INTRODUCTION

The prevention and control of infectious diseases during Skylab missions are directly related to crew safety and mission succuess. Within the Skylab Orbital Assembly, crewmembers will exist in a dynamic relationship with their own microflora, that of other crewmembers, and those organisms which are generated from growth within the spacecraft. Under those conditions it is expected that man-microbe ecological patterns will tend to reach new equilibria. Such modifications and microbial alterations will be the complex resultant of the combined physical and biological factors that prevail.

Man has evolved in intimate and constant association with a complex microflora. Those organisms which are normally present in and on the body are referred to as the indigenous microflora. Most organisms which produce disease, usually referred to as pathogens, are normally not part of the indigenous flora and are referred to as exogenous in origin. The modes of transmission and pathological processes induced by the common pathogens are, for the most part, well understood. However, mechanisms operating in the production of disease by the indigenous flora, referred to as endogenous disease, are poorly understood. It is well established, however, that damaging effects of the indigenous microflora become manifest chiefly when individuals are placed under conditions that differ from those under which the normal equilibrium between host and microbes became established. The spacecraft environment represents a new and unique environment. Therefore, the major objective was the qualitative

and quantitative identification of microbial alterations mediated by such factors as confinement, diet, restricted activity and environmental parameters which are characteristic of Skylab but are not peculiar to space flight. Analysis of microbial alterations at one G will aid in the interpretation of alteration mediated by the reduced G environment of Skylab.

A second facet of the problem related to habitability and microbial contamination of the chamber interior. Data on aerosol and surface bio-burdens were required to evaluate the effectiveness of Skylab baselined microbial contamination control procedures. This information has direct bearing on crew health and materials degredation within the orbital assembly.

Crew microbiological investigations were performed before, during and after the SMEAT and chamber environmental microbial monitoring was performed during and after SMEAT. The overall purpose was to obtain data on man-microbe-environment interactions which reflect the effects of ground-based Skylab environmental parameters on crew microbial burdens and the microbial ecology of the SMEAT chamber. The functional objectives of the Detailed Test Objectives covering Crew Microbiology (71-19) and Chamber Environmental Microbial Monitoring (71-28) were as follows:

1. Evaluate the Skylab crew microbiology protocols.

2. Determine the aggregate effects of Skylab environmental parameters on the microflora of crewmembers.

3. Identify SMEAT, chamber-mediated alterations not related to zero gravity.

4. Ascertain and determine the significance of transfer of microflora between crewmembers.

5. Observe overt effects of microflora changes on crew health and/or identify biological trends having significance for Skylab and other extended duration missions.

6. Define the normal fecal flora of test subjects and determine changes occurring during specific time periods relative to diet and/or chamber confinement.

7. Determine the microbial content of Skylab food items for comparison with fecal flora data.

8. Obtain data on the habitability of the environment as influenced by aerosol and surface burdens of viable microorganisms.

The major portion of the studies were carried out by the Medical Support Branch, Bennie C. Wooley, Ph. D., Chief, in the MSC Lunar Receiving Laboratory under the direction of James L. McQueen, D.V.M., Dr. P. H.; Richard C. Graves, B.S.; Bernard J. Mieszkuc, M.S.; and Gerald R. Taylor, Ph. D. Laboratory analyses were performed by Northrop Services, Inc., under the supervision of Cyril J. Hodapp, Ph. D.; Walter L. Ellis, M.A.; Mary Henney, Ph. D.; Florence Pipes, M.S.; Riley Wilson, B.S.; and Paul Phillips, M.S. and with the participation of Mary Arredondo, B.S.; Glee Decelle, B.S.; Nem Bryan, B.S.; Carolyn Carmichael, B.S.; Edward Carter, M.S.; Robert David, B.S.; Sara Fan, B.S.;

Theron Groves, B.S.; John Harrison, B.S.; Donald Johnson, M.S.; Kathryn Kropp, M.S.; Richard Long, B.S.; Thomas Molina, B.S.; Mike Parson, B.S.; Ronald Wrenn, B.S.; Gloria Hayes; Tal Holmes, B.S.; C. Park; James Edwards; Bryon Gammage, B.S.; Marian Lewis, M.S.; Jim Romero, B.S.; Marge Arseno; Glenn Burks; Clara Lartigue; Doris Morris; Charles Neal; Marcello Noyola; Tommie Thompson; Russell Watson; and Alice Whittington.

In this report the results of DTO's 71-19 and 71-28 have been combined. Studies of the anaerobic fecal flora were conducted by Edwin C. Moore, under Contract NAS9-12601 and are presented as written by the Principal Investigator in a separate section.

MATERIALS AND METHODS

The following is intended to give the reader only a general understanding of the methods and materials used during collection and processing of specimens and environmental samples collected before, during and after the SMEAT. All evaluations were performed according to detailed test plans on file in the Medical Support Branch.

Specimen collection regimens and processing schema are summarized in Table I. Sterile phosphate buffered saline (PBS) was used throughout the study as the initial collection medium for bacteriology and mycology. Earle's Balanced Salt Solution containing 0.5 percent gelatin was used as the diluent for specimens for virologic analyses. Gargle samples were obtained by having the crewmembers gargle and "wash" the oral cavity with

TABLE I. - SMEAT MICROBIOLOGY-SAMPLING SCHEDULE

	Pre-Chamber												Intra-Chamber								Post-Chamber		
Julian Date	131	138	145	152	159	166	173	180[a]	187	194	201	208[b]	215	222	229	236	243	250	257	264[c]	271	278[d]	306
Specimen																							
Gargle	XO	X	X	X	XO	X	X	X	X	X	X	XO	X	X	X	X	XO	X	X	XO	X	XO	X
Throat Swab	O				O							O					O			O		O	
Nasal Swab			X		X		X		X			X	+		X		X		X	X		X	X
Skin Swabs	X		X		X		X		X			X	+		X		X		X	X		X	X
Fecal	ZO			Z	O		Z				Z	O		Z			ZO			ZO		ZO	Z
Urine	XO				XO		X		X			XO			X		XO			XO		XO	X
Strip Samples													X	X	X	X	X	X	X	A			
Air Samples													B	B	M	B	B	B	B	B			
Chamber Swab Samples																				A			

a - Start Skylab diet - June 28, 1972

b - Chamber close - July 26, 1972

c - Chamber open - September 20, 1972

d - End Skylab diet - October 7, 1972

X - Bacteriology (aerobes, anaerobes) fungi and yeast

\+ - Medically significant organisms only

B - Bacteriology medium used

M - Mycology medium used

A - Aerobic bacteria, yeasts and fungi

O - Viruses and Mycoplasma

Z - Anaerobic and Facultative bacteria, yeasts and fungi

30 cm^3 of PBS and return the material to the original container. Skin and nasal sampling was accomplished using two premoistened calcium alginate swabs for each designated area. After sampling an approximate 13 cm^2 area, exceptions being the external nares and external auditory canals, swabs were placed individually in tubes containing five cm^3 of PBS. Mid-stream urine samples of approximately 60 cm^3 were collected in sterile containers. Fecal samples were collected in sterile "poly" bags contained within a sterile carton. Two stainless steel strips measuring 2.54 cm x 5.08 cm were collected weekly from each of eight locations. The strips were placed individually in sterile metal screw cap containers. Ten minute air samples were collected using a standard six-stage Anderson Cascade Air Sample calibrated for one CFM (0.028 m^3 per min) of air at 5 psia, (35.2 gm per cm^2 absolute). Before chamber swab samples were taken the area was first outlined with a 13 cm^2 template. The area was then scrubbed with a moistened swab and dried with a second swab. Both swabs were placed in a single tube containing 5 cm^3 of PBS.

Specimens collected during the prechamber period were processed as soon as possible, usually within 30 minutes, after collection. Specimens collected during the chamber run were passed out thru the transfer lock as soon as possible after collection. Ordinarily, about one hour elapsed between the time of collection and laboratory processing of intra-chamber samples.

Crew Mycology - Samples were collected from each crewmember according to the schedule shown in Table I and employing the methods outlined above.

One-tenth cm^3 aliquots of each sample were inoculated for quantitation onto: (1) corn meal/malt-extract/yeast-extract agar with antibiotics (CMMY), (2) Sabouraud's dextrose agar with antibiotics (SAB), and (3) Czapek-Dox agar (CD). Following incubation at 25°C (298°K) for 120 hours, isolated colonies were counted and recovered from the agar surfaces for identification.

In addition to the above quantitative isolation scheme, a regimen was employed to recover fungi present in low numbers. Four cm^3 of diluent of skin-swab samples and urine were each centrifuged at 5,000 rpm (12,000 x G) for 15 minutes. The supernatents were discarded and the sediments were inoculated onto CMMY, SAB, and CD agar. After incubation at 25°C (298°K) for 120 hours, those species which were not recovered from the first set of isolation plates were removed for identification. All filamentous fungi and yeasts were identified to species where possible.

Crew Bacteriology - Samples were collected from each crewmember as previously described. One-tenth cm^3 aliquots of each sample were inoculated onto quantitation agar. The specimens and the media utilized were as follows. (1) skin samples: blood agar, Staphylococcus - 110 agar, and letheen agar for aerobes; blood agar containing vitamin K and hemin for anaerobes, (2) throat-mouth gargle: blood agar, Staphylococcus-110, Mitis - Salivarius agar, and chocolate agar containing bacitracin for aerobes; Rogosa agar, Paromomycin - Vancomycin - Menadione agar, and blood agar with vitamin K and hemin for anaerobes, (3) urine: blood

agar, Staphylococcus - 110 agar, MacConkey agar, and chocolate agar containing bacitracin for aerobes. Following incubation at 35 °C (308°K) for 24 hours, isolated colonies were counted and recovered from the agar surfaces for identification.

In addition to the above quantitative isolation scheme, a regimen was employed to recover aerobic bacteria in low numbers. A standard loop of each sample was inoculated into trypticase soy broth. This broth was incubated at 35°C (308°K) for 24 hours. A loop full of the broth was then streaked on the above media for isolation. After incubation of the plates, those species which were not recovered from the first set of isolation plates were removed for identification. All bacteria were identified to species where possible.

Crew Virology - Specimens were obtained for analysis for viruses and mycoplasma are shown in Table I. Twenty percent (w/v) stool suspensions were prepared by homogenizing the specimen with Earle's Balanced Salt Solution in a Sorvall omnimixer. The suspension was centrifuged for 30 minutes at 2500 rpm (6000 x G). The supernatant was adjusted to neutrality and treated with 5000 units of penicillin, 5000 µg of streptomycin, and 5 µg of fungizone per cm^3.

The pharyngeal swab gargle specimens were processed by expressing the medium from the swab and transferring it to the gargle. A portion of the specimen was reserved for mycoplasma analysis and the remainder was centrifuged for 30 minutes at 2500 rpm (6000 x G). The supernatant was adjusted to neutrality. The portion used for tissue culture challenge

was treated with 1,000 units of penicillin, 1,000 µg of streptomycin, and 5 µg of fungizone per cm^3 and the portion used for embryonated egg challenge was treated with 1,000 units of penicillin and 1,000 µg of streptomycin per cm^3. The portion reserved for mycoplasma analysis was treated with 10,000 units of staphcillin and 10,000 units of penicillin per cm^3.

