

METHODS OF TESTING A GERMICIDE INCORPORATED IN SOAP*

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OUR STUDIES on this problem extended over a number of years and totaled in the neighborhood of 200 washing experiments. The compound studied was 2,2'-dihydroxy-3,5,6-3',5',6'-hexachloro-diphenyl-methane, hereafter called for brevity "G-11." Before the preparation had been submitted to us, various *in vitro* studies had been done using the F.D.A. standard tests for germicides as outlined in Circular 198, U. S. Department of Agriculture. Also the wet filter paper technique described in the same pamphlet had been done and the rate of killing of the organisms had been studied. The phenol coefficient had been determined and the effect of the compound in the presence of serum had been studied. In view of the favorable reports, the next question that it was necessary to settle was whether or not the preparation was a skin sensitizer. We performed a series of over 200 patch tests using

0.5 per cent G-11 in petrolatum and 1 per cent G-11 in petrolatum, allowing the closed patch tests to remain on the skin for forty-eight hours. As the results were negative, the preparation did not seem to be a primary irritant. The same subjects were then re-tested ten days to two weeks later at approximately the same sites. Similar tests were allowed to remain in place for forty-eight hours, and were read. The readings were all negative, indicating that G-11 was non-irritating to the skin and was not a skin sensitizer. Subsequently, several hundred more patch tests were done with similar results. A hand-washing experiment was then done. The procedure was described in detail as follows:

Hands were rinsed with 70 per cent alcohol (by weight) and allowed to dry in air. One cubic centimeter of 24-hour culture of F.D.A. standard strain of *Staphylococcus aureus* was added to the palm of one hand, and culture was spread over the surface by means of a sterile tooth

* Presented at the May 19, 1948. Meeting of The Society of Cosmetic Chemists, in New York City.

brush, and allowed to dry for five minutes. Three-tenths gram of the soap to be tested was dissolved in 3 cc. of sterile distilled water and added to the hand with the dried culture on it, rubbing up into a good lather on the palm by means of another sterile brush for a period of two minutes. The total lather was removed from the hand by means of the brush and dissolved in 500 cc. of sterile distilled water. One cubic centimeter of this solution was plated in agar and the number of bacteria present determined by counting the colonies that appeared in standard beef broth agar after incubating the same in a Petri dish for a period of forty-eight hours. The possibility of bacteriostatic effects here were excluded by streaking the sterile plates containing the G-11 with a fresh staphylococcus culture and observing its normal growth. Under these conditions of testing we found: (A) Using ordinary soap for the washing, the plate count showed 110 staphylococci and a few spore formers present; (B) using soap of the same composition as in (A) but containing 2 per cent of Compound G-11, the plate was sterile; (C) using the same amount of "Neko" soap (Parke, Davis 2% HgI_2 soap), there were no staphylococci on the plate, but approximately a dozen spore formers or "spreaders" were present.

This was not the type of hand-washing experiment carried out in our remaining tests. All of our washing experiments were conducted in the following manner:

1. Ten sterile basins used.
2. Each contained 2000 c.c. freshly distilled H_2O at 23–28°C.
3. Measured lengths of hands and forearms (14 inches from tip of middle finger).
4. Marked off with "wet-proof" tape.
5. By stop watch:
 - 25 seconds to lather.
 - 75 seconds to scrub (no brush).
 - 20 seconds to rinse in basin.

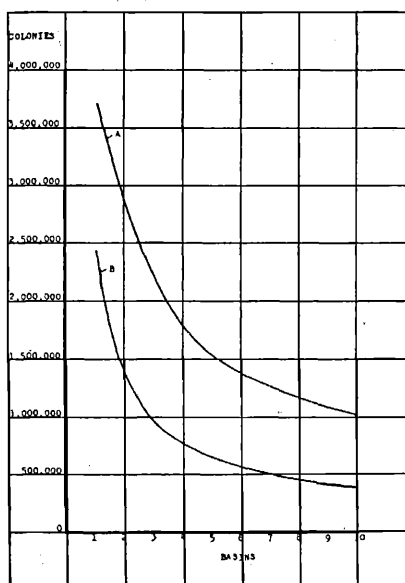


Figure 1.—*A*, washings, control soap used in all basins. Average of 25 subjects. *B*, washings, 2 per cent G-11 soap used in all basins. Average of 6 subjects.*

