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

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Abstract

Experiments have continued directed toward a solution of the problem of maintaining the body temperature of small experimental animals in a helium atmosphere, and toward an analysis of the significance of a lowered body temperature in susceptibility to influenza viral infection. Maintenance of the gas phase (1% O_2 in He, 500 psig) at 35°C enabled mice to preserve a body temperature within 2-3°C of normal. Additional experiments point toward an adverse effect of a normoxic O_2-He atmosphere, at normal or increased barometric (95 psig) pressures, even when the temperature of the gas phase is raised to a point (30°C) that enables mice to maintain a normal, or nearly normal, body temperature.

An experiment utilizing 11% O_2 in N_2 at one atmosphere for exposure of mice following inoculation with Coxsackie virus has confirmed the earlier observation of a more severe infection due to hypoxia as provided by an atmosphere of air at 7.3 psia.

An in vitro cell culture system has been set up by which the transforming effect of SV40 virus will be studied under parabarosis. Although definitive tests on the incidence of transformation in altered atmospheres are not yet complete, an increased resistance of transformed cells to O_2 toxicity has been observed.

1. Observations on temperatures of mice in parabaric environments.

An additional experiment has been completed, designed to determine the effect of an environmental temperature of 35°C on rectal temperatures of mice exposed to a 1% O_2 in He atmosphere at 500 psig. This is to be compared to a similar experiment in which the environmental temperature was 30°C, as summarized in Fig. 3, QSR No. 19 (1 January - 30 March, 1970). With the chamber elevated to 35°C, rectal temperatures of the lightly restrained mice became fairly well stabilized between 35.5 and 36.5°C after a sharp initial rise during compression when the internal chamber temperature increased to 37°C (Fig. 1). After the four hour exposure interval in 1% O_2 - He atmosphere at 500 psig (34 atm), decompression was initiated. Rectal temperatures rose steadily to original values during decompression to a condition of ambient pressure with normal pO_2 in He, even though the environmental chamber temperature was decreased to slightly below 35°C during the 80 minute decompression interval.

The results depicted in Fig. 1 may be compared with the rectal and subcutaneous temperatures recorded in mice maintained in a 2.8% O_2 in He atmosphere at 95 psig as presented in Fig. 5 of QSR No. 14 (1 October - 31 December, 1968). Although a compensating chamber temperature of 33-35°C in the 1968 experiments was able to maintain mouse subcutaneous and rectal temperatures at approximately 37°C, in the present experiment utilizing 1% O_2 in He atmosphere at 500 psig, the rectal temperatures stabilized only about 1°C lower when the environmental chamber temperature was maintained at 35°C. These observations would suggest that elevation of the environmental temperature of mice to 35°C, under an essentially normoxic pO_2 in He environment at 500 psig, is able to compensate for a large fraction of the body temperature loss due to the compressed helium atmosphere in the range from 95-500 psig. Such compensation will eliminate a significant portion of stress due to body heat loss, and will increase the possibility of identifying other variables causing alterations in susceptibility to experimental infections in mice under parabaric conditions.

2. Effect of chilling, in O_2 - He or air, on influenzal pneumonia in mice.

Previous experiments, PR8-30, 31, and 33, reported in QSR No. 19, gave evidence that chilling of mice by maintaining them in air at 15°C results in a greater mortality from influenza viral infection. Chilling may be one of the factors responsible for the increased mortality observed in similar groups maintained in hyperbaric (95 psig) - normoxic O_2 - He gas mixtures as described in QSR No. 12, experiments PR8-4 and 5. While complete

isolation of this factor was not achieved, the evidence derived indicated that a 5°C elevation of the ambient 20% O_2 - He environment would decrease the observed mortality to a level equal to or slightly less than that observed in a 25°C ambient air atmosphere. Since a 10°C elevation of chamber environment to 35°C has enabled mice to compensate for body heat loss when maintained in a normoxic (2.8% O_2 - He) environment at 95 psig, QSR No. 14 (1 October - 31 December, 1968) the outcome of influenza viral infection of mice kept at 95 psig in O_2 - He should not be influenced by loss of body heat per se if the ambient temperature is kept at 35°C.

The following experiments were designed to test the effect of a normoxic O_2 - He mixture at 95 psig on influenzal pneumonia in mice when loss of body heat was prevented by raising the temperature of the gas phase. Controls were maintained at ambient temperature (25°C) in either at 20% O_2 - He or air atmosphere.

