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Optimization of oxygen tolerance extension in rats by intermittent exposure

J. M. Clark, C. J. Lambertsen, R. Gelfand, and A. B. Troxel

University of Pennsylvania School of Medicine, Environmental Biomedical Stress Data Center, Institute for Environmental Medicine, Department of Biostatistics and Epidemiology, Philadelphia, Pennsylvania

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Clark, J. M., C. J. Lambertsen, R. Gelfand, and A. B. Troxel. Optimization of oxygen tolerance extension in rats by intermittent exposure. *J Appl Physiol* 100: 869–879, 2006. First published November 23, 2005; doi:10.1152/japplphysiol.00047.2005.—Optimization of oxygen tolerance extension by intermittent exposure was studied in groups of 20 rats exposed to systematically varied patterns of alternating oxygen and normoxic breathing periods at 4.0, 2.0, and 1.5 ATA. Oxygen periods of 20, 60, and 120 min were alternated with normoxic intervals that provided oxygen-to-normoxia ratios of 4:1, 2:1, 1:1, and 1:3. In general, median survival times had nearly linear relationships to increasing normoxic intervals with oxygen period held constant. Exceptions occurred at 4.0 and 2.0 ATA where a 5-min normoxic interval was too short for adequate recovery even with a 20-min oxygen period, and an oxygen period of 120 min was too long even with a normoxic interval of 30 min. These exceptions did not occur at 1.5 ATA. Survival time for many intermittent exposure patterns was equivalent to that for continuous exposure to an oxygen pressure definable as a time-weighted average of the alternating oxygen and normoxia periods. However, this predictive method underestimated the degree of protection achieved by several of the intermittent exposure patterns, especially those performed at 4.0 ATA. Results provided guidance for selection of intermittent exposure patterns for direct evaluation in humans breathing oxygen at 2.0 ATA. Definition of intermittent exposure patterns and conditions that produced prominent gains in oxygen tolerance can also facilitate the performance of future experiments designed to study potential mechanisms for oxygen tolerance extension by intermittent exposure. Heat shock and oxidation-specific stress proteins that are induced by exposure to oxidant injury are suggested for emphasis in such investigations.

O_2 toxicity; O_2 tolerance extension; biochemical mechanisms for O_2 tolerance extension

IT HAS BEEN DEMONSTRATED in human subjects (23), small mammals (20–22, 28), and insects (15) that systematic interruptions of toxic degrees of hyperoxic exposure can extend tolerance to the composite effects of oxygen poisoning. This concept of periodically interrupting hyperoxic exposure (alternation of hyperoxic and normoxic cycles) as a basis for delaying the development of neurological and pulmonary toxic effects has been elaborated in a dedicated Symposium (6), in reviews of oxygen toxicity (8, 10, 26), and in experiments designed specifically to study oxygen tolerance extension by intermittent exposure in guinea pigs (20–22) and rats (21). With the exception of the early work of Hall (20), however, there has been no attempt to determine optimal combinations of oxygen exposure periods and normoxic recovery intervals that would provide maximal extensions of neurological and pulmonary tolerance over a useful range of oxygen pressures. Hall exposed guinea pigs to several patterns of intermittent oxygen exposure at 3.0 ATA. Selected results of the guinea pig experiments, in conjunction with a study of human pulmonary tolerance to continuous oxygen breathing at 2.0 ATA (9), were purposely incorporated into the design of a related human study by Hendricks et al. (23) in which prominent extension of pulmonary tolerance at 2.0 ATA was achieved by alternation of 20-min oxygen exposure periods with 5-min normoxic intervals.

The present studies of extreme degrees and durations of intermittent oxygen exposures in rats were performed in preparation for planned measurements of oxygen tolerance extension in human subjects exposed to additional patterns of intermittent oxygen breathing at 2.0 ATA. A major objective was the selection of intermittent exposure patterns that would complement the initial study by Hendricks et al. (23) and provide information relevant to determination of optimal patterns for intermittent oxygen exposure at 2.0 ATA. The rat studies were purposely designed to extend in degree and duration well beyond current and likely future human exposure conditions. To identify promising intermittent exposure patterns for selective evaluation in human subjects, rats were exposed systematically to alternating periods of hyperoxia and normoxia at 4.0, 2.0, and 1.5 ATA. Hypothetically, it was expected that an excessively long oxygen period would cause toxic effects that would not readily reverse during the subsequent normoxic interval. It was also considered likely that a very brief normoxic interval would not allow adequate reversal of toxic effects from even a relatively short oxygen period. These expectations were combined to form an overall hypothesis that some optimal combination of oxygen exposure and normoxic recovery periods will provide maximal extension of neurological and pulmonary oxygen tolerance. The selected range of oxygen pressures allowed comparison of results obtained at 4.0 ATA, where there are prominent interactions between pulmonary and central nervous system effects of oxygen toxicity, with similar data obtained at 2.0 and 1.5 ATA, where effects of pulmonary oxygen toxicity are not influenced by concurrent convulsions. It was recognized that development of maximal tolerance to this variable blend of neurological and pulmonary effects of oxygen toxicity might require a unique combination of oxygen-normoxia exposure periods at each level of hyperoxia.

Another objective of the present investigation was the facilitation of future experiments designed to study potential mechanisms for oxygen tolerance extension by intermittent exposure. The identification of intermittent exposure patterns and conditions that produce prominent gains in oxygen tolerance

Address for reprint requests and other correspondence: J. M. Clark, Institute for Environmental Medicine, Rm. 1, John Morgan Bldg., Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104-6068 (e-mail: jmclark@mail.med.upenn.edu).

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should guide the design of such experiments. The potential occurrence of increased antioxidant enzyme activities during intermittent oxygen exposures was previously evaluated with inconclusive results (21). However, the recent evolution of information regarding the "heat shock response" and similar "stress protein" responses to a variety of noxious stimuli, including oxidant injury, identifies several potential candidates for protective roles in research concerning oxygen tolerance extension (4, 29). These possibilities have not been investigated to date.

METHODS

To determine rates of recovery from different degrees of oxygen poisoning, oxygen exposure periods of 20, 60, or 120 min were each systematically alternated with constant normoxic intervals the durations of which were also varied systematically at oxygen pressures of 4.0, 2.0, and 1.5 ATA (Table 1). Durations of normoxic intervals were selected to provide the same hyperoxic-to-normoxic ratios for each of the three oxygen exposure periods. This was done to determine whether the toxic effects accumulated over a relatively long oxygen exposure (120 min) reversed on return to normoxia at the same rate as those that accumulated during shorter oxygen exposures (60 or 20 min).

Exposure Conditions

For each intermittent pattern, a group of 20 rats, housed individually in wire and Plexiglas cages, was exposed in a steel hyperbaric chamber with large viewports. Chamber concentrations of oxygen and carbon dioxide were monitored continuously. During oxygen periods, oxygen concentration was maintained at 99–100%. Carbon dioxide concentrations were near zero during both oxygen and normoxic periods. Ambient temperature was maintained within a range of 22–25°C. High gas-flow rates were used at the start of each oxygen or normoxic period to provide a 98% change of inspired gas within 90 s.

