AMRL-TR-66-59

PRELIMINARY STUDY ON THE MICROBIAL FLORA OF NORMAL HUMAN SUBJECTS IN A RESTRICTED ENVIRONMENT

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DECEMBER 1965

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600 - October 1966 7-169

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FOREWORD

This is a technical report of a preliminary study conducted from 18 February to 15 July 1964 at the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio under Air Force Contract AF 33(657)-11716 with Dr. R. E. Zipf, Miami Valley Hospital Department of Research, Dayton, Ohio, as principal investigator, and K. J. Smith, First Lieutenant, USAF, Biospecialties Branch, as contract monitor. This investigation was conducted in conjunction with the National Aeronautics and Space Administration Defense Purchase Request R-85, "Research on the Nutritional Requirements of Man Under Simulated Space Flight Stress." This study was initiated in support of Project 7164, "Biomedical Criteria for Aerospace Flight," Task 716405, "Aerospace Nutrition."

The authors acknowledge with thanks the technical assistance of Mr. Arselus West, Biospecialties Branch, Aerospace Medical Research Laboratories.

This technical report has been reviewed and is approved.

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ABSTRACT

Microorganisms were recovered from selected skin and mucosal surfaces of men in groups of four living in a restricted environment. Of several sampling methods studied, the use of cotton swabs proved to be the technique most appropriate for the scope of this study. Differences between the microbial flora of the test subjects and major alterations in microbial populations were observed. These alterations could not be definitely attributed to the direct influence of the MA-10 full pressure suit. However, three of the pressure suited subjects exhibited foot lesions, initiated by a bacterium apparently indigenous to those individuals, during the period when the suits were worn. These data indicated that the carrier state may be of greater significance to the carrier than to other individuals in the restricted environment. Although transference of certain microbes was noted, the exchange of microorganisms generally considered to be pathogenic was not observed.

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SECTION I

INTRODUCTION

This report is concerned with a microbiological investigation of human subjects participating in a series of nutrition experiments in which the precise caloric, protein, and water requirements are being determined. To accomplish the goals of the nutrition study, experimental protocols were established, consisting of confinement within a controlled activity facility, wearing the MA-10 full pressure suit, repeated consumption of a restricted dietary regimen, and performance of assigned physiological tasks. The experimental design is shown in tables I and II. The purpose of the microbiological investigation reported herein was to obtain preliminary data on the microbial flora of man's skin and mucosal surfaces and to establish guidelines for determinating personal hygiene criteria for aerospace missions. These studies were initiated during the third and fourth experimental periods of the nutrition study. Experiment III was conducted during the period 18 February to 31 March 1964 and Experiment IV during the period 4 May to 15 June 1964.

Although studies have revealed that man naturally harbors a wide variety of indigenous microorganisms in varying quantities, depending on his physiologic condition, his environment, and other factors, virtually nothing is known regarding the effect of those constraints noted above on his microbial flora (ref 5, 7). The general experimental approach of this investigation was to consider the microorganisms usually thought to be pathogenic, and also to determine those microbes that might serve as marker microorganisms or indicators of microbial build-up or transfer between individuals isolated in a simulated space environment. Such microbial markers could be used as monitors of deleterious changes that might forecast a pathogenic event. In addition, the data obtained from this type of investigation should also lend pertinent information pertaining to the importance of microbiological carrier states of future astronauts. Even if a microbial build-up or transfer does not occur among astronauts in flight, such a carrier state could be dangerous to the individual harboring potentially pathogenic microorganisms, if his susceptibility to these microbes is altered due to the stresses of space flight. If the events described above occur, then microbiological standards for selecting, monitoring, and protecting future astronauts will have to be developed.

SECTION II

METHODS

BODY SAMPLING PROCEDURES

Since quantitation of the microbial population from various body areas is inherently difficult and was not feasible due to lack of established methods, attempts were made to develop a more acceptable and standardized method of sampling the microbial flora of the skin. Four methods were evaluated:

TEMPLATE METHOD

Templates with a central hole about 2.5 cm in diameter and slightly smaller than a standard petri dish were prepared from aluminum foil. These templates (previously sterilized by autoclaving in petri dishes) were placed aseptically against the axilla and groin regions. The body areas exposed through the holes in the templates were thoroughly swabbed with cotton-tipped applicator sticks and were then streaked on Bacto-Staphylococcus No. 110 medium.

AGAR IMPRESSION METHODS

Two different methods were used. In the first method Millipore plastic petri dishes, approximately 5 cm in diameter, were filled nearly to capacity with sterile Staphylococcus No. 110 medium. These agar plates were then placed aseptically against axillary and groin areas, after first applying the sterile aluminum templates described above. This allowed a quantitative comparison of the impression plate and template techniques because the same size area was sampled.

Sterile plastic screw caps (the same diameter as the hole in the aluminum template) filled with Staphylococcus No. 110 medium were also placed aseptically against the axillary and groin regions without prior application of the aluminum template. In both agar impression techniques the inoculated plates were incubated 24 hours at 37°C.

REPLICA SAMPLING METHOD

A small glass rod approximately 8 to 10 cm long was inserted in the narrow end of each of six No. 6 rubber stoppers to act as a handle. A 10 cm^2 velveteen cloth was attached, nap up, to each of the six rubber stoppers with a rubber band. These were then wrapped in aluminum foil and sterilized in an autoclave. A volunteer subject was sampled as follows on 6 consecutive days after daily bathing in the evening. Each of the previously sterilized velveteen samplers was placed aseptically against the axillary area of the subject and twisted 3 times, a predetermined amount in opposite directions. The velveteen sampler was then gently placed on media selected for culturing the microorganisms. For each sample taken, six replicas were made, three on each of two plates with the desired media to be tested. A sample from both right and left axillary areas was placed on Staphylococcus No. 110 medium, blood agar (Bacto-Tryptose Blood Agar Base plus 5% citrated human blood) and Trypticase Soy Broth (BBL) with 1.5% agar.

REPLICA PLATING FOR ISOLATION FROM A PRIMARY OR INITIAL PLATE

Runnels and Wilson (ref 6), adapted the replica plating technique of Lederberg and Lederberg (ref 3), to facilitate a range of tests on all staphylococcal colonies on an initial plate. The present investigation was an attempt to test the feasibility of such an approach to select the largest percentage of staphylococcal colonies from primary plates with a mixed variety of microorganisms.

Velveteen replicators were prepared as described previously, with the exception that the No. 6 rubber stoppers were replaced with No. 13 stoppers.

The sterile velveteen replicator was first placed on the initial plates to be replicated and then gently touched against the desired media. Five different initial plates were replicated in triplicate on Staphylococcus No. 110 medium, except that replica No. 1 of initial plates 1 and 3 were made on Bacto-Coagulase-Mannitol medium. Plates 1, 2, 4, and 5 contained throat cultures, whereas plate 3 contained a nose culture. The former cultures were isolated on blood agar and the latter on Staphylococcus No. 110 medium. Plates 1, 3, and 5 were 3 days old when replicated, whereas plates 2 and 4 were 5 days old (Table XIII).

BODY AREA SAMPLING AND MEDIA EMPLOYED

Microbial samples were taken from the eye, ear, nose, throat, axilla, umbilicus, groin, and foot by means of sterile cotton swabs and were cultured on a variety of media. The exact sizes of the areas sampled were not measured, and the cultures were taken only from the right side of the body. The eye was sampled by having the subject pull the lower lid down, and the inner mucosal surface was then swabbed. The other areas were sampled by thorough swabbing; all swabs were streaked on appropriate media. Staphylococcus No. 110 medium was used for the eye, ear, nose, axilla, umbilicus, groin, and foot; blood agar for the throat; Bacto-Sabouraud Dextrose medium and Bacto-Littman Oxgall medium for the foot. Sabouraud Dextrose and Littman Oxgall media were also tested once in conjunction with routine samples taken from the ear, throat, axilla, and groin. No attempt was made to classify the fungi that were isolated. The general protocol of these experiments consisted of screening for selected microorganisms, such as staphylococci, streptococci, neisseriae, and diphtheroid-like Gram-positive rods. In addition to the routine screening for the above microorganisms, any other predominating or unusual forms that appeared were noted. Details of the experimental design are shown in Tables I and II.

ATMOSPHERIC SAMPLING

In Experiment III, atmospheric samples from the controlled activity facility were taken once in order to determine if potentially pathogenic microorganisms were present. These samples were obtained by exposing blood agar plates as follows: (1) central location in the controlled activity facility for 1 hour, (2) pressure suit air inlet for 5 minutes, (3) pressure suit exhaust for 5 minutes, and (4) controlled activity facility ventilator for 5 minutes. In addition, the laboratory air was sampled as in (1) above, and the inside and outside of the filter core for the pressure suit air supply was sampled using a cotton swab and streaking on blood agar and Littman Oxgall medium.

SECTION III

RESULTS AND DISCUSSION

BODY SAMPLING PROCEDURES

Reference was made earlier to the difficulty of detecting quantitative changes in the indigenous flora of man because of the lack of a standardized method of measuring such changes. An example of this difficulty is illustrated by reference to tables II through IX. For instance, a rating of 4, which refers to maximal recovery or 1 which indicates minimal recovery, applied to a particular organism is only valid when this relative number is compared to other microorganisms on the same plate. A rating of 4 for staphylococci on one plate is of questionable value when compared with a 4 rating for staphylococci on another, even when the sample is taken from the same body region and subject. Obviously, then, the validity of comparing ratings from different body areas and between subjects is even less reliable. However, the method of recovery used in this study of sampling with cotton swabs followed by streaking on proper media, and Gram-staining of such cultures was meaningful or interpretable by the worker evaluating it. The reason for this is that observations made from such stained smears, when combined with observations of the types of colonies appearing on the original plates, permitted a comparison of the microorganisms that were being studied. Such a rating method, is considered the best that could be applied to data obtained by the swabbing technique used and within the scope of this study. Therefore, the data in tables III through X are comparable only when consideration is given to the proportions of particular microbial types isolated from individual samples. Since the method employed is not in reality quantitative, comparisons of various sampling periods or body areas must be made only with respect to changes in the proportions of isolated microbes.

