Comparative antibacterial activity of hexachlorophane in different formulations used for skin disinfection

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SYNOPSIS Two formulations of hexachlorophane have been compared for their antibacterial effects in respect of skin disinfection. It was found that the activity of hexachlorophane is dependent upon its vehicle of formulation. A 2.5% soap gel possesses broad-spectrum bactericidal activity with a remarkable speed of kill, whereas a 3% detergent formulation has no bactericidal action against Gram-negative bacteria and only a very slow action against Gram-positive bacteria.

In practice the rapid action of the 2.5% soap gel against both Gram-negative and Gram-positive transient skin bacteria can be achieved by correctly applying the preparation directly to the dry hands.

It appears that the 2.5% soap gel does not need to rely on mechanical removal of transient organisms as does the 3% detergent.

The 2.5% soap gel is more dependable in its action on the resident bacteria than the 3% detergent. It controlled the resident flora in the skin of all subjects tested whereas the latter appeared to be potentiated on the skin of certain individuals only.

It has been possible to distinguish between the antibacterial effect on the resident organisms and the mere removal of transient bacteria by mechanical action of the 3% detergent as opposed to the antibacterial effect on residents and rapid antibacterial effect on transients by the 2.5% soap gel.

Many publications in Britain and the United States have described the value of hexachlorophane preparations as skin degerming agents. Various methods have been employed to determine the reduction of organisms, but not all distinguished between antibacterial action and mechanical removal due to the detergency of the preparation. Further, little work has been published on the reasons for using particular vehicles to carry the hexachlorophane. Hufnagel, Walter, and Howard (1948) proposed the use of alkylphenoxypolyether sulphonate as a vehicle for 3% hexachlorophane. This was claimed to be more effective than the bar soap preparations of Traub, Newhall, and Fuller (1944) and confirmed by Udinski in 1945 and Seastone in 1947. Although Price (1951) had made such statements as 'the presently popular brief period of hand washing was not as efficacious as had been previously claimed', 'hexachlorophane does not disinfect the skin quickly,

as alcohol does, but much more slowly . . .', 'the slow degerming is attributed to a film of the agent left on the hands after washing with hexachlorophane', 'it is necessary to use hexachlorophane preparations exclusively and frequently', Smylie, Webster, and Bruce in 1959 confirmed the findings of Hufnagel *et al.*

PROPERTIES OF HEXACHLOROPHANE PRODUCTS

The properties of the various hexachlorophane products should therefore be considered.

SOLID BAR SOAP A known amount of the product cannot be applied and due to its hardness only a very small fraction of the antiseptic is transferred to the skin. This is further complicated by a subject's individual method of washing. A bar soap can also be contaminated when passed from hand to hand, especially if the antiseptic is specific against Gram-positive organisms.

LIQUID PREPARATIONS BASED ON DETERGENTS OR SOAP These have the advantage that the skin surface may be subjected to the maximum amount of hexachlorophane before the preparation is diluted with water. It should also be noted that the 3% hexachlorophane/detergent of Hufnagel *et al* contained the water-repellent substances lanolin, cholesterol, and white soft paraffin; thus the hexachlorophane may not be reduced in activity by subsequent dilution with water.

Lowbury, Lilly, and Bull (1963) found the 3% hexachlorophane detergent product superior to a 2% hexachlorophane bar soap but also found a 2% hexachlorophane liquid soap equal to the 3% hexachlorophane detergent. Since the method of test involved washing under running water, the 2% hexachlorophane liquid soap, not containing water-repellent substances, could well have been diluted more quickly than the 3% hexachlorophane detergent, yet worked as well. Lowbury *et al* concluded that the vehicle for the hexachlorophane seemed to have some effect.

Chemically there is a significant difference between a colloidal suspension of hexachlorophane and a solubilized hexachlorophane in liquid soap, the latter being a molecular dispersion in the soap micelle. The work of Bean and Berry (1953) indicated that the bactericidal activity of a phenol (and hexachlorophane is a phenol) solubilized by a soap is a function of the concentration of phenol in the soap micelle and is independent of the total phenol in the system, provided that micellar saturation is satisfied. It follows that increasing the concentration of a phenol may not improve the bactericidal activity of a formulation and in fact it is the experience of the author that random increase may indeed reduce bactericidal activity.

