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EVALUATION OF PROPOSED SKYLAB AND SSP SOAP PRODUCTS

FINAL REPORT

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SUMMARY

Three personal hygiene cleansing agents (Neutrogena bar soap, Miranol JEM concentrate, and Olive Leaf) and one laundry detergent (sodium dodecyl benzene sulfonate), which are candidates for use on long-duration space missions, have been evaluated in terms of dermatological effects on human subjects and effects on microbiological species. None of the four materials exhibited adverse dermatological effects from skin patch tests of two weeks duration, and Neutrogena and Miranol JEM exhibited no adverse dermatological effects in a simulated Skylab personal hygiene regimen of up to four weeks duration (the other two products were not tested in this manner). Neutrogena and Miranol JEM also produced no significant alterations in skin microflora during the use regimen.

None of the four materials were found to serve as microbiological support media for the species tested, but a species of air-borne mold was observed to grow rapidly in a neutralized aqueous solution of Neutrogena. None of the candidate agents was found to be strongly biocidal.

Of the four agents, only Neutrogena was found to exhibit gellation and/or precipitation in aqueous solutions over the pH range of 2.5 to 11.0. This solution instability could pose problems if local pH excursions are allowed to occur in a washwater reclamation system in which Neutrogena is removed.

TABLE OF CONTENTS

LIST OF TABLES

1.0 INTRODUCTION AND BACKGROUND

The overall objective of this program was to identify and evaluate any potential hazards to crew and to a washwater reclamation system which might result from the use of Miranol JEM and Neutrogena, the personal hygiene cleansing agents currently proposed by NASA for Skylab and SSP. Types of potential hazards which were to be considered included:

(1) Adverse dermatological effects — Allergic and sensitization reactions, drying, and other irritant reactions, etc.;

(2) Adverse effects on skin microflora — neither complete removal nor growth enhancement of the skin microflora would be desirable;

(3) Enhanced growth of potentially pathogenic species — on the skin, on the surface of the Neutrogena, in the personal hygiene area, or in the washwater;

(4) Incompatibility of the personal hygiene agents with reverse osmosis membranes — causing hydrolysis, excess swelling, or decreases in throughput due to suspended solids, colloids, or gels; and

(5) Other potential hazards associated with a washwater recovery system — salting-out of solids, line plugging, chemical instability leading to gas formation, etc.

The program was to be conducted in three phases, with Phase I the dermatological and microfloral studies, Phase II the identification and evaluation of potential hazards, and Phase III the testing of compatibility of Miranol JEM and Neutrogena with reverse osmosis membranes. Dr. Jack M. Spurlock was the Principal Investigator for the program, with Dr. Frank C. Whitmore in charge of the effort on Phases I and II and Dr. Robert L. Durfee in charge of the Phase III effort. The microbiological preparations and analyses for Phase I were performed under the direction of Dr. William F. Enos and Mr. William Sellers, at the Pathology Laboratory, Northern Virginia Doctors Hospital, Arlington, Virginia.

At the request of the Contracting Officer, Phase III of the program was deleted and a preliminary screening of Olive Leaf and Sodium Dodecyl-Benzene Sulfonate for acute dermatological effects and for the ability of these cleansing agents to support growth of potentially pathogenic species of microflora was substituted. A detailed evaluation of the potential system hazards from any of the four cleansing agents and from mixtures of these materials is not complete, in the sense that items (4) and (5) above remain to be evaluated.

2.0 EXPERIMENTAL RESULTS FROM SOAP EVALUATION STUDIES (Phase I)

2.1 Characterization of Personal Hygiene Soap Products

Although a number of cleansing agents are being used by the various NASA centers and contracts in programs concerned with the personal hygiene regimen, washwater characterization, hardware design and development, and washwater reclamation, we have concluded that Miranol JEM concentrate and unscented Neutrogena are the two personal hygiene products most likely to be used on Skylab.

The Skylab regimen calls for use of 8 gm of the Miranol JEM concentrate in a weekly shower with about 6 lbs. of water. The Miranols are amphoteric surface active agents having caticnic and anionic groups of equal strength (isoelectric point at pH 7.0) and a pH of slightly above 9.0 when made up as aqueous solution.

Neutrogena is a proprietary formulation of a "super-fatted" soap. It is widely used by persons with dermatological and allergic problems. It produces the alkaline aqueous solution typical of soaps, but its structure and physical-chemical properties are not generally known. It is a mixture of several components. However, the manufacturers of Neutrogena, like the Miranol Co., have been very cooperative in furnishing samples and available technical information. The aqueous solutions of Neutrogena have a normal pH of about 9.3.

2.2 Test Methods and Protocol for Dermatological and Skin Microfloral Testing

2.2.1 Selection of Use Regimen and Schedule

The bathing regimen (using Neutrogena and Miranol JEM concentrate finally selected represents something of a compromise between the several possible Skylab protocols and the practical necessities inherent in this typ

of an experiment. The instruction sheet issued to each subject at the outset of the test program is included on Table I. This sheet summarized the final form of the regimen.

2.2.2 Selection of Subjects

It was determined that twenty subjects divided into two groups, Group A with twelve members and Group B with eight members, represent a sample that is large enough for statistical purposes, and yet small enough to generate a manageable number of bacteriological samples. The actual subjects represent a rather wide selection of skin types, including several blacks, and are of both sexes. Each subject signed a permission sheet and had the program and its purpose carefully and fully explained.

Group A (twelve) were medical technicians and students from a local college who were involved in the skin flora/bath regimen for approximately six weeks. Group B were mostly older persons with widely diverse environmental backgrounds who were involved in the skin flora/bath regimen for approximately four weeks. Each group of subjects was studied for normal skin flora for approximately one week while maintaining their normal personal hygiene regimen. After a base line was established for each subject, Group A followed the simulated Skylab personal hygiene regimen for a total of four weeks during which skin flora measurements were taken for the first two weeks. The extent of the test was such that any evidence of adverse dermatological response to chronic use should have shown up. At the conclusion of the four week simulation, the subjects of Group A returned to their normal hygiene regimen. Data on the return to "normal" microflora were taken during this last week. Group B followed essentially an identical regimen except for the shorter period on the Skylab regimen (one week). This group was also tested for acute dermatological effects.

2.2.3 Sampling and Test Procedures

A total of six body sites were selected for sampling:

TABLE I

INSTRUCTIONS FOR PARTICIPANTS IN THE SKYLAB PERSONAL HYGIENE PROGRAM

In order to determine the efficiency of the soap compounds to be use in the 28-day and the 56-day flights of Skylab scheduled to occur within the next few years, Versar has contracted to NASA to carry out this preliminary study. You, as a volunteer participant, will be asked to follow the regimen for personal hygiene that is outlined below. We hope that in the interests o carrying out a definitive study, you will follow the procedure as closely as is possible:

Sunday morning:

Shower using 6 pounds (about 1 gal.) of 105°F water and 8 grams Mira JEM. The subject should wet down, rub on the Miranol and work into the skin several moments. (Hair should be washed at this time). Use the remainder of allotted water to rinse off. Towel dry with a clean terry cloth towel.

Tuesday and Friday morning:

Sponge bath using moistened wash cloth coated with Neutrogena for full body bath. Rinse with moistened cloth until soap is removed.

Monday, Wednesday, Thursday and Saturday morning:

Wipe off using almost dry cloth with Neutrogena for whole body wipe-down. Wipe off with nearly dry cloth.

Hands & Face:

Hands and face may be washed with dampened cloth and Neutrogena at any time but not more often than five (5) times per day.

At the end of the first week of this regimen, each subject will be asked to report for skin swabs at 1300 hours for each day of the next seven days. The sampling sites and procedures will be identical to those for the baseline data.

Group A will continue the hygiene regimen for a total of 28 days and will be examined biweekly for signs of dermatological effects. Group B will continue the specified regimen for two weeks or until a satisfactory picture of their skin flora is obtained.

a. Left ear canal

b. Right axillary

c. Back of left hand

d. Upper right thigh at crotch

e. Upper left thigh at rectum

f. Bottom of right foot

Sampling was accomplished by wiping an area of approximately 3 square cm. with a cotton swab moistened with buffered sterile saline solution. Tubes and swabs were made available for the subject to make his (or her) own sampling at approximately 1300 hours on the test days.

On return to the laboratory the 0.5 ml water and swab was diluted to 1.0 ml with sterile saline and plated on Blood Agar, EMB, Mannitol Salt, Thioglycolate broth and Sabourand. These cultures are incubated at 37°C and read at 24 and 48 hours. Data were obtained in terms of general types of organisms and approximate numbers of each.

2.3 Results of Simulated Skylab Regimen — Microfloral Effects

The data obtained from this portion of the program are too voluminous to be included in the text; therefore, they are included as Table VII in Appendix I to this report. Table VII contains both baseline data and data obtained during the simulated Skylab regimen for each subject tested. A discussion of the results is presented below.

In order to present the experimental data of Appendix I in a form which most easily lends itself to interpretation, the individual sets of triplicate plates for each medium and each site have been averaged. In addition, although a more detailed identification was made in many cases, the

numerical results are presented as average numbers of gram negative cocci, gram negative rods, yeasts and molds. In only one case was a gram positive cocci observed. Although this method of presentation serves to suppress much of the detail of the experiment, it does adequately and correctly express the general results. A few cases wherein significant changes in the complexion of the microfloral population appear to have occurred are presented in detail in Table VIII of Appendix I. In addition, several plots of the total population vs. time are presented on Figures 1-5 of Appendix I. These plots show the surprisingly large daily variations in microfloral population in addition to the lack of significant variations associated with the experimental bath regimen.

The gram negative cocci were overwhelmingly staphylococci and α streptococci. The gram negative rods were predominantly proteus, E. Coli, diptheria and enterobacter. The rarely found yeasts were not identified specifically. In a number of cases wherein no growth (-) is reported in Appendix I, colonies were found in the thioglycolate broth even though there was no growth in the solid media.

The computation of the average number of organisms per unit area of the sample site cannot be very precisely computed from the data in Table VII of Appendix I, since there was apparently considerable variation in the degree to which the subject expressed the saline solution from the swab prior to taking the swab sample. This variation could be expected to alter the dilution factor which, coupled with the statistical variations in taking the samples for culturing, introduces considerable uncertainty in reducing the actual numbers to estimates of total surface population.

