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THE POTENTIAL HAZARD OF STAPHYLOCOCCI AND MICROCOCCI TO HUMAN SUBJECTS IN A LIFE SUPPORT SYSTEMS EVALUATOR AND ON A DIET OF PRECOOKED FREEZE DEHYDRATED FOODS

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FOREWORD

This research was initiated by the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, and was accomplished by the Department of Research of the Miami Valley Hospital, Dayton, Ohio, and the Biotechnology Branch, Life Support Division, Biomedical Laboratory, Aerospace Medical Research Laboratories. This effort was supported jointly by the USAF under Project No. 7164, "Biomedical Criteria for Aerospace Flight," Task No. 716405, "Aerospace Nutrition," and NASA Manned Spacecraft Center, Houston, Texas, under Defense Purchase Request R-85, "The Protein, Water, and Energy Requirements of Man Under Simulated Aerospace Conditions." This contract was initiated by 1st Lt John E. Vanderveen, monitored by 1st Lt Keith J. Smith, and completed by Alton E. Prince, PhD, for the USAF. Technical contract monitor for NASA was Paul A. Lachance, PhD. The research effort of the Department of Research of the Miami Valley Hospital was accomplished under Contract AF 33 (657)-11716. Bernard J. Katchman, PhD, and George M. Homer, PhD, were technical contract administrators, and Robert E. Zipf, MD, Director of Research, had overall contractual responsibility.

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ABSTRACT

Two groups of four human male subjects participated in 6-week simulated aerospace studies. The subjects were confined under controlled metabolic conditions; during this time, 28 consecutive days were spent in a Life Support Systems Evaluator. The subjects ate diets composed of either fresh foods or precooked freeze dehydrated foods. The subjects were exposed to simulated aerospace stress of confinement wearing an unpressurized MA-10 pressure suit, experimental diet, and minimal personal hygienic conditions. Body and environmental areas were sampled and the catalase-positive, gram-positive cocci isolated were tested for production of coagulase, deoxyribonuclease, hemolysin, gelatinase, and utilization of mannitol. The results showed no significant differences in frequency of occurrence of biochemical types among the subjects or among the environmental areas during the chamber period. There were significant differences in the frequency of occurrence of biochemical types in microbiological specimens from the eye, ear, nose, throat, mouth, axilla, umbilicus, groin, glans penis, and anus. There was no buildup of biochemical types with time in any test condition. There was no difference in the frequencies of biochemical types when either the coagulase-mannitol marker or the deoxyribonuclease marker was used to indicate the potentially pathogenic type. The subjects remained healthy without any decrease in resistance to infection throughout all the test conditions. Those body areas most likely to harbor potentially pathogenic staphylococci are the nose and groin. In concurrent metabolic studies, the physiological, biochemical, and nutritional parameters investigated were all in the normal range of clinical values. Confinement under simulated aerospace conditions for at least 28 consecutive days and conditions of minimal personal hygiene show that no unique set of circumstances are operable that would require the establishment of special biomedical criteria.

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SECTION I

INTRODUCTION

Biomedical criteria required to establish the necessary personal hygiene and sanitation procedures for long term flights in space are not available. Of considerable import would be the buildup of microbial populations and the development of deleterious effects on personnel as a consequence of stress induced conditions of long term space flight derived from a variety of parameters.

Several stressful factors have increased occurrence of staphylococcal pathogenicity in man and animals. Starvation, vitamin deficiencies, and protein deficient diets are examples of nutritional stresses that have predisposed man and animals to staphylococcal infection (1-4). Mice fed a protein deficient diet (5% casein) succumbed to infection by Staphylococcus aureus, while those on 20% casein did not (3). The same authors (4) reported that coagulase-negative staphylococci readily infected mice fed another protein deficient diet (corn or gluten-lysine) in contrast to a casein enriched diet. These data suggest that nutritional balances are important in the resistance of man and animals to microbial infection.

Stresses such as burns (5), traumatic shock (6), fatigue (7), extensive body irradiation (8), hyposecretion or hypersecretion of hormones (9), and diabetes mellitus, tuberculosis, and kidney damage (7, 10, 11) have been shown to reduce resistance to infection. Although any of these factors might lower the resistance of astronauts to microbial infection during prolonged space travel, those pertaining to the nutritional status are probably more germane to the problem of space travel stress.

Micrococci, especially S. aureus, have been reported as predominant colonizers on human skin and body surfaces and rank foremost among the potential pathogens (12). Various products or properties of S. aureus have been associated with virulence; for example, the production of coagulase, alpha-toxin and hemolysins, leukocidin, lipase, deoxyribonuclease, phosphatase, hyaluronidase, and other enzymes, and the ability to resist phagocytosis (13). Of these properties, coagulase activity has been regarded as the main determinant of staphylococcal pathogenicity (14-17).

The purpose of this study was to determine the distribution of staphylococci indigenous to humans and their environment in a controlled ecological system and to ascertain if the associated biochemical markers provide reliable criteria of pathogenicity. A buildup of these organisms or their transfer among humans and their environment, or even among specific body regions, may pose a threat to the health of humans during long term space flight and require the establishment of biomedical criteria.

This report describes the results obtained from two 6-week experiments during which time two groups of four human male subjects were confined under simulated aerospace and controlled metabolic conditions; during this time the subjects spent 28 consecutive days in the Life Support Systems Evaluator (LSSE)* and wore the unpressurized MA-10** pressure suit for at least 8 hours a day. The subjects ate either a fresh food diet or an experimental diet composed of precooked freeze dehydrated foods. The results of the basic nutritional program are reported elsewhere (18, 19).

In these studies, selected body areas and the environment were sampled by means of dry cotton swabs which were applied to appropriate culture media. Staphylococci or micrococci were isolated from the culture media and tested for their characteristic biochemical reactions. The bacterial and fungal flora excluding the Micrococcaceae were investigated as part of the overall program (20).

