The Alteration of Superoxide Dismutase, Catalase, Glutathione Peroxidase, and NAD(P)H Cytochrome *c* Reductase in Guinea Pig Polymorphonuclear Leukocytes and Alveolar Macrophages during Hyperoxia

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ABSTRACT Superoxide dismutase, catalase, glutathione peroxidase, and NAD(P)H cytochrome *c* reductase were quantitated in polymorphonuclear leukocytes (PMN) and alveolar macrophages (AM) obtained from guinea pigs exposed up to 90 h to 85% oxygen. PMN and AM were sonicated and separated into a 16,000-*g* pellet, a 100,000-*g* pellet, and a 100,000-*g* supernate. Superoxide dismutase activity increased in both cells within 18 h, persisted for 66 h and decreased by 90 h. The highest rate of increase was in the 100,000-*g* pellet containing 3.4% of total enzyme activity in PMN but 28% in AM. The enzyme induction in PMN and AM was partially inhibited by daily intracardiac injections of 50 mg/kg actinomycin D.

During oxygen exposure, catalase activity in PMN and AM decreased to 60% of its original activity, and glutathione peroxidase was reduced in PMN to 60% and in AM to 20% of control values. Although NAD(P)H cytochrome *c* reductase decreased to 50% in PMN, no change was noted in AM. Upon exposure to superoxide anion, purified catalase, the glutathione peroxidase of the 100,000-*g* supernate, NADH, and NADPH cytochrome *c* reductases of the 16,000-*g* pellet decreased to 66±5%, 72±4%, 52±8%, and 40±9%, respectively, of their original activity. This inactivation was prevented by 0.1 mg superoxide dismutase. These in vitro observations could explain the decreased catalase and glutathione peroxidase activity demonstrated in vivo that may lead to an intracellular accumulation of hydrogen peroxide. Increased hydrogen peroxide concentrations have been found to inactivate superoxide dismutase thus impairing the first defense mechanism against superoxide anion.

INTRODUCTION

Aerobic organisms require oxygen, but when they are exposed to hyperoxia, they die. The irreversible pulmonary and other tissue damage upon breathing high concentrations of oxygen is well documented (1). It is thought that activated metabolites of oxygen, e.g. superoxide anion (O_2^-)[1], hydroxyl radical, oxygen singlet, and hydrogen peroxide are the sources of damage (2, 3). Protection against one of the reduction products, O_2^-, arises from the metalloenzyme, superoxide dismutase (SOD) which catalyzes the dismutation of O_2^- to hydrogen peroxide (4):

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

SOD is widely distributed throughout all aerobic organisms but is lacking in strict anaerobes (5). Four kinds of SOD have been identified: a copper-zinc form localized in the cytosol of eukaryotic cells, a manganese-containing SOD found in mitochondria or in the matrix of bacteria (6), and an iron-bound enzyme in the periplasmic space of *Escherichia coli*. (7). Only the copper-zinc enzyme is inhibited by cyanide (8).

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Abbreviations used in this paper: AM, alveolar macrophages; FI O_2, fraction of inspired oxygen; O_2^-, superoxide anion; PMN, polymorphonuclear leukocytes; SOD, superoxide dismutase.

Several unspecific oxidases are known to generate O_2^- (3). Some of the O_2^- generating enzyme systems are dependent on the ambient oxygen concentration. For instance, the oxidation of xanthine by xanthine oxidase and the autooxidation of ferridoxin produce increased amounts of O_2^- in the presence of higher oxygen tension (9, 10). Since a high oxygen environment increases the rates of O_2^- production, it seems possible that, in turn, the increased substrate, O_2^-, would induce SOD. This has been demonstrated in studies performed on yeast and bacteria (11). *Saccharomyces cerevisiae* and *E. coli* grown under 100% or hyperbarric oxygen contain elevated levels of SOD compared to anaerobically grown bacteria (12). Rats, guinea pigs, and hamsters exposed to 85% oxygen also had increased SOD activity in their lung tissue (13). In these studies no attempt, however, was made to isolate the cellular source of the increased SOD from the lung, known to contain 40 different cell types (14). Therefore, we determined SOD in isolated alveolar macrophages (AM) and peritoneal polymorphonuclear leukocytes (PMN) from guinea pigs to assess the influence of the oxygen environment on a given phagocytic cell type. In a previous study, we demonstrated that AM contain five times more SOD activity than peritoneal PMN (15). This difference in SOD activity could be explained by the difference in oxygen tension between the lung and peritoneal cavity. To investigate how high oxygen tension alters in vivo SOD and other enzymes related to oxygen and its reduction products in AM and PMN, we studied SOD, catalase, glutathione peroxidase, and NAD(P)H cytochrome *c* reductase activities after the exposure of guinea pigs to a fraction of inspired oxygen (FI O_2) of 85%.