The urine specimens were adjusted to neutrality and a portion reserved for mycoplasma analysis. The remainder, used for tissue culture challenge, was centrifuged for 30 minutes at 2500 rpm (6000 X G). The antibiotic treatment for the urine specimens was similar to the pharyngeal swab-gargle specimens.

Primary rhesus monkey kidney (RhMK), primary human embryonic kidney (HEK), and diploid semicontinuous human embryonic lung (WI38) were utilized. Six culture tubes of each cell culture type were inoculated with 0.2 cm^3 of treated specimens as shown in Table II.

Table II.
Cell Culture Inoculation Scheme and Incubation Temperatures for Analysis of SMEAT Crewmember Specimens

Cell Culture	Specimen		
	Urine	Feces	Pharyngeal swab/gargle
HEK	X(36°C; 309°K)	X(36°C; 309°K)	X(36°C; 309°K)
RhMK		X(36°C; 309°K)	X(33°C; 206°K)
WI38	X(36°C; 309°K)		X(33°C; 306°K)

The cultures were rolled in roller drums at the designated temperatures and were examined daily by direct microscopic examination. At 10 day

intervals, 2 blind subpassages were made. At each passage, control and inoculated tubes were tested for hemadsorption using guinea pig erythrocytes at ambient temperature and chicken erythrocytes at 4°C (277°K). The final cultures were challenged with vesicular stomatitis virus to detect inapparent infection.

Treated stool specimens were inoculated into suckling mice. Each specimen was inoculated intracerebrally, intrascapularly, and intraperitoneally into a litter of mice, less than 24 hours old. The mice were observed daily and subpassaged after 14 days.

The treated pharyngeal swab/gargle specimens were inoculated into the amniotic and allantoic sacs of embryonated eggs from White Leghorn chickens. The inoculated eggs were incubated at 36°C (309°K) in increased humidity and observed daily for evidences of infection. At 4 day intervals, 2 blind subpassages were made. Subpassage material from cell cultures, suckling mice and embryonated eggs was tested for hemagglutinins using chicken, guinea pig and human O erythrocytes at ambient temperature and 4°C (277°K).

Mycoplasma isolations were attempted from treated pharyngeal swab-gargle and urine specimens. The medium developed by Hayflick and modified by Barile was used as an agar and a broth. Shepard's medium for the primary isolation of small colony mycoplasma (T-strains) was also used. The plates were incubated at 36°C (309°K) in a humidified 5 percent CO_2 - 95 percent air environment. The cultures were examined daily for growth and the isolants were identified. The specimens

inoculated into Hayflick's Broth (pH 7.2) were subpassaged 3 times at 4 and 7 days into broth and agar. A-3 and U-9 broths and A-6 agar plates of Shepard's Medium (ph 6.0) were utilized. The broth cultures were subpassaged into A-6 agar plates after 18 hours incubation

Environmental Microbiology - Each stainless steel strip was transferred to a flask containing 16 cm^3 of a 0.2 percent solution Tween 80. The strip was insonated for 2 minutes. Eight cm^3 of the suspension were then transferred to a screw cap tube and heat shocked at 80°C for 20 minutes. The remaining eight cm^3 were processed in the following manner. Serial dilutions of 1/10 through 1/10,000 were prepared in the Tween 80 solution. For each dilution, 2 aliquots of 0.2 cm^3 were placed on 2 Trypticase Soy Agar (TSA) plates and 2 aliquots of 0.2 cm^3 were placed on blood agar plates. The TSA plates were incubated under aerobic conditions and the blood plates were incubated under anaerobic conditions at 37°C (310°K). The heat shocked portion was handled in the same manner as the non-heat shocked portion. Aerobic plates were counted after 48 hours, anaerobic plates were counted after 96 hours. Following quantitation all plates were examined for different colony types. All representative colony types were isolated and identified to species by standard procedures when possible.

After using the Anderson Air Sampler the unit was passed out of the chamber and the plates removed. The six plates were incubated 48 hours at 37°C (310°K) and colonies were counted. Each plate was then examined qualitatively in the same manner as those from the stainless strips.

Each swab sample was taken at chamber close-out and vortexed for two minutes in an attempt to disolve the swabs. Based on results from the stainless steel strips, it was deemed not necessary to heat shock or analyze the swab samples for anaerobes.

After insonation serial dilutions of 1/10, 1/100 and 1/1000 were made using 0.5 cm^3 of sample in sterile phosphate buffer. Duplicate blood agar spread plates were prepared for each dilution using 0.5 cm^3 per plate. All plates were incubated at 37°C (310°K) for 48 hours and then examined quantitatively. Following quantitation the plates were examined for different colony types and treated in the same manner as the aerobic, non-heated treated stainless steel strip plates.

Original material from both the strip samples and swab samples were examined for fungi and yeasts using CMMY containing antibiotics. All plates were incubated at room temperature for 7 days. All filamentous fungi and yeasts were isolated and identified using standard procedures.

RESULTS AND DISCUSSION

SKIN BACTERIAL FLORA STUDIES

The combined incidence of the predominant bacteria found on seven skin sites from all SMEAT crewmembers is presented in Table III. It will be noted from Table III that the gram positive bacilli and the gram negative flavobacteria, occurring naturally in water and soil, were not, with one exception, recovered from the skin after the crewmembers were

TABLE III.- INCIDENCE OF PREDOMINANT BACTERIA ON SEVEN SKIN SITES[a] DURING THE PRECHAMBER, INTRA-CHAMBER, AND POSTCHAMBER PERIODS

Astronauts 1, 2, & 3	Prechamber (6)[b] C-77 thru C-0[f]		Intra-chamber (4) C+21 thru C+56		Postchamber R+14
	No. Isolants	Mean[d]	No. Isolants	Mean	No. Isolants
Bacillus spp. (10)[c]	49	8	1	--[e]	7
Flavobacterium sp.	29	5	0	--	0
Corynebacterium spp. (15)	198	33	133	33	26
Micrococcus spp. (7)	113	19	92	23	12
Staphylococcus epidermis (5)	141	23	73	18	12
Streptococcus spp. (6)	24	4	10	2	2
Mima spp. and *Moraxella spp.* (5)	15	2	7	2	1
Gram negative enterics[g]	17	3	1	--	1
Anaerobic cocci (7)[h]	41	7	3	--	1
Propionibacterium spp. (5)	100	17	39	10	6

[a] Neck, ear, axilla, hands, umbillicus, groin, toe web

[b] Number of times sampled

[c] Number of species, varieties or subgroups

[d] $\frac{\text{No. isolants}}{\text{No. samples}}$ = mean rounded to nearest whole number

[e] Less than 1

[f] Chamber minus 77 days thru chamber close

[g] Escherichia, Enterobacter, Klebsiella

[h] Peptococcus and Peptostreptococcus

isolated inside the chamber. These organisms, recovered during the prechamber period in approximately equal proportions from all crewmembers, are obviously not part of the indigenous flora and represent contamination of the skin by ubiquitous organisms. The species which were isolated are almost never involved in pathological processes. This represents an example of microflora alteration resulting from isolation in a closed environment.

The largest proportion of the indigenous aerobic skin flora of all crewmembers was found to be comprised of numerous species or subgroups of corynebacteria, micrococci and *Staphylococcus epidermis*. It may be noted that the incidence of these organisms on the skin was not affected by chamber confinement and/or chamber environmental factors.

The corynebacteria are widely distributed in nature and, except for *Corynebacterium diphtheriae*, are rarely implicated in human disease. *Corynebacterium spp.*, Evan's groups D and G and the lipophilic group VII accounted for 65 and 57 percent of the corynebacteria isolated from the skin during the prechamber and intra-chamber periods respectively.

S. epidermis, a part of the normal skin flora, produces disease less frequently than *Staphylococcus aureus* but may be responsible for cutaneous lesions of both minor and relatively severe consequence. *S. epidermis* was isolated in almost pure culture from an acne pustule of one crewmember during the prechamber period. Although the eruptions partially receeded following tetracycline therapy, these pustules continued to be present during the chamber run and recurrence of lesions

followed return to the normal postchamber environment. In debilitated and/or stressed individuals, *S. epidermis* has been incriminated as a causative agent of bacteremia and endocarditis. The incidence of *S. epidermis* types II and VI showed a relative decrease during the period of confinement.

The aerobic, gram positive micrococci from the third major group of indigenous skin flora common to all three crewmembers. These normal skin inhabitants have not been incriminated in disease. Subgroups 1, 2, 3 and 7 formed 85 and 76 percent of the micrococci recovered from the skin surfaces during the prechamber and intra-chamber periods respectively.

Various species of streptococci were found with approximately equal frequencies on all crewmembers. Group A, beta-hemolytic streptococci were not recovered from skin surfaces. During chamber confinement the incidence of *Streptococcus faecalis*, *Streptococcus mitis*, *Streptococcus salivarius*, and the alpha- and gamma-hemolytic streptococci showed a decrease in occurrence to four from a prechamber value of 21. Beta-hemolytic, not Group A streptococci increased in occurrence from three to six. Members of this genus are considered to form the dominant aerobic bacterial flora of the mouth. The strains recovered from the skin are a part of this normal flora and are not those normally responsible for acute disease. However, beta-hemolytic, non-Group A streptococci have been incriminated in septicemia and meningitis.

Sixteen of the 17 prechamber isolants of gram negative enterics, including species of *Escherichia* and *Enterobacter* and *Klebsiella*

pneumoniae were recovered from one crewmember. The significant decrease of these organisms during chamber confinement suggests that their occurrence on the skin may be dependent on continued exposure to the outside environment including varied social contact. Alternatively, one may postulate that chamber environmental factors are not favorable for survival of these organisms once shed from their normal habitat in the oral cavity and intestinal tract. In either case, the fact that these enterics did not increase on skin surfaces during chamber confinement is a suggestion that the personal hygiene regimens were adequate. Any significant build-up of these organisms in the oral cavity or skin would have been indicative of "bacterial flooding" (movement from the gut to the oral cavity and skin) and of serious concern. The gram-negative enterics are causal agents in a wide variety of pathological processes and the lack of build-up on the skin surfaces is encouraging.

It will be noted from Table III that during chamber confinement recovery of anaerobic cocci (*Peptococcus spp.* and *Peptostreptococcus spp.)* and *Propionibacterium spp.* was significantly decreased. Using Wilcoxon's Rank-Sum Test, one would reject the Null Hypothesis that the distribution of the counts before and during the chamber run have the same mean at a 0.01 level of significance. This appears to represent a rather striking example of microbial imbalance. Alternatively, it must be suggested that the difference may be only apparent and due primarily to the increased length of time that occurred between sample collection

and sample processing during the chamber run. Specimens collected during the prechamber period were always processed within one-half hour from the time of collection, whereas samples collected in the chamber were processed approximately one hour after collection. In examining this alternative, it must be pointed out that both gargle and skin-swab samples were collected, handled and processed in an identical manner. Further, all samples were subject to the same time lag in processing. An analysis of the oral flora data tends to refute the above as a viable alternative explaination for an inchamber decrease in anaerobic skin flora since those organisms were recovered from the oral cavity with equal frequency during both prechamber and intra-chamber periods. The decreased incidence of recovery of anaerobic skin flora may be attributed to either the effects of environmental factors (e.g., elevated pp O_2) or to personal hygiene regimens or both. Further testing using either human or animal subjects could be used for clarification.

