J. Hyg., Camb. (1960), **58**, 159
Printed in Great Britain

Disinfectants for use in bar-soaps

BY A. HURST, L. W. STUTTARD AND R. C. S. WOODROFFE
Unilever Limited, Food Research Department, Colworth House, Sharnbrook, Bedford

(*Received* 21 *December* 1959)

Part I

IN VITRO SCREENING TESTS

From time to time the chemical industry produces disinfectants which, it is hoped, will be useful in soaps. These substances are evaluated for their effect on bacteria and on the persons using them. The toxicological, pharmaceutical and synthetic chemical side of this work is outside the scope of these papers which are concerned solely with the bacteriological evaluation of disinfectants for use in bar soaps. Disinfectants are used also in liquid soaps, in household, farm and other detergent preparations, but the methods for evaluating these products are rather different (Hirsch & Muras, 1955). Thus restricted, the field is still very wide; G 11, (2,2′-dihydroxy-3,5,6-3′5′6′-hexachlorodiphenylmethane (also known as Hexachlorophene)) was the first disinfectant which could be combined successfully with soap and was first described by Gump (1945). We propose to deal only with modern disinfectants which have been described since the advent of G 11 and these include, Actamer (2,2′-thiobis, 4-6-dichlorophenol, also known as Bithional), DCMX (dichloro-*m*-xylenol), TMTD (3:4:5-tetramethyl thiuram disulphide), TCC (3:4:4′-trichlorocarbanilide), TBS (3:4:5-tribromsalicylanilide), and TCS (3:3′:4′:5-tetrachlorsalicylanilide) (Shumard, Beaver & Hunter, 1953; Egan & Reed, 1953; Monsanto, 1953; Firmenich, 1954; Gemmell, 1952; Vinson, 1954; Baer & Rosenthal, 1954; Traub, Newhall & Fuller, 1944; Monsanto, 1957).

The antibacterial success of these substances depends, first, on their effectiveness as disinfectants, secondly, on whether they are compatible with soap, which is the carrier vehicle, and finally on the interaction between the disinfectant and the skin. It is intended that this part of our work, viz. the retention of disinfectants on skin, should form the subject of a later paper.

Disinfectant potency can be measured by bactericidal or bacteriostatic tests. Bactericidal tests are difficult to perform and often give unreproducible results, and for routine use a bacteriostatic test was employed. This was an end-point dilution method in a fluid medium and the minimum concentration of disinfectant to inhibit the growth of a test organism was found. This test has the advantage of simplicity and the results are a useful guide for comparing various disinfectants. The test, however, takes no account of the product in which the disinfectant may be used.

Soap can have an adverse effect on the action of disinfectants, and soap alone has some disinfectant property which may be reduced by incompatible substances.

The antibacterial power of soap is not clearly understood but it is probably due to several factors (Diasis, 1934).

Bean & Berry (1950) report that the bactericidal activity of low concentrations of potassium laurate or of benzylchlorophenol is negligible when they are mixed together in a constant proportion; the bactericidal activity increases as the concentration of the solution increases, followed by an abrupt fall in activity and finally by a second gradual increase. An increase in the proportion of benzylchlorophenol to potassium laurate in the solution produces a marked increase in bactericidal activity, the maximum activity being exhibited at the critical micelle concentration (C.M.C.) of the soap. Above the C.M.C. soap molecules in water become arranged in a micelle structure and, with increasing soap concentration, the micelles undergo alteration in their shape and structure. The solubility of water-insoluble compounds such as benzylchlorophenol depends on the micelle structure. The solubility rises sharply as micelles are formed and some disinfectants may then lose their potency.

It has been estimated that during washing of the hands a soap solution is formed containing 6–10 % soap. This concentration is well above the C.M.C. of soap and is therefore capable of reducing the effect of disinfectants which may be incorporated into the soap. A test is described to measure this interaction.

It is now well established that substances from the human skin, such as sebum, may possess bactericidal properties (Burtenshaw, 1938; Ricketts, Squire, Topley & Lilly, 1951). If these substances are soap-soluble they may enhance or reduce the potency of applied disinfectants. A test will be described which is designed to estimate the effect of such substances as well as that of dirt.

METHODS

Bacteriostatic test

Nutrient broth was distributed in 4 ml. amounts in test-tubes. The disinfectant was dissolved in a solvent (dilute NaOH, alcohol, acetone, etc.) from which dilutions were made in nutrient broth. The highest concentration generally made was 100 p.p.m.; from this halving dilutions were prepared, also in nutrient broth. The tubes were capped and sterilized by autoclaving at 120° C. for 15 min. After cooling, each tube was inoculated with 0·1 ml. of 18 hr. culture of either *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 8196) or some other test organism. The tubes were incubated at 37° C. overnight and then examined for growth.

Test for the effect of soap on disinfectant action

The effect of the soap on the disinfectant was measured by an agar diffusion test. Disinfectants were prepared in soap and soap-free solutions and the zones of inhibition in agar were compared. Solutions were defined as equally potent when the zones were of equal size; the ratio of the concentrations at which two solutions were equally potent was termed the soap inactivation coefficient (S.I.C.). The S.I.C. is thus defined as the concentration of the disinfectant in soap solution divided by its concentration in an equally potent soap-free solution.

An S.I.C. of 1 indicates that the activity of the disinfectant is unimpaired by soap. A figure greater than unity indicates that the activity is reduced and a figure of less than unity indicates that the activity is enhanced.

The test was carried out as follows. A strain of *Staph. aureus* (NCTC 6571) was subcultured at daily intervals into nutrient broth and incubated at 37° C. for 18 hr. before use. Assay plates ($8\frac{3}{4}$ in.) were sterilized by autoclaving. To 100 ml. nutrient agar at approximately 47° C., 1·0 ml. bacterial culture was added which was then poured into a plate. After the agar had set, Heatley cylinders were sealed on the surface.

