SECTION 11

EFFECT OF DIET AND ATMOSPHERE ON INTESTINAL AND SKIN FLORA: (SUMMARY REPORT)*

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SECTION 11

GENERAL DISCUSSION OF RESULTS AND CONCLUSIONS

INTRODUCTION

The health and welfare of the astronaut is of prime importance during space flight so that he is capable of rendering peak performance at all times during his misssion. One factor that can impair the health of the astronaut is related to the microbial flora which live in or on his body or which are present in his environment. For this reason it is necessary to study the effect of conditions of space flight upon these indigenous microorganisms, since knowledge of the alterations in the occurrence, and metabolism of individual microorganisms, or in the balances of organisms under these conditions is necessary in maintaining the health and welfare of these astronauts.

Certain conditions of space flight such as personal hygiene procedures, wearing of space suits, atmospheric composition and pressure, confinement and a space-type diet are particularly apt to influence the microbial flora of the astronaut. For example, the type and frequency of personal hygiene procedures such as bathing, brushing of teeth, shaving and toilet routines play an important role in the establishment of the resident flora of the exposed areas of the human body. A requirement of space travel is the minimal use of personal hygiene procedures, which often are limited to the use of face cloths without a cleansing agent and brushing the teeth without a dentrifrice. The effect of the less complete hygiene methods on the resident microflora of men living under these conditions is important. Microbial studies underway at Wright-Patterson under contract AF33(615)-1814, Biomedical Criteria for Personal Hygiene(1), where men have been confined to a chamber under ambient conditions for four weeks and have used minimal personal hygiene procedures indicate that certain bacterial populations tend to build up in some body areas, particularly in areas of heavy perspiration; but that no serious health problems have developed in these men. However, potentially pathogenic bacteria have been isolated, and further study superimposing certain space stresses are needed to evaluate this finding as related to space flight.

The wearing of a closely fitting space suit, with air or oxygen circulating through it for prolonged periods, will create an altered environment for the microorganisms on the skin of the astronaut. Problems may arise both with respect to possible areas of contact irritation and to the effects of humidity. The close-fitting suit may rub a certain body area, creating an abrasion ideal for microbial infection. Areas of the body where perspiration is normally heavy may remain damp, while areas near the air intake may be abnormally dry. The variable humidity plus the altered atmospheric flow may well influence the bacterial population. In fact the high oxygen atmosphere itself coupled with reduced atmospheric pressure may also influence the character of the bacterial flora.

The close confinement imposed by space flight will cause considerable interaction between the microbial flora of the several astronauts with each other and with their environment. The spread of potentially pathogenic microorganisms from a "well" carrier to another astronaut directly or by way of the common environment presents a problem and microbiological studies

conducted on men in simulated space chamber experiments under contracts NASr-92 (2) and AF33(615)-1814 (1) have shown the probable transfer of pathogenic bacteria from one subject to the others. The mode and frequency of such transfer of potential pathogens is of importance in maintaining the flight crew efficiency.

Diet is known to influence the intestinal flora of animals and recent studies conducted under Wright-Patterson contract AF33(615)-1748 (3) at Republic Aviation Corporation have shown that a space-type diet affects the human fecal flora, especially with respect to the increased occurrence of a group of gas-forming, proteolytic anaerobes. Since flatulence and odors from intestinal gas may be a problem in space flight, the influence of diet on the intestinal flora must be clarified.

From this discussion it is evident that several questions remain to be answered relating to the microbiological aspects of space flight.. Specifically, does the bacterial population of the astronaut or his environment build-up during space flight and if so, with respect to which type of organisms? Do any of the factors of space flight favor this buildup? Is this buildup harmful to the health and well-being of the astronauts? Do any of the pathogenic organisms present a problem during space flight? Are the astronauts healthy carriers of potentially pathogenic organisms and do they transfer these organisms to fellow crew members? If so, does this create a health hazard? If any of the foregoing considerations create problems, what can be done to alleviate them? Only a comprehensive microbiological study on humans under simulated space conditions can offer the answers to these questions.

For these reasons an extensive study was done on the types and numbers of microorganisms present and the frequency of their occurrences in six body areas of six young men subjected to certain conditions of space flight and their two controls for a 34 day period in a recent study in the Aerospace Crew Equipment Laboratory chamber at the Naval Air Engineering Center, Philadelphia, Pa., 19112. Under contract NAS-9-4172, samples for microbiological culturing were taken at the start of the trial and at frequent intervals during the test, and the kinds and numbers of bacteria isolated in each sampling period were studied. The bacteria present in their environment were also determined in the same manner.

The experiment was designed in such a way that the six experimental subjects and their controls used the same minimal personal hygiene procedures and ate the same dehydrated foods throughout the entire experiment. Both the test subjects and their controls lived in confinement which in the case of the controls was maintained at ambient conditions. This was in contrast to the test subjects who were maintained at an altitude of 27,000 feet under 100% oxygen for a total of three weeks and wore space suits during the last two weeks at altitude and for one week post-altitude. The control subjects wore space suits during the last three weeks of the trial.

Since the microbiological determinations were carried out throughout the entire experimental period, the effect of the various experimental conditions can be evaluated in terms of their influence on the various members of the microbial population.

