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

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Abstract.

Experiments with mice in pressure chambers have been performed continually during the period of improvement of the chambers in terms of prevention of undesirable concentrations of water vapor, CO_2, NH_3. As these improvements have been put into practice, however, the earlier results with infectious agents and observations on fecal flora have been confirmed. However, increasing difficulty has been encountered in monitoring mice in He-O_2 mixtures, attributable to increased loss of body heat. New chambers with temperature controls, now on order, are expected to remove this difficulty. The difficulties, however, are encountered under hyperbaric conditions, and the remarks above do not describe the situation with simulated altitude.

Attention was called previously to the greater mortality in mice held at simulated altitude with an ambient normal pO_2 (160 mm Hg) following aerosol exposure to the mouse pneumonitis strain of Chlamydia. If the Bohr equation for estimating alveolar pO_2 for man is used, it is clear that an alveolar hypoxia occurs under these conditions, raising the question whether hypoxia can account for the observed results. An experiment (Mopn 36) in which this hypothesis was tested directly by holding mice in air at 7.3 psia (pO_2, 80 mm Hg) after aerosol exposure to the mouse pneumonitis agent provided evidence that hypoxia per se does not account for the greater mortality observed at simulated altitude. Although a slightly greater degree of infection probably occurred in the hypoxic mice, compared to controls, their motality rates were essentially the same.

The increased mortality observed previously in influenza virus-infected mice held at simulated altitude with 100% ambient O_2 might be due to increased multiplication of virus, an increase in inflammatory reaction in the host, or to a reduced ability of the mouse at altitude to tolerate normal levels of the two preceding factors. Exp. PR-8 #7 was designed primarily to measure the level of influenza virus in test and control mice. A preliminary experiment provided information for designing a suitable protocol, the results of which gave no evidence for increase in virus levels. The somewhat crude methods of assessing extent of inflammatory response also showed no striking differences between test and control groups.

Two experiments were performed in which mice were held at simulated altitude (37,000 ft) with normal ambient pO_2, following intraperitoneal inoculation of Chlamydia psittaci. The results confirmed previous observations in which a greater survival rate occurred in the normal pO_2 group at simulated altitude, compared to controls, and a greater mortality was seen in the hypoxic group. As noted in QSR No. 12, the former effect is in direct contrast to the results seen with a similar disease agent, C. trachomatis (mouse pneumonitis strain) administered by aerosol.

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

Experiment 16.-In our previous experiments of this type the control of CO_2 tension was not entirely satisfactory. An improved system has been devised in which the CO_2 concentration in the hyperbaric chamber (2.8% O_2 in He, 95 psig) was maintained at 0.03 - 0.08 vol. percent by use of an absorbent hydroxide bed within the chamber (Baralyme, National Gas Co.). Control animals were maintained at 1 atm in flowing air in an identical chamber. At 95 psig (7 atmospheres) the CO_2 concentration noted above is equivalent to 0.21 - 0.56 vol. percent at one atmosphere, an acceptable figure.

As before, samplings were obtained at -2 week and 0 week before starting parabaric exposure and at bi-weekly intervals after maintenance under altered atmosphere. Alterations were similar to those seen before and the experiment was discontinued after the 6th week.

In the present experiment, typical E. coli were not present in detectable numbers at any sampling interval in either experimental or control groups. A single mouse was found to be excreting low numbers of Klebsiella-aerogenes at the -2 week sampling interval but none was present in the lowest dilutions plated at any subsequent sampling interval. In the 95 psig He-O_2 atmosphere, slow lactose fermenters and atypical E. coli, both decreased by orders of 1 to 2 magnitudes below concentrations observed in 1 atmosphere control animals. On the other hand, Group D fecal streptococci again sharply increased (to a level two orders of magnitude greater than controls) following both 2 and 4 weeks exposure to the altered atmosphere.

In the anaerobic series of organisms enumerated, all three Bacteroides strains remained at a slightly higher level than controls at both +2 and +4 weeks sampling intervals but the differences were only statistically significant ($.01 > P > .001$) in 3 instances. Numbers of both strains of obligate anaerobic lactobacilli remained very uniform throughout in both experimental and control groups.

2. Effect of parabarosis on infections of mice with chlamydial agents.

In the preceding QSR, an increased mortality, in comparison to controls, was demonstrated in mice exposed to either hyper- or hypobaric atmospheres with normal ambient pO_2, and challenged with the mouse pneumonitis agent. It can be argued that mice in such a hypobaric environment are slightly hypoxic even though the ambient gas phase provides a normal pO_2, if the Bohr equation for estimating alveolar pO_2 is used. In the present experiment (Mopn #36), the parabaric condition was limited to post-challenge exposure to decreased pressure with hypoxia (pO_2, 80 mm Hg; tank air, 7.3 psia). Controls were maintained at one atmosphere pressure in flowing line air. Two groups of 22 mice each were exposed to an infecting aerosol and each group was then separated into parabaric and control subgroups and placed in the environments described above. Group 1 was held for observation of mortality rates only. Mice of Group 2 were killed at intervals and various

observations were recorded. The results of this experiment are shown in Table 1.

