Report No. IITRI-L6031-14
(Final Report)

EFFECTS OF SPACE CABIN ENVIRONMENTS ON INFECTION

National Aeronautics
and Space Administration
Manned Spacecraft Center
Houston, Texas

Attention: Dr. W. W. Kemmerer, EC641
Crew Systems Division

IIT RESEARCH INSTITUTE

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FOREWORD

The project entitled "Effects of Space Cabin Environments on Infection" was initiated by IIT Research Institute for the National Aeronautics and Space Administration, Manned Spacecraft Center, Houston, Texas. This is Report No. IITRI-L6031-14 (Final Report) on IITRI Project L6031, Contract No. NAS9-4978. The report covers the period from July 19, 1965, through September 18, 1966, and describes the research conducted to study the effects of exposure to a space cabin environment on the resistance of mice to infection. The simulated space cabin environment was represented by an altitude of 27,000 ft (5 psi), 98% oxygen atmosphere, 25 $\pm$ 2°C, and 50 $\pm$ 10% relative humidity.

John Findlay and Herbert Logan participated in the technical performance of the program. Gail Butler conducted the irradiation exposures. William Blair conducted the enzyme analyses and the histopathological examinations with the assistance of Kenneth Chunn and Betty Larson. William Grizzle, Ronald Rummage, Anthony Segredo, Russell Neckorcuk, and William Lubera participated at various times in the operation of the high-altitude environmental facility.

The experimental data are recorded in IITRI Logbooks C16170, C16404, C16585, C16669, C16688, C16886, and C16942.

In conducting the research reported, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Respectfully submitted,

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ABSTRACT

EFFECTS OF SPACE CABIN ENVIRONMENTS ON INFECTION

The effects of a simulated space cabin environment of 27,000-ft altitude (5 psi), 98% oxygen atmosphere, 25°C, and 50% relative humidity on the resistance of mice to infection were studied.

Mortality increased significantly when mice were challenged with Klebsiella pneumoniae aerosols 3, 14, or 30 days after exposure to the space cabin environment. Mortality also increased significantly when mice were challenged at ambient conditions (approximately 600-ft altitude) 24 hr after 30 or 45 days of exposure in the space cabin environment. However, mortality did not increase when mice were kept in the space cabin environment for 14 days and challenged 24 hr after return to ambient conditions.

Exposure of mice to the space cabin environment before and after exposure to 300 to 500 rads of gamma radiation resulted in a significant increase in mortality. Exposure of mice to the space cabin environment only or to both the space cabin environment and radiation had no effect on resistance to Staphylococcus aureus infection or on effectiveness of treatment of this infection with tetracycline.

The hemoglobin, hematocrit, and differential blood cell characteristics of mice kept in the space cabin environment for 3 to 30 days did not differ significantly from those of mice kept at ambient conditions. However, a decrease in white blood cell count was observed in mice kept in the space cabin environment for 3 and 14 days.

Histopathological examination of mice exposed to the space cabin environment for up to 21 days and to gamma radiation indicated radiation damage exemplified by gastric erosion and intestinal edema. These changes were not observed in mice exposed to space cabin environment for up to 30 days before Co^{60} gamma radiation.

Lactic acid dehydrogenase isoenzyme patterns in mice exposed to the space cabin environment for 14 to 30 days showed increases in band M in kidney tissue and decreases in band M in heart tissue.

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EFFECTS OF SPACE CABIN ENVIRONMENTS ON INFECTION

I. INTRODUCTION

The purpose of this program was to study the effects of space cabin environment on the susceptibility of small laboratory animals to infection. Previous work at IIT Research Institute (ref. 1-3) indicated that exposure of mice to simulated altitudes of 18,000 ft (7.3 psi) at ambient gaseous atmosphere or to 35,000 ft (3.5 psi) at 85% oxygen, 10% carbon dioxide, and 5% nitrogen atmosphere reduced their resistance to infection produced by Klebsiella pneumoniae aerosols. Also, the efficiency of lincomycin therapy for staphylococcal infection in mice was reduced when the mice were kept at simulated 35,000-ft altitude (ref. 4).

This report describes studies on the effects of a simulated space cabin environment of 27,000-ft altitude (5 psi), 98% oxygen atmosphere, 25 $\pm$ 2°C, and 50 $\pm$ 10% relative humidity on the resistance of mice to infection. The data summarize studies designed to determine the effects of this space cabin environment on:

(1) Susceptibility to bacterial respiratory infection initiated by aerosol challenge with K. pneumoniae

(2) Susceptibility to and effectiveness of antibiotic treatment of bacterial infection initiated by challenge with Staphylococcus aureus

(3) Susceptibility to viral infection produced by challenge with the Venezuelan equine encephalomyelitis (VEE) virus.

