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Study of hexachlorophene and its use in treating burns

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A STUDY OF HEXACHLOROPHENE AND ITS USE IN TREATING BURNS

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I. INTRODUCTION

Infection is a major problem in the treatment of burns. This problem must be controlled during the early treatment of a burn, otherwise all phases of recovery are delayed. Many cases result in months of hospitalization, poor grafting results, multipal cicatrix, and also neurosis from repeated dressings and surgery.

A method in treating burns prophalactally has been brought to my attention deserving careful study because it seems to give a lower incidence of infection in burn wounds.

After control of any shock phase, the burn wound is gently washed for ten minutes (under proper anesthesia) with a liquid detergent containing hexachlorophene (G-11). This procedure requires repeated saline flushings. The final sudsing is blotted and not flushed off with saline. It is usual after this ten minute period to find remarkable hemostasis. Then the use of pressure dressings or open-air method may be used as long as the burn wound is not exposed to further contamination.

(a) Factors Involved in Technic

This type of study requires a knowledge of the physiology of a burn wound, normal flora contained on

skin surfaces and what flora remains after a burn. As to the use of a liquid detergent with G-11, one has to know what the mechanical effect of the detergent and hexachlorophene has on the burn and bacteria present.

(1) Mechanical Effect

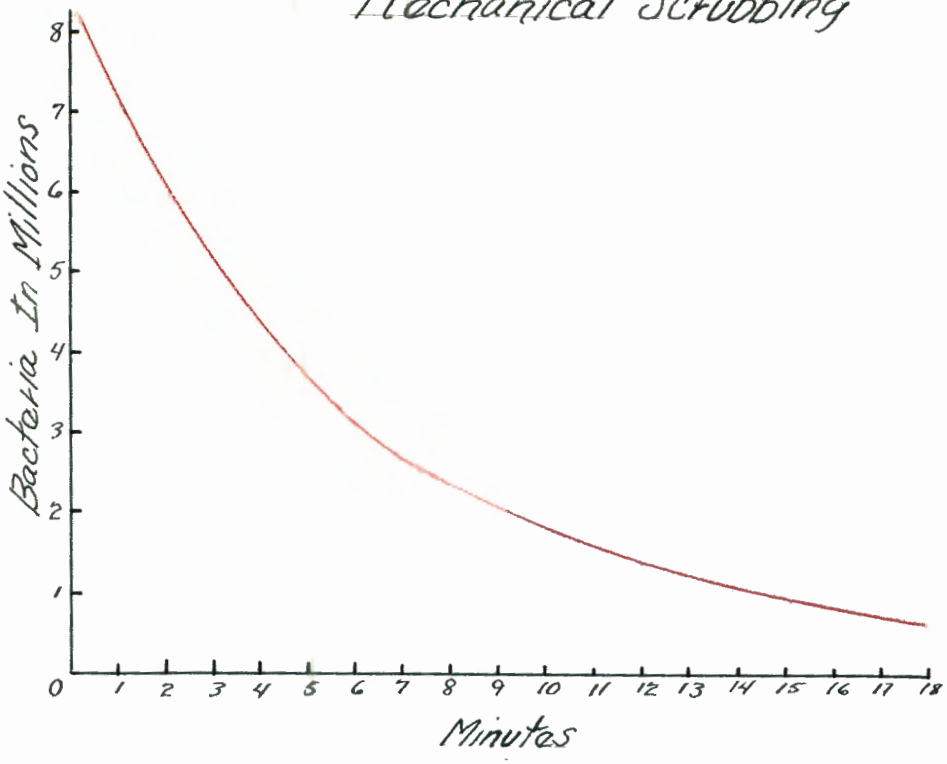
The Council on Pharmacy (1) of the A.M.A. suggests that neither hexachlorophene nor any other chemical agent should be relied on as a subsitute for mechanical cleansing of the skin.

Mechanical cleansing according to Price (2) with ordinary nonmedicated soap removes dirt, oils, and fats, as well as many loosely attached contaminating microorganisms; but the resident bacteria flora is not easily reduced. He amply displays the results of mechanical cleansing and the re-establishment of bacteria flora in graphs A and B.

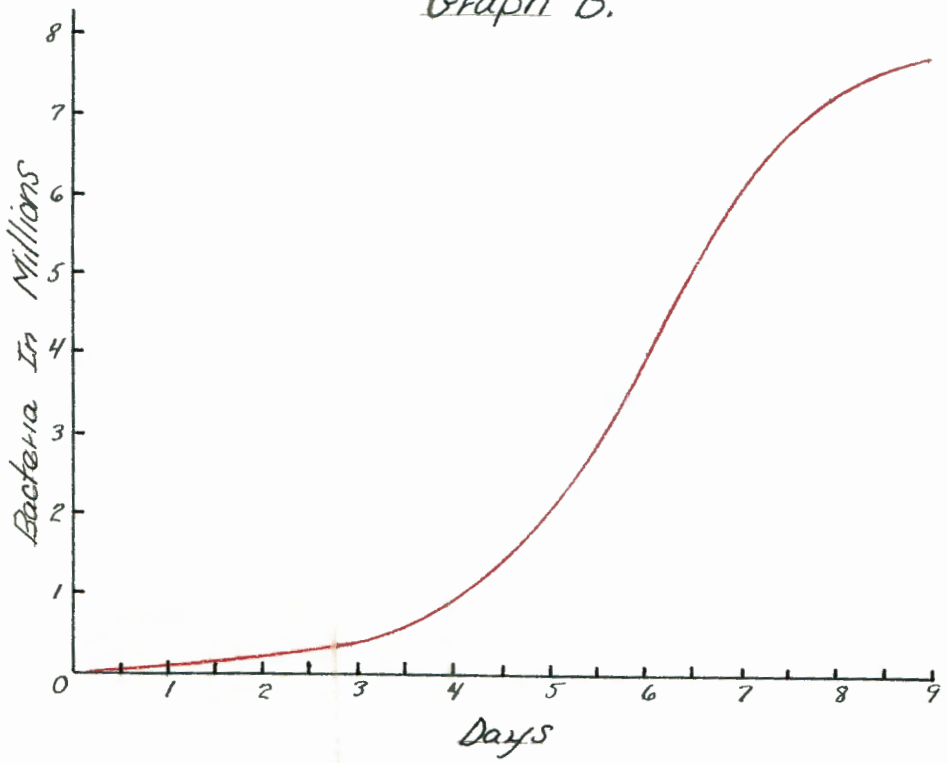
Hufnagel (3) reports the effect of mechanical scrubbing. The distilled water, or control curve, demonstrates the effect of mechanical scrubbing alone. The curve, graph C, expresses the average of nineteen experiments. It corresponds with that of the rectangular hyperbola $y=f(x)^{-1}$. This curve does not appear to agree with the curve found by Price (2) because the tested area is much smaller (4x4cm). If

Graph A.

