factor is taken into account in the design of the study or the analysis of the data. This conclusion is reinforced by the occurrence of most of the deaths from rheumatic fever (10 of 14) among cases with pre-existing heart disease, and the absence of deaths among the cases without heart involvement at start of treatment.

No comparisons have been made with the conflicting reports of results obtained with large-dose hormone therapy,⁷⁻¹¹ the most recent of which shows no advantage in a well-controlled study.¹¹ A firm decision on the efficacy of large-dose hormone treatment of rheumatic fever will depend on controlled studies of adequate size in which the initial status of the heart is taken into account.

Summary

A study has been made at the end of five years after the conclusion of treatment of the 497 children who were admitted to the U.K./U.S. co-operative clinical trial of the relative merits of A.C.T.H., cortisone, and aspirin in the treatment of acute rheumatic fever.

Four hundred and forty-five of the cases (89.5%) were followed for the complete five years, and the status of the heart was known for 426 of them. Only 16 (3.2%) had died, 14 of them from rheumatic heart disease; 36 (7.2%) were untraced. The very low fatality rate is striking.

At the end of five years there is no evidence, on the treatment schedule used in this study, that the prognosis has been influenced more by one treatment than another. This confirms the findings reported at one year.

The major factor in determining the incidence of rheumatic heart disease at the end of five years is the status of the heart at the time treatment was begun. For cases without carditis initially the prognosis was excellent, since in 96% there was no residual heart disease. In cases with carditis initially, but without pre-existing heart disease, the proportion without residual heart disease decreased progressively from 82% for those with only a grade 1 apical systolic murmur to 30% for those with failure and/or pericarditis. In cases with pre-existing heart disease the prognosis was poor. Only 30% of those without pericarditis or failure and none of those with pericarditis and/or failure were without heart disease at five years.

Cases with carditis and without pre-existing heart disease which had recurrences demanding retreatment during the follow-up period had on the average a more severe cardiac status at start of treatment than did those without recurrences requiring re-treatment. At five years a larger proportion of these re-treated cases had murmurs.

These results make it clear that treatment of acute rheumatic fever cannot be properly evaluated unless the status of the heart of the patients at the start of treatment is taken closely into account.

The National Heart Institute of the United States Public Health Service supported the study with grants to the co-ordinating centre and to the co-operating centres in the United States and by a travel grant to the Medical Research Council of Great Britain. The Medical Research Council provided support for the six centres and for a co-ordinating registrar in the United Kingdom. The centre in Toronto received a grant from the Canadian Arthritis and Rheumatism Society. In the United States the costs of the medical care of its patients were met by each co-operating centre,

and in Great Britain by the National Health Service. The American Heart Association provided office space for the American Co-ordinating Centre and a grant for statistical services.

This co-operative clinical trial was first proposed by Dr. John R. Mote, then assistant general manager of the Armour Laboratories. For the planning of the study, funds were provided by Armour Laboratories and Merck and Company, and space and services by the Helen Hay Whitney Foundation.

In the planning and conduct of this trial much is owed to the wise advice and guidance of the late Dr. T. Duckett Jones and the late Sir James Spence.

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DISINFECTION OF THE SKIN OF OPERATION SITES

BY

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Disinfection of the skin has been assessed in terms of the removal of superficial organisms only or of organisms from the deeper as well as the more superficial layers. Gardner and Seddon (1946), Gardner (1948), and Story (1952) found that several antiseptics (notably 1% iodine in alcohol) could, in 20 to 30 seconds, bring about "virtual disinfection"—that is, removal of 99.9% or more—of organisms previously deposited and allowed to dry on the skin; in their view the dangers of wound infection come largely from the superficial or "transient" flora which they simulated in their tests. Price (1938, 1957) described a method of serial hand-washing by means of which estimates of the deeper (or "resident") flora could be made; by this method considerable numbers of resident organisms were found to persist after two minutes' exposure to various antiseptics, including 1% iodine in alcohol. Most of the deeper bacteria proved to be harmless commensals, but pathogenic organisms were also found among them.

The surgeon's knife will carry surviving bacteria from all levels of the skin to the tissues, so it is desirable to know the effects of antiseptics on the bacteria of the deeper layers as well as on the transient bacteria of the

surface. Moreover, as the deeper bacteria are not completely removed even by the most effective antiseptic, it is possible to make quantitative comparisons of the relative effectiveness against them of different antiseptic applications; this cannot be done satisfactorily in tests on the superficial flora.

