases with growing chain length of the alcohol. Whereas, with ethanol and propanol, penetration of mouse or guinea pig skin was not detected by the methods employed, increased penetration was measured with higher alcohols [234]. With human abdominal skin, the following permeability constant (cm/h) were established in vitro [235]: water, 10^{-3}; methanol, 10^{-3}; ethanol, 1.2×10^{-3}; and propan-1-ol, 1.4×10^{-3}. Thus, the short-chain alcohols used for disinfection of hands and skin behave like water. The permeability of human skin for water was measured to be 0.2 mg/cm² h^{-1} [235].

Methanol may be absorbed through the skin. The consequences would be similar to those observed after ingestion; namely, metabolic acidosis and effects on sight (temporary or permanent blindness) and on other functions of the central nervous system.

Ethanol can also be absorbed through the skin. In mammals, however, it is quickly degraded so that below 10 mg/L the plasma concentration decreases exponentially [236]. At higher concentrations, the metabolizing enzyme systems are saturated. Consequently, the alcohol is eliminated at a rather constant rate of 0.5 mg/L min^{-1} [236]. Alcohol-soaked (200-ml) dressings placed on both legs in humans did not cause blood levels that were detectable after 3 h [237]. Therefore, the amount and rate of absorption must have remained well below the respective values for enzyme saturation and for elimination of the alcohol.

In very young (< 1 year) children, however, abdominal alcohol dressings were reported to have caused intoxication [238]. However, 25 of the 28 children involved had skin lesions; therefore, it cannot be excluded that the alcohol at least partially penetrated the skin through these lesions.

As can be seen from Table 24, the acute toxicity of ethanol, propan-2-ol, and propan-1-ol—alcohols that are actually used on the skin as disinfectants, as antiseptics, or in cosmetics—is very low. Taken together with the small absorption rate through intact skin, it may be assumed, therefore, that toxicological arguments are of little importance in decisions concerning the choice of these alcohols for the foregoing purposes. And, indeed, this has been the general experience ever since these alcohols have been used by health care personnel, usually many times a day for many years. Contact with large areas of diseased or lesioned skin, however, should be permitted only with caution.

B. Inhalation Toxicity

According to Lester and Greenberg [239], ethanol is tolerated by the human respiratory tract without any reaction at 100 mg/m³, whereas 300 mg/m³ causes permanent irritations, and 400 mg/m³ is intolerable. To give a picture of the whole range of harmful inhalatory exposure to alcohols, lethal concentrations (LCLO)

Table 24 Acute Oral Toxicity (Rat) of Alcohols

Alcohol	LD_{50} (g/kg)
Methanol	5.6
Ethanol	10.4–18.0
Propan-1-ol	1.9
Propan-2-ol	4.6–5.8
Butan-1-ol	0.8
Pentan-1-ol	2.2
Hexano-1-ol	0.7
Heptanol-1-ol	0.5
Benzyl alcohol	1.2

Source: Refs. 240, 251.

for mice are given as examples [240]: methanol, 50 g/m³; ethanol, 39 g/m³; and propan-2-ol, 31.5 g/m³. As can be deduced from these values (a) toxicity increases with the growing chain length of the alcohol, and (b) lethal effects are observed only with enormously high concentrations of these alcohols.

Short chain alcohols evaporate easily when used on the skin surface. Therefore, some inhalatory exposure is unavoidable. The following calculation may give an estimate of the effect of this exposure. Ten milliliters of 80% (w/w) ethanol consists of 6.3 g alcohol. When this amount has been rubbed onto the hands, has evaporated, and is completely distributed in a room of 10 m³, the minimum volume usually required for a working person, the alcohol concentration will then be 0.63 g or 630 mg/m³. Comparing this value with the occupational exposure limit (see TWA and MAK-values in Table 23), which is 1880 mg/m³, it is evident that inhalation toxicity need not be considered as a problem in using ethanol for hand or skin disinfection. With 70% propan-2-ol, the corresponding values are 550 mg/m³ and a TWA/MAK of 983 mg/m³. Propan-1-ol 60% (w/w) produces a concentration of 480 mg/m³, thus nearly reaching the TWA-limit of 492 mg/m³ (OSHA). Because of natural or artificial ventilation, however, the estimated alcohol concentrations will be of only short duration. Therefore, the comparative basis should be the STEL/MAK-ST, rather than TWA/MAK values. For propan-1-ol the STEL is 614 mg/m³.

Similar to absorption through the skin, the uptake of alcohol by the lungs is small. A concentration of 0.5 g/m³ inhaled during 10 min causes an alcohol uptake of 10–100 mg. The blood level remains far below 0.1% [239]. Only concentrations of 15 g/m³ inhaled for several hours at a breathing volume of 15 L/min will produce alcohol blood levels of 0.8–0.9% [239]. Thus, toxic effects by the

inhalation of alcohol from disinfection of hands and skin with alcoholic solutions can reasonably be expected not to occur, as the amounts of alcohol employed are far below the thresholds of toxicity [54,240].

C. Acceptability

The assessment of acceptability of hand disinfectants and antiseptics is still a problem of methodology. Among other reasons, the small extent of objectively quantifiable changes in skin condition, the paucity of techniques for objective assessment, and the possible bias of prejudice in self-assessment may be listed. Therefore, the results of relatively few studies are available [35,217,241–243]. Usually, both methods of assessment, subjective and objective, are employed. The latter may be carried out by a dermatologist or by biophysical measurements of parameters, such as transepidermal water loss [242–244], skin temperature and skin blood flow [245], or the change in desquamation of the stratum corneum [246] as a consequence of skin disinfection. Another approach is the examination of silicone rubber templates of the skin by computer-assisted microimage analysis of the shadows generated from the skin profile by oblique illumination [247]. Because of the usually very slight deterioration of skin condition, the sensitivity of assessment by a dermatologist has not been found satisfactory [35,217,242,243]. In contrast, the results of semiquantitative self-assessment proved to be a sensitive and reliable indicator of product acceptability [35,217]. Evaluation of the acceptability of frequent application of alcohols on the hands, "intactness" (judging the roughness of skin), "turgor," and "sensation" proved to be very sensitive markers [217], whereas "appearance" was unaffected and, therefore, not usable as a distinguishing feature. To eliminate bias, however, an intelligent experimental design and blinding of the test subjects are of utmost importance. If it is not possible to conceal the identity of the test and placebo products from the subjects, objective methods of assessment are a better choice.

The most common effects of brief exposure of skin to alcohols are signs and symptoms of mild irritation, such as slight redness and sensations of heat and burning owing to removal of the water–lipid layer and dehydration, followed by toxic damage to living cells of the epidermis by the alcohol [248]. Symptoms of allergic contact dermatitis, such as contact urticaria at the exposed site, are extremely seldom [240,248]. Extended, repeated exposure to high concentrations of alcoholic solutions may cause drying of the skin, with roughness and reddening. In a comparative study involving daily surgical scrubbing for 5 consecutive days, Larson et al. [35] found preparations of unmedicated soap and of a triclosan-containing detergent rated as the mildest. Ethanol (70%) plus chlorhexidine gluconate (0.5%) was less harsh than a povidone–iodine liquid soap, but not as mild as a chlorhexidine detergent. Leyden [249] compared the acceptability of propan-1-ol and propan-2-ol with that of povidone–iodine soap. On the whole, he,

OCT-17-1995 17:15 P&G 3WTC CLIN RSCH & EID. 513 626 3275 P.23

found that alcohols caused skin irritation more often than soap. The climate seems to play an important role, however. With low air temperatures and humidity, alcoholic handrubs seem to be better tolerated than the otherwise allegedly more accepted chlorhexidine gluconate preparation [241].

In a prospective, randomized, double-blind study, Rotter et al. [217] demonstrated that the only slight deterioration of skin condition diagnosed after 14 days of frequent (15 times per day) application of a high-concentration (50% + 30% v/v) mixture of propan-1-ol and propan-2-ol was significantly less if the antiseptic contained suitable emollients. Therefore, alcoholic solutions for frequent-use purposes, such as hand disinfection, should not be employed without emollients, thereby preventing skin problems and ensuring compliance of usage.

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248

Journal of Hospital Infection (1981) 2, 273–280

Letters to the Editor

Sir,

Povidone-iodine and chlorhexidine gluconate containing detergents for disinfection of hands

I should like to comment on 'Letters to the Editor' from two industrial laboratories concerning our publication in this Journal (Rotter, Koller and Wewalka, 1980). One of these letters (R. Hall, 1980) raises some relevant questions which cannot all be answered unequivocally at the present time. The results of the writer's own investigations are presented, but although they may be valid cannot be judged because of insufficient information on the methods.

The objections raised may be summarized as follows:

(1) methods;
(2) our results as compared to those of other investigators;
(3) interpretation of the results.

(1) *Methods.* At the time of the investigation that led to our publication, the test methods for assessing the efficacy of hygienic and surgical hand disinfection were not standardized. Therefore, there was no formal reason to adhere to techniques we might have used in earlier investigations. It is true, however, that with slight modifications the method proposed by Rotter *et al.* (1977) became the standard method for testing procedures for hygienic hand disinfection as published by the Austrian Society of Hygiene, Microbiology and Preventive Medicine (OGHMP, 1981), and by the German Society of Hygiene and Microbiology (DGHM, 1981). The method was adopted by the Federal Office of Health of the FRG (Bundesgesundheitsamt, 1980).

The new standard method of OGHMP (1981) and DGHM (1981) for tests of surgical hand disinfection is based on the method that has been the standard test procedure of the DGHM since 1972. It has been improved by an additional test for substained action under the surgical glove and by adjusting the evaluation of the results to the method used for testing hygienic hand disinfection.

Both test methods will be published in this Journal with comments.

Recent investigations with chlorhexidine gluconate-containing detergents Rotter, Koller and Wewalka, 1981) that were performed according to the new standards and with the same neutralizers in all sampling fluids (for pre- and post-values as well as for alcohol and the disinfectant under test), showed no significant differences from earlier results (Rotter, Koller and Wewalka, 1980).

(2) *Our results as compared to those of other investigators.* It is not reasonable to compare results on the efficacy of chlorhexidine gluconate-containing detergents if the assessment is based on different parameters, i.e. in terms of mean log reduction of the release of skin bacteria, proportion of hands giving negative cultures in finger streak cultures, or viable bacterial counts from the inside of surgeons' gloves, especially if comparative results with alcohols are not available. Therefore, a

synopsis of results from the literature has been prepared from studies using similar parameters (Table I). Apart from a few values, the reported mean log reductions of release of skin bacteria are reasonably comparable despite differing methods. Much better results were only obtained when chlorhexidine was not neutralized in the sampling fluid.

Table I. *Efficacy of surgical scrub with chlorhexidine-detergent (Hibiscrub)*

Investigators	Duration of scrub (min)	Mean log reduction after disinfection	
		Immediately	3 h later
Ojajärvi, 1976	0·5	1·56*	
Sevens, Malcom and Lockwood, 1973	1	0·74	
Lowbury, Lilly and Bull, 1963	2	0·89	1·59
Lowbury and Lilly, 1973	2	0·88	
Lilly and Lowbury, 1974	2	0·89	
Ayliffe *et al.*, 1975	2	0·92	
Lilly, Lowbury and Wilkins, 1979 *a*	2	0·83	
Lilly, Lowbury and Wilkins, 1979 *b*	2	0·87	
Furuhashi and Miyamae, 1979	2	0·30	
Furuhashi and Miyamae, 1979	3	0·97	
Rotter, Koller and Wewalka, 1980	3	0·78	0·75
Rotter, Koller and Wewalka, 1981	3	0·80	1·00
Rotter, Koller and Wewalka, 1981	5	0·90	0·90
Rosenberg, Alatary and Peterson, 1976	6	1·94*	1·53*
Peterson, Rosenberg and Alatary, 1978	6	3·64*	3·99*
Furuhashi and Miyamae, 1979	6	1·21	

* No inactivation of chlorhexidine in sampling fluid.

(3) *Interpretation of results.* As shown in the graph (Fig. 1) the efficacy of the more modern agents used in surgical hand disinfection (chlorhexidine gluconate detergents, hexachlorophane liquid soap, povidone-iodine liquid soap) differs in some respects such as velocity of onset of action and presence or absence of a sustained effect, but their order of magnitude is reasonably similar. Suitable concentrations of iso- or n-propanol on the other hand reduce the release of skin bacteria to a degree of one or two powers of ten greater than the agents mentioned. Similarly, no acceptable antibacterial substance is known for hygienic hand disinfection, with an efficacy comparable to that of alcohol in the right concentration.

How the results of the tests are interpreted or what is taken as a yardstick is, of course, a matter for discussion, but once the necessity for disinfection is recognized it seems sensible to use the most efficient procedure.

Therefore, the OGHMP and DGHM have chosen n-propanol (60 per cent, ml/ml) applied for 5 min as the standard for surgical hand disinfection and iso-propanol (60 per cent, ml/ml) rubbed on to the hands for 1 min as the standard for hygienic hand disinfection.

Inadequately controlled in-use tests may have some advantages, but testing the antibacterial effect of a disinfectant on the hands is a scientific experiment in which

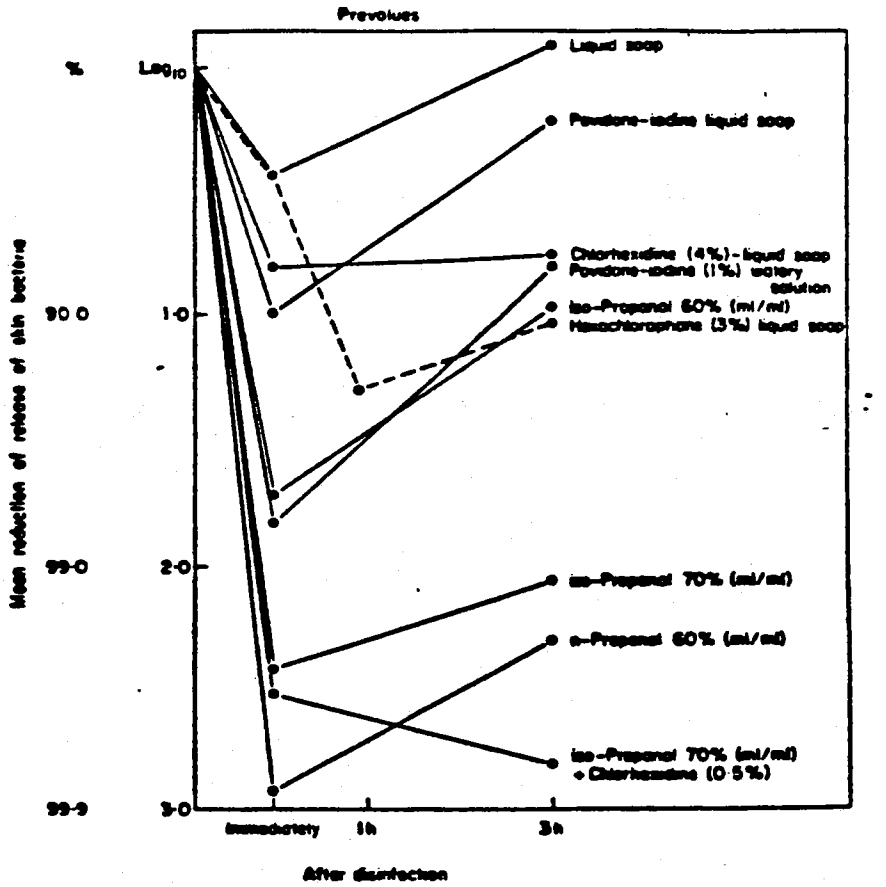


Figure 1. Mean reduction of release of skin bacteria immediately and 1 or 3 h after a surgical scrub (5 min, apart from chlorhexidine detergent: 3 min). ———, Results of Rotter's group; - - - -, after results of Michaud, McGrath and Goss (1976).

as many conditions as possible must be under the control of the investigator. This, in our opinion, is only possible in an experiment that as far as possible simulates real conditions and minimizes uncontrolled influences. The laboratory tests with volunteers have been performed by other workers for many years. In our test model effects of individual influences are kept to a minimum as each volunteer acts as his own control, and the results are compared directly with the results obtained with a standard disinfection procedure in the same experiment.

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Sevens, F., Malcom, J. & Lockwood, P. U. (1973). Clinical evaluation of Hibiscrub. Medikon 2, 369 (cited after Peterson, Rosenberg and Alatary, 1978).

Sir,

Hazards of naso-enteric feeds

I am writing to draw attention to an infection hazard presented by naso-enteric feeds. Although this hazard is not a new one (Casewell, Cooper and Webster, 1981),

248

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(2) *Our results as compared to those of other investigators.* It is not reasonable to compare results on the efficacy of chlorhexidine gluconate-containing detergents if the assessment is based on different parameters, i.e. in terms of mean log reduction of the release of skin bacteria, proportion of hands giving negative cultures in finger streak cultures, or viable bacterial counts from the inside of surgeons' gloves, especially if comparative results with alcohols are not available. Therefore, a

synopsis of results from the literature has been prepared from studies using similar parameters (Table I). Apart from a few values, the reported mean log reductions of release of skin bacteria are reasonably comparable despite differing methods. Much better results were only obtained when chlorhexidine was not neutralized in the sampling fluid.

Table I. *Efficacy of surgical scrub with chlorhexidine-detergent (Hibiscrub)*

Investigators	Duration of scrub (min)	Mean log reduction after disinfection	
		Immediately	3 h later
Ojajärvi, 1976	0·5	1·56*	
Sevens, Malcom and Lockwood, 1973	1	0·74	
Lowbury, Lilly and Bull, 1963	2	0·89	1·59
Lowbury and Lilly, 1973	2	0·88	
Lilly and Lowbury, 1974	2	0·89	
Ayliffe *et al.*, 1975	2	0·92	
Lilly, Lowbury and Wilkins, 1979 *a*	2	0·83	
Lilly, Lowbury and Wilkins, 1979 *b*	2	0·87	
Furuhashi and Miyamae, 1979	2	0·30	
Furuhashi and Miyamae, 1979	3	0·97	
Rotter, Koller and Wewalka, 1980	3	0·78	0·75
Rotter, Koller and Wewalka, 1981	3	0·80	1·00
Rotter, Koller and Wewalka, 1981	5	0·90	0·90
Rosenberg, Alatary and Peterson, 1976	6	1·94*	1·53*
Peterson, Rosenberg and Alatary, 1978	6	3·64*	3·99*
Furuhashi and Miyamae, 1979	6	1·21	

* No inactivation of chlorhexidine in sampling fluid.

(3) *Interpretation of results.* As shown in the graph (Fig. 1) the efficacy of the more modern agents used in surgical hand disinfection (chlorhexidine gluconate detergents, hexachlorophane liquid soap, povidone-iodine liquid soap) differs in some respects such as velocity of onset of action and presence or absence of a sustained effect, but their order of magnitude is reasonably similar. Suitable concentrations of iso- or n-propanol on the other hand reduce the release of skin bacteria to a degree of one or two powers of ten greater than the agents mentioned. Similarly, no acceptable antibacterial substance is known for hygienic hand disinfection, with an efficacy comparable to that of alcohol in the right concentration.

How the results of the tests are interpreted or what is taken as a yardstick is, of course, a matter for discussion, but once the necessity for disinfection is recognized it seems sensible to use the most efficient procedure.

Therefore, the OGHMP and DGHM have chosen n-propanol (60 per cent, ml/ml) applied for 5 min as the standard for surgical hand disinfection and iso-propanol (60 per cent, ml/ml) rubbed on to the hands for 1 min as the standard for hygienic hand disinfection.

Inadequately controlled in-use tests may have some advantages, but testing the antibacterial effect of a disinfectant on the hands is a scientific experiment in which

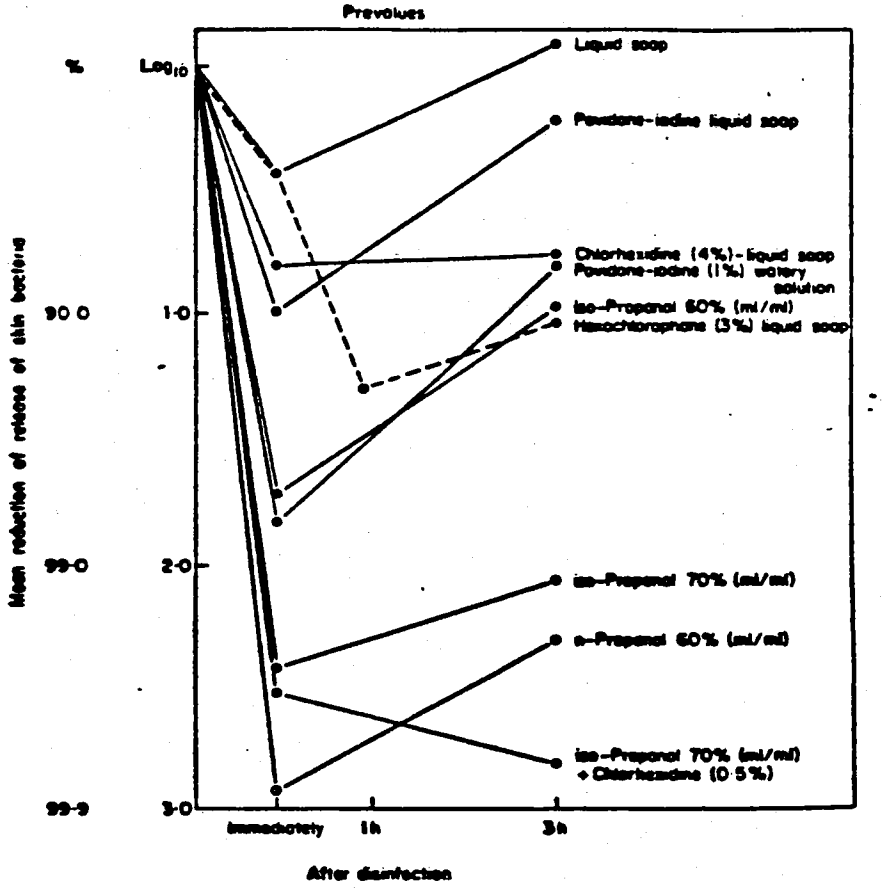


Figure 1. Mean reduction of release of skin bacteria immediately and 1 or 3 h after a surgical scrub (5 min, apart from chlorhexidine detergent: 3 min). ———, Results of Rotter's group; – – – –, after results of Michaud, McGrath and Goss (1976).

as many conditions as possible must be under the control of the investigator. This, in our opinion, is only possible in an experiment that as far as possible simulates real conditions and minimizes uncontrolled influences. The laboratory tests with volunteers have been performed by other workers for many years. In our test model effects of individual influences are kept to a minimum as each volunteer acts as his own control, and the results are compared directly with the results obtained with a standard disinfection procedure in the same experiment.

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Sir,

Hazards of naso-enteric feeds

I am writing to draw attention to an infection hazard presented by naso-enteric feeds. Although this hazard is not a new one (Casewell, Cooper and Webster, 1981),

Hygienic Hand Disinfection

M.L. Rotter, MD

ABSTRACT

The purpose of hygienic hand disinfection is to render hands safe after contact with pathogens. Comparing effects of disinfection procedures on infection ratios is too difficult for routine purposes but the degerming efficacy may be determined in laboratory tests with volunteers. In the Vienna test model the efficacy of a specific procedure being tested is compared to that of a standard disinfection (rubbing into hands 3 ml of isopropanol 60% v/v, 30 seconds, twice) tested in parallel with the same volunteers. This ensures standardization, thus comparability of results between laboratories, and provides the investigator with a yardstick for efficacy. The model includes artificial contamination, assessment of the release of test bacteria (*E. Coli* ATCC 11229) before and after disinfection by the finger tip method, and addition of neutralizers to sampling fluids. Alcohols in appropriate concentrations are highly effective (log reductions: >4.0) whereas procedures employing disinfectant detergents act like soap (log reductions: ≤ 3.2) and may cause dissemination of pathogens. [Infect Control 1984; (5)1:18-22.]

RATIONALE AND DEFINITION

Disinfection is only one of several methods involved in hand hygiene: others include no-touch techniques, wearing of gloves, and washing procedures. Here, only disinfection procedures will be considered.

In German-speaking countries disinfection of hands is, according to different objectives, strictly separated into two kinds of procedures, namely "surgical" and "hygienic" disinfection. Whereas the aim of surgical hand disinfection is to minimize the release of any kind of microorganism, extraneous as well as autochthonous, from the surgeon's hands into the surgical wound, hygienic hand disinfection is intended to block the transmission of pathogens via contaminated hands. Thus, the purpose of hygienic disinfection is to render hands safe after contact with dangerous pathogens. This implies that hygienic disinfection is directed only against the transient, usually loosely-adhering flora. An effect against the resident skin flora is not usually necessary or even desired. A sustained action as might be required under a surgeon's glove is of no advantage as the hands are expected to be safe immediately after disinfection. For practical reasons hygienic disinfection must be effective within as short a time as possible, ie, 1 minute maximum.[1,2] Furthermore, in order to prevent dissemination of dangerous pathogens during and by the disinfection procedure itself, microorganisms must be inactivated on the skin. Thus, in German-speaking countries any procedure that involves splashing or other dispersal of pathogens is regarded undesirable.[1,2] Washing hands with soap or with a disinfectant detergent may be sufficient in everyday hospital work but is a debatable procedure when, after contamination, hands have to be rendered free of pathogenic organisms. Instead, procedures like rubbing a fast-acting disinfectant onto the hands should be used. This method, of course, can also be advantageous in situations that do not really require a hygienic disinfection (for instance if a washbasin is not readily available).

EFFECTS

The positive effect of hygienic hand disinfection was convincingly demonstrated in Vienna by Semmelweis[3-6] when he succeeded in lowering the maternal mortality quota by 90%, from an average of 13.7% in 1846 to 1.3% in 1848 by introducing his rigid regimen of hand disinfection with a solution of chlorinated lime (Figure 1). In addition, this finding implicitly proved the role of the hands of medical staff in transmitting hospital infections. This fact had already been suspected before Semmelweis by O.W. Holmes. Although or even because this is still being accepted, the effects of different methods of hand disinfection are not easily demonstrable. Today, hand hygiene is more or less included in most procedures involving direct and indirect patient contacts, so that the variation of methods is unthinkable to provoke effects as dramatic as they were in the days of Semmelweis. This is especially true for the parameter he had to use, namely mortality.

EVALUATION OF THE EFFECTS

The effect of a disinfection procedure can be assessed either, directly, by determining its influence on the frequency of infections or, indirectly, by testing the disinfection power (efficacy) of the procedure itself.

The former, the assessment of the effect of a disinfection procedure by epidemiological analysis is tedious and time-consuming. As an example, the following case might be constructed:

In a surgical department:

Total ratio of nosocomial infections a[illegible] with hygienic hand disinfection proc[illegible] 10%

infections by exoge[illegible] 5%

From the Hygiene-Institute of University Vienna, Austria.

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infections by hand transmission 2%
Desired decrease of hand-transmitted infections by change-over to an allegedly better hand disinfection procedure, number 2 by 50% (2% − 1%)
Assumed effect on the total infection ratio: 10% − 9%
Further pre-settings
desired level of significance (directional) 5%
desired power of statistical test 90%

Power analysis by arcsine transformation of the proportions[7] reveals that for demonstration of a significant difference under the above assumptions the sample size of each of the two groups would have to include 2,428 patients. This example illustrates that the expense to demonstrate a significant difference between the effects of two disinfection procedures is even then very high if their effects are assumed unrealistically different. Thus, direct assessment of the influence on the infection ratio can not be the parameter in routine testing of disinfectants. Instead, test procedures for hand disinfection will have to concentrate on the measurement of their disinfection power. This indirect way is compatible with scientific proceeding as the degree of degerming activity may be assumed somehow related to the effect of disinfection on the incidence of hospital infections.

The following requirements, however, must be obeyed:
1) The antimicrobial efficacy of a procedure for hand disinfection must be assessed according to the rules of a scientific experiment. Thus, excepting the variables under test, as many conditions as possible must be under the control of the investigator. Therefore, laboratory tests with volunteers are the most appropriate and economic way to obtain objective information about the efficacy of a disinfection procedure.
2) Although such experiments should be performed in a controlled fashion, the test model should simulate practical conditions as far as possible. For example, hands should be used instead of other carriers, concentrations of the disinfectant, its action time, and the mode of application should be those intended for real use.
3) The test model must allow for individual attributes of the volunteers that may influence the test results (eg, size of the hands and skin quality) as well as the way the procedure is being performed. We have therefore suggested that each volunteer acts as his own control by performing a standard disinfection procedure in the same experiment.[8]
4) With new disinfectants the range of organisms that can be inactivated by the substance also must be assessed in vitro.

THE VIENNA TEST MODEL

In 1974 we proposed a test model for the evaluation of procedures for hygienic hand disinfection.[9] In the meantime this model (Figure 2) has been adopted by the Austrian and German Societies for Hygiene and Microbiology and by the Federal Office of Health in the Federal Republic of Germany.

In principle the release of test bacteria from the finger tips of artificially contaminated hands is determined before and after disinfection, first with a standard disinfection and then with the disinfection procedure under test using the same volunteers, on the same day, and under similar environmental conditions. The ratio of bacterial release before and after disinfection, the so-called reduction factor, is a direct measurement for the efficacy of the procedure, the latter being defined as the combination of all factors, as there are: disinfectant, its concentration, time of action, and mode of application. For interpretation of the results the mean reduction factor of the procedure being tested is compared to that of the standard by a pairwise statistical procedure.

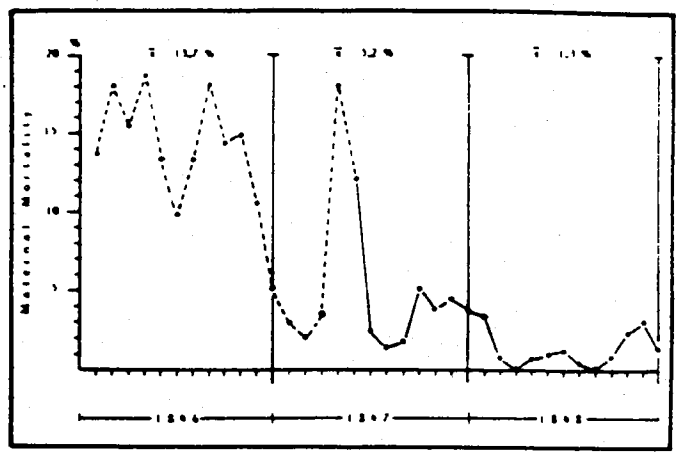


Figure 1. Effect of hygienic hand disinfection as introduced by Semmelweis in May 1847 on the maternal mortality at the k.k. I. Obstetric University Clinic in Vienna. •—— • = before introduction; •— • = after introduction.

As a test bacterium, *E. coli* ATCC 11229 had been chosen. Sampling of test bacteria was done by the finger tip method. If, according to the nature of the disinfectant being tested, neutralizers are necessary to inhibit prolonged action after termination of the disinfection they must be added to the sampling fluids and their diluents, but not to the culture media. As a standard disinfection it had been agreed that 3 ml of isopropanol 60% (by volume) be rubbed onto the hands; after 30 seconds this procedure is repeated with another 3 ml during the following 30 seconds. The disinfection procedure being tested is always done as specified by the producer. Regimens that require more than one minute action time or the addition of water before or during application were not regarded suitable for hygienic hand disinfection.

THE VIENNA MODEL AS COMPARED TO OTHER MODELS

In the Vienna model principles and methods studied by others are contained, eg, the principle of evaluation in laboratory tests with volunteers, artificial contamination, sampling before and after disinfection, and the finger tip method.[10] The Vienna model, however, is the only one which allows for the influence of the test population in the results. It is hardly possible to compare the test results from different groups of people without relating these results to those obtained with a parallel standard disinfection, ie, by standardization. This is evident from the results of parallel experiments in Vienna and Mainz with four different disinfection procedures comprising the use

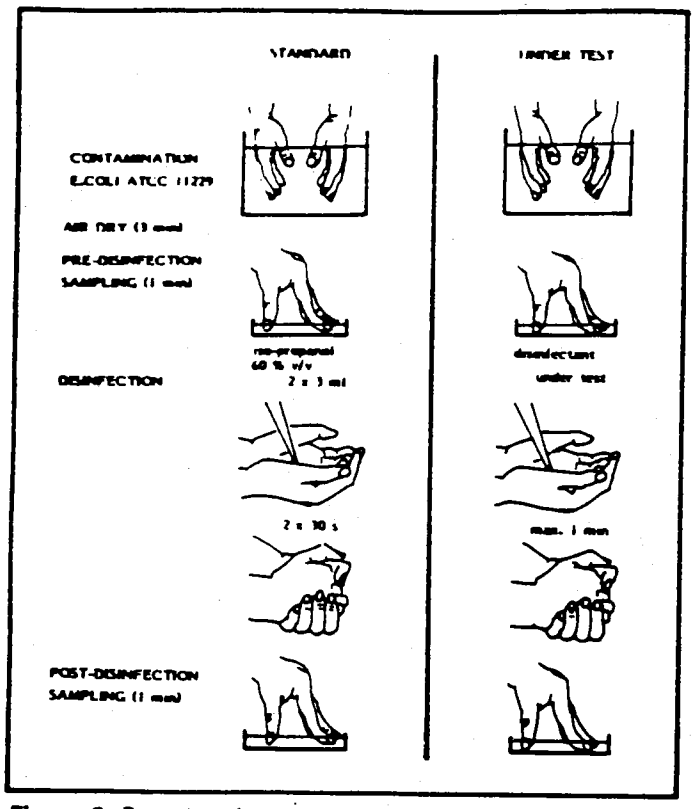


Figure 2. Principles of the Vienna model for testing procedures for hygienic hand disinfection.

TABLE 1

ANALYSIS OF VARIANCE ON THE NON-STANDARDIZED RESULTS (LOG P_i) OBTAINED WITH FOUR PROCEDURES FOR HYGIENIC HAND DISINFECTION

(Tests fivefold, in parallel at two locations with 15 volunteers each)

Source of Variation	DF	Mean Squares	$\hat{F}$	P
Procedures (P)	3	102.4	327.5	<0.0001
Volunteers (V)	14	2.6	8.1	<0.0001
Locations (L)	1	5.8	18.6	<0.0001
P x V	42	0.7	2.2	<0.0001
V x L	3	3.4	10.8	<0.0001
P x L	14	3.1	10.0	<0.0001
P x V x L	42	0.7	2.4	<0.0001
Error	470	0.3		

Procedures (all 1 minute): Rubbing-in: n-propanol 50% (v/v), ethanol 70% (v/v) + chlorhexidine 0.5% (g/v). Washing with povidone-iodine liquid soap, phenolics in detergent.
Volunteers: 15 at each location.
Locations: Vienna, Mainz.

of n-propanol 50% (v/v), ethanol 70% (v/v) + chlorhexidine gluconate 0.5% (g/v), a povidone-iodine liquid soap, and a disinfectant detergent on the basis of a mixture of phenolics. Each disinfection procedure was tested five times together with the standard disinfection on 15 volunteers in Vienna and on 15 in Mainz. After transformation of the measured reduction factors into units of $\log_{10}$, analysis of variance was carried out on both, the non-standardized values (log P_i) obtained with each disinfection procedure being tested and the standardized ones being the differences of log P_i to those as observed with the appropriate standard disinfection (log ST_i), thus log P_i-log ST_i. The analysis of variance performed on the non-standardized log P-values (Table 1) revealed a significant difference between the means of all factors. If, however, the standardized values (log P_i-log ST_i) were analyzed (Table 2), only the disinfection procedures and volunteers rather than the location of the experiments were indicated as factors that contributed significantly to variation. This signifies that the differences between the two groups of test persons in Vienna and Mainz were abolished by standardization and that the results were thus comparable between different laboratories.

For the purpose of standardization the degerming activity of the standard disinfection is of no special interest. For purposes of evaluating new methods of hand disinfection, however, it is wise to choose a procedure whose antimicrobial efficacy is such that it can be agreed upon as a standard for effectiveness, as well. This has been done in Austria and the Federal Republic of Germany. It is curious that all relevant test methods state how the efficacy of hand disinfectants should be assessed but very few indicate how good it should be!

Test results may, of course, be influenced by methodical details of different models. For this reason standard test methods have to be agreed upon. Examples for such are the "guidelines" of the Societies for Hygiene and Microbiology in Austria[2] and in the Federal Republic of Germany[1] or the rules proposed by the Food and Drug Administration.[11] The latter, the test whose intention comes closest to what is known in Central Europe as hygienic hand disinfection is the "test for health care personnel handwash effectiveness."

We have evaluated the influence of some methodical details that are different in the Vienna test model and are proposed by the US Food and Drug Administration.

One striking difference is the technique for sampling the test bacteria from the artificially contaminated hands. Whereas the Vienna model utilizes the finger tip method,[9] in the FDA-proposals the glove juice procedure is mentioned. As an example, results from comparative experiments with both sampling techniques are shown (Table 3). There was no difference of mean log reductions when hand washing with povidone-iodine liquid soap was used as a degerming procedure, but a significantly lower reduction of test bacteria was measured with the glove juice method than with the finger tip technique when isopropanol was used according to our standard disinfection procedure. An exact analysis of both sampling techniques revealed that with the glove-juice technique the

TABLE 2

ANALYSIS OF VARIANCE ON THE STANDARDIZED RESULTS (LOG P_i-LOG ST_i) OBTAINED WITH 4 PROCEDURES FOR HYGIENIC HAND DISINFECTION

(Tested fivefold, in parallel at two locations with 15 volunteers each)

Source of Variation	DF	Mean Squares	F	P
Procedures (P)	3	90.2	117.5	<0.00001
Volunteers (V)	14	2.7	3.5	<0.00001
Locations (L)	1	3.5	4.6	n.s.
P x V	42	0.9	1.1	n.s.
V x L	3	1.6	2.1	n.s.
P x L	14	1.7	2.1	<0.01
P x V x L	42	0.9	1.2	n.s.
Error	466	0.8		

n.s.—p>0.01
Procedures (all 1 minute): Rubbing-in: n-propanol 50% (v/v); ethanol 70% (v/v) + chlorhexidine 0.5% (g/v). Washing with: povidone-iodine liquid soap; phenolics in detergent.
Volunteers: 15 at each location.
Locations: Vienna, Mainz.

TABLE 3

EFFECT OF SAMPLING METHOD ON THE TEST RESULTS OBTAINED WITH PROCEDURES FOR HYGIENIC HAND DISINFECTION

Sampling Method	Log Reduction Caused by: Rubbing-in isopropanol 60% (v/v)		Log Reduction Caused by: Washing with povidone-iodine liquid soap
Finger tip	4.1 ± 0.7	**	3.3 ± 0.6
Glove juice	3.4 ± 0.9	n.s.	3.2 ± 0.5

** 2 p = 0.05*
*** p < 0.01*
n.s.—2 p (p) > 0.05
Disinfection time: 1 minute
Test bactenum: E. coli ATCC 11229
Volunteers: 15

TABLE 4

EFFECT OF CONDITION OF HANDS, DRY OR WET, ON THE TEST RESULTS OF HYGIENIC HAND DISINFECTION WITH ISOPROPANOL 60% (v/v)

Condition of Hands Before Disinfection	Log Reduction x̄		s
dry	4.3	±	0.6
wet	3.7	±	0.6

** p < 0.05*
Disinfection time: 1 minute
Test bactenum: E. coli ATCC 11229
Sampling method: finger tip method
Design of study: cross-over, 15 volunteers

volunteers had applied the alcohol on their hands which were still wet from the pre-disinfection sampling. Thus, we suspected that dilution of the alcohol on the hands might have impaired its activity. Therefore, other experiments were performed[12] using isopropanol on wet and dry hands with the finger tip method that, excepting the finger tips, keeps hands dry. The results (Table 4) clearly prove that alcohol should not be used on wet hands and that the glove-juice method creates problems if this is ignored.

Another important difference in both test models is the usage of substances neutralizing the antimicrobial action of the disinfectant after termination of disinfection. While the Vienna model requires neutralizers to be incorporated into the sampling fluids and their diluents, the FDA rules must be interpreted such that neutralizers are to be added to the diluents and to the culture plates. Following the FDA method we have performed comparative tests[12] using a disinfectant detergent on the basis of chlorhexidine gluconate that exerts a marked sustained action. When neutralizers were added only to the diluents the log reductions of test bacteria (*Serratia marcescens* ATCC 30121) were significantly higher (log RF: 4.8) than with neutralizers incorporated additionally into the sampling solutions used for assessment of bacterial release after disinfection (log RF: 3.0; Table 5). These facts clearly demonstrate that the excellent results reported with chlorhexidine-based disinfectants may easily be artifacts generated by the prolonged action of chlorhexidine in the sampling fluids until processing.[13,14]

EFFICACY OF VARIOUS DEGERMING PROCEDURES AS ASSESSED BY THE VIENNA MODEL

In this presentation only a few examples may be shown of the efficacy of degerming procedures as assessed by the Vienna model. In fact, after all that has been said about standardization of results obtained with different groups of volunteers the results stemming from many experiments should be presented in a standardized manner. Unfortunately, such values would not give an idea about the real magnitude of the reduction of the release of test bacteria. Therefore, the results have been made comparable by selecting the values from experiments in which the mean log reduction factor of the parallel standard disin-

TABLE 5

EFFECT OF NEUTRALIZER IN SAMPLING FLUIDS FOR POST-DISINFECTION SAMPLING ON THE TEST RESULTS WITH CHLORHEXIDINE GLUCONATE (CHX)

Neutralizer* in Sampling Fluid	Log Reduction x̄	±	s
absent	4.8 **	±	0.5
present	3.0	±	0.5

** per liter: 150 ml Tween 80 + 15 g lecithine + 5 g histidine*

*** 2 p < 0.01*

Disinfection: 1 min hand-wash with CHX (4%) detergent
Test bacterium: Serratia marcescens *ATCC 30121*
Sampling method: glove juice method
Design of study: cross-over, 16 volunteers

TABLE 6

EXAMPLES OF THE EFFICACY OF VARIOUS DEGERMING PROCEDURES AS ASSESSED BY THE VIENNA MODEL

Procedures (all 1 minute)	Conc %	Log Reduction
Rubbing-in		
n-propanol	60 v/v	5.5
	50 v/v	4.9
	40 v/v	4.3
isopropanol	60 v/v	4.2
ethanol	80 v/v	4.5
	70 v/v	4.0
	60 v/v	3.8*
povidone-iodine solution	1 g/v	4.0
Washing with		
povidone-iodine soap	0.75 g/v	3.2*
chlorhexidine soap	4.0 g/v	2.9*
phenolic soap	mixed	2.9*
liquid soap ÖAB 9	20 v/v	3.0*

** Significantly less effective than standard.*

Values derived from different experiments but from one laboratory; the mean log reduction of standard disinfection always being 4.1 and 4.3.

fection was between 4.1 and 4.3. Table 6 shows that procedures based upon rubbing appropriate disinfectants onto the hands are better than washing procedures.

Excepting ethanol at a concentration of 60% v/v all substances summarized in the first group are at least not significantly inferior to the efficacy of isopropanol 60% v/v, our standard. Among the three alcohols there exists a clear range of order as far as efficacy is concerned, namely n-propanol > isopropanol > ethanol. With *E. coli* as a test bacterium a watery solution of povidone-iodine with 1% available iodine is about as effective as isopropanol 60% when rubbed onto the skin.

All washing procedures with disinfectant detergent are significantly inferior to the standard. In fact, they are not or hardly better than the degerming activity of ordinary liquid soap. In this context it is important to realize that the efficacy of povidone-iodine together with a detergent is much worse than its counterpart, the watery solution.

In summary, our findings demonstrate that disinfectant detergents available at present do not fulfill the requirements of hygienic hand disinfection, not only because they imply undesired washing procedures, but also because their degerming activity exceeds hardly that of ordinary detergents. So, we regard alcohol in appropriate concentrations as the most suitable substance for hygienic hand disinfection, at the moment.

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Povidone-iodine and chlorhexidine gluconate-containing detergents for disinfection of hands

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Summary: The efficacy of a povidone-iodine and a chlorhexidine gluconate detergent was evaluated for 'hygienic' and 'surgical' hand disinfection. Rubbing 60 per cent *iso*-propanol on to the hands for 1 min was used as a standard for 'hygienic' disinfection, with *Escherichia coli* as the test organism. The mean log reduction obtained was 3·16 with povidone-iodine and 2·92 with chlorhexidine. These were considerably less than obtained with *iso*-propanol which produced a reduction of 4·23 and 4·30 orders of magnitude. In the 'surgical' hand disinfection tests, a 5 min wash with povidone-iodine and a 3 min wash with chlorhexidine detergent were compared with rubbing 60 per cent *n*-propanol or *iso*-propanol on to the hands for 5 min; *n*-propanol was taken as the standard preparation. Immediately after disinfection the log reduction in the resident flora was 0·92 with povidone-iodine and 0·78 with chlorhexidine, whereas *n*-propanol gave a log reduction of 3·43. After wearing a surgical glove for 3 h a log reduction of 0·24 was obtained with povidone-iodine, 0·75 with chlorhexidine and 2·94 with *n*-propanol. Treatment with *iso*-propanol gave a log reduction of 1·04. Thus, alcohols are shown by these tests to be far more effective than povidone-iodine or chlorhexidine detergent preparations in 'hygienic' as well as 'surgical' hand disinfection.