Mechanical Scrubbing

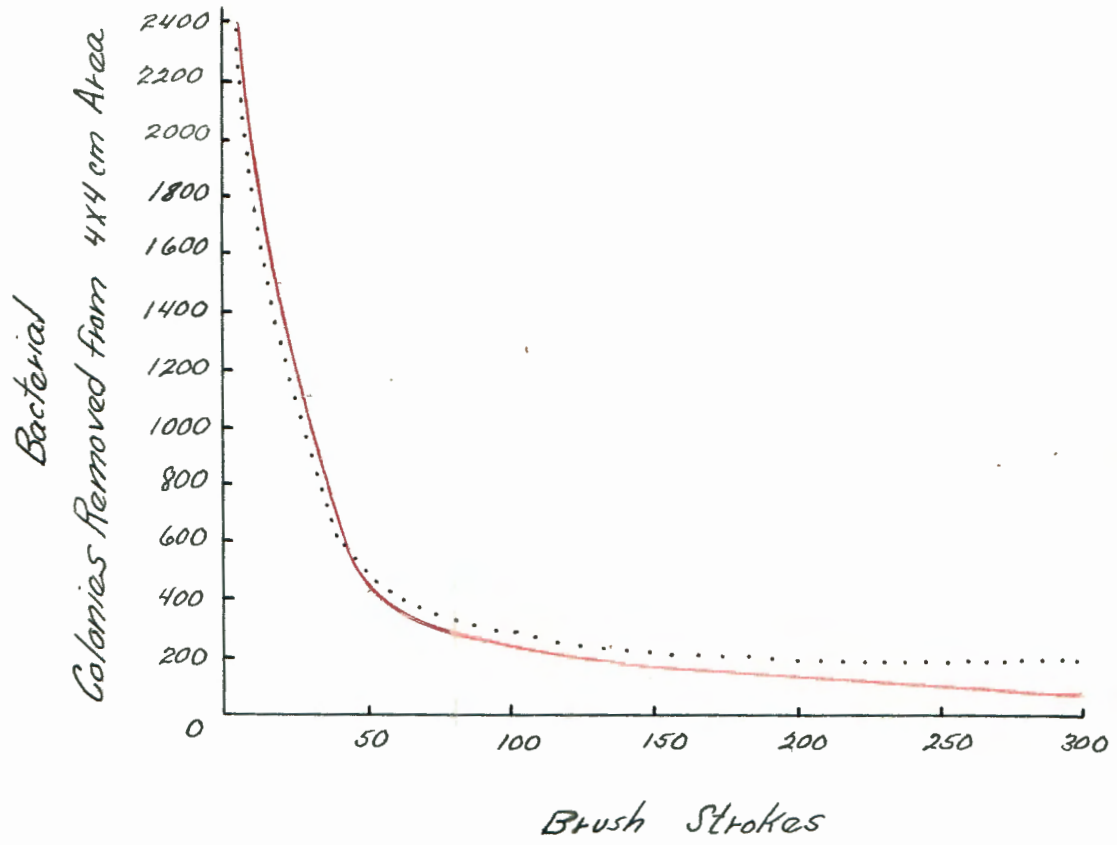


Graph B.



Re-establishment of Bacteria Flora

Mechanical Scrubbing



Graph C.

comparable areas were used, a like curve would probably result.

(2) Detergent or Soap

Synthetic detergents, Rosenberg (4), are particularly fitted for the cleansing and debridment of burns contaminated with and covered by a layer of grease, oil, and particulate matter. Most of them are non-irritating and have a pH which varies from neutrality to moderate alkalinity. Many have been used repeatedly and for long periods as soap substitutes for sensitive skin. Nearly all of them have bacteriostatic and some bacteriocidal power, especially for the cocci found on the skin. Cleland (5) reports that the detergents seem to aid considerably in decreasing the bacteria flora of the skin.

The subject of effectiveness of soap alone is difficult to explain at this point because of the controversy; some studies show that soap is much less effective than hexachlorophene soap. The answer lies in the testing technic and will be discussed later in this thesis.

(b) Material to be Studied

Most of the material pertaining to the use of hexachlorophene has been in its use with the surgical

scrub. Much of this type of material can be directly related to its use in washing burns. Very little has been written in the literature pretaining to studies of hexachlorophenes' use in washing burns. The treatment in burns deals directly with the remaining skin and bacteria present; so the primary interest in this thesis is the part which hexachlorophene plays in the healing process of burns.

(c) Skin Physiology and Infection in Burns

Meleney (6) gives a very good discription of bacterial location in burns and the physiology present. He believes that infection has always been a serious problem in burns. This infection is of importance prophylactically from the moment of injury until the entire area is again covered with skin. Burns differ from other wounds in two important respects: first, they are usually extensive but not deep, while other wounds are relatively deep but not extensive; second, it is not often possible or advisable to remove the dead tissue at the surgical procedure as it is with other wounds. These two facts are important from the point of view of infection because the contamination with organisms is greater and impossible to remove and the medium which sustains their growth; that is, dead

tissue remains to favor their development. Meleney feels that the bacteria causing infection in burns may be those residing normally in the hair follicles or sweat glands or those deposited on the surface subsequent to the burn. He feels that a superficial burn caused by a low temperature applied for only a short duration may not kill all of the organisms in the hair follicles. A deep burn caused by a high temperature applied for a longer time probably kills all the bacteria in the skin in the central areas, but at the margin there must always be areas where the burn becomes superficial and the organisms which are there remain viable and capable of growth. He sums his feelings up by saying that in every case of burn there are three zones: one in which the tissue is killed directly by the heat, one in which it is injured but not immediately killed, and the third in which it is not injured at all.

