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A comparison of four skin cleansing products.

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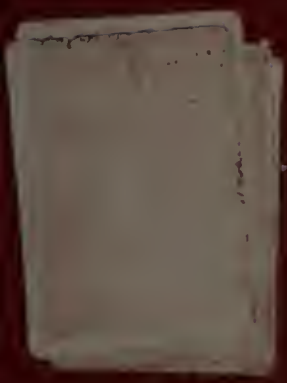
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A COMPARISON
OF
FOUR SKIN CLEANSING PRODUCTS

A thesis presented

by

ELI BORODA

Submitted to the Graduate School of the
University of Massachusetts in
partial fulfillment of the requirements for the degree of
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Department of Plant and Soil Sciences


A COMPARISON OF FOUR
SKIN CLEANSING PRODUCTS

A Dissertation


By

ELI BORODA

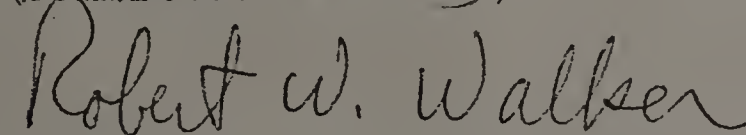
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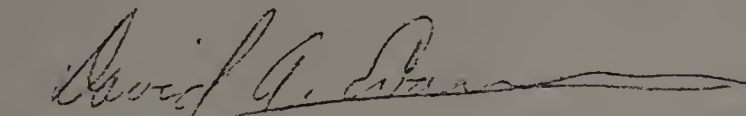
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ABSTRACT OF THESIS

There have been many reports in the literature evaluating the skin degerming ability of several hand washing products. The aim of this investigation is to define the shortest time necessary for maximum degerming of the hands during ordinary washing using four popular skin cleansers. For this purpose, eight normal subjects were selected (4 males and 4 females). It was found that after 20, 40 and 90 seconds washing with sterile water, Green Soap and Ivory Soap no significant reduction of the bacterial population on the palmar surface of the hands was evident, whereas Hexachlorophene (3%) liquid preparation showed an 85% reduction. Povidone-Iodine (7.5%) surgical scrub preparation, however, reduced the bacterial counts by at least 99.5%. It appears that Povidone-Iodine (7.5%) surgical scrub preparation will eliminate the majority of the bacteria from the palmar surface of the hands using a 20 second wash.

INTRODUCTION

The palmar surfaces of normal hands contain numerous bacteria that range from non-pathogenic to highly virulent strains. The ability of several products to reduce the number and type of bacteria present on the surface of the hands is very diverse and inconsistent. There have been many reports in the literature pertaining to the various hand cleansing products and methods for their evaluation. With the increasing need in the medical profession for a fast acting, efficient hand degerming agent, these seperate studies have produced data that are confusing and difficult to evaluate.

The object of this thesis was to evaluate the activity of each of four commonly used skin cleansers. Since, the persistence of a particular group of organisms, primarily the gram positives, is considered serious, it was important that a product show a broad spectrum of activity and reduce the bacterial flora on the palms of the hands to a very low number. Employing an adequate testing procedure, information was sought which reflected the effectiveness and rapid degerming capability of these products.

LITERATURE REVIEW

Flora of the Skin

The importance for washing the hands was advocated by Semmelweis at the middle of the 19th Century when he directed his medical students to wash with solution of chlorinated lime.³¹ He had observed that students attending deliveries after dissecting cadavers were responsible for the spread of puerperal fever among women after childbirth.¹⁰

Joseph Lister,¹⁵ concerned over the high rate of infected surgical wounds, attempted to eliminate the introduction of all bacteria in the operative field. He wrote on the subject of hand degerming, "This is done by cleansing it (the hand) with an antiseptic solution, making sure that it passes into the folds of the skin about the nail".

In a study conducted at the Denver V.A. Hospital in 1968 concerning the spread of Klebsiella, Eickhoff¹¹ reported that 61 of 162 patients harbored Klebsiella in their intestinal tracts. The organism was found to be present on admission in 30 patients while 31 had acquired it in the hospital. An extensive survey revealed the absence of these organisms in nasogastric suction and inhalation therapy.

Salzman²⁸ while studying drug resistant enteric bacteria, found that 20% of patient care personnel showed the presence of these organisms on their hands. He demonstrated the possibility of cross infection in a hospital environment by

way of the attending medical personnel.

Since the hands are subjected to constant activity and various environments, there is no way to determine their flora at any specific time. There have been many reports pertaining to the predominant group of bacteria usually found on the hands, however, these reports generally lead to false conclusions. Engley¹² studied the survival of various organisms on the skin and reported the following:

Factor	Survival	Reference
<u>Brucella milentis</u> 34% inhibited on normal human skin.		Hill 1933
<u>Proteus spp</u> Proteus survived longer on skin than on filter paper.		Hellat 1948
1.6% inhibited on normal skin.		Hill 1933
<u>Pseudomona aeruginosa</u> survived longer on skin than on filter paper.		Hellat 1948
4.4 inhibited on normal skin.		Hill 1933
<u>Serratia marcescens</u> survived longer on skin than on filter paper.		Hellat 1948
16.1% inhibited on normal skin.		Hill 1933

Factor	Survival	Reference	
palmar surface, clean hand	Inoc. 1200, Recov. 0 after 2 min.	Norton	1932
palms of hands	Inoc. 770 Recov. 112 after 1 min.	Norton	1932
<u>Escherichia coli.</u>			
<u>Aerobacter and Paracolobacter</u>			
palmar surface, clean inoc. 4,000	Recov. 1 after 10 min.	Arnold	1930
<u>Mycobacterium tuberculosis</u>			
human tubercle bacillus in reed capsule placed under skin of cattle	7 yrs.	Heymans	1927
<u>Micrococcus pyogenes</u>			
var. aureus			
palms, clasped	53% recov. in 30 min.	Cornbleet	1932
palms, open	6% recov. in 30 min.	Cornbleet	1932
<u>Salmonella enteritidis</u>			
palmar surface, clean R.T.	Recov. 0, 10 min.	Arnold	1930
dirty hands, body temp.	5% gone, 20 min.	Arnold	1930
hands after washing	100% gone, 20 min.	Arnold	1930
palm of hand	destroyed in 10 min.	Krueger	1942
<u>Streptococcus pyogenes</u>			
fingers, exposed 2 min.	Inoc. 1270 wash off 329	Burtenshaw	1938

Factor	Survival	Reference
fingers, exposed 54 min.	Inoc. 1228 wash off 190	Burtenshaw 1938
fingers, exposed 118 min.	Inoc. 339 wash off 3.1	Burtenshaw 1938
palm, exposed 2 min.	Inoc. 1270 wash off 50.6	Burtenshaw 1938
palm, exposed 54 min.	Inoc. 1228 wash off 0.32	Burtenshaw 1938
palm, exposed 118 min.	Inoc. 339 wash off 96	Burtenshaw 1938
<hr/>		
Influenza Virus		
human skin, inoc. 0.2cc virus susp.	50% recov. in 10 min.	Krueger 1942

Weaver,⁴⁰ in his attempt to relate contamination of the hands with the spread of diphtheria, found the presence of Bacillus diphtheria and Streptococcus hemolyticus on the skin of hospital attendants.