Hexachlorophane has been stated to be predominantly effective against Gram-positive organisms yet it is frequently used in situations where Gram-negative organisms are of importance. The present paper shows that the vehicle for the hexachlorophane plays a major part in determining its antibacterial activity (even against Gram-negative organisms) and that it is unjust to define the activity of a group of hexachlorophane products on the basis of having tested one number assumed to be representative of the group.

PART I

In considering relationships between detergent and soap vehicles we carried out simple culture mortality counts comparing a detergent-hexachlorophane formulation with soap gel formulations containing hexachlorophane.

BROAD SPECTRUM EVALUATION Of the formulations, 2 g was inoculated with 0.2 ml of a 24-hour broth culture of *Pseudomonas aeruginosa* NCTC 7244, *Escherichia coli* NCTC 9002, *Salmonella typhi* NCTC 8393, and *Staphylococcus aureus* NCTC 9789 (phage 80/81).

The formulation and culture were mixed and subcultures taken at intervals of 0.5, 2, 4, 8, and 16 minutes and serially diluted to give 1 part of hexachlorophane to 333,000 in quarter strength Ringer's solution containing 1% Tween 80. The decimal dilutions were plated onto nutrient Tween 80 agar. A loopful of the formulation-culture mixture was also taken straight on to Tween 80 agar and no differences in the results were found from those of the serial dilution method. (Complete inhibition was found with agar containing no Tween 80.)

RESULTS A 3% hexachlorophane detergent formulation required a period of between eight and 16 minutes to destroy the staphylococcus whilst no reduction in the numbers of the Gram-negative organisms was discerned even over 30 minutes' contact. A 2.5% hexachlorophane to 23% potassium soap gel completely killed all cultures in under two minutes, only 30 seconds being necessary against the staphylococcus and pseudomonas. A 3% hexachlorophane-23% potassium soap gel, although highly active against the staphylococcus, was not as effective against the Gram-negative organisms as was the 2.5% hexachlorophane soap gel.

The possibility of the 2.5% hexachlorophane soap gel deriving activity from something other than hexachlorophane was excluded by testing the formulation from which the hexachlorophane had been omitted. This was found devoid of activity against staphylococcus, but some slight activity was found against pseudomonas and salmonella. On comparing minimal inhibition concentrations of the two formulae a very much higher level of activity was found when hexachlorophane was present. The activity of the hexachlorophane soap gel could be neutralized with Tween 80 whereas that of the soap gel without hexachlorophane could not. We were satisfied that the antiseptic effect of a 2.5% hexachlorophane-23% potassium soap gel was essentially a function of the hexachlorophane complex.

Since the results obtained for this gel were surprising on the basis of available literature we decided to extend the preliminary investigation over a wider range of organisms. There seemed little point in evaluating the 3% hexachlorophane detergent or 3% hexachlorophane soap gel further since they were so obviously inferior in bactericidal activity.

The method employed was as described above with direct plating of the formulation-culture mixture on to nutrient or blood agar containing Tween 80. The

organisms tested and the results obtained are shown in Table I.

TABLE I

Organism	Maximum Time (min) for 100% Bacterial Kill
<i>Staphylococcus aureus</i> (7 strains)	0.5
<i>Corynebacterium diphtheriae</i>	0.5
<i>Escherichia coli</i> (6 strains)	2
<i>Aerobacter aerogenes</i>	2
<i>Klebsiella pneumoniae</i>	4
<i>Pseudomonas aeruginosa</i> (5 strains)	0.5
<i>Pseudomonas fluorescens</i>	0.5
<i>Proteus mirabilis</i> , <i>flexneri sonnei</i>	0.5
<i>Salmonella typhi</i> (5 strains)	2
<i>Salmonella dublin</i> , <i>enteritidis</i> , <i>typhimurium</i> , <i>pullorum</i> , <i>cholerae suis</i>	2
<i>Salmonella paratyphi A</i> and <i>paratyphi C</i>	2
<i>Shigella dysenteriae</i> , <i>flexneri sonnei</i>	2
<i>Streptococcus agalactiae</i> , <i>faecalis</i> , <i>hominis</i> , <i>lactis</i> , 'Viridans', <i>pyogenes</i> (3 strains)	0.5
<i>Diplococcus pneumoniae</i>	0.5

The encouraging results obtained against both Gram-positive and Gram-negative organisms with this gel prompted us to investigate its effect upon organisms on the skin. The 3% hexachlorophane detergent formulation was also used for comparison.