6. Subject moved to next basin and procedure repeated.
7. Within two minutes after subject finished at each basin two 0.1-cc. samples removed and each placed in sterile Petri dish, 15 cc. of melted beef heart infusion hormone agar pH 7.2 at 45°C. added to each dish.

8. Incubated forty-eight hours and then counted on Quebec colony counter.

1. In the first series of experiments—Fig. 1:

A. Control soap was used to wash through all 10 basins on an average of 25 subjects.

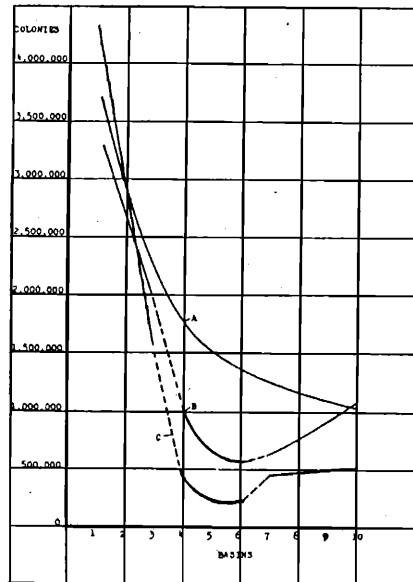


Figure 2.—*A*, initial washings, control soap used in all basins. Average of 25 subjects. *B*, 2 per cent G-11 soap used in basins 4, 5, and 6. Control soap used in all other basins. Average of 16 subjects. *C*, 4 per cent G-11 soap used in basins 4, 5, and 6. Control soap used in all other basins. Average of 6 subjects.

B. Two per cent G-11 soap was used in all 10 basins on an average of six subjects.

This was done to determine the immediate killing effect of G-11. On counting the colonies cultured from the various basins it will be seen that

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the curve when G-11 was used began at a slightly lower level and remained slightly lower than the control curve, but the result was not striking.

2. In the second experiment—Fig. 2:

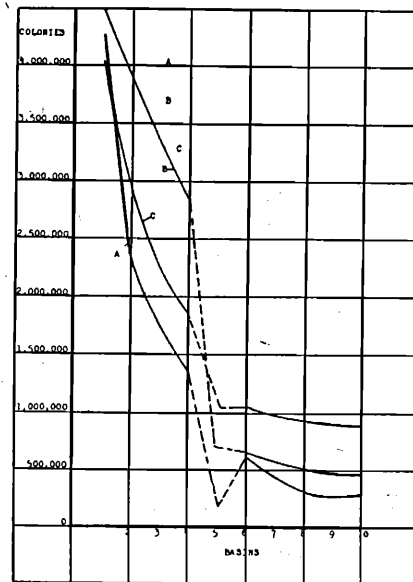


Figure 3.—*A*, 70 per cent ethyl alcohol used in basin 5. Control soap used in all other basins. Average of 9 subjects. *B*, 70 per cent ethyl alcohol used in basin 5 for 50 seconds only, followed by washing with control for 5 seconds. Average of 6 subjects. *C*, 2 per cent G-11 soap used in basin 5. Control soap used in all other basins. Average of 9 subjects.

A. Control soap was used in all 10 basins for an average of 25 subjects.

B. Two per cent G-11 soap was used in basins 4, 5, and 6. Control soap in all other basins on an average of 16 subjects.

C. Four per cent G-11 soap was used in basins 4, 5, and 6. Control

soap in all other basins for an average of six subjects.

The drop noted in basins 4, 5, and 6 was only slight from 2 per cent G-11 and only very little greater from the 4 per cent G-11 soap. The accuracy of the method is demonstrated in noticing slight effects and that the resident flora is reached in about the seventh basin, after which little change is noted. The result of this experiment was not striking.

