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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 LIQUID 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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This report has been reviewed and is approved.

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ABSTRACT

Two groups of 4 human male subjects participated in 6-week simulated aerospace studies. The subjects were confined and kept under controlled metabolic conditions; during this time, 28 consecutive days were spent in the Life Support Systems Evaluator. The subjects ate diets composed either of fresh food or liquid food. The subjects were exposed to simulated aerospace stress of confinement, wearing an unpressurized MA-10 space 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 show that there were no significant differences in the frequency of occurrence of biochemical types among subjects and among environmental areas during the chamber period. There were significant differences in frequency of occurrence of biochemical types on ear, nose, throat, mouth, axilla, groin, and glans penis. There was no buildup of biochemical types with time in any test condition. Two phage types, UC-18 and 79, were recovered. Phage type UC-18 was transferred from subject to environment but not vice versa or among other subjects. Phage type 79 was not transferred at all. Despite the fact that cultures tested by the coagulase plate method were shown to be false positive when tested by the coagulase tube method, in either case the frequencies of occurrence of biochemical types did not differ significantly. The same fact was observed when the deoxyribonuclease marker was used to indicate the potentially pathogenic type. The subjects remained healthy without any decrease in resistance to infection throughout all test conditions. Those body areas most likely to harbor potentially pathogenic staphylococci are the ears and nose. In the 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 flight 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 are known to increase the 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 maintenance of nutritional balances are important in the resistance of man and animals to microbial infection.

Other stresses such as burns (5), traumatic shock (6), fatigue (7), extensive body irradiation (8), hyposecretion and hypersecretion of hormones (9), and diabetes mellitus, tuberculosis, and kidney damage (7,10,11) have been shown to reduce resistance to infection. Although any one 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 instance, 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).

Phage typing represents an ancillary approach in identifying potentially pathogenic staphylococci. Blair (18) claimed that only coagulase-positive staphylococci are phage typable, although 20% to 30% of these are not lysed by typing phages. Lysogeny which confers specific prophage immunity may be responsible for insensitivity to these phages (19). Most nosocomial strains of staphylococci are phage sensitive and resistant to one or more antibiotics (18).

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. In an earlier report Lotter, Horstman, and Rack observed in healthy male human subjects neither a decrease in resistance to staphylococcal infection nor a buildup or dissemination of these organisms among subjects and their environment (20). During that time subjects were maintained on a diet composed of either fresh foods or precooked freeze dehydrated foods under simulated aerospace conditions.

This report describes the results obtained from two 6-week experiments during which time two groups of 4 human male subjects were confined under simulated aerospace and controlled metabolic conditions. The subjects wore unpressurized MA-10 pressure suits* for part of the time and were given a liquid diet. The results of the basic nutritional study are reported elsewhere (21, 22). 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 (23).

SECTION II

EXPERIMENTAL METHODS AND PROCEDURES

During each of two 42-day experiments 4 healthy male subjects were confined in a controlled activity facility (CAF)** for a period of one week, then transferred to the Life Support Systems Evaluator (LSSE)** for 28 days, followed by a final week in the

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

** 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.

CAF. Table I contains the experimental design for both experiments. Throughout the experiment all personal contacts with the subjects were rigidly minimized. Only personnel wearing sterile surgical clothing were permitted to enter the CAF, and no one except the subjects entered the chamber. Transfer of subjects to and from the chamber was strictly controlled, including the wearing of sterile surgical apparel. Part of the daily activity schedule (table II) of the subjects involved microbiological and chemical tests, physiological measurements, psychological tests, exercise, and free activity periods (21,22).

During experiment 1, a 1-day cycle diet composed of fresh foods was served for 3 weeks followed by a nutritionally matched liquid diet prepared by the Pillsbury Company of Minneapolis, Minnesota. The daily menu contained approximately 2700 calories and was divided into 4 meals per day. The fresh food diet consisted of a meat sandwich, a lettuce salad, fruit, and tea. The 4 flavors of the liquid formula were cherry, vanilla, chocolate, and strawberry, rotated daily (21). In experiment 2, a fresh food diet was served for 3 weeks, followed by a nutritionally matched liquid diet for the same period of time. The fresh food diet was divided into 4 meals per day, served at 4-hour intervals. The fresh food diet consisted of a meat sandwich, canned peaches, pudding, and Kool-Aid. The liquid diet consisted of six flavors: strawberry, cherry, vanilla, chocolate, raspberry, and butterscotch served on a rotated cycle (22). Subjects were required to consume all food served to them.

For experiments 1 and 2, the CAF and the chamber were disinfected by sponging and spraying with benzalkonium chloride (BAC) solution. During experiment 1, all parts of the body of every subject were thoroughly cleansed with pHisoHex before entering either the CAF or the chamber. The ears and nose of each subject were cleansed with sterile cotton swabs. After using sterile washcloths and towels, the subjects donned sterile surgical clothing for transfer to the CAF and chamber. They were not permitted to bathe, groom hair, clean or cut nails, shave, change or remove clothes. Wipes saturated with sodium lauryl sulfate and wipes saturated with the quarternary amine, *p*-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride were used for personal hygiene. Subjects 25 and 28 wore the MA-10 space suite, unpressurized, with boots, helmet, and gloves at two different 8-hour periods per day for the 28 days in the chamber. Use of a regular toothbrush with water was the only oral hygiene practiced. During experiment 2, the same minimal hygienic practices were followed with the exception that plain wipes were substituted for the chemically impregnated wipes. These were moistened before use with water that was supplied for this purpose. Subjects 29 and 32 wore the torso for the unpressurized MA-10 space suit with boots continually, and helmet and gloves part time during the entire 28-day confinement in the chamber. Oral hygiene involved the use of a regular toothbrush with an edible dentifrice provided by the School of Aerospace Medicine, Brooks Air Force Base, Texas. Sweat tests were performed during the prechamber and postchamber periods (22).