Introduction

Semmelweis (Anon., 1849) and Lister (1890) were among the first to prove that hands are the main mode of spread of bacteria from surgical and nursing personnel. Measures which prevent hand transfer of organisms are considered to be of major importance in the control of hospital infection. Disinfection is only one of several methods involved in hand hygiene; others include no-touch techniques, the wearing of gloves, and simple washing procedures. Although disinfection of the hands is carried out by the surgical team before operating, and by nurses and other personnel in wards, the objectives are different.

Before operating, a surgeon requires to remove extraneous organisms (transient flora) and also his own skin organisms (resident flora) (Price, 1938). In many instances, he is prepared to spend 5 min on hand disinfection. In contrast to this, if hands are accidentally contaminated with a dangerous pathogen, these must be inactivated on the skin to avoid further dissemination. It is important to realise that dissemination can occur during the disinfection procedure and any method that involves splashing or other dispersal of pathogens is undesirable. Washing the hands with a disinfectant detergent is an example of this. In 'hygienic' hand disinfection, the antimicrobial effect of a suitable disinfectant must be directed against

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a wide range of micro-organisms, and be effective within 1 min. An effect against the resident skin flora, or a sustained action as might be required under a surgeon's glove, is not necessary.

According to these requirements 'hygienic' hand disinfection is distinguished from 'surgical' hand disinfection in several European countries. As well as on the rare occasion that 'hygienic' disinfection is required for safety, it can also replace routine washing for practical reasons. Rubbing alcohol on to the skin takes less time and is particularly useful if a wash-basin is not readily available (see Kurtz & Boxhall, 1976; Meers & Yeo, 1978). Increasingly, antiseptic detergents containing povidone-iodine or chlorhexidine are being introduced into hospitals. Although the literature contains many reports describing their effectiveness, there are important indications that the efficacy of both antiseptics may have been over-estimated and that they may not necessarily fulfil the objectives of hand disinfection.

In the study reported here, the efficacy of two of these preparations is compared with two alcohols accepted as standard in several European countries. Comparisons are made under the conditions of both 'hygienic' and 'surgical' hand disinfection.

Materials and methods

Preparations

1. Povidone-iodine liquid soap ('Betaisodona'™, liquid soap, Mundipharma) containing 0·75 per cent (g/ml) free iodine.
2. Chlorhexidine gluconate disinfectant-detergent ('Hibiscrub'™, ICI) containing 4 per cent (g/ml) chlorhexidine gluconate.
3. *iso*-propanol 60 per cent (ml/ml) as a standard in 'hygienic' hand disinfection.
4. *n*-propanol 60 per cent (ml/ml) as a standard in 'surgical' hand disinfection.

The times of application of agents 1 and 2 were those recommended by the manufacturers.

'Hygienic' hand disinfection

Principle of test method

To assess the efficacy of the substances listed, the test method employed by our Viennese group was used (Koller, Rotter, Kundi, Mittermayer & Wewalka, 1977; Rotter, Koller, Kundi, Wewalka & Mittermayer, 1977). The hands were artificially contaminated, creating conditions that would require 'hygienic' hand disinfection in practice. The release of test bacteria from the finger-tips before and after disinfection was determined by quantitative culture of the sampling fluid. The quotient of the pre- and post-samples, the reduction factor, is a measure of the degerming power of the procedure and is expressed as a logarithm. As the results of tests may be influenced by differences between test persons, and the circumstances of the experiment (Rotter, Mittermayer & Kundi, 1974), control tests with a standard agent are necessary. In these tests, the comparison was made with *iso*-propanol used before the test agent, on the same day and the same test persons under comparable environmental conditions (Rotter, Koller & Kundi, 1977; Rotter, Koller, Kundi, Wewalka & Mittermayer, 1977).

Application of test organisms
The hands were washed with non-medicated soap for 2 min and dried with paper towels. They were then immersed up to the mid-metacarpals in 2 l of a broth culture of *Escherichia coli* for 5 s. The hands were left to dry in the air for 3 min in a horizontal position with the fingers spread out and rotated to and fro to avoid the formation of droplets.

Test organism
Escherichia coli ATCC 11229 was grown for 18 h in Tryptic Soya Broth (Oxoid) at 35°C.

Assessment of pre-treatment values
After contamination, the release of test bacteria from the finger-tips of contaminated hands before disinfection (pre-treatment value) was assessed by rubbing the finger-tips of each hand for 1 min on the base of a Petri dish which contained 10 ml of a solution of 0·1 per cent (g/ml) Tryptone (Oxoid) in isotonic phosphate-buffer (pH 7·0) and suitable neutralizing agents.

Neutralisers were added to inhibit the bacteriostatic effect of disinfectants carried over to the counting plates from the sampling fluids. For the neutralization of povidone-iodine, a mixture of Tween 80 (3 per cent), lecithin (0·3 per cent), histidine (0·1 per cent), sodium-thiosulphate (0·5 per cent) and bovine albumin (freeze-dried, 0·1 per cent) was used, and for chlorhexidine gluconate, Tween 80 (3 per cent), lecithin (0·3 per cent) and histidine (0·1 per cent). Although the sampling fluids obtained from experiments with the standard procedure (*iso*-propanol) were not expected to show bacteriostatic effect, the corresponding neutralizers were used for methodological reasons.

Disinfection of hands
Five ml of povidone-iodine liquid soap were poured into the wetted cupped hands, distributed evenly over them and then mixed with small quantities of tap water to produce a lather. This was done for 2 min, and was followed by rinsing for 20 s under running tap-water. Chlorhexidine detergent was used in a similar way but only for 1 min. The standard disinfection procedure with *iso*-propanol was always performed before the test procedure. Three ml of *iso*-propanol 60 per cent were poured into the cupped hands and rubbed on to the skin; after 30 s this procedure was repeated for another 30 s.

Assessment of post-treatment values
To assess the release of test bacteria from the finger-tips of contaminated hands after disinfection (post-treatment values), the same procedure was used as for the recovery of test bacteria before disinfection.

Viable counts
Volumes of 0·1 ml of the sampling fluids and of appropriate dilutions made in an isotonic solution of phosphate-buffered (pH 7·0) Oxoid Tryptone (0·1 per cent g/ml), were spread on the surface of an agar plate (Oxoid).

'Surgical' hand disinfection

Principle of test method

The degerming effect of a 'surgical' hand disinfection procedure was assessed by measuring the reduction in resident bacteria released by clean hands after treatment with the disinfectant. Counts were made before, immediately after, and 3 h after disinfection. The immediate effect of treatment was assessed on one hand and the sustained effect after wearing a glove for 3 h on the other hand. The glove prevented extraneous contamination and also corresponded to the surgeon's hand during an operation. The reduction factors obtained from measuring the immediate and the sustained effects (3 h) were compared with *n*-propanol used on the same test persons and under similar environmental conditions.

Design of test

The four disinfection procedures were included in a latin square experiment involving 15 volunteers. Each procedure was performed once on each subject, and in any one experiment each subject used a different disinfectant. One week elapsed between experiments to allow the recovery of the normal flora.

Assessment of pre-treatment values

After a social hand-wash with a non-medicated soap for 2 min volunteers dried their hands with paper towels. They then proceeded as for the assessment of the pre-treatment values with the 'hygienic' hand disinfection method.

The sampling fluid consisted of isotonic peptone-water (1 per cent g/ml, neutral bacteriological peptone, Oxoid). The neutralizers for povidone-iodine liquid soap were Tween 80 (3 per cent ml/ml), lecithin (0·3 per cent g/ml), histidine (0·1 per cent g/ml), sodium-thiosulphate (0·5 per cent g/ml), and bovine albumin (freeze-dried, 0·1 per cent g/ml), and for chlorhexidine detergent Tween 80 (3 per cent ml/ml), histidine (0·1 per cent g/ml), saponin (3 per cent g/ml), and l-Cystein (0·1 per cent g/ml). No neutralizer was included in sampling fluids used in tests with alcohols.

Disinfection of hands

Five ml of povidone-iodine liquid soap was applied to the hands and further water was added to produce a lather. After 2·5 min this procedure was repeated for a similar period followed by rinsing for 20 s. Chlorhexidine detergent was used in a similar way with a 1 min initial application followed by a further application for 2 min. *Iso*-propanol 60 per cent (ml/ml) was applied in several lots of 3 ml poured into the cupped hands, and constantly rubbed-on for 5 min. Throughout the period the hands were kept wet with the alcohol. Depending on the size and skin-temperature of the hands, 9–18 ml were necessary per person. The standard disinfection procedure with *n*-propanol 60 per cent (ml/ml) was used in the same manner as described for *iso*-propanol.

Assessment of post treatment values
Immediately after disinfection the volunteers shook their hands to remove excess water and sampled the finger-tips as already described to assess the immediate effect, but using only one hand. Whether this was the right or left hand was determined by a randomization procedure before the experiment. The other hand was carefully kept free from contamination until dry. A surgical glove was then worn on this hand for 3 h. It was then sampled in the same way.

Viable counts
Volumes of 1·0 and 0·1 ml of the sampling fluids as well as 0·1 ml of appropriate dilutions in isotonic peptone-water were spread on the surface of an agar plate (Oxoid) without any additives. All plates were incubated at 35°C for 48 h and colonies were counted.

Handling the data

'Hygienic' hand disinfection
Viable counts per ml of sampling fluid were obtained if possible from plates showing 30–300 colonies. Average log values of the counts from the right and left hands of each volunteer were calculated for pre- and post-values, and the difference, the log reduction factor (RF), established. The mean of the RFs of all the volunteers provided a measure of the degerming power of the disinfectant procedure.

'Surgical' hand disinfection
The procedure is as described above, but the values of the two hands were not averaged. Log pre- and post-treatment values were calculated for the immediate and for the sustained effect.

Statistical tests
Mean RFs were tested for significance by Wilcoxon's matched-pairs signed rank test (Siegel, 1956). For analysis of the 'hygienic' hand disinfection experiments a 'one-sided' test was used as it was only necessary to determine whether the test result was significantly less than the standard. The same applied to the comparison with the standard in the 'surgical' hand disinfection tests. Otherwise the two-sided test was used. The significance level was set at P or $2P = 0{\cdot}05$. Under these conditions, for the results of the 'hygienic' hand disinfection tests, the power of the test was estimated at 95 per cent at an effect size of 1·4 with 14 volunteers. This signifies that with an estimated population standard deviation of 0·6–0·7, the difference between the means would be significant in 95 per cent of the experiments if the mean RFs of the test procedure was more than 0·5 log steps less than the standard. For the 'surgical' hand disinfection tests with 14 volunteers a difference of 1·0 log step would be necessary for significance at a power of 85 per cent when the standard deviation of the population is 1·0.

Results

Washing artificially contaminated hands with either povidone-iodine liquid soap for 2 min, or with chlorhexidine detergent for 1 min reduced the release of test bacteria from finger-tips significantly less than rubbing the hands with *iso*-propanol for 1 min. In this application of 'hygienic' hand disinfection, the washing procedures produced mean log RFs of only 3·16 and 2·92 respectively, whereas factors of 4·30 and 4·23 were obtained with *iso*-propanol (Table I).

Table I. *Hygienic hand disinfection. Reduction of the release of test bacteria from finger-tips of artificially contaminated hands by various procedures*

Disinfection procedure	Time taken (min)	Number of subjects	Mean log reductions (± s.d.) of test bacteria	Significance of difference
Washing with povidone-iodine liquid soap (0·75%)	2	15	3·16 ± 0·46	<0·005
Rubbing-in *iso*-propanol (60%)	1		4·30 ± 1·00	
Washing with chlorhexidine detergent (4%)	1	14	2·92 0·82	<0·005
Rubbing-in *iso*-propanol (60%)	1		4·23 0·68	

The results of the experiments for 'surgical' hand disinfection show that rubbing *n*-propanol on to the skin was by far the most effective method of reducing the release of skin bacteria from finger-tips, either immediately or after 3 h. The respective mean log RFs of 3·43 and 2·94 (Table II) differ significantly from those of all other procedures. Using *iso*-propanol in the same manner and concentration gave log RFs of 1·65 and 1·04 respectively. These values are larger than those obtained with povidone-iodine liquid soap (0·92 and 0·24) or with chlorhexidine detergent (0·78 and 0·75), although the differences were not significant. Comparison of the two detergent preparations shows a statistically significant difference at 3 h.

Table II. *Surgical hand disinfection. Reduction of the release of skin bacteria from finger-tips by various procedures*

Disinfection procedure	Time taken (min)	Number of subjects	Mean log reductions (± s.d.) after disinfection	
			Immediately	After 3 h
Rubbing-in *n*-propanol (60%)	5	15	3·43 ± 0·9	2·94 ± 1·4
iso-propanol (60%)	5	15	1·65 ± 1·6	1·04 ± 1·4
Washing with povidone-iodine (0·75%)	5	15	0·92 ± 0·6	0·24 ± 0·4
Washing with chlorhexidine detergent (5%)	3	15	0·78 ± 0·7	0·75 ± 0·7

Discussion

The outcome of experiments designed to investigate the efficacy of disinfection procedures under practical conditions is highly dependent on the method used, and the results may be difficult to interpret. We have, therefore, compared the results with those obtained with a standard disinfection procedure performed under similar conditions with the same volunteers (Rotter, Koller & Kundi, 1977; Rotter, Koller, Kundi, Wewalka & Mittermayer, 1977). The reference method should correspond with the test method, especially in its efficacy and mode of application. The effectiveness of a preparation is regarded as satisfactory if the transmission of pathogens via the hands is prevented. Unfortunately, epidemiological data showing the effectiveness of modern preparations, as was obtained by Semmelweis, are rarely available.

The required effect and choice of a standard is therefore arbitrary. We have chosen alcohols, since they are the most rapidly acting and most effective skin disinfectants known today. Their application by rubbing on the skin is ideal for 'hygienic' hand disinfection because there is no uncontrolled dispersal of organisms during the procedure. The drying effect of alcohols on the skin can be overcome by adding protective substances which are usually present in commercially available preparations. Most of the arguments and explanations pertaining to our methods have been described elsewhere (Rotter *et al.*, 1974; Kundi, Koller, Mittermayer & Rotter, 1975; Mittermayer & Rotter, 1975; Koller, Rotter, Mittermayer & Wewalka, 1976; Koller *et al.*, 1977; Rotter, Koller & Kundi, 1977), and only the choice of *Esch. coli* as the test organism will be discussed. *Escherichia coli* and *Staphylococcus aureus* are the commonest causes of hospital infection (Center for Disease Control, 1978) so there is little doubt that *Esch. coli* is an important pathogen. We believe it is more suitable than *Staph. aureus* as the test organism because it is easily distinguished from the skin flora and does not have the problem of aggregate dispersal as does *Staph. aureus*, it is more resistant than staphylococci to hexachlorophane, chlorhexidine and quaternary ammonium compounds and it is safer than *Staph. aureus*. *Staph. aureus* used as the test organism has caused sepsis in similar experiments (Ayliffe, Babb & Quoraishi, 1978). Its main disadvantage is a relatively high death rate on drying, but under standardized conditions this is not a great problem (Koller *et al.*, 1976; Ayliffe *et al.*, 1978).

From our results it is clear that in 'hygienic' hand disinfection both povidone-iodine liquid soap and chlorhexidine detergent are far less active than *iso*-propanol. Indeed, the measured effects of both substances (RFs 3·16 and 2·92 respectively) are not any better than those achievable with liquid soap and water, which tested by our method gave RFs of 2·99 and 3·27 (Mittermayer & Rotter, 1975). These results simply reflect the physical action of hand washing. Applying tests comparable to our method, Lowbury, Lilly and co-workers (Lowbury, Lilly & Bull, 1964; Ayliffe, Babb & Quoraishi, 1978; Lilly & Lowbury, 1978) came to similar results and conclusions. We consider the reduction of micro-organisms achieved by a simple ablution is sufficient to prevent infection-generating transmission of pathogens via hands in most situations, and we have no indication that povidone-iodine or chlorhexidine added to detergents offer any advantage. The addition of

these agents is not only unnecessary, since they are relatively ineffective, but possibly introduce a risk of allergic or toxic reactions. Furthermore, for true 'hygienic' hand disinfection, as might be necessary in the microbiology laboratory after contamination with dangerous pathogens, washing procedures may not be suitable.

Pre-operative preparation of the surgeon's hands should reduce the release of both transient and resident flora for the period of the operation. To examine this, a test is required that evaluates the efficacy of a procedure for 'surgical' hand disinfection not only immediately after disinfection, but also after wearing gloves for a period which should cover the duration of most operations. In contrast to this sustained effect, the so called 'cumulative' action of an antimicrobial substance, the progressive reduction of the release of skin bacteria by multiple applications, in our opinion is of minor importance. The surgeon's hands must be safe immediately after the first disinfection and this includes Monday morning. For this reason, we do not consider a cumulative action is worth investigating for 'surgical' hand disinfection. Our results (Table II) agree with those of other authors who have used similar methods. Michaud, McGrath & Goss (1976) have reported immediate log reductions of between 1·01 and 1·10 after a surgical scrub of 6 min using the iodophor preparation, 'Betadine'. One application of chlorhexidine detergent for 2 to 2·5 min was estimated to reduce the release of resident bacteria immediately after disinfection by log reductions of between 0·83 and 0·92 (Ayliffe *et al.*, 1976; Lilly, Lowbury & Wilkins, 1979*a*,*b*). From these and our results it appears that the immediate effect of both compounds (RFs of 0·92 and 0·78) is poor and almost negligible in comparison to that of the alcohols (RFs of 3·43 and 1·65). One has to accept, however, that treatment of hands with any simple detergent and water often not only fails to reduce the release of skin bacteria, but even increases it for a while (Speers, Bernhard, O'Grady & Shooter, 1965; Meers & Yeo, 1978; Lilly *et al.*, 1979*a*). In this respect, the moderate but significant ($2P < 0{\cdot}004$ and $2P > 0{\cdot}02$, respectively) reduction is definitely attributable to the antimicrobial activity of both compounds. Lack of epidemiological data concerning the size of the bacterial reduction needed to reduce infection prevents any evaluation on the basis of absolute values, so only a comparison with other procedures can serve as a guideline. Taking this into consideration, povidone-iodine liquid soap and chlorhexidine detergent must be regarded as considerably less safe than alcohols.

With a mean RF of only 0·24 after 3 h no sustained effect can be attributed to the povidone-iodine liquid soap used in these tests. For 'Betadine', Michaud *et al.*, (1976) found a log reduction of 0·28 after 3 h using a similar method, and Muntener, Schwarz & Reber (1972) could not find any true sustained effect. In contrast, chlorhexidine detergent exerted a marked sustained effect that, though insignificantly inferior to that of *iso*-propanol ($P > 0{\cdot}1$), was significantly better ($P < 0{\cdot}01$) than that of the povidone-iodine containing detergent.

While the powerful immediate antimicrobial action of alcohols has always been accepted, their unexpectedly good sustained action might seem surprising. This was explained by Lilly, Lowbury, Wilkins & Zaggy (1979) as being due to a large immediate effect with added sublethal damage to some bacteria. These damaged

cells can recover if promptly inoculated onto culture media, but not under a surgical glove. The greater effectiveness of *n*-propanol over ethanol or *iso*-propanol has been reported by others as well as by our own group (Rotter, Koller & Kundi, 1977).

There can be no doubt that at present alcohols are the most effective agents for hand disinfection, and that their efficacy should serve as a yardstick for any other antiseptics.

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The Antibacterial Activity of a New Chloroxylenol Preparation Containing Ethylenediamine Tetraacetic Acid

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A new chloroxylenol preparation (DC) containing ethylenediamine tetraacetic acid (EDTA), has been shown to be an effective bactericidal agent against various Gram positive and Gram negative bacteria. It possessed significant and rapid activity against two strains of *Pseudomonas aeruginosa* as well as against chlorhexidine- and chloroxylenol-resistant derivatives of these strains. DC showed high activity at 20° and 30 °C, and (unlike chlorhexidine) when prepared in hard water or when used in the presence of organic matter. Repeated challenge testing at 20 °C of DC (but not of chloroxylenol) with *Ps. aeuruginosa* indicated that contamination of DC will not prove to be a problem in practice and that the activity of solutions will be retained during storage.

CHLOROXYLENOL as a disinfectant has been used for many years (Colebrooke & Maxted 1933). It shows a fairly broad spectrum of activity, but strains of *Pseudomonas aeruginosa* may be resistant (Lowbury 1951; Gray & Wilkinson 1965*a*,*b*). Recently, interest in its antibacterial properties has been renewed following the findings that activity against *Ps. aeruginosa* of chloroxylenol plus ethylenediamine tetraacetic acid (EDTA) was greater than that of chloroxylenol alone (Gray & Wilkinson 1965*a*; for reviews on EDTA, see Russell 1971, and Wilkinson 1975).

A new chloroxylenol formulation containing EDTA is shortly to be marketed in this country, and the present paper describes its antibacterial activity against various organisms.

Materials and Methods

Organisms

These consisted of hospital isolates (termed RF) of the following: *Ps. aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella agona*, *Klebsiella aerogenes* and *Shigella sonnei*. The following strains were also used: a methicillin-resistant *Staph. aureus* (WH 68, kindly provided by Mrs C. H. O'Callaghan), and R^+ *E. coli* (strain R1, kindly provided by Dr N. Datta), *Ps. aeruginosa* NCTC 6750, *P. morganii* NCTC 232, *P. vulgaris* NCTC 4175, and a pigmented strain (NCTC 8706) and a non-pigmented strain (NCTC 9741) of *Serratia marcescens*.

Disinfectants

Three disinfectants were used: (a) a concentrate of chloroxylenol plus EDTA (DC; Reckitt & Colman, Hull), which in its recommended in-use form consists of 0·12% (w/v) chloroxylenol plus 0·165% (w/v) (*ca.* $4{\cdot}4 \times 10^{-3}$ M) EDTA and is prepared by

adding 10 ml of a concentrate to 1 litre of water; (b) Dettol (Reckitt & Colman, Hull) which in its recommended in-use concentration consists of 5% (equivalent to 0·24% (w/v) chloroxylenol) of the concentrate; (c) chlorhexidine diacetate, used within the range 0·01–0·1% (w/v), and prepared from a concentrated 20% solution (Hibitane: I.C.I. Ltd., Macclesfield).

Nutrient media

Organisms were grown in 10 ml Nutrient Broth No. 2 (Oxoid Ltd., Basingstoke). Recovery media consisted of Letheen Broth (Difco, Detroit, Michigan, U.S.A.). This medium was effective in neutralizing DC (maximum final EDTA concentration was 8×10^{-5} M: see below), chloroxylenol or chlorhexidine, as indicated by the finding that inoculation of 5 ml Letheen Broth containing a concentration of disinfectant equivalent to maximum 'carry-over' (Testing procedure, below) with *ca.* 20 viable cells of *Ps. aeruginosa* RF4 or *Staph. aureus* RF3 allowed growth to proceed.

Testing procedure

Volumes (0·5 ml) of overnight 37 °C broth cultures of the organisms were added to 10 ml volumes of appropriate dilutions, previously equilibrated at 30 °C, of the test disinfectants. Disinfectants were prepared in sterile glass distilled water, or in sterile hard water (containing 0·304 g/l anhydrous calcium chloride and 0·139 g/l magnesium chloride hexahydrate). In some experiments, organic matter in the form of 10% serum (Wellcome Research, London: horse serum No. 3), final concentration, was included in the testing procedure. 0·1 ml samples were removed in duplicate after 10 min and transferred to 5 ml Letheen broth. The maximum 'carry-over' of disinfectant was thus: (a) DC: 0·0024% chloroxylenol + 8×10^{-5} M-EDTA; (b) 0·0048% chloroxylenol; (c) 0·002% chlorhexidine. Media were incubated at 37 °C and the presence or absence of growth noted daily for up to 3 d.

In some experiments, exposure to disinfectants was carried out at 20 °C instead of 30 °C. One series of tests was also made to determine the minimum lethal time of various dilutions of DC acting against *Ps. aeruginosa* RF4 at 20 ° and 30 °C.

Repeated challenge testing

Volumes (50 ml) of 5% Dettol and DC were stored at 20 °C. On various days, 0·05 ml of an overnight 37 °C broth culture of *Ps. aeruginosa* RF4 was added, and after 10 min at 20 °C, 0·1 and 0·5 ml volumes were removed and inoculated into 5 ml Letheen Broth. This was incubated at 37 °C and the presence or absence of growth observed for up to 3 d. In some experiments, *Ps. aeruginosc* was not added but a sample was removed (a) for inoculation into Letheen Broth, (b) to determine the number of viable cells present by means of the pour plate method.

Resistance studies

Reistance studies were made using the gradient plate technique devised by Szybalski (1952) and by continuous subculturing on to agar containing gradually increasing concentrations of chlorhexidine (0·02–0·1%) or chloroxylenol (0·112–0·48%). Two strains of *Ps. aeruginosa* (RF4 and NCTC 6750) were used for this purpose. Derived strains resistant to chlorhexidine were designated R4H and R67H, and to chloroxylenol R4D.

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MIC determination

Nutrient agar plates containing appropriate concentrations of chloroxylenol (0, 0·024–0·24%) or chlorhexidine (0–0·05%) were dried for 3 h at 37 °C, and overnight 37 °C broth cultures streaked on the surface. Presence or absence of growth was noted after incubation of the plates at 30 or 37 °C. The minimum inhibitory concentration (MIC) is the lowest concentration completely preventing growth.

Results

MIC values

These are listed in Table 1, which indicates that most strains tested were inhibited by 0·024–0·048% (w/v) chloroxylenol except for the two *Ps. aeruginosa* strains, although at the highest chloroxylenol concentrations tested, only very few colonies (<10 with

TABLE 1

MIC values of chlorhexidine and chloroxylenol against various strains

Organism	Strain	MIC of chlorhexidine (%)		MIC of chloroxylenol (%)	
		30 °C	37 °C	30 °C	37 °C
E. coli	RF1	<0·01	<0·01	<0·024	<0·048
E. coli	R1	<0·01	<0·01	<0·024	<0·048
P. morganii	NCTC 232	<0·01	ND	<0·024	ND
P. mirabilis	RF2	0·02	0·02	<0·024	<0·048
P. vulgaris	NCTC 4175	<0·01	ND	<0·024	ND
S. aureus	RF3	<0·01	<0·01	<0·024	<0·048
S. aureus	WH68	<0·01	<0·01	<0·024	<0·024
Ps. aeruginosa	RF4	0·04	0·03	>0·24	0·19–0·24
Ps. aeruginosa	NCTC 6750	0·03	0·03	>0·24	0·19–0·24
Strep. faecalis	RF5	<0·01	<0·01	<0·024	<0·048
K. aerogenes	RF6	<0·01	<0·01	<0·024	<0·048
Salm. agona	RF7	<0·01	<0·01	<0·024	<0·024
Shig. sonnei	RF8	<0·01	<0·01	<0·024	<0·024
S. marcescens	NCTC 8706	<0·01	0·01	<0·024	<0·024
S. marcescens	NCTC 9741	0.02	0·02	<0·024	<0·024

ND, not done.

RF4 or NCTC 6750) developed. When chlorhexidine was used, a concentration of 0·01% (w/v) was inhibitory to most strains, with the two *Ps. aeruginosa* strains again being the most resistant, closely followed by *P. mirabilis* RF2 and the non-pigmented strain (NCTC 9741) of *S. marcescens*. Results at 30 °C were generally similar to those obtained at 37 °C.

MLC values

MLC values for the various products tested at 30° and at 20 °C are listed in Tables 2 and 3, respectively. Generally, high concentrations (often 20–40 × MIC) of chlorhexidine were needed for a complete kill within 10 min. Effective killing concentrations of DC varied from a 1 in 2 dilution (chloroxylenol 0·06% (w/v) + EDTA 0·0825%

TABLE 2

Minimum lethal concentration (MLC) of products after 10 min at 30 °C

Organism	Strain	MLC of chlorhexidine (%)	MLC of chloroxylenol (%)	DC* (1 in)
E. coli	RF1	0·02–0·04	0·048	<1 in 5
E. coli	R1	0·02–0·04	0·048	<1 in 5
P. morganii	NCTC 232	0·03–0·04	<0·048	<1 in 5
P. mirabilis	RF2	>0·05	<0·048	1 in 4
P. vulgaris	NCTC 4175	<0·01	<0·048	<1 in 5
S. aureus	RF3	0·02	<0·048	<1 in 5
S. aureus	WH68	0·02	<0·048	1 in 3
Ps. aeruginosa	RF4	0·03	0·12–0·24	1 in 5
Ps. aeruginosa	NCTC 6750	>0·05	>0·24	<1 in 5
Strep. faecalis	RF5	0·04	<0·048	1 in 5
K. aerogenes	RF6	0·04	<0·048	1 in 5
Salm. agona	RF7	0·02	0·096	<1 in 5
Shig. sonnei	RF8	0·02	<0·048	1 in 4
S. marcescens	NCTC 8706	0·02–0·03	<0·048	<1 in 5
S. marcescens	NCTC 9741	0·05	<0·048	<1 in 5

* DC (neat, i.e. 10 ml/l) contains 0·12% (w/v) chloroxylenol + 0·165% (w/v) EDTA.

TABLE 3

Minimum lethal concentration (MLC) of products after 10 min at 20 °C

Organism	Strain	MLC Chlorhexidine (%)	MLC Chloroxylenol (%)	DC* (1 in)
S. aureus	RF3	0·05	0·048	1 in 5
Ps. aeruginosa	RF4	0·05	0·144	1 in 2

* Dettol chelate (neat, i.e. 10 ml/l) contains 0·12% (w/v) chlorhexidine + 0·165% EDTA.

TABLE 4

Minimum lethal concentrations (MLC) of the products against resistant strains of Ps. aeruginosa

Strain	Resistance induced to	MLC after 10 min at 30 °C of chlorhexidine (%)	MLC after 10 min at 30 °C of chloroxylenol (%)	DC* (1 in)
R4H	Chlorhexidine (MIC > 0·05%)	0·1	0·29	1 in 2
R4D†	Chloroxylenol (MIC > 0·48%)	0·02	0·19–0·29	Neat
R67H	Chlorhexidine (MIC 0·05%)	0·05	0·19	1 in 3

* DC (neat, i.e. 10 ml/l) contains 0·12% (w/v) chloroxylenol + 0·165% (w/v) EDTA.
† The R4D strain did not show stable resistance to chloroxylenol and after growth overnight in plain nutrient broth No. 2 would not grow on agar containing 0·48% chloroxylenol.

(w/v)) to <1 in 5. A distinct increase in activity of DC was noted, as compared with that of chloroxylenol alone, against *Ps. aeruginosa* and possibly against some of the other strains also, e.g. *E. coli* RF1 and the R⁺ strain, R1. Other Gram negative bacteria and the Gram positive strains tested were sensitive to Dettol alone.

As expected, substances were generally less active at 20 than at 30 °C, although half the recommended in-use concentration of DC was still lethal to *Ps. aeruginosa* RF4 after 10 min.

MLC values of the test disinfectants against strains R4H, and R4D, both derived from RF4, and R67H, derived from NCTC 6750, are shown in Table 4. DC remained highly active against all the *Ps. aeruginosa* strains including the chlorhexidine resistant (R4H and R67H) and chloroxylenol resistant (R4D) strains. Colonies of R4D on agar containing 10% Dettol were whitish and highly mucoid. Resistance to chloroxylenol was not stable.

Death rate of Ps. aeruginosa

Two strains, RF4 and its chlorhexidine-resistant derivative R4H, were used in tests involving various dilutions (neat, 1 in 2, 1 in 3) of DC at 20° and 30 °C. No viable cells could be recovered after treatment at either temperature even when exposure of the cells was for only 1 or 2·5 min. This suggests that the mixture has an immediate, irreversible effect on the cells.

Effect of organic matter

The activity of all three products was reduced in the presence of 10% serum (Table 5). Chlorhexidine was particularly affected, with 'precipitation' occurring at all concentrations. Although the effectiveness of DC was reduced, it was still highly active at half of its recommended in-use concentration against both *Ps. aeruginosa* and *S. aureus*.

TABLE 5

Effect of organic matter (as 10% serum) on the minimum lethal concentration (MLC) of products after 10 min at 30 °C

Organism	Strain	MLC of chlorhexidine (%)	MLC of chloroxylenol (%)	MLC of DC* (1 in)
S. aureus	RF3	>0·05	0·096	2
Ps. aeruginosa	RF4	>0·05	>0·24	2

* DC (neat, i.e. 10 ml/l) contains 0·12% (w/v) Chloroxylenol + 0·165% (w/v) EDTA.

Effect of hard water

Artificial hard water, prepared to the specifications of the World Health Organisation, was used to prepare all disinfectants. Its influence on the activity of the three products at 20° and 30 °C against *Ps. aeruginosa* and *S. aureus* is illustrated in Table 6. The activity of all three products was generally markedly reduced, but even so, DC at its recommended in-use concentration was still highly active against *Ps. aeruginosa*.

TABLE 6

Effect of hard water on the minimum lethal concentration (MLC) of products after 10 min at 20 or 30 °C

		MLC of					
		chlorhexidine (%)		chloroxylenol (%)		DC* (1 in)	
Organism	Strain	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
S. aureus	RF3	>0·05	0·02–0·03	<0·048	<0·048	1 in 3	1 in 5
Ps. aeruginosa	RF4	>0·05	>0·1	>0·24	>0·24	Neat	Neat

* DC (neat, i.e. 10 ml/l) contains 0·12% (w/v) chloroxylenol + 0·165% (w/v) EDTA.

Repeated challenge testing

Table 7 presents the results of tests in which DC and 5% Dettol solutions were repeatedly inoculated with *ca.* 10^6 viable cells/ml of *Ps. aeruginosa* RF4. Dettol rapidly became infected, and on day 12 the number of viable cells/ml was $>5 \times 10^6$/ml. On the other hand, DC at its in-use concentration rapidly killed (<10 min) all the *Ps. aeruginosa* cells added to it: even at day 48, no viable cells could be recovered, Thus, whereas active proliferation in 5% Dettol is a likely possibility (Lowbury 1975), the same untoward result is not seen when EDTA is present.

TABLE 7

Repeated challenge testing of DC and Dettol at 20 °C with ca. 10^6 viable cells/ml of Ps. aeruginosa *RF4*

	Complete kill (+) or not (–) after exposure of cells for 10 min to	
Day No.	Dettol* (5%)	Dettol chelate† (10 ml/l)
1	+	+
2	–	+
3	–	+
4	ND	+
6	–	ND
9	–	+
10	–	+
11	ND	+
28	ND	+
48	ND	+

* 5% Dettol contains 0·24% (w/v) chloroxylenol.
† DC (neat, i.e. 10 ml/l) contains 0·12% (w/v) chloroxylenol + 0·165% (w/v) EDTA.
ND, not done.

Discussion

The present investigation has shown that the new chloroxylenol preparation containing EDTA is an effective antibacterial mixture at concentrations ranging from ½ to ⅓ of its in-use level. As would be expected from the conditions tested, its activity is most marked at 30 °C and is reduced somewhat by usage at 20 °C, in the presence of organic matter (Russell 1974) or when diluted in the hard water. The latter result is not surprising, since the effective EDTA concentration will be decreased as a result of its interaction with the cations present. EDTA reacts with all metal ions in a 1:1 ratio (West 1969). This means that with the hard water used about 75% of the EDTA in in-use DC is involved in complexation with Mg^2 and no 'free' EDTA is available in dilutions of DC. Consequently, the chloroxylenol-EDTA mixture is less effective under these conditions. Even so, the in-use concentration remained fully active at 20° and 30 °C against *Ps. aeruginosa*. Chlorhexidine, in particular, was greatly affected by all of these factors.

The results have also demonstrated: (i) that the combination of chloroxylenol and EDTA is effective not only against the two strains (RF4 and NCTC 6750) of *Ps. aeruginosa* used throughout this work but also against the two chlorhexidine-resistant derivatives, R4H and R67H, respectively, and against the chloroxylenol-resistant derivative R4D. Although changes in the outer cell membrane are likely to have occurred in these cells, with a consequent barrier to the entry of chlorhexidine and/or chloroxylenol, EDTA or chloroxylenol could alter the envelope thereby permitting the other to enter the cell. Experiments reported previously from other laboratories (Russell 1971; Wilkinson 1975) in which cells were pretreated with EDTA before being exposed to antibacterial agents would suggest that envelope alteration is achieved by EDTA. (ii) An increase in activity of DC over chloroxylenol alone also occurs to some extent against the two *E. coli* strains tested, although this is less marked than with *Ps. aeruginosa*. EDTA has been found to potentiate the activity of other substances against *E. coli* (Gray & Wilkinson 1965*a*,*b*; Russell 1967, 1971; Wilkinson 1975).(iii) Other Gram negative organisms appeared to be unaffected by EDTA, at least as far as the test methods were concerned. Most of these strains were also highly sensitive to chloroxylenol alone (i.e. EDTA absent). (iv) There was no increase in activity of the EDTA-chloroxylenol combination as compared with the Gram positive bacteria tested which follows previously published patterns of EDTA activity (Russell 1971; Wilkinson 1975). These strains were highly sensitive to chloroxylenol alone.

Repeated challenge testing of both Dettol and DC with *Ps. aeruginosa* at 20 °C indicated that the former rapidly became infected (see also Lowbury 1951) whereas DC was highly active against far higher levels of contaminating bacteria than could be expected in practice. Thus, anxiety at the possibility of DC becoming contaminated during storage is likely to be unfounded.

'Carry-over' of a toxic amount of EDTA into the recovery medium is not borne out by the finding that small numbers of viable cells can grow in Letheen Broth containing DC (equivalent to 8×10^{-5} M-EDTA); thus, lack of available iron or magnesium as a result of EDTA chelation of the medium (Melling & Brown 1975) cannot be put forward as being a factor in preventing bacterial growth. Moreover bacteria can, in fact, grow in nutrient medium containing much higher concentrations of EDTA (Russell 1967) than those carried over into recovery-media in our testing procedure.

DC is, thus, a potentially useful new disinfectant preparation with sustained and high activity against pseudomonads. It is likely to find use as a skin disinfectant (Dankert & Schut 1976), as well as for wound cleansing and general antiseptic application.

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Review article

Atopic dermatitis

Hugh A. Sampson, MD

INTRODUCTION

Atopic dermatitis is a form of eczema that is most prevalent during the childhood years. Approximately 80% of pediatric cases develop by 1 year of age, and 95% of all cases are apparent by 5 years of age.[1] Recent surveys indicate that the prevalence of atopic dermatitis has increased over the past several decades and now affects 10% to 15% of the population at some point during their lifetime.[2] Over 10% of pediatric dermatology visits and approximately 1% of general pediatric visits are reportedly related to atopic dermatitis. While less common in adults, atopic dermatitis is one of the most challenging (and often frustrating) disorders treated by the allergist. The pathogenesis, clinical course, therapy, and natural history of atopic dermatitis will be reviewed. Practical therapeutic modalities and possible future therapies are presented.

BACKGROUND

The first comprehensive description of atopic dermatitis was put forth a century ago by Besnier,[3] who noted the hereditary nature, chronically recurring course, and association with hayfever, asthma, and rarely gastrointestinal disturbances. Wise and Sulzberger[4] later coined the term "atopic dermatitis" to emphasize further the relationship between atopic eczema, hayfever, and asthma (the "allergic triad"). Atopic dermatitis is one form of eczema, all of which are characterized by erythematous, pruritic skin lesions that may peel, blister, and weep. Other forms of eczema include seborrheic dermatitis, dyshydrotic eczema, irritant contact dermatitis, and allergic contact dermatitis. Like asthma, atopic dermatitis might be divided into "extrinsic" and "intrinsic" forms of the disorder. Patients with extrinsic atopic dermatitis are generally younger (ie, less than 20 years of age), have significant allergen-specific IgE antibodies, and have specific food and/or airborne allergens that exacerbate flares of eczema. Patients with intrinsic atopic dermatitis tend to be older and have no evidence of allergen-induced flares.

PATHOPHYSIOLOGY

Atopic dermatitis generally presents as an erythematous papulovesicular eruption that evolves into scaly, lichenified plaques over time (Fig 1). Histologic features of atopic dermatitis lesions are nonspecific and vary with the stage of the lesion.[5,6] Acute lesions are characterized by intracellular edema (ballooning of the keratinocytes) and intercellular edema (spongiosis) with vesiculation and infiltration of lymphocytes and monocytes. In the dermis, lymphocytes, monocytes, and occasional neutrophils and eosinophils are prominent in perivenular areas. Chronic lesions are characterized by marked epidermal hyperplasia, dyskeratosis and fibrosis at all levels of the dermis, increased numbers of mast cells and Langerhans cells, and demyelination of cutaneous nerves.

Immunohistochemical studies have shown that infiltrating lymphocytes are predominantly $CD4^+$ cells, Langerhans cells bear high-affinity Fc, receptors (Fc,RI) and allergen-specific IgE on their surface[7,8] a significant proportion of mast cells in lesional skin are lymphocyte-dependent M_T type (instead of M_{TC} type)[9] and eosinophil major basic protein (MBP) and cationic protein (ECP) are deposited in the dermis of eczematous lesions in most patients.[10] Recent studies suggest that the majority of infiltrating $CD4^+$ T cells are antigen-specific TH_2 cells.[11,12] Activated TH_2 cells secrete IL-4, IL-5, IL-6, IL-10, IL-3, and GM-CSF.[13] These cytokines promote B cell responses, especially IgE production, up-regulation of IgE receptors on Langerhans' cells[7] and local infiltrating cells, and down-regulation of TH_1 activity[14] and IL-1 receptors on monocytes.[15] The latter effects may account for the lack of delayed skin test reactivity and the cutaneous anergy seen in most patients with atopic dermatitis.

A number of abnormal physiologic and immunologic phenomena have been delineated in patients with atopic dermatitis, most of which vary in direct proportion to the extent of cutaneous lesions.[16] Physiologic abnormalities include a decreased itch threshold, paradoxical cutaneous vascular responses including white dermographism, increased transepidermal water loss secondary to decreased and/or

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Figure 1. Young child with facial involvement typical of the childhood form of atopic dermatitis.

structurally altered ceremides from keratinocytes, increased plasma linolenic acid levels and abnormal red cell phospholipids, and increased cyclic-AMP phosphodiesterase activity in mononuclear cells.

"Itch" is the dominant symptom in atopic dermatitis ("the itch that rashes") and the major cause of damaging excoriations, erosions, and lichenification, which are characteristic features of atopic dermatitis.[17] The etiology of this increased cutaneous pruritus is unknown. Vasodilation has been shown to precede the itch suggesting that local release of mediators may be responsible for the increased pruritus. The increased number of mast cells and elevated tissue histamine in chronically involved areas support this hypothesis.

Increased transepidermal water loss is believed to be secondary to decreased sebum production.[18] A number of abnormal cutaneous responses have been noted in patients with atopic dermatitis[19] including increased sweating and a variety of abnormal vascular responses involving exaggerated constrictor response of cutaneous vessels and poor adaptability (vascular hyperactivity), white dermographism, delayed blanch to cholinergic stimuli, and a paradoxical response to application of nicotinic acid. None of these responses is specific for atopic dermatitis.

Atopic dermatitis patients have several features suggesting the presence of β-blockade. Their skin lacks the expected inhibition of DNA synthesis following treatment with beta-adrenergic agonists and their leukocytes show functional responses that correlate with subnormal cellular cyclic-AMP levels following beta-adrenergic stimulation.[20] Some studies have shown a consistent increase in cyclic-AMP phosphodiesterase activity in untreated mononuclear leukocytes from patients with atopic dermatitis, but not in patients with contact dermatitis.[21] Increased phosphodiesterase activity could account for the reduced cellular cyclic-AMP levels seen in patients with atopic dermatitis. Other investigators, however, have failed to demonstrate a significant difference in leukocyte phosphodiesterase activity between children with atopic dermatitis and normal controls[22] and phosphodiesterase activity was found to return to normal in many adults with atopic dermatitis following topical steroid therapy.[23]

Immunologic abnormalities are characterized by overproduction of IgE and diminished T cell functions. Two lines of evidence suggest an underlying abnormality in some bone marrow-derived cell(s) or factor(s); patients with Wiskott-Aldrich syndrome experience clearing of their eczematous rash following successful reconstitution by bone marrow transplantation.[24] and latent atopy has been transferred to the recipients of bone marrow from atopic donors.[25, 26] Abnormalities of both humoral and cellular immunity have been described in patients with atopic dermatitis[16]: (1) elevated serum IgE concentrations in approximately 80% of patients, (2) decreased anamnestic antibody levels of recall antigens (eg, tetanus, polio, etc), (3) defective delayed-type skin responsiveness with increased viral infections of the skin and decreased ability to sensitize patients to contact antigens such as DNCB, (4) variably decreased lymphocyte response to mitogens, recall antigens, and alloantigens in vitro, (5) decreased $CD8^+$ cells and increased CD4/CD8 ratio in the peripheral blood, (6) defective generation of cytotoxic T lymphocyte response in vitro, and (7) variably decreased phagocytic capacity and chemotaxis of neutrophils and monocytes. These defects do not appear to be central to the disorder since they fluctuate with disease activity and may revert to normal during long remissions.

