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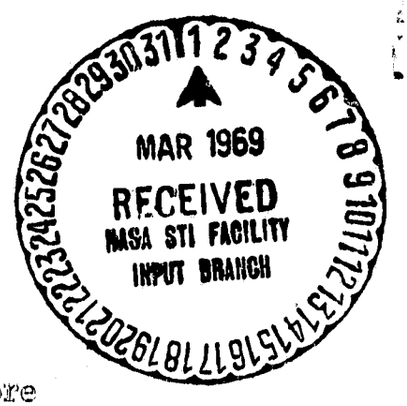
"Effects of High and Low Barometric Pressures on Susceptibility and Resistance to Infection"



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Abstract.

With the animal chambers used to date it has not been possible to maintain a normal body temperature of experimental animals (mice) in a He atmosphere under pressure or at one atmosphere in contrast to an N_2 atmosphere under pressure. Body temperatures of mice in such environments have now been determined by means of thermister probes inserted intrarectally and subcutaneously, and were found to be several degrees below normal in He atmospheres. The degree of heating of the ambient gas needed to allow the mouse to maintain a normal body temperature in He was also determined. This information will be applied in the design of experiments to be done in new pressure chambers (maximum, 1,000 psig) in which temperature, humidity, CO_2, O_2, gas recirculation, can all be carefully controlled. One such chamber has been delivered; 2 more are expected soon.

The alteration in enteric flora described previously in mice subjected to 100% O_2 at 3.3 psia has been confirmed and found to return to normal levels when the mice are returned from the abnormal to normobaric environment.

Exposure of mice to 100% O_2 at 3.3 psia only before aerosol challenge with Chlamydia trachomatis (mouse pneumonitis strain) does not result in the increased mortality rate seen in mice exposed to this parabaric environment after aerosol challenge, as reported previously. Pre-challenge exposure only for two weeks to hypoxic environment (air at 7.3 psia) also did not significantly affect the infection.

A reduction in mortality in mice injected I.P. with C. psittaci is present in those groups exposed to 77% O_2 after, or before and after, injection. But exposure to the hyperoxic environment only before inoculation of the infective agent does not alter the mortality rate significantly from that of the controls.

Confirmation of a previous experiment was obtained when mice were placed in 100% O_2 at 3.3 psia following aerosol challenge with influenza virus. Although titers of virus in the lungs, and degrees of lung consolidation were the same as in controls under normobaric conditions, the mortality rate in the parabaric group was greatly increased.

Exposure of strain A/HeJ mice to hyperoxia and hypoxia following intravenous inoculation of the carcinogen, dibenz-anthracene, resulted in a significantly greater incidence of pulmonary adenomas in the hyperoxic group than in the hypoxic. The normoxic controls showed a rate lying between the two but not significantly different than either. Nevertheless this is the type of result reported by others and indicates that our test system is working satisfactorily for further investigations on the effect of parabarosis on the incidence of pulmonary adenomas in these mice.

Another host-virus test system has been developed for use in this study, i.e., Coxsackie B virus-adult mouse. The first exploratory experiment gave no evidence for modification of the host-virus relationship under parabaric conditions.

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

Attention was directed in QSR No. 13, July-Sept., 1968, towards efforts for improvement in limiting undesirable concentrations of gases accumulating in chamber atmospheres during long term exposures. Predictable alterations in the normal fecal flora of mice exposed to simulated altitude in 100% O_2 with normoxic pO_2 (37,000 ft, 3.3 psia) have been repeatedly demonstrated, but great difficulty has been experienced in maintaining experimental animals at simulated depth sea water under helium-oxygen atmosphere (2.8% O_2 in He, 95 psig) for over one or two week intervals.

In experiment No. 17, with animals exposed to the same hyperbaric, hypobaric and control atmospheres with sampling performed at two week intervals, significant elevations (> 2 logs) in concentrations of both typical E. coli and Klebsiella-Aerogenes were again obtained at both 2 and 4 week intervals following exposure to 100% O_2 at 3.3 psia. Enterococci also were found to increase in greater than 1 log magnitude after 4 weeks at altered atmosphere. The observed concentrations all returned to control levels when the parabaric mice were returned to ambient atmosphere for two weeks and remained constant at an additional two weeks sampling period. Only three mice survived 2 weeks' exposure to the 95 psig He-O_2 atmosphere and this portion of the experiment was discontinued.

In the preceding experiments, the humidity, CO_2, NH_3 and H_2S concentrations were all maintained within acceptable limits at the 95 psig operating pressure by an adequate He-O_2 flow rate and adsorbent agents (Baralyme, Boric acid) within the chambers. However, with the present equipment, we have been unable to compensate for the considerable loss of body heat within the pressurized helium atmosphere.

2. Observations on temperature of mice in parabaric environments.

Because of the problem described above, precise measurements have been made on the body temperature of mice, and the ambient temperature, at simulated depth and altitude. Thermister probes were inserted subcutaneously and intrarectally in restrained mice, and placed in the ambient atmosphere near the mouse.

Figure 1 shows the effect of compression to 95 psig in N_2 plus 2.8% O_2 (normoxic condition). Relatively little effect on body temperature is apparent. In contrast, Fig. 2 shows the effect of 95 psig in He plus 2.8% O_2 (normoxic condition). A profound and sustained drop in body temperature occurs. This effect no doubt accounts for the persistant loss of experimental animals, reported previously, during the first two weeks' exposure to hyperbaric He-O_2 atmosphere. Mice that were able to survive stress due to body heat loss for the first two weeks continued to tolerate the environment equally as long as mice held at the same pressure of O_2 in nitrogen for periods of up to 14 weeks in some experiments.