Litsky¹⁹ reported that hands, not degermed with a surgical scrub and conventionally washed, contained an average of 12,000 to 15,000 organisms per millimeter of test rinse water before washing. It was also reported that on occasion there was no reduction after washing. She stated that personnel using a surgical scrub regularly averaged less than 50 colonies before and 0-10 colonies immediately after scrubbing.

Davis⁹ explained that although skin is considered by many as a single site, its flora is not always similar at different anatomical areas. In the facial region, for instance, the bacterial flora reflects that of the oropharynx. Vigorous scrubbing of the skin with soap and water (or other disinfectants) can temporarily eliminate most of the surface bacteria. This condition is not lasting since organisms sequestered in hair follicles and sweat glands, soon recolonize the surface. Dineen's¹⁰ view on the subject of microorganism removal is more explicit than the one presented by Davis, since he is of the opinion that the bacterial recovery rate is related to the method of removal, substance used, and the nature and degree of recontamination of the skin.

Dissemination of bacteria from the nurse to the patient is an important factor in the origin of cross infection. In 1956 a report was presented concerning a controlled experiment where nurses were asked to talk to and handle newborns for differing periods of time after which cultures of the infants' skin were taken. It was found that the infants acquired the nurses' strain more readily when they were handled.

Effect of Different Topical Cleansers

Death rate

Chick⁶ showed that there is a predictable and orderly

course that is followed when a lethal substance comes in contact with a bacterial population. The death rate would depend on the number of living cells at any given time. Mathematically this is expressed as:

$$\frac{db}{bd t} = K$$

Upon integration and evaluation of the constant of integration the equation becomes:

$$K = t^{-1} \ln a^{-1}$$

a= represents the initial number of cells
 b= the number of cells living after the time involved
 t= the time
 K= the rate of death

Soaps

Norton²⁶ investigated the claim of various soaps to eliminate bacteria from the skin. He found that there was no difference between antiseptic soaps and regular toilet soaps. Most astounding was his finding that regular toilet soap could eliminate bacteria more efficiently than the Green Soap, that had been widely used in hospitals. He also stated that residue of the soap on the hands had no germicidal power.

Walker³⁷ in 1924 presented data pertaining to the germicidal properties of pure Sodium and Potassium salts of fatty acid soaps on the "typhoid bacillus" (sic), Staphylococcus aureus, Pneumococcus and Streptococcus. With the exception of Staphylococcus aureus all the above micro-

organisms were affected in varying degrees by the soaps. He further stated that the effect of the soap is dependent on the length of the fatty acid chain used in its manufacture. In another study reported in 1925, Walker³⁸ stated that cotton seed oil used in the manufacture of soap would aid in the prevention of the spread of typhoid fever by hand contamination. Davis³⁹ in the third of his series of reports published in 1926 indicated that various types of soaps at differing concentrations were effective against Meningococcus and Gonococcus.

Hexachlorophene

Hexachlorophene is a bisphenol containing two hydroxyl groups, one of which is neutralized by alkalies of soaps and detergents. The second hydroxyl group usually remains free and active against some bacteria. Designated as 2,2'-dihydroxy-3,3',5,5',6,6'-hexachlorodiphenylmethane, Hexachlorophene has been many times referred to as bis-(3,5,6-trichloro-2-hydroxyphenyl)methane.⁸

Stetler,³³ recently reporting on Neonatal Mastitis in two cases caused by Escherichia coli, explained that the facilitation of gram-negative skin colonization was due to Hexachlorophene baths used as antistreptococcal measures in nurseries. After washing the skin of newborn with soap and water followed by a single application of Hexachlorophene, a marked suppression of diphtheroids, Staphylococcus albus

and Staphylococcus aureus was noted by Sarkany.²⁹ There was no significant effect on Streptococci or Escherichia coli.

Light¹⁸ indicated that the ratio of Staphylococcus aureus to Pseudomonas was inversed after handwashing by attending personnel and infant bathing, in that a reduction of Staphylococci was associated with an increase in gram-negative Pseudomonas.

Forfar¹⁴ reported that while daily applications of Hexachlorophene decreased the coagulase-positive Staphylococci population, a definite rise in the incidence of infection from gram-negative organisms was observed. There also was a predominance of Escherichia coli, Proteus, Pseudomonas and other coliform organisms. Stratford³⁵ in 1963 stated that the eradication of Staphylococcus aureus could be predicted with certainty when Hexachlorophene is used. Unfortunately he added that little is gained since colonization with resistant gram-negative strains occurs.

In an attempt to identify the causes of an epidemic, Fierer¹³ noted that the hands of the attending personnel were heavily contaminated with Pseudomonas after changing the diapers of fecal carriers. Moreover, he found that this contamination was not completely eliminated by handwashing with a detergent containing 0.25% Hexachlorophene.

It was demonstrated that burn patients were also victims

of infection carried by the hands of the medical staff.

Lowburry²¹ reported that Hexachlorophene resistant coliforms were found on the hands of the nurses in the burn unit.

Larson¹⁷ presented evidence in which eight patients were found to have dangerous levels of Hexachlorophene in their body fluids following treatment with Hexachlorophene solutions. Experiments with rats and pigs indicated the rapid penetration of Hexachlorophene into the body.

Iodine compounds

Dineen¹⁰ stated that Iodine has a rapid bactericidal activity against a wide spectrum of vegetative microorganisms. As a skin antiseptic, Iodine in an alcohol and aqueous vehicle has been used, but there have been reports of skin irritation. In recent years Iodine has been complexed with surfactive agents resulting in a water soluble Iodophor compound, i.e. Povidone-Iodine, which possesses the antiseptic activity of Iodine but is largely free of adverse skin and staining properties.