The scope of the work can probably be best appreciated by quoting several figures: 1378 samples were taken from the body areas and the environment as well as from the urine bottles, wash water and the suits, which resulted in 18,500 odd primary cultures. To study these cultures, over 150,000 plates and tubes of media were used in secondary culturing and over 10,000 slides were made and observed. The results of these studies have been summarized in table form (reference 11) and present the total numbers and occurrences of aerobic and anaerobic bacteria found on the various body areas tested and in the environmental areas of the chamber and cottage.

METHODS

Eight young men from the United States Armed Forces were subjects in a chamber trial to determine the effect of a pure oxygen atmosphere at altitude combined with the wearing of space suits, minimal personal hygiene procedures, and eating a space-type diet upon their health and well-being. Six men were confined to the Aerospace Crew Equipment Laboratory chamber for a period of 34 days and two men served as controls in a nearby installation termed the "cottage". The exact experimental conditions have been defined elsewhere, but in brief the subjects in the space chamber were under ambient conditions for one week, at altitude with no space suits for one week and with space suits for two weeks, after which the chamber was maintained under ambient conditions, but the subjects wore the space suits. The men in the cottage were under ambient conditions at all times but wore space suits for the last three weeks of the trial. All eight men ate the dehydrated food and used the minimum personal hygiene procedures of washing only the face and hands with a face cloth with no cleanser and brushing the teeth with no dentrifrice for the entire experimental trial.

The microbiological samples were taken according to the schedule indicated in Table 11-1. All eight men were scrubbed thoroughly, as were the chamber and cottage, prior to the first sampling. The exact details of the collection and processing of the samples are contained in Appendix A, but in brief two swabs--one for aerobic and one for anaerobic culturing, were taken from the following body areas: throat, buccal area, axilla, groin, glans penis, and eye (first sampling only) for a total of 10 samples from each area and fecal samples were obtained approximately twice a week as indicated in Table 11-2. Fourteen samples were taken from each of several environmental areas in both the chamber and the cottage as indicated in Table 11-1, by means of sedimentation plates or swabs. In addition cultures were made from the urine collection bottles, water squeezed from face cloths and from certain areas of the suit prior to donning and from the suit vents after donning at stated intervals indicated in Table 11-1.

The detailed anaerobic and aerobic experimental procedures for obtaining each sample and the technique and media used for the primary culturing for each of the body areas is included in Appendix A. The culturing of the samples from the body areas was done at the site of the Aerospace Crew Equipment Laboratory chamber and the culturing was done immediately following the collection of the samples by the subjects. The fecal samples were cultured immediately following elimination on an individual basis. After proper incubation the primary cultures on solid media were then transferred to Republic Aviation Corporation laboratories for processing according to the schema set forth in Appendix A. Selected broth cultures from the anaerobic series were transferred into agar shakes at the primary culturing site for transport to Republic Aviation Corporation's laboratories. The cultures that were made from the environmental areas and from the miscellaneous items were treated in the same manner as the cultures from the body areas.

Slides were made from all aerobic and anaerobic cultures showing growth and slides were also made of all original samples at the time of primary culturing. None of the samples collected appeared to be abnormal in character.

The data from both the aerobic and anaerobic culturing (when done) for each body area sampled, from the feces, the environmental areas, and the miscellaneous items are recorded in tabular form and are considered both with respect to the microflora of each subject or environmental area and with respect to each sampling period which reflects the effect of the various test conditions.

The data recorded in the tables refers to the subjects by number as shown in the following list:

Subject Number	Area
1	Chamber
2	Chamber
3	Chamber
4	Chamber
5	Chamber
6	Chamber
7	Cottage
8	Cottage

RESULTS

1. The total number of colonies on aerobic blood plates in all body areas and in the environment increased as the experiment progressed. The buildup in the axilla, groin, glans penis, and buccal area reached a plateau by the mid-point of the experiment and then stayed relatively constant or decreased, while the buildup in the throat flora was more variable. Although the counts from all body areas fluctuated, consideration of the colony counts from the blood plates incubated under CO_2 from the skin areas would eliminate many of these fluctuations. Bacterial buildup was larger and more irregular in the chamber than in the cottage.

2. The types of microorganisms found in each of the body areas and feces were in good agreement with those regarded in published reports as normal body microflora for that area of the body. Whereas the same types of bacteria usually grew under both the aerobic and anaerobic conditions used in this experiment, several predominating types grew better under CO_2 incubation on primary isolation. The kinds of microorganisms found in the environment reflected the hardier types of body microorganisms isolated from the subjects.

3. The bacteria involved in the buildup of microflora in the axilla, groin and glans penis were staphylococci or micrococci and corynebacteria, with the corynebacteria predominating in the groin and glans penis of the subjects and in the axilla of four of the subjects in the last sampling periods. Streptococci, and to some extent staphylococci or micrococci, were involved in the increase in microorganisms in the throat and buccal area. The buildup of corynebacteria in most instances represented a relative increase of the rod over the cocci toward the end of the trial while the increase of streptococci seemed only to indicate an increase in the numbers of the type of bacteria that had predominated throughout the experiment. The buildup was most marked in the body areas were sweat is a factor. The strict anaerobes peptococcus and veillonella may have built up in the glans penis and throat, respectively. The bacteria involved in the buildup in the environment were largely staphylococci or micrococci, gram negative rods and, to a lesser extent, streptococci.

4. The buildup of bacteria on the body areas occurred in such a pattern that it appears to be the result of the minimal personal hygiene procedures with the subjects in a confined area and the increase in bacteria was of such a nature that it does no appear harmful for a 34 day period.