Groups of 20-30 mice each, following aerosol challenge with influenza virus, were held in the parabaric atmospheres described below for determination of mortality rates. In one instance, infectious titers of lung pools were also determined. For PR8-35, the three experimental groups consisted of 20 mice each and were exposed post-challenge to a 2.8% O_2 - He, 95 psig, atmosphere at 30°C with one control group being exposed to a 20% O_2 - He atmosphere at 25°C and ambient pressure and the second control group to ambient air at 25°C. In this experiment (not illustrated) the challenge aerosol from a newly prepared purified pool of PR-8 virus caused over 95% mortality in all three experimental groups and little difference could be discerned under the various environmental conditions.

In PR8-37, the experimental conditions consisted of exposure of 20 mice to a 2.8% O_2-He atmosphere at 35°C, an environment enabling the experimental mice to maintain nearly normal body temperatures. An earlier and more severe (95%) mortality occurred in the hyperbaric mice than in the control group (75%), as shown in Fig. 2. Similarly, in PR8-38 (Fig. 3), the hyperbaric group (95 psig, 2.8% O_2 - He) maintained in a 35°C environment, again demonstrated more than a 25% greater mortality than occurred in the corresponding control group held in an ambient 20% O_2 - He mixture at 25°C, even though this latter atmosphere stresses the mice by causing an approximate 5°C reduction in intrarectal or subcutaneous temperature as described in Fig. 3, QSR No. 14 (1 October - 31 December, 1968).

The parabaric conditions for PR8-39 for both hyperbaric-normoxic O_2 - He and ambient O_2 - He atmospheres were as described for the previous experiment, PR8-38. In this instance, the parabaric and

mortality groups were increased to 25 mice each with an additional eight in each group similarly exposed and maintained for sacrifice and preparation of 10% lung pools from four mice each at the fifth and seventh day post-challenge exposure interval. Earlier deaths and a greater mortality rate (84%) was again observed (Fig. 4) in the 35°C temperature compensated hyperbaric group, when compared with the 20% O_2 - He group (at 25°C) in which the mortality rate was 56%. Infectivity (EID_{50}) titrations performed on the fifth and seventh day lung pool preparations, however, again showed only minimal differences between test and control mice, as calculated by Karbers method and shown in Table 1. The gross pathology scores for lung consolidation were also found to be almost identical.

Table 1. Experiment PR8-39. Effect of parabaric conditions on mouse lung infection following aerosol challenge with PR-8 influenza virus.

Environment	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	5	4	15.6	0.18	0.2	$10^{7.3}$
2.8% O_2 in He					(0.3, 0.3, 0.1, 0.1)	
95 psig	7	4	12.9	0.23	2.1	$10^{5.7}$
35°C					(3.0, 2.5, 2.0, 1.0)	
Group B	5	4	15.9	0.20	0.15	$10^{7.1}$
20% O_2 in He					(0.3, 0.2, 0.1, 0.0)	
1 atm	7	4	14.0	0.24	2.1	$10^{5.3}$
25°C					(3.0, 3.0, 2.0, 1.5)	
Group D	5	4	15.0	0.20	0.2	$10^{7.1}$
Tank Air					(0.3, 0.3, 0.2, 0.1)	
1 atm	7	4	13.4	0.28	2.8	$10^{5.5}$
25°C					(3.5, 3.0, 2.5, 2.0)	

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

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

Aerosol exposure: 25 min. using 1:450 dilution Pool A (PR8), 0.25 ml/min. R.H. 88%

One additional experiment, PR8-40, was performed to determine again whether the reported mortality increase in mice exposed to ambient 20% O_2 - He atmosphere at 25°C as compared to ambient air at the same temperature could be demonstrated. The experimental groups in this instance consisted of 30 mice each and again a 25% greater mortality was observed in the O_2 - He mixture as compared with the air control group, as presented in Fig. 5.

The data from these and earlier experiments indicate that in normobaric, normoxic helium (20% O_2 - He) atmospheres at 25°C, the stress from loss of body heat in this artificial environment contributed significantly to a decrease in resistance to influenzal infection in the mouse. The loss of body heat can be prevented by elevation of the environmental chamber temperature to a sufficient level, i.e., 30°C. Elevation of environmental temperature to 35°C in a hyperbaric normoxic (2.8% O_2 - He) environment, will maintain mouse intrarectal or subcutaneous temperatures close to normal limits. But even with body temperatures approximately normal, such parabaric conditions still result in a greater mortality in influenzal mice than in appropriate control groups. It is, therefore, reasonable to postulate that some other factor(s) in the hyperbaric, normoxic - He environment accounts for the increased mortality observed repeatedly in influenza virus infected mice kept under this condition.

