

Superoxide dismutase and pulmonary oxygen toxicity

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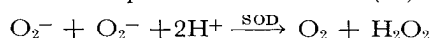
CRAPO, JAMES D., AND DONALD F. TIERNEY. *Superoxide dismutase and pulmonary oxygen toxicity*. Am. J. Physiol. 226(6): 1401-1407. 1974.—Superoxide (O_2^-) is a highly reactive free radical produced by the one electron reduction of oxygen. Superoxide dismutase (SOD) dismutates this free radical to less toxic forms. After exposure to 85% oxygen for 7 days, 250- to 350-g rats had a 50% increase of SOD activity in their lungs and became "tolerant" (survived more than 4 days) to 100% oxygen. Control rats died within 72 h in 100% oxygen. The rate of development of oxygen "tolerance" closely parallels the time course for the increase in pulmonary SOD activity. Following return to air, rats made tolerant by prior exposure to 85% oxygen show decreases in pulmonary SOD activity which parallel their progressive loss of tolerance over 30 days. Guinea pigs, hamsters, and mice did not develop oxygen tolerance under similar circumstances and did not have as large a change in pulmonary SOD as did the rat. These observations lend support to the postulate that O_2^- contributes in the development of pulmonary toxicity and that SOD provides an in vivo defense against this radical.

oxygen tolerance; free radicals; superoxide free radical

MOST MAMMALIAN SPECIES die after prolonged exposures to 100% oxygen (1 atm). The primary damage is to the lung, and the signs are those of progressive respiratory distress until death. Ultrastructural studies indicate that the major insult is to the alveolar capillary endothelium (31). The mechanism of this toxicity remains unresolved. Although increased biological production of free radicals has been proposed as a possible mechanism of toxicity (9), no specific free radical has yet been shown to be responsible.

The superoxide anion (O_2^-) is an extremely potent free radical which is produced by the single electron reduction of oxygen. It is produced by a number of biologically important reactions including those of the enzymes xanthine oxidase (18), ferredoxin (23), and flavodoxin (24). The production of O_2^- by xanthine oxidase and ferredoxin is increased by increasing the oxygen tension (7, 23), and therefore, increased concentration of inspired oxygen may lead to increased production of O_2^- .

Since the superoxide anion is an unstable free radical, it can be expected that even low concentrations of it would be intolerable to the living cell. Apparently cells can tolerate the production of superoxide anion because it is so rapidly removed and high concentrations do not develop. Superoxide dismutase (SOD), a metalloprotein, catalyzes the dismutation of the superoxide free radical (19).



It has been postulated that the presence of SOD is protective against the toxic effects of oxygen and that it is necessary for survival in an oxygen atmosphere. In studies of bacteria it was found that their ability to live in and to utilize oxygen correlates with the level of SOD (6, 20).

There are no reports to date which evaluate the role of SOD in pulmonary oxygen toxicity at atmospheric pressure. The phenomenon of oxygen tolerance may offer an opportunity to evaluate the biological function of this enzyme. When large rats are exposed to 100% oxygen, they generally die between 60 and 72 h of exposure, but if they are first exposed to 85% oxygen for 7 days they become tolerant and can then be transferred to 100% oxygen and survive for prolonged periods (27).

We postulated that oxygen toxicity is at least in part caused by the free radical superoxide which may be biologically produced in increased amounts in the presence of high oxygen tension. The development of tolerance may be related to an increase in the activity of SOD which could be induced during a sublethal exposure to oxygen.

METHODS

Materials and equipment. DL-Epinephrine and cytochrome *c* type III were obtained from Sigma Chemical Company and xanthine oxidase was obtained from Worthington Biochemical Corporation. SOD, purified from bovine liver, was obtained from Diagnostic Data Incorporated. Liquid oxygen came from North Carolina Welding Supply.

Equipment used included a Beckman Acta CIII recording spectrophotometer, a Shimadzu model MPS-50L dual-beam recording spectrophotometer, a Gilford 300-N spectrophotometer, Dwyer Visi Float air flowmeters, a Corning model 165 pH blood gas analyzer, and a Branson model 140-D sonifier.