Animals

Male, specific pathogen-free, Charles River CD rats maintained on Ziegler rat and mouse diet were used in these exposures. Average weights of the different exposure groups ranged from □300 to 400 g with an overall average of □350 g. The experiment protocol was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Oxygen Tolerance Indexes in the Rat

Survival time. Elapsed oxygen time before cessation of breathing was determined by 24-h visual monitoring of all 20 rats in each intermittent oxygen exposure. Although it is recognized that many interacting factors determine the lethal duration of exposure to any toxic oxygen pressure, survival time in a sufficiently large animal population is an important systemic index of cumulative, composite effects of oxygen toxicity. At oxygen pressures of 1.5 and 2.0 ATA, pulmonary oxygen poisoning occurs in the absence of convulsions, whereas at 4.0 ATA there are prominent interactions between pulmonary effects of oxygen toxicity and the violent sympathetic nervous system activity associated with convulsions.

Convulsion time. The elapsed oxygen time before initial onset of seizures is also affected by many variables other than inspired oxygen pressure. During continuous or intermittent oxygen exposures at 4.0 ATA, however, onset of convulsions is a definite and usually an early manifestation of central nervous system oxygen poisoning.

Table 1. *Intermittent oxygen exposure patterns for optimization of tolerance extension in rats*

Oxygen Period		Normoxic Interval, min							
ATA	Minutes	5	10	15	20	30	60	120	180
4.0	20	4:1	2:1		1:1		1:3		
	60			4:1		2:1	1:1		1:3
	120					4:1	2:1	1:1	
2.0	20	4:1	2:1		1:1				
	60			4:1		2:1	1:1		1:3
	120					4:1	2:1	1:1	
1.5	20	4:1	2:1						
	60			4:1		2:1			
	120					4:1	2:1	1:1	

Values are ratios of oxygen-to-normoxia interval durations.

Model Analysis

The data set consisted of survival times for all of the exposed rats; some animals were right-censored if they had not expired by the end of the observation time. We fit both parametric and semiparametric survival analysis models to the data, using well-known approaches (25). Different models were chosen for the hazard function, leading to different functions and properties of the survival and failure functions. The likelihood function was then constructed as the product of a contribution from each rat. An animal whose death was observed contributed the probability of death at time t, whereas animals who were right-censored contributed the probability of living at least as long as time t. Parameter estimates were obtained by maximizing the log of the likelihood function as a function of the unknown model parameters. We accomplished this via iterative programs written in the statistical freeware package R using nonlinear optimization functions. Automated searches of the likelihood surface using multiple starting values were employed to avoid finding local maxima. Because the specific determinants of survival time are likely multiple, complex, and varied at different oxygen pressures, we stratified each model by estimating three sets of parameters, one for each of the three pressures employed.

Models

We fit three different models for the hazard function, beginning with two proposed by Harabin et al. (22). *Model 1* was a power curve in which

$$r\ t \square\square nc_i^{\,n}\ t_{O_2}\square^{n\square 1} \qquad (1)$$

where t_{O_2} was the length of time spent on oxygen, and c_i was a gain parameter for the ith oxygen schedule. The second model was an autocatalytic model in which the risk of death at time t was proportional to the buildup and breakdown of some toxic substance X, determined by the differential equation

$$dX/dt \square aO_2 \quad k\ O_2\square O_s\square X \qquad (2)$$

where a is a scale factor and k is a rate constant, and O_s is a parameter that allows for a change in behavior at different levels of oxygen; the hazard function is

$$r\ t\square\square \begin{cases} X\square\text{thr} & \text{if } X\square\text{thr} \\ 0 & \text{if } X\square\text{thr} \end{cases} \qquad (3)$$

Finally, we fit a proportional hazards model (11). This is a semiparametric model in which the baseline hazard function was estimated nonparametrically, and a parametric function was used to relate predictors Z to the overall hazard function. We maximized the partial (or conditional) likelihood, which corresponds to the likelihood function defined above for fully parametric models. The specific equation for *model 3* is as follows:

$$r\ t\ Z\square\square r_0\ t\square e^{\square\square_1\text{-ratio}\square\square_2\square\text{normtime}\square\square_3\ \square\ \text{ratio}\square\text{normtime}\square\square\square_4\square\text{conv}\square\square_5\square\text{convtime}\square} \qquad (4)$$

Here ratio is the ratio of normoxic time to oxygen time, normtime is the length of the normoxic interval, conv is a binary variable indicat-

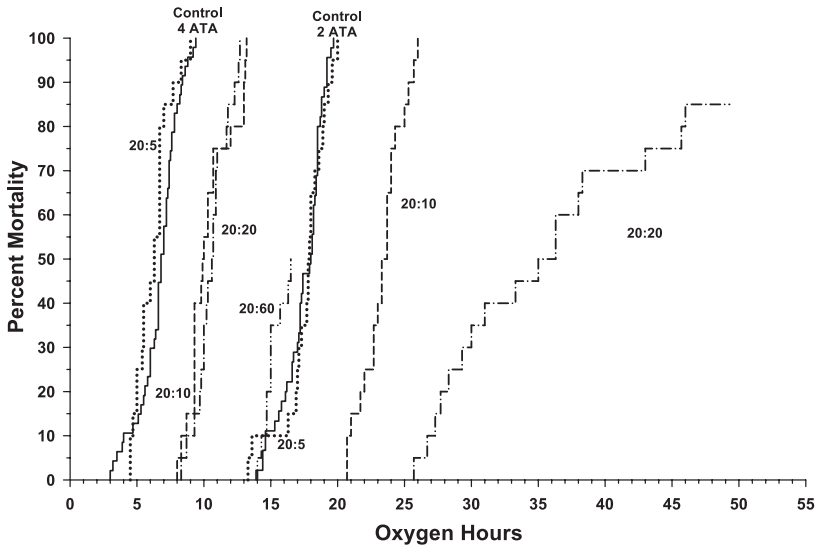

Fig. 1. Survival time responses to intermittent exposure patterns with 20-min oxygen periods at 4.0 and 2.0 ATA, plotted against accumulated oxygen hours. Solid lines represent continuous control exposures at 4.0 (*n* 47) and 2.0 (*n* 45) ATA. Each intermittent exposure contained 20 rats. Each step indicates the death of one or more rats. Survival times for rats that died during normoxic intervals are plotted at the end of the previous oxygen period. Magnitude of oxygen tolerance extension for each intermittent exposure pattern is indicated by a shift to the right of the continuous exposure control curve. All 4.0 ATA data are on the left of the 2.0 ATA control. The 20:60 pattern, performed only at 4.0 ATA, was terminated at 16.5 oxygen hours when half of the rats had died. The 10 surviving rats were killed for other studies. Three rats remained alive when the 20:20 intermittent exposure at 2.0 ATA was stopped at 49.3 oxygen hours.

ing whether a rat suffered convulsions, and convtime is the time at which a convulsion occurred. Note that for the 1.5 and 2.0 ATA programs, conv 0 for all rats and thus the last two terms drop out of the model. This approach is similar to the autocatalytic model in that it can be useful for prediction once the characteristics of a given program are known.