Disinfectant solutions containing 1000 p.p.m. were made in 10% aqueous solution of flaked soap and in a suitable solvent such as dilute sodium hydroxide, or alcohol. Serial twofold dilutions were then made in soap solution or solvent down to 31·25 p.p.m. The soap solution was kept in a waterbath at 45° C. to prevent setting; the solutions were then placed in the cups using six replicates at each dilution.

The order of working and location on the plates were randomized; the plates were then refrigerated to allow the disinfectants time to diffuse before bacterial growth started. The plates were then incubated at 37° C. for 24 hr. and the zone sizes measured using dividers and a steel rule.

Test for the effect of dirt and skin substances on disinfectant action

A disinfectant in soap may be inactivated by either skin proteins or dirt. A modification of the serial dilution test was used for measuring the protein effect and horse serum was employed to simulate the effect of skin protein, as follows. Sterile serum (Burroughs Wellcome and Co.) was added aseptically to sterile nutrient broth to give a final concentration of 10%. This was then dispensed in 4 ml. amounts into sterile test-tubes. The solution of the disinfectant under test generally at 100 p.p.m. in nutrient broth was autoclaved at 120° C. for 15 min. After cooling, halving dilutions of the disinfectant were made in the serum broth, inoculated, incubated and read as already described.

The effect of dirt was followed by a zone diffusion test similar to that described for the S.I.C. test, but dilutions were made in powers of 1·5 instead of powers of 2.

Two litres of a 10% solution of a toilet soap was prepared; 100 ml. was removed immediately and dilutions of the disinfectant under test made in this soap solution. The remaining soap solution was used by a number of subjects for washing their hands, thus leaving skin substances and dirt in this soap solution.

Generally six subjects used the same soap solution but the number varied slightly in the different tests; subjects continued to use the same solution until it became discoloured and obviously dirty. A sample was then removed and used for making dilutions of the disinfectant. Heatley cups, etc., were set up as already described for the S.I.C. test and a comparison was made between the zone diameters obtained in fresh soap solution and used soap solution.

RESULTS

Bacteriostatic potency

Table 1 gives the minimum inhibitory concentration (M.I.C.) of each disinfectant. DCMX is the weakest disinfectant against *Staph. aureus*. All the others are effective at high dilution, TCS being the most active. None of the disinfectants is sufficiently active for practical use against the Gram-negative *Esch. coli*.

Table 1. *Bacteriostatic levels (p.p.m.) of different disinfectants*

Disinfectant	*Staph. aureus*	*E. coli*
G 11	0·5	50·0
Actamer	1·0	50·0
DCMX	2·5	50·0
TMTD	0·25	25·0
TCC	0·2	25·0
TCS	0·1	12·5
TBS	1·0	25·0

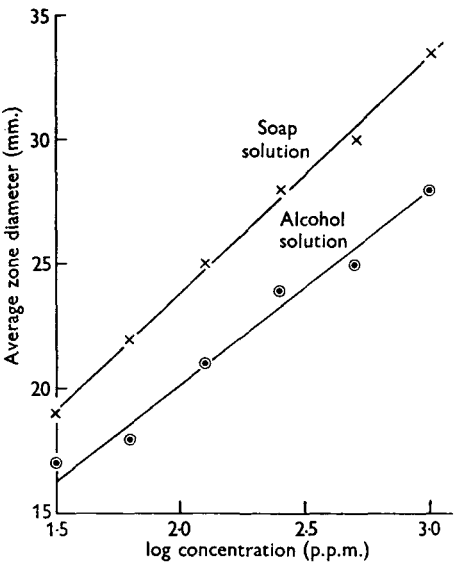

Fig. 1. Soap inactivation coefficient of tribromosalycilanilide (TBS).

Soap inactivation coefficient (S.I.C.)

Fig. 1 gives some typical results obtained with TBS. The average zone diameter obtained in soap solution and solution of alcohol are plotted against concentration. As with other graded response assays, statistical techniques may be applied to this data, or a simple graphical method can be used to find the ratio of equally potent concentrations.

Table 2 shows the S.I.C. of TBS at various disinfectant concentrations. It can be seen that there is a slight increase as the concentration of TBS is lowered.

Many disinfectants, including G 11, show a marked lack of parallelism of the

two response curves and, for this reason, the S.I.C. should either not be expressed as a single figure or the concentration at which the S.I.C. is given should be stated. As a convenience in comparing the behaviour of different disinfectants the S.I.C. is given as a single figure at 100 p.p.m. The results are shown in Table 3.

It can be seen from this table that DCMX would not be expected to act in the presence of soap and even the activity of G 11 is much reduced. Other disinfectants such as TCS and TBS are more active in the presence than in the absence of soap.

Table 2. *Soap inactivation coefficients* (S.I.C.) *of TBS at different concentrations*

TBS (p.p.m.)	S.I.C.
1000	0·29
500	0·39
250	0·50
125	0·64

Table 3. *Soap inactivation coefficients of different disinfectants at* 100 *p.p.m.*

Disinfectant	S.I.C.
G 11	208
Actamer	1·4
DCMX	∞
TMTD	2·6
TCC	0·98
TCS	0·32
TBS	0·64

Effect of dirt and skin substances

Table 4 gives the results of the dilution assays with nutrient broth containing 10 % serum. These results were compared with those of Table 1 and a column introduced which gives the number of times the presence of 10 % serum has reduced the activity of the disinfectants.

Table 4. *Effect of serum on disinfectant activity by dilution assay*

	Bacteriostatic concentration (p.p.m.) in 10 % serum broth		Ratio of concentration serum broth/nutrient broth	
	Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*
G 11	12·5	125	25	2·5
Actamer	12·5	100	12·5	2
DCMX	50	50	2	1
TMTD	0·5	100	2	4
TCC	50	100	250	4
TCS	25	100	250	8
TBS	100	100	100	4

From these results it is evident that all the disinfectants lose a lot of activity in the presence of 10 % serum. Although the M.I.C. in the absence of serum varies widely, this difference disappears when serum is used in the medium. Moreover, the difference between the sensitivity of the gram-positive and the gram-negative test organism becomes less marked.