Consideration should also be given to those microorganisms that are inhabitants of the hair follicles, sebacious glands, and deep epithelial surfaces. The literature contains many approaches to these problems and the variability in the data reflects differences in the sampling procedures. Evans *et al.* (ref 1) attempted to determine the microflora residing in deeper portions of human skin surfaces by grinding skin scrapings with alundum. Pachtman, Vicher, and Brunner (ref 4) cultured scrapings obtained by pressing 23-mm glass tubing against the nasolabial skin. They also studied the use of 2% Na_2CO_3 to liberate subsurface organisms. Grubb and Puetzer (ref 2) and Watson *et al.* (ref 8) used a washing technique to obtain nasal cultures.

Thus the approaches studied in this investigation did not recover those microorganisms that require more rigorous manipulations for their isolation. These techniques, such as skin scraping and punch biopsy, could not be applied because of the resultant destruction of skin integrity. Therefore, only those methods that entailed minimal alteration of the test areas were employed. Since several possible sampling methods were examined, primarily to select the one most applicable for this study, it is unnecessary to present detailed results but rather a general evaluation of each approach is given below.

TEMPLATE METHOD

This method of sampling was tested to see if more quantitative data could be obtained by swabbing a defined area, but the results obtained indicated that this technique was no more quantitative than ordinary methods. The main difficulty lies with the swab itself. Results have shown that regardless of the method of swabbing, the quantitative recovery of all or even a consistent part of the resident microorganisms is doubtful, if not impossible to accomplish by this technique. In addition, it is inconvenient and difficult to use the tem-

plate aseptically. The method required two technicians to properly insure against contamination from the environment.

AGAR IMPRESSION METHODS

The plastic petri dish method, or "impression plate" or "pressure plate" technique of sampling revealed large numbers of well-defined, countable colonies, presumably a direct and relatively quantitative impression or copy of the microbial population from the body area sampled. In theory, data obtained in this manner are more valid because a known area of the skin is sampled. There is a more complete recovery of the microbes present because the media are directly inoculated and there is no loss via the cotton swabs. However, there are many inherent difficulties in the method. Because the petri dishes cannot be filled completely, the surface just next to the edge of the plastic dish does not contact the skin. To compensate for this, a template was used to insure that a precise area of the skin was sampled. The difficulties in using the template in the preceding procedure were also incurred.

The plastic screw-cap method seemed to be the most efficient procedure as compared to the template or plastic petri dish method. It also revealed large numbers of well-defined, countable colonies, presumably a direct and relatively quantitative impression or copy of the microbial flora able to grow on the medium provided in the screw cap. The method is good in that it is direct. No cotton swab or template is involved to introduce the difficulties mentioned above and the technique can be performed easily and aseptically by one technician. Obviously, similar containers of various sizes and materials could be used. The major problem of this or any similar method of sampling is that direct impression on the skin will result in a deposition of media on the body area sampled. In a short period of time, a build-up of the medium would have a gross effect on both the qualitative and quantitative interpretation of the microbial flora from the body regions sampled. The method could be successfully employed if one were sampling to determine the relative number of microorganisms present before daily bathing. However the experimental protocol required that the subjects not bathe for 16-day periods, thus precluding use of this technique.

REPLICA SAMPLING METHOD

Since the basic idea of the agar impression methods was successful, the best approach toward a relative quantitation of the microbial flora seemed to be a method similar to these, but with the difference that no culture medium would be deposited on the skin. Such an approach was developed in this investigation by modifying a technique developed by Lederberg and Lederberg (4) to study microbial mutants.

The basic idea behind the technique used in this study centers on the velveteen sampler. When the velveteen is placed against the skin surface it comes in contact with hundreds of clones or microcolonies. Thus, the fabric is imprinted with a certain portion of each clone. The imprinted fabric now can act as a pattern or replica of the clones contacted on the skin surface sampled. Each thread in the pile fabric acts as an inoculating needle for transfer to the surface of agar in a plate. Replica-inoculating can be made from one imprinted velveteen sample onto plates containing different media if so desired.

Six replicas were made from each sample taken, and originally it was hoped that there would be a dilution in the numbers of colonies appearing with each replica. If this occurred, it was thought that after standardization of the technique, a particular dilution could be chosen with a countable number of colonies. After each sampling of the same subject and body area, the proper dilution would be chosen, microorganisms counted, and then compared with previous results.

Data obtained indicated that such a technique could be used successfully, if standardiza-

tion on subjects in a controlled environment were accomplished. Standardization may be fulfilled by using one of two techniques: (1) finding which dilution or replica is countable for a given body region that is being sampled; or (2) merely assigning a rating for a particular replicate from a given area, such as 1, 2, 3. On future sampling of the same area, the rating on the dilution previously chosen could be compared with the rating given for the same dilution at the second sampling. However, this method did not prove applicable for this study because (1) attempts to formulate standardized techniques were not successful, and (2) the number of replicates required would be too cumbersome for the extent of the effort.

REPLICA PLATING FOR ISOLATION FROM A PRIMARY OR INITIAL PLATE

This investigation was an attempt to test the feasibility of using a replica plating technique to select out the largest percentage of staphylococcal colonies from primary or initially isolated cultures. Two important benefits of such a method would be to recover a larger percentage of staphylococci for further analysis, and to prevent overlooking any potentially pathogenic strains of *Staphylococcus aureus*. It was found that the success of the replication directly depends on the age of the primary or initial culture. When plastic disposable petri dishes are used, the media dry out rapidly. Therefore, the maximum age of a plate to be replicated is 3 days and care should be taken to have the plates to be used for primary isolation as fresh as possible. When the initial plates are in proper condition, three or more replicas can successfully be made from one primary plate. This emphasized the feasibility of using different media.

Although data were not extensive enough to draw final conclusions, it does not seem to be of value to replicate directly from a primary plate to Coagulase Mannitol medium when the primary plate contains a mixed culture, such as from the throat. Often other microorganisms besides staphyloccoci cause a positive mannitol reaction, and 15 per cent undiluted plasma must be added to show coagulase production. The plasma enhances the growth of many additional microbes, and makes interpretation of the coagulase and mannitol reactions most difficult. However, when the replication is made from a medium such as Staphylococcus No. 110, which allows staphylococci to predominate, the recovery is very successful and the reactions on the medium already mentioned are easy to interpret.

BODY AREA SAMPLING

The general protocol of this investigation consisted of screening for selected microorganisms, such as staphylococci, streptococci, neisseriae, and diphtheroid-like Gram-positive rods. Because of their frequency of appearance, these microorganisms were selected as markers to determine if a general build-up of the normal flora was occurring in a particular body region. Consideration of the anaerobic bacteria and many aerobic and facultative microorganisms was omitted, not because of a lesser importance, but in most cases because these areas of investigation were beyond the scope of the present study.

The relative occurrence or recovery of the selected microorganisms is presented in tables III through X. Data shown in tables III, IV, V, and VI allow certain comparisons of these selected microorganisms recovered from specific body areas of subjects 9, 10, 11 and 12 respectively (Experiment III). Data found in tables VII, VIII, IX, and X allow such a comparison of the microorganisms recovered from subjects 13, 14, 15 and 16 respectively (Experiment IV). The relative recovery of the microorganisms indicated was based on an arbitrary rating system of 1, 2, 3 and 4, as described previously, the latter representing the maximum recovery of the microorganism in question. Considerable care must be taken in evaluating these data because of the many inadequacies of both the isolation procedures and this method of estimation.

In this investigation the term "staphylococci" refers to all microorganisms that appear-

ed as irregularly clustered, Gram-positive cocci upon microscopic observation. In keeping within the scope of the study, it was not possible to perform further analysis to distinguish the staphylococci from the micrococci.

The term "diphtheroid-like, Gram-positive rods" also requires definition. Some workers apply the nebulous term of "diphtheroid" to almost any aerobic Gram-positive, tapered-rod, without any biochemical, serological, or other data to confirm such a classification. However, in this study the term diphtheroid or diphtheroid-like is used knowingly as a relative term only to describe morphologically, a Gram-positive, aerobic, somewhat pleomorphic rod. No detailed identification was attempted. Although tables III through X indicate the presence of diphtheroids in most of the body areas tested, this does not imply that they were of the same microbial species. In keeping with the scope of this study, only microscopic observation could be performed which strongly suggested the presence of definite morphologic types in a particular body region. For example, Gram-positive rods isolated from the ear of a particular individual were especially characteristic as compared with Gram-positive rods from other body regions or even from the ear of another individual. In fact, in both Experiment III and Experiment IV, certain individuals exhibited characteristically different Gram-positive rods in the same body region. Smears of ear cultures grown on Staphylococcus No. 110 medium revealed that certain subjects would never exhibit such diphtheroid-like rods, whereas others would always have one or two specific morphologic types. These morphologic types most often seemed to be pure cultures with respect to contamination by staphylococci. In the ear of other individuals, however, specific diphtheroid-like rods always appeared with staphylococci. Such data, although based on a relatively small number of test subjects, seems to justify strongly further investigation. Photomicrographs of representative microorganisms isolated from the ear are shown in Figure 1. Development of a scheme of identification of these Gram-positive rods could provide an important tool or marker of microbial build-up or transfer during future space flights.

Microscopic examination of smears prepared from cultures taken from the groin and feet of certain subjects and grown on Staphylococcus No. 110 medium, revealed, a variety of fungal forms in addition to staphylococci and diphtheroid-like Gram-positive rods. Photomicrographs of representative microorganisms from the feet are shown in figure 2. These cultures regularly contained very characteristic types. The unusual feature was that these fungal forms were so small that in order to observe them carefully a magnification of 970X was needed. These microorganisms did not appear to be actino mycetes. Since they appeared in smears of groin and foot cultures containing the diphtheroids previously described, further investigation will be needed to determine if these forms are of the same species. The appearance of these fungal forms necessitated the use of Sabouraud Dextrose medium and Littman Oxgall medium to establish the presence of fungi in such body regions as the ear, throat, axilla, groin, and foot. After incubation for several days, various fungi and yeast-like forms developed which did not appear on media used for routine sampling, especially from samples taken from the throat. This revealed the importance of incorporating fungal media for future sampling of all the body regions studied.