In the studies reported here we have tested a number of antiseptics both against pathogens deposited on the surface and against the normal skin flora; in the latter study a hand-washing method of sampling was used, and the comparative value of the antiseptics was statistically assessed by the use of a Latin square design. This study included a quantitative comparison of selected modern antiseptics with alcohol and iodine, an assessment of the value of repeated disinfection, and tests of the degree of association between counts of total organisms and of *Staphylococcus aureus* in handwash-sampling before and after disinfection.

Materials and Methods

Antiseptics Tested.—The following antiseptics were tested: ethyl alcohol (70% w/v in water); 1% iodine in 70% ethyl alcohol; 1% iodine with potassium iodide in water (Lugol's iodine); "virac," an iodophore consisting of a quaternary ammonium compound coupled with 0.2% available iodine (Frisch, Davies, and Krip-paehne, 1958); 0.5% chlorhexidine ("hibitane") digluconate in 70% ethyl alcohol; 0.5% chlorhexidine diacetate in water; 0.1% phenylmercuric dinaphthyl methane disulphonate ("penotrane") in water; and cetrimide (1% and 2%) in water. Hexachlorophane soaps, which reduce the skin flora on repeated application (see Lowbury and Lilly, 1960), were not included in this study, because their action is slow and therefore less appropriate for operation sites than for the hands of surgeons and nurses.

Tests on Transient Flora

Tests were made on members of the laboratory staff by a method based on that of Story (1952). Three circular areas 4 cm. in diameter were marked out on the front of each forearm. One drop (0.02 ml.) of a washed suspension in Ringer's solution of an overnight nutrient broth culture of a penicillin-resistant *Staph. aureus* was spread in each of these areas. When dry, four of the sites were treated with antiseptic, three drops of which were spread with a wire loop over the whole area and allowed to dry; one or, more often, two control areas were left untreated. Aqueous solutions dried in about two minutes. Alcohol and alcoholic solutions, which dried in about 20 seconds, were tested in this way, and also after repeated applications, to keep the surface moist for about two minutes. Virac, which is a viscous fluid, dried very slowly, and the surface treated with this material was therefore gently blotted dry after two minutes; because of the difficulty of spreading virac, tests were done with six drops as well as three drops applied to the test area.

Bacterial sampling was done by firmly applying truncated glass cylinders, 4 cm. across and about 2.5 cm. deep, to the test areas, measuring 5-ml. amounts of Ringer's solution with neutralizers (see below) into these "cups," and rubbing the skin in a standard way with a glass spreader for 15 seconds. In the tests on the controls, and on all the antiseptics except penotrane, pour-plates were prepared with 1 ml. of tenfold dilutions of the washings in nutrient agar containing the appro-

priate neutralizers and 0.3 unit of penicillin per ml. to inhibit the growth of normal skin flora. Colonies were counted after 48 hours' aerobic incubation at 37° C. Some tests (on alcoholic iodine and chlorhexidine) were also made with a strain of *Pseudomonas pyocyanea* (1/100 dilution of overnight broth culture) as the test organism.

The following neutralizers were included in the culture medium: for iodine, sodium thiosulphate, 0.04%; for chlorhexidine, "lubrol W" 1% and lecithin 0.5%; for virac, sodium thiosulphate 1% and "tween 80," 1%; for cetrimide, sterile milk (an equal volume added to the washings before pouring plates); for penotrane, sodium thioglycollate 0.15% (the highest concentration which was not itself inhibitory to the staphylococcus). To confirm the effectiveness of neutralization, samples were taken as described above from control areas of skin which had been treated with antiseptics, and small inocula (0.02 ml. of 1/500,000 dilution) of broth cultures of *Staph. aureus* were seeded in duplicate, (1) in the sampling fluids and media, and (2) in control fluid and medium. Viable counts showed that neutralization was complete for all the antiseptics except cetrimide and penotrane.

For penotrane, which appeared in preliminary tests to have destroyed the staphylococci deposited on the skin, we adopted a modified sampling method in which 1-ml. washings were diluted (1/10) and divided between the smallest number of pour-plates (about 20) which showed no inhibition of added staphylococci.

