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"Effects of High and Low Barometric Pressures on

Susceptibility and Resistance to Infection"

Francis B. Gordon and James D. Gillmore
Department of Microbiology
Naval Medical Research Institute
Bethesda, Maryland 20014

Abstract.

1. An additional experiment has been completed in which observations were made at 2 week intervals on mice being maintained under simulated "space cabin" atmosphere (70% O_2 in N_2, 5 psia), hypoxia (air, 7.3 psia), or air at ambient pressure. An increased incidence (> 1-5 logs) of various types of bacteria was seen in both parabaric groups. The increase was in same types of bacteria seen in either pressure groups under O_2 in He or O_2 in N_2 or at simulated altitude of 37,000 ft (100% O_2, 3.2 psia). The incidence of the various types returned to normal levels when mice were put back to air at ambient pressure in the same chambers following 6 weeks parabaric exposure.

2. In an additional experiment the earlier onset and increased mortality observed in hypoxic mice (77% O_2, 1 atm) following challenge with influenza virus (PR8) was confirmed. Differences between hypoxic and control lung infectivity titers were small and mortality did not occur in mice exposed to a diluent control aerosol (0.5 BPA in PBS) and maintained under the same atmosphere.

   Exposure to hyperbaric-normoxic environments (2.8% O_2 in He, 95 psig) following influenzal aerosol challenge increased the mortality and differences in degree of lung consolidation between test and control groups were in agreement. Titers of lung tissue, however, were nearly the same, showing a possibly significant difference (0.5 - 1.0 unit) in only the later stages of lung involvement.

3. The effect of hypoxia following intraperitoneal challenge with Coxsackie virus was investigated in mice exposed to simulated 18,000 ft altitude (air, 7.3 psia) for comparison with previous alterations observed in 11% O_2 at 1 atm. Enhancement of Coxsackie virus infection due to hypoxia was demonstrated by an almost 2 log virus titer increase over ambient air control mice. Pancreatic tissue for histopathologic examination, however, showed only normal pathologic changes associated with this type infective agent in both hypoxic and control groups.

1. Effect of parabarosis on enteric bacterial flora of mice.

The effect of exposure to either hyper- or hypobaric atmospheres with essentially normal pO_2 has been described in several previous QSR's. (Nos. 11, 12, 13, and 14, 1 Jan. through 31 Dec. 1968.) Altered environments in all the experiments cited were limited to 2.8% O_2 in He (95 psig), and 100% O_2 (3.2 psia), and were compared with control groups in air at normal pressure. In experiment 20, described below, the effect of two additional types of parabarosis is reported, i.e., hypoxia, and a simulated space cabin atmosphere.

Exp. Enteric flora #20. Experimental groups of 10 mice each were exposed in 30 L, Plexiglass chambers to 70% O_2 in N_2, 5 psia (space cabin atmosphere), air at 7.3 psia (hypoxic atmosphere) and flowing air at 1 atm (controls). Fecal samples were taken for culture at 2-week intervals before and after exposure to altered atmosphere. Manostat controls installed in the system represented an improvement in the equipment and allowed precise continuous exposure to the 5 psia and 7.3 psia environments for three samplings covering six weeks of parabarosis. At the end of this time the mice were all placed under normobaric conditions (flowing air at one atmosphere of pressure) in the same chamber. Two additional samplings were obtained at two-week intervals from the experimental animals in order to differentiate between any effect of chamber residence only from that of the parabaric atmospheres. In this experiment the alterations in concentrations of the principal aerobic microflora present were found to be similar or even more pronounced than those observed under essentially normoxic hyper- or hypobaric conditions presented previously.

The numbers of slow lactose fermenting organisms (SLF) increased sharply in both groups of mice exposed to either space cabin or hypoxic environments in comparison to the 1 atm air controls. At +4 and +6 weeks sampling intervals, mean values for SLF in the mice maintained under the simulated space cabin atmosphere were found to be one to two logs higher than corresponding 1 atm control animals. The difference noted at the end of 6 weeks exposure to 70% O_2 in N_2 at 5 psia declined rapidly on return to flowing air at 1 atm in the same chamber and reached a concentration almost identical with that of control mice at the 10-week sampling interval.