SECTION II

EXPERIMENTAL METHODS AND PROCEDURES

EXPERIMENTAL PROTOCOL

During each 42-day experimental period, four healthy male subjects were confined in the controlled activity facility (CAF)* 7 days, then transferred to the LSSE for 28 days, followed by a final week in the CAF. During the experiments all contacts with the subjects were limited. Personnel gowned in sterile surgical attire were permitted to enter the CAF; only the subjects entered the chamber. Transfer of subjects to and from the chamber was strictly controlled, including the wearing of sterile surgical apparel. A 4-day cycle diet of fresh foods served at room temperature was used in experiment 1 and a 3-day cycle diet of precooked freeze dehydrated foods was used in experiment 2. Subjects were required to consume all food served. All subjects followed a regulated daily activity schedule involving the collection of

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* The controlled activity facility (CAF) and the Life Support Systems Evaluator (LSSE) at the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, were used to provide a simulated space cabin environment.

** The MA-10 pressure suits were furnished for these experiments by the Manned Spacecraft Center, NASA, Houston, Texas.

biological samples for hematological and chemical analysis, physiological measurements, psychological, tests, exercise, and free activity periods. During the chamber period of experiment 1 each subject wore the MA-10 pressure suit with helmet, gloves, and boots for at least 8 hours a day for 14 consecutive days. Table I contains the experimental design for experiments 1 and 2.

PERSONAL HYGIENE

Minimal hygienic procedures were established during experiment 1 to obtain baseline microbiological data for later experimental variations. The subjects showered and washed with soap before entering the CAF and the chamber. Approximately five wet wipes impregnated with sodium lauryl sulfate were used to cleanse body areas before meals and after defecation. The subjects were not permitted to bathe, shower, groom hair, clean or trim nails, brush teeth, or change clothes while in a test condition.

In experiment 2 only minimal hygienic care was permitted. At the beginning of both the prechamber and the chamber periods the subjects washed all parts of their bodies with pHisoHex. Ear and nose areas were cleaned by the subjects with sterile cotton swabs; sterile wash cloths, towels, and garments were provided for their use. During the experiment, approximately five wipes per day were available to cleanse body areas before meals and after defecation. An electric tooth brush was used during the prechamber period and a regular tooth brush with water was used in the chamber period for oral hygiene. A daily activity schedule for all subjects from both experiments appears in table II.

The CAF and chamber were thoroughly decontaminated by sponging and spraying with benzalkonium chloride (BAC) solution before use to provide as sterile a physical environment as possible at the start of each experiment.

AREAS SAMPLED

Body areas sampled during both experiments were divided into primary regions designated "A" areas, and secondary regions designated "B" areas.

Experiment 1 - Areas "A" included the eye, ear, nose, throat, axilla, umbilicus, groin, and anus. Areas "B" included the scalp, mouth, glans penis, forearm and toes.

Experiment 2 - Areas "A" included the ear, nose, throat, mouth, axilla, groin, and glans penis. Areas "B" included the scalp, eye, forearm, umbilicus, anus, and toes.

Data obtained during experiment 1 indicated the need for a redistribution of certain

TABLE I

EXPERIMENTAL DESIGN

Experimental day	Condition (period)	Metabolic diet*		Body areas "A"		Body areas "B"		Environment	
		exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
1	Prechamber	2	1	x	x		x	x	x
2		3	2						
3	7 days	4	3		x	x			x
4		1	1	x			x	x	
5		2	2						
6		3	3						
7		4	1		x				x
8	Chamber	1	2						
9	28 days	2	3	x				x	
10		3	1		x	x			x
11		4	2						
12		1	3	x				x	
13		2	1						
14		3	2		x				x
15		4	3	x				x	
16		1	1						
17		2	2	x				x	
18		3	3		x				x
19		4	1						
20		1	2						
21		2	3		x				x
22		3	1	x			x	x	
23		4	2						
24		1	3			x			
25		2	1	x	x			x	x
26		3	2						
27		4	3						
28		1	1		x				x
29		2	2	x				x	
30		3	3						
31		4	1	x				x	
32		1	2		x				x
33		2	3	x				x	
34		3	1						
35		4	2						
36	Postchamber	1	3						
37	7 days	2	1						
38		3	2	x	x			x	x
39		4	3			x			
40		1	1	x	x		x	x	x
41		2	2						
42		3	3						
43									

* A 4-day cycle metabolic diet composed of fresh foods served at room temperature was used in experiment 1.

A 3-day cycle metabolic diet composed of precooked freeze dehydrated foods served at room temperature was used in experiment 2.

TABLE II

DAILY ACTIVITY SCHEDULE

Time	Subject No. 17, 21	Subject No. 19, 22	Subject No. 18, 23	Subject No. 20, 24	Time
0730	Wake; void; physiological measurements. Transfer food and other items into chamber. Biological specimens collected and returned to laboratory.				0730
0900					0900
1000	Eat meal A				1000
1100	Don MA-10 pressure suit 21 and 22				1100
1200	Psychological testing 17 and 22				1200
1300	Exercise 19 and 21				1300
1400	Eat meal B		Sleep		1400
1500	Psychological testing 19 and 21				1500
1600	Exercise 17 and 22				1600
1700	Free time				1700
1800	Eat meal C				1800
1900	Free time and exercise				1900
2000					2000
2100					2100
2200	Eat meal D		Eat meal B		2200
2300	Free time				2300
2400			Don MA-10 pressure suit 23 and 24		2400
0100			Free time		0100
0200			Eat meal C		0200
0300			Free time		0300
0400	Sleep		Psychological testing 18 and 24		0400
0500			Exercise 20 and 23		0500
0600			Eat meal D		0600
0700			Psychological testing 20 and 23		0700
0730			Exercise 18 and 24		0730

of the body regions to be sampled among the primary and secondary regions. In addition to this change, the frequency of samples taken was decreased during experiment 2. In both experiments, areas "A" were sampled at least twice a week during the 42-day test period, whereas areas "B" were sampled only four times during the entire experiment (tables I and II).

Experiment 1 - Environment included bed, dining table, window, and personal hygiene area floor for prechamber and postchamber periods and bed, fore table, aft table, personal hygiene area floor, and filter for the chamber period.

Experiment 2 - Environment included bed, dining table, work table, and personal hygiene area floor for the prechamber and postchamber periods, and bed, fore table, aft table, and personal hygiene area floor for the chamber period.

The environmental samplings were made whenever areas "A" were sampled.