RESULTS

Results are described initially with respect to survival time responses to intermittent exposure patterns having the same "oxygen period" (20, 60, or 120 min). Survival times of individual animals within groups of 20 rats exposed intermittently to oxygen pressures of 4.0 or 2.0 ATA are shown as "mortality curves" in Figs. 1–3 along with corresponding curves for continuous exposures of larger groups at the same pressures (7). Survival time data for continuous and intermittent exposures at 1.5 ATA are shown separately in Fig. 4 to avoid overlap with the 2.0 ATA data. Survival times for the intermittent exposures are expressed as cumulative oxygen time while excluding the cumulative duration of normoxic exposure. Percent changes in median survival times for all of the intermittent exposure patterns with respect to continuous exposure control values are summarized in Table 2. Convulsion incidence and latency during continuous and selected intermittent oxygen exposures at 4.0 ATA are described after presentation of the survival time data.

Intermittent Exposure Patterns with 20-Min Oxygen Periods

Alternation of 5-min normoxic intervals with 20-min oxygen exposure periods (20:5) did not increase survival time at either 4.0 or 2.0 ATA (Fig. 1), but median survival time was increased by 24% for the same intermittent oxygen exposure pattern at 1.5 ATA (Fig. 4). Doubling the normoxic interval to 10 min (20:10) increased median survival time by 47% at 4.0 ATA, 30% at 2.0 ATA, and 65% at 1.5 ATA. Again doubling the normoxic interval to 20 min (20:20) resulted in only a 56% increase in survival time at 4.0 ATA, but median survival time at 2.0 ATA was nearly doubled (□98%), and 3 of 20 rats were still alive at 49.3 h of oxygen exposure (Fig. 1). The 20:20 oxygen-to-normoxic pattern was not evaluated at 1.5 ATA for reasons that are given below. Alternation of 20-min oxygen

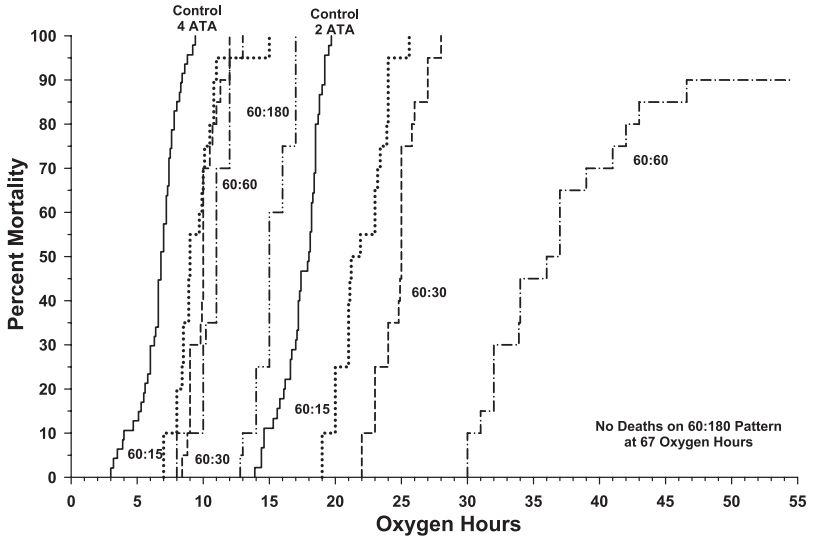

Fig. 2. Survival time responses to intermittent exposure patterns with 60-min oxygen periods at 4.0 and 2.0 ATA. See Fig. 1 legend. Two rats remained alive when the 60:60 intermittent exposure at 2.0 ATA was stopped at 54.4 oxygen hours. All 20 rats remained alive when the 60:180 exposure pattern was stopped at 67 oxygen hours (11 days total).

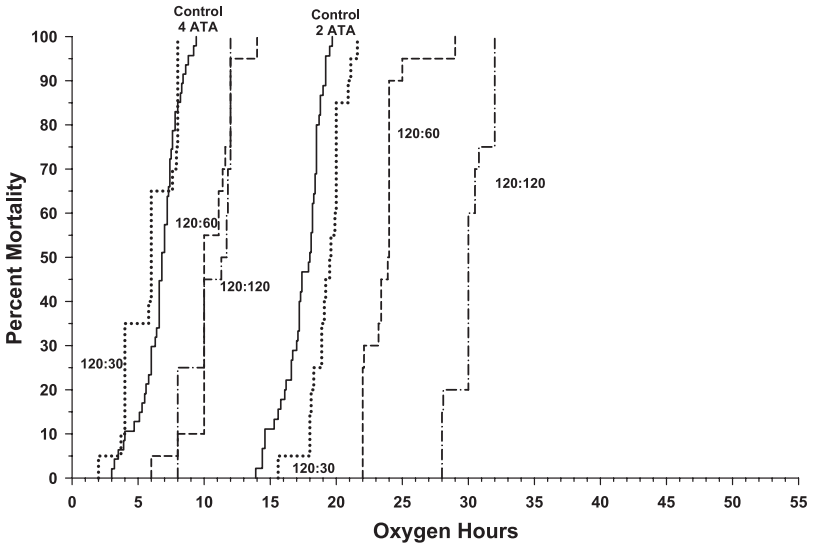

Fig. 3. Survival time responsesto intermittent exposure patterns with 120-min oxygen periods at 4.0 and 2.0 ATA. See Fig. 1 legend.

periods with 60-min normoxic intervals (20:60) at 4.0 ATA increased median survival time by 143%. This exposure was stopped at 16.5 oxygen hours when half of the animals had died. The remaining 10 rats were killed for other project purposes. The 20:60 pattern was not evaluated at lower oxygen pressures.

Intermittent Exposure Patterns With 60-Min Oxygen Periods

Combination of 60-min oxygen periods with normoxic intervals of 15 and 30 min (60:15, 60:30) increased median survival time, respectively, by 32 and 47% at 4.0 ATA, 20 and 39% at 2.0 ATA, and 27 and 43% at 1.5 ATA (Figs. 2 and 4). The 60:60 oxygen-to-normoxia pattern, which was not evaluated at 1.5 ATA, increasedsurvival time by 62 and 103% at 4.0 and 2.0 ATA, respectively. Two of 20 rats remained alive at 54.4 oxygen hours on the 60:60 pattern at 2.0 ATA (Fig. 2). When 60-min oxygen periods were alternated with 180-min normoxic intervals (60:180) at 4.0 ATA, survival time increased by 120%, but the same exposure pattern at 2.0 ATA allowed all 20 rats to tolerate 67 oxygen hours (3.7 median survival time for continuous exposure) over a total time of 11 days without a single death. Electron microscopy of the lungs from six randomly selected rats revealed only minimal alterations of pulmonary ultrastructural constituents. The absence of pulmonary pathology appeared to indicate essentially complete tolerance to a cumulative oxygen exposure at 2.0 ATA that was nearly four times the normally lethal duration. Similar tolerance to oxygen breathing at 1.0 ATA has been produced in rats by prior exposure to a sublethal level (12) or duration (16) of hyperoxia.