Similar experimental groups maintained in flowing air at 7.3 psia (hypoxic) showed an earlier and higher increase in SLF organisms (1 to 3 logs) but also began to decrease following the +4 weeks sampling interval, and exhibited almost identical concentrations as controls at both +8 and +10 week intervals following return to ambient pressure. Complete data are presented in Fig. 1. Atypical fecal coli, always associated with SLF organisms, exhibited a very similar trend at all sampling intervals during parabaric exposure and also quickly reverted to concentrations similar to those of control animals when returned to ambient chamber atmosphere.

In this experiment, both the appearance and observed concentrations of Klebsiella-Aerogenes organisms were extremely pronounced and sharply defined. Initial concentrations were found to be less than 10^4/gm at -2 weeks exposure to altered atmosphere and none was found in the lowest dilution plated (0.1 ml of 10^{-3} dilution) at week 0, the start of parabaric exposure. While detectable numbers were found in air controls after both 2 and 4 weeks of chamber exposure, but not at 6, 8, or 10 weeks, both groups of experimental animals, exposed to either 5 psia (70% O_2) or 7.3 psia (air), showed a 4 to 5 log increase in this type at every sampling interval during parabaric exposure (Fig. 2). Following return to ambient air pressure in the same chamber, numbers of Klebsiella-Aerogenes in mice exposed to "space cabin" atmosphere decreased by almost 3 logs, the incidence in hypoxic mice was below detectable limits, and after 4 weeks at ambient chamber air, none was detected in any group.

It is of interest to note that although detectable numbers of typical E. coli were not found in any experimental group at any sampling interval before, during parabaric exposure and for the first two weeks after return to ambient pressure, all mice in the hypoxic group after 4 weeks at ambient pressure were shown to harbor readily detectable numbers of typical E. coli. Values ranged from 10^4 to 10^6 for each animal with a mean of 10^5. At the same time, the hypoxic mice were shown to have an earlier and more rapid decline in atypical E. coli, SLF and Klebsiella-Aerogenes members than either control or "space cabin" mice in return to ambient atmosphere. This appears to be an excellent example of the type of population dynamics described by Dubos, et al,* in their explanation of "indigenous microbiota" which include both the symbiotic and potentially pathogenic microorganisms. Dubos, et al, have good reason to believe that changes in indigenous microbiota can affect favorably or unfavorably, the nutritional state of their host and its resistance to infection.

The group D fecal streptococci (enterococci) in experiment No. 20 were also found to increase by order of 3-4 logs magnitude by the 4th week under both space cabin and hypoxic atmospheres. Initial values were elevated at the -2 week interval before the start of parabarosis and declined steadily until after the second week of parabaric exposure (Fig. 3). Following this, values increased sharply and remained elevated until return to ambient chamber air. Concentrations in hypoxic mice reached control levels at 8 weeks, and at 10 weeks in the space cabin (5 psia) mice.

It is not surprising that the hypoxic condition employed represents a type of stress reflected in alterations in fecal flora. More important, the conclusion seems justified that exposure to 70% O_2 in N_2 at 5 psia (simulated space cabin environment) in our experiments has a similar effect on the fecal flora of mice. As noted above, this result was also shown in our previously used condition of simulated altitude (100% O_2 at 3.2 psia).

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

PR8-17 was designed to investigate the effect of post-challenge exposure to hyperoxia (77% O_2, 1 atm) on aerosol infected mice. In addition to the usual infected control groups

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* Dubos, R., R. W. Schaedler, R. Costello, and P. Holt. Indigenous, normal and autochthonous flora. J. Exp. Med. 122:67, 1965.

maintained in air at 1 atm, a non-infected control group was included in this protocol. Twenty-two additional mice from the same experimental group were exposed for the same time to an aerosol of virus diluent only [0.5% bovine plasma albumin (BPA) in phosphate buffered saline (PBS)]. Lung pools from each experimental group were prepared on post-challenge days 6 and 8 and two additional pools were made at 16 days post-challenge on diluent control mice only.