We can understand the habitat of skin bacteria by the work of Lovell (7) who feels that under normal conditions skin bacteria are located on the surface embedded in the horny fat, in the crypts and crevices, hair follicles, and sebacious glands. Most of the transient organisms of the skin are quite superficial

and can be removed by mechanical and chemical cleansing. The resident bacteria are situated so deep in the hair follicles and sebaceous glands that they cannot be removed by mechanical means without injuring the skin; the generally used antiseptics do not penetrate sufficiently to reach the organisms located in the deeper parts of these structures. Although the surface of the skin may be rendered relatively free of bacteria by mechanical cleansing and chemical cleansing before and during operation, the resultant bacteria rise to the surface, multiply, and thus constitute an important source of wound contamination.

From these opinions one would conclude that the only way hexachlorophene lowers the bacteria count directly is by its penetrating these recesses to cause bacteriostasis and thus lower the bacterial multiplication ratio. The lower counts would occur with increased amount of hexachlorophene absorbed with frequent applications and time factor of prolonged use.

Meleney (6) presents some interesting data to the onset of infection in burns. In 232 cases the serious burn cases developed a peak of infection in the second five day period while in the trivial burn cases the onset of infection was fairly evenly distributed

through the first fifteen days. Of these infections he is struck by the large number of cases in which organisms are originally found which failed to develop infection and also by the comparatively large numbers of cases in which they appeared as new cultures. Meleney (6) also has a short study as incidence of infection in 591 burn cases. Of these 129 or 22% had serious infection and 146 or 25% had trivial infection; thus giving a total of 47% of 591 burn cases which were infected clinically.

Rosenberg (4) claims the common contaminants of burns are the various forms of particulate soil and the charred remnants of clothing. Other contaminants are oils and grease applied by first aid.

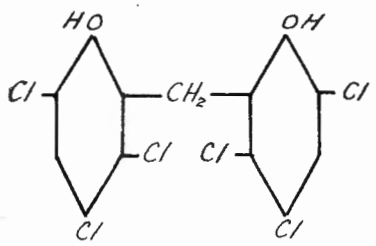
Price (8) thinks that use of G-11 soap does not prevent multiplication of bacteria and increased flora beneath rubber gloves. (This is comparable to an air-tight bandage over a burn wound.) Price (9) also claims skin is never sterilized by heat; so burn wounds always are covered by burned or charred skin containing bacteria. Whether the wound is left exposed or is dressed, the bacterial flora remaining on the burn skin multiply during the next few days, often with tremendous increases so that the bacterial counts

far exceed the counts of normal skin. Pathogens participate in this increase.

Wright (10) had trouble with persistent infection. In his series of five cases, preliminary cleansing of the burned area was done with a detergent containing G-11. Despite every effort to keep the operative site free of contamination, each case in this series revealed pathogenic or potentially pathogenic bacteria.

II. HEXACHLOROPHENE

(a) Description and Chemistry



Gump (11) in 1941 filed a patent for hexachlorophene. He says, "This compd., m. 161-2°, and suitable for use as an antiseptic, bactericidal, fungicidal or preserving agent, as in toothpowders and pastes, ointments, (creams), cosmetics or rubber goods is made by treating a solu. contg. 2 mols. of 2,4,5-trichlorophenol, 'mol of CH_2O and MeOH at a temp. of 0-5° in the presence of H_2SO_4, and may be purified by crystn. from benzene, toluene or ethylene dichloride."

The Council on Pharmacy and Chemistry of the A.M.A. (12) voted and approved in 1948 the term "hexachlorphene" as a generic designation for his (2-hydroxy-3,5,6-tri-chlorphenyl) methane.

The Council on Pharmacy and Chemistry of the A.M.A. (1) in 1951 accepted the use of hexachlorophene. They described its topical application to reduce the numbers and to inhibit the metabolism of microorganisms which occur naturally and pathogenically in the skin flora. Tests and standards are given in their report and would be of importance to anyone doing research in this field.

In Blanks report (13), the hexachlorinated compound know as G-11 or hexachlorophene has received extensive investigation as a cutaneous disinfectant. This compound does not appear to lose its germicidal property in the presence of soap. This is said to result from the fact that one of the two phenolic groups in the compound remains uncompounded in the presence of soap.

Artz (14) describes hexachlorophene as being a white crystalline, oderless powder which is insoluble in water but is soluble in alcohol, acetone, and dilute alkalies. It is more soluble in alkaline than in

neutral or acid media. This compound is usually incorporated in bar soap, liquid soap, or a water miscible emollient detergent. In these media it is stable and bacteriostatically active.

Solubility of Hexachloropene

Solvent	Gm. of G-11 Dissolved in 100 cc at 25°C
H_2O	0.0012
10% Ethyl alcohol	0.013
20% " "	0.02
50% " "	0.15
75% " "	4.94
Propylene glycol	24.5
Isopropyl alcohol	31.6
Methyl ethyl Ketone	77.0

(from Chisholm (15))

(b) Film and Accummulation

Early studies of hexachlorophene indicate that this substance tends to remain upon the skin and thus lowers the bacterial potential by several possible means: one, by producing a continuous film which seals in the bacteria and kills them; second, that G-11 may attach its self to the bacteria and prevent multiplication; and thirdly, some believe that G-11 is absorbed by the skin and prevents bacteria from growing there.

The A.M.A. Council on Pharmacy (1) claims that residual amounts of hexachlorophene, which are absorbed on the skin, maintain a reduction in numbers of bacteria. Optimum results are obtained only with exclu-

sive application of the agent to the skin surface. Substitution of other cleansing agents, including water, removes the absorbed G-11 with resultant rapid increase in numbers and metabolism of microorganisms.

Fahlberg (16) studied the skin level of G-11 over a period of time and puts his data in form of a chart.

Amount of Extractable Hexachlorophene at Varying Time Intervals After Three Consecutive Six-minute Daily Washes

Time After G-11 Applied	Inhibitory Dilution of Extract	Average Calculated Amt. of G-11 ug	Average Amt. of G-11/sq in of Skin ug
Immediately	1/1000 dil.	100	8.9
24 hours	1/50 dil.	5	0.4
48 hours	1/10 dil.	1	0.08
72 hours	No Inhibition	None	-
96 hours	No Inhibition	None	-

1.0 ug - (0.0001 mg)

Duncan (17) feels that a protective film is formed on the skin which has a persistent antibacterial effect. This is confirmed by the failure to detect viable organisms following mechanical removal of the film. This study provides a corollary to the findings of Fahlberg (16) who was unable to release viable bacteria in significant quantities by experiments devised to remove chemically G-11 from the skin. Both of these authors were sincere, but they were using faulty culture technic. The bacteria were there but would not grow due to the presence of G-11 in culture media.