5. In the feces strict anaerobes represented over 95 percent of the predominating bacteria and outnumbered the aerobes by more than 1000 to 1. In general, the types of fecal anaerobes isolated, as well as the frequency of their occurrence, agreed well with the distribution of the bacteria described as FA types on the basic NASA study with one significant exception. After the subjects had been on the experimental diet for about two weeks, the type of fecal anaerobes designated as GD started to increase and continued to be isolated frequently for the remainder of the trial. These GD types of organism which form gas, black-slime and are proteolytic, were previously found associated with a space-type diet eaten by subjects on a study conducted by Wright-Patterson Air Force Base under contract AF33(615)-1748[3]. This change in fecal anaerobes probably is diet connected. Another interesting finding was an increase in the diversity of fecal anaerobes, starting about a week after the men changed to the experimental diet and continuing for about two weeks, after which the variety of fecal anaerobes decreased to the original level, a finding similar to that observed in primates when their diet was shifted on a nutrition study conducted by 6571st Aeromedical Research Laboratory at Holloman Air Force Base under contract AF33(600)-4124(7).

6. Other experimental conditions, such as the 100% oxygen at 5 psia and the wearing of space suits did not seem to affect the body flora materially, as there was no marked difference in the microflora of the subjects when these experimental conditions prevailed.

7. The potentially pathogenic bacteria, Shigella Poly B, Bethesda-Ballerup and coagulase-positive phase tyable staphylococci were isolated from certain subjects during the experiment; but these bacteria did not cause overt illness and did not appear to transfer readily from one subject to another.

8. Only one apparent transfer of a bacterium from one subject to another occurred when Bethesda-Ballerup was isolated from Subject 4 after having been isolated from Subject 6 previously. The organism did not seem to implant well, as it was isolated only once from the second subject. The Shigella Poly B and phage typable staphylococci did not transfer to other subjects.

9. The types and numbers of bacteria found in the wash water from the "space" sink contributed to the abandonment of the use of these sinks. The microbiological examination of the wash water from the wash cloths used in the latter part of the experiment indicated they were unsatisfactory also when used continuously.

10. The kinds and numbers of bacteria isolated from the neck of the urine bottles indicated that these bottles could serve as a source of undesirable contamination of the environment.

11. The method of cleaning the space suits left a sizeable residual contamination of typical body organisms which could be transferred to the body of the wearer. The air coming from the vent of the space suits during wearing is contaminated, but not excessively, with body microflora.

DISCUSSION

The microbiological determinations carried out in this study were designed to detect the effect of various conditions typical of space flight including minimum personal hygiene procedures, confinement, space type diets, space suits and 100% oxygen at 5 psia upon the microflora of the subjects and their environment. To do this both aerobic and anaerobic cultural procedures were used to study the kinds and numbers of bacteria present in several body areas including the axilla, groin, glans penis, throat and buccal area as well as the feces and in several areas of the chamber and cottage. The samples were taken frequently throughout the experimental period, at intervals planned to reflect the changing conditions of the experiment, especially the effect of 100% oxygen at altitude to which the six men in the chamber were subjected. Two men living in the cottage served as controls with respect to the 100% oxygen at altitude.

The first question involved the effect of the minimal hygiene procedure coupled with confinement on the numbers of bacteria on the man and his environment, and both plate counting and serial dilution techniques were employed to determine whether the total numbers of bacteria increased during the trial. Both methods showed that there was a build up of bacteria in all body areas and in both environments as the experiment progressed. The build up was greatest in the body areas where sweating occurred, the axilla and groin, and was also pronounced in the buccal cavity where the effects of minimal oral

hygiene probably were felt. The build up was greater in the chamber where six men lived than in the cottage, and the numbers of microorganisms in the chamber fluctuated more than in the cottage for an unexplained reason. This build up usually occurred gradually and then tended to plateau or even to fall off somewhat rather than to continue rising at each testing period. This suggested that more prolonged confinement per se probably would not result in a much greater build up of microorganisms.

The next important factor to be considered is the types of bacteria involved in the buildup in each body area and the environment. To do this, it is first necessary to establish what bacteria are present initially in these areas and then to follow the increase or decrease of each type of microorganism.

To establish the types of microorganisms present, appropriate microbiological procedures were employed to identify the various bacteria cultured from the body areas and environment. The results of these studies showed that the aerobic microorganisms found on the various body areas were in general in good agreement with those reported in the literature for normal adults and that the microflora of the chamber and cottage reflected to a certain degree the more hardy microorganisms such as staphlococci, or micrococci, streptococci and gram negative rods present on the body of the occupants. Certain types of bacteria such as neisseria and hemophilus often reported as part of the normal flora were isolated infrequently from these subjects, as were certain fungi such as Pityrosporum ovale. The failure to culture P. ovale was probably due to the deficiency of an essential oil in the medium. Corynebacterium acnes was not identified among the isolants in this study, probably because the definition of this organism as an obligate anaerobe was strictly adhered to. Of interest also was the lack of yeasts in the axilla, although these organism were isolated from other body areas.