Another significant observation made concerning the characteristics of cultures from the groin and feet was the appearance of a brown color in the Staphylococcus No. 110 medium used for cultivation. Whether this color was due to a true pigment secreted into the medium or merely a change in color induced by some alteration of the medium such as pH, has not been determined. Coloring of Staphylococcus No. 110 medium occurred consistently in cultures from the feet of subjects 13 and 16, and only once in cultures from the groin, umbilicus, and nose of the latter. A definite but transient transfer of the characteristic was apparent from cultures taken on 5 consecutive days of sampling from the groin of subject 15. The significance of the appearance of this brown color may be quite important, if

the property can be attributed to a specific strain or strains of microorganisms. This marker could then be used as a rapid, simple, and readily visible indicator of microbial transfer between astronauts or between them and their space environment. Investigation of this phenomenon has indicated that the color appeared only in certain cultures of the diphtheroid-like Gram-positive rods already discussed At the present time, no correlation has been made between the brown color and any specific morphologic type of diphtheroid-like rod observed.

In addition to the selected microorganisms that were recovered during Experiment III and Experiment IV, an unusual pleomorphic Gram-negative, strongly hemolytic rod was detected in throat cultures of several subjects and in the controlled activity facility. This rod often contained typical spores located subterminally and frequently exhibited filaments of various lengths. The filaments often contained large swollen portions and frequently many large, free, Gram-negative spheres were present. Besides these unusual features, there was a possible connection between this microorganism and an illness experienced by two of the test subjects and the bacteriologist performing the sampling. Although in this instance, the vomiting and/or nausea suffered by the three individuals could not be directly attributed to the Gram-negative rod, personal communication with Republic Aviation Corporation, Farmingdale, New York, revealed that a similar microorganism had been detected during their studies on human subjects. They reported that the unusual forms were isolated from an ear infection of one individual and were later found in the throats of their subjects This information, plus data obtained in this experiment seem to implicate this organism as a potentially pathogenic entity. At this time, insufficient information has been obtained for classification. Photomicrographs of this Gram-negative rod and the unusual forms associated with it are shown in figure 3.

Data obtained during Experiments III and IV indicated that certain individuals in the group of test subjects normally carried beta-hemolytic coagulase positive staphylococci of the type generally considered to be pathogenic, in addition to other staphylococci here regarded as part of their normal flora. One significant alteration of the staphylococcal population occurred during Experiment III when the level of coagulase-positive staphylococci increased steadily from a very small percentage to approximately 98 per cent in nose cultures taken from subjects 10 and 11. The surprising point was that no pathogenic effect in the nasal areas of these individuals was detected by the attending physician. Coagulase-positive staphylococci were also recovered in cultures from other body regions, but no particular build-up of these types could be detected. However, the importance of the presence of such potentially hazardous microorganisms was emphasized when subject 10 was removed after he had worn the full-pressure suit for 16 days. He exhibited a rather severe lesion in the top center of the metatarsal region of the right foot The minor infection apparently followed a blister caused by rubbing from the suit. Although the lesion seemed to be primarily of staphyloccoccal origin, an unidentified fungus was also recovered.

Another shift in bacterial population was noted in cultures taken from throats of subjects 13 and 16. This was a change from a previously high percentage of streptococci to a predominating percentage of staphylococci followed by a return to the high numbers of streptococci. The important aspect of this population change was that although a rather large portion of both the streptococci and staphylococci were of the beta-hemolytic variety, no pathological effect was detected.

During Experiment IV, the use of bactericidal soap, containing 3% hexachlorophene, on the feet of test subjects was attempted because of the lesion which occurred on the foot of Subject 10 during Experiment III. It was hoped that such an agent might reduce the chance of infection of the feet of those individuals who naturally harbored potentially

pathogenic microorganisms. The results were very significant, since subjects 13 and 15 revealed a marked difference in the condition of the left foot which was thoroughly scrubbed with the bactericidal agent, and the right foot, which was not scrubbed before entering the full-pressure suit. The left foot of both subjects had little or no signs of infection, whereas, the right foot of each was dirtier and had many pustules of apparent staphylococcal origin. There was a much less marked difference between the left and right feet of subjects 14 and 16 who were treated in the same manner. However, both subjects 13 and 15 had a much poorer skin condition at the onset of the experiment than did subjects 14 and 16. In addition, hemolytic coagulase-positive staphylcocci were repeatedly recovered from the various body regions of subjects 13 and 15; however, such staphylococci were only occasionally recovered from subjects 14 and 16. Thus, this portion of the experiment was successful in two ways: (1) it further emphasized the importance of the "carrier state" of certain individuals subjected to simulated space environment; and (2) it pointed out a procedure that might well be used as a personal hygiene preventative measure before subjecting individuals to the stresses of space travel. Although the microbial population changes in cultures from the nose and throat that were cited previously could not be attributed to a direct influence of the MA-10 full-pressure suit, other qualitative and quantitative effects seemed to have been influenced by it. As was discussed earlier, it is particularly hard to detect quantitative changes in the microbial flora, regardless of the cause, since a standardized method of measuring the endogenous microflora of man is lacking. However, by the techniques used in this study, there was a recognizable build-up of the selected microorganisms on the groin and feet.

The foot seems to be an especially critical portion of the body, particularly where astronauts will be subjected to prolonged periods in full-pressure suits of the type used in this experiment. In this study, the influence of the MA-10 full-pressure suit was more qualitative than quantitative. Because of the limited aeration of the feet of the suit, subjects reported they were literally standing in water, especially after periods of exercise. Continuous exposure of their feet to such an environment could well have been an important factor in the infections which were experienced by subjects 10, 13 and 15.

ATHOSPHERIC SAMPLING

The possible etiological relationship between the unusual Gram-negative microorganism that was detected, and the ill effects experienced by subjects 9 and 10 prompted this investigation. The organism was suspected as the causative agent since it was detected at the same time the illness occurred and also because it was markedly hemolytic on blood agar. Naturally, it was important to find out if the bacilli were part of the normal flora of the throat, or pathologic or saprophytic contaminants of the environment.

The data revealed that not only the Gram-negative rods, but also a rather startling number and variety of other fungi and hemolytic bacteria were present in the limited environments of the subjects being studied. Although a hemolytic capability does not necessarily mean that a microorganism is a pathogen, it is a characteristic which is generally associated with many pathogens. Since our work on the personal hygiene of the astronaut is concerned with the pathogenic potential of his normal flora and even contaminants of his "closed" environment, the general approach to the screening and evaluation of the microbial flora must differ considerably from the typical clinical procedures. In these situations, microorganisms of the less typical pathogenic variety are usually missed or disregarded.

The data obtained indicate that the subjects were being exposed directly to many possible pathogens through the pressure suit air supply and from other sources in their limited environment as shown in table XI. Because of the scope of this experiment, we could not identify all of the hemolytic colonies selected. Different colonial morphologic types were

picked to show their relationship to each other for later identification or recognition. However, the colonial types arbitrarily designated as Types C, H, K, M, O, and T (see table XII), although exhibiting many colonial similarities, differed markedly in certain cellular characteristics. Because of the presence of aberrant forms that were not typical of either bacteria or fungi, and the lack of further evidence, these organisms could not be designated bacteria, actinomycetes, or fungi.

On the basis of the above observations, it will be necessary in future tests to scrutinize thoroughly the microbial environment not only to make a valid interpretation of the microbial flora of the astronaut, but also to permit early detection of microorganisms potentially hazardous to his health. Data also indicated the possible importance of a re-evaluation of the approach to the study and detection of the pathogenic potential of the so-called saprophytes in a closed space environment. An example of this was the tremendous hemolytic activity of some of the fungi recovered, as compared to the relatively small hemolytic activity of such recognized pathogens as *Staphylococcus aureus*.

SECTION IV

SUMMARY AND RECOMMENDATIONS

Preliminary data obtained in this study have indicated many difficulties which should be considered before man can be safely subjected to the stresses of aerospace flight. Basic to all the problems is the need for a thorough knowledge of the entire human microbial population, including both the interactions between individual microbial components of the microflora and interactions between the individual microbial components of the microflora and interaction between the microflora and the host. The first step in accomplishing this is to select suitable sampling techniques to insure the complete recovery of the microbes characteristic of the body region being sampled. Those methods must be chosen which will not disturb greatly the integrity of the ecological environment and also permit repeated sampling over extended periods. This necessitates the use of more topical procedures rather than the use of skin scrapings or biopsy. However, this may sacrifice recovery of those microbes inhabiting the deeper tissues. In addition, it must be realized that those methods which reveal the quantitative aspects of the biota will not always be suitable for the recovery of microbes present in lesser numbers or those requiring special physiological conditions.

In future tests, efforts should be made to study as many different body areas as possible (1) to obtain reliable baseline data and (2) to determine if the microbial flora of any particular region or regions is especially sensitive to environmental changes. All body regions sampled should be subjected to as thorough a microbiological evaluation as possible before attempting to determine the effect of aerospace stresses on the population dynamics of the indigenous flora. Consideration must be given to both aerobic and anaerobic bacterial flora as well as pleuro-pneumonia-like organisms (PPLO), protozoa, fungi, and viruses. Although potentially pathogenic microorganisms are of significance, those microbes not considered pathogenic by the accepted clinical criteria may be important as marker or indicator strains that might forecast deleterious population changes.

Particular attention should be given to staphylococcal strains recovered from the specific body areas not only because of their ubiquitous numbers and pathogenic potential, but also because of the many biochemical tests and detailed bacteriophage typing methods available. These procedures should be valuable aids in determining the extent of microbial build-up or transfer between astronauts in a limited space environment.