In order to give some estimate of the variations introduced by the experimental procedure, a known concentration of lactobacteria suspended in sterile buffered saline was spread over a 10 cm^2 area on a clean glass plate. Swabs prepared as in the skin microflora experiment were used to wipe an area of 3 cm^2. Subsequent treatment exactly as used in the skin microflora experiment suggest variations in count by an average factor of

three to four high for the computed surface concentration. On the other hand, the sampling error anticipated from taking three successive 0.001 ml samples from a suspension in 1.0 ml of sterile buffered water is (for the low concentrations of organisms expected) probably of the same order of magnitude in the opposite direction (a factor of three or four too low).

In spite of the uncertainties in numbers, repeated experiments with specific subjects indicate a rather constant error in sampling indicating that the relationship between successive samples taken by a particular subject are consistent and therefore comparable.

The experimental results obtained indicate (as shown in the Appendix, particularly Table IX) that there is no significant variation in the distribution or total population of skin microflora associated with the simulated Skylab personal hygiene regimen.

2.4 Results of Dermatological Testing

2.4.1 Dermatological Effects of Miranol and Neutrogena

The two cleansing agents selected by NASA for use on Skylab, Miranol JEM Concentrate and Neutrogena, have been tested for dermatological effects. Miranol is a liquid amphoteric surfactant (Miranol Chemical Co., Livingston, N.J.) used as an ingredient in baby shampoos and other related products, while Neutrogena is a bar soap (Neutrogena Co., Santa Monica, Calif.) advertised as "hypoallergenic" and suitable for persons with some dermatological problems ("sensitive skin"). Commercial Neutrogena is available in scented and unscented forms. Thus, both candidates appear to be excellent choices in terms of minimal direct dermatological effects — a point which was verified by test results from some twenty subjects for either acute or chronic exposure effects. These tests indicated no adverse effects for any subject.

Twelve subjects were tested for reaction to the two soaps following

routine exposure of the hands, arms, and axillae. Each subject received a detailed skin examination prior to testing to identify any condition which would bias the result. Weekly examinations were made to identify any apparent reaction. Use was as prescribed by NASA for Skylab and SSP. Since no reaction occurred for any subject, testing on that subject was continued for 28 days after which a complete examination of the skin was performed. No adverse reaction of any kind was observed during these examinations. As an aside, one male subject who has had a chronic localized foot infection reported that the Neutrogena/Miranol regimen offered him some relief from this condition, but this observation did not correspond to any observable change in his typical microflora at the designated sampling areas.

A second series of tests were performed on the other eight subjects. These tests consisted of skin patches on the arms and back of each subject, using a 2 per cent aqueous solution of the test soap. These tests were started on the first day of a week and subjects were examined for reactions on the third and fifth day. In the absence of a reaction, the patches were removed for the weekend, and fresh patches were applied on the first day of the following week. The cycle was repeated for a second week.

This second group showed no adverse reaction to the acute exposure. The absence of reaction was determined in each case by the subjective reaction of the subject coupled by examination by a competent authority. Incidentally, the twenty subjects included six (6) females and four (4) blacks and represented a wide variation in skin types.

2.4.2 Acute Dermatological Effects of Sodium Dodecyl-Benzene Sulfonate (NDBS) and Olive Leaf (OL)

A mixed group of subjects was tested for dermatological reaction

to the test soaps by a regimen essentially identical to that described in the preceding subsection (Section 2.4.1). Each subject had a 1" x 1" sterile gauze patch, moistened in the center with 0.25 ml of a 2 per cent (2 grams agent in 100 ml water) solution of the cleansing agent affixed to his upper left arm. The patches were affixed on Monday and allowed to remain until Friday for a total exposure of five days. The identical test was repeated for a second five day period. Upon removal the affected area was examined for reaction and rated on a numerical scale ranging from 0 - no observable reaction to 5 - extreme dermatitis.

The results of these tests for NDBS and OL have shown a consistent 0 rating for all tested subjects (19 in all).

2.5 Effects of Miranol JEM and Neutrogena on Growth of Microbiological Species

2.5.1 Test Matrix for Effects of Soaps on Microbiological Species

In order to determine, in at least a gross manner, the direct pgysiological effects of Miranol and/or Neutrogena on skin microflora and other microbiological species a test matrix was established using: (1) Blood agar plus 5% sheep cells, (2) EMB and (3) Mannitol salt plates as standard growth support media. The soaps were used as aqueous solutions made up in concentrations of 1%, 3%, 5%, and 10% by weight. The experimental protocol was established according to the guidelines set up as a test matrix shown in Table II.

The test media used were Blood Agar with 5% Sheep Erythrocytes, mannitol salt and eosin methylene blue agar. In the case of Test Series A, an attempt was made to identify, at least by species, the organisms which developed following a twelve (12) hour exposure to ambient laboratory air.

2.5.2 Results of Series A

Six BAP plates and six EMB/MS biplates were covered with 1.0 ml of the appropriate soap solution in each of the made-up concentrations and subsequently exposed to the laboratory airborne organisms for twelve hours. After exposure, the contaminated plates were incubated at 37°C. Plates of the same media covered with 1.0 ml sterile water served as controls. The incubated plates were read at 24 hours and at 48 hours for number and type of colonies present. A new set of test and control plates were exposed on each of three successive days.

A set of typical data from this exposure are shown below (only the 48-hour incubation is shown) as developed colonies:

Control BAP		12 Staph; 6 Dipth; 1 Yeast; 1 Mold
Control EMB/MS Biplate		3 Staph; 1 Mold
Neu rogena	1% BAP	10 Staph; 9 Dipth; 3 Yeast
"	3% "	19 Staph; 14 Dipth
"	5% "	8 Staph; 12 Dipth
"	10% "	2 Staph;
"	1% MS/EMB Biplate	3 Staph; 1 Yeast
"	3% "	No growth
"	5% "	No growth
"	10% "	1 Mold
Miranol	1% BAP	10 Staph; 1 Yeast
"	3% "	9 Staph; 5 Dipth; 2 Mold
"	5% "	13 Staph; 2 Mold
"	10%	1 Staph; 2 Dipth
"	1% MS/EMB Biplate	1 Staph; 2 Coag(-) Staph; 2 Mol
"	3% "	2 Coag(-) Staph
"	5% "	1 Mold
"	10% "	No growth

TABLE II

Test Matrix for Cleansing Agent Effects on Microbiological Growth Rates

Test Series A - Open Exposure of Culture Medium to Airborne Organisms (Clinical Environment)

Control - Sterile culture media; no cleansing agent.

Test Conditions - Sterile culture media plus addition of cleansing agent solutions (in distilled water) at concentrations up to ten per cent by weight; ambient-temperature culturing.

Test Results - Colony counts by gross species.

Test Series B - Exposure of Culture Medium to Washwater Samples from Selected Human Subjects

Control - Sterile culture media plus addition of washwater samples (washwater consists of distilled water only).

Test Conditions - Sterile culture media plus addition of cleansing agent-washwater samples (washwater consists of two per cent by weight cleansing agent); ambient-temperature culturing.

Test Results - Colony counts by gross species.

Test Series C - Inoculation of Support Medium with Selected Organisms to Determine the Ability of the Soaps to Serve as Growth Media

Control - Sterile support media inoculated with desired species; no cleansing agent (standard incubation conditions).

Test Conditions - Sterile support media plus cleansing agent solutions (up to ten per cent) inoculated with:

(a) Predominant and growth-accelerated species from Test Series A;

(b) Predominant and growth-accelerated species from Test Series B;

(c) Other species selected for potential significance.

Standard incubation conditions.

Test Results - Colony counts by gross species.

The results of this experiment indicate no significant difference between the controls and the treated plates. The conclusions resulting from this test suggest that neither Miranol nor Neutrogena exhibit biocidal effects. There is some slight indication of a longer range retardation of growth observed with the more concentrated Miranol solutions (5% or higher) suggesting a slight biostatic effect.

2.5.3 Results of Series B

In order to refine the results of Test Series A, six BAP plates and six EMB/MS biplates were inoculated (by streaking) with pure cultures of representative organisms and subsequently covered with 1.0 ml of the test soap solutions (using the same soap concentration range as simulated washwater as in Test Series A). The test organisms and their standard concentration, as determined by serial dilution, were as follows:

E. coli	~ 10^5/ml
pseudomonas	~ 10^5/ml
α streptococci	~ 2.6 x 10^4/ml
coagulase positive staphylococci	~ 2.6 x 10^4/ml
coagulase negative staphylococci	~ 1.5 x 10^4/ml
yeast	10^5/ml

After inoculation (standard loop 0.001 ml) and the introduction of the test washwater solution, the plates were incubated at 37°C for 48 hours before reading. The controls were treated as in Test Series A. In all cases, there were no statistically significant differences between the controls and the test plates.

2.5.4 Results of Series C

In order to determine the extent to which Miranol JEM and Neutrogena in aqueous solution can serve as biological support media, 1.0 ml of the same standard inoculation as in Test Series B was introduced with 5.0 ml of the standard soap solutions at each concentration. A similar inoculation with 5.0 ml of sterile buffered water served as control. The inoculated tubes were incubated at 37°C for 48 hours and read by the removal of 0.001 ml of the suspension which was streaked on the basic (EMB) support media. A typical set of results for the streaked plates is shown in Table III, which shows that neither soap is capable of serving as a microfloral support medium.

There is underlying these conclusions a possible loophole that should be more deeply explored. The aqueous solutions of both Miranol JEM and Neutrogena have pH values above 9. In the presence of typical nutrient culture media (as in Test Series A and B), the buffering capabilities of the media are sufficient to maintain the whole system (aqueous soap solution/culture medium) at a pH near 7.0. On the other hand, the incubation of the organisms in the soap solution for 24 or 48 hours prior to their inoculation onto culture media as was the case in Test Series C raises the question of the relative effect of the soap as compared to that of the high pH value. In order to test this, 2 per cent solutions of Neutrogena and of Miranol JEM in water were neutralized by the addition of 0.3 N HCl to a final pH of 7.0. Tubes were inoculated with test cultures, incubated for 24 and 48 hours at 37°C, then plated on EMB plates — following exactly the procedure of Test Series C. The results of such an experiment are

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TABLE III. Results from Exposure of Various Microorganisms to Soap Solutions - Culture on EMB Plates

Soap Solution	24 hr. Reading 1%	3%	5%	10%	48 hr. Reading 1%	3%	5%	10
Species	Neutrogena				Neutrogena			
E. coli	NG	NG	NG	NG	NG	NG	NG	NG
Pseudo	1	NG	NG	NG	1	NG	NG	NG
α-strep	NG	NG	NG	NG	NG	NG	NG	NG
⊕ staph	NG	100	100	α50	NG	∱100	∱100	∱10
⊖ staph	α50	α50	NG	NG	∱100	∱100	α50	α2
Yeast	α50	2	NG	NG	∱100	2	NG	NG
Species	Miranol JEM				Miranol JEM			
E. coli	1	NG	NG	NG	1	NG	NG	NG
Pseudo	NG	NG	NG	NG	NG	NG	NG	NG
α-strep	NG	NG	NG	NG	NG	NG	NG	NG
⊕ staph	NG	NG	NG	NG	NG	NG	NG	NG
⊖ staph	NG	NG	NG	NG	1	5	9	NG
Yeast	NG	NG	NG	NG	NG	NG	NG	NG
Species	Control				Control			
E. coli	over 100,000				over 100,000/cc			
Pseudo	over 100,000				over 100,000/cc			
α-strep	over 100,000				over 100,000/cc			
⊕ staph	26,000				26,000/cc			
⊖ staph	15,000				22,000/cc			
Yeast	over 100,000				over 100,000			

shown in Table IV for Neutrogena.

