NASA CR 105586

A-3061A(AS-1)

Former designations: ~~L-97,464~~
R-21-010-010

Quarterly Status Report No. 16 to

National Aeronautics and Space Administration

1 April - 30 June 1969

"Effects of High and Low Barometric Pressures on Susceptibility and Resistance to Infection"

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Abstract.

1. Three experiments in which mice were subjected to simulated space cabin atmospheres (70% O_2 in N_2, 5 psia) before or after exposure to aerosol challenge with influenza virus did not confirm the impression obtained from an earlier experiment (PR8-10), i.e., that the 70% O_2 induced a more severe disease. The last 3 experiments gave evidence for a less severe infection in the simulated space cabin environment. A comparison of the evolution of the infection in the experiments with discrepant results shows a difference, and suggests that an unrecognized factor may be responsible, rather than random chance.

2. Groups of hypoxic mice (air, 7.3 psia) were included in the experiments mentioned above, and additional evidence was obtained for protection against influenzal infection by this environmental condition.

3. In a single experiment influenza-infected mice exposed to hyperoxia (77% O_2, 1 atm) had a more severe infection than did the controls in air.

4. By careful attention to standardization of all possible controllable factors in an experiment with A/HeJ mice in which pulmonary adenomas were induced by intravenous inoculation of dibenz-anthracene, a much better within-group range of variability has been achieved. There was a 30% greater incidence of tumors in the mice exposed to 100% O_2 for 48 hours, a result in the direction of that reported by Heston, but not significant in itself ($0.1 > P > 0.05$).

5. Improved methods for titration of Coxsackie virus (B-1) have been learned, and an experiment was performed in which adult mice were exposed to hyperoxia (77% O_2, 1 atm), hypoxia (11% O_2, 1 atm), and normoxia (controls in air, 1 atm) following intraperitoneal inoculation of Coxsackie virus. As expected in adult mice, no deaths occurred in the controls. This was also true of the hyperoxic group, but 3 of 10 mice died in the hypoxic group. Weights of pancreas tissue were much less in sacrificed hypoxic mice and titers of virus in pancreas were approximately 1 log unit greater than in the other two groups. The results suggest that hypoxia enhances Coxsackie virus infection.

6. Newly acquired hyperbaric chambers, and an improvement in control of pressure in hypobaric chambers, are briefly described.

1. Effect of parabarosis on pulmonary infection of mice with influenza virus.

In QSR #15 an experiment (PR8-10) was reported in which influenza infected mice kept in a simulated space cabin atmosphere (70% O_2 in N_2, 5 psia) had a somewhat greater mortality rate than controls. We tended to regard the difference in mortality rates (50% & 30%) as significant mainly because of their similarity to results obtained in earlier experiments in which mice were kept in an environment with an even greater ratio of O_2 (100% O_2, 3.3 psia). Repeated experiments have shown the latter environment to be responsible for significantly greater mortality rates in influenza-infected and in mouse pneumonitis-infected mice.

It now appears that the general conclusion drawn from PR8-10 may have been incorrect. Additional experiments of the same design have given results suggesting that influenza-infected mice exposed to 70% O_2 in N_2 at 5 psia can have a somewhat lower mortality rate than do the controls. In 2 instances (PR8-14 and PR8-16) exposure to the altered environment took place only after viral challenge, and in one case (PR8-12) only before. In all 3 instances the mortality rates of the test mice were less than those of the controls. These experiments are described briefly below. They also show again the protective effect of an hypoxic environment against influenzal infection.

PR8-14. Mice were exposed to influenza virus by aerosol challenge and 2 groups of 12 mice each were placed in each of the 3 environments: 70% O_2 in N_2, 5 psia (simulated space cabin); air, 7.3 psia; air at one atmosphere. Altered parabaric conditions were limited to post-challenge exposure only. Lung pools for determination of infectivity titers were prepared as before on mice of one group in each environment sacrificed on the 4th, 5th, 6th, and 7th day post-challenge.

In this experiment (Figure 1), a 50% mortality rate was observed in the one atmosphere control animals; deaths occurred between the 11th and 14th day. In the simulated space cabin atmosphere, deaths began earlier (7th-9th day), but then remained unchanged through the 19th day to give a total mortality of only 20%. As depicted in Figure 1, the first death in the hypoxic mice was not observed until the 10th day post-challenge and increased to 30% mortality by the 14th day.

