EFFECTS OF SPACE CABIN ENVIRONMENTS ON RESISTANCE OF MICE TO INFECTION WITH KLEBSIELLA PNEUMONIAE

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FOREWORD

This report was prepared in the Life Sciences Research Division of IIT Research Institute, Chicago, Illinois, by—

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The technical assistance of H. Logan and G. Jedlicka is gratefully acknowledged.

The strain of *Klebsiella pneumoniae*, type A-D, was obtained from Dr. R. S. Speck, University of California School of Medicine.

CORRECTION

("Effects of Space Cabin Environments on Resistance of Mice to Infection with Klebsiella Pneumoniae," by Bernard J. Mieszkuc and Richard Ehrlich, SAM-TDR-64-9, March 1964)

ABSTRACT: Sentence 2 should be changed to read--

"Mice exposed to 35,000 feet for 14 days prior to challenge and then returned to 35,000 feet exhibited an increase in mortality from 37% to 76%."

insert as indicated in abstract p. iii

Followed by (sentence to be added)--

"Mice exposed to 35,000 feet for 14 days prior to challenge and then kept at ambient altitude exhibited an increase in mortality from 37% to 53%."

Then--

"There was an increased neutrophilic percentage . . . "

add from correction notice attached to inside front cover.

ABSTRACT

This study shows the effect of exposure of mice to an altitude of 35,000 feet and to an atmosphere consisting of approximately 85% oxygen, 10% carbon dioxide, and 5% nitrogen for 3, 7, 14, and 30 days on susceptibility to respiratory infection caused by aerosols of *Klebsiella pneumoniae*. Mice exposed to 35,000 feet for 14 days prior to challenge and then returned to 35,000 feet exhibited an increase in mortality from 37% to 52%. There was an increased neutrophilic percentage accompanied with a lower white cell count in the blood of mice exposed to 35,000 feet for 14 days. Mice lost weight initially when placed at 35,000 feet. They started gaining weight after about 2 weeks, and the rate of weight gain was approximately that of mice kept at ambient altitude. Mice kept at 35,000 feet consumed more food than mice kept at ambient altitude.

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This technical documentary report has been reviewed and is approved.

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1. INTRODUCTION

Since the space traveler may be exposed to stresses which may lower his resistance to infection, stress factors in spacecraft must be controlled. The effects of diet, temperature, relative humidity, ionizing radiation, noxious gases, fatigue, and seasonal variation on susceptibility to infection are well documented. The effect of altitude, however, has received little attention. Berry (2) found that mice exposed to an altitude of 20,000 feet for 3 to 4 months were more susceptible to *Salmonella typhimurium* infection than mice kept at ambient altitude. Ehrlich and Mieszkuc (3) found that mice exposed to an altitude of 18,000 feet for 3 days were more resistant to *Klebsiella pneumoniae* infection than mice kept at ambient altitude; the increased resistance disappeared after 7 days of exposure to 18,000 feet. After 30 and 90 days' exposure to 18,000 feet, the mice were significantly more susceptible to infection.

This report describes the response of Swiss albino mice to respiratory infection caused by aerosols of *K. pneumoniae* after exposure to an altitude of 35,000 feet for different periods of time. Studies are also described on the changes in weight, food consumption, and blood, and on possible cross infection with *K. pneumoniae* under environmental stress.

2. METHODS

Mice

Swiss albino mice of either sex, 8 to 10 weeks old and weighing 18 to 20 gm., were used. The mice were randomly placed in cages, 10 mice of the same sex per cage and quarantined for 2 weeks prior to experimental use. After exposure to the experimental conditions, the mice were kept in an isolation room and were protected from possible cross infection by ultraviolet germicidal lamps. They were maintained at all times on diets that were nutritionally adequate.