In the work of Artz (14), he claims that the thin layer of retained G-11 does not obscure the bacteria of the skin beneath the imperceptible film but actually kills the bacteria. Traub (18) in his experiments proves that 2% G-11 soap does not form a film which would retain a large number of live bacteria beneath it.

Price (2) thinks that washing or scrubbing leaves on the skin a film of soap which is not entirely removed by ordinary rinsing. Price (8) later in his work with G-11 analized the bacteria that are taken off of the skin after washing with G-11. These bacteria appear to have some of the substance absorbed on the bacteria cells. These bacteria do not grow very well, even if you wash them with water. A diluting technic does not eliminate the bacteriostatic effect.

Best (19) indicates that the cummulative factor and sustained action of hexachlorophene are important in maintaining a low skin bacterial flora.

(c) Testing Methods of Efficiency of G-11

This is one of the most important items in the study of hexachlorophene, and anyone interested in original work in this field must study this subject

with complete thoroughness. This is particularly true due to the high incidence of misconceptions already published on this subject.

An attempt will be made to describe in general the various methods used to determine the bacteria present on skin surfaces after exposure to hexachlorophene.

(1) Direct Contact

The usual procedure is to apply and fix a culture plate to the tested area for a definite period and then remove and place in proper incubation. Best (19) and his group applied this technic to their study, and they felt that G-11 lowered the bacterial flora of the skin. The validity of the results will be expressed later. Other groups have used a similar technic by pressing the test area for an instant to the culture plates; their results were encouraging for the use of G-11.

(2) Culture Washings

The greatest tendency in this type of technic has been to test an area with G-11 and rinse and then rinse the area again in a sterile water basin. From this, serial dilutions with cultures are determined. Some of the literature results were judged on the original rinse water; this sample especially contains

the very transient flora and high concentration of G-11 in the sample to be cultured. Price (2) particularly thought that this technic would lead to misinterpretation and would not reflect the change in resident flora. Because of the effects of G-11 on culture media and bacteria present, Price (2) developed the serial basin test where the area tested is then submitted to multiple rinses to dilute the G-11 present to the point where it will not disturb the results. Then cultures are made of the bacteria present on the skin, and the efficiency of G-11 is plotted. He concluded that a single scrub with G-11 was not much superior to ordinary non-medicated soap. If G-11 is used continuously and for prolonged periods, it does lower the normal bacterial count.

(3) Biopsy Method

This technic requires surgical removal of a portion of skin which has been exposed to the effects of hexachlorophene. This biopsy may then be: (a) examined microscopically for reactions to G-11 as Best (20) did (his group found no tissue damage signs that could be attributed to G-11); and (b) the biopsied skin can be placed in culture environment and the resulting colonies counted. Both of these methods have little

control over the quantitative angle and do not tend to be conclusive.

Nungester (21) contaminated the shaved abdomens of mice and then applied various agents - G-11, Iodine, or nothing to the skin area. This was then biopsied and implanted intraperitoneally in the same mouse. He gives this data in the following chart.

	No. of Animals	Death	Mortality Per Cent
Iodine	36	15	42
G-11	36	24	67
Control	16	15	94

G-11 was better than the control with less reaction than iodine on skin.

This method of Nungester may have some value in approaching a clinical situation.

(4) Animal Injections

Nungester (21) states that in this type of study animal inoculation is used to evaluate the agents tested. The examination of hand washings by animal inoculation tends to: (a) differentiate potentially pathogenic from purely saprophytic organisms, and (b) to eliminate false bactericidal effects of chemicals only bacteriostatic in nature. From this study it was felt that G-11 seems to be a factor in aiding the resistance of the animal to infection.

Best (20) and his group decided to evaluate the

use of hexachlorophene soaps in wounds contaminated with a virulent strain of Streptococcus and in which blood serum is always present. This virulent culture was applied to open wounds of 70 white mice which were then scarified. The various groups were treated to various soap washes with or without G-11 added. Then the mice were studied for survival length and recorded in the following chart.

Survival Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control (no soap)	9	8	6	5	5	3	1	0	0	0	0	0	0	0
Bar Soap (no G-11)	9	9	9	7	7	6	5	4	4	4	4	4	3	2
Bar Soap (ē G-11)	10	9	9	8	8	6	5	3	3	2	1	1	0	0
Liquid Soap (no G-11)	10	9	7	7	5	4	4	3	3	2	1	1	0	0
Liquid Soap (ē G-11)	10	10	10	9	9	8	8	7	4	4	3	3	2	2
Liquid Soap (ē alcohol 5%)	10	10	10	10	7	7	7	7	6	5	4	3	2	2
Saline	10	8	8	6	4	2	1	-	-	-	-	-	-	-

From this study the only indication is that soap with or without G-11 is best to wash contaminated wounds as compared to no treatment or saline flushes.

(5) Controlled Washings

This technic developed from the need of more scientific control over the testing technic. Price (8) is one of those who devised a cone which was applied to

the tested area. Then a sterile brush is inserted and the proper amount of strokes, definite area, and approximate equal culture samples can be obtained. Conclusions from this technic indicated that single washings with G-11 soap have the same effect as non-medicated soap. The effective action of G-11 was felt to be a slow degerming action attributed to a film of the agent left on the hands which lowered the size of the bacterial flora.

Hufnagel (3) has improved upon this technic to permit multiple determinations of the bacterial flora of the human skin under standardized mechanical conditions. This apparatus was designed and built to standardize the various mechanical factors involved in scrubbing techniques. This device isolates adequate areas of skin and scrubs it at a constant rate with a specially designed brush (definite number of hairs and tensile strength) pressed against the skin by a constant force. The machine has three components: a mechanical reciprocating scrubber, a stable brush, and a vacum seal to isolate and hold the skin against the brush.