Animals. We used specific pathogen-free male rats (Charles River) which weighed 250-350 g. To establish that the procedures did not lead to lung infection, 20 rats were removed from the barrier and 10 were kept in air for 37 days while 10 were exposed to 85% oxygen for 7 days and then kept in air for 30 days. At the end of this 37-day period, fluid obtained by bronchial lavage of all 20 animals was cultured, and the lungs were examined by light microscopy. Cultures were done for viral, bacterial, and mycoplasma pathogens and none was found. The lungs had no histological evidence of infection.

Hartley guinea pigs were obtained from Camm Research, Syrian hamsters were obtained from the Lakeview Hamster

Colony, and Swiss Webster strain mice were obtained from Hilltop Laboratory Animals.

Exposure. All exposures were continuous and done at 23°C in polystyrene chambers 37 x 47 x 41 cm. The concentration of oxygen was controlled by mixing pure oxygen from a liquid oxygen tank with air using air flowmeters. Flow rates sufficient to provide five to seven air changes per hour were used. Throughout the period of exposure, the animals were allowed free access to food and water. The oxygen and carbon dioxide concentrations in the chamber were monitored by sampling with a syringe and the PO_2 and PCO_2 were determined on a blood gas analyzer. Using this system an oxygen concentration of $85 \pm 2\%$ or a concentration equal to that in the liquid oxygen source could be sustained. Carbon dioxide concentration was maintained less than 0.6%. Control animals were exposed to air at similar flow rates in similar chambers for each experiment.

Four types of exposures were performed: 1) Rats, guinea pigs, hamsters, and mice were exposed to 85% oxygen for 7 days. 2) Rats, hamsters, and mice were exposed to 85% oxygen for 7 days and then tolerance was determined by exposure to 100% oxygen for 4 days. 3) Rats were exposed to 85% oxygen for variable numbers of days between 0 and 8, and then a portion of each group was exposed to 100% oxygen for 4–21 days. 4) Rats were exposed to 85% oxygen for 7 days and then kept in air for 6–30 days after which a portion of each group was exposed to 100% oxygen for 4 days.

Tissue preparation. Rats and guinea pigs were anesthetized with an intraperitoneal injection of 30 mg pentobarbital. Tracheostomy was performed and ventilation was provided with a respirator (Harvard Apparatus) using a maximum pressure of 10 cmH₂O. In rapid sequence we transected the inferior vena cava and aorta, cannulated the pulmonary artery, excised the left atrium, and perfused the lungs with 10 ml of 0.9% NaCl solution. The lungs were then blotted dry, weighed, and homogenized in 4 vol of 0.01 M potassium phosphate buffer containing 0.1 mM EDTA at pH 7.8. We used eight strokes with a Teflon-pestled Potter-Elvehjem type homogenizer attached to a $\frac{3}{8}$ -inch drill spun at 1,150 rpm. After centrifugation at 20,000 *g* for 10 min, the supernatant was removed and used for SOD and protein analyses. Hamsters and mice were treated in a similar fashion except that the lungs were not perfused with saline solution prior to their excision.

Brain, heart, liver, and kidney were removed without being perfused and were otherwise treated in the same fashion as was lung. Blood was obtained by direct aortic puncture. Since high concentrations of hemoglobin interferes with the assay for SOD, we removed hemoglobin from packed red cells by lysis with an equal volume of deionized water and precipitation by addition of 0.25 vol of ethanol and 0.15 vol of chloroform in the cold. The resulting supernatant obtained by centrifugation was used for SOD determination and was assumed to contain all of the original SOD activity (19).