Tests on Resident Flora

The method of sampling was similar to one previously used for the assessment of hexachlorophane soap (Lowbury and Lilly, 1960). The hands were first washed with ordinary bar soap and water under a running tap for 30 seconds, and dried on a sterile towel. A sample of hand-washings was then taken in 100 ml. of a sampling fluid in a bowl (Ringer's solution with a mixture of neutralizers described below). Both hands were thoroughly moistened and then rubbed firmly three times palm to palm, three times with the right palm over the left dorsum, three times with the left palm over the right dorsum, and three times with fingers interlacing; the hands were thoroughly rinsed before and after each of these procedures, and an effort was made to keep the technique as consistent as possible.

After this sampling the hands were rinsed in running water and dried on a sterile towel, and the antiseptic was applied with a gauze swab to the whole surface of both hands, the skin being kept visibly moist for approximately two minutes; to achieve this a single application of aqueous solutions and one or two reapplications of alcoholic solutions were required; virac was blotted dry after two minutes. A second sample of hand-washings was then taken by the same method as initially. In the control experiments two successive samplings were made, with a rinse only between the samplings. Tenfold dilutions of the washings were made in sampling fluid, and pour-plates were prepared from 1-ml. amounts of each dilution. After 48 hours' aerobic incubation at 37° C. colonies were counted.

The sampling fluid was Ringer's solution containing sodium thiosulphate (1%), lubrol W (1%), lecithin (0.5%), and tween 80 (1%); the same neutralizers were included in the nutrient agar used for pour-plates. In

this way all the antiseptics which were studied by this method were completely neutralized in the inocula used for counting, as shown by duplicate test inoculation of staphylococci on to the plates used for sampling and on to control plates. In each experiment tests were made by this method for inhibitory traces of antiseptic in the sample plates used for counting.

Since only one treatment can be satisfactorily tested at a time, and since personal variation might be important, a Latin square design was used which permitted the separate calculation of the contributions of effect of treatment, person, and days (Fisher, 1942).

Results

Tests on Transient Skin Flora

The effects of antiseptics on the cultures of *Staph. aureus* deposited on the skin are summarized in Table I.

TABLE I.—Survival of Added *Staph. aureus* After Treatment of Skin with Different Antiseptics

Antiseptic	Approx. Duration of Treatment (min.)	Tests	Viable Counts per ml. Washings from Untreated Areas (Range)	% Organisms Surviving after Treatment†
Ethyl alcohol (70%)	0.4*	4	39,520–122,000	0.008–1.2
Iodine (1%) in alcohol (70%)	2.0	3	236,000	<0.0004
Chlorhexidine (0.5%) in alcohol (70%)	0.4*	7	1,260–7,860	<0.012–0.24
Lugol's iodine	0.4*	2	122,000	0.002–0.03
Virac 3 drops	2.0	2	282,000	<0.0003
(6 drops)	2.0	2	282,000	<0.0003–0.007
Chlorhexidine (0.5%) in water	2.0	2	282,000	<0.0003
Cetrimide (2%) in water	2.0	2	209,000	>0.09–>0.14
Penotrane (0.1%) in water	2.0	4	1,600–81,000	6.4–12.2

* Until evaporation of the fluid.

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

No bacteria were isolated from samples taken after two minutes' treatment with 70% ethyl alcohol, with virac, with aqueous chlorhexidine, and with aqueous iodine (Lugol's); after 20 seconds' treatment—that is, until drying—with alcohol and with alcoholic iodine or chlorhexidine, very small numbers of staphylococci were still present, the proportion of survivors being slightly higher with alcohol than with the alcoholic

solutions. A much larger proportion of survivors was found after two minutes' treatment with penotrane. Some survivors were found after two minutes' treatment with 1% and 2% aqueous cetrimide, and the effect of this antiseptic was clearly smaller than these tests would suggest, for the milk which was used as a neutralizer did not, in fact, prevent some inhibition by cetrimide transferred to the culture plates.

Quantitative measures of the relative value of different skin antiseptics could not be obtained by this method, and we used it as a screening method for the exclusion of less effective antiseptics. Penotrane and aqueous cetrimide were clearly less effective than the other antiseptics in our series; they were therefore excluded from the next stage of this study—the comparative assessment of antiseptics on the resident skin flora.

