of cortisone daily by intramuscular injection. In eight of these skin sensitivity to tuberculin was detected at intervals ranging from 6 to 17 days after the first injection of cortisone; in six the reaction produced by tuberculin during the period of sensitivity persisted, with induration, up to 48 hours, and in two it consisted of erythema, evident at 24 hours but fading at 48 hours. In those patients tested repeatedly this sensitivity was transient, even though the cortisone was continued.

In nine patients normally not reacting to at least 100 T.U., intradermal injection of 1.25 mg. cortisone with a dose of tuberculin equal to the greatest to which the patient had previously failed to respond produced a reaction with induration and erythema persisting at least 48 hours in seven, and no response in two.

In three patients normally sensitive to 100 T.U. or less, similar local administration of cortisone with tuberculin inhibited the reaction produced by tuberculin alone.

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TESTING OF SKIN DISINFECTANTS

BY

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Skin disinfectants may be compared by their killing effect on normal skin flora or bacteria applied to the skin, and numerous techniques are based on this idea. Pathogenic bacteria are nearly always transient on the skin surface, where they can be killed by suitable chemicals, although in special circumstances pathogens such as *Staph. pyogenes* become established as part of the resident flora in deeper layers of the skin, where they may not be reached by disinfectants (Price, 1938). Normally there are resident bacteria deep in the skin which are mainly inaccessible to disinfectants or bacteriological sampling, but they are non-pathogenic organisms of little or no practical importance. Lovell (1945) has ingeniously demonstrated these deeply situated bacteria by sectioning and staining excised skin after a period of incubation in a moist chamber to allow organisms to multiply and become visible histologically.

Although there is general agreement that skin cannot be chemically sterilized throughout its thickness without destroying it, Gardner and Seddon (1946) have shown that the skin cocci can sometimes be eliminated completely. They excised and cultivated pieces of normal skin at the beginning and end of surgical operations; after preliminary treatment with 2% aqueous iodine 58%

yielded no growth of skin cocci; corresponding results with 5% and 1% aqueous cetrimide were 34% and 25%.

This last method could not be applied safely to skin contaminated with known pathogens, and to test a disinfectant's action against such organisms a practical method is to apply bacteria to the skin followed by disinfectant and recovery of any surviving bacteria after varying periods. This does at least supply the information that a disinfectant will destroy pathogenic bacteria known to be on the skin surface, where, indeed, they are most likely to be found under normal circumstances. Although open to the objection that bacteria placed on the skin may not be affected by disinfectants in the same way as natural transient flora, it is probably the best method of testing available.

In the investigation reported here this technique was used to test the efficacy of several skin disinfectants against strains of *Staph. pyogenes*, *Bact. coli*, *Ps. pyocyanea*, and *Proteus vulgaris* isolated from wound swabs. These organisms were all penicillin-resistant, and penicillin was added to the culture medium to suppress growth of normal skin flora. Most of the tests were carried out against a strain of *Staph. pyogenes* which grew freely in the presence of 0.5 unit of penicillin per ml. In experiments on quaternary ammonium compounds, such as cetrimide, the recovered organisms were mixed with sterile milk before cultivation, since it has been shown (McCulloch, 1947) that this substance will neutralize the bacteriostatic action of cetrimide. Bacteria were recovered from the skin by a method similar to Burtenshaw's (1938).

Numerous techniques have been employed in recovering bacteria from skin surfaces; they include rubbing the skin with a moist swab and then agitating the swab in broth and making pour plates (Colebrook, 1930) or streaking out the swab on an agar plate (Gardner and Seddon, 1946). Price (1938) devised the method of washing the hands and forearms in a series of bowls of water and cultivating this water; he has recently described a method for repeated bacteriological sampling of a small skin area which he calls "spot testing" (Price, 1951). Burtenshaw (1938) applied a rectangular glass chamber to the skin, added saline, and scraped the skin with a glass slide to make a suspension of organisms from which he made viable counts. Brown *et al.* (1944) filled watch-glasses to the brim with agar and placed them on skin to collect the bacteria. Most elaborate, perhaps, is the method of Nungester and Kempf (1942), who rubbed a mouse's tail with pneumococcal culture, dipped the tail into disinfectant for two minutes, and then cut off $\frac{1}{4}$ in. (1.3 cm.) and placed it in the mouse's peritoneal cavity. Any pneumococci surviving exposure to the disinfectant caused a fatal peritonitis.