Data obtained in this investigation have also pointed out the importance of close scrutiny and further study of the physiological characteristics of the Gram-positive rods recovered from the various body areas. Special attention should be given to those pleomorphic rods often labeled with the nebulous term of "diphtheroids." In addition, the actinomycetes and the lactobacilli should also be considered.

Data obtained stressed the importance of a complete microbiological monitoring of the controlled activity facility or space environment evaluator in which human subjects will be studied. Cultures of various strategic locations should be made at regular intervals and also of the air supply input and exhaust. Only when significant baseline information has been obtained will it be possible to determine validly the effect of such stresses as a limited environment, freeze-dehydrated or liquid diets, full-pressure suits, and altered temperature and atmospheric conditions. Each of these changes could have dynamic effects on a specific portion of the host biota.

The limited data obtained concerning the efficacy of hexachlorophene soap warrant that a study be initiated on various topical disinfectants to control the indigenous microflora. This investigation should include (1) possible noncompatibility of the selected anti-

microbial agent with the subject, (2) the degree of control required, and (3) the frequency and mode of application necessary to obtain the desired level of control. Consideration should also be given to the incorporation of stable disinfectants into undergarments as a substitute or additional method to maintain adequate personal hygiene requirements.

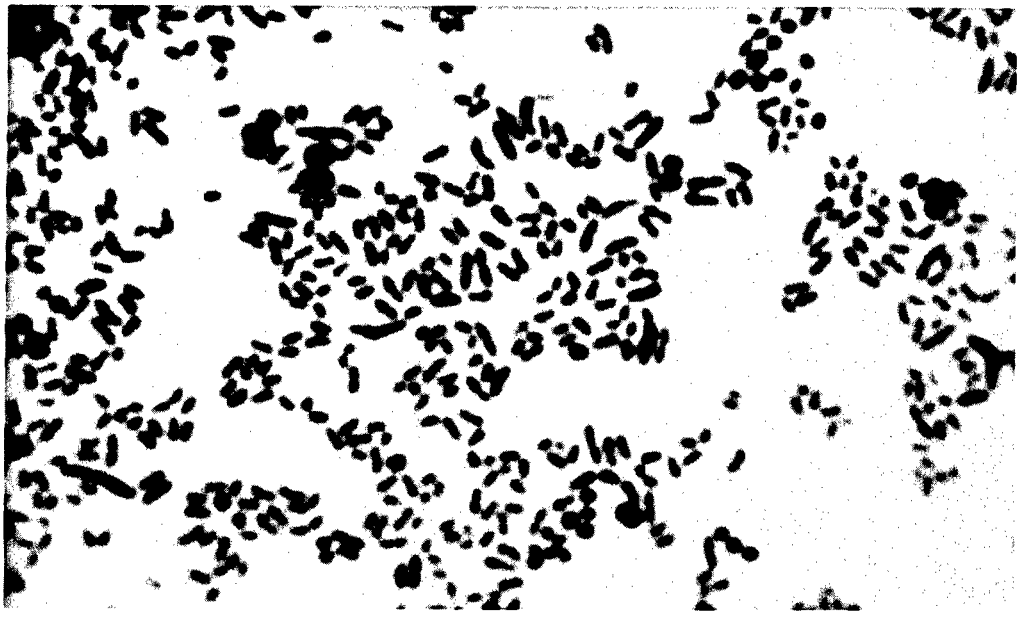
As was demonstrated in this study, proper fitting of the pressure suit is especially critical. Any alteration of the integrity of skin may upset the host microbial balance resulting in an infection at the site of the damaged tissue. This suggests that the microbiological carrier state of individuals could be of the utmost importance in selecting future space crews. Future investigation must determine not only the potential pathogenicity for the astronauts but also the interactions of his microflora with the biota of other members of the crew. If such a problem exists, it may be necessary to select crews and substitutes on the basis of their microbial compatabilities.

SECTION V

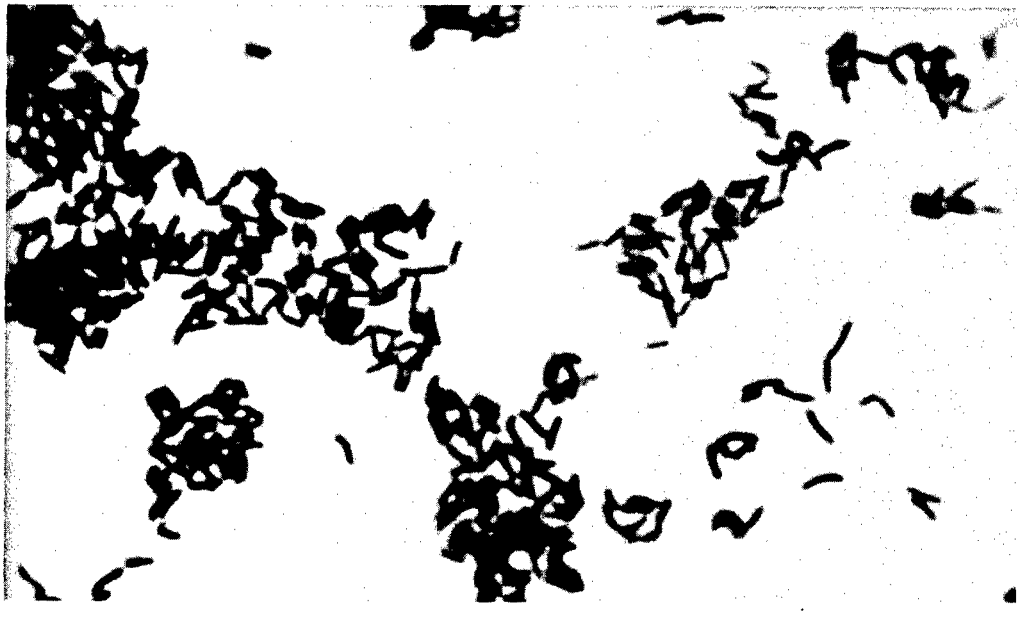
ILLUSTRATIONS

FIGURE 1

Representative Microorganisms Isolated from the Ear.



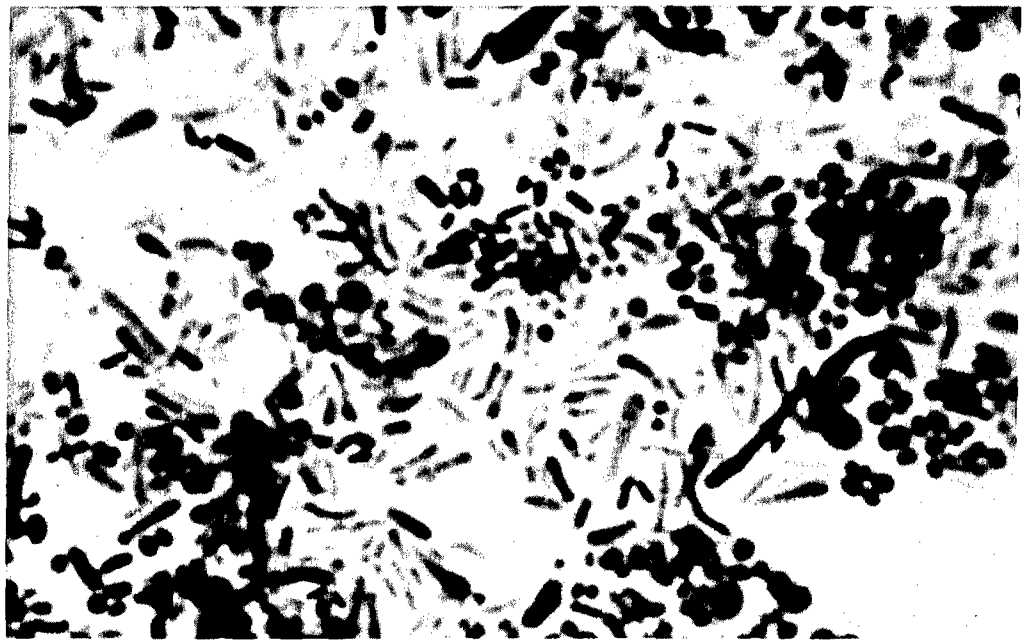
A. Gram stain of ear culture exhibiting diphtheroid-like Gram-positive rods and yeast-like coccal forms. X2000.



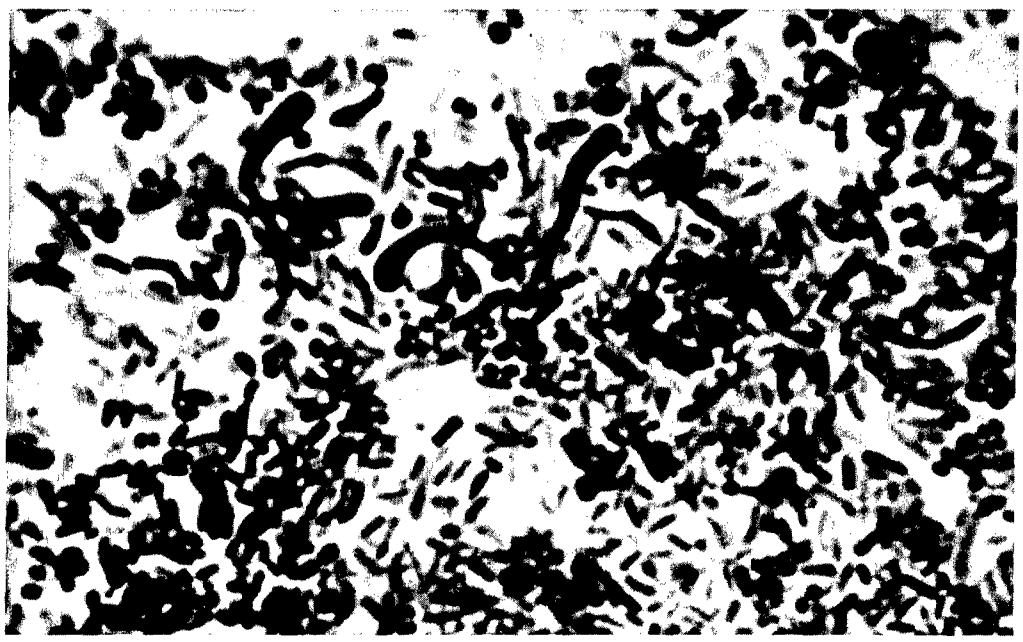
B. Gram stain of ear culture exhibiting one morphologic type of diphtheroid-like baccillus. X2000.