The effect of dirt from the skin, as measured by zone diffusion tests, is illustrated in Fig. 2. This type of experiment has been done with other disinfectants (Table 5) but TMTD was chosen for illustrating this technique because, of the disinfectants tested, it was the only one which suffered a marked loss of potency by this test. In Fig. 2 the zone diameters are plotted against log concentration. In the preparation of the dilutions, the disinfectant/dirt ratio is displaced in favour of the dirt which is at a constant concentration; thus, with increasing dilution the effect becomes more obvious and there is a loss of parallelism. On the average 1/3 of the TMTD potency was lost.

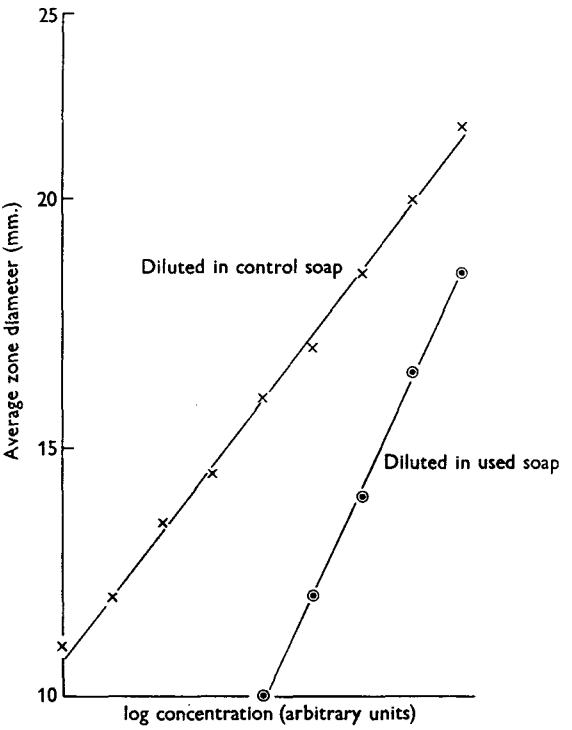

Fig. 2. Effect of soap-soluble skin substances on tetramethylthuramdisulphide (TMTD)

Table 5. *Effect of skin substances and dirt on disinfectant potency by the zone diffusion test*

Disinfectant*	Activity in presence of soap-soluble skin substances (%)
G 11	100
Actamer	120
TMTD	66
TCC	100
TCS	100
TBS	100

* DCMX could not be tested by this method as it does not give zones in 10 % soap solution.

https://doi.org/10.1017/S0022172400038249 Published online by Cambridge University Press

A summary of the effect of dirt on all the disinfectants is given in table 5.

The potency of G 11, TCC, TBS and TCS was not affected by skin substances; that of Actamer was slightly increased and TMTD was reduced by about 33 %.

Part II

IN VIVO TESTING (HAND WASHING TEST)

The bacterial flora of skin varies with the site from which samples are taken. Evans, Smith, Johnston & Giblet (1950) found that the count from the ear concha, where there are no sweat glands but many sebaceous glands, was 10^4 times the count from the palm of the hands, where there are numerous sweat glands but no sebaceous glands. Lovell (1945) also observed bacteria in sebaceous glands but not elsewhere. He examined tissue sections obtained at operations and from cadavers. Strauss & Kligman (1956) isolated micrococci, diphtheroids, *Esch. coli*, *Proteus vulgaris*, and *Sarcina* from skin and produced characteristic odours by inoculating them into sterile sweat; all types were active except *Sarcina*. Shelley, Hurley & Nichols (1953) observed that sweat is non-odorous when delivered at the skin surface and the odour develops when bacteria grow. Other workers have found that the gram-negative intestinal flora can survive on skin as long as it is kept moist. Investigating the influence of atmospheric drying on the survival of hand flora, Lowbury & Fox (1953) prepared suspensions of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas pyocyanea*, and micrococci in serum, distilled water, saline, and oleic acid and allowed them to dry on cover-slips; all the organisms showed after drying a drop in viability, although serum afforded some protection.

Ricketts *et al.* (1951) studied the self-sterilizing property of skin. *Strep. pyogenes* added to skin disappeared after 1 day, and *Staph. aureus* after 3 days; *Esch. coli* and *P. pyocyanea* disappeared in 1 day if drying was permitted, but persisted on the skin if drying was prevented. The same micro-organisms were tested *in vitro* for their sensitivity to oleic acid and other fatty acids, because extracts of human skin made with acetone contained 35–45 % free fatty acids, mostly oleic. The sensitivity of *Strept. pyogenes* and *Staph. aureus* to the unsaturated fatty acids ran parallel with their rate of disappearance on moist or dry skin. *P. pyocyanea* and *Esch. coli*, which failed to disappear from skin when drying was prevented, were extremely resistant to fatty acids.

Burtenshaw (1942) has also demonstrated the mortality of *Strept. pyogenes* on skin, while Arnold (1942) has pointed out that bacteria added to clean hands disappear more rapidly than those added to dirty hands. Hellatt (1948) confirmed some of the work of Ricketts *et al.* by showing that gram-negative bacteria survive only on moist skin. Under very dry conditions, Staphylococci are able to multiply hence they predominate on skin. However, Bryan & Mallmann (1932), point out that desiccation cannot fully explain the disappearance of *Esch. coli* from skin, because these organisms disappear more rapidly from skin than from glass or filter-paper under similar conditions of humidity. The resistance of *Staph. aureus* to the antibacterial action of the skin has lately assumed greater importance

because it is the main organism concerned in cross-infections (Miles, Williams & Clayton-Cooper (1944), Williams (1946), Hare & Thomas (1956), Gillespie, Simpson & Tozer (1958), Hare & Ridley (1958)).