The results shown on Table IV did raise the question of possible support by Neutrogena for the growth of pathogenic organisms at pH levels near neutral. Repetition of the neutral solution tests of Table IV utilizing serial dilution techniques yielded quantitative results showing that the organisms per unit volume in the Neutrogena solutions were the same as those (controls) in which no Neutrogena was present. Thus, it appears that Neutrogena at neutral pH was only an inert ingredient causing no effect on microbiological growth.

In direct contrast, Miranol JEM solutions at pH 7.2 showed exactly the same results as at pH 9 (shown in Table III) — the biostatic effect is clearly a function of the cleansing agent and not of the solution pH.

2.6 Effects of Sodium Dodecyl Benzene Sulfonate and Olive Leaf on Microbiological Species

In order to determine, in at least a gross manner, the direct physiological effect of Sodium Dodecyl Benzene Sulfonate NDBS) and Olive Leaf on representative skin microflora, a test matrix was established using Blood Agar with 5% sheep cells, EMB and Mannitol salt plates as standard growth media. The soaps were prepared as aqueous solutions in concentrations of up to 10% by weight in distilled water. The format of the test matrix was shown in Table II.

2.6.1 Results of Series A

Six BAP plates and six EMB/MS biplates were covered with 1.0 ml of the appropriate soap solution in each of the made-up concentrations and subsequently exposed to the laboratory air-borne organisms for twelve hours. After exposure, the contaminated plates were incubated at 37°C. The incubated plates were read at twenty four and forty eight hours

TABLE IV.

The Effect of pH on the Ability of a Soap Solution to Support Bacterial Growth

	2% Neutrogena in Water at pH 9.3									
Organism	24	48	24	48	24	48	24	48	24	48
A	12	15	25	32	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-
D	-	1	-	-	1	2	-	-	-	-
E	13	50	-	10	24	57	-	-	-	-
F	-	-	-	-	-	-	-	-	-	-
G	3	4	-	-	11	12	-	-	-	-

	2% Neutrogena in Water at pH 7.2									
Organism	24	48	24	48	24	48	24	48	24	48
A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
B	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
C	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
D	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
E	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
F	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
G	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Key: A - E coli
B - α Strep
C - β Strep
D - ⊕ staph
E - ⊖ staph
F - Pseudomonas
G - Proteus

for number and type of colonies present. A new set of test and control plates (control plates were covered with 1.0 ml sterile water) were exposed on each of three successive days.

The results of this experiment indicate no significant difference between the controls and the treated plates. These results lead to the conclusion that both components were essentially inert components of the medium during this test series.

2.6.2 Results of Test Series B

Six BAP plates and six EMB/MS biplates were covered with 1.0 ml of cleansing agent-wash water samples and incubated at 37°C. The wash water sample was prepared by having several subjects repeatedly wash their hands in a 2% (by weight) solution of the appropriate cleansing agent. The control plates were covered with 1.0 ml of distilled water. The contaminated plates were read after 24 and 48 hours of incubation.

The results of this experiment again showed no significant differences between wash water samples and controls.

2.6.3 Results of Test Series C

In order to determine the extent to which the test cleansing agents, NDBS and OL, in aqueous solutions can serve as biological support media, a series of test organisms were introduced into various concentrations of the cleansing agent, held at incubation temperature for 24 hours, then streaked on Blood Agar plates.

The test organisms used were as follows:

A - E coli
B - α Strep
C - β Strep
D - + staph

E - - staph
F - pseudomonas
G - Proteus

A suspension of each of the test organisms was prepared by inoculating 1.0 ml of sterile water with 0.002 ml of the culture. Sterile tubes containing 1.0 ml of each of the cleansing agents at each concentration were then inoculated with 0.001 ml of the test suspension. Controls consisted of sterile tubes with only the cleansing agent solutions without the inoculation and a set containing 1.0 ml of the bacterial suspension. The tubes, contaminated and controls, were then incubated at 37°C for 24 hours.

After incubation, blood agar plates were streaked with 0.001 ml of each test solution. The contaminated plates were incubated at 37° and read after 24 and 48 hours. The same procedure was followed after a 48-hour incubation of the original sample tubes.

The results of these experiments are tabulated in Table V wherein the data have been averaged over each duplication.

The results of this experiment indicate that both cleansing agents exhibit biocidal activity toward gram-positive organisms at all concentrations of the cleansing agents and the sodium dodecyl benzene sulfonate had no effect on the concentration of the gram-negative organisms. On the other hand, the Olive Leaf solution at concentrations at 3 per cent or above seems to be uniformly an inhospitable medium for all of the tested organisms.

The data presented in Table V could be somewhat misleading in that there is a considerable difference in the pH of aqueous solutions of Olive Leaf as compared to those of the sodium dodecyl benzene sulfonate. The latter solutions have an adjusted pH of about 7.0 to 7.2 whereas the Olive Leaf solutions typically exhibit pH values above 9.0 and perhaps as high as 9.3. Since the test organisms generally prefer pH values near 7.0, there may be concern that the high pH values of the soap solution, coupled

TABLE V

Test Soaps as Bacterial Support Media

Organism (see key)	1% NDBS		3% NDBS		5% NDBS		10% NDBS		1% OL		3% OL		5% OL		10% OL		Soap trol	Con- NDBS	Soap trol	Con- OL	Water Control	
	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48
A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	4	4	-	-	-	-	-	-	-	-	>100	>100
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>100	>100
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	>100	>100
D	-	-	-	-	-	-	-	-	-	-	14	15	4	5	13	16	-	-	-	-	>100	>100
E	-	1	-	-	-	-	-	-	67	>100	1	1	-	-	-	-	-	-	-	-	>100	>100
F	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	9	25	-	-	-	-	-	-	-	-	>100	>100
G	>100	>100	78	78	90	93	63	70	>100	>100	17	25	-	-	-	-	-	-	-	-	>100	>100

The column headings 24 and 48 refer to incubation times of the test suspensions.

with the lack of buffering associated with sterile water systems, could account for the apparently biocidal effects of Olive Leaf solutions as compared to the NDBS. To test for such a pH effect, a 2 per cent by weight solution of Olive Leaf in distilled water was neutralized by the addition of 0.1N HCl to a final pH of 7.0. The results of Test Series C on such a solution are shown in Table VI.

A comparison of the 1 and 3 per cent solution data in Table V with the data in Table VI suggests that, in strong contrast to the results with Neutrogena, there appears to be little pH effect on the biocidal activity of Olive Leaf.

2.7 Other Tests of Microbiological Support with Candidate Cleansing Agents

2.7.1 Possible Contamination of As-Received Neutrogena

Thin slices were taken from five samples of as-received Neutrogena bars to determine if any microbiological contamination was present. These samples were imbedded in various media and incubated for 48 hours. No evidence of any as-received contamination was found.

2.7.2 Exposure of Neutralized Aqueous Soap Solution to Laboratory Air

Several samples of each of the four cleansing agents (as 2 per cent aqueous solutions neutralized to an approximate 7.0 pH) were exposed to laboratory air for one month. At the end of this period one sample of Neutrogena solution exhibited a fairly large spherical growth (about 1 cm in diameter) which appeared to be some type of dark-colored mold. The growth was floating on the surface of the liquid.

In order to further investigate the phenomenon described above, five samples of 2 per cent Neutrogena in distilled water, with pH reduced

TABLE VI

2% Olive Leaf Solution in Water at Adjusted pH of 7.0

Organism	24	48	24	48	24	48	24	48	24	48	24	48
A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
B	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-
D	-	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-
F	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
G	-	-	1	1	-	-	2	2	-	-	-	-

to 7.8 by the addition of HCl solution, were exposed for five days to ambient air at about 75° in our analytical chemistry laboratory. After this exposure, the samples were sealed and allowed to stand at ambient temperature for three weeks. At the end of three weeks one of the samples contained a large roughly spherical growth of a mold on the bottom of the plastic bottle. The growth was about 1.5 cm. in diameter and was black in color. The rapid growth rate of the mold and the size attained appear to indicate that the Neutrogena was being utilized to support its growth, since few other potential nutrients were available.

The mold described above appeared similar in growth habit and color to the specimen described previously. However, the new sample grew at the bottom of the container and was more dense than the solution whereas the previous sample was less dense than the solution and grew at the water-air interface.

The mold required several weeks to grow to a significant size in various culture media (apparently, it grew more quickly in the soap solution than in the various media used). Although preliminary indications were that it was not an Aspergillus, further investigations indicated that, to approximately 90 per cent confidence, the growth was some specific type of Aspergillus, but was not an A. Niger.

We must conclude from this result that there are specific microorganisms which can utilize Neutrogena in aqueous solution as a growth medium. The mold described above is the only species for which this has been demonstrated.

3.0 EVALUATION OF POTENTIAL HAZARDS FROM THE USE OF MIRANOL JEM AND NEUTROGENA (PHASE II)

Work on this phase was subdivided into the following subtask headings:

(1) Interpretation of the results from Phase I and the assignment of any potential health hazards to crew members;

(2) Investigation and evaluation of possible hazards to reverse osmosis (RO) membranes with inputs from Phase III; and

(3) Definition and evaluation of other possible hazards to the washwater recovery system.

3.1 Potential Health Hazards to Crew Members

The results of Phase I indicate that there should be no adverse dermatological effects following the use of either or both of these personal hygiene agents. No significant changes in the normal skin microflora were noted. In addition, in spite of the rather restricted use of water and the infrequency of whole body showers, all of the test subjects indicated a surprising feeling of being clean. Clearly, the potential health hazards to crew members associated with the simulated Skylab personal hygiene regimen appear to be minimal.