FIGURE 2

Representative Microorganisms Isolated from the Feet.



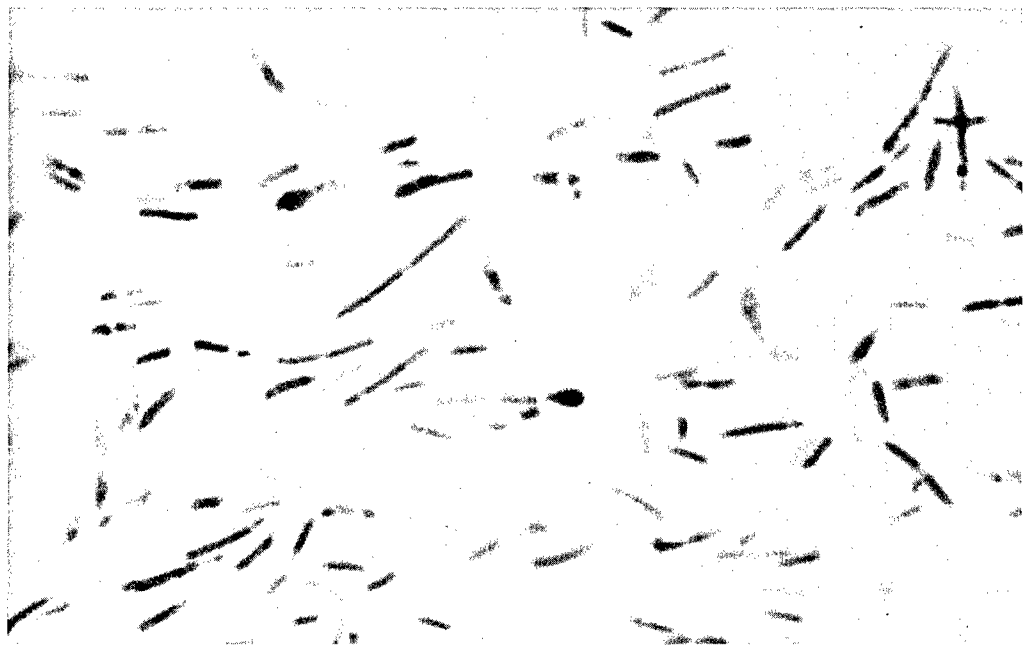
A. Gram stain of foot culture exhibiting coccal and yeast-like forms, diphtheroids, and various fungal forms. X2000.



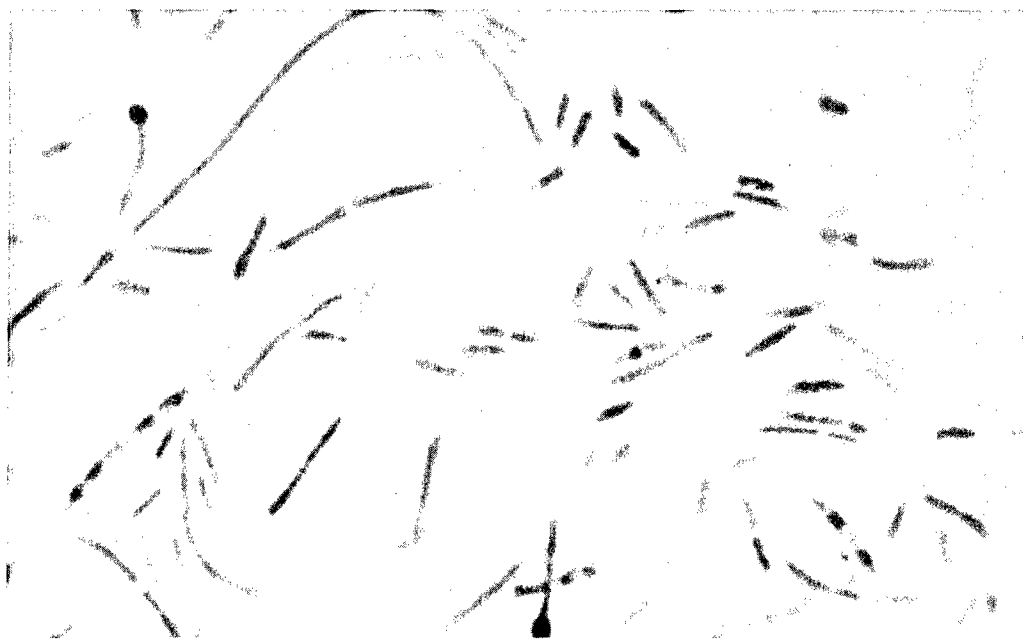
B. Gram stain of foot culture exhibiting coccal and yeast-like forms, diphtheroids, and various fungal forms. X2000.

FIGURE 3

Unusual Gram-Negative Rods Isolated from the Throat.



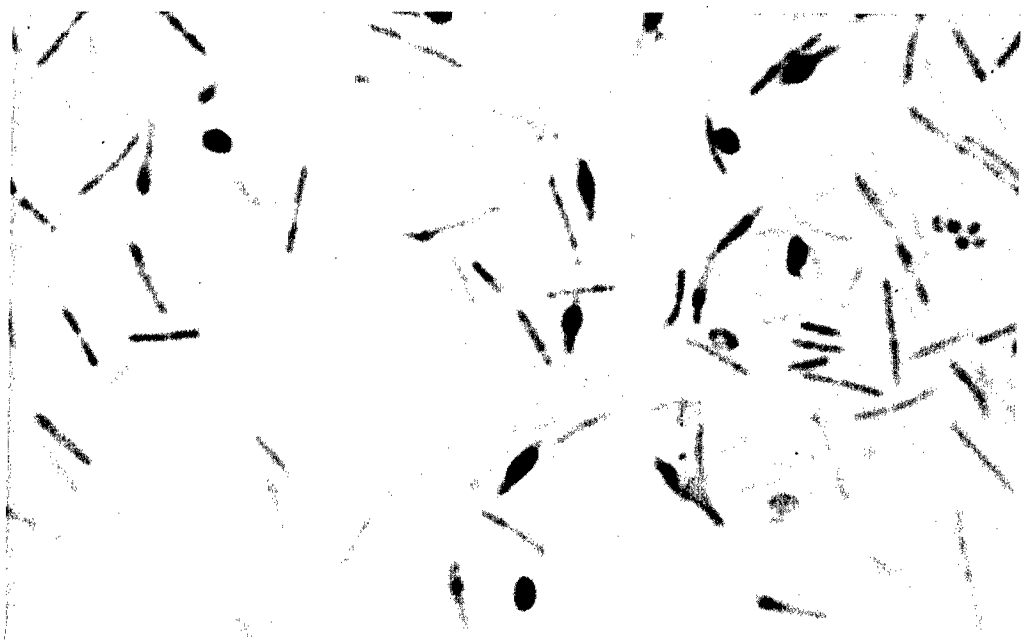
A. Gram stain of mixed throat culture showing the most frequently found form of the unusual Gram negative rod. X2000.



B. Gram stain of mixed throat culture showing Gram negative rods, long felaments, and sperm-like forms. X2000.

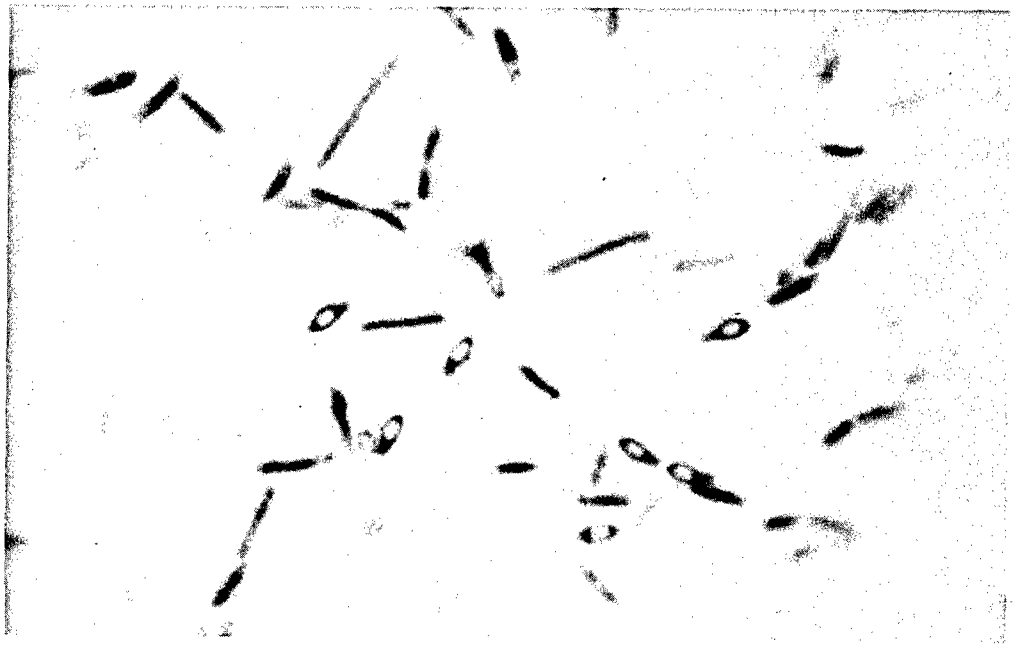
FIGURE 3 (Continued)