The effect of exposure to a normoxic, normobaric helium atmosphere (20% O_2, 80% He at one atmosphere) in lowering both subcutaneous and rectal temperatures is shown in Fig. 3. Although the observed temperature reduction observed at ambient atmosphere appears comparable to that seen in mice under pressure, the caloric demand required to compensate for heat loss under pressure is greatly increased. For this reason "controls" for the hyperbaric He-O_2 conditions are not provided by He-O_2 gas mixture at one atmosphere.

In order to determine whether a controlled elevation of environmental temperature would decrease stress due to body heat loss, a small table model pressure chamber (Bethlehem Corp.) was modified by installing a hot water circulating copper coil internally and connecting with an externally controlled hot water supply. Rectal and subcutaneous thermisters were again implanted in restrained mice with an additional thermister available for recording chamber temperature.

In Fig. 4 the stabilizing effect on rectal and subcutaneous animal temperatures of raising the environmental chamber temperature is shown during compression to 95 psig in 2.8% oxygen in nitrogen. Fig. 5 shows the same effect in 2.8% O_2 in helium. In several experiments involving elevation of chamber temperatures death of the experimental animals occurred when chamber temperatures accidentally reached 41-42° C. Attempts to escape restraint in the ventilated plastic holder within the heated helium-oxygen atmosphere may have stressed the animal too severely.

Observations have begun on the body temperature of mice subjected to simulated altitude.

3. New equipment.

The first of three, 5.3 cu ft (150 L), hyperbaric chambers capable of simulated altitude (vacuum to 1 mm Hg) or pressure to 1000 psig has been delivered. Two more with a connecting 4" ball valve are nearing completion so that animals may be transferred from chamber to chamber without altering the parabaric conditions. This will allow, for the first time, planning for long term experiments without alterations in simulated altitude or depth. A built-in recycling system with CO_2 adsorbent chamber and automatic temperature control between 0-40° C will help eliminate previous uncontrolled variables, including low body temperature, encountered during previous long term exposures.

4. Effect of parabarosis on infection of mice with Chlamydia trachomatis (mouse pneumonitis strain).

The effect of post-challenge exposure to air at 7.3 psia (simulated altitude with hypoxia; pO_2 80 mm Hg) on mortality and severity of mouse pneumonitis infection, as measured by

gross pathology and lung infectivity titers, has been described in QSR No. 13, July-Sept., 1968. Although the post-challenge exposure to hypobaric-hypoxic atmosphere appeared to favor pulmonary infection to some degree (increased infectivity titers, $.01 > P > .001$, at only the 16-day interval of sacrifice), mortality rates of both parabaric and control animals were essentially the same. This is in contrast with the increased mortality rates observed when mice were exposed post-challenge or both pre- and post-challenge to hypobaric-normoxic atmosphere (100% O_2, 3.1 psia). (Fig. 3 of QSR No. 11, Jan.-March, and Fig. 3, QSR No. 12, April-June, 1968.)

The present experiments were designed to determine the effect of pre-challenge exposure only to hypobaric-normoxic (100% O_2, 3.3 psia), hypobaric-hypoxic (pO_2 80 mm Hg, 7.3 psia) and normobaric-normoxic (line air, 1 atm.) atmospheres on both mortality rates and severity of lung infection.

Experiment Mopn #37 was limited to determining the cumulative mortality rates obtained when groups of mice were pre-exposed for 10 days to the described simulated altitude and control conditions, challenged with the infecting aerosol, and then held on the shelf in room air at 1 atm. Although average survival time differed to some extent (greatest in the hypoxic group) the final mortality rates were essentially the same for all 3 groups. These results are shown in Fig. 6.

In Mopn #38 mice were exposed for 2 weeks to the same environmental conditions as described for the preceding experiment, then challenged similarly with an infective aerosol and placed on the shelf. Lung pools were harvested from each of the experimental groups at 11, 15 and 21-day intervals following challenge. Average mouse weights, lung weights, gross pathology scores and lung infectivity titers expressed as inclusion forming units (IFU) per ml are summarized in Table 1.

The interval to first sacrifice was chosen on the basis of the onset of illness in the mice, which was not apparent until the second week. This long interval was probably not a wise choice because individual differences between mice are more apparent at the later stages of the infection, particularly when some of the mice are beginning to recover. This is reflected in the great diversity in degree of lung involvement recorded for individual mice. The differences seen between groups at 11 days with respect to lung weights, gross pathology give some evidence for a greater level of infection in the two parabaric groups, as compared to the controls, but this cannot be regarded as certain. It is clear that pre-challenge exposure to 100% O_2 at 3.3 psia does not affect the outcome of mouse pneumonitis infection as does this parabaric environment <u>after</u> challenge, when markedly increased mortality is observed.

5. Effect of parabarosis on infection of mice with Chlamydia psittaci.

In QSR's No. 12 and No. 13, the effects of parabaric exposure on a second chlamydial infection, C. psittaci, were described. Challenge was by the I.P. route to produce a systemic infection. A decrease in mortality was obtained when mice were exposed either post-challenge or both pre- and post-challenge to simulated altitudes with normoxic pO_2 (37,000 ft, 100% O_2). In the same experiments, a definite increase in mortality was obtained when hypobaric-hypoxic atmospheres (pO_2 80 mm Hg, 7.3 psia) were utilized. Because hypoxia at simulated altitude (18,000 ft, pO_2 80 mm Hg) increased mortality, a hyperoxic environment might be expected to decrease mortality, and the effects following exposure to altered environments before or after infectious challenge could indicate whether the effect was directly on the host, or on the infective agent-host interaction.