3. Effect of parabarosis on mouse lung influenzal infection; microscopic study.

In a single experiment PR8-34, the experimental conditions were designed to evaluate the influenzal process by measuring either altered mortality rates or changes in the lung pathologic process by routine light microscopy section supplemented with electron microscopy sections if indicated. The parabaric exposure was limited to 77% O_2 at one atmosphere following aerosol challenge with either PR-8 influenza virus diluted in 0.5% bovine plasma albumin in phosphate buffered saline, or to the diluent alone (controls). The resultant mortality curves are depicted in Fig. 6 and may be observed to confirm previously reported experiments, PR8-11, 13, and 17, where increased mortalities were always seen in mice exposed to hyperoxic environments. Conventional H & E staining has been performed on representative sections prepared for light microscopy and their interpretation utilized to select the more promising areas to prepare for ultramicroscopic sectioning and interpretation to be reported later.

4. Preparation of influenza virus pool.

Experiments PR8-1 through 33 depleted the original PR-8 pool (A). An additional partially purified high titer PR-8 pool B has been prepared and stored as aliquots for future experiments. Titrations have been performed to allow the selection of doses of aerosolized virus to induce greater or less severe infections, depending upon the objective of the experiments. Titrations have also been performed to provide positive controls in the usual EID_{50} and hemagglutination tests.

5. Experiments with Coxsackie virus.

Experiment Coxsackie #12 has been completed with post-challenge parabaric exposure limited to hyperoxia (77% O_2, one atmosphere), hypoxia (11% O_2, one atmosphere), in contrast to the hypoxic environment of air at 7.3 psia utilized in Coxsackie #10, and to an ambient air control group. The results confirm the results of earlier experiments.

Groups of 20 mice each were challenged i.p. as before with 0.25 ml of 1:500 dilution of pool A (equivalent to 2500 LD_{50} suckling mouse doses). Four mice from each group were sacrificed on day 3 post-challenge and three mice in each group on days 5 and 7 for preparation of 10% pancreatic pool suspensions. The remaining 10 mice in each group were maintained under the parabaric atmospheres for observations on mortality.

A definite increase in pancreatic tissue viral titer, as determined by standard plaquing procedures using LLC-MK2, rhesus monkey kidney cells, was observed in animals exposed to the decreased pO2 at ambient pressure. As presented in Table 2, a plaque titer of 8800 x 10^3/gm pancreatic tissue was observed in hypoxic animals on day 3 post-challenge as compared to 1100 x 10^3/gm for the hyperoxic group and 570 x 10^3/gm for ambient air controls. Although the plaque titers were sharply reduced at sacrifice on day 5 post-challenge, the values for the hypoxic animals were still elevated (48 x 10^3/gm) over either hyperoxic (5.8 x 10^3/gm), or air in the control animals (3.3 x 10^3/gm). Plaque assays were not performed on the seventh day post-challenge pancreatic pools because of the abrupt decrease observed at day 5. No deaths occurred in any of the mouse groups during the 21 days of the experiment.

6. Effect of increased oxygen tensions on latent virus infections (by E. Zebovitz and S. Bond)

The microbial flora of the human body is composed not only of bacterial species, but of a number of viruses which are present as latent

or masked infections within the cells of their host. Normally, the host animal does not exhibit disease symptoms and appears healthy, and as a consequence, the presence of the virus usually is unsuspected. Certain viruses which can establish latent infections in man are oncogenic, i.e., they produce tumors in various animal species and transform cells in tissue culture.

Table 2. (Coxsackie Exp. #12) Effect of parabarosis on susceptibility of adult mice to Coxsackie B-1 infection; parabaric exposure followed challenge.

Group and environment (1 atm pressure)	Mortality	Observation 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[b]	3	0.93	1100
		5	0.63	5.8
		7	1.45	ND[c]
C, 11% O_2 in N_2, (hypoxia)	0/10	3	0.42	8800
		5	0.32	48
		7	0.45	ND
D, line air (normoxia)	0/10	3	0.30	570
		5	0.19	3.3
		7	0.28	ND

[a] Four mice sacrificed on day 3 and three mice on day 5 and 7 for each pancreatic pool.

[b] No deaths in any group during 21 days exposure.