3.2 Hazards to RO Membranes

Due to the alteration of the Work Statement which substituted a preliminary evaluation of acute dermatological effects and laboratory testing for microbiological compatibility of a laundry detergent and another personal hygiene agent, the RO membrane compatibility work was discontinued.

3.3 Possible Hazards to Washwater System

Neutrogena is a complex mixture exhibiting significant buffering capability over a rather wide pH range (9 to 5.5) with several breaks throughout this range — suggesting the differing isoelectric points of the several components. Below a pH of approximately 5.0, the solubility of at least one component is sharply reduced, and a gel is produced which gradually coagulates into a surface scum. At very low pH (3.0 or below) the precipitate rapidly becomes flocculated and is easily filtered. Subsequent to filtration, the resulting solution exhibits little or no buffering capability and no soap action. If the initial solution of Neutrogena is adjusted to high pH (about 11.0), a semi-rigid gel is formed which can be dispensed by subsequent reduction of the pH. These observations would suggest that Neutrogena could cause serious problems in an RO washwater recovery system especially under conditions where the pH is adjusted to match the (low) pH range that is optimum for RO membrane performance.

The behavior described above seems to be inherent to the formulation of Neutrogena. Composition information from the basic Neutrogena patent* is presented below, but significant deviations from this formulation cannot be ruled out.

Component	Percentage by Weight
Sodium soap (saponified tallow, coconut oil and castor oil)	35-40
Triethanol ammonium salt of Stearic Acid	35-40
Triethanolamine, glycerine, perfume	unspecified

In contrast to Neutrogena, Miranol JEM, Sodium Dodecyl benzene sulfonate and Olive Leaf show much simpler behavior under wide pH ranges. All

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*"Soaps and Their Method of Preparation", U.S. Patent No. 2,820,768, 21 Jan. 1

these materials exhibit considerable buffering action at or near the normal pH values of their aqueous solutions. None of these cleansing agents showed any significant tendencies toward gel formation, at least in concentrations of up to 2% by weight in water, over the pH range 2.5 to 11.

In summary, a tentative study of physical chemical properties of aqueous solutions for the four candidate cleansing agents indicates that Neutrogena alone of the four is subject to precipitation and gel formation over the pH range of 2.5 to 11.0 which might be encountered in a washwater reclamation system. These results suggest that pH control might be important in a system used to reclaim washwater containing Neutrogena. They also suggest that further effort in defining the physical chemical properties of aqueous solutions of candidate cleansing agents should be performed in order to identify more precisely the nature and severity of the potential problems which could arise in washwater recovery.

4.0 CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Analysis of the results obtained from the program have led to the following conclusions:

(1) The simulated Skylab personal hygiene regimen used in the program, including the use of Neutrogena bar soap for sponge bathing and hand washing and Miranol JEM for showering, does not appear to cause any significant changes in skin microflora.

(2) None of the four cleansing agents tested (Neutrogena, Miranol JEM, Olive Leaf, and Sodium dodecyl benzene sulfonate), in the form of two per cent aqueous solutions, were found to cause any adverse dermatological effects after direct contact with the skin for up to two weeks. Neutrogena and Miranol JEM were also found to have no adverse dermatological effects on human subjects during up to four weeks of use in the simulated Skylab personal hygiene regimen (the other two cleansing agents were not tested in this manner).

(3) None of the four cleansing agents were found to serve as general support media for microbiological growth. However, one specific type of a mold (presumably an Aspergillus) was found to utilize a neutralized two per cent aqueous solution of Neutrogena.

(4) None of the four cleansing agents tested were found to exhibit definitive biocidal activity in concentrations appropriate to washwater (0-2 per cent in water).

(5) Except for Neutrogena, the cleansing agents tested exhibited stable aqueous solution properties over the pH range of 2.5 to 11.0. The formation of stable gels and/or precipitates in aqueous solutions of Neutrogena, near the extremes of the pH range covered, suggest that potential system performance limitations could arise in the reclamation of washwater containing Neutrogena.

4.2 Recommendations

The following recommendations are submitted on the basis of the program results presented in this report:

(1) Testing of Olive Leaf and sodium dodecyl benzene sulfonate for possible adverse dermatological and other effects in a use regimen should be performed in order to bring the state of knowledge about these two cleansing agents to the same level as for Neutrogena and Miranol JEM.

(2) Other candidate cleansing agents for use on long-duration space missions should also be tested similarly so that valid comparison with those already tested can be made.

(3) The physical chemical properties of candidate cleansing agents in aqueous solution and in representative washwater solutions should be investigated in order to more clearly define potential problem areas in washwater reclamation.

(4) In the design of a reclamation system for washwater containing Neutrogena, care must be exercised to prevent gellation or precipitate formation due to local or general pH excursions.

APPENDIX I

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TABLE VIIA. SUBJECT A - MALE

	Baseline			Shower Regimen			Basel	
	1	2	3	4	5	6	7	8
Site - Left Ear Canal								
Cram (-) cocci	150	150	60	150	34	30	125	150
Gram (-) rods	150	150	40	-	150	-	100	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Right Arm Pit								
Gram (-) cocci	73	20	2	10	900	5	140	1
Gram (-) rods	55	20	-	4	-	120	900	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Back of Left Hand								
Gram (-) cocci	-	-	-	-	11	-	-	1
Gram (-) rods	27	-	-	-	-	100	-	900
Yeast/molds	-	-	-	-	-	-	-	-
Site - Crotch Right Side								
Gram (-) cocci	125	2	35	125	5	-	120	15
Gram (-) rods	4	-	3	35	900	300	120	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Rectum Left Side								
Gram (-) cocci	15	9	-	20	6	-	25	-
Gram (-) rods	125	-	6	2	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Bottom Right Foot								
Gram (-) cocci	-	-	-	10	900	2	900	900
Gram (-) rods	-	9	-	9	9	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-

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TABLE VIIB. SUBJECT B - MALE

	Baseline			Shower Regimen			Baseline	
	1	2	3	4	5	6	7	8
Site - Left Ear Canal								
Gram (-) cocci	1	90	90	90	190	120	110	165
Gram (-) rods	-	-	80	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Right Arm Pit								
Gram (-) cocci	-	-	12	90	20	-	-	-
Gram (-) rods	4	98	90	90	75	13	6	1300
Yeast/molds	-	-	-	-	-	-	-	-
Gram (+) cocci	-	-	-	-	-	4	-	-
Site - Back of Left Hand								
Gram (-) cocci	-	-	2	-	900	-	-	-
Gram (-) rods	1	-	-	1	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Crotch Right Side								
Gram (-) cocci	-	-	16	50	5	-	2	3
Gram (-) rods	-	20	3	900	1	13	-	-
Yeast/molds	-	-	-	-	-	-	-	-
Site - Rectum Left Side								
Gram (-) cocci	2	90	5	105	18	40	-	-
Gram (-) rods	-	90	90	10	18	40	-	-
Yeast/molds	-	-	-	-	-	5	-	-
Site - Bottom Right Foot								
Gram (-) cocci	120	125	23	110	110	90	34	110
Gram (-) rods	-	-	-	-	-	-	-	-
Yeast/molds	-		-	-	-	-	-	-

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TABLE VIIC. SUBJECT C - MALE

	Baseline			Shower Regimen			Baseline		
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	110	110	-	110	120	30	15	-	-
Gram (-) rods	120	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	-	-	-	2	-	-	-	-
Gram (-) rods	115	1	-	-	-	-	1	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	-	-	-	9	-	-	-	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	110	8	-	-	-	-	-	-	-
Gram (-) rods	950	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	900	-	-	-	900	-	-	-	-
Gram (-) rods	125	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	200	-	-	3	-	-	-	-	100
Gram (-) rods	120	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VIID. SUBJECT D - MALE

	Baseline			Shower Regimen			Baseline		
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	110	110	3	14	5	30	-	2	-
Gram (-) rods	-	-	14	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	-	-	35	-	25	-	-	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	-	-	-	1	-	-	-	-
Gram (-) rods	1	-	-	-	-	-	-	-	-
Yeast/molds	-	-	1	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	120	100	5	90	16	2	2	25	-
Gram (-) rods	-	-	-	-	-	2	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	70	110	11	-	1	-	1	1	-
Gram (-) rods	80	-	-	1	-	25	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	43	110	14	92	92	1	-	5	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	15	-

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TABLE VIIE. SUBJECT E - MALE

	Baseline			Shower Regimen			Baseline		
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	38	9	-	25	16	92	22	16	6
Gram (-) rods	-	-	-	-	-	1	-	-	-
Yeast/molds	-	-	1	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	2	-	-	-	4	-	-	-
Gram (-) rods	110	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	9	-	-	1	-	-	-	-
Gram (-) rods	-	-	-	1	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	-	25	80	45	900	1	2	28	-
Gram (-) rods	-	-	-	-	900	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	-	7	-	12	10	5	4	-	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	-	-	-	-	-	-	7	-	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VIIF. SUBJECT F - FEMALE

	Baseline			Shower Regimen			Baseline		
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	-	16	-	40	-	-	850	-	-
Gram (-) rods	-	-	-	-	-	2	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	45	-	-	5	3	85	4	110	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	15	1	-	-	-	-	-	-	-
Gram (-) rods	-	2	-	90	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	-	6	-	1	-	-	8	1	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	-	900	-	-	1	3	9	-	-
Gram (-) Rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	-	14	2	-	900	-	-	15	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	1	-	-	-	-	-	-

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TABLE VIIG. SUBJECT G - MALE

	Baseline			Shower Regimen			Baseline		
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	-	-	6	950	-	-	-	-	-
Gram (-) rods	-	-	1	-	-	-	-	-	800
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	-	-	-	-	-	-	-	-
Gram (-) rods	4	90	3	40	3	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	-	-	-	-	-	-	-	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	-	4	-	-	90	-	-	-	-
Gram (-) rods	19	5	-	160	30	-	-	-	105
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	-	95	-	2	12	-	-	-	-
Gram (-) rods	-	875	-	875	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	255	125	-	130	130	-	-	-	840
Gram (-) rods	250	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VIIH. SUBJECT H.- MALE