Alveolar macrophages were obtained by inflating the lungs of rats 10 times with 7 ml of 0.9% NaCl solution and collecting the fluid after each rinse (3). These alveolar washings were centrifuged at 1,000 *g*, and the cellular frac-

tions were combined and then resuspended in 4 ml of 0.9% NaCl solution. Since erythrocytes were found in the alveolar washings of oxygen-exposed rats, they were lysed in all washings by addition of 20 ml of deionized water. After mixing for 30 s, 10 ml of 2.7% NaCl solution were immediately added to restore the solution to normal tonicity. Macrophages were recovered by centrifugation at 1,000 *g* and washed 3 times with 20 ml of 0.9% NaCl solution. Cells were recovered by centrifugation after each wash, and following the final wash they were resuspended in 1–4 ml of 0.9% NaCl solution. Cell counts of viable and non-viable cells were done using trypan blue exclusion as an index of viability. The cells were disrupted by sonication for 45 s at a power of 70 W, and the sonicate was centrifuged at 20,000 *g* for 10 min. The supernatant was used for SOD determination. Sonification was found to disrupt all cells and did not affect the activity of purified SOD.

Chemical analysis. Unless otherwise stated SOD activity was determined by the epinephrine-adrenochrome assay (25). One unit of SOD activity was defined as the amount which produced a 50% inhibition of the rate of change in absorbance in a 2.5-ml assay. In the presence of tissue supernatant, there was a 3- to 5-min lag period followed by a linear rate of change in absorbance which we accepted as the reaction rate. This lag period was much longer than that occurring either in the absence of homogenate or when assayed with the purified enzyme, and it was not affected by prior dialysis of the supernatant. The activity of SOD was also determined by inhibition of cytochrome *c* reduction by xanthine and xanthine oxidase (19). The supernatant was dialysed against 0.01 M potassium phosphate containing 0.1 mM EDTA at pH 7.8 prior to this assay, and no lag period was detected. The enzyme was found to be stable for at least 2 wk when the supernatant was refrigerated or frozen.

DNA was determined by the diphenylamine method of Schneider (28), and succinate cytochrome *c* reductase activity was estimated by the method of Singer and Kearney (29). Hemoglobin was determined on rat lung homogenate by the carbonmonoxyhemoglobin minus oxyhemoglobin difference spectrum and was quantitated by comparison to standard hemoglobin solutions (13). Hemoglobin was determined on blood and packed red cells by the cyanomethemoglobin method (17). Protein was assayed by the method of Lowry et al. (15).

Statistical analysis. While most comparisons were made using a Student *t* test, with a logarithmic transformation being used when necessary to achieve homoscedasticity, analysis of variance procedures and multiple-comparison techniques such as Dunnett's and William's tests (34) were also employed. With the exception of Table 3 and Fig. 1, all tests were two-sided tests.

RESULTS

Effects of 85% oxygen on pulmonary SOD activity. Eight rats were exposed to 85% oxygen for 7 days, and eight controls from the same group of rats were exposed to air. The activity of pulmonary SOD is shown in Table 1 and the results are related to several parameters. The oxygen-exposed animals developed gross pulmonary edema with increased lung weight and protein content which obscured the change

TABLE 1. Increased activity of superoxide dismutase in lungs of rats exposed 7 days to 85% oxygen

	Controls	Exposed, g	Percent Increase
Perfused lung weight	1.19 ± .03	1.74 ± .10	45 (P < 0.01)
Protein/whole lung, mg	112 ± 8	188 ± 15	68 (P < 0.01)
DNA/whole lung, mg	7.07 ± .40	7.81 ± .58	9 (NS)
SOD/whole lung, U	777 ± 48	1,130 ± 87	46 (P < 0.01)
SOD/mg DNA, U	110 ± 4	146 ± 7	33 (P < 0.01)
SOD/unit succinate cytochrome c reductase, U	139 ± 15	201 ± 17	45 (P < 0.01)

Control and exposed values are means ± SE. n = 8 for both groups. NS = not significant.

in SOD when it was related to these parameters. DNA showed a small increase during the oxygen exposure and an unrelated mitochondrial enzyme, succinate cytochrome c reductase, showed no statistically significant change.

The rats ate and drank poorly during the 7-day oxygen exposure and weighed 28% less than their matched controls. To evaluate the effects of starvation on the activity of pulmonary SOD, four rats were deprived of food and given 320 ml of water over the 7-day period. They weighed 35% less, their lung weight decreased 26%, and the activity of SOD in their lungs decreased 37% (P < 0.01) when compared to matched control animals.