Inspection of Table 1, where data on the sacrificed mice can be compared, shows that the simulated space cabin group had essentially similar lung weights and infectivity titers as the controls, with the highest titers on days 4 and 5, and the development of consolidation lagging by 1 or 2 days. Estimates of extent of gross pathology (consolidation) were only slightly greater for the test group. The same table shows data for the hypoxic group of mice (air at 7.3 psia) also to be similar to that for the controls, except for a consistent slightly lower average degree of consolidation in the test group.

PR8-16. This was identical in design to PR8-14. As shown in Figure 2, the first deaths occurred in the control mice on the 8th day post-challenge and a maximum mortality of 78% was observed on the 13th day. The first mortality in the simulated space cabin atmosphere (70% O_2 in N_2, 5 psia) occurred on the 10th day and maximal

mortality was only 40% by the 14th day. The hypoxic group again showed a mortality curve less severe than that of the controls; there was a final 56% mortality in mice in the hypoxic environment.

The data on lung weights, gross pathology scores and infectivity titers are presented in Table 2. It may be noted that the highest titers are again found on the first two days of sacrifice as in PR8-14, with development of lung consolidation occurring later than the peak viral titer. Inspection of gross pathology scores suggests that the lower mortality rates in the 2 test groups are reflected in somewhat lower levels of lung involvement in these groups when compared with the controls.

PR8-12. This was designed similarly to the 2 experiments (PR8-14 and PR8-16) described above, except that the exposure to altered environments took place over a 2-week period before viral challenge only. Figure 3 depicts the 50% mortality observed in control mice, absence of mortality in hypoxic mice, and only a 10% mortality observed in the simulated space cabin atmosphere. The data on sacrificed mice (Table 3) show again only a slight indication of less severe infection in the test groups than in the controls.

A partial explanation for the discrepancy noted earlier between the results of PR8-10 (QSR #15) and the 3 experiments just described may be found in the patterns of viral growth as revealed by titrations of lung pools of sacrificed mice. In the last 3 experiments the titers were high on days 4 and 5 and dropped approximately one log unit on day 6 or 7. In contrast, the observations made in PR8-10 (QSR #15, Table 3) indicate that the level of virus was rising over days 4-7. The gross pathology scores also indicate a slower evolution of the disease. Thus it appears that there was a somewhat different pattern of pathogenesis between the experiments that show discrepant results. Why there should be this difference in evolution of the infection (survival periods and final mortalities in control groups are all similar), and a difference in the effect of the special parabaric condition, is yet unknown.

Effect of hyperoxia. In PR8-13 the experimental groups of mice were limited to post-challenge exposure only to hyperoxic-normobaric (77% O_2 in N_2, 1 atm), hypoxic-normobaric (11% O_2, 1 atm) and control (air, 1 atm) environments. The observed mortality rate was 90% in the hyperoxic group as compared with only 8% in the controls, and there was a complete absence of mortality in mice under the hypoxic environment. These results are presented in Figure 4.

The increased mortality of influenza-infected mice in 77% O_2 is of interest, and a repeat of this experiment is planned. In a sense the result is compatible with the decreased mortality in influenza-infected mice, induced by exposure to hypoxia. Observations on gross pathology and viral titer (Table 4) both show moderate but consistent evidence of a more severe disease in the mice exposed to hyperoxia.

The zero mortality observed in mice exposed to 11% O_2 in N_2, 1 atm., in PR8-13 is only slightly decreased from that of the controls (8%; 1 mouse) and is not significant by itself. This portion of the experiment, too, will be repeated.

2. Effect of parabarosis on induction of pulmonary tumors in A/HeJ strain mice following i. v. challenge with chemical carcinogens.

Difficulty was encountered in the past in obtaining consistent results with a reasonable in-group variation in this type of experiment. The protocol for the presently reported cancer experiment (#6) was designed to control more vigorously several factors possibly responsible for the large standard errors experienced within the various parabaric groups as reported in QSR #15, 1 Jan - 31 Mar 1969. The experimental groups consisted of equal-weight ranges of 23 or 24 mice exposed to each parabaric condition following i. v. challenge with 0.1 mg of dibenz-anthracene. All syringes were filled by one individual, the colloidal suspension maintained in continuous motion by a second individual until challenge by a third who performed all i. v. injections. The parabaric conditions were designed to include two weeks exposure to 2.8% O_2 in He (hyperbaric-normoxic), tank air, 1 atm (normobaric-normoxic) controls and 100% O_2 at 1 atm for 48 hours (Heston control). Unfortunately, the group (A) of mice in the $He-O_2$ atmosphere were lost due to pressure failure and only the normoxic controls (E) and Heston (100% O_2) controls (F) remained for evaluation. The 2 remaining groups of mice were returned to the shelf and sacrificed at the end of 4 months. The lungs were examined for tumor incidence. The experiment is summarized in Table 5.

