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THE STUDY OF MAN DURING A 56-DAY EXPOSURE
TO AN OXYGEN-HELIUM ATMOSPHERE
AT 258 mm. Hg TOTAL PRESSURE

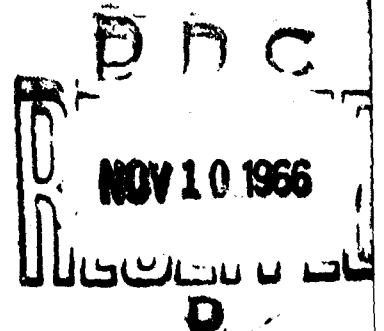
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USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

XI. Oral, Cutaneous, and Aerosol Bacteriologic Evaluation

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Studies were initiated to determine the numbers, distribution, and types of microorganisms encountered under conditions of a sealed environment for extended periods of time, such as would occur during space explorations. A 56-day experiment, utilizing four test subjects confined within a double-walled test cell in an oxygen-helium atmosphere at 258 mm. Hg, was performed. Quantitative counts of the aerobic microorganisms present in the circulating atmosphere as well as those present on the skin of the subjects were established. Distribution of coagulase positive, phage typable *Staphylococcus aureus* strains and predominant microbial types in throat, nasal, skin and aerosol samples was determined. Evidence of a staphylococcal transfer between subjects was obtained. Implications of these findings, as related to the utilization of the two-gas atmosphere for future space flights, are discussed.

THE STUDY of microbiological problem areas that might be of concern to the health and well-being of astronauts during long-term habitation of outer space, either in orbiting or land-based stations, is essential. Many of the anticipated problems are amenable to examination through the use of environmental test cells specifically designed and constructed for prolonged human occupancy. The considerations for utilizing a two-gas atmosphere for extended manned space flight, with helium as the inert diluent, have been reviewed by Welch and Robertson,⁵ and Clamann.³ The microbiological investigations described were undertaken to determine the effects of a 56-day exposure to an oxygen-helium atmosphere at 258 mm. Hg total pressure⁴ on the oral and cutaneous microbial populations of man. Additionally, aerosol studies were performed to determine the numbers and types of microorganisms present in the circulating atmosphere.

METHOD

Briefly, the experiment was conducted on four test

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subjects confined within the double-walled environmental chamber in operation at the USAF School of Aerospace Medicine, Brooks AFB, Texas. Experimental parameters, along with a description of the test cell and subjects, are detailed elsewhere.⁴

Nasal, throat, and skin samples were collected from each individual prior to, during, and following confinement in the test cell and subjected to bacteriological analyses to determine any significant alterations in the aerobic microflora. Subjects collected all specimens during the test period. During the interval of confinement nasal, throat, and skin samples were collected twice weekly on Monday and Thursday. During the pre-experimental and post-experimental periods, specimens were taken more frequently to establish adequate baseline data.

a. Throat swabs, immersed in trypticase soy broth as a preservative, were streaked to 5 per cent sheep blood agar plates, incubated at 35°C. for 24 hours, and observed for gross changes in the total flora.

b. Nasal swabs, also immersed in trypticase soy broth, were streaked to sheep blood agar as well as mannitol salt agar to detect the presence of *Staphylococcus aureus* organisms. Suspicious staphylococci (at least 3 colonies) were picked to trypticase soy agar (TSA), and retained for bacteriophage typing.

c. Skin specimens were collected from the medial aspect of the upper arm. These samples were obtained by means of a specially designed contact plate (Rodac, Falcon Plastics) containing 5 per cent sheep blood agar in which the surface of the agar is pressed directly against the skin area to be studied. Subjects alternated arms so as to minimize errors in the sampling technic. All plates were incubated aerobically for 48 hours at 35°C. before counts were performed.

Five aerosol sampling stations were established within the test cell. Blood agar petri plates were opened by the test subjects and exposed to the cabin atmosphere for periods of one hour. An unexposed blood agar plate was passed into the chamber to serve as a control for changes in atmospheric pressure. The plates were removed from the test cell and incubated aerobically at 35°C. until colony counts could be made. Predominant microbial species were determined and hemolytic *Staph. aureus* colonies (3 from

each plate) were streaked to TSA and retained for phage typing.