PART II

Throughout the remainder of this paper the 3% hexachlorophane detergent formulation will be referred to as the 3% detergent and the 2.5% hexachlorophane-23% potassium soap gel as the 2.5% soap gel.

REDUCTION OF TRANSIENT ORGANISMS Lowbury, Lilly, and Bull (1964) stated that *Staphylococcus aureus* is the only common resident pathogen. This organism and others such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*, which become deposited on the skin, may be regarded as representatives of pathogenic transient organisms.

We decided to test the effect of the 2.5% soap gel and 3% detergent as rapid antiseptics on transient organisms, excluding their action against the resident flora, and selected *Staphylococcus aureus* NCTC 9789, *Escherichia coli* NCTC 9002, *Salmonella typhi* NCTC 8393, and *Pseudomonas aeruginosa* NCTC 7244 as test bacteria. Experiments showed the *Escherichia* to be extremely susceptible to drying; frequently it was impossible to recover viable organisms even from untreated skin, and we discontinued its use. *Salmonella* and *pseudomonas* also showed some tendency to die naturally when in contact with a dry skin. This effect was overcome by moistening the skin with sterile distilled water and allowing it to dry in air before inoculating with

Salmonella typhi, or by allowing a very short time for drying between inoculation with *Pseudomonas aeruginosa* and application of the antiseptic.

METHOD Before each test the hands were swabbed with 70% alcohol and allowed to dry. A 1 mm loopful of the appropriate culture was spread over the finger tips of each hand and when dry the formulations were spread over the inoculated areas of the left hand and allowed to act for 30 seconds before swabbing with calcium alginate swabs. The swabs were immediately immersed in 10 ml of Calgon Ringer Tween 80 solution.¹ The right hand was similarly inoculated, but without any antiseptic being applied and served as a control. It was calculated that 0.025 g was picked up by a swab which diluted to 1/400 on immersion in the 10 ml of Calgon Ringer-Tween 80 solution. This was serially diluted 10, 100, and 1,000-fold and pour plates were prepared from 1 ml of each dilution in 15 ml of nutrient agar containing 1% Tween 80. From earlier investigation we were satisfied that any bacteriostatic effect of carried over antiseptic would be completely neutralized at the first stage, that is, on placing the swab in the 10 ml of Calgon Ringer-Tween solution. By adopting the above method, applying the formulations without a washing action we were able to distinguish between true antiseptic effect and mechanical reduction of organisms.

RESULTS Tables II and III show the results of the experiments. As may be seen, the 2.5% soap gel largely reduced the numbers of all three test organisms. It may also be seen that the effect of the 3% detergent formulation was comparatively insignificant except for a small reduction against *Staphylococcus aureus* only.

PART III

SUPPRESSION OF RESIDENT ORGANISMS A use for hexachlorophane preparations is the maintenance of near sterility inside the gloves of surgeons. Even though transient organisms may be controlled by scrubbing up before donning gloves deep-seated resident staphylococci are constantly reaching the skin surface by perspiration and movement. On the hypothesis that hexachlorophane deposits as a film on the skin and perhaps in the pores, any staphylococci reaching the skin surface will come into contact with it and be destroyed before escaping through a damaged glove. It was decided to test the efficiency of the 2.5% soap gel and the 3% detergent over a contact time of one hour in surgical gloves.

METHOD A timetable was devised employing the two

¹Calgon Ringer-Tween 80 solution was prepared from Calgon Ringer solution tablets (Oxoid code no. BR.49) which provide a solution containing 1% Calgon in quarter strength Ringer solution (Higgin, 1950). One per cent of Tween 80 was added to this.