The combined quantitative values from all crewmembers of the predominant bacteria recovered from seven skin sites during the prechamber, intra-chamber, and postchamber periods are shown in Table IV. With respect to the corynebacteria, the micrococci and *S. epidermis*, the ranges of values during all periods remained fairly constant and there was essentially no change in the number of these organisms recovered during the prechamber and intra-chamber periods. Analysis of data from individuals reveals that this pattern was characteristic of all three crewmembers. The numbers of streptococci recovered from the skin were

decreased by approximately one-log during the chamber run. Further analyses of the data reveal a slight increase in the Beta-hemolytic, non Group A streptococci and an overall decrease of one log in all other streptococci. There were no consistent patterns among the three individual crewmembers. Species of *Mima* and *Moraxella* showed similar patterns.

Although a significant decrease in the incidence of anaerobic cocci was shown in Table III, a large quantitative decrease is not reflected in Table IV. The mean values of anaerobic cocci recovered prechamber from the skin of crewmembers 1, 2 and 3 were 4.0×10^4, 1.1×10^4 and 1.4×10^4 respectively. The mean values of anaerobic cocci recovered from crewmembers 1, 2 and 3 during the chamber run were 9.7×10^3, 1.5×10^1 and zero respectively. The total burden of anaerobic cocci recovered from skin surfaces during the chamber run was due to the isolation of *Peptostreptococcus anaerobius* from one crewmember's umbilicus and the isolation of *Peptococcus prevotii* and *P. anaerobius* from the hands of another crewmember. These organisms were recovered only during the fifth week of the chamber run.

The incidence of isolation of Propionibacteria from the skin surface during the chamber run was reduced approximately 50 percent from the prechamber baseline. From Table IV it may be seen that this was not accompanied by a large reduction in the average number of propionibacteria recovered during the chamber run. Although the *Propionibacterium spp.* were isolated from fewer sites during the chamber

TABLE IV. - QUANTITATIVE VALUES OF PREDOMINANT BACTERIA ON SEVEN SKIN SKITES[a] DURING THE PRECHAMBER, CHAMBER AND POSTCHAMBER PERIODS

Isolants from Astronauts 1, 2 and 3	Prechamber (6)[b] C-77[c] thru C-0 Range[d]	Mean	Intra-chamber (4) C+21 thru C+56 Range	Mean	Postchamber R+14 No. Isolants	Postchamber R+42 No. Isolants
Corynebacterium spp.	9.5x5[e] to 7.3x6	2.2x6	5.0x5 to 7.5x5	6.5x5	1.1x6 1.1x6	
Micrococcus spp.	8.7x4 to 7.2x5	3.6x5	1.5x5 to 3.0x5	2.2x5	8.9x4	
Staphylococcus epidermis	7.3x4 to 4.2 x 5	1.8x5	4.9x4 to 1.1x5	7.7x4	2.2x5	
Streptococcus spp.	1.0x2 to 4.8x3	2.0x3	1.0x2 to 4.2x2	2.4x2	2.0x2	
Mima spp. and *Moraxella spp.*	7.2x2 to 5.0x4	1.1x3	6.0x1 to 4.9x2	2.5x2	5.4x2	
Anaerobic cocci[f]	3.7x4 to 1.6x5	6.6x4	0 to 3.9x5	9.7x4	9.0x3	
Propionibacterium spp.	2.3x4 to 1.6x5	9.3x4	9.3x3 to 9.0x4	4.71x4	1.6x3	

[a] Neck, ear, axilla, hands, umbillicus, groin, toe web

[b] Number of times sampled

[c] Chamber minus 77 days thru chamber close

[d] Range of total values from all crewmembers

[e] 9.5×10^5 viable cells per cm^3 of diluent

[f] Peptostreptococcus and Peptococcus

run, the total numbers remained almost constant due to the continued isolation of *P. acnes* from all three crewmembers. *P. granulosum*, recovered in comparable numbers and in frequency of occurrence during the prechamber period, was recovered only during the third week (first sampling period) of the chamber run.

Analyses of data relating both to the kinds and numbers of bacteria present on skin surfaces suggests that chamber confinement had little, if any, effect on the indigenous aerobic skin flora. There was a significant decrease in both the kinds and numbers of anaerobic bacteria recovered from skin surfaces during the period of chamber confinement. This decrease was due mainly to the almost complete disappearance of anaerobic cocci and significant reductions in two species of *Propionibacterium*. This may or may not be a reflection of changes occurring in the deep layers of the skin where anaerobic conditions, which favor the survival of these organisms, are maintained. It is entirely possible that the microbial burden is unchanged in the deeper layers of the skin and that the changes noted during the chamber run are only the result of accelerated inactivation of anaerobic bacteria due to the slightly elevated partial pressure of oxygen. Alternatively, it may be suggested that a reduction in whole-body washing resulted in changes in the skin surface environment which mediated towards lower skin surface burdens of viable anaerobic bacteria. Detailed data analyses reveal that decreases in anaerobic skin flora were not accompanied by increases in other genera or species of aerobic bacteria.

Nasal and Oral Bacterial Flora Studies - The total numbers of viable aerobic bacteria recovered from the nasal samples from the three astronauts during each sample period are presented in Figures 1a, b, c and d. When the counts recovered from all three subjects are summed, the value lies quite close to the median of 3×10^5 viable cells per cm^3 of diluent throughout the study. Although the total quantitation gradually decreased through day 227 and thereafter increased through day 263, such movements are not statistically significant (Figure 1a). Analysis of Figures 1b, 1c and 1d does not reveal changes which can be directly related to chamber confinement or consumption of flight food. The observed quantitations are largely due to corynebacteria which are generally present in concentrations of about 2×10^5 viable cells per cm^3 of diluent. The quantitations of this species remained fairly constant except for the decrease at day 208 and day 227 for Astronaut 1 and 2, respectively. These changes, however, cannot be shown to be due to chamber of flight food conditions.

Whereas *Staphylococcus epidermidis* quantitations remained very stable at 3×10^4 viable cells per cm^3 of diluent, the number of recovered viable *Staphylococcus aureus* cells increased from a prechamber high of 2×10^4 to about 2×10^5 during the chamber confinement period. The majority of *Staphylococcus aureus* isolations were made from throat-mouth gargle specimens. Isolations of strains reacting with Group I phages, primarily 52, 52A and 29 were made from crewmember 1 throughout the duration of the study. Isolation of strains typeable as

Figure 1. Aerobic Microorganisms Recovered from SMEAT Crew Nasal Samples

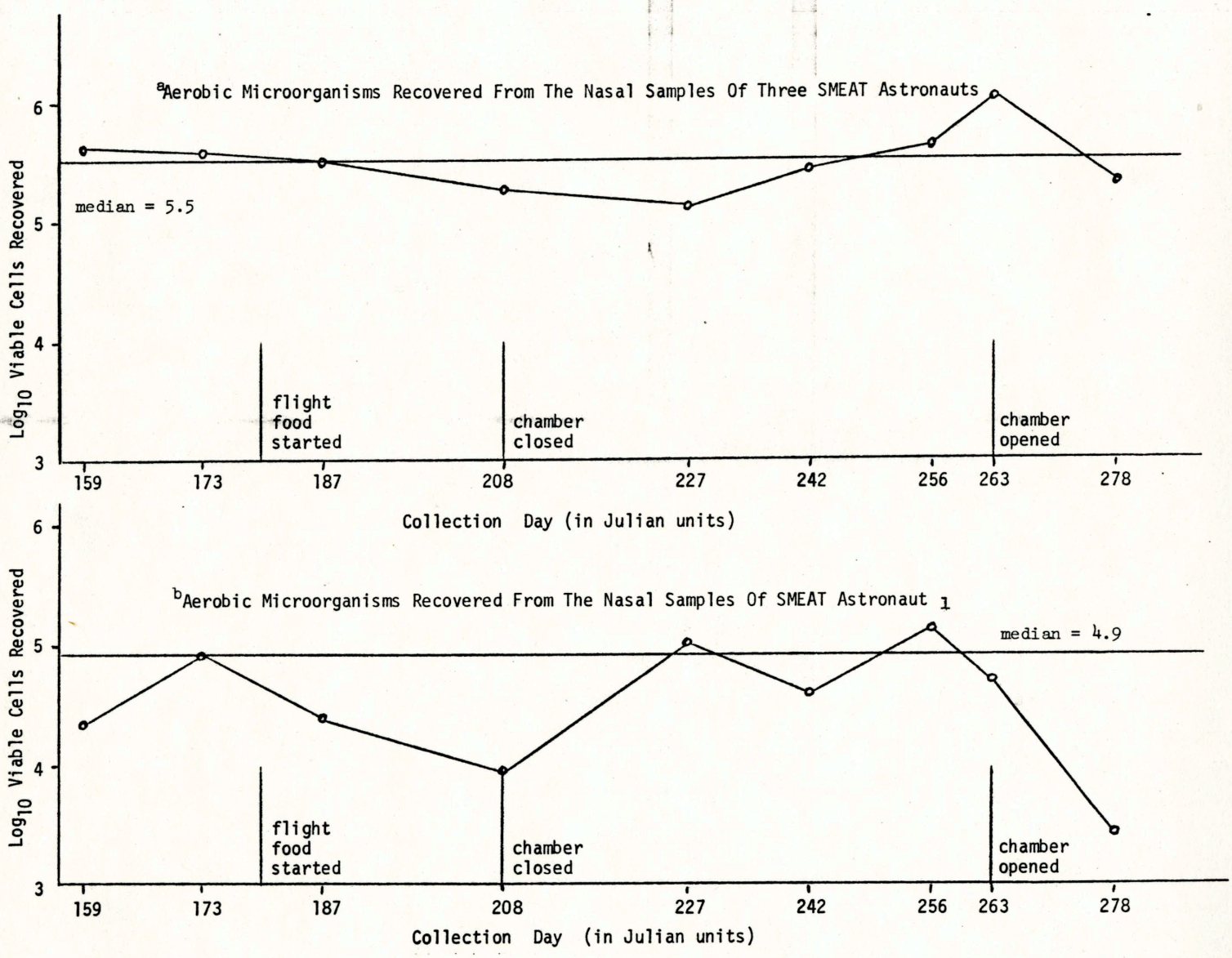
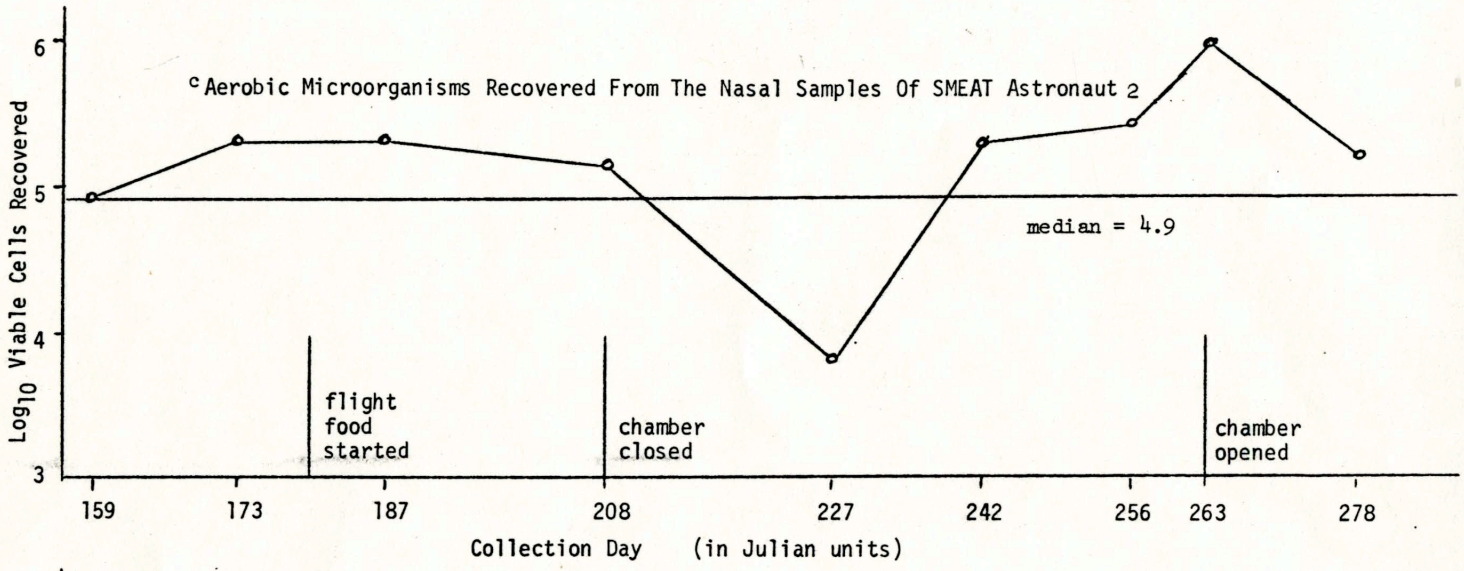
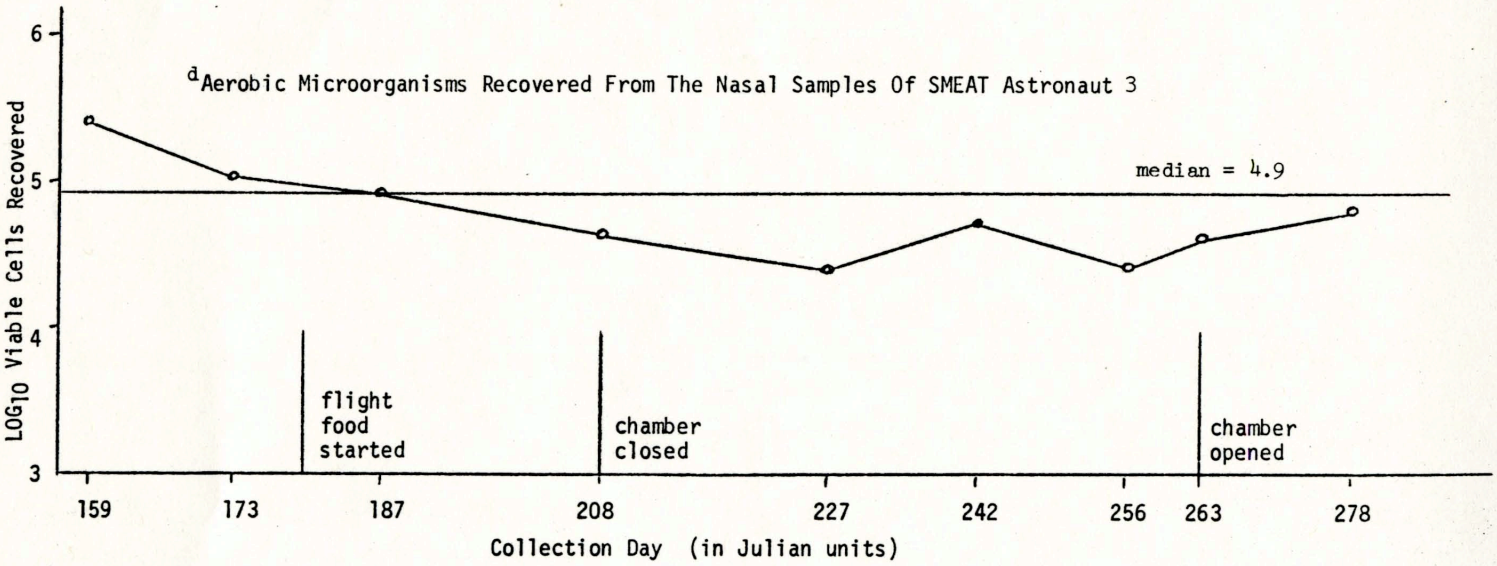


