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NASA CR-
147570

Final Report Submitted To
National Aeronautics and Space Administration
Manned Spacecraft Center
Houston, Texas 77058

(NASA-CR-147570) [INCREASED CONCENTRATION OF PSEUDOMONAS AERUGINOSA AND STAPHYLCCOCCUS SP. IN SMALL ANIMALS EXPOSED TO AEROSPACE ENVIRONMENTS] Final Report, 1 Jul. 1971 - 31 Dec. 1972 (Clemscn Univ.) 28 p HC $4.00 G3/51

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work accomplished for the period
July 1, 1971 through December 31, 1972
at Clemson University

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Abstract of Project

The purpose of this contract was to evaluate the effects of increased concentration of Pseudomonas aeruginosa and Staphylococcus sp. in the total bacterial flora of small animals exposed to simulated spacecraft environments. This has been done by tests to detect (1) changes in infectivity, (2) effects of antibiotic treatments, (3) immune responses to bacterial antigens, and (4) effectiveness of immune response in the experimental environment.

The experimental plans, methods of investigation, results, and conclusions are reported in the following sections. The most significant results appear to be the differences in immune responses at simulated altitudes and the production of infection in the presence of specific antibody.

Introduction

The report of work covering the period January 28, 1970 through March 15, 1971 concluded that (1) untreated rabbits and guinea pigs in simulated spacecraft environments show a reduction in bacterial intestinal flora in total counts and in the streptococcus, coliform and staphylococcus segments of that flora. (2) Exposure of Salmonella california infected animals to simulated spacecraft environments enhances the virulence of this otherwise low grade pathogen, as measured by death rates in exposed animals compared to control animals. (3) S. california is transmitted from infected to uninfected animals in the simulated spacecraft environment, as measured by serological responses, although enhanced invasiveness is not observed under these conditions. (4) Pre-treatment of experimental animals with ampicillin or terramycin lowers the survival rate in animals exposed to simulated spacecraft environments. (5) Exposure of experimental animals to intestinal infection with Pseudomonas aeruginosa does not appear to reduce survival rates unless combined with antibiotic treatment. (6) Infection of the skin of experimental animals with Staphylococcus aureus results in larger lesion development although the infections do not become generalized. (7) The primary immune response of infected or artificially immunized experimental animals appears to be suppressed when antigen exposure occurred immediately prior to environmental exposure. The secondary immune response did not appear to be affected under these conditions.

As a result of the above conclusions a statement of work, and time lines were developed for the current project which included the following major studies: (1) The effects of simulated spacecraft environment exposure on the primary and secondary response of experimental animals. (2) The effects of simulated spacecraft environment exposure on lesion development and immune response in S. aureus skin infections. (3) The effects of

combined exposure to P. aeruginosa and antibiotics when experimental animals are exposed to simulated spacecraft environments. (4) The effects of ampicillin treatment on intestinal bacterial flora in rabbits which results in toxicity to the animals exposed to simulated spacecraft environments. (5) The effects on experimental animals of dual infections with S. aureus and P. aeruginosa in simulated spacecraft environments.

Experimental Procedures

All experiments were performed under similar conditions of isolation, barometric pressure and oxygen tension in altitude chambers of approximately 3.5 cubic feet of space for each animal. Chamber pressure was maintained at 380mm Hg by continuous negative pressure exhaust, with continuous supply of 100 percent oxygen. Temperature was ambient room temperature of approximately 75°F. Control animals at ground level were maintained in identical chambers at ambient pressure and room air. Exposures were generally for one week periods.

Intestinal flora determinations were made by rectal swab cultures on general, selective, and differential media.

Blood samples were taken by cardiac puncture or from ear veins immediately prior to exposure, immediately following exposure and at various intervals for serological testing of serum.

Results

1. Immune Responses:

In the previous report, results indicated that there was some inhibition, at altitude, of the primary immune response to injection of Salmonella california heat killed antigen, as compared to ground control animals. There appeared to be less effect on the primary

immune response in animals fed living S. california and exposed to the simulated spacecraft environment. In those results a mean primary peak titer of 1:160 was reached at 14 days in ground controls, and a mean primary peak titer of 1:88 was reached at 7 days in animals in the experimental environment.