It may be seen that mortality rates of test and control subgroups were essentially the same. Deaths occurred over the period of the 12th to 21st day. Average mouse weights in hypoxic infected animals were consistently less than controls after 8 or more days exposure to altered atmosphere. Average lung weights at sacrifice in general followed the severity of infection as indicated by the gross pathology score (extent of consolidation). Lung infectivity titers, expressed as numbers of inclusion-forming units (IFU) per ml, determined in cell cultures, showed an almost 15-fold increase in the hypoxic exposed animals as compared to only a 5-fold increase in the ambient air mice. While differences in lung infectivity titers were too small to be of significance at either the 8 or 12 day sacrifice intervals, the observed increased in the hypoxic group at 16 days was significant to the $.01 > P > .001$ level.

Taken together, these several types of observations indicate a slightly greater infection in the hypoxic mice, or perhaps more precisely, a delayed recovery from this intermediate level of exposure dose. The significant finding is, as expected, that exposure to hypoxia, for a period of time, favors greater infection with this bacterium-like agent, but that hypoxia at this level does not result in immediate greater mortality, as has been observed repeatedly in mice exposed to simulated 37,000 ft altitude but with normal ambient pO_2 (100% O_2, 3.0 psia). (See, for example, Exp. Mopn #33, Fig. 3 of QSR No. 11, Jan. - March, 1968). The greater mortality seen in mice at simulated altitudes in 100% O_2, therefore, cannot be due to a lowered alveolar pO_2.

C. psittaci infection. To confirm and extend the results of Exp. Psitt-1, described in the previous QSR, a similar experiment (Psitt-2) has been completed with the same parabaric groups and i.p. challenge with 1 LD_{50} dose of C. psittaci. The parabaric exposure was initiated two weeks prior to challenge and continued for 10 days thereafter. Since only 3 mice survived the two weeks exposure to 2.8% O_2 in He, 95 psig, before i.p. challenge, they are omitted from data presented in Fig. 1. The greater survival in Group B (simulated altitude, normal pO_2), previously described, was again evident as well as an increased mortality in the hypoxic group (7.3 psig, air) over line air control animals at 1 atmosphere.

An additional experiment (Psitt-3) utilizing the same parabaric conditions before and after challenge was also performed in an attempt to provide more reliable data on the 2.8% O_2 in He, 95 psig, Group A animals. Although the hyperbaric, normoxic group again did not survive the 2 weeks pre-exposure interval, confirmation was again obtained of decreased mortality in the hypobaric, normoxic Group B animals, and an increased mortality in Group C, hypobaric (7.3 psia), hypoxic (pO_2 80 mm Hg) animals. Although the differences between controls and test animals are not great in any single experiment of this series, they are consistently in the same direction and are therefore considered to be representative and significant.

The loss of mice in the He-O_2 atmosphere, as mentioned before, is attributed to loss of

body heat in He as compared with N_2. Larger animal holding pressure chambers, in which the internal temperature can be controlled, are expected to compensate for this factor. Delivery is expected soon.

3. Effect of parabarosis on pulmonary infection of mice with PR-8 influenza virus.

In previous QSR's attention was directed towards demonstration of altered mortality rates by pre- or post-challenge exposures to parabaric conditions utilizing PR-8 influenza aerosols as the challenge virus. If we postulate that local alteration in lung tissue due to the type of parabaric exposure is a significant factor, we must ask also whether an altered host factor is responsible for the different clinical outcome, or whether altered viral multiplication within the pulmonary tissue is a contributing factor.

Experiment PR-8 #6 was exploratory, and designed to determine the lung infectivity titers of mice, expressed as EID_{50} (50% egg infective dose) following aerosol challenge with PR-8. An additional group of mice exposed to the same challenge was included for observation of mortality rate. The first lung tissue pool prepared from 4 mice on the 6th day following challenge had a titer of $10^{6.75}$ EID_{50} per gram of lung tissue, although no deaths had occurred in any of the exposed animals. EID_{50} titer rose to $10^{7.5}$ on the 7th day and 25% mortality was observed in the control group. On the 8th day the titer fell to $10^{6.5}$, but the mortality rate had risen to 48%. The lung pool infectivity titer on the 9th day was $10^{5.7}$ with a corresponding mortality of 55%. The final mortality rate was 82% on the 12th day post-challenge. In our test system therefore there is a rapid multiplication of virus in the lungs during the first 5 days post-challenge with the peak concentration being present at the onset of mortality. Following this, lung infectivity titers decline while mortality steadily increases towards 100%.

Experiment PR-8 #7 was therefore designed to measure any alteration in lung infectivity titers of mice exposed to 100% oxygen atmosphere at 3.3 ± 0.1 psia following aerosol challenge, in comparison with similar animals maintained at normal ambient atmosphere. Ten percent lung pool suspensions were prepared from 3 mice of each group on the 5th, 6th, 7th and 8th day following challenge and EID_{50} titrations again were performed in fertile eggs. Results are given in Table 2.