Figure 1. Aerobic Microorganisms Recovered from SMEAT Crew Nasal Samples (Continued)





55 or 47/55 were made from crewmember 2 throughout the study period. Analyses of the phage typing data reveals that there was no interchange of *S. aureus* between crewmembers 1 and 2 with each crewmember carrying only his particular phage types on the skin, in the external nares or in the throat and mouth. Strains of *S. aureus* were not isolated from crewmember 3 until the seventh and eighth weeks of chamber confinement. Type 52 was isolated from the nasal swab from crewmember 3 on day 257 and from the skin on day 264. Thereafter all specimens from crewmember 3 were negative for *S. aureus*. During the 12 week prechamber period *S. aureus* was recovered from the gargle, skin and nasal area of crewmember 1 a total of 9 times. During the eight week intra-chamber period the incidence of recovery increased to 14. Similar totals for crewmember 2 during the prechamber and intra-chamber periods were 3 and 8 respectively.

At the beginning of the chamber study the number of viable corynebacteria recovered from the nasal samples decreased. Much of this loss was replaced with an increased number of viable *S. aureus* cells up to day 227. From day 242 until the end of the chamber confinement the *S. aureus* maintained a high incidence and the corynebacteria regained their former high quantitation, resulting in the highest quantitation of the entire study at day 263 (chamber opening day).

As with the nasal samples, aerobic bacteria recovered from the gargle samples demonstrated very little variation around a prechamber median of 2.3×10^7 viable cells per cm^3 and an interchamber median of

5.6×10^7 viable cells per cm^3 of gargle. *Streptococcus mitis* generally accounted for the high quantitations as it was recovered in concentration of up to 2.6×10^8 viable cells per cm^3 of gargle. Other species which were also present in high numbers were *Streptococcus salivarius* (max. of 8.5×10^7 cells/cm^3), *Haemophilus parainfluenzae* max. of 8.5×10^7 cells/cm^3) and *Neisseria perflava* (max. of 2.8×10^7 cells/cm^3). Although there were no obvious changes in these species throughout the study, the following points require attention: (1) *S. salivarius, S. mitis, Staphylococcus epidermidis, N. perflava* and *H. parainfluenzae* were always recovered from every sample period. No significant change could be detected in the incidence of these species resulting from test conditions. (2) *Klebsiella pneumoniae* was present in prechamber gargles but was not recovered from intra-chamber samples. (3) *Pseudomonas maltophalia* was recovered from a day 264 sample. (4) Beta-hemolytic streptocci and *Haemophilus influenzae* were nearly always recovered. (5) The quantitation of *Escherichia coli,* which was recovered from most sample periods, remained very constant at 100 cells per cm^3 of gargle.

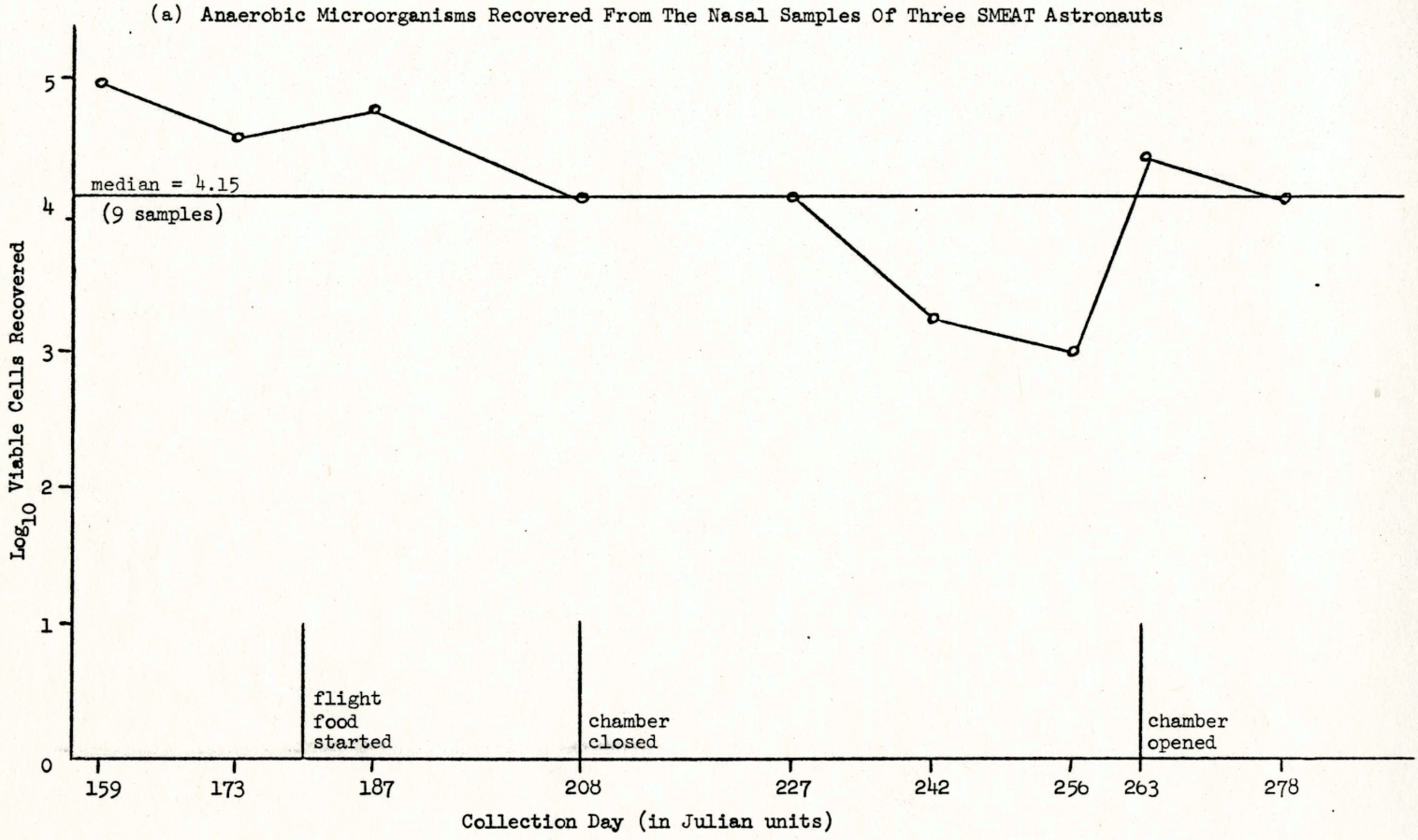
The above data indicate that whereas the astronauts were burdened with several species of medically important microorganisms in the nasal and oral cavity, no alterations could be detected in their microbial flora which could be directly related to flight food or chamber conditions.

The total number of viable anaerobic bacteria recovered from the nasal passages of the three crewmembers during each sample period is presented in Figure 2. The most commonly isolated organism were members of the genera *Propionobacterium* which decreased through day 256 and disappeared altogether from Astronauts 1 and 2 on day 242. This decrease was not matched by an increase in other anaerobic bacteria but does coincide with the increase in aerobic bacterial quantitation noted earlier. This significant alteration in the incidence of propionobacteria at day 242 could be the reflection of chamber mediated stresses on this species.