Sixteen additional animals have been tested in the current contract period at ground control, and have developed a mean peak titer of 1:1599 at 11 days. Fourteen additional animals have been tested in simulated spacecraft environments with this antigen, and have developed a mean peak titer of 1:558 at 9 days. These results are in agreement with those reported earlier, and do appear to indicate an inhibition of the primary immune response under these experimental conditions.

The secondary immune response appeared to be little affected, or even possibly stimulated in previously reported results. In the current study, eighteen additional animals have been immunized at both ground control and simulated spacecraft environments. Ground control animals had a mean initial titer of 1:141. These animals developed a mean peak titer of 1:3779 at 12 days following immunization. Altitude test animals had a mean initial titer of 1:205, and developed a mean peak titer of 1:4107 at 10 days following immunization. Again these results are compatible with those reported previously, and indicate little effect of the experimental environment on the secondary immune response.

Table 1. Heat Killed S. california Antigen

A. Primary response ground 16 animals	Initial titer - mean - 0 Highest titer - mean - 1599 at 11 days
B. Primary response altitude 14 animals	Initial titer - mean - 0 Highest titer - mean - 558 at 9 days
C. Secondary response ground 18 animals	Initial titer - mean - 141 Highest titer - mean - 3779 at 12 days
D. Secondary response altitude 18 animals	Initial titer - mean - 205 Highest titer - mean - 4107 at 10 days

Using the same antigen dose and immunizing methods, responses to other antigen types have also been tested in this contract period, to determine whether the effect observed above is one which will occur in all responses. Antigens were prepared from S. california by two additional methods; formalin killed (H antigen) and nitrous acid killed somatic antigen. Results are seen below.

Table 2. Formalin Killed S. california Antigen

	Day	Mean Titer
Simulated spacecraft environment: 9 animals	0	0
	7	2664
	11	1227
	15	1600
	19	2133
	23	3840
	27	3893
Ground Level controls: 5 animals	0	0
	7	1536
	11	1152
	15	896
	19	1504
	23	1568
	27	2920

It is apparent in these results that there was no inhibition of the primary immune response to the "h" antibody production in these animals. Whether there was inhibition in production of "o" antibody is not known. There is in both groups, a phasic antibody production curve with two peak titer periods. The explanation of these phases is not apparent.

Table 3. Nitrous Acid Killed S. california Antigen

	Day	Mean Titer
Simulated spacecraft environment: 3 animals	0	0
	7	747
	11	533
	15	320
	19	160
	23	160
Ground Level controls: 3 animals	0	0
	7	640
	11	320
	15	160
	19	160

It is apparent in the above results of immunization with nitrous acid killed S. california antigen that there is again no inhibition of the immune response developed in these animals. Previous reports of use of this type of antigen indicate that the antibodies produced in response to its injection are most likely to be bactericidal antibodies, and that much of the surface cellular antigens may be stripped off in the antigen preparation. In these results it is observed that the titers are drastically lower than in either "h" or "o" animals.

To further test antigen effect on this response in a simulated spacecraft environment, heat killed Staphylococcus aureus antigen was used. Results, seen below, indicate that there is essentially no difference in the primary immune responses seen at ground level and in simulated spacecraft environments.

Table 4. Staphylococcus aureus 6538 Antigen

Primary Ground Controls

Rabbit No.	Initial titer	Peak titer (days post inject)	
59	0	1:5120	(13)
60	0	1:2560	(8)
61	0	1:1920	(8)
64	0	1:1920	(13)
66	0	1:1920	(8)
77	0	1:2560	(8)
81	0	1:1280	(8)
82	0	1:1280	(8)

mean - 1:2320 at 9 days

Primary Altitude

Rabbit No.	Initial titer	Peak titer (days post titer)	
56	0	1:2560	(7)
57	0	1:3840	(7)
62	0	1:1920	(7)
67	0	1:1280	(7)
68	1:10	1:1920	(7)
69	1:60	1:1280	(7)
76	0	1:2560	(7)
78	0	1:5120	(7)
79	0	1:5120	(7)
80	0	1:2560	(7)

mean - 1:2816 at 7 days

In the secondary response using the S. aureus killed antigen, there may be some interference with antibody production if one looks at the change in titers of individual animals. A doubling of titer in serial dilution titrations is not significant: two of three animals at altitude doubled titers only at 7 days while three of four animals had true secondary response rises at ground level. The one animal showing a true secondary response at altitude had a 4 fold rise, while at ground level there were 8 fold to 10 fold increases.