The retained staphylococcal colonies were re-streaked for purity on a blood agar plate, and single colonies were tested for coagulase reaction by the tube method. All coagulase positive staphylococci were phage-typed by the procedure of Blair and Williams.² The phages employed were the international basic set: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 83A, 42D, 187, and 81. The experimental phage UC-18¹ was also used. All phages and propagating strains were obtained from the Communicable Disease Center, Atlanta, Georgia. The routine test dilution of each phage was employed; that is, the highest dilution of phage lysate which just failed to give confluent lysis. Only reactions of 2+ (more than 50 plaques) were considered in reporting a phage pattern.

RESULTS

Throat Cultures. No shift in the normal distribution of microorganisms was noted. The subjects harbored the usual predominant flora consisting of alpha streptococci, neisseria, and *Staphylococcus epidermidis* species. Occasionally, *Staph. aureus* colonies were observed in small numbers. Though specifically looked for, beta hemolytic streptococci were not isolated.

Nasal Cultures. Table I shows the staphylococcal

TABLE I. STAPHYLOCOCCAL PHAGE TYPES FROM NASAL CULTURES

Day of Experiment	Subject Number			
	66	69	67	68
-28	29/52/80	7/53	*	None
-27	29/52/80/81	7/53	29	None
-26	29/52/80/81	7/53	29	None
-23	29/52/80/81	*	None	None
-22	29/52/80/81	7/53	29	None
-20	*	7/53	29	*
-16	29/52/80/81	7/52A	None	None
-13	29/52/80/81	7	29	None
-9	29/52/80/81	7;7/52A	None	None
-6	29/52/80/81	7/52A	None	None
-5	29/52/80/81	7	None	None
1	29/52/80/81	7	29	None
2	29/52/80/81	7;7/53	None	None
3	29/52/80/81	7/53	None	None
8	29/52/80/81	7;7/53	29	None
12	29/52/80/81	7/53	29;7	None
15	29/52/80/81	7/53;29	29	29
19	29/52/80/81	7/53	29	29
22	29/52/80/81	7/53	29	29
26	29/52/80/81	7/53	29	29
29	29/52/80/81	7/53	29	29
33	29/52/80/81	7;7/53	29	29
36	29/52/80/81	7	None	29
40	29/52/80/81	7;7/53	None	None
43	29/52/80/81	7	None	None
47	29/52/80/81	7/47	29	29
49	29/52/80/81	None	None	29
54	29/52/80/81	7/53;29	29	29
+1	29/52/80/81	29	29	29
+2	29/52/80/81	7/53;29	29	29
+3	29/52/80/81	7/53;29	29	29
+5	29/52/80	7	29	29
+6	*	*	None	29
+7	*	*	None	None
+8	29/52/80	7/53	None	None

*No specimen received

phage types isolated from each of the four test subjects during the course of the experiment. The minus and plus signs in the left hand column (Day of Experiment) refer to pre- and post-experimental periods when the subjects were at ground level under normal atmospheric conditions. Three of the subjects harbored specific, identifiable staphylococcal strains, while the fourth subject (Number 68) apparently was not a carrier of any *Staph. aureus* strains. Both blood agar and mannitol salt agar plates from nasal cultures of this individual were negative for this organism. To assure that no *Staph. aureus* organisms were present, colonies were streaked to TSA, tested for coagulase reaction, and phage typed. All cultures proved to be coagulase negative, nontypable *Staph. epidermidis* strains.