The part played by gram-negative intestinal bacteria in body-odour development is uncertain and it has been claimed that these organisms do not occur on hands. However, Howard & Minch (1951) and Källander (1953) examined food handlers and found the organisms on 50 % of the subjects. Payne (1949) considers that such bacteria die on the skin because of drying out; the rate of drying also plays a part.

Methods for estimating the effect of disinfectants on the bacterial flora of the skin have either relied on measuring the effect on cultures of bacteria deliberately added to skin for testing, or determining the effect of disinfectants on those bacteria normally present on skin.

Various techniques have been evolved in which bacteria have been added to skin. Kempf & Nungester (1942) and Nungester, Thirlby & Vial (1949) used pathogenic organisms applied to the tails of mice. The tails were then treated in various ways with disinfectants, amputated and planted in the peritoneal cavity of the animal. The percentage mortality of the animals was an indication of the effectiveness of the disinfectant treatment. Story (1952) isolated an area of the skin by using a glass ring and placed certain recognizable bacteria on the skin. After application of a disinfectant, he recovered the bacteria to determine the numbers of survivors. Sykes (1955) applied known dilutions of disinfectant to small marked areas of the skin and then, at selected time intervals up to several hours, infected the test areas with a culture of *Staph. aureus* and assessed the survivors after 10 min. contact by swabbing and plating.

The recovery of the indigenous flora of the skin was attempted by Killian (1950) who described a direct culture technique which relies either on an agar disc placed in contact with the skin for a certain length of time, or the application of a cylindrical tube to a small area of the skin. A volume of solution, poured into the cylinder, was continuously stirred so that organisms could be removed from the skin for culture. Laurie & Jones (1952) also used the agar disc method, the disc being placed on skin and removed by a gauze tab.

Price (1938) distinguishes two sorts of skin bacteria. Those which lie on the surface, or are loosely attached with dirt, he calls 'transients'; the deeper-seated ones, which are not necessarily different in kind, he calls 'residents'. Resident bacteria form a comparatively stable population and are characteristic of the individual. There appears to be a dynamic equilibrium between the host and the resident bacteria; the self-sterilizing power of the skin is an obvious factor which might affect this equilibrium, but there are probably other factors not at present understood, such as climate, hormone balance, age, etc.

Price (1938) studied skin sterilization by the 'multiple basin handwashing technique'. Hands were cleansed by scrubbing with soap in a series of bowls containing sterile water, so that the numbers of bacteria removed at each wash could be estimated from the wash-water. The counts showed that the reductions for successive basins occurred in a definite proportion. Price plotted cumulative totals of organisms against the number of the basins and obtained a logarithmic

curve. He estimated that, by using ordinary, disinfectant-free soap, with vigorous lathering and scrubbing, 50% of the organisms remaining on the skin were removed at each cleaning.

Pohle & Stuart (1940) and Traub, Newhall & Fuller (1944) reported some variability in the Price technique and made certain alterations, and Hufnagel, Walker & Howard (1948) thought that the variability reported by the previous authors was due to the difficulty of washing and scrubbing in a standardized manner. They prepared a machine for standardized mechanical scrubbing and rinsing using isolated areas of skin scrubbed at a constant rate with a specially designed brush pressed against the skin with constant force. Bacterial counts were done on the rinse water.

Price (1954) applied his original technique to the testing of disinfectant soaps, by using soap and a brushing technique in eight consecutive basins; after drying the disinfectant was applied to the hands and rinsed off. The hands were then soaped and brushed in a further eight bowls. From the bacterial counts, Price constructed curves and estimated the antibacterial activity of the disinfectant.

Cade (1950) adapted Price's original technique and introduced a new approach to the testing of disinfectant soaps. Subjects were asked to use the test soap for 1 or 2 weeks in a normal way. Control soaps only were used in the laboratory for collecting rinse-water for sampling. In this way, Cade calculated the effect of continual use of a disinfectant soap and suggested a method of testing which closely resembled actual user conditions.

It appeared to us from this review of the literature that the Cade modification of Price's technique offered the best way of assessing the performance of disinfectant soaps under conditions closely resembling those of actual use. The object of this paper is to examine the variables of this technique and to carry out such further modifications as become necessary to improve the tests, and finally to report the results obtained with a number of disinfectants.

METHOD FOR HANDWASHING

Subjects attended the laboratory to carry out the following procedure. The hands were wetted to the wrist line and disinfectant-free soap rubbed on for 15 sec., followed by 60 sec. lathering. Both hands were then rinsed in 2000 ml. sterile, lukewarm tap-water. This process was repeated in consecutive bowls depending upon the design and purpose of the experiment. Immediately after washing and rinsing, the water was stirred and three 1·0 ml. samples pipetted into sterile Petri dishes (neat). A fourth 1·0 ml. sample was mixed with 9·0 ml. sterile Ringer's solution and three 1·0 ml. samples were placed in sterile Petri dishes (10^{-1}); a further 1 in 10 dilution was made in Ringer's and added to sterile Petri dishes in triplicate (10^{-2}). To each of these dishes about 15 ml. melted nutrient agar at 48° C. was added and, after thorough mixing, the plates were incubated at 37° C. for 48 hr. Only those plates which contained 20–200 colonies were counted.

RESULTS

(i) *Bacteria removed by successive washes*

With the standardized washing procedure it was hoped that a constant proportion of bacteria would be removed from the hands by successive washes (Price, 1954). It should then be possible to make a straight line plot in which the ordinates would be the number of the basin against a function of the bacterial count. If in the washing sequence a disinfectant soap were substituted for the control soap a break in the line would be anticipated from which the disinfectant effect could be calculated.