High-altitude chamber

The high-altitude chamber used for exposure of the mice to a simulated space cabin environment consists of two connected chambers—the main chamber and the air lock. Each is approximately a 6-ft. cube. The rear portion of the main chamber is compartmented from the work space and contains a heating unit, refrigeration coils, and circulating fans. The main chamber is capable of attaining an altitude of 20,000 feet in 5 minutes and 150,000 feet in 30 minutes. The temperature range possible is $-10°$ to $+95°$ C. The relative humidity is automatically controlled at ambient temperatures in the range of 20% to 90%. Recorders monitor and control the altitude and the temperature of the dry bulb and wet bulb. A communication system is provided, consisting of microphones, speakers, and amplifiers. The oxygen supply system consists of a standard 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 are employed. Analyzers and recorders continuously monitor the concentrations of oxygen and carbon dioxide. A Beckman model F3 oxygen analyzer is used for oxygen determinations, and a Beckman model L/B 15-A infrared analyzer is used for carbon dioxide determinations.

The mice were exposed to an altitude of 35,000 feet in the following manner: In step 1 the chamber containing the mice was evacuated to a pressure of 250 mm. Hg. Oxygen was then fed into the chamber until a pressure of approximately 510 mm. Hg was obtained. The chamber atmosphere at the time contained approximately 60% oxygen. In step 2 the chamber was re-evacuated to a pressure of 180 mm. Hg, and oxygen was then fed into the chamber until the pressure was 380 mm. The chamber atmosphere now contained 80% oxygen. By repeating the second step twice, an atmosphere was obtained that contained approximately 95% oxygen and 5% nitrogen at 179 mm. Hg.

After the presence of the mice caused the carbon dioxide concentration in the chamber to increase to a value of 10% to 12%, the atmosphere was controlled by slowly feeding in oxygen and evacuating to a pressure of 179 mm. Hg. Thus, the conditions simulated in the chamber were as follows: barometric pressure, 179 mm. Hg; oxygen concentration, 80% to 85%; carbon dioxide concentration, 10% to 12%; nitrogen concentration, 5%; relative humidity, 50%; and temperature, 25° ± 1° C.

Personnel entered the main chamber three times a week—on Monday, Wednesday, and Friday—for observation and maintenance of the mice. The personnel breathed 100% oxygen for at least 15 minutes before they entered the air lock. The atmosphere in the lock was evacuated to 179 mm. Hg and then replaced with oxygen until a pressure of 380 mm. was obtained. This procedure was repeated four times producing an atmosphere similar to the one in the main chamber.

The mice were kept at the simulated altitude at all times except when they were challenged with *K. pneumoniae* aerosols. The time interval between the removal from the high-altitude chamber and return was kept as short as possible and did not exceed 1 hour.

K. pneumoniae aerosol and infectious challenge

K. pneumoniae, type A-D, was used. The bacteria were grown on blood agar base medium, harvested with sterile 10% Difco dehydrated skim milk, placed in ampuls, quickly frozen, and stored at −10° C. until used. For aerosolization, the frozen stock culture was thawed at room temperature. Then blood agar base plates were inoculated with the culture and incubated at 37° C. for 24 hours. The growth was washed from the surface with distilled water, and a diluted suspension was used for aerosolization. Usually the culture was diluted to give a reading of 35 units on the Coleman Nepho-colorimeter, using a nephelos standard of 74.

The aerosol chamber was a plastic hood, 23 x 23 x 27 in., installed within a biologic safety hood. A University-of-Chicago type of atomizer was used to produce the aerosol. The bacterial culture was fed to the atomizer by a 50-ml. syringe, activated by a motor-driven piston, which delivered the diluted culture to the atomizer at a rate of 0.4 ml./minute. Filtered air was supplied to the primary and secondary inlets of the atomizer at a constant pressure of 10 p.s.i. and at a flow rate of 1 c.f.m. The air within the chamber was sampled at the beginning and the end of each exposure by drawing air through 20 ml. of 0.1% gelatin phosphate solution contained in an all-glass impinger with a critical orifice of 12.5 liters/minute.