Intermittent Exposure Patterns With 120-Min Oxygen Periods

When 120-min oxygen periods were alternated with 30-min normoxic intervals (120:30) at 4.0 ATA, survival times for many rats were actually shorter than for rats exposed to continuous hyperoxia (Fig. 3). Evidently, the long oxygen exposure periods at 4.0 ATA, with only 30-min recovery periods, produced enough lung damage to cause fatal hypoxemia upon return to a normoxic atmosphere. At 2.0 and 1.5 ATA, the 120:30 program increasedmedian survival time by 9 and 20%, respectively (Figs. 3 and 4). When the normoxic

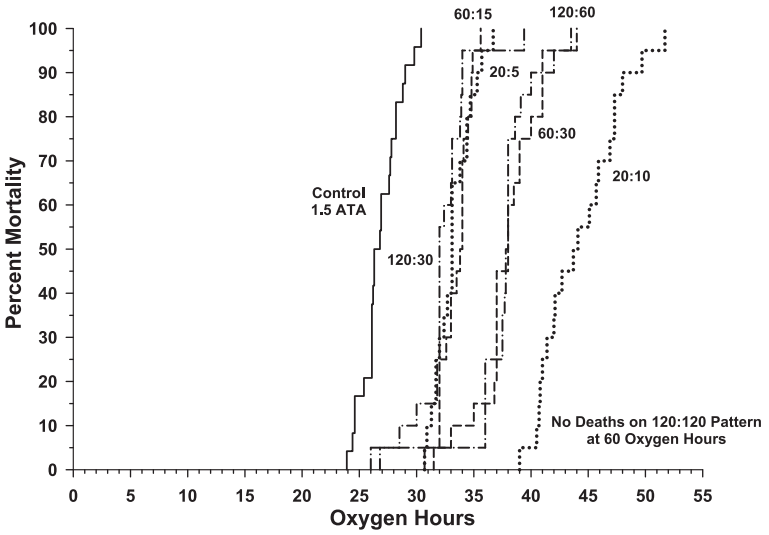

Fig. 4. Survival time responsesto intermittent exposure patterns at 1.5 ATA. See Fig. 1 legend. The solid, continuous exposure curve representssurvival times in 24 rats. All 20 rats remained alive when the 120:120 intermittent exposure pattern was stopped at 60 oxygen hours.

Table 2. *Percent change in median survival time during intermittent oxygen exposure at 4.0, 2.0, and 1.5 ATA (with respect to continuous exposure control value)*

Oxygen Period		Normoxic Interval, min							
ATA	Minutes	5	10	15	20	30	60	120	180
4.0	20	□6	47		56		143		
	60			32		47	62		120
	120					□12	47	69	
2.0	20	□1	30		98				
	60			20		39	103		2 7 2
	120					9	33	67	
1.5	20	24	65						
	60			27		43			
	120					20	42	126	

Values are percent change in median survival time (oxygen hours).

interval was lengthened to half the duration of the oxygen period (120:60), median survival time increased by 47, 33, and 42%, respectively, at 4.0, 2.0, and 1.5 ATA.

Alternation of 120-min oxygen periods with 120-min normoxic intervals (120:120) at 4.0 and 2.0 ATA increased survival time by 69 and 67%, respectively (Fig. 3). At 1.5 ATA, however, the same intermittent exposure pattern was continued for 60 oxygen hours without a single death (Fig. 4). The experiment was discontinued at this time, because the rats did not appear to be in a preterminal state at an exposure duration that already represented a 126% increment in median survival time. Given the reasonable expectation that shorter oxygen exposure periods, when paired with equal recovery intervals, would yield the same result, the 60:60 and 20:20 intermittent exposure patterns were not evaluated at 1.5 ATA.

Relationships of Survival Times to Normoxic Interval Durations

Median survival times for all of the intermittent exposure patterns that were evaluated at 1.5, 2.0, or 4.0 ATA are plotted against durations of the corresponding normoxic intervals in Fig. 5. At each oxygen pressure, connecting lines are drawn from the survival time point for continuous exposure through survival times for all of the intermittent exposures that have the same oxygen period. Slopes of these lines indicate the rates at which survival times were lengthened by progressively increasing the durations of normoxic intervals while holding the alternating oxygen exposure periods constant at 20, 60, or 120 min. The slopes of the lines should also reflect the relative rates at which toxic effects were reversed upon termination of the preceding oxygen exposure.

Comparison of the slopes in Fig. 5 indicates that recovery from oxygen poisoning occurs most rapidly after the 20-min (shortest) oxygen exposures and least rapidly after the 120-min (longest) exposures. It also indicates that recovery from a given oxygen exposure duration occurs more rapidly at a lower oxygen pressure. These relationships were anticipated qualitatively. However, the consistent data now provide a quantitative description of the rate of recovery under each set of experimental conditions, extending well beyond potential ranges of human exposures.

Convulsion Incidence and Latency During Continuous and Intermittent Oxygen Exposures

Relationships of convulsion incidence to duration of oxygen breathing for continuous and selected intermittent exposure patterns at 4.0 ATA are shown in Fig. 6. Median convulsion time for continuous exposure was 3.0 h with an overall convulsion incidence of 66% for 47 rats. About one-third of the continuously exposed rats died without ever having a convulsion. The general effects of intermittent exposure patterns with hyperoxic-to-normoxic ratios of 4:1 or 2:1 (60:15, 20:5, 60:30, 20:10) were to delay the onset of convulsions in the more susceptible rats and increase the overall incidence of convulsions within each group of 20 rats. As the duration of the normoxic interval was increased to equal or exceed the duration of the associated oxygen period (60:60, 60:180, 20:20, 20:60), the onset of convulsions was further delayed and the incidence of convulsions was progressively decreased. Among the intermittent exposure patterns that were evaluated, the most effective was the 20:60 pattern in which no convulsions occurred during a 4.0 ATA exposure that continued for 16.5 oxygen hours (Figs. 1 and 6). For intermittent exposure patterns with the same oxygen-to-normoxia ratio, those with 20-min oxygen periods appeared generally to be more effective than corresponding patterns with 60-min oxygen periods (Fig. 6).

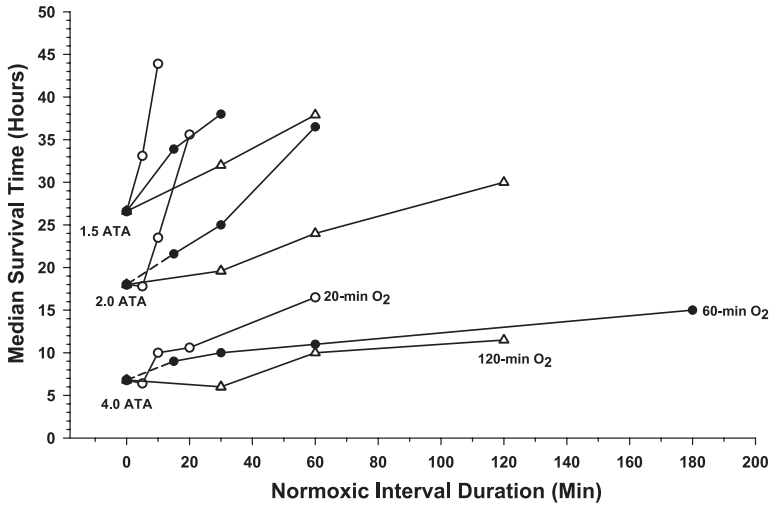

Fig. 5. Relationships of median survival times to normoxic interval durations for oxygen periods of 20 min (E), 60 min (F), and 120 min (,) at 1.5, 2.0, and 4.0 ATA. The dashed lines in the curves for 60-min oxygen periods at 2.0 and 4.0 ATA reflect the fact that a 5-min normoxic interval was not evaluated under those conditions. In general, there was a nearly linear increase in survival time as normoxic interval was lengthened whereas the oxygen period remained constant.