Unlike the nasal samples, the number of viable anaerobic bacteria recovered from the gargle specimens remained quite constant around a prechamber media and an intra-chamber media of 4×10^6 cells per cm^3. Likewise no significant alterations in individual species were detected.

Crew Mycological Studies - A list of the filamentous fungi recovered from the three SMEAT crewmembers at each of the major sampling periods is presented in Table V (fecal data are deleted from this table as these samples often were collected at a different time). These data show that of the 52 species recovered during the 182 day sampling period, 35 (67 percent) were isolated only once and 42 (81 percent) were isolated no more than twice. This reflects the ephemeral nature of the relationship between the human body and most filamentous fungi.

Figure 2. Anaerobic Microorganisms Recovered From SMEAT Crew Nasal Samples



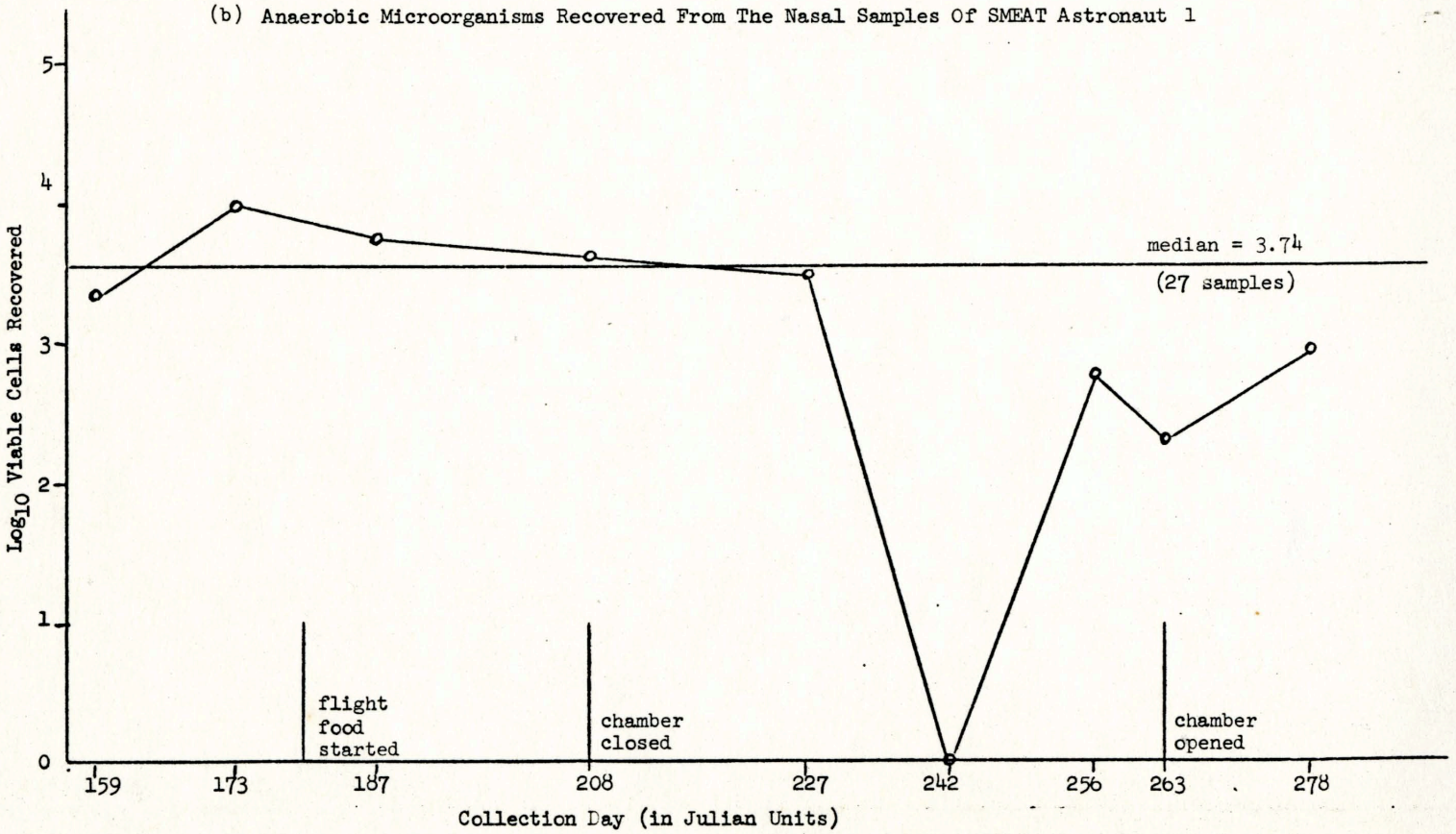
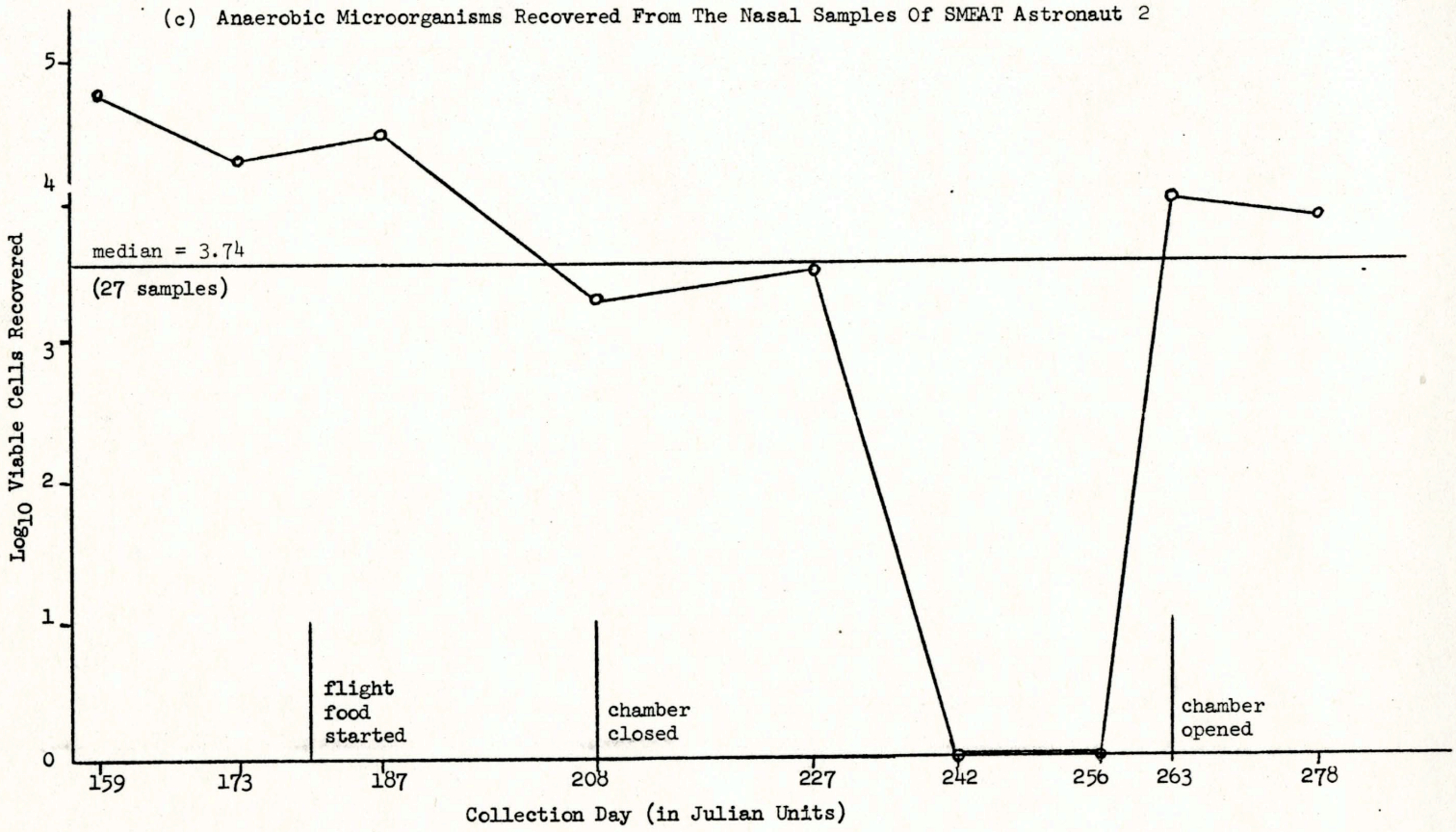


Figure 2. Anaerobic Microorganisms Recovered From SMEAT Crew Nasal Samples (Continued)