Twenty mice exposed to 35,000 feet and 20 kept at ambient altitude were challenged simultaneously in the aerosol chamber. Ten of the mice exposed to a 35,000-foot altitude and 10 kept at ambient altitude were returned to an altitude of 35,000 feet; the rest were kept at ambient altitude. The period of respiratory challenge, usually 10 minutes, was determined by the virulence and the concentration of the bacterial aerosol. After the challenge, all mice were observed daily for 14 days, and mortality and survival time were recorded. Animals dying during this period were autopsied, and their lungs and hearts were cultured on blood agar base medium to confirm the cause of death. Animals surviving through the 14th day were sacrificed and autopsied and their lungs and hearts were cultured.

Cross infection

To determine whether cross infection occurs when mice are exposed to a 35,000-foot altitude, 60 mice were challenged with *K. pneumoniae* aerosol. Of these, 20 had been exposed to 35,000 feet for 14 days and 20 others for 30 days, while the remaining 20 had been kept at ambient altitude. From each group of challenged mice, 10 were placed at an altitude of 35,000 feet, and the other 10 were kept at ambient altitude. Five noninfected mice which had been kept at ambient altitude were added to each group. In other experiments 5 uninfected mice kept at 35,000 feet for 14 and 30 days were added to each group.

All of the mice were observed daily for 4 weeks, and the mortality was recorded. Mice that died were autopsied, and their lungs and hearts were cultured to confirm the cause of death. The surviving mice were sacrificed and autopsied, and their lungs and hearts were cultured.

Hematology

Blood was obtained by decapitating the mice and collecting blood in small vials containing heparin. The Hellige procedure was used to determine hemoglobin content. Hematocrit, sedimentation rates, red and white blood cell counts, and differential white cell counts were obtained by standard procedures.

Weight gain and food consumption

The effect of high altitude on weight gain and food consumption of mice was determined by placing 50 mice at a simulated altitude of 35,000 feet and 50 at ambient altitude. Weight and food consumption of the mice were determined weekly.

3. RESULTS AND DISCUSSION

Effect of altitude on resistance to infection

On the assumption that mortality accurately reflects the effects of respiratory infection, efforts were made to produce 30% to 50% mortality in the control mice kept at ambient conditions. Mortality rate rather than LD_{50} dose was selected because of the inherent variability in aerosol experimentation. Data given in tables I and II show the response of mice to challenge with *K. pneumoniae* aerosol after exposure to 35,000 feet. Experiments falling outside the 30% to 50% range in the control mice were not used for statistic analysis. The significance of the results was determined by the t-test (1). Before the t-test was administered, the mortalities were subjected to an arc sine transformation.

The experimental results indicate that the resistance of mice to respiratory infection is reduced by altitude stress. The decrease in resistance to respiratory infection was most noticeable in mice exposed to 35,000 feet for 14 days before infectious challenge and kept at 35,000 feet after challenge. As shown in table I, the mortality increased from 37% to 76%. Significant difference in mortality was also demonstrated in mice kept at 35,000 feet for 14 days before infectious challenge and at ambient altitude after challenge. A comparison of the mice kept at 35,000 feet before infectious challenge and at ambient altitude after challenge, with mice kept at 35,000 feet before and after infectious challenge, indicates an increase in mortality from 53% to 76%. The difference is significant at $P < .001$. A comparison of the mice kept at ambient altitude before challenge and at 35,000 feet after challenge, with the mice kept at 35,000 feet before and after challenge, indicates an increase in mortality from 42% to 76%. The difference is significant at $P < .001$.