TABLE II

RANGE OF VIABLE COUNTS AFTER 30-SECOND CONTACT WITH 2.5% HEXACHLOROPHANE-23% POTASSIUM SOAP GEL
Five Tests for Each Subject

	J.W.L.	J.M.C.	J.A.	J.S.	K.W.
<i>Staphylococcus aureus</i> NCTC 9789 and 2.5% Hexachlorophane-23% Potassium Soap					
Neat	0.2	0.3	0.3	0.8	0.3
There were no viable counts for dilutions 1/10, 1/100, 1/1,000					
Control ¹					
Neat	5+>5+	3+>5+	2+>5+	>5+	3+>5+
1/10	60-2+	39->5+	17->5+	98->5+	34-5+
1/100	4-30	4-3+	5-4+	10-5+	1-45
1/1,000	1-8	0-12	0-40	2-9	0-3
<i>Pseudomonas aeruginosa</i> NCTC 7244 and 2.5% Hexachlorophane-23% Potassium Soap					
Neat	0.1	0	0.6	0.2	0
There were no viable counts for dilutions 1/10, 1/100, 1/1,000					
Control					
Neat	>5+	200->5+	4+>5+	4+>5+	3+>5+
1/10	2+>5+	6->5+	35->5+	30->5+	43->5+
1/100	20->5+	0->5+	2-3+	1-5+	0-3+
1/1,000	0-228	0-5+	0-40	2-50	0-24
<i>Salmonella typhi</i> NCTC 8393 and 2.5% Hexachlorophane-23% Potassium Soap					
Neat	0	0	0.4	0.2	0
There were no viable counts for dilutions 1/10, 1/100, 1/1,000					
Control					
Neat	79-4+	31-3+	104-5+	22->5+	33-93
1/10	4-32	2-27	10->5+	6-4+	3-7
1/100	0-4	0-6	0-3+	0-2+	0
1/1,000	0	0	0-20	0-27	0

>5+ = considerably greater than 500; 5+ = 500 approximately; 4+ = 400-499; 3+ = 300-399; 2+ = 200-299.

'Neat' = swab immersed in 10 ml Calgon/Ringer/Tween 80, 1 ml for pour plates.

'Neat' serially diluted to 1/10, 1/100, 1/1,000 (see text for actual dilution of formulations), 1 ml of each for pour plates.

¹Control was inoculated with culture only.

hexachlorophane preparations and an unmedicated bar soap. The timetable is set out below.

Formulation	No. of Days of Test
Bar soap	4
2.5% Soap gel	6
Bar soap	5
3% Detergent	6
Bar soap	5

Experiments were as continuous as possible, but no work was done on Saturdays or Sundays.

1 Unmedicated bar soap The hands were washed for two-and-a-half minutes with the soap, then rinsed for a further minute. Sterile distilled water at approximately 40°C was used for both washing and rinsing. We have preferred this, since earlier examination of some hospital and laboratory water supplies had shown us that they might be grossly contaminated with organisms which could confuse viable counts, or be mistaken for staphylococci.

2 2.5% Soap gel The preparation was added to the dry hands from a specially made dispenser (1 g gel to two hands), rubbed into the skin for 30 seconds, and then rinsed off in the sterile distilled water at 40°C.

3 3% Detergent The hands were wetted with sterile distilled water and the 3% detergent preparation added (approximately 1 g) from an appropriate dispenser (as

recommended and supplied by the manufacturer). The hands were washed by gradually adding more sterile distilled water until all of the preparation appeared to be washed off, a procedure which took about two-and-a-half minutes.

After rinsing in sterile distilled water, the hands were dried with a sterile towel.

Sterile rubber gloves were worn for one hour.

After one hour the gloves were removed, excess glove powder was shaken from the fingers and the finger tips were streaked over nutrient agar containing 1% Tween 80 (one plate/hand).

The plates were incubated at 37°C for 24 hours and the colonies counted. After re-incubation for a further 24 hours the colonies were counted again.

Plates showing sterility or near sterility were streaked with 1 in 100,000 dilutions of 24-hour broth cultures of *Staphylococcus aureus* and *Escherichia coli*. No inhibitory effects were noted.

RESULTS The results are shown in Table IV. Colonies were not typed biochemically, but after 24 hours' incubation there appeared to be a pre-dominance of staphylococci except in the case of R.G. whose colonies were more varied. After 48 hours' incubation the numbers of staphylococci had not appeared to increase, but increases were recorded in the numbers of non-staphylococcal colonies.