The possibility that erythrocytes trapped in the oxygen-exposed lungs could account for some of the change in activity of SOD was evaluated by determining the hemoglobin content on the homogenate of saline-perfused lungs. In both the exposed and the control groups, whole blood accounted for less than 1% of the weight of all lungs which had been perfused with saline solution. SOD activity attributable to erythrocytes in each whole lung was 5 U for the exposed lungs and 2 U for the control lungs.

Leucocytes are known to migrate into the lung during the development of oxygen toxicity. To evaluate their possible role in changing the activity of pulmonary SOD activity, macrophages and leucocytes free in the air spaces were obtained from 16 rats exposed to 85% oxygen for 7 days and from three groups of control animals. We obtained concentrations of 30–50 × 10⁶ cells per milliliter with 65–72% viable. These cells contained 7.6 × 10⁻¹² g DNA per cell, and 1.4 × 10⁶ cells had 1 U of SOD activity. There was no significant difference in SOD activity per cell between the exposed and control groups. Therefore, 1.3 × 10⁸ alveolar leukocytes would contain 1 mg of DNA and would have 93 U of SOD.

Effects of 85% oxygen on SOD activity in different tissues. Four rats were exposed to 85% oxygen for 7 days, and the activities of SOD in brain, heart, lung, liver, kidney, erythrocytes, and plasma are shown in Table 2. The SOD activity in these lungs was evaluated by both the epinephrine-adrenochrome assay and the xanthine-xanthine oxidase assay, and the increase in activity of SOD was found with both techniques. Only the lung and brain had an increase in SOD.

Development of tolerance and time course of SOD changes. The time course of the development of tolerance was compared to the time course of the increase in activity of pulmonary SOD. In separate experiments, each using 14–16 animals,

TABLE 2. Superoxide dismutase activity in different tissues after 7-day exposure to 85% oxygen

	Organ Wt, g		SOD/Organ, U	
	Control	Exposed	Control	Exposed
Lung				
Epinephrine assay	1.33 ± .01	2.12 ± .09	590 ± 17	833 ± 62†
Xanthine oxidase assay			340 ± 14	570 ± 33‡
Brain	1.88 ± .06	2.10 ± .06	744 ± 20	831 ± 17§
Heart	.96 ± .01	.86 ± .03	485 ± 10	393 ± 11‡
Liver	14.8 ± 1.0	10.9 ± .2	46,200 ± 1,100	28,300 ± 770‡
Kidney	2.74 ± .11	2.43 ± .06	4,590 ± 200	3,770 ± 160§
Whole blood			799 ± 89*	636 ± 33*
Plasma			31 ± 2†	28 ± 2†

Values are means ± SE. n = 4 for each group. * U SOD/ml whole blood. † U SOD/ml plasma. ‡ Two-sided, P ≤ 0.01. § Two-sided, P ≤ 0.05.

rats were exposed to 85% oxygen for 1, 2, 3, 4, 5, 7, and 8 days. At the end of each of these time periods, 10 rats were placed in 100% oxygen to test for oxygen tolerance, and the remaining four to six animals were sacrificed and the activity of SOD in the lungs was determined. To determine the reproducibility of the results obtained from this group of exposures, 24 additional rats were exposed to 85% oxygen and groups of four were sacrificed after 2, 3, 4, 5, 6, and 8 days, and their pulmonary SOD activity was compared to that of 12 matched control animals. The results of this group of experiments closely reproduced those obtained from the initial exposures; therefore, the results obtained from each different exposure period were combined and are shown in Fig. 1. The increase in activity of pulmonary SOD occurs between days 3 and 5 of the 85% oxygen exposure. This correlates well with the development of tolerance after 3 days of the 85% oxygen exposure. Those animals which did not demonstrate tolerance died 24–72 h after being transferred to 100% oxygen. Several of the groups of animals showing tolerance were exposed to 100% oxygen for periods up to 21 days. Of those animals which survived 72 h in 100% oxygen, only one died subsequently.