Tests on Resident Skin Flora

A comparative study was made on the following antiseptics: 70% ethyl alcohol, 1% iodine in 70% ethyl alcohol, 0.5% chlorhexidine digluconate in 70% alcohol, Lugol's iodine, 0.5% chlorhexidine diacetate in water, and virac. Each of these agents, and a control rinse in running water, were tested on the hands of each of seven subjects; a minimum of one week was left between tests. Since the treatments were randomly allotted in a Latin square design, each treatment had equal chances of being affected by possible variation due to differences such as room temperature or humidity on different days. The performance of each agent was measured by the reduction in bacterial counts after its use, expressed as a percentage of the initial counts. Table II shows these results and averages derived from them. Analysis of variance (Table III) demonstrates a marginally significant variance due to differences

TABLE III.—Analysis of Variance

Item	Sum of Squares	Degrees of Freedom	Mean Square	Significance
"Persons"	378	6	63	Not sigt. (p>0.2)
"Days"	2,164	6	361	Borderline p=0.05
"Treatment" ..	21,318	6	3,553	Sigt., p<0.001
Residual	4,823	30	160.7	(S.D. of single observation=12)
Residual + "Persons" = "Error" ..	5,201	36	145	

TABLE II.—Reduction in Bacterial Counts from Hand-washes expressed as Percentage of Initial Count (Measured at 10⁻² Dilution)

	Days							Totals (Treatments)	Mean % Reduction
	1	2	3	4	5	6	7		
Alcohol	(5) 72	(1) 53	(6) 59	(3) 60	(4) 67	(7) 93	(2) 76	480	68.6
Alcoholic iodine	(2) 72	(5) 83	(3) 93	(7) 78	(1) 81	(4) 83	(6) 75	565	80.7
Lugol's	(6) 67	(2) 73	(7) 35	(4) 76	(5) 70	(1) 89	(3) 62	472	67.4
Alcoholic chlorhexidine ..	(3) 81	(6) 84	(4) 70	(1) 82	(2) 80	(5) 93	(7) 79	569	81.3
Aqueous	(7) 60	(3) 79	(1) 64	(5) 35	(6) 76	(2) 66	(4) 45	425	60.7
Virac	(4) 57	(7) 70	(5) 55	(2) 64	(3) 67	(6) 66	(1) 29	408	58.3
Control	(1) —4	(4) 46	(2) 12	(6) 12	(7) 9	(3) 16	(5) 12	103	14.7
Total (days)	405	488	388	407	450	506	378		
Total (persons)	(1) 394	(2) 443	(3) 458	(4) 444	(5) 420	(6) 439	(7) 424		

Numbers in parentheses indicate experimental subjects.

between days. These differences bear no obvious relationship to temperature or humidity, nor to mean initial counts. The method of analysis excludes this variation from the estimate of error. No significant difference of effectiveness was found between persons.* Pooling the variance attributable to persons with the residual, the derived standard error can be used for comparisons between treatments. Two antiseptics—alcoholic chlorhexidine and alcoholic iodine—were clearly better than the rest, with no significant difference between them. Alcohol, Lugol's iodine, aqueous chlorhexidine, and virac were effective in this order, but on the data of this experiment the differences between any pair are not significant. All are less effective than alcoholic iodine or alcoholic chlorhexidine and significantly more effective than the control rinse in running water.

Effect of Penotrane on the Resident Flora

It has been reported that penotrane applied to the surface will penetrate through all the layers of the skin (Goldberg, Shapero, and Wilder, 1950). Although in our tests (see above) its bactericidal effect on superficial organisms was rather poor, it seemed possible that penotrane might have more effect against the deeper flora than other antiseptics which are more active against bacteria deposited on the surface. We therefore tested its disinfectant action on the resident flora by the method described above, modified as follows: (1) the sampling fluid and the culture medium used for pour-plates contained 0.15% sodium thioglycollate as a neutralizer; (2) the sample of hand-washings taken after the treatment with penotrane was diluted 1 in 1,000 in Ringer's solution, and the diluted sample was distributed in 0.1-ml. amounts in 15 to 20 pour-plates, this degree of dilution having previously been found the lowest which did not allow the transfer of bacteriostatic levels of penotrane to the culture plates. Viable counts were made after 48 hours' incubation.

Results.—The reduction in bacteria on the skin after treatment with penotrane (expressed as percentage of the pre-treatment counts) was 28% in the first and 37.7% in the second experiment. This effect was smaller than the reduction in count obtained from any of the antiseptics included in the comparative study reported above (see Table II). In its high bacteriostatic and relatively low bactericidal action penotrane is typical of the mercurial group of antiseptics.