TABLE III

RANGE OF VIABLE COUNTS AFTER 30-SECOND CONTACT WITH 3% HEXACHLOROPHANE DETERGENT FORMULATION

Five Tests for Each Subject

	J.W.L.	J.M.C.	J.A.	J.S.	K.W.
<i>Staphylococcus aureus</i> NCTC 9789 and 3% Hexachlorophane Detergent					
Neat	10-4+	96-3+	99->5+	66->5+	8-2+
1/10	0-34	4-30	22-4+	2-2+	0-25
1/100	0-40	0-1	0-17	0-5	0-5
1/1,000	0-1	0	0-1	0-3	0
Control ¹					
Neat	104->5+	5+>5+	>5+	2+>5+	2+>5+
1/10	10->5+	64-3+	138-5+	34-2+	32-2+
1/100	0->3+	4-58	10-66	0-36	3-37
1/1,000	0-6	1-11	0-16	0-7	0-5
<i>Pseudomonas aeruginosa</i> NCTC 7244 and 3% Hexachlorophane Detergent					
Neat	88->5+	68->5+	>5+	3+>5+	2+>5+
1/10	9-3+	5-5+	136-5+	34->5+	24-2+
1/100	1-33	2-4+	15-77	2-32	0-42
1/1,000	0-1	0-54	1-10	0-5	0-3
Control					
Neat	55->5+	5+>5+	>5+	>5+	2+>5+
1/10	7-5+	64->5+	4+>5+	172->5+	18-3+
1/100	0-115	6-2+	48-3+	20-5+	1-53
1/1,000	0-28	0-26	10-46	1-44	0-4
<i>Salmonella typhi</i> NCTC 8393 and 3% Hexachlorophane Detergent					
Neat	76->5+	76-3+	45->5+	48->5+	13->5+
1/10	0-2+	6-36	4-113	3-3+	2-88
1/100	0-12	0-10	0-13	0-26	0-22
1/1,000	0-4	0	0-3	0-12	0-10
Control					
Neat	111->5+	38->5+	66->5+	43->5+	29->5+
1/10	7-5+	10->5+	5->5+	1-3+	1-74
1/100	1-80	0-72	2-2+	0-40	0-25
1/1,000	0-12	0-2	0-34	0	0-4

>5+ = considerably greater than 500; 5+ = 500 approximately; 4+ = 400-499; 3+ = 300-399; 2+ = 200-299.

'Neat' = swab immersed in 10 ml Calgon/Ringer/Tween 80, 1 ml for pour plates. 'Neat' serially diluted to 1/10, 1/100, 1/1,000 (see text for actual dilution of formulations), 1 ml of each for pour plates.

¹Control was inoculated with culture only.

The results show very low counts from all subjects using the 2.5% soap gel with the possible exception of R.G. It was discovered that R.G. had been using a water-resistant barrier cream at this time which might have prevented the hexachlorophane preparation from penetrating the pores. The results for the 3% detergent show moderately low counts on J.A., J.S., and R.G. but J.W.L. and J.M.C. were different in showing (1) a higher total number of survivors, and (2) an increase in survivors between 24 and 48 hours' incubation.

It is interesting to note that both preparations gave low viable counts compared with bar soap at the first and subsequent washings, which is against the accepted thinking that most hexachlorophane preparations have a delayed effect due to cumulative action of the antiseptic on repeated application. The reason for this rapid action is not clear, but may be due to the wearing of gloves when skin secretions solubilize available hexachlorophane making it more antiseptic, or more probably to an additional antiseptic effect exerted by accumulated perspiration. In

presenting the results we have preferred to record the average viable counts from the three treatments rather than to show percentage reductions in the counts of the hexachlorophane preparations over the bar soap. We believe that the latter presentation frequently gives a false picture of activity. Suffice it to say that the 2.5% soap gel maintained near sterility over a contact time of one hour whilst the 3% detergent reduced the viable count considerably.