To determine which of the types of bacteria were involved in the build up of microorganisms two procedures were used. The characteristics of distinctive colonies found in the greatest numbers on the blood plates were linked to bacteria selected for identification, and using this as a basis, estimates were made of the predominating organism on each plate. To check this estimate, microscopic observations were made to determine the morphological types of bacteria occurring in the highest broth culture in the dilution series of each sample. Excellent agreement was obtained between these two methods and from these observations, it was evident that staphlococci or micrococci and corynebacteria were the organisms involved in the buildup of bacteria in the axilla, groin and glans penis, with corynebacteria being the most predominant in the latter part of the experiment in the groin and glans penis and on four subjects in the axillar area. Streptococci with some staphylococci or micrococci were principally involved in the buildup in buccal area and the throat.

The organisms which built up in the chamber and to a lesser extent in the cottage were staphylococci, gram negative rods and streptococci, probably from the bodies of the occupants.

Because of the aerobic techniques used in enumerating the bacteria, no strict anaerobes were specified as being involved in a bacterial buildup on any of the body areas, excluding the feces and during the studies on strict anaerobes, only the peptococci isolated from the glans penis offer any evidence of increasing in prevalence as the experiment progressed. However, since strict enumeration of the obligate anaerobes is difficult, the increased incidence of this type of bacterium in the glans penis area of several subjects during the last few sampling periods may not indicate a true numerical buildup.

From the data presented and discussed, it is evident that the minimum hygiene procedures employed in this experiment coupled with confinement did bring about the buildup of microorganisms both in the body areas tested and in the environment, which reached a plateau about midway in the experiment and remained on this plateau or declined. This increase in bacteria seemed to be associated with the microflora usually found in that area of the body and did not seem to harm the subjects. The bacteria in the environment also reached a plateau, after which no further substantial increase occurred and the most numerous bacteria in the environment apparently came from the subjects.

These findings are strengthened by their general agreement with the results of the series of similar tests conducted at Wright-Patterson Air Force Base (1).

The strict anaerobes isolated in this study were principally from the feces, where they comprise over 95% of the predominating flora. Anaerobes were shown to outnumber aerobes by about 1000 fold and for this reason the work on fecal bacteria in this study emphasizes the strict anaerobes. The techniques for isolating these organisms and the key for grouping similar bacteria has been devised under NASA contract NASw-738(4), and the discussion of the results involving the fecal anaerobes will be based on the data from the NASA study on normal males.

In general the fecal anaerobes isolated during this experiment correspond well both with respect to kinds and frequency of distribution to the NASA study. Most of the FA types defined in the NASA experiment were found in this study and the most and least prevalent types were similar on both studies. There was more variation between individuals than between sampling periods in the occurrence of the FA types.

There was one notable point of divergence between the two studies in the increased occurrence on this experiment of strict anaerobes of the GD type, a group of black slime, gas forming proteolytic bacteria that were seldom found in the NASA study. The GD types occurred more frequently in the latter part of the trial in a pattern similar to, but not as marked as that which was found in subjects fed a space-type diet at Wright-Patterson Air Force Base (3). The pattern in both of these trials suggests that diet was involved in the increase in isolations of the GD types.

Also worthy of comment is the increase in diversity of the fecal anaerobes after the men had been on the space type diet for about a week followed by a decrease in the diversity about two weeks later. This increase in the variety of fecal anaerobes present in a group of subjects has been noted before, during a nutrition study conducted with primates under contract AF29(600)-4555(5) which occurred whenever there was a shift from one diet to another.

Thus the findings with respect to the fecal anaerobes in this study are in good agreement with other similar studies and indicate that diet is the most important factor influencing the fecal anaerobes.

The effect of 100% oxygen at altitude and the wearing of space suits during certain periods in the trial were other experimental variables. There was little, if any evidence that either of these factors influenced either the body, fecal or environmental microflora. The possible exception was the seemingly smaller fluctuations in the bacterial counts of the axilla and groin of the cottage inhabitants as well as in the environmental bacterial counts in the cottage itself. This may have been due to the smaller number of men in the cottage with the resultant quieter conditions or to a more careful sampling technique practiced by these men.

An important microbiological consideration in the confined environment of a space capsule is the presence of potential or frank pathogens which could affect the well-being of the space travelers. During the course of this study particular attention was paid to the isolation of any such bacteria, and three types of potentially dangerous bacteria were isolated. From the first fecal sample from Subject 6, as well as from the seventh fecal sample, the potentially disease-producing Bethesda-Ballerup was isolated and typed, and this same subject repeatedly showed the pathogen, shigella, during the most of the experiment. The shigella was not found in any other subject, but the Bethesda-Ballerup was isolated from Subject 4 in the second fecal sample. Neither subject appeared to be ill.

The other incident involved the isolation of several coagulase positive, phage typable staphylococci from the bodies of several of the subjects as well as from their environment. One subject developed staphylococcus-infected pustules, but the potentially pathogenic staphylococci were not involved. Thus dangerous bacteria were present throughout the experiment but fortunately in no instance did the possibly dangerous bacteria cause an overt illness in the subject, and these bacteria did not build in the subject's bodies or their environment. However, a microbiological examination of the subjects prior to the experiment, would have revealed the presence of these bacteria and proper treatment could probably have been instituted to eliminate the possibility of trouble from these bacteria.

Another important microbiological consideration is the transference of bacteria, particularly pathogens, from one subject to another. In this experiment there were three good opportunities to determine whether bacteria are transferred, two of which involved the fecal bacteria, Bethesda-Ballerup and Shigella Poly B. There was no transfer of the shigella, but it appears that one instance of the transfer of the Bethesda-Ballerup did occur. However this bacterium apparently did not implant firmly, as it was isolated from the second subject only once.