Six subjects were chosen for this test and they each carried out the hand washing procedure in twelve successive basins. Basins 7 and 8 were not sampled. The count from each basin was averaged and the log of these was plotted against basin

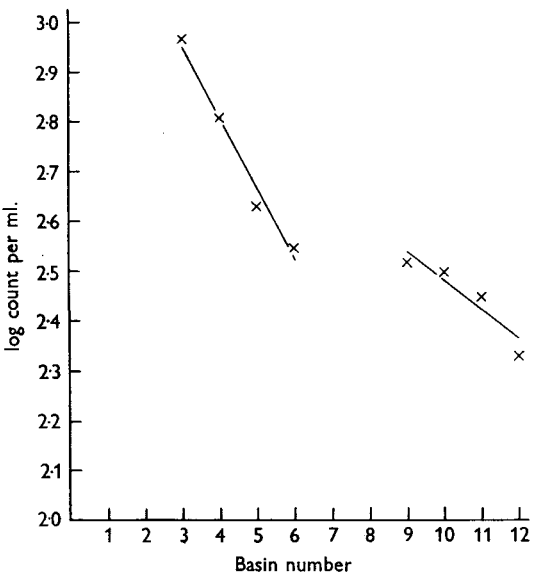

Fig. 3. Removal of bacteria by successive washes.

numbers. The results shown in Fig. 3 strongly suggest that bacteria were not removed at a constant rate and that more were removed at the beginning of the washings than at the end. This agrees with Meyer & Vichet (1943) results. This method was therefore not pursued and further investigations were concerned with the Cade test.

There are additional reasons why the Price test is not attractive. Disinfectants such as G 11, which are not fully compatible with soap, would erroneously appear ineffective by this test; other workers and ourselves have shown that a single wash with G 11 soap is no more effective than a wash with a control soap, yet G 11 soaps are effective when tested by the Cade test (Table 9). Furthermore, the washing procedure is very drastic; one could argue that this procedure is representative of surgical scrub-up but it does not simulate normal washing habits, and therefore volunteer panels for these tests were frequently difficult to find.

(ii) *Variables of the Cade test*

It can be anticipated that the major sources of variation in the Cade test will be due to individual variation and to differences between samples taken at different times (days or weeks). Individual variation in count over a period of 16 months is shown in Table 6; three subjects showed a variation of 319 to 3773 in the count of

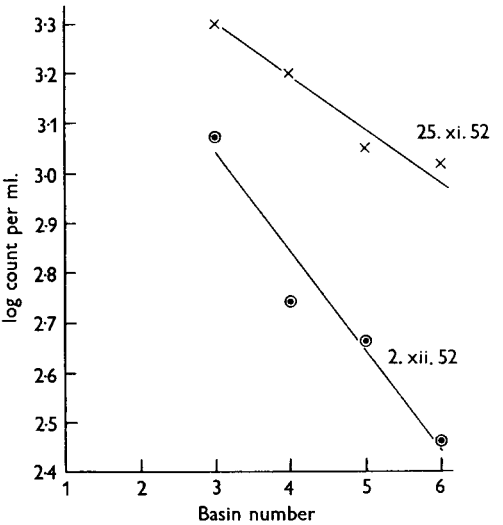

Fig. 4. Variation in count of one subject.

Table 6. *Variation in initial count on individuals over a period of* 16 *months*

Subject	No. of counts	Average	Count in fourth and 5th washes (number per ml.)* Range
1	7	1205	437–3711
2	9	2892	1297–5816
3	4	2376	319–3773
4	4	1127	185–1957
5	3	2219	1213–3856
6	7	2235	839–3474
7	5	2762	687–5000
8	3	1277	1008–1686
9	6	1847	739–3228
10	3	1345	616–1852
11	3	1135	579–2209
12	7	2186	772–3645
13	9	2009	1098–3521
14	4	2480	1206–4625
15	4	1735	1270–2520
16	7	2304	424–5366
17	6	3577	1909–4826
18	6	1031	773–1380
19	4	2587	1524–4039
20	10	876	607–1246

* For an average count per hand these figures should be multiplied by 10^3.

https://doi.org/10.1017/S0022172400038249 Published online by Cambridge University Press

wash water. This difference is of the same order as that caused by the action of a good disinfectant soap.

Counts from the same individual vary not only from time to time, but the rate of removal of the bacteria from the skin may vary on different occasions. This is shown in Fig. 4 where log count is plotted against basin number for the same individual sampled at 1 week's interval. The slopes of the lines are significantly different.

Although the factors causing this variation are not understood at present and merit considerably more work, two practical conclusions are apparent. First, test and control soaps should be used at the same time and not one after the other. In practice this means that the subjects taking part in the test are divided into two groups, one using the control and the other test soap. After a period (say 1 week) the control and the test soaps are exchanged. In this way, allowance can be made for unforeseen weekly variations. Secondly, the subjects themselves must be selected from panels of persons whose previous counts are known.

(iii) *A modified Cade test*

The minimum number of subjects for test was arbitrarily set at twelve so that individuals representing a good cross-section of initial counts could be used. These volunteers used control soap at least three times daily for 1 week in preparation for the following 2 weeks of trial when the subjects were divided into two groups, as follows. Second week of test group A changes to disinfectant soap and B continues with control soap. Third week of test group A returns to control soap and B changes to disinfectant soap.

Counts on the hands from the fourth and fifth washes were made daily during the second and third weeks.

This procedure was used with a soap containing 3·0% mercuric chloride. The results, shown in Fig. 5, show a decrease in the bacterial count during the second week of group A, when this group was using the disinfectant soap. During the corresponding period, group B used control soap only and wide variations in the count were obtained, without a consistent trend. During the third week of the test, group B showed a decrease in the count while group A returned to its former level of bacterial population.

These results have been analysed in two ways. First, similarly to the original Cade test, by calculating the percentage reduction of the log count by comparing the count on the day before washing with the disinfectant soap with the count obtained on the last day of washing with disinfectant soap. Secondly, the calculation was repeated to take account of the refinements of the modified technique.

The percentage reduction was calculated for the two groups and the means were pooled. For group A the percentage reduction was calculated by comparing the mean log count for the last 3 days in which the control soap was used with the last 3 days in which the disinfectant soap was used. In group B the counts of the last 8 days on the control soap were compared to the counts of the last 3 days on disinfectant soap.