	Baseline			Shower Regimen			Baseline		
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	6	-	-	7	3	-	-	-	-
Gram (-) rods	-	-	-	-	-	-	125	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	-	-	950	19	-	-	-	-
Gram (-) rods	18	3	-	-	-	-	900	6	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	1	-	-	1	-	-	-	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	30	50	-	58	10	-	4	-	-
Gram (-) rods	120	1	-	900	900	-	850		
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	125	120	-	15	950	-	-	-	-
Gram (-) rods	15	2	-	-	-	-	900	2	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	45	1	-	5	890	-	920	950	-
Gram (-) Rods	3	-	-	-	900	-	-	1	-
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VII. I SUBJECT I - MALE

	Baseline	Shower Regimen					Baseline		
	1	2	3	4	5	6	7	8	9	1
Site - Left Ear Canal										
Gram (-) cocci		120	175	160	120	20	46	125	125	12
Gram (-) rods		-	-	-	-	-	34	-	-	-
Yeast/molds		-	-	-	-	-	-	-	-	-
Site - Right Arm Pit										
Gram (-) cocci		25	125	8	135	25	26	120	125	-
Gram (-) rods		-	-	-	-	-	13	-	-	-
Yeast/molds		-	-	-	-	-	-	-	-	-
Site - Back of Left Hand										
Gram (-) cocci		3	-	-	-	-	3	-	-	-
Gram (-) rods		-	-	-	-	-	-	-	-	-
Yeast/molds		-	-	-	-	-	-	-	-	-
Site - Crotch Right Side										
Gram (-) cocci		23	18	5	45	1	6	-	125	
Gram (-) rods		-	-	15	-	-	2	-	-	-
Yeast/molds		-	-	-	-	-	-	-	-	-
Site - Rectum Left Side										
Gram (-) cocci		150	165	120	120	250	210	120	210	
Gram (-) rods		-	-	-	115	125	-	-	-	
Yeast/molds		-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot										
Gram (-) cocci		15	27	-	128	130	8	950	210	11
Gram (-) rods		21	135	130	-	145	41	1000	150	12
Yeast/molds		-	-	-	-	-	-	-	-	-

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TABLE VII. J. SUBJECT J. - FEMALE

	Baseline		Shower Regimen				Baseli	
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	5	15	3	-	1	-	-	-	
Gram (-) rods	-	-	3	-	-	-	5	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Right Arm Pit									
Gram (-) cocci	15	2	5	-	-	-	-	-	
Gram (-) rods	-	-	20	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Back of Left Hand									
Gram (-) cocci	5	6	5	-	-	14	3	-	
Gram (-) rods	-	-	1	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Crotch Right Side									
Gram (-) cocci	30	125	7	1	-	13	-	-	
Gram (-) rods	-	-	1	-	-	4	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Rectum Left Side									
Gram (-) cocci	30	125	3	46	41	109	18	45	
Gram (-) rods	-	-	20	-	-	53	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Bottom Right Foot									
Gram (-) cocci	11	30	8	215	15	25	5	9	
Gram (-) rods	-	4	33	230	9	108	3	-	
Yeast/molds	-	-	-	-	-	-	-	-	

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TABLE VIIK. SUBJECT K - FEMALE

	Baseline	Shower Regimen						Baseline	
	1	2	3	4	5	6	7	8	9	1
Site - Left Ear Canal										
Gram (-) cocci	5	92	12	45	2	11	68	6	1
Gram (-) rods	-	-	-	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Right Arm Pit										
Gram (-) cocci	3	4	5	10	12	4	-	5	
Gram (-) rods	5	6	23	2	125	16	130	1	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Back of Left Hand										
Gram (-) cocci	1	3	-	-	35	-	-	1	
Gram (-) rods	-	-	-	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Crotch Right Side										
Gram (-) cocci	15	15	125	1	6	54	9	-	
Gram (-) rods	-	-	-	-	1	4	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Rectum Left Side										
Gram (-) cocci	125	125	120	120	78	110	88	190	
Gram (-) rods	-	-	-	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Bottom Right Foot										
Gram (-) cocci	125	160	8	230	215	111	215	230	5
Gram (-) rods	17	-	109	115	195	115	108	125	13
Yeast/molds	-	-	-	-	-	-	-	-	

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TABLE VII.L SUBJECT L - FEMALE

	Baseline	Shower Regimen						Baseline		
	1	2	3	4	5	6	7	8	9	1
Site - Left Ear Canal										
Gram (-) cocci	60	30	5	33	25	4	1	145	55	
Gram (-) rods	-	1	-	-	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	-	
Site - Right Arm Pit										
Gram (-) cocci	8	-	-	-	-	-	-	-	-	
Gram (-) rods	-	-	-	-	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	-	
Site - Back of Left Hand										
Gram (-) cocci	125	-	-	-	-	-	-	-	-	
Gram (-) rods	-	4	-	-	-	-	-	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	-	
Site - Crotch Right Side										
Gram (-) cocci	130	20	112	50	24	19	7	31	60	
Gram (-) rods	-	30	-	23	-	17	145	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	-	
Site - Rectum Left Side										
Gram (-) cocci	115	12	40	4	15	125	4	130	-	
Gram (-) rods	-	-	-	-	-	-	7	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	-	
Site - Bottom Right Foot										
Gram (-) cocci	-	21	240	110	100	55	12	1	250	3
Gram (-) rods	1100	1100	60	21	-	-	130	1	110	
Yeast/molds	-	-	-	-	-	-	-	-	-	

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TABLE VII.M. SUBJECT M - MALE

	Baseline	Shower Regimen							Base
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	20	20	18	6	-	-	-	-	1
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	17	-	-	9	1	6	6	-
Gram (-) rods	110	20	26	45	3	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	7	8	6	-	-	2	-	-	11
Gram (-) rods	-	-	-	-	-	-	-	-	9
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	135	120	130	15	9	2	36	135	36
Gram (-) rods	-	-	-	2	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	125	130	125	135	120	4	3	125	100
Gram (-) rods	-	-	-	-	-	-	4	16	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	-	16	100	20	125	130	125	120	120
Gram (-) rods	110	68	42	110	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VII. O. SUBJECT O. - FEMALE

	Baseline	Shower Regimen						Baseline	
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	135	140	135	70	40	23	22	100	125
Gram (-) rods	9	-	-	65	-	-	125	30	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	7	9	4	-	-	-	10	125	8
Gram (-) rods	7	-	-	6	-	-	1	5	110
Yeast/molds	1	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	4	5	110	4	1	30	-	5	28
Gram (-) rods	-	-	-	3	1	-	3	16	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	114	60	130	110	125	4	3	19	17
Gram (-) rods	200	25	-	40	109	45	120	10	33
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	110	108	65	8	15	15	33	110	125
Gram (-) rods	20	-	-	11	-	-	45	21	90
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	150	90	60	130	110	120	25	250	240
Gram (-) rods	30	60	210	55	108	250	130	125	230
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VII.P SUBJECT P - FEMALE

	Baseline	Shower Regimen						Baseli	
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	35	-	8	-	-	-	-	19	-
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	-	5	-	-	-	-	17	2
Gram (-) rods	-	-	-	125	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	1	4	-	-	-	-	11	50
Gram (-) rods	-	-	-	-	-	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	125	15	35	45	-	-	-	15	30
Gram (-) rods	110	-	-	10	-	-	-	1	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	115	25	21	25	-	-	-	-	2
Gram (-) rods	100	-	-	-	-	-	-	7	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	-	110	115	-	-	-	-	175	250
Gram (-) rods	125	135	120	250	-	-	-	-	115
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VII.Q. SUBJECT Q - MALE

	Baseline	Shower Regimen							Baseli
	1	2	3	4	5	6	7	8	
Site - Left Ear Canal									
Gram (-) cocci	15	120	-	-	70	12	170	150	11
Gram (-) rods	120	-	200	210	-	125	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Right Arm Pit									
Gram (-) cocci	45	50	60	40	1	24	125	10	2
Gram (-) rods	-	25	20	15	1	-	-	-	1
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Back of Left Hand									
Gram (-) cocci	-	11	-	-	-	30	-	5	
Gram (-) rods	-	-	6	7	-	-	-	-	1
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Crotch Right Side									
Gram (-) cocci	125	60	150	150	-	2	5	15	
Gram (-) rods	-	-	-	-	1	-	4	1	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Rectum Left Side									
Gram (-) cocci	30	30	25	-	10	7	125	4	6
Gram (-) rods	120	125	130	140	2	27	21	13	7
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Bottom Right Foot									
Gram (-) cocci	55	62	60	8	18	40	16	76	12
Gram (-) rods	30	35	45	10	-	-	-	-	13
Yeast/molds	-	-	-	-	-	-	-	-	

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TABLE VII.R. SUBJECT R. - MALE

	Baseline		Shower Regimen						Baseline
	1	2	3	4	5	6	7	8	9
Site - Left Ear Canal									
Gram (-) cocci	130	145	135	150	100	135	240	125	130
Gram (-) rods	-	-	-	-	10	-	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Right Arm Pit									
Gram (-) cocci	-	-	-	-	-	-	7	6	20
Gram (-) rods	125	130	-	-	-	1	1	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Back of Left Hand									
Gram (-) cocci	-	25	-	-	6	2	125	1	160
Gram (-) rods	-	-	-	-	-	-	2	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Crotch Right Side									
Gram (-) cocci	115	125	110	-	7	250	130	18	120
Gram (-) rods	120	-	8	-	-	125	-	-	-
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Rectum Left Side									
Gram (-) cocci	115	95	125	120	250	240	119	120	240
Gram (-) rods	8	6	30	150	110	125	10	110	50
Yeast/molds	-	-	-	-	-	-	-	-	-
Site - Bottom Right Foot									
Gram (-) cocci	2	85	240	-	100	29	110	210	135
Gram (-) rods	125	40	115	125	110	8	125	22	60
Yeast/molds	-	-	-	-	-	-	-	-	-

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TABLE VII.S. SUBJECT S - MALE

	Baseline	Shower Regimen						Baselin
	1	2	3	4	5	6	7	8	
Site - Left Ear Canal									
Gram (-) cocci	15	65	18	15	-	-	-	4	
Gram (-) rods	25	-	110	106	3	-	-	115	10
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Right Arm Pit									
Gram (-) cocci	-	-	-	-	-	1	-	-	
Gram (-) rods	30	-	-	-	-	4	-	-	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Back of Left Hand									
Gram (-) cocci	8	15	3	-	34	215	-	15	1
Gram (-) rods	11	-	2	-	50	108	-	1	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Crotch Right Side									
Gram (-) cocci	15	125	130	130	240	210	-	115	
Gram (-) rods	6	5	-	-	125	130	-	108	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Rectum Left Side									
Gram (-) cocci	110	240	118	135	220	230	-	225	
Gram (-) rods	100	5	-	-	120	108	-	100	
Yeast/molds	-	-	-	-	-	-	-	-	
Site - Bottom Right Foot									
Gram (-) cocci	15	220	108	-	240	230	-	225	13
Gram (-) rods	125	8	125	135	110	128	-	110	
Yeast/molds	-	-	-	-	-	-	-	-	

TABLE VIII constitutes a reiteration of some of the data recorded in Tables VII except that more detailed information as to the specific organisms found is reported for selected subjects and sites. The striking feature of these data lies in the rather wide variations in numbers of specific organisms found, coupled with the surprisingly small variety of organisms found.