All coagulase positive staphylococci were studied for phage type and among eight typable cultures, five different phage types were found. No instance of transfer of a typable staphylococci from one subject to another was demonstrated.

Another possible instance of transference involved the peptococci. Peptococcus 1 was found only once (in Subject 5) prior to the fifth sampling period, after which this type of organism was isolated in several subjects. However, this "spread" of Peptococcus 1 from a subject confined in the chamber to two men living in the cottage weakens the transference concept in this instance, unless the transfer was effected in the two weeks prior to the start of the experiment by a light inoculum of Peptococcus 1 that required several weeks to implant firmly.

From these data, it would appear that transference can occur under certain circumstances, but it did not seem to occur frequently nor to have lasting effect in this trial.

Additional studies were made on the bacterial content of the wash water coming from the face cloths of the subjects. During the initial week of the experiment two men shared each of four "space sinks" for washing purposes. When the wash water from these space sinks was tested microbiologically, the numbers of bacteria found coupled with the presence of many coliform organisms contributed to the decision to abandon this method of washing. The substitution of clean face cloths twice during the trial which were rinsed in the regular wash bowl temporarily cleared up this undesirable situation, but as soon as the wash cloths were used continuously, the same unsatisfactory condition developed, although not quite as acutely.

CONCLUSIONS

The experimental conditions imposed on the subjects in this trial did not appear to create a microbiological situation that would be harmful to the subjects for the length of time encompassed in this test. A build-up of bacteria did occur both with respect to the various body areas examined and to the environmental areas in the chamber and cottage, but the increase in bacteria appeared to have plateaued or decreased before the end of the trial and most of the bacteria involved in the build-up are generally not considered to be pathogenic. Pathogenic bacteria were brought to the experimental site in or on the subjects, but these bacteria did not cause a frank illness and with one possible exception did not appear to be transferred from one subject to another.

The predominating fecal flora was affected to some extent apparently by the diet eaten, and the types of bacteria that occurred after the subjects had been eating the experimental diet for several days were heavy gas-formers which might produce increased flatulence.

The bacterial build-up in the wash water and on the neck of the urine bottle was caused by generally undesirable types of bacteria, and the suits showed many residual microorganisms of the type associated with the human body.

As the result of these studies, recommendations for consideration in future studies include a thorough microbiological study to be done on future subjects to detect carriers of potentially pathogenic bacteria prior to the experiment, better methods of cleaning the face and hands, collecting urine and cleaning the suits to prevent microbiological contamination.

APPENDIX A

TECHNIQUES

Collection of Samples

The procedure for the collection of samples from the body areas, feces, environmental and miscellaneous areas are described for each class of samples.

Body areas.- Two swabs from each body area were collected by subjects in the chamber and cottage at 7-8 AM on specified sampling days (see Table 11-1). One swab was placed in 10 ml of Gall's broth plus cysteine for anaerobic culturing and one was placed in 10 ml of heart infusion broth for aerobic culturing. Collection was made by swabbing a 1" x 1/2" area as follows:

(1) Eye (first sample period only) - Evert lower eyelid and swab confunctiva gently, following contour of eyelid with swab.

(2) Groin - Swab from front toward rear.

(3) Axilla - Swab with care to get specimen from skin below hair area.

(4) Throat - While depressing tongue, swab tonsillar area.

(5) Buccal Area - Swab gingival margin adjacent to the last upper right molar.

(6) Glans Penis - Swab specified area of skin of glans, or between glans and foreskin.

For purposes of approximate quantitation each swab was considered to contain about 0.01 gm of sample.

Feces. - Fecal samples were eliminated into sterile containers and were cultured immediately. Composite samples were taken by inserting a standard loop into five separate areas of the fecal mass, and the 0.01 gm sample was placed into 10 ml Gall's broth plus cysteine, representing a 10^{-3} dilution of the feces. Samples were received as indicated on Table 11-2.

Environmental areas. - Aerobic cultures were made from several room areas, using two procedures:

(1) Sedimentation plates of blood, MacConkey's actinomyces agar, and phytone yeast were made from the following room areas as indicated on Table 11-1 by exposing the plates for thirty minutes.

TV
Table
Bed
Personal hygiene area

(2) Swabs were taken from the following areas of the chamber and cottage, placed into 10 ml broth and incubated aerobically as indicated in Table 11-1

Telephone (chamber only)
Filter (cottage only)
Toilet seat
Transfer lock handle
Two buttons (chamber only)
Table top (cottage only)
Water faucet
Bed post
Floor area
Chair

Miscellaneous items. - Cultures were made from the lips of the urine collection bottles, water squeezed from face cloths, from three areas of the suit prior to donning and from the air coming from the suit vents after donning. Samples were taken at the intervals indicated in Table 11-1.

(1) Urine Bottles - The urine bottles were cultured by swabbing around the outside rim of the urine bottle and placing the swab into 19 ml of Gall's broth plus cysteine, which represented a 10^{-3} dilution of the original samples.

(2) Wash Water - The wash water was cultured by taking 0.5 ml of the squeezed water from the wash cloth and adding it to 10 ml of Gall's broth.

(3) Suit Areas - The suit areas were sampled at the axilla, crotch, and right boot prior to donning by taking two swabs, one of which was placed in 10 ml of Gall's broth plus cysteine for anaerobic culturing and the other in 10 ml of heart infusion broth for aerobic culturing.