In some experiments acute exposure to ionizing (gamma) radiation was used as an additional environmental stress.

II. METHODS

A. Mice

Male Swiss albino Ha/ICR strain mice (A. R. Schmidt Co., Madison, Wis.) each weighing 20 ± 2 g were used. Groups of 15 mice were selected at random, placed in a cage, and quarantined for a 1-week period prior to experimental use. Food and water were supplied ad libitum. The mice were maintained on Purina laboratory chow throughout the experiments. Food consumption or weight data were not determined on the mice during the experiments.

B. High-Altitude Chamber

The high-altitude chamber used for exposure of mice to a simulated space cabin environment consisted of two connecting chambers: the main chamber and the air lock. Each was approximately a 6-ft cube. The rear portion of the main chamber was

isolated from the work area and contained heating and refrigeration units and air circulating fans. Chart recorders were used to monitor the pressure, temperature, and humidity in the chamber. A communication system was provided.

The space cabin environment was 5 psi pressure, 98 ± 1% oxygen atmosphere, 25 ± 2°C, and 50 ± 10% relative humidity. The oxygen supply system consisted of a 2-bottle oxygen manifold located externally to the chamber and connected to the interior of the chamber and the air lock. Standard diluter demand-type oxygen regulators were employed. The oxygen atmosphere in the chamber was maintained by constantly flushing the chamber with fresh oxygen to purge it of contaminating gases produced by respiration of the mice and leakage in the chamber. Constant monitoring with a Beckman model F3 oxygen analyzer assured that the desired oxygen atmosphere was maintained.

Personnel entered the chamber three times a week for observation, maintenance, and experimentation with the mice. The personnel breathed pure oxygen through face masks for at least 15 min before they entered the air lock. After they entered the air lock, the environment was equilibrated with that in the main chamber.

The mice were kept at the 5 psi pressure environment at all times except when they were challenged with K. pneumoniae aerosols or exposed to the ionizing radiation. The time interval between removal from the chamber and return was not more than 1 hour.

C. Infectious Challenge

The K. pneumoniae culture used to produce infectious aerosols was originally obtained from the University of California, School of Medicine, San Francisco, California. The bacteria were grown on blood agar base medium, harvested with sterile water, placed in ampoules, quickly frozen in an acetone dry ice bath, and stored at -70°C until used.

For aerosolization, the frozen stock culture was thawed at room temperature (25 ± 2°C). Blood agar base plates were inoculated with the culture and incubated at 37°C for 18 hour. The growth was washed from the surface with distilled water. The suspension, after appropriate dilution in distilled water, was used for aerosolization.

The aerosol chamber was a plastic hood of approximately 230 liters volume (58 x 58 x 69 cm) installed within a microbiological safety hood. A University of Chicago Toxicity Laboratory-type nebulizer was used to produce the aerosol. The liquid culture was fed to the atomizer by a 50-ml syringe, activated by a motor-driven piston that delivered the culture to the

atomizer at a rate of 0.4 ml/min. Filtered air was used as the primary and the secondary air for the nebulizer at a flow rate of 28 liters/min. The aerosol chamber temperature was 24 ± 2°C, and the relative humidity was 85 ± 5%. The air within the aerosol chamber was sampled for 1 min at the beginning and the end of challenge of mice. All-glass impingers with jets 30 mm from the bottom of the bottle and an airflow of 12.5 liters/min were used for aerosol sampling.

Staphylococcal infections were produced by intraperitoneal inoculation with S. aureus, Smith strain, obtained from Lederle Laboratories, Pearl River, New York. S. aureus was grown in trypticase soy broth (TSB) containing 2% defibrinated rabbit blood. For maintenance, 1.0 ml of the stock culture in TSB was transferred to 9.0 ml of TSB, incubated for 3 hr at 37°C and diluted to 10^{-1} in TSB. For duplicate cultures, 2 mice were injected intraperitoneally. The mice were sacrificed 5 hr after injection, and a loopful of heart blood was transferred from each mouse to TSB tubes, which were incubated for 18 hr at 37°C. The 18-hr cultures were checked for purity and refrigerated until used.

For experimental mouse inoculation, 0.2 ml of the stock cultures was transferred to TSB and incubated for 18 hr at 37°C. Then 1 ml of this culture was passed into TSB to obtain a 5-hr culture, which, with appropriate dilution in TSB, was used to inject mice intraperitoneally.