3. Figure 3:

A. In this experiment 70 per cent ethyl alcohol was used in Basin 5. Control soap was used in all other basins on an average of nine subjects.

B. Seventy per cent ethyl alcohol was used in Basin 5 for fifty seconds, only followed by washing with control soap for five seconds on an average of six subjects.

C. Two per cent G-11 soap was used in Basin 5. Control soap was used in all other basins for an average of nine subjects.

In noticing the curve of this experiment it will be noted that when 70 per cent ethyl alcohol was used in Basin 5 (A), a decided drop occurred, but the count again rose in Basin 6 indicating that the alcohol probably did not kill as many bacteria as was apparent from the drop, but rather had a coating effect which permitted the bacteria to be washed off in the next basin. The G-11 did not cause as large a drop, but neither was there the rise in the basin following its use.

4. In this series of experiments—
Fig. 4:

A. Initial washings were done in all control basins for an average of six subjects.

B. Control soap was used in all basins for an average of three subjects, who had used a control soap for a period of one week.

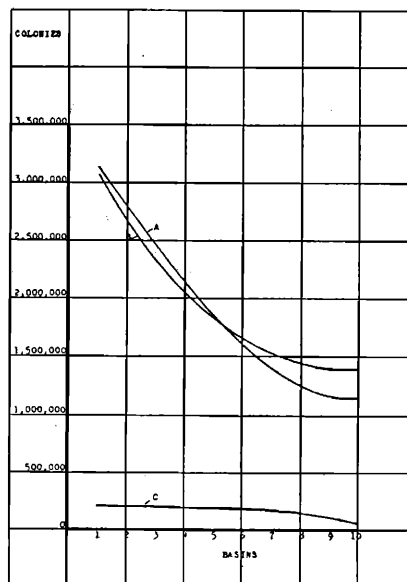


Figure 4.—A, initial washings, control soap used in all basins. Average of 6 subjects. B, control soap used in all basins. Average of 3 subjects who had used control soap for the period of one week. C, control soap used in all basins. Average of 3 subjects who had used 2 per cent G-11 soap for a period of one week. Figures indicate that resident level was reached practically from second basin.

C. Control soap was used in all basins on an average of three subjects, who had used 2 per cent G-11 soap for a period of one week.

This experiment gave a striking result. It indicated that G-11 had not only wiped out the transient flora, but had greatly reduced the level of the resident flora and had brought the count down to an ex-

tremely low level. Price, in his experiments on commercial soaps, had noted that none of those tested by him had had any germicidal action on the resident flora of bacteria.

5. Figure 5: Because of the striking result of Experiment 4, it

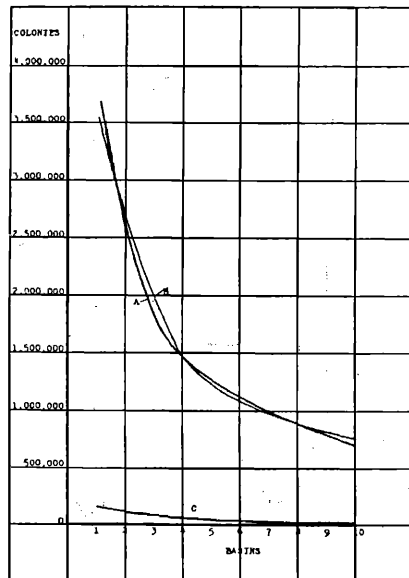


Figure 5.—*A*, initial washings, control soap used in all basins. Average of 14 subjects. *B*, control soap used in all basins. Average of 4 subjects who had used control soap for a period of one week. *C*, control soap used in all basins. Average of 10 subjects who had used 2 per cent G-11 soap for a period of one week.

was repeated using a larger average group of subjects and the results of the preceding experiments were confirmed.