In Experiment Psitt #6, six groups of mice were used to determine the effects of pre-, post-, and pre- & post-challenge exposures to an ambient hyperoxic atmosphere (77% O_2) with corresponding flowing line air and shelf (room air) controls. The experimental design was as follows, and survival times and mortality rates were determined.

Group A-15 mice: 77% O_2 for 2 weeks - challenge - 77% O_2 for 2 weeks

" B-15 " : 77% O_2 for 2 weeks - challenge - shelf for 2 weeks

" C-15 " : Shelf for 2 weeks - challenge - 77% O_2 for 2 weeks

" D-15 " : Flowing line air for 2 wks - challenge - flowing line air for 2 wks

" E-15 " : Flowing line air for 2 wks - challenge - shelf for 2 weeks

" F-15 " : Shelf for 2 weeks - challenge - flowing line air for 2 wks

The challenge (one LD_{50} dose) consisted of I.P. inoculation with 0.25 ml of a 2×10^{-5} dilution in phosphate-buffered saline of C. psittaci, strain 6BC-#3805. The survival rates for each parabaric group and corresponding controls are presented in Figures 7, 8, and 9.

The reduced mortality in mice exposed to a hyperoxic atmosphere is readily apparent and this effect is obtained either with post-challenge exposure alone (Fig. 7), or with both pre- and post-challenge exposure (Fig. 8). In contrast, when exposure to the hyperoxic condition was limited to the two weeks' pre-challenge period only, the observed survival rates and corresponding controls are found to be almost identical (Fig. 9).

When the chlamydial agent of mouse pneumonitis was used for challenge by means of infective aerosol, a similar decrease in mortality was observed in mice when exposed to the ambient 77% O_2 atmosphere following challenge (previously reported). Ambient

hypoxic atmospheres (11% O_2) similarly tended to increase mortality following aerosol challenge but in contrast, exposures to simulated 37,000 ft altitude in 100% O_2 (normoxic pO_2) immediately following challenge, significantly increased the mortality. The present report confirms our earlier conclusion that the host response to challenge with closely related infective agents may be influenced by the altered parabaric conditions in different ways depending upon the type of infection induced in the host.

6. Effect of parabaresis on pulmonary infection of mice with PR-8 influenza virus.

Experiment PR-8 #8 was designed to measure both lung infectivity titers and corresponding mortality rates in mice exposed to 100% oxygen atmospheres at 3.8 (±) 0.1 psia following aerosol challenge, in comparison with similar animals maintained under normal ambient atmospheres. Twenty mice were observed for determination of mortality rate during a 20-day post-challenge interval, and 24 mice similarly challenged were sacrificed for determination of lung infectivity titers. Ten percent lung pool suspensions were prepared from 3 mice of each infectivity group on the 4th, 5th, 6th and 7th day following challenge and EID_{50} titrations performed in fertile eggs. Titration results are presented in Table 2 and the cumulative mortalities in both groups (hypobaric with 100% O_2, and 1 atm controls) are presented in Fig. 10.

The evidence confirms a previous finding, and again supports the observation that viral concentration is high before there is gross evidence of lung involvement. Although the degree of lung involvement was most pronounced at the 7th day sacrifice post-challenge, a 5 to 10-fold decrease in EID_{50} was observed in both hypobaric and control groups on that day, compared to the 4th day observation. The mortalities in the experimental group exposed to hypobaric environment with 100% oxygen were first apparent on the 8th day post-challenge, immediately following peak lung infectivity titers, and steadily continued until the 12th day when survival was only 30%. In this experiment the aerosol exposure for 20 minutes at a rate of 0.84 ml/min at 91.2% R.H. using a 1:100 dilution of our PR-8 virus pool, was not quite infective enough to cause mortality in the ambient atmosphere control group.

If we postulate that an altered host factor is responsible for the altered clinical outcome, because viral multiplication within the pulmonary tissue is equal in both groups, a local alteration in lung tissue due to parabaric exposure may be a significant factor. Pre-challenge exposure only to parabaric conditions may assist in elucidating the mechanism of this effect. Additional experiments are programmed to investigate the effects of not only pre-challenge exposures but also the various parameters of space cabin atmosphere exposures (70% O_2 in nitrogen, 5 psia).

7. The effect of parabarosis on induction of pulmonary tumors in A/HeJ strain mice following I.V. challenge with chemical carcinogen.

Conflicting results on the incidence of lung tumors in mice exposed to 100% O_2 atmospheres at 3.3 (±) 0.1 psia immediately after I.V. challenge with chemical carcinogen were reported in tumor experiments #1 and #3, QSR No. 12, April-June, 1968. In order to augment and clarify the previous data, experiment #4 was designed to expose equal weight groups of 2-3 month old A/HeJ strain mice to various parabaric conditions, as recorded in Table 3, immediately following I.V. injection of 0.1 mg of dibenz-anthracene.

Following the I.V. inoculation and exposure to the various altered atmospheres for the times indicated, the mice were held in ambient room air for 4 months. The experimental groups were sacrificed under ether anaesthesia. The lungs were infiltrated in situ with fixative, removed intact, and the tumor nodules were counted under 10 X magnification with dissecting microscope.