These are extrememly interesting technics and appealing because of some control over the highly

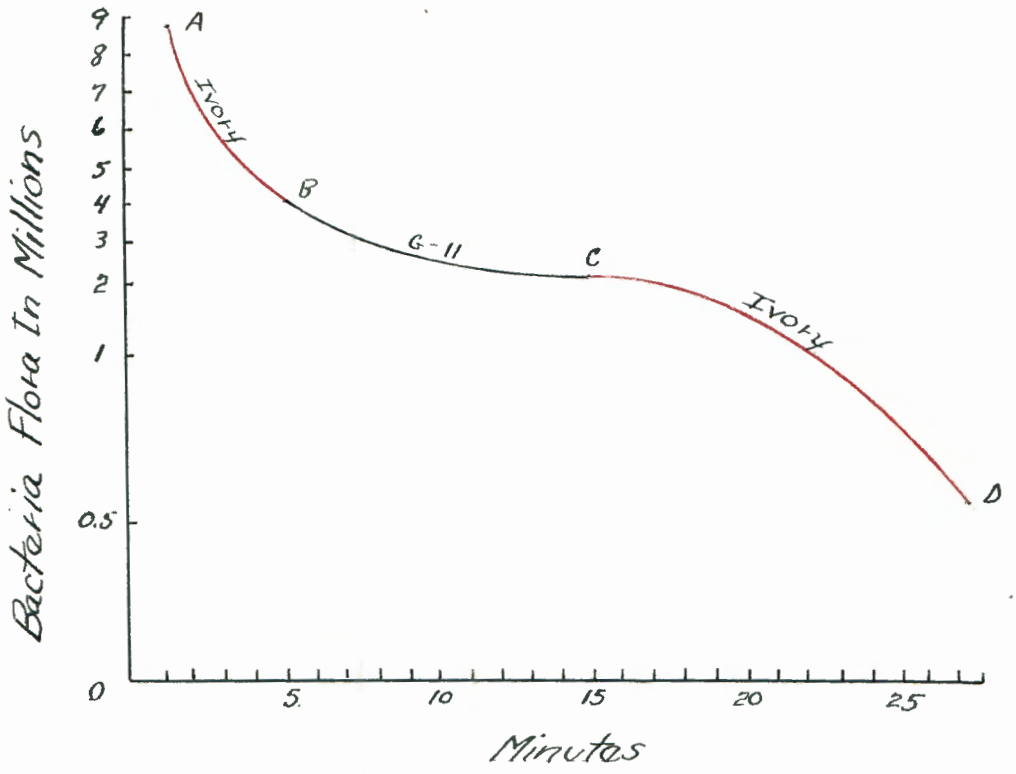
variable situation in the study of G-11's effect on skin flora.

(6) Predicted Curves

This is a device supported by Price (8) which compares the immediate effects of one material to another under actual testing conditions. An example is the charted effect of a ten minute wash with G-11 in graph D. A liquid soap containing 2% G-11 was used without a brush. Obviously the immediate effect (curve BC) was less than the effect of scrubbing with Ivory soap for an equal length of time (extension of curve AB). The duration of the first few points from curve AB is due to the presence of transient bacteria which come away more easily than the resident flora.

Hufnagel (3) used a comparable study of difference substances in lowering the bacterial count in a limited number of brush strokes. This graph C was a comparison of the effect of scrubbing, detergents, and germicides. The distilled water curve is plotted cummulatively rather than in a conventional fashion. The total counts for the agents are given at ten brush strokes. The second period of ten strokes is that used to apply the agent. The third period, therefore, rep-

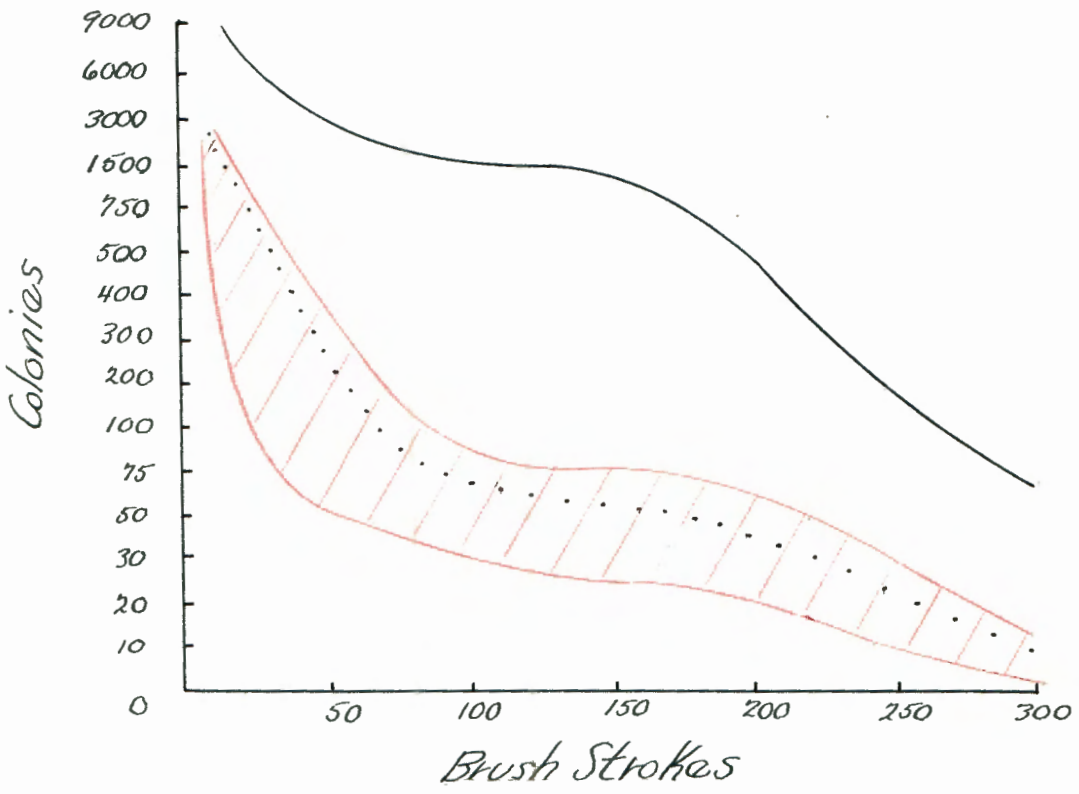
Graph D1



Predicted Curve With
Ivory Soap Compared To
G-11 Soap

Graph D 2

Predicted Curve



Comparison of (—) Distilled Water

Detergent

Detergent + 15% G-11

Zephiran 0.1%

.... Plain Soap

resents the total number of organisms removed following the application of the agent under study.