During 85% oxygen exposures the increase in pulmonary SOD activity was found to closely correlate with the increase in lung weight. To evaluate the direct role that the formation of edema might have in changing the activity of pulmonary SOD, four rats were exposed to 100% oxygen, and after 66 h when they were moribund with cyanosis and respiratory distress, they were sacrificed. They were found to have a 30% increase in lung weight over that of matched controls, but had a 25% decrease per whole lung (P < 0.05) in pulmonary SOD activity. This is compatible with the finding that during exposure to 85% oxygen significant increases in pulmonary SOD activity do not occur until the 4th day, and with our postulate that this is necessary for adaption to 100% oxygen. Perhaps control animals which die between 60 and 72 h in 100% oxygen do so in

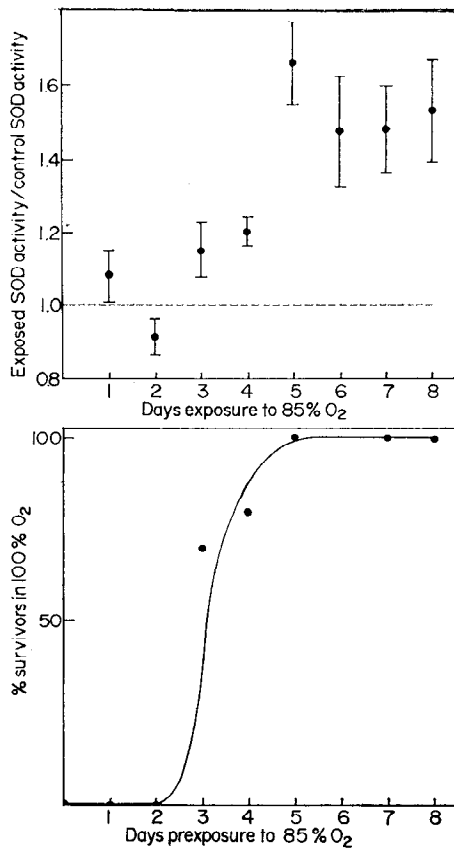


FIG. 1. Time course of changes in pulmonary SOD activity and development of tolerance during 85% oxygen exposure. Top figure is a composite of several individual experiments and is graphed as composite mean \pm SD for each time period. Each individual experiment showed a significant increase in pulmonary SOD levels among exposed animals by 4th day that persisted throughout day 8. In each instance beginning with day 4, $P < 0.05$ and often $P < 0.01$. $n = 10$ for bottom figure.

part because by that time period the activity of the enzyme SOD has not yet changed to higher and therefore more protective levels in the lung. The finding of a decrease in pulmonary SOD in the presence of acute increases in lung weight does demonstrate that significant amounts of SOD do not enter the lung in the edema fluid. During the 85% oxygen exposures, slight decreases were found in pulmonary SOD activity only at 48 h. After 96 h of exposure those factors causing a decrease in pulmonary SOD activity were dominated by those factors leading to an increase in the enzyme activity. The decrease in SOD activity could be related to the general effects of sickness, starvation, and dehydration on the lung and is similar to the effects of 85% oxygen exposure on heart, liver, and kidney.

Oxygen tolerance has previously been shown to persist for periods up to 1 mo after return to air (6). We, therefore, related the duration of the increase in pulmonary SOD activity with the duration of the tolerant state. Eighty rats were exposed to 85% oxygen for 7 days and then kept in air for 0, 6, 13, 20, and 30 days. At each of these time periods, pulmonary SOD activity was determined on 6 rats, and 10 rats were exposed to 100% oxygen for 96 h. Matched controls were used for each time period, and the results are contained in Fig. 2. This figure shows that SOD activity per