PART IV

Although we believe that the preceding parts of the paper have indicated that the 2.5% soap gel has satisfactory action against both the transient and resident skin flora and is superior to the 3% detergent which only acts slowly upon the transients and erratically against the residents we decided to investigate further. Streak plates were taken immediately after washing with bar soap or the hexachlorophane preparations. This method fraught with difficulties as attempts must be made to

TABLE IV

RESULTS

Material Used	Average Count per Person			
	Name	24 Hours	48 Hours	Increase over 48 Hours
Soap (4 tests)	J.W.L.	79.3	79.3	0
	J.M.C.	244.5	244.5	0
	J.A.	102.8	106.0	3.2
	J.S.	15.5	37.6	22.1
	R.G.	225.0	225.0	0
	B.D.	117.8	117.8	0
	Total	784.9	810.2	25.3
	Average	130.8	135	4.2
2.5% Hexachlorophane-23% potassium soap (6 tests)	J.W.L.	3.9	4.0	0.1
	J.M.C.	4.5	5.7	1.2
	J.A.	3.0	3.3	0.3
	J.S.	2.2	5.8	3.6
	R.G.	13.8	19.4	5.6
	B.D.	2.6	3.7	1.1
	Total	30.0	41.9	11.9
	Average	5	7	2
Soap (5 tests)	J.W.L.	776.4	825.2	48.8
	J.M.C.	209.8	211.8	2.0
	J.A.	139.0	155.2	16.2
	J.S.	13.4	53.8	40.4
	R.G.	99.6	166.6	67.0
	Total	1,238.2	1,412.6	174.4
	Average	247.6	282.5	34.9
3% Hexachlorophane detergent (6 tests)	J.W.L.	31.3	58.4	27.1
	J.M.C.	86.7	111.4	24.7
	J.A.	4.4	5.8	1.4
	J.S.	—	2.2	2.2
	R.G.	9.6	14.1	4.5
	Total	132.0	191.9	59.9
	Average	26.4	38.4	12.0
Soap (5 tests)	J.W.L.	110.2	214.8	104.6
	J.M.C.	494.4	593.4	99.0
	J.A.	273.4	278.4	5.0
	J.S.	16.6	78.4	61.8
	R.G.	157.0	318.4	161.4
	Total	1,051.6	1,483.4	431.8
	Average	210.3	296.7	86.4

¹R.G. had a 17-day gap of testing between 2.5% hexachlorophane-23% potassium soap tests 3 and 4.

detect (a) the normal levels of both transient and resident bacteria; (b) the period taken to return to normal levels of skin flora after discontinuing medication; and (c) the genuine antibacterial action against both transient and resident organisms as well as mechanical reduction.

The viable counts obtained after washing with unmedicated bar soap represent survivors which have escaped mechanical removal. They may show a preponderance of resident organisms, the transient being removed first, though this may be erratic. Price (1951) and Lowbury *et al* (1963), amongst others, have stated that a single wash with hexachlorophane is not enough, the reason being invariably attributed to the hypothesis of hexachlorophane forming a film on the skin which, being insoluble, can only act slowly, or not at all if hexachlorophane is only present in insufficient quantity.

If sufficient hexachlorophane can be supplied at one application it might follow that a dramatic reduction in organisms would be immediately obvious depending upon the speed of antiseptic action on the bacteria in direct contact with the preparation. It is important to exclude confusing effects of mechanical reduction and this, we contend, can only be done by employing the method described in Part II.

METHOD The hands were treated with the three preparations as described under the method in part III. The period of washing and rinsing with the 2.5% soap gel was extended to two minutes (following the initial 30-second application) to bring it into line with the procedures of the other two preparations. The hands were gently dried on a sterile towel and five minutes after the initial contact with the preparation the finger tips were streaked over nutrient agar-1% Tween 80 plates, which were incubated at 37°C, and the colonies counted after 24 and 48 hours.

Increases in viable counts between 24 hours' and 48 hours' incubation varied considerably, from several hundred in the case of bar soap, to about 30 for the 2.5% soap gel. We therefore decided to analyse the 48-hour counts only. As a general rule counts over 200 have been approximated to the nearest 100, all counts being carried out by a single technician.

RESULTS The two main blocks of results, the 2.5% and 3.0% hexachlorophane preparations, were examined (Table V). The 10 test results on each of the six subjects were analysed: test number 1 for each preparation was discarded (see below), and the remaining nine tests on six subjects were treated collectively, *ie*, $6 \times 9 = 54$ tests, but two tests were excluded from the 2.5% soap gel and one test from the 3% detergent as sporing organisms were present which confused the result. Although the data are not normally distributed the standard deviation gives a measure of spread for comparison purposes.