In this experiment, the individual distribution of tumors encountered was very uniform in both experimental groups. Numbers of tumor nodules in each animal in the air control group varied from 0 to 11 with a mean of 3.54 and varied from 1 to 11 in the Heston 100% O_2 controls with a mean of 5.13. The validity of the difference in tumor incidence, as measured by application of Students' t test, gives a P value of $0.10 > P > 0.05$ which is slightly higher than the 5% level of confidence, and not to be considered significant by itself.

Additional A/HeJ strain mice have been injected with carcinogen, exposed to simulated space cabin atmosphere for two weeks (70% O_2 in N_2, 5 psia) with appropriate 1 atm air and Heston control groups for determination of resulting tumor incidence at the end of the four month holding interval.

3. Experiments with Coxsackie virus.

Following the initial experiments with Coxsackie B-1 virus reported in QSR #14, 1 Oct - 31 Dec 1968, suitable methods of viral titration in the MK-2 cell line have been developed. Both tube dilution and plaque assay methods are available. The 10% suckling mouse tissue pool A, prepared in experiment Coxsackie #1, gave values of $10^{6.25}$ ID_{50} per g by tube dilution and 9.5×10^6 PFU per g by the plaque assay method.

An experiment (Coxsackie #9) was designed to measure mortality and viral content of pancreas tissue in parallel groups of adult mice exposed to either 77% O_2 at 1 atm, 11% O_2 at 1 atm, or line air at 1 atm (controls) for periods up to 16 days following i.p. challenge with 0.25 ml of 1:500 dilution of pool A (equivalent to 2500 LD_{50} suckling

mouse doses).

Results of observations on mortality, weights of pancreatic pools at 3 intervals of sacrifice, and corresponding viral titrations by plaque assay are presented in Table 6.

Total weights of pancreatic pools (5 mice each) were found to be slightly less at sacrifice in hypoxic mice at the 5th day post-challenge period than in either 77% O_2, 1 atm, or line air 1 atm control groups. At the 7th and 16th day, post-challenge intervals, the pancreatic pool weights of mice exposed to the hypoxic environment were only about one-half of either hyperoxic or control pancreatic pools.

The only observed mortalities occurred in the experimental 1 atm hypoxic mice. The initial death occurred on day 9, post-challenge exposure, and an estimated plaque assay titer of 3×10^6 per g was obtained for the single pancreas available. No demonstrable virus could be found in the lowest dilution tested with either heart or spleen suspension prepared from the same mouse. Both the second and third deaths occurred overnight at day 12, post-challenge, and organ tissue was not satisfactory for plaque assays.

As indicated in Table 5, the highest plaque titers were obtained on the 5th day of sacrifice. A uniform decrease of at least two log titer was demonstrated for pancreatic pools from each parabaric group at day 7 of sacrifice and virus could not be demonstrated by plaque assay at day 16 sacrifice, post-challenge. While titers for both hyperoxic and control groups were found to be very comparable at both day 5 and 7, post-challenge sacrifice, the corresponding titers for the hypoxic groups were quite uniformly 1 log higher at both intervals. Since the observed deaths occurred when pancreatic viral titers and pancreatic weights were decreasing, pancreatic pools prepared at earlier intervals of sacrifice may show higher initial involvement following challenge of animals stressed under parabaric conditions. Histopathologic examination of pancreatic tissue at the various intervals following challenge and exposure to altered atmosphere may help clarify the reasons for mortality and increased viral production in the adult mice subjected to hypoxia.

4. New equipment.

All three large hyperbaric chambers, on order for the past year, have finally been delivered. To permit planning of long-term uninterrupted exposure experiments, two of the chambers were leveled and exactly aligned so that they could be joined together by means of a 6" helium tight ball valve with 180° turning radius. A one-way clear lucite gate has been installed within the ball valve so that experimental animals can be trained to enter the opposite chamber on signal and not be able to return. Work is underway to familiarize personnel with proper methods for compression cycles without hyperoxia and decompression schedules without hypoxia or decompression damage. Plans for design and construction of similar chambers to serve for holding control animals under identical physical environments but in normal atmospheres are underway. The latter will be designed to allow their use also for simulated altitude.