Hrsuzek¹⁶ reported on the effectiveness of Iodine against herpes virus. He stated that the application of tincture of Iodine to the skin before the application of the virus prevented the development of the infection. However, no interference with the growth of the virus was observed when the application of the Iodine was made after the inoculation of the sacrificed area. He also found that

tincture of Iodine diluted 1:400 inactivated the virus of fowl pox while 1:800 failed.

McCullock²³ explained that Iodine is not very active against the tobacco mosaic virus. However, he further stated that it is not known how closely this virus is related to animal viruses.

In a comprehensive study by Simmons,³⁰ it was found that Iodine killed Staphylococcus aureus in 50% of the tests; Streptococcus pyogenes in 60% of the tests; Escherichia coli in 50% of the tests; while it only reduced the numbers of Clostridium welchii.

In 1967, Price²⁷ disclosed that Iodine is known to be extremely effective when used as a wide-spectrum local antiseptic and applied to healthy skin. He commented that Iodophors are free from allergic or toxic effects and yet retain the advantages of Iodine as an antiseptic. Moreover, Iodophors have the added virtue of cleansing the skin at the same time that Iodine is acting.

Environmental Controls

Burke⁵ reported that over the past 50 years, the emphasis has been to regulate the measures to prevent airborne and contact bacterial spread. Coriell⁷ revealed that with the use of laminar flow, a unidirectional flow of air, airborne particles of 4 to 24u in diameter which can carry

Staphylococcus aureus made a single traverse of a room prior to its entrapment in the filter mechanism. Whereas, using a radioactive tracer method of detection, similar particles have been observed to make from 8 to 12 traverses in a room with turbulent air and no laminar flow.

With the emphasis presently being made on environmental controls, the role of the attending nurse and physician as well as other medical personnel is being scrutinized insofar as the spread of microorganisms is concerned. Since there is a need to effectively degerm the hands of the medical staff in order to protect the patients with whom they come in daily contact, there is a demand for a skin antiseptic that has the ability to kill bacteria in the shortest period of time and will not cause irritation or sensitization. In this regard, Litsky and Litsky²⁰ found that ordinary washing with a Povidone-Iodine surgical scrub preparation for 10 to 20 seconds was sufficient to effectively degerm the hands.

EXPERIMENTATION

Selection of Subjects

In order to prevent the interference with the activity of the preparations used in this study by the daily use of skin cleansing products by the volunteers, a rigorous selection of subjects was conducted. To qualify, a subject had to be a user of a non-germicidal soap and shampoo exclusively. These individuals had to agree not to use any product that was or claimed to be antimicrobial. Everyday tasks such as dishwashing and car washing that need the use of soap-detergent solutions, were performed with the use of rubber gloves.

Because of the differences that exist in the bacterial flora of the distinctive sexes and in an attempt to make this study as close to reality as possible, equal numbers of subjects were selected from each gender. Of the eight subjects selected, four were males and four were females. The selection of eight volunteers insured accurate and significant statistical analysis of the resulting data. All volunteers were college students over 18 years of age.

Test Products

In view of the conflicting reports, the testing was conducted using four brands of popular skin cleansers. Ivory Soap was selected because of its wide cosmetic use. The choice of Tincture of Green Soap was made because of its

preference in many hospitals. pHisoHex, a 3% preparation of Hexachlorophene, was chosen due to its prevalent use as a skin degorming agent. A 7.5% Povidone-Iodine preparation sold under the brand name of Betadine and adopted by the United States National Aeronautic and Space Administration as a skin cleanser for its apollo moon mission, was chosen as a result of its reported efficacy.²⁰

To avoid any preferential treatment, the products used with the exception of water were purchased on the open market in bulk form and the required amounts aseptically measured in the laboratory before dispensing.

1. One single use bar of Ivory Soap was provided to each subject prior to testing when the schedule required it.
2. 10 mls of water were dispensed in 15 ml screw cap test tubes and sterilized prior to use.
3. 10 mls of Tincture of Green Soap were dispensed in 15 ml screw cap test tubes.
4. 13 mls of pHisoHex were measured in 15 ml sterile screw cap test tubes. This amount was chosen so as to deliver 10 mls to the palms. The viscosity of the product makes adhesion to the sides of the vessel possible and decreases the amount being poured.
5. In a sterile screw cap test tube 12 mls of Betadine

were quantified. The added volume was for the same purpose as described above.

Hand Washing Using the Test Products

The routine chosen for the experiment encompassed three important phases of the wash. The moistening of the hands must use sufficient water to allow the substance used to form suds and be transported to all the folds of the hands including below the nails. The second phase which includes the application of the product is the rubbing of the hands for the prescribed period of time and the formation of suds. This step does not present any problem when the test product used is a liquid, however with the use of a solid, extra time must be allocated for the soap to start dissolving with the water used for moistening. The final step includes the rinsing and drying of the hands.

Using the above criterias as guidelines, the following routine was used by each subject:

1. The hands were moistened with approximately 10 to 15 mls of sterile water maintained at about 37 C.
2. 10 mls of the test product, sufficient to give ample sudsing, were applied to the palms of the hands from a test tube. Because Ivory Soap was used as a solid instead of a liquid, 5 seconds were added to each washing interval to insure proper dispersion of the

soap on the hands and wrists.

3. After the test product was applied to the palms of the hands, the hands and wrists were rubbed for 20, 40, and 90 seconds according to the randomized schedule in Table 1.
4. Immediately following the test washing, both hands were rinsed with 800 mls sterile distilled water to remove the residual of the test product. This rinse was approximately 10 seconds in duration.
5. Using aseptic techniques both hands were dried employing a sterile barrier towel for 10 to 15 seconds and sampled.

Sampling and Counting of Bacteria

Immediately after drying both hands as described above (section 5), one hand was pressed, palm down, for 5 seconds on the appropriate agar. This was done to allow time for the bacteria still residing on the hands to be transferred to the surface of the media. The plates were incubated for 24 hours at 37 C. Several trials with differing periods of incubation revealed that more than 24 hours did not yield higher numbers.

Two counts were performed on each hand. The first involved the sum of all the colonies found on the tips of the

Table 1. Washing Schedule Indicating Subject, Test, Product and Washing Times in Seconds.