E. Hematology

Blood for hematology was obtained by decapitation. It was collected in small vials containing heparin. Hemoglobin was determined by using Drabkin's solution which converts hemoglobin to cyanmethemoglobin. The optical density of the solution was measured on a Coleman colorimeter and the percent hemoglobin was read from the standard curve. The hematocrits were determined by drawing blood in capillary tubes and centrifuging for 10 min at approximately 2000 x g. The packed volume of red cells was read on a microscale and expressed in percent of total volume.

The white blood cell counts were obtained using a brightline hemocytometer. The counts were expressed as number of cells per cubic millimeter. Differential counts were made utilizing Wright's standard procedure. One hundred cells were counted and the number of each type was expressed as percent of the total.

F. Lactic Acid Dehydrogenase Isoenzymes

The following procedure was used to determine lactic acid dehydrogenase (LDH) isoenzyme patterns in heart, lung, kidney, and liver tissues. Mice were sacrificed by cervical decapitation to minimize excess blood in the tissues. The tissues were removed by rapid dissection and weighed on a torsion balance.

One ml of veronal buffer per 100 mg of tissue was added and the tissues were homogenized while in an ice bath. The ionic strength of the veronal buffer was 0.1 and the pH was 8.6. Solids were separated by ambient centrifugation for 5 min at 3,000 rpm, and clear supernatants were removed and stored frozen at -20°C until analyzed.

Electrophoresis was carried out on agar-coated microscopic slides. A current flow of 15 ma per slide at 120 volts was used, and the slides were kept cool in a petroleum ether bath. For electrophoresis, 10 microliters of extract was used. The time required was 1 hr. The slides were then incubated at 37°C in the following mixture:

Nitroblue tetrazolium 0.5%	12 ml
Sodium lactate 6%	12 ml
Sodium cyanide 0.1 M	12 ml
Phosphate buffer (pH 7)	20 ml
Phenazine methosulfate 0.1%	12 ml
Diphosphopyridine nucleotide	40 mg
Water	52 ml

After 45 min, the slides were washed in an acetic alcohol solution, (500 ml of water, 450 ml of 95% ethyl alcohol, and 50 ml of glacial acetic acid), eluted for 24 hr, and dried with filter paper and a fan.

The purple-blue isoenzyme bands were assayed with a Spinco model RB Analytrol, which was modified to scan microscope slides, and the area densities were calculated. Evaluations of isoenzyme patterns were made from these data. Bands 1 and 2 were summed and designated as H isoenzyme, bands 4 and 5 were summed and designated as M isoenzyme, and band 3 was treated separately. On a functional basis, H bands are found in highly aerobic tissues such as heart and brain and M bands are found in tissues with anaerobic metabolism, i.e., skeletal muscle and liver.

G. Histopathology

For histopathological analysis, the mice were sacrificed by cervical decapitation. Heart, lung, spleen, kidney, stomach, intestines, and liver tissues were fixed in Bouin solution. After fixation, the tissues were cut on a rotary microtome at 6 μ and stained with hematoxylin and eosin in an Autotechnicon.

III. RESULTS AND DISCUSSION

A. Susceptibility to Klebsiella pneumoniae Infection

To study the effects of space cabin environment on susceptibility to respiratory infection, mice were challenged with K. pneumoniae aerosols. For each aerosol challenge, 10 mice kept at the space cabin environment and 10 kept at ambient environmental conditions were used. The ambient environment present

in our laboratories was approximately 600 ft altitude, 25 ± 2°C, and 50 ± 10% relative humidity. A minimum of seven replicates was obtained for each time exposure. The infectious challenges were made 3, 7, 14, 21, or 30 days after continuous exposure to the space cabin environment. Immediately after the aerosol challenge carried out at ambient environment, the mice were returned to their respective environments. Mice that died during the 14-day observation period following the infectious challenge were autopsied, and their hearts and lungs were cultured on blood agar medium to confirm the cause of death.

Table 1 summarizes the data. For statistical analysis, the mortality data were subjected to an arc sine transformation and the significance of the differences was determined by the t test (ref. 5). Increased mortality was observed in all groups of challenged mice exposed to the space cabin environment as compared with the challenged controls kept at ambient animal room environment. The increase in mortality was significant at the 5% probability level for mice exposed to the space cabin environment for 3, 14, or 30 days. When the mortalities in the two groups were compared without regard to the duration of the exposure to the space cabin environment, the mortality was 37.1 and 55.2% for the challenged control and the challenged space cabin group, respectively. This difference in mortality was significant at the 5% probability level.