INITIAL RECOVERY OF CULTURES FROM SPECIFIC BODY AREAS AND THE ENVIRONMENT

In experiment 1 samples were taken from the various body and environmental areas with cotton swabs and placed in brain-heart infusion broth tubes. Aliquots from these tubes were diluted through a series of four ten-fold dilutions estimated to yield the most desirable distribution of microorganisms. The dilutions were then plated on phenylethyl alcohol agar (PEA; Baltimore Biological Laboratories (B.B.L.) and incubated aerobically at 37°C. This medium was chosen, because Lilley and Brewer (21) reported that media containing 0.25% PEA can be used to isolate S. aureus selectively from mixed flora. These initial sampling procedures were performed by personnel from Republic Aviation Corporation who participated in the joint microbiological investigation.

In experiment 2 cultures were obtained by streaking cotton swabs directly on PEA agar or 5% sheep blood agar and incubating aerobically at 37°C. This change in procedure was necessary because at the high dilutions used during experiment 1 only predominant strains of staphylococci were being recovered. Since one of the major goals was to detect qualitative changes or transfer of staphylococci or micrococci among the subjects or between them and the environment, even those strains present in low numbers were important.

PROCEDURES AND MEDIA USED FOR INITIAL SCREENING OF CULTURES

During both experiments much effort was devoted to obtaining a consistent and qualitative selection of all staphylococci and micrococci recovered from the specific areas sampled. To accomplish this task all bacterial colonies on the initial plates were subjected to a thorough macroscopic examination based upon morphology, pig-

mentation, and degrees of hemolysis, followed by the culturing of at least two of each colonial type observed.

In experiment 1 the initial isolation was made on PEA medium. Colonies were chosen by the criteria already mentioned and transferred to 5% sheep blood agar to insure purity. Gram stains of every culture were made and all gram-positive cocci were transferred to Bacto Staphylococcus 110 medium (B.B.L.). This medium contains 7.5% sodium chloride and is selective for staphylococci. It was used to determine if any indigenous staphylococcal strains were not salt-tolerant. All gram-positive cocci were tested for catalase activity.

After the 25th day of experiment 2, PEA was replaced by 5% sheep blood agar for initial isolation to insure that no staphylococcal strains were being inhibited in growth on the PEA medium. Trypticase Soy Broth (B.B.L.) plus 1.5% agar was used to purify selected colonies further, and to allow gram staining and performance of the catalase test.

PHYSIOLOGICAL STUDIES OF GRAM-POSITIVE, CATALASE-POSITIVE COCCI

All cultures that were considered gram-positive cocci after microscopic observation and found to be catalase-positive after addition of 3% hydrogen peroxide were studied for further biochemical reactions.

REPLICA METHOD

In order to facilitate the biochemical study of the large number of cultures recovered the replica plating technique of Lederberg and Lederberg (22) was adopted. Replicators slightly smaller than the 100 x 15 mm plastic petri dish were fabricated from aluminum alloy stock and covered with velveteen. The fiber pile of the velveteen functioned as a battery of inoculating needles when the replicators were pressed against bacterial colonies growning on an agar surface. Ten cultures to be tested were inoculated on small areas of blood agar plates in such a way as to be equally spaced. After proper incubation, 10 cultures at a time were then transferred to a series of other biochemical test media by first pressing the velveteen replicator against the colonies on the blood agar and then touching the test media.

MEDIA USED FOR THE BIOCHEMICAL STUDIES

The 5% sheep blood agar, from which cultures were replicated to the biochemical test media, was also used to determine hemolysis after 24 hours incubation at 37° C. Coagulase production and mannitol utilization were determined on Coagulase Mannitol Agar Base (B.B.L.) (23) to which 15% sterile horse coagulase plasma (B.B.L.) (24) had been added. Deoxyribonuclease production was determined on DNAase Test Medium

(B.B.L.) (25). Bacto Chapman-Stone Medium (B.B.L.) (26) containing 5.5% sodium chloride was used to show gelatinase production.

CONTROL CULTURES

Staphylococcus aureus strains 3A, 3B, and 3C were supplied by the Communicable Disease Center, Atlanta, Georgia. Micrococcus roseus strain 516 was obtained from the American Type Culture Collection. These cultures were tested for production of hemolysin, coagulase, deoxyribonuclease, gelatinase, and mannitol utilization. The staphylococci were positive for each marker, whereas M. roseus was uniformly negative. All control cultures were maintained on Brain-Heart Infusion (Difco) plus 1.5% agar slants and transferred every two months to fresh slants.

STATISTICAL TREATMENT OF DATA

Statistical tests included analysis of variance, χ^2, and Student's t-test. The factors of body areas "A": subjects, time, body areas, and interaction were tested by analysis of variance at 0.01 level of significance (27). For these body areas fourteen samplings were taken during experiment 1 and twelve samplings during experiment 2. In each case, the first and last halves of the sampling periods were summed. Thus two measures for each subject and body area were obtained. To simplify statistical handling of the data for the analysis among subjects as a function of time and test conditions, the staphylococci were grouped into three catagories on the basis of biochemical reactions: CM isolate produced coagulase and utilizated mannitol; D isolate produced deoxyribonuclease; X isolate were positive for all biochemical types except CM; and Y isolate were positive for all biochemical types except D. A separate analysis was run on the CM frequencies, another on the D, another on the X (all positives except CM), and another on the Y (all positives except D). The tests of body areas "A" follow: A test was carried out on the four subjects of both experiments to determine if a significant difference in frequency of biochemical types occurred between the two time periods. The test for body areas was made to determine if one or more of the body areas had a significantly higher frequency than the other body areas considered. The test for interaction was made to determine the effect when two or more factors change at the same time. Two types of interaction considered were subject versus time and body area versus time. For example, let us examine subject versus time interaction. If both subjects A and B possess a higher number of types by the same relative amount, no interaction can be concluded. If subject A was higher and subject B was lower however (for the second time period), then a significant interaction would probably exist.

In the case of body areas "B", subjects and body areas were analyzed by the χ^2 test at the 0.01 level of significance (28). The CM, D, X, and Y frequencies were summed for each body area and subject.

Time and location in the prechamber, chamber, and postchamber periods of the environment were analyzed by Student's t-test (28). This test was applied to the proportion of frequencies observed to the total possible for CM, D, X, and Y. An 0.01 level of significance was selected. The first and last halves of the sampling periods in the chamber were compared. Chamber results were matched with prechamber and postchamber results.