Figure 4 depicts the rapid 100% mortality rate observed between the 6th and 8th day post-challenge exposure to 77% O_2. The first deaths in control mice at 1 atm began one day later on day 7 and did not reach a maximum (70%) until day 15 post-challenge. No mortality or gross evidence of illness was observed in mice exposed to the diluent control aerosol. The earlier onset of infection and increased mortality confirms the results of PR8-13 reported in QSR No. 16, 1 April - 30 June 1969. The average mouse and lung weights, along with gross pathology scores and lung infectivity titers as determined by EID_{50} titration, are presented in Table 1.

A distinct decrease in average mouse weight between day 6 and 8 may be noted in both hyperoxic and 1 atm air control mice as compared with the diluent control mice. Average lung weight and gross pathology score increased in a parallel manner in both infected groups, but did not do so in the aerosol diluent control mice.

The 6-day lung infectivity titers in both hyperoxic and control mice were very similar and while the 8th day infectivity titer decreased more in the hyperoxic mice it could possibly be due to the fact that only 2 mice remained for sacrifice at this interval. Of the 6 mice sacrificed in the diluent control groups on day 6 post-challenge, only one had a slight degree of consolidation. This particular pool gave a low infectivity titer ($10^{2.6}$) as determined by standard hemagglutination procedure on allantoic fluid from inoculated fertile eggs. No detectable titer was present in the 8-day pool prepared from 6 mice; all with lungs of normal appearance. At day 16 post-challenge 10 mice were sacrificed in the diluent control group. One pool was prepared from 7 mice with normal gross lung appearance and a separate pool prepared from lungs of 3 mice having small areas of visible consolidations. Lung infectivity titers could not be demonstrated in either pool.

PR8-18. Because of the evidence for low infectivity in the lung of one diluent control mouse sacrificed on day 6 in PR8-17, experiment PR8-18 was designed to determine whether lung pools prepared from the NMRI albino mouse colony would induce a hemagglutinating response following allantoic inoculation of fertile chick embryos and whether lung pools from similar mice aerosol challenged with virus diluent only might also induce a similar response at various intervals of sacrifice.

The primary lung pool was prepared from 10 random mice of 40 as delivered directly from the animal house. The thirty remaining mice were all exposed for 20 minutes to an aerosol of 0.5% BPA in PBS at a rate of 0.34 ml/min and R.H. of 81%. At 4, 7, and 9-day intervals following challenge, 10 mice were sacrificed for preparation of lung pools. At each interval 2 mice were found to have slight hemorrhagic lung areas, not resembling the usual consolidation observed following challenge with PR8 influenza virus. Separate lung

pools were prepared from the abnormal lungs.

Infectivity tests of the primary and all 6 post-aerosol challenge pools (uninfected mice) did not yield any evidence of a hemagglutinating agent. Although evidence was obtained about 5 years ago, that the NMRI mouse colony does have some latent Sendai viral infection, a HA response from lung pools of mice this age (6-8 weeks) due to this agent seems remote under normal conditions. The possibility must be borne in mind, however, that under the various conditions of physical and physiological stress imposed by the parabaric conditions utilized in our experiments, we may activate, accentuate, or alter the usual host response to the particular infective agent. Identification of the HA agent recovered from one diluent control mouse in PR8-17 remains to be done.

PR8-19. This was designed to investigate the effect of post-challenge exposure to 2.8% O_2 in He at 95 psig, a condition used earlier, but in chambers in which the environmental temperature could not be satisfactorily controlled. Four groups of 12 mice each were employed; two were exposed to infective virus aerosol and two were exposed to diluent control. Immediately following challenge the mice were placed in the new 150 L hyperbaric chambers, one with 2.8% O_2 in He at 95 psig, and one with air at 1 atmosphere pressure. Chamber temperatures, both 95 psig and 1 atm, were maintained at 28 C, while chamber gas was continuously recycled through Baralyme absorbent. A 1.5 L/min replacement flow was provided through chamber to oxygen analyzer to monitor O_2 concentration. At 6 and 8-day post-challenge intervals chambers were opened (40-45 min required for 95 psig chamber maintaining normal pO_2 during decompression) and lung pools were prepared from each experimental group. Lung infectivity titers were performed as with previous PR8 lung pools. The results are reported in Table 2.