(4) Suit Vent - Forty-eight hours following the donning of the suit, samples were taken by holding a blood plate approximately one foot from the vent of each suit for about fifteen seconds. This was done subsequently two more times at approximately weekly intervals.

Primary Culturing

Primary culturing of body areas (other than feces).

Aerobic: The aerobic swab collected by each subject for each body area was emulsified in 10 ml of broth into which it had been placed when collected and serial dilutions in 4-6 tubes were made in heart infusion broth diluting by 1:10, 1:20, or 1:40 depending upon the numbers of organisms expected to be present in the sample based on previous experience. The exact procedure for culturing is shown in Figure 11-1. The heart infusion broth series was incubated aerobically and observed for growth at 24 and 48 hours. All cultures showing growth were smeared. Aerobic plates were made on the media listed in Table 11-3, for each of the body areas by spreading 0.1 ml of broth from the lead tube plus one on the plate using a glass spreader, and additional blood agar plate was made in the same manner from the lead tube. Aerobic count was taken from a blood plate.

Anaerobic: The anaerobic swab from each body area collected by each subject in the chamber or cottage was emulsified in 10 ml of broth into which the swab was placed when collected and the sample was then serially diluted through 4-6 tubes of Gall's broth containing cysteine by making dilutions of 1:10, 1:20, or 1:40 depending rupon the numbers of organisms expected to be found in that particular sample. The procedure is essentially the same as the aerobic method is depicted in Table 11-1. The cultures were then incubated in a CO_2 anaerobic incubator at 37°C and were observed after 24 and 48 hours for growth. Agar shakes in Gall's agar were made from the top 2 or 3 dilutions showing growth and slides were made on all cultures that showed growth. The agar shakes were then transported from the site of primary culturing to Repuclic Aviation Corporation's laboratories where the cultures were further studied. Anaerobic Brewer plates were made with 1.0 ml of the appropriate dilution of the throat, buccal and glans penis samples using Gall's agar with cysteine. A blood agar plate, and where indicated a chocolate agar plate, was inoculated with 0.1 ml from the lead tube plus one and spread over the surface of the plate with a sterile, bent-glass rod. A pour plate of Rogosa's agar, when appropriate, was inoculated with 1.0 ml of the lead tube plus one. These plates were incubated in the CO_2 anaerobic incubator. Deep blood agar shakes were made from the buccal sample only by placing 1 ml of blood into a cooled Gall's agar shake and inoculating with 0.2 ml of the lead tube plus one of the buccal sample.

Primary culturing of feces.

Aerobic: The aerobic plates from the fecal sample were taken from the anaerobic broth series. One-tenth ml from the lead tube plus one was spread on one blood plate, and all other aerobic plates listed in Table 11-3 under media for feces including the second blood plate were made by spreading - .1 ml of the lead tube plus two on the plate with a glass rod. 0.1 ml of the lead tube plus two was also used as inoculum for a pour plate for aerobic count. One ml of the lead tube plus two was used as inoculum for the Rogosa's pour plate.

Anaerobic: The anaerobic broth series for the primary culture of the fecal sample was essentially the same as that used previously by Gall, et al.[8] for culturing rumen anaerobes, and which has been recently successfully adapted in the Republic laboratories to the culture of human feces.[9] This is a technique that can be adapted easily for work under field conditions. Figure 11-3 gives a schematic representation of the primary culturing technique, which is modified to culture from a standard loopful (0.01 gram) of freshly eliminated fecal material. Samples were cultured within fifteen minutes of elimination.

The fecal material on the standard loop was placed directly into a tube containing 10 ml of Gall's broth prepared with two drops of cysteine and one drop of sodium bicarbonate. This tube was considered to represent roughly a 10^{-3} dilution to the fecal contents. Serial dilutions were made into 11 additional tubes containing 9 ml of Gall's broth prepared as above by transferring 1 ml from the inoculated tube into the next tube, etc. The top 10 tubes were labeled 1 to 10 and were incubated anaerobically in a CO_2 incubator until growth occurred usually within 48 hours. Observations were made at 16 and 24 hours and daily thereafter.

These ten tubes were considered to approximate a dilution of the sample from 10^{-4} to 10^{-13}. No dilution blanks were used, as each tube containing broth acts as a dilution blank for the next tube in the series. From tubes 5 and 6 pour plates were made into anaerobic Brewer dishes using Gall's medium with cysteine and bicarbonate added.

The top three tubes showing growth were subcultured into agar shakes using Gall's medium to observe the anaerobic or aerobic character of the growth and to preserve the cultures for transport and for purification and study. Each culture was stained by Hucker's modification of the Gram stain and the slide was observed microscopically.

In addition, blood plates were made from the 10^{-3} and 10^{-4} dilution of the fecal sample by the same technique as the aerobic plates from the other body areas and were incubated in the same manner as the anaerobic broth series. Growth was recorded after 24 hours and the plates were treated in the same manner as the aerobic blood plates to be described below.

Primary culturing of environmental areas. - The sedimentation plates made from the several room areas indicated above were exposed for 30 minutes, incubated at 37°C and were observed for growth at the end of 24 hours. The swab cultures taken from the various environmental areas were placed in broth, incubated aerobically at 37°C and smears were made of all broths that grew.

Primary culturing of miscellaneous items.