The graph curve by Hufnagel (3), graph C, compares mechanical scrubbing with plain soap, pHisoderm, 0.1% Zephiran, and pHisoderm with G-11 added. This chart allows for good evaluation of poor culture technics. Yet even with the presence of G-11 in the media as compared to plain soap, the slight increase in lower counts is due to the carry over of G-11 into culture media. The resulting conclusion is that G-11 soap is not any better than plain soap within a time limit of 300 brush strokes.

(d) Pitfalls of Testing Methods

Price (8) emphasizes very strongly that in hexachlorophene studies one must be cautious. Studies of G-11 germicidal activity are complicated by the fact that extremely minute traces of the substance, as little as one part in many millions, carried over into subcultures are sufficient to cause significant bacteriostatic effects. Failure to recognize this characteristic has led many investagators to erroneous conclusions.

Price (2) in earlier studies of disinfection thinks that the pittfall lies in mistaking bacterio-

stasis for bacteriocidal effect. Unless a specific antidote is employed, which effectively removes or neutralizes the disinfectant being tested, gross misconception of its germicidal value may be obtained. He observed that Seastone had suggested the use of serum in media as an antidote for G-11. Although serum probably does not neutralize these agents chemically, it does liberate some of the bacteria as shown by significantly higher bacterial counts in serum agar plates.

These statements are very important because many of the previous studies on hexachlorophene have shown remarkable results due to poor culture technic. Due to invalid conclusions, their results should be analized carefully.

(e) Inactivators of Hexachlorophene
(1) Serum

The description of hexachlorophene in the A.M.A. Council of Pharmacy (1) indicates the activity of hexachlorophene, like that of other antibacterial agents, is considerably reduced by blood serum and other organic matter. In the actual washing of burns with G-11, Price (9) thinks a G-11 soap may be used for this purpose, but it should be remembered that G-11 acts only very slowly and that it is largely neutra-

lized by the presence of serum.

Fahlberg (16) studied the depressing effect of serum components on bacteriostatic action of hexachlorophene. He summarizes these findings in a chart.

Hexachlorophene	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Control	-	-	-	+	+
1% Serum	-	+	+	+	+
0.0483% Albumin	-	+	+	+	+
0.0189% Globulin	-	+	+	+	+

- Equals growth of standard innoculum of Staph. albus in 72 hrs.
- Equals no growth in 72 hrs.

The effect of whole serum in reducing the bacteriostatic effect of G-11 can be duplicated by egg albumin or serum globulin.

Price (8) believes although ordinary cultures may produce little growth because of bacteriostatic effects of the chemical present. The addition of serum which has some neutralizing action to culture media will permit abundant growth. These same organisms falling into a wound where serum is present are therefore potentially infectious.

Best (20) records that the study with Armour Lab, as to the serum aspect, showed somewhat higher counts but respective authors feel that this is not conclusive for conditions as exist in wound healing.

Seastone (22) notes that the bacteriostatic

effectiveness of G-11 is decreased a hundredfold by the presence of 1% serum in culture medium.

(2) Tween-80

A recent article by Lawrence (23) possibly will clarify the uncertain aspects in culture technic. To count the bacteria remaining after the effects of hexachlorophene, representative amounts of the wash or rinse water from the basins are plated in appropriated media containing blood, serum or serum globulin and albumin. From this method of testing it has not been felt that a true interpretation with the use of serum has resulted. Conceivably, bacteria may also become coated by a film of hexachlorophene in the same manner in which normal human skin is coated by the use of the compound in soap wash experiments and thus the organisms would not develop in a nutrient medium to which only serum was added as the inactivating agent.

Tween-80 is a polyoxyakylene der. of sorbitan monooleate (Product of Atlas Powder Company, Wilmington, Del.), and of all the chemicals tested it showed the highest degree of ability to inactivate hexachlorophene. In comparison of inactivation properties of serum, albumin and Tween-80, it was shown that 5 - 1.3% of Tween-80 completely inactivated G-11 in 0.1%

concentration. Where as 5% serum and albumin inhibited G-11 of only 0.01% or less G-11. The Tween-80 is approximately up to fifty times more effective.

In culture technics the plating of G-11 wash waters in plain agar resulted in no growth. This same wash-water with serum added grew an average of 500,000 colonies. The addition of Tween-80 grew an average two million colonies.

The studies of prolonged use of G-11 (six to ten days) show that cultures on serum agar plates produce an average 30,000 to 56,000 colonies. This same sample water when influenced by Tween-80 grew an average 120,000 to 340,000 colonies. Thus in this study, hexachlorophene had an "apparent" rather than "real" killing effect.

The comparison of colonies after one G-11 wash and prolonged G-11 use showed an average drop in counts from 1-2 million to 100-500,000 colonies. The drop in bacterial count found by the author is partly due to prevention of multiplication of bacteria. Also there is a definite decrease of skin flora bacteria, but the remaining number is still a long ways from being sterile.

In all the studies of hexachlorophene's effect

upon the skin flora, one is interested primarily in the number of bacteria present at a specific time upon that specific area and not interested in the effects of G-11 in the bacteria in the media after washing a skin area. Tween-80 would seem to be the answer to this problem and thus demands a re-examination of earlier observations pertaining to bacterial counts during, after, and with prolonged use of hexachlorophene in the study of washing burns, wounds, and surgical scrub.

(f) Sensitivity

The A.M.A. Council on Pharmacy (1) when it officially accepted hexachlorophene mentions its toxic effects. These effects of hexachlorophene on the skin surface, even after long-continued daily use, have been infrequently reported. Udinsky (24) observed the use of G-11 for two years on his personnel, none of whom showed any untoward reaction. In addition, a group of over 200 individuals were patch tested with an 8% solution of a standard bar soap containing 2% G-11. The same type of soap without the G-11 was used as the control. Positive reaction of the same nature developed in about 4% of individuals for both the G-11 soap and control. There were no reactions attributable to the

presence of G-11 in the soap. Traub (18) in similar testing claims that G-11 was non-irritating to the skin as judged by more than four hundred patch tests. These were repeated on the same subjects after ten to fourteen days and were again found to be negative showing that no sensitivity of the skin had been produced by the first tests. Subjects using 2% G-11 soap regularly for one year have shown no evidence of irritation.