As is seen in the results reported in the infection experiments using S. aureus the development of titer at altitude did not prevent the progression of lesions in infected animals. These results, taken in that light, indicate that the potential of antibody to protect against infection in this experimental environment requires additional study for adequate determination. In view of the differences in immune responses and the lack of protection observed in one instance, the effectiveness of this resistance mechanism requires additional study. Such studies will be done in the next contract period.

Table 5. Staphylococcus aureus 6538 Antigen
Secondary Response

Secondary Ground Controls

Rabbit No.	Initial titer	Peak titer	at 8 days
56	1:1280	1:2560	
60	1:640	1:5120	
65	1:320	1:3840	
83	1:320	1:2560	
		mean - 1:3520	

Secondary Altitude

Rabbit No.	Initial titer	Peak titer	at 7 days
77	1:2560	1:10,240	
81	1:1280	1:5120	
82	1:1280	1:5120	
		mean - 1:6826	

II. Staphylococcus aureus infections:

In the prior report, it appeared that skin infections resulting from Staphylococcus aureus injections developed into larger, more progressive lesions in animals at altitude than at ground control environments. Results of previous tests are shown in the tables below.

Table 6. Staphylococcus aureus Injection

Animal No.	Route	Lesion Measurement, mm	Lesion Description
Controls - Ground Level			
19	SC	0	None
4	SC	4 x 4	Closed, cystic, no inflammation
41	SC	5 x 5	Closed, cystic, firm, inflamed
22	ID	2 x 4	Closed, cystic, firm, inflamed
3	ID	8 x 5	Open, dry, inflamed
6	ID	10 x 10	Closed, cystic, firm, no inflammation
11	ID	5 x 5	Open, dry, cystic, no inflammation
12	ID	5 x 5	Closed, cystic, firm, inflamed
Average lesion area 29 mm			
Simulated Spacecraft Environment			
20	SC	10 x 15	Closed, elevated, cystic, inflamed
2	SC	10 x 10	Closed, cystic, firm, no inflammation
40	SC	10 x 10	Closed, cystic, firm, inflamed
42	SC	15 x 15	Closed, cystic, firm, inflamed
21	ID	10 x 15	Closed, cystic, inflamed
1	ID	15 x 15	Open, cystic, purulent, inflamed
8	ID	15 x 15	Closed, cystic, firm, inflamed
9	ID	4 x 5	Open, cystic, firm, inflamed, elevated
10	ID	6 x 5	Open, cystic, firm, inflamed, elevated
Average lesion area 136 mm			

In the data compiled below, this development of more extensive lesions in rabbits kept in a simulated spacecraft environment after infection with S. aureus is borne out. These data include results from the previous report, combined with experimental results from the current contract period. The lesion area average of 80 mm^2 as compared to 34 in controls points up the difference. Only five experimental animals developed lesions of 25 mm^2 or less as compared to eleven controls with these small lesions. The larger lesions developed at altitude although in four animals tested for antibody response, average peak titer of 1:2560 was reached as compared to an average peak titer of 1: 2080 at ground level. These results indicate a need for additional information dealing with the effectiveness of the immune response in simulated spacecraft environments.

Table 7. Characteristics of Lesions Developed on Control Rabbits Injected Intradermally with S. aureus ATCC 6538.