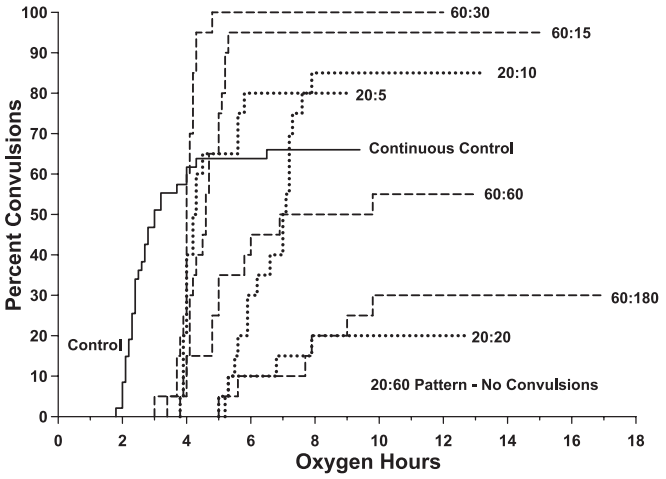

Fig. 6. Effects of intermittent oxygen exposure at 4.0 ATA on convulsions in rats. The curve for continuous exposure represents convulsions in 31 of 47 exposed rats. For all intermittent exposures, *n* 20 rats. No convulsions occurred on the 20:60 pattern which was terminated at the median survival time of 16.5 oxygen hours.

Relationships of both survival and convulsion times to normoxic interval durations at 4.0 ATA are shown in Fig. 7. The survival time data are identical to those shown in Fig. 5 on a more compressed scale. Because of the low incidence of convulsions for some exposure patterns (Fig. 6), oxygen exposure times for a 20% convulsion incidence are compared rather than the 50% incidence used for survival times. In general, the convulsion time slopes for 20-, 60-, and 120-min oxygen exposure periods are nearly parallel to the slopes for the corresponding survival time curves (Fig. 7).

Effectiveness of Intermittent Exposure Patterns With Varying Oxygen-to-Normoxia Ratios

The combinations of oxygen exposure period and normoxic interval duration shown in Table 1 were selected to determine whether rates and degrees of recovery from oxygen poisoning during the normoxic intervals were equivalent during intermittent exposures to oxygen periods that were relatively short, intermediate, and long. The mortality curves in Fig. 3 show that an oxygen period of 120 min at 4.0 ATA was too long to allow adequate recovery to occur during a 30-min normoxic interval. Many of the rats on the 120:30 pattern actually died earlier in terms of oxygen hours than control rats that were exposed continuously. With respect to survival time at 4.0 and 2.0 ATA, the mortality curves in Fig. 1 also indicate that a 5-min normoxic interval was too short for significant recovery, even after an oxygen exposure of only 20 min. At 1.5 ATA, however, all three patterns with an oxygen-to-normoxia ratio of 4:1 produced similar extensions of survival time, with relative increments of 20 to 27% (Fig. 4). These results indicate that significant recovery from 20-min oxygen exposure periods at 1.5 ATA can occur with normoxic intervals as short as 5 min and also show that 120-min oxygen exposure periods at 1.5 ATA are not too long to allow significant reversal of toxic effects during 30-min normoxic recovery intervals. Both observations are consistent with development of cumulative oxygen poisoning effects less rapidly at 1.5 ATA than at higher oxygen pressures.

Results obtained at 1.5 ATA (Fig. 4) also show clearly that intermittent exposure patterns with a 4:1 oxygen-to-normoxia ratio were less effective than corresponding patterns with a 2:1 ratio that, in turn, were much less effective than the 1:1 ratio, 120:120 pattern that caused no deaths during an intermittent exposure that was continued for 60 oxygen hours. Although the 120:120 pattern was not unusually effective at either 4.0 or 2.0 ATA (Fig. 3), it is of interest that both the 20:20 and 60:60 patterns at 2.0 ATA (Figs. 1 and 2) allowed some of the rats to have extremely long survival times. Overall, the data are consistent with the possibility that rats exposed to a sufficiently toxic, sublethal level of oxidant stress can, by some as yet undefined defense mechanism, develop an extreme degree of oxygen tolerance. The observation that the 20:10 intermittent exposure pattern at 1.5 ATA was significantly more effective than the other 2:1 patterns (Fig. 4) suggests that such a "defense mechanism" was also triggered by this pattern but not to the degree manifested in the 120:120 pattern.

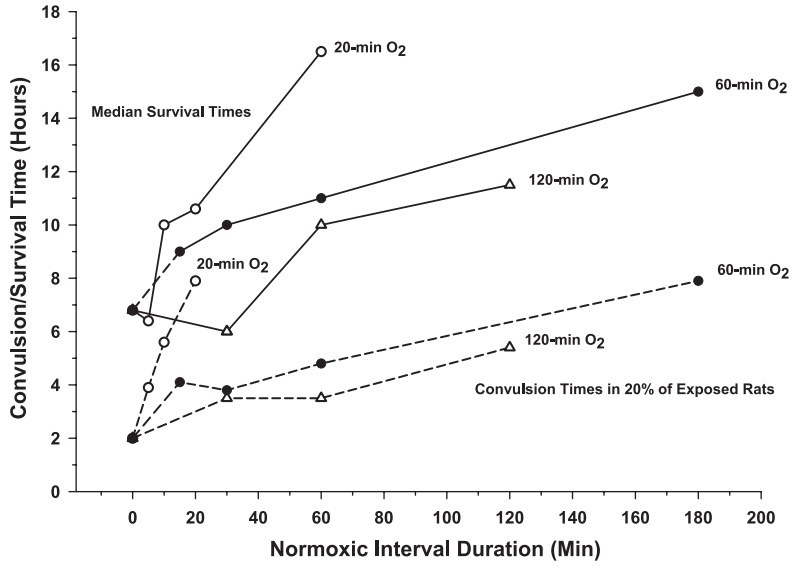

Fig. 7. Relationships of median survival time and 20% convulsion incidence to normoxic interval duration for oxygen periods of 20, 60, and 120 min at 4.0 ATA. The survival time data are identical to those shown on a different scale in Fig. 5. Solid lines represent survival data and dashed lines represent convulsion data. As in Fig. 5, the dashed line on the left end of the 60-min survival curve indicates that the slope of that segment may be inaccurate because a 60:5 exposure pattern was not evaluated.