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TABLE VIII

DETAILED DATA ANALYSIS OF SELECTED SUBJECTS

SUBJECT SITE	ORGANISM	BASELINE 1	2	3	REGIMEN 4	5	6	BASELINE 7	8
A - Ear	Staph (-)	150	150	60	150	34	30	125	150
	Dipth (-)	150	150	40	-	-	-	100	-
	E.Coli	-	-	-	-	-	-	-	-
	Enterobacter (-)	-	-	-	-	150	-	-	-
A - Crotch	Staph (-)	25	2	35	125	5	-	120	15
	Dipth (-)	-	-	3	-	-	155	-	-
	Bacillus (-)	4	-	-	-	-	-	-	-
	Proteus	-	-	-	35	-	145	120	-
	Strep (α)	100	-	-	-	-	-	-	-
	Enterobacter (-)	-	-	-	-	900	-	-	-
B - Rectum	Staph (-)	2	90	5	15	30	2	19	12
	Strep (-)	-	-	-	90	-	-	-	-
	Dipth (-)	-	-	-	-	-	-	-	-
	Enterobacter (-)	-	90	-	-	-	-	-	-
	E. Coli	-	-	90	10	-	-	-	-
	Bacillus	-	-	-	-	18	-	-	-
	Yeast	-	-	-	-	-	5	-	-
	Proteus	-	-	-	-	-	40	-	-
D - Foot	Staph (-)	43	110	14	92	92	1	-	5
	Yeast	-	-	-	-	-	-	-	15
E - Ear	Staph (-)	38	9	-	25	16	92	22	16
	Proteus	-	-	-	-	-	1	-	-
	Yeast	-	-	1	-	-	-	-	-
H - Foot	Staph (-)	45	1	-	5	890	-	920	950
	Bacillus	3	-	-	-	900	-	-	1
G - Foot	Staph (-)	255	125	-	130	130	-	-	-
	Neisseria	130	-	-	-	-	-	-	-
	Enterobacter (-)	120	-	-	-	-	-	-	-

TABLE VIII

DETAILED DATA ANALYSIS OF SELECTED SUBJECTS

SUBJECT SITE	ORGANISM	BASE 1	REGIMEN 2	3	4	5	6	7	BASELI 8	
I - Rectum	Staph (-)		130	29	120	120	145	100	120	
	Strep (-)		20	136	-	-	155	110	-	
	E.Coli		-	-	-	115	-	-	-	
	Dipth (-)		-	-	-	-	125	-	-	
	Citrobacter		-	-	-	-	-	-	-	
J - Rectum	Staph (-)		30	125	3	46	41	109	15	
	Dipth (-)		-	-	20	-	-	-	-	
	Citrobacter		-	-	-	-	-	53	-	
	Enterococcus		-	-	-	-	-	-	3	
K - Foot	Staph (-)		125	144	8	120	105	111	105	
	Strep (-)		-	16	-	110	110	-	110	11
	Dipth (-)		17	-	-	115	120	-	108	12
	E.Coli		-	-	109	-	75	115	-	
	Klebsiella		-	-	-	-	-	-	-	
M - Arm Pit	Staph (-)	-	17	-	-	9	1	6	6	
	Dipth (-)	-	-	-	-	-	-	-	-	
	Proteus	110	20	26	45	3	-	-	-	
O - Crotch	Staph (-)	114	25	130	110	125	4	3	19	
	Strep (-)	-	35	-	-	-	-	-	-	
	Dipth (-)	-	25	-	-	109	45	120	10	
	Enterobacter	200	-	-	-	-	-	-	-	
	E.Coli	-	-	-	40	-	-	-	-	

TABLE IX presents the numerical data which has been summarized with plots of 1 + log N vs. time. From these plots, which are typical of all the data, it is clear that there is no significant variation in the total microfloral populations introduced by the simulated Skylab personal regimen.

TABLE IX

SKIN MICROORGANISM TOTALS - SELECTED DATA

SUBJECT	SITE	SAMPLE	TOTAL NO.ORGANISMS N	1 + LOG N	PLOT
A	Crotch	Base 1	129	3.11	
		2	2	1.30	
		3	38	2.58	
		Reg. 4	160	3.20	
		5	905	3.96	Figure 1
		6	300	3.48	
		Base 7	240	3.38	
		8	15	2.18	
		9	-	1.00	
A	Ear	Base 1	300	3.48	
		2	300	3.48	
		3	100	3.00	
		Reg. 4	150	3.18	
		5	184	3.26	Figure 1
		6	30	2.48	
		Base 7	225	3.35	
		8	150	3.18	
		9	150	3.18	
B	Rectum	Base 1	2	1.30	
		2	180	3.26	
		3	95	2.98	
		Reg. 4	115	3.06	
		5	48	2.68	Figure 2
		6	37	2.67	
		Base 7	19	2.28	
		8	12	2.08	

TABLE IX

SKIN MICROORGANISM TOTALS - SELECTED DATA

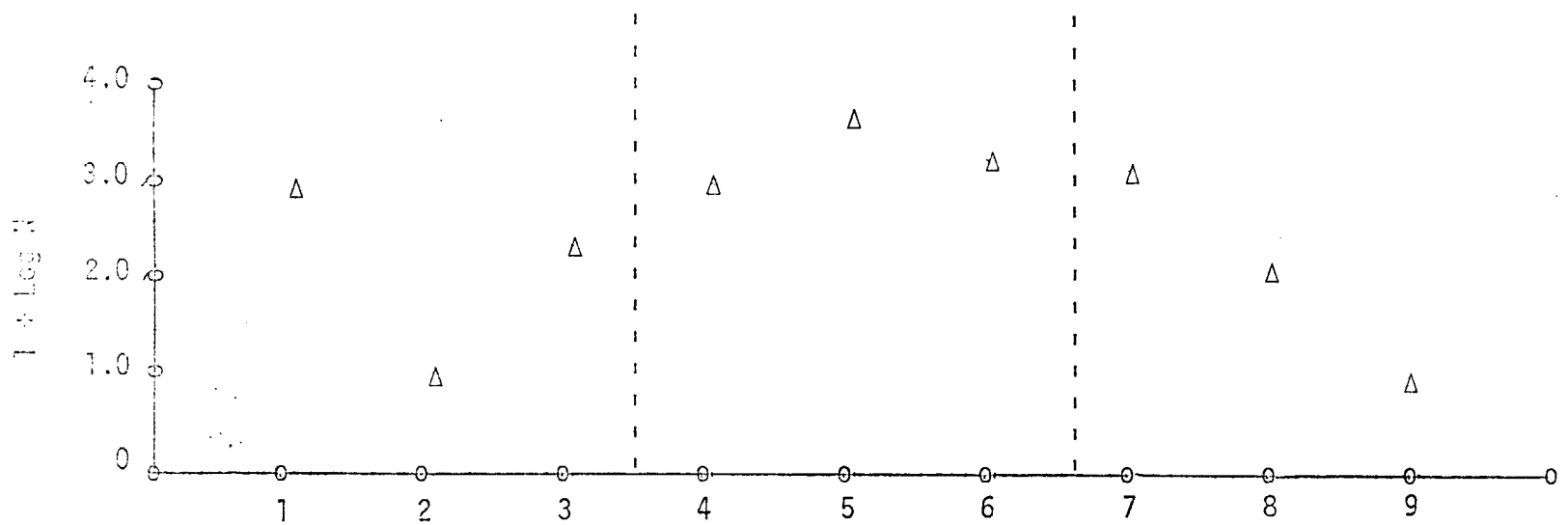
SUBJECT	SITE	SAMPLE	TOTAL NO. ORGANISMS N	1 + LOG N	PLOT
D	Foot	Base 1	43	2.63	
		2	110	3.04	
		3	14	2.15	
		Reg. 4	92	2.96	
		5	92	2.96	Figure 2
		6	1	1.00	
		Base 7	-	1.00	
		8	20	2.30	
		9	1	1.00	
E	Ear	Base 1	38	2.58	
		2	9	1.95	
		3	-	1.00	
		Reg. 4	25	2.40	
		5	16	2.20	Figure 3
		6	93	2.97	
		Base 7	22	2.34	
		8	16	2.20	
		9	6	1.78	
H	Foot	Base 1	45	2.65	
		2	1	1.00	
		3	-	-	
		Reg. 4	5	1.70	Figure 3
		5	1970	4.25	
		6	-	-	
		Base 7	920	3.96	
		8	951	3.98	
		9	940	3.97	

TABLE IX

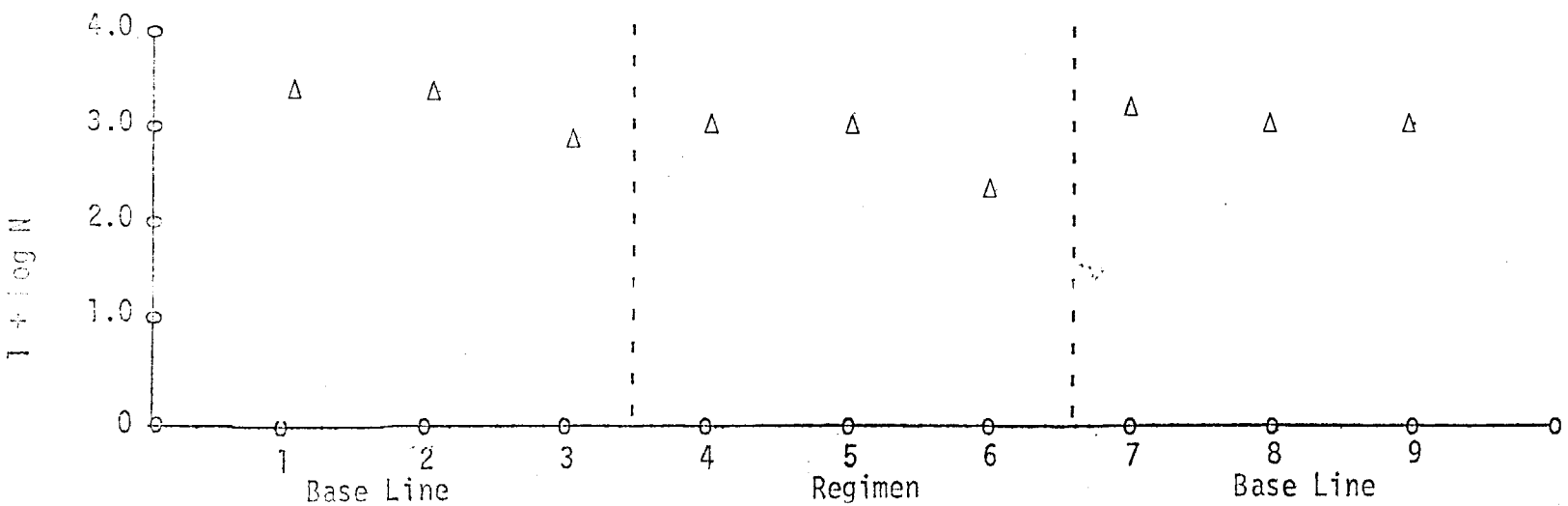
SKIN MICROORGANISM TOTALS - SELECTED DATA