An accelerated mortality rate is immediately noted in aerosol-infected animals maintained in a normoxic pO_2 at 95 psig in He. The first death occurred before the 6th day and no mice survived for preparation of lung pools on day 8. All control mice held in an identical chamber under flowing tank air, survived for 6 days but only 1 was available for lung pool preparation at day 8. Mice exposed only to diluent aerosol survived throughout, whether held at 95 psig or ambient atmosphere. Average mouse weights, where available, decreased in infected mice and increased in all diluent controls. Average lung weights, as indicated in previous experiments, were higher when consolidation was present.

The lung infectivity titer for hyperbaric mice was found to be almost 0.5 log higher at day 6 post-challenge than corresponding 1 atm air controls. No mice remained on day 8 for comparison with probable falling titer of day 8 air controls.

No infectivity was detected in either 6 or 8-day pools prepared from diluent control mice maintained at ambient pressure. However, the diluent control mice maintained at 95 psig under 2.8% O_2 in He did show an appreciable titer ($> 10^{3.5}$) when sacrificed at day 6 though gross lung pathology was not observed. On sacrifice at day 8, the lung pool prepared from 5 mice without evidence of lung involvement also did not exhibit infectivity on egg inoculation. A single mouse showing significant consolidation (score, 1.5) gave an EID_{50} of $> 10^{4.5}$.

End points were not obtained in either instance because log dilutions inoculated were restricted to lowest dilutions possible in order to detect unsuspected minimal infectivity.

It is possible of course that the evidence for virus in the uninfected control group results from an occasional occurrence of natural transmission from infected mice in the same chamber.

PR8-22. This is a repetition, in part, of PR8-19. After infection by aerosol mice were held in the 150 L chambers, one group in 2.8% O_2 in He, 95 psig, and one in air at one atmosphere. Chamber temperature, recycling atmosphere, and flow rate through oxygen analyzer were as previously described in PR8-19. Groups of 12 mice each were used for determination of mortality rates and preparation of pools for lung infectivity titrations on the selected days of sacrifice.

The observed mortality rates are presented in Fig. 5. The first death in the hyperbaric mice occurred on day 5 and in the control mice on day 8 post-challenge. Mortality in both groups continued in a linear manner reaching 100% in the parabaric and 50% in the control group at day 10.

The data for average mouse and lung weights, degree of lung consolidation, and infectivity titer at each interval of sacrifice are presented in Table 3. The average mouse weight decreases slightly as disease progresses and average lung weights increase in both parabaric and control mice with the maximum occurring at about onset of mortality. The degree of lung involvement is more rapid and extensive in the hyperbaric mice. These data are all consistent with the observed differences in mortality rates. However, when we examine the lung infectivity titers little difference is noted. Titers are identical at day 4 of sacrifice and steadily decrease in both hyperbaric and control groups through day 7. The rate of decline in titer does appear to be more rapid in the control mice and is over 1 log titer lower by the 7th day post-challenge. This, however, is coincidental with onset of mortality in the control mice while corresponding hyperbaric mice have already experienced 35% mortality.

3. Experiments with Coxsackie Virus.

Coxsackie #10. To confirm the reported increase in viral content of pancreatic tissue from hypoxic mice in experiment Coxsackie #9, QSR No. 16, 1 April – 30 June 69, an additional, slightly modified hypoxic experiment was designed. Parabaric exposure was again limited to the post-challenge interval and i.p. challenge was as before, 0.25 ml of 1:500 dilution of pool A (equivalent to 2500 LD_{50} suckling mouse doses). Only two groups of mice were utilized, 14 for hypoxic exposure and 13 as ambient flowing air controls in 30 L Plexiglass chambers. Hypoxia in this experiment was induced by maintaining the chamber under tank air at 7.3 psia (simulated 18,000 ft altitude) in contrast to ambient pressure 11% oxygen in nitrogen employed for Coxsackie #9. Seven mice from each group were sacrificed at day 5 post-challenge for preparation of pancreatic tissue for plaque assay titrations. Two mice, each group, on both days 5 and 7 post-challenge,

were sacrificed for preparation of pancreatic sections for histopathology. Sections were also prepared from 3 hypoxic mice surviving at 13 days post-challenge. Separate experimental groups for mortality determinations could not be included because of limitations in capacity of simulated altitude and control chambers.