Rabbit	Index of Lesion Area (mm^2)*	Lesion Description
1	8	Closed, cystic, firm, inflamed
2	40	Open, dry, inflamed
3	100	Closed, cystic, no inflammation
4	25	Open, dry, cystic, no inflammation
5	25	Closed, cystic, firm, inflamed
6	12	Open, cystic, purulent, inflamed, elevated
7	6	Closed, cystic, inflamed
8	30	Open, cystic, elevated, inflamed, purulent
9	130	Open, inflamed, purulent
10	100	Open, inflamed, elevated, cystic
11	40	Open, elevated, cystic
12	25	Closed, elevated, cystic
13	4	Closed, elevated, cystic
14	25	Closed, elevated, cystic
15	25	Closed, cystic, inflamed, elevated
16	4	Open, dry, inflamed
17	0	No lesion

* Average lesion area of control rabbits - 34 mm^2

Table 8. Characteristics of Lesions Developed on Experimental Rabbits Injected Intradermally with S. aureus ATCC 6538.

Rabbit	Index of Lesion Area (mm^2)*	Lesion Description
1	150	Closed, cystic, inflamed
2	225	Open, cystic, purulent, inflamed
3	225	Closed, cystic, firm, inflamed
4	20	Open, cystic, inflamed, elevated, firm
5	30	Open, cystic, inflamed, elevated, firm
6	16	(scab scratched off) open
7	63	Open, dry, inflamed
8	16	Open, dry, inflamed
9	140	Open, cystic, inflamed, elevated
10	100	Open, cystic, inflamed, elevated
11	56	Open, cystic, firm, elevated
12	9	(scab scratched off) open
13	100	(scab scratched off) open
14	25	Open, elevated, cystic, purulent
15	56	Closed, cystic, elevated
16	48	Closed, cystic, elevated

* Average lesion area of experimental rabbits - 80 mm^2

III. Combined Antibiotic Treatment and P. aeruginosa Infection:

In the previous annual report, experimental results had indicated that: (1) pre-treatment of rabbits with terramycin increased death rate in a simulated spacecraft environment, (2) pre-infection with P. aeruginosa had no detrimental effects in this environment, and (3) that combined terramycin and P. aeruginosa pre-treatments also resulted in some deaths in a simulated spacecraft environment. Additional experiments have been performed in this research period to test these conclusions.

While no apparent toxicity or detrimental effects are observed when rabbits are treated with 1000 mg of terramycin at ground level, there is a general reduction in total flora of the intestinal tract of 15% as has now been observed in twelve animals tested for a one week period. When animals are treated with this dosage and exposed to simulated spacecraft environments, however, this is not the case. In these test animals, those surviving (5/12) also show approximately a 15% reduction in total flora. No signs of infection was observed in any animals so treated.

Infection with P. aeruginosa(1×10^{10} cells, orally) has not been observed to produce an infection, or any increase in death rate at either ground level or in simulated spacecraft environments in any of twenty-three animals tested, although two additional animals with a pre-existing diarrhea did die in the test environment.

In this research period 8 additional rabbits have been treated with terramycin and given 1×10^{10} cells of P. aeruginosa orally prior to exposure to the simulated spacecraft environment for one week. In prior results this procedure resulted in a 40% increase in total aerobic flora in six test animals. In that work 2/6 animals died during the simulation period.

In the results of this more recent work, there was approximately a 60% increase in total aerobic flora in the 8 test animals, and 1/8 rabbits died during simulation. Although no obvious infection was apparent, even at autopsy, there was a dramatic change in all measurable components of the aerobic flora although the test organism was not frequently isolated (2/8) in the post simulation cultures. In general, the gram positive components changed most as in other tests involving terramycin treatment, however, there was also an increase in the gram negative (coliform) segments.

Table 9. Pre-Treatment With Terramycin and P. aeruginosa

	Mean Pre	Mean Post	% Change	Animals Number Increasing	Animals Number Decreasing	Animals Total** Number
Total Count	4.0*	6.5	+63	7	4	11
Staphylococcus	1.8	8	+300	6	4	11
Streptococcus	1.4	1.8	+28	4	7	11
Bacillus	3.0	5.0	+66	3	2	11
Coliform	6.0	11.0	+83	7	4	11

* All counts x 10^8
**Numbers unchanged included in total.