Table 7 shows that the percentage reduction figure is substantially the same by the two methods of calculation. However, the confidence limits are much closer with the modified method when the calculations are based on additional information which enables any anomalies to be duly weighed.

Table 7. *Estimation of percentage reduction by two methods*

	Calculation similar to Cade test	New calculation
Best estimate of percentage reduction	92·0	95·6
(Confidence limits $P = 0{\cdot}95$)	5·3 and 99·3	72·3 and 99·3

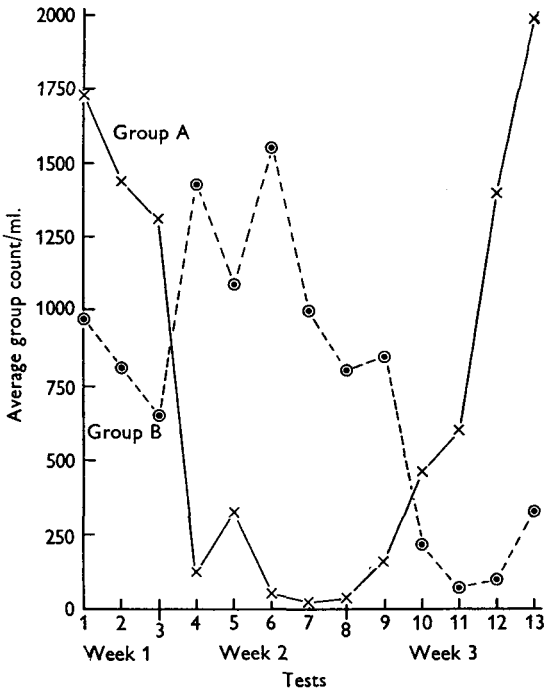

Fig. 5. Disinfectant activity of soap containing 3·0 % mercuric iodide.

Table 8. *Design of handwashing test*

Subject no.	Week 1	Week 2	Week 3	Week 4
1	A	C	B	D
2	C	D	B	A
3	D	A	B	C
4	B	A	C	D
5	B	D	A	C
6	D	B	C	A
7	C	B	A	D
8	A	D	C	B
9	A	B	C	D
10	B	C	D	A
11	D	C	A	B
12	C	A	D	B

https://doi.org/10.1017/S0022172400038249 Published online by Cambridge University Press

(iv) *Extension of the modified Cade test to include several test soaps*

The above experimental design only permits one soap to be examined and it is clear that if several experimental soaps are to be examined this should be done simultaneously rather than sequentially. This can be achieved with a multiple Latin-square design in which the rows represent subjects and the columns represent weeks.

As an example, an experiment is shown in which three disinfectant soaps (B, C, D) were tested against a control soap (A). Twelve subjects were available, so three Latin squares were needed.

Latin squares

A, B, D, C	A, C, B, D	A, D, C, B
B, A, C, D	B, D, A, C	B, C, D, A
C, D, B, A,	C, A, D, B	C, B, A, D
D, C, A, B	D, B, C, A	D, A, B, C

The rows of these squares were re-arranged in random order, giving the design shown in Table 8, so that in any week each of the soaps was used by the same number of subjects.

In these experiments the bacterial count was estimated daily. Calculation of weekly totals on a subject's hands was expressed by the total of the logarithms of counts from Tuesdays to Fridays. Experience has shown that counts were consistently higher on Monday than during the rest of the week; therefore the handwashing procedure was done on Mondays but not set up for counting. The weekly totals were analysed by the standard method for multiple Latin squares.

(v) *Results of handwashing tests with various disinfectants*

Table 9 shows the reduction in cutaneous flora caused by various disinfectant soaps.

Table 9. *Percentage reduction of cutaneous flora after using disinfectant soap for 7 days*

Disinfectant	Disinfectant conc. in soap			
	2·0%	1·0%	0·5%	0·2%
Dihydroxy-hexachlorodiphenylmethane (G 11)	81	—	—	—
Thiobis dichlorophenol (Actamer)	77	64	—	—
Tetramethyl thiuram disulphide (TMTD)	—	76	—	66
Trichlorcarbanilide (TCC)	72	—	—	—
Tribromsalicylanilide (TBS)	—	—	65	56
Tetrachlorsalicylanilide (TCS)	—	—	84	76

DISCUSSION

The first part of this paper has been concerned with *in vitro* bacteriological methods of evaluating disinfectants for use in soap. The tests have been selected from a wide variety of tests for their convenience, simplicity and reproducibility. The conditions laid down should be adhered to if similar results are to be obtained.

For instance, the minimal inhibitory concentration (M.I.C.) can be altered by the weight of the inoculum and the composition of the medium.

All the compounds described, with the exception of DCMX, were active at high dilution against *Staph. aureus* and this activity was reduced in the presence of 10 % serum. None of the disinfectants was very active against the gram-negative test-organism *Esch. coli*. This lack of activity is not very important so long as the application is in soap because soap alone is a powerful bactericide against *Esch. coli* provided it has the right fatty acid composition (Walker, 1924, 1925, 1926). This may be one of the reasons why this organism is rarely found on skin.

G 11 has a high soap-inactivation coefficient, possibly because the soap micelles appear to trap the compound which cannot then diffuse through agar. Chemical investigations (Evans & Jones, personal communication) have shown that G 11 is dissolved in the soap micelle and probably not available for antibacterial activity. This is confirmed by other observations; e.g. Price & Bonnett (1948) have shown that a single wash with a soap containing G 11 is no more effective than a single wash with soap alone. The fact that G 11 is effective in practice is best explained by its retention on the skin after the soap has been rinsed off (Annotation 1959).

The other disinfectants probably act at the time when the soap is used, although with Actamer and TMTD there is evidence that soap reduces this activity. The S.I.C. of TCC, TBS and TCS is less than unity and soap increases the zone diameters obtained with these disinfectants.