Week	Day	Subject							
		A	B	C	D	E	F	G	H
1	M	PH-90	GS-90	PH-90	IV-40	W-90	GS-40	PH-20	B-40
	T	c	c	c	c	c	c	c	c
	W	GS-90	PH-40	GS-90	PH-90	W-40	IV-20	W-40	PH-90
	T	-	-	-	-	-	-	-	-
	F	B-40	W-40	W-40	W-40	IV-40	PH-90	IV-20	IV-90
2	M	PH-20	W-20	IV-40	IV-20	PH-40	PH-20	B-40	GS-40
	T	c	c	c	c	c	c	c	c
	W	W-20	IV-20	B-40	GS-20	B-40	B-40	PH-40	GS-90
	T	-	-	-	-	-	-	-	-
	F	B-20	PH-90	W-90	GS-90	PH-90	W-20	IV-40	GS-20
3	M	IV-40	B-40	B-20	B-20	B-90	W-40	W-20	W-20
	T	c	c	c	c	c	c	c	c
	W	PH-40	PH-20	GS-40	W-90	PH-20	B-20	B-20	IV-20
	T	-	-	-	-	-	-	-	-
	F	IV-90	IV-40	W-20	B-40	W-20	PH-40	GS-90	IV-40
4	M	W-90	B-20	PH-20	GS-40	IV-90	IV-40	PH-90	W-40
	T	c	c	c	c	c	c	c	c
	W	W-40	GS-20	IV-90	W-20	GS-40	IV-90	GS-20	PH-40
	T	-	-	-	-	-	-	-	-
	F	IV-20	GS-40	PH-40	PH-20	B-20	GS-90	IV-90	B-20
5	M	B-90	B-90	IV-20	IV-90	GS-90	W-90	W-90	W-90
	T	c	c	c	c	c	c	c	c
	W	GS-40	IV-90	GS-20	PH-40	IV-20	GS-20	B-90	PH-20
	T	-	-	-	-	-	-	-	-
	F	GS-20	W-90	B-90	B-90	GS-20	B-90	GS-40	B-90

W= Water

GS= Tincture of Green Soap

IV= Ivory Soap

PH= pHisoHex

B= Betadine

c= Control

-= Not a Test Day

Numbers indicate washing times in seconds

fingers. The count for the entire hand (Total) included the finger tips as well as the remaining sections of the palm.

Baseline counts were made on Tuesday of each week by having every volunteer print their hands on the plates without washing. This was performed at least 24 hours after the Monday test periods.

Gloving of the Hands

Because of insufficient data concerning the presence or absence of residual action in the products used, the hand not printed was inserted in a surgical glove for one hour. The purpose therefore was to determine the effect of one hour gloving on the residual microflora of the hands washed with the test products. Since the bacterial flora varies between hands, the hands to be gloved were alternated on successive days.

Media Employed for Various Test Products

Trypticase soy agar was used as a basic media for the enumeration of the bacteria found on the hands. In the cases of water, Ivory Soap, and Tincture of Green Soap no neutralizer was added to the basic agar media, since previous experiments indicated the absence of germicidal residual due to formulation or rinse.

Litsky and Litsky,²⁰ using a similar procedure as the one indicated in this paper, showed that there were no significant differences between the observations of Povidone-Iodine washed hands subjected to inactivator and hands not subjected to inactivator. After following a normal routine of washing the hands with Betadine and immersing them in inactivator, they found, after conducting a statistical analysis, that there was no significant difference between the two treatments.

In order to remove the remote possibility of the delayed action caused by any residue which might be left on the hands and then transferred to the culture plates, neutralizer was added at the rate of 3.0% Tween 80 for the Hexachlorophene and 1.0% Sodium Thiosulfate for the Povidone-Iodine preparation.

The printing of the palms was conducted in 9 inch Alcoa aluminum pie plates used to contain the media, similar to the Petrie dish. Two plates were used; one to contain the media and one as a cover. To form a better seal, the aluminum pie plates used as covers had the ridge removed from their periphery. After several trial runs, it was found that 250 mls were sufficient to maintain bacterial growth on each plate. Amounts less than 250 mls lead to splits and tears in the media due to drying. Overage of media in the plates caused the content to fall when the inverted plates were

incubated.

To sterilize, the plates were stacked vertically in the autoclave to allow for adequate steam penetration. Using horizontal laminar air flow, melted agar 46 C was poured into the sterile aluminum plates. After 24 hours of refrigeration to allow for proper solidification of the media, the plates were incubated at 37 C for 24 hours prior to use. This step ensured sterility and drying of the agar, in order to minimize confluence of developing colonies. Periods longer than 24 hours were found to be excessive and unnecessary.

Method for Counting

Conventional methods of counting the colonies growing on the surface of the agar plates were found to be inadequate due to the large numbers in many cases. An automatic colony counter was therefore used to assure consistency and accuracy in counting.

RESULTS AND DISCUSSION

The numbers of bacteria found on the total surface of the ungloved hands are tabulated in Table 2. With the use of pHisoHex there is an observable drop in the count from the average of the untreated hands shown in Table 6. With the exception of individuals E and H, all counts show a drop from the 20 seconds to the 90 seconds washes. The percent reduction of colonies for the 20, 40, and 90 seconds washes was 56.5% to 77.6% and 85.0% respectively and indicated the scope of activity of the Hexachlorophene preparation as presented in Table 9. Table 7 also shows a constant and observable decrease in the mean, median and range for this product.

The geometric mean was calculated for all 8 subjects for the purpose of determining the percent reduction in bacterial counts (Table 6). With the exception of individual E, all volunteers were subjected to five control counts. The average values for the palmar counts of any single individual ranged from a low of 55 colonies to a high of 627. Females having been assigned letters A, C, E and G; it must be noted that counts for all the male subjects were considerably higher. Individual F consistently showed colonies that spread over the total surface of the plate during the allotted period of incubation. After thorough questioning it was found that this subject always played billiards one hour before the

testing. These spreading colonies were only found on the control plates and water washes. Upon discontinuing his billiard playing, this subject's plates were found to no longer contain spreaders.

Tincture of Green Soap, water and Ivory Soap showed a rather insignificant drop in the bacterial number on the surface of the palms of the ungloved hands. The results presented in Table 2 indicate this fact. Tincture of Green Soap seemed to be more inefficient than the water or Ivory Soap. The 20 and 40 seconds washes for the Tincture of Green Soap reduced the bacterial colony counts by 31.7% and 20.0% accordingly. Whereas the water showed a 55.5% reduction for the 20 seconds wash and 37.0% for the 40 seconds wash. The Ivory Soap for the same washing intervals indicated a 44.5% drop at the 20 seconds test and 60.0% at the 40 seconds test.