Table 1

MORTALITY OF MICE EXPOSED TO SPACE CABIN ENVIRONMENT AND CHALLENGED WITH KLEBSIELLA PNEUMONIAE

Days at Space Cabin Environment, before Challenge	Ambient Mortality Dead/Total	Ambient Mortality %	Space Cabin Mortality Dead/Total	Space Cabin Mortality %
3	69/160	43.1	91/160	56.8[a]
7	36/80	45.0	48/80	60.0
14	30/80	37.5	54/80	67.5[a]
21	27/70	38.5	39/70	55.7
30	9/70	12.8	22/70	31.4[a]
	171/460	Avg 37.1	254/460	Avg 55.2[a]

[a]Significant at $P < 5\%$.

To elucidate one possible mechanism involved in the increased susceptibility to infection, hematological examinations were made of blood samples of mice exposed to the space cabin environment for 3, 7, 14, 21, or 30 days. Table 2 summarizes the results, each value representing an average of at least five mice. Hemoglobin, hematocrit, and differential counts did not appear to be affected by the environmental stress. However, a 24 and a 41% decrease in white blood cells (WBC) was observed after 3 and 14 days at the space cabin environment, respectively, when compared with control WBC counts of mice maintained at ambient conditions. These two exposure periods coincide with those in which significant increases in mortalities were observed after respiratory challenge with K. pneumoniae.

Table 2

HEMATOLOGY OF MICE EXPOSED TO SPACE CABIN ENVIRONMENT

Days at Space Cabin Environment	Hemoglobin, g/100 ml	Hematocrit, %	White Blood Cells no./mm^3	Lympho-cytes	Neutro-phils	Baso-phils	Eosino-phils	Mono-cytes
0	14.8	42	8000	69	25	1	2	3
3	15.3	48	6070	75	21	1	2	1
7	14.9	46	7420	49	48	0	2	0
14	13.3	44	4790	67	31	0	2	0
21	14.4	44	7420	72	25	0	0	3
30	13.9	46	10520	71	27	0	0	2

The 30-day exposure period, which also resulted in a significant increase in mortality, however, showed a 32% increase in WBC over the control values.

Concurrent LDH isoenzyme studies were conducted on selected tissues of groups of 10 mice exposed to space cabin environment for 3, 14, or 30 days. The lung and liver tissues appeared to be normal at all exposure periods, with some expected variations in the LDH isoenzyme distribution. After 14 days of exposure the kidney tissue LDH isoenzyme patterns revealed an increase in band M in one half of the mice, and two mice also had an elevated band 3. Exposure to the space cabin environment for 30 days resulted in elevated band M isoenzyme in kidney tissue of three mice, and an elevation of band 3 in two out of ten mice. Heart tissue variations, mainly a depression of band M isoenzyme, were seen in the mice exposed to the space cabin environment for 14 or 30 days.

To determine the persistence of the increase in susceptibility to respiratory infection after exposure to space cabin environment, groups of mice were kept at this environment for 14, 30, or 45 days. At the end of this exposure period they were returned to ambient environment and challenged with K. pneumoniae aerosol after 1 or 24 hr. After the infectious challenge the mice were kept at ambient environment for the 14-day observation period. The data, shown in Table 3, indicate that the mortality increased as the result of space

cabin environment exposure when the infectious challenge occurred within 1 hr after termination of the exposure. Significantly higher mortality was observed in the 30- and 45-day exposure groups after the 24-hr period at ambient conditions. The 14-day group, however, showed a recovery within the 24-hr period - the increased susceptibility was not apparent.

Table 3

MORTALITY OF MICE EXPOSED TO SPACE CABIN ENVIRONMENT AND CHALLENGED WITH KLEBSIELLA PNEUMONIAE AFTER RETURN TO AMBIENT CONDITIONS

Exposure to Space Cabin Environment, days	Time of Challenge after Exposure, hr			
	1		24	
	Dead/Total	%	Dead/Total	%
Ambient control	6/40	15	16/50	32
14	18/40	45[a]	11/50	22
30	22/40	55[a]	28/50	56[a]
45	22/40	55[a]	24/50	48[a]

[a]Significant from corresponding ambient controls at $P < 5\%$.

A potential additional stress in a space cabin is the presence of ionizing radiation. Under normal environment conditions exposure to ionizing radiation is known to reduce resistance to infection. Thus it was of interest to determine whether a potentiation of susceptibility to respiratory infection occurs upon exposure to the combined stress of space cabin environment

and gamma radiation. Initially the effect of radiation only was studied in mice exposed for 7, 14, 21, or 30 days to the space cabin environment. The gamma radiation levels used were 100, 300, and 500 rads. As seen in Table 4, a synergistic effect resulted in increased mortality due to the combined stress of radiation and exposure to space cabin environment. At each radiation level the mortality was higher upon exposure to the space cabin environment than without this exposure. An analysis of variance performed on the data indicated that the increased mortality was significant at the 5% probability level.