Urine Bottles: Using the 10^{-3} broth dilution containing the swab, serial dilutions representing 10^{-5} and 10^{-7} dilution were made by taking 0.1 ml from the previous tube into 10 ml of Gall's broth. In addition 0.1 ml from the 10^{-3} tube was streaked by the usual procedure on MacConkey's plates, Mitis salivarius and two blood plates, one of which was incubated aerobically and one under CO_2. Pour plates using Gall's agar were made using 1 ml of the 10^{-3} and 10^{-7} dilutions are inoculum.

Wash water: Using the lead tube containing the wash water as inoculum, 0.5 ml was transferred serially into three more tubes containing 9 ml of broth. These broths were incubated aerobically. Pour plates were made using Gall's agar inoculated with 1 ml of the first and third tube in the series with Gall's broth.

Suit areas: Using the lead tube as inoculum one more serial dilution was made from each suit area and blood plates were streaked from this dilution from each suit area and incubated aerobically.

Suit vent: The exposure plates made by holding the blood plates 1 foot for 30 seconds were incubated aerobically and were observed for growth.

Subject 4: Approximately one week after donning the suit Subject 4 noticed a bloody discharge near the area of the buttocks and at that time several other pustules were noted on his upper torso near the vent in the suit. Swabs for culture were taken from the pustules in both areas and placed on blood plates.

Secondary Culturing

Aerobic. - All the cultures from the Petri dishes incubated aerobically and under CO_2 from all body areas, feces, environmental areas and miscellaneous items, were returned to the Republic Aviation Corporation laboratories and selected colonies were picked into broth. Cultures picked from the anaerobically incubated plates were incubated in the CO_2 incubator while all other colonies from the aerobic plates were processed by the usual aerobic methods. The cultures were smeared, stained, observed microscopically, separated according to morphological types, and processed according to the schema if applicable.

(1) Staphylococci and Micrococci

Mannitol salt agar
All positives confirmed with coagulase test
Phage typing on selected cultures

(2) Streptococci

Alpha hemolysis
Beta hemolysis
Gamma hemolysis
Differential sugars
Typing

(3) Pneumococci

Pneumococcus broth - bile solubility

(4) Haemophilus

Identified with typing antisera

(5) Neisseria

Sugar screen test

(6) Lactobacillus

pH in glucose broth

(7) Gram Positive Rods

Loeffler's
Ziehl Neelsen
Sporulation
Gelatin
Sugar screen
Hydrolysis of starch
Detection of hyphae (Proact. or Nocardia groups)
Tellurite
Catalase
Hemolysis on sheep blood
CO_2 requirement

(8) Gram Negative Rods

TSI
Indol
Methyl red
Voges-Proskauer
Simmon's citrate
Urease
Nitrate
Litmus milk
Motility
Gelatin
KCN
Phenylalanine

(9) PPLO

Dienes' stained agar technique

(10) Fungi and Actinomyces

WET mount
Lactophenol cotton blue
Corn meal agar
Fermentation series

(11) Spirochetes

Blood broth (morphology)
Darkfield when indicated
Vincent's stain

(12) Protozoa

Identification by selective stains

Phage typing: Phage typing of staphlococci isolated in the course of this contract were done by Dr. John E. Blair, Head, Department of Microbiology, Chariman International Subcommittee of Phage Typing of Staphylococcus. The Roosevelt Hospital, Long Island, New York.

The cultures of staphylococci submitted for examination were isolated from various body surfaces of the test subjects or from environmental sources in the chamber and the cottage. The 33 cultures received included 25 coagulase-positive strains and 8 coagulase-negative strains. One culture proved unsuitable for study because of poor growth.

For typing, the 22 standard phages recommended by the International subcommitted on Phage typing of Staphylococcus were used.[10] The phages are: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 83A, 42D, 81 and 187. In addition, four "experimental" phages from Dr. Blair's personal collection were used which sometimes have proved useful for the identification of certain strains that are not typable with the standard phages; these phages were B5, D, 77ad and UC18.

The methods recommended by the Subcommittee were employed. Cultures were typed first with the routine test dilutions (RTD) of the phages. Those cultures which showed no significant lytic reactions at RTD were then retyped with the phages in concentrations 10000 times stronger than RTD. The phage pattern, or "type", of a culture is reported by listing those phages that produced significant lysis. Cultures showing no significant lysis at either RTD or 1000 x RTD were recorded as nontypeable.

Although it is well known that coagulase-negative strains of staphylococci are not susceptible to lysis by the typing phages, the seven viable coagulase-negative cultures nevertheless were submitted to typing together with the coagulase-positive cultures because these strains had been isolated from Subject 4 who had pustules and from Subject 7 because of the high frequency of isolation of staphylococci from this subject. None of the coagulase-negative cultures was typable.

Anaerobic

Body areas other than feces: The agar shakes made from the dilution series and the colonies picked from the Brewer plate (when made) were separated into two groups depending upon the degree of anaerobiosis. The obligate anaerobes were processed in the same way as the fecal anaerobes described below with the exception that many of the cultures particularly from the buccal area, throat and glans penis were identified from Bergey's manual (6) rather than from the anaerobic "key". The facultative anaerobes were grouped according to morphology and were processed as indicated for the aerobes of similar morphology.

