Variation in Response to Hyperbaric Oxygen among Inbred Strains of Mice* (33398)

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The therapeutic application of hyperbaric oxygen (HBO) in certain disease states is limited by oxygen toxicity. The two primary manifestations of oxygen toxicity stem from the central nervous system and the lungs (1). Small laboratory animals are generally quite susceptible to oxygen toxicity, and thus are useful in investigating this phenomenon (1, 2). However, oxygen toxicity in small experimental animals limits other areas of hyperbaric research such as testing the effectiveness of HBO therapy for infections and therefore must be circumvented if possible (3–5).

During the course of investigating HBO treatment of a model anaerobic infection in mice (6), both central nervous system and pulmonary forms of oxygen toxicity were observed. The development of oxygen toxicity in the HBO-treated animals was an experimental liability. In attempting to alleviate this liability another mouse strain was tested and found to have a greater tolerance to HBO than the strain previously used. Information concerning variation in response to HBO exposure among the many different inbred strains of laboratory mice was not available in the literature. However, if significant differences in oxygen tolerance exist among inbred strains, such information would permit a more intelligent selection of the strain of mouse best suited for any particular investigation utilizing HBO environments. Thus, a survey of the tolerance of various inbred strains of mice to HBO exposure was undertaken.

Materials and Methods. Eighteen inbred strains of male mice (approximately 11 weeks of age) were used in the study.[1] Survival times were used as the end point for comparing the responses of the different strains. An oxygen pressure at 3 atmospheres absolute pressure (ATA) was used in the initial studies since this pressure is commonly used to treat patients. Ten hyperbaric experiments were performed in which a single mouse from each of the 18 different strains was visually followed until death as indicated by cessation of respiration.

Hyperbaric exposures were carried out in a variable-temperature pressure chamber (80 × 50 cm outside dimensions) with viewing windows at each end of the cylinder. The mice were placed in small individual cages (7 × 9 cm cylinder) of wire mesh which were fastened to an expanded-metal rack resting over a pan containing soda lime. The arrangement allowed individual observations of each mouse and free diffusion of gases around the mice. The mice were given Purina lab chow and water *ad libitum* before the experiments, but were not given food or water during HBO exposures. Oxygen used was USP medical oxygen, moisture free, purity 99.5% or over (National Welders Supply Co., Inc.). The hyperbaric chamber was flushed with oxygen prior to pressurization to remove ambient air, and a continual controlled flow of oxygen was maintained while the chamber was at pressure to negate any accumulation of expired CO_2. Analyses of the O_2 and CO_2 content of the chamber during its operation showed no inert gas and negligible CO_2 ($<0.1\%$). The temperature within the chamber was maintained at 21° ($\pm 1°$). The relative humidity in the chamber was monitored, and the values ranged from approximately 85% during the early part of the

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[1] Furnished by the Jackson Laboratory of Bar Harbor, Maine.

TABLE I. Survival Times of 18 Inbred Mouse Strains in 100% O_2 at 3 ATA.

Rank[a]	Strain	Mean survival time (hr)[b]	N
1	CBA/J	9.8 ± 4.8	9
2	C3H/HeJ	10.4 ± 2.3	10
3	C3HeB/FeJ	14.1 ± 3.1	10
4	C57BL/6J	14.5 ± 1.2	10
5	RF/J	15.2 ± 3.0	6
6	DBA/2J	15.5 ± 0.8	9
7	SWR/J	15.5 ± 4.2	10
8	AKR/J	15.6 ± 1.2	10
9	C57BL/10J	16.2 ± 1.6	10
10	129/J	16.6 ± 2.1	10
11	C57L/J	17.5 ± 2.9	10
12	A/HeJ	17.8 ± 3.0	10
13	DBA/1J	18.0 ± 2.2	10
14	SJL/J	18.2 ± 1.5	10
15	A/J	18.9 ± 2.6	9
16	BALB/cJ	19.1 ± 2.4	10
17	C58/J	19.8 ± 1.4	9
18	C57BR/cdJ	21.7 ± 2.3	9

[a] Increasing mean survival time.
[b] Mean ± SD.

exposure to a low of approximately 45% after 24 hr.

Results and Discussion. The results of these experiments are shown in Table I. An analysis of variance was performed comparing strains and days. There was no significant difference between days; however, there were significant differences between strains. The Studentized Range Statistic was used to investigate the differences between specific pairs of means. In this analysis the mean of strain no. 1 was found to be significantly different ($p < .05$) from all other strains except no. 2, whereas the mean of strain no. 18 was only significantly different from the means of the five least resistant strains. Thus, the data readily indicates that strain no. 1 (CBA/J) is the least resistant to oxygen toxicity, but is less clear in indicating the most resistant strain.