	2.5% Hexachlorophane- 23% Potassium Soap	3% Hexachlorophane Detergent
No. in sample	52	53
Average	6.6	33.1
Standard deviation	7.4	46.9
Range	39	212

A frequency distribution diagram is shown in Figure 1.

It is apparent that neither preparation has a strictly normal statistical distribution. The 2.5% soap gel is skewed in the direction of sterility whilst the 3% detergent has a wider and flatter distribution. As the results of these two preparations are not normally distributed, statistical analyses beyond averages and standard deviations have not been attempted.

The statistical appraisal of the results may be summarized as follows: on average the level of bacterial survivors after washing with the 2.5% soap

gel is about one fifth of the average for the 3% detergent; the spread of results around the average is narrower for the 2.5% soap gel.

Without reference to any other results so far appraised in this paper, the statistical inference is that, not only is 2.5% soap gel more effective than the 3% detergent but its action is more regular and precise. In more general terms, the results indicate that the 2.5% soap gel gave low counts which were in fact, near to sterility. The first wash figures were always higher than subsequent counts. This we attribute to deepseated flora being brought to the surface throughout the washing process and at a time when the hexachlorophane has been diluted to a level where rapid antibacterial action cannot be expected. (It could be that a repeat wash would eliminate these emerging bacteria as do subsequent washes at a later time, but we have not followed this up.) For this reason we discarded the results of first washes from the analyses. We could not confirm chemically whether an inhibitory concentration of hexachlorophane was left in the skin pores, or whether re-establishment of deepseated flora takes longer than the six hours between the two washes, *ie*, morning and afternoon viable counts. A fair supposition is that after the first wash using the 2.5% soap gel only surface bacteria are encountered and erratic reductions due to mechanical removal do not count. The reduction found can be attributed to rapid antibacterial action as would be expected on consideration of the results in part II.

Whether or not the staphylococcus becomes re-established as a resident organism has posed a problem, hence washes with the 2.5% soap gel after two days without treatment were begun again. With the exception of J.A., who had a lacerated finger, the counts were still remarkably low. Fahlberg, Swan, and Seastone (1948) stated that the effects of hexachlorophane may persist for several days after

TABLE V
AVERAGE VIABLE COUNTS FIVE MINUTES FROM START OF WASH TREATMENTS ON LEFT AND RIGHT HANDS
AFTER 48 HOURS' INCUBATION OF PLATES

Formulation	No. of Tests	Subject					
		J.W.L.	J.M.C.	J.A.	J.S.	R.G.	B.D.
Bar soap (A)	4	700	207	650	114	402.3	216.5
2.5% Hexachlorophane-23% potassium soap	10	18	20	20	5.9	12.1	29.1
2.5% Hexachlorophane-23% potassium soap (after two days' rest)	2	8.5	15	104	9	7.5	6.5
Bar soap (B)	6	355	167.8	53.8	51.5	132	57.5
Bar soap (C)	10	511.7	268.3	51.4	43.9	110.4	203.3
(after one week's rest)							
3% Hexachlorophane detergent	10	57.3	25.4	48.1	39.8	58.6	31.2
3% Hexachlorophane detergent (after two days' rest)	2	21.5	282	12.5	3	42	42
Bar soap (D)	8	520	249.6	41.3	17	134.8	189.1

FIG. 1. Frequency distribution.

cessation of use, and this seems evident in the case of J.S., J.A., and D.B. but not in J.W.L. or J.M.C.

The persistent inhibition of resident staphylococci has two indivisible effects: (1) the speed of re-establishment of the staphylococci and (2) the antibacterial effect of residual hexachlorophane in the pores. Since we obtained the impression that certain subjects were not re-establishing resident flora rapidly, we allowed a resting period of one week between soap washes B and C in order (see Table V) that relatively normal levels of both resident and transient bacteria could re-establish.

The results obtained with the 3% detergent were again erratic as shown by statistical appraisal. The first count was high (compared with the 2.5% soap gel) and has been excluded from the analysis, but subsequent counts are erratic and unpredictable which implies variable antibacterial and mechanical action. After the one week rest the results were equally erratic.