TABLE I

EXPERIMENTAL DESIGN

Test days	Condition	Metabolic diet	MA-10 pressure suit Subject No.	Sweat test	Microbiological sampling			
					Body areas "A"	Body areas "B"	Environment	Feces
6	Prechamber	Fresh food	None	x	xx	x	xx	xxx
14	Chamber	Fresh food	25, 28 29, 32		xxxx		xxxx	xxxx
14	Chamber	Liquid food	25, 28 29, 32		xxxx		xxxx	xxxxx
6	Postchamber	Liquid food	None	x	xx	x	xx	xx

TABLE II

DAILY ACTIVITY SCHEDULE

Time	Subject No. 25, 29 / 26, 30	Subject No. 27, 31 / 28, 32	Time
0700			0700
0715	Wake; void; physiological measurements. Transfer food and other items into chamber. Biological specimens collected and returned to laboratory.		0715
0800			0800
0900	Eat meal A		0900
1000	Physiological testing and exercise		1000
1100			1100
1200			1200
1300	Meal B		1300
1400			1400
1500	Testing period I		1500
1600		Sleep	1600
1700	Meal C		1700
1800	Testing period II		1800
1900			1900
2000	Television available		2000
2100	Meal D	Meal B	2100
2200			2200
2300	Television available		2300
2315			2315
2400		Free time	2400
0100		Meal C	0100
0200			0200
0300		Testing period III	0300
0400	Sleep		0400
0500		Meal D	0500
0600			0600
0700		Testing period IV	0700
0730			0730

Body areas sampled during both experiments were divided into primary regions designated areas "A", and secondary regions designated areas "B". Areas "A" included the ear, nose, throat, mouth, axilla, groin, and glans penis; areas "B" included the scalp, eye, forearm, umbilicus, anus, and toe. In both experiments, areas "A" were sampled 12 times and areas "B" were sampled 3 times. The environmental areas were sampled 13 times in experiment 1 and 11 times in experiment 2 (tables I and II).

Samples of areas "A" and "B" were taken during both experiments with sterile dry cotton swabs which were then streaked on 5% sheep blood agar (Baltimore Biological Laboratories, B.B.L.) and then incubated aerobically at 37°C for 24 hours, followed by incubation at 30°C for 48 hours. The latter incubation enhanced colonial morphology and pigmentation. Fecal plates were provided by personnel of Republic Aviation Corporation. One loop of fecal material was inoculated into Gall's broth (23) and a dilution series prepared. One-tenth of a milliliter of the 10^{-5} and 10^{-6} dilution was plated on 10% sheep blood agar (B.B.L.) and incubated aerobically at 37°C for 24 hours. Environmental areas were sampled by exposing 10% sheep blood agar plates (B.B.L.) to the air of each environment for one-half hour.

The indirect mutant selection technique of Lederberg and Lederberg (24) was utilized to simplify the biochemical study. Replicators slightly smaller than the standard 100 x 15 mm plastic petri dish were cast from aluminum alloy stock and covered with velveteen. The velveteen replicator was pressed against 10 colonies grown on blood agar plates and then applied to the test medium surface.

The bacterial colonies on the initial 5% sheep blood agar plates were thoroughly examined for colonial morphology, pigmentation, and hemolysis. One of each colonial type observed was streaked on a plate of Trypticase Soy Broth (B.B.L.) plus 1.5% agar (Difco). Three percent H_2O_2 was applied to colonies of gram-positive cocci to detect catalase production and catalase-positive cocci were further tested for several biochemical reactions as shown below.

All cultures considered gram-positive cocci after microscopic observations and found to be catalase-positive were accepted for further biochemical studies. Before replication to biochemical test media, staphylococci were grown on 5% sheep blood agar plates and their colonies showed hemolysis after 48 hours of aerobic incubation at 37°C. Coagulase production and mannitol utilization were determined on Coagulase Mannitol Agar Base (B.B.L.) (25) to which 15% sterile horse coagulase plasma (B.B.L.) (26) had been added. Deoxyribonuclease production was detected on DNAase Test Medium (B.B.L.) (27), and gelatinase production on Chapman-Stone Medium (B.B.L.) plates (28). Lotter and Horstman (29) found that results of the coagulase plate method (25) are unreliable after all coagulase-positive cultures had been tested by the coagulase tube method of Fisk (30).

The test for free coagulase (30) was performed by combining aseptically 0.05 ml. of a 24-hour TSB culture with 0.5 ml of 1.5 dilution of citrated horse plasma (B.B.L.) in a sterile 12 x 75 mm serological test tube. The plasma was diluted with sterile distilled water. The tubes were incubated in a 37°C water bath. Clots formed usually within 3 hours but occasionally were delayed until 18 hours. The method of Blair and Williams (31) was employed for phage typing coagulase-positive isolates. These isolates as well as host strains were grown in TSB for 6 hours at 37°C. Phage routine test dilutions were applied to the bacteria coated surface of TSA plates by sterile 2.5 cc disposable syringes.