The most drastic reductions were noted with the Betadine. In the majority of the cases zero counts were obtained for the palmar surfaces of the ungloved hands. Early in the testing it was feared that the medium employed using thio-sulfate did not support growth. However, upon inoculation of the surface of the plates with organisms obtained from the control plate normal bacterial growth was observed.

The counts conducted on the finger tips of the ungloved hands were expectedly lower than the palms. All the results

revealed drops in bacterial counts similar to those obtained with the palmar surfaces. The order of efficacy remained similar to that of the ungloved palms. Tincture of Green Soap, water and Ivory Soap, in this case, again acted in a similar manner. pHisHex, though, showed a marked improvement. However, many subjects' fingertips showed large amounts of microorganisms still remaining. Counts with Betadine were drastically reduced since individuals, with the exception of one, had absolutely no bacterial growth on the plates as indicated by Table 3.

Consistently lower results were obtained for the gloved hands. The belief that the powder within the gloves was germicidal was discounted after testing. Pieces of glove were laid on top of inoculated plates for 24 hours of incubation resulting in good growth on most plates with only minimal zones of inhibition on two. Upon inspection of the results it was found that the reduction was expressed on all the products used. Tincture of Green Soap, Ivory Soap and water were again grouped together. With the Hexachlorophene preparation next to best, Betadine showed its capability of reducing the counts to zero in most cases.

The results of the control counts are found in Table 6. The geometric mean of the finger tip counts for all 8 subjects was 62, while that of the total count was 227 colonies. All the percent reductions for the four products and water are

included in Table 9 for both the gloved and the ungloved hands. Hexachlorophene reduced the counts by a maximum of 85.0% for the ungloved hand, while Povidone-Iodine showed a maximum of 99.6%. Reduction for Hexachlorophene after one hour of gloving was drastically increased to a maximum of 98.5% whereas Povidone-Iodine remained at a high of 99.5% reduction level.

As a first step in the statistical analysis of the data, the bacterial counts were converted to squares as is customary with data of this nature. An analysis of variance was then completed by computer in Tables 10 and 11. A copy of the computerized statistical analysis printout for both the gloved and ungloved total palmar surface counts are included in Tables 12 and 13.

This analysis of variance showed no significant differences among subjects or washing times as well as no significant interaction between any of the effects included in the analysis. However, there were highly significant differences among the products to which the subjects' hands were exposed.⁴⁰

In view of the significant differences among the test products employed, a Duncan's Multiple Range Test was conducted to differentiate these factors. This test showed that the counts of the ungloved hand obtained after washing with Tincture of Green Soap, Ivory Soap and water yielded maximum counts but did not differ significantly from one another

($P < 0.05$). Moreover, it was found that lower counts were obtained from subjects using pHisoHex which differed significantly from the above two test products. Betadine, however, lowered the counts of the hand to the extent that, on the average, less than one colony was observed and, likewise, these observations proved to be statistically significant. Similar results and conclusions were obtained when data from the gloved hands was subjected to statistical analysis.

Continued absence of organisms on the plates printed with Povidone-Iodine washed hands made it necessary to run a control streak of organisms obtained from plates stamped with unwashed hands. It was observed that the growth of microorganisms on the plates was not impeded by the neutralizer, sodium thiosulfate.

The variations in the results obtained from the water and Ivory soap were possibly due to the fact that no germicidal properties were involved in either case. The high concentration of bacteria in small areas of the hands was therefore due to a simple mechanical shedding of the bacteria on the hands being tested, thus leaving sections on the palms with high numbers of microorganisms.

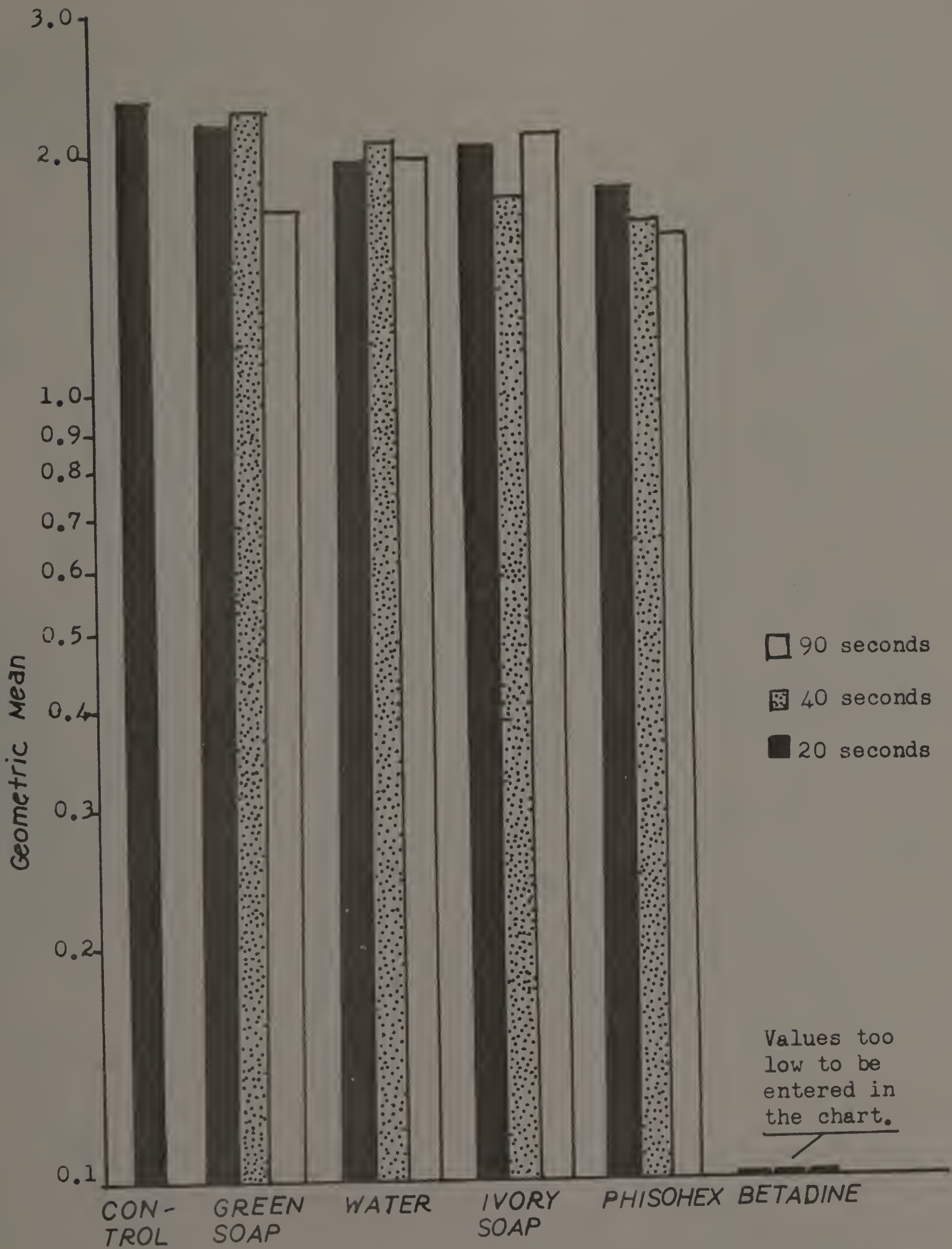


Fig. 1. Averages of bacterial counts on the total palmar surfaces of ungloved hands.