Feces: The agar shakes from the top three tubes of the cultural series were processed in the following manner. The agar shake cultures were transferred to Gall's broth plus cysteine and incubated anaerobically until growth occurred. Gram stains were made, and if the cultures were pure, they were immediately screen tested as described below. Cultures showing two or more distinct morphological types of bacteria were purified by plating using the following anaerobic technique. A needle of the impure broth culture was spread on a bed of Gall's agar which was then covered with a layer of Gall's agar with added cysteine. The plates were incubated anaerobically in a Torbal jar with hydrogen and 10% CO_2 and discrete colonies were picked. Selected colonies on the anaerobic Brewer dishes originating from tubes 5 and 6 were picked and treated like the subcultures from the agar shakes as described above. The physiological studies of the pure cultures isolated from the feces included the following screen tests:

(1) Gram stain to observe morphology
(2) Final pH in 0.1% glucose broth
(3) Fermentation of the following sugars in Gall's media with glucose omitted (Glucose, Sucrose, Lactose, Dextrin - sugars added at 0.1% level aseptically after autoclaving)
(4) Growth in Gall's broth with no carbohydrate added
(5) Liquefaction of 12% gelatin in Gall's media minus carbohydrate
(6) Growth and reaction in litmus milk (to which 0.05% bovine albumin and 0.1% of peptone have been added)
(7) Growth in agar shake containing Gall's Media

All media contained bicarbonate and all media except the agar shake contained cysteine to produce an Eh of about -200 mv. The results of the screen tests on each anaerobic culture were compared with a "key" derived from tests done on NASA study (4). This "key" consists of the results of the screen tests from the most frequently occurring fecal anaerobic cultures and is designed to group similar bacteria. Each different screen test pattern is assigned an FA, FN or GD number. The GA and GD types are used to designate obligate anaerobes.

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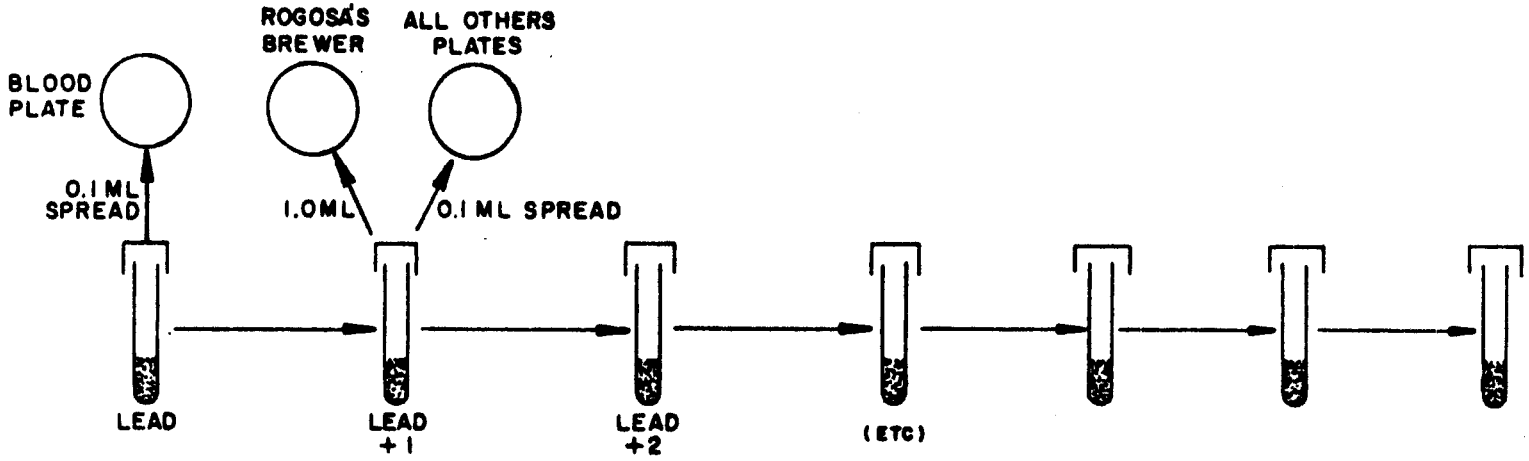
TABLE 11-1

SCHEDULE OF SAMPLES FROM THE BODY AREAS AND THE ENVIRONMENT

AREAS \ DATE	TEST CONDITION: AMBIENT								ALTITUDE - NO SUITS							ALTITUDE PLUS SUITS														AMBIENT PLUS SUITS							
	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ALL BODY AREAS	1							2			3				4			5				6			7				8			9				10	
ENVIRONMENTAL CHAMBER & CONTROL COTTAGE	1							2		3		4			5		6		7			8		9		10			11		12		13		14	
MISCELLANEOUS SAMPLES WASH WATER OR CLOTHS								1							2							3							4							
URINE BOTTLES										1								2							3											
SUIT	SAMPLES TAKEN FROM SUITS AFTER CLEANING AND PRIOR TO DONNING																																			
SUIT VENT																		1								2							3				

NUMBERS REFER TO SAMPLE NUMBER

CONTROLS WERE AT AMBIENT AT ALL TIMES



Plates made from Lead tube + 1 listed for each body area in Table 47.

All plates except fungi incubated at 37°C. Fungi incubated at room temperature.

Sample No. 1, 2, 3 - amount transferred - 1 ml
Sample No. 4 - amount transferred - 0.5 ml
Sample No. 5 and thereafter - amount transferred 0.5 ml first tube;
0.25 ml thereafter.

Number of tubes in culture series:

Eye	1 - 4	Buccal	1 - 6	Groin	1 - 5
Throat	1 - 7	Axilla	1 - 4	G.P.	1 - 4

Figure 11-1 Aerobic or Anaerobic Cultural Series for All Body Areas