TABLE IV.—Effects of Repeated Disinfection with 70% Ethyl Alcohol (2 Minutes) on Six Subjects

Experiment	Reduction in Bacterial Counts from Hand-washings expressed as % 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	68	81
6	74	90	91

Effect of Repeated Antiseptic Treatment of the Skin

The possibility of improving on a single antiseptic treatment by repeating it once or twice, or by doubling

*One subject (No. 3) had to drop out of the trial after two experiments owing to illness. His place was taken by a new volunteer, who continued to the end of the series. Scrutiny of the results reveals no appreciable disturbance due to this change and suggests that it has not seriously impaired the reliability of the trial.

or trebling the time of treatment, was tested with 70% ethyl alcohol. After a 30-seconds wash with soap and water the skin was sampled as above for resident organisms. Alcohol treatment for two minutes was followed by another sampling. This was followed immediately by a second and a third two minutes' treatment with alcohol, after each of which the resident organisms were sampled. Six experiments were made, each on a different subject.

Results.—In all but one of these experiments (see Table IV) the reduction in bacterial counts was greater after the first treatment than after the second or third treatment, and in all but one (which showed a marginal effect) there was no further reduction in bacterial count after two periods of treatment; in two of the experiments the counts after two and after three periods of treatment were as high as they were after a single treatment of two minutes.

Effect of Skin Disinfection on Total Organisms and on *Staph. aureus*

To find whether our hand-washing technique gave some indication of the effect of antiseptic treatment on skin-borne *Staph. aureus*, we made the following tests on eight nurses in the burns unit of this hospital. The nurses were asked to wash their hands with soap and water for 30 seconds. The hands were then dried on a sterile towel, sampled by the hand-washing technique described above, treated with 70% alcohol for one minute, and then sampled again. Tenfold dilutions of the first and second washings were spread on the surface of phenolphthalein diphosphate agar plates (Barber and Kuper, 1951). Total and presumptive *Staph. aureus* counts (the latter after inversion over ammonia) were made after 48 hours' incubation of the plates.

Results.—Table V shows the estimated proportion of total organisms and of *Staph. aureus* persisting after skin

TABLE V.—Counts of Bacteria on Nurses' Hands Before and After Disinfection with 70% Alcohol (1 Minute)

Subject	Totals (per 0.5 ml. Washings)		<i>Staph. aureus</i> (per 0.5 ml. Washings)	
	Before	After	Before	After
1	375	275	4	0
2	1,875	700	38	14
3	850	212	27	1
4	2,387	1,000	8	0
5	1,112	762	34	2
6	3,200	2,237	287	187
7	4,712	275	1,087	112

treatment with alcohol in the seven nurses from whom staphylococci were isolated. Of these, two nurses showed very small numbers of *Staph. aureus*, and two showed a more complete removal of staphylococci than of total organisms from the hands; in three nurses the proportions of staphylococci and of total organisms removed by the antiseptic were of the same order. We inferred from this observation that staphylococci were represented among the deeper as well as the superficial flora of the skin in more than half of the subjects investigated.

Evidence that Sampling Technique Showed Effect of Antiseptics on Resident Flora

The method which we used for sampling the deeper flora was less arduous than that of Price (1938), who scrubbed the skin with brush and soap in a series of bowls of sampling fluids. For the following reasons, however, it seemed that our method was giving us a fair sample of the resident flora: (1) the percentage

reduction in bacterial flora after treatment with 70% alcohol and with iodine in alcohol was no greater in our experiments than it was in those reported by Price (1957); (2) the percentage reduction in the counts of bacteria deposited on the skin and then exposed to antiseptics was much greater; (3) the removal of staphylococci was more complete than that of the total organisms after the treatment of the hands of two out of seven nurses with 70% ethyl alcohol. This was consistent with the view that in these nurses most of the staphylococci were superficial, and the total flora included representatives of the deeper layers.

The organisms which were sampled after three successive treatments with alcohol appear to have come from a still deeper layer, where they were completely protected from the antiseptic, for their numbers were as great as those present in the samples taken after two such treatments.