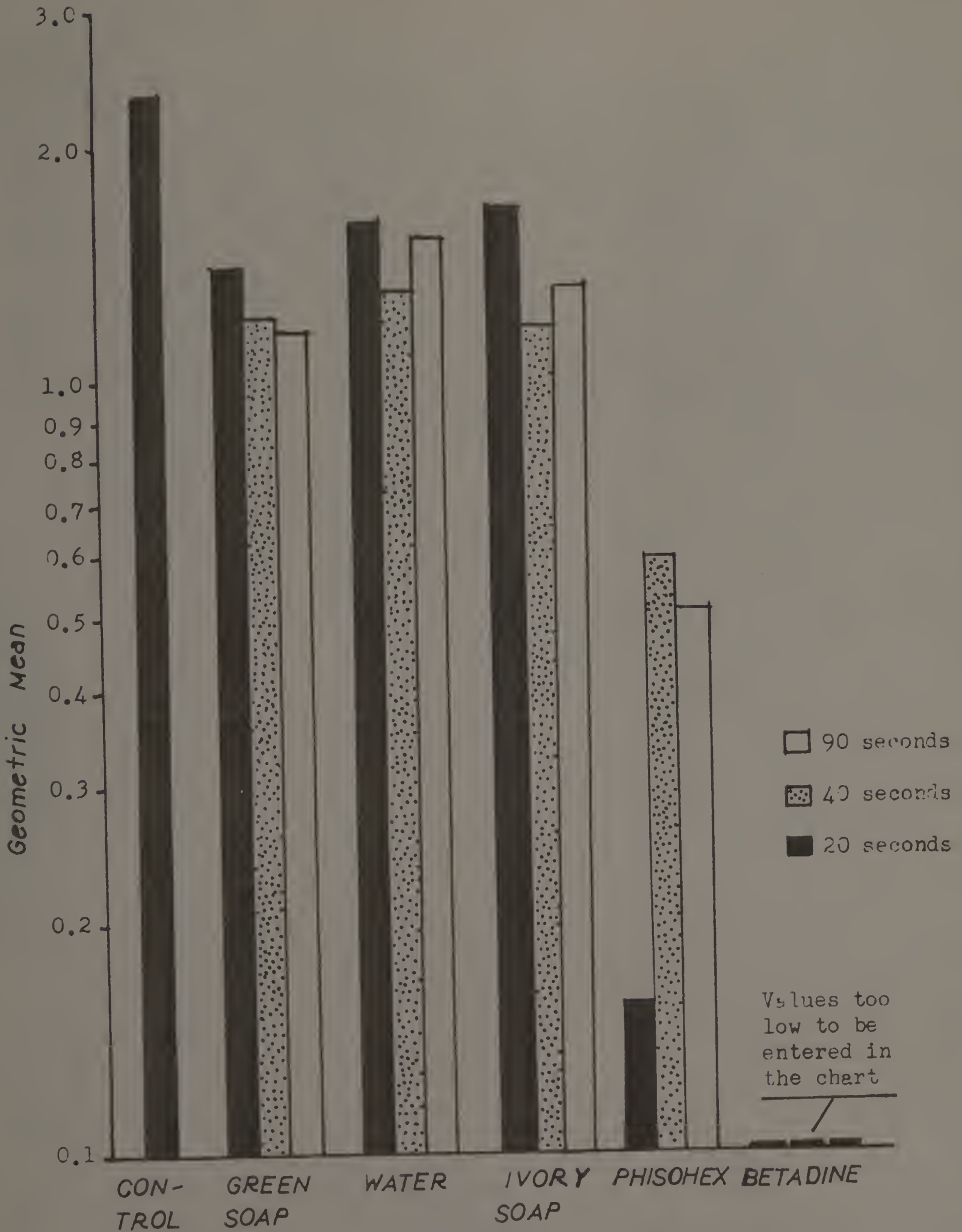


Fig. 2. Averages of bacterial counts on the total palmar surfaces of gloved hands.

Table 2. Numbers of bacteria on the total palmar surfaces of ungloved hands exposed to the test products for 20, 40, and 90 seconds.

Product	Time	Numbers of Bacteria (Colony Counts)							
		Subjects							
		A	B	C	D	E	F	G	H
pHisoHex	20	115	115	329	217	4	14	183	54
	40	115	31	82	68	15	12	179	56
	90	44	29	20	19	80	14	135	144
Ivory Soap	20	731	511	37	165	12	120	212	100
	40	652	24	87	98	35	19	251	175
	90	237	418	263	34	49	10	1001	582
Water	20	68	494	67	54	16	45	277	381
	40	151	174	61	112	100	117	340	225
	90	128	564	38	175	25	15	261	278
Tincture of Green Soap	20	1001	81	20	184	78	242	189	280
	40	905	431	403	55	32	28	388	361
	90	60	16	72	126	34	12	634	84
Betadine	20	0	0	0	0	0	1	0	1
	40	0	0	0	0	0	0	0	0
	90	0	1	0	0	0	1	0	3

Table 3. Numbers of bacteria on the finger tips of ungloved hands exposed to the test products for 20, 40, and 90 seconds.

Product	Time	Numbers of Bacteria (Colony Counts)							
		A	B	C	D	E	F	G	H
pHisollex	20	90	87	124	47	1	4	116	29
	40	52	8	47	11	3	5	65	18
	90	9	12	10	6	69	3	49	57
Ivory Soap	20	403	45	31	32	9	52	97	15
	40	319	5	61	51	16	0	71	54
	90	95	70	203	11	25	1	192	139
Water	20	22	53	13	7	6	19	46	190
	40	55	61	24	32	44	46	145	50
	90	31	117	1	37	5	3	12	21
Tincture of Green Soap	20	173	42	12	83	35	71	124	36
	40	422	26	186	15	23	3	103	173
	90	34	3	10	46	11	2	330	20
Betadine	20	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0
	90	0	1	0	0	0	0	0	0

Table 4. Numbers of bacteria on the total palmar surfaces of the gloved hands exposed to the test products for 20, 40, and 90 seconds.