In all animals tested in this manner the death rate was 3/14 as compared to a rate of 5/12 for terramycin treatment alone.

When terramycin treatment was given via drinking water during simulation after feeding of 1×10^{10} P. aeruginosa prior to simulation, the results were somewhat different. There was a net decrease of 46% in total count and a general decrease in all segments of the aerobic intestinal flora.

Table 10. Pre-Treatment With P. aeruginosa Plus Simulation Treatment With Terramycin

	Mean Pre	Mean Post	% Change	Animals: Number Increasing	Animals: Number Decreasing	Animals: Total** Number
Total Count	4.3	2.3	-46	5	4	11
Staphylococcus	2.0	0.7	-65	4	4	11
Streptococcus	10.0	4.0	-60	4	6	11
Bacillus	1.7	0.7	-58	2	4	11
Coliform	1.4	0.8	-43	5	6	11

* All counts x 10^8
**Numbers unchanged included in total

In all animals tested in this manner the death rate was 1/12 as compared to 5/12 for terramycin treatment alone.

From the above experiments it must be concluded that although oral feeding of P. aeruginosa in this dosage is not apparently detrimental to animals exposed to simulated spacecraft environments, this feeding when combined with terramycin treatment appears to result in danger to these experimental animals. In view of previously reported results from ampicillin and terramycin treatments alone, it must be considered that there is some danger of difficulty in these experimental animals following such treatment. Since there is relatively little disruption of the intestinal flora, and no apparent damage to the animal following treatment with terramycin at ground level, it must at least be considered as possible that the flora distortions produced with these treatments at altitude may be responsible for the observed increases in death rates. In view of this possibility there is a need to do additional testing to detect the mechanism of effects of antibiotic treatment of experimental animals at altitude.

In limited testing, oral administration of additional antibiotic was not found to produce the desired effects of lowering intestinal flora counts and was not, therefore, included in this study as a major effort.

IV. Intestinal Bacterial Flora in Ampicillin Treated Rabbits:

The apparent extreme toxicity of ampicillin at either ground or simulated spacecraft environments prompted additional studies to attempt determination of the cause. In the results reported in the previous annual report, it was seen that only 3/22 animals tested survived the one week test period, and that two of the survivors were ground control animals. Since there was relatively little change detected in total or segmental aerobic bacterial flora, tests were run to determine the effects of ampicillin treatment on the anaerobic bacterial flora.

The anaerobic set-up included Brain Heart Infusion Agar (BHI) (Difco) with added cysteine-HCl and sodium thiosulfate as reducing agents and resazarin as an indicator. Medium was prepared by autoclaving and was poured in an atmosphere of CO_2 and after solidification and inoculation of the test sample, was overlayed with thioglycallate agar to maintain the reduced state of the BHI medium at below -42 mv. The inoculum was diluted prior to plating fluid thioglycollate agar medium for plating. Culture flasks were incubated at 39°C for one week.

Cultures were taken from the five groups of rabbits listed below.

I. Ground Controls, no treatment
II. Ground Controls, ampicillin treatment (500 mg)
III. Oxygen Ground Control, ampicillin treatment (500 mg)
IV. Test Group, simulated spacecraft environment, ampicillin treatment (500 mg)
V. Simulation Control, chamber environment, no treatment.

Cultures taken from rabbits four days after treatment.

In fourteen tests of groups I and V animals, anaerobic counts varying from 10×10^3 to 30×10^4 were repeatedly obtained. The heavily predominant organisms, as expected, were Bacteroides sp., however Sphaerophorus sp., and Streptococcus sp. were also isolated. The CDC "Laboratory Methods in Anaerobic Bacteriology" identification scheme was used.

In four tests of Group II animals, counts were increased approximately 100 fold. The same species of bacteria were present, including the predominant types and with the addition of numerous Clostridium sp. colonies.

In four tests of Group III and IV animals, total counts were comparable to those of Group II and the same species were isolated, including Clostridium sp. In these groups, however, the numbers of Clostridium sp. colonies was reduced more than 10 fold as compared to Group II cultures.