Clinically, patients with atopic dermatitis experience increased numbers of skin infections. *Staphylococcus aureus* colonizes the skin of over 95% of atopic dermatitis patients (<5% of normal controls), with particularly high concentrations of the organism in areas of active dermatitis.[27] The nearly constant presence of *Staphylococcus aureus* suggests some microbicidal dysfunction, possibly due to the abnormal fatty acid content of sebum[28] and/or intermittent depression in neutrophil and monocyte chemotaxis. Superficial pustules, which are extremely pruritic and consequently are rapidly excoriated, usually are associated with active or flaring dermatitis. Septicemia generally is not a problem, and deep cutaneous infections are rare in contrast to patients with the hyper-IgE syndrome. The means by which *Staphylococcus aureus* causes flares of the dermatitis have not been fully delineated. It has been suggested that various enzymes and toxic products which can activate mast cells may be introduced into the skin by scratching, leading to further pruritus and inflammation. Recently, it has been shown that some patients with atopic dermatitis have IgE antibodies specific to toxins secreted by *Staphylococcus aureus* (eg, Staphylococcal exotoxins A, B, and C; TSST-1)[29] that may lead to allergic inflammation.

Cutaneous anergy is the most striking immunologic dysfunction during active atopic dermatitis. This anergy is transient; it resolves with resolution of the dermatitis. Patients are at increased risk of developing cutaneous infections with herpes simplex (eczema herpeticum—Fig 2), verruca vulgaris (common warts), molluscum contagiosum (Fig 3), and previously vaccinia. Dermatophyte infections are reportedly more common in atopic dermatitis patients and *Pityrosporum ovale* colonization recently has been associated with chronic flares of eczema.[30] Patients are less easily



Figure 2. Patient with eczema herpeticum (disseminated cutaneous herpes simplex inf of the skin). Lesions about eye indicate the need for intravenous acyclovir therapy.

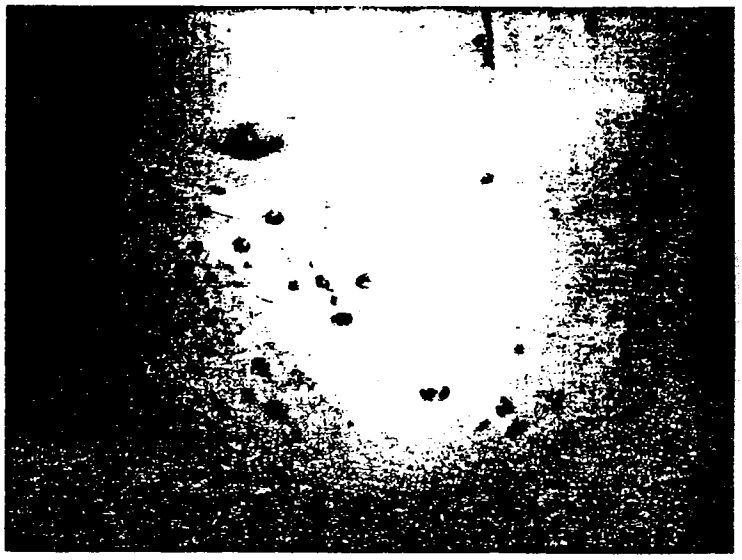


Figure 3. Patient with molluscum contagiosum, an epidermotropic DNA poxvirus, is commonly seen in the axillae, neck, or lateral aspects of the trunk.

sensitized to Rhus (poison ivy) and dinitrochlorobenzene and show decreased delayed skin test reactivity to a variety of antigens.

For years various lines of evidence have been advanced supporting the role of allergen-specific IgE antibodies in the pathogenesis of atopic dermatitis[16]: at least 80% of patients have elevated serum IgE concentrations and positive immediate skin tests and RASTs to various dietary and environmental allergens, family history of atopy is positive in 70% to 90% of patients, and other atopic disorders (allergic rhinitis and asthma) develop in most children with atopic dermatitis.[31,32] Although the histologic appearance of the eczematous lesion appears most consistent with a classical cell-mediated hypersensitivity response,[33] the terminal stages of IgE-mediated hypersensitivity reactions also are characterized by infiltration of lymphocytes and monocytes.[34] During the early phase of mast cell activation, cytokines are generated that upregulate vascular endothelial cell adhesion molecules and direct eosinophils and mononuclear cells to the area.[35-37] Approximately four to eight hours following initial cutaneous mast cell activation, eosinophils and neutrophils infiltrate the dermis. Biopsies obtained eight hours after initiation of the reaction revealed 48% lymphocytes, 27% eosinophils, 9% neutrophils, and 7% monocytes.[34] Lymphocytes and monocytes predominated at one to two days. Skin biopsies from chronic eczematous lesions of patients with atopic dermatitis contain large quantities of MBP in the superficial dermis. Major basic protein is not present in noneczematous skin indicating selective deposition of MBP by infiltrating eosinophils.[38]

Food and airborne allergens may reach cutaneous mast cells, lymphocytes, monocytes, and Langerhans' cells through breaks in the skin or by way of the circulation after entering at mucosal surfaces. The interaction of allergens with allergen-specific IgE on the surface of mast cells activates the cells to release histamine, LTC_4, platelet-activating factor, IL-4 and other cytokines that may attract other cells (eg, eosinophils, lymphocytes, and monocytes) found in an IgE-mediated late-phase response. Recent studies have demonstrated the presence of allergen-specific $CD4^+TH_2$ cells in the skin of atopic dermatitis patients.[11,39-41] Repeated allergen exposure provokes chronic inflammation secondary to IgE-mediated mast cell and lymphocytic responses, and contributes to the pathogenesis of atopic dermatitis.

Utilizing double-blind placebo-controlled food challenges (DBPCFC), 320 children and adolescents with atopic dermatitis were evaluated for food hypersensitivity.[42] Patients developing a pruritic, erythematous, macular or morbilliform rash following allergen exposure in a positive DBPCFC were found to have a rise in plasma histamine,[43] but no change in complement activation products, circulating basophil number or total basophil histamine content.[44] Skin biopsy specimens obtained from involved sites four and 14 hours later revealed an infiltration of eosinophils and early MBP deposition. Taken together, these studies demonstrated that food allergens could trigger both "immediate" and "late-phase" responses in the skin.

Patients with atopic dermatitis and newly diagnosed food hypersensitivity were found to have high "spontaneous" basophil histamine release (SBHR) from peripheral blood basophils in vitro compared with normal controls and patients with atopic dermatitis and no food allergy.[45] When food-allergic patients were placed on an appropriate elimination diet for at least 1 year, most experienced good clearing of their eczema and a significant fall in SBHR. In food allergic subjects with high SBHR, unstimulated peripheral blood mononuclear cells produced a histamine-releasing factor in vitro that could activate basophils from other food sensitive individuals (ie, patients with IgE^+), but not food nonsensitive subjects (ie, individuals with IgE^-). The ingestion of food allergens appeared to provoke histamine-releasing factor production in vivo, which then could activate, or lower the "threshold" of activation, of mast cells. It also may account for the increased basophil "releasability" in some patients with atopic dermatitis and the high "spontaneous" basophil histamine release seen in food sensitive subjects in vitro. The loss of "spontaneously" generated histamine-releasing factor appeared to correlate with the loss of "cutaneous hyperreactivity" (increased pruritus due to a variety of minor stimuli such as heat, stress, or irritants) and inversely with the improvement in the patients' skin. Similarly, Alam et al[46] reported an association between bronchial hyperreactivity in patients with asthma and the spontaneous generation of histamine-releasing factor by peripheral blood mononuclear cells.

Several lines of evidence also suggest a pathogenic role for inhalant allergens (pollens, molds, dust mites) in atopic dermatitis. Cohen confirmed that pollen allergen was readily absorbed through respiratory mucosa and transported to distal skin sites[47] and Tuft[48] demonstrated eczematous skin changes following allergen inhalation in some adult patients with *Alternaria* or ragweed sensitivity. Mitchell produced eczematous skin changes in patients with IgE antibodies to dust mite utilizing a patch test technique on lightly abraded skin.[49] Repeated application of antigen P_1 resulted in peak infiltration of basophils, eosinophils, and mononuclear cells by 48 hours, and a significant increase in mast cells by six days.[50] Similar findings have been reported by other investigators utilizing different aeroallergens[51,52] and aeroallergen-specific TH_2 lymphocytes have been

isolated from lesional skin of patients with atopic dermatitis.[39-41]

DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS

The skin symptoms of atopic dermatitis generally present as an erythematous, papulovesicular eruption that progresses to a scaly, lichenified dermatitis secondary to persistent scratching. The distribution of the rash typically varies with age.[53] In infancy (3 months to 2 years), the cheeks, wrists, and extensor surfaces of the arms and legs typically develop papulovesicular, often weeping lesions that occasionally develop fine scaling or mild lichenification. The scalp and postauricular area frequently are affected with dermatitis. The eczematous dermatitis may involve the entire body, but generally the diaper area is spared. Traumatic lesions from scratching and secondary infection are common. In young children (2 to 12 years) flexor surfaces, the neck, wrists, and ankles are generally involved with dry maculopapular lesions being a more prominent feature. Pruritus and scratching lead to excoriations, hyperpigmentation, and lichenification. In teenage patients and young adults, flexural surfaces, face (especially periorbital), hands, and feet frequently are involved. Extreme xerosis, marked papulation, and lichenification are common in these patients. Older patients often have symptom-free periods that may last for months, but even during periods of remission, these patients retain a tendency toward dry, sensitive skin.

Atopic dermatitis has no primary skin lesion, but is identified by a constellation of symptoms. Hanifin and Rajka's[54] criteria (Table 1) for the diagnosis of atopic dermatitis are internationally accepted. A modification of these criteria (Table 2) for the young infant was subsequently proposed.[55] The main features include the extremely pruritic nature of the rash, its typical morphology and distribution, and its tendency toward a chronic or relapsing course. Some features, such as orbital darkening, Dennie-Morgan infraorbital fold, and hyperlinearity of the palms are common but nonspecific, whereas others such as anterior subscapsular cataracts, nipple eczema, and upper lip cheilitis are uncommon but relatively specific for the diagnosis of atopic dermatitis.

Seborrheic dermatitis and allergic contact dermatitis are most frequently confused with atopic dermatitis. Seborrheic dermatitis may be indistinguishable from atopic dermatitis in some cases, but generally can be differentiated by its more frequent distribution in the axillae and diaper area, less prominent pruritus, and general absence of elevated serum total IgE and positive skin tests to foods and inhalants. Other less common disorders that may be mistaken for atopic dermatitis include hyper-IgE syndrome, Wiskott-Aldrich syndrome,

Table 1. Diagnostic Features of Atopic Dermatitis

Must have 3 or more major features:
Pruritus
Typical morphology and distribution
Flexural lichenification or hyperlinearity in adults
Facial and extensor involvement in infants and children
Chronic or chronically relapsing course
Personal or family history of atopy (asthma, allergic rhinitis, or atopic dermatitis)
Must also have 3 or more minor features:
Xerosis
Ichthyosis/palmar hyperlinearity/keratosis pilaris
Immediate (type I) skin test reactivity
Elevated serum IgE
Early age of onset
Tendency toward cutaneous infections (esp. *Staphylococcus aureus* and herpes simplex)/ impaired cell-mediated immunity
Tendency toward nonspecific hand or foot dermatitis
Nipple eczema
Cheilitis
Recurrent conjunctivitis
Dennie-Morgan infraorbital fold
Keratoconus
Anterior subcapsular cataracts
Orbital darkening
Facial pallor/facial erythema
Pityriasis alba
Itch when sweating
Intolerance to wool and lipid solvents
Perifollicular accentuation
Food hypersensitivity
Course influenced by environmental/emotional factors
White dermographism/delayed blanch

Table 2. Modified Criteria for Diagnosis of Atopic Dermatitis in Infants

Three major features:
Family history of atopic disease
Typical facial or extensor eczematous or lichenified dermatitis
Evidence of pruritus
Three minor features:
Xerosis, icthyosis, hyperlinear palms
Perifollicular accentuation
Postauricular fissures
Chronic scalp scaling

and a variety of genetic disorders such as phenylketonuria, biotinidase deficiency, and erythrokeratoderma variabilis, and histiocytosis X.

No routine laboratory test is helpful in making the diagnosis of atopic dermatitis. Peripheral blood eosinophilia (5% to 20%), elevated total serum IgE concentrations, and specific IgE antibodies to foods and inhalants (eg, prick skin tests, radioallergosorbent tests) are present in about 80% of patients. An intracutaneous injection of acetylcholine (0.1 mL of 1:1000) provokes increased sweating and delayed blanching at the injection site (normal response: erythema, sweating, and piloerection).

THERAPY

Atopic dermatitis is characterized by dry and easily irritated skin with intermittent flares of eczema. The exacerbations may be infrequent with prompt resolution and healing, but more commonly, exacerbations occur regularly. A variety of "trigger" factors are known to exacerbate flares: irritants (eg, soaps and harsh chemicals), heat and humidity, stress and anxiety, infection, and allergens. The importance of these triggers differs in individual patients and therefore must be delineated in each patient for successful management.

A number of general measures may be taken to reduce pruritus and scratching which lead to skin damage. Fingernails should be trimmed short and cotton gloves may be worn at night. Since dry skin is more prone to itch, efforts to obtain maximal skin hydration are necessary. Bathing for hydration, soaking in tepid water for 25 to 30 minutes 2 to 3 times per day, followed by the immediate application of a lubricating ointment or cream (eg, Aquaphor®, Acid Mantle®, Unibase®, petrolatum, etc) is the most effective form of therapy. Lotions should be avoided since they contain alcohol and water which may sting and provide limited moisture. Lubricants should be applied within three minutes after the patient gets out of the tub in order to prevent evaporation of water absorbed into the stratum corneum. In patients with marked excoriation and/or weeping lesions, initial wet wraps with Burrow's solution (1:40) will avoid the stinging or burning sensation sometimes seen with bathing. Addition of oil to the bath water generally has little effect.

Patients with atopic dermatitis have a decreased "itch" threshold and therefore are much more sensitive to a variety of cutaneous irritants. Bathing in hot water and scrubbing vigorously with soap are two of the most frequent sources of irritation. Patients should be encouraged to bathe in tepid water (especially for hydration), avoid soap, and pat dry with soft absorbent towels. Clothing should be double-rinsed after washing to remove all residual detergent.

Whether induced by high temperatures, exercise, or anxiety, sweating generally leads to cutaneous pruritus, scratching, and subsequent skin changes characteristic of atopic dermatitis. Measures to reduce sweating such as avoiding excessive room temperature, wearing light, nonocclusive clothing (eg, cotton instead of polyester) and keeping the bedroom cool and avoiding excessive bed clothing will help reduce sweating.

Topical corticosteroids are a mainstay of therapy in atopic dermatitis, probably due to their broad immunomodulatory effects on Langerhans' cells, lymphocytes, and the IgE-mediated late-phase response. For general management, mid-strength corticosteroids such as 0.1% triamcinolone cream or ointment are optimal. Occasionally more potent fluorinated steroids are required to suppress an acute flare. Use of these potent agents should be limited because of their accompanying side-effects. In general, the least potent steroid that controls a patient's symptoms should be employed (Table 3). The use of systemic corticosteroids should be avoided if possible since many patients experience a rebound flare after a short course, which only leads to further requests for systemic therapy. A course of antibiotics or "simulated hospitalization" should be considered before utilizing systemic corticosteroids.

Staphylococcus aureus infections of the skin frequently induce acute flares in atopic dermatitis. Infection should be suspected in the presence of acute weeping or crusted lesions, small superficial pustules, or recalcitrant erythematous patches. In

Table 3. Potency of Representative Topical Steroids

Class		Brand Name	Generic Name
Ultra high potency	1	Diprolene Ointment 0.05%	Betamethasone dipropionate
		Temovate Ointment and Cream 0.05%	Clobetasol propionate
High potency	2	Diprosone Ointment 0.05%	Betamethasone dipropionate
		Halog Cream 0.1%	Halcinonide
		Lidex Cream 0.05%	Fluocinonide
	3	Aristocort Cream (HP) 0.5%	Triamcinolone acetonide
		Diprosone Cream 0.05%	Betamethasone dipropionate
		Valisone Ointment 0.1%	Betamethasone valerate
Mid potency	4	Westcort Ointment 0.2%	Hydrocortisone valerate
		Aristocort Ointment 0.1%	Triamcinolone acetonide
		Cordran Ointment 0.05%	Flurandrenolide
	5	Diprosone Lotion 0.2%	Betamethasone dipropionate
		Valisone Cream and Lotion 0.1%	Betamethasone valerate
Mid-Low	5-6	Elocon Cream and Ointment 0.1%	Mometasone furoate
Low potency	6	Synalar Solution 0.01%	Fluocinolone acetonide
		Tridesilon Cream 0.05%	Desonide
	7		Hydrocortisone 1-2%

over 300 patients evaluated by the author, over 95% of patients were colonized (infected) with *Staphylococcal aureus* organisms which were resistant to penicillin in 90% patients and to erythromycin in 31%. Although ideally guided by results of skin culture and sensitivity, antibiotic coverage may be initiated with erythromycin or cephalexin, since the presence of a resistant strain may be suspected in the absence of a prompt clinical response. Oral dicloxacillin may be substituted. Bactroban®, a topical antibiotic shown to be effective for superficial *Staphylococcus aureus* infections, may be used when infection is localized.

If crusted lesions fail to respond to oral antibiotics, herpes simplex infection should be considered. A giemsa stained Tzanck smear or viral culture will indicate the presence of a herpes infection. Patients at risk for ocular involvement, widespread dissemination or systemic involvement with herpes simplex should be treated with intravenous acyclovir. Less extensive involvement may be treated with topical acyclovir. Occasionally patients are flared from superimposed dermatophyte infections. These infections respond readily to either locally applied imidazole creams or oral griseofulvin daily for one month. *Pityrosporum ovale* may be treated with clotrimazole.[30]

Studies over the past decade have clearly demonstrated a pathogenic role for food allergy in some patients with atopic dermatitis. Eggs, milk, peanut, soy, and wheat are the most commonly offending foods. Overall, one-third of children with atopic dermatitis have been shown to have food hypersensitivity contributing to their symptoms.[56] Recent studies have suggested a significant role for inhalant allergens in the pathogenesis of atopic dermatitis.[49-52] There is, however, *no* controlled trial to support the use of immunotherapy in atopic dermatitis. In many cases, the eczema flares when allergy shots are initiated. Some attempt to reduce dust mite and pet exposure does appear warranted.

Allergic contact dermatitis is uncommon in patients with atopic dermatitis, but patients occasionally become sensitized to topical medications, preservatives, or the lanolin base. Patch testing may be helpful in detecting the offending contact allergen.

A number of studies investigating the antipruritic effect of antihistamines have shown that histamine plays a minor role in the itchy skin of atopic dermatitis patients and that other mediators (eg, neuropeptides, cytokines) are primarily responsible for the pruritus. Many patients experience some symptomatic relief with antihistamines, whether due to an antipruritic or soporific effect remains unclear. Response to various antihistamines differs among individual patients but two antihistamines, hydroxyzine and cetirizine, are generally effective for reducing pruritus and have several unique properties that may favor their use in atopic dermatitis. Not only are these drugs effective H_1-receptor antagonists, they also block IgE-mediated late-phase mast cell release of PAF[57] and the recruitment of neutrophils, eosinophils, and basophils into allergen-challenged skin blisters.[58] Doxepin, a tricyclic antidepressant with antihistaminic effects, has also been reported to be effective. Large doses of hydroxyzine (2 mg/kg up to 75 to 100 mg before bedtime) or doxepin as a single bedtime dose generally provides a prolonged antihistaminic effect and circumvents the problem of daytime sedation and facilitates nighttime sleep.

Patients (or their patients) often note that anxiety, anger, and frustration provoke pruritus and flares of atopic dermatitis. Earlier studies have demonstrated the role of anxiety in inducing pruritus in patients with atopic dermatitis.[59] Patients should be encouraged to verbalize their emotional conflicts and, occasionally, psychologic counseling should be recommended. In children, potential stressful situations at home or school should be evaluated and discussed.

Tar preparations provide a useful nonseroidal approach to therapy; however, gel preparations are frequently very irritating to dry skin and the odor is unacceptable except to the most motivated patients. Several uncontrolled trials of topical or oral sodium cromoglycate or the newer nedocromil sodium suggested some beneficial results. Most recent controlled trials have failed to show any beneficial effects in the treatment of patients with atopic dermatitis.[60] Phosphodiesterase inhibitors also have been promoted for their antipruritic effect, but in a recent double-blind controlled trial of papaverine (the most promising agent), no beneficial effect was detected.[61]

Hospitalization, or a "simulated hospitalization" at home, is a highly effective but often overlooked therapeutic modality. Whether removal from daily stresses or environmental factors, a brief period of bedrest often leads to considerable symptomatic improvement.

Several immunomodulatory approaches to the treatment of atopic dermatitis show promise and may eventually lead to more effective forms of therapy. Ultraviolet light therapy and psoralen photochemotherapy (PUVA) have been shown to induce remission in many patients with recalcitrant atopic dermatitis[62] probably due to its suppressive effect on Langerhans' cells and possibly on cutaneous mast cells. The almost inevitable relapse following treatment, and the promotion of skin aging and increased risk of squamous cell carcinoma of the skin with prolonged therapy discourage the use of PUVA in children and limit its use in adults.

A recent double-blind, placebo-controlled cross-over trial of cyclosporin (5 mg/kg/day) in adults with refractory atopic dermatitis revealed

significant benefit when compared with placebo.[63] Pruritus was noted to decrease dramatically within 2 to 4 days, suggesting the inhibition of a "pruritogenic cytokine."[64] Unfortunately the hepatotoxic and immunosuppressive effects of this drug will preclude its use except in the most refractory cases of adults with atopic dermatitis. Early enthusiastic reports on the beneficial effects of topical cyclosporin have not been borne out in a larger controlled trials.

Thymopentin (TP5) is a synthetic pentapeptide corresponding to amino acid residues 32 through 36 of human thymopoietin. It has been shown to promote differentiation of thymocytes and enhance T lymphocyte function. In a 6-week double-blind, controlled trial, 48 patients with atopic dermatitis received daily subcutaneous TP5 injections and 52 received placebo.[65] Subjects treated with TP5 experienced a significant decrease in their total severity scores compared with the placebo-treated group. Significant differences were not seen until the 6-week examination suggesting a delayed onset of action for TP5. No significant adverse effects were seen.

Interferon-gamma (IFN) inhibits TH_2 cell functions including IgE synthesis and upregulation of CD23 antigens (FC,RII) on a variety of cells (eg. Langerhans' cells, monocytes, lymphocytes, etc). In a double-blind, controlled trial of recombinant IFN, 40 patients with moderate atopic dermatitis received daily subcutaneous injections of IFN, and 43 received placebo for 12 weeks.[66] Physician and patient severity scores were significantly improved in subjects treated with IFN compared with placebo. In addition, blood eosinophil counts and spontaneous in vitro IgE synthesis by peripheral blood lymphocytes were decreased in the treated group. Phase 3 trials are needed to confirm these promising results before IFN can become available for general use.

Injection therapy with mite allergen-antibody complexes appeared to have some beneficial effect in an uncontrolled study of 10 adults with atopic dermatitis and dust mite sensitivity.[67] Significant improvement was noted in all patients after 3 to 4 months of therapy, and 6 of 10 were reportedly in complete or near-complete remission at 1 year. The lack of placebo control and the complexity of preparing specific antibody-antigen complexes for each patient temper the enthusiasm for this approach.

A recent double-blind placebo-controlled cross-over trial of a formulation of Chinese medicinal plants for the treatment of atopic dermatitis demonstrated benefit.[68] Thirty-seven of 47 children completed a trial consisting of active therapy and placebo for 8 weeks separated by a 4-week washout period. Skin erythema and surface damage were scored before, during, and after each treatment phase. Although considerable placebo effect was noted in many patients, the active therapy induced a significant decrease in skin erythema and surface damage compared to placebo (median decrease: erythema—51% versus 6.1%; surface damage—63.1% versus 6.2%). The active components of the various herbs utilized are unknown. While no short-term toxicity was noted, the investigators caution that careful longterm follow-up will be necessary to determine the safety of this form of therapy.

PREVENTION, COURSE AND PROGNOSIS

Several studies suggest that strict dietary elimination of major food allergens from infants at high risk for atopy can prevent some atopic dermatitis. The neonate may be exposed and sensitized to food allergens crossing the placenta in utero, secreted into maternal breast milk, and/or from ingestion of infant formulas and solid foods. A number of studies have shown that eliminating major allergens (ie, egg, milk, and fish) from the maternal diet during the third trimester of pregnancy does not prevent the development of atopy in their offspring.[69,70] On the other hand, removing major food allergens from the maternal diet during lactation appears to have beneficial effect. In a follow-up study of 65 infants whose mothers were placed on a restrictive diet (prophylactic group) and 50 infants whose mothers consumed a normal diet, children in the prophylactic group had significantly less atopic dermatitis at 18 months and 4 years of age than children whose mothers received a regular diet during lactation.[71,72] Zeiger and coworkers[73,74] compared the potential benefit of placing both the lactating mother and her infant on food allergen elimination diets. Overall, 103-high risk infant-mother pairs were compared with 185 high risk controls. Infants placed on strict allergen avoidance diets were found to have significantly less atopic dermatitis at 1 year of age compared to infants of no dietary restrictions, but this difference was no longer apparent beyond 2 years of age (follow-up to 4 years of age). Chandra compared a number of feeding regimens in infants at high risk for developing atopic disorders.[75] The prevalence of atopic dermatitis at 18 months of age was lowest (22%) in infants receiving breast milk from mothers who were eliminating major food allergens from their diets or in infants receiving a casein hydrolysate formula. Infants receiving breast milk from mothers on no dietary restrictions had a higher prevalence of atopic dermatitis then the first two groups, but significantly less than infants receiving cow milk or soy formulas. The late addition of solid foods to the diet (after 6 months) was shown to delay (or possibly prevent) the development of atopic dermatitis.[76] At 1 year of age, infants receiving solid foods after 6 months had less atopic dermatitis (14% versus 35%; $P < .01$) and food

hypersensitivity (7% versus 37%; $P < .001$) than infants started on solids at 3 months.

The course of atopic dermatitis is highly variable and frequently marked by unexplained exacerbations and remissions. The relatively late acceptance of standardized diagnostic criteria for atopic dermatitis has left most epidemiologic studies of little value. Based on these studies, it is often stated that most children "outgrow" atopic dermatitis. While eczematous lesions may be less prominent as the patient gets older, some skin symptoms generally persist well into adulthood. Rystedt and coworkers reevaluated over 900 patients seen by the Dermatology Department at Karolinska Hospital between 1952 and 1956.[77] Patients were divided into those seen as inpatients—group I, and those seen only as outpatients—group II. Group I consisted of 549 patients, 90% of whom were followed-up 24 years later and 189 (35%) of whom were examined personally by the investigator, while group II consisted of 395 patients, 80% of whom were reevaluated and 162 (41%) of whom were examined by the investigator. Over half the subjects followed-up after 24 years had persistent atopic dermatitis; 25% had lost all symptoms during childhood but had a recurrence in adulthood. Several risk factors were identified which appeared to affect prognosis: (1) severe dermatitis in childhood, (2) family history of atopic dermatitis, (3) associated asthma and/or allergic rhinitis, (4) female, and (5) and onset before 1 year of age. If all risk factors were present, greater than 80% of patients had persistent atopic dermatitis whereas if no risk factors were present, less than 15% of patients had persistent disease.

Atopic dermatitis is a disorder that affects about 10% of the population and causes immeasurable patient and family morbidity. When given the theoretic option of eliminating either their atopic dermatitis or asthma, an informal survey of children and their parents revealed that all but one parent felt they could deal with the asthma but wished to eradicate the eczema. While the exact pathogenic mechanisms involved in atopic dermatitis remain unknown, it is clear that IgE-mediated immediate and cellular responses contribute to the chronic inflammation of this disorder and therefore place it under the purview of the allergist. At present therapeutic modalities for atopic dermatitis are limited and less than adequate. However, a systematic evaluation of triggering factors and comprehensive approach to management generally provides tremendous relief to these patients.

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CME Examination
Identification No 012-012
Questions 1–21. Sampson HA: Ann Allergy 1992;69:469–481.

CME Test Questions

1. Most recent studies indicate that the following percentage of the population is affected by atopic dermatitis:
   a. 2% to 3%
   b. 5% to 7%
   c. 10% to 12%
   d. 18% to 20%
   e. 25% to 30%

2. Which one of the following is not considered a major criterion of atopic dermatitis:
   a. personal history of asthma and/or allergic rhinitis
   b. pruritus
   c. flexural lichenification or hyperlinearity
   d. elevated serum IgE concentration
   e. chronically relapsing course

3. Immunohistochemical studies of skin biopsies from eczematous lesions of patients with atopic dermatitis have demonstrated all of the following *except*:
   a. a predominance of $CD4^+$ cells
   b. increased numbers of IgE-bearing Langerhans' cells
   c. a predominance of TH2 lymphocytes
   d. a paucity of mast cells
   e. increased number of eosinophils

4. Physiologic abnormalities found in patients with atopic dermatitis include all except the following:
   a. increased itch threshold
   b. increased transepidermal water loss
   c. increased linoleic acid levels in plasma
   d. increased cyclic-AMP phosphodiesterase activity in mononuclear cells
   e. increased sweating

5. Abnormal vascular responses in patients with atopic dermatitis include all except the following:
   a. white dermographism

EVALUATION OF ANTIE

Diana D. Scala, M.S., George E. Fischler, M.S
Raza Aly, Ph.D., Howard I. Maibach.

ABSTRACT

Handwashing with soap and water is the most important step in preventing the spread of nosocomial infections. The antibacterial efficacy of soap bars containing 0.8% and 1.5% 3,4,4'- trichlorocarbanilide (triclocarban) have been demonstrated by Yackovich *et al.* (1986) and Finkey *et al.* (1984) against organisms representative of skin microflora and common skin pathogens. We have modified the methods used by these investigators to simulate normal washing conditions and to evaluate the residual antibacterial efficacy of up to 4 soap bars simultaneously. Using this modified method, we demonstrated that soap bars containing 0.7% and 1.5% triclocarban are effective against *S. aureus* as well as axillary bacteria. Our results are in agreement with those found by other investigators thereby validating our test method. Additionally, we showed that a 0.3% triclocarban containing soap was as effective as a soap containing higher levels of triclocarban. The method is reproducible and can be used effectively to evaluate the efficacy of triclocarban containing soap bars.

INTRODUCTION

Epidemiological and clinical studies have shown that washing hands with soap and water is important in helping remove bacteria responsible for spreading diseases caused by poor hygienic practices (1,2,3). Washing hands with a non-antibacterial soap has been suggested to decrease the incidence of such diseases (1,2). However, investigators have shown that antibacterial soaps are significantly more effective than plain soap in reducing and inhibiting the growth of bacteria (4,5,6,7). This is presumably due to the substantive properties of the agents in the antibacterial soap which remain on the skin after handwashing.

The selection of antimicrobial agents, their concentration, and soap vehicle is continually being revised. The antibacterial efficacy of 3,4,4'- trichlorocarbanilide (triclocarban) was first reported in 1958 (8) and is currently one of the most commonly used antibacterial ingredient in bar soaps. In this paper, we describe a modification of a previously published method to evaluate the antimicrobial efficacy of up to 4 bar soaps and demonstrate its value in optimizing the concentration of triclocarban in a soap bar.

METHOD

The method is a modification of that described by Finkey *et al.* (5).

The number of subjects for each study varied and ranged from 4 to 24. Volunteers above the age of 18 participated in each study after giving their informed written consent.

After undergoing a one-week washout period in which the panelists refrained from using any antibacterial products such as soaps, shampoos, lotions etc., the panelists forearms were washed with the placebo and up to 3 test soaps sequentially by a technician. Each of the panelists inner volar forearms were divided into the upper and the lower aspect for a total of four test sites. Each site was washed for 60 seconds with either a test soap or the placebo based on a randomized schedule. For studies with an untreated control site, one site was not washed. After each wash, the sites were rinsed and dried with paper towels. Each site was washed a total of 7 times over 3 days (3 washes each on Days 1 and 2 and one wash on Day 3). Each daily wash was separated by at least 1 hour. Ten minutes after the last wash, bacteria (inoculum size = 10^6-10^7) were applied to each site and kept in place for 5 hours with a plastic chamber and adhesive tape. After that time, the bacteria were harvested by a scrub cup technique and the skin degermed with 70% alcohol.

Harvesting involved positioning a hollow cylinder upon the site, pipetting in 1 ml of buffer containing an appropriate neutralizer (0.1% Triton X-100) or culture media containing a neutralizer (Letheen® broth); massaging the skin for 60 seconds with a sterile glass rod; removing the fluid; adding a second ml of buffer or media and scrubbing for 30 or 60 seconds; and removing and pooling the fluid. Dilutions were made in the appropriate media containing a neutralizer. After 48 hour incubation at 35° to 37° C, the colonies were counted and expressed as colony-forming units.

Figure

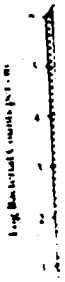
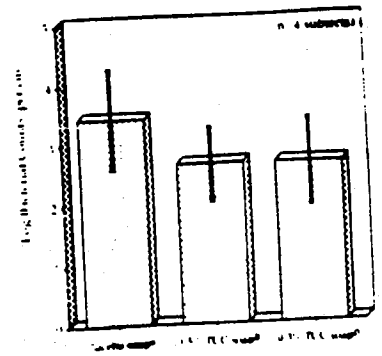


Figure 3. Antibacterial Efficacy (Soap Bars Against Axillary Bacte



Treatments with the same superscripts are statistically equiva

ALUATION OF ANTIBACTERIAL BAR SOAPS CONTAINING TRI

S., George E. Fischler, M.S., Boyce M. Morrison, Jr., Ph.D., Colgate-Palmolive Tec
Ph.D., Howard I. Maibach, M.D., Department of Dermatology, University of Califo

Figure 1. Antibacterial Efficacy of Soap Bars against *S. aureus* (ATCC # 27217)

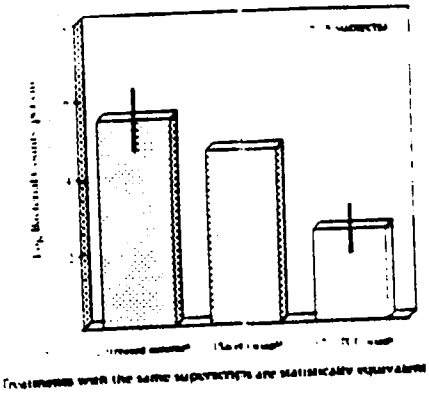
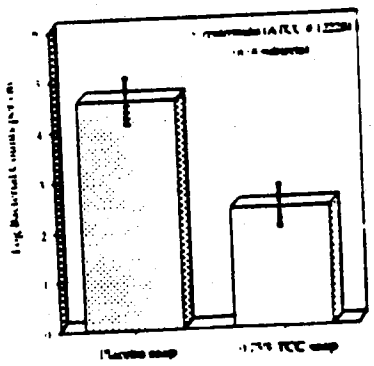


Figure 2. Antibacterial Efficacy of Soap Bars Containing TCC against Staphylococci



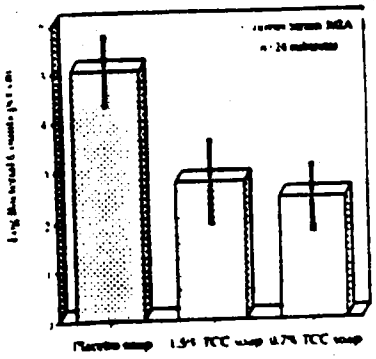


Figure 3. Antibacterial Efficacy of Soap Bars Against Axillary Bacteria

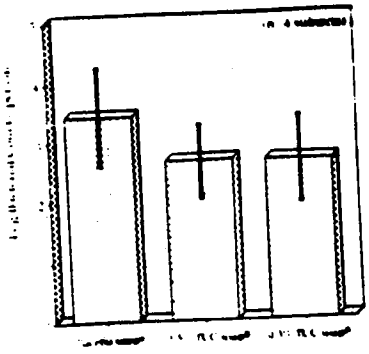


Figure 4. Reproducibility of Test Method Using the Same Bacterial Strain and Same Laboratory (Soaps A, B, and C contain TCC)

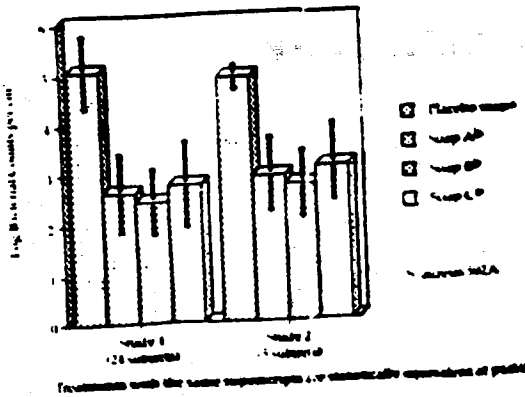
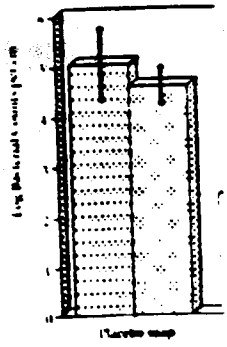


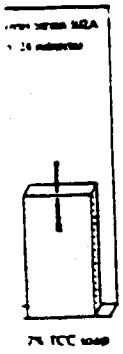
Figure 5. Reprodu Using the Same Ba Different Test Labs



NING TRICLOCARBAN

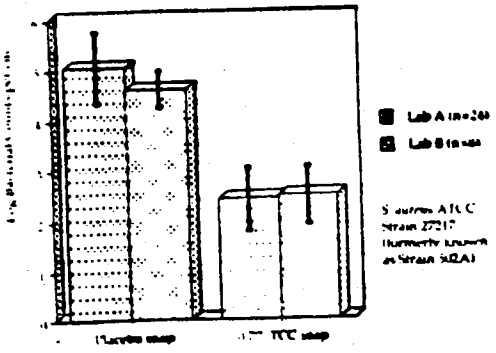
almolive Technology Center, Piscataway, NJ,
sity of California, San Francisco, CA.

phylococci



7% TCC soap

Figure 5. Reproducibility of Test Method Using the Same Bacterial Strain but Different Test Labs



STATISTICAL ANALYSIS

The effectiveness of the test soaps was determined by how well they inhibited bacterial growth compared to the placebo or to each other.

For each study the $\log_{10}$ colony forming units (CFUs) for each site was calculated and the efficacy of the test products were compared using a within subject ANOVA. If an overall difference was detected at the 5% significance level then Fisher's PLSD was used to compare each product against each other to determine where the significant difference existed.

RESULTS AND DISCUSSIONS

- Figure 1 shows that washing the skin with a non-antibacterial soap is effective in inhibiting the growth of bacteria applied to the skin (untreated control vs placebo). However washing with a soap containing 0.7% triclocarban (TCC) is even better at controlling bacterial growth.
- We slightly modified published methods evaluating the efficacy of TCC in bar soaps to better simulate normal conditions. To validate our method, we selected levels of TCC previously shown to be effective in bar soaps and evaluated their efficacy using our method. Results showed that skin washed with soaps containing 0.75% and 1.5% triclocarban (TCC) are effective in inhibiting the growth of staphylococci (Figure 2). These findings are in agreement with those found by other investigators (5,6) thereby validating our method.
- Figure 3 shows that skin washed with soaps containing 0.3% and 1.5% TCC are equally effective against axillary bacteria. This supports the finding of Sauermann et al (9) who reported that soap containing 0.26% TCC has the same deodorant effect as soap containing 1.3% TCC.
- Results from our method are very reproducible within and between laboratories (Figures 4 and 5).
- Because of the test method's high reproducibility, we can use relatively small panel sizes (n=8 subjects) to statistically discriminate between products that have at least 1 $\log_{10}$ difference in efficacy. The efficacy of up to 4 products can be examined simultaneously compared with only 2 products using previously published methods.

CONCLUSIONS

We have modified previously published methods to evaluate the antibacterial efficacy of up to 4 soap bars simultaneously. Our method has good reproducibility between and within laboratories. Because of this, relatively small panel sizes can be used to detect 1 log bacterial reductions. Therefore, this method can be used to rapidly evaluate the antibacterial efficacy of TCC containing soap bars.

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COMPARATIVE ANTIBACTERIAL EFFICACY OF HIBICLENS® AND BETADINE® IN THE PRESENCE OF PUS DERIVED FROM HUMAN WOUNDS

WAHEED SHEIKH

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ABSTRACT

The bacteriostatic and bactericidal activity of 4% chlorhexidine gluconate (Hibiclens®) and 7.5% povidone-iodine (Betadine®) were studied in the presence of pus derived from human wounds containing *Morganella morganii* and upon inocula of the same organism without pus. In the absence of pus, with an inoculum of 8.5×10^6 viable cells, the maximum effective dilution of chlorhexidine gluconate was 1/400; for povidone-iodine it was 1/8. In the presence of 5% pus and the same number of viable cells, the maximum inhibitory dilutions for the two agents were 1/75 and 1/4, respectively. In the absence of pus, both agents were highly bactericidal (>6 $\log_{10}$ reduction within one minute). As the ratio of antiseptic to infected pus was decreased, the contact time required to achieve a given $\log_{10}$ reduction increased. However, at all except one level tested (antiseptic: infected pus ratios of 19:1, 9:1, 4:1, and 1:1 were evaluated), chlorhexidine gluconate always achieved a given reduction in bacteria more quickly than povidone-iodine. We thus conclude that the presence of pus reduces the bactericidal activity of povidone-iodine to a much greater extent than that of chlorhexidine gluconate.

INTRODUCTION

Despite the proliferation of antibiotics and topical antimicrobial agents, gram-negative bacteria still pose a serious threat to medical and surgical patients. While a wide variety of antimicrobial surgical scrubs are currently available, two of the most widely used are based on chlorhexidine gluconate (Hibiclens®,* a detergent solution containing 4% chlorhexidine gluconate and povidone-iodine (Betadine®,† a detergent solution containing about 7.5% povidone iodine). The antimicrobial efficacy of both of these agents has been amply demonstrated in a number of experimental studies.[1-9]

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* Stuart Pharmaceuticals, Wilmington, Delaware.
† The Purdue Frederick Company, Norwalk, Connecticut.
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computing the mean $\log_{10}$ surviving bacteria and the percent kill for each contact time.

In an additional control experiment, the test solutions were inoculated with an aqueous washed suspension of *M morganii* that had approximately 10^8 viable cells/ml of reaction mixture. These solutions were evaluated using methods identical to those described above.

RESULTS AND DISCUSSION

The results of both the bacteriostatic and bactericidal experiments were obtained both in the presence and absence of pus. The findings from the bacteriostatic experiments are presented first.

In the absence of pus, the highest dilution of the chlorhexidine gluconate that prevented bacterial growth was 1:400. The highest dilution of the povidone-iodine that produced similar results was 1:8. In the presence of 5% pus, the highest effective dilution of the chlorhexidine gluconate was 1:75, and that of the povidone-iodine was 1:4. Thus, in the absence of pus, the chlorhexidine gluconate was 50 times more effective than the povidone-iodine and, in the presence of pus, the former was almost 19 times more effective than the latter.

The results of bactericidal experiments were consistent with those of the bacteriostatic experiments described above. Four dilutions containing antiseptic to infected pus ratios of 19:1, 9:1, 4:1, and 1:1 were used. At all except one of the dilutions tested, the chlorhexidine gluconate invariably required less contact time than the povidone-iodine to achieve a given $\log_{10}$ reduction in the number of bacteria present in the test solution. The data from these experiments are summarized in the table and presented graphically in the figure. In the absence of pus, both test solutions exceeded a 99.9999% kill and reduced the $\log_{10}$ mean surviving bacteria to <1.0 within one minute of contact time. Thus, within the limits of our measurements, both solutions had equivalent bactericidal potency in the control situation.