SECTION III

RESULTS

The data obtained in these experiments are shown in tables III through VIII. Table III shows the biochemical types recovered from selected environmental areas in experiment 1. Areas sampled included bed, dining table, window, and personal hygiene area floor for the prechamber and postchamber sampling days. During these periods subjects were confined to the CAF. While the subjects were confined to the chamber, the bed, fore table, aft table, personal hygiene area floor, and filter were sampled. Potentially pathogenic staphylococci were detected by one or more of the following indices: C = coagulase production; M = mannitol utilization; D = deoxyribonuclease production; G = gelatinase production; H = hemolysis on 5% sheep blood agar. The (x) in the table indicates the occurrence of a particular biochemical type no matter how many times it was isolated. Table IV shows the biochemical types recovered from the environment in experiment 2. The environment encompassed bed, dining table, aft table, and personal hygiene area floor for prechamber, chamber, and postchamber periods. Table V shows the biochemical types recovered from selected body areas "A" of test subjects during experiment 1, which were eye, ear, nose, throat, axilla, umbilicus, groin, and anus. Table VI shows the biochemical types isolated from selected body areas "B" of the test subjects during experiment 1 which were scalp, mouth, glans penis, forearm, and toes. Table VII illustrates the biochemical types isolated from selected body areas "A" of test subjects during experiment 2, which were ear, nose, throat, mouth, axilla, groin, and glans penis. Body areas "B" of test subjects in experiment 2 shown in table VIII were scalp, eye, forearm, umbilicus, anus, and toes.

The number of catalase-positive cocci, presumably staphylococci, totaled about 700 cultures in experiment 1 and 1300 cultures in experiment 2.

Tables IX and X summarize results of the statistical analysis of the biochemical type

recovered from the environment and selected body areas of test subject in both experiments. The frequency of biochemical types from body areas "A", body areas "B", and environment of each experiment was analyzed, respectively, by analysis of variance, χ^2, and the Student's t-test. The data show that under body areas "A" the body area factor was significant for CM, D, X, and Y types. This means that in one or more body areas there occurred considerably larger frequencies of biochemical types than in other body areas (table XI). Time was not a significant factor; the frequency of occurrence of CM, D, X, and Y types in the first 7 (experiment 1 or 6 (experiment 2) sampling periods was not markedly different from the frequency of occurrence in the second 7 (experiment 1) or 6 (experiment 2) sampling periods. There was no buildup of any biochemical type as the experiment progressed. No significant difference was observed when the frequency of biochemical types was compared among subjects. All subjects exhibited similar distribution patterns. Neither subject versus time nor body area versus time interactions were significant. The results indicate that the change in frequency among biochemical types from the time period 1 to time period 2 was relatively the same for all body areas and all eight subjects. Body areas "B" show that similar frequencies of biochemical types were recovered from body areas and subjects. The CM, D, X, and Y types did not increase in frequency of occurrence with time. The analysis of the environment was accomplished by making seven separate statistical tests. When the prechamber period is compared to the postchamber period in experiment 1, it was found that the CM and D types occurred significantly more frequently in the postchamber period (90% of the time) than in the prechamber period (50%). The X and Y types did not occur more frequently in one period than in the other period. In experiment 2 (table X) CM, D, X, and Y types did not occur more frequently in one period than in the other period. In the prechamber and postchamber periods of both experiments there were no significant differences in the frequency of occurrence of biochemical types among the bed, dining table, window, or personal hygiene area while the subjects were in the chamber. There were no significant differences in frequencies of occurrence of biochemical types when the frequencies in the first 6 sampling periods were compared to the second 6 sampling periods in experiment 1 (chamber) and the first 4 sampling periods were compared to the second 4 sampling periods in experiment 2 (chamber). No significant differences were observed in the chamber when bed, fore table, aft table, personal hygiene area floor, and filter were compared to each other (experiment 1 and experiment 2). No significant differences were observed in the frequency of occurrence of a biochemical type when the chamber and the CAF (prechamber) were compared to each other (tables IX and X). When the chamber and CAF (postchamber) were compared, however, the CM and D types occurred more frequently in the postchamber period (CM type, 100% of the time; D type, 90%) than in the chamber (CM type, 60% of the time and D type, 20%) of experiment 1. A comparison of chamber and CAF (prechamber and postchamber) in experiment 2 showed no significant differences in occurrence of types. Only in experiment 1 was there an apparent buildup of CM and D types in the CAF (postchamber). Since there was no similar buildup of

types on body areas and since it occurred in the CAF in only one experiment, the apparent buildup of these types may have resulted from incomplete decontamination of the CAF during experiment 1.

Table XI shows distribution of the frequencies of biochemical types recovered from particular body areas designated as significant body areas "A" in table IX and X. Underlined numbers refers to those types found to be significantly higher when the averages were compared by the Duncan Multiple Range Test (27). The nose and groin showed the largest frequency of CM and Y types; the groin, the largest frequency of D types; and nose and axilla, the largest frequency of X types in experiment 1. In experiment 2, axilla and glans penis showed the largest frequency of CM types and nose, the largest frequency of D types. No particular body areas "A" in experiment 2 showed the largest frequency of X and Y types. Of all the body areas "A" listed, the nose and groin (experiment 1 and experiment 2) stand out as those areas most likely to carry potentially pathogenic staphylococci (when the total number of CM, D, X, and Y types was averaged and compared among body areas for each experiment).

SECTION IV

DISCUSSION

Two groups of four human male subjects were confined for a 6-week experimental period during which time they ate either a diet composed of fresh foods or a diet composed of precooked freeze dehydrated foods. For 28 days the subjects lived in a simulated aerospace environment provided by the LSSE. It is tacitly assumed that a certain degree of stress is induced by confinement, in general by confinement in the LSSE, by the experimental diet, by wearing unpressurized MA-10 pressure suit, by minimal personal hygienic conditions, and by the overall restrictive nature of the 6-week experimental protocol. Under this particular set of circumstances, there were no changes found in biochemical, physiological or nutritional parameters as evaluated among the subjects. These data obtained in the basic nutritional study are in accord with the results obtained in the microbiological study; namely, that confinement even under minimal hygienic conditions did not cause any buildup of potentially pathogenic organisms nor did it cause lowered resistance to infection. These results are in accord with those of Sladen (29) who studied the effect of isolation of humans upon their bacterial flora. He found that during prolonged contact the subjects retained rather than exchanged

phage types; after 12 months of isolation in the Antarctic, the total carrier rate was lowered because of the decrease in the intermittent and occasional carrier rates. Even the persistent carriers who harbored S. aureus for as long as 2 years in the Antarctic never developed infection. It is apparent that a more definitive measure of stress, especially as related to its role in enhancing susceptibility to infection in human subjects is needed if one is to evaluate stress.