From these results it can be concluded that (1) ampicillin treatment of rabbits, with this 500 mg dose, increases the anaerobic intestinal bacterial flora, and stimulates the growth of Clostridium sp. to the level of a major component of this flora; (2) Oxygen concentration in the environment, reduces the stimulation of Clostridium sp. growth at either ground level or in a simulated spacecraft environment, but does not affect the increased presence of total anaerobic bacterial flora resulting from ampicillin treatment.

Since the aerobic bacterial flora was largely unaffected by ampicillin treatment it must be concluded that if the intestinal bacterial flora is responsible for the detrimental effects, then effects must result from the distortions produced in the increases in anaerobic flora and types. Additional studies are needed to fully elucidate the observations made in this work.

V. Dual Identification With S. aureus and P. aeruginosa

An attempt was made to study the effects of subcutaneous injections of 1×10^{10} cells of S. aureus and P. aeruginosa in separate sites, 4 cm apart on the shoulder area of experimental animals. Both immune and non-immunized animals were included in this study. P. aeruginosa was not isolated from the lesions or from blood cultures although all ground controls developed lesions as shown below, and one experimental animal

developed a lesion at this site. Only a non-immune animal, in simulated spacecraft environment developed a lesion at the S. aureus injection site in these animals. In all other animals no lesion developed at this site. Non-immunized animals developed antibody specific for S. aureus to a good level, while as expected, there was little antibody response to the P. aeruginosa under any conditions. It is worthy of note that the low titers produced specific for P. aeruginosa averaged 1:47 at ground level and 1:20 at altitude. The primary and secondary immune responses to S. aureus are difficult to analyze in these data, and are considered more fully in another section of this report.

These results indicate that there is a very complex response to these dual infections which would be very difficult to interpret and would require extensive pathological and physiological experimentation. Additional study of these factors is considered to be beyond the scope of this contract.

Table II. Dual Infection

(all post titers read at 7 or 8 days)

Ground Controls*

Rabbit No.	Prior Immunization	Titers Pre S	Titers Pre P	Titers Post S	Titers Post P	Lesion Description
71	None	0	0	640	80	Large inflamed "lump" in center. P. aeruginosa
66	Primary immunization at ground. S. aureus	2560	20	2560	40	Large inflamed "lump" in center. P. aeruginosa
67	Primary immunization at altitude. S. aureus	1280	0	1280	20	Large inflamed "lump" in center. P. aeruginosa

Altitude*

Rabbit No.	Prior Immunization	Titers Pre S	Titers Pre P	Titers Post S	Titers Post P	Lesion Description
84	None	0	0	2560	10	Small, closed, cystic lesion from S. aureus
64	Primary immunization at ground. S. aureus	320	0	640	40	Large, closed, non-inflamed "lump" in center. P. aeruginosa
76	Primary immunization at altitude. S. aureus	2560	0	2560	10	None

* Thioglycollate and TS blood culture were negative for all 6 rabbits at 5 days.

VI. Work accomplished on funds from NASA Contract #NAS 9-10494 in the period of July 1, 1972 to December 31, 1972.

Pursuant to the Statement of Work submitted in response to RFP 9-BB 321-81-3-2-P, work was continued on the above contract to the limit of funds allocated for NAS 9-10494. Results of experimental procedures carried out in this period are detailed in the sections following.

A. Challenge of immunized animals with homologous organisms.

Six rabbits (3 ground control, 3 altitude exposed) remained from primary immunization with Salmonella california in the previous period. The ground control animals had titers of 1:80, 1:640, and 1:320. Altitude exposed animals had titers of 1:80, 1:1280, and 1:160. Each animal was challenged orally, with $1X10^9$ live cells, taken from an 18 hour culture, washed and suspended in saline. No infection occurred after one week, and no S. california was recovered from any animal. Two weeks after the first challenge, the same animals were subjected to a second challenge consisting of an oral dose of $3X10^9$ living cells. Again no infection occurred and the organism was not recovered.