Soap may aid the solution of the disinfectant which will then become more active than a corresponding amount of disinfectant in a non-soapy solvent. Whether the dissolved disinfectant becomes trapped by the micelle probably depends on the chemical structure of the disinfectant. There is also a pH difference between soapy and non-soapy solutions which could affect the medium near the cups.

Objections have been raised to agar diffusion tests because they have apparently little bearing on what happens during the use of a disinfectant soap. Thus it is said that a disinfectant, which may have no practical application but is diffusible, could give rise to greater zones of inhibition than another disinfectant which is effective in practice but is only slowly diffusible through agar. This objection has been eliminated from these tests because disinfectants are not compared by zones of inhibition, but by inactivation coefficients which are obtained by comparing the potency of two solutions of one disinfectant. It is clear, however, that the soap inactivation coefficient alone cannot be used to judge new disinfectants and that the bacteriostatic potency must also be noted. For instance, G 11 would have been rejected on the results of the S.I.C. test alone.

On the basis of the four *in vitro* screening tests, the disinfectants can be arranged in the following ascending order of suitability for use in soap: DCMX, G 11 or Actamer, TMTD, TBS, TCC, TCS.

The second part deals with the bacterial flora of the skin and the reduction of the resident populations of these bacteria by various methods of handwashing. Most of the investigations in this field have been concerned with helping the surgeon and

his assistants in the effective preparation of their hands before a surgical operation by reducing the time spent in 'scrubbing-up'.

We have done a small-scale investigation to discover the time usually taken for handwashing. We found the period varied between 10 and 30 sec. Such superficial cleansing bears very little relationship to the intensive cleansing undertaken by a surgeon and will probably remove only a small percentage of so-called transient bacteria. It therefore appears necessary, in the future, to determine the effect of disinfectant soaps on skin flora as a whole. Bacteria removed from washes 1, 2 and 3 in the laboratory should be enumerated, as well as those from 4 and 5. The handwashing test described in this paper, with the subjects using the test soap in their habitual manner, resembles use conditions more closely than the original technique of Price. However, for surgical use, the results are more difficult to interpret.

Transient bacteria are also important from the point of view of hospital cross-infection and contamination of food. An ideal disinfectant should attack transient bacteria and break the vicious circle of contamination and recontamination.

As regards the resident deep-seated bacteria of the skin they are of interest not only from the point of view of the surgeon but also as producers of body odour.

In this connexion, the use of nutrient agar as the only plating medium can be questioned. Evans *et al.* (1950) have shown that *Corynebacterium acnes*, an anaerobe, is the most numerous organism on some individuals. This organism does not grow on nutrient agar but may do so on serum agar. It may be that this organism is important in body-odour production.

The confidence limits of the percentage reductions given in Table 7 are of interest. Even with the improved experimental design they range from 72 to 99 and the handwashing test results may vary by 20 % even when the same soaps and identical techniques are employed.

Because it is not yet practicable to sterilize skin by washing alone, the aim has been to achieve as high as possible a reduction of the flora. It is therefore difficult to decide on a standard of satisfactory performance, especially when the same handwashing procedure can give a 20 % difference between laboratories. In our experience, the regular use of a soap containing no disinfectant can cause a reduction of up to 50 %; this can be accentuated by the use of brushing and scrubbing technique. On the other hand, the highest reduction ever obtained in a handwashing test was about 95 % when a soap containing 3 % mercuric iodide was tested. Between these two results fall disinfectant soaps which are fairly good, such as 2 % G 11 soaps causing reductions of 70–80 %. For the moment, it would appear reasonable to use a 2 % G 11 soap as a standard and to aim in the test for a reduction which is not less than that given by the G 11 soap.

SUMMARY

Part I

1. The following disinfectants were investigated for use in bar-soaps: Hexachlorophene (G 11) (2:2′-dihydroxy-3:5:6:3′:5′:6′-hexachlorodiphenylmethane); Actamer (2:2′-thiobis, 4-6-dichlorophenol); DCMX (dichloro-*m*-xylenol); TMTD

(3:4:5-tetramethyl thiuram disulphide); TCC (3:4:4′-trichlorcarbanilide); TBS (3:4′:5-tribromsalicylanilide) and TCS (3:3′:4′:5-tetrachlorsalicylanilide).

2. The concentration required for bacteriostasis of *Staph. aureus* was 2·5 p.p.m. for DCMX and 0·1 p.p.m. for TCS, the other disinfectants falling between these values. None were effective against *E. coli*, 12·5–50 p.p.m. being required for bacteriostasis (Table 1).

3. In the presence of serum the concentration required for bacteriostasis was increased (Table 4).

4. The soap inactivation coefficient (S.I.C.) is a value derived from zone diffusion tests. Zone diameters of the same disinfectant are compared in soap and soap-free solution so that the S.I.C. measures the effect of soap on disinfectant activity.

5. DCMX is inactivated by soap (S.I.C. = ∞). The activity of G 11 is much reduced by soap (S.I.C. = 200). Actamer and TMTD are active in soap, whereas TCC, TBS and particularly TCS is more active in soap than soap-free solution (S.I.C. = 0·32) (Table 3).

6. Another test based on zone diffusion is described which measures the effect of soap-soluble skin substances on disinfectant activity. Zone diameters of the same disinfectant are compared in fresh soap and used soap solutions.

7. Of the disinfectants tested only TMTD lost some of its activity in the presence of soap-soluble skin substances (Table 5).

Part II

8. Conventional bacteriological plate-counting technique was used to enumerate bacteria in wash-water. The bacteria were removed from hands by using a standardized washing procedure with bar-soap but without scrubbing.

9. Bacteria were not removed uniformly when washing was carried out twelve times in succession, significantly more bacteria being removed at the beginning of of the washing procedure.

10. When the same individuals were tested at weekly intervals the rate at which bacteria were removed in successive washes was found to vary significantly.

11. Using a single standardized wash the variation in the count of twenty individuals was followed for a period of 16 months. The count from one individual could vary by a factor of 10.