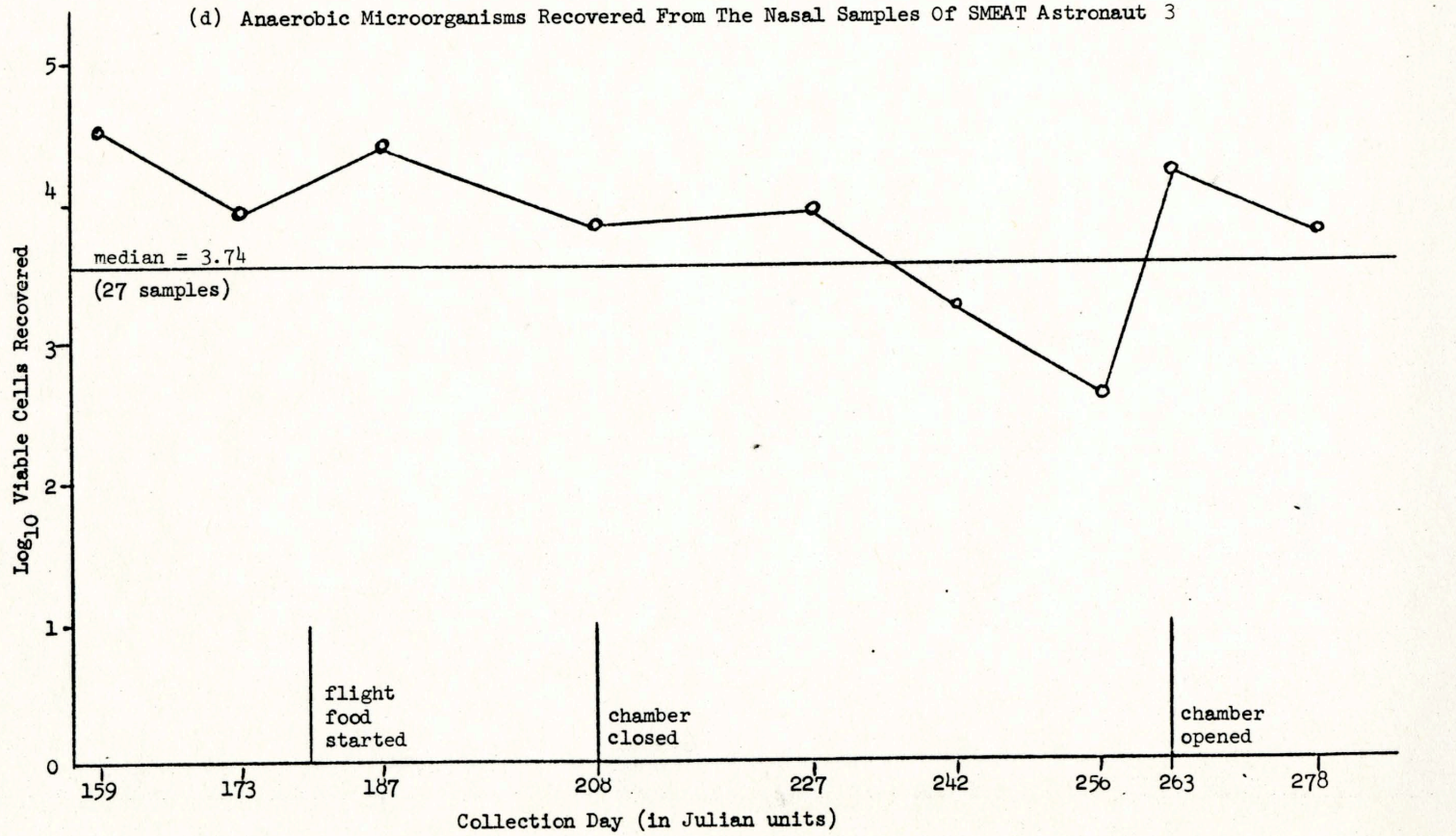


TABLE V. - NUMBER OF FILAMENTOUS FUNGI RECOVERED FROM SMEAT CREWMEMBERS OVER AN 182-DAY PERIOD

Genus	Species	Prechamber[b] 1[d]	2	3	Intra-chamber[b] 1	2	3	Total
Acremonium	*species*			1				1
Alternaria	*citri*	1						1
	longipes		1	1				2
	tenuissima		1	1				2
Aspergillus	*fumigatus*	1						1
	niger			1				1
	terreus			1				1
	tonaphilus	1						1
	unquis			1				1
	versicolor		1					1
	flavus			2				2
Aureobasidium	*pullulans*			6			2	8
Bipolaris	*maydis*			2				2
Cephalosporium	*acremonium*			3				3
	species	1						1
Chrysosporium	*keratinophilum*		1					1
	tropicum			2			1	3
Cladosporium	*cladospoioides*	1	5	1	1	1		9
	elatum			1				1
	herbarum			1				1
	sphaerospermum		1	1	3			5
Curvularia	*lunate*			1			1	2
Drechslera	*hawaiiensis*			1				1
Epicoccum	*nigrum*	2		3				5
Epidermophyton	*floccosum*			1				1
Fusarium	*lateutium*			1				1
Geotrichum	*candidum*	1						1
Haplobasidion	*thalictri*			1				1
	lelebae		1					1
Micromonospora	*fusca*			2				2
Nigrospora	*sphaerica*						1	1
Paecilomyces	*terricola*			1				1
	varioti			1				1
Penicillium	*corymbiferum*			5				5
	expansum		1					1
	frequentans					1		1
	granulatum						1	1
	lanoso-coeruleum				1			1
	notatum		1	2				3
	steckii	1						1
	tardum	1						1
	species		1					1
Phoma	*species*			1				1
Pithomyces	*atro-olivaceus*			4				4
Sporothrix	*species*		1					1
Streptomyces	*section spira*	1		1				2
	section rectus flexibilis	1	1					2
	section retinaculum-apertum			1				1
	species			1				1
Syncephalastrum	*racemosum*			1				1
Vermiculatum	*species*	1						1
Wallemia	*ichthyophage*			4				4
Total Occurrence		13	15	57	5	2	6	
Combined Total			85			13		98

a - From all sampling sites except fecal samples

b - Combined data for 6 sample periods

c - Combined data for 4 sample periods

d - Astronaut Number 1, 2, or 3

Of those species that were recovered more than twice, special note should be made of *Aureobasidium pullulans* and *Cladosporium cladospoioides*. These species were both present in high numbers before chamber closure and remained in the population under chamber conditions. This is the type of pattern expected of species that are not merely transients. The fact that *C. cladospoioides* was likewise isolated from all three crewmembers makes this a good candidate for consideration as part of the commonly isolated fungal flora.

Attention should also be given to *Epicoccum nigrum*, *Penicillium corymbiferum*, *Pithomyces atro-olivaceus*, and *Wallemia ichthyophage*. These species were all present in high numbers before chamber closure but, were entirely absent from specimens collected within the chamber. These species are undoubtedly transients that are present in some segment of the environment of Astronaut three but were not sustained within the chamber.

A list of the yeasts recovered from the three SMEAT crewmembers at each of the major sampling periods is presented in Table VI. As with the filamentous fungi, a large portion (72 percent) of the species were isolated no more than two times. This indicates that most of the recovered species were simple transients and cannot be considered as part of the commonly isolated flora.

Even though *Pityrosporum ovale* and *Torulopsis famata* were isolated in large numbers before chamber closure they could not be recovered from the samples collected in the chamber. *Candida albicans* and

TABLE VI. - NUMBER OF YEASTS RECOVERED[a] FROM SMEAT CREWMEMBERS OVER AN 182-DAY PERIOD

		Prechamber[b]			Intrachamber[c]			
Genus	Species	1[d]	2	3	1	2	3	Total
Bullera	*alba*			1				1
Candida	*albicans*			5			4	9
	guilliermondii	1		1				2
	parapsilosis	2		4	7			13
	claussenii	1					1	2
	scotti				1			1
	solani	1						1
Cryptococcus	*albidus*		1	1				2
	luteolus		1					1
	uniguttulatus		2					2
Pityrosporum	*ovale*			5				5
Rhodotorula	*glutinis*	1		1				2
	rubra		2				1	3
	mucilaginosa	1						1
	flava	1						1
Torulopsis	*famata*			3				3
Trichosporon	*cutaneum*		1	1				2
	pullulans				1			1
Total Occurrence		8	7	22	9	0	6	
Combined Total			37			15		52

a - From all sampling sites except fecal samples

b - Combined data for 6 sample periods

c - Combined data for 4 sample periods

d - Astronaut Number 1, 2, and 3

Figure 4. Total Number Of Yeasts Recovered From Each Set Of SMEAT Crew Samples

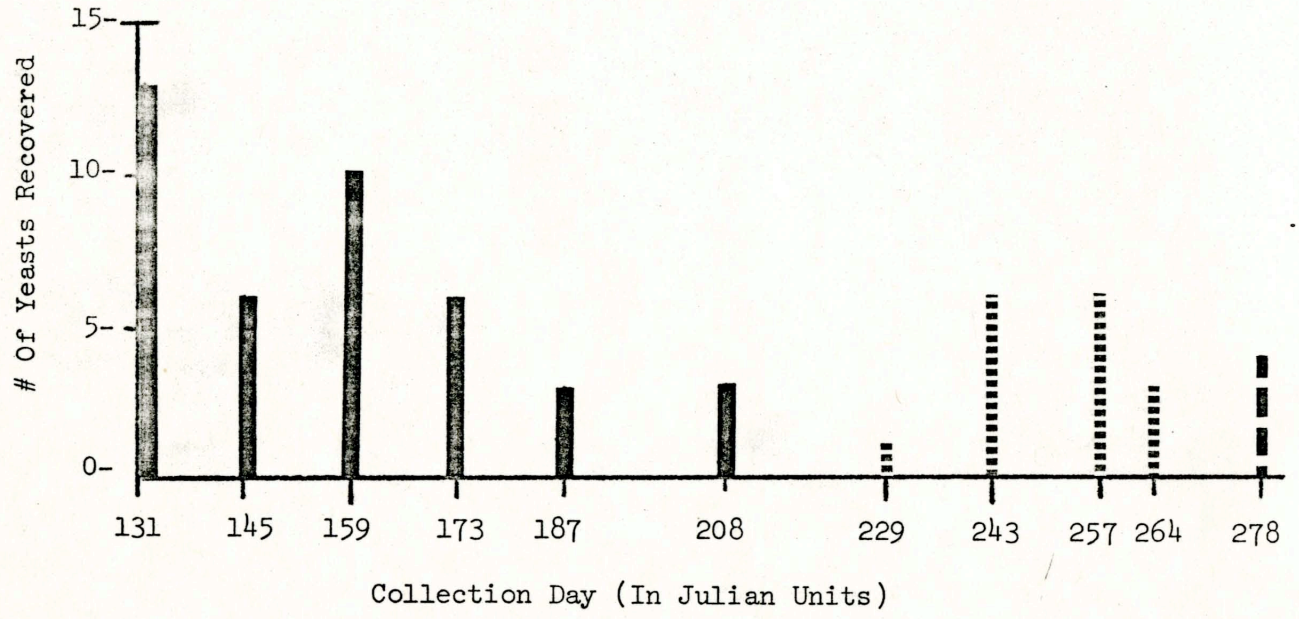
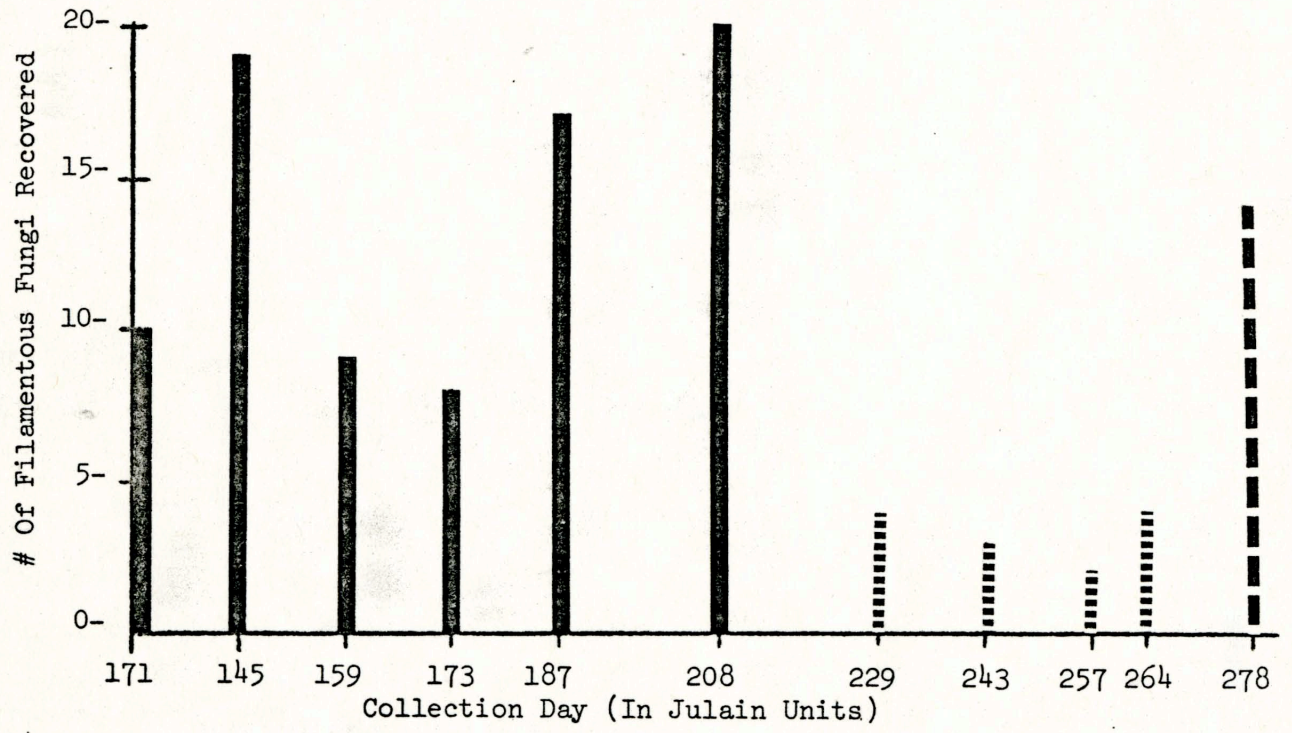


Figure 3. Total Number Of Filamentous Fungi Recovered From Each Set Of SMEAT Crew Samples



Prechamber Samples

Intra-chamber Samples

Postchamber Samples

C. parapsilosis, however, were recoverable from within the chamber. *C. albicans* must be considered as part of the permanent oral flora of Astronaut three as this species was isolated from all but one of the gargle samples obtained from this subject.