DISCUSSION

The toxic biochemical effects caused by continuous exposure to high oxygen pressures are initiated by reactive oxygen species that simultaneously affect multiple intracellular and membrane enzyme systems (17, 24). As the oxygen partial pressures of intrinsic cellular environments are increased, rates of enzyme inactivation are accelerated and, with extended duration of exposure, there are consequent failures of multiple cell and organ system functions. Prediction of tolerance to such continuous oxygen exposures would be relatively simple if all cells and enzymes were both equally sensitive to oxygen toxicity and exposed at their cellular locations to the same partial pressures of oxygen. However, sensitivity to oxygen poisoning varies among different enzyme systems, and even the same enzyme systems in different organs (lung, brain, eye) will have diverse intracellular chemical environments and be exposed to different oxygen partial pressures (26, 27).

During intermittent exposure to oxygen at any ambient pressure, the cyclical insertion of normoxic recovery periods provides another source of toxic effects modification by superimposing the influences of varying rates of onset and recovery from diverse effects of oxygen toxicity in multiple organ systems (27). Therefore, degrees of oxygen tolerance extension for a single pattern of intermittent exposure at a given oxygen pressure may vary for different toxic effects and among different organ systems (27). Nevertheless, as stated previously, survival time in a sufficiently large population is a useful systemic index of cumulative, composite effects of oxygen toxicity.

Descriptive Model of Results

In attempting to develop a mathematical description of our data, we evaluated three different models. The first was a power curve identical to that used by Harabin et al. (22). As noted by the previous authors, this approach provided a very good fit because it could use parameters determined by each individual exposure group. However, it did not provide a means for predicting the efficacy of an intermittent exposure pattern that was not evaluated empirically.

Harabin et al. (22) also developed an autocatalytic model consisting of a general expression that described their continuous and intermittent exposure data as well as fitting individual power curves for each set of exposure conditions. The data pool available to these investigators was limited to one continuous exposure control curve and six intermittent exposure patterns at an oxygen pressure of 2.8 ATA. Our more extensive data set (Table 1) provided a more rigorous test of an autocatalytic or any other descriptive model. Although the autocatalytic model afforded reasonably accurate descriptions of survival time responses to some of the exposure patterns defined in Table 1, it was clearly not equivalent to individual power curves in many cases.

As an alternative to an autocatalytic model, we developed a Cox proportional hazards model that incorporates the normoxia-to-oxygen ratio, normoxic interval duration, and latency of convulsions when they occur. Parameter estimates for the Cox model at 1.5, 2.0, and 4.0 ATA are summarized in Table 3. We found that the ratio and normoxic interval parameters interacted significantly at 1.5 and 4.0 ATA but not at 2.0 ATA. These statistical results are consistent with other indications

Table 3. *Cox model parameter estimates*

		1.5 ATA	2.0 ATA	4.0 ATA
Ratio	Estimate	−15.692	−7.693	−1.773
	SE	1.362	0.539	0.186
	P value	<0.0001	<0.0001	<0.0001
	Relative risk	1.5E−7	0.000	0.170
Normtime	Estimate	−0.040	−0.011	−0.009
	SE	0.022	0.004	0.003
	P value	0.063	0.002	0.005
	Relative risk	0.961	1.011	0.991
Ratio Normtime	Estimate	0.147		0.006
	SE	0.046		0.002
	P value	0.001		0.001
Convtime	Estimate			−0.184
	Relative risk	1.16		0.832
	SE			0.056
	P value			0.001
Conv	Estimate			2.091
	SE			0.394
	Relative risk			8.094
	P value			<0.0001

Normtime, length of normoxic interval; conv, binary variable indicating whether a rat suffered convulsions; convtime, time at which a convulsion occurred.

that lethal durations of exposure at different oxygen pressures are determined by a complex and variable blend of direct oxidant effects that are exacerbated by cellular and tissue reactions, ameliorated by concurrent repair processes, and opposed by both latent and induced antioxidant defenses (4, 8, 10, 17, 18, 24, 27).

Selected examples of goodness of fit for both the autocatalytic and Cox models are shown in Fig. 8. The data selected for comparison include the continuous exposure control curve and three patterns of intermittent exposure at 1.5 ATA. The Cox model provides a much closer fit to the control curve, a slightly better fit to the curve for the 60:15 intermittent exposure, and much better fits to the 120:60 and 20:10 curves for which the autocatalytic model predictions show no separation and are nearly superimposed on each other.

In addition to the comparison in Fig. 8, we assessed goodness of fit for the autocatalytic and Cox models more objectively by calculating mean squared error (MSE) values for each continuous and intermittent oxygen exposure in which deaths occurred. The MSE value for each exposure was defined as the mean of the squared vertical distances between each observed data point and the corresponding model prediction. In general, MSE values were much smaller for the Cox model. Although some degree of improvement in predicted values would be expected on the basis that the Cox model is semi- rather than fully parametric, the Cox MSE values were smaller, sometimes dramatically so, for 25 of the 29 exposures in which deaths occurred. The four intermittent exposures that had smaller MSE values for the autocatalytic model were the 20:20, 120:30, and 120:120 programs at 4.0 ATA and the 20:5 program at 2.0 ATA. The differences in MSE values for the two models were smallest at 4.0 ATA and greatest at 1.5 ATA. In general, goodness of fit for the autocatalytic and Cox models was similar for steep mortality curves, whereas Cox model predictions were superior for more shallow curves, as illustrated respectively by the 60:15 and 20:10 curves in Fig. 8.

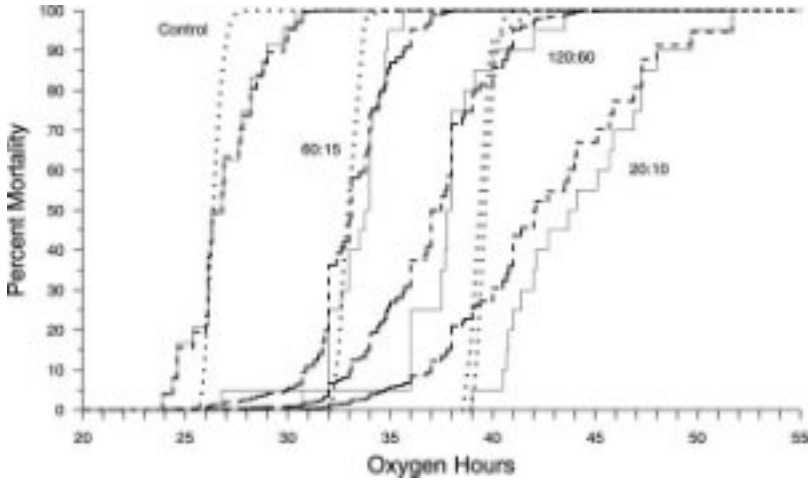

Fig. 8. Comparison of autocatalytic and Cox model predictions for continuous oxygen exposure and selected intermittent exposure programs at 1.5 ATA. Observed survival time data for the selected exposures are shown as thin solid lines. Autocatalytic and Cox model estimates are shown as dotted and heavy dashed lines, respectively. Time scale expanded to provide better resolution for comparisons. See text for additional discussion.