[c] ND = Not done

The response of latent viruses to alterations in the external environment to which man may be subjected is little known, except for the well-recognized effects of fever, ultraviolet irradiation, etc., on herpes virus. It is possible that

unusual environmental conditions will trigger a normally latent virus to express its oncogenic capacity. From what is known about oxygen toxicity and other effects of abnormal oxygen tensions, it is reasonable to postulate that environmental changes of this type can activate latent oncogenic viruses.

A model system has been devised to study the effect of oxygen concentration on the transforming activity of an oncogenic virus. The virus selected was SV_{40} (Simian vacuolating) since it has the highest transforming activity of any known oncogenic virus. An epithelial line of cells was derived in this laboratory from an inbred strain of mice, the AL/N strain, which has a low susceptibility to the development of spontaneous tumors.

Approximately 1-2% of cells exposed to SV_{40} virus were transformed under normal oxygen tensions. Preliminary experiments show no dramatic enhancement or suppression of the frequency of transformation when infected cells were exposed to one or more 18 hr pulses of 95% oxygen at 4 day intervals. Oxygen, however, is deleterious to normal cells and lowers their plating efficiency 5 to 10 fold below normal. Transformed cells on the other hand appear to be resistant to high levels of oxygen.

The effect of prolonged exposure to high oxygen tensions on the frequency of viral induced transformations is being investigated further.

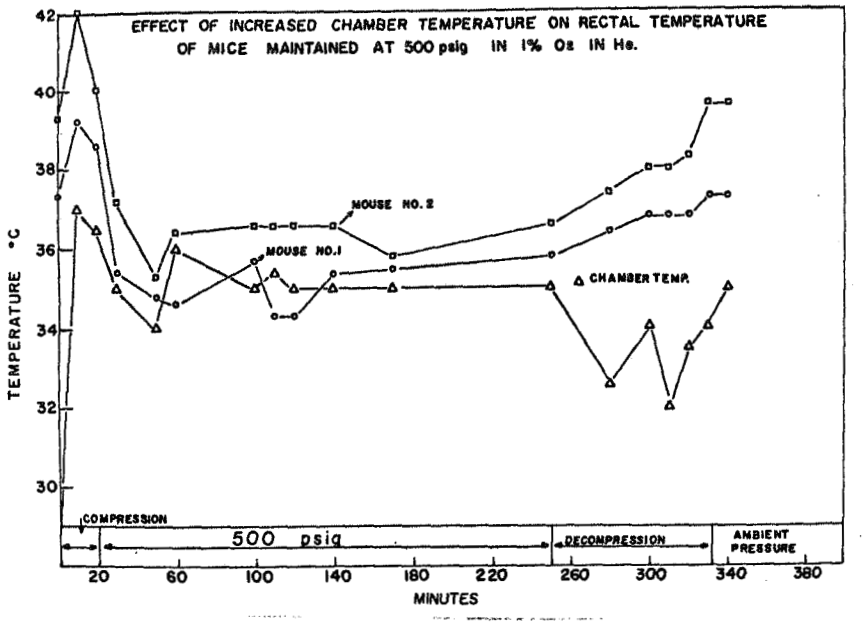

Figure 1. Ability of increased temperature (35°C) of gas phase to enhance maintenance of normal body of mice in normoxic (1% O_2) - He environment as compared to a 30°C temperature of the gas phase as depicted in Fig. 3 of QSR-19.