With the addition of pus, however, the activity of the two agents quickly diverged. When the ratio of antiseptic to pus was 19:1, the chlorhexidine gluconate achieved a greater than 99.9999% kill within five minutes of contact. The povidone-iodine approached this value only when contact with the target bacteria was extended to 60 minutes. As the ratio of antiseptic to pus was further decreased, the differences in the efficacy of the chlorhexidine gluconate and the povidone-iodine became more pronounced. For example, when the ratio of antiseptic to pus was 4:1, the chlorhexidine gluconate still achieved greater than 99.9999% kill and reduced $\log_{10}$ mean survivors to less than 1.0, with contact times of 30 minutes or greater. At the same concentration and contact time, the povi-

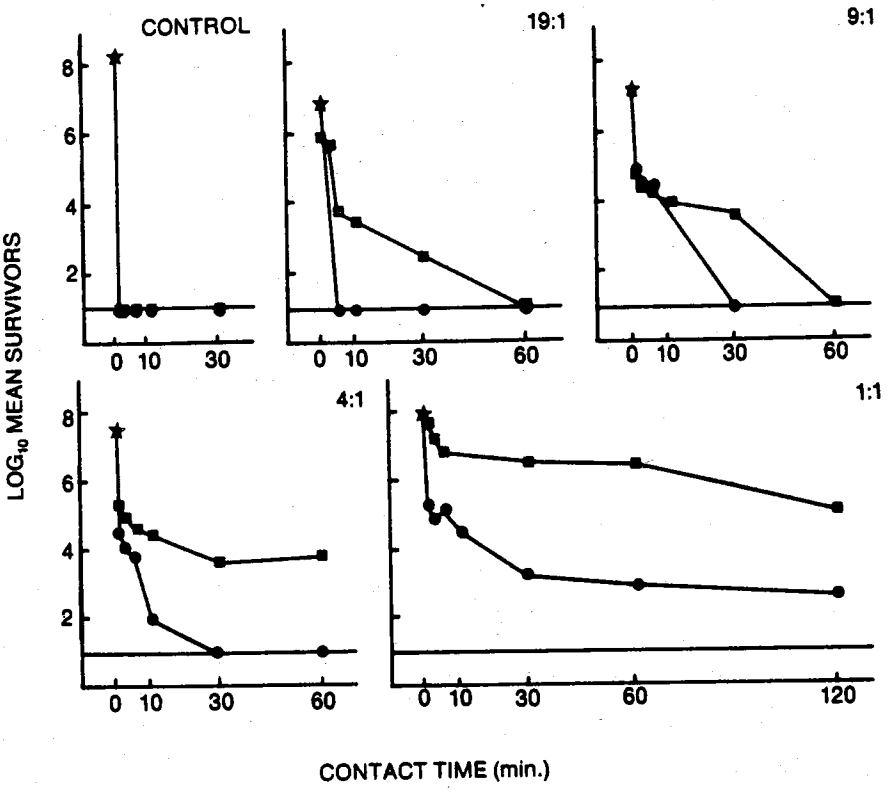


Figure. Results of the bactericidal experiments with chlorhexidine gluconate (Hibiclens®) and povidone-iodine (Betadine®). Each graph depicts the results for a given antiseptic:pus mixture (the ratio is indicated at the upper right of each panel). ★ = no. of bacteria present at the beginning of each trial; ● = chlorhexidine gluconate; ■ = povidone-iodine; line at $\log_{10}$ = detectability limit of our assay.

effect of each antiseptic was achieved within the first minute of exposure, at each of the ratios tested in the study. With the chlorhexidine-gluconate, increased contact time resulted in small, but consistent, additional reductions in surviving bacteria. The single exception to this generalization is the five-minute contact time in the experiments when the ratio of the chlorhexidine-gluconate to pus was 1:1. With the povidone-iodine, however, increasing the contact time from 30 to 60 minutes had virtually no effect when the ratios of antiseptic to pus were either 4:1 or 1:1. In the latter case, an increase to 120 minutes of contact time produced a further reduction in the number of surviving bacteria.

The fact that the chlorhexidine gluconate had greater antibacterial efficacy than the povidone-iodine in our in vitro system is consistent with the results of several clinical trials. For example, Goldblum et al[12]

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164

Efficacy of Germicidal Hand Wash Agents in Use in a Meat Processing Plant

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ABSTRACT

The in-use efficacy of a selected range of germicidal hand wash agents was tested in a meat processing plant. The hand washes included non-germicidal soaps and germicidal agents containing chlorhexidine, iodophor and Irgasan DP 300 as active ingredients. A laboratory study was done under controlled conditions with standardized procedures for hand washing; in the meat plant, "normal" (unstandardized) hand wash procedures were followed. Levels of contamination on hands varied markedly between work units. Only in the meat cutting area could a significant difference be attributed to hand wash agents against transient-type bacteria on workers' hands. The hand wash agent with 4% chlorhexidine gluconate, the iodophor with 0.75% available iodine and the gel containing 0.3% Irgasan DP 300 were the only products that gave a significantly better reduction of transient bacteria than non-germicidal soap. Transient bacteria were detected on hands after washing, indicating that under the in-use conditions in the meat processing plant, hand wash techniques did not remove all of these bacteria from hands. The plant workers generally indicated a dislike for the iodophor products as hand germicides.

Hand washing is an important component of a hygiene program for food handlers. Hands can be a source of direct or indirect contamination of foods with pathogenic microorganisms *(3,11,18)*. It is generally considered that a germicidal hand wash agent is preferable to a non-germicidal agent *(2)*. This principle was developed with exclusive use of germicidal soaps such as those containing 2 or 3% hexachlorophene *(12)*. However, other studies revealed that intermittent use *(28)* or a single application *(13)* of hexachlorophene soaps was no more effective than non-germicidal soap. Despite certain limitations, hexachlorophene was a popular germicidal agent for hand hygiene *(8,17)*. Subsequently, the possibility of toxic absorption of hexachlorophene through the skin led to restrictive regulation of its use *(6,10,16)*.

Based on criteria for satisfactory hand hygiene, Crisley and Foter *(4)* selected iodine compounds (iodophors) as the best agents for hand washing by food handlers. Since then, several other germicidal agents have gained acceptance for use in hand hygiene, including chlorhexidine *(23)*, triclosan or Irgasan DP 300 *(9)* and para-chloro-meta-xylenol *(5)*. In our studies *(19-22,27)*, the only agents tested that met our criterion of reduced numbers of bacteria released from hands after a 15-s standardized hand wash were a 4% chlorhexidine gluconate liquid detergent (4% chlorhexidine) and an iodophor product containing 0.75% available iodine (0.75% iodophor). Products containing Irgasan DP 300 and para-chloro-meta-xylenol did not achieve a significant reduction in numbers of bacteria released from hands when they were compared with a non-germicidal soap. Products containing low concentrations of available iodine also failed to meet our experimental criterion *(27)*. The purpose of this study was to compare the efficacy of a selected range of chlorhexidine, iodophor and Irgasan DP 300 germicidal hand wash products with two non-germicidal products under in-use conditions in a meat processing plant.

MATERIALS AND METHODS

Two experiments were done concurrently, *(a)* a laboratory study with four volunteers who washed their hands according to a standardized hand wash procedure, and *(b)* an in-use study in a meat processing plant in which four workers in each of three work units washed their hands in their "normal" way. The three work units were selected to represent workers with heavily soiled hands (meat cutting), moderately soiled hands (kitchen area) and clean hands protected with plastic gloves (processed meat packaging area).

Hand wash agents tested

A total of nine agents was tested: 4% and 2% chlorhexidine gluconate liquid detergents (Hibitane, Ayerst Laboratories, Montreal, Canada); iodophor hand wash agents containing 0.02% (theoretically 0.005%) and 0.75% available iodine (West Chemicals Ltd., Montreal, Canada) and 0.10% available iodine (West Design Chemical Group, West Agro-Chemicals Inc., Westwood, Kansas, U.S.A.); a bacteriostatic hand soap containing 0.5% Irgasan DP 300 (Bacto-Wash, Du Bois Chemicals of Canada Ltd., Weston, Ontario, Canada); a non-germicidal gel skin cleanser (Sceptre) and the same product containing 0.3% Irgasan DP 300 (Florafree, Deb Swarfega Inc., Waterford, Ontario, Canada); and a non-germicidal hand "cleansing lotion" (West Chemicals Ltd., Montreal, Canada). The available iodine concentration of the iodophor products was deter-

mined by titration of available iodine against standardized sodium thiosulfate solution *(1)*.

Laboratory (controlled) study

The efficacy of the hand wash agents was tested against transient bacteria, *Escherichia coli* and *Pseudomonas fluorescens*, inoculated onto hands from ground beef containing approximately 1×10^7 and 1×10^8 colony forming units (cfu) per gram, respectively. Inoculation and hand wash procedures were outlined in our previous study *(27)*. Four volunteers washed their hands with the test agent on two successive days of each week. Hands were sampled before (X_0) and after (X_1) washing, by immersion and rinsing of the selected hand in 100 ml of letheen broth (Difco), as described by Sheena and Stiles *(19)*, except that the glass beads were excluded from the rinse solution.

In-use study

Four randomly selected workers in each of the three main work units of a meat processing plant were used for this study. Hand wash agents were placed in 1-L capacity dispensers (Hypor Soap Cartridge System, Deb Swarfega Inc., Waterford, Ontario, Canada) installed on the wall adjacent to the hand wash facilities in each section of the plant. The dispensers had a locking mechanism which made them tamper resistant. Dispensers were filled with the new agent to be tested, immediately after the final sampling of the previous agent had been completed. Therefore, workers used the new agent for 2 d before sampling was started. Sequencing of the agents was selected to avoid cross-reactions or to minimize the possible influence of one agent on another, as follows: *(a)* non-germicidal (lotion) soap; *(b)* 2% and *(c)* 4% chlorhexidine gluconate; iodophor products containing *(d)* 0.02%, *(e)* 0.10%, and *(f)* 0.75% available iodine; *(g)* non-germicidal gel; *(h)* 0.3% Irgasan DP 300 gel (Deb Swarfega); and *(i)* 0.5% Irgasan DP 300 (Du Bois). Clean, previously unused cartridges were used for dispensing agents 1, 2, 4, 7, 8 and 9. When cartridges were re-used, they were thoroughly rinsed before adding the new agent. The assigned agents were placed in the dispensers and used by all workers in that section for the full week (5 working days).

Workers participating in the trial were instructed to wash their hands in their "normal" manner. Hands were sampled before (X_0) and after (X_1) washing, as described for the laboratory study, on 3 successive working days (Monday to Wednesday), after the plant had been in operation for 3 h. Hand rinse samples were taken to the laboratory for bacteriological analysis within 30 min of collection. The amount of hand wash product used each week was recorded. Workers were asked about the acceptability of each product.

Bacteriology

The same bacteriological analyses were done on the laboratory and in-use hand rinse samples. In the laboratory study, X_0 samples were plated onto growth media without delay, X_1 samples were held at room temperature (22°C) for 1.5 h before plating to allow for resuscitation of injured cells. The meat plant samples were handled in the same way after they were received at the laboratory. The different microbiological parameters were determined by surface plating appropriate dilutions of each sample onto Standard Plate Count agar (SPC, Difco), Baird-Parker medium (B-P, Difco) and CFC agar prepared in the laboratory with Heart Infusion agar (Difco) supplemented with 50 μg cephaloridine, 10 μg fucidin and 10 μg cetrimide per ml *(14;* antibiotics obtained from Sigma Chemicals, St. Louis, U.S.A.). Appropriate dilutions were also pour-plated onto two sets of Violet Red Bile agar (VRB, Difco), overlayered with an additional 5 ml of VRB. Inoculated plates were incubated as follows: SPC plates at 35°C for 24 h for the total aerobic colony count; B-P plates at 35°C for 48 h for the Micrococcaceae-type count *(27)*; CFC plates at 22°C for 72 h for a presumptive *Pseudomonas* count; and the two sets of VRB plates at 45 and 35°C for 24 h to obtain presumptive fecal and total coliform counts, respectively. Isolated colonies were picked from the VRB plates incubated at 45°C and identified using the Minitek identification technique *(26)*.

Statistical analyses

Data were calculated as the $\log_{10}$ ratios of the number of microorganisms released from hands after washing (X_1) to the number released before washing (X_0). The data were analysed in a Repeated Measures analysis of variance (BMDP2V, Biomedical Computer Program, P-Series, 1983, University of California Press) to determine the effect of agents and repeated use of the agents on the microorganisms released from hands. The significance of the differences among treatment means was determined using Duncan's multiple range test.

RESULTS

Laboratory study

Hands contaminated with *E. coli* and *P. fluorescens* were washed under the standardized conditions for 15 s. Mean percentage reductions in counts for *E. coli* and *P. fluorescens* (transient) and mean percentage change in counts for Micrococcaceae-type (resident) bacteria are shown in Table 1. Initial mean counts were calculated from the initial counts of the four volunteers for the two successive testing days. Mean percentage reductions or changes in counts were calculated from the percentage reduction or change in count observed for each hand washing. All hand wash agents tested, including the non-germicidal products, achieved 99% reduction in *E. coli* and *P. fluorescens* counts. Results for the change in resident-type bacteria varied markedly between agents. The 4% chlorhexidine gluconate and 0.75% iodophor products generally caused a decrease in numbers of microorganisms released from hands. Other agents gave very variable results. Non-germicidal soaps generally caused an increased number of microorganisms to be released from hands after the 15-s wash.

Results for the analyses of variance using $\log_{10}$ reduction or change ratios indicated significant effects attributable to agents against *E. coli* ($P=0.04$) and *P. fluorescens* ($P<0.001$), but not against resident bacteria measured on B-P medium ($P=0.27$). Variances of data for resident bacteria were large, contributing to the non-significant effect. Differences among reduction ratio means determined for *E. coli* using 0.75% iodophor resulted in a significant reduction in number of bacteria released from hands compared with 0.02 and 0.1% iodophor products, 2% chlorhexidine or the non-germicidal soaps. For *P. fluorescens*, the 0.3% Irgasan DP 300, 4% chlorhexidine and 0.75% iodophor products gave significant reductions compared with all other products.

TABLE 1. *Mean initial counts of bacteria released from hands and percentage reduction or change in counts as a result of 15 s exposure to hand wash agents under standardized, laboratory conditions.*

Hand wash agent	*E. coli*[a] Initial count ($\times 10^3$)	*E. coli*[a] Reduction (%)	*P. fluorescens*[b] Initial count ($\times 10^3$)	*P. fluorescens*[b] Reduction (%)	Micrococcaceae[c] Initial count ($\times 10^1$)	Micrococcaceae[c] Change (%)
Non-germicical soap	7.6	(99.0)	4.8	(98.9)	6.3	(135)
Chlorhexidine (2%)	6.5	(99.0)	3.9	(99.2)	5.4	(92)
Chlorhexidine (4%)	6.3	(99.7)	5.0	(99.7)	3.8	(53)
Iodophor (0.02%)	5.2	(99.2)	3.7	(99.1)	4.2	(100)
Iodophor (0.12%)	7.6	(99.4)	3.5	(99.2)	3.0	(97)
Iodophor (0.75%)	6.5	(99.8)	3.4	(99.6)	2.2	(73)
Non-germicidal gel	6.3	(99.3)	3.1	(99.0)	2.5	(103)
Irgasan DP300 (0.3%)	7.5	(99.5)	4.2	(99.8)	8.0	(93)
Irgasan DP300 (0.5%)	6.8	(99.6)	3.8	(99.2)	5.9	(102)

[a]Counts obtained on VRB agar at 45°C.
[b]Counts obtained on CFC agar at 22°C.
[c]Counts obtained on B-P medium at 35°C.

TABLE 2. *Initial contamination of hands of workers in three departments of the meat processing plant over the nine-week study period.*

Microbial count[a]	Meat cutting	Kitchen	Packaging
	(cfu/ml of hand rinse solution)		
VRB (45°C)	76	1.4	<1.0
VRB (35°C)	1.5×10^4	5.1×10^2	2.0
CFC (22°C)	5.8×10^5	8.7×10^3	50
B-P (35°C)	1.3×10^5	8.5×10^3	3.9×10^3
SPC (35°C)	1.2×10^6	5.6×10^4	1.1×10^4

[a]VRB (45°C), counts of presumptive "fecal" coliform bacteria.
VRB (35°C), counts of total coliform bacteria.
CFC (22°C), counts of presumptive pseudomonad bacteria.
B-P (35°C), counts of Micrococcaceae-type bacteria.
SPC (35°C), total aerobic colony count.

In-use study

Hand wash procedures for the in-use study were not standardized. Workers were requested to wash their hands in the "normal" way. This generally involved 8 to 10 s to wash and rinse hands. Workers in the meat cutting unit generally washed their hands so that the visible soil was removed. Hand wash samples were taken immediately before the morning break on three successive days at the beginning of each week. Treatment means are the result of testing one wash by each of the four workers on each of the three test days. The marked differences in the degree of hand soiling of workers in the three work units (shown in Table 2) led to the results for each work unit being analyzed separately. Workers in the meat cutting area had heavily soiled hands, yielding a total aerobic bacterial count approximately 1×10^6 cfu/ml of hand rinse solution. High counts on the selective growth media indicated not only coliform and pseudomonad-type bacteria as transient microorganisms on hands, but also an elevated gram-positive Micrococcaceae-type count. In contrast, workers in the processed meat packaging area wore plastic gloves, and the microbial load on their hands resembled that of "socially" clean hands. Workers in the kitchen area had moderately soiled hands. Mean SPC counts for these workers ranged from 3.1×10^4 to 1.0×10^5 cfu/ml of hand rinse solution over the 9 weeks of the study.

Initial mean counts and percentage reduction of presumptive fecal coliform bacteria (VRB at 45°C) and pseudomonads (CFC at 22°C) on hands of workers in the meat cutting area are shown in Table 3. Percentage reductions in counts varied between agents, ranging from 77.6 to 96.7% for fecal coliform bacteria and 85.6 to 99.6% for pseudomonads. Analyses of variance of the data indicated significant differences attributable to hand wash agents for each parameter measured: VRB (45°C) $P=0.02$; CFC $P=0.002$; B-P $P=0.006$; and SPC $P=0.003$. Differences among reduction ratio means were determined using Duncan's multiple range test at the 95% confidence level. The data shown in Table 4 indicate that 4% chlorhexidine gave a significantly greater reduction in fecal coliform and pseudomonad counts than other agents, except for 0.75% iodophor and Irgasan DP 300 (0.3%) products against fecal coliform bacteria, and 0.75% iodophor against pseudomonads. The 0.75% iodophor product was more effective than many of the other agents used. The remaining agents, with a few exceptions, were similar to the non-germicidal soaps in their action against coliform and pseudomonad organisms. No effect of repeated use of the agents (from day to day) was detected. None of the germicidal agents, under these conditions of use, eliminated the fecal coliform or pseudomonad bacteria from hands. Residual fecal coliform bacteria on hands after washing ranged from a mean count of 0.7 cfu/ml of rinse solution after the 4% chlorhexidine wash to 27 cfu/ml after the 0.1% iodophor wash. Similar data for the pseudomonad bacteria were 7×10^2 cfu/ml of rinse solution after 4% chlorhexidine and 3.5×10^5 cfu/ml after 0.1% iodophor.

TABLE 3. *Mean initial counts of bacteria released from hands and percentage reduction in counts as a result of the use of the hand wash agents in the meat cutting area.*

Hand wash agent	VRB 45°C[a] Initial count ($\times 10^1$)	VRB 45°C[a] Final count ($\times 10^0$)	VRB 45°C[a] Reduction (%)	CFC 22°C[b] Initial count ($\times 10^4$)	CFC 22°C[b] Final count ($\times 10^3$)	CFC 22°C[b] Reduction (%)
A Non-germicidal soap	8.4	17	(83.5)	6.6	11	(85.6)
B Chlorhexidine (2%)	11	21	(80.0)	77	32	(95.8)
C Chlorhexidine (4%)	2.2	0.7	(96.7)	17	0.7	(99.6)
D Iodophor (0.02%)	2.3	5.1	(78.0)	28	14	(95.4)
E Iodophor (0.12%)	12	27	(78.5)	263	357	(86.7)
F Iodophor (0.75%)	7.6	4.6	(94.2)	198	20	(99.1)
G Non-germicidal gel	15	14	(89.9)	69	71	(89.8)
H Irgasan DP 300 (0.3%)	17	8.5	(96.2)	56	34	(94.4)
I Irgasan DP 300 (0.5%)	7.3	16	(77.6)	132	185	(86.3)

[a]Counts on VRB at 45°C indicate fecal coliform bacteria.
[b]Counts on CFC at 22°C indicate pseudomonad bacteria.

TABLE 4. *Significance of differences among hand wash agent means (for meat cutters) determined at the 95% confidence level using Duncan's Multiple Range test.*[a,b]

	Rank order of treatment means								
VRB, 45°C	C	H	F	G	A	B	E	D	I
CFC, 22°C	C	F	B	D	H	G	E	I	A

[a]For key to product codes see Table 3.
[b]Agents underlined with an unbroken line are not statistically different.

The percentage reduction in total (SPC) and Micrococcaceae-type (resident) bacterial counts are shown in Table 5. The 4% chlorhexidine product gave >90% reduction in counts. The 0.75% iodophor product gave >90% reduction for the total (SPC) count and 86.7% reduction in Micrococcaceae-type count. However, most agents were better than the non-germicidal (lotion) soap against the gram-positive, Micrococcaceae-type bacteria that grow on B-P medium.

RESULTS

For workers in the kitchen and processed meat packaging units, no significant differences in reduction of bacteria released from hands could be attributed to hand wash agents. Mean percentage decrease in number of bacteria released from hands was less when compared with results for workers in the meat cutting area. However, hands were less soiled, both visibly and bacteriologically. Initial mean counts and mean percentage reductions for four of the microbial parameters (excluding fecal coliform bacteria) for workers in the kitchen area are shown in Table 6. Reductions in microbial counts were poor, ranging from 58.3 to 91.3% for the different parameters with 4% chlorhexidine and 50.6 to 88.3% with 0.75% iodophor. Transient bacteria were detected on hands after washing, including fecal coliform bacteria, despite the fact that the level of contaminating fecal coliforms was low (see Table 2). Similar results were obtained for hand washing in the processed meat packaging area.

A total of 72 random colonies growing on VRB at 45°C was selected for identification by the Minitek iden-

TABLE 5. *Mean initial counts of bacteria released from hands and percentage change in counts as a result of the use of the hand wash agents in the meat cutting area.*

Hand wash agent	SPC 35°C[a] Initial count ($\times 10^5$)	SPC 35°C[a] Final count ($\times 10^4$)	SPC 35°C[a] Reduction (%)	B-P 35°C[b] Initial count ($\times 10^4$)	B-P 35°C[b] Final count ($\times 10^3$)	B-P 35°C[b] Reduction (%)
Non-germicidal soap	2.9	16	(41.8)	1.5	23	(159)[c]
Chlorhexidine (2%)	45	78	(82.8)	38	76	(79.7)
Chlorhexidine (4%)	10	2.2	(98.2)	2.0	0.4	(98.0)
Iodophor (0.02%)	4.6	24	(49.0)	6.5	55	(16.0)
Iodophor (0.12%)	26	8.4	(66.6)	55	184	(66.6)
Iodophor (0.75%)	21	0.7	(96.8)	32	42	(86.7)
Non-germicidal gel	8.4	14	(84.0)	28	73	(74.3)
Irgasan DP 300 (0.3%)	17	22	(86.5)	19	52	(71.7)
Irgasan DP 300 (0.5%)	14	29	(79.6)	18	37	(81.1)

[a]Counts on SPC at 35°C indicate total aerobic cfu.
[b]Counts on B-P medium.
[c]( ) indicates percent change in count.

TABLE 6. *Mean initial counts of bacteria released from hands and percentage reduction in counts as a result of the use of the hand wash agents in the kitchen area.*

Hand wash agent[e]	VRB 35°C[a]		CFC 22°C[b]		B-P 35°C[c]		SPC 35°C[d]	
	Init. count ($\times 10^2$)	Reduction (%)	Init. count ($\times 10^4$)	Reduction (%)	Init. count ($\times 10^3$)	Reduction (%)	Init. count ($\times 10^3$)	Reduction (%)
A	2.6	(80.9)	2.2	(63.7)	11.0	(8.0)	3.1	(20.6)
B	3.6	(72.2)	11.8	(85.6)	6.2	(48.0)	4.8	(54.6)
C	11.5	(84.9)	5.8	(91.3)	9.0	(58.3)	9.5	(71.4)
D	7.7	(60.6)	38.5	(71.9)	6.4	(28.0)	10.3	(31.0)
E	3.7	(83.8)	6.2	(73.8)	11.0	(40.9)	5.0	(37.2)
F	2.1	(81.8)	4.2	(88.3)	5.3	(50.6)	6.5	(63.3)
G	6.7	(79.8)	6.5	(67.5)	8.7	(23.3)	3.2	(26.4)
H	5.4	(82.6)	16.5	(87.6)	11.0	(48.6)	6.1	(60.3)
I	9.8	(81.0)	18.7	(76.2)	10.3	(44.5)	6.3	(45.9)

[a]Counts on VRB at 35°C indicate coliform bacteria.
[b]Counts on CFC at 22°C indicate pseudomonad bacteria.
[c]Counts on B-P medium indicate Micrococcaceae-type bacteria.
[d]Counts on SPC indicate total aerobic colony count.
[e]For key to product codes, see Table 3.

TABLE 7. *Acceptability and volume of each hand wash agent used during a five work day period.*

Hand wash agent	Acceptability[a] No. of workers			Meat cutting	Kitchen	Packaging	Total
	A	NC	U	[volume (ml) hand wash used]			
Non-germicidal soap	16	0	0	325	225	250	800
Chlorhexidine (2%)	13	1	2	550	170	450	1170
Chlorhexidine (4%)	12	2	2	460	245	550	1255
Iodophor (0.02%)	6	4	6	400	225	420	1045
Iodophor (0.12%)	2	4	10	240	170	300	710
Iodophor (0.75%)	0	3	13	250	140	250	640
Non-germicidal gel	15	1	0	380	400	400	1180
Irgasan DP 300 (0.3%)	14	2	0	350	410	410	1170
Irgasan DP 300 (0.5%)	3	3	10	300	275	280	855

[a]Acceptability recorded from opinions expressed by workers: A = acceptable, NC = no comment; U = unacceptable.

tification technique. Of the 72 isolates, 49 (68%) of the isolates were *E. coli* that grew in EC broth at 45°C. An additional 11 isolates grew at elevated temperature in EC broth, including 9 *Klebsiella pneumoniae* and 2 *Enterobacter* isolates. A total of 12 isolates failed to grow in EC broth at 45°C; these were identified as *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* (4 isolates) and *Serratia rubidaea*. These data indicate that colonies growing on VRB at 45°C should not be considered as fecal "*E. coli*."

The volumes of each agent used per week and the responses to questions about the acceptability of the hand wash products are shown in Table 7. The opinions expressed about the hand wash agents are generally supported by the volumes used. An exception to this is the non-germicidal (lotion) soap which was ranked highly acceptable, but a relatively low total volume was used. This might be attributed to the fact that this was the first agent tested, and workers were still becoming accustomed to the hand wash experiment. Specific objections accounting for the poor acceptability of 0.1 and 0.75% iodophor products were their color and smell. The product containing 0.5% Irgasan DP 300 was also criticized for its color and smell. The non-germicidal gel and 0.3% Irgasan DP 300 products were generally well accepted, with favorable comments about color and "softness" of the product.

DISCUSSION

Most studies of germicidal agents for hand hygiene have been done under standardized "laboratory" conditions, using controlled exposure times and specified hand wash procedures. Efforts to simulate practical conditions by contaminating hands with a transient microflora, apart from contaminating hands with specific bacteria, have included contamination of nurses' hands when changing diapers in a neonatal ward *(24)*, changing of wound dressings *(15)* and, in our previous studies, contamination of hands with ground beef inoculated with specific bacteria *(21,27)*.

The laboratory trial was included in this study to pro-

vide a cross-reference to our previous studies in which Latin Square experimental designs were used to compare hand wash agents. A Latin Square design was impractical for use in the meat plant because of the number of workers that would be entailed and the problem of using different hand wash agents in the plant at the same time. A sequential-type study was planned, in which the order of use of the agents was rationalized, and all workers used the same agent in each test location at the same time. In fact, all workers in a unit used the experimental hand wash agent, but only four workers in each unit were included in the study.

Results for the laboratory study were consistent with our previous report for transient bacteria inoculated onto hands *(27)*. Although some agents gave a significantly greater reduction in number of test organisms released from hands after the 15-s hand wash, the practical significance of these differences may be questioned. However, both the 4% chlorhexidine and the 0.75% iodophor products gave greater than 99.5% reduction in number of transient bacteria released from hands after washing. Products with Irgasan DP 300 showed more promise against transient bacteria than we reported in an earlier study *(21)*. The same Irgasan DP 300 (0.3%) product was used in the earlier and current studies. The non-germicidal and germicidal gels (products G and H) have the same basic formula, except for inclusion of 0.3% Irgasan DP 300 in the germicidal agent. Hence, a definite effect can be ascribed to Irgasan DP 300 as the active ingredient, but the practical importance of this effect is limited.

In the meat processing plant, large differences in level of contamination of workers' hands with transient microorganisms were associated with differences in type of work. The visible contamination of hands of meat cutters might have caused a more thorough hand wash by these workers, resulting in greater reductions in bacterial count and in significant differences attributable to hand wash agents. No apparent improvement in efficacy of the agents (substantive effect) could be associated with continuous use of the agents from day-to-day. The reductions in fecal coliform and pseudomonad counts (VRB 45°C and CFC 22°C, respectively) were smaller than noted for the controlled laboratory study. This might be caused by shorter washing time, unstandardized washing procedures, and heavier load of meat soil on hands. Considerable numbers of transient bacteria remained on the hands of meat cutters after washing, indicating that the hand wash procedures were not sufficient to eliminate contaminating bacteria. The meat cutters also had an elevated gram-positive microflora on their hands, probably as a part of the transient flora from meats, as evidenced by the elevated B-P counts. The resident Micrococcaceae-type flora (B-P count) of socially clean hands in the laboratory study was usually about 50 cfu/ml of hand rinse (see Table 1), whereas B-P counts for meat cutters were 2×10^4 to 6×10^5 cfu/ml of hand rinse solution (Table 5). Hand washing also failed to remove these transient bacteria.

For workers in the kitchen and meat packaging areas, initial contamination of their hands with transient microorganisms was much lower than workers involved in meat cutting. This could be attributed to the nature of the work, and the fact that in the processed meat packaging area the workers wore plastic gloves. The type of plastic glove did not occlude the skin, which might cause an increase in the resident microflora *(7,12,25)*. However, hand washing in both the kitchen and processed meat packaging areas did not eliminate transient microorganisms contaminating hands.

In the meat plant, the only unit in which differences could be attributed to hand wash agents was in the meat cutting area. The 4% chlorhexidine gluconate liquid detergent and the iodophor product containing 0.75% available iodine were the hand wash agents of choice. However, in some instances the 0.3% Irgasan DP 300 gel also showed promise. The iodophor product was poorly accepted by the workers, in comparison with the 4% chlorhexidine and 0.3% Irgasan DP 300 gel products. Lower concentration iodophor products were developed to reduce user objections to iodophor compounds. The product containing 0.1% available iodine did not overcome the user objections, and neither of the lower concentration iodophor products in this study could be recommended bacteriologically for control of bacteria on workers' hands. Under the conditions of this study, therefore, the lower concentration iodophor products, the Irgasan DP 300 formulation containing 0.5% active ingredient and the 2% chlorhexidine gluconate product were similar in effect to the non-germicidal products.

The in-use study in a meat processing plant indicated that the initial step to effective hand hygiene would be improved hand washing practice. The washing time of 8 to 10 s is too short for the hand wash agents to be effective. The effectiveness of some of the agents in the meat cutting area can be attributed to the longer washing time necessary to remove the meat soil on hands. Some of the agents were more effective than others in reducing "transient" bacteria on hands, and may be considered more effective than non-germicidal hand wash agents in achieving hand hygiene.

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Efficacy of Germicidal Hand Wash Agents Against Transient Bacteria Inoculated onto Hands

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ABSTRACT

The efficacy of germicidal hand wash agents against transient bacteria (*Escherichia coli* and *Pseudomonas fluorescens*) in ground beef rubbed onto hands was determined using a hand rinse sampling technique. The reduction in *E. coli* and *P. fluorescens* counts on selective growth media and the change in count on Baird-Parker medium were used to indicate action against transient and resident bacteria, respectively. Most of the agents tested, including 4% chlorhexidine gluconate, iodophor (0.75% available iodine), Irgasan DP 300, *para*-chloro-*meta*-xylenol (PCMX) as well as the non-germicidal soap, gave marked reduction in counts of *E. coli* and *P. fluorescens* (>90% reduction), even after one 15-s wash. The hand dip treatments with iodophor (25 ppm available iodine), hypochlorite (50 ppm available chlorine) or QAC (930 ppm benzalkonium chloride) were generally less effective than hand wash treatments, especially against *P. fluorescens*. Iodophor (0.75% available iodine) and 4% chlorhexidine gluconate significantly reduced more *E. coli* on hands than the other agents.

Earlier studies by Sheena and Stiles (*18,19*) assessed the efficacy of a range of germicidal hand wash agents against the total microflora occurring naturally on hands. Dividing the skin microflora into transient and resident microorganisms has long been accepted (*15*). Transient microflora are those organisms acquired from the surroundings, which are superficially located on the skin, generally do not colonize the skin to become part of the resident microflora, and are readily removed by washing (*10,11,17*). Aerobic isolates identified after germicidal hand washing (*18*) were 85% *Staphylococcus epidermidis* and *Micrococcus* spp., indicating that the principal survivors after washing were organisms typically associated with the resident microflora of skin (*16,17*).

The method of application of transient microorganisms to the skin (*9,13*) and the species or strains of test organisms selected (*2*) play an important role in efficacy testing. Koller et al. (*8*) observed differences in the level of contamination between finger tip and hand immersion contaminating techniques, but no significant difference in the efficacy of hand disinfection attributable to the method of contamination. In earlier studies, the transient microorganisms were spread on the skin (*1,6,7,11,21*); more recently, the natural work environment has been used to contaminate skin with a transient microflora (*13,20*). The natural environment approach was used in this study, with meat as the suspending agent.

Most studies have used germicidal products intended for medical use, including 70% ethyl alcohol, 70% isopropanol, alcoholic preparations of chlorhexidine, 4% chlorhexidine gluconate liquid detergent, 0.75% povidone iodine, 2% Irgasan DP 300 and 3% hexachlorophene (*1,2,9,11,13*). Information on the use of these agents in food handling using short exposure times is scant. In 1965, Crisley and Foter (*4*) reviewed antibacterial soaps for hand washing in foodservice establishments. They concluded that hand washing with non-germicidal soap was required to prevent transmission of possible pathogens from hands to foods during handling and preparation. Frequent hand washing by food handlers is considered mandatory to maintain hygienic conditions (*5,14,17*).

The object of this study was to evaluate and compare the efficacy of germicidal hand wash agents for control of transient microorganisms inoculated onto hands from meat.

MATERIALS AND METHODS

Two separate experiments were conducted, a 7×7 and a 5×5 Latin Square design, involving the exposure of each subject to each agent included in the experiment. The sequence in which agents were used by subjects was randomly assigned by a specified procedure (*12*). Each subject was exposed to all of the agents over the period of the experiment. Subjects used the assigned agent on two occasions, one 15-s exposure in the morning and two successive 15-s exposures in the afternoon. The hand washing procedure was detailed in our previous study (*18*). There were two testing days per week (Monday and Thursday) so that two different products were tested on each subject each week.

In the first experiment, seven agents were tested: (a) non-germicidal liquid hand soap, (b) chlorhexidine gluconate (4%) detergent solution (Hibitane, Ayerst Laboratories, Montreal) as a positive control; (c) an antibacterial gel skin cleanser containing 0.3% 2,4,4′trichloro-2′-hydroxy

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diphenyl ether (Irgasan DP 300); (d) germicidal liquid soap diluted to 0.25% Irgasan DP 300; (e) antiseptic liquid hand soap containing 0.65% *para*-chloro-*meta*-xylenol (PCMX) diluted to 0.325% active ingredient at the use concentration; (f) iodophor ("Tamed Iodine" Scrub) containing 0.75% available iodine; and (g) germicidal bar soap containing 1.0% trichlorocarbanilide (TCC).

In the second experiment, five agents were tested: (a') non-germicidal liquid hand soap (same as "a" above); (h) iodophor hand wash containing 0.005% available iodine; and three hand dips consisting of (i) iodophor solution containing 25 ppm available iodine, (j) sodium hypochlorite solution containing 50 ppm available chlorine, and (k) quaternary ammonium (QAC) solution containing 930 ppm benzalkonium chloride. Hand wash agents were used in 5-ml amounts, the hand dip solutions were freshly prepared in 1.5 L of deionized water.

Two bacterial strains were isolated from ground beef for use in this study: *Escherichia coli* and *Pseudomonas fluorescens*. The *E. coli* isolate was identified and confirmed by comparison with a standard strain of *E. coli* from the American Type Culture Collection (ATCC; strain 11229) and a strain (1840) previously isolated from ground beef (M.E.S.). The *P. fluorescens* isolate was similarly compared to a strain of *P. fluorescens* from the National Collection of Type Cultures (NCTC) strain 10038 and a strain (R639) from the Alberta Laboratory for Public Health, Edmonton. Cultures were carried in nutrient broth. *E. coli* was incubated at 35°C for 18 h and *P. fluroescens* was incubated at 20°C for 30 h for use in the experiments. Final concentrations for *E. coli* averaged 5×10^8 CFU/ml and *P. fluorescens* averaged 1.1×10^8 CFU/ml.

A ground beef inoculum was prepared to inoculate the test organisms onto subjects' hands. Freshly prepared ground beef from a local retail store was inoculated with the *E. coli* and *P. fluorescens* test cultures to give counts of 10^6 and 10^7 CFU/g of ground beef, respectively. This necessitated a 10^{-1} dilution of the *E. coli* culture in 0.1% peptone water, whereas the *P. fluorescens* culture was inoculated without dilution. The ground beef was checked each day to determine its microbiological quality, including total aerobic plate count, coliform, *E. coli*, *P. fluorescens* and total "gram-positive cocci" counts. The levels of *E. coli* and *P. fluorescens* inocula in the ground beef were also determined. The inoculated ground beef was dispensed in two 50-g amounts in separate petri dishes to use as inocula for the fingertips.

To reduce the microbial load on hands in preparation for the experiment, subjects' rinsed their hands with 5 ml of 95% ethanol containing glycerol, and the hands were rubbed together until they were dry (*3,13*). The finger and thumb tips were pushed into and held in the ground beef inoculum for 5 s and the inoculum was distributed over the hands by rubbing up to the wrists until the hands were dry. The hands were washed on two separate occasions with the same agent (morning and afternoon wash). One of the hands was randomly selected for sampling for the initial count (X_0) by rinsing in 100 ml of letheen broth (LB; Difco) in a plastic bag (28.5 × 12.5 × 7.5 cm, 25 mil, Polyrama Plastics Ltd., Edmonton) using the standard hand rinse method described in our previous study (*18*). Hand washing and dipping were also done according to the procedures previously described (*18*). The sample for the first 15-s wash (X_1) was taken at the morning wash. The sample for the two successive 15-s washes (X_2) was taken at the afternoon wash and compared with the initial (X_0) count for the afternoon testing period. Subjects rinsed their hands with the glycerol in ethanol solution after the sampling procedure had been completed.

Bacteriological testing of the hand rinse samples was done by plating in duplicate onto the following Difco media: standard plate count agar (SPC), violet red bile agar (VRBA), Pseudomonas agar F (PAF) and Baird-Parker agar (B-P). Two sets of VRBA plates were prepared for comparison of the differential incubation temperatures. After washing hands with germicidal agents, such as chlorhexidine gluconate (4%) liquid detergent or iodophor (0.75% available iodine), injury levels between 50 and 90% were observed when the organisms were grown on selective media compared to growth on tryptic soy agar (TSA). Holding LB samples at room temperature for 1 to 2 h allowed resuscitation of injured organisms for growth on selective media. Holding LB samples for greater than 2 h resulted in growth of the microorganisms.

The X_0 LB samples were plated immediately onto agar media; the X_1 and X_2 LB samples were held 1.5 h at 20°C for recovery of any injured cells. Initial studies indicated that this treatment of samples reduced the level of injury for growth on selective media. X_0 samples were diluted 1:10 with 0.1% peptone water for pour plating with VRBA and overlayered with 5 ml of the VRBA medium. X_1 and X_2 samples were pour plated on VRBA without dilution. Prepoured plates of SPC, PAF and B-P were surface streaked with 0.1-ml portions of samples. The two sets of VRBA plates were incubated separately at 35 and 45°C for 24 h. SPC and B-P plates were incubated at 35°C for 48 h, and PAF plates were incubated at 20°C for 72 h.

Data were calculated as ratios of the number of organisms released from hands after washing compared to the number released before washing. Mean counts and mean percentage change in number of bacteria released from hands were based on individual changes in count for each subject. The data were analyzed using $\log_{10}$ transformed ratios in a statistical computer package for Latin Square designs (BMDP2V, Biomedical Computer Programs, P-Series, 1979, University of California Press).

RESULTS

The microbiological techniques allowed five microbial parameters to be monitored, including transient and resident microflora. The SPC count was intended to determine the total transient and resident flora. The VRBA counts determined the efficacy of the agents against *E. coli* (incubated at 45°C) and total coliform-type bacteria (incubated at 35°C). The correlation between VRBA counts at 45 and 35°C was $r>0.98$, indicating that the transient *E. coli* strain inoculated onto hands predominated the VRBA counts at both temperatures. Only data for VRBA at 35°C are presented. PAF counts were used to indicate the efficacy of the agents against *P. fluorescens*. The *P. fluorescens* strain grew as distinctive fluorescent yellow colonies on PAF medium. Only typical colony types on the PAF medium were included in the presumptive *P. fluorescens* count. Initially, typical colonies were isolated from the PAF plates and confirmed as *P. fluorescens* by comparison with reference strains. B-P medium was used to monitor *Micrococcaceae*-type organisms released from the hands, which were considered to represent typical resident microflora as well as organisms possibly acquired from the ground beef. A summary of the probabilities of a significant effect attributable to agents is shown in Table 1.

Data for the percentage mean reduction in total count of transient and resident flora on hands monitored using SPC counts at 35°C are shown in Table 2. The reduction in number of bacteria released from hands as a result of one and two successive 15-s washes was not impressive. All products gave reduced counts as a result of the treatments, implicating the transient flora in this measure. Only three agents (4% chlorhexidine gluconate and the iodophor products containing 0.75 or 0.005% available iodine) achieved 80% or slightly greater reduction in count with one 15-s wash. Non-germicidal soap achieved a 75% reduction. After two successive 15-s washes, most agents (including the non-germicidal soap) achieved virtually 80% reduction in SPC counts. Only the 4% chlorhexidine gluconate and Irgasan DP 300 (0.25%) washes gave better than 90% reduction. In Experiment 1 there was a significant effect attributable to agents (see Table 1).

Duncan's multiple range tests for differences among treatment means are shown in Table 3. On SPC medium,

TABLE 1. *Summary of probabilities (P) of a significant effect attributable to agents as a result of Latin Square design analyses of variance.*

Medium[a]	Experiment I		Experiment II	
	After 1 × 15-s wash	After 2 × 15-s washes	After 1 × 15-s wash	After 2 × 15-s washes
SPC	0.0415[b]	0.0468[b]	0.4218	0.4946
VRBA (35)	0.0239[b]	0.0290[b]	0.1612	0.3188
VRBA (45)	0.0450[b]	0.0061[c]	0.5113	0.5211
PAF	0.1743	0.3336	0.9876	0.0246[b]
B-P	0.3382	0.4651	0.2412	0.0534[b]

[a]Microbiological media: SPC, standard plate count agar; VRBA, violet red bile agar; PAF, Pseudomonas agar F; B-P, Baird-Parker agar.
[b]Significant at the 95% confidence level ($P<0.05$).
[c]Significant at the 99% confidence level ($P<0.01$).

after one 15-s wash, only 4% chlorhexidine gluconate and iodophor (0.75% available iodine) showed a significantly greater decrease in count compared to the Irgasan DP 300 wash and the TCC bar soap. After two successive 15-s washes, only PCMX gave a significantly poorer result than the chlorhexidine and iodophor (0.75% available iodine) products. The rest of the agents, including the non-germicidal soap, were not significantly different from these two agents.