6. Figure 6: In this experiment one of the subjects who had used G-11 soap for a week reported for his washing experiment directly from football practice with his hands very much soiled. Even in

this subject an unexpectedly low count was encountered in the first basin and a reduction to the low level typical of all other subjects was found in all the other basins.

7. In this series of experiments the effect of 2 per cent mercuric

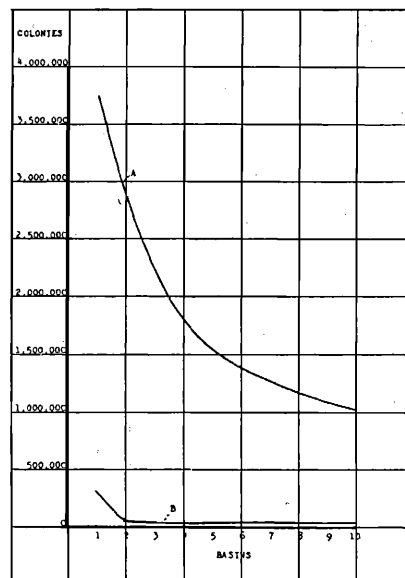


Figure 6.—*A*, initial washings, control soap used in all basins. Average of 25 subjects. *B*, colony counts on one subject who had used 2 per cent G-11 soap for a period of one week. Control soap used in all basins.

iodide contained in Neko soap was compared to the effect of 2 per cent G-11 soap (Figure 7):

A. There was an initial washing with control soap in all basins for an average of six subjects.

B. Control soap was used in all basins for an average of six subjects, who had used 2 per cent Neko soap for a period of one week.

C. Control soap was used in all basins on an average of three sub-

jects, who had used 2 per cent G-11 soap for a period of one week.

The resultant curve showed that the Neko soap curve varied very little from the control soap curve and did not compare at all to that of G-11 soap.

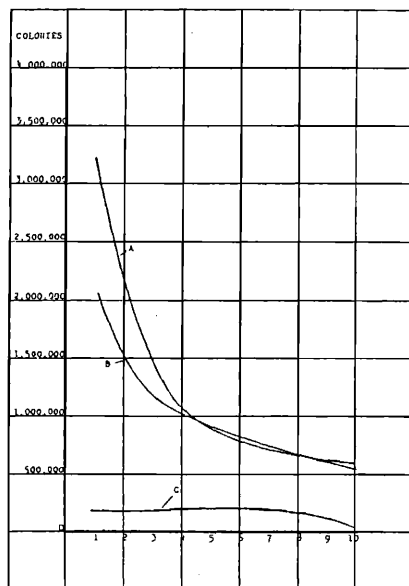


Figure 7.—*A*, initial washings, control soap used in all basins. Average of 6 subjects. *B*, control soap used in all basins. Average of 6 subjects who had used 2 per cent neko soap for a period of one week. *C*, control soap used in all basins. Average of 3 subjects who had used 2 per cent G-11 soap for a period of one week.

In general, therefore, the above experiments showed that three 2-minute washings or even ten 2-minute washings with 2 per cent G-11 soap had slight effect in depressing the bacterial flora of skin as compared with the great reduction noted after one week's regular use of the same soap. Instantaneous killing of bacteria obviously took

place to a small degree only; the effect of 2 per cent G-11 soap apparently is accumulative.

8. In this experiment the regular surgical scrub-up technique was compared to a modified scrub-up technique using G-11 soap (Fig. 8):