The Communicable Disease Center, Atlanta, Georgia, supplied 22 strains of S. aureus from the International set for phage typing. These control cultures included strains 3a, 3b, 3c, 6, 7, 29, 42d, 42e, 47, 52, 52a, 53, 54, 55, 71, 75, 77, 79, 80, 81, 83a, and 187. Strain UC-18 was supplied by Dr. E. O. Hill, Surgical Bacteriology Department, Cincinnati General Hospital, Cincinnati, Ohio. Micrococcus roseus strain 516 and Sarcina lutea strain 533 were obtained from the American Type Culture Collection. These cultures were tested for production of hemolysis, coagulase, deoxyribonuclease, gelatinase, and mannitol utilization. The staphylococci were positive for each marker, although M. roseus and S. lutea were uniformly negative.

All control cultures were maintained on Brain-Heart Infusion (Difco) plus 1.5% agar slants and transferred every 2 months.

Statistical tests included analysis of variance, χ^2, Student's t-test. The factors of body areas "A": subjects, time, body areas, and interaction were tested by analysis of variance at the 0.01 level of significance (32). In each case, the first and last halves of the sampling periods were summed. Thus 2 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 3 catagories on the basis of biochemical reactions: CM-isolate produced coagulase and utilized mannitol; D-isolate produced deoxyribonuclease; X-isolates were positive for all except CM and Y-isolates were positive for all except D. A separate analysis was run on the CM frequencies, another on the D, another on the X (all biochemical types positive except for CM), and another on the Y (all biochemical types positive except for D). The factor in body areas "A" were tested as follows: a test was carried out on the 4 subjects of both experiments to determine if a significant difference existed among subjects. The test for time was made to determine if a significant difference in frequency of biochemical types occurred between the 2 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 2 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 were higher and subject B were 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 a χ^2 test at the 0.01 level of significance (33). 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 (33). 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 presented 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, work table, and personal hygiene area floor for prechamber and postchamber sampling days in the CAF. While the subjects were confined to the chamber, the bed, fore table, and personal hygiene area floor were sampled. Potentially pathogenic staphylococci were detected by one or more of the following indices: C = coagulase production, M = mannitol utilization, D = DNAase production, G = gelatinase production, and 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 included the same areas as is described for experiment 1. Tables V and VI, respectively, show the biochemical types recovered from selected body areas "A" of test subjects during experiments 1 and 2, which were ear, nose, throat, mouth, axilla, groin, and glans penis. Body areas "B" of test subjects in experiments 1 and 2 were scalp, eye, forearm, umbilicus, anus, and toes (tables VII and VIII).

The number of catalase-positive cocci, presumably staphylococci, totaled 1165 cultures in experiment 1 and 995 cultures in experiment 2.

TABLE III

RECOVERY OF BIOCHEMICAL TYPES FROM SELECTED ENVIRONMENTAL AREAS DURING EXPERIMENT 1

Area sampled	Biochemical type	Sampling day												
		Prechamber			Chamber								Postchamber	
		1	2	3	4	5	6	7	8	9	10	11	12	13
Bed	C M D G H											×		
	C M D G -								×					
	C M D - H						×	×	×	×			×	
	C M D - -											×		×
	C M - - H	×		×		×	×	×		×	×	×		×
	C M - - -		×			×		×				×		×
	- - D G -									×				
	- - D - H								×					×
	- - - G H	×	×			×		×	×				×	×
	- - - - H		×					×					×	
Dining table	C M D - -												×	×
	C M - - H	×	×	×										×
	C M - - -		×											×
	- - D G H												×	
	- - D - H													×
	- - - G H			×									×	×
	- - - - H		×	×										×
Work table	C M D - H												×	
	C M - - H	×												×
	C M - - -			×										×
	- M D G H			×										
	- - - G H		×	×									×	×
	- - - G -												×	
	- - - - 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.

TABLE III, continued

Area sampled	Biochemical* type	Sampling day												
		Prechamber			Chamber								Postchamber	
		1	2	3	4	5	6	7	8	9	10	11	12	13
Floor	C M D - H					×		×		×				
	C M D - -				×					×				
	C M - - H	×			×	×	×	×	×		×			
	C M - - -			×		×	×		×		×			
	- - D G H	×												
	- - D G -			×				×						
	- - D - H		×								×			
	- - - G H	×	×		×	×			×	×				
	- - - G -			×		×								
	- - - - H		×	×	×		×	×	×					
Fore table	C M D G H				×									
	C M D G -						×							
	C M D - H						×		×		×	×		
	C M D - -				×				×					
	C M - - H						×	×	×	×				
	C M - - -					×	×	×		×				
	- - D - - H										×	×		
	- - - G H				×				×		×	×		
	- - - G -									×				
	- - - - H							×		×				
Aft table	C M D G H											×		
	C M D - H								×	×	×			
	C M D - -					×			×					
	C M - - H				×			×		×	×	×		
	C M - - -				×	×		×						
	- - D G H									×				
	- - - G H				×	×		×		×		×		
	- - - G -								×					
	- - - - H					×				×				