Other authors who mention sensitivity to G-11 are: Seastone (25) used G-11 in preparation of the skin of more than fifty surgical patients with no evidence of irritation or delay of healing; Clark (26) found no evidence of skin irritation in following repeated use of soap 2% G-11 by twenty subjects; Price (2) claims G-11 was quite innoculous as far as irritating the skin; Artz (14) experienced in over a five year period the claim that G-11 is non-irritating and hypo-allergenic; Walter (27) states that no skin sensitivity to G-11 has been demonstrable, but those who react to soaps cannot use the mixture; Kraissl (28) reports of one case in 1,500 cases where there was noticed a general skin irritation. Kraissl reports of another case (from personal communication with another

doctor) of skin irritation encountered in a child where a cast was applied over prepared area. He mentions that G-11 should not be allowed to get into the eyes because of reaction. Best (20) in the study of burns on rabbits gives histological evidence that the presence of 2% G-11 in bar soap or 5% in liquid soap exerts no influence on the amount of tissue reaction. Also there was no evidence of delayed wound healing. None of his animals gave evidence of any toxic effects from G-11. Gump (11) states that G-11 injected in 0.01N NaOH solution is a highly toxic agent. As little as 35 mg. in dogs weighing 7-8 kilograms usually caused death within a few minutes.

This presents a very interesting theoretical problem. What would be the total dosage absorbed from a 70 kg. man, 170 cm tall, who had a 100% burn? Then purpose the man was able to maintain life and also absorb all of the G-11 on his skin. From Fahlberg (16) we use the 9.0 ug of G-11/sq. inch which he found present after routine scrubbing. This concludes that our theoretical man would receive 2.6 mg. of G-11 to his venous system which is far below the 35 mg. toxic dose in a 7 kg. dog. Also the usual survival patient does not have over 50% burns, and the absorption from burn

surfaces are complicated by effusion of serum externally and edema of extracellular tissues. This indicates that even with a marked difference to toxicity between man and dog, the absorption dosage a burn patient would get would be 1.3 mg. of G-11. Therefore, toxicity of G-11 through burn wounds is not predicted.

(g) Bacteriostasis Value

The A.M.A. Council of Pharmacy (1) reports G-11 to be effective against gram-positive bacteria; the gram-negative are much more resistant to its action.

In the studies by Lawrence (23), it was found that one part G-11 in two and one half million parts broth inhibited the Staph. aureus strain. In another portion of their study, strains of bacteria were exposed to hexachlorophene and then later to Tween-80. The cultures not exposed to Tween-80 remained high in bacteriostasis effects (1:5,000 to 1:80,000). The cultures with Tween-80 all needed G-11 concentrations of 1:500 or more to be effective. These conclusions by the author would indicate that hexachlorophene plays a definite bacteriostatic part upon bacteria and that this effect can be released by G-11 inactivators.

Price (2) reports that G-11 in dilutions as high

as 1:5 to 1:8 million in agar media causes complete or nearly complete inhibition of Staph. aureus. This bacteriostatic effect disappears when the dilutions are in the neighborhood of 1:20 million. E. coli grows with slight reduction in 1:1 million media. The author feels this is conclusive and at the same time is a major factor in false reports because G-11 is transfered from test areas to culture media and causes further depression of bacterial growth.

Chisholm (15) compares a good detergent with the same detergent plus G-11 in bacteriostatic studies. This is carried out by the agar-cup plate method against Staph. aureus. The detergent alone displayed a 1 mm. zone of inhibition; with the detergent containing 3% G-11, there was a 12 mm. zone of inhibition. Phenol coefficient studies further illustrate that the hexachlorophene mixtures are effective in low dilutions.

Both Seastone (22) and Clark (26) report that G-11 exercises a marked bacteriostatic effect - even in high dilution. Both authors were reading these results from culture plates taken from scrub washings and not seeing the bacteria on the culture media because of the G-11 inhibition there.

(h) Antiseptic or Bacteriocidal Value

Price (8) reports hexachlorophene does not disinfect the skin quickly as alcohol does but much more slowly. This slow degerming action is attributed to a film of the agent left on the hands after washing with the medicated soap. The cutaneous flora of different persons varies in susceptibility to G-11. The fact that some individuals harbor a bacterial flora that is resistant to the action of G-11 injects a disturbing element of uncertainty into its exclusive use in preparing the skin.

There is in Blank's (13) opinion less evidence that a "one-time" surgical scrub with a G-11 detergent preparation does much more in reducing the bacterial population of skin than will be done by a surgical count with a detergent containing no hexachlorophene.

Price (2) in his earlier work found 1:1 million G-11 in 0.1 N NaOH appears to kill suspension of Staph. aureus after 30 minutes exposure at room temperature; but with exposures of only ten minutes, normal counts are obtained. Udinsky (24) reported that 1:1000 G-11 will kill Staph. aureus in 5 minutes at 37°C, but his cultures were taken from direct swabbing of hands thus resulting in a culture technic error.

Chisholm (15) reports that G-11 accomplished the bacteriocidal effect shown in his study of comparisons to mechanical cleansing, other detergents and G-11 soaps; but he concludes that the detergent and G-11 is best. The author conveniently leaves out the study of plain soap which he has done and which almost is as effective. The only reason it is not as effective is due to the defective culture technic used giving an "apparent" more effectiveness to the detergent G-11 technic.

Nungester (21) took a known virulent strain of Streptococcus and applied it to human skin. These areas were then washed with G-11 soap or with a routine surgical scrub with plain soap. A sample of these washings was then injected into mice. The wash was repeated once a day for five days and each time a sample injected into mice. This is shown in the following chart.

	Hexachlorophene 3 minute wash (6 subjects)			Routine Surgical Scrub - 10 minutes (7 subjects)		
	# of mice injec.	Dead	Per Cent	# of mice injec.	Dead	Per Cent
1st day	60	59	98.5	70	69	97.0
2nd day	60	7	"11.7"	70	27	"38.6"
3rd day	60	9	15.0	70	32	45.7
4th day	60	5	10.0	70	9	15.0
5th day	60	8	16.0	70	13	21.7

The effects of G-11 on the bacterial strain appear after twenty-four hours with a noticeable drop in death incidence of mice. Also this study would indicate that an intial G-11 use without time for the G-11 to be effective had no difference from plain soap.

Fishbein (29) supports the germicidal effect of soaps containing G-11. The bacterial flora seem to continually be effected by G-11 after actual use. This germicidal substance therefore seems to be the most promising preparation so far available for incorporation into soap used in conjunction with surgery. The validity of these statements is not known because the test technic or basis of his opinions is not given.