If the number of different types of fungi that are isolated from each sample site of each crewmember is measured, this factor may be used as a means of establishing the mycological load of each crewmember. If the values obtained for all three crewmembers are added together it is possible to establish the mycological load of the population (of three crewmembers) at any particular sample time. The values resulting from this type of analysis are presented in Figures 3 and 4 for the filamentous fungi and yeasts respectively.

The values presented in Figures 3 and 4 reveal that the total number of yeast and of filamentous fungal recoveries in the population prior to entry into the chamber was quite variable. Values range from a low of 7 to a high of 20 filamentous isolates, and yeast values range from a low of 3 to a high of 13 isolates from the three crewmembers. A similar variability is demonstrated within the chamber. This variability demonstrates the normal ephemeral relationship between integumentary fungi and the healthy body.

Even with this wide variability, the incidence of prechamber recovery of filamentous fungi values are significantly different (to the 0.005 level) than the intra-chamber values as calculated by the Wilcoxon Rank-Sum Test. This demonstrates that even with the high degree of

variability, normally inherent in total body microflora studies, it is possible to demonstrate a significant decrease in the fungal load of crewmembers. The data presented in Figure 4 demonstrate that there is a similar depression of the total number of yeasts recovered from crewmembers in the chamber, although this depression is not statistically significant at the 0.1 level.

A superficial analysis of the reported depressions might lead to the conclusion that the probability of infection within the chamber is decreased by virtue of the fact that there are fewer types of fungi available. In reality, the probability of mycotic disturbance may actually be increased in the chamber. The loss of fungi from the body surface can upset the microbial balance of these areas and provide a more favorable environment to those species which remain. For example, the well known pathogenic yeast, *Candida albicans* which Astronaut three carried in his oral cavity, remained throughout the chamber study. In addition, different species of *Candida* began to appear near the middle of the chamber period signaling a build-up of these species. Likewise, the odious fungus *Aureobasidium pullulans* remained through the end of the chamber study. In fact, *Candida* and *Aureobasidium pullulans* comprised 88 percent of the recovered fungal flora by the end of the chamber study. The number of fungi recovered returned to normal upon removal of the crewmembers from the chamber (Figures 3 and 4).

It is of interest to ascertain the level at which each crewmember and each sample site contributed to the total fungal load. Astronaut three contributed 59.2 percent of the recovered fungi whereas Astronaut

one contributed 22.4 percent and Astronaut two only 18.4 percent. The recovery frequency for each sample site is given in Table VII. The values on this table indicate that 28.5 percent of all reported isolates were recovered from nasal swabs, with about half that many being recovered from the fecal samples, the gargles, and the toe swabs. Twenty-eight percent of all recovered samples were obtained from the remaining seven sites combined.

Crew Virological and Mycoplasma Studies - There was no evidence of viral growth in any of the host systems inoculated with specimens obtained from the SMEAT crew before, during or after chamber exposure. Since the crew remained healthy throughout the duration of the study, these results were expected. There was a possibility that the environment of SMEAT might induce the appearance of viruses that are not isolated normally. This did not occur. *Mycoplasma orale 1* was repeatedly isolated from the throats of Astronauts 2 and 3 before, during, and after chamber exposure. These results are similar to the results obtained in monitoring the Apollo space flight crews. Usually, *Mycoplasma salivarium* and *M. orale 1* are isolated from the throat and *M. hominis 1* is isolated from the urine. There were no mycoplasma isolation from the urines during SMEAT. The fact that Astronaut one did not carry *M. orale 1* before chamber exposure afforded an excellent opportunity to demonstrate cross infection during chamber residence but this did not occur.

TABLE VII. - RECOVERY FREQUENCY OF FUNGI FROM THREE SMEAT CREWMEMBERS

Sample Type	Percent of Whole
Nasal Swab	28.5
Feces	15.5
Gargle	14.5
Toes	13.5
Hands	7.8
Scalp	5.7
Ears	5.2
Groin	3.1
Axilla	2.6
Umbilicus	2.6
Urine	1.0
Total	100.0

Environmental Microbiological Studies - Levels of bacterial contamination which accumulated on stainless steel strips located in eight different areas in the SMEAT chamber are shown in Figure 5. This represents total contamination from five horizontal strips for each of the eight weeks. All vertical strips, three per week, remained negative for the same eight weeks. As indicated by this figure maximum loading on the strips was obtained by week one. The predominate organisms found on the strips are shown in Table VIII. The total number of organisms recovered did not vary materially from week to week. However, variance did occur in the types of organisms isolated. As an example, species of *Bacillus* were in high numbers after the first week, decreased during the next two weeks and were not recovered again for three weeks. A possible explaination for this could be that only general house cleaning, not decontamination, was done in the chamber prior to closure. After closure all items being passed into the chamber through the transfer lock were decontaminated or sterilized. More use was made of the man lock for equipment transfers during the last two weeks. Contamination control for items being passed into the chamber in this manner was more difficult and offered a better opportunity for organisms of the *Bacillus* type to gain entrance in the chamber.

Corynebacterium, Micrococcus spp. and *Staphylococcus epidermidis* were recovered in relatively high numbers from every horizontal location throughout the eight weeks. After the first week the total counts varied as the numbers of these three organisms varied. This was a direct

Figure 5. - Quantitative Weekly Totals of Bacteria Recovered From Stainless Steel Strips

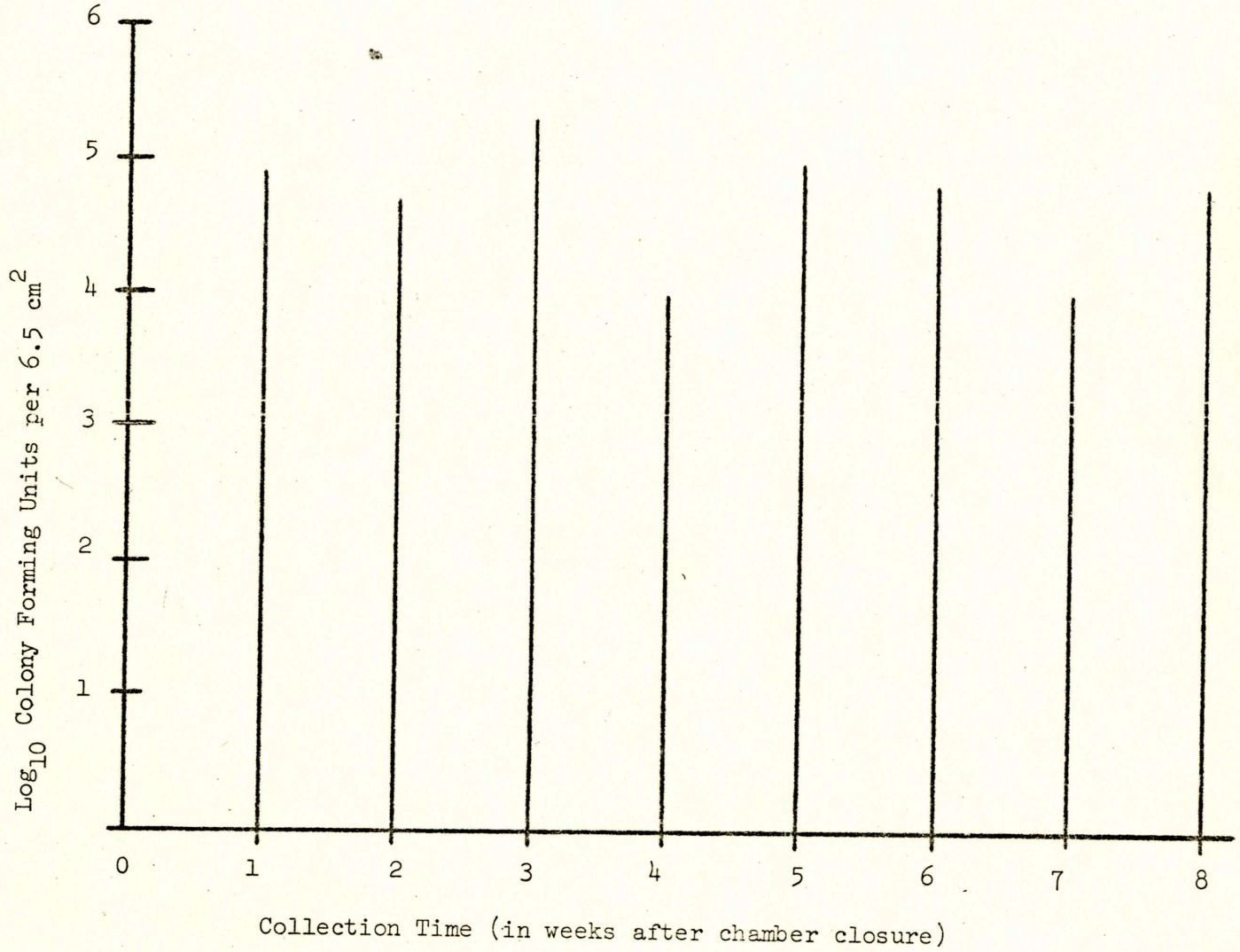


TABLE VIII.- QUANTITATIVE VALUES OF PREDOMINANT BACTERIA RECOVERED FROM STAINLESS STEEL STRIPS

Julian Date	215	222	229	236	243	250	257	264
Bacillus spp.	$8x10^{4}$*	$1x10^{1}$	$2x10^{1}$	0	0	0	$6x10^{2}$	$2x10^{3}$
Corynebacterium spp.	$2x10^{3}$	$2x10^{4}$	$3x10^{4}$	$3x10^{3}$	$3x10^{4}$	$9x10^{3}$	$1x10^{3}$	$9x10^{3}$
Micrococcus spp.	$5x10^{2}$	$2x10^{4}$	$1x10^{5}$	$4x10^{3}$	$5x10^{4}$	$5x10^{4}$	$9x10^{3}$	$2x10^{4}$
Mima	0	0	0	$8x10^{2}$	0	0	0	0
Staphylococcus epidermidis	$8x10^{3}$	$7x10^{3}$	$1x10^{4}$	$3x10^{3}$	$1x10^{4}$	$1x10^{4}$	$3x10^{3}$	$3x10^{4}$
Staphylococcus aureus	$2x10^{2}$	0	$8x10^{2}$	$8x10^{2}$	$4x10^{3}$	0	0	$2x10^{2}$
Streptococcus spp.	$5x10^{1}$	$8x10^{2}$	$1x10^{4}$	0	0	0	0	0
Propionebacterium spp.	$8x10^{2}$	0	$2x10^{3}$	$1x10^{3}$	0	0	0	0
Yeast and Fungi	$2x10^{2}$	$4x10^{2}$	$2x10^{3}$	$9x10^{2}$	$5x10^{3}$	$7x10^{0}$	$3x10^{0}$	$2x10^{2}$
Totals	$9x10^{4}$	$5x10^{4}$	$2x10^{5}$	$1x10^{4}$	$1x10^{5}$	$7x10^{4}$	$1x10^{4}$	$6x10^{4}$

*Sum of 5 strips expressed as organisms per square inch.

reflection of the microflora of the skin of the three SMEAT crewmembers.