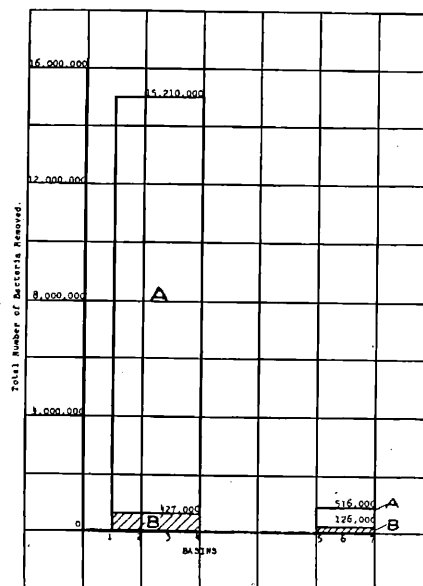


Figure 8.—*A*, subjects washed according to a routine surgical wash-up technique which included the use of iodine and alcohol after Basin 4. *B*, results of washings after subjects had used 2 per cent G-11 soap for one week. Here the surgical wash-up technique was modified by substituting control soap for hospital soap and omitting iodine and alcohol. Forty-five minutes' time elapsed between Basins 4 and 5. During this period subjects wore sterile rubber gloves and manipulated surgical instruments.

A. The subjects washed according to the following procedure:

Basin 1. The subject scooped up hospital soap and washed his hands only for thirty seconds. He then rinsed in the basin for twenty seconds.

Basin 2. The subject picked up a sterile brush, wet it with hospital soap, and then washed the skin test areas of one limb, successively scrubbing the backs and fronts of each digit, the palms and wrists, the knuckles, and the forearms for a period of three minutes and rinsed twenty seconds.

Basin 3. The subject repeated the performance of Basin 2 on the other limb, again washing three minutes and rinsing twenty seconds. At this point the subject put aside the brush, laying it in a sterile Petri dish cover and cleansed his nails with the point of a nail file and an orange stick. No time limit was put on this action.

Basin 4. All the tests areas of both limbs were scrubbed with the brush, soap being used as needed for a period of four minutes, devoting half of the time to each limb. The subject rinsed for twenty seconds and discarded the brush.

The ends of the digits to the distal interphalangeal joints were dipped into half strength tincture of iodine and withdrawn at once. The hands were briefly rinsed in 75 per cent (by volume) ethyl alcohol which was slightly tinged with iodine, and the arms were raised to allow the excess alcohol to run down the forearms. The subject wiped his hands and arms with a sterile towel, pulled on sterile muslin sleeves, powdered his hands with sterile talc and put on sterile rubber gloves.

The subject manipulated surgical instruments for a period of forty-five minutes. The rubber

gloves were aseptically removed and, the subject washed in Basins 5, 6 and 7, two minutes in each without a brush and using a bar of control soap.

B. The subjects had used 2 per cent G-11 soap for one week. Here the surgical scrub-up technique was modified by substituting control soap for hospital soap and omitting iodine and alcohol. Forty-five minutes' time elapsed between Basins 4 and 5 during which the subjects wore sterile rubber gloves and manipulated surgical instruments.

It was shown that an individual using 2 per cent G-11 soap regularly had a lower resident count after two minutes of washing than an individual who had washed for twenty minutes with ordinary soap. In the surgical scrub-up the additional use of alcohol and iodine did not reduce the count or hold it as well as had the regular use of 2 per cent G-11 soap with only a two-minute washing. We did not confirm Price's observation that the bacterial count was doubled in approximately forty to fifty minutes under the rubber gloves. It is possible that had our subjects actually been operating with the additional manipulation, tension, and sweating that the results might have been different.

The Question of Film Formation. Miller and his associates had shown by experiments that washing the hands with the use of a germicidal cationic detergent such as Zephiran chloride left the skin apparently free from bacteria, but subse-

quent washings with an anionic soap released large numbers of live bacteria, thus suggesting the presence of an imperceptible film of the cationic substance. Alcohol, alum and mercuric salts had also been observed to produce protective films masking viable organisms.

We reasoned that if a film formed by a cationic soap is broken by the use of an anionic soap, the opposite should also take place. Therefore, if anionic soap containing Compound G-11 would form a film on the skin, then this film should be removable by subsequent use of cationic soap, and this should result in a flora of normal numbers. We set up an experiment on this basis.

Six subjects washed through the standard 10-basin washing procedure, using control soap (anionic). Three of the subjects substituted Onyxsan S, 1 per cent (a surface active agent of the alkyl amidoalkyl amidoazoline type), and the remaining three subjects Zephiran chloride 1 per cent in Basin 5 for the control soap. Onyxsan S and Zephiran chloride are cationic detergents; the former is non-bactericidal, while the latter is a potent germicide.