Animals remaining from secondary immunization with Staphylococcus aureus consisted of 4 ground controls and 4 altitude exposed with titers of 1:1280, 1:2560, 1:640, 1:640, 1:160, 1:640, 1:2560, and 1:640 respectively. These animals were challenged by the subcutaneous injection of $1X10^9$ living cells of an 18 hour S.aureus culture. No lesions were produced in any animal after one week. The same animals were then challenged for a second time by injection of $2X10^9$ living S. aureus cells by the intraperitoneal route. No apparent lesions developed in any animal and upon sacrifice and post mortem examination, no infection was found after two weeks.

B. Production of vaccines.

Two separate lots, each, of O antigen vaccine were prepared for both S. Aureus and S california as described in earlier reports. These vaccines were prepared in a heavy concentration to permit proper dilution for immunization and for in vitro testing. One lot of S. aureus vaccine was used in the work described in section C below. Immunizations were all done by injection of 1 ml ($1X10^9$ cells) total antigen dose. This was accomplished by injection of 0.5 ml intravenously and 0.5 ml subcutaneously, just prior to the beginning of altitude chamber exposure.

C. Results of Immunizations

1. Animals were divided into three groups: control at ground level, altitude exposed (380 mm Hg) with 100% oxygen atmosphere, and altitude exposed (380 mm Hg) with 70% oxygen and 30% nitrogen atmosphere. Following injection of antigen, animals were exposed to test conditions for one week, removed and titered. No animals were lost during the chamber exposure period and all remained in good physical condition. Titers at 8 days are shown in Table 12 for animals immunized with the 0 antigen preparation of S. aureus.

Table 12

	Ground Control		Altitude (100% O_2)		Altitude (70% O_2)	
Number	Pre	Post	Pre	Post	Pre	Post
3			1:0	1:5120		
			1:0	1:2560		
			1:10	1:2560		
5	1:10	1:5120				
	1:10	1:10,240				
	1:0	1:5120				
	1:0	1:20				
	1:0	1:5120				
2					1:0	1:20
					1:0	1:20

Following titer, animals were exsanguinated, and serum was collected and stored for use in mouse protection tests in future work. In the animals immunized the mean ground control titer was 1:5160; altitude (100%, 0_2) 1:3412; and altitude (70% 0_2) 1:20. The extremely low titers of the altitude (70% 0_2) animals was not expected, however, conclusions can not be drawn from this very small group of animals. The mean titers of the other two groups should be considered in association with the titers reported in Table 4 and the conclusion must follow that there is very little effect of this environment (altitude, 100% 0_2) on the production of the immune response to this antigen. When the mean of all titers in these two tests is calculated the ground control titer is 1:3400 and the altitude (100 0_2) titer is 1:2953.

The failure of two animals exposed to altitude (70% 0_2) to produce meaningful immune response to this antigen appears to warrant further investigation, however, time and funds on this contract did not permit this work.

D. Immune Response to S. california antigens.

Work was continued on the immunization of animals to the H antigen of S. california and to the nitrous acid antigen of S. california. Results of titers in these animals are shown in Tables 13 and 14.

It can be concluded from these results that animals in this environment produced higher titers to the H antigen preparation and much lower titers to the nitrous acid antigen preparation of S. california. Serum was collected from these animals for future use and aliquots were tested in an effort to determine the globulin class of antibodies produced. From limited numbers of tests involving both heat treatment and mercaptoethanol treatment, it appears that

Table 13. Antibody response of rabbits to S. california H antigen preparation.

Environment	Rabbit ID	Antibody titer at day 0	7	11	15	19	23	27
Altitude simulation	CF	Neg.	1:5120	1:2560	1:640	1:640	1:640	1:640
	DF	Neg.	1:1280	1:640	1:640	1:640	1:640	1:320
	GF	Neg.	1:1280	1:320	1:640	1:640	1:640	1:640
	HF	1:80			DIED			
	IF	1:80	1:2560	1:1280	1:2560	1:640	1:640	1:1280
	JF	Neg.			DIED			
	LF	Neg.			DIED			
	MF	Neg.	1:5120	1:1280	1:2560	1:5120	>1:10,240	>1:10,240
	NF	Neg.	1:640	1:1280	1:2560	1:5120	1:10,240	1:10,240
Ground control	AF	Neg.	1:1280	1:1280	1:640	1:640	1:1280	1:640
	BF	Neg.	1:1280	1:640	1:640	1:1280	1:640	1:640
	EF	1:80	1:1280	1:1280	1:320	1:160	1:160	1:160
	FF	Neg.	1:2560	1:1280	1:320	1:320	1:640	DIED
	KF	Neg.	1:1280	1:1280	1:2560	1:5120	1:10,240	>1:10,240