Hemoglobin, hematocrit, and differential counts were made on the mice exposed both to the space cabin environment and to radiation. As seen in Table 5, the differences were not significant. The WBC of the same mice showed a decrease related to the radiation dose, ranging from 50 to 300 rads. The radiation effect on WBC appeared to be the predominant factor that overshadowed any possible effects of space cabin environment.

Histopathological examination was made of tissues of mice exposed first to the space cabin environment for 0, 3, 7, 21, or 30 days and then to 50, 100, or 300 rads of gamma radiation. The mice were sacrificed 3 days after the radiation exposure. All the mice showed moderate edema of the lung tissue. Lymphocytic infiltration was also seen. Many mice exhibited cells with pyknotic nuclei in the spleen. Mild lymphocytic infiltration was noted in otherwise normal cardiac tissue in the mice

Table 4

MORTALITY OF MICE EXPOSED TO SPACE CABIN ENVIRONMENT AND TO CO^{60} GAMMA RADIATION

Days at Space Cabin Environment before Radiation	0 Rads		100 Rads		300 Rads		300 Rads	
	Ambient	Space Cabin	Ambient	Space Cabin	Ambient	Space Cabin	Ambient	Space Cabin
	Mortality, dead/total							
7	0/40	0/40	0/40	1/40	0/40	8/40	15/40	29/40
14	0/80	0/80	0/80	4/80	1/80	11/80	8/80	16/80
21	0/80	0/80	0/80	1/80	0/80	4/80	0/80	5/80
30	0/40	0/40	0/40	0/40	1/40	6/40	5/40	11/40
Total, %	0	0	0	2.5	0.8	12.1	11.7	25.4

Table 5

BLOOD CHARACTERISTICS OF MICE EXPOSED TO IRRADIATION AND TO SPACE CABIN ENVIRONMENT

Days at Space Cabin Environment before Radiation	Radiation Dose, rads	Hemoglobin, g/100 ml	Hematocrit, %	White Blood Cells	Lympho-cytes	Neutro-phils	Baso-phils	Eosino-phils	Mono-cytes
0	0	15.8	47	4950	84	14	0	2	0
	50	14.5	47	5460	71	26	0	3	0
	100	13.8	43	2980	71	28	0	1	0
	300	13.5	40	830	75	23	0	2	0
3	0	15.7	49	6770	63	35	0	2	0
	50	13.7	45	4800	76	24	0	0	0
	100	14.8	47	2840	61	35	1	1	2
	300	15.1	49	2800	56	42	0	1	1
7	0	14.4	46	6320	68	28	0	0	4
	50	14.6	50	4020	65	32	0	0	3
	100	15.4	49	3720	43	53	0	1	3
	300	14.0	41	1220	58	39	0	0	3
21	0	15.6	53	4170	74	23	1	1	1
	50	14.4	51	3860	78	24	0	0	2
	100	13.3	49	2620	77	21	1	1	0
	300	13.1	48	1650	67	30	1	1	1
30	0	14.5	45	8060	73	22	0	3	3
	50	13.4	44	4460	69	27	0	2	2
	100	13.9	44	2720	68	30	0	1	1
	300	13.1	43	1650	65	32	0	1	2

[a]Mice irradiated at this time. Hematology performed 3 days later.

exposed to the space cabin environment and 0, 100, or 300 rads of radiation. Lymphocytic infiltration was noted in the mice exposed to the space cabin environment. The gastrointestinal tract appeared normal in the mice kept at ambient and the space exposed mice receiving 0 to 50 rads of radiation. The mice exposed to the space cabin environment and receiving 100 rads exhibited erosion of the stomach wall (glandular portion) and edema, pyknosis, and necrosis of the intestine. The mice exposed to the space cabin environment and 300 rads exhibited severe erosion of the stomach after 3 days of space cabin exposure, hyperplasia after 7 days, and mild erosion at 21 days. The mice exposed for 30 days did not show stomach erosions. The intestinal tract of mice exposed to the space cabin environment for 3, 7, or 21 days prior to radiation exposure exhibited edema, but the intestinal tract of mice exposed to this environment 30 days did not exhibit edema.