The reproducibility of the results for the two extreme strains was verified by repeat experiments. In two successive experiments nine animals from each of the two strains were simultaneously observed in the chamber until their death. The Days-Strains analysis of variance showed no difference between days with a marked difference between strains. The means and standard deviations are given in Table II. These results indicate a remarkable agreement with the initial set of data.

A second interesting observation in these studies was the difference in the form of oxygen toxicity which generally prevailed in a strain. The three most susceptible strains of mice (nos. 1, 2, and 3, Table I) showed the highest incidence of central nervous system involvement including convulsive episodes (grand mal convulsions with clonic and tonic features) and lesser symptoms (tremors). Occasional convulsions and tremors were seen in some of the strains of mice falling within the middle range of tolerance; however, the primary manifestation of oxygen poisoning in these strains was pulmonary distress as the exposure progressed. The most resistant strains of mice generally showed few central nervous system symptoms but instead finally succumbed with pulmonary toxic manifestations. The strain (C57BR/cdJ) with the greatest mean survival time was never observed to convulse during the initial experiments or the repeat studies at 3 ATA.

There is a general demarcation at approximately 3 ATA of 100% O_2 beyond which central nervous system manifestations of oxygen toxicity increase in prominence with increasing oxygen tensions. At 3 ATA of 100% O_2 strain CBA/J (no. 1) was very susceptible to central nervous system toxicity whereas C57BR/cdJ (no. 18) was resistant to both pulmonary and central nervous system manifestations. Further experiments were performed at 4 ATA of 100% O_2 to determine whether the differences in forms of oxygen toxicity and survival times would persist in these two strains. Nine mice of each

TABLE II. Survival Times of the Two Extreme Strains (from Table I) in a Repeat Experiment at 3 ATA of 100% O_2.

No.	Strain	Mean survival time (hr)[a]	N
1	(CBA/J)	9.6 ± 3.1	18
18	(C57BR/cdJ)	21.4 ± 3.1	18

[a] Mean ± SD.

TABLE III. Survival Times of the Two Extreme Strains (from Table I) at 4 ATA of 100% O_2.

No.	Strain	Mean survival time (hr)[a]	N
1	(CBA/J)	4.6 ± 0.6	18
18	(C57BR/cdJ)	9.4 ± 3.6	18

[a] Mean ± SD.

the C57BR/cdJ strain maintained its significantly greater resistance (using a rank test, $p < .001$).

strain were simultaneously exposed in two successive experiments and the particular symptoms of oxygen toxicity were recorded as well as survival data.

At 4 ATA of oxygen all (18) of the CBA/J mice convulsed, and the time to convulsion decreased sharply from an average of approximately 3.4 hr at 3 ATA to 0.7 hr at this pressure. Eight of the C57BR/cdJ mice convulsed violently as was typical of CBA/J, but the time to convulsion for this strain averaged approximately 3.4 hr. Six mice in this resistant group had lesser indications of central nervous system involvement, but there were still four animals in which pulmonary distress was the only obvious sign of toxicity. In both strains the mean survival time at 4 ATA was approximately halved (see Table III) as compared with 3 ATA, but the C57BR/cdJ strain maintained its significantly greater resistance (using a rank test, $p < .001$).

Beyond the experimental advantage of isolating inbred strains which have greater tolerance to hyperbaric oxygen, these widely divergent strains could be useful in investigating mechanisms of oxygen toxicity. Comparative studies of the innate biochemical and physiological characteristics of these two extreme strains might delineate some of the determinant factors in the development of oxygen toxicity. It is interesting to note that the three most susceptible inbred strains (nos. 1, 2, and 3, Table I) were derived from an identical genetic line (7). However, these strain differences also emphasize the error that may be encountered in expecting similar experimental results from different strains of a species.

Summary. Eighteen inbred mouse strains were exposed to 100% oxygen at 3 ATA to determine whether significant differences in oxygen tolerance exist among highly inbred strains. Significantly different survival times (mean survival time range = 9.8–21.7 hr) were demonstrated for certain strains. The form of toxic response to oxygen (pulmonary or central nervous system toxicity) varied between strains. At higher oxygen pressure (4 ATA) the proportional differences in mean survival times and variation in the types of toxic response to oxygen were maintained. Oxygen resistant mouse strains useful in evaluating HBO as a therapeutic agent for certain disease states have been delineated. Comparative studies of the inherent biochemical and physiological characteristics of these susceptible and resistant inbred strains could be useful in efforts to elucidate mechanisms of oxygen toxicity.

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