TABLE IV

RECOVERY OF BIOCHEMICAL TYPES FROM SELECTED ENVIRONMENTAL AREAS DURING EXPERIMENT 2

Area sampled	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	Postchamber 10	11
Bed	C M D G H									x		x
	C M D G -				x			x				
	C M D - H	x					x	x				
	C M - - H	x				x			x			x
	C M - - -				x		x		x	x		
	- - D G H		x									
	- - D - H			x	x	x						
	- - D - -		x									
	- - - G H									x		x
	- - - - H	x				x	x	x				
Dining table	C M D G H	x										x
	C M D - H	x	x									
	C M - - H	x	x									
	- - D G H	x										
	- - D - H											x
	- - D - -										x	
	- - - G H											x
	- - - - H		x									
Work table	C M - - H										x	x
	C M - - -											x
	- - D - H											x
	- - - - H											x
Floor	C M D G H		x		x							
	C M D G -							x		x		
	C M - G -										x	
	C M D - H				x	x			x			
	C M D - -					x						
	C M - - H					x	x			x		x
	C M - - -					x	x	x		x		x

TABLE IV, continued

Area sampled	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	Postchamber 10	11
Floor	C - - - H	x										
	- M D G -									x		
	- - D G -				x							
	- - D - H			x			x					x
	- - - G H			x			x		x	x		x
	- - - G -				x							
	- - - - H	x					x					
Fore table	C M D G -				x		x					
	C M D - H			x	x							
	C M D - -				x							
	C M - - H				x	x			x	x		
	C M - - -					x			x			
	C - D - H					x						
	- - D G H						x					
	- - D - H					x						
	- - - G H			x					x	x		
	- - - G -									x		
Aft table	C M D G H					x	x					
	C M D G -						x					
	C M D - H			x		x			x			
	C M D - -	x						x				
	C M - - H	x			x				x	x		
	C M - - -					x		x				
	- - D G H	x										
	- - D - H			x	x		x					
	- - - G H	x			x			x	x			
	- - - - H		x							x		

TABLE V

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

Body areas	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	Postchamber 11	12
Subject 25													
Ear	C M D G H					×		×	×	×	×	×	
	C M D - H				×		×			×			
	C M D - -	×	×										×
	C M - - H			×	×	×	×		×	×	×		
	C M - - -		×										
	- - D G H							×					
	- - D - H										×		
	- - - G H								×				
	- - - G -		×										
	- - - - H						×	×					
Nose	C M D - H							×			×		
	- - D G H									×			
	- - - G H	×	×	×	×	×	×	×	×	×	×	×	×
	- - - G -	×		×				×					×
Throat	C M - - H						×					×	
	C M - - -							×					
	- - D - H							×					
	- - - G H					×							
Mouth	- M D G -								×				
	- - D G H										×		
	- - D G -								×				
	- - - G H								×				
Axilla	C M D G H							×		×			
	C M D G -	×		×		×			×				
	C M - - H	×	×			×	×	×			×	×	
	- - D G H									×			
	- - D - H								×				
	- - D - -							×					
	- - - G H		×		×	×	×	×	×	×	×	×	×
	- - - G -	×		×			×	×	×		×	×	×
Groin	C M D G H				×		×	×		×	×	×	
	C M D G -		×	×	×	×	×		×	3(			
	- - D G H						×						
	- - D - H										×		
	- - - G H		×		×		×	×	×	×	×	×	×
	- - - - H					×				×			
Glans penis	C M D G H					×	×	×			×		
	C M D G -				×	×	×		×	×		×	×
	C M D - H							×					
	C M - G -											×	
	C M - - H				×						×	×	
	- - D G H									×			
	- - D - H							×					
	- - - G H	×		×	×	×	×		×	×	×		×
	- - - - H							×					

TABLE V, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	Postchamber 11	12
						Subject 26							
Ear	C M D - H			x	x	x	x	x	x	x		x	x
	C M D - -				x								
	C M - - H				x	x			x			x	
	- - - G H	x							x	x			x
Nose	C M D - H									x			x
	C M - - H												x
	- - D G -				x								
	- - - G H	x		x	x	x	x	x	x	x	x	x	x
	- - - G -							x			x		
	- - - - H	x		x						x			
Throat	C M D - -											x	
Mouth	C M D - H									x			
	C M - - H							x					
	- - - G H							x	x		x		
Axilla	C M D - H							x			x		
	C M - - H								x	x		x	
	- - D G H						x						
	- - D - H										x		
	- - - G H		x	x	x	x	x	x	x	x	x	x	x
	- - - - H								x			x	x
Groin	C M D - H							x					
	C M D - -							x					
	C M - - H										x	x	x
	C M - - -		x	x	x	x	x		x	x	x		
	- - D - H								x			x	
	- - - G H			x	x	x	x		x			x	x
Glans penis	C M D - H						x					x	
	C M - - H						x	x	x	x			x
	C M - - -		x	x	x	x	x			x	x		
	- - D - H								x	x	x	x	
	- - - G H		x	x	x	x	x				x		
						Subject 27							
Ear	C M D G H					x				x	x		
	C M D G -				x								
	C M D - H					x		x	x	x	x	x	x
	C M D - -		x	x	x	x	x				x	x	
	C M - - H			x			x				x		x
	C M - - -			x									
	- - D G H										x		
	- - - G H	x	x	x	x		x	x	x	x	x		x
	- - - G -												x
Nose	C M D G H								x		x	x	
	C M D - H					x		x	x			x	x
	C M D - -			x			x		x	x		x	
	C M - - -												
	- - D - -			x									
	- - - G H	x	x	x	x	x	x	x		x	x		x
	- - - G -												x
	- - - - H							x		x	x	x	x