The control systems on plexiglass chambers utilized for simulated altitude studies with altered gaseous environments, (i.e., 100% O_2, 3.3 psia; 70% O_2 in N_2, 5 psia; and air at 7.3 psia) have recently been modified by the addition of cartesian manostats (Manostat Corp., New York, N.Y.) between the chambers and hi-vac pump. With control of the vacuum regulator on the gas supply, flow rate on the gas flow meter and accurate vacuum setting on the manostat, we now have a much safer and stable system for controlling altitudes within a few mm of mercury.

Table 1. Experiment PR8-14. Effect of parabaric conditions on mouse lung infection following aerosol challenge with PR8 influenza virus.

Environment	Day of sacrifice post-challenge	No. of mice	Observations on sacrificed mice: Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol. [a]	Infectivity titer of lung (EID_{50};Kärber) [b]
Group B 70% O_2 in N_2 5 psia	4	3	15.8	0.17	1.0 (1.5, 1, 0.5)	$10^{7.6}$
	5	3	15.2	0.19	0.6 (1, 0.5, 0.3)	$10^{7.0}$
	6	3	17.3	0.20	0.4 (0.3, 0.3, 0.6)	$10^{6.9}$
	7	3	14.8	0.28	2.3 (3.5, 2, 1)	$10^{6.7}$
Group C Air, 7.3 psia	4	3	12.2	0.17	0.1 (0.3, 0, 0)	$10^{7.0}$
	5	3	15.2	0.18	0.1 (0.3, 0, 0)	$10^{7.8}$
	6	3	12.0	0.18	0.2 (0.3, 0.3, 0)	$10^{6.8}$
	7	3	15.8	0.24	1.0 (1.5, 1, 0.5)	$10^{6.9}$
Group D Tank air, Control, 1 atm	4	3	14.5	0.20	0.2 (0.3, 0.3, 0)	$10^{7.7}$
	5	3	17.5	0.19	0.4 (0.6, 0.3, 0.3)	$10^{7.3}$
	6	3	17.0	0.21	0.6 (1, 0.3, 0.5)	$10^{6.7}$
	7	3	14.7	0.24	1.5 (2, 2, 0.5)	$10^{6.1}$

[a] Arbitrary 0-5 scoring for degree of lung involvement; average and (individual) scores.

[b] Expressed as 50% egg infective dose per 0.1 ml inoculum. (Kärber)

Aerosol exposure: 20 minutes using 1:100 dilution of PR8, pool A, at 0.28 ml/min, RH 83%.

Table 2. Experiment PR8-16. Effect of parabaric conditions on mouse lung infection following aerosol challenge with PR8 influenza virus.

Environment	Day of sacrifice post-challenge	No. of mice	Observations on sacrificed mice: Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol.[a]	Infectivity titer of lung (EID_{50};Kärber)[b]
Group B 70% O_2 in N_2 5 psia	4	3	14.8	0.19	0.0 (0, 0, 0)	$10^{7.7}$
	5	3	15.0	0.19	0.1 (0, 0, 0.3)	$10^{7.6}$
	6	3	16.6	0.23	0.6 (1, 0.5, 0.3)	$10^{6.6}$
	7	3	15.3	0.31	0.7 (1, 0.5, 0.6)	$10^{5.9}$
Group C Tank air, 7.3 psia	4	3	13.0	0.18	0.2 (0.3, 0.3, 0)	$10^{7.7}$
	5	3	15.0	0.19	0.1 (0.2, 0.1, 0)	$10^{7.3}$
	6	3	13.8	0.21	0.2 (0.3, 0.2, 0.1)	$10^{6.9}$
	7	3	12.8	0.22	1.1 (2, 1, 0.3)	$10^{6.1}$
Group D Tank air, Control, 1 atm	4	3	14.8	0.17	0.4 (1, 0.2, 0)	$10^{7.4}$
	5	3	16.2	0.22	0.3 (0.5, 0.3, 0.1)	$10^{6.7}$
	6	3	14.3	0.31	1.7 (2.5, 2, 0.6)	$10^{6.8}$
	7	3	13.3	0.25	2.0 (2.5, 2.2, 1.3)	$10^{5.8}$

[a] Arbitrary 0-5 scoring for degree of lung involvement; average and (individual) scores.