C. Gram stain of mixed throat culture showing Gram negative rods and large swollen forms. X2000.

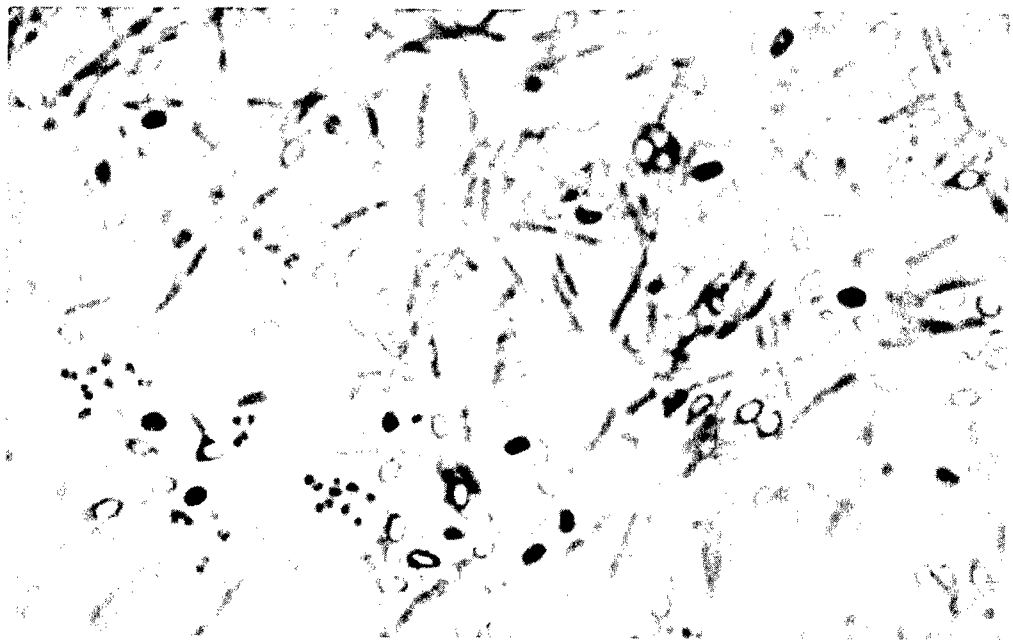


D. Gram stain of mixed throat culture showing Gram negative rods and spores that have retained the stain. X2000.

FIGURE 3 (Continued)



E. Gram stain of mixed throat culture showing Gram negative rods containing subterminal spores. X2000.



F. Gram stain of mixed throat culture showing Gram negative rods with profuse numbers of free spores. X2000.

SECTION VI
TABLES

TABLE I
EXPERIMENTAL DESIGN
EXPERIMENT III

<table>
<tr><td rowspan="2">Days on Test</td><td colspan="4">Subject</td></tr>
<tr><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td>1
2
3
4</td><td colspan="2">DEHYDRATED DIET</td><td colspan="2">FRESH DIET</td></tr>
<tr><td>5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20</td><td>SUIT</td><td>NO SUIT</td><td>SUIT</td><td>NO SUIT</td></tr>
<tr><td>21
22
23
24</td><td colspan="2">FRESH DIET</td><td colspan="2">DEHYDRATED DIET</td></tr>
<tr><td>25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40</td><td>NO SUIT</td><td>SUIT</td><td>NO SUIT</td><td>SUIT</td></tr>
<tr><td>41
42</td><td colspan="2">FRESH DIET</td><td colspan="2">FRESH DIET</td></tr>
</table>

TABLE II
EXPERIMENTAL DESIGN
EXPERIMENT IV

Days on Test	*Subject*			
	13	*14*	*15*	*16*
1 2 3 4	FRESH DIET		DEHYDRATED DIET	
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20	SUIT	NO SUIT	SUIT	NO SUIT
21 22 23 24	DEHYDRATED DIET		FRESH DIET	
25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40	NO SUIT	SUIT	NO SUIT	SUIT
41 42	FRESH DIET		FRESH DIET	

TABLE III

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*
SUBJECT 9, EXPERIMENT III

Body Area	*Microorganism*	2-21	2-24	2-26	2-28	3-1	3-4	3-6	3-9	3-9	3-11	3-13	3-16	3-18	3-20	3-23	3-25	3-27	3-29
		In Suit							1	1	1	1	1	1	1	1	1	1	1
EYE	Staphylococci	2	1	1	1	1	1	1	1										
	"Diphtheroids"**				1														
EAR	Staphylococci	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	"Diphtheroids"**	2							1										
NOSE	Staphylococci	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2
	"Diphtheroids"**		1			1		1	1						1	1		1	1
THROAT	Staphylococci	1	2	2	2	1	1	2	1	1	2	1	1	1	2	1	1	1	1
	Streptococci	2	1	1	1	1	1	1	2	1	2	1	1	1	2	1	1	1	1
	"Diphtheroids"**	1										1							
	Neisseria	3	2	3	3	3	3	3	3	3	3	2	2	3	2	2	2	3	3
	Gram-Neg. Bacilli											t	t	t	t				
AXILLA	Staphylococci	3	NOT TESTED						3	1	3	3	3	3	3	3	3	2	2
	"Diphtheroids"**																		
UMBILICUS	Staphylococci	2	NOT TESTED						2	2	2	3	2	2	2	3	3	3	2
	"Diphtheroids"**		NOT TESTED									1			1				
GROIN	Staphylococci	4	NOT TESTED						3	4	4	4	4	4	4	4	4	4	4
	"Diphtheroids"**	1	NOT TESTED									1	1	1	2	1	1	1	1

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

t Less than one percent (1%) recovery.

TABLE IV

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*

SUBJECT 10, EXPERIMENT III

Body Area	*Microorganism*	2-21	2-24	2-26	2-28	3-1	3-4	3-6	3-9	3-9	3-11	3-13	3-16	3-18	3-20	3-23	3-25	3-27	3-29
													In Suit						
EYE	Staphylococci	2	2	2	1	1	1	1	1	2	2	1	1	2	1	1	1	1	1
	"Diphtheroids"**																		
EAR	Staphylococci	3	2	2	2	1	1	3	1	1	2	1	2	1	1	2	2	2	2
	"Diphtheroids"**	1	3	3	3	2	2	1	3	3	3	3	2	3	2	3	2	2	2
NOSE	Staphylococci	2	2	3	3	3	2	2	2	3	2	3	2	3	2	3	2	3	3
	"Diphtheroids"**	2																	
THROAT	Staphylococci	1	1	2	2	2	1	1	2	1	1	1	1	1	1	1	2	2	1
	Streptococci	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1
	"Diphtheroids"**	1																	
	Neisseria	2	3	3	3	3	3	3	3	2	3	2		2	2	3	3	3	3
	Gram-Neg. Bacilli											t	t	t	t				t
AXILLA	Staphylococci	2	4	2	3	3	1	2	3	1	2	2			NOT TESTED				3
	"Diphtheroids"**						1	1				1			NOT TESTED				
UMBILICUS	Staphylococci	2	2	1	1	1	1	2		1	1	1			NOT TESTED				3
	"Diphtheroids"**	1	1		1		1			2		1			NOT TESTED				
GROIN	Staphylococci	4	4	4	4	4	4	4	3	2	4	4			NOT TESTED				3
	"Diphtheroids"**	1	1	1	1	1	1	1		1		1			NOT TESTED				3

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

t Less than one percent (1%) recovery.

TABLE V

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*

SUBJECT 11, EXPERIMENT III

Body Area	*Microorganism*	2-21	2-24	2-26	2-28	3-1	3-4	3-6	3-9	3-9	3-11	3-13	3-16	3-18	3-20	3-23	3-25	3-27	3-29
													In Suit						
EYE	Staphylococci	1	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1
	"Diphtheroids"**												1						
EAR	Staphylococci	1	1		2	2	2	1	1	1	2	1	1	1	1	1	1	2	1
	"Diphtheroids"**	2	2	2	2	1	2	2	2	3	3	1	2	2	2	2	2	2	2
NOSE	Staphylococci	2	4	3	2	3	2	2	3	3	3	3	3	3	3	2	3	3	2
	"Diphtheroids"**																		
THROAT	Staphylococci	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	1	2	1
	Streptococci	1		1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1
	"Diphtheroids"**	1	1			1													
	Neisseria	2		3	3	3	2	3	2	3	3	2	2	2	2	2	2	3	3
	Gram-Neg. Bacilli											t	t		t				t
AXILLA	Staphylococci	2	2	2	2	3	2	2	2	2	3	3			NOT TESTED				3
	"Diphtheroids"**																		
UMBILICUS	Staphylococci	2	1		1	1	1	1	2	1	1	1			NOT TESTED				1
	"Diphtheroids"**					3									NOT TESTED				
GROIN	Staphylococci	3	4	3	3	4	3	4	4	2	3	4			NOT TESTED				3
	"Diphtheroids"**	1	1	1	1	1	1	1	1		1	1			NOT TESTED				3

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

t Less than one percent (1%) recovery.

TABLE VI

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*
SUBJECT 12, EXPERIMENT III

Body Area	*Microorganism*	2-21	2-24	2-26	2-28	3-1	3-4	3-6	3-9	3-9	3-11	3-13	3-16	3-18	3-20	3-23	3-25	3-27	3-29
		In Suit																	
EYE	Staphylococci	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	"Diphtheroids"**							2		1					2				
EAR	Staphylococci	1	2	2	2	2	2	2	2	1	2	2	2	2	2	2	1	2	2
	"Diphtheroids"**	2	1	1	2				2	3	1	3	2	2	3	3	2	2	
NOSE	Staphylococci	2	2	2	2	3	2	3	3	3	2	2	2	2	3	2	3	2	2
	"Diphtheroids"**	2		1		1	1	1	1	2	1		1		1				
THROAT	Staphylococci	2	2	1	2	1	1	2	1	2	2	1	1	1	1	1	1	1	1
	Streptococci	1	1	1	1	1	2	1	1	1	2	1	1	1	1	1	1	1	1
	"Diphtheroids"**	1											1						
	Neisseria	2	2	3	3	2	2	3	3	3	3	2	2	2	2	2	2	2	2
	Gram-Neg. Bacilli											t	t		t		2	t	t
	Hemophilus																	1	
AXILLA	Staphylococci	2		NOT TESTED					3	2	1	2	2	2	2	2	3	2	1
	"Diphtheroids"**			NOT TESTED						1									
UMBILICUS	Staphylococci	1		NOT TESTED					2	2	1	3	2	2	3	1	1	2	2
	"Diphtheroids"**			NOT TESTED						1			1					2	
GROIN	Staphylococci	3		NOT TESTED					3	2	2	4	3	4	4	3	3	4	3
	"Diphtheroids"**	1		NOT TESTED					1	1	1	1	1	1	1	1	1	1	1

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

t Less than one percent (1%) recovery.