A definite increase in pancreatic tissue viral titer, as determined by standard plaquing procedures using LLC-MK2, Rhesus Monkey Kidney cells, was again observed in animals exposed to a decreased pO_2. A plaque titer of 7400×10^3/gm pancreatic tissue from hypoxic mice was obtained as compared to 99×10^3/gm pancreas ambient air control animals. Corresponding values as reported in Coxsackie #9 for 1 atm hypoxic mice and ambient air controls were 2900×10^3 and 280×10^3/gm pancreas.

On routine histopathologic sections, pancreatic tissue following either Zenker's or buffered formalin fixation, showed only the normal pathologic changes associated with this type of infective agent. Pancreatic sections obtained on the 5th and 7th day post-challenge, showed only slight edema; moderate lymphocytic infiltration composed mainly (90%) of mature lymphocytes and scattered immature lymphocytes; peripancreatic retroperitoneal adipose tissue also showed slight edema with a similar infiltrate as described above. In this early stage, the pancreatic infiltrate in hypoxic mice appeared to contain more immature lymphocytes in the control mice, but differences were not great.

The pancreas from hypoxic mice sacrificed at 13 days post-challenge appeared normal. A slight edema and inflammatory infiltrate appearing in the peripancreatic retroperitoneal adipose tissue was composed solely of small mature lymphocytes. (The collaboration of Dr. G. W. Bailey and Dr. L. G. Dickson of the Experimental Pathology Division, Clinical Investigation Department, NMRI, is appreciated.)

Table 1. Experiment PR8-17. Effect of parabaric conditions on mouse lung infection following aerosol challenge with PR8 influenza virus or 0.5% bovine plasma albumin in phosphate buffered saline.

| Environment | Day of sacrifice post-challenge | No. of mice | Observations on sacrificed mice | | | Infectivity titer of lung $(EID_{50};Karber)$[b] |
			Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol.[a]	
Group B 77% O_2 1 atm[d]	6	6	16.0	0.31	1.5 (2.5,0.4,0.2,3, 1.5,1.5)	$10^{6.5}$
	8	2	14.5	0.34	3.2 (4,2.4)	$10^{5.5}$
Group D Line Air 1 atm[d]	6	5	18.2	0.28	0.6 (0.5,0.5,0.5,1,0.3)	$10^{6.3}$
	8	4	14.5	0.32	2.3 (3,2.5,2,1.5)	$10^{6.0}$
Group E Line Air 1 atm[e]	6	6	19.4	0.17	0.05 (0,0,0,0,0,0.3)	$10^{2.6}$
	8	6	19.6	0.29	0.0 (0,0,0,0,0,0)	$<10^{1.5}$
	16	10	–	0.18	0.0 (0,0,0,0,0,0,0)[c]	$<10^{1.5}$
				0.19	0.2 (0.3,0.1,0.2)	$<10^{1.5}$

[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 (Karber).

[c] Separate lung pools prepared at 16 day sacrifice on 3 mice showing a slight degree of lung consolidation.

[d] Virus aerosol: 20 min using 1:100 dilution pool A (PR8), 0.40 ml/min, R.H. 85%.

[e] Aerosol exposure: Diluent aerosol 0.5% BPA in PBS 20 min, 0.39 ml/min, R.H. 85%.

Table 2. Experiment PR8-19. Effect of parabaric conditions on mouse lung infection following aerosol challenge with either PR8 influenza virus or 0.5% bovine plasma albumin in phosphate buffered saline.