TABLE V, continued

Body areas	Biochemical type	Prechamber 1	Prechamber 2	Chamber 3	Chamber 4	Chamber 5	Chamber 6	Chamber 7	Chamber 8	Chamber 9	Chamber 10	Postchamber 11	Postchamber 12
Subject 27													
Throat	C M D - H			×	×	×	×	×				×	×
	- - - G H								×		×		
Mouth	- - - G H					×				×	×		
Axilla	C M D - H					×							
	C M - - H								×	×		×	×
	- - - G H	×	×			×	×	×	×	×		×	×
Groin	C M D G H										×		
	C M D G -	×				×		×	×	×	×		
	C M - - H		×	×	×	×	×			×	×	×	×
	- - - G H						×				×		
Glans penis	C M D G H										×		
	C M D G -			×			×					×	
	C M - - H										×		
	- - - G H						×		×				
Subject 28													
Ear	C M D G H				×		×	×	×		×		
	C M D G -	×	×			×							
	C M D - H		×	×	×	×				×		×	
	C M D - -								×	×	×		×
	C M - G H												×
	C M - - H											×	
	- - D - H										×		
	- - - G H		×					×	×	×			
	- - - - H											×	
Nose	C M D G H							×					
	C M D - H	×	×	×	×	×	×	×	×		×	×	×
	- - - G H	×	×	×	×	×	×	×	×		×		
Throat	C M D G H							×					×
	C M D - H			×	×					×			
Axilla	C M D - H											×	
	- - - G H				×	×		×		×	×		
	- - - - H			×	×	×	×	×	×	×	×	×	×
Groin	C M D G H					×		×		×			
	C M D G -				×		×			×		×	
	C M D - H			×									
	C M - - H			×	×								
	C M - - -							×					
	- - D G -			×					×				
	- - D - H				×								
	- - - - H	×	×		×	×	×	×	×	×	×		×
Glans penis	C M D G H							×					
	C M D G -			×		×	×		×	×		×	×
	C M - - -			×									
	- - - G H					×						×	
	- - - - H		×		×	×	×	×	×		×	×	×

TABLE VI

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

Body areas	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	Postchamber 11	12
Subject 29													
Ear	C M D G H	×		×		×		×		×		×	
	C M D G -				×		×		×		×		
	C M D - H		×	×									
	C M D - -								×				
	C M - - H							×		×			×
	- M D G -				×								
	- - D G H	×	×										
	- - D - H			×		×				×			
	- - - G H	×			×		×	×			×	×	×
	- - - - H	×			×	×		×	×		×		
Nose	C M - - H												×
	- - D - H				×								
	- - - G H	×	×	×	×	×	×	×	×	×	×	×	×
	- - - - H	×						×	×	×	×		
Throat	C M D - H								×				
	- - D G H									×			
	- - - G H									×			
Mouth	C M D G H				×		×			×		×	×
	C M D - H					×			×				
	C M - - H											×	×
	- - - G H						×		×	×			
	- - - G -							×					
	- - - - H			×									
Axilla	C M D G -										×		×
	C M D - H	×											
	- - - G H										×		
	- - - - H	×	×	×	×	×	×	×	×	×		×	
Groin	C M - - H	×		×	×	×	×	×	×		×	×	×
	- - D - H			×									
	- - - - H								×		×		
Glans penis	C M D G H									×			
	C M D G -				×		×						
	C M D - H	×											
	C M - - H	×	×	×				×	×	×	×	×	×
	C M - - -								×			×	
	C - D G -			×									
	- - - G H	×										×	
Subject 30													
Ear	C M D G -		×					×					
	C M D - H											×	
	C M D - -									×			×
	C M - - H					×						×	
	- - D - H						×						
	- - - - H		×	×				×		×	×	×	×
	- - - G -							×					
	- - - - H						×						
	C M D - H								×				
	C M - - H								×			×	
	C M - - -								×				
	- - D G H	×		×	×			×		×			

TABLE VI, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	Postchamber 11	12
						Subject 30							
Nose	- - - G H	x	x	x	x	x	x	x	x	x	x	x	x
	- - - G -				x								
Throat	- - D - -					x							
	- - - - H	x											
Mouth	- - D G H				x								
	- - - G -											x	
Axilla	- - D G H						x						
	- - D G -					x							
	- - - G H			x				x					
	- - - - H	x	x	x	x	x	x	x	x	x	x	x	x
Groin	C M D G H			x									
	C M D G -					x				x			
	C M - - H		x		x				x		x	x	
	C - D G -	x											
	- M D G -							x					
	- - D G H							x					
	- - D G -				x								
	- - D - H	x		x		x		x					
	- - D - -										x		
	- - - - H	x	x	x			x						x
Glans penis	C M D G H			x									
	C M D G -				x	x				x			x
	C M D - H											x	
	C M - - H		x				x	x					x
	C - D G H	x											
	- M D G -										x		
	- - D G H		x			x							
	- - D G -		x										
	- - D - H							x	x				x
	- - - G H							x					
	- - - - H	x		x					x	x	x	x	
						Subject 31							
Ear	C M D G -											x	
	C M D - H						x						
	C M D - -						x						
	C M - - H							x	x	x	x	x	
	- - D - -			x	x								
	- - - G H	x	x			x	x		x		x	x	x
	- - - - H									x			
Nose	C M D G H					x							
	C M D G -							x					
	C M D - H			x									
	C M - - H		x				x						
	C M - - -				x								
	- - D G H				x		x	x	x		x	x	x
	- - D - H	x								x	x		x
	- - - G H	x	x	x	x	x		x	x	x	x	x	x
	- - - - H								x				
Mouth	C M - - H										x		
Axilla	- - D G H	x			x		x						
	- - D G -	x			x								
	- - - G H		x		x	x	x		x	x	x	x	x
	- - - - H	x	x	x	x	x	x	x	x	x	x	x	x