During the two weeks parabaric exposure and subsequent 4 months holding period, cumulative loss of mice in Groups B and C left too few survivors for valid statistical evaluation. In the other experimental groups, 12 mice remained for tumor counts in Group D (11% O_2, 1 atm), 15 mice in Group E (air controls) and 9 mice in Group F (100% O_2, 48 hours. The results are summarized in Table 3.

The incidence of tumors was found to be considerably lower in all groups than was observed in preceding experiments, but the variation within groups was sufficiently limited to provide a significant difference between groups D (hypoxic) and F (hyperoxic).

This result is in agreement with the original observations of Hester and Prah (PSEBM 92:451-454, 1956) on similar ambient hypoxic and hyperoxic groups. The difference between hypoxic-ambient air and hyperoxic-ambient air were not significant for this experiment. Additional A/HeJ strain mice have been injected with the carcinogen and exposed to these parabaric atmospheres. They are awaiting tumor count determinations upon completion of the 4 months shelf period and results will be reported in the next QSR.

8. Experiments with Coxsackie virus.

Exploratory experiments have been performed by which orienting information has been obtained for use of an adult mouse-Coxsackie B virus system for testing effect of parabarosis. Assays of virus in the pancreas of adult mice were made by suckling mouse inoculation and by plaquing on cell monolayers. In control mice virus was found and titrated in the pancreas up to 10 days following I.P. inoculation.

One experiment (Coxsackie #5) was performed with adult mice under parabaric conditions following I.P. inoculation. The details are given in Table 4. No deaths occurred in any of the groups and the slight differences in viral titer of pancreas pools of Groups D and E are not considered to be significant. Additional experiments of this type utilizing other dosages, time intervals, and parabaric conditions are planned.

Table 1 (Exp. Mopn #38). Effect of two weeks pre-challenge exposure to parabaric conditions on mouse lung infection with a chlamydial agent (mouse pneumonitis)

Environment	Day of sacrifice post-challenge	No. mice	Av. mouse wt. (gm)	Av. lung wt. (gm)	Gross pathology[a]	Lung titer IFU/ml
B	11	3	16.0	0.50	+2.0 (2,2,2)	$5.3(\pm)1.1\times10^{6}$
2 wks in 100% O_2 at 3.3 psia before challenge	15	4	21.6	0.32	+0.5 (0,0.5,0.5,1)	$< 2.66 \times 10^{4}$
	21	3	22.5	0.34	+0.7 (0,0,2)	$< 5.33 \times 10^{3}$
C	11	3	17.5	0.44	+2.75 (1,2,4)	$4.4(\pm)0.7\times10^{6}$
2 wks in tank air at 7.3 psia (pO_2 80 mm Hg)	15	4	19.6	0.44	+1.75 (0,2,2,3)	$6.0(\pm)0.37\times10^{4}$
before challenge	21	4	22.9	0.41	+1.25 (0,0.5,1,3)	$< 5.33 \times 10^{3}$
D	11	3	19.2	0.35	+1.0 (0,1,2)	$0.4(\pm)0.1\times10^{6}$
2 wks in line air at 1 atm (pO_2 160 mm Hg)	15	4	20.6	0.40	+1.87 (0.5,1,2,2)	$5.33(\pm)2.9\times10^{4}$
before challenge	21	3	21.8	0.38	+0.33 (0,0,1)	$< 5.33 \times 10^{3}$

[a] Arbitrary 0–5 scoring for degree of lung involvement: Mean scores are indicated, with individual mouse scores in parentheses.

Aerosol exposure 30 min using 1:5 dilution of 50% mouse pneumonitis pool at rate of 0.30 ml/min and relative humidity of 91.6%.

$t_6\ B_{11}$ & D_{11} $\quad 0.01 > P > 0.001$

$t_6\ C_{11}$ & D_{11} $\quad 0.01 > P > 0.001$

These probabilities are based only on the degree of variation in inclusion counts among the 4 replicate coverslips used for each assay.

Table 2 (Influenza PR-8, Exp. #8). Observations on lungs of mice sacrificed at intervals after aerosol challenge, exposed to parabarosis after challenge.

Lung pools 3 mice each	Day of sacrifice	Average consolidations	EID_{50} of influenza virus[a]
B 100% O_2 at 3.3 psia	4	0	$10^{-6.7}$
	5	0	$10^{-6.9}$
	6	1.3	$10^{-6.7}$
	7	2.3	$10^{-5.7}$
C Line air 1 atm	4	0	$10^{-6.7}$
	5	0.3	$10^{-6.9}$
	6	1.2	$10^{-6.7}$
	7	2.7	$10^{-5.7}$

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

Table 3. Pulmonary adenomas in A/HeJ mice subjected to parabaric environments following I. V. injection of dibenz-anthracene.

Group	No. mice at challenge	Type and duration of environment	No. mice at sacrifice	Average No. tumors
B	15	100% O_2, 3.3 psia 2 weeks	0	-
C	15	Tank air, 7.3 psia 2 weeks	2	-
D	15	11% O_2 in N_2 2 weeks	12	0.9 (±) 0.3
E	15	Tank air, 1 atm 2 weeks	15	1.9 (±) 0.6
F	15	100% O_2, 1 atm 2 weeks	9	3.9 (±) 1.3

Level of significance by Student's t.

t_{18} = D--F $\quad 0.02 > P > 0.01$
t_{24} = D--E $\quad 0.3 > P > 0.1$
t_{22} = E--F $\quad 0.3 > P > 0.1$

Table 4. Test for effect of parabarosis on Coxsackie virus infection in adult mice.