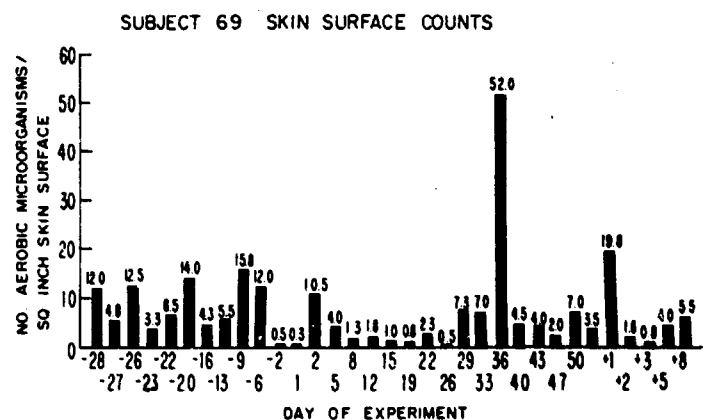
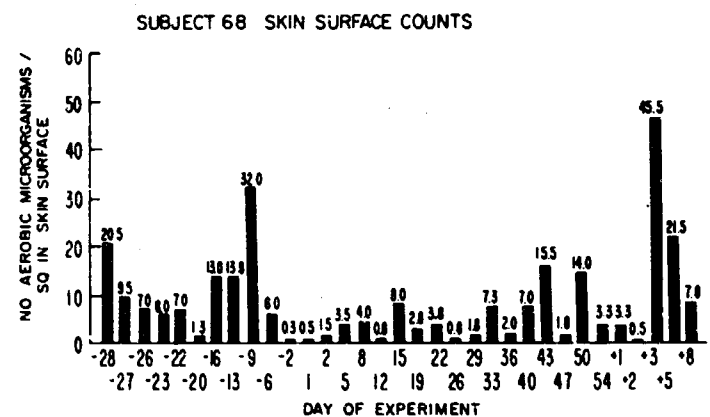
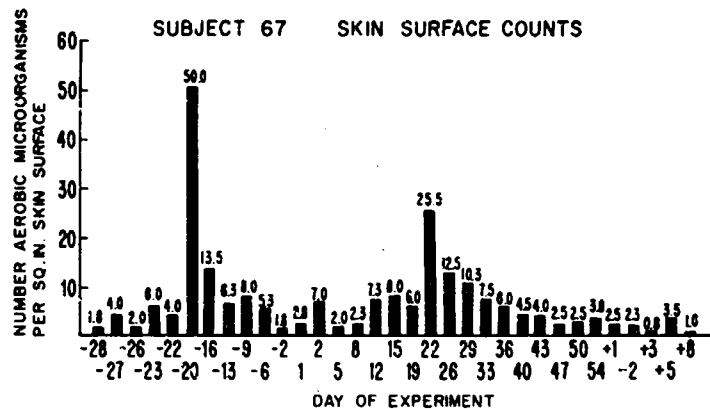
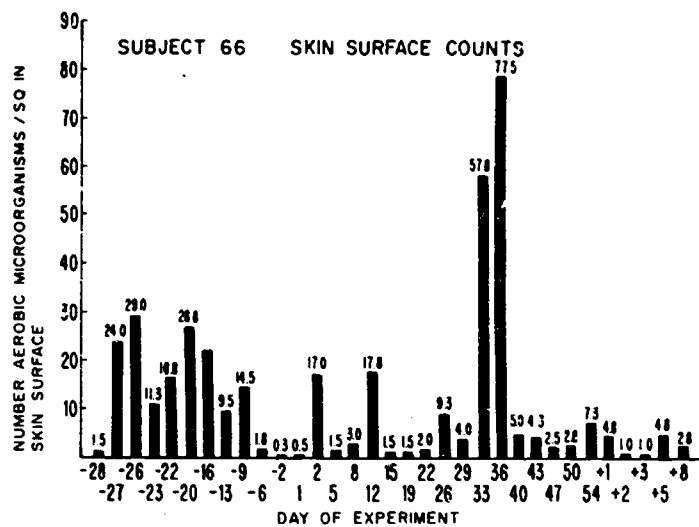
Following two weeks confinement at the simulated altitude, a transfer of staphylococcal phage type 29 from subject 67 to subject 68 was evident. Nasal cultures streaked to mannitol salt agar revealed large numbers of these microorganisms. The transfer persisted throughout the course of confinement and into the post-experimental period. It is of interest to mention that subjects 67 and 68 were on the same sleep/rest/work cycle; hence, were closely associated with one another.

Lesser evidence for a transfer of phage type 29 from subject 67 to 69 is present. Although this phage type was isolated from the nasal cultures of subject 69 on five occasions (15th, 54th, +1, +2, and +3 days), the transfer would appear to be of a transitory nature. Not enough post-experimental cultures were obtained, however. Subject 66 retained his phage pattern throughout the experiment.

Staphylococcal Phage Types from Aerosols. Table II presents the staphylococcal phage types isolated from blood agar plates exposed to the test cell atmosphere for periods of one hour while all four subjects were awake. It is interesting to note that all phage patterns harbored by the subjects were isolated from aerosol samples at least once during the period of confinement. The more frequent isolation of phage

TABLE II. STAPHYLOCOCCAL PHAGE TYPES FROM AEROSOLS

Day of Expt.	Phage Type(s)
-7	None
-5	7
5	29/52
7	None
12	None
14	29
16	29;29/52
26	29;29/52/80/81
28	29;7/53
30	29
33	29;7/29
35	None
37	None
47	29
49	7/53
51	29
54	29
56	29
+2	None



type 29 (10 of 19 sampling days) may account for its greater transmissibility among subjects.