Product	Time	Numbers of Bacteria (Colony Counts)							
		Subjects							
		A	B	C	D	E	F	G	H
pHisoHex	20	2	0	1	9	0	0	0	1
	40	6	1	22	6	2	0	1	37
	90	3	1	0	8	2	7	3	10
Ivory Soap	20	192	1001	1	16	5	58	264	109
	40	71	6	6	10	55	35	55	0
	90	16	45	14	3	12	3	221	183
Water	20	51	204	8	214	15	4	113	67
	40	33	63	5	27	4	9	269	41
	90	32	1001	13	152	25	4	41	69
Tincture of Green Soap	20	184	144	26	25	1	5	78	20
	40	71	25	42	18	1	9	65	53
	90	36	15	0	28	3	172	26	8
Betadine	20	0	0	0	1	0	0	0	0
	40	1	0	2	0	0	0	0	0
	90	0	0	0	0	1	0	1	1

Table 6. Numbers of bacteria on the fingertips and total palmar surface of untreated hands of all 8 subjects.

Control Number	Hand	Section	Numbers of Bacteria (Colony Counts)							
			Subjects							
			A	B	C	D	E	F	G	H
1	Left	Fingertips	22	749	66	81	5	98	19	62
		Total Palm	70	1001	144	229	23	349	37	324
	Right	Fingertips	9	77	30	73	15	80	4	65
		Total Palm	80	552	200	552	47	33	19	722
2	Left	Fingertips	111	48	40	91	6	66	25	132
		Total Palm	291	100	170	330	28	188	80	603
	Right	Fingertips	461	489	12	56	58	131	40	73
		Total Palm	760	1001	292	450	66	264	82	220
3	Left	Fingertips	58	47	37	508	53	77	15	501
		Total Palm	337	417	456	746	612	269	50	1001
	Right	Fingertips	58	131	102	321	1	60	41	501
		Total Palm	309	364	257	804	229	293	71	1001
4	Left	Fingertips	41	163	28	154	29	501	61	501
		Total Palm	133	1001	52	366	237	1001	107	1001
	Right	Fingertips	66	191	16	93	17	81	41	501
		Total Palm	357	518	51	477	30	352	103	1001
5	Left	Fingertips	87	96	67	118	14	184	10	72
		Total Palm	109	299	147	430	83	544	28	319
	Right	Fingertips	261	133	20	104	-	501	34	12
		Total Palm	1001	1001	105	235	-	1001	53	95
Average	Both	Fingertips	67	142	34	142	13	128	23	126
		Total Palm	241	514	153	426	79	312	55	627
Average of all Subjects		Fingertips	62							
		Total Palm	227							

Table 7. Means, medians, and ranges of bacterial numbers on the fingertips and total palmar surfaces of ungloved hands exposed to test products for 20, 40, and 90 seconds.

Product	Time (sec.)	Numbers of Bacteria (Colony Counts)					
		Finger Tips			Total Palmar Surface		
		Mean	Median	Range	Mean	Median	Range
pHisoHex	20	29.7	67.0	123	71.0	115.0	325
	40	15.8	14.5	62	49.5	62.0	167
	90	15.7	11.0	66	40.8	36.5	130
Ivory Soap	20	43.4	38.5	388	127.0	142.5	719
	40	27.2	52.5	319	89.2	92.5	633
	90	42.1	82.5	202	150.0	250.0	991
Water	20	23.9	20.5	184	99.0	67.5	478
	40	49.8	48.0	121	141.0	134.0	279
	90	12.2	16.5	116	103.0	151.5	549
Tincture of Green Soap	20	54.7	56.5	161	153.0	186.5	981
	40	50.3	64.5	459	180.0	374.5	877
	90	17.8	15.5	328	60.9	66.0	622
Betadine	20	0.0*	0.0	0	<1.0*	0.0	1
	40	0.0*	0.0	0	0.0*	0.0	0
	90	0.0*	0.0	0	<1.0*	0.5	3

*Arithmetic mean used instead of geometric

Table 8. Means, medians, and ranges of bacterial numbers on the fingertips and total palmar surfaces of gloved hands exposed to test products for 20, 40, and 90 seconds.

Product	Time (sec.)	Numbers of Bacteria (Colony Counts)					
		Finger Tips			Total Palmar Surface		
		Mean	Median	Range	Mean	Median	Range
pHisoHex	20	<1.0*	0	3	1.43	0.5	9
	40	1.4	0	11	3.95	4.0	37
	90	<1.0*	0	4	3.16	3.0	10
Ivory Soap	20	13.2	27.5	205	47.4	84.0	1000
	40	2.5	1.5	30	15.1	22.5	71
	90	9.3	10.0	81	21.4	15.0	218
Water	20	13.8	22.5	74	41.1	59.0	210
	40	6.1	5.0	60	24.0	30.0	265
	90	11.2	7.0	501	34.1	36.5	997
Tincture of Green Soap	20	4.79	8.5	100	24.6	25.5	183
	40	7.03	15.5	51	15.9	33.5	70
	90	5.59	5.5	51	14.2	20.5	172
Betadine	20	0.0*	0.0	0	<1.0*	0.0	1
	40	<1.0*	0.0	1	<1.0*	0.0	2
	90	0.0*	0.0	0	<1.0*	0.0	1

*Arithmetic mean used instead of geometric

Table 9. Percent reduction in bacterial counts on the palmar surface of the hands.

Product	Time	Percent	
		Ungloved	Gloved
pHisoHex	20	56.5 %	98.9 %
	40	77.6 %	97.7 %
	90	85.0 %	98.2 %
Ivory Soap	20	44.5 %	76.4 %
	40	60.0 %	92.9 %
	90	32.5 %	89.6 %
Water	20	55.5 %	80.9 %
	40	37.0 %	88.6 %
	90	53.7 %	79.0 %
Green Soap	20	31.7 %	87.7 %
	40	20.0 %	89.5 %
	90	72.4 %	94.3 %
Betadine	20	99.5 %	97.5 %
	40	99.6 %	99.5 %
	90	99.4 %	99.4 %

Table 10. Duncan's Multiple Range Test for the analysis of the total palmar surface of ungloved hands.