(i) Liquid Versa Solid Media

Some difference of opinion is expressed of which media liquid or solid soap is best for using hexachlorophene.

In the works of Fahlberg (16), he observed when a solid soap vehicle was substituted for a liquid that the amount of retained hexachlorophene was reduced about tenfold. This was of interest in view of a similar observation by Seastone (25) based on skin counts in which it was shown that the solid soap vehicle is also less effective in reducing the resident bacterial skin

flora. The most obvious explanation for this difference lies in the fact that a much larger amount of the agent comes into contact with the skin when it is dissolved in a liquid soap vehicle.

Best (19) feels in general that all liquid soaps are more irritating to the skin than bar soaps because of their cocoa oil and potash content.

From an economical standpoint, the liquid form would seem to be a preference in that not as much would be wasted and that a higher concentration is applied to the skin.

(j) Speculation

Growth stimulation and biochemical action are two of the possible many questions about hexachlorophene which have not been answered. Certainly if hexachlorophene is going to be used on areas which have a possibility of further skin growth, G-11 should be tested for its possibility as a skin growth stimulator. In 1906 the initial studies on scarlet red observed that such a substance when injected into the germinal area of the skin produced abnormal increase in the number of germinal cells. Could possibly hexachlorophene be tested in the same manner?

No one in the study of hexachlorophene has yet

produced conclusive evidence of the biochemical and physiological action of hexachlorophene upon bacteria or its persistant presence on skin for several days.

III. CLINICAL NOTES AS TO USE

Rosenberg (4) supports the opinion of many workers that vigorous cleansing methods have no place in the preliminary treatment of burns because: (a) anesthesia is required, (b) shock is thereby increased and (c) injruy to remaining healthy tissue results from scrubbing and the use of irritating cleansing agents. Certainly the author has some argument to the danger of anesthesia, but the shock and traumatic picture are of different opinion. Fishbein (29) reports in the washing of a patient's burn with water and soap in one hundred cases has given considerable evidence that "thermal shock" and complex blood concentration syndromes are prevented. Freeman (30) thinks that despite the reported inactivation of hexachlorophene by serum, he has used it in the mechanical cleansing and debridement of extensive burns, with resultant kindly wound healing, no evidence of tissue trauma, and no infection.

Several men have devised some clinical test evi-

dence that are of interest. Nungester (21) injected intraperitoneally washings of skin to susceptable (Mucin added lowers animal resistance one million fold) mice. These washings were of skin either using G-11 soap or routine surgical scrub technic. Of the animals injected with G-11 soap washings, 8% died; and of those injected with plain soap washings, 12.5% died. It is felt that this is statistically significant. (Serum environment would be present - yet G-11 still seemed to be effective somewhat). Fahlberg (16) found an unexpected enhancement of bacteriostasis following defatting (ether) of the skin. This may indicate that a larger reservoir becomes available for the retention of hexachlorophene following this treatment. It implies that the residual hexachlorophene activity is not due to solution and retention in the lipoids of the skin.

Price (9) thinks it is of advantage in the initial treatment of burns to reduce the bacterial flora present on the burned skin and in the surrounding area by surgical cleansing. Removal of dirt and dead skin can only do good provided it is carried out gently and with good technic. G-11 soap may be used for this purpose, but it should be remembered that hexachlorophene

acts only very slowly and that it is largely neutralized by the presence of serum. Best (20) felt that the use of G-11 in contaminated wounds will promote better control of any potential infection. A single wound washing does not permit the cummulative effect that is so important in the surgical scrub, but it still can have value. The usual technic of final irrigation of the wound with sterile water or saline solution is recommended. One would think that the final application of G-11 soap to the skin area would be more effective if not diluted by water.

Rousselot (31) in fifty-two to the most severe cases of second and third-degree burns used the method of washing gently the burned wounds with G-11 detergent. Freeman (32) in his control series of 368 patients prepared by a 10 minute scrub with soap followed by alcohol, ether, and zephiran chloride (routine scrub) found the incidence of wound infection was 8%. In a strictly comparable series of 328 patients prepared by a 4 minute scrub with G-11 detergent, wound infections were seen in only 2%. Kraissl (28) used G-11 soap in 1,500 preoperative skin preparations and felt that there are no practical disadvantages to its general use.

Up to the time of preparation for this paper, very

little has yet been introduced into the literature pretaining to clinical results of hexachlorophene's part in the treatment of burns; many men now use such technic but the conclusive answer lies in the future clinical and scientific evidence.

IV. CONCLUSION

1. If feasible wash all burns with hexachlorophene soap or detergent for ten minutes. This must be done gently with repeated sudsings and saline flushing. The final application is blotted and allowed to remain on the burn wound.
2. Independently the mechanical effect, detergent, soap, and hexachlorophene play a part in lowering the transient and resident flora which are <u>always</u> present in burns.
3. Hexachlorophene seems readily adaptable to use in soaps and detergents although latest studies might indicate that detergents may hinder the effects of G-11. The method of action of G-11 is unknown; it is believed that a free chloride in its chemical structure explains its activity against bacteria. After a washing with G-11, this activity is present on the skin with a certain degree up to two days.

4. Methods for testing the efficiency of G-11 on the skin flora are presented - several of which show promise for future use in the study of hexachlorophene. The short comings of these tests are explained: mainly the fault of carrying hexachlorophene over into culture media and thereby getting lower counts with a false impression of excellent results of the test.
5. Improvements in culture technic with addition of serum to try and inactivate the G-11 have given the impression that with correction of technic, G-11 does lower the bacteria some with the element of time present. Latest tests with a new G-11 inactivator Tween-80 give the impression that serum is a poor inactivator. Tween-80 apparently will allow for more concise test results and possibly will indicate that the study of G-11 in clinical situations has just begun. Many of the original articles should be re-examined for validity.
6. Sensitivity to hexachlorophene is rarely reported.
7. Hexachlorophene is highly bacteriostatic invitro and moderately effective invivo as to time of exposure and concentration. Primary washings as a general consensus of opinion have minimal G-11

effect upon the skin flora. This has been only crudely determined and needs further study. Hexachlorophene is a poor antiseptic.

8. Hexachlorophene in liquid media permits a more effective environment to lower bacterial flora.
9. The few clinical reports of hexachlorophene soap used in washing burns have indicated excellent results with less infection and complications.
10. A re-evaluation of hexachlorophene's efficiency is in demand due to improved testing technics.

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