In the second experiment six subjects washed through the standard 10-basin washing procedure, using control (anionic) soap and each subject was given a bar of 2 per cent G-11 (anionic) soap with instructions to use only this soap for all washings of the skin for the following week and then to return for the final washings.

The final washings were con-

ducted as follows: Three subjects washed for a two-minute period, using a 1 per cent aqueous solution of Onyxsan S, and rinsed in Basin 1. Washing with control (anionic) soap then followed in the other nine basins. The remaining three subjects went through this same routine except that 1 per cent Zephiran chloride was used instead of Onyxsan S.

It would appear that Zephiran chloride killed those bacteria which were removed in Basin 5, but, as was shown, the count returned to a high level as soon as the cationic film was removed by anionic soap in Basins 6 to 10. Thus Zephiran chloride apparently had only a superficial action and simply covered the bacteria temporarily with a removable film. These results agreed with the findings of Miller and his associates. Onyxsan S, which is non-germicidal, had an action similar to that of the ordinary anionic soaps. After G-11 (anionic) soap was used for one week, it did not matter whether Zephiran chloride or Onyxsan S was used before the final washing. The counts were equally low and not higher than in the previous experiments, in which no cationic detergent was applied prior to the washings with control soap.

Obviously, if our premise was correct, G-11 did not exert its apparent action in reducing the resident bacterial flora of the skin by obscuring the bacteria beneath a film. This substantiated the opinion that G-11 acted by actually killing the bacteria on and in the skin; in

other words, 2 per cent G-11 soap did not produce a protectant film on the skin, a condition which would give rise to a false sense of security.

The Role of Bacteriostasis. We felt certain that any trace of G-11 soap left on the skin following its routine use over one week and possibly carried over in the rinsings obtained from control soap washings would be so negligible that it could exert no bacteriostatic effect in the still more dilute cultures made from these rinsings.

However, in order to prove this point conclusively, cultures were made of the rinsings obtained in the control soap washings after the routine use of 2 per cent G-11 soap for one week, by plating 1 cc. and 0.1 cc. samples from each basin. If bacteriostasis is not a factor, then the ratio of the number of colonies obtained in these two plates should be of the order of 10:1. If G-11 carried over does cause bacteriostasis in a certain dilution, then the bacteriostasis should be lessened by increasing the dilution of G-11. Therefore if we can obtain counts of the same ratio, regardless of the dilution, bacteriostasis is not involved.

It was readily seen in tabulation that the ratio obtained in the two dilutions after the routine use of 2 per cent G-11 soap was approximately the same as the ratio obtained in the two dilutions prior to the use of the 2 per cent G-11 soap.

The remote possibility that the extremely small amount of Compound G-11, which could be carried

over from the skin to the basins and then to the cultures, exerted a bacteriostatic action was entirely excluded by these findings; hence the low counts obtained in control-soap washings following the routine use of 2 per cent G-11 soap were not due to bacteriostasis.

Effect on Pathogens. Until now no attempt had been made to differentiate between pathogens and saprophytes. In order to determine whether the 2% G-11 soap killed pathogens, the skin on the dorsal surface of the hands on 207 industrial employees was swabbed to determine the presence of *Staphylococcus aureus*. *S. aureus* was selected as the example of a pathogenic flora because it was thought to be present on approximately 10 per cent of human skins, because it was an accepted organism in evaluating the potency of germicides, and because of the relative ease with which it is identified culturally. Eight persons were found to be carriers of *S. aureus*. This was determined by doing frequent swabbings and repeatedly finding the organism to be present. This excluded the possibility that the organism was only incidentally present on one occasion.

The eight subjects were given the use of G-11 soap for a ten-day period and again swabbed and cultured. One subject ran out of the soap three days before the swabbing was done and this subject was the only one on whom the organism was found. The results showed that seven out of eight cases were free and warranted the assumption that

the routine use of G-11 soap was equally effective in removing pathologic and non-pathologic bacteria from human skin. Pathogens were found to return to the skin surface after a period of seven days or more when G-11 soap had been discontinued.