Results of the efficacy of the germicidal agents against *E. coli* are shown in Table 4. All agents, including the non-germicidal soap, gave greater than 90% reduction in count. Only 4% chlorhexidine gluconate achieved 99% reduction after two successive 15-s washes. A significant effect was attributed to agents in Experiment I (see Table 1). Duncan's multiple range tests for differences among treatment means (see Table 3) revealed that, after one 15-s wash, the iodophor (0.75% available iodine) gave a significantly greater reduction than all other agents, except 4% chlorhexidine gluconate. After the two 15-s wash sequences, significant differences were observed. However, 4% chlorhexidine gluconate was not significantly more effective against *E. coli* than iodophor (0.75% available iodine), 0.3% Irgasan DP 300 gel and PCMX (0.325%) antiseptic hand soap. These agents, except PCMX, gave a significantly greater reduction of *E. coli* compared to the non-germicidal soap.

Data for *P. fluorescens* are shown in Table 5. Results similar to those for *E. coli* were observed, except that the TCC bar soap (one 15-s wash) and dip methods of hand washing only gave 80 to 90% reduction in *P. fluorescens* count, whereas other agents gave greater than 90% reduction. The only significant effect attributable to agents in Experiment II was against *P. fluorescens* (see Table 1). Duncan's multiple range tests on these data indicated a significantly greater decrease in *P. fluorescens* count attributable to the iodophor wash (0.005% available iodine) and non-germicidal soap compared to iodophor and hypochlorite dips (Table 3).

Changes in the "resident" microflora measured on B-P medium are shown in Table 6. These changes showed a marked difference to the trends observed for the "transient" microflora (Tables 2, 4 and 5). The non-germicidal soap caused a marked increase in the number of microor-

TABLE 2. *Reduction in total number of bacteria (counts on SPC) released from hands as a result of one or two successive 15-s hand wash treatments.*

Agent[a]	Initial count	After 1 × 15-s wash [Mean count × 10^2 (percent)][b]	Initial count	After 2 × 15-s washes
Experiment I				
A. Control soap	11.0	2.9 (75)	10.0	2.1 (79)
B. Chlorhexidine	11.0	1.8 (85)	9.4	0.3 (96)
C. Irgasan gel	7.6	2.7 (66)	8.5	1.6 (80)
D. Irgasan wash	9.9	4.3 (58)	9.4	1.0 (90)
E. PCMX	8.1	3.2 (65)	7.8	2.4 (74)
F. Iodophor	11.0	1.8 (81)	8.2	0.7 (88)
G. TCC	7.4	3.0 (56)	8.6	1.8 (80)
Experiment II				
A'. Control soap	8.5	3.5 (48)	11.0	1.6 (82)
H. Iodophor wash	12.0	2.5 (80)	9.9	0.9 (84)
I. Iodophor dip	12.0	3.4 (66)	11.0	1.8 (84)
J. Hypochlorite dip	8.7	3.4 (62)	13.0	2.4 (81)
K. QAC dip	9.9	3.3 (67)	10.0	2.6 (76)

[a]Hand wash agents: A and A', non-germicidal liquid hand wash; B, 4% chlorhexidine gluconate (Hibitane); C, Irgasan DP 300 (0.3%) gel; D, Irgasan DP 300 (0.25%) hand wash; E, *Para*-chloro-*meta*-xylenol (PCMX) (0.325%) hand wash; F, Iodophor (0.75% available iodine); G, 1% Trichlorocarbanilide (TCC) bar soap; H, Iodophor hand wash (0.005% available iodine); I, Iodophor hand dip containing 25 ppm available iodine; J, sodium hypochlorite dip containing 50 ppm available chlorine; K, Quaternary ammonium (QAC) dip containing 930 ppm benzalkonium chloride.
[b]Mean counts and mean percentage change in number of bacteria released from hands are based on individual changes in count for each subject.

TABLE 3. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means on different microbiological media*[a,b,c].

Experiment I	
(i) SPC	
After 1 × 5-s wash	F B A C E D G
After 2 × 15-s washes	B F D A C G E
(ii) VRBA	
After 1 × 15-s wash	F B E G D A C
After 2 × 15-s washes	B F C E D G A
Experiment II	
(i) PAF	
After 2 × 15-s washes	H A′ K I J
(ii) B-P	
After 2 × 15-s washes	K J H I A′

[a]For key to product codes see Table 2.
[b]Microbiological media: SPC, standard plate count agar; VRBA, violet red bile agar; PAF, Pseudomonas agar F; B-P, Baird-Parker agar.
[c]Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

ganisms released from hands after one or two successive 15-s washes. This applied also to most of the germicidal washes or dips except 4% chlorhexidine gluconate, iodophor (0.75% available iodine) and the QAC dip. QAC dip treatment gave a significant reduction in number of microorganisms released from hands compared to iodophor dip and non-germicidal soap.

DISCUSSION

Results of this study on transient bacteria inoculated onto hands from meats contrasts markedly with our earlier results for the total hand microflora (*18,19*). However, the previous studies involved primarily the resident microflora. Both test organisms included in this study were gram-negative bacteria originally isolated from meat. *E. coli* was selected because of its role as an indicator organism and as a possible indicator of what might happen to related pathogenic bacteria, such as *Salmonella* (*14*). *P. fluorescens* was selected to indicate activity against spoilage-type bacteria. This particular strain was selected because of its pigmentation on PAF that made it easy to detect in mixed culture.

These transient bacteria from meats inoculated onto hands were markedly reduced by the short-exposure washes used in this study. Effective reduction or elimination of transient microflora by non-germicidal soap has been widely reported (*2,11,13,20*); however, more recent studies with agents such as alcohol, povidone-iodine and chlorhexidine have given better results than non-germicidal soaps (*3,6,9,13*). Our results confirmed the improved action of 4% chlorhexidine gluconate and iodophor (0.75% available iodine) against *E. coli* compared to non-germicidal soap.

In our studies on resident microflora (*18,19*), the efficacy of germicidal products containing Irgasan DP 300 or *para*-chloro-*meta*-xylenol (PCMX) for short-exposure hand washing was not satisfactory. However, against the transient bacteria in this study, these agents were far more effective. Trichlorocarbanilide (TCC) bar soap and hand dips gave the least effective results against transient bacteria. Results of this and our earlier study (*18*) favor hand washing over hand dipping techniques both for the bacteriological results as well as practical control.

The use of Baird-Parker (B-P) agar to monitor resident flora was justified by the similarity of these results compared to those obtained on the non-selective medium used in our previous study (*18*). Coagulase-negative

TABLE 4. *Efficacy of germicidal hand wash agents against* Escherichia coli *artificially inoculated onto hands from ground meat (based on count on VRBA incubated at 35°C)*[a].

Agents	Initial count	After 1 × 15-s wash	Initial count	After 2 × 15-s washes
	[Mean count × 10^2 (percent)]			
Experiment I				
A. Control soap	8.4	0.6 (94)	6.0	0.4 (95)
B. Chlorhexidine	7.9	0.2 (97)	6.5	0.1 (99)
C. Irgasan gel	6.3	0.3 (92)	11.0	0.2 (98)
D. Irgasan wash	6.8	0.4 (93)	5.7	0.2 (97)
E. PCMX	5.0	0.2 (95)	3.4	0.1 (98)
F. Iodophor	6.3	0.1 (98)	3.6	0.1 (98)
G. TCC	6.1	0.3 (94)	7.8	0.2 (97)
Experiment II				
A′. Control soap	7.2	0.3 (95)	8.8	0.2 (97)
H. Iodophor wash	9.7	0.4 (96)	5.8	0.1 (98)
I. Iodophor dip	11.0	0.5 (96)	8.4	0.2 (97)
J. Hypochlorite dip	5.7	0.4 (93)	10.0	0.4 (96)
K. QAC dip	8.9	0.3 (97)	8.2	0.4 (97)

[a]See footnotes of Table 2.

TABLE 5. *Efficacy of germicidal hand wash agents against* Pseudomonas fluorescens *artificially inoculated onto hands from ground meat (based on count on PAF)*[a].

Agent	Initial count	After 1 × 15-s wash	Initial count	After 2 × 15-s washes
		[Mean count × 10^2 (percent)]		
Experiment I				
A. Control soap	4.3	0.2 (96)	3.4	0.1 (98)
B. Chlorhexidine	3.2	<0.1 (98)	2.5	<0.1 (98)
C. Irgasan gel	2.4	0.2 (93)	3.3	0.2 (95)
D. Irgasan wash	2.9	0.2 (95)	3.7	0.2 (93)
E. PCMX	2.9	0.1 (97)	2.6	<0.1 (99)
F. Iodophor	2.6	0.1 (96)	2.5	<0.1 (99)
G. TCC	2.7	0.4 (88)	3.2	0.2 (92)
Experiment II				
A'. Control soap	3.5	0.2 (93)	3.6	0.2 (96)
H. Iodophor wash	4.1	0.2 (95)	3.1	0.1 (96)
I. Iodophor dip	3.9	0.3 (90)	3.8	0.5 (83)
J. Hypochlorite dip	3.9	0.6 (87)	4.2	0.8 (80)
K. QAC dip	3.5	0.5 (88)	4.4	0.5 (90)

[a]See footnotes of Table 2.

TABLE 6. *Mean count and percentage change in residual-type* (Micrococcaceae) *bacteria released from hands after use of germicidal hand wash agents measured by growth on Baird-Parker agar (based on count on B-P)*[a].

Agent	Initial count	After 1 × 15-s wash	Initial count	After 2 × 15-s washes
		[Mean count × 10^2 (percent)]		
Experiment I				
A. Control soap	0.9	1.2 (291)	0.7	0.4 (113)
B. Chlorhexidine	1.5	0.5 (83)	0.2	<0.1 (57)
C. Irgasan gel	0.6	0.4 (88)	0.2	0.3 (119)
D. Irgasan wash	0.4	0.6 (246)	0.1	0.3 (192)
E. PCMX	1.1	2.4 (275)	0.6	0.7 (160)
F. Iodophor	2.1	1.4 (83)	0.5	0.2 (74)
G. TCC	1.9	2.6 (134)	0.4	0.9 (158)
Experiment II				
A'. Control soap	0.9	2.3 (313)	0.2	0.7 (263)
H. Iodophor wash	0.7	1.5 (226)	0.4	0.7 (155)
I. Iodophor dip	1.3	1.5 (137)	0.1	0.4 (242)
J. Hypochlorite dip	1.6	1.7 (246)	0.7	0.4 (234)
K. QAC dip	1.5	1.9 (84)	0.7	0.2 (68)

[a]See footnotes of Table 2.

staphylococci are part of the resident microflora of skin (*15,17*). The preferential activity of QAC's against gram-positive bacteria might account for the favorable result of the QAC dip against "resident" flora. This confirmed our previous observation of the efficacy of a QAC dip (*18*).

Coliform bacteria and associated enteric pathogens are generally absent from skin, except in some special studies of food handlers (*14*). *Staphylococcus aureus* is generally associated with the nasal cavity (*22*) but it may be carried on skin as part of the transient microflora (*11*). The incidence of coagulase-positive staphylococci is greater among meat handlers, with a tendency for these organisms to become part of the resident skin microflora (*16,17*). This weighs in favor of the selection of germicidal hand washes as opposed to non-germicidal soaps for hygienic hand disinfection of food handlers. Bacteriologically, 4% chlorhexidine gluconate and iodophor (0.75% available iodine) remain the agents of choice for their better action against resident and transient skin bacteria.

The 4% chlorhexidine gluconate product (Hibitane) was selected as a reference agent for this study because of its use in medical practice. It is probably unsuitable for food handlers in this formulation. However, our studies with 4% chlorhexidine gluconate detergent solution confirmed its marked residual (substantive) effect (*19*) and a need for special care in neutralizing its antibacterial activity for efficacy testing (*18*). Iodophor (0.75% available iodine) and equivalent products with relatively high concentrations of iodine are used in medical practice and by food handlers. There is resistance to the latter products because of color

and odor, hence the low iodine products, such as product H (0.005% available iodine), warrant further study.

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Application of a new microbiological technique to the study of antiperspirant and deodorant soap efficacy

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Synopsis

A new technique for sampling cutaneous microorganisms has been used to study the effects of deodorant products on aerobic axillary microbial populations. Initially the Thran spray gun was compared to the mechanical scrub method and was found to compare favorably in terms of reproducibility and sensitivity. When a commercial stick antiperspirant was evaluated, both methods demonstrated similar log reductions in axillary bacteria when compared to controls. The Thran spray gun was also used to study the efficacy of a commercial deodorant soap containing 3,4,4'-trichlorocarbanilide. A 55% reduction in aerobic axillary microorganisms was demonstrated 24 hours after washing with the soap.

INTRODUCTION

Many locations on the surface of human skin emit distinctly different odors. However, of all the human scents, those emanating from the axilla are regarded by society as some of the most offensive. In recent years the accumulation of a significant body of scientific evidence has traced the source of axillary odors to the action of bacteria on secretions from the apocrine gland (1-4). In a number of recent publications it has been suggested that a class of Gram positive microorganisms, the diphtheroids, are responsible for the selective generation of the distinctly pungent axillary odors, while the micrococci are responsible for the generation of sweaty, acid odors (2-4). Therefore, numerous manufacturers of deodorant products have marketed formulations with an active ingredient which reduces axillary microbial populations for the purpose of reducing the intensity of axillary odor.

Since bacterial reduction is related to a product's deodorant efficacy, numerous methods have been developed for qualitatively and quantitatively monitoring the distribution and population of skin flora. Among these are mechanical scrub techniques (5-6), swabbing procedures (7-9), tape stripping and contact plates (7-13), as well as basin scrubbing methods (14-15). Among these techniques, a widely employed procedure for quantitating aerobic skin microflora has been the mechanical scrub technique which involves a timed scrub of the skin surface with a blunt teflon scrubber

in the presence of a buffer containing surfactant. Certain problems are encountered with the mechanical scrub procedure, particularly in situations in which a panelist's axillae must be repetitively extracted. In these cases mechanical scrubbing can result in localized edema and irritation of the test site.

In the present investigation we describe the use of a more gentle, pressurized spray technique (Thran bacterial sampler) to quantitatively evaluate total aerobic axillary microbial populations (16). The Thran bacterial sampler was compared to the mechanical scrub procedure, and was subsequently employed to demonstrate the efficacy of a solid stick antiperspirant as well as a deodorant soap containing trichlorocarbanilide (TCC).

EXPERIMENTAL

A. BACTERIAL SAMPLING

Bacterial sampling of the axillary vault was performed utilizing both the mechanical scrub procedure of Williamson and Kligman (5) and the pressurized spray gun technique of Thran (16). In the case of the mechanical scrub method, a glass cylinder which circumscribed a 3.8 cm^2 area was firmly placed onto the exposed skin of the axilla, and 3 ml of 0.075 M phosphate buffer pH 7.9 containing 0.1% Triton X-100 was added. The circumscribed skin surface was rubbed with moderate pressure for one minute with a blunted teflon scrubber; sampling fluid was removed by aspiration, and the procedure repeated. The two samples containing extracted bacteria were pooled and stored on ice prior to serial dilution. Recoveries for the sampling fluid averaged 90%.

The Thran spray gun bacterial sampler was obtained from Dr. Med Vet. Volker Thran, Smetslaan 9, B 1900 Overijse-Maleizen, Brussels, Belgium. In principle, the Thran spray gun technique involves the use of air pressure to aspirate a sampling solution over an isolated region of the skin surface (see Figure 1—a complete description of the apparatus can be found in reference 16.) A jar containing a 100 ml. aliquot of bacterial sampling fluid consisting of 0.075 M phosphate buffer pH 7.9 containing 0.005% Triton X-100 was attached to the spray gun along with an empty sterile collection jar. To extract bacteria the silicon rubber tip of the spray gun was firmly pressed against the axillary vault, and the sampling solution was simultaneously sprayed onto and aspirated from a 1.77 cm^2 area. With a compressed air pressure of 1.8 bars, aspiration times averaged 65 seconds and sampling fluid recovery averaged 98% for the 100 ml sample. Following extraction, bacterial collection jars were removed from the spray gun and stored on ice prior to serial dilution. The Thran spray gun was sterilized between uses by consecutive rinses with sterile water (15 ml), 3A alcohol (15 ml), and one final sterile water rinse (25 ml).

Total aerobic bacterial count was determined by serially diluting 1 ml aliquots from each sample in 10 fold steps with 0.0375 M phosphate buffer, pH 7.9 containing 0.05% Triton X-100. In deodorant soap and antiperspirant experiments 0.5% lecithin and 5.0% Tween-80 were added to this dilution buffer to neutralize any antimicrobial activity associated with the active ingredients. Diluted samples were plated in duplicate in trypticase soy agar supplemented with 0.25% dextrose, 0.1% yeast extract, and 0.2% Tween-80 to facilitate growth of diphtheroids (17). Following a 48 hour aerobic

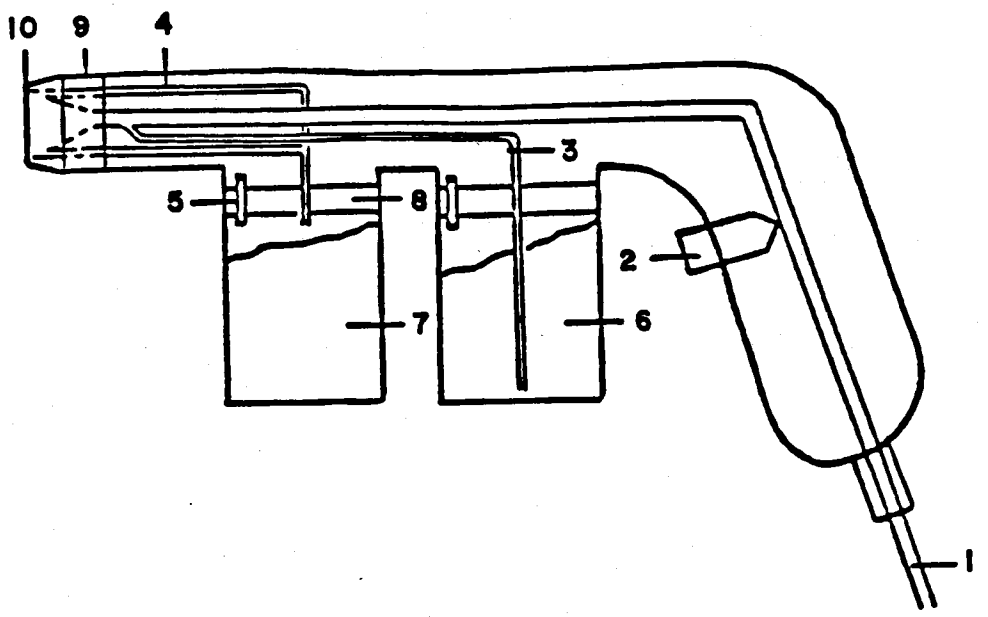


Figure 1. Schematic drawing of the Thran spray gun bacterial sampler. 1. Compressed air entry; 2. Valve; 3. Entry to spray head; 4. Exit from spray head; 5. Air vent; 6. Sampling fluid reservoir (detachable); 7. Collecting reservoir (detachable); 8. Special fastening; 9. Spray head; 10. Sealing band.

incubation at 37°C, plates were counted under a dissecting microscope and colony forming units (CFU) per plate converted to log CFU per cm^2 of axilla sampled. Average log CFU's per axilla were calculated for the duplicate plates and paired t-tests conducted when appropriate as a test of statistical significance of differences in axillary aerobic bacterial populations.

B. CLINICAL TESTING PROCEDURES

Male and female subjects over 18 years of age were recruited for clinical studies. All subjects were in excellent health and free of visible dermatitis. Prior to laboratory testing all subjects participated in a one week washout period with placebo soap (containing no germicide, fragrance, or color) during which time they were restricted from the use of all deodorant or medicinal soaps, antiperspirants or deodorants, and antibiotics. In addition, subjects were restricted from swimming in chlorinated water within three days of the test period. Female subjects were instructed to stop underarm shaving three days before bacterial extraction.

Initial experiments were designed to compare the reproducibility of bacterial extraction using the Thran spray gun or the mechanical scrub procedure. Twenty-four hours after the final placebo soap wash, two adjacent sites on a subject's left and right axillae were extracted—the first site via mechanical scrubbing, the second site using the Thran spray gun. In antiperspirant experiments, following a one week washout period and a placebo soap wash, a commercial antiperspirant stick based on an active ingredient aluminum zirconium tetrachlorohydrex glycine, cyclomethicone, stearyl alcohol, water, glyceryl monostearate, quaternium-18 hectorite, talc, fragrance and SD alcohol 40, was applied

(0.5 gm per male/0.3 gm per female) to either the left or right axilla based on a computer randomized assignment. The corresponding placebo stick, in which the active ingredient was removed and replaced with talc and cyclomethicone, was applied to the opposite axilla. Twenty-four hours later adjacent sites of antiperspirant and placebo treated axillae were extracted, the first site with mechanical scrubbing and the second site with the Thran spray gun.

In clinical experiments designed to evaluate the *in situ* antibacterial efficacy of a commercial deodorant soap based on an active ingredient (3,4,4',Trichlorocarbanilide), sodium tallowate, sodium cocoate, water, glycerin, fragrance, sodium chloride, preservatives, and colors, only the Thran spray gun procedure was employed. Panelists participated in a one week washout with placebo soap (with the same restrictions listed previously) prior to reporting to the laboratory. Placebo and deodorant soaps were assigned to opposite axillae using a computer generated randomization schedule, and panelists participated in four supervised washes as described below. Panelists were instructed to wet their right axilla with a clean washcloth (15 seconds), after which the assigned soap bar was wet under running water (15 seconds) and applied directly to the axilla (20 seconds). Following a 15 second hand rinse, panelists wet a clean washcloth and removed all remaining soap lather from their axillae (45 seconds). Panelists' axillae were patted dry with a clean towel, and the procedure was repeated for the opposite axilla with the other test soap using a fresh supply of linen. This process was repeated each morning for four mornings. Twenty-four hours following the last wash each axilla was extracted using the Thran spray gun, and samples were serially diluted for plating and analysis of total aerobic bacterial count as previously described.

RESULTS AND DISCUSSION

The Thran spray gun (TSG) and mechanical scrub (MS) techniques represent distinctly different methods of bacterial extraction. The TSG delivers a fine mist of buffer and surfactant which we believe removes only surface bacteria. For TSG sampling, the surfactant concentration (Triton X-100) was reduced to 1/20th of the level used with the MS procedure to reduce foaming during extraction. The MS procedure involves extensive agitation of the skin surface resulting in the removal of greater amounts of the desquamating stratum corneum. In contrast to the MS method where microorganisms present in lower levels of the stratum corneum might also be removed, the tendency of the TSG is to only remove surface microorganisms. For this reason there was concern about the percentage of total axillary skin bacteria extracted utilizing either method.

To answer this question, initial experiments focused on evaluating the *in situ* axillary bacterial levels on human subjects 24 hours following a supervised wash with placebo soap. For purposes of comparison, adjacent areas in the axillary vault of each panelist were extracted, with the first site using the MS method and the second site using the TSG. This process enabled a comparison of total aerobic bacteria extracted and an examination of the reproducibility of both methods. In addition, total aerobic bacteria could be compared between left and right axillae. Experimental results for five panelists are illustrated in Table I with bacteria levels for each subject expressed in log units per cm^2. Based on the MS method, total axillary bacterial count was found to range from 10^5 to 10^7 per cm^2 which is in agreement with previously published information (18-19).

Table I
A Comparison of Mechanical Scrub (MS) and Thran Spray Gun (TSG) Methods of Bacterial Extraction on Placebo Soap Washed Axillae

	Mechanical Scrub[a] log CFU/cm²			Thran Spray Gun[a] log CFU/cm²		
Panelist	Left Axilla	Right Axilla	Difference	Left Axilla	Right Axilla	Difference
1	5.72	7.03	−1.31	5.42	5.16	0.26
2	6.26	6.14	0.12	5.61	5.07	0.54
3	6.32	6.11	0.21	5.59	4.66	0.93
4	6.87	6.68	0.19	5.53	5.67	−0.14
5	6.67	6.89	−0.22	5.98	6.25	−0.27
Average			−0.20			0.26
Std. Dev.			0.64			0.49
P			≤0.60			<0.30

[a]Comparison of MS to TSG for total bacteria extracted, as follows: ave. $\Sigma_{n=1}^{n=5}$ (log MS_{left} − log TSG_{left}) = (log MS_{right} − log TSG_{right})/2 resulting in $\bar{X}$ = 0.98, std. dev. = 0.21, $P < 0.001$.

With either method when bacterial concentrations from left versus right axilla were compared, no significant differences were observed. This suggests that the random assignment of two test products represents an appropriate testing model. However, significant differences were observed between numbers of total bacteria extracted using the two procedures. The MS method removed more than five times the number of microorganisms as compared to extraction with the TSG. This difference was highly significant ($p < 0.001$), and was apparently due to more vigorous scrubbing and hence the removal of greater numbers of microorganisms. In these experiments a significant number of subjects developed erythema as a result of the pressure applied during scrubbing (approximately 100–120 g. pressure). In subsequent bacterial extraction on one axilla of 20 panelists the physical pressure exerted during scrubbing was reduced to approximately 60–70 g. pressure, resulting in a concomitant reduction in the incidence of erythema and a reduction in the total number of bacteria extracted (Table II).

Table II
A Comparison of Gentle Mechanical Scrub (MS) and Thran Spray Gun (TSG) Methods of Bacterial Extraction on Placebo Soap Washed Axillae

Panelist	Log CFU/cm² MS minus TSG	Panelist	Log CFU/cm² MS minus TSG
1	0.52	11	0.08
2	−1.38	12	−0.45
3	0.86	13	−0.68
4	−1.60	14	−0.71
5	−0.18	15	1.37
6	0.04	16	0.31
7	0.12	17	0.21
8	0.91	18	−0.81
9	0.59	19	−0.68
10	−0.53	20	3.34
		Average	0.07
		Std. Dev.	1.08
		P	<0.8

Experimental results indicated that if very gentle mechanical scrubbing is performed nearly equivalent numbers of bacteria are extracted with the two methods.

A number of experiments were conducted which involved repetitive extraction of the same axillary skin site. As illustrated in Table III, regardless of the number of extractions, the MS method removed more total bacteria than did the TSG. In addition, the first extraction with the MS method tended to remove a greater percentage of the total *in situ* axillary bacteria than did the TSG. While the TSG tended

Table III
Quantitation of Aerobic Axillary Bacterial Populations as a Function of the Number of Extractions

Extraction #	log CFU/cm² Panelist # 1	2	3	4	Ave. log CFU/cm²	CFU/cm²	% of Total Bacteria Per Extraction
Mechanical Scrub[a]							
1	6.66	7.01	6.36	5.87	6.48	3.02×10^6	79.68
2	5.98	6.44	5.85	4.52	5.70	5.01×10^5	13.22
3	5.77	5.89	5.50	3.86	5.26	1.82×10^5	4.80
4	5.40	5.78	5.00	3.64	4.96	9.12×10^4	2.41
Total						3.79×10^6	
Thran Spray Gun							
1	6.86	6.42	5.32	4.97	5.89	7.76×10^5	44.34
2	6.46	6.18	5.27	4.42	5.72	5.25×10^5	30.00
3	6.30	5.86	4.93	4.48	5.39	2.45×10^5	14.00
4	6.05	5.90	4.96	4.31	5.31	2.04×10^5	11.66
Total						1.75×10^6	

[a]Each extraction consisted of a single 60 second scrub with 3 ml. of buffer.

to extract fewer bacteria, this does not suggest the procedure is any less sensitive or statistically less reliable in detecting reductions in a given microbial population. As a test of reproducibility and sensitivity, both test methods were used to evaluate the germicidal efficacy of a stick antiperspirant versus a placebo stick (no antimicrobial agent). Immediately following a placebo soap wash, a placebo stick or an active containing stick antiperspirant was applied to the axillae of seven subjects. Using both bacterial extraction methods, adjacent locations in each axilla were extracted 24 hours after treatment with the antiperspirant sticks. As illustrated in Table IV, a significant reduction in bacteria was observed for axillae treated with the active containing antiperspirant 24 hours following product application. Both methods detected log reductions in CFU's. The TSG demonstrated a 1.82 log decrease in axillary bacteria or 98.49% reduction in the geometric mean bacteria per cm², compared to a 1.02 log reduction for the MS or 90.45% reduction in geometric mean bacteria per cm². Despite the fact that the MS harvested three times the number of bacteria per cm², both methods demonstrated log reductions in axillary bacteria as well as similar percent errors and statistical probabilities. These results suggest that either method represents a satisfactory technique for measuring *in situ* reductions in axillary microorganisms.

In a final series of experiments the TSG was utilized to evaluate the effect of a germicide containing soap bar on the levels of *in situ* axillary microflora. Following a one week washout period, 20 subjects participated in four days of supervised washes with placebo soap and an antimicrobial soap containing 3,4,4′-Trichlorocarbanilide.

Table IV
Reduction of Aerobic Axillary Bacterial Populations by a Solid Antiperspirant as Measured by Mechanical Scrub and Thran Spray Gun Methods of Bacterial Extraction

	Mechanical Scrub log CFU/cm²			Thran Spray Gun log CFU/cm²		
Panelist	Placebo Treated Axilla[a]	Antiperspirant Treated Axilla	Difference	Placebo Treated Axilla[a]	Antiperspirant Treated Axilla	Difference
1	5.73	4.96	0.77	5.39	3.92	1.47
2	5.27	4.18	1.09	5.23	3.44	1.79
3	6.71	5.88	0.83	6.29	4.85	1.44
4	6.59	4.16	2.43	6.21	3.20	3.01
5	6.17	5.47	0.70	5.73	3.38	2.35
6	6.06	5.37	0.69	4.73	3.23	1.50
7	6.24	5.60	0.64	5.85	4.68	1.17
			1.02			1.82
Average			0.64			0.64
Std. Dev.			<0.005			<0.0005
P			90.45			98.49
% Reduction						

[a]Comparison of MS to TSG total bacteria extracted on placebo treated axillae yields $\bar{X} = 0.48$, Std. Dev. = 0.40, $P < 0.01$.

Table V
Reduction of Aerobic Axillary Bacterial Populations by a Deodorant Soap as Measured by the Thran Spray Gun Method of Bacterial Extraction

Panelist	Placebo Soap Treated Axilla log CFU/cm²	Deodorant Soap Treated Axilla log CFU/cm²	Difference
1	4.50	4.53	−0.03
2	7.01	5.96	1.05
3	5.14	4.35	0.79
4	5.71	6.39	−0.68
5	5.66	5.85	−0.19
6	5.76	4.72	1.04
7	5.29	5.42	−0.13
8	4.33	5.71	−1.38
9	5.51	4.63	0.88
10	5.83	5.24	0.59
11	3.83	3.26	0.57
12	5.37	4.91	0.46
13	4.85	3.79	1.06
14	5.80	6.05	−0.25
15	5.14	4.82	0.32
16	5.97	5.29	0.68
17	4.96	4.64	0.32
18	6.23	4.85	1.38
19	4.88	4.88	0.00
20	3.12	2.55	0.57
			0.35
Average			0.67
Std. Dev.			<0.025
P			55.36
% Reduction			

Twenty-four hours following the last supervised wash, placebo and antimicrobial soap washed axillae were extracted with the TSG and total aerobic bacteria counted. Experimental results revealed a significant reduction in recoverable aerobic axillary microbial population by the antimicrobial soap when compared to the placebo soap (Table V). The antimicrobial soap was shown to reduce bacterial populations an average 0.35 log units ($p < 0.03$), representing a 55% overall reduction in recoverable aerobic axillary bacteria.

In conclusion, these results demonstrate that the Thran spray gun can be employed to quantitate the *in vivo* antimicrobial efficacy of antiperspirant and bar soap products. This technique compares favorably with the mechanical scrub method in terms of both reproducibility and in its ability to detect changes in the population levels of cutaneous microorganisms. The mechanical scrub method, however, extracts larger numbers of bacteria per cm^2 in a smaller sampling volume and is recommended for studies requiring concentrated bacterial samples such as those involving quantifying pathogens or other low level ($<1 \times 10^3$ CFU/cm^2) bacterial populations. The Thran spray gun offers several advantages over the mechanical scrub including ease of sampling, increased sampling volume with recoveries of 98%, and the reproducibility of sampling pressure which allows collection of several samples on the same site without inducing localized erythema. This method should have broad application in assessing the antimicrobial efficacy of topically applied products.

ACKNOWLEDGEMENT

The authors thank Dr. J. E. Heinze, Dr. F. Yackovich, and Ms. M. B. Finkey for their assistance during the course of the present investigation.

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RB39

Van der Wyk, R.W., 1974

KILLING EFFICIENCY OF BETADINE PREPARATIONS

in vitro and in vivo laboratory study of the microbicidal activity of these povidone-iodine formulations

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AEROSPACE MEDICAL ASSOCIATION
MAY 6-9, 1974
WASHINGTON, D.C.

SUMMARY & CONCLUSIONS

- BETADINE SOLUTION IS A POTENT MICROBICIDAL AGENT, ACTIVE IN USE DILUTIONS AGAINST COMMONLY-ENCOUNTERED MICROORGANISMS.

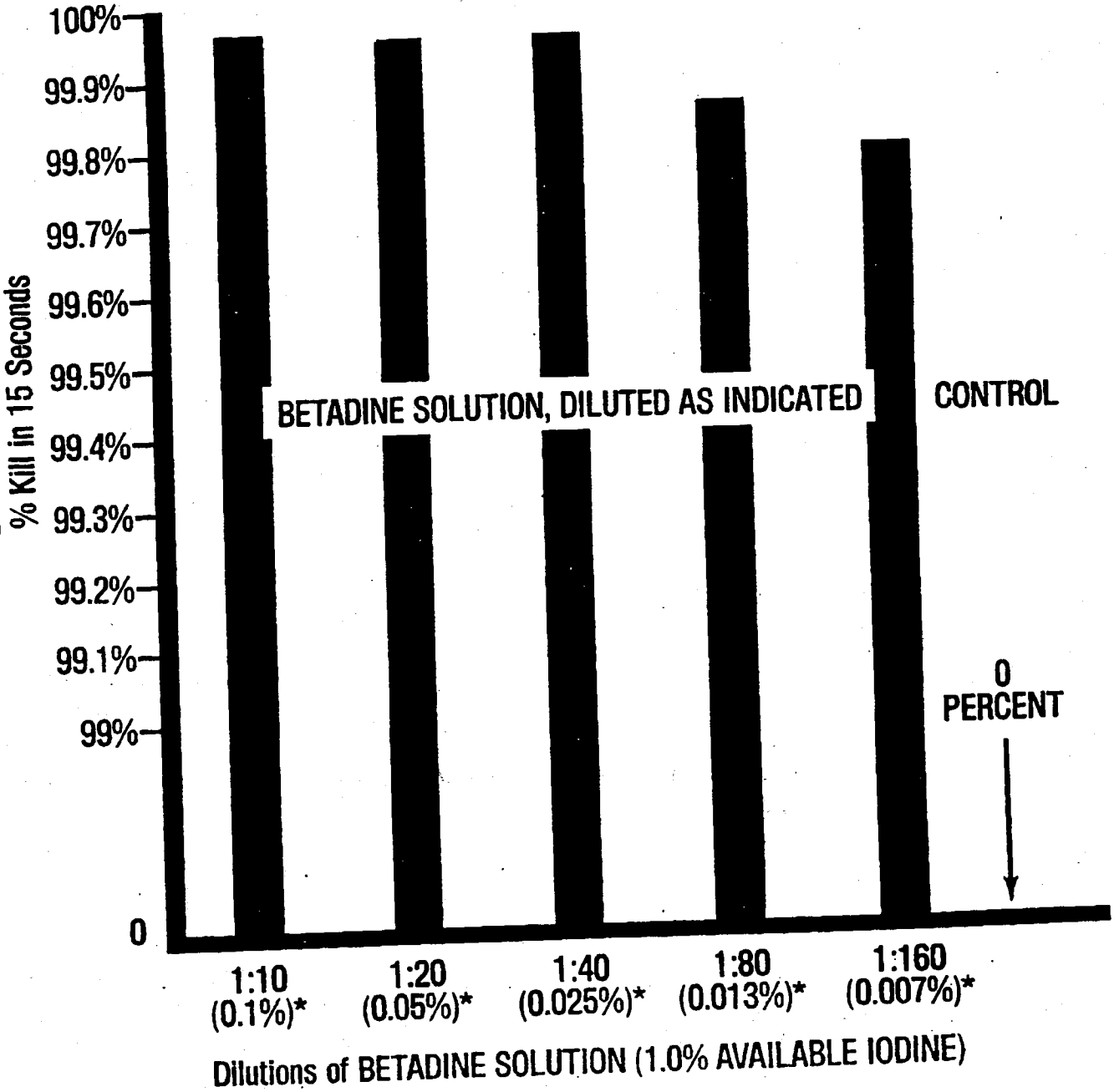
- BETADINE SOLUTION WAS LETHAL, IN VITRO, TO STRAINS OF COMMONLY-ENCOUNTERED ORGANISMS RESISTANT TO MOST CHEMOTHERAPEUTIC AGENTS TESTED.

- BETADINE SOLUTION, IN DILUTIONS UP TO 1:10,000 AVAILABLE IODINE, KILLED STAPH. AUREUS (FDA 209) WITHIN 15 SECONDS.

- BETADINE SOLUTION,CONTAINING 1% AVAILABLE IODINE, REDUCED BACTERIA 93.4% 15 SECONDS AFTER SWABBING OF GUMS.

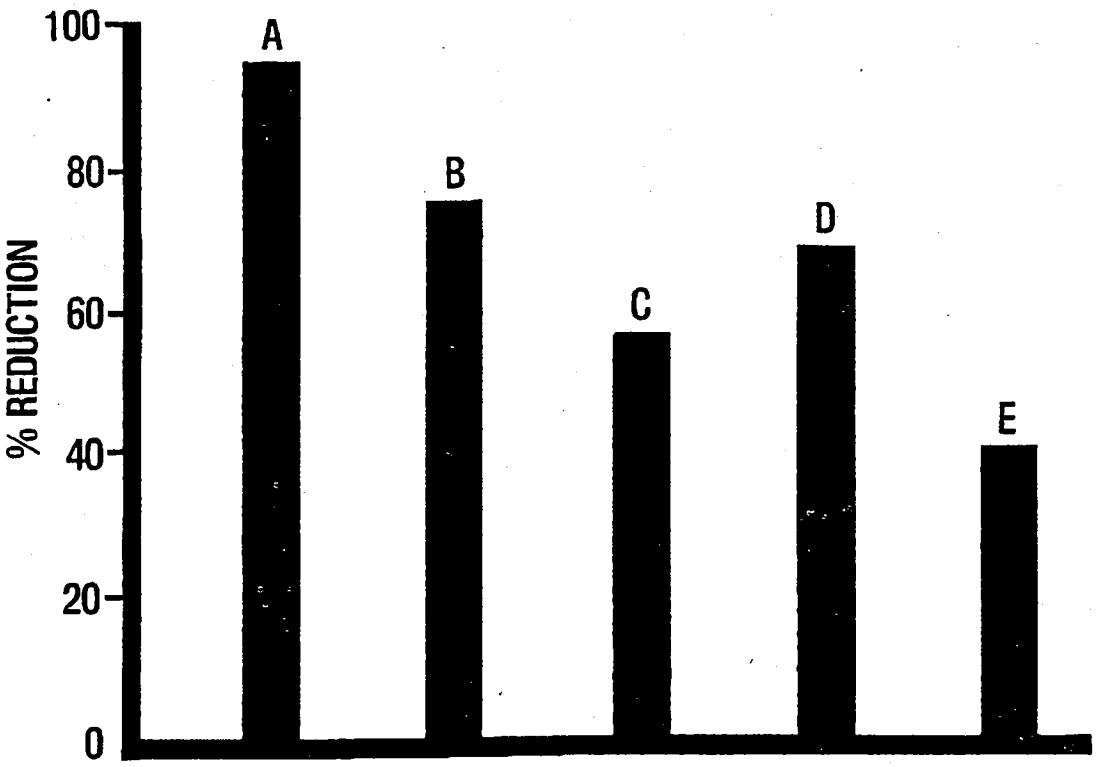
- BETADINE MOUTHWASH/GARGLE WHEN USED AS A GARGLE AND MOUTHWASH (0.5% AVAILABLE IODINE), REDUCED BACTERIA ON GINGIVAL TISSUE:
  - 71.6% 1/2 HOUR AFTER GARGLE
  - 38.8% 1-1/2 HOURS AFTER GARGLE

% KILL, IN VITRO, OF VARIOUS DILUTIONS OF BETADINE SOLUTION AGAINST STAPH. AUREUS IN 15 SECONDS



*Available iodine

IN VIVO STUDIES OF BACTERIAL REDUCTION ON GUMS AND IN ORAL CAVITY



A. BETADINE SOLUTION (1.0% AVAILABLE IODINE): GUMS SWABBED AND BACTERIAL COUNTS MADE 15 SECONDS LATER.

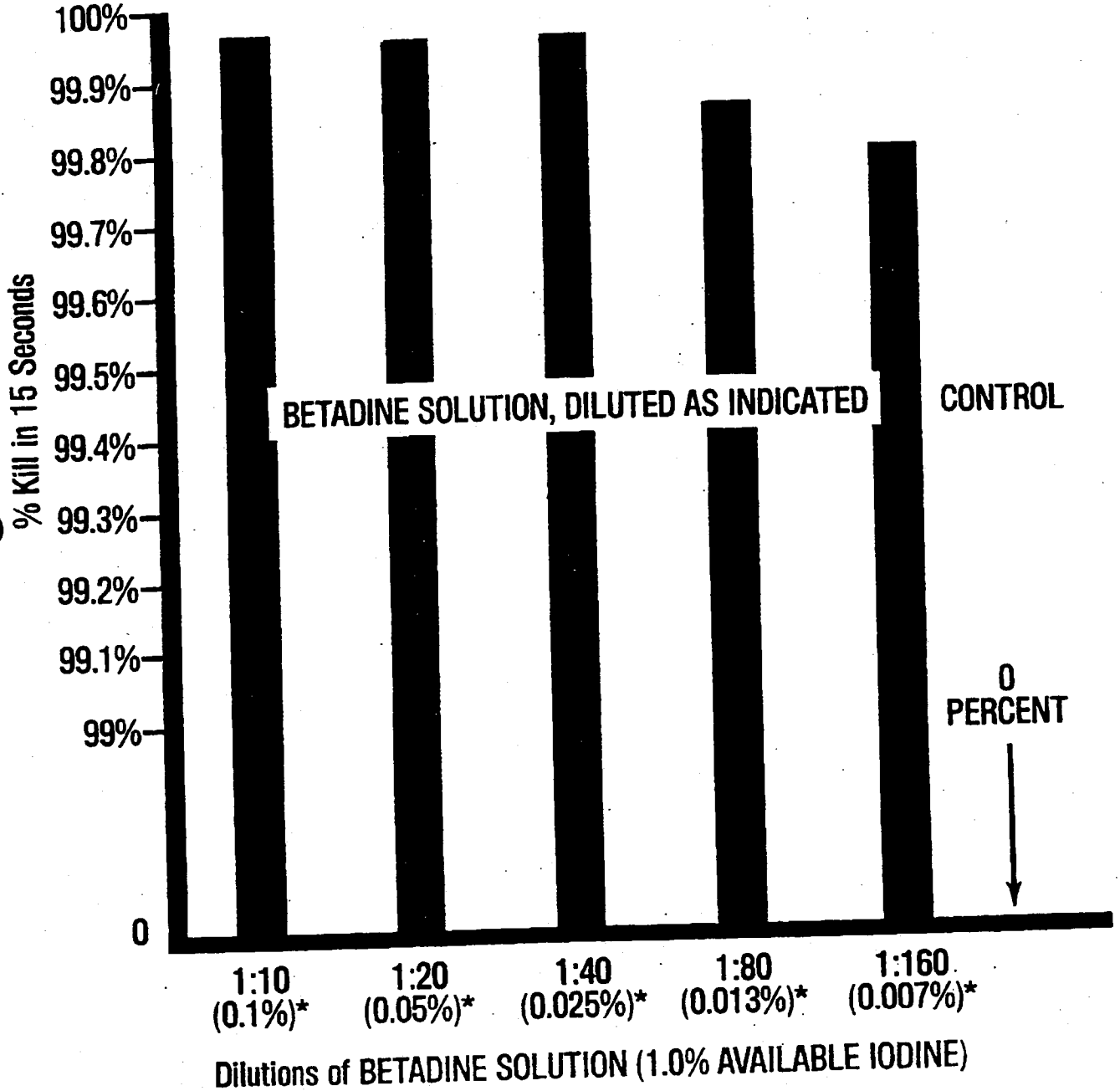
B. BETADINE SOLUTION (DILUTED TO PROVIDE 0.1% AVAILABLE IODINE): ORAL CAVITY RINSED FOR 15 SECONDS, WITH BACTERIAL COUNT 30 MINUTES LATER.