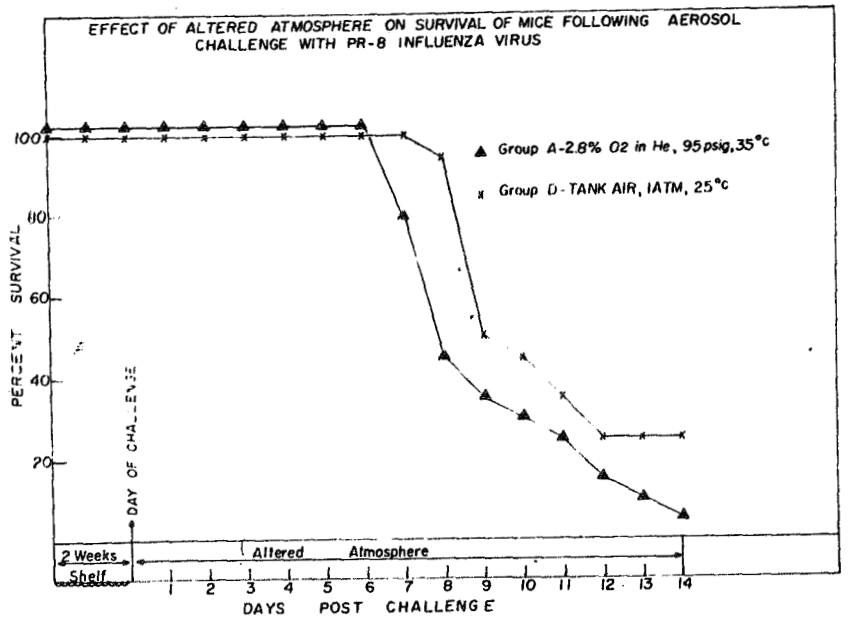

Figure 2. Exp. PR8-37. Failure of increased temperature (35°C) of gas phase to prevent increased mortality in influenzal mice exposed to normoxic (2.8% O_2) - He environment at 95 psig as compared with ambient air (25°C) influenzal controls.

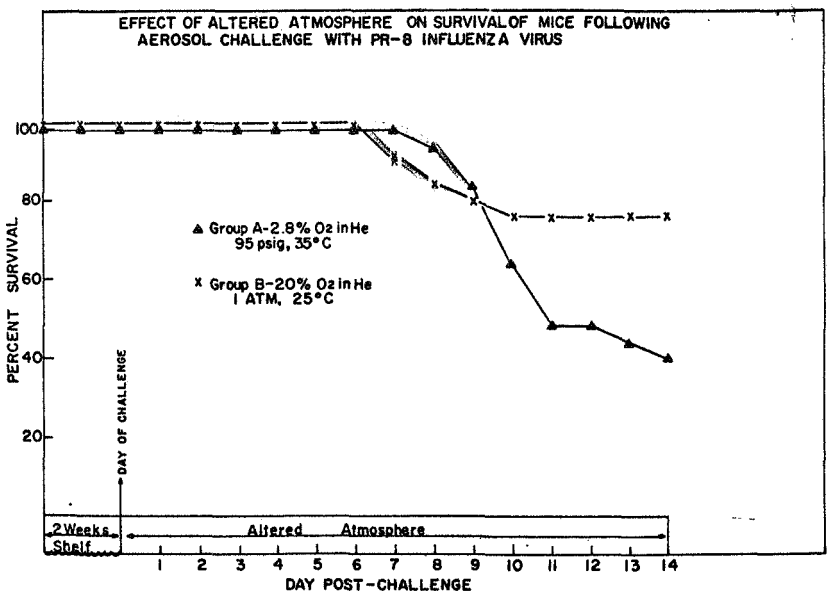

Figure 3. Exp. PR8-38. Failure of increased temperature (35°C) of gas phase to prevent increased mortality in influenzal mice exposed to normoxic (2.8% O_2) - He environment at 95 psig as compared with ambient 20% O_2-He (25°C) influenzal controls.

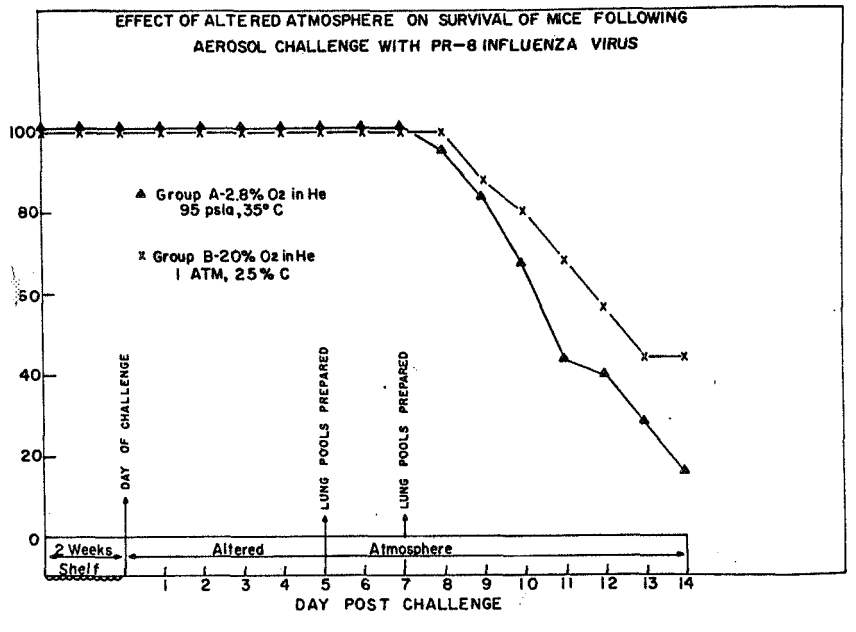

Figure 4. Exp. PR8-39. Failure of increased temperature (35°C) of gas phase to prevent increased mortality in influenzal mice exposed to normoxic (2.8% O_2) - He environment at 95 psig as compared with ambient 20% O_2-He (25°C) influenzal controls. Note minimal differences in lung consolidation and infectivity titration between test and control mice at sacrifice as presented in Table 1.