It is evident that during this interval following aerosol challenge, when viral concentration was high and degree of lung involvement was increasing, that the actual lung infectivity titers in both groups of mice (hypobaric with 100% oxygen, and 1 atm control animals) show little difference. This result suggests that the greatly increased mortality from influenza virus, observed in hypobaric mice exposed to hypobaric 100% O_2 atmospheres, is not due to an increase in the level of virus in the lung, but supports the hypothesis that an altered host factor is responsible. This experiment provides little evidence that a greater inflammatory response occurs in the test mice, so that the factor accounting for greater mortality has not yet been identified. As discussed briefly, in a section above, there may be a slight alveolar

hypoxia in mice maintained at 100% oxygen and 3.3 ± .1 psia, as indicated for man by the Bohr equation. However, previous observations (see QSR No. 12, April-June, 1968) indicate that hypoxia has the effect of reducing the mortality of mice, the opposite of the effect seen in our hypobaric normoxic tests.

Additional experiments to examine this effect are programmed.

Table 1 (Exp. Mopn 36). Effect of hypobaric, hypoxic environment on mouse lung infection with a chlamydial agent (Mouse pneumonitis).

Environment (post-challenge exposure)	Group 1 (Accumulated mortality at 24 days)	Group 2 Day of assay	No. of mice	Av. mouse wt.	Av. lung wt.	Gross pathol. of lung[a]	Lung titer IFU/ml X 10^5
Tank air 7.3 psia (pO_2 80mm Hg)	6/11	8	4	13.7	0.39	1.5	0.7 (+) 0.15
		12	4	13.0	0.38	2.0	1.8 (+) 0.2
		16	3	13.8	0.40	2.0	10.0 (+) 3.7
Line air 1 atm. (pO_2 160mm Hg)	5/11	8	4	17.7	0.28	1.3	0.6 (+) 0.15
		12	4	16.1	0.55	3.0	2.3 (+) 0.1
		16	2	21.0	0.31	0.5	3.0 (+) 0.4

[a] Arbitrary 0-5 scoring for degree of lung involvement.

Aerosol exposure 20 minutes using 1:5 dilution of 50% mouse pneumonitis pool at rate of 0.35 ml/min and relative humidity 91.2%.

Table 2 (Exp. PR-8 #7). Estimation of lung involvement in parabaric and control mice, following aerosol exposure to influenza virus.

Environment (following challenge)	Day after challenge	Pool	Av. mouse wt. (g)	Av. lung wt. (g)	Degree of consolidation (0-4 scale)	Titer; EID_{50} per g
100% O_2, 3.3 ± 0.1 psia	5	B-1	14.6	0.32	0.3	$10^{7.5}$
	6	B-2	14.0	0.35	1.3	$10^{7.5}$
	7	B-3	13.6	0.39	1.3	$10^{7.7}$
	8	B-4	13.4	0.48	2.5	$10^{7.3}$
Flowing air, 1 atmosphere (controls)	5	C-1	17.8	0.36	0.0	$10^{7.5}$
	6	C-2	15.4	0.38	2.0	$10^{7.3}$
	7	C-3	14.9	0.44	2.3	$10^{7.3}$
	8	C-4	15.6	0.44	2.3	$10^{7.5}$

Each pool was composed of 4 mice, except B4 with 2 mice.

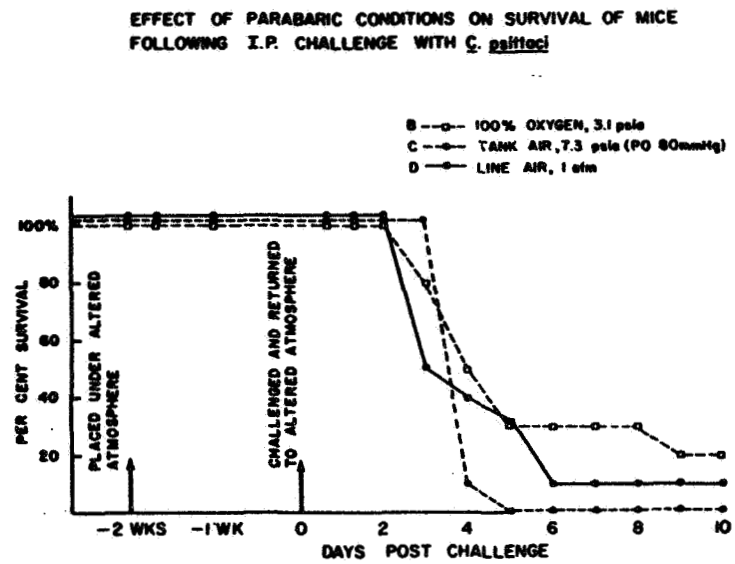

Fig. 1. (Exp. Psitt-2). Each group of 10 mice received 0.5 ml intraperitoneal inoculations of Chlamydia psittaci diluted to contain approximately one LD_{50}.