[b] Expressed as 50% egg infective dose per 0.1 ml inoculum. (Kärber)

Aerosol exposure: 20 minutes using 1:100 dilution of PR8, pool A, at 0.30 ml/min, RH 88%.

Table 3. Experiment PR8-12. Effect of pre-exposure to altered atmospheres on mouse lung infection following aerosol challenge with PR8 influenza virus.

Environment	Day of sacrifice post-challenge	No. of mice	Observations on sacrificed mice: Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol. [a]	Infectivity titer of lung (EID_{50};Kärber) [b]
Group B 70% O_2 in N_2 5 psia	4	3	23.0	0.21	0.2 (0, 0.3, 0.3)	$10^{7.0}$
	5	3	19.0	0.22	0.2 (0, 0, 0.6)	$10^{7.6}$
	6	3	17.7	0.23	1.1 (2, 1, 0.3)	$10^{6.5}$
	7	3	19.7	0.38	2.0 (2.5, 2, 1.5)	$10^{5.8}$
Group C Tank air, 7.3 psia	4	3	19.5	0.21	0.1 (0, 0, 0.3)	$10^{7.3}$
	5	3	21.5	0.23	0.1 (0, 0, 0.3)	$10^{6.3}$
	6	3	22.5	0.30	0.6 (1, 0.5, 0.3)	$10^{6.9}$
	7	3	20.3	0.32	1.3 (1.5, 1.5, 1)	$10^{5.7}$
Group D Tank air, Control, 1 atm	4	3	18.3	0.19	0.1 (0, 0, 0.3)	$10^{7.3}$
	5	3	21.7	0.20	0.3 (0.3, 0.3, 0.3)	$10^{7.3}$
	6	3	19.5	0.27	1.2 (1.5, 1, 1)	$10^{6.4}$
	7	3	17.5	0.28	2.2 (3, 2.5, 1)	$10^{6.3}$

[a] Arbitrary 0-5 scoring for degree of lung involvement; average and (individual) scores.

[b] Expressed as 50% egg infective dose per 0.1 ml inoculum. (Kärber)

Aerosol exposure: 20 minutes using 1:100 dilution of PR8, pool A, at 0.27 ml/min, RH 83%.

Table 4. Experiment PR8-13. Effect of parabaric conditions on mouse lung infection following aerosol challenge with PR8 influenza virus.

Environment	Day of sacrifice post-challenge	No. of mice	Observations on sacrificed mice: Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol.[a]	Infectivity titer of lung (EID_{50};Kärber)[b]
Group B 77% O_2 1 atm	4	3	18.8	0.18	0.2 (0, 0.3, 0.3)	$10^{7.6}$
	5	3	18.7	0.21	1.5 (2, 1.5, 1)	$10^{7.5}$
	6	3	15.2	0.22	1.5 (2, 1.5, 1)	$10^{7.3}$
	7	3	16.3	0.29	3.0 (3, 3, 3)	$10^{6.9}$
Group C 11% O_2 1 atm	4	3	16.2	0.18	0.1 (0, 0, 0.3)	$10^{6.8}$
	5	3	16.2	0.19	0.1 (0, 0, 0.3)	$10^{7.3}$
	6	3	16.8	0.23	0.7 (1, 0.6, 0.6)	$10^{6.5}$
	7	3	14.8	0.23	1.3 (1.5, 1.5, 1)	$10^{6.5}$
Group D Air control 1 atm	4	3	19.2	0.20	0.1 (0, 0, 0.3)	$10^{7.3}$
	5	3	19.2	0.21	0.3 (0.3, 0.3, 0.3)	$10^{6.9}$
	6	3	16.0	0.20	1.0 (1.5, 1, 0.5)	$10^{6.7}$
	7	3	16.5	0.23	1.0 (1.5, 1, 0.5)	$10^{6.3}$

[a] Arbitrary 0-5 scoring for degree of lung involvement; average and (individual) scores.

[b] Expressed as 50% egg infective dose per 0.1 ml inoculum. (Kärber)

Aerosol exposure: 20 minutes using 1:100 dilution of PR8, pool A, at 0.31 ml/min, RH 87.5%.

Table 5. (Cancer Exp. 6) Effect of parabarosis on chemical induction of pulmonary tumors in A/HeJ mice.