Environment	Challenge	Day of sacrifice Post-challenge	No. of mice	Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol.[a]	Infectivity titer of lung (EID$_{50}$; Kärber)[b]
Group A 2.8% O$_2$ in He 95 psig	PR8 virus[d]	6	5	15.0	0.29	2.0 (4,3,1.5,1,0.5)	$10^{7.5}$
		8	0	–	–	–	–
Group A$_1$ 2.8% O$_2$ in He 95 psig	Diluent control[e]	6	6	15.2	0.16	0.0 (0,0,0,0,0,0)	$> 10^{3.5}$
		8	6	19.3	0.15	0.0 (0,0,0,0,0)[c]	$< 10^{1.5}$
				17.0	0.27	1.5 (1.5)	$> 10^{4.5}$
Group D Line Air 1 atm	PR8 virus[d]	6	6	15.0	0.33	2.1 (4,3,2,2,1,0.5)	$10^{6.9}$
		8	1	13.0	0.29[c]	3.0 (3)	$10^{5.7}$
Group D$_1$ Line Air 1 atm	Diluent control[e]	6	6	16.4	0.16	0.0 (0,0,0,0,0,0)	$< 10^{1.5}$
		8	6	20.9	0.14	0.0 (0,0,0,0,0,0)	$< 10^{1.5}$

[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).

[c] Separate lung pool prepared from single lung showing consolidation.

[d] Virus aerosol: 20 min using 1:100 dilution pool A (PR8), 0.375 ml/min, R. H. 96%.

[e] Aerosol exposure: Diluent aerosol 0.5% BPA in PBS 20 min, 0.38 ml/min, R. H. 92%.

Table 3. Experiment PR8-22. Effect of altered atmosphere on mouse lung infection following aerosol challenge with PR8 influenza virus.

| Environment | Day of sacrifice post-challenge | No. of mice | Observations on sacrificed mice | | | Infectivity titer of lung $(EID_{50}; Kärber)$[b] |
			Av. mouse wt. (g)	Av. lung wt. (g)	Lung consol.[a]	
Group A 2.8% O_2 in He 95 psig	4	3	18.0	0.23	0.5 (1,0.3,0.2)	$10^{8.1}$
	5	3	17.5	0.34	2.0 (2,2,2)	$10^{7.6}$
	6	3	15.5	0.38	2.4 (3.2,2,2)	$10^{7.3}$
	7	2	17.3	0.32	2.4 (1.8,3)	$10^{6.9}$
Group D Air Control, 1 atm	4	3	19.5	0.25	0.4 0.8,0.2,0.2)	$10^{8.1}$
	5	3	19.5	0.28	,0.3 (0.5,0.2,0.2)	$10^{7.1}$
	6	3	17.0	0.31	1.2 (2,1,0.6)	$10^{6.9}$
	7	3	16.2	0.36	1.4 (2,1,1.2)	$10^{5.5}$

[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).

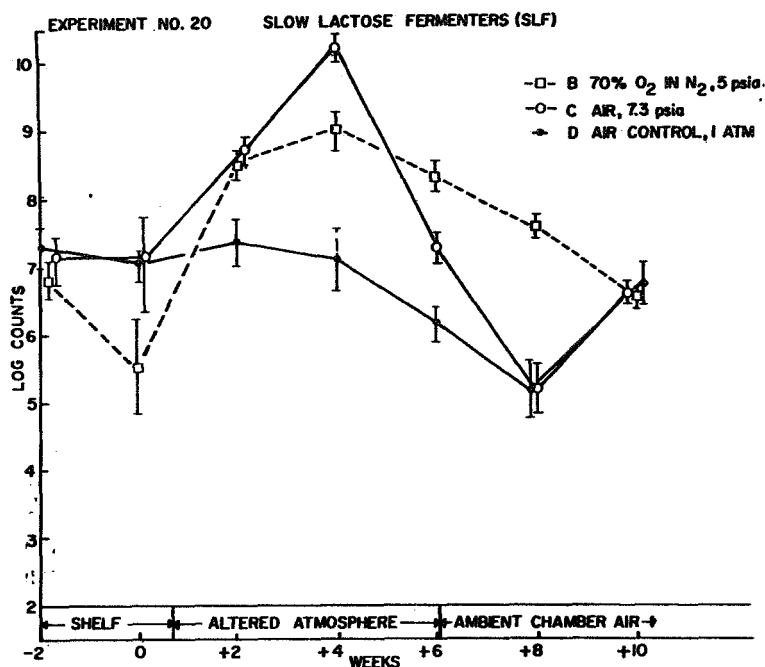Aerosol exposure: 20 minutes using 1:200 dilution of PR8, pool A, at 0.24 ml/min, R.H. 95.2%.