TABLE VI, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	2	Chamber 3	4	5	6	7	8	9	10	Postchamber 11	12
Subject 31													
Groin	C M D G H						×		×			×	×
	C M D G -			×	×	×		×		×			
	C M D - H			×	×		×			×	×		
	C M - - H	×											
	- - D G H		×	×				×					
	- - - G H	×	×			×				×		×	×
	- - - - H		×										
Glans penis	C M D G H		×	×			×	×	×	×		×	
	C M D G -			×		×	×						×
	C M D - H			×				×			×	×	
	- - D G H	×	×					×					
	- - - G H	×	×	×		×					×	×	×
	- - - - H	×											
Subject 32													
Ear	C M D - H		×			×	×		×	×	×	×	
	C M - - H			×					×				
	C - D - H								×				
	- - D G H			×			×						
	- - D - H												
	- - D - -									×			
Nose	- - - G H	×	×	×	×	×				×	×	×	
	C M D G H	×	×	×	×						×	×	×
	C M D - H		×				×	×	×				
	C M D - -						×						×
	C M - - H												
	- - D G H		×			×		×					
	- - - G H	×	×	×	×	×		×	×	×	×	×	×
	- - - G -									×			
Throat	C M D - H						×						
	C M - - H									×			
	- - - G H						×						
	- - - - H					×			×				
	- - - - H					×							
Mouth Axilla	C M - - H			×	×			×	×	×	×	×	×
	C M - - -		×										
	- - D G H	×		×			×						
	- - - G H		×		×			×	×	×	×	×	×
	- - - - H		×	×	×	×	×	×	×	×			×
Groin	C M D G -											×	
	C M D - H												×
	C M - - H			×	×	×	×	×	×	×	×	×	
	C M - - -	×	×										
	- - D G -	×								×			
	- - D - H										×	×	×
	- - - G H	×				×							
	- - - - H		×	×									
Glans penis	C M D G H				×				×			×	×
	C M D G -			×	×	×	×	×	×	×	×	×	×
	C M - - H							×	×		×		×
	- - D - H						×		×	×	×	×	×
	- - - G H	×											
	- - - - H	×	×	×									

TABLE VII

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

Body areas	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Postchamber 3
		Subject 25		
Scalp	C M D G H			×
	C M D G -			×
	C M D - H	×		
	C M - - H			×
	C M - - -	×		×
	- - - G H	×		
Eye	- - - G H			×
Forearm	C M D G -			×
	C M - - H		×	×
	- - - G H	×		
Umbilicus	C M D G -			×
	C M D - H			×
Anus	- - - G H		×	
	- - - - H			×
Toe	C M D G -		×	
	C M - - H			×
	- - - G H	×	×	
		Subject 26		
Scalp	C M D - H	×		×
	C M - - H		×	×
	- - - G H		×	
Eye	- - D G H	×		×
	- - - G H	×	×	×
Forearm	C M D - H		×	
	C M - - H		×	×
	C M - - -		×	
	- - D G H			×
Umbilicus	C M - - H		×	×
	- - - G H		×	
	- - - G -		×	
Anus	C M - - H		×	×
	C M - - -		×	
	- - D - H			×
	- - - G H		×	×
	- - - - H		×	×

TABLE VII, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Postchamber 3
		Subject 26		
Toe	C M - - -			×
	- - D - H			×
	- - D - -	×		
	- - - G H	×		×
		Subject 27		
Scalp	C M D - H	×	×	×
	C M D - -	×	×	
	C M - - H	×		
	- - - G H			×
Eye	- - - G H			×
	- - - - H			×
Forearm	C M D G -		×	
	C M D - H		×	
	C M D - -			×
	C M - - H		×	
	- - - G H		×	×
Umbilicus	- - - G H			×
	- - - - H			×
Anus	C M - - H			×
Toe	C M - - H			×
	- - - G H	×		×
	- - - - H	×		
		Subject 28		
Scalp	C M D G -	×		
	C M D - H		×	×
	C M D - -	×		
	C M - - H		×	×
	- - - - H	×		
Eye	C M D - H			×
	C M - - H			×
	- - - G H	×		
Forearm	C M D - H			×
	- - - G H	×		×
Umbilicus	- - - G H		×	
Anus	C M - - H			×
	- - - - H	×	×	×
	C M - - H			×
	- - - G H	×		
	- .. - G -			×

TABLE VIII

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

Body areas	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Postchamber 3
		Subject 29		
Scalp	C M D - H	x	x	
	C M D - -		x	x
	C M - - H		x	
	- - D G H			x
	- - - G -			x
	- - - - H			x
Eye	- - D G H	x		
	- - D G -	x		
	- - - G H	x	x	x
	- - - G -			x
Forearm	C M D G -		x	
	C M D - H	x		x
	C M D - -			x
	C M - - H	x		
	C M - - -		x	
Umbilicus	C M D - H			x
	C M - - H		x	
	C M - - -		x	
	- - - G H			x
Anus	C M - - -	x	x	
	- - - G H			x
Toe	C M - - H	x		x
	- - D - H			x
	- - - G H			x
		Subject 30		
Scalp	C M D G -	x		
	C M D - H	x	x	x
	C - D - H	x		
	- - D - H			x
	- - - G H		x	
	- - - - H			x
Forearm	- - - - H		x	
Umbilicus	C M D G -		x	
	C M - - H	x		
	C - D - H	x		
	- - - G H		x	
Anus	- - D G H		x	
	- - D G -			x
	- - - - H	x	x	x
Toe	- - - G H	x	x	x
	- - - G -	x		
		Subject 31		
Scalp	C M D G H	x		
	C M D - H	x	x	
	C M - - H			x
	C M - - -			x
	- - - G H			x