Relationships of Survival Times to Time-Weighted Average Oxygen Pressures During Intermittent Exposure

Berghage (1, 2) has proposed that the rate of development of oxygen poisoning during intermittent exposure is equivalent to that which occurs during continuous exposure to a constant oxygen pressure that is definable as a time-weighted average of the alternating hyperoxic and normoxic exposure periods. His hypothesis was generally supported by the observed total exposure times for convulsions to occur in 50% of the rats exposed continuously or intermittently to oxygen pressures of 3.2, 3.9, and 4.2 ATA (2). We have further examined this hypothesis over a wider range of oxygen pressures by comparing the relationships of median survival times to time-weighted average oxygen pressures for the intermittent oxygen exposure patterns shown in Table 1 with those for continuous exposures to oxygen pressures of 1.0, 1.5, 2.0, 3.0, and 4.0 ATA (7). These data are plotted on log-log coordinates (Fig. 9) because the log survival time-log oxygen pressure relationship for continuous exposure is linear over the stated range of pressures (8, 10).

The log-log plot in Fig. 9 has a regression line fitted to the five points that represent continuous oxygen exposures. Plotted on linear coordinates, the regression curve is a hyperbola with a vertical survival time asymptote at zero hours and a horizontal, oxygen pressure asymptote at 0.7 ATA. The survival time asymptote is consistent with the prior reasonable assumption that "death" would occur almost immediately as inspired oxygen pressure approaches infinity (8, 26). The inspired P_{O_2} asymptote at 0.7 ATA, implying indefinite survival at this pressure, provided a better fit to the continuous exposure data than either 0.8 or 0.6 ATA. It is considered to be reasonable because rats exposed to oxygen at 0.6 ATA for 64 days did not appear to be distressed, maintained normal food and water consumption, and had no demonstrable pathological changes in multiple vital organs (3).

Except for the two intermittent exposure patterns in which no deaths occurred (120:120 at 1.5 ATA and 60:180 at 2.0 ATA), median survival times (expressed as total oxygen and normoxia exposure hours) at the calculated time-weighted average oxygen pressures for all of the intermittent patterns at 1.5, 2.0, and 4.0 ATA lie on or near the regression line for continuous oxygen exposure. However, many points lie to the right of the line for continuous exposure indicating that the observed survival times exceeded the predictions based on calculation of the time-weighted average oxygen pressures. On the far right side of Fig. 9, survival time extensions by

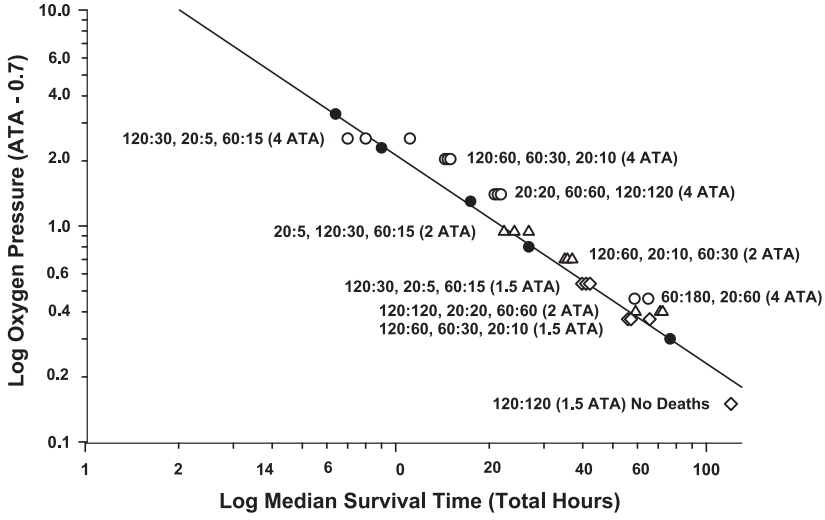

Fig. 9. Log median survival times for continuous and intermittent exposures to oxygen pressures of 4.0, 3.0, 2.0, 1.5, and 1.0 ATA, plotted against log oxygen pressure (ATA □0.7). The regression line is fitted to 5 points (F) that represent survival times for continuous exposures to oxygen pressures of 4.0, 3.0, 2.0, 1.5, and 1.0 ATA (7). Inspired oxygen pressures for intermittent exposures are calculated as time-weighted averages (1, 2). Median survival times for intermittent exposures at 4.0, 2.0, and 1.5 ATA are represented, respectively, by E, ., and {. See text for additional discussion.

intermittent exposure are visually narrowed by logarithmic compression of the time scale.

The point for intermittent exposure to a time-weighted average oxygen pressure of 0.65 ATA (60:180 pattern at 2.0 ATA) is not shown on Fig. 9 because it is below the assumed pressure asymptote of 0.7 ATA. Representing the only other intermittent exposure pattern in which no deaths occurred, the point for the 120:120 intermittent exposure at 1.5 ATA (120 total hours or 60 oxygen hours at a time-weighted average oxygen pressure of 0.85 ATA) represents the time at which the exposure was terminated. It is of interest that rats exposed continuously to 0.85 ATA oxygen for at least 120 h have increased activities of pulmonary superoxide dismutase and can survive subsequent multiday exposures to 1.0 ATA oxygen (12).

The observation that intermittent exposure patterns with the same oxygen-to-normoxia ratios often provide similar survival time extensions (Fig. 4) may be related, at least in part, to the fact that time-weighted average inspired oxygen pressures are identical for such patterns (Fig. 9). However, it is likely that other factors related to the alternating onset and reversal of complex toxic effects are also involved in extension of tolerance. Although the proposed calculation of time-weighted average oxygen pressures (1, 2) provides a useful approximation for survival time extension by intermittent exposure, as a predictive method it underestimates the degree of protection achieved by many of the intermittent exposure patterns. The magnitude of underestimation is most prominent for the patterns studied at 4.0 ATA where, with the exception of the 120:30 and 20:5 combinations, median survival times for all of the other patterns fall well to the right of the curve for continuous oxygen exposure (i.e., extended survival) (Fig. 9).

By using the regression line in Fig. 9 to calculate predicted median survival times corresponding to the time-weighted average oxygen pressures for all of the intermittent exposure patterns at 1.5, 2.0, and 4.0 ATA, the differences between observed and predicted survival times (in total hours) can be determined quantitatively. Percent changes in observed median survival times with respect to predicted values are summarized in Table 4 for all of the intermittent patterns except the two (120:120 at 1.5 ATA and 60:180 at 2.0 ATA) in which no deaths occurred. The overall average deviation from the regression line in Fig. 9 for the six patterns at 1.5 ATA in which deaths occurred was □2%. The average deviation at 2.0 ATA for the nine patterns in which deaths occurred was 12%, whereas the same patterns at 4.0 ATA had an average deviation of 28%. When the results obtained for the 20:5 and 120:30 patterns at 2.0 and 4.0 ATA are excluded, the average deviations are 16 and 38%, respectively, for the remaining seven patterns at each pressure. These results are consistent with the conclusion that some protective influence is activated more effectively by intermittent exposures to higher oxygen pressures.