It appears that altitude stress before infectious challenge has a greater effect than altitude stress after infectious challenge. However, the continued exposure of mice kept at 35,000 feet for 14 days prior to infectious challenge to a simulated altitude of 35,000 feet further decreases their resistance to respiratory infection. Table II shows that approximately 50% of the mice exposed to 35,000 feet for 14 days prior to challenge and kept at 35,000 feet after challenge were dead within 8 days after infectious challenge. It also shows

TABLE I

Effect of 35,000-foot altitude on mortality of mice challenged with K. pneumoniae

Altitude		Mortality		Average survival time (days)
Before challenge	After challenge	Deaths/total	Percent	
Ambient for 3 days	Ambient	20/50	40	7.4
7		32/70	46	7.5
14		55/150	37	7.2
30		47/130	36	6.8
Ambient for 3 days	35,000 feet	19/50	38	7.0
7		29/70	41	7.1
14		63/150	42	7.2
30		51/130	39	6.9
35,000 feet for 3 days	35,000 feet	22/50	44	7.7
7		27/70	39	6.7
14		114/150	76*	7.6
30		56/130	43	7.0
35,000 feet for 3 days	Ambient	19/50	38	6.8
7		33/70	47	8.1
14		79/150	53†	6.5
30		51/130	39	7.3

*Significant at $P < .001$.

†Significant at $P < .01$.

that 40% exposed to 35,000 feet for 14 days prior to challenge and at ambient altitude after challenge died within 8 days after infectious challenge as did 25% kept at ambient altitude before challenge and either at ambient altitude or at 35,000 feet after challenge.

The experimental results are in agreement with those of Berry (2); the mean survival time was reduced by approximately 30% in mice exposed to 20,000 feet for as long as 4 months and challenged with *S. typhimurium*. In Berry's experiments the mice were brought to sea-level pressure daily for 15 to 20 minutes. In the present experiments the mice were kept continuously at 35,000 feet.

In our previous work (3) exposure of mice to 18,000 feet for 30 and 90 days prior to infectious challenge significantly decreased their resistance to infection. It is interesting that no decreased resistance to respiratory infection was observed after 3, 7, and 30 days' exposure to an altitude of 35,000 feet before infectious challenge. Apparently more than 7 days are required to reduce resistance to infection. Results of the 30-day experiments could indicate that the mice adapted to altitude stress. Immunologic and histopathologic studies must be conducted to adequately explain the mechanism of the observed reduced resistance. Apparently mice react more quickly to a stress of 35,000 feet, and the recovery period also appears to be shorter.

Autopsy of the mice that died during the 14-day observation period showed a high incidence of purulent exudate in the pleural cavities. The lungs were consolidated and reddish brown and often showed white plaques. The lungs and heart contained *K. pneumoniae*, confirming the cause of death. No *K. pneumoniae* was isolated from the heart, lungs, spleen, liver, kidneys, or bladder of surviving and noninfected mice.

Effect of altitude on cross infection

No cross infection was observed when infected mice (which had been kept at ambient

TABLE II

Effect of 35,000-foot altitude on survival of mice challenged with K. pneumoniae

Time at altitude before challenge (days)	Time after challenge (days)	Percent of mice surviving			
		Ambient* Ambient†	Ambient* 35,000†	35,000* 35,000†	35,000* Ambient†
7	1	100	100	100	100
	2	100	100	100	100
	3	100	94	100	100
	4	91	93	97	97
	5	90	90	93	97
	6	84	81	83	86
	7	79	77	70	83
	8	67	70	66	74
	9	64	64	64	67
	10	57	63	63	57
	11	57	61	61	54
	12	57	59	61	54
	13	54	59	61	53
	14	54	59	61	53
14	1	100	100	100	100
	2	100	100	100	100
	3	100	99	100	99
	4	95	93	87	88
	5	88	91	84	79
	6	83	82	65	70
	7	77	76	58	64
	8	75	73	51	59
	9	73	72	47	57
	10	68	67	41	49
	11	66	64	34	49
	12	65	63	29	47
	13	64	62	26	47
	14	63	58	24	47
30	1	100	100	100	100
	2	100	100	98	99
	3	95	94	92	96
	4	90	88	87	95
	5	89	85	85	92
	6	85	83	79	87
	7	77	78	75	81
	8	71	69	70	72
	9	69	66	62	68
	10	65	65	61	66
	11	65	63	59	65
	12	64	62	58	62
	13	64	62	58	61
	14	64	61	57	61

*Altitude before challenge.