The overall impression in the ambient mice is minimal radiation response characterized by pyknotic nuclei in the spleen and no gastrointestinal tract involvement. The mice irradiated with 0 or 50 rads and exposed to the space cabin environment appeared to be harboring a generalized infection or inflammatory process with lymphocytic infiltration of the liver, heart, and lungs. There were no unique alterations suggestive of radiation sickness. Mice exposed to 100 or 300 rads and the cabin environment for 3, 7, or 21 days showed evidence of radiation damage exemplified by gastric erosion and intestinal edema.

Further studies were conducted to determine the effect of the combined stress of space cabin environment and gamma radiation on susceptibility to respiratory infection. Mice were kept at the space cabin environment for 3, 7, or 14 days and then were exposed to a single acute dose of 50, 100, or 300 rads of gamma radiation. Seven days after the radiation exposure the mice were challenged with K. pneumoniae aerosols. Mice kept at ambient conditions were treated in the same manner and served as controls. Both the exposure to radiation and the infectious challenge were made at ambient environment. Each aerosol challenge consisted of four groups of 10 mice: ambient mice, ambient mice exposed to radiation, space cabin environment mice, and space cabin environment mice exposed to radiation.

The data from these experiments are summarized in Table 6. The data were tested for significance by utilizing a t test, the tests for significance being based upon the theoretical variance under binomial sampling (ref. 6). Several comparisons among the data can be made. Mice kept at space cabin environment for 10 days before the infectious challenge showed higher mortality than those kept at ambient conditions. Radiation alone increased susceptibility of mice to infection; higher mortality was observed. The combined stress of space cabin environment and gamma radiation produced the highest mortality.

Table 6

MORTALITY OF MICE EXPOSED TO SPACE CABIN ENVIRONMENT, 50 TO 300 RADS OF GAMMA RADIATION, AND CHALLENGED WITH KLEBSIELLA PNEUMONIAE

Days at Space Cabin Environment		Ambient		Ambient Irradiated		Space Cabin		Space Cabin Irradiated	
Before Radiation	Before Challenge	Mortality Dead/Total	%	Mortality Dead/Total	%	Mortality Dead/Total	%	Mortality Dead/Total	%
3	10	86/120	71.6[a]	101/120	84.1[b]	113/120	94.1[b]	116/120	96.6[b]
7	14	44/120	36.6[a]	70/120	58.3[b]	52/120	43.3[ab]	38/120	31.6[a]
14	21	20/120	16.7[a]	19/120	15.8[a]	33/120	27.5[a]	23/120	19.2[a]

Percentages in the same horizontal line with common superscripts are not significantly different at $P < 5\%$.

In the group of mice kept in the space cabin environment for 14 days before challenge some increase in mortality occurred as a result of the space cabin environment stress. A significant increase was observed in mice kept at ambient environment and exposed to radiation. However, comparison of the ambient-radiation and the space cabin radiation mice shows a significant decrease in mortality in the mice kept in the space cabin environment. Thus it appears that under the experimental conditions the space cabin environment might have some protective effect that nullifies the effect of gamma radiation exposure.

In the group of mice infected after 21 days at the space cabin environment significant differences in mortality were not observed in either the radiation- or space-exposed mice.

B. Susceptibility to Staphylococcus aureus Infection

Data reported in previous publication (ref. 4) showed that no significant differences were observed between the mortality of mice infected with S. aureus and kept under ambient conditions and those kept at 3.5 psi environment with 85% oxygen and 10% carbon dioxide. At the same time, previous experiments suggested that the efficiency of treatment of staphylococcal infection with lincomycin is reduced under the space cabin environmental conditions.

Experiments were conducted to determine the susceptibility to S. aureus infection and the effectiveness of treatment of the infection with tetracycline in mice kept under the space cabin environment. Mice were kept in the space cabin environment for 3, 7, 14, 21, or 30 days and then infected by the intraperitoneal route with S. aureus in various concentrations embracing the LD_{50}. LD_{50} values were calculated by the Reed and Muench method (ref. 5). The data shown in Table 7 indicate that no significant differences were found in the LD_{50} for the mice kept in the space cabin as compared with those kept in ambient environment. The control LD_{50} was 32×10^7, and the mean for the space cabin environment was 25×10^7.

Table 7

STAPHYLOCOCCUS AUREUS LD_{50} OF MICE EXPOSED TO SPACE CABIN ENVIRONMENT

Days at Space Cabin Environment before Challenge	LD_{50}
Ambient control	32×10^7
3	22×10^7
7	24×10^7
14	35×10^7
21	21×10^7
30	23×10^7

Mice kept at the space cabin environment for 3, 7, 14, 30, or 45 days were inoculated intraperitoneally with 0.5 ml of a S. aureus culture containing 10 to 20 x 10^6 organisms by plate count. This concentration is usually fatal for mice within 24 hr. Tetracycline therapy consisted of a single subcutaneous inoculation of tetracycline administered within 30 min after infection. Five different doses of tetracycline were utilized - 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg, and 10 mice were used for each dosage level. The infection and treatment took place at the space cabin environment. Mice kept at ambient conditions were treated similarly to the mice exposed to the space cabin environment and served as controls. All the experiments except the 45-day exposure were conducted in replicate.