Group	No. mice	Environmental condition, after inoculation	Mortality	Titer of pancreas pools[a]	
				Suckling mouse LD_{50}/0.05 ml	Plaque-forming units/0.1 ml
A	10	100% O_2 3.3 psia	0	-	-
B	10	Air, 7.3 psia	0	-	-
C	10	Air at 1 atm (controls)	0	-	-
D	15	77% O_2, 1 atm	0	$10^{3.1}$	1.3×10^5
E	15	Air at 1 atm (controls)	0	$10^{3.9}$	0.8×10^5

[a] Harvested from 5 mice of groups D and E on day 6.

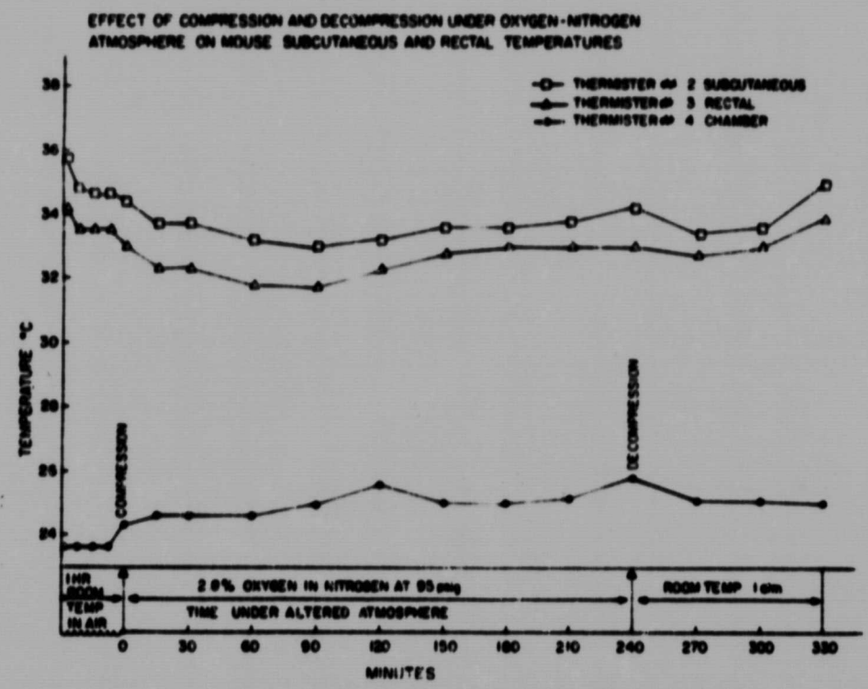

Fig. 1. Compression with 2.8% O_2 in N_2, 95 psig. In the tests depicted in Figs. 1-5 observations were made on one mouse restrained in a plastic holder with openings to allow contact with ambient gas phase. Thermisters were placed subcutaneously, intrarectally, and in the gas phase near the mouse.

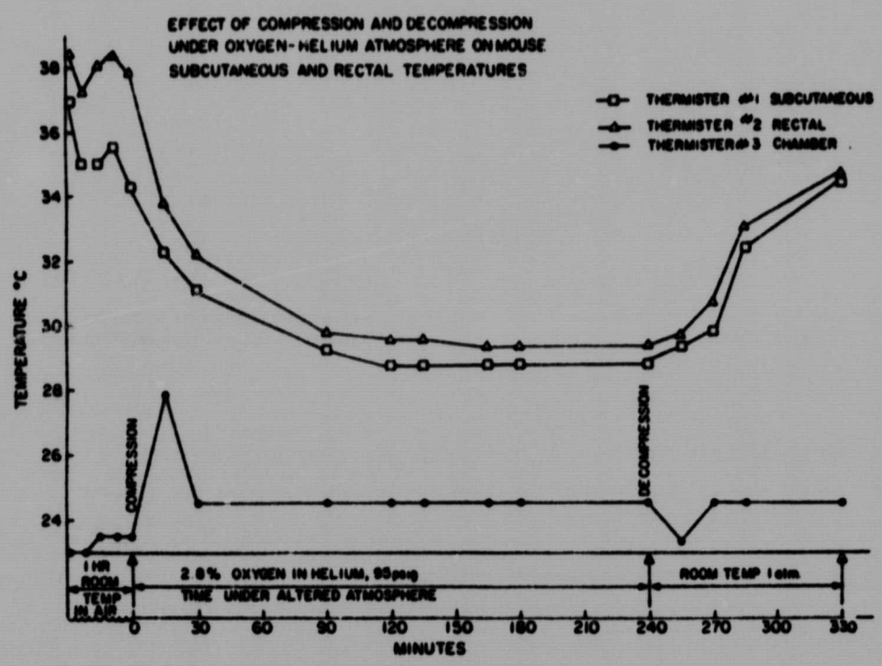

Fig. 2. Compression with 2.8% O_2 in He, 95 psig.