In general, staphylococci are dispersed in the environment by air, direct contact, and contaminated objects (30). In the present study no data were obtained on the transmission of staphylococci among body areas within the same subject, among subjects, and between subjects and their environment because phage typing studies were not employed. From data obtained in these studies no particular biochemical marker can be selected as the main index of staphylococcal potential pathogenicity; a subsequent report will reveal that the coagulase-mannitol plate medium (23) has caused false-positive reactions and that the coagulase tube test (31) should have been used instead. In any case, the results and conclusions drawn would not change.

TABLE III

RECOVERY OF BIOCHEMICAL TYPES FROM SELECTED ENVIRONMENTAL AREAS DURING EXPERIMENT 1

Area sampled	Biochemical* type	Sampling day: Prechamber 1	2	3	4	5	Chamber 6	7	8	9	10	11	12	13	14	15	16	17	Postchamber 18	19
Bed	C M D G -											×							×	×
	C M - G H		×											×						
	C M - - H			×						×	×	×		×		×				×
	C M - - -	×															×		×	
	- - D G -																×			
	- - D - -																×			
	- - - G H				×	×							×					×		
	- - - - H				×				×		×		×					×		
Dining table	C M D G H																			×
	C M - - H	×		×															×	
	C M - - -	×																	×	
	- - D G H		×																	
	- - D G -																		×	
	- - - G H																			×
	- - - - H		×		×	×														×
Window	C M D G H																		×	
	C M - - H			×															×	×
	C M - - -																			×
	- - D - -																		×	
	- - - G H																		×	
	- - - - H																			×
Personal hygiene area	C M D G -				×															
	C M D - H										×									
	C M - - H				×	×			×	×		×	×			×		×		
	- - D G -									×										
	- - - G H				×	×		×		×		×				×				
	- - - - H				×				×	×	×		×	×				×		
Fore table	C M D G H										×									
	C M D - H																×	×		
	C M - - H							×	×	×	×		×		×	×	×	×		
*	C M - - -												×				×			
	- - D G -															×				
	- - D - -															×				
	- - - G H								×			×		×			×			
	- - - - H						×	×		×	×	×	×	×						
Aft table	C M - - H									×	×		×	×		×	×	×		
	C M - - -									×		×								
	- - D G -									×	×									
	- - D - -							×										×		
	- - - G H													×		×				
	- - - - H							×	×					×	×					
Filter**	- - - G H													×						
	- - - - H													×						

* Biochemical type refers to those cultures with any positive reaction for the series of biochemical criteria used and are coded throughout the tables as follows: C = coagulase production; M = mannitol utilization; D = DNAase production; G = gelatinase production; H = hemolysis on 5% sheep blood agar.

** Filter was sampled only once.

TABLE IV

RECOVERY OF BIOCHEMICAL TYPES FROM SELECTED ENVIRONMENTAL AREAS DURING EXPERIMENT 2

Area sampled	Biochemical type	Sampling day									
		Pre-Chamber	Chamber							Post-Chamber	
		1	2	3	4	5	6	7	8	9	10
Bed	C M - - H		x	x		x					x
	C M - - -					x					
	- - D G -		x							x	x
	- - - G H		x				x		x		
	- - - - H	x		x		x	x	x	x		
Dining table	C M D G H	x									
	- - - G H	x								x	x
	- - - - H	x								x	x
Fore table	C M - - H		x	x	x	x			x		
	- - D G -				x						
	- - - G H			x	x	x		x	x		
	- - - - H		x	x	x	x	x	x			
Aft table	C M D G H	x							x		
	C M - - H	x			x	x	x			x	
	C M - - -				x						
	- - - G H	x	x	x		x			x		
	- - - - H	x	x	x	x	x	x	x	x	x	
Personal hygiene area	C M - - H					x	x		x	x	
	- - D - -	x									
	- - D G -						x	x		x	x
	- - - G H	x				x					x
	- - - - H	x	x	x		x	x	x		x	

TABLE V

RECOVERY OF BIOCHEMICAL TYPES FROM BODY AREAS "A" OF TEST SUBJECTS DURING EXPERIMENT 1

Body area	Biochemical type	Sampling day: Prechamber 1	Prechamber 2	Chamber 3	Chamber 4	Chamber 5	Chamber 6	Chamber 7	Chamber 8	Chamber 9	Chamber 10	Chamber 11	Chamber 12	Postchamber 13	Postchamber 14
						Subject 17									
Ear	- - - G H					x									
	C M D G H							x		x	x	x			
	C M D - H									x				x	x
	C M - - H								x						
	- - D - H														x
	- - - G H	x	x				x	x	x		x	x	x	x	
	- - - G -										x				x
Throat	C M D G H												x		
	C M D G -														x
	- - - G H										x				
Axilla	C M D G -														x
	C M - - H											x			
	C M - - -									x				x	x
	- - D G H		x												
	- - D - H													x	
	- - - G H	x				x	x	x			x		x		
	- - - - H					x			x		x			x	
Umbilicus	- - D G -								x						
	- - - G H			x											
Groin	C M D G -											x			
	C M D - H	x			x								x		
	C M D - -						x		x						x
	C M - - H	x		x	x		x		x		x				
	C M - - -											x			
	- - D G -		x								x			x	
	- - D - H												x		
	- - D - -									x					
	- - - G H					x	x	x	x				x	x	x
Anus	C M D - -														x
	C M - - H	x													
	- - D G -														x
	- - - - H								x	x					
	- - - G -						x								
	- - - - H		x										x		
						Subject 18									
Eye	- - - G H	x	x			x	x	x	x			x			x
	- - - - H										x				
Ear	C M D - H											x			
	C M D - -											x			
	- - D G -		x												
	- - - G -						x				x		x		
	- - - - H														