C. BETADINE SOLUTION (DILUTED TO PROVIDE 0.1% AVAILABLE IODINE): ORAL CAVITY RINSED FOR 15 SECONDS, WITH BACTERIAL COUNT 90 MINUTES LATER.

D. BETADINE MOUTHWASH/GARGLE (0.05% AVAILABLE IODINE): ORAL CAVITY RINSED FOR 15 SECONDS, WITH BACTERIAL COUNT 30 MINUTES LATER.

E. BETADINE MOUTHWASH/GARGLE (0.05% AVAILABLE IODINE): ORAL CAVITY RINSED FOR 15 SECONDS, WITH BACTERIAL COUNT 90 MINUTES LATER.

% KILL, IN VITRO, OF VARIOUS DILUTIONS OF BETADINE SOLUTION AGAINST STAPH. AUREUS IN 15 SECONDS



*Available iodine

COMPARATIVE ANTIBACTERIAL ACTIVITY IN VITRO, OF VARIOUS CHEMOTHERAPEUTIC AGENTS AND BETADINE ANTISEPTIC PRODUCTS

Agent	Pseudomonas aeruginosa*	Aerobacter aerogenes*	Escherichia coli*
Penicillin	R	R	R
Oleandomycin	R	R	R
Methicillin	R	R	R
Erythromycin	R	R	R
Tetracycline	R	R	R
Oxytetracycline	R	R	R
Lincomycin	R	R	R
Neomycin	S	S	S
Vancomycin	R	R	R
Oxacillin	R	R	R
Novobiocin	R	R	R
Kanamycin	R	R	R
Sulfisoxazole	R	R	S
Nafcillin	R	R	S
Methenamine mandelate	S	S	R
Cephalothin	S	S	R
Ampicillin	R	R	S
Chloramphenicol	R	R	R
Demethyl chlortetracycline	R	R	R
Polymyxin	S	S	S
Colistin	S	S	S
Nitro furantoin	R	R	S
Povidone-Iodine Surgical Scrub[a]	Killed in 30 seconds	Killed in 30 seconds	Killed in 3 minutes
Povidone-Iodine Solution[b]	Killed in 30 seconds	Killed in 15 seconds	Killed in 5 minutes

*Strains isolated form hospitalized patients
[a])contains 0.75% available iodine and detergents
[b])contains 1% available iodine

R = Resitant
S = Sensitive

IN VITRO COMPARISON STUDY OF THE KILLING TIMES OF BETADINE ANTISEPTIC PRODUCTS* AGAINST VARIOUS ORGANISMS

BETADINE ANTISEPTIC PRODUCTS	Available iodine	MICRO-ORGANISMS: Staphylococcus aureus	Corynebact. diphtheriae	Salmonella typhosa	Clostridium perfringens	Candida albicans	Saccharomyces carlsbergensis
SURGICAL SCRUB	0.75%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.
SHAMPOO	0.75%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	30 SEC.	15 SEC.
SOLUTION	1.0%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.
DOUCHE	1.0%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.
MOUTHWASH/ GARGLE	0.05%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.	1 MIN.
AEROSOL SPRAY	0.5%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.	15 SEC.
OINTMENT	1.0%	15 SEC.	15 SEC.	15 SEC.	15 SEC.	1 MIN.	15 SEC.
VAGINAL GEL	1.0%	2 MIN.	15 SEC.	15 SEC.	5 MIN.	2 MIN.	15 SEC.

*THE PURDUE FREDERICK COMPANY, NORWALK, CONNECTICUT

BETADINE PREPARATIONS

COMPLEXES CONTAINING POLYVINYLPYRROLIDONE (POVIDONE) AND IODINE

- POLYVINYLPYRROLIDONE REPORTED TO POSSESS DETOXIFYING ACTION AND PROLONG ACTIVITY OF MANY DRUGS
- IODINE AMONG OLDEST AND MOST EFFECTIVE ANTISEPTICS

CHARACTERISTICS OF BETADINE PREPARATIONS

- HAS THE SAME BROAD SPECTRUM OF ANTIMICROBIAL ACTIVITY AS ELEMENTAL IODINE
- PROVIDES RAPID KILL AGAINST BACTERIA, VIRUSES, FUNGI, PROTOZOA, YEASTS
- FURNISHES PROLONGED GERMICIDAL ACTIVITY

ADVANTAGES

- BETADINE SOLUTION/AEROSOL SPRAY PRODUCE NO APPRECIABLE PAIN EVEN ON DENUDED SURFACES
- BETADINE ANTISEPTIC PRODUCTS:
  - CAUSE VIRTUALLY NO SKIN IRRITATION
  - CAN BE EASILY WASHED OFF SKIN AND NATURAL FABRICS
  - RESULT IN MARKEDLY REDUCED VAPOR PRESSURE
- SKIN TREATED WITH BETADINE SOLUTION/AEROSOL SPRAY/OINTMENT MAY BE COVERED WITH GAUZE OR BANDAGE

Reference 1

D 1279

Zbl. Bakt. Hyg., I. Abt. Orig. A 226, 376–389 (1974)

Biological Research Laboratories of the Pharmaceutical Division of CIBA-GEIGY Limited, Basle

Antimicrobial Spectrum of Triclosan, a Broad-Spectrum Antimicrobial Agent for Topical Application

Antibakterielles Wirkungsspektrum von Triclosan, einem Breitband-antimicrobicum zur lokalen Anwendung

W. A. VISCHER and J. REGÖS

With 3 Figures · Received September 8, 1973

Summary

The minimum inhibitory concentrations (MIC) of triclosan (trichlorohydroxydiphenylether) for a wide range of bacteria and fungi are compared with those of hexachlorophene and, in many instances, neomycin. Triclosan has a very much more potent effect than the other two substances against Staphylococcus aureus and against gram-negative microorganisms, especially Enterobacteriaceae, but is less active against Serratia and Pseudomonas.

The clinical isolates show the same degree of sensitivity as the laboratory strains.

In view of its broad spectrum of activity and its potency of effect, triclosan could prove suitable for the treatment of skin infections caused by Staphylococci, Enterobacteriaceae, Candida or dermatophytes.

Zusammenfassung

An einem breiten Spektrum von Bakterien und Pilzen werden die minimalen Hemmkonzentrationen von Triclosan (Trichlor-hydroxydiphenylaether) mit denjenigen von Hexachlorophen und z.T. mit Neomycin verglichen. Gegenüber Staphylococcus aureus und ganz besonders bei Enterobacteriaceen ist Triclosan dem Hexachlorophen stark überlegen. Bei gram-positiven Keimen, mit Ausnahme von Staphylococcus aureus, ist Hexachlorophen aktiver als Triclosan. Nur in sehr hohen Konzentrationen hemmt Triclosan Alcaligenes, Serratia und Pseudomonas.

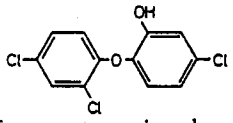
Aus klinischem Material frisch isolierte Stämme waren gegen Triclosan gleich empfindlich wie Laborstämme.

Triclosan könnte sich deshalb dank seinem breiten Spektrum und seiner intensiven Wirkung besser als Hexachlorophen zur Bekämpfung von Hautinfektionen eignen, die durch Staphylococcen, Enterobacteriaceen, Candida oder Dermatophyten hervorgerufen werden.

Synthetic substances belonging to a variety of chemical classes, e.g. sulphonamides, nitrofuranes, oxyquinolines and diphenols, to mention only the most important, are employed as remedial or prophylactic agents against bacterial infections of the skin. Owing to the selection of resistant micro-organisms, however, or to certain deficiencies in the spectrum of activity, tolerability, pharmaceutical characteristics or physical properties of these substances, the need has arisen to find new and better substitutes.

The present paper is concerned with a compound synthesized in the research laboratories of CIBA-GEIGY that has already proved useful in cosmetics (SAVAGE, 1) and hygiene (JUNGERMANN and TABER, 2).

Chemically, this compound, known as triclosan, is 5-chloro-2- (2, 4-dichlorophenoxy) phenol. It is a finely crystalline, colourless and odourless substance with the following structure:



Triclosan dissolves readily in most organic solvents and is soluble to the extent of 2.35% in an excess of 0.1 n sodium hydroxide; in water, however, it is virtually insoluble. Detailed information on the solubility and other physicochemical characteristics of the substance has been published by ZINKERNAGEL and KOENIG (3).

In the present communication, the antimicrobial spectrum of triclosan is compared with two other topically applied antimicrobial agents, namely hexachlorophene and neomycin.

Material and Methods

Micro-organisms. The micro-organisms tested were obtained from internationally known collections, or from various clinics and institutes. Some strains were isolated in our own laboratories.

Substances. Hexachlorophene, obtained from Givaudan S.A., Geneva, and neomycin in the form of neomycinum sulfuricum Ph.H. V supplied by Siegfried A.G., Zofingen, Switzerland, were used as reference compounds.

Nutrient media. The minimum inhibitory concentrations were determined in nutrient media generally containing between 1.5% and 2% agar, details of which are listed below. The particular medium used is always indicated together with the results (cf. Table 1).

Abbreviation	Medium	Composition or Supplier	
NA	Nutrient agar	Bacto peptone (Difco No. B 118)	10 g
		Na Cl	3 g
		$Na_2HPO_4 \cdot 12\ H_2O$	2 g
		Liebig beef extract	10 g
		Bacto agar	20 g
		water, sufficient to produce	1000 ml
BA	Blood agar	NA + 10% (v/v) preserved human blood	
MACA	Micro assay cul. agar	Difco No. B 319	
BHI-A	Brain-heart inf. agar	Difco No. B 37	
EA	Eugon agar	BBL 01-265	

Br. A. A.	Brucella agar "Albimi"	Albimi Laboratories Inc., N. Y.
YA	Youmans' agar	l-asparagine 5 g KH_2PO_4 5 g K_2SO_4 0.5 g water 1000 ml — basal medium Basal medium 270 ml — sterilize and add 30 ml Noble agar 4.5 g — bovine serum
PPLO-A	PPLO agar	BHI-A + 15% calf serum
SMA	Sabouraud maltose agar	Difco No. B 110
ACM	AC medium	Difco No. B 316 + 5% bovine serum

Determination of minimum inhibitory concentration (M.I.C.) by the agar-dilution method

Triclosan and hexachlorophene were dissolved in ethyl alcohol and further diluted with the same solvent. One part of the antimicrobial solution was mixed with 99 parts of molten agar to give the desired final concentrations, and the medium was then poured into Petri dishes or test tubes. Neomycin was dissolved in water. Once solidified, the media were inoculated with one drop of bacterial suspension and then incubated at the optimal temperatures for the micro-organisms concerned (as a rule, 25 °C for fungi and 37 °C for other organisms). The growth of the cultures was estimated when the controls displayed satisfactory growth (usually after 2 days for bacteria, 2 weeks for fungi, 3 weeks for tubercle bacilli, and 1 day for trichomonads).

Inoculum. Bacteria from freshly grown agar cultures were suspended in physiological saline at a density corresponding to the turbidity of the McFarland No. 1 barium sulphate standard (approx. 1–4 × 10^8 organisms per ml). In the case of fungi, 5 ml of physiological saline were added to 3–6-week-old slant agar cultures, and spores and mycelia suspended with the aid of a loop. To study the activity against trichomonads 0.2 ml of a 24-hour culture in AC-medium was inoculated into 10 ml of semisolid medium containing the test substances. The trichomonads thus developed under partially anaerobic conditions.

The lowest concentrations of the test substances at which the growth of the cultures was completely suppressed or was negligible in comparison with the controls were taken to the minimum inhibitory concentrations.

Results

The minimum inhibitory concentrations of triclosan, hexachlorophene and, with some exceptions, neomycin are shown in Table 1. In comparing these figures, it should be noted that the microorganisms were not invariably grown in the relatively simple nutrient agar medium; more complex media, including some containing blood or serum, had to be used for certain strains.

Among the Micrococcaceae, it is particularly noticeable that the potentially pathogenic strains, e.g. Staphylococcus aureus, were considerably more sensitive than the saprophytes. No such difference was evident with hexachlorophene, which was usually less active.

Lactobacillaceae, Corynebacteriaceae, Bacillaceae and Clostridia were roughly 10 times more susceptible to hexachlorophene than to triclosan.

The two Alcaligenes strains tested proved only slightly sensitive to hexachlorophene and even less so to triclosan. Both Achromobacters, on the other hand, were relatively sensitive to triclosan.

Table 1. Antimicrobial spectrum of triclosan, hexachlorophene and neomycin

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Staph. aureus	99	NCTC 7447	NA	0.01	1	1
Staph aureus	121	NCTC 4163	NA	0.01	1	1
Staph. aureus	122	NCTC 6571	NA	0.03	1	3
Staph. aureus	123	NCTC 6966	NA	0.1	0.3	3
Staph. aureus	322	ATCC 13709	NA	0.01	0.3	0.3
Staph. aureus	438	ATCC 6538	NA	0.01	0.1	1
Staph. albus	120	NCTC 7292	NA	0.1	0.3	0.1
Staph. albus	742	C.-G.	NA	0.03	0.1	0.3
Staph. lactis	461	NCTC 8340	NA	3	0.2	1
Micrococcus lysodeikticus	440	NCTC 2665	BHI-A	3	0.1	3
Sarcina lutea	119	NCTC 196	BHI-A	3	0.1	3
Streptococcus haemolyt. A	109	A	BHI-A	1	0.3	33
Streptococcus haemolyt. A	113	A	BHI-A	3	0.3	10
Streptococcus faecalis	114	NCTC 8619	BHI-A	10	0.3	100
Streptococcus faecalis	1071	ATCC 10541	BHI-A	3	0.3	33
Streptococcus agalactiae	486	NCTC 8181	BHI-A	3	0.3	10
Diplococcus pneumoniae	118	NCTC 7465	BHI-A	3	0.3	33
Lactobacillus sp.	129	A	MACA	33	3	10
Lactobacillus casei	435	ATCC 7469	MACA	33	3	10
Lactobacillus arabinosus	485	CITM 706	MACA	33	3	3
Lactobacillus arabinosus	611	ATCC 8014	MACA	33	3	10
Lactobacillus fermenti	501	CITM 707	MACA	33	3	-
Lactobacillus leichmannii	623	ATCC 7830	MACA	33	3	33
Corynebact. kutscheri	547	A	BHI-A	3	0.03	3
Corynebact. kutscheri	547	A	BA	100	-	-
Corynebact. kutscheri	248	C.-G.	BHI-A	3	0.1	0.1

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Corynebact. diphtheriae	57	NCTC 3984	BHI-A	3	0.03	3
Corynebact. diphtheriae	666	A	BHI-A	3	0.1	0.3
Corynebact. diphtheriae	667	A	BHI-A	3	0.03	0.3
Listeria monocytogenes	60	NCTC 7973	EA	3	0.3	0.3
Erysipelothrix rhusiopath.	58	NCTC 8163	EA	3	0.3	-
Bacillus subtilis	65	NCTC 8236	NA	0.1	0.03	0.1
Bacillus megatherium	63	A	NA	3	1	0.3
Bacillus cereus	134	A.	NA	3	0.3	3
Bacillus cereus var. myc.	133	A	NA	3	0.3	3
Bacillus macerans	238	A	NA	3	0.3	3
Bacillus circulans	239	A	NA	1	0.03	1
Clostridium botulinum	66	NCTC 3805	EA	3	1	> 100
Clostridium histolyticum	69	NCTC 503	EA	3	0.3	> 100
Clostridium welchii	72	NCTC 8359	EA	10	1	> 100
Clostridium welchii	73	NCTC 3110	EA	10	1	> 100
Clostridium sporogenes	195	A	EA	10	1	> 100
Clostridium butyricum	67	NCTC 7423	EA	10	1	-
Clostridium tetani	71	NCTC 9571	EA	3	1	-
Alcaligenes faecalis	305	A	NA	> 100	33	> 100
Alcaligenes sp.	44	A	NA	100	33	> 100
Achromobacter sp.	304	A	NA	0.3	100	10
Achromobacter sp.	476	A	NA	3	10	33
Escherichia coli	2	NCTC 86	NA	0.1	100	10
Escherichia coli	3	A	NA	0.3	33	10
Escherichia coli	5	NCTC 8196	NA	0.03	100	10

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Escherichia coli	6	ATCC 9663	NA	0.3	100	10
Escherichia coli	7	ATCC 9661	NA	0.3	33	10
Escherichia coli	15	NCTC 9001	NA	0.1	100	3
Escherichia coli	259	A	NA	0.3	33	3
Escherichia freundii	766	A	NA	> 100	100	3
Escherichia freundii	767	A	NA	3	100	10
Cloaca cloacae	329	NCTC 8155	NA	0.3	33	10
Cloaca sp.	429	C.-G.	NA	0.3	100	10
Aerobacter aerogenes	242	CITM 413	NA	1	100	10
Aerobacter aerogenes	298	CITM 812	NA	1-3	100	10
Klebsiella edwardsii	22	NCTC 7242	NA	0.3	100	10
Klebsiella aerogenes	23	NCTC 8172	NA	0.3	100	10
Klebsiella sp.	243	C.-G.	NA	1	100	33
Serratia marcescens	343	A	NA	> 100	100	10
Serratia marcescens	344	A	NA	> 100	100	33
Serratia marcescens	345	A	NA	> 100	10	10
Proteus vulgaris	47	NCTC 8313	NA	0.1	10	10
Proteus vulgaris	48	NCTC 4636	NA	0.3	10	100
Proteus vulgaris (Neotype)	872	NCTC 4175	NA	0.01	10	1
Proteus mirabilis	316	A	NA	0.1	33	33
Proteus mirabilis	564	A	NA	0.3	33	100
Proteus mirabilis	241	NCTC 8309	NA	0.3	33	10
Proteus rettgeri	856	A	NA	33	10	33
Proteus rettgeri	969	C.-G.	NA	> 1000	10	33
Proteus morganii	855	A	NA	10	10	10

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Salmonella enteritidis	77	C.-G.	NA	0.1	100	10
Salmonella enteritidis	542	A	NA	0.1	100	10
Salmonella enteritidis	659	A	NA	0.1	100	10
Salmonella enteritidis	660	A	NA	0.3	100	10
Salmonella typhimurium	31	NCTC 5710	NA	0.3	100	10
Salmonella typhimurium	412	C.-G.	NA	0.3	100	10
Salmonella typhimurium	661	A	NA	0.1	100	10
Salmonella typhimurium	662	A	NA	0.3	100	10
Salmonella typhi	27	NCTC 8384	NA	0.3	100	10
Salmonella typhi	30	NCTC 786	NA	0.3	33	1
Salmonella typhi	570	A	NA	0.1	100	10
Salmonella typhi	573	A	NA	0.1	100	10
Salmonella typhi	575	A	NA	0.3	33	10
Salmonella paratyphi A	28	NCTC 5322	NA	0.3	33	10
Salmonella paratyphi B	29	NCTC 3176	NA	0.3	100	33
Salmonella paratyphi B	580	NCTC 5704	NA	0.1	100	10
Salmonella paratyphi B	541	A	NA	0.1	100	10
Shigella flexneri	33	NCTC 8192	NA	0.3	100	10
Shigella flexneri	34	NCTC 8204	NA	0.1	100	10
Shigella flexneri	35	NCTC 8205	NA	0.1	100	33
Shigella sonnei	36	NCTC 7240	NA	0.1	100	33
Shigella dysenteriae	32	NCTC 2249	NA	0.1	100	10
Pseudomonas aeruginosa	25	NCTC 1999	NA	> 100	33	100
Pseudomonas aeruginosa	306	A	NA	> 1000	33	33
Pseudomonas aeruginosa	388	CITM 419	NA	> 1000	33	-
Pseudomonas aeruginosa	389	CITM 427	NA	> 1000	-	-

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Pseudomonas aeruginosa	799	ATCC 12055	NA	> 1000	100	100
Pseudomonas aeruginosa	838	NCTC 8060	NA	> 1000	33	10
Pseudomonas fluorescens	26	NCTC 4755	NA	> 100	33	1
Aeromonas sp.	375	A	NA	10	33	10
Aeromonas sp.	376	A	NA	10	33	10
Loefflerella mallei	954	NCTC 10230	NA	0.3	100	33
Loefflerella pseudomallei	949	NCIB 9674	NA	1	10	100
Moraxella duplex	552	A	NA	0.01	0.1	3
Moraxella duplex	554	A	NA	0.01	0.1	3
Moraxella glucidolytica	553	A	NA	0.3	10	10
Moraxella lwoffi	562	A	NA	0.1	10	-
Pasteurella septica	46	NCTC 948	NA	0.1	1	3
Pasteurella pseudotuberc.	392	C.-G.	NA	10	10	-
Haemophilus influenzae	921	A	BA	33	-	-
Brucella intermedia	40	A	Br. A. A.	0.1	0.3	-
Brucella abortus	37	NCTC 8226	Br. A. A.	0.1	0.3	-
Brucella melitensis	41	A	Br. A. A.	0.1	1	-
Brucella melitensis	41	A	BA	1	100	-
Brucella suis	43	A	Br. A. A.	0.03	0.1	-
Neisseria catarrhalis	117	NCTC 3622	BA	33	10	-
Vibrio cholerae	877	A	NA	10	10	33
Vibrio cholerae	879	A	NA	10	10	33
Vibrio eltor	874	A	NA	10	10	100
Vibrio eltor	62	NCTC 8457	NA	10	10	-

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Nocardia asteroides	229	NCTC 6761	BHI-A	3	1	33
Nocardia asteroides	557	A	BHI-A	1	1	100
Nocardia mexicana	658	A	BHI-A	3	1	> 100
Nocardia brasiliensis	675	A	BHI-A	3	1	100
Nocardia brasiliensis	676	A	BHI-A	1	1	100
Actinomyces israelii	694	NCTC 8047	NA	1	< 0.1	10
Actinomyces israelii	604	A	NA	10	< 0.1	1
Actinomyces bovis	605	A	NA	1	< 0.1	10
Actinomyces naeslundii	603	A	NA	1	< 0.1	10
Dermatophilus congolensis	887	A	BHI-A	1	-	-
Actinobacillus israelii	602	A	NA	1	< 0.1	1
Streptomyces coelicolor	722	A	SMA	3	10	-
Streptomyces coelicolor	722	A	BHI-A	1	-	-
Streptomyces sp.	682	A	BHI-A	1	-	-
Micromonospora chalcea	677	A	BHI-A	0.1	-	-
Mycobacterium tubercul.	203	A	YA	100	-	33
Mycobacterium smegmatis	140	CITM 78	BHI-A	3	0.3	0.3
Mycobacterium smegmatis	141	CITM 170	BHI-A	1	0.3	0.3
Mycobacterium smegmatis	256	NCTC 8152	BHI-A	1	0.3	0.3
Mycobacterium smegmatis	366	A	BHI-A	3	0.3	0.3
Mycobacterium phlei	199	A	BHI-A	0.3	0.3	-
Mycobacterium phlei	361	A	BHI-A	0.3	3	-
Mycoplasma orale	800	A	PPLO-A.	1000	-	-
Mycoplasma hominis	802	A	PPLO-A.	1000	-	-
Mycoplasma pneumoniae	804	A	PPLO-A.	1000	-	-

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Aspergillus fumigatus	76	ATCC 9197	SMA	10	> 100	> 1000
Blastomyces dermatitidis	382	ATCC 10225	SMA	3	-	-
Candida albicans	75	A	NA	3	33	> 1000
Candida albicans	75	A	SMA	33	> 100	-
Candida brumptii	342	A	SMA	10	10	-
Candida guillermondi	334	A	SMA	33	> 100	-
Candida parakrusei	647	A	SMA	3	1	-
Candida parapsilosis	332	A	SMA	33	> 100	-
Candida pulcherrima	643	A	SMA	33	> 100	-
Candida stellatoidea	645	A	SMA	10	10	-
Candida tropicalis	644	A	SMA	10	> 100	> 1000
Candida utilis	482	A	SMA	33	33	-
Cryptococcus neoformans	646	A	SMA	10	10	-
Epidermophyton floccosum	327	ATCC 10227	SMA	1-10	1-10	> 100
Hansenula anomala	483	A	SMA	10	1	-
Keratinomyces ajelloi	466	A	SMA	10	33	> 1000
Microsporum canis	240	ATCC 10214	SMA	1-10	10	> 1000
Microsporum cookei	406	A	SMA	33	33	> 1000
Microsporum nanum	408	A	SMA	10	33	-
Microsporum gypseum	413	A	SMA	10	100	> 1000
Neurospora sitophila	86	A	SMA	0.1	-	-
Penicillium egyptiacum	431	A	SMA	33	33	-
Pichia farinosa	642	A	SMA	33	> 100	-
Piedraia hortai	464	A	SMA	10	100	-
Rhodotorula mucilaginosa	331	A	SMA	33	> 100	-
Schizosaccharomyces pombe	651	A	SMA	3	33	-
Sporotrichum schenckii	83	ATCC 10212	SMA	3-10	6.2	> 100

Microorganisms	Code-No.*	Source**	Medium	Triclosan	Hexachlorophene M.I.C. mcg./ml.	Neomycin
Trichophyton mentagrophytes	84	ATCC 9533	SMA	1	3	> 1000
Trichophyton rubrum	351	A	SMA	10	100	> 1000
Trichophyton tonsurans	396	A	SMA	10	100	> 1000
Trichophyton concentricum	395	A	SMA	10	10	-
Trichophyton quinckeanum	397	A	SMA	10	33	-
Trichophyton schoenleinii	398	A	SMA	3	10	> 1000
Trichophyton terrestre	410	A	SMA	10	33	-
Trichosporon cutaneum	333	A	SMA	10	-	-
Torulopsis dattila	336	A	SMA	10	> 100	> 1000
Torulopsis glabrata	588	A	SMA	-	> 100	> 1000
Torulopsis famata	338	A	SMA	10	> 100	-
Trichomonas foetus	837	A	ACM	100	-	> 100

* Number of internal strain collection.
** A = from outside institutes / C.-G. = Ciba-Geigy.

ATCC = Amer. Type Culture Collection
NCTC = National Collection of Type Cultures (London)
CITM = Centre Intern. de Distribution de Souches (Lausanne)
NCIB = National Collection of Industrial Bacteria (U.K.).

Triclosan was about 100 times as effective as hexachlorophene against the strains of Escherichia, Cloaca, Aerobacter, Klebsiella, Proteus vulgaris, Proteus mirabilis, Salmonella, Shigella, Loefflerella and Moraxella.

In cultures of Proteus morganii, Pasteurella, Brucella, Vibrio and Nocardia, no difference in activity between triclosan and hexachlorophene was demonstrable.

The tested strains of Serratia, Proteus rettgeri, Pseudomonas and Actinomyces, however, were inhibited to a greater degree by hexachlorophene.

Among the fungi, yeasts (Blastomyces, Candida, Torulopsis, etc.) and dermatophytes tended to be more sensitive to triclosan, whereas the others tested were affected to very much the same extent by both substances.

The sensitivity of strains isolated from clinical specimens is illustrated in Figs. 1–3. Care was taken to ensure that these strains were all derived from different patients. Altogether 28 strains of Staphylococcus aureus and 30 each of E. coli and Proteus sp. were isolated and tested.

As is evident from the figures, triclosan proved very much more active than hexachlorophene against these clinical strains also. It is notable that the degree of sensitivity to both substances displayed by these organisms was highly consistent.

All the strains of Staphylococcus aureus were inhibited by concentrations of 0.005–0.02 mcg/ml triclosan, as compared with 0.4–1.6 mcg/ml hexachlorophene. For E. coli, the minimum inhibitory concentrations of triclosan and hexachlorophene were 0.1–0.2 and 25–100 mcg/ml respectively. Proteus sp. was suppressed by triclosan in a concentration between 0.4 and 0.8 mcg/ml and by hexachlorophene in a concentration of 12 mcg/ml .The tested strains reacted less consistently to neomycin, a small percentage (less than 10%) proving resistant.

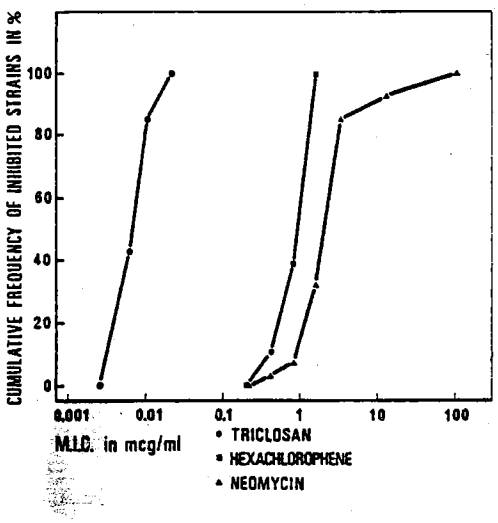


Fig. 1. Sensitivity of clinical isolates of Staph. aureus strains.

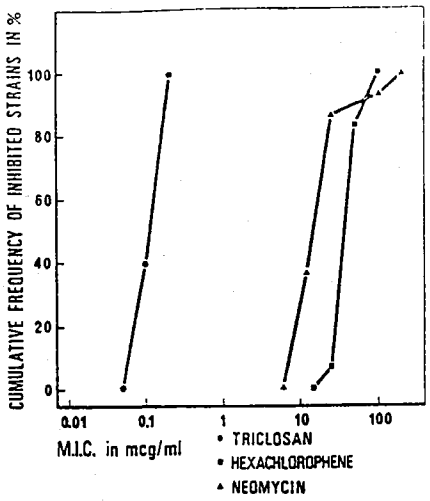


Fig. 2. Sensitivity of clinical isolates of E. coli strains.

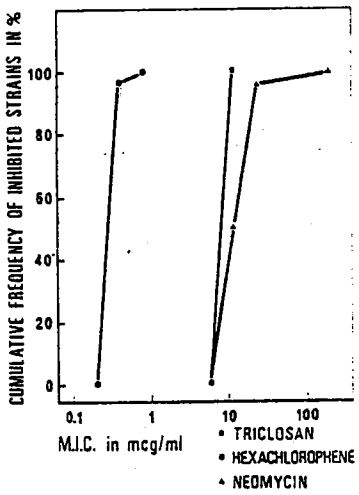


Fig. 3. Sensitivity of clinical isolates of Proteus sp. strains.

Discussion

The results of these tests show that triclosan is highly active in vitro against a wide range of microbial species. It displayed a very much (10–100 times) more potent inhibitory effect than hexachlorophene against the following micro-organisms: Staphylococcus aureus, Escherichia, Cloaca, Aerobacter, Klebsiella, Proteus mirabilis, Proteus vulgaris, Salmonella, Shigella, Loeffflerella and Moraxella. It

proved slightly less active, but still considerably more so than hexachlorophene, against yeasts and dermatophytes. Alcaligenes, Serratia and Pseudomonas showed little or no sensitivity to triclosan. Hexachlorophene was about ten times more active than triclosan against Streptococci, Lactobacilli, Corynebacteria, Listeria, Erysipelothrix, Bacilli, Clostridia, Alcaligenes, Serratia, Pseudomonas and Actinomyces.

This may mean that triclosan would be particularly effective against infections due to pathogenic organisms such as Staphylococci, Enterobacteriaceae, Candida and dermatophytes, whereas hexachlorophene would chiefly act against the other gram-positive species.

Neomycin was found to be 10–30 times less active than triclosan against Staphylococci and Enterobacteriaceae and had no effect at all on fungi.

Minimum inhibitory concentrations of triclosan for a limited range of organisms have already been reported by ZINKERNAGEL and KOENIG (3) and FURIA and SCHENKEL (4). The concentrations determined by these investigators for bacteria, in particular Staphylococcus aureus, are higher than those found in our experiments, whereas the active antifungal concentrations correspond fairly well. The discrepancies are presumably due to differences in the methods and the criteria adopted: it will be remembered that in our experiments, concentrations which inhibited the growth of the cultures drastically without completely arresting it were also taken as the minimum inhibitory concentrations. This concession was necessary because cultures grown from inocula of the order of 10^8 still could show a very slight growth, in the form of either individual colonies or a delicate film, at several consecutive concentrations. This may be attributed to the presence of isolated, less sensitive organisms or to a delay in the onset of the bacteriostatic effect.

In our tests, Neisseria catarrhalis cultured on blood agar was not inhibited by triclosan at concentrations of less than 33 mcg/ml, whereas the minimum inhibitory concentration found by DEVINE and HAGERMAN (5) for 39 clinical isolates of Neisseria meningitidis grown on Mueller-Hinton agar lay between 0.00195 and 0.031 mcg/ml. The results obtained with Corynebacteria also indicate that the addition of blood to the culture medium diminishes the action of triclosan in vitro. In determining minimum inhibitory concentrations, blood or serum should therefore, whenever possible, not be added to the medium: for fastidious organisms, brain-heart infusion agar or Mueller-Hinton agar may suitably be used.

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The evaluation of residual antimicrobial activity on hands and its clinical relevance

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Summary: A model for evaluation of the residual activity of agents used for hygienic hand disinfection, which relates closely to the 'in-use' setting, is described. We looked for residual activity following hand disinfection by a standard method with soap and water and four skin disinfectants in common use. Two test organisms known to have caused cross-infection and having clinically-significant antibiotic resistance were used: a strain of vancomycin-resistant *Enterococcus faecium* and one of gentamicin-multiply-resistant *Enterobacter cloacae*. Both organisms survived well on artificially inoculated hands. Residual activity on previously disinfected hands against both test organisms was most marked for alcoholic chlorhexidine ('Hibisol') which showed a 4 $\log_{10}$ reduction within 1 min of inoculation. Chlorhexidine digluconate skin cleanser ('Hibiscrub') and povidone–iodine surgical scrub ('Betadine') also had marked residual activity, but less than alcoholic chlorhexidine. 60% Isopropanol showed no residual activity and, except for marginal activity against *E. cloacae*, soap and water showed little residual activity. The residual activity of alcoholic chlorhexidine may be useful to control cross-infection with those multiply-resistant strains which survive well on fingers previously washed with soap and water.

Keywords: Hand disinfection; residual activity.

Introduction

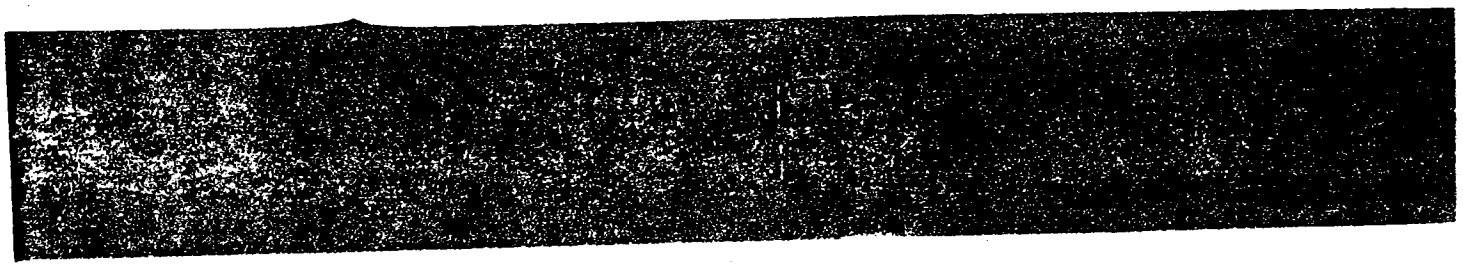
The requirements for hygienic hand disinfectants include a broad spectrum of activity, rapid action and user acceptability. The value of residual activity is less clear,[1–3] although widely accepted as being useful for agents used in the preoperative disinfection of surgeons' hands.

Although previous studies demonstrated the residual activity of chlorhexidine and povidone–iodine preparations, these have often employed National Collection of Typed Cultures (NCTC) strains which may not share the survival or antiseptic susceptibility characteristics of isolates known to cause serious cross-infection problems.[4–7]

We have already described a model to evaluate residual activity after hand disinfection.[8] We report here the results of a study, using two epidemic strains of particular importance, to evaluate residual activity after hand disinfection with soap and water and four commonly used skin disinfectants.

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Materials and methods

Test organisms

Two organisms that have caused hospital outbreaks of cross-infection and cross-colonization were used. The first, kindly provided by Dr A. H. C. Uttley of Dulwich Public Health Laboratory, London, was a strain of *Enterococcus faecium* with high level plasmid-mediated vancomycin resistance which infected 27 patients on a renal unit.[9] The second test organism, kindly provided by Dr A. J. Howard, was a gentamicin-resistant, multiply-resistant strain of *Enterobacter cloacae*, also bearing plasmid-mediated resistance, which infected or colonized 60 patients on a geriatric unit in Wales over a 7-week period.[10]

Volunteers

Three Caucasian female volunteers were used. None had evidence of skin disease, were receiving antibiotics, or had used hand disinfectants in the 48 h preceding each experiment.

Hygienic hand disinfectants tested

The aqueous agents tested were: non-medicated bar soap and water, 4% chlorhexidine digluconate skin cleanser ('Hibiscrub', ICI Pharmaceuticals plc), and 7·5% w/v povidone–iodine surgical scrub ('Betadine', Napp Laboratories Ltd). Volunteers washed their hands, using Ayliffe's standard method,[11] for 30 s with either bar soap or 5 ml of one of the disinfectants. They then rinsed their hands in running water for 15 s and dried them with clean paper towels for a further 15 s.

The alcohol-containing agents tested were 60% isopropanol (BDH Ltd) and 0·5% w/v chlorhexidine digluconate in 70% w/w isopropanol ('Hibisol', ICI Pharmaceuticals plc). Five ml of these agents were rubbed into the hands (again in a standard fashion) for 30 s and then allowed to dry.

Experimental procedure

A slight modification of our previous method[8] was used. Survival of the test strains on the untreated hands of volunteers was determined. Then the survival on hands, previously washed with each agent three times (as described above) at five min intervals, was assessed in experiments separated by at least 3 days.

The inoculation and subsequent counting method were the same for the control and residual activity experiments. After inoculation of the test organism, individual fingers were sampled immediately (0 min) and 1, 3, 10 and 30 min later. Bacterial counts were made on finger washings as previously described[8] except that for spiral plating an additional 10^{-2} dilution was counted. The counts for 0 min were adjusted to 10^4 colony forming units (cfu) per finger and all other values adjusted by the same factor.

For each experiment, the median value for the three volunteers at each sampling interval was plotted against time, allowing direct comparison of

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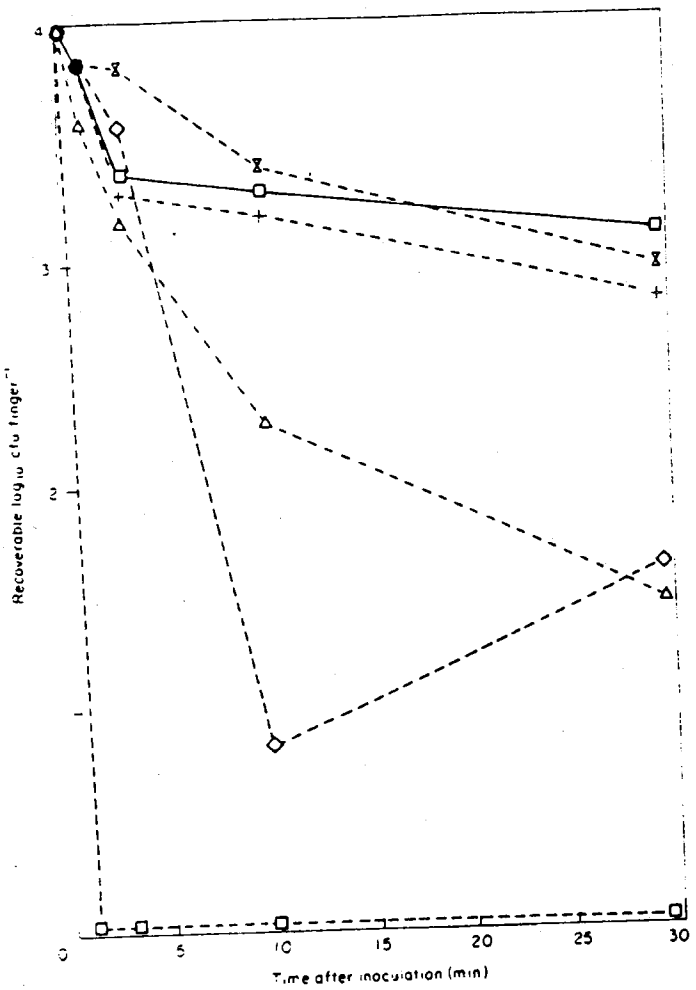


Figure 1. Persistent effect of antiseptic on vancomycin-resistant *E. faecium* following hand disinfection. Key: —=— control, no previous hand disinfection; -◇- 'Hibiscrub'; -□- 'Hibisol'; -△- 'Betadine'; - - + - - 60% IPA; -⧖- soap and water.

control (no pretreatment of hands) and residual activity (pretreated) survival curves.

Results

Figure 1 shows the results for the test strain of *E. faecium*. In the control experiment, when the organism was inoculated onto fingers that had not been pre-treated, there was less than 1 $\log_{10}$ decrease in recoverable cfu after 3 min and more than 10^3 cfu were still recoverable 30 min after inoculation. On hands previously washed with soap and water the survival curve of the enterococcus was similar to the control, i.e. no residual activity was

detected. Similarly, pre-rubbing with 60% isopropanol showed little, if any, residual activity. The two aqueous disinfecting agents, chlorhexidine digluconate and povidone–iodine, resulted in counts consistently less than those for the control: 10 min after inoculation there was a clear reduction in counts compared with the control. The residual action following alcoholic chlorhexidine hand rub was striking. Figure 1 shows that for all intervals after inoculation there was a decrease of 4 $\log_{10}$ in recoverable cfu. For two of the three volunteers the enterococcus could not be recovered, even by enrichment.

Figure 2 shows that the results for the test strain of gentamicin-resistant *E. cloacae* were similar to those for the enterococcus. No residual activity was detected for 60% isopropanol but for soap and water some residual activity in samples taken 10 min after inoculation of pre-washed fingers was shown. There was approximately a 1 $\log_{10}$ difference by 30 min. For chlorhexidine skin cleanser and povidone–iodine, moderate residual action was demonstrated. As with the test strain of enterococcus, alcoholic chlorhexidine showed by far the greatest residual activity with a 4 $\log_{10}$ reduction in viable counts within 1 min (see Figure 2).

Discussion

This model has proved useful for detecting important differences in the survival of organisms on skin. We have argued elsewhere about the importance of testing clinically relevant organisms and extending finger sampling for longer than 5 or 10 min after inoculation.[8] Another advantage of this model is that it permits evaluation of residual activity following skin disinfection.

The test strains were chosen because they had already caused cross-infection in hospitals. We know of no other studies showing the survival characteristics of *E. faecium* or *E. cloacae* on human skin. The control experiments show that these organisms are easily recoverable 30 min after inoculation on normal skin. The degree of survival is similar to that of epidemic strains of *Klebsiella*[12,15] and *Acinetobacter* spp.[13] and suggests that the strains are likely to be readily transmissible from patient to patient via staff hands. The risk is highest if the patient's skin is colonized, yet ward personnel may be performing 'clean' procedures for which handwashing is not thought to be necessary.