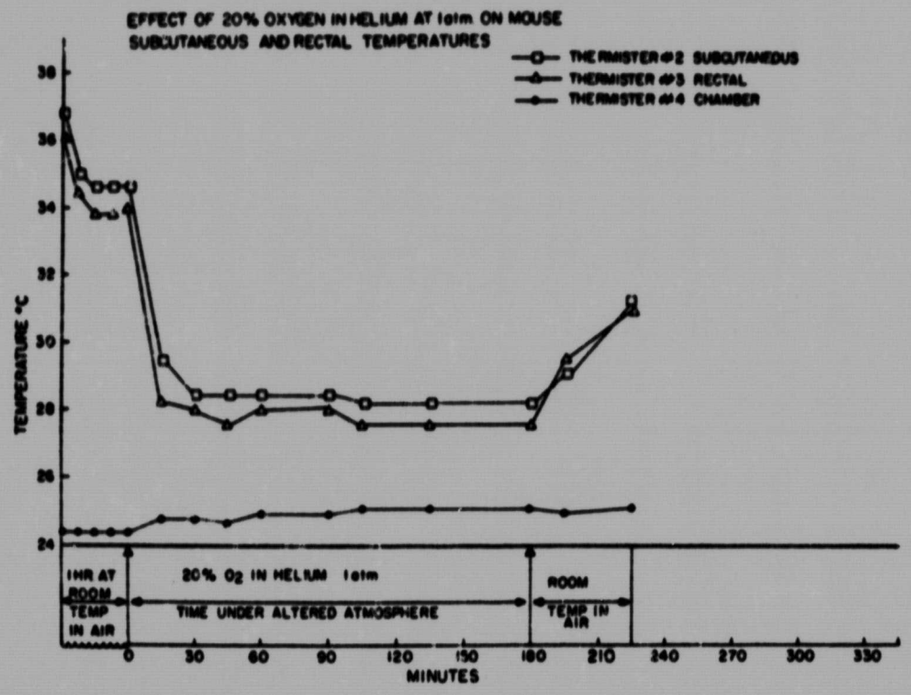

Fig. 3. Gas phase was 20% O_2 in He at one atmosphere.

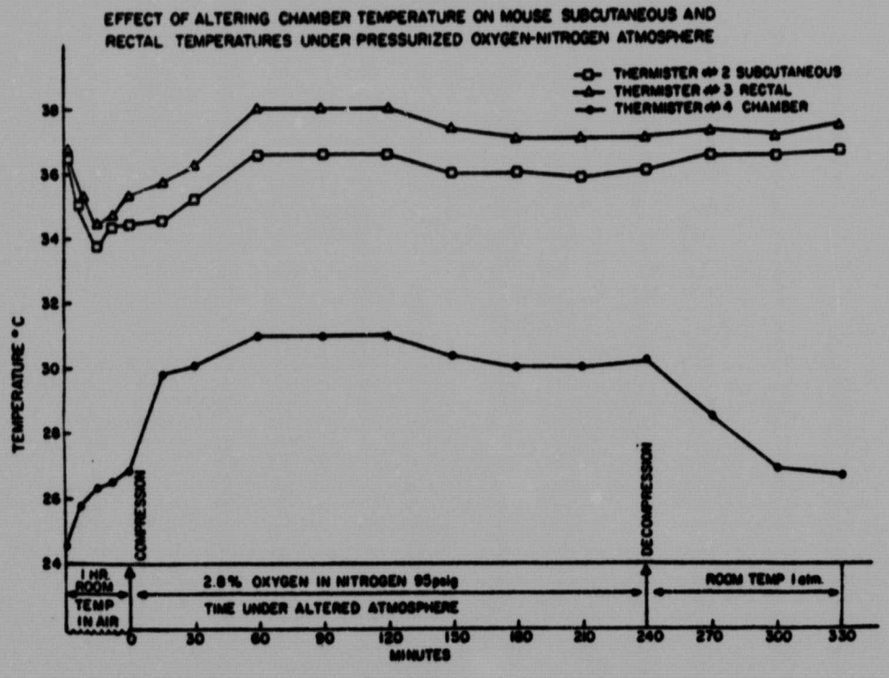

Fig. 4. Compression with 2.8% O_2 in N_2 at 95 psig. Temperature of gas phase raised by coil circulating hot water. Compare with Figure 1.

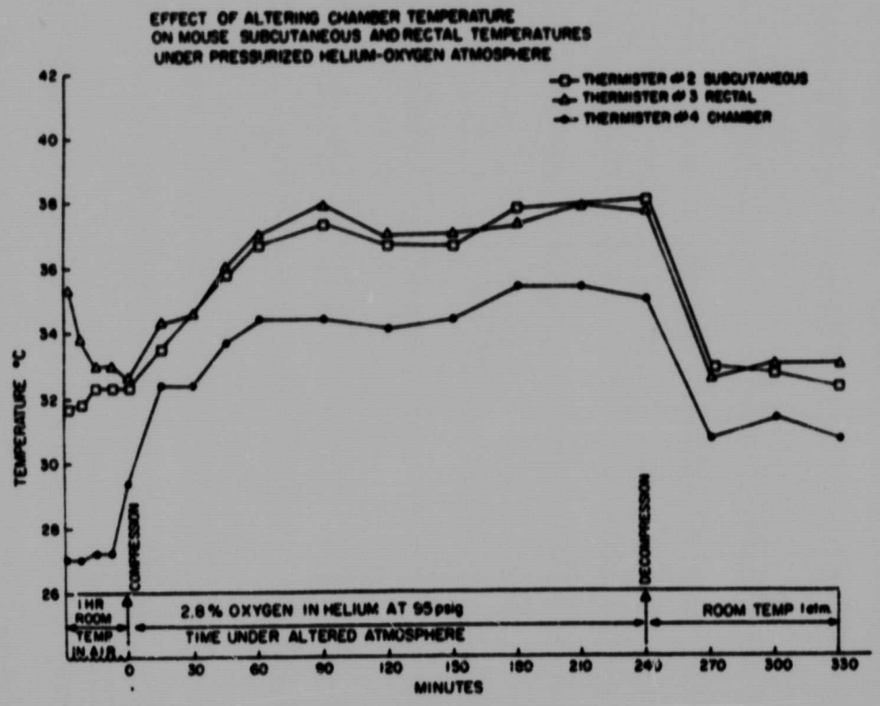

Fig. 5. Compression with 2.8% O_2 in He at 95 psig. Temperature of gas phase raised. Compare with Figs. 2 and 4. Note higher ambient temperature needed to maintain body temperature in He than in N_2 (Fig. 4).