Figure 1. Experiment 20. Average numbers/g of SLF in stools of mice before, during parabaric exposure and after return to ambient chamber air.
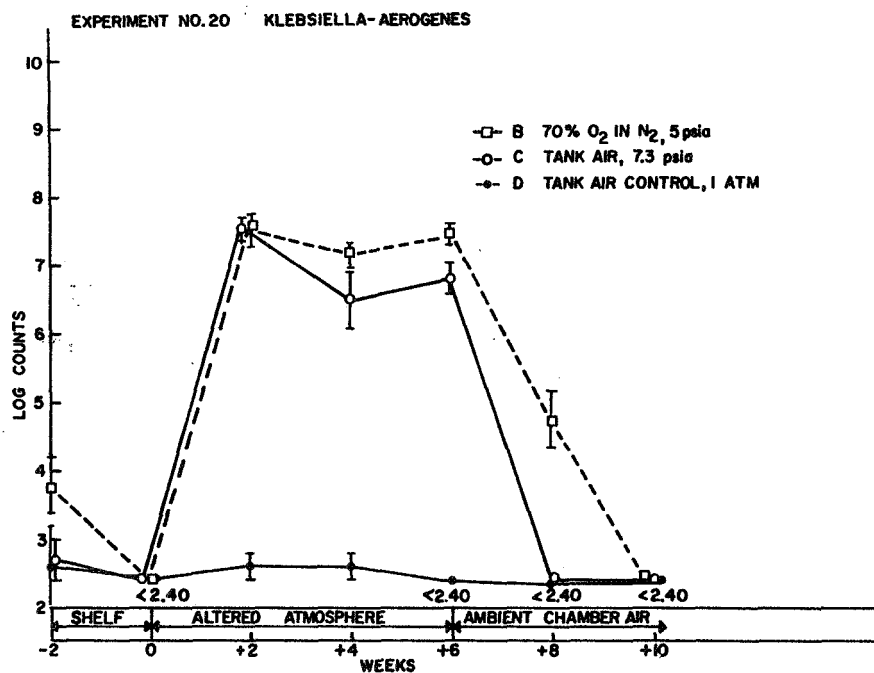