The case already made elsewhere[8] that the residual activity of a skin disinfecting agent is of clinical significance and should form part of the assessment of agents in common use, is supported by the remarkable differences in residual activity shown in this study. As anticipated, there was minimal, if any, residual activity on hands that have been previously washed with soap and water. The residual activity of chlorhexidine that has been described for certain organisms is confirmed. Few studies of hygienic handwashing have reported the residual action of povidone–iodine, which in our study was comparable to that found with chlorhexidine skin cleanser;

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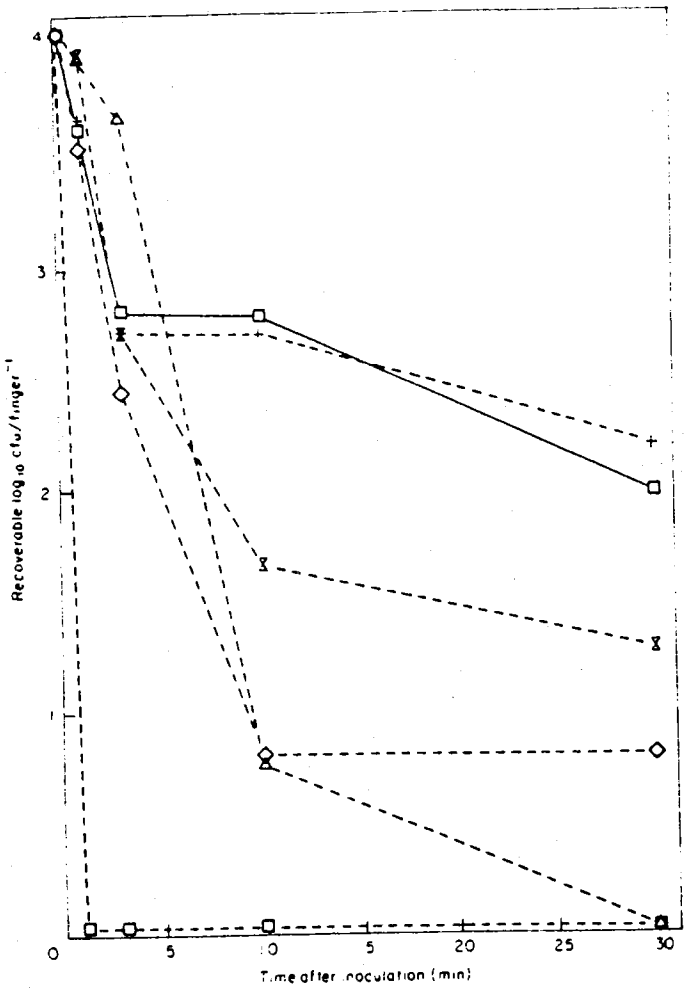


Figure 2. Persistent effect of antiseptics on gentamicin-resistant *E. cloacae* following hand disinfection. Key: ⇌ control, no previous hand disinfection; -◇- 'Hibiscrub'; -□- 'Hibisol'; -△- 'Betadine'; - - + - - 60% IPA; ⋯ soap and water.

those that have reported residual action with this agent used NCTC strains of unknown epidemicity.[2,4]

The residual activity following hand rubbing with alcoholic chlorhexidine is impressive. This had been noted previously, using similar techniques, for *Acinetobacter* sp.[14] and *Klebsiella* sp.[8] On the basis of our results it seems that alcoholic chlorhexidine is the agent of choice when faced with outbreaks of vancomycin-resistant *E. faecium*, or gentamicin-resistant *E. cloacae*, and probably other related organisms.

The use of various alcohols for hygienic hand disinfection is commonplace in some parts of Europe.[16] The two alcoholic preparations in

this study, alcoholic chlorhexidine and 60% isopropanol, have quite different residual activities thus it seems that the chlorhexidine component of 'Hibisol' is responsible for its residual activity. Possibly the alcohol acts as a vehicle to incorporate the disinfectant into the skin.

Although the method is tedious to perform, the semi-quantitative results obtained are helpful in selecting antiseptic agents. Whilst such marked differences in agents exist, we consider it important that further studies, perhaps by a central reference laboratory, are conducted using clinically relevant test organisms which are known to be transmitted in outbreaks via the hands of ward personnel.

Clearly, residual activity has to be considered in conjunction with the efficacy of the agents in decontaminating hands and we are presently completing a study of the five agents described above.

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303

Hygienic hand disinfection for the removal of epidemic vancomycin-resistant *Enterococcus faecium* and gentamicin-resistant *Enterobacter cloacae*

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Summary: An outbreak strain of *Enterococcus faecium*, bearing plasmid-mediated vancomycin resistance, and an epidemic gentamicin-resistant, multiply-resistant strain of *Enterobacter cloacae*, both survived well on fingertips of three volunteers for up to 30 minutes after inoculation. Hand disinfection of inoculated fingers with 60% isopropyl alcohol, or with alcoholic chlorhexidine, reliably gave a 4 $\log_{10}$ reduction of both test organisms. *Enterobacter cloacae* could not be detected in any finger washings, even by enrichment culture. Chlorhexidine digluconate and povidone–iodine were also effective against *E. faecium* and *Ent. cloacae*, giving 4 $\log_{10}$ reductions, but finger washings taken after disinfection yielded low counts of the test strains, e.g. less than 45 recoverable colony forming units (cfu) per finger. Handwashing with soap and water was the least reliable method. The epidemicity, serious antimicrobial multiple resistance and survival on fingertips of these two strains justifies the use of handwashing agents which have maximum effect. The rapid bactericidal (and residual) activity of alcoholic chlorhexidine suggests that, on the basis of present evidence, it is the preferred agent for hygienic hand disinfection against such strains.

Keywords: *Enterococcus faecium*; vancomycin resistance; *Enterobacter cloacae*; handwashing; disinfection.

Introduction

The occurrence in a neighbouring hospital of the world's first documented outbreak of hospital-acquired infection with *Enterococcus faecium* bearing plasmid-mediated vancomycin resistance,[1] in which hand transmission was implicated, drew our attention to the lack of published data on the survival of enterococci on human skin and, most importantly, any data to indicate which of the commonly-used skin disinfectants might be most effective for hygienic hand disinfection during such outbreaks. Similarly, although it is increasingly recognized that species of opportunistic Gram-negative bacilli show variable survival on skin,[2–5] little is known about the hand survival of,

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or optimal skin disinfectants for, *Enterobacter* species which may express Richmond and Sykes Class I beta-lactamase, acquire plasmids conferring gentamicin resistance and cause hospital outbreaks. We therefore studied the survival on fingers of, and the efficacy of various skin disinfection procedures against, vancomycin-resistant *E. faecium* and gentamicin-resistant *Ent. cloacae*.

Methods

Volunteers

Three female Caucasian volunteers aged 24–35 years were used throughout. None of the volunteers had evidence of skin disease, were receiving antibiotic therapy, had used any disinfectant skin preparation for the 48 h preceding each experiment or had any contact with patients. At least three days elapsed between experiments on the same subject.

Test strains

The two strains used as test organisms were as previously described[6]: an *E. faecium* bearing high-level vancomycin resistance (minimum inhibitory concentration > 64 mg l^{-1}) and a gentamicin-resistant, multiply-resistant *Ent. cloacae*. The vancomycin-resistant *E. faecium*, kindly provided by Dr Anne Uttley, was one of 27 patient isolates of this species isolated at Dulwich Hospital, South London over a 2-year period in which 39 patients were infected with vancomycin-resistant enterococci.[7] Hand transmission was implicated, although not proven, in the outbreak. The strain used in this study was multiply resistant (including resistance to ampicillin, erythromycin, tetracycline and chloramphenicol) and harboured a 24 Md plasmid which conferred resistance to vancomycin, erythromycin and chloramphenicol and was transferable to *E. faecalis*.[7] The gentamicin-resistant, multiply-resistant *Ent. cloacae* caused an outbreak on a geriatric unit involving 60 patients within a 7-week period; a common source was identified and, again, hand transmission was implicated. This strain bore a transferable plasmid conferring resistance to gentamicin, kanamycin, streptomycin, ampicillin, tetracycline, trimethoprim and chloramphenicol.[8]

Inoculation and recovery of test strains from fingertips

An overnight nutrient broth culture (0·02 ml) that had been incubated at 37°C was inoculated by means of a standard calibrated Pasteur pipette on the palmar surfaces of the distal phalanges of the fingers and thumb of the right hand which were immediately apposed to the corresponding fingertips of the left hand. The apposed fingertips were rubbed together for 30 s in order to spread the inoculum evenly. This inoculation procedure provided 10 fingertips which could be sampled at increasing time intervals to determine the survival of the test strains and the efficacy of subsequent hand disinfection procedures.

The methods for sampling the inoculated fingertips, with and without disinfection, and for the determination of recoverable colony forming units (cfu) in fingertip washings have been described in detail elsewhere.[4] Essentially, finger washings were obtained by rotating the fingertip for 30 s in 20 ml of Ringer's solution immediately (0 min) and 1, 3, 10 and 30 min after inoculation. Double strength MacConkey broth [20 ml (Oxoid Ltd)] was added immediately to each 20 ml finger washing. The number of cfu per washing was determined by standard quantitative methods, including surface-count plates, a spiral plater and enrichment cultures.

For the recovery and quantitation of vancomycin-resistant *E. faecium*, 5 mg l^{-1} of vancomycin, to inhibit normal flora, was added to the MacConkey agar in plates used for surface-counts and spiral plating. This inhibition was confirmed in every experiment by plating a pre-inoculation finger washing on MacConkey agar, with and without 5 mg l^{-1} vancomycin. To detect any inhibition of the test strain of enterococcus, colony counts of five serial ten-fold dilutions of an overnight broth culture of the enterococcus were made by means of spiral plating on MacConkey agar, with and without added vancomycin.

For all experiments the number of recoverable cfu for the first sample (0 min) was adjusted to 10^4 cfu per fingertip and the counts for the subsequent samples for that experiment adjusted by the same factor and plotted against time. Adjustment to 10^4 cfu at time 0 was necessary in order to permit comparison between experiments and the reproducibility of replicate experiments using this method has been described previously.[5]

Disinfection procedures

The survival characteristics of the test strains (without handwashing) were first determined for each subject by taking finger washings at 0, 1, 3, 10 and 30 min after inoculation and determining the number of recoverable cfu.

To evaluate the effects of 4% w/v chlorhexidine digluconate skin cleanser ('Hibiscrub', ICI Pharmaceuticals plc) and 7·5% w/v povidone–iodine surgical scrub ('Betadine', Napp Laboratories Ltd), the fingers were inoculated and samples taken at 0 and 1 min. Both hands were then washed with 5 ml of these preparations for 30 s according to the standard hygienic procedure described by Ayliffe *et al.*[2] The hands were then rinsed for 15 s in running tap water and dried for 15 s with two clean paper towels. The 3, 10 and 30 min post-inoculation samples were then taken from different fingers. The effect of non-medicated bar soap (Jeyes Hygiene Ltd) was determined using the same washing, sampling and culture protocol.

To evaluate the effects of 0·5% w/v chlorhexidine digluconate in 70% w/v isopropyl alcohol ('Hibisol', ICI Pharmaceuticals plc) and of 60% isopropyl alcohol (BDH Ltd), after taking the 0 and 1 min post-inoculation samples, 5 ml of the alcoholic preparation was rubbed into the inoculated hands for

30 s. After air drying for 30 s the 3, 10 and 30 min post-inoculation finger washings were taken as before. The number of recoverable cfu from the finger washings was determined as before.

In each experiment the median recoverable cfu value for the three volunteers at a given sampling time was recorded and plotted against time.

Results

Overnight nutrient broth cultures of both test organisms yielded *c.* 10^8 cfu ml^{-1} (*E. faecium*: range $7{\cdot}6 \times 10^6$ to 3×10^8; *Ent. cloacae*: range $2{\cdot}3 \times 10^7$ to $6{\cdot}3 \times 10^8$). In all experiments, a factor of less than 3 $\log_{10}$ was required to correct the recoverable cfu per finger at 0 min to 10^4.

MacConkey agar containing 5 mg l^{-1} vancomycin did not inhibit the test strain of vancomycin-resistant enterococcus but completely inhibited the normal skin flora in finger washings from all three volunteers.

Vancomycin-resistant E. faecium

Figure 1 shows that, in the absence of hand disinfection, vancomycin-resistant *E. faecium* survived well on the fingers of all subjects. Although there was approximately 1 $\log_{10}$ reduction in the first 3 min, 10^3 cfu could still be recovered from the initial inoculum of 10^4 cfu at 10 min after inoculation. Thirty minutes after inoculation more than 10^3 cfu per fingertip could be recovered from two of the three subjects.

Figure 1 also shows the median numbers of recoverable cfu in fingertip washings taken at increasing intervals up to 30 min after hygienic hand disinfection. After hand disinfection with chlorhexidine skin cleanser, alcoholic chlorhexidine or 60% isopropyl alcohol, there was a prompt 4 $\log_{10}$ fall in recoverable cfu. For the total of nine washings taken after the 0 min sample (3, 10 and 30 min post-disinfection samples from each of the three subjects) the number that did not yield any cfu after chlorhexidine skin cleanser, alcoholic chlorhexidine or 60% isopropyl alcohol was 7, 6 and 7, respectively. Of the remaining washings only one yielded > 2 cfu. The results for povidone-iodine were very similar except for one subject from whom 1·1 cfu were recovered from the finger washing taken at 30 min after inoculation. Figure 1 also shows that the median values indicate that washing with soap and water was less reliable; for two of the three volunteers *E. faecium* was recovered in all post-handwashing samples.

Gentamicin-resistant Ent. cloacae

Figure 2 shows the median results for the gentamicin-resistant *Ent. cloacae*. In the absence of hand disinfection there was, for all three volunteers, a 1–2 $\log_{10}$ reduction in the number of recoverable cfu by 3 min. The median number of recoverable cfu showed almost a further one $\log_{10}$ reduction

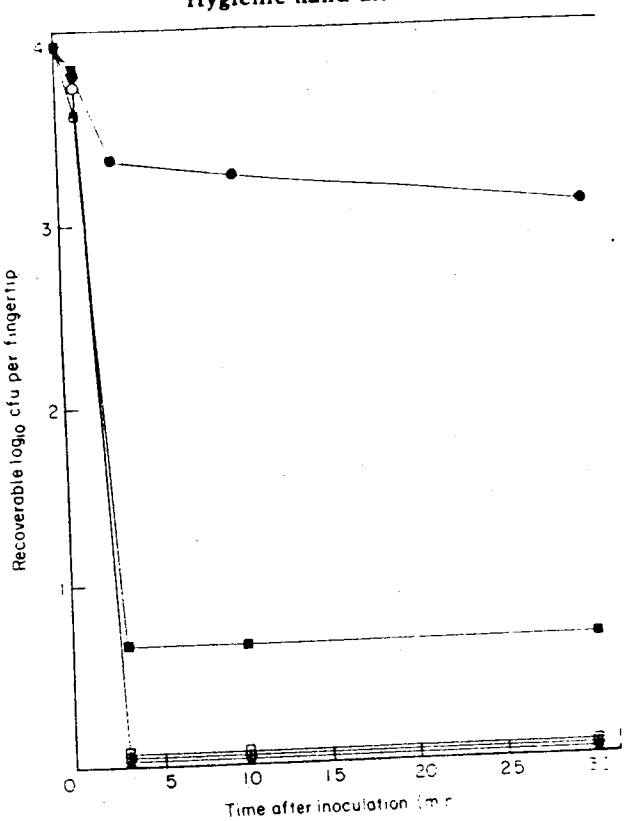


Figure 1. Effects of washing with five agents on the recovery of *E. faecium*. Median values for three volunteers. ●, Control; ■, soap and water; △, alcoholic chlorhexidine; ○, chlorhexidine skin cleanser; ▲, povidone-iodine; □, 60% isopropyl alcohol.

30 min after inoculation. Alcoholic chlorhexidine or 60% isopropyl alcohol most reliably eliminated an inoculum of 10^4 cfu of *Ent. cloacae* (Fig. 2); after handwashing with either of these agents, *Ent. cloacae* was not detected, even by enrichment, in finger washings taken from any of the subjects. The results were similar for chlorhexidine digluconate except for the recovery of 45 cfu from one subject 30 min after handwashing. After disinfection with povidone–iodine *Ent. cloacae* was detected in the finger washing taken from one subject 3 min after disinfection and in another subject in the 30 min sample. The median counts for the three volunteers shown in Figure 2 indicate that handwashing with soap and water was less likely to eliminate completely an inoculum of 10^4 cfu of *Ent. cloacae*. The 3 min finger washings of all three volunteers yielded 8–110 cfu per fingertip. Thirty-six and 110 cfu of *E. cloacae* were still detected in two subjects in the 30 min sample after handwashing with soap and water.

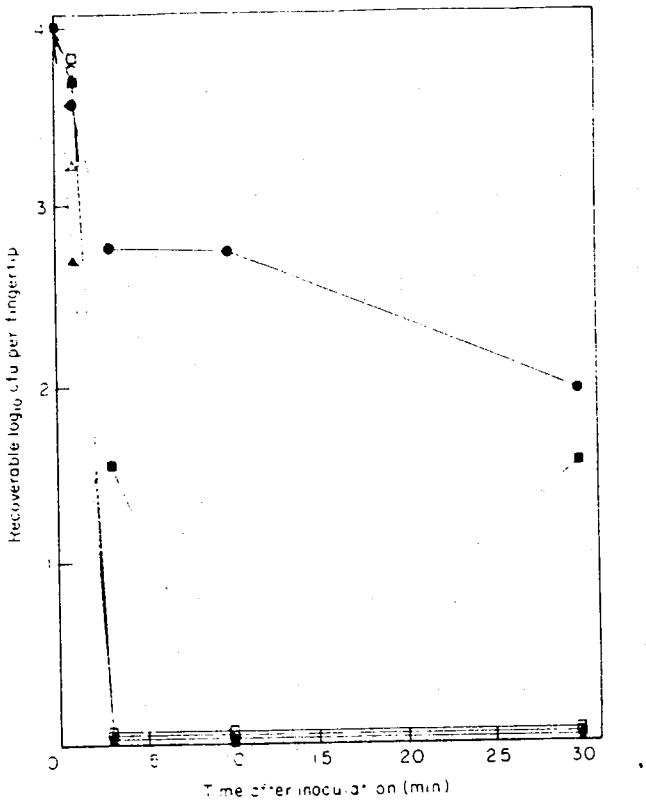


Figure 2. Effect of washing with five agents on the recovery of *Ent. cloacae*. Median values for three volunteers. ●, Control; ■, soap and water; △, alcoholic chlorhexidine; ○, chlorhexidine skin cleanser; ▲, povidone-iodine; □, 60% isopropyl alcohol.

Discussion

We have emphasized elsewhere the value, and limitations, of the testing of skin disinfectants by methods that attempt to reproduce closely the events that occur in clinical practice.[9] Most importantly, the selection of the test organisms and a comparison of their recoverability with, and without, subsequent skin disinfection seems important when formulating ward procedures for hand decontamination during outbreaks.

The survival, in the absence of handwashing or skin disinfection, of the outbreak strain of vancomycin-resistant *E. faecium* in this study is similar to the survival of *Staphylococcus aureus* described by Ayliffe *et al.*[10] This supports the potential for hand transmission by ward personnel and thus the need for effective hand disinfecting agents. For the complete removal of the test strain of *E. faecium*, there seems little to choose between alcoholic chlorhexidine, 4% chlorhexidine skin cleanser or 60% isopropyl alcohol, all of which more reliably yielded finger washings free of *E. faecium* than soap

and water. The convenience of alcoholic chlorhexidine hand rub encourages hand hygiene when access to hand basins is limited and we have described elsewhere[6] its striking residual activity against this strain of *E. faecium*. Residual activity was not found with 60% isopropyl alcohol alone,[6] and on the basis of the present evidence, alcoholic chlorhexidine is the agent of choice.

The survival of *Ent. cloacae* shown in this study is similar to that for outbreak strains of *Klebsiella aerogenes*[5] and *Acinetobacter calcoaceticus*.[11] Again, this supports the suggestion of Howard & Grinter[8] that this strain of *Ent. cloacae*, and probably others, may be transmitted easily by contaminated hands of staff and that such multiply-resistant strains warrant optimal staff hand disinfection. The results of this study show that all the procedures using a chemical disinfectant were more effective against *Ent. cloacae* than washing with soap and water alone. Handwashing with chlorhexidine digluconate skin cleanser, or hand rubbing with alcoholic chlorhexidine, most reliably eliminated the outbreak strain of *Ent. cloacae* that had been artificially inoculated on the fingertips of volunteers. Again, the residual activity of alcoholic chlorhexidine that we demonstrated previously against the same strain of *Ent. cloacae*[6] leads us to conclude that this is the agent of choice.

It remains possible that different strains within a given species will show different survival characteristics on contaminated hands and will show differing susceptibilities to commonly used agents. Nevertheless, we propose that the results of this, and other studies, show correlation between survival on artificially inoculated fingertips and epidemic potential, and that there are differences in susceptibility to various commonly used disinfecting procedures. There is a clear need for more studies in order to define further optimal disinfecting procedures that will minimize the spread of such important multiply-resistant organisms and their transmissible plasmids.[7,12,13]

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THE STAPHYLOCOCCI

Importance of Their Control in the Management of Skin Disease

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Members of the staphylococcal family of gram-positive cocci play a central role in the microbiologic ecosystem of human skin. The coagulase-positive *Staphylococcus aureus* is an important cutaneous pathogen, whereas the coagulase-negative staphylococci such as *S. epidermidis* and *S. hominis* are part of the normal flora and are not usually considered pathogenic. Control of *S. aureus* colonization and infection is important not only for the direct benefit of patients themselves but also for their immediate contacts because *S. aureus* skin carriage is a potential cross-infection hazard.[1,2]

Persistent, asymptomatic nasal carriage of *S. aureus* occurs in 20% to 40% of normal individuals,[3,4] with the organism being isolated from the perineum and axilla in a smaller proportion of the general population.[5,6] This colonization usually represents asymptomatic carriage for the individual concerned, but it can result in recurrent episodes of boils or folliculitis. For some health care workers the increased nasal carriage presents a cross-infection risk, and attempts at eradication of the carrier state become necessary.

S. aureus is clearly a primary pathogen in conditions such as impetigo and furunculosis, in which specific antistaphylococcal treatment results in resolution of the infection. However, when *S. aureus* is isolated from an underlying cutaneous disease process such as atopic dermatitis, its exact pathogenic role is less well defined. Attempts to control *S. aureus* colonization in these circumstances have yielded clinical benefit, but without resolution of the underlying skin disease.[7]

SKIN DISEASES OF PRIMARY *S. AUREUS* ORIGIN

In conditions such as impetigo, furunculosis, and the staphylococcal scalded skin syndrome, it is accepted that the presence of *S. aureus* is the primary cause of the disease process.

Impetigo

Traditionally impetigo is divided into bullous and nonbullous variants. The bullous variant, which is less common and tends to occur in clusters, is considered to be a relatively pure staphylococcal infection, but the cause of the nonbullous type is a subject of current controversy. This controversy tends to be reported in varying terms in the dermatologic and pediatric literature. In a paper regarded as a classic published in 1970,[8] the authors produced microbiologic evidence that the majority of cases of nonbullous impetigo were caused by group A beta-hemolytic streptococci, although in a small proportion *S. aureus* could also be grown from the lesions. Further evidence that streptococci were the primary cause was the fact that the lesions responded to therapy with penicillin and that *S. aureus* could no longer be cultured from lesions after treatment with penicillin even though staphylococci are resistant to this antibiotic. The problem with this paper is that the culture conditions were designed to promote growth of streptococci rather than staphylococci. In modern studies of the microbiology of impetigo

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and of the relative contributions of staphylococci and streptococci, it is recommended that cultures be established in at least two media, one favoring the growth of staphylococci and the other streptococci.

With this proviso, it appears that the majority of reports of nonbullous impetigo from temperate countries in the 1980s concern primarily staphylococcal infection,[8,10] whereas streptococcal infection appears to be reported more commonly in tropical climates. In bullous impetigo, *S. aureus* can be cultured from the blister fluid, and if injected into neonatal mice this fluid causes a high-level epidermal split as a result of the action of an epidermolytic toxin produced by the staphylococci. This toxin is believed to be responsible for cleavage of the epidermis and bulla production. The staphylococcal phage most commonly responsible for bullous impetigo is phage type 2.

Clinical Features

The clinical feature of bullous impetigo is the relatively rapid appearance of clusters of fragile blisters, which quickly rupture to leave a circular raw area surrounded by a ragged fringe of epidermis. These may be seen on any body site. There is no systemic upset, and enlarged lymph nodes are unusual.

In nonbullous impetigo, outbreaks are relatively frequent, particularly in hot humid environments where there is overcrowding and poor hygiene. Reports are more frequent of cases involving infants and children, but this may result from a relative under-reporting of cases in adults.

Histopathology

At the histologic level the differences between the two conditions are relative rather than absolute. High-level epidermal splits are also seen in the nonbullous variety, but the clinical presence of vesicles is transient. As with the bullous variety, neutrophils may be seen in the underlying epidermis, and a mixed neutrophil/lymphocyte infiltrate is seen in the dermis.

The clinical features in nonbullous impetigo are the presence of heavy yellow-brown (honey-colored) crusts, most commonly around the nose and mouth. This type of impetigo may complicate preexisting atopic eczema or herpes simplex infection. Temporary enlargement of the local draining lymph nodes is a frequent phenomenon.

Treatment

Swabs should be taken for culture, identification, and sensitivities of microorganisms in all cases of suspected impetigo, and, as stated above, culture media should be used to promote growth of both staphylococci and streptococci. Treatment must be undertaken before the results of bacteriologic examination are known but should of course be reviewed and if necessary modified when these results are available.

The treatment options for impetigo presumed to be caused by staphylococcal infection are topical or systemic antistaphylococcal agents. In the past 3 years, a number of clinical studies and controlled trials have reported a high clearance rate—both clinical and bacteriologic—using topical mupirocin.[3] In addition, there is a low recurrence rate with mupirocin and it therefore appears to be the current topical therapy of choice.

Studies comparing topical mupirocin with systemic antistaphylococcal antibiotics such as erythromycin show a comparable response rate.[13] A high proportion of staphylococci isolated from patients with impetigo in developed countries are penicillin-resistant; therefore, if systemic therapy is chosen, the choice lies between erythromycin and penicillinase-resistant antibiotics such as cloxacillin and the cephalosporins. In general, however, if the patient is otherwise fit and neither very young, very old, nor immunocompromised, topical mupirocin is recommended as the initial treatment of choice.

In view of the controversy concerning the pathogenicity of staphylococci and streptococci in nonbullous impetigo, controversy exists concerning the need for rapid systemic antistreptococcal therapy. The reason for concern here is the possibility of post-streptococcal glomerulonephritis. This entity has been very rarely reported in the past 20 years, but it is not clear whether this is due to a genuinely low incidence of this complication, possibly owing to the predominance of cases caused by staphylococci rather than streptococci, or due to under-reporting. Recent case report and review of treatment options for nonbullous impetigo suggest that oral or intramuscular penicillin should remain a treatment option for this condition and that the switch to more expensive systemic alternatives is not justified on bacteriologic sensitivity grounds.[10] Because a large proportion of cases of nonbullous impetigo show a mixed growth of staphylococci and streptococci on culture, additional topical mupirocin seems a prudent and reasonable addition.

Investigation of an outbreak of impetigo in a school, hospital, or institution should involve bacteriologic examination of both sufferers and all other individuals in that community in order to identify asymptomatic *S. aureus* carriers. This involves swabbing the nasal vestibule and perineum and treating any identified carrier with topical antistaphylococcal therapy until bacteriologic clearance is achieved. Once again, topical mupirocin is currently the treatment of choice.

Furunculosis

Furunculosis is an infection, often relatively chronic, of the deeper components of the hair

follicle, caused by *S. aureus*.[9] The clinical feature of furunculosis is the appearance of deep-seated tender nodules on hair-bearing areas, particularly those subjected to friction, such as the perineum, the collar area, and the beard area. It appears to be more common in Afro-Caribbeans than in Caucasians, possibly due to anatomic differences in the pilosebaceous duct. The available evidence suggests that the predisposition to furunculosis is due to local factors rather than to a general immunologic deficiency. The majority of sufferers are, however, staphylococcal carriers, and the same strains of staphylococci that are found in the lesions can be cultured from the nose and groin.

Affected individuals should be screened for diabetes, as furunculosis is seen more commonly than expected in association with the diabetic state. In recurrent cases, the possibility of HIV infection should also be considered and screening undertaken if appropriate. Thereafter, appropriate systemic antistaphylococcal therapy should be started without delay and adjusted if necessary when bacteriologic results and antibiotic sensitivities are available. Oral flucloxacillin in full doses would be reasonable initial therapy. Furunculosis causes destruction of the hair follicle resulting in scarring, and prompt treatment minimizes this effect.

Furunculosis tends to be a chronic and recurrent problem. Few adequate studies have been done of the value of prophylactic low-dose antibiotic therapy continued for some months or of the use of topical antibiotics or antiseptics. If the causative staphylococci are penicillin sensitive, low-dose oral therapy for 6 to 8 weeks after an acute episode is a reasonable approach. However, the use of long-term topical mupirocin is to be discouraged. This is because topical mupirocin is at present an extremely valuable antibiotic for use against methicillin-resistant strains of staphylococci, and there are fears that continuous use may encourage the development of mupirocin-resistant strains. Topical antiseptic washes such as chlorhexidine may be of value, and reduction of physical trauma has been reported to coincide with improvement in many cases. This may involve discontinuing wet shaving if the affected area is the face, or even discontinuing all shaving and growing a beard.

Staphylococcal Scalded Skin Syndrome (SSSS)[24]

This entity is due to an epidermolytic toxin produced by certain strains, most commonly of phage type group 2, of *S. aureus*.[9]

SSSS is most commonly seen in infants and young children and tends to occur in outbreaks in neonatal nurseries or in day nurseries, owing to the presence in the community of an asymptomatic adult carrier of the causative staphylococcus.

The clinical presentation is that of a fretful, often febrile child. The skin is bright red, and rapidly, over a period of hours, separation and peeling of the outermost layers of the epidermis occurs, leaving a raw and glistening surface. This is most striking on the trunk. The epidermolytic toxin cleaves the epidermis at the level of the granular layer, which determines the site of the split. Around the edge of the raw area, the ragged remains of the epidermis curl in a manner resembling fine tissue paper.

SSSS is rare in adults, and the few reported cases have been in elderly immunocompromised patients who may also have had impaired renal function.[13,14]

An important differential diagnosis in children is nonaccidental injury. It is possible that social workers and other nondermatologic professionals involved in the care of children may not be aware of the existence of SSSS and may believe that a true scald due to application of hot water has occurred. The author has twice in the past 3 years been confronted with such situations.

All patients with suspected SSSS should be swabbed for identification and sensitivities of the causative organism, and the possibility of a staphylococcal carrier in the environment should be investigated. Patients should be started on systemic antistaphylococcal therapy; the current treatment of choice is probably flucloxacillin. SSSS is a serious problem in neonatal nurseries and may be fatal in premature or otherwise immunosuppressed infants.

ROLE OF *S. AUREUS* IN ESTABLISHED SKIN DISEASE

S. aureus may be isolated from the skin surface in a number of skin diseases whose primary cause is not thought to be infectious. The most widely known example of this is atopic dermatitis (AD), in which isolation rates approaching 100% from clinically involved skin and over 50% from clinically uninvolved skin have consistently been reported.[3,15,25] Other conditions in which cutaneous *S. aureus* colonization is increased are allergic contact dermatitis,[26] patients receiving isotretinoin therapy,[16] leg ulcers, and psoriasis.[3] Increased adherence between *S. aureus* and corneocytes has been demonstrated in AD,[4] in allergic contact dermatitis (Williams REA, accepted for presentation at 1992 meeting of ESDR), and in patients receiving retinoids.[25] This increased adherence is likely to be at least partly responsible for the increased isolation rates.

The consequences of the increased colonization rate in all the above conditions are not entirely clear, and the boundaries between "colonization" and "infection" become blurred. Certainly, a greater incidence of frank staphylococcal infections such as boils and folliculitis is seen in patients with AD[11] and in those receiving isotretinoin.[16] These phenomena resolve, at least

emporarily, with appropriate antistaphylococcal reatment and are likely to be a direct result of he increased colonization rate. However, the effect of the increased *S. aureus* colonization rate on he underlying activity of a cutaneous disease such s AD has never been definitively investigated ecause of the difficulty in reliably removing *S ureus* from the skin surface for a prolonged period f time. The available evidence, however, does uggest that *S. aureus* colonization has a detrimenal role on the underlying disease activity.

Atopic Dermatitis

The density of *S. aureus* colonization varies proportionately with the severity of AD affecting he area of skin sampled.[10] Areas of skin with exudative AD carry the highest density of *S. ureus*. It has previously been suggested that bacterial counts greater than 10^5 should be considered "infection" and those below "colonization," with a corresponding break point for the initiation of antistaphylococcal therapy.[10] Such a break point is unlikely to exist, however, if the first of the two currently proposed mechanisms for staphylococcal damage in AD is accepted. The proposed hypothesis is that the presence of *S. aureus* has a direct inflammatory effect on the underlying AD, perpetuating the itch–scratch–skin damage–inflammation–infection cycle. This is supported experimentally by the observation that protein A, a component of staphylococcal cell walls, has a direct inflammatory action when applied to skin in which the stratum corneum has been damaged or partly removed.[11] If this hypothesis is accepted, then antistaphylococcal treatment would be beneficial at any level of colonization density, although removal of dead staphylococcal cell wall material from the skin surface would also be important.

The alternative hypotheses to explain staphylococcal damage in AD are immunologic. The possibilities include a type 1 reaction producing erythematous flares, altered humoral immune responses, and altered cell-mediated effects. Studies examining the possibility of a type 1 reaction have focused on the incidence of specific antistaphylococcal IgE. High levels of specific antistaphylococcal IgE would cause triggering of mast cells, leading to the release of inflammatory mediators with a subsequent flare of the dermatitis. In spite of a large volume of evidence, the verdict is unclear, possibly because of the technical problems associated with measuring specific antistaphylococcal IgE in the presence of very high total IgE levels. The earlier studies suggested a high level of antistaphylococcal IgE in AD patients, but no difference was shown between patients with eczema and staphylococcal infections; patients who were atopic did not have eczema but did have staphylococcal infections.[1, 12] More recent studies, such as those by Friedman et al[12] and Hauser et al.[13] have used more specific antigens and have found much lower levels of antistaphylococcal antibodies in patients with AD. The significance of these findings is therefore uncertain. The humoral immune response in AD has been investigated by Ventura et al.[12] This group concluded that an impaired humoral response to *S. aureus* is probably not the cause of staphylococcal damage in AD. The studies looking at the cellular immune response in AD are also contradictory to the intradermal injection of staphylococcal antigen showing hyporesponsiveness,[14] whereas in vitro work by Higaki et al suggests that chronic colonization with *S. aureus* may stimulate a cellular immune response.[15] Whichever arm (if any) of the immune system is responsible for staphylococcal damage in AD, most immunologic reactions are dose independent; therefore, if antistaphylococcal treatment is to be beneficial, it needs to reduce the antigenic load below a critical "trigger" level.

Whatever the mechanism, the benefit of treating staphylococcal colonization and infection in AD seems to be widely accepted. Attempts to control *S. aureus* colonization in AD are part of most clinicians' armamentarium in the management of patients with AD. Nearly all dermatologists can identify patients for whom the intermittent prescribing of an antistaphylococcal antibiotic results in rapid but temporary improvement.

Formal studies have tended to look at the effect of antistaphylococcal therapy during exacerbations of AD with good evidence from David et al[7] and Ventura et al[12] that some flares of atopic dermatitis settle only after the staphylococcal load has been reduced. Some evidence is also available on the effect of antistaphylococcal therapy on AD not in exacerbation. Lever et al[17] showed a significant reduction in disease activity associated with a reduction in staphylococcal colonization produced by the use of topical mupirocin ointment, which is highly active against gram-positive cocci.

We have also used mupirocin in an attempt to completely eradicate *S. aureus* from the skin surface of patients with AD. Six inpatients were treated with the application of 2% mupirocin in white soft paraffin to their entire body surfaces, including the scalp and anterior nares twice daily for 3 days. No topical corticosteroids were applied either during their 3-day admission or until 1 week following the first application of mupirocin. Bacterial colonization was assessed at six body sites using the method of Williamson and Kligman.[9] All patients showed a total eradication of *S. aureus* colonization on the day following the final mupirocin application, with only a slow recolonization rate. Densities of colonization at 1 week after mupirocin treatment were much lower than those originally present, and in the one patient followed extensively over a 4-week period the mean log count at 4 weeks after mupirocin treatment was 1.61 colony forming units (CFU)/cm² compared with 4.92 CFU/cm² before treatment. The same patient's eczema activity was assessed over the 4

weeks using a scoring system with a maximum value of 288. The pretreatment value of 98 fell to 34 by the end of the study period, and the patient reported that his eczema was much more easily controlled than before the mupirocin treatment. "Mupirocin immersion" has also been used to eradicate a multiply-resistant *S. aureus* in a patient with AD.[35]

The prolonged use of antibiotics, both systemic and topical, is complicated by the potential development of resistance and the possibility of systemic side effects. The use of antiseptics would avoid this problem, although most of the commonly used preparations are irritant in nature, making their use in AD difficult. The only published work did not show them to be bacteriologically effective in AD,[36] but we are currently conducting a controlled trial of a soap solution containing chlorhexidine to examine the same problem. Many clinicians advise their patients to add a small amount of antiseptic to their bath water in a similar attempt to control staphylococcal colonization.

It therefore seems that the control of staphylococcal colonization with specific antistaphylococcal treatment can benefit patients with AD. An interesting phenomenon, however, is that decreases in *S. aureus* counts in AD may also be produced by the use of topical corticosteroid preparations. White and Noble[34] showed a significant decrease in aerobic bacterial counts in patients with AD treated over a 7-day period with betamethasone 0.1% diluted 1:4 in cetomacrogol. In this case one is presumably attacking the underlying disease process that allows staphylococcal colonization to take place, but the exact mechanism of this reduction in colonization is not known.

Other Forms of Dermatitis

Although, with respect to *S. aureus* colonization, AD is the most widely studied form of dermatitis, good evidence also exists of colonization in allergic contact dermatitis, discoid eczema, and stasis dermatitis.[37] Both in the published reports and in our own series, approximately 50% of eczematous areas sampled in patients with allergic contact dermatitis grew *S. aureus*. The same arguments concerning the potential for staphylococcus-induced damage as detailed above for AD can be applied to these other forms of dermatitis. We have demonstrated that *S. aureus* adherence to corneocytes is increased in the eczematous areas of patients with allergic contact dermatitis in a way similar to AD (Williams REA, accepted for presentation at 1992 meeting of ESDR), but less information is available concerning the correlation of colonization densities and clinical state. The use of antibiotics has, however, again become a routine part of management of these patients, with apparently beneficial results.

Patients Receiving Isotretinoin

It has been recognized almost from its introduction into clinical use that isotretinoin causes an increase in *S. aureus* carriage.[38] Sites particularly prone to colonization are the anterior nares, lips, and facial skin.[39] Interestingly, *S. aureus* adherence to corneocytes is increased by the addition of acitretin, a related retinoid, to the in vitro assay system.[39] The increased colonization in patients receiving isotretinoin results in an increased risk of staphylococcal infections such as boils or folliculitis. In these patients, the colonization of the anterior nares seems to be particularly important, and control of nasal colonization using an ointment containing neomycin, bacitracin, and polymyxin resulted in a significant reduction in the incidence of boils and folliculitis.[38] Its use can be recommended especially in patients who have had recurrent problems with *S. aureus* infections prior to commencing isotretinoin. It has also been suggested that the *S. aureus* colonization might have a detrimental effect on the severity of isotretinoin-induced side effects such as dry nasal mucosa, cheilitis, and facial eczema in a way analogous to its effect in AD. We found no such evidence in our recent clinical trial, although the numbers studied were relatively small.[39]

Venous Leg Ulcers

S. aureus is frequently isolated from venous leg ulcers. The clinical significance of this is doubtful in the absence of surrounding signs of erythema, pain, and tenderness. A recent study by Goodfield[12] demonstrated the use of estimation of C-reactive protein in helping to clarify the clinical importance of an isolate from leg ulcers and the subsequent need for antibiotic treatment. Certainly, antibiotics do not accelerate the healing of uncomplicated leg ulcers and merely serve to expose the patient to potential side effects, which include, for the use of topical antibiotics such as neomycin, the possibility of developing allergic contact dermatitis.

SUMMARY

For skin diseases such as impetigo and furunculosis in which infection with *S. aureus* is the primary pathologic event, control of that infection is obviously paramount in the management of the clinical situation. The available antistaphylococcal agents are highly effective, and topical mupirocin has been a valuable recent addition. In conditions such as AD the position is less clear. Because the presence of the underlying disease is a prerequisite for the staphylococcal colonization, the exact role of staphylococcal damage is difficult to separate from the underlying disease activity. The definitive experiment, whereby staphylococcal colonization is eradicated over a prolonged period of time by a nonirritant method, remains to be performed. Until that time it is reasonable to

pursue a policy of treating *S. aureus* colonization when it is believed to be of clinical significance and not simply on the basis of bacteriologic findings.

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A NEW METHOD FOR THE QUANTITATIVE INVESTIGATION OF CUTANEOUS BACTERIA*

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Over the past 25 years, many different methods have been used for sampling the bacterial flora of the human skin (1–3). The results obtained have for the most part lacked reproducibility, and have been extremely divergent. We have embarked upon an intensive study of the bacterial ecology of normal and abnormal skin. The development of a better quantitative sampling technic was an indispensable step. The method to be described is a modification of the cup scrub technic of Pachtman *et al* (4). The principal refinements which have resulted from our study have to do with the composition of the wash fluid which is placed in the cup. This study deals exclusively with the aerobic microflora.

MATERIALS AND METHODS

Subjects.—Subjects were healthy adult Negro men, inmates of Holmesburg Prison, Philadelphia, Pa.

Bacteriological technics.—Bacteria were counted in pour plates of appropriately diluted samples using 15–20 ml of Tryptic Soy Agar (Difco) per plate. After 36–48 hours of aerobic incubation at 37° C., the colonies were counted on a Quebec counter.

Selection of surfactants.—Of the three classes of surfactants, the cationics, anionics and nonionics, the first was dismissed because it is made up largely of quaternary ammonium compounds; these are potent bactericides *in vitro*. Anionic surfactants are among the best known and most effective detergents in use today; for instance, the alkyl benzene sulfonates, the alkyl sulfates, and the soaps (salts of long chain fatty acids). Ten representative compounds of this type were tested and rejected as too toxic to bacteria; *viz.* alkyl benzene sulfonates and soaps.

Non-ionic detergents seem suited for skin sampling because of low irritancy, low bacterial toxicity, high stability, relative purity of commercial products and great effectiveness as detergents and dispersants. In the present study sixteen non-ionic detergents were chosen to give a representative sample of the various chemical types (Table I). The usefulness of a detergent was estimated in two different ways:

(1) *In vitro screening.*—Both in culture and especially on the skin, bacteria grow in clusters, not as single entities. Unless effectively dispersed, colony counts reflect aggregates, not individual cells, leading to underestimations. To study dispersing ability, aqueous solutions of surfactants were added to water suspensions of an 18 hr. broth culture of *Staphylococcus albus* having a bacterial density of 5–30 × 10^4 organisms/ml to make final surfactant concentrations of 0.1%. These were briefly shaken mechanically (Vortex Jr. Mixer) and incubated for 10 minutes at room temperature. After re-shaking, aliquots were removed, diluted in water in 10-fold steps, and plated as described above. Water controls were included and treated in the same manner. The effectiveness of a detergent was estimated by taking the ratio of the test count to the water control count, times 100. We have termed this the 'dispersal efficiency'.

(2) *In vivo* testing.—Those surfactants having the highest dispersal efficiency were tested for their ability to remove bacteria from the glabrous region of the forearm. One ml of 0.1% detergent solution was pipetted into the sterile glass cylinder circumscribing an area of 3.8 cm sq and the surface rubbed as evenly as possible with a blunted Teflon 'policeman' (Arthur H. Thomas) for two minutes. Pilot studies made it clear that hard rubbing or mechanical disruption of the horny layer were superfluous. The wash solution was aspirated, an aliquot diluted, and pour plates prepared; after 48 hour aerobic incubation, the colonies were counted. Each test was done in triplicate.

Effect of pH.—Arnold's (5) observations that strongly basic solutions removed far more bacteria from the skin than did acidic solutions were confirmed later by Blank and Coolidge (6). The latter suggested that bacteria were held to keratinized cells by electrostatic charges that were strongest at low pH and could be negated at highly alkaline pH. Preliminary studies with aqueous salts, acids and bases in the pH range 4–10 indicated a preferential removal of bacteria in the strongly alkaline range, but in no case was aqueous alkali alone more effective than aqueous non-ionic detergent in the slightly acid range. Consequently, 0.1% solutions of the most effective non-ionic detergents were made in 0.067 M (physiologic) phosphate buffer ranging in pH from 6–10, and examined for optimal efficiency using the *in vivo* method described above.

Efficacy of surface sampling.—The scrub technic is valuable only if it can be shown that the great

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Vol. 45
1976

majority of organisms are actually located on or near the surface and are hence removable. Past teaching emphasizes a follicular situation. Histologic studies now in progress in this laboratory indicate that aerobic bacteria are found neither in the follicles nor deeply within the horny layer; only anaerobic bacteria of the *C. acnes* type are regularly found in deep follicular recesses of certain areas.