Group	No. mice	Av. wt.	Parabaric environment	Av. no. tumor nodules at 4 mos. ± SE
A	24	20 g	2.8% O_2 in He, 95 psig, 2 wks	(lost due to tank leak)
E	24	20 g	Tank air, 1 atm, 2 weeks (normal controls)	3.54 ± 0.57
F	23	20 g	100% O_2, 1 atm, 48 hrs (Heston controls)	5.13 ± 0.67

Table 6. (Coxsackie Exp. #9) Effect of parabarosis on susceptibility of adult mice to Coxsackie B-1 infection; parabaric exposure followed challenge.

Group and environment (1 atm pressure)	Mortality	Observations on sacrificed mice		
		Day of sacrifice[a]	Wt. (g) of pancreatic pool	Viral titer of pancreatic pool PFU/g x 10^3
B; 77% O_2 in N_2, (hyperoxia)	0/10	5	0.56	300
		7	0.40	2
		16	0.40	0.5
C, 11% O_2 in N_2, (hypoxia)	3/10[b]	5	0.45	2900
		7	0.25	19
		16	0.27	< 0.5
D, Line air (normoxia)	0/10	5	0.56	280
		7	0.50	3
		16	0.65	< 0.5

[a] Five mice sacrificed for each pancreatic pool.

[b] First death in Group C occurred on 9th day, post-challenge. Plaque assay of pancreas from first mouse dying, estimated at 30 x 10^5 PFU/g. Heart & spleen titer, <0.5 x 10^3. Second and third deaths both occurred on day 12.