Table 8 presents the results of a probit analysis performed on the ED_{50} (effective dose protecting 50% of the mice) data. In the 3-day experiment a higher dose was required to protect 50% of the mice exposed to the space cabin environment. However, this increase was not significant at the 95% confidence level on the basis of a t test. On the basis of this experiment, it appears that exposure to the space cabin environment does not alter the response of the mice to tetracycline therapy for staphylococcal infection.

Table 8

EFFECTIVENESS OF TETRACYCLINE THERAPY
IN STAPHYLOCOCCUS AUREUS INFECTED MICE
EXPOSED TO SPACE CABIN ENVIRONMENT

Days at Space Cabin Environment, before Challenge and Treatment	Ambient ED_{50}, mg/kg		Space Cabin ED_{50}, mg/kg	
	Mean	95% C.L.	Mean	95% C.L.
3	0.78	0.51-1.20	1.28	0.97-1.68
7	0.66	0.40-1.09	0.70	0.45-1.09
14	1.23	0.86-1.78	1.13	0.81-1.58
30	1.50	1.10-2.05	1.01	0.78-1.31
45	1.27	0.86-1.88	1.38	0.96-1.98
Mean	1.05	0.89-1.24	1.06	0.92-1.23

Mice kept at the space cabin environment for 3, 7, 14, 21, or 30 days were exposed to 50, 100, or 300 rads of Co^{60} gamma radiation. Three days later the mice were infected with S. aureus and treated with tetracycline as described in the previous study. Five different doses of tetracycline were used, and 6 mice were used for each dosage level. The probit analysis for the summarized data is presented in Table 9. For the purpose of this analysis, the data for the ambient controls and the data for the various radiation exposures were grouped together.

Table 9

EFFECTIVENESS OF TETRACYCLINE THERAPY
IN STAPHYLOCOCCUS AUREUS INFECTED MICE
EXPOSED TO SPACE CABIN ENVIRONMENT AND TO ACUTE RADIATION

Days at Space Cabin Environment		Space Cabin ED_{50}, mg/kg		Space Cabin Irradiated ED_{50}, mg/kg	
Before Radiation	Before Challenge	Mean	95% C.L.	Mean	95% C.L.
Ambient Control		0.50	0.23-1.05	0.90	0.64-1.28
3	6	0.42	0.09-1.85	0.83	0.53-1.29
7	10	0.80	0.02-3.30	1.41	0.37-5.46
14	17	0.19	0.01-6.19	0.82	0.45-1.46
21	24	1.76[a]	0.98-3.16	1.80[a]	1.27-2.55
30	33	1.47[a]	0.88-2.44	0.62	0.42-0.94

[a]Significant at $P < 5\%$.

Significant differences at the 5% probability level were obtained in the 21-day experiments in both the simulated space cabin exposed mice and the simulated space cabin and radiation-exposed mice. A significant difference ($P < 5\%$) was also present in the simulated space cabin exposed mice in the 30-day experiments. These differences were not present in the previous experiments, in which exposure to the space cabin environment was the only experimental parameter.

C. Susceptibility to Venezuelan Equine Encephalomyelitis Virus Infection

Mice were exposed to the space cabin environment and then inoculated intraperitoneally with three different doses of VEE virus at 1, 3, 24, or 48 hr after return to ambient conditions. Ten mice were used at each dilution. The mice were observed for mortality for 14 days. The data were summarized and are presented in Table 10. An analysis of variance performed on the data indicated that there were no significant differences between the control and the experimental mice. Apparently, previous exposure to the space cabin environment does not alter the resistance of mice to VEE virus infection after they return to ambient conditions.

IV. SUMMARY AND CONCLUSIONS

Exposure of mice to a simulated space cabin environment reduced their resistance to Klebsiella pneumoniae infection. A significant increase in mortality occurred when mice were challenged by the respiratory route with airborne K. pneumoniae 3, 14, or 30 days after they were exposed to the space cabin environment. After 7 or 21 days of exposure the mortality was also higher than in the control mice kept at ambient conditions, but the increase was not statistically significant.