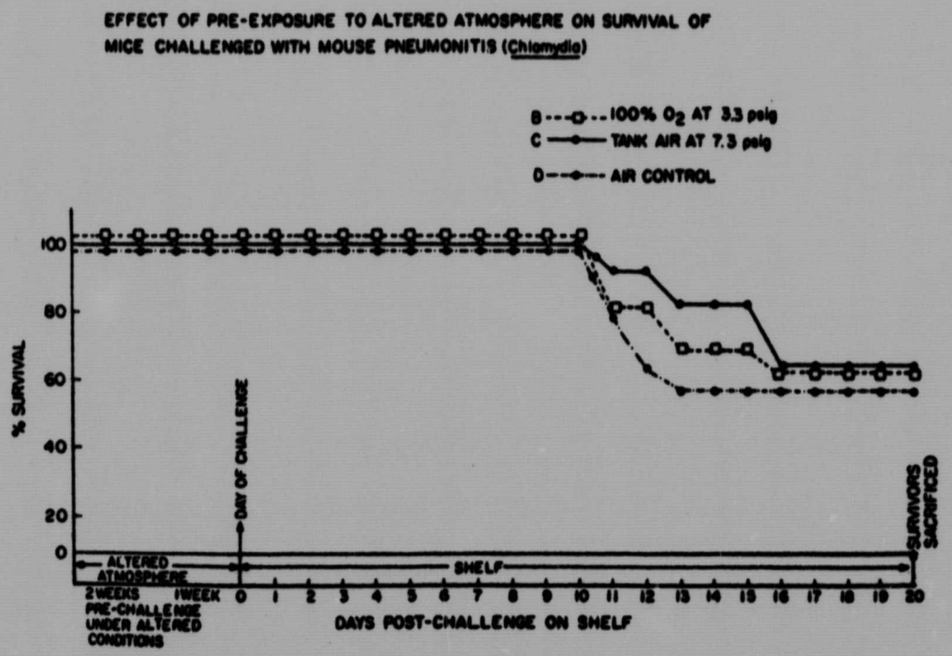

Fig. 6. Exp. Mopn #37.

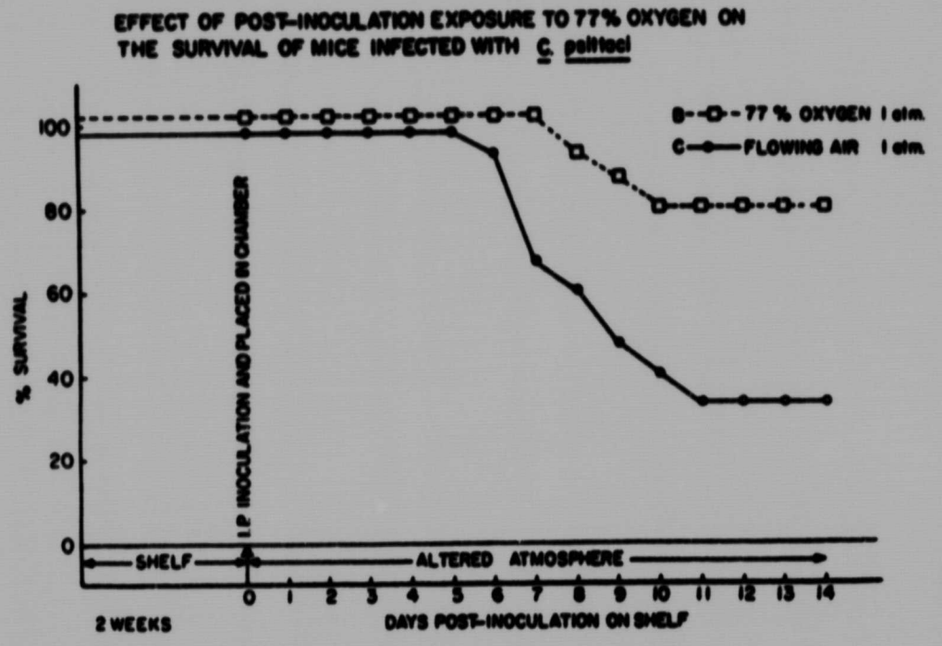

Fig. 7. Exp. Psitt #6, Groups C and F.