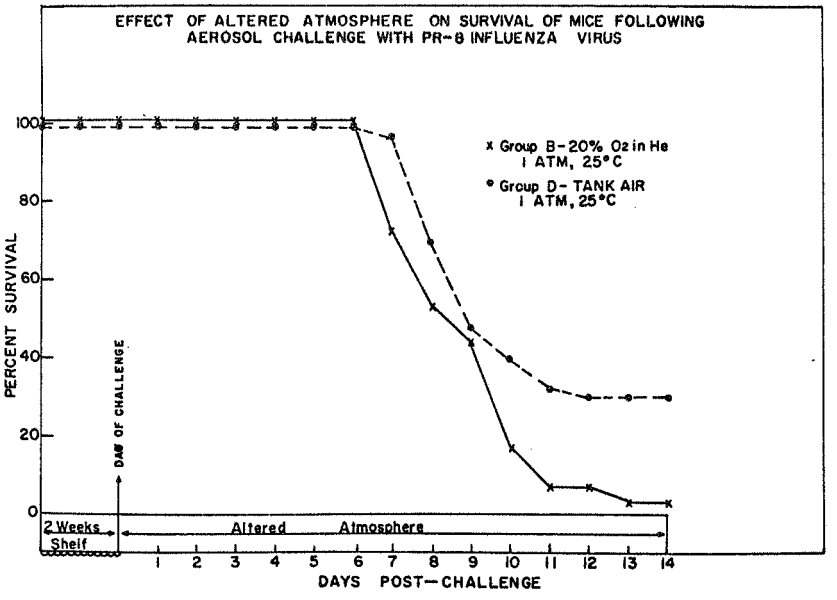

Figure 5. Exp. PR8-40. Effect of chilling in ambient gas phase of 20% O_2 in He (25°C) on mouse influenzal pneumonia.

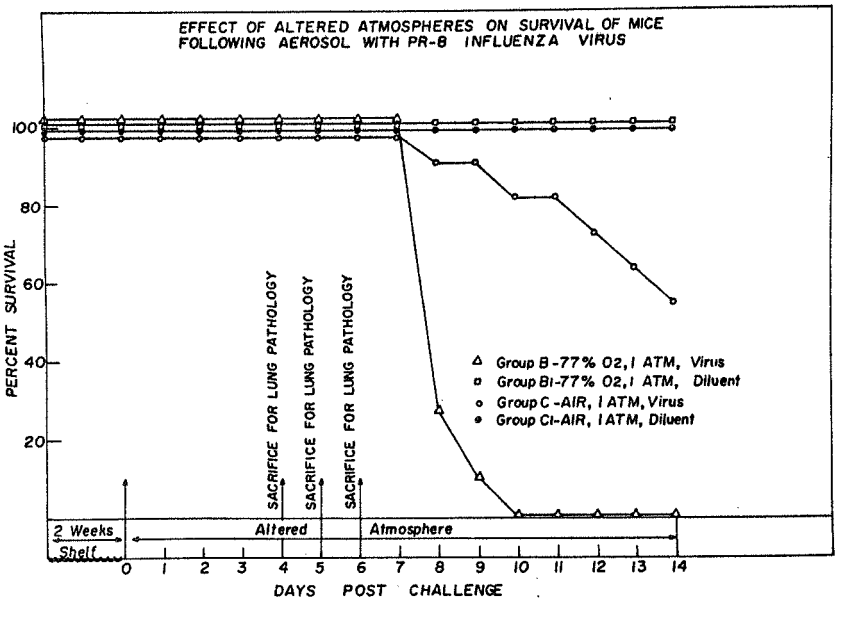

Figure 6. Exp. PR8-40. Effect of ambient hyperoxic or normoxic environmental atmospheres on mouse lung influenza as compared with uninfected diluent control mice. Note intervals of sacrifice for preparation of lung histopathologic sections.