TABLE V, continued

Body area	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	11	12	Postchamber 13	14
Subject 18															
Nose	C M D G H														×
	C M D - H							×							
	C M - - H							×	×	×					×
	- - D - -										×				
	- - - G H		×			×	×	×	×	×	×			×	
	- - - G -	×									×	×		×	
	- - - - H	×													
Throat	- - - G H						×								
	- - - - H			×											
Axilla	C M D - -									×					
	- - D G -					×									
	- - - G H	×							×						
	- - - - H						×		×						
Umbilicus	C M D G -					×									×
	- - D G H							×							
	- - - G H		×	×	×	×	×		×	×	×	×	×	×	×
	- - - G -						×								
	- - - - H								×						
Groin	C M D G -	×					×	×	×				×	×	×
	C M - - H						×						×		
	- - D G -								×	×	×				
	- - - G H										×	×		×	
	- - - G -														×
	- - - - H	×	×				×								
Anus	C M D G -									×	×		×		
	C M - - H	×				×									
	C M - - -												×		
	- - - - H								×						
Subject 19															
Eye	C M D G -						×								
	C M D - H	×													
	- - - G H		×										×		×
	- - - - H		×												
Ear	C M D G H		×	×				×				×			
	C M D G -														×
	C M - G H	×													
	C M - - H	×											×		×
	C M - - -									×					
	- - D - -								×						
	- - - G H		×												
	- - - - H					×									
Nose	C M - - -			×											
	- - - G H	×							×	×	×	×	×		×
	- - - G -		×						×						×
	- - - - H		×					×						×	

TABLE V. continued

Body area	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	11	12	Postchamber 13	14
							Subject 19								
Throat	C M D - H										×	×			
	- - D - H		×												
	- - - G H								×				×		
Axilla	C M - - H						×					×	×		
	- - - G H	×	×			×	×	×	×	×			×		×
Umbilicus	- - - G H		×				×							×	
	- - - G -	×									×				
Groin	C M D G -								×						
	C M - - H	×	×	×								×			
	- - D - -	×													
	- - - G H						×		×						
	- - - - H	×													
Anus	C M - - H					×					×				
							Subject 20								
Eye	- - - G H														×
	- - - G -												×		
Ear	- - D - -														×
	- - - G H												×		
	- - - G -												×		
	- - - - H	×													
Nose	C M D G -										×				
	C M D - H													×	
	C M D - -	×	×			×			×						
	- - D - -		×												
	- - - G H	×						×		×	×	×			
	- - - G -	×				×									
Axilla	C M - - H											×			
	- - - G H	×	×		×	×	×	×	×	×	×		×	×	×
	- - - - H							×							
Umbilicus	C M - - H											×			
	C M - - -	×				×									
	- - - G H								×	×					
Groin	C M D G -													×	
	C M - - H						×					×	×		
	C M - - -	×													
	- - D G -													×	
	- - - G H			×	×									×	
	- - - G -							×	×		×				
	- - - - H	×	×								×				
Anus	C M D G -										×				
	C M - - -		×			×								×	
	- - - G H				×										
	- - - - H											×			

TABLE VI

RECOVERY OF BIOCHEMICAL TYPES FROM BODY AREAS "B" OF TEST SUBJECTS DURING EXPERIMENT 1

Body area	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Chamber 3	Postchamber 4
		Subject 17			
Scalp	C M D - H		×	×	
	C M - G H				×
	C M - - H	×			×
	- - - G H			×	
Mouth	- - D - H				×
	- - - G H		×		×
Glans penis	C M D - H		×		
	C M D - -	×			
	C M - - H	×			
	- - D G -			×	
	- - D - H	×			
	- - - G -			×	
	- - - - H				×
Toe	C M - - -				×
	- - D - H	×			
	- - - G H	×			
		Subject 18			
Scalp	- - - G H	×			
	- - - - H	×			
Mouth	- - - G H	×			
Arm	- - - G H	×	×	×	
Glans penis	C M D - -	×			
	- - - G H				×
Toe	C M D - H	×			×
	- - D G -	×			
	- - D - -				×
	- - - - H	×			
		Subject 19			
Scalp	C M D - H	×			
	- - - G H	×			
Mouth	C M - - -				×
Glans penis	C M - - H	×			
Toe	C M - - H	×			
	- - - G H	×			×
		Subject 20			
Scalp	C M D - -	×			
	- - - - H	×			
Mouth	- - D G H	×			
	- - D G -				×
Arm	- - - - H	×			
Glans penis	C M - - H	×			
	- - - - H	×			
Toe	C M - - H			×	
	- - - G H	×		×	
	- - - G H		×		

TABLE VII

RECOVERY OF BIOCHEMICAL TYPES FROM BODY AREAS "A" OF TEST SUBJECTS DURING EXPERIMENT 2

Body area	Biochemical type	Sampling day: Prechamber 1	2	3	Chamber 4	5	6	7	8	9	10	Postchamber 11	12
					Subject 21								
Ear	C M - G H			x	x	x	x	x				x	x
	C M - G -								x				
	C M - - H						x					x	
	- - D G H		x										
	- - - G H	x				x	x	x	x			x	x
	- - - G -	x				x	x						
	- - - - H				x				x	x	x		
Nose	C M - - H	x		x		x		x					
	- - D G H			x					x	x		x	x
	- - D - H		x								x		
	- - - G H	x		x	x	x	x	x	x	x	x	x	x
	- - - - H	x		x	x					x	x		
Throat	C M - - H								x				
	- - D G H		x									x	x
	- - D - H									x		x	
	- - - - H	x											
Mouth	C M - - H	x		x								x	
	- - D G H			x								x	
	- - - G H			x								x	
	- - - G H							x					
Axilla	C M - - H	x		x	x		x	x	x	x	x	x	x
	- - - G H								x	x	x	x	
	- - - - H									x			
Groin	C M - - H	x	x		x	x	x	x	x	x	x	x	x
	- - D G H		x										
	- - - G H	x						x	x	x	x	x	x
	- - - - H	x	x	x			x	x					
Glans penis	C M - - H	x	x	x	x	x	x	x	x	x	x	x	
	- - D G H			x									
	- - D G -											x	
	- - - G H			x	x	x	x	x	x	x	x	x	
	- - - - H	x	x		x	x						x	
					Subject 22								
Ear	C M - G H												x
	C M - - H									x		x	x
	C M - - -										x		
	- - D G H			x									
	- - D G -										x		
	- - D - H												x
	- - - G H		x				x	x		x		x	
	- - - G -	x											
	- - - - H		x	x	x	x		x			x	x	
Nose	C M D G H	x		x									
	C M D G -											x	
	C M - - H											x	
	- - D G H		x	x			x	x	x				
	- - D G -									x			
	- - D - H										x	x	x