TABLE VIII, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Postchamber 3
		Subject 31		
Eye	- - - G H	x		
	- - - - H		x	
Forearm	C M D G H		x	
	C M D - H		x	
	C M - - H	x		
	- - D - H		x	
	- - - G H		x	x
	- - - - H	x	x	x
Umbilicus	C M D G H	x		
	C M D G -		x	
	C M D - H	x		
	C M - - H	x		
	C - D G H	x		
	- - - - H			x
Anus	C M D G -		x	
	C M D - H		x	
	- - D - H	x		
	- - - G H		x	
	- - - - H	x		
Toe	C M - - -			x
	- - D - H			x
	- - - G H	x		x
	- - - G -			x
	- - - - H	x		
		Subject 32		
Scalp	C M D G H	x		
	C M D - H	x	x	x
	C M - - H		x	
	- - - - H	x		
Eye	C M - - H		x	
	C M - - -		x	
	- - - G H		x	x
Forearm	C M - - H		x	x
	- - D - H		x	x
	- - - G H			x
Umbilicus	C M D - H			x
	C M - - H		x	
	C M - - -			x
	- - D - H			x
	- - - G H	x		x
Anus	C M - - H	x	x	
	- - D G H	x		x
	- - - G H		x	
	- - - - H	x	x	x
Toe	- - D G H			x
	- - D - -	x		
	- - - G H			x
	- - - - H		x	x

Tables IX and X summarize results of the statistical analysis of the biochemical types recovered from the environment and selected body areas of test subjects 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 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 area there occurred considerably larger frequencies of biochemical types than in (see table XII) other body areas. Time was not a significant factor; the frequency of occurrence of CM, D, X, and Y types in the first 6 sampling periods did not differ strikingly from the frequency of occurrence in the second 6 sampling periods. There was no buildup of any biochemical type as the experiment progressed with time. No significant difference was observed when the frequency of biochemical types was compared among subjects. Neither subject versus time nor body areas versus time interactions were significant. The results indicate that the change in frequency among biochemical types from time period 1 (first 6 sampling periods) to time period 2 (second 6 sampling periods) was relatively the same for all body areas and all 8 subjects. Body areas "B" show that comparable frequencies of biochemical types were isolated for all subjects in both experiments. The body area factor however, for CM and D biochemical types (both experiments) was significant, indicating that these types occurred more frequently in one or more body areas than in others. CM and D types, respectively, occurred 92% and 83% of the time on the scalp in experiment 1, but 100% and 92% of the time on the scalp in experiment 2. A buildup of CM, D, and Y types took place with increasing time in experiment 1, but no buildup of any type was observed in experiment 2. It seems surprising that time (experiment 1) under body areas "B" was significant for CM, D, and Y types, whereas the same factor under body areas "A" was not. Perhaps this is due to the fact that analysis of variance was used to analyze body areas "A", and χ^2 test was used on body areas "B". Because of the limited data in body areas "B", these areas could not be analyzed by analysis of variance. These statistical tests probably differ from each other in their sensitivity. The analysis of the environment was accomplished by making 7 separate statistical tests. Biochemical types CM, D, X, and Y did not occur more frequently in one test period than in any other test period, when the first 6 sampling periods are compared to the second 6 sampling periods. There was no apparent buildup of biochemical types in the environment as both experiments progressed with time.

The coagulase reaction on the coagulase-mannitol plate medium of Esber and Faulconer (25) produced false positive results. This was shown to be the case when Lotter and Horstman (29) tested all the coagulase-positive (plate method) by the tube method of Fisk (30). Statistical analysis was carried out on C types determined by the tube method (20) and by the plate method (25). The results of this analysis appear in table XI which shows statistical agreement between both methods in the frequency of occurrence of C types under body areas "A" in both experiments. Analysis on C types for body areas "B" and environment could not be accomplished because of a limited amount of data.

Table XII shows the distribution of the frequencies of biochemical types recovered from particular body areas designated as significant "A" body areas in tables IX and X. Underlined numbers refer to those types found to be significantly higher than the other types when their averages were compared by the Duncan Multiple Range Test (32). In experiment 1 the ear and groin displayed the largest frequency of CM types as determined by the plate method of Esber and Faulconer (25); the nose and throat, the largest frequency of C types as determined by the tube method of Fisk (30); the ear, the largest frequency of D types; nose and axilla, the largest frequency of X and Y types. In experiment 2 the ear, groin, and glans penis yielded the largest frequency of CM types as determined by the plate method of Esber and Faulconer (25); the nose, the largest frequency of C types; ear, nose, and glans penis, the largest frequency of D types; ear, nose, and axilla, the largest frequency of X types; nose and axilla, the largest frequency of Y types. Of all body areas "A" listed, the ear and nose (experiments 1 and 2) are the areas most likely to carry pathogenic staphylococci.

Phage typing of coagulase-positive isolates by the tube method was employed to determine which strains of staphylococci were identical and if exchange of strains occurred between subjects and their environment. In experiment 1 phage type UC-18 was recovered twice. It was obtained from the bed in the first postchamber period and was transferred to the throat of subject 28 in the second postchamber period. Only one phage type was recovered during experiment 2, type 79 from the throat of subject 29 which failed to be reisolated in subsequent periods.