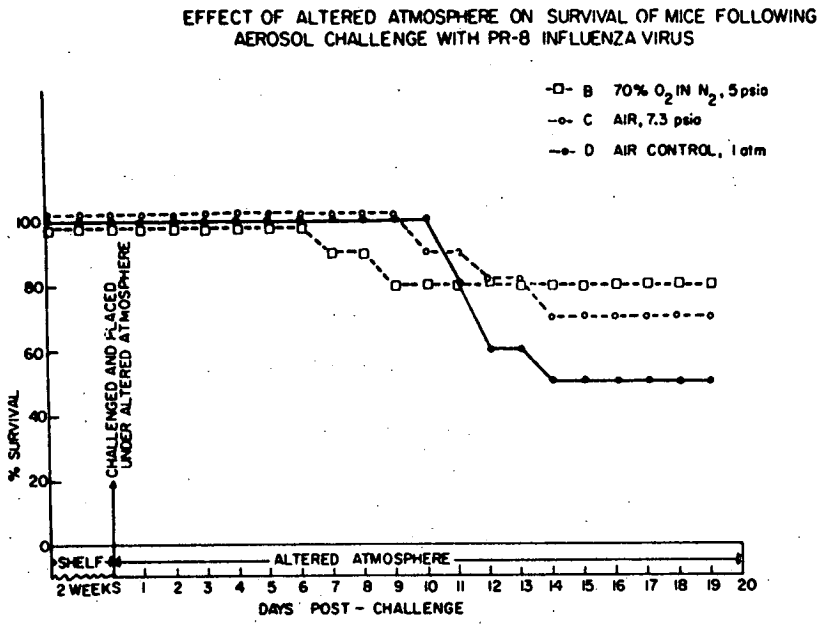

Figure 1. Experiment PR8-14

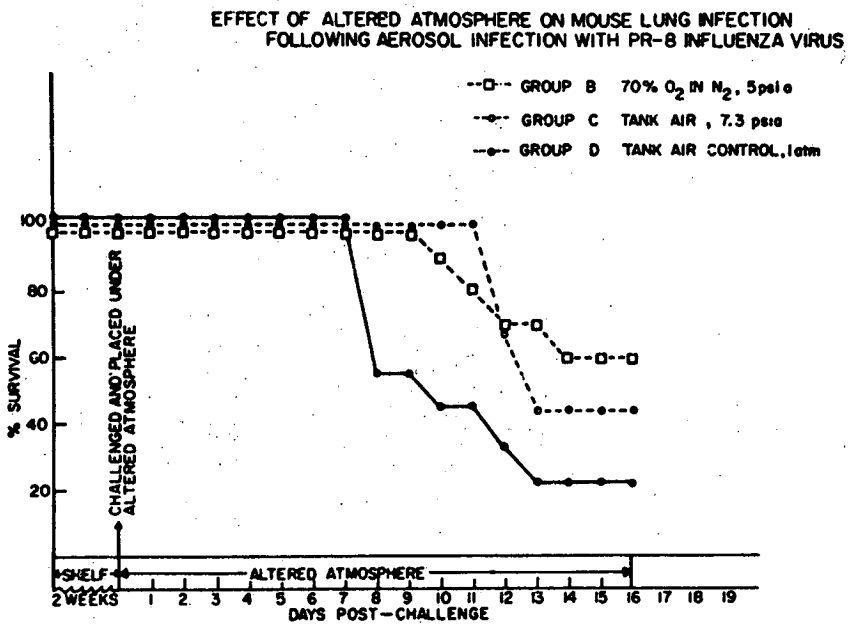

Figure 2. Experiment PR8-16

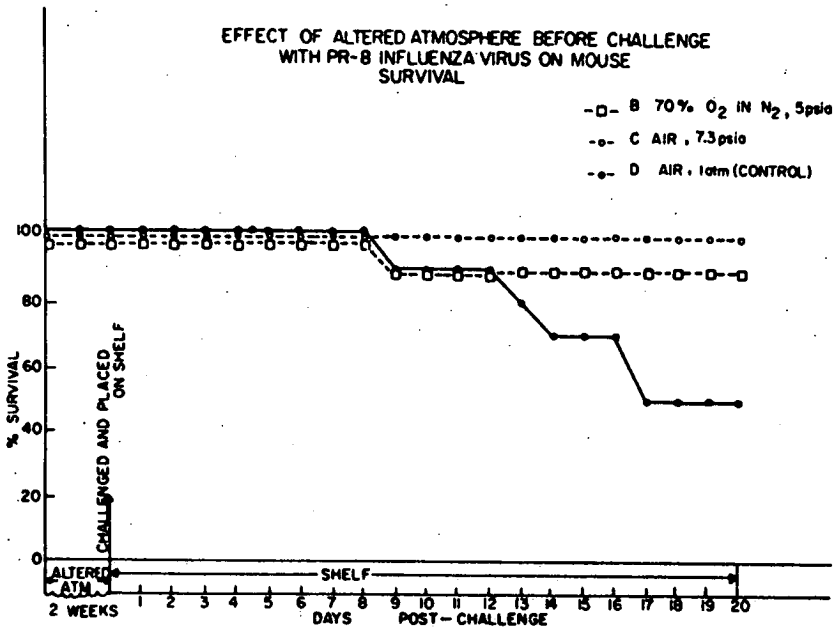

Figure 3. Experiment PR8-12

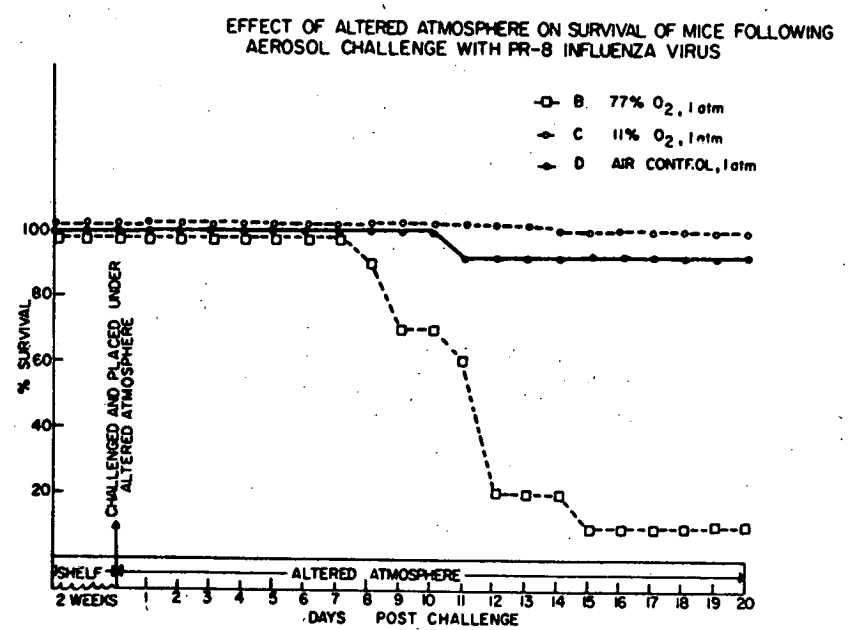

Figure 4. Experiment PR8-13