Methods

For each disinfectant test six circular areas 4 cm. in diameter were outlined on the forearm or thigh, using a glass ring and skin pencil. Most tests were done on the forearm, the skin being unwashed. The bacterial inoculum to each test area consisted of 1 drop, from a pipette delivering 40 drops/ml., of a 1/10 dilution of 20-hour broth culture (in the case of *Ps. pyocyanea* only a 1/100 dilution was used). Each drop was spread over its circular area with a wire.

Bacteria were added to the first five circular areas. When dry the first four circles and the last (containing no added bacteria) were treated with disinfectant applied with a cotton-

wool-tipped throat swab in as uniform a manner as possible, fairly freely, but not enough to run. A stop-watch was started at the first application of disinfectant and areas were sampled as described after half, one, two, and five minutes. The fifth area, carrying added bacteria but no disinfectant, was then sampled; it served as a control against autogenous disinfection, and several dilutions were made so as to obtain a total count. The sixth and last circle contained disinfectant but no added bacteria; it acted as a carry-over control and was sampled in the same way as the other areas, and 1 ml. was added to a pour plate which was then inoculated with the test organism. This showed whether an inhibitory concentration of disinfectant was being carried over into the culture medium during sampling from the test areas.

In those experiments in which Gram-negative bacilli were used as test bacteria they were exposed to disinfectant for a half and two minutes only (Tables I and II).

TABLE I.—Detailed Results of Two Tests Against *Staph. pyogenes* Giving Numbers of Organisms Recovered from Each Test Area and Controls

Disinfectant	Test Areas (1/10)					Control Area		Carry-over Control
	½ min.	1 min.	2 min.	2 min.*	5 min.	1/200	1/2,000	
1% cetrimide in 70% spirit	0	0	0	0	0	380	40	Satisfactory
1/1,000 zephiran in 50% alcohol ..	40	8	1	0	0	120	—	"

* Culture containing no milk.

TABLE II.—Detailed Results of Two Tests Carried Out Against *Ps. pyocyanea* Giving Numbers of Organisms Recovered from Each Test Area and Controls

Disinfectant	Test Areas (1/10)		Control Area		Carry-over Control
	½ min.	2 min.	1/50	1/500	
1% iodine in 70% spirit	0	0	+	136	Satisfactory
1% aqueous cetrimide	160	48	+	160	"

Bacteria were recovered from the skin by means of glass rings 4 cm. in diameter, 2.5 cm. deep, and 1.5 mm. thick. One of these rings was placed over a test area, held in position by the subject, and 5 ml. of sterile distilled water was added; the operator quickly and systematically rubbed the skin for 15 seconds, using a glass spreader with an arm 1.5 cm. long (see Fig.), and then took a 1-ml. sample of fluid. The aim was to get bacteria on the skin surface into uniform suspension in the water. Viable counts were made by a pour-plate method after 48 hours' incubation at 37° C. Using this technique, we got a higher recovery of bacteria

Glass ring held on skin contains water and glass spreader. Note the adjacent test areas outlined by skin pencil.

from adjacent areas of normal skin and of bacteria placed on the skin than we did by either of the swabbing methods.

In all experiments on quaternary ammonium compounds 1 ml. of fluid from the glass ring was added to 1 ml. of sterile milk, mixed, and 1 ml. of the mixture was used for a pour plate or further dilutions (necessary in the control) which were made

in distilled water. In the same way 1 ml. of milk was added to the fluid from the carry-over control. When iodine was being tested enough sodium thiosulphate was placed in the Petri dishes to neutralize any iodine carried over.

Four plates were made by placing in a Petri dish 0.5 ml. of penicillin solution (10 units/ml.), 1 ml. of fluid from the glass ring, and about 14 ml. of agar melted and cooled to 48° C.; the final concentration of penicillin was thus 0.3 unit/ml. The normal flora from the forearms of several individuals was sampled, 1 ml. of fluid from each glass ring being added to melted agar containing 0.3 unit of penicillin per ml. and 1 ml. into agar with no penicillin; altogether 36 skin areas were examined in this way. In the absence of penicillin an average of 231 colonies per 1/5 test area was obtained, in contrast to 8 when penicillin was present. Usually with the antibiotic there were 0 to 3 colonies, but one subject carried very large numbers of penicillin-resistant organisms, which raised the average.

McCulloch (1947) has shown that the bacteriostatic action of cetrimide on *Staph. aureus* is neutralized by skim milk. In preliminary experiments with the strain of *Staph. pyogenes* used in these tests 10% skim milk and 10% human plasma were about equally effective in this respect for cetrimide and also for "bradosol," "zephiran," and dynium chloride. In order to demonstrate this anti-bacteriostatic action of milk during skin experiments a second 1-ml. sample was taken after the 2-minute test, diluted with 1 ml. of water instead of milk, and made into a pour plate; counts were consistently higher in the milk-containing plates.