Mass Experiment in determining the effectiveness of 2 per cent G-11 soap in reducing pyogenic skin infections in a state asylum: The entire population of the Brandon State School caring for mental defectives ranging in age from six months to over fifty years and including an average of 389 persons—179 males and 210 females—comprised the experimental group.

The incidence of all types of pyogenic infections in the group were recorded for one year—June 1, 1945, through May 31, 1946. During this period no special medications or precautions were taken. The only change made from the period of June 1, 1946, through May 31, 1947 was that instead of using ordinary soap 2 per cent G-11 soap was substituted. In order to avoid suggestion effects, increased incidence of washings, or other factors that might influence the result, neither the employees or the inmates were advised and only the medical staff and the head nurse who made daily inspections of the population knew of the experiment.

The skin infections were classified in the following groups:

1. *Carbuncles*. Acute pyogenic inflammations of the skin and subcutaneous tissues characterized by multiple foci of necrosis and sloughing.
2. *Large furuncles*. Acute circumscribed pyogenic infections of sebaceous glands of hair follicles, usually more than 2 cm. in diameter.
3. *Small furuncles*. Less than 2 cm. in size.
4. *Styes* and other hair follicular infections of the eyelid.
5. *Cellulitis*, including felons and other infections about the nails and nail beds.

Bacteriological Controls. In order to provide an objective check control, hand-washing experiments were performed on a group of 10 subjects chosen from the entire population because they had had the highest incidence of skin infections during the twelve-month control period. Hand-washing experiments with bacteriologic colony counts were performed approximately every two to three months during the experimental period of one year using the same routine except that it was changed to the use of three basins instead of the regular 10-basin method.

The results indicated a corresponding decrease of bacterial skin flora which paralleled closely the clinical results. The total reduction in colony counts over the period of the year was 80 per cent. The clinical results indicated that the incidence of carbuncles was reduced 89 per cent during the experimental period; that of large furuncles 78 per cent; while at the same time there was an increase of 100 per cent of pyogenic infections classified as small furuncles. A decrease in cellu-

litis from 81 cases to 30 during the experimental period or a decrease of 63 per cent was noted. The data concerning styes indicated a slight increase during the experimental period. The increase in small furuncles may be explained on the basis of aborted infections which might have developed into large furuncles or carbuncles had the G-11 soap not been used, and also possibly due to the fact that the population was combed more carefully during the experimental period for the slightest evidence of any type of skin infection.

Its use to prevent folliculitis, boils, and irritations from cutting oils in industrial plants: Cutting oils during the war period and since were known to cause many pustular infections particularly about the hair follicles of the forearms and on body areas generally if the workers were careless and wore soiled clothing. The effectiveness of G-11 soap was studied in a number of rather large plants and it was found that in those individuals suffering from folliculitis and boils that G-11, while not curative, did seem to control the infection somewhat. Where it was used prophylactically it definitely appeared to reduce the incidence of such pyogenic infections.

Summary and Conclusions. A series of experiments have been performed for the purpose of observing the effect of continuing use of soap containing compound G-11 in reducing the bacterial flora of the skin and decreasing the incidence of pyogenic skin infections. The subjects of the experiment were 389 mentally defective inmates of the Brandon State School on whom detailed records concerning the incidence of skin infections were available for a control period of one year. During the experimental period, the only change in normal institutional routine was the substitution of G-11 soap for ordinary toilet soap previously used. Clinical results indicated a significant decrease in the number and severity of carbuncles, furuncles, and cellulitis. Control bacterial colony counts performed at intervals during the experiment on a group of 10 inmates who had the highest incidence of individual skin infections during the control period indicated that a corresponding decrease of bacterial skin flora paralleled the clinical results. It is concluded that the continuous use of G-11 soap is of marked value in the prevention of serious pyogenic skin lesions in institutional or other populations.