We included bar soap essentially as a qualitative control, but statistical appraisal of the results would serve no useful purpose, and it is sufficient to say that the distribution is similar to that of the 3% detergent, but flatter and wider, indicative of mechanical removal only.

We consider that great care must be shown when testing numerous skin preparations within a short time due to unpredictable residual effects and erratic re-establishment of resident organisms. As an

example, the 2.5% soap gel was used to wash the hands and counts were made. The following day a non-medicated liquid soap was employed, the counts being very low as compared with those from the hexachlorophane wash. The reason for this is most probably due to the unmedicated soap merely having to remove transient organisms mechanically, deep-seated residents having been previously eliminated by the hexachlorophane. The dangers of misinterpretation from such results are all too obvious.

DISCUSSION

The vehicle in which hexachlorophane is suspended appears to be of the utmost importance. It is difficult to decide whether the detergent formulation depresses potential activity of hexachlorophane, but that soap activates the antibacterial action would seem irrefutable on the evidence presented. It is unfortunate that the main types of hexachlorophane formulation previously investigated, *ie*, bar soap and types of detergent are what we consider abnormal by virtue of their chemistry, methods of application and, in the 3% detergent preparation, ability to repel water.

To obtain the maximum concentration of hexachlorophane for surface disinfection of transient organisms we have applied the 2.5% soap gel neat; in effect we have used it as a cream. It is also our

belief that this maximum of hexachlorophane should be available in a suitable antibacterial form. It appears that the 3% detergent demands some intrinsic property of the skin for optimum action, a property not present in all subjects. The soap gel hexachlorophane does not appear to depend on such phenomena.

Although it seems difficult to distinguish between a residual effect of hexachlorophane film and lack of re-establishment of deep-seated organisms it appears that the soap preparation possesses all the properties of the detergent preparation but without its erratic nature. This may be because the soap removed grease from the pores more efficiently, facilitating a better film coating, and/or the hexachlorophane is deposited more finely and is more easily solubilized by the skin secretions. Again, since the 2.5% soap gel is a rapid antiseptic due to its solubilized state, it may be that the hexachlorophane is deposited on the skin in a solubilized form.

The previous statements that hexachlorophane is effective only against Gram-positive cocci is fallacious, since it really means that hexachlorophane preparations so far investigated have shown a Gram-positive specificity. We contend that hexachlorophane is a chlorphenolic substance and as such would obey the rules of phenolic formulations and is not a law unto itself. For example, coal tar fractions, whilst having some intrinsic antibacterial activity, are essentially activated by the medium in which they are suspended: cresols, xyleneols, and their halogenated derivatives obey the same rules. An excellent summary of these phenomena is given by Sykes (1965).

The statistical appraisal of the results obtained in part IV reveals a constant low number of surviving bacteria on using the 2.5% soap gel, indicative of antibacterial action (superior to that of the 3% detergent) against both transient and deep-seated organisms. We have further shown that the 2.5% soap gel can rapidly kill Gram-negative organisms on the skin as well as the Gram-positive flora when applied as we suggest.

Throughout this work the 2.5% hexachlorophane-23% potassium soap gel has referred to a commercial formulation produced by the action of the alkali upon a suitable blend of fatty acids and hexachlorophane. It is impossible to say that the antibacterial action evidenced in this paper could be obtained

simply by adding hexachlorophane to potassium soap or by using other grades of fatty acids.

A certain amount of skin irritation and chapping after the application of the 2.5% soap gel has been reported, and since the original work in this paper a 2.5% hexachlorophane-23% potassium soap gel containing emollients has become available which we have examined by many of the methods described above and found to be at least as rich in germicidal properties as those of the non-emollient preparation. Despite frequent use, there have been very few complaints regarding skin irritation. One must realize, however, that whatever skin preparation used there is bound to be someone who is 'sensitive'. It is known that some people are sensitive to hexachlorophane, so that any preparation containing it would cause irritation. It is important that an excessive amount is not used at each application. The products have been tested repeatedly on laboratory and hospital staff with no sign of local irritation.

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¹The 2.5% hexachlorophane-23% potassium soap gel used in this work is marketed as Zalpon antibacterial washing cream and the emollient formulation as Steridermis.