Recovery of bacteria from control areas was always satisfactory with Gram-negative organisms. On a few occasions recovery of *Staph. pyogenes* was poor; this did not occur intermittently but in small batches of tests towards the end of the investigation. These tests were considered to be invalid. Several subsidiary experiments failed to show a cause for this.

At the end of an experiment skin which had been contaminated with bacteria was treated with 1% iodine in spirit and subsequently washed well with soap and water. This precaution is, of course, essential when working with a test organism such as a penicillin-resistant strain of *Staph. pyogenes*. After an experiment a skin area was not used again until at least two to three days had passed.

Results

The result of a disinfectant test was regarded as satisfactory when not more than one colony grew on the pour plates; this represented not more than 5-10 surviving bacteria in a test area. At least 10,000 organisms were recovered from each staphylococcal control area, and often 50,000-100,000; recoveries of Gram-negative bacilli were generally higher. This is in line with Gardner's (1948) conception of "virtual disinfection" involving the death of at least 99.9% of bacteria on the skin surface.

Of the disinfectants tested against *Staph. pyogenes* the following were found effective within 30 seconds: 1% iodine, 1% and 10% cetrimide, or 1/1,000 dynium chloride in 70% industrial spirit (74 O.P.). In all these tests there were no surviving bacteria. Seventy per cent. industrial spirit alone was effective in 30 seconds: a few of the plates grew one colony; after two minutes all plates were sterile. Four per cent. aqueous cetrimide was not completely effective in 30 seconds, but was after two minutes. With 10% cetrimide carry-over controls were unsatisfactory, showing either no growth or considerable inhibition.

Other disinfectants tested which were not consistently effective even after five minutes included 1% and 2% aqueous cetrimide, 1% aqueous bradosol, and 1/1,000 zephiran in 50% alcohol. Only a few tests were carried out with the last two substances.

Bact. coli, *Ps. pyocyanea*, and *Proteus vulgaris* behaved alike against the disinfectants tested. Completely effective within

30 seconds were 1% iodine or 1% cetrimide in 70% spirit or 70% industrial spirit alone. One per cent. aqueous iodine was effective against *Ps. pyocyanea* in 30 seconds; no other organisms were tested with this disinfectant. One per cent. aqueous cetrimide was ineffective against all the Gram-negative bacilli; after two minutes' exposure to disinfectant there were large numbers of surviving bacteria. No tests on Gram-negative bacteria were carried beyond two minutes.

A total of 157 tests were made by the method described here; Table III gives the numbers of experiments for each disinfectant and test organism, with the proportion yielding "virtual disinfection."

Discussion

It will be seen from Table III that, in the case of some disinfectants, the numbers of tests do not permit any firm conclusions to be drawn; generally, however, results agree with those of other workers using different methods.

TABLE III.—Proportion of Tests Resulting in "Virtual Disinfection"

Disinfectant	<i>Staph. pyogenes</i>				<i>Ps. pyocyanea</i>		<i>Bact. coli</i>		<i>P. vulgaris</i>	
	1/2 min.	1 min.	2 min.	5 min.	1/2 min.	2 min.	1/2 min.	2 min.	1/2 min.	2 min.
1% iodine in 70% spirit	15/15	15/15	15/15	15/15	16/16	16/16	5/5	5/5	5/5	5/5
1% aqueous iodine	—	—	—	—	5/5	5/5	—	—	—	—
1% aqueous cetrimide	6/9	6/9	6/9	6/9	0/5	1/5	0/5	0/5	0/5	0/5
2% aqueous cetrimide	3/5	3/5	3/5	3/4	—	—	—	—	—	—
4% aqueous cetrimide	4/5	4/5	5/5	5/5	—	—	—	—	—	—
1% cetrimide in 70% spirit	10/10	10/10	10/10	10/10	5/5	5/5	5/5	5/5	5/5	5/5
10% cetrimide in 70% spirit*	10/10	10/10	10/10	10/10	—	—	—	—	—	—
1% aqueous bradocool	1/6	0/6	2/6	5/6	—	—	—	—	—	—
1/1,000 zephiran in 50% alcohol	3/6	2/6	3/6	4/6	—	—	—	—	—	—
1/1,000 dynium chloride in 70% spirit	5/5	5/5	5/5	5/5	—	—	—	—	—	—
70% industrial spirit	10/10	10/10	10/10	10/10	5/5	5/5	5/5	5/5	5/5	5/5

Denominator represents total number of tests with adequate controls.
* Carry-over control unsatisfactory.