SECTION IV

DISCUSSION

Two groups of 4 human male subjects were confined for a 6-week experimental period and ate a controlled metabolic diet composed of either fresh food or liquids. For 28 days the subjects lived in a simulated aerospace environment provided by the LSSE and during part of this time the subjects wore unpressurized MA-10 pressure suits. It is tacitly assumed that a certain degree of stress is induced by confinement in general, by confinement in the chamber, by the wearing of space suits, 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 (21, 22). The 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 a previous investigation. The staphylococcal flora of subjects who ate either fresh food or precooked freeze dehydrated food under simulated space conditions was analyzed (20). Results of both investigations agree with those of Sladen (34) 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 aerospace stress, especially as related to enhancing susceptibility to infection in human subjects is needed if one is to evaluate conditions related to stressful environments.

In general, staphylococci are dispersed in the environment by air, direct contact and contaminated objects (35). Several investigators have employed phage typing to study the mode of transmission in staphylococcal infection. Mortimer, et al. (36) studied staphylococcal infections in newborns and noted that the airborne rate of transmission was 8%, whereas that by direct contact through nurses was 43%. Greendyke, et al. (37) stated that more organisms are released to the environment by fecal rather than nasal carriers. The risk of transmission of staphylococci from carriers may be considerable. In one hospital study, a single carrier infected a new patient every 14 days. If 2 carriers of a particular staphylococcus were present, a new infection occurred about every 10 days; the rate amounted to one every 7 days with 3 or more carriers (38). In the present study, phage type UC-18 was obtained in experiment 1 and phage type 79 in experiment 2. Phage type UC-18 was transferred from subject to environment but not vice versa or among other subjects, whereas type 79 was not transferred at all. These data indicate little danger in the transmission of these few potentially pathogenic staphylococci.

In the present investigation coagulase production detected by the modified tube method of Fisk (30) was selected as the main index of staphylococcal potential pathogenicity. Lotter and Horstman (29) found that about one-tenth as many coagulase-positive cultures are detected by the tube method of Fisk (30) as by the plate method of Esber and Faulconer (25). However, the factor(s) in the plate medium causing the discrepancy between the methods have not been identified.

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	S	S	NS
Subjects	NS	NS	NS
Time	S	S	S, 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 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	S	S	S
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 area	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

SUMMARY OF STATISTICAL ANALYSIS OF BIOCHEMICAL TYPE "C" RECOVERED FROM BODY AREAS "A" OF TEST SUBJECTS AND THE ENVIRONMENT

Factors		C*		C**	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Body areas		S	S	S	S
Subjects		NS	NS	NS	NS
Time		NS	NS	NS	NS
Interaction:	subject vs. time	NS	NS	NS	NS
	body area vs. time	NS	NS	NS	NS

* Coagulase production determined by modified tube method of Fisk (30).

** Coagulase production determined by coagulase-mannitol plate method of Esber and Faulconer (25).

TABLE XII

FREQUENCY OF BIOCHEMICAL TYPES RECOVERED FROM SIGNIFICANT BODY AREAS "A" DURING EXPERIMENT 1

Biochemical type	Body areas*							
	Ear	Nose	Throat	Mouth	Axilla	Groin	Glans penis	Ratio**
C, M†	44	25	16	2	20	40	31	25.43
C‡	0	12	11	0	1	0	0	3.43
D	43	27	14	3	12	27	26	21.71
X	24	44	4	8	45	30	32	26.71
Y	33	44	5	7	43	40	36	29.71

* Sum of observations for all subjects in all sampling periods.

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

† Coagulase production detected by coagulase-mannitol plate method of Esber and Faulconer (25).

‡ Coagulase production detected by modified coagulase tube method of Fisk (30).

TABLE XIII

FREQUENCY OF BIOCHEMICAL TYPES RECOVERED FROM SIGNIFICANT BODY AREAS "A" DURING EXPERIMENT 2

Biochemical types	Body areas*							
	Ear	Nose	Throat	Mouth	Axilla	Groin	Glans penis	Ratio**
C, M†	33	20	3	8	12	41	41	22.57
C‡	0	11	1	7	0	0	1	2.85
D	28	30	4	8	11	23	33	19.57
X	42	47	6	8	48	28	32	30.14
Y	39	46	6	10	47	38	38	32.00

* Sum of observations for all subjects in all sampling periods.

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

† Coagulase production detected by coagulase-mannitol plate method of Esber and Faulconer (25).

‡ Coagulase production detected by modified coagulase tube method of Fisk (30).

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13. ABSTRACT

Two groups of 4 human male subjects participated in 6-week simulated aerospace studies. The subjects were confined and kept under controlled metabolic conditions; during this time, 28 consecutive days were spent in the Life Support Systems Evaluator. The subjects ate diets composed either of fresh food or liquid food. The subjects were exposed to simulated aerospace stress of confinement, wearing an unpressurized space 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 show that there were no significant differences in the frequency of occurrence of biochemical types among subjects and among environmental areas during the chamber period. There were significant differences in frequency of occurrence of biochemical types on ear, nose, throat, mouth, axilla, groin, and glans penis. There was no buildup of biochemical types with time in any test condition. Two phage types, UC-18 and 79, were recovered. Phage type UC-18 was transferred from subject to environment but not vice versa or among other subjects. Phage type 79 was not transferred at all. In the 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 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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