†Altitude after challenge.

TABLE III

Effect of 35,000-foot altitude on blood of mice

Days at 35,000 feet	Hematocrit (%)	Hemoglobin (gm./100 ml. blood)	Red blood cells (10^6/mm.^{3})	White blood cells (10^3/mm.^{3})	Lymphocytes (%)	Neutrophils (%)
0	45.4	13.8	8.5	5.0	87	13
3	42.2	12.8	8.5	3.8	84	14
7	44.0	14.1	9.8	2.6	81	17
14	42.0	13.2	9.9	2.3*	67	32*
30	46.0	14.4	10.5	3.7	82	16

*Significant at $P < .001$.

altitude, at 35,000 feet for 14 days, or at 35,000 feet for 30 days prior to infection) were placed with noninfected mice (also previously subjected to 1 of the 3 altitude treatments). Half of the mice in the 9 groups were kept at ambient altitude, and the remainder at an altitude of 35,000 feet; all were observed for 4 weeks. Since it is apparent that *K. pneumoniae* is not suitable for cross-infection studies, other microorganisms will be employed in future studies.

Effect of altitude on blood

The data on the hematology of mice exposed to 35,000 feet for 3, 7, 14, and 30 days are given in table III. At least 20 mice were studied in each altitude-exposure group. The sedimentation rate showed no significant changes within 1 hour in any of the mice. In those exposed to 35,000 feet for 14 days, the neutrophilic percentage increased and the white cell count decreased. Many blast cells were present in the blood of the altitude-stressed mice. This may indicate a rapid outpouring of younger leukocytes from the marrow in response to an urgent need. It also suggests failure of the leukocytes to mature as a result of marrow depression.

Effect of altitude on weight gain and food consumption

The effect of altitude on weight gain and food consumption of mice is shown in figure 1. The mice initially lost weight when kept at 35,000 feet. They started to gain weight after

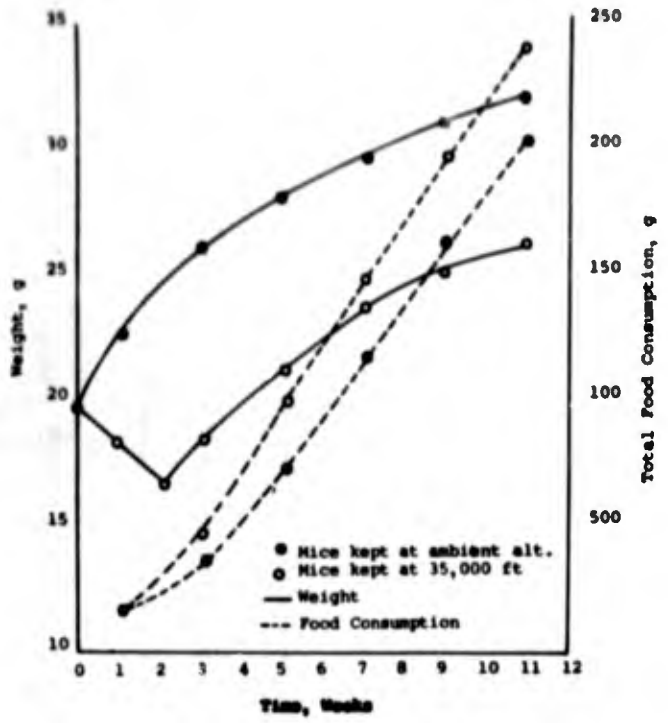

FIGURE 1

Effect of altitude on weight gain and food consumption of mice.

2 weeks and gained at approximately the same rate as the mice kept at ambient altitude. Mice kept at 35,000 feet did not gain as much weight as those kept at ambient altitude. There does not appear to be any correlation between weight gain and food consumption in mice kept at 35,000 feet nor in those kept at ambient altitude. After an initial adaptation period, mice kept at 35,000 feet consumed more food than those kept at ambient altitude but gained approximately the same amount of weight.

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