TABLE VII

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*

SUBJECT 13, EXPERIMENT IV

Body Area	*Microorganism*	5-4	5-6	5-8	5-11	5-13	5-15	5-18	5-20	5-22	5-24	5-25	5-27	5-28	5-29	6-1	6-3	6-5	6-8	6-10	6-12	6-13	6-14
					In Suit																		
EYE	Staphylococci		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1
	"Diphtheroids"**			1																			
EAR	Staphylococci	2	3	3	2	3	2	2	2	2	3	2	2	3	3	3	3	3	2	3	3	3	3
	"Diphtheroids"**														1								
NOSE	Staphylococci	2	3	2	2	2	2	3	2	3	2	2	3	2	2	3	3	3	3	3	2	3	2
	"Diphtheroids"**			1								1	1		1								
THROAT	Staphylococci	2	2	2	1	2	1	1	1	2	1	2	2	1	1	1	1	1	2	1	1	1	2
	Streptococci	2	2	2	2	1	1	2	1	1	1	1	1	1	2	1	1	1	2	1	1	1	1
	Neisseria	1	1	3	2	2		2	3	2	3	1	3	3	3	3	3	3	3	3	2	2	3
	"Diphtheroids"**																						
	Gram-Neg. Bacilli						3																
AXILLA	Staphylococci	1	2	2		NOT TESTED					3	3	3	3	3	3	2	3	3	3	2	2	2
	"Diphtheroids"**			1		NOT TESTED					1				1	1				1		1	1
UMBILICUS	Staphylococci	1	2	1		NOT TESTED					1	1	2	1	2	2	2	2	3	3	2	3	2
	"Diphtheroids"**	1	1	1		NOT TESTED					1	1	2	1	1	1	1	2	1	1	1		1
GROIN	Staphylococci	2	3	2		NOT TESTED					3	3	2	2	2	3	2	3	3	3	3	3	3
	"Diphtheroids"**	1	1	2		NOT TESTED					1	1	2	1	2	1	2	1	1	2	2	1	2
RIGHT FOOT	Staphylococci	3	3	3		NOT TESTED					2	3	2	2	2	3	3	3	2	2	3	3	3
	"Diphtheroids"**		1	1		NOT TESTED					3	3	3	1	3	2	3	3	3	3	3	3	3
LEFT FOOT	Staphylococci	NT	NT	NT		NOT TESTED					4	NT	NT	3	NT	NT	NT	NT	NT	NT	NT	3	3
	"Diphtheroids"**	NT	NT	NT		NOT TESTED					1	NT	NT	1	NT	NT	NT	NT	NT	NT	NT	2	2

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

NT Body Area was not tested.

TABLE VIII

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*

SUBJECT 14, EXPERIMENT IV

Body Area	*Microorganism*	5-4	5-6	5-8	5-11	5-13	5-15	5-18	5-20	5-22	5-24	5-25	5-27	5-28	5-29	6-1	6-3	6-5	6-8	6-10	6-12	6-13	6-14
															In Suit								
EYE	Staphylococci		1	1	1	1	1	1	1	1	1	1		1	1	1	1	2	1	1		2	1
	"Diphtheroids"**		1					1															
EAR	Staphylococci	2	2	1	1	2	1	1	1	2	1	1	2	2	2	1	1	2	2	1	2	2	3
	"Diphtheroids"**	2	3	2	3	3	3	2	2	1	3	3	2	2	3	3	3	3	2	3	3	3	1
NOSE	Staphylococci	2	2	2	1	2	2	2	2	2	3	2	3	2	2	2	2	2	2	2	2	2	2
	"Diphtheroids"**											1			1					1			
THROAT	Staphylococci	1	2	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	2	1	1	1	1
	Streptococci	2	2	2	1	2	2	2	1	2	2	1	1	1	1	1	1	1	1	2	1	2	1
	Neisseria	2	1	1	3	1	1	2	2	2	3	3	2	2	2	3	3	3	3	3	2	3	3
	"Diphtheroids"**																						
	Gram-Neg. Bacilli					1			1														
	Hemophilus	NT	NT	NT	NT	NT	2	NT	NT	NT	NT	NT	NT	NT			NOT TESTED					NT	NT
AXILLA	Staphylococci		1	1	1	1	1	1	1	1	1	1	1	2			NOT TESTED					3	2
	"Diphtheroids"**		1	1			1	1	1	1	1	1	1	1			NOT TESTED						
UMBILICUS	Staphylococci	1	2	2	1	1	2	2	3	3	2	2	2	1			NOT TESTED					1	1
	"Diphtheroids"**			2	1			1	1		1	1	1	1			NOT TESTED					1	
GROIN	Staphylococci	2	3	3	2	2	3	3	3	3	3	2	2	2			NOT TESTED					3	3
	"Diphtheroids"**	1	1	2	2	2	1	2	1	1	1	2	1	1			NOT TESTED					1	2
RIGHT FOOT	Staphylococci	3	3	3	3	2	2	2	2	3	1	2	1	2			NOT TESTED					2	2
	"Diphtheroids"**		2	1	1	2	3	2	3	2	3	2	2	3			NOT TESTED					1	3
LEFT FOOT	Staphylococci	NT	NT	NT	NT	NT	NT	NT	NT	NT	1	NT	NT	3			NOT TESTED					2	2
	"Diphtheroids"**	NT	NT	NT	NT	NT	NT	NT	NT	NT	3	NT	NT	2			NOT TESTED					1	2

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

NT Body Area was not tested.

TABLE IX

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*

SUBJECT 15, EXPERIMENT IV

Body Area	*Microorganism*	5-4	5-6	5-8	5-11	5-13	5-15	5-18	5-20	5-22	5-24	5-25	5-27	5-28	5-29	6-1	6-3	6-5	6-8	6-10	6-12	6-13	6-14
		In Suit																					
EYE	Staphylococci	1	1	1	1	1	1			1	1	1	1		1		1	1	1		1	1	
	"Diphtheroids"**														1						1	1	
EAR	Staphylococci			1		1		1	1				1	1	2	1	1	1	2	1	3	1	1
	"Diphtheroids"**		2	2	1	2	1	1		1	2	1	2	1	3	2	2	2	1	2	1	1	3
NOSE	Staphylococci	3	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	3	3	2	2	3
	"Diphtheroids"**											1	1										
THROAT	Staphylococci	1	2	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	2	1	1	1	2
	Streptococci	2	2	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	1	1	1	2	1
	Neisseria	1	1		2	1	2	1	2	3	1	2	3	3	2	2	2	2	3	3	2	3	3
	"Diphtheroids"**						1											3					
	Gram-Neg. Bacilli	1		3	2	2	3	3	1														
AXILLA	Staphylococci	1	1	1		NOT	TESTED				1	1	1	1	3	2	1	1			1	1	1
	"Diphtheroids"**			1		NOT	TESTED				1	1	1	1	1	1	1	1	1				1
UMBILICUS	Staphylococci	1		1		NOT	TESTED				1	1	1	1	2	1	1	1		1	1	1	1
	"Diphtheroids"**		1	1		NOT	TESTED					1	1	1	2	1	1	1		1	1	1	
GROIN	Staphylococci	2	2	2		NOT	TESTED				1	2	2	2	2	3	3	2	1	3	2	3	3
	"Diphtheroids"**	1	1	1		NOT	TESTED				3	2	1	2	2	1	2	3	2	1	2	1	1
RIGHT FOOT	Staphylococci	3	3	3		NOT	TESTED				4	2	2	3	3	4	3	3	3	3	3	3	3
	"Diphtheroids"**		1	1		NOT	TESTED					1		2	2	1			1		1		1
LEFT FOOT	Staphylococci	NT	NT	NT		NOT	TESTED				3	NT	NT	2	NT	NT	NT	NT	NT	NT	NT	3	3
	"Diphtheroids"**	NT	NT	NT		NOT	TESTED				1	NT	NT	1	NT	NT	NT	NT	NT	NT	NT	1	2

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

NT Body Area was not tested.