Table 10

MORTALITY OF MICE CHALLENGED
WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS
AFTER EXPOSURE TO SPACE CABIN ENVIRONMENT

Exposure to Space Cabin Environment, days	Time of Challenge after Exposure, hr	Ambient Mortality Dead/Total	Ambient %	Space Cabin Mortality Dead/Total	Space Cabin %
3	1	10/30		15/30	
	3	10/30		8/30	
	24	13/30		11/30	
	48	1/30		1/30	
		Total	28.3		29.2
7	1	11/30		5/30	
	3	4/30		3/30	
	24	14/30		8/30	
	48	11/30		13/30	
		Total	33.3		24.2
14	1	10/30		9/30	
	3	10/30		13/30	
	24	13/30		12/30	
	48	1/30		9/30	
		Total	28.3		35.9
45	1	6/30		10/30	
	3	12/30		8/30	
	24	24/30		22/30	
	48	17/30		21/30	
		Total	49.2		50.8

When mice were exposed to the space cabin environment for 14, 30, or 45 days and challenged with the K. pneumoniae aerosol 1 hr after return to ambient conditions, significant increases in mortality occurred in comparison with mice not exposed to the space cabin environment. Similar significant increases in mortality occurred when the mice kept in the space cabin environment for 30 or 45 days were challenged with the infectious aerosol 24 hr after return to ambient conditions. However, increased mortality was not observed in mice kept in the space cabin environment for 14 days and challenged with the infectious aerosol 24 hr after return to ambient conditions.

Hematological studies of mice kept in the space cabin environment for 3 to 30 days did not show any significant differences in hemoglobin, hematocrit, or differential blood cell count, but did reveal a decrease in white blood cell count after 3 and 14 days and an increase after 30 days. In exploratory studies, the lactic acid dehydrogenase isoenzyme patterns of mice exposed to the space cabin environment appeared to differ from those of mice kept at ambient conditions. Elevation of bands M and 3 were observed in kidney tissue of mice kept in the space cabin environment for 14 and 30 days, and a depression of band M isoenzyme was observed in heart tissue.

Experiments were conducted to determine the effect of space cabin environment, single exposure to gamma radiation, and K. pneumoniae challenge on mortality of mice. The combined stress of space cabin environment and radiation resulted in increased mortality in mice receiving doses of 100 to 500 rads. The additional stress of the infectious challenge produced varied results, which cannot be fully interpreted at the present time. When the mice were kept in the space cabin environment for 3 days before radiation exposure and an additional 7 days before the infectious challenge mortality increased significantly. However, in the experimental group maintained at the space cabin environment for 14 days before exposure to the radiation and an additional 7 days before the infectious challenge, mortality did not increase significantly. This finding suggests a possible protective role of space cabin environment in radiation exposure; more experimental data are required to confirm this observation.

When mice exposed to the space cabin environment from 3 to 30 days were challenged by the intraperitoneal route with Staphylococcus aureus, the LD_{50} was not significantly different from that of control mice kept under ambient conditions. Comparison of mortalities resulting from intraperitoneal injection of Venezuelan equine encephalomyelitis virus in mice kept in the space cabin environment and ambient conditions also did not disclose any significant difference.

Exposure of mice to the space cabin environment or to both the space cabin environment and radiation prior to infection with S. aureus and treatment with tetracycline did not appear to alter the response to the therapy. In one experiment, a significant increase occurred in the 50% protective dose of mice exposed to space cabin environment for 21 and 30 days. In another experiment, however, no increase occurred. Experiments utilizing larger numbers of animals would establish the significance of the observation.

Since the experimental data pertain to mice, extrapolation of the observed effects to man can be, at best, speculative. However, the data suggest that resistance to respiratory infection is reduced upon exposure to a space cabin environment. Additional studies are necessary to define more closely the conditions under which this reduced resistance occurs and to determine the mechanisms involved. Such studies should include challenges with respiratory viruses, biochemical studies of the respiratory system, and studies on the effects of the space cabin environment on immunological responses and processes.

REFERENCES

1. Ehrlich, R. and Mieszkuc, B. J. J. Infect. Dis. 110, 278, 1962.
2. Mieszkuc, B. J. and Ehrlich, R. Technical Documentary Report SAM-TDR 64-9.
3. Ehrlich, R. and Mieszkuc, B. J. Developments Ind. Microbiol. 5, 207, 1964.
4. Ehrlich, R. and Mieszkuc, B. J. Nature 207, 1109, 1965.
5. Batson, H. C., An Introduction to Statistics in the Medical Sciences. Burgess Publishing Co., Minneapolis, 1957.
6. Brownlee, K. A., Statistical Theory and Methodology in Science and Engineering. John Wiley and Sons, New York, 1960.

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