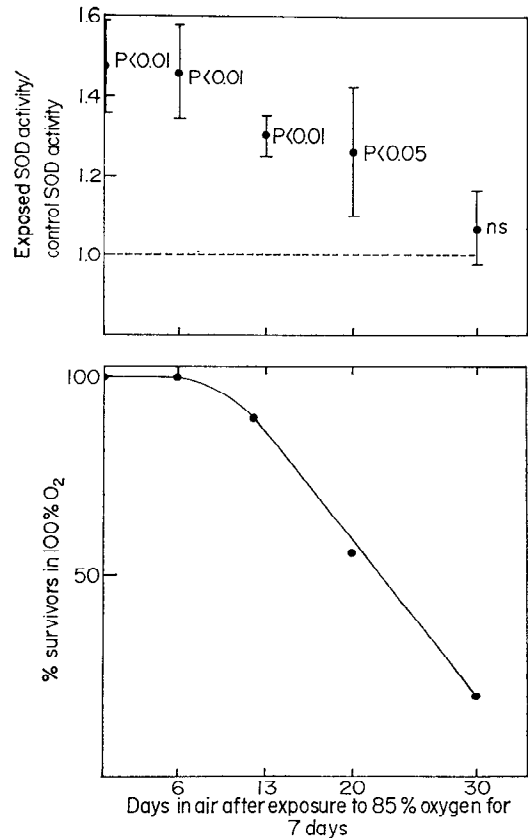


FIG. 2. Duration of increased pulmonary SOD activity after return to air compared to duration of the tolerant state. In top figure, all data are means \pm SD with $n = 6$. $n = 10$ for bottom figure.

whole lung remained elevated for 6 days after return to air and then progressively decreased over the 30-day period. Tolerance to 100% oxygen similarly remained unchanged for 6 days and then gradually decreased over the remainder of the 30-day period.

Comparisons of different species. Fourteen guinea pigs were exposed to 85% oxygen; after 7 days 10 had died and the SOD activity was determined on the four survivors (Table 3). Fourteen hamsters and 16 mice were exposed to 85% oxygen for 7 days, and then 10 of each species were further exposed to 100% oxygen, and the remainder were used to determine activity of pulmonary SOD (Table 3). Only the rat demonstrated both an increase in SOD and tolerance to pure oxygen. Guinea pigs and hamsters showed no change in SOD activity and did not develop tolerance. Mice showed a small increment in SOD activity and were not tolerant to 100% oxygen.

DISCUSSION

When an animal is exposed to pure oxygen at 1 atm for prolonged periods, the primary site of injury is the lung. Under these conditions the P_{O_2} to which the lung is exposed is markedly higher than that of other organs. While the lung would have a P_{O_2} close to 700 mmHg, there is very little change in tissue P_{O_2} of most other tissues. Hemoglobin is nearly fully saturated with oxygen when it is exposed to air at 1 atm, and the oxygen dissolved in blood with a P_{O_2} of 700 mmHg is relatively small. Only a slight change in

TABLE 3. Pulmonary superoxide dismutase activity in different species after 7-day exposure to 85% oxygen

	Lung Weight	SOD/Lung, U	Percent Increase	Percent Survival in 100% O ₂ (7 days)
<i>Rat</i>				
Control	1.20 ± .03	777 ± 48	46	0
Exposed	1.74 ± .08	1,130 ± 87		100
				(<i>P</i> < 0.01)
<i>Guinea pig</i>				
Control	2.61 ± .15	1,142 ± 64	9	Died in
Exposed	5.18 ± .3	1,247 ± 89		85% O ₂
				(NS)
<i>Hamster</i>				
Control	.588 ± .01	250 ± 3	0	0
Exposed	.562 ± .03	249 ± 13		0
				(NS)
<i>Mice</i>				
Control	.17 ± .01	122 ± 3	18	0
Exposed	.21 ± .03	145 ± 3		0
				(<i>P</i> < 0.01)

Lung weight and SOD/lung values are means ± SE. NS = *P* > 0.10 in two-sided test.

mixed venous PO₂ occurs when breathing 100% oxygen at 1 atm, and the mixed venous PO₂ reflects the tissue PO₂ under most conditions.