TABLE VII, continued

Body area	Biochemical type	Sampling day: Prechamber 1	2	3	Chamber 4	5	6	7	8	9	10	Postchamber 11	12
					Subject 22								
Nose	- - - G H	x	x		x	x	x	x	x	x	x		x
	- - - G -												x
	- - - - H									x			
Throat	C M D G H		x	x	x	x	x	x	x				
	C M D - H										x		
	- - D G H	x	x										
Mouth	C M D G H					x							
	C M D - H					x							
	C M - - H	x											
	- - D G H			x	x								
	- - - G H	x											
	- - - G -					x							
Axilla	C M - - H								x	x	x		
	C M - - -			x									
	- - D - H											x	
	- - - G H	x	x	x	x	x	x	x	x	x	x	x	x
	- - - G -	x											
	- - - - H							x			x	x	x
Groin	C M - - H	x					x					x	x
	C M - - -	x		x						x			
	- - D G H										x		
	- - D - H			x									
	- - - G H		x				x		x		x		x
	- - - - H	x	x	x	x	x	x	x		x		x	x
Glans penis	C M - - H	x		x	x	x		x	x		x	x	x
	- - D - H			x									
	- - - G H	x	x	x	x	x	x	x	x		x	x	x
	- - - - H	x	x			x	x	x		x	x	x	
Ear	C M D G H							x			x		
	C M D - H	x											x
	C M D - -						x		x		x		
	- - D G H			x									
	- - D G -								x		x	x	
	- - D - H			x									
	- - - G H		x			x	x	x	x	x		x	x
	- - - G -									x			
	- - - - H					x	x	x					
Nose	C M D G H	x	x	x					x	x	x		
	C M D G -								x				
	C M - - H											x	
	- - D G -											x	
	- - - G H	x	x	x	x	x	x			x	x		x
	- - - G -		x										
	- - - - H								x				
Throat	- - D - -								x				
	- - - G H				x								x
Mouth	C M D - H											x	
	- - - G H					x		x			x	x	
	- - - - H						x						
Axilla	C M - - H	x										x	
	- - D - -							x					
	- - - G H			x	x	x	x	x	x	x	x	x	x
	- - - - H												x

TABLE VII, continued

Body area	Biochemical type	Sampling day: Prechamber 1	2	3	Chamber 4	5	6	7	8	9	10	Postchamber 11	12
					Subject 23								
Groin	C M D G -							×					
	C M - - H								×			×	
	- - D G -											×	×
	- - - G H				×						×		
	- - - - H	×	×	×	×	×	×		×	×	×	×	×
Glans penis	C M D G -							×	×				
	C M - - H							×				×	
	C M - - -												×
	- - D G -						×					×	
	- - D - H	×											
	- - - G H				×	×	×			×		×	
	- - - - H	×		×	×			×		×	×		
					Subject 24								
Ear	C M D G H	×											
	C M D - H	×											
	C M - G H											×	
	C M - - H							×					
	- - D G H						×						
	- - D G -										×	×	
	- - - G H	×	×	×	×	×	×	×	×	×	×		
	- - - G -								×				
Nose	C M D G H	×	×	×		×	×						
	C M D - H							×	×	×		×	×
	C M - - H				×				×		×		
	- - D G H		×										
	- - D G -								×			×	
	- - - G H	×	×	×	×	×	×	×			×	×	×
	- - - - H							×					
Throat	C M D G H	×											
	C M D - H	×											×
	C M - G H					×							
	- - - G H												×
	- - - G -						×						
Mouth	C M D G H					×							
	C M - - H						×						
	- - - - H		×									×	
Axilla	C M - - H		×			×	×	×					×
	- - D G H		×		×								
	- - - G H	×		×	×	×	×	×	×	×	×	×	×
	- - - - H	×	×	×	×	×			×			×	
Groin	C M - - H		×			×	×	×	×	×	×	×	×
	- - D G H				×								
	- - D G -	×				×	×	×			×		×
	- - - G H		×			×	×			×		×	×
	- - - - H	×		×	×	×	×	×	×				
Glans penis	C M - - H							×	×	×	×	×	×
	C M - - -						×						
	- - D G -		×						×	×	×		×
	- - - G H	×		×	×	×	×	×	×	×	×	×	×
	- - - G -	×											
	- - - - H	×				×	×	×			×		

TABLE VIII

RECOVERY OF BIOCHEMICAL TYPES FROM BODY AREAS "B" OF TEST SUBJECTS DURING EXPERIMENT 2

Body area	Biochemical type	Sampling day: Pre-Chamber 1	Pre-Chamber 2	Chamber 3	Post-Chamber 4
		Subject 21			
Scalp	C M - - -	x			
	- - D G H				x
	- - - G H	x	x	x	x
	- - - - H		x	x	x
Eye	- - D - H				x
	- - - G H		x		x
	- - - - H				x
Arm	C M - - H			x	
	- - - - H	x			x
Umbilicus	C M - - H		x		x
	- - - G H			x	
	- - - - H	x	x	x	
Anus	- - D G H	x	x		x
	- - D - H		x		
	- - - G H	x	x	x	x
	- - - - H	x	x	x	
Toe	- - D G H		x		
	- - - G H	x		x	x
	- - - - H	x	x		x
		Subject 22			
Scalp	C M - G H	x	x		
	C M - - H	x			x
	C M - - -	x	x		
	- - D G H				x
	- - D - H		x		x
	- - - G H	x		x	
	- - - - H		x	x	
Eye	- - - G H				x
Arm	C M - - H	x			
	C M - - -		x		
	- - D G -				x
	- - - - H			x	x
Umbilicus	C M - - H	x		x	x
	C M - - -	x	x		
	- - - G H		x		
	- - - - H	x	x		
Anus	C M - - -		x		
	- - - - H	x	x	x	
Toe	C M - - H	x			
	- - D G H	x			
	- - D - -	x			
	- - - G H	x			x
		Subject 23			
Scalp	C M D G -	x			x
	C M - - H			x	