In these experiments 70% industrial spirit (74 O.P.) alone produced "virtual disinfection" within 30 seconds with the four test organisms. This is a more rapid disinfectant action on skin than has generally been attributed to 70% alcohol; Gardner (1948), for example, found that it required two minutes to kill organisms on the skin surface. It may be that a more liberal application of disinfectant was made to test areas in this investigation. However, as described above, care was taken not to add an excess which would run on the skin, and certainly less was applied than would normally be put on the skin before a surgical incision.

As was to be expected, 70% spirit containing other disinfectants such as 1% cetrimide or 1/1,000 dynium chloride also produced "virtual disinfection" of skin areas within 30 seconds. The performance of 70% spirit alone in these experiments was so good that it is difficult to decide whether the addition of other disinfectants improves it. On testing 1% cetrimide in 70% spirit and 70% spirit against the Gram-negative bacteria both produced completely sterile test areas after 30 seconds. When *Staph. pyogenes* was tested 5 to 10 organisms (which is "virtual disinfection") survived on four of the areas exposed to 70% spirit for 30 seconds, whereas all plates from areas treated with 1% cetrimide in spirit were sterile. As only 10 tests were done with each of these disinfectants against *Staph. pyogenes* this cannot be regarded as significant, but if similar results were repeated it would suggest that 1% cetrimide in spirit is a somewhat better skin disinfectant than 70% spirit alone.

Gardner (1948) found that 10% cetrimide in 70% alcohol killed *Ps. pyocyanea* on the skin in 30–45 seconds. When this disinfectant was tested against *Staph. pyogenes* by the technique described here an inhibitory concentration of

cetrimide was carried over on sampling into the cultures (even when milk was added) so that they were inevitably sterile.

In 29 experiments 1% and 2% aqueous cetrimide were not completely effective within two minutes against any of the test organisms, but they performed best against *Staph. pyogenes*. Gardner and Seddon (1946) found that even 5% aqueous cetrimide did not produce "virtual disinfection" of *Ps. pyocyanea* in one and a half minutes.

Some importance is attached to neutralization of the bacteriostatic action of cetrimide. This has not been done in other investigations, though controls have usually been designed to demonstrate carry-over of an inhibitory concentration of disinfectant which does not serve the same purpose. Failure to prevent bacteriostasis might make a disinfectant appear more effective than it is if some surviving organisms were unable to grow into visible colonies. As milk and human plasma are almost equally effective in reversing the bacteriostatic effect of cetrimide and some other quaternary ammonium compounds it seems possible that, in a wound, any organisms exposed to but not killed outright by cetrimide could be revived by the plasma which is present.

In common with numerous other investigations, iodine in spirit was found consistently effective. Forty-one tests were carried out with 1% iodine in 70% industrial spirit against the four test organisms, and after 30 seconds' exposure there were no survivors.

Summary

A method of testing skin disinfectants is described which includes: (1) using penicillin-resistant test organisms with penicillin in culture medium to suppress natural skin flora; (2) counteraction of the bacteriostatic effect of quaternary ammonium compounds with sterile skim milk; and (3) recovery of skin bacteria by use of a glass ring

Several disinfectants have been compared by their ability to kill *Staph. pyogenes*, *Bact. coli*, *Ps. pyocyanea*, and *Proteus vulgaris* on the skin.

One per cent. iodine or 1% cetrimide in 70% industrial spirit (74 O.P.) killed all test organisms within 30 seconds, and 70% spirit alone produced "virtual disinfection" within that time. Ten per cent. cetrimide or 1/1,000 dynium chloride in 70% spirit and 1% aqueous iodine were effective within 30 seconds against the bacteria tested; some other disinfectants were only partially effective.

I am indebted to Professor L. P. Garrod for suggesting this investigation and for advice during its progress. Miss M. Rhodes gave me valuable technical assistance and carried out many of the tests.

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A medical gastronome, Dr. Paul Romain, from Douvaine, in Savoy, has won a prize of 100,000 francs for the best book of the year on food (*The Times*, November 14). His book, which is not yet published, describes 336 new ways of cooking and serving edible fungi. The prize was awarded by a jury of members of the Club des Cent, a gourmets' association. Dr. Romain is clearly a man of parts: his name is also associated with a small Alpine butterfly which he classified.