Effect of Alcoholic Chlorhexidine and Alcoholic Iodine on *Ps. pyocyanea*

The experiment reported above (Table III) showed that alcoholic chlorhexidine (0.5%) was as effective as alcoholic iodine (1%) in disinfectant action against the resident flora of the skin. As chlorhexidine is less active *in vitro* against Gram-negative bacilli, and especially against pseudomonas, than against the Gram-positive cocci, we compared the effect of alcoholic iodine and of alcoholic chlorhexidine against *Ps. pyocyanea* deposited on areas of skin, using the method described above for transient flora, but with the suspension of *Ps. pyocyanea* diluted 1 in 100, as recommended by Story (1952); tests were made with a single application of the antiseptic, which dried in about 20 seconds, and with repeated applications to keep the contaminated surface moist for two minutes.

Results.—These are summarized in Table VI. No *Ps. pyocyanea* could be detected after two minutes' treatment with alcoholic iodine or alcoholic chlorhexidine, or after 20 seconds' treatment with alcoholic chlor-

TABLE VI.—Survival of Added *Ps. pyocyanea* After Treatment of Skin with Alcoholic Iodine (1%) and Alcoholic Chlorhexidine (0.5%)

Antiseptic	Duration of Treatment	Tests	Viable Counts per ml. Washings from Untreated Areas	% Organisms Surviving after Treatment*
Alcoholic iodine (1%)	2 minutes	3	24,000	<0.004 0.05–10.8
	20 seconds	2		
Alcoholic chlorhexidine (0.5%)	2 minutes	3	36,800	<0.0027 <0.0027
	20 seconds	2		

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

hexidine; in both experiments with alcoholic iodine some *Ps. pyocyanea* survived 20 seconds' exposure to the antiseptic.

Discussion

Of the two methods of bacteriological sampling used in this study, one gave information about the most superficial organisms of the skin, and these could be virtually eliminated by several alternative methods of disinfection; the other method included in its sample some of the deeper organisms, and the best forms of skin disinfection reduced the skin flora estimated in this way only by about 80%. In disinfection of the surgeon's or the nurse's hands the important object is to prevent the transfer of bacteria from these surfaces to the

patients, for which purpose the removal of superficial flora is presumably of first importance. Gardner and Seddon (1946) consider the removal of superficial organisms to be the main concern of disinfection at operation sites, too, because, in their opinion, pathogenic organisms, including *Staph. aureus*, are generally transient. In the majority of our tests on nurses' hands, staphylococci were removed from the skin in approximately the same proportion as the total organisms, from which we inferred that they were present in the deeper as well as the superficial layers. No antiseptic can be claimed to achieve "virtual disinfection" against organisms at this level, and tests with indicator organisms deposited on the skin exaggerate the value of antiseptics by disregarding the deeper flora.

Iodine in alcohol appeared, from the work of Gardner and Seddon (1946) and of Price (1957), to be the most effective skin antiseptic available at the time, but sensitivity of some patients to iodine has caused many surgeons to avoid using it. In our tests, 1% iodine in alcohol and 0.5% chlorhexidine in alcohol showed approximately the same effectiveness in reducing the resident flora, and chlorhexidine does not appear to cause sensitization (Calman and Murray, 1956); ethyl alcohol (70%), Lugol's iodine, virac, and aqueous chlorhexidine (0.5%) were significantly less effective. All of these antiseptics, however, were shown to be highly effective in removing superficial or "transient" staphylococci from the skin, and alcoholic chlorhexidine was at least as effective as alcoholic iodine in removing strains of *Ps. pyocyanea*. In their activity against surface organisms, 1% and 2% aqueous cetrimide and 0.1% aqueous penotrane (especially the latter), were less satisfactory.

Both penotrane and cetrimide, however, had strong bacteriostatic activity, which made it difficult to prevent inhibition by small amounts of these agents transferred to culture media. It might be argued that this inhibitory mechanism should be enough also to prevent the growth of bacteria transferred from the skin treated with such an agent to an operation wound. By suitable dilution during sampling, however, organisms exposed to such skin treatment were found to grow in culture media, and the use of disinfectants which kill the skin flora must be regarded as preferable. In their detergent action cetrimide and other quaternaries have an advantage which is independent of their bactericidal action and is not shared with alcoholic chlorhexidine or iodine; we would suggest that such compounds should be used in place of soap and water for the mechanical cleansing of the skin before the application of the chosen skin disinfectant, which is left on the surface and allowed to dry.