The mechanism by which oxygen causes injury to tissue is not understood, but several theories have been proposed. Evidence from both in vivo and in vitro experiments demonstrates that increased oxygen tensions produce alterations in cellular metabolism (11). A number of enzymes containing sulfhydryl groups are inactivated by oxygen, and the sulfhydryl groups of several smaller compounds, such as reduced glutathione, lipoic acid, and coenzyme A are oxidized. These phenomena have been proposed as important components of oxygen toxicity (12, 22). Oxygen also may damage cell membranes by lipid peroxidation which may lead to mitochondrial swelling and release of protein (27). One toxic product of oxygen metabolism is H₂O₂, and the presence of catalase or peroxidase has been proposed to be important in protecting against oxygen toxicity (4, 21).

Gershman (9) first proposed that oxygen toxicity may be related to formation of free radicals, chain reactions, and destructive oxidations. Gershman noted that the damage caused to tissue by ionizing radiation and that caused by increased pressures of oxygen were similar. Many agents protect against damage from both sources. Antioxidants, such as alpha-tocopherol, protect against uncontrolled oxidations due to free radicals by acting as scavengers and have been shown to protect against some of the toxic effects of oxygen at high pressures. Gershman suggested that aerobic animals developed defenses to enable them to survive in a normal atmosphere of 20% oxygen. These mechanisms of protection from harmful oxidations could be overwhelmed by exposure to irradiation or high tensions of oxygen (9). While indirect evidence has been obtained to support this theory (9, 12, 22), the specific free

radicals involved and their source of production in vivo have not been established.

Molecular oxygen has two unpaired electrons and is paramagnetic. This presents a spin restriction to an interaction with an exogenous electron pair and would make it likely that oxidation reactions involving oxygen would proceed by one-electron steps and would produce free radical intermediates such as the superoxide radical (8). Fridovich (8) has suggested that this free radical is biologically significant and that aerobic cells must have a defense system to protect against its potential toxicity. The superoxide radical has been documented to have a fleeting existence in aqueous media after being produced by pulsed radiolysis, flash photolysis, irradiation with an electron beam, and exposure to an alpha-ray source (19). Its presence in biological systems is dependent on its enzymatic production, and several enzyme systems involved in oxygen metabolism have been associated with production of the superoxide free radical. A rapid freezing technique has been used with electron spin resonance to confirm the production of the superoxide radical by xanthine oxidase (14). Reduced flavins and quinones and clostridial and spinach ferredoxins have been shown to carry out the univalent reduction of oxygen (23, 24), and the slowly occurring autoxidation of hemoglobin results in the generation of superoxide radicals (26) as does the autoxidation of the reduced form of the antibiotic streptonigrin (33). The rate of production of the superoxide radical for some enzymes (but not all) has been shown to be dependent on the PO₂. Xanthine oxidase and the ferredoxins produce increased amounts of univalently reduced oxygen in the presence of higher oxygen tensions (7, 23). Since it is a free radical, the superoxide anion should be cytotoxic. It has been shown to interact with H₂O₂ to form the hydroxyl radical (OH⁻) (2), to hydroxylate some aromatic compounds (30), and to be involved in lipid peroxidation (36). It has the ability to be both a potent oxidizing and a reducing species, since it can either accept another electron or give up its extra electron. Superoxide dismutase, a blue, copper and zinc containing enzyme with a molecular weight of 34,000, was first isolated in 1939 from bovine erythrocytes (16), but its enzymatic function was not appreciated until McCord and Fridovich (19) in 1969 demonstrated its ability to dismutate superoxide free radicals. Two assays have been described for SOD. The first is the xanthine-xanthine oxidase system in which SOD prevents cytochrome *c* reduction by O₂⁻ produced by xanthine oxidase (19). The second is based on the observation that epinephrine spontaneously oxidizes to adrenochrome (25). At a high pH this oxidation is thought to be dependent on O₂⁻, since it can be inhibited by extremely small quantities of SOD. SOD is remarkably stable, retaining a large fraction of its activity in 9.0 M urea at neutral pH. It is primarily found in the supernatant fraction of the cell, but SOD activity has also been found in the mitochondria. This form of SOD contains manganese rather than copper and zinc and represents about 10–20% of the total SOD activity in cells (32).