Streptococcus spp. and *Propionlabacterium spp.* were recovered from the strips only for the first few weeks. This correlates with the results obtained from the crew wherein it was shown that the skin burdens of these organisms decreased during chamber confinement.

No gram-negative organisms were obtained from any location during the eight weeks. This is a reflection of the microflora of the crew-members as these reflect the types of microorganisms were rarely recovered from skin swabs. It is evident from the data obtained that under the conditions of SMEAT the environmental microbiological burden rapidly became a duplication of the skin and oral flora of the personnel in the environment. This could become significant in transmittal of infectious organisms between crewmembers as well as between crews.

The recovery of yeast and fungi (Table VIII) in relative large numbers throughout the chamber over the eight weeks could have significant implications. Yeasts such as *Candida* and *Rhodotorula*, both of which were recovered from the environment are recognized as "opportunistic" microorganisms with significant pathogenic potential. As a result of chemotherapy and during periods of stress these types of microorganism can become medically important as infectious agents. Filamentous fungi like *Aspergillus* or *Penicillium* can become medically significant and also may degrade hardware. In a reduced gravity environment where aerosol burdens are expected to be quite high, large

quantities of fungal spores could be inhaled and cause infectious and/or allergic reactions in the crewmembers. Filamentous fungi could destroy or degrade a wide variety of materials throughout the cluster.

Numerous yeasts and fungi were recovered from contingency samples taken from around the food chiller. This was an area of high humidity and visible growth was observed on the chamber wall. A list of organisms recovered are shown on Table IX. These types of organisms can be expected to be present in Skylab. If areas of high humidity, 65 percent or above, are maintained these organisms can be expected to proliferate.

The exhalation hose of the metabolic analyzer (MA) was examined for microbial contamination. This was done during, and at the end of the chamber study. Two types of yeast, *Rhodotorula* and *Candida* were recovered both times. In addition another yeast and two filamentous fungi were recovered in the last sample. The inside of the MA hose is an area of high humidity and gives ideal conditions for growth of yeasts and fungi.

Immediately after crew exist from the chamber, 30 areas within the chamber were sampled using the swab-rinse technique. Table X indicates the types of organisms found. *Micrococcus spp.*, the predominate organism, occurred in 20 of the 30 samples. *Staphylococcus epidermidis* and *Corynebacterium spp.* were isolated 17 and 4 times, respectively. These results, like those obtained from the stainless strips, show direct correlation to the SMEAT crewmembers skin flora.

TABLE IX. - ORGANISMS ISOLATED FROM CONTINGENCY HARDWARE SAMPLE

Chamber Wall Behind Food Chiller

Fungi	Yeasts
Alternaria sp.	Rhodotorula sp.
Epicoccum nigrum	Cryptococcus sp.
Aspergillus flavus	
Penicillum notatum	
Nigrospora sp.	
Fusarium sp.	
Curvularia lavata	
Trichoderma sp.	
Cladosporium sp.	
Gliodadium sp.	

Metabolic Analyzer Hose

Fungi	Yeasts
Penicillium sp.	Rhodotorula glutinis
Unknown filamentous	Candida claussinii
	Candida albicans
	Candida sp.
	Aureobasidium sp.
	Botrytis cinerea

TABLE X . - DISTRIBUTION OF PREDOMINANT BACTERIA, FUNGI AND YEASTS ISOLATED FROM CHAMBER SWAB SAMPLES

Isolants	Swab Sample Sites																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Corynebacterium			+[a]											+							+								+	
Micrococcus	+	+	+	+			+	+	+			+	+		+		+	+	+		+	+	+	+		+		+	+	
Staphylococcus epidermidis	+	+			+		+					+		+		+		+		+		+					+	+		+
Staphylococcus aureus			+																							+				
Bacillus																	+													
Candida	+					+		+					+	+							+		+	+		+		+		
Aureobasidium		+												+																
Penicillum						+																								
Aspergillus																				+										
Cladosporium																							+							
Alternaria																								+						
Rhodotorula																								+				+		
Geotrichum																											+			
Unidentified fungi					+						+		+				+									+		+		

a = Denotes recovery of organism

Yeast or fungi were recovered from 17 of the 30 locations, with eight locations having more than one type.

Levels of organisms obtained from the swabs were lower than those obtained on the stainless steel strips. A possible explaination is that the strips were not disturbed during the eight weeks except for times of sampling, while the majority of the swabed areas had undergone some form of housekeeping, vacuuming or washing, during the chamber test.

The Anderson Air Sampler, which was run for a 10 minute period each week at a rate of 0.028 m^3/min indicated a microbial burden in the chamber atmosphere of 8-10 organisms.per 0.028 m^3. These organisms were predominately micrococci and staphylococci These counts were quite high when compared to normal environments where 2-4 organisms per m^3 are expected.

CONCLUSIONS

Genera and species comprising the major portion of the indigenous aerobic bacterial skin flora were not affected as a result of chamber confinement.

Chamber confinement resulted in an almost complete loss of ubiquitous "soil" organisms which normally contaminate the skin surfaces.

Gram negative enterics, isolated frequently from the skin of one crewmember during the prechamber period, were not recovered in any significant amounts from skin surfaces of any crewmembers during the intra-chamber period.

A significant qualitative and quantitative decrease in recovery of indigenous anaerobic skin flora occurred during chamber confinement.

Decreases in anaerobic skin flora were not accompanied by increases in other genera or species of aerobic bacteria.

Microbial imbalance of the normal skin flora was indicated only by a loss of "soil" contaminants and a significant decrease in recovery of indigenous anaerobes.

Nasal and oral aerobic bacteria remained quite stable during the chamber period with the exceptions of decreases in the total quantitative values of corynebacteria and increases in *S. aureus*.

The incidence of isolation of *S. aureus* from two crewmembers increased during chamber confinement.

Transfer of strains of *S. aureus* between crewmembers did not occur. One crewmember who did not carry *S. aureus* was not colonized by this organism during the chamber run.

The anaerobic bacterial load of the nose and throat remained quite stable during chamber confinement with the exception of the propionobacteria which showed a significant decrease and disappeared from two crewmembers.

Only 19 percent of all species of filamentous fungi and 28 percent of all species of yeasts were recovered more than twice from all crewmembers during the study duration. This indicates the extremely transient nature of most fungi and yeasts recovered from crewmembers.

Most species of fungi and yeast present in high numbers before chamber closure were not recovered after the crewmembers were isolated in the chamber.

The intra-chamber decrease in the isolation of filamentous fungi was statistically significant at the 0.01 level whereas the decrease in yeasts, although quite obvious, was not statistically significant.

Aureobasidium pullans, *Cladosporium cladospoioides*, *Candida albicans*, and *Candida parapsilosis* were recovered from the crewmembers throughout the duration of the chamber study.

C. albicans was recovered from the oral flora of crewmember 3 at every sampling interval except one.

No alterations in the viral or mycoplasma flora of crewmembers could be demonstrated.

A variety of fungi and yeasts were recovered throughout the chamber from both stainless steel strips and swab samples. There were no significant differences in the types of organisms recovered from the strips and swab samples.

Analyses of one contingency sample taken from an area of obvious fungal growth within the chamber revealed a large variety of fungi and yeast.

Chamber air sampling yielded from 8-10 organisms per 0.028 m^3 of air sampled.

Rates of recovery of bacteria from strip, swab and air samples were approximately 2 to 3 times higher than that found in the average home or office environment.

Maximum microbial loading of the chamber interior had occurred after one week.

Soil type organisms decreased after chamber closure and remained at near zero levels until the final two weeks when the air lock had to be utilized for outgoing and ingoing transfers of large equipment items.

Organisms typical of the crewmembers predominant skin and oral flora became the predominating chamber contaminants within one week of chamber closure.

Gram negative organisms were not recovered on any strip sample at any location.

SKYLAB SIGNIFICANCE

States of microbial imbalance as a result of chamber confinement occurred, for the most part, only in those genera and species of bacteria, yeast and fungi which are classified as transients and are not part of the true indigenous flora of the crewmembers.

In as much as no crew illness events occurred and only subtile changes in the indigenous flora were noted, it appears that 56 days confinement in a Skylab simulated environment does not mediate towards shifts in bacterial populations which have obvious clinical significance.

The lack of build-up of skin flora, particularly gram negative species and enteric bacteria, suggests that the personal hygiene regimens are adequate.

The increased incidence of recovery of *S. aureus* and the true quantitative increase in this organism is cause for concern. It is recommended that all *S. aureus* cultures recovered from Skylab prime and backup crewmembers during the preflight period be subjected to antibiotic sensitivity testing so that profiles are available to the mission medical managers. Such data would be extremely valuable as an adjunct for therapeutic decisions should *S. aureus* be incriminated or suspect in inflight disease processes. Due to the persistance of *S. epidermidis*, such rationale should also be applied to this species.

Careful analyses of preflight Skylab data should be accomplished for medical management purposes. As an example, any crewmember carrying a burden of *C. albicans* similar to that seen for crewmember 3 would represent a poor candidate for high-level oral antibiotic therapy. Such a treatment regimen could modify the bacterial flora and result in an overgrowth of *C. albicans* with potentially severe consequences.

It is probable that latent or "hidden" viruses were present among the crewmembers even though this was not demonstrated. The Skylab simulated environment did not result in the activation of such latent agents. This would suggest that viral infections may be of minimal consequence providing crewmembers are not actually in the incubation phase of a viral disease prior to launch.

The finding of bacteria, yeasts and fungi in high numbers, relative to what one would expect in a normal environment, throughout the

chamber both on surfaces and in the air is highly significant. In the Zero G situation it can be expected that the majority of the microbial burden of the cluster will exist for long periods as aerosolized particles. It is anticipated the atmosphere will contain viable particles several orders of magnitude higher than in the normal environment.

The SMEAT results clearly demonstrate that potential pathogens are indeed present in significant amounts. Continued long-term exposure and inhalation of these organisms could result in clinical manifestations ranging in severity from frank pneumonia to allergic responses.

The results of SMEAT also clearly demonstrate that a variety of filamentous fungi will survive in the environment. Condensate formation resulting in local areas of high relative humidity will almost certainly result in focal areas of growth. These organisms then could be disseminated throughout the cluster. If the relative humidity of the cluster were to exceed 65 percent for long periods (3 to 7 days) the fungi could begin to overgrow with the resultant degradation of a wide variety of materials (optics, electrical connections, etc.) within the cluster.

Control of microbial growth within the cluster will be primarily dependent upon the faithful execution of baselined housekeeping tasks. The results of SMEAT demonstrate that the performance of these tasks with rigor is fully justified.