It has been customary in many hospitals, and especially in orthopaedic practice, for the skin to be disinfected on three successive days as part of the pre-operative preparation. In our study we found that more than two treatments, each of two minutes, with 70% alcohol did not appear to add anything to what was achieved by two successive treatments (or sometimes even a single treatment) with alcohol. The rationale of disinfecting on successive days is uncertain. Staphylococci which are not removed at the first treatment may be regarded as resident organisms and can probably grow in the intervals between disinfection; for these, two treatments on the same day seem to be more useful than one treatment on each of two successive days. But if the skin is dirty and perhaps heavily

contaminated with spore-bearers that resist any form of skin disinfection, it is clearly desirable to allow time for the horny layer carrying these organisms to be completely removed before an incision is made. Under these conditions, the repeated application of a hexachlorophane soap or "phisohex" might be useful as a component of skin preparation. We did not include these agents in the present study because their action is slow, and speed is essential in an antiseptic for use on operation sites; for repeated use on the hands of surgeons and nurses a cumulative action such as that provided by hexachlorophane is more suitable (Lowbury and Lilly, 1960).

The part which skin disinfection plays in preventing sepsis and the importance of choosing the best antiseptic are matters for speculation. We do not know how often the introduction of skin flora is to blame for the appearance of post-operative sepsis. No antiseptic can be expected to remove all the resident bacteria, or even all of the staphylococci when these are present in the deeper layers of the skin; some of the resident flora are probably transferred to the wound at every operation. Normally these organisms cause no clinical infection, and even when *Staph. aureus* is introduced into a wound it seems that a million or more cocci must be present before pus can be formed (Elek and Conen, 1957). It is rational, therefore, to use an antiseptic which, without injuring the patient, will cause the largest reduction in the skin flora.

Summary

The effect on antiseptics on "transient" skin flora was studied by a method in which test organisms (*Staph. aureus*), allowed to dry on areas of skin, were exposed to the action of antiseptics. After two minutes' treatment no test organisms were found in areas treated with 70% ethyl alcohol, with aqueous solutions of chlorhexidine (0.5%), with Lugol's iodine (1%), or with an iodophore (virac). Appreciable numbers of test organisms survived after treatment with 0.1% aqueous phenylmercuric dinaphthyl methane sulphate (penotran) and with 1% or 2% aqueous cetrimide.

Six antiseptics and a control were tested for their action in two minutes' treatment against "resident" skin flora by a hand-washing technique on seven subjects, using a Latin square design. Iodine (1%) in alcohol had approximately the same effect and were significantly more effective than the other antiseptics tested. In contrast with iodine, chlorhexidine does not appear to cause sensitivity reactions, and therefore seems to be more suitable for routine use as a skin disinfectant.

A majority of nurses carrying *Staph. aureus* on their hands lost approximately the same proportion of staphylococci as of total organisms when their hands were disinfected for one minute with 70% ethyl alcohol, suggesting that these organisms were present in the deeper as well as the more superficial layers of the skin.

Three successive treatments of the hands for two minutes with 70% ethyl alcohol produced no greater reduction in hand flora than two such treatments, and in some subjects no further disinfection was obtained after a single treatment.

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HYDROFLUMETHIAZIDE DIURESIS IN HOSPITALIZED PATIENTS

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Since the discovery of the diuretic properties of chlorothiazide (Ford *et al.*, 1957) a number of chemically related compounds have been developed and introduced in the continuing search for an ideal non-mercurial oral diuretic agent. One of these new compounds is hydroflumethiazide, a trifluoromethyl derivative of a benzothiadiazine dioxide. Hydroflumethiazide differs from chlorothiazide in the presence of a trifluoromethyl group in place of the chloride group and two hydrogen atoms replacing the double bond in the thiadiazine ring. As may be seen from the formulae, hydroflumethiazide bears the same relationship in chemical structure to flumethiazide as hydrochlorothiazide does to chlorothiazide.

Experimental studies have demonstrated that hydroflumethiazide, administered orally to rats, induces a marked increase in the urinary excretion of sodium and chloride with much less effect on potassium, and that, on a weight basis, hydroflumethiazide is approximately 15 times as potent as chlorothiazide in producing a similar saluretic effect (Kobinger and Lund, 1958). Moreover, hydroflumethiazide possesses very low acute toxicity by oral administration to both mice and rats,

FLUMETHIAZIDE

HYDROFLUMETHIAZIDE

CHLOROTHIAZIDE

HYDROCHLOROTHIAZIDE