McCord et al. (20) have postulated that SOD protects cells from the deleterious actions of the superoxide radical. They tested this thesis by relating the SOD activity of microorganisms to their capacity to tolerate oxygen in the environment. They found that all aerobes contained sig-

nificant levels of SOD and that all strict anaerobes contained no SOD. Microaerotolerant anaerobes, those which tolerate exposure to air and grow in air, but do not utilize oxygen as their major electron receptor, were found to contain low levels of SOD activity. The single exception to this was *Lactobacillus plantarum* which contained no SOD, but it was found to consume no detectable amounts of oxygen even when growing in air. This theory has been further tested in bacteria by Gregory and Fridovich (10) by exposing *Streptococcus faecalis* and *E. coli* to hyperbaric oxygen and demonstrating a large increase in SOD activity. *S. faecalis* grown in 100% oxygen was also shown to be more resistant to the deleterious effects of 46 atm of oxygen than were bacteria which had been grown under nitrogen.

We considered that the superoxide free radical may fulfill some of the characteristics suggested by Gershman and that it may be one of the important causes of the toxic effects of oxygen in mammals. In the presence of increased oxygen tensions, increased amounts of this reactive radical may be produced enzymatically and lead to harmful oxidations and reductions of cellular components. Since rats are capable of undergoing an adaptive change if first exposed to sublethal doses of oxygen which can subsequently protect against a lethal exposure, we postulated that adaptive changes may increase the activity of superoxide dismutase in the involved tissue.

We tested this postulate by using rats made tolerant to oxygen and found that the activity of SOD did increase when compared per whole lung and per milligram DNA. These increases in SOD activity changed in parallel with the development of oxygen tolerance, and they remained elevated for a time period corresponding to the duration of the tolerant state. Among the organs evaluated, only the lung responded to the oxygen stress with a statistically significant increase in SOD. We did not find other species which developed oxygen tolerance under the same conditions as the rat, and during a sublethal oxygen exposure none of the other three species studied developed as large a change in pulmonary SOD activity as did the rat.

Although these results are consistent with the postulate that SOD activity may relate to oxygen tolerance, the change in activity of SOD with exposure to 85% oxygen could be coincidental and relate to other effects of oxygen. We have found that the increased number of erythrocytes or leucocytes in the lung is probably not sufficient to produce the increased SOD activity. Erythrocytes can be effectively washed out of the lung, and alveolar macro-

phages and other cells free in the air spaces do not contain enough activity of SOD to account for the change noted. Also, other species which have been shown to have the same histological response did not have changes in their levels of pulmonary SOD activity. A humoral or general body response to the sublethal stress is unlikely to have caused the change in activity of SOD because no other organ was similarly affected.

Two important possibilities are: 1) that there is a general increase in SOD content of some cell types by induction of the enzyme or by decreased degradation, and 2) the increased content of SOD could be due to a change of cell population patterns. In several species alveolar type I cells have been shown to be destroyed during the development of oxygen toxicity, and alveolar type II cells markedly proliferate (1, 31). If type II cells do proliferate in the rat exposed to 85% oxygen and if they contain significantly more SOD than other lung cells, this may account for the increase in SOD activity. If so, it may be the presence of increased numbers of a more oxygen-resistant metabolically active cell type which is responsible for the development of tolerance, and this could be consistent with the postulate that SOD provides increased resistance to oxygen toxicity.

Since so many different metabolic systems have been shown to be influenced by high tensions of oxygen, it is not likely that oxygen toxicity is due to a single toxic action of oxygen. It is most reasonable to suppose that a number of actions and reactions of oxygen will be shown to be involved to different degrees, and this may vary depending on the tissue involved and the tension of the oxygen. The development of tolerance is probably multifactorial. The ability to eliminate the toxic species, the ability to repair the injured cell, and the ability to remove cellular debris may be involved.

The data reported in this paper indicate that SOD may play a role in pulmonary oxygen toxicity by reducing the concentration of the free radical superoxide.

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