Figure 2. Experiment 20. Average numbers/g of Klebsiella-Aerogenes in stools of mice before, during parabaric exposure and following return to ambient chamber air.
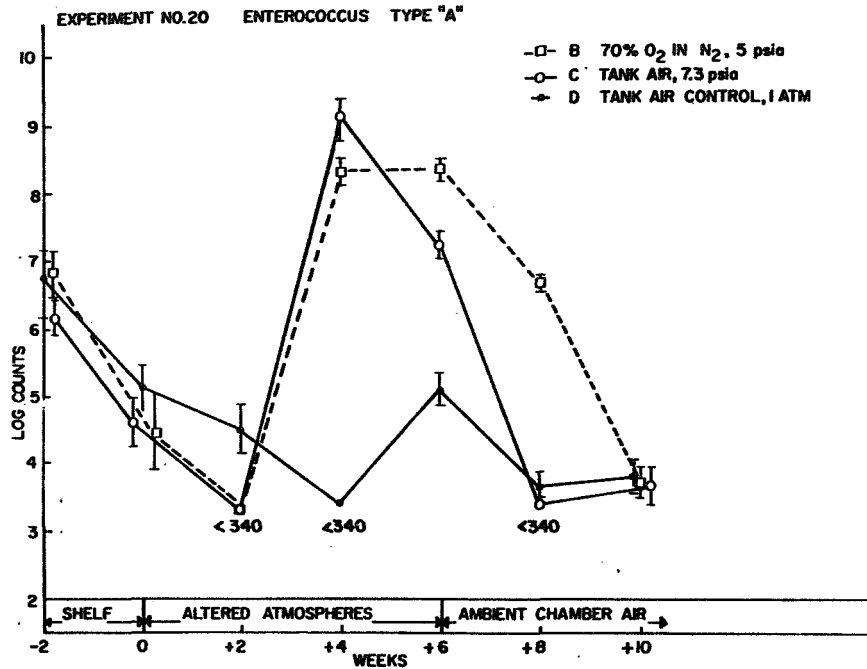
Figure 3. Experiment 20. Average numbers/g of enterococcus, colony type A, in stools of mice before, during parabaric exposure and following return to ambient chamber air.
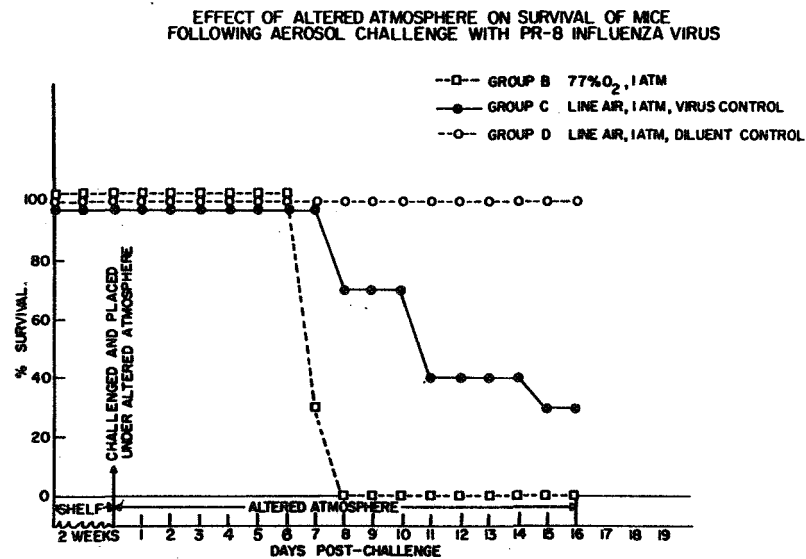

Figure 4. Experiment PR8-17.

EFFECT OF ALTERED ATMOSPHERE ON SURVIVAL
OF MICE FOLLOWING AEROSOL CHALLENGE
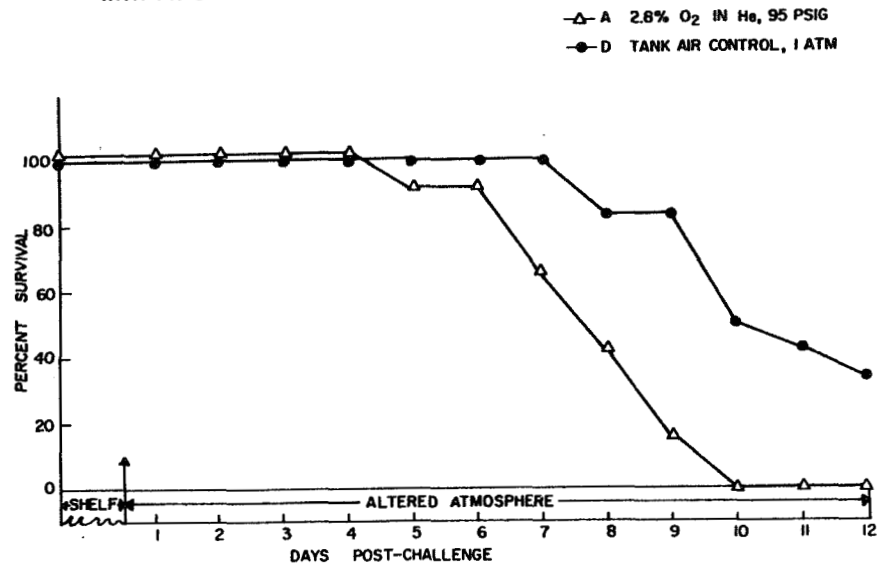WITH PR-8 INFLUENZA VIRUS

—△— A 2.6% O_2 IN He, 95 PSIG
—●— D TANK AIR CONTROL, I ATM

Figure 5.   Experiment PR8-22.