SUBJECT	SITE	SAMPLE	TOTAL NO. ORGANISMS N	1 + LOG N	PLOT
I	Rectum	Reg. 2	36	2.56	
		3	162	3.21	
		4	130	3.11	
		5	128	3.10	
		6	175	3.24	Figure 4
		7	49	2.69	
		Base 8	1950	4.29	
		9	360	3.56	
		10	235	3.37	
J	Rectum	Base 1	30	2.48	
		Reg. 2	125	3.10	
		3	23	2.36	
		4	46	2.66	
		5	41	2.61	Figure 4
		6	162	3.21	
		7	18	2.25	
		Base 8	45	2.65	
		9	235	3.37	
J	Foot	Reg. 2	132	3.12	
		3	160	3.20	
		4	107	3.03	
		5	345	3.54	
		6	410	3.61	Figure 5
		7	225	3.35	
		Base 8	323	3.51	
		9	355	3.55	
		10	180	3.26	

SUBJECT A - CROTCH



SUBJECT B - EAR



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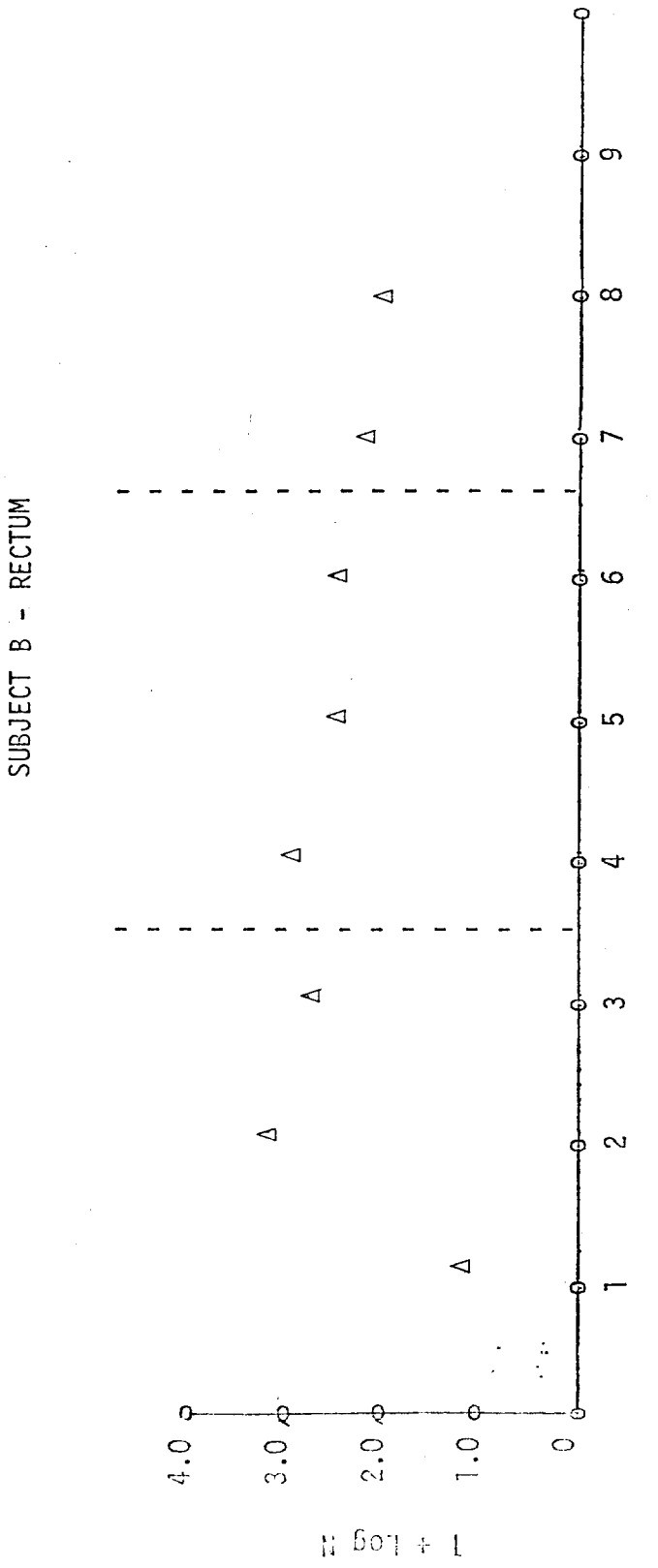
SUBJECT B - RECTUM
1 + log N
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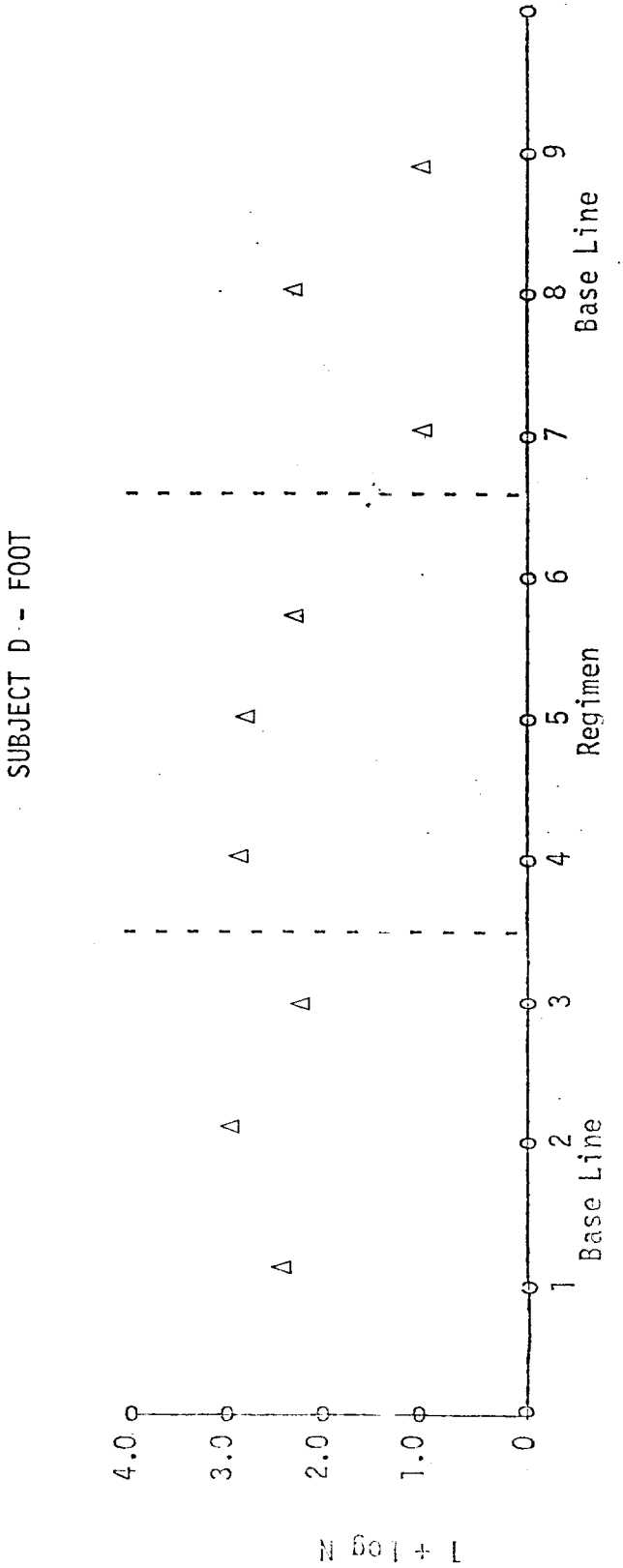
3.0
2.0
1.0
0
1
2
3
4
5
6
7
8
9


SUBJECT D - FOOT
1 + log N
4.0
3.0
2.0
1.0
0
1
Base Line
2
3
4
5
Regimen
6
7
8
Base Line
9