0.05 level

1) Treatment

$$s\bar{y} = \sqrt{\frac{0.1988}{24}} = 0.0910$$

d.f. = 28

	2	3	4	5
rp	2.90	3.04	3.14	3.20
Rp	0.264	0.277	0.285	0.291

Green Soap	2.1237	
Ivory Soap	2.4029	
Water	2.0184	
pHisoHex	1.7447	
Betadine	0.0752	

2) Times

$$s\bar{y} = \sqrt{\frac{0.1964}{40}} = 0.0701$$

d.f. = 14

	2	3
rp	3.03	3.18
Rp	0.212	0.223

20 seconds	1.6750	
40 seconds	1.5658	
90 seconds	1.5621	

Any two means included by the same straight line do not differ significantly from one another. Any two means not included by the same straight line do differ significantly from one another. ($P < 0.05$)

Table 11. Duncan's Multiple Range Test for the analysis of the total palmar surface of gloved hands.

0.05 level

1) Treatment

$$s_{\bar{y}} = \sqrt{\frac{0.4161}{24}} = 0.135$$

d.f.=28

	2	3	4	5
rp	2.90	3.04	3.14	3.20
Rp	0.382	0.400	0.412	0.421

Water	1.5872	
Ivory Soap	1.4362	
Green Soap	1.3363	
pHisoHex	0.5696	
Betadine	0.0826	

2) Times

$$s_{\bar{y}} = \sqrt{\frac{0.2390}{40}} = 0.0773$$

d.f.=14

	2	3	4
rp	3.03	3.18	3.27
Rp	0.234	0.246	0.253

20 seconds	1.0473	
90 seconds	0.9985	
40 seconds	0.9614	

Any two means included by the same straight line do not differ significantly from one another. Any two means not included by the same straight line do differ significantly from one another. (P < 0.05)

Table 12. Copy of the computer printout of the statistical analysis of the results of the ungloved hands. (Total)

INDEX	P	T	S
NUMBER OF LEVELS	5	3	8
POPULATION SIZE	5	3	INF

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

SOURCE	SUM OF THE SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	EXPECTED MEAN SQUARE
1 MEAN	307,5752	1	307,5752	120,000 (1) 15,000 (4) 1,000 (8)
2 P	71,7892	4	17,9473	24,000 (2) 3,000 (6) 1,000 (8)
3 T	0,3288	2	0,1644	40,000 (3) 5,000 (7) 1,000 (8)
4 S	6,4201	7	0,9172	15,000 (4) 1,000 (8)
5 PT	1,6974	8	0,2122	8,000 (5) 1,000 (8) 1,000 (9)
6 PS	5,5652	28	0,1988	3,000 (6) 1,000 (8)
7 TS	2,7502	14	0,1964	5,000 (7) 1,000 (8)
8 (PTS)	11,4542	55	0,2045	1,000 (8) 1,000 (9)
9 PTS	0,0000	0	0,0000	1,000 (8) 1,000 (9)

MEAN	1,60098							
CELL MEANS	1	2	3	4	5	6	7	8
P	1,74468	2,04285	2,01838	2,12370	0,07525			
T	1,67498	1,55584	1,56212					
S	1,84741	1,70345	1,58913	1,56605	1,25271	1,22627	1,83880	1,79299

Table 13. Copy of the coputer printout of the statistical analysis of the results obtained from the gloved hands. (Total)

INDEX P T S
 NUMBER OF LEVELS 5 3 8
 POPULATION SIZE 5 3 INF

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	EXPECTED MEAN SQUARE			
1 MEAN	120,5769	1	120,5769	120,000 (1) 15,000 (4) 1,000 (8)			
2 P	40,2021	4	10,0505	24,000 (2) 3,000 (6) 1,000 (8)			
3 T	0,1485	2	0,0743	40,000 (3) 5,000 (7) 1,000 (8)			
4 S	7,0513	7	1,0073	15,000 (4) 1,000 (8)			
5 PT	2,1682	8	0,2710	8,000 (5) 1,000 (8) 1,000 (9)			
6 PS	11,6512	28	0,4161	3,000 (6) 1,000 (8)			
7 TS	3,3463	14	0,2390	5,000 (7) 1,000 (8)			
8 (PTS)	12,8198	56	0,2289	1,000 (8) 1,000 (9)			
9 PTS	0,0000	0	0,0000	1,000 (8) 1,000 (9)			
MEAN	1,00240						
CELL MEANS	1	3	4	5	6	7	8
P	0,56962	1,43629	1,58718	1,33633	0,08259		
T	1,04729	0,96137	0,99854				
S	1,20182	1,20055	0,71169	1,13945	0,62785	0,74977	1,26546 1,12260

CONCLUSIONS

After careful consideration it appears that there is no difference in bacterial counts after washing for 20, 40, or 90 seconds with an individual test product and leaving the hands ungloved. Similarly, the gloved hand, after the application of the Duncan's Multiple Range Test, illustrated the lack of difference among washing times. Any variation among the results indicates either differences among subjects or among test products. Since the analysis revealed that there is no significant difference among individuals, the variance could only be due to the products used.

The 20, 40, or 90 seconds wash with the non-germicidal soaps (Ivory Soap and Tincture of Green Soap) did not appreciably reduce the microflora when compared to water washes of the same duration. In many circumstances these values were higher than the water. The possibility that these soaps would break up the grease and oils on the hands to allow for more mechanical removal of the bacteria did not materialize. A comparison between Ivory Soap and Tincture of Green Soap showed practically no difference in counts. In many cases higher bacterial counts were obtained after using the latter product.

While significantly lower counts were obtained from ungloved hands when using pHisoHex as compared to the water,

Ivory Soap and Tincture of Green Soap, a more striking reduction was observed on gloved hands washed with pHisoHex. These results may be explained by the fact that a residual amount of pHisoHex on the gloved hand may have exerted its antimicrobial activity during the one hour between the application of the product and the culturing of the hand. Results from the finger tip counts confirm these findings.

The microflora of both the ungloved and gloved hands after 20, 40, or 90 seconds washing with Betadine was reduced by 99.5%. The Duncan's Multiple Range Test indicated that these counts were significantly lower than when other products were used.

It appears again, as stated by Litsky and Litsky,²⁰ that a 20 second wash with the Povidone-Iodine is the most efficient method for degerming hands. The order of product efficiency agrees with Dineen¹⁰ in that Povidone-Iodine preparation was considerably better in reducing bacteria on the hands than pHisoHex or the non-germicidal soaps.

No skin irritation was noted at any time with the usage of any of the test products.

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