Table 14. Antibody response of rabbits to a nitrous acid treated preparation of S. california.

Environment	Rabbit ID	Antibody titer at day 0	7	11	15	19	23	27
Altitude simulation	AN	1:320	1:640	1:640	1:640	1:320	1:320	1:320
	BN	1:320	1:640	1:640	1:640	1:320	1:320	1:320
	CN	1:40	1:320	1:320	1:320	1:160	1:160	1:160
	IN				DIED			
	GN	1:20	1:1280	1:640	1:640	1:640	1:640	1:320
	HN	1:80	1:640	1:640	1:640	1:320	1:320	1:320
Ground control	DN	Neg.	1:640	1:320	1:160	1:160	*	1:80
	EN	1:320	1:640	1:640	1:320	1:320	1:160	1:160
	FN	1:320	1:640	1:640	1:640	1:640	1:320	1:320
	JN	1:80	1:640	1:640	1:640	1:320	1:320	1:320
	KN	1:40	1:2560	1:1280	1:2560	1:1280	1:640	1:640
	LN	1:20	1:1280	1:320	1:640	1:320	1:320	1:320

*Indicates no blood obtained.

there is a greater gamma M globulin antibody production under altitude simulation conditions than under ground control conditions.

Conclusions and Recommendations

I. The effect of a simulated spacecraft environment on the immune responses of rabbits varies with the type of antigen, and the method of administration of that antigen. Feeding of living S. california cells as a primary antigen stimulus provides little antibody response at either experimental or control environment. Production of antibody in the primary response to either formalin killed or nitrous acid killed S. california antigen, or heat killed S. aureus appears to develop at approximately equivalent rates at either experimental or control environments. Feeding or injection of living P. aeruginosa cells results in a low antibody titer, however, in both cases titers have been lower at altitude than at ground control conditions. There is a consistent inhibition of the primary immune response in rabbits at altitude when immunized with heat killed S. california antigen.

II. The presence of an apparently significant antibody titer does not prevent the development of more progressive and larger lesions in a subcutaneous infection with S. aureus. Such infection lesions are significantly larger in animals in simulated spacecraft environments than at ground control as seen in these studies.

III. When animals were infected with both S. aureus and P. aeruginosa by subcutaneous injection of 1×10^{10} living cells each, S. aureus lesions failed to develop in 5 of 6 animals at both experimental and control environments. P. aeruginosa lesions developed in 5 of 6 animals in these tests, but did not progress or spread.

IV. Combined feeding of living P. aeruginosa and terramycin in animals exposed to simulated spacecraft environments there is a general distortion of the total aerobic bacterial flora of the intestinal tract, as well as the relative numbers of major segments.

These changes were not noted when P. aeruginosa alone was fed. It appears that there is a possibility that this bacterial flora distortion may be involved in the somewhat increased death rate when the combined treatment is given or when terramycin alone is given in the experimental environment.

V. Ampicillin appears to be very toxic to rabbits under all test conditions used in this study. In results reported here, it appears that treatment with these doses of ampicillin results in an increase in the anaerobic bacterial flora under all conditions and also appears to lead to a selective increase in the numbers of Clostridium sp. in this flora.

From results reported in this contract period it appears that the areas which show most promise for future studies are: (1) testing the protective capacity of immunity to bacterial pathogens in animals exposed to simulated spacecraft environments, (2) testing the immune response production and the protective capacity of immunity at altitude, 70% O_2, 30% N_2 simulation, and (3) complete testing to determine gamma G and gamma M globulin production at altitude simulation.

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