TABLE X

RELATIVE OCCURRENCE OF MICROORGANISMS FROM SPECIFIC BODY AREAS*

SUBJECT 16, EXPERIMENT IV

Body Area	*Microorganism*	5-4	5-6	5-8	5-11	5-13	5-15	5-18	5-20	5-22	5-24	5-25	5-27	5-28	5-29	6-1	6-3	6-5	6-8	6-10	6-12	6-13	6-14
EYE	Staphylococci	1	1	1	2	1	1	1	2	1	2	1	1	1	2	2	2	2	1	1	1	2	2
	"Diphtheroids"**																						
EAR	Staphylococci	2	2	2	2	3	2	1	2	1	1	1	1	1	1	1	3	2	2	2	2	2	3
	"Diphtheroids"**		1	2	1		2	1			2	1	1	2	3	3	1	3	1		1		1
NOSE	Staphylococci	3	2	2	2	2	3	2	2	3	3	3	3	2	3	3	2	3	3	2	2	2	2
	"Diphtheroids"**	1	2	1	1	1	1		1		1	1	1	1		1	1		1	1	1	1	1
THROAT	Staphylococci	2	2	1	1	1	1	1	1	1	1	2	1	2	2	1	1	1	2	1	1	1	1
	Streptococci	2	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
	Neisseria	2	1	2	3	2	3	2	2	3	3	3	3	3	3	2	2	3	3	2	2	2	2
	"Diphtheroids"**		2			1	1	2	3	1	2		2	2		2	1	2		2		2	2
	Gram-Neg. Bacilli																						
AXILLA	Staphylococci	3	2	3	3	2	3	2	3	3	3	3	3	3			NOT TESTED					3	3
	"Diphtheroids"**											1		1									
UMBILICUS	Staphylococci	2	1	2	1	1	1		1	1	2	1	1	1			NOT TESTED					3	2
	"Diphtheroids"**		1	2	2	2	1	1	2	1	1	1	1	1			NOT TESTED					1	2
GROIN	Staphylococci	3	3	3	2	2	3	3	2	3	3	3	2	2			NOT TESTED					3	2
	"Diphtheroids"**		1	2	2	2	1	1	2	1	1	1	1	1			NOT TESTED					1	2
RIGHT FOOT	Staphylococci	3	3	1	2	3	3	3	3	3	2	2	2	3			NOT TESTED					3	1
	"Diphtheroids"**	1	2	3	2	2	1	1	3	2	2	1	4	2			NOT TESTED					3	3
LEFT FOOT	Staphylococci	NT	NT	NT	NT	NT	NT	NT	NT	NT	2	NT	NT	2			NOT TESTED					3	1
	"Diphtheroids"**	NT	NT	NT	NT	NT	NT	NT	NT	NT	3	NT	NT	3			NOT TESTED					3	3

*Recovery of microorganisms is evaluated on the basis of a 1 - 4 rating. All blank spaces indicate no recovery made.

**"Diphtheroids" as a grouping is explained in detail in the text.

NT Body Area was not tested.

TABLE XI

RECOVERY OF MICROORGANISMS FROM THE METABOLIC WARD AND PRESSURE SUIT AIR INTAKE AND EXHAUST*

Area Sampled	*Exposure Time*	*Total Bacteria*	*Hemolytic Bacteria*	*Total Fungi*	*Hemolytic Fungi*
SUBJECT'S ROOM (Blood Agar)	1 Hour	27	4	3	
SUIT INTAKE (7-10 cfm) (Blood Agar)	1-2 cm from Agar for 5 Minutes	11	4	1	1
SUIT EXHAUST (Blood Agar)	1-2 cm from Agar for 5 Minutes	160-170	5	20-25	2
VENTILATOR SUBJECT'S ROOM (Blood Agar)	5 Minutes	3	2	2	
FILTER, INSIDE (Blood Agar)	Swab	NOT DETERMINABLE*			
FILTER, INSIDE (Littman oxgall)	Swab	NOT DETERMINABLE*			
FILTER, OUTSIDE (Blood Agar)	Swab	NOT DETERMINABLE*			
(LITTMAN) negative	Swab	NOT DETERMINABLE*			
LAB CONTROL (Blood Agar)		25	9	2	2

*See "Results" for complete description or explanation.

TABLE XII

SELECTED HEMOLYTIC MICROORGANISMS RECOVERED FROM THE METABOLIC WARD AND PRESSURE SUIT AIR INTAKE AND EXHAUST*

Area Sampled	*Colonial Morphologic Type**	*Size***	*Color*	*Shape*	*Elevation*
SUBJECT'S ROOM (Blood Agar)	A	Average	White, dark center	Circular	Slight, umbonate, striations
	B	Average	White	Circular	Slight, umbonate
	C*	Large	Greenish	Irregular	Flat*
	D	Average	White	Circular	Raised wing formation
SUIT INTAKE (Blood Agar)	E	Average	Grayish	Irregular	Umbonate
	F	Large	Chalk white	Circular	Generally pulvinate
	G	Average	White	Circular	Umbonate
SUIT EXHAUST (Blood Agar)	H*	Very large	Greenish	Irregular	Flat, raised concentric rings
	I	Average	Greenish-gray	Irregular	Umbonate, beaded center
	J	Average	Beige	Circular	Pulvinate, large beads
VENTILATION	K*	Large	Greenish-gray	Irregular	Flat, depressed concentric rings*
SUBJECT'S ROOM (Blood Agar)	L	Very small	White	Circular	Umbonate
FILTER, INSIDE (Blood Agar)	K*	SEE TYPE "K" ABOVE			
	M*	Large	Opaque to slight green	Circular	Slightly raised, depressed center
	N	Small to average	White	Circular	Raised, finely beaded
FILTER, INSIDE (Littman oxgall)	P	Minute	White	Circular	Pulvinate, beaded
FILTER, OUTSIDE (Blood Agar)	K	SEE TYPE "K" ABOVE			
	O*	Large	Opaque to translucent	Slightly irregular	Flat, finely beaded
(LITTMAN) negative					
LAB CONTROL (Blood Agar)	R	Average	White, dark center	Circular	Slightly umbonate
	S	Very large	Brown center, White periphery	Circular	Raised center
	T	Large	Opaque	Irregular	Flat

*See "Results" for complete description or explanation.

**Size is defined as follows: Small = under 2 mm., Average = 2-6 mm., and Large = over 6 mm.

TABLE XII (Continued)

Area Sampled	*Colonial Morphologic Type**	*Margin*	*Bacteria or Fungi*	*Hemolytic Activity***	*Gram Reaction, Morphology*
SUBJECT'S ROOM (Blood Agar)	A	Undulate	Bacteria	2	Small Gram positive cocci
	B	Entire	Bacteria	1	Average to large Gram positive cocci
	C*	Undulate	*	4	Various Gram negative forms*
	D	Entire	Bacteria	2	Average Gram positive cocci
SUIT INTAKE (Blood Agar)	E	Entire	Bacteria	3	Average Gram positive cocci
	F	Periphery embedded	Fungi	5*	
	G	Undulate	Bacteria	2	Average Gram positive cocci
SUIT EXHAUST (Blood Agar)	H*	Lobate	Bacteria		Large: Gram positive rods, various forms*
	I	Entire	Bacteria	2	Minute Gram negative cocci
	J	Slightly lobate	Yeast-like	2 (Diffused)	Gram negative cocci, tetrads, etc.
VENTILATOR SUBJECT'S ROOM (Blood Agar)	K*	Lobate	*	1 (Diffused)	Gram negative rods, various forms*
	L	Undulate	Bacteria	1	Irregular Gram negative cocci
FILTER, INSIDE (Blood Agar)	K*	SEE TYPE "K" ABOVE			
	M*	Lobate to erose	*	1 (Diffused)	Gram negative, mainly filamentous*
	N	Lobate	Bacteria	1	Gram positive cocci
FILTER, INSIDE (Littman oxgall)	P	Entire	Bacteria		Large Gram positive cocci, tetrads
FILTER, OUTSIDE (Blood Agar)	K	SEE TYPE "K" ABOVE			
	O*	Slightly undulate	Bacteria	4	Few Gram negative rods, 99% spores
(LITTMAN) negative					
LAB CONTROL (Blood Agar)	R	Entire	Bacteria	2	Small Gram positive cocci
	S	Filamentous	Fungi	3 (Under Colony)	
	T	Filamentous	Bacteria	4	Gram variable rods*

*See "Results" for complete description or explanation.

**Hemolysis is evaluated on the basis of a 1 - 5 rating.

TABLE XIII

RESULTS OF REPLICATION FROM A PRIMARY OR INITIAL PLATE

Primary Plate No.	*Age in Days*	*Culture Source*	*Primary Isolation Media*	*Replication Media**			*Remarks*
				Replica 1	*Replica 2*	*Replica 3*	
I	3	Throat	Blood Agar	C-M** 3	110** 2	110 2	Microorganisms other than staphylococci transferred to C-M media so C-M reaction difficult to interpret.
II	5	Throat	Blood Agar	110 1	110 1	110 1	Primary plate very dry, poor replication.
III	3	Nose	Staphylococcus	C-M 4	110 4	110 4	Primary plate still moist. Very good replication. C-M reaction very good.
IV	5	Throat	Blood Agar	110 1/2	110 (-)	110 (-)	Primary plate very dry. Very poor replication.
V	3	Throat	Blood Agar	110 3	110 3	110 3	Primary plate fairly dry. Good replication.

*Replication media is evaluated on the basis of a 1 - 4 scale (4 = maximum transfer or replication).

**C-M and 110 = BBL-Coagulase-Mannitol agar base plus 10% undiluted human outdated donor plasma and Bacto-Staphylococcus 110 medium respectively.

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Security Classification

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(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Miami Valley Hospital, Dept of Research, Dayton, Ohio and Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

PRELIMINARY STUDY ON THE MICROBIAL FLORA OF NORMAL HUMAN SUBJECTS IN A RESTRICTED ENVIRONMENT

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (Last name, first name, initial)

Rack, Joseph V.
London, Sheldon A., PhD

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1965	39	8

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
in part under AF 33(657)-11716	
b. PROJECT NO. 7164	
c. Task No. 716405	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) AMRL-TR-66-59
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13. ABSTRACT

Microorganisms were recovered from selected skin and mucosal surfaces of men in groups of four living in a restricted environment. Of several sampling methods studied, the use of cotton swabs proved to be the technique most appropriate for the scope of this study. Differences between the microbial flora of the test subjects and major alterations in microbial populations were observed. These alterations could not be definitely attributed to the direct influence of the MA-10 full pressure suit. However, three of the pressure suited subjects exhibited foot lesions, initiated by a bacterium apparently indigenous to those individuals, during the period when the suits were worn. These data indicated that the carrier state may be of greater significance to the carrier than to other individuals in the restricted environment. Although transference of certain microbes was noted, the exchange of microorganisms generally considered to be pathogenic was not observed.

DD FORM 1 JAN 64 1473

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	ROLE	WT	ROLE	WT	ROLE	WT
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