Table 4. *Percent change in median survival time during intermittent oxygen exposure at 4.0, 2.0, and 1.5 ATA (with respect to interpolated value on regression line for continuous exposure)*

Oxygen Period		Normoxic Interval, min							
ATA	Minutes	5	10	15	20	30	60	120	180
4.0	20	□4	42		36		35		
	60			34		40	37		20
	120					□16	39	40	
2.0	20	□4	11		26				
	60			15		17	28		*
	120					3	10	5	
1.5	20	0	7						
	60			2		□7			
	120					□4	□9	*	

Values are percent change in median survival time (total hours). Time-weighted average oxygen pressure calculated for each intermittent exposure pattern. See Fig. 9. *No deaths.

There are other indications that some as yet unexplained type of protective influence may be conferred by a brief period of oxygen breathing at 4.0 ATA. The onset of convulsions in rats exposed to 4.0 ATA oxygen for 1 h followed by continued exposure without interruption to 3.0 ATA oxygen was significantly delayed compared with convulsion time in rats exposed directly to oxygen at 3.0 ATA (14). The animals that were initially exposed to 4.0 ATA oxygen also had a transient reversal of the progressive hypothermia that occurs in rats during exposure to hyperbaric hyperoxia (30). Although intermittent exposure to 4.0 ATA oxygen is not practical for diving operations or therapy purposes, investigation of the biochemical basis for the increased oxygen tolerance associated with brief exposures to this level of hyperoxia may ultimately provide more effective methods than those now available for optimization of oxygen tolerance extension by intermittent exposure.

Evidence for Involvement of Antioxidant Enzymes as Factors in Oxygen Tolerance Extension by Interrupted Exposure

It is now well established that exposure to hyperoxia increases the rates of production of reactive oxygen species, which are concurrently opposed by a variety of antioxidant defenses (12, 16–18, 24). At a sufficiently high oxygen pressure for a sufficiently prolonged duration of exposure, oxidant damage to cell and tissue functions occurs when antioxidant defenses are overwhelmed. Periodic interruption of less toxic degrees of hyperoxic exposure by restoration of inspiratory normoxia could allow antioxidant defenses to be maintained or possibly even enhanced. Frank et al. (16) found that tolerance to oxygen exposure at 1.0 ATA could be greatly increased in rats by a "type" of intermittent exposure in which the rats were preexposed to oxygen for 48 h, returned to air for 12 to 24 h, then reexposed to oxygen for 72–168 additional hours. Whereas continuous oxygen exposure for 72–168 h (3–7 days) was lethal for all control rats, the preexposed rats survived the additional exposure with only slight pulmonary edema. The increased tolerance to 1.0 ATA oxygen was associated with statistically significant increments in pulmonary concentrations of superoxide dismutase, catalase, and glutathione peroxidase.

Harabin et al. (21) studied the relations of antioxidant enzyme activities to the increased oxygen tolerance afforded to guinea pigs and rats exposed intermittently to oxygen at 2.8 ATA in a cycle that alternated 10-min oxygen periods with 2.5-min intervals of air breathing (0.59 ATA P_{O_2}). Only one pattern of intermittent exposure was studied at the single pressure. Intermittent exposure significantly delayed onset of convulsions and increased survival times in both species. Brain

antioxidant enzyme activities were not significantly increased in either species. Lung superoxide dismutase activities were significantly increased during intermittent exposure in both species and were also increased in the rat during continuous exposure. In the guinea pig, activities of glutathione peroxidase in both lung and brain and lung catalase activities were reduced more during continuous than during intermittent exposure. The authors concluded that these complex results did not fully explain the observed increments in oxygen tolerance.

Potential Involvement of Stress Proteins in Oxygen Tolerance Extension by Interrupted Exposure

It is well documented that exposure of a living organism to a sublethal chemical or physical stress, followed by a period of recovery, induces a sequence of cellular defense mechanisms that transiently protect the organism from normally lethal exposures to the same stress (29). This "stress response" occurs in bacteria, plants, and all animals that have been studied, including humans. Although the initial studies of this phenomenon focused on the heat shock response and the development of thermotolerance, it is now known that the toxic effects of reactive oxygen species can also induce cellular and molecular responses that include alterations in the gene expression of antioxidant enzymes, stress-response genes, and various cytokines (4).

Choi and Alam (5) reviewed the evidence in support of their proposal that heme oxygenase 1, which is highly induced by a variety of agents causing oxidant stress, may have an important protective function against oxidant-induced lung injury and thereby supplement the protective roles of classical antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Potential mechanisms for an antioxidant action of heme oxygenase 1, also called heat shock protein 32, include catalysis of the oxidative degradation of heme, which can function as a cellular prooxidant, and production of bilirubin as an end product that has antioxidant properties.

The well-documented augmentation of classical antioxidant enzyme activities in response to oxidant injury (12, 16–18, 24) does not exclude the possibility that other responses to oxidant stress may also be involved. Speit et al. (31) provided evidence of such involvement by demonstrating increased levels of heme oxygenase 1 in lymphocytes obtained from normal humans 24 h after a single exposure to 2.5 ATA oxygen for 60 min on a 20:5 intermittent schedule. Reversible breakage of DNA strands found in lymphocytes after an initial exposure to this therapeutic level of hyperoxia did not occur after the second or subsequent exposures. In addition, lymphocytes isolated from blood obtained 24 h after the initial exposure were protected against DNA damage caused by hydrogen peroxide in vitro. A related investigation (13) showed that synthesis of heat shock protein 70 was also significantly induced in lymphocytes after a single 3
20-min exposure at
2.5 ATA whereas red cell concentrations of superoxide dismutase, catalase, and glutathione peroxidase were not changed. Evidence that heat shock proteins may provide cross-protection against oxidant injury is afforded by the observation that hyperthermic preconditioning of cultured human umbilical vein endothelial cells significantly reduced the cellular damage caused by subsequent exposure to hydrogen peroxide (19).

Although our results provide no direct evidence that heat shock or oxidation-specific stress proteins are involved in oxygen tolerance extension by intermittent exposure, the data summarized in Table 4 reflect an interesting pattern of responses. With respect to median survival times predicted by the regression line in Fig. 9, survival times for the intermittent exposures at 1.5 ATA fall on or near the curve, whereas survival times for intermittent exposures at 2.0 and 4.0 ATA exceed the predicted values by average deviations of 16 and 38%, respectively. This response pattern resembles the development of thermotolerance in which induction of a protective response requires a threshold level of stress and the magnitude of protection is proportional to the severity of the inducing stress (29).

With the exception of the brief intermittent exposures to 2.5 ATA oxygen that were cited above (13, 31), previous studies of stress protein responses to sublethal injury involve a single exposure to a stressful condition followed by a prolonged period of recovery. Responses to sustained alternating periods of stress and recovery have apparently not been studied to date.

Additional information about the nature, kinetics, and potency of heat shock and oxidation-specific stress protein responses to oxidant injury may provide insights into mechanisms for the established benefits of intermittent hyperoxia and thereby provide opportunities for continued improvement of this practical means for extending human oxygen tolerance in diving, aerospace activity, and hyperbaric oxygen therapy. The incorporation of intermittent exposure patterns and conditions that produce prominent gains in oxygen tolerance of laboratory animals should allow studies of the underlying biochemical mechanisms for such gains to be designed efficiently and with a high probability of success.

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