Four quantitative methods were utilized to demonstrate the superficial location of the aerobic microflora. (1) Cellophane tape stripping: Röckl and Muller (7) and Updegraff (2) have previously shown by qualitative methods that the majority of bacteria are present in the outer layers of the stratum corneum; these investigators relied on culturing bacteria directly from the tape strips. In the present study, bacterial counts were made on adjacent areas scrubbed before and after firm serial stripping with 'Scotch' brand cellophane tape (Minnesota Mining and Manufacturing Co.). (2) A comparison of scrubbing to soaking: Two minutes of normal manual scrubbing was compared on adjacent sites to gentle agitation of the wash fluid alone, with no manipulation of the skin surface. (3) Normal cleansing: Subjects washed their own foreheads and forearms with Ivory soap and warm tap water for approximately 15 seconds following a control sample and then were resampled. (4) Serial scrubbing: Successive one minute washes were taken at the same site with as short an interval as possible between each scrub. Each sample was then worked up separately. Preliminary studies were done on the forearms of five men, three with high average bacterial counts and two with low. Similar experiments were then performed on areas of high bacterial population such as forehead and axilla.

RESULTS

1. Dispersion.—The concept of dispersal efficiency has been of considerable assistance in the selection of detergents. As can be seen in Table I, the list of 16 non-ionic detergents can be narrowed to four worthy of further study, those having a dispersal efficiency of 170 or greater. This value indicates that these particular detergents disperse the test bacteria 1.7 times greater than water, thereby nearly doubling the count. When the most effective substances were buffered to pH 7.9, Plurafac A-26 and Triton X-100 gave dispersal efficiency values ranging from 205 to 220 which were consistently higher than those of Surfonic N-95 and Triton N-101. In general, *in vivo* studies with these compounds paralleled *in vitro* results; Triton X-100 however appeared to be more satisfactory in regard to reproducibility and to maximal removal of microorganisms.

TABLE I

Nonionic detergents

Chemical Type	Trade Name	Manufacturer[1]	Dispersal Efficiency[2]
Polyoxyethylene aliphatic ethers	Plurafac A-16	(1)	139
	Plurafac A-26	(1)	182
	Poly-Tergent J-400	(2)	147
	Brij-35	(3)	80
	Sterox AJ-100	(4)	120
	Surfonic LF-7	(5)	150
Polyoxyethylene sorbitan fatty acid esters	Tween 20	(3)	139
	Tween 40	(3)	122
	Tween 60	(3)	126
	Tween 80	(3)	127
	Tween 81	(3)	119
Alkyl phenoxy polyoxyethylene ethers	Poly-Tergent B-300	(2)	156
	Surfonic N-95	(5)	186
	Triton X-100	(6)	180
	Triton N-101	(6)	173
Polyoxyalkalene block copolymer	Plurafac RA-10	(1)	138

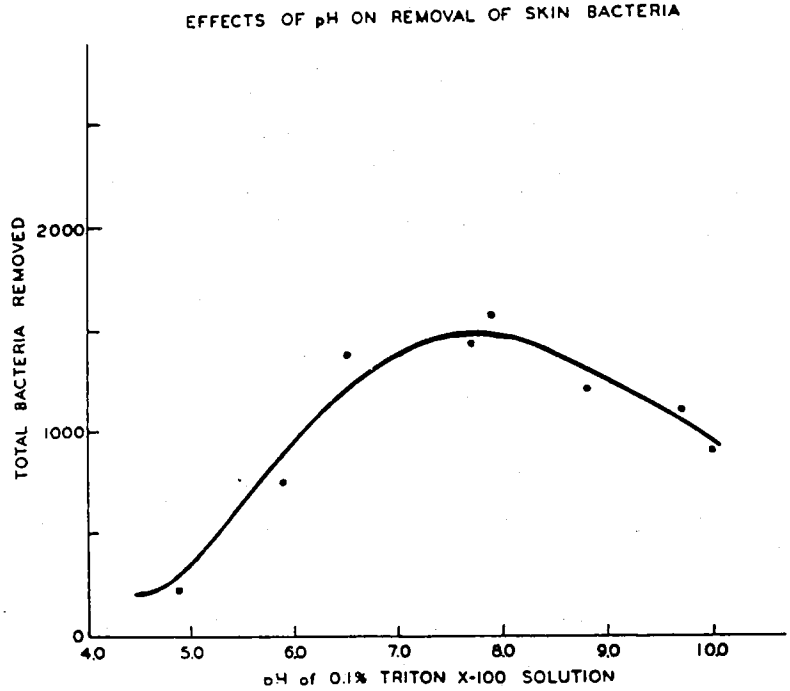
[1] Manufacturers listed according to the following scheme: (1) Wyandotte Chemical; (2) Olin Mathieson; (3) Atlas Chemical; (4) Monsanto; (5) Jefferson Chemical; (6) Rohm and Haas.

[2] Dispersal Efficiency

$$= \frac{\text{Bacterial count, detergent-treated sample}}{\text{Bacterial count, water control}} \times 100$$

2. pH.—The effect of pH on the efficiency of a skin sampling solution is apparently related to some phenomenon of the cutaneous surface, since pH changes in the range of 4–10 have no effect on bacteria in suspension when examined by the method described above. *In vivo* studies, on the other hand, show a small but definite increase in the number of organisms recovered with aqueous alkali over neutral or acid solutions, but it must be emphasized that alkali alone does not approach a slightly acidic aqueous detergent in its ability to recover bacteria from the skin. Examination of buffered solutions of effective detergents has shown in the case of Triton X-100 a definite increase in bacterial removal as the pH increases from 6 to 8, followed by a slight decline which may be due to a

PAGE G



deleterious effect of the alkali alone. The peak efficiency from the five experiments summarized in Fig. 1 is at approximately 7.9. Because of these findings, it was decided that the standard wash fluid be 0.1% detergent (Triton X-100) in 0.075 M phosphate buffer, pH 7.9. The deliberately high buffer concentration was chosen to prevent any lowering of the pH during sampling by the strongly acid buffered skin. Thus, the change in pH of the wash solution after a two-minute scrub was less than 0.05 pH units.

5. Superficial location of aerobic organisms.—

(a) Scotch Tape. Regardless of area, 95% of the organisms were removed after four strips. Ten strips were no more effective. This suggests that the bacteria are in the very outermost horny layer. The almost complete removal by four strips holds whether a normal area having 1,000 bacteria per sq cm is studied or an experimentally hydrated one having 50×10^6 organisms per sq. cm. These results are in accord with recent findings (8) which indicate that the major portion of the horny layer is a coherent membrane which does not provide crevices for the enlodgement of bacteria. The sloughing of the horny layer is a surface phenomenon restricted to the outer three to four layers.

(b) If the wash fluid is left in contact with the skin of the forearm and merely agitated, it was found, in ten different cases, that the percentage recovery was 42.6% of that obtained by manual scrubbing. The aerobic organisms are not only superficial, but also rather weakly adherent.

(c) The same conclusions can be drawn from examining the effect of simple cleansing of the skin with soap. An average of several experiments show a 60–80% reduction in the bacterial flora of the forearm, and a 65–95% reduction on the forehead.

(d) The results of nine serial scrub experiments on three individuals of widely varying bacterial counts are given in Table II: 85% of the organisms are removed in a single one minute scrub

Vol. 45
1976

TABLE II

Serial sampling

Subject	% Organisms removed during each wash			Average
	A[1]	B[2]	C[3]	
1st Wash	81.6	86.5	87.4	85.1
2nd Wash	18.4	8.7	10.6	12.7
3rd Wash	0	4.8	2.0	2.2
Average total organisms recovered per experiment	69	21,665	229	

[1] Average of four experiments.
[2] Average of three experiments.
[3] Average of two experiments.

and 97–98% in two. This emphasizes the necessity of utilizing two scrubs to provide near complete removal.

The efficiency of a detergent-based sampling solution can be appreciated by considering the great ease with which bacteria are removed from keratinized cells not attached to the cutaneous surface. Specimens taken in buffered detergent which have heavy tissue sediments, *e.g.* from the axilla, were found microscopically to contain only disaggregated organisms; subsequent homogenation of the sediment in a glass tissue homogenizer frees no additional bacteria. This is most easily shown with scurf samples from the scalp. Table III summarizes an experiment in which weighed scurf samples were exposed to buffered Triton X-100 for a short period of time and the dispersed bacteria counted. The counts per mg scurf were very much greater than those reported in a similar experiment by VanderWyk and Roia (9), the essential difference between the two studies being the presence of a surfactant. The increase in counts points out the effectiveness of the buffered Triton sampling without the use of severe mechanical methods.

DESCRIPTION OF METHOD AND REPRESENTATIVE RESULTS

The method routinely used in our laboratory is as follows:

(1) The area to be scrubbed (3.8 sq cm) is delineated by a sterile glass cylinder held firmly to the skin by two attached handles.
(2) One ml of wash solution—0.1% Triton X-100 in 0.075 M phosphate buffer, pH 7.9—is pipetted in and the area scrubbed with moderate pressure for one minute using a sterile Teflon 'policeman.'
(3) The wash fluid is aspirated, replaced with a fresh 1 ml, and the scrub repeated.
(4) The two washes are then pooled and an aliquot diluted in 10-fold steps using as diluent 0.05% Triton X-100 in 0.0375 M phosphate buffer to prevent any reaggregation of organisms.
(5) The appropriate dilutions (usually 10^0, 10^{-1}, 10^{-2} for normal skin; 10^{-3} and 10^{-4} for areas of high bacterial density) are plated in 15–20 ml Tryptic Soy Agar per plate.
(6) After 48 hour aerobic incubation at 37° C, colonies are counted and viable cells in the original sample calculated by standard methods.

It must be noted however that in spite of the improved sampling method, variation in bacterial density is still encountered. Table IV lists results obtained with multiple scrubs of adjacent sites on the back of one man and the forearm of another. The similarity of multiple counts on a given day is obvious, as is the fact that day-to-day variations in a single subject are pronounced. These data emphasize the necessity for a minimum of triplicate samples at a given site on a given day.

The possibility of standardizing test conditions of the skin by pretreatment has been considered, in the hope of reducing the variability between counts. Certain treatments most assuredly change the bacterial count; for instance, the use of occlusive dressings or a sustained rise in temperature and humidity, but these serve to increase the population above so-called normal levels, with no decrease in variability between

TABLE III

Dispersion of bacteria from scurf samples

Technique	Number of Subjects	Average bacterial counts per milligram scurf
10 minute soak; one minute mechanical shake in detergent	2	530,000
30 minute mechanical shake in 0.85% saline[1]	9	7558 (Range: 1340–22,970)

[1] For reference, see text.

Vol. 45
1976

TABLE IV

Representative counts from multiple skin samples on adjacent sites

Area Sampled	Day	Bacterial Counts per sq cm		Average
Back	1	110	120	181
		233	253	
		95	276	
Forearm				
	1	1937		2621
		3521		
		2404		
	2	7974		6640
		7263		
		4684		
	3	3947		3273
		2599		
	4	6200		6287
		6354		

samples. Soap and water washes 5 to 24 hours before sampling, quick scrubbing with 95% ethanol 20 hours before sampling, normal cleansing of the skin followed by non-occlusive covering with sterile gauze for as much as 50 hours before sampling, all had no effect on the total number of bacteria obtained or on the variability between samples if triplicate samples were taken. One cause of innocent error, resulting in low counts, is the use of antibacterial soaps by the subject.

TABLE V

Representative aerobic bacterial counts obtained using buffered Triton X-100 scrub technic

Area	Number of Samples	Counts/sq cm	Range
Scalp	24	1.46×10^6	0.22×10^6-14.3×10^6
Axilla	31	2.41×10^6	6300-16.7×10^6
Forearm[1]	17	4500	400-19,000
	15	105	25-300
Back	19	314	50-1450
Forehead	19	0.20×10^6	0.03×10^6-0.55×10^6

[1] Individual forearm results could be conveniently grouped into high and low counts.

As reported by many other investigators (3, 10, cf. 1), there are great regional variations in the quantity of organisms. Table V shows some representative data obtained using the Triton scrub technic described here. As expected, the moist intertrigenous areas and hairy sebaceous areas have the highest counts, the normal glabrous areas of back and forearm having counts lower by a factor of 10^2 to 10^4. The range in counts is given in the last column of Table V to indicate the large differences in bacterial numbers between different individuals. It should be noted that the ranges listed include the extreme values which make up only a minority of the subjects for all test sites. For instance, the axilla figures include out of 31 subjects only two with counts in the $6–7 \times 10^7$ range and two in the $12–17 \times 10^6$ range.

SUMMARY

A simple, reproducible procedure has been described for accurate sampling of the cutaneous microflora. This method utilizes a buffered non-ionic detergent as sampling fluid in order to assure complete removal of bacteria from the skin and to disperse the removed microorganisms so that subsequent colony counts reflect single bacterial cells rather than aggregates. Supporting data from various areas of the body are also given to show the efficiency of a detergent-based sampling fluid.

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Vol. 45
1976

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DISCUSSION

DR. IRVIN H. BLANK, Boston, Mass.: Dr. Williamson, in your curve relating pH to the number of organisms removed with a non-ionic solution, as I remember it, somewhere above a pH of 8, the curve tends to fall slightly. Do you feel that this fall is real and if so, do you think that it is due actually to a decrease in the number of viable organisms removed or is it due possibly to death, because of the high pH, of some of those which are removed.

PETER WILLIAMSON (in closing): We have done a number of these pH curves, and the decrease at high pH seems to be real. What it is due to, we don't really know, but I am inclined to believe that it is due to some sort of selective killing because these studies were done on an arm with a series of different types of organisms, some of which may be more susceptible to the effects of alkali than others.

Vol. 45
1976

PAGE GR

J. Soc. Cosmet. Chem., 36, 231–236 (May/June 1985)

A84

Evaluation of substantivity and antibacterial activity of soap bars on human skin by an *in vivo* agar patch method

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Synopsis

An *in vivo* method, the "agar patch test," has been developed for determining the efficacy of antibacterial agents deposited on human skin from soap bars. This method has the advantage over previously published methods of eliminating the need for germicides to migrate through agar to reach the bacteria since the test bacteria are streaked on the surface of the agar medium and placed in direct contact with the soap-washed skin. This method was used to demonstrate the buildup of germicide activity as a function of the number of washings on forearms washed with a deodorant soap containing 1.5% 3,4,4′-trichlorocarbanilide (triclocarban) compared to forearms washed with a placebo soap (no active ingredient). The results indicate that the extent of triclocarban residual activity deposited on skin washed with the deodorant bar (1.5% triclocarban) in the agar patch test reaches a statistically significant level after 7 washes and remains at that level at least through 13 washes.

INTRODUCTION

There are a number of *in vivo* methods for measuring the number of bacteria on skin, the substantivity of germicides on skin, or the degerming activity of soaps and soap germicides (1–9). Eigen *et al.* (9) stated that their *in vivo* seeded contact plate method could demonstrate differences in residual antibacterial activity remaining on skin after washing with a liquid antibacterial cleanser or antibacterial soap and thoroughly rinsing with water. The advantages of this method over previously published methods were that it allowed free movement of the panelists, controlled the size and type of bacterial population, measured efficacy and not quantity of antibacterial agents found on the skin, and was easy to perform. The disadvantage is that only soap germicides that readily desorb off the skin and migrate through the agar to reach deeply seeded bacteria can be tested by this method. Thus, this method does not work well with the triclocarban (3,4,4′-trichlorocarbanilide) because it has very low water solubility and does not rapidly diffuse in agar media. The "agar patch test" was developed as a modification of the method of Eigen *et al.* (9). The newly developed method eliminates the need for an antibacterial agent to migrate through agar to reach the bacteria since the bacteria are surface-streaked on the agar which is in contact with soap-washed skin.

EXPERIMENTAL

AGAR PLATES

Falcon 1008 petri dishes (35 × 10 mm) without lids containing 11 ml of nutrient agar (Difco), mannitol salt agar (Difco), or trypticase soy agar (BBL) with 1 mg/ml

streptomycin sulfate (Sigma) were placed in 150 × 25-mm petri dishes and dried overnight at 35°C prior to inoculation. The convex surface of the agar plates was streaked with the diluted cultures and allowed to air dry for 30 minutes before attaching to panelists' forearms.

CULTURES

The bacterial cultures were grown overnight in AATCC bacteriostasis broth (Difco) at 35°C. The third to seventh consecutive overnight cultures were used to prepare the diluted inoculums for each experiment.

(A) *Staphylococcus epidermidis* ATCC 155 was maintained on trypticase soy agar (BBL). A 4-mm loopful of an appropriate dilution of a 23-hour-old culture was streaked on the dried agar plates to obtain the desired number of colony-forming units (CFU) per plate.

(B) *Staphylococcus epidermidis* JEH 51 was maintained on trypticase soy agar containing 1 mg/ml streptomycin sulfate. This culture was isolated as a spontaneous streptomycin-resistant colony from *S. epidermidis* ATCC 14990 and has the same agar media growth rate and biochemical properties on Staph-Indent Strips (API) as the parent strain. A 4-mm loopful of the diluted 23-hour-old culture in 0.1% peptone broth was streaked on trypticase soy agar containing 1 mg/ml streptomycin sulfate to obtain approximately 1000–2000 CFU per plate.

PROCEDURE

Six to eight panelists were used for comparing two soap bars in each test. The panelists were instructed to use a placebo soap for all bathing and washing of hands and forearms for 1 week prior to the test. On test days supervised washings of the forearms with the randomly assigned soap bars were conducted in the laboratory. Three agar plates streaked with *S. epidermidis* ATCC 155 were attached to the volar surface of the washed forearms with Blenderm® Surgical (3M) or Dermilite II® (Johnson & Johnson) tape for either 2 or 4 hours. For later experiments trypticase soy agar + 1 mg/ml streptomycin sulfate plates streaked with JEH-51 were taped to the volar surface of the forearms with 1″ by 3″ strips of Microfoam® tape (3M) and wrapped with an Ace® type elastic bandage (Becton-Dickinson) for 30 minutes.[1] The plates were removed, placed in 150 × 25-mm petri dishes, and incubated at 35°C. Control plates were not attached to panelists' forearms but were placed directly in the 35°C incubator. After 24 hours, colonies were counted using a 10× dissecting microscope.

The regimen for the supervised laboratory washings consisted of washing the hands with the placebo soap bar, wetting the volar surface of the forearms and the assigned soap bar under the tap, rubbing the soap bar to the volar surface of the forearm for 15 seconds, lathering for 30 seconds, rinsing under tap water for 30 seconds, blotting forearms dry with paper towels, and repeating the whole procedure for the other forearm and soap bar. In all the experiments performed there was at least a 2-hour drying out time between the different washes conducted over a 2 to 4 day period.

[1] All three taping methods held the entire inoculated portion of the agar plates firmly to the panelists' forearms. However, the Microfoam strips were the easiest and least painful to remove from panelists' skin.

For these experiments, a deodorant soap containing 1.5% triclocarban, sodium tallowate, sodium cocoate, water, glycerin, fragrance, sodium chloride, preservatives, and color was compared to non-germicide placebo bar soap.

CALCULATION

The number of CFUs (colony forming units) on each plate was converted to logs, and mean log CFU/plate determined for each panelist's forearms (treatment). Statistical differences between treatments were determined using the Student's paired t-test. The geometric mean CFU/plate is the antilog of the mean log CFU/plate.

RESULTS AND DISCUSSION

The results of three experiments using the agar patch method are shown in Table I. Regardless of whether nutrient agar or mannitol salt agar was used, the residual antibacterial activity of deodorant soap (1.5% triclocarban)-washed forearms after eight supervised washings showed a 79 to 88% reduction in the geometric mean CFU count compared to the placebo soap-washed forearms. All reductions were statistically significant at the $\leq .025$ probability level. Note that the coefficient of variation (standard deviation/mean) of the log CFU/plate of the control plates ranged from 3.3 to 6.4%. These values are comparable to those obtained in previous experiments using pipets (10), indicating that the use of an inoculation loop is as reproducible as the use of a pipet to inoculate the agar plates.

Table I

A Comparison of Residual Antibacterial Activity of a Deodorant Soap (1.5% Triclocarban) vs. a Placebo Soap (no Triclocarban) by the *In Vivo* Agar Patch Method[a]

Test Number	Panelist Initials/ Soap Used	Mean Log CFU/Plate								$\bar{d}$[b] ±S.D.	% Reduction[c]	p-Value[d]
1[e]	Initials	LS	RD	WF	PF	RB	RC					
	Placebo	1.9318	1.8949	2.3514	2.3784	1.9898	1.8647			.6874 ±.3165	79.5	<.005
	Deodorant	1.1127	1.7703	1.3809	1.7807	1.0249	1.2173					
2[f]	Initials	RL	RD	PF	PW	BB	GG	CS	MH			
	Placebo	1.0887	2.2535	2.2503	2.5692	2.087	1.8221	1.5519	1.8345	.732 ±.7120	81.5	<.025
	Deodorant	1.1937	1.5576	0.8844	2.2329	0	1.6806	1.1032	0.9488			
3[g]	Initials	GG	CS	SE	JM	PF	RB	MH	RC			
	Placebo	1.2915	1.2896	1.1286	1.7654	1.9211	1.7472	1.4053	1.5853	.9401 ±.3916	88.4	<.001
	Deodorant	0.4771	0.7101	0.6990	0.6667	0.2817	1.0195	0.4407	0.3181			

[a] All panelists washed with the test bars for 8 applications. The placebo soap had a different base composition from the deodorant soap. The test bacterium was *S. epidermidis* ATCC 155.

[b] $\bar{d}$ = mean difference of the placebo mean log CFU/plate—the deodorant mean log CFU/plate. S.D. = standard deviation.

[c] The % reduction = (1 − 1/antilog $\bar{d}$) 100.

[d] p-value = the smallest alpha level at which the null hypothesis of no differences can be rejected.

[e] The desired control inoculum was 100 CFU/plate on nutrient agar using a 1/10,000 dilution of the overnight culture in AATTC bacteriostasis broth. Actual control mean log CFU/plate ± S.D. = 2.0188 ± 0.1137 (n = 7). The contact time was 4 hours between the washed forearms and the inoculated plate.

[f] The desired control inoculum was 200 CFU/plate on mannitol salt agar using a 3/10,000 dilution in 0.1% peptone. Actual control mean log CFU/plate ± S.D. = 2.2703 ± .1460 (n = 12). The contact time was 4 hours. Because some of the test plates contained no surviving CFU, "1" was added to all CFU/plate counts to allow mean log CFU/plate to be calculated.

[g] The desired control inoculum was 400 CFU/plate on mannitol salt agar using a 6/10,000 dilution in 0.1% peptone. Actual control mean log CFU/plate ± S.D. = 2.4642 ± .0810 (n = 8). Contact time = 2 hr.

Results from the series of tests shown in Table I indicate that the percent reduct could be duplicated between tests when 6 to 8 panelists were used. Reducing contact time between the skin of the forearm and the streaked plate from 4 hours hours did not affect the residual antibacterial activity of the skin and did red discomfort to the panelist. In later experiments a 30-minute contact time of the streak petri dishes with the skin was shown to be equally effective.

During these first experiments, some of the skin-attached streaked plates became c taminated with resident skin bacteria. To avoid such problems in subsequent exper ments, a spontaneous streptomycin-resistant colony from *Staphylococcus epidermidis* ATC 14990 was isolated. This bacteria was designated as *S. epidermidis* JEH-51. One mi ligram of streptomycin sulfate was also incorporated per milliliter of trypticase soy ag to inhibit the growth of resident skin bacteria on the streaked plates. In an attempt improve test sensitivity and to further decrease the coefficient of variation, the inoculum on the streaked agar plates was also raised from 100–300 CFU to 1000–2000 CFU

The next series of experiments was designed to determine if a buildup of triclocarban germicidal activity on the deodorant soap-washed forearms compared to placebo soap (no triclocarban)-washed forearms could be shown as a function of the number washings. Seven or eight different panelists were used for each experiment. The panelist washed for an average of 5 to 13 washes over a period of 2 to 4 days, with at least 2-hour drying out time between washes. The data for these runs are presented in Table II.

The reduction in bacterial levels observed with the deodorant soap after 5 washing

Table II
A Comparison of the Residual Antibacterial Activity of a Deodorant Soap (1.5% Triclocarban) as a Function of the Number of Washings[a]

Test Number	No. of Panelists[b]	No. of Applications Mean (range)[c]	$\bar{d}$ ± S.D.	% Reduction	p-Val
4	8	5.25 (4–7)	0.1722 ± .5172	32.7	N.S.
5	8	5.13 (5–6)	0.0821 ± .1548	17.2	N.S.
4 + 5[e]	16	5.19 (4–7)	0.1271 ± .3717	25.4	N.S.
6	8	7.25 (7–8)	0.1400 ± .1574	27.6	<.05
7	7	9.57 (8–10)	0.2429 ± .2520	42.8	<.05
8	8	13.0 (13)	0.1439 ± .1170	28.2	<.02

[a] The placebo soap used had the same base composition as the deodorant soap. The test bacterium was *S. epidermidis* JEH 51. The desired control inoculum was 1,000–2,000 CFU/plate using a 3/2,000 dilution of the overnight culture. Actual control mean log CFU/plate ranged from 3.0365 to 3.2764 with coefficients of variation of 1.21 to 3.38%. The contact time of the inoculated plates on the washed forearms was 30 minutes. Other abbreviations are given in Table I.

[b] Different panelists used in each test.

[c] All panelists participated in a minimum number of supervised washings (the minimum number given under "Range") over a 2–4 day period. In addition, panelists were issued color-coded test bars, and instructed to perform any *ad. lib.* washings of their forearms with the test bars and to record the number of such washings. The total of the supervised plus *ad. lib.* washings is the maximum number of applications for any panelist in the test (the maximum number under "Range"). The "mean" is the average number of applications per panelist.

[d] N.S. = nonsignificant ($p > .05$).

[e] Data from test 4 and 5 combined.

was not statistically significant, even when the results from two independent tests were combined. This finding suggests that the magnitude of the reduction after 5 washings is less than that observed with 7 or more washings, which gave significant reductions. Since the magnitude of the reductions after 7, 9, and 13 washes are statistically indistinguishable, the extent of triclocarban residual antimicrobial activity deposited on deodorant soap (1.5% triclocarban)-washed skin in the agar patch test apparently reaches a maximum after 7 washes and remains at that level at least through 13 washes. A plausible interpretation of this result is that after 7 washes, the amount of antimicrobial activity that is absorbed on the skin is in equilibrium with the amount of antimicrobial material that desorbs off the skin with additional washings.

Increasing the number of CFU/plate in the experiments in Table II did reduce the coefficients of variation of the control plate counts. However, the magnitude of the reductions observed in Table II are smaller than those observed in Table I. Since there were a number of differences in experimental procedures between the two sets of tests, described in the table footnotes, the actual reason(s) for the smaller magnitudes observed in Table II remain(s) an area for further investigation. Nevertheless, statistically significant reductions were observed with 6–8 panelists in both sets of tests after 7–8 applications of the deodorant bar soap.

CONCLUSION

The agar patch test is a useful method for determining residual antimicrobial activity on soap-washed skin. This method eliminates the need for chemicals to migrate through agar to reach the deep-seeded organisms, since the organisms are streaked on the surface of the agar which is in direct contact with the soap-washed skin. Contamination of the contact plates by skin bacteria can be reduced by using a streptomycin-resistant strain and streptomycin-containing media. This method can be used for evaluating antimicrobials or antimicrobial products which are intended to be washed off the skin after use. In principle, any relatively flat area of the human body could be used in this test, although the volar surfaces of the forearms are the most conveniently studied site. Using this method, it was shown that repeated washing with a deodorant soap containing 1.5% triclocarban deposits measurable germicidal activity on panelists' skin.

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Validation of the agar patch test with an antibacterial liquid soap and comparison with the finger imprint method

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Synopsis

The purposes of this investigation were: (1) to evaluate the ability of the agar patch test to detect the antibacterial activity in a liquid hand soap containing 2,4,4′-trichloro-2′-hydroxydiphenylether (triclosan) and (2) to compare the results from the agar patch test with those obtained using the finger imprint test. Both test methods were developed to evaluate the bacteriostatic activity deposited on panelists' skin by washing with antibacterial soap bars; neither test had been previously used to evaluate liquid hand soaps or triclosan-containing products. In the present investigation both test methods detected bacteriostatic activity on panelists' skin after one wash with a detergent-based liquid hand soap containing triclosan. As expected, no such activity was detected after washing with nonantimicrobial detergent-based or soap-based liquid soaps or with a nonantimicrobial bar soap. The results with triclosan are in marked contrast to the previously published results with 3,4,4′-trichlorocarbanilide (triclocarban), the most commonly used bar soap antimicrobial. Triclocarban is poorly detected with the finger imprint test but easily detected with the agar patch test. The conclusion of this investigation is that both the agar patch test and the finger imprint test are suitable for evaluating triclosan-containing liquid soaps.

INTRODUCTION

The agar patch test (1,2) is an *in vivo* method for evaluating the skin substantivity and antibacterial activity of bar soaps containing triclocarban (3,4,4′-trichlorocarbanilide). The test method was developed as a modification of the method of Eigen *et al.* (3), which does not readily detect the activity of triclocarban since this antimicrobial has very low water solubility and does not rapidly diffuse through agar to reach seeded bacteria. The agar patch test uses surface-streaked bacteria to overcome this limitation of the Eigen *et al.* method (3) and allows the detection of triclocarban activity deposited on forearms during washing with soap bars containing this antimicrobial (1,2).

Workable tests, such as the agar patch test, may be most useful in the development and evaluation of new products. Liquid "soaps," whether detergent-based or soap-based, have become a large and important segment of the consumer market in the US and abroad. Recently a detergent-based antibacterial liquid hand soap was developed and marketed which contains triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) as the

antibacterial. Consequently, we wished to determine if the agar patch test could detect the skin substantivity and antibacterial activity of triclosan-containing liquid soaps. An additional purpose was to compare the results from the agar patch test on this soap with those from the finger imprint test (4). This test, like the agar patch test, was developed to evaluate antibacterial soap bars by measuring the bacteriostatic activity deposited on panelists' skin. In contrast to the agar patch test, triclocarban is poorly detected in the finger imprint test (4). Liquid soaps or triclosan-containing products have not been previously evaluated by either test method.

EXPERIMENTAL

SOAPS

1. Liquid soap A (antibacterial, commercial product) contained water, ammonium lauryl sulfate, lauramide DEA, sodium laureth sulfate, disodium ricinoleamido MEA-sulfosuccinate, citric acid, DMDM hydantoin, tetrasodium EDTA, FD&C yellow #5, and FD&C red #4. The active ingredient was triclosan.
2. Liquid soap B (nonantibacterial, made in the Dial Technical Center pilot plant) was the same formula as liquid soap A except that it contained no triclosan.
3. Liquid soap C (nonantibacterial, commercial product) contained water, lauric acid, oleic acid, potassium hydroxide, glycerin, fatty acids, preservative, and fragrance.
4. Soap bar D (nonantibacterial, commercial product) contained sodium tallowate, sodium cocoate, potassium tallowate, potassium cocoate, sodium chloride, alcohol insolubles (inorganic material), glycerin, magnesium silicate, chelating agent, and fragrance.
5. Soap bar E (nonantibacterial, made in Dial Technical Center pilot plant) contained sodium tallowate, sodium cocoate, water, sodium chloride, glycerin, preservative, and color.

PANELISTS

Informed consent was obtained from all panelists. Only volunteers with undamaged skin were studied. The panelists participated in a one-week washout (handwashing and bathing) with bar soap E (placebo) prior to beginning the study. They were instructed to refrain from using cosmetic products such as lotions or perfumes on the hands or forearms for 24 hours prior to the test. They were also told not to use antibacterial antiperspirants, deodorants, soaps, or shampoos. Nonpowdered rubber gloves were provided for panelists to use when hands came in contact with acids, bases, or solvents. Panelists were required to refrain from swimming in biocide-treated pools, hot tubs, spas, etc., for 24 hours prior to the test. Panelists with open cuts or wounds on their hands or forearms on the day of the test were excluded from participating in the study.

At the conclusion of each test, the panelists washed their hands with antibacterial liquid soap A to remove any bacteria that might have been picked up during the test.

AGAR PATCH TEST

Test culture. A streptomycin-resistant derivative of *Staphylococcus epidermidis* ATCC

14990 was maintained on trypticase soy agar (BBL) plates containing 1 mg/ml of streptomycin sulfate. The third consecutive overnight culture of the streptomycin-resistant *S. epidermidis* grown in tryptic soy broth (Difco) and diluted with 0.1% peptone was used in this test. Falcon 1008 petri dishes (35 × 10 mm) containing 11 ml of trypticase soy agar with 1 mg/ml streptomycin sulfate were streaked with 1000–2000 CFU/plate using a 4-mm loop. The streptomycin in the media prevents contamination of the plates by resident skin bacteria during the test procedure (1).

Wash procedure. The hands and forearms were wetted under warm, running tap water. The hands were washed with placebo soap bar E to remove transient bacteria. In the first test, one pump of liquid soap B (nonantibacterial) was added to one hand and carefully rubbed over the volar surface of the opposite forearm for 15 seconds. From the tap, a small volume of water was added to the same hand, and the volar surface of the opposite forearm was lathered for 30 seconds. The hand and forearm were rinsed for 30 seconds and blotted dry with paper towels. The identical procedure was repeated with liquid soap A (antibacterial) using the other hand and forearm. The assignment of the soaps to panelists' hands and forearms followed a computer-generated randomization table.

In the second test, commercial liquid soap C (soap-based) was used instead of pilot plant-made liquid soap B (detergent-based). Also, eight panelists were used in the second test instead of the four used in the first test. Otherwise, the two tests were conducted identically.

Test procedure. Three *S. epidermidis* streaked trypticase soy agar plates ("agar patches") containing 1 mg/ml streptomycin sulfate were attached to the volar surface of each forearm with 1-inch × 3-inch strips of Microfoam® tape (3M) and wrapped with an elastic bandage (Becton-Dickinson) for 30 minutes. The plates were then removed from the forearms, placed in 150 × 25-mm petri dishes, and incubated at 35°C. Streaked controls, which were not attached to the forearms, were incubated in the same way. Twenty-four hours later, the colonies in all plates were counted with the aid of a 10X dissecting microscope.

FINGER IMPRINT TEST

Test cultures. ***Staphylococcus epidermidis*** ATCC 14990 and *Escherichia coli* ATCC 11229 were individually grown in brain heart infusion broth (BBL), and a 1% inoculum of each bacterium was prepared in individual bottles of melted and cooled (48°C) trypticase soy agar. Bacteria-seeded plates were prepared by placing 15 ml of trypticase soy agar in sterile petri dishes and allowing the agar to harden. Then 5 ml of the 1% culture in the melted trypticase soy agar was carefully spread over the surface of the hardened trypticase soy agar and allowed to solidify.

Wash procedure. Panelists first washed their hands with a placebo soap bar (E) to remove transient bacteria. In the first test, two pumps (3.7 g) of either liquid soap A or B were delivered into panelists' wet hands from a dispenser. The panelists then applied the soap by rubbing it over their hands for 15 seconds. Panelists then wetted their hands with a small amount of tap water and lathered the soap for 60 seconds. Finally the panelists rinsed their hands under the tap for 30 seconds and blotted both hands dry with paper towels.

The second experiment was identical to the first except that (1) commercial liquid soap C (soap-based) was used instead of pilot plant-made liquid soap B (detergent-based) and (2) commercial soap bar D was also tested. Panelists who were assigned soap bar D followed the same washing procedure except that they picked up the soap bar and wetted it under warm running tap water before rubbing the soap over their hands for the 15-second application step.

Test procedure. Immediately after washing and drying his/her hands, each panelist gently but firmly pressed the four fingers (thumb not used) of one hand to the surface of an *S. epidermidis*-containing plate for 30 seconds and the four fingers of the other hand to an *E. coli*-containing plate for 30 seconds. The assignment of the plates to panelists' hands, as well as the assignment of soaps to the panelists, was determined by a computer-generated randomization table. After the last panelist touched his/her plate, all the plates were incubated at 35°C for 24 hours.

The plates were then examined for clear areas underneath the panelists' finger imprints. Three trained judges independently rated the clarity using the following scale:

Overgrowth (no clearing) = 0
Turbid area (slight clearing) = 1
Clear area with growth = 2
Clear area with rough edge = 3
Clear area with smooth edge = 4

The judges were not informed of the identity of the test soap used by individual panelists until after the evaluation.

RESULTS

AGAR PATCH TEST

The purpose of this investigation was to determine if the antibacterial activity of a detergent-based liquid soap could be tested by the agar patch test method. Previously, the method has been validated only with soap bars containing different concentrations of triclocarban (1,2).

In these studies, liquid soap A (detergent-based) containing triclosan was compared with two nongermicide liquid soaps against a streptomycin-resistant derivitive of *Staphylococcus epidermidis* ATCC 14990. The data for these tests are presented in Table I and Table II. Analyses of the data in both tables showed that complete inhibition (100% reduction compared to either nonantibacterial liquid soap) was obtained with liquid soap A against *S. epidermidis*. The results in Table I and in Table II were both significant at a p-value of less than 0.0001. These results showed that the residual antibacterial activity of a triclosan-containing liquid soap can be successfully demonstrated by the agar patch test. Moreover, the results demonstrate that the residual antibacterial activity of liquid soap A is due to the triclosan, since liquid soap B shows no residual antibacterial activity and is identical to liquid soap A except that liquid soap B contains no triclosan. The results also show that both detergent-based and soap-based liquid soaps can be tested using the agar patch method. Based on these and previously published results (1,2), direct comparisons between the residual antibacterial effectiveness of bar and liquid soaps could readily be made using the agar patch test.

Table I
Agar Patch Test: A Comparison of Residual Bacteriostatic Activity Against *Staphylococcus epidermidis*[a] of Commercial Liquid Soap A (Detergent-Based, Containing Triclosan) Versus Pilot Plant-Made Liquid Soap B (Same Formula but No Triclosan) After One Application

Soap used	Mean log CFU/plate[b] Panelist: SC	EL	JF	PW	$\bar{d} \pm SD$[c]	% Reduction[d]	p-Value[e]
A	0.0000	0.0000	0.0000	0.0000	1.981 ± .0914	100	<.0001
B	2.1270	2.0132	2.0190	1.7652			

[a] A spontaneous streptomycin-resistant isolate from *S. epidermidis* ATCC 14990, which has the same agar media growth rate and biochemical properties on Staph-Ident strips (API) as the parent strain.
[b] Contact time between forearms and streaked plates was 30 minutes.
[c] $\bar{d}$ = mean of (soap A mean log CFU/plate minus soap B mean log CFU/plate). SD = standard deviation.
[d] The % reduction = (1- 1/antilog $\bar{d}$) × 100.
[e] p-Value = the smallest alpha level at which the null hypothesis of no difference can be rejected.

FINGER IMPRINT TEST

The purpose of this investigation was to compare the residual antibacterial activity of detergent-based antibacterial liquid soap A with nonantibacterial liquid soap B (detergent-based), and nonantibacterial liquid soap C (soap-based) and nonantibacterial soap bar D against *Staphylococcus epidermidis* ATCC 14990 and *Escherichia coli* ATCC 11229. Only soap and detergent bars had been tested in the original publication describing this method (4).

The data for these experiments are presented in Table III and Table IV. An examination of the test plates revealed clear zones under the imprints of the fingers washed with antibacterial liquid soap A and no zones under the imprints of the fingers washed with the nonantibacterial liquid or bar soaps. The results demonstrate that the antibacterial agent (triclosan) deposited on the fingers of the panelists who had washed with liquid soap A had transferred from their fingers to the seeded agar and prevented the growth of the bacteria. These results imply that the antibacterial agent deposited on the fingers before imprinting was at a high enough concentration to prevent the multiplication of such bacteria on fingers.

Statistical analyses of the data using Student's t-test for independent data were performed. Fingers washed with liquid soap A produced higher mean clarity scores than

Table II
Agar Patch Test: A Comparison of Residual Bacteriostatic Activity Against *Staphylococcus epidermidis*[a] of Commercial Liquid Soap A (Detergent-Based, Containing Triclosan) Versus Commercial Liquid Soap C (Soap-Based, No Antibacterial Agent) After One Application

Soap Used	Mean log CFU/plate Panelist: DK	RK	JW	BC	MW	JF	MB	PD	$\bar{d}$ ± SD	% Reduction	p-Value
A	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.3019 ± .3100	100	<.0001
C	2.4048	2.4752	2.5303	2.3100	2.2596	2.5661	1.5894	2.2797			

[a] See footnotes in Table I for explanation of terms.

Table III

Finger Imprint Test: Evaluation of Commercial Liquid Soap A (Detergent-Based, Containing Triclosan) With Pilot Plant-Made Liquid Soap B (Same Formula but No Triclosan) Against *Staphylococcus epidermidis* ATCC 14990 and *Escherichia coli* ATCC 11229

		Staphylococcus epidermidis					*Escherichia coli*				
			Rating of imprint zone[a]					Rating of imprint zone[a]			
Panelists	Soap	Hand	Judge 1	Judge 2	Judge 3	p-Value[b]	Hand	Judge 1	Judge 2	Judge 3	p-Value[b]
EM	A	R	2	2	2		L	0	0	0	
KO	A	R	2	3	2		L	0	0	0	
JF	A	R	2	2	2	<.0001	L	0	0	0	.3409
EL	A	L	2	3	2		R	0	0	0	
JM	A	R	2	4	4		L	2	2	2	
PHy	A	L	1	3	2		R	0	0	0	
6 Panelists	B	2R/4L	0	0	0		4R/2L	0	0	0	

[a] Scale used: 4 = Clear area with smooth edges. 3 = Clear area with rough edges. 2 = Clear area with colonies in area. 1 = Turbid area. 0 = Completely overgrown.
[b] p-Value = the smallest alpha value at which the null hypothesis (no difference between soap A and soap B) can be rejected.

fingers washed with the three other soaps. These differences were significant in both tests with *S. epidermidis* ($p < .0001$) and in the second test with *E. coli* ($p = .02$).

DISCUSSION AND CONCLUSION

The agar patch test and the finger imprint test are both methods that can be used for testing antibacterial soaps that have residual antibacterial properties.

Table IV

Finger Imprint Test: Evaluation of Commercial Liquid Soap A (Detergent-Based, Containing Triclosan) With Commercial Liquid Soap C (Soap-Based, No Antimicrobial) and Commercial Soap Bar D (No Antimicrobial) Against *Staphylococcus epidermidis* ATCC 14990 and *Escherichia coli* ATCC 11229

		Staphylococcus epidermidis					*Escherichia coli*				
			Rating of imprint zone[a]					Rating of imprint zone			
Panelists	Soap	Hand	Judge 1	Judge 2	Judge 3	p-Value[b]	Hand	Judge 1	Judge 2	Judge 3	p-Value[b]
MW1	A	R	4	2	3		L	0	0	0	
MS	A	R	4	4	4		L	2	1	1	
CM	A	L	4	4	4	<.0001	R	0	0	0	014
VC	A	R	4	4	4		L	0	0	0	
WJ	A	R	4	4	4		L	0	0	0	
JF	A	R	4	4	4		L	1	1	1	
6 Panelists	C	4L/2R	0	0	0		4R/2L	0	0	0	
6 Panelists	D	5R/1L	0	0	0		5L/1R	0	0	0	

[a] See Table III for scale used.
[b] p-Value = the smallest alpha value at which the null hypothesis (no difference between soap A and soap B or C) can be rejected.

Both tests use relatively simple microbiological procedures, although the finger imprint test is less time-consuming to perform than the agar patch test. The results of the agar patch test are more quantitative than those from the finger imprint test since the agar patch test quantitates the reduction in bacterial counts while the finger imprint test uses a qualitative scale to grade zones of inhibition. Also, the sites on the panelists' skin that are tested differ in the two tests: the agar patch test uses the inside or volar surface of the forearms, while the finger imprint tests uses the finger tips. Despite some differences in the tests, the results of this investigation demonstrate that both test methods can be successfully used to evaluate the skin substantivity and antimicrobial activity of triclosan-containing liquid soaps.

Previous results (1,2) have indicated that the agar patch test can also be used for detecting triclocarban, the most commonly used antimicrobial in bar soaps. However, triclocarban is reported to be poorly detected with the finger imprint test (4). Possible factors in causing this difference in the spectrum of soap antimicrobials detected in the two methods include: (1) different methods for inoculating the test plates (agar overlay in the finger imprint test; surface streaking in the agar patch test); (2) different skin surfaces used (finger tips in the finger imprint test; forearms in the agar patch test); (3) different contact times between the soap-washed skin and the agar test plates (30 seconds in the finger imprint test; 30 minutes in the agar patch test); and (4) different criteria used for detecting antimicrobial activity (zones of inhibition in the finger imprint test; bacterial colony counts in the agar patch test). The relative importance of factor number one is suggested by our experience with the Eigen *et al.* method (3), which also poorly detects triclocarban despite the use of forearms as the test site, long contact times (four hours), and quantitation of bacterial colonies as the test parameter. Indeed, the only modification of the Eigen *et al.* method (3) we found necessary to detect triclocarban was switching from the use of an agar overlay to surface streaking (1). Whether or not soaps containing triclocarban could be detected in the finger imprint test if it were modified to incorporate surface streaking of test bacteria is a matter for further investigation.

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