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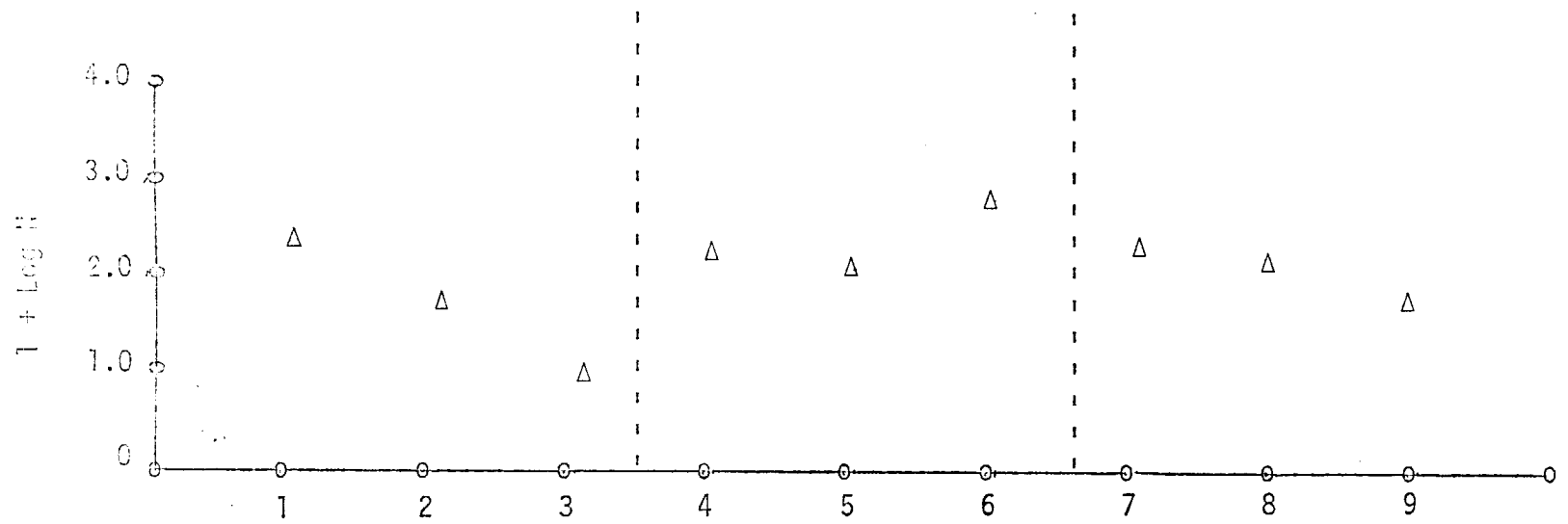
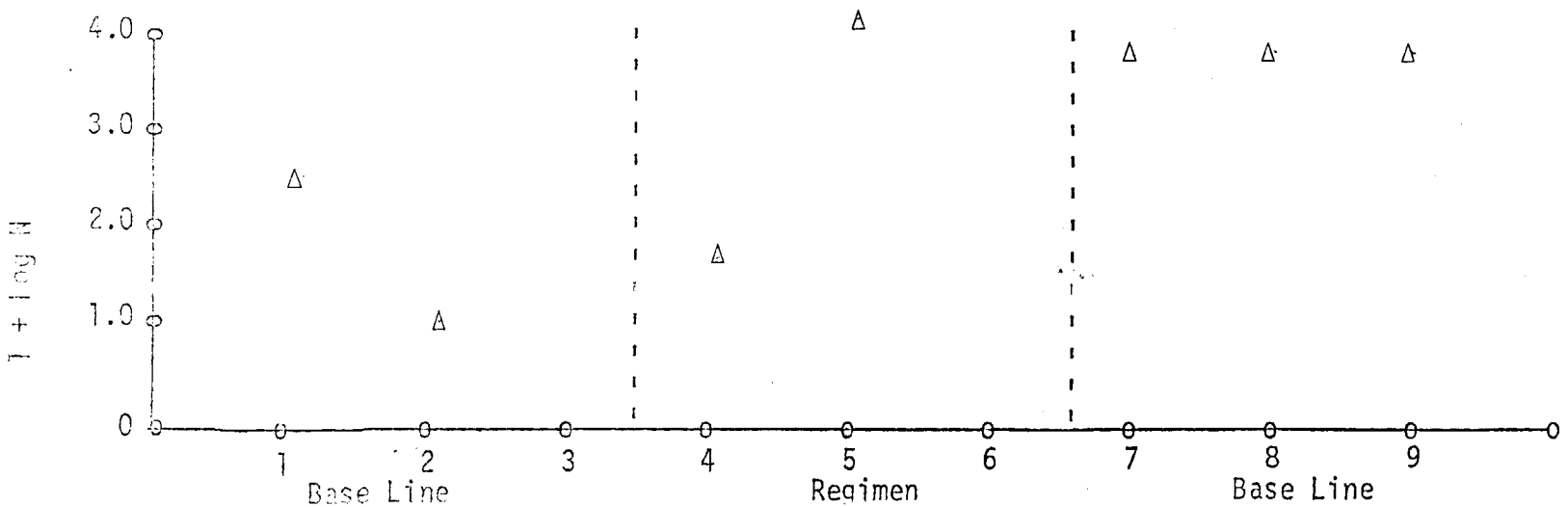
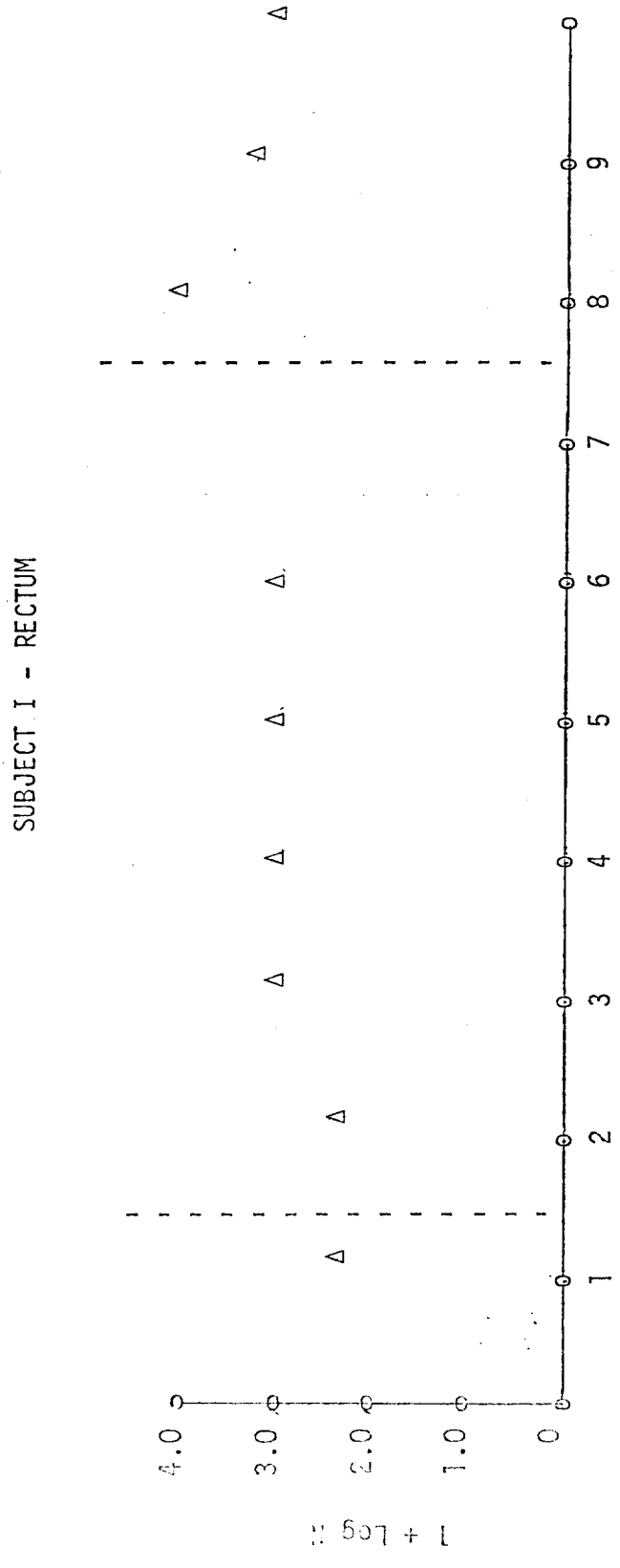
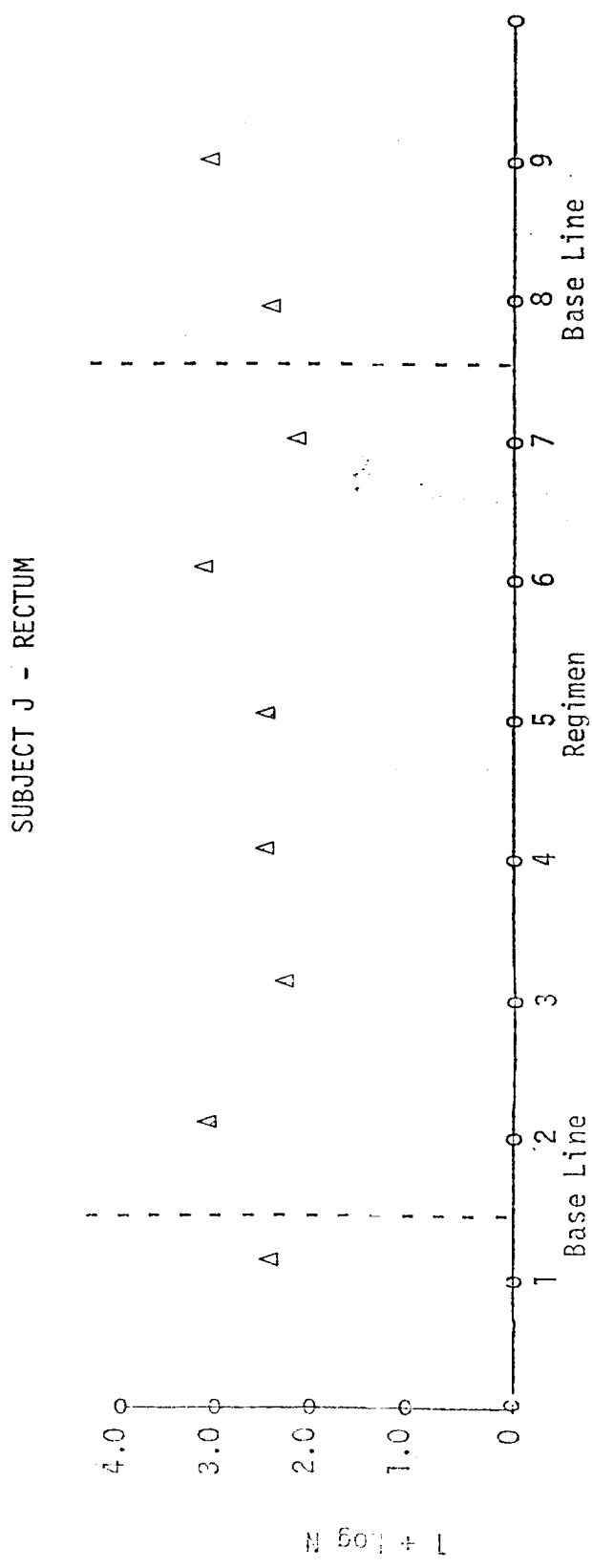
SUBJECT E - EAR



SUBJECT H - FOOT




SUBJECT I - RECTUM
1 + Log N
4.0
3.0
2.0
1.0
0
1
2
3
4
5
6
7
8
9


SUBJECT J - RECTUM
1 + Log N
4.0
3.0
2.0
1.0
0
1
Base Line
2
3
4
5
Regimen
6
7
8
Base Line
9

VERFAR INC.

SUBJECT K - FOOT