Skin Samples. Figures 1 through 4 represent data obtained on the numbers of aerobic microorganisms isolated per square inch skin surface of the area tested. The medial aspect of the upper arm, an area relatively free of hair, was chosen as the skin sampling site, since previous studies of other areas had shown that the number of microorganisms present was directly related to the hirsuteness of the individual. During the period of confinement the subjects took one sponge bath per day prior to their sleep cycle. *Staph. epidermidis* was always greatly in the predominance of the organisms isolated from the skin cultures. Other organisms found in small numbers included *Staph. aureus*, an unidentified gram positive rod, probably a diphtheroid, and various saprophytic fungi.

Aerosol Counts. The total number of aerobic microorganisms present per square inch cabin surface area per hour may be found in Figure 5. Air flow through the cabin during the sampling period was approximately 250 std. cu. ft. per min.; however, some variation was present depending upon the number of blowers in operation. The individual counts shown in Figure 5 were obtained by averaging the total number of microorganisms found at the five aerosol sampling stations and dividing by the area of the blood agar plate. Data for the 40th, 42nd, and 44th days are not included, since the blood used to prepare the agar plates that week was contaminated. While the predominant organism isolated from aerosol samples was *Staph. epidermidis*, many more *Staph. aureus* colonies were noted than in the skin counts. In several instances *Staph. aureus* colonies comprised one half or more of the total colonies counted. Various *Bacillus* sp. and saprophytic fungi were found in lesser numbers.

DISCUSSION

Immediate postexperimental radiographic studies of subject 68 revealed changes in the left frontal and left maxillary sinuses, and the subject was placed on a two-week regimen of erythromycin therapy. Throughout the entire experimental period, however, the subject was asymptomatic, with no clinical signs of an acute

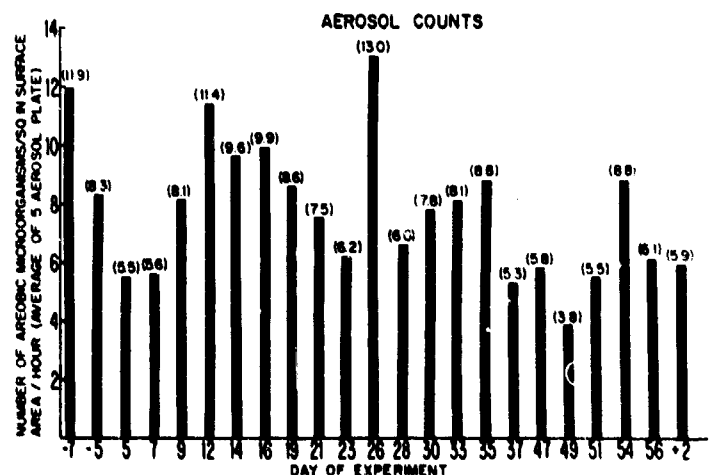


Fig. 5. Aerobic microorganisms isolated from circulating atmosphere during fifty-six day experiment in an oxygen-helium gas mixture at 258 mm. Hg total pressure.

sinusitis.⁶ Subsequent radiographic studies on this individual since his return to his duty station have been negative; however, nasal cultures reveal that he still harbors phage type 29. These findings appear to indicate that the radiographic changes initially noted were not related to the presence of the phage typable staphylococci.

The cutaneous studies suggest that no build-up of the aerobic microflora of the skin occurred during the course of the experiment. The counts are quite uniform with few exceptions. The occasional high counts found for each subject probably occurred when for some reason the subjects were unable to wash adequately. The unusually low counts found on minus 2 days are adequately explained by an entry in one subject's diary in which he mentions that all the subjects received two showers the previous day.

As regards the aerosol studies, no cumulative increase or decrease in the numbers of aerobic microorganisms present in the circulating atmosphere was noted. While it is realized that the open plate method of sampling atmospheres has limitations, plates exposed by this technic consistently showed higher counts and more representative types of microorganisms than those obtained during the same time interval by a commercially available impinger-type sampler placed externally. Moreover, it is believed that the open plate method is of considerable value in determining relative numbers of microorganisms over an extended period of time.

Portions of the cabin air were passed through a lithium hydroxide-activated charcoal filled cannister for removal of CO₂. This device, fitted on both ends with a fiberglass filter (Aerosolve 95, Cambridge Filter

Corp.), no doubt acted as a microbiological filter, reducing the total count.

From a microbiological viewpoint there appears to be no serious objections to the use of an oxygen-helium atmosphere at 258 mm. Hg total pressure. The one demonstrated instance of staphylococcal interchange was not occasioned by any adverse clinical symptoms of consequence. For aesthetic purposes and odor problems, a sponge bath daily is considered sufficient to keep the resident skin microorganisms in check.

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