TABLE VIII, continued

Body area	Biochemical type	Sampling day: Pre-Chamber 1	Pre-Chamber 2	Chamber 3	Post-Chamber 4
			Subject 23		
Scalp	- - D G -				x
	- - - G H	x	x	x	x
	- - - G -	x			
Eye	C M D G H				x
	C M D G -			x	x
	- - D G H		x		
	- - - G H			x	x
	- - - - H			x	
Arm	C M D G -				x
	C M - - H		x		x
	- - D G -				x
	- - D - -		x		
	- - - G H			x	
Umbilicus	C M D G -				x
	- - - G H			x	x
	- - - - H			x	
Anus	- - - - H	x	x		
Toe	- - D G H	x			
	- - - G H		x		
	- - - - H	x			
			Subject 24		
Scalp	C M - - H	x	x		x
	- - D G H		x		
	- - - G H			x	x
	- - - - H		x		
Eye	- - D G H		x		
	- - D - H	x			
	- - - G H	x		x	x
	- - - G H	x	x		
	- - - G -	x		x	x
Arm	C M - - H				x
	- - D G -				x
	- - D - H		x		
	- - - G H			x	x
	- - - - H			x	
Umbilicus	C M - - H				x
	- - D G -			x	
	- - - G H		x	x	x
	- - - - H	x	x	x	
Anus	C M - - H			x	
	- - D G -	x	x	x	
Toe	- - D G H	x	x		
	- - - G H	x			
	- - - - H	x			

TABLE IX

SUMMARY OF STATISTICAL ANALYSIS OF BIOCHEMICAL TYPES RECOVERED FROM SELECTED BODY AREAS OF TEST SUBJECTS AND THE ENVIRONMENT DURING EXPERIMENT 1

Factors	Biochemical types*		
	C, M	D	X, Y
Body areas "A"			
Body areas	S	S	S
Subjects	NS	NS	NS
Time**	NS	NS	NS
Interaction: subject vs. time	NS	NS	NS
body area vs. time	NS	NS	NS
Body areas "B"			
Body areas	NS	NS	NS
Subjects	NS	NS	NS
Time	NS	NS	NS
Environment			
Prechamber vs. postchamber time	S	S	NS
Prechamber physical areas	NS	NS	NS
Postchamber physical areas	NS	NS	NS
Chamber time	NS	NS	NS
Chamber physical areas	NS	NS	NS
Chamber vs. prechamber time	NS	NS	NS
Chamber vs. postchamber time	S	S	NS

* X = all positives except for C and M; Y = all positives except for D; S = significant; NS = not significant.

** Time period 1 compared to time period 2.

TABLE X

SUMMARY OF STATISTICAL ANALYSIS OF BIOCHEMICAL TYPES RECOVERED FROM SELECTED BODY AREAS OF TEST SUBJECTS AND THE ENVIRONMENT DURING EXPERIMENT 2

Factors	Biochemical types*		
	C, M	D	X, Y
Body areas "A"			
Body areas	S	S	S
Subjects	NS	NS	NS
Time**	NS	NS	NS
Interaction: subject vs. time	NS	NS	NS
body area vs. time	NS	NS	NS
Body areas "B"			
Body areas	NS	NS	NS
Subjects	NS	NS	NS
Time	NS	NS	NS
Environment			
Prechamber vs. postchamber time	NS	NS	NS
Prechamber physical areas	NS	NS	NS
Postchamber physical areas	NS	NS	NS
Chamber time	NS	NS	NS
Chamber physical areas	NS	NS	NS
Chamber vs. prechamber time	NS	NS	NS
Chamber vs. postchamber time	NS	NS	NS

* X = all positives except for C and M; Y = all positives except for D; S = significant; NS = not significant.

** Time period 1 compared to time period 2.

TABLE XI

FREQUENCY OF BIOCHEMICAL TYPES RECOVERED FROM SIGNIFICANT BODY AREAS

Biochemical type	Experiment	Body areas*										
		Eye	Ear	Nose	Throat	Mouth	Axilla	Umbilicus	Groin	Glans penis	Anus	Ratio**
C, M	1	2	9	18	4		9	5	27		13	10.9
	2		21	26	12	8	33		29	32		23.0
D	1	2	8	15	5		7	6	21		7	8.9
	2		14	34	16	7	4		13	13		14.4
X	1	14	12	37	7		34	23	28		8	20.4
	2		42	45	13	14	38		44	44		34.3
Y	1	16	13	36	12		28	25	35		14	22.4
	2		41	43	7	13	45		47	44		34.3

* Sum of observation for all subjects in all sampling periods. Blank spaces indicate no samples collected during experimental period.

** Ratio $= \frac{\text{number of types}}{\text{sum of body areas}}$.

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13. ABSTRACT

Two groups of four human male subjects participated in 6-week simulated aerospace studies. The subjects were confined under controlled metabolic conditions; during this time 28 consecutive days were spent in a Life Support Systems Evaluator. The subjects ate diets composed of either fresh foods or precooked freeze dehydrated foods. The subjects were exposed to simulated aerospace stress of confinement wearing an unpressurized MA-10 pressure suit, experimental diet, and minimal personal hygienic conditions. Body and environmental areas were sampled and the catalase-positive, gram-positive cocci isolated were tested for production of coagulase, deoxyribonuclease, hemolysin, gelatinase, and utilization of mannitol. The results showed no significant differences in the frequency of occurrence of biochemical types in microbiological specimens from the eye, ear, nose, throat, mouth, axilla, umbilicus, groin, glans penis, and anus. There was no buildup of biochemical types with time in any test condition. There was no difference in the frequencies of biochemical types when either the coagulase-mannitol marker or the deoxyribonuclease marker was used to indicate the potentially pathogenic type. The subjects remained healthy without any decrease in resistance to infection throughout all the test conditions. Those body areas most likely to harbor potentially pathogenic staphylococci are the nose and groin. In concurrent metabolic studies, the physiological, biochemical, and nutritional parameters investigated were all in the normal range of clinical values. Confinement under simulated aerospace conditions for at least 28 consecutive days and conditions of minimal personal hygiene show that no unique set of circumstances are operable that would require the establishment of special biomedical criteria.

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Phage type						
Hygiene						
Diet						
Confinement						