12. A design for a handwashing test, to take account of these variables is described. The bacterial counts of the wash-water when subjects used control soap for 1 week was compared with counts obtained from subjects using disinfectant soap. Subjects and soaps were randomized according to multiple Latin squares.

13. This handwashing technique was used to test the disinfectants described in Part I of this paper. All these disinfectants were effective but tetrachlorsalycilanilide (TCS) was the best. Soap containing 0·5 % TCS caused an 84 % reduction (Table 9).

The authors wish to thank Dr H. Wilkinson for the ideas he contributed and the interest he has shown in this work, and the Directors of Unilever Limited for permission to publish this paper.

REFERENCES

ANNOTATION (1959). *Brit. med. J.* i, 1398.
ARNOLD, L. (1942). *J. invest. Derm.* **5**, 207.
BAER, R. L. & ROSENTHAL, S. A. (1954). *J. invest. Derm.* **23**, 193.
BEAN, H. S. & BERRY, H. (1950). *J. Pharm. Pharmacol.* **2**, 484.
BRYAN, C. S. & MALLMANN, W. L. (1932). *J. Lab. clin. Med.* **18**, 1249.
BURTENSHAW, J. M. L. (1938). *J. Hyg., Camb.*, **38**, 575.
BURTENSHAW, J. M. L. (1942). *J. Hyg., Camb.*, **42**, 184.
CADE, A. R. (1950). *J. Soc. cosmet. Chem.* **2**, 281.
DIASIS, F. A. (1934). *Med. Rev.* of *Revs., N.Y.*, **40**, 421.
EGAN, R. R. & REED, M. D. (1953). *Soap, N.Y.*, **29**, 42.
EVANS, C. A., SMITH, W. M., JOHNSTON, S. H. & GIBLET, E. R. (1950). *J. invest. Dermatol.* **15**, 305.
FIRMENICH & CO. LTD. (1954). *Soap, Perfum. Cosm.* **27**, 165.
GEMMELL, J. (1952). *Mfg. Chem.* **23**, 63.
GILLESPIE, W. A., SIMPSON, K. & TOZER, R. C. (1958). *Lancet*, ii, 1075.
GUMP, W. S. (1945). *Soap, N.Y.*, **21**, 36.
GUMP, W. S. & CADE, A. R. (1952). *Soap, N.Y.*, **28**, 52.
HARE, R. & RIDLEY, M. (1958). *Brit. med. J.* i, 69.
HARE, R. & THOMAS, C. G. A. (1956). *Brit. med. J.* i, 840.
HELLATT, A. (1948). *Ann. Med. exp. Fenn, Suppl.* 26.
HIRSCH, A. & MURAS, R. (1955). *J. appl. Bact.* **18**, 425.
HOWARD, M. P. & MINCH, V. A. (1951). *Food Res.* **16**, 133.
HUFNAGEL, C. A., WALKER, C. W. & HOWARD, R. W. (1948). *Surgery*, **23**, 753.
KÄLLANDER, A. (1953). *Nordisk hyg. Tidskr.* nos. 1–2, 1–8.
KEMPF, A. H. & NUNGESTER, W. J. (1942). *J. infect. Dis.* **71**, 174.
KILLIAN, J. A. (1950). *Proc. sci. Sect. Toilet Goods Ass.* **34**, 13.
LAURIE, P. & JONES, B. (1952). *J. Pharm.* **168**, 288.
LOVELL, D. L. (1945). *Surg. Gynec. Obstet.* **81**, 174.
LOWBURY, E. J. L. & FOX, J. (1953). *J. Hyg., Camb.*, **51**, 203.
MEYER, E. & VICHER, A. E. (1943). *Arch. Surg.* **47**, 468.
MILES, A. A., WILLIAMS, R. E. O. & CLAYTON-COOPER, R. (1944). *J. Path. Bact.* **56**, 513.
MONSANTO CHEMICAL COMPANY (1953). *Technical Bulletin* ODB-53-12.
MONSANTO CHEMICAL COMPANY (1957). *Technical Bulletin* O-4.
NUNGESTER, W. J., THIRLBY, R. L. & VIAL, A. B. (1949). *Surg. Gynec. Obstet.* **88**, 639.
PAYNE, A. M. M. (1949). *Mon. Bull. Minist. Hlth Lab. Serv.* 263.
POHLE, W. D. & STUART, L. D. (1940). *J. infect. Dis.* **67**, 275.
PRICE, B. & BONNET, A. (1948). *Surgery*, **24**, 542.
PRICE, P. B. (1938). *J. infect. Dis.* **63**, 301.
PRICE, P. B. (1954). *Antiseptics, Disinfectants, Fungicides and Sterilisation*, ed. Reddish, p. 321. London: Henry Kimpton.
RICKETTS, C. R., SQUIRE, J. R., TOPLEY, E. & LILLY, H. A. (1951). *Clin. Sci.* **10**, 89.
SHELLEY, W. B., HURLEY, H. J. & NICHOLS, A. C. (1953). *Arch. Derm. Syph., N.Y.*, **194**, 430.
SHUMARD, R. S., BEAVER, D. J. & HUNTER, M. C. (1953). *Soap, N.Y.*, **29**, 34.
STORY, P. (1952). *Brit. med. J.* ii, 1128.
STRAUSS, J. S. & KLIGMAN, A. M. (1956). *J. invest. Derm.* **27**, 67.
SYKES, G. J. (1955). *J. Pharm. Pharmacol.* **7**, 561.
TRAUB, E. F., NEWHALL, C. A. & FULLER, J. R. (1944). *Surg. Gynec. Obstet.* **79**, 205.
VINSON, L. J. (1954). *Soap, N.Y.*, **30**, 44.
WALKER, J. E. (1924). *J. infect. Dis.* **35**, 557.
WALKER, J. E. (1925). *J. infect. Dis.* **37**, 181.
WALKER, J. E. (1926). *J. infect. Dis.* **38**, 127.
WILLIAMS, R. E. O. (1946). *J. Path. Bact.* **58**, 259.