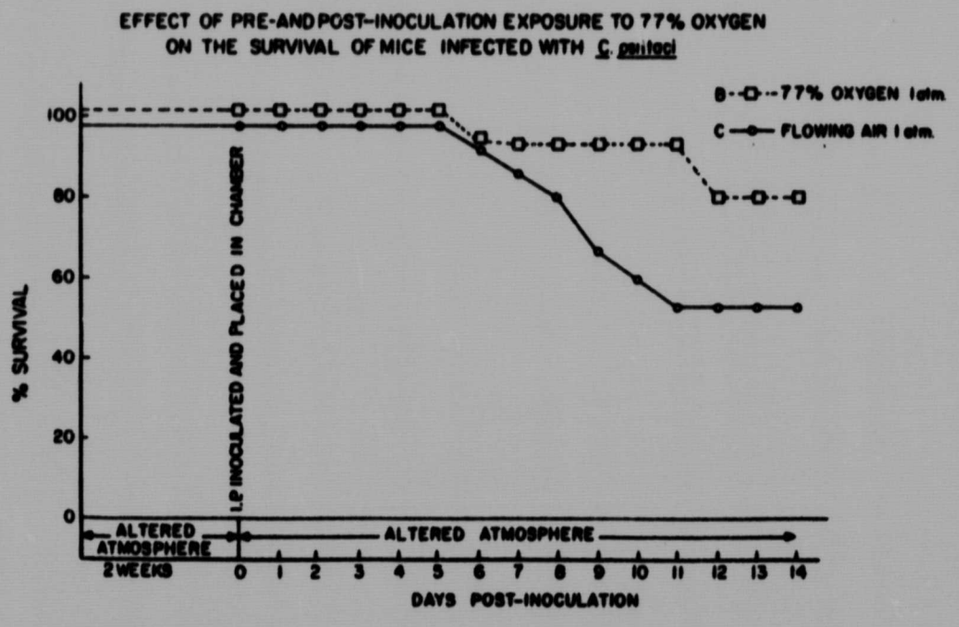

Fig. 8. Exp. Psitt #6, Groups A and D.

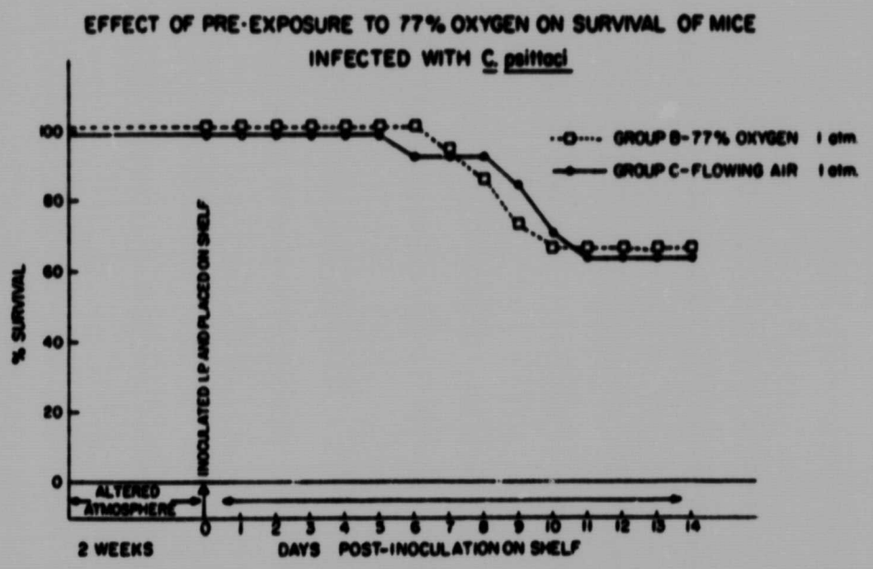

Fig. 9. Exp. Psitt #6, Groups B & E.

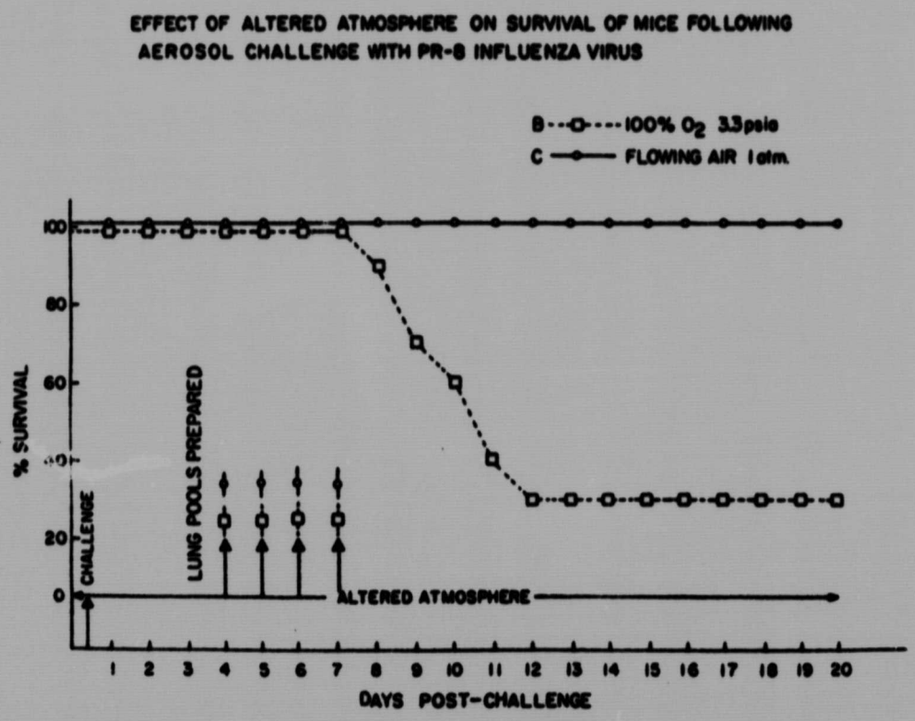

Fig. 10. Exp. PR8 #8. (See Table 2 for titers of virus in lung pools harvested at indicated times.)