METHODS

Exposure of guinea pigs to 85% oxygen. Guinea pigs were maintained in a 35 × 40 × 86 cm plastic chamber at a temperature of 24°C. The FI O_2 of 85% was generated by flowing 8 l/min of humidified oxygen (Aquapak Respiratory Care, Inc., Arlington Heights, Ill.) through the chamber. The oxygen concentration was monitored continuously with a Beckman oxygen monitor, model OM 10 or OM 12. (Beckman Instruments, Inc., Fullerton, Calif.) Control animals were kept under similar conditions at room air.

Preparations of PMN and AM. PMN and AM were obtained by a method previously described (15). In brief, PMN were harvested 18 h after intraperitoneal injection of 12% casein. AM were obtained from the same guinea pigs by bronchopulmonary lavage. The cells were suspended in 0.1 M phosphate buffered saline, pH 7.4, warmed to 37°C (16). The cell suspensions were centrifuged at 200*g* at 4°C and washed thrice in Krebs Ringer phosphate buffer, pH 7.4. The AM suspension was further purified on a Ficoll-Hypaque gradient (17) which achieved a purification of 95% viable macrophages documented by the exclusion of 0.2% Trypan blue and a Wright's or monocyte-specific α-naphthyl esterase stain (18). Erythrocytes were lysed by exposure to deionized water for 20 s. Isotonicity was then restored by the addition of an appropriate volume of 3.5% saline. The final samples of PMN and AM were resuspended in Krebs Ringer phosphate buffer to a concentration of 2×10^8 cells/ml and 5×10^6 cells/ml, respectively. To insure that all enzyme within the cell was accessible to substrate, the cells were sonicated at 4°C for 1 min with a sonifier cell disruptor, model W 140 by Branson Power Corporation with an output control setting of three which disrupted greater than 99.7% of the cells. The sonicates were centrifuged at 16,000-*g* for 20 min, and the resultant supernate was again centrifuged at 100,000-*g* for 1 h in a Beckman ultracentrifuge, model L3-50, with a fixed angle rotor, type 30. The 16,000-*g* and 100,000-*g* pellets were resuspended in ice cold 0.2% (vol/vol) Triton X 100 to lyse mitochondria, lysosomes, and other cell organelles. The 16,000-*g* and 100,000-*g* pellets and the supernate were assayed for cyanide sensitive and cyanide insensitive SOD, catalase, glutathione peroxidase, and NAD(P)H cytochrome *c* reductase. The protein concentration of each cell fraction was determined with the Folin phenol reagent and compared to human albumin as a standard (19).

Enzyme assays

SOD. All SOD data reported in this paper were obtained by the assay method of McCord and Fridovich (20). This assay depends upon the capacity of SOD to inhibit the cytochrome *c* reduction mediated by O_2^- generated during the oxidation of xanthine catalyzed by xanthine oxidase. Xanthine (grade 3), xanthine oxidase (grade 4), and horse heart cytochrome *c* (type VI) were obtained from Sigma Chemical Corporation, St. Louis, Mo. The assays were performed at room temperature in 0.05 M potassium phosphate buffer, pH 7.8, containing 0.1–1.0 mg of protein. At pH 7.8 the manganomitochondrial type enzyme is as active as the copper-zinc cytosol type enzyme (21). Total SOD activity was measured without cyanide. The manganese containing enzyme was determined by observing the SOD activity remaining after the addition of 1 mM cyanide. Superoxide radical was generated by incubating 50 μM xanthine with 0.01 U of xanthine oxidase. Since SOD and cytochrome *c* both compete for O_2^- generated by this system, maximum sensitivity of the SOD activity was obtained with 5 μM cytochrome *c*(22). Bovine erythrocyte SOD obtained from Truett Laboratories, Dallas, Tex. was used as a standard. The change of the optical density was read at 550 nm with the Gilford model 2400 recording spectrophotometer. (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) Since azide does not interfere with this assay, each reaction mixture contained 1 mM sodium azide to inhibit cytochrome *c* oxidase which would produce a false inhibition rate of cytochrome *c* reduction (8, 23). 1 U of bovine erythrocyte SOD/ml of the reaction mixture diminished the cytochrome *c* reduction to 30% of control. We compared the SOD activity found in each sonicate to the amount of protein causing a 30% inhibition and converted this activity in units/milligram protein. Since the percentage of inhibition was not linear over a wide range of protein concentrations, we utilized several different protein concentrations of cell sonicates to obtain the inhibition rate within a few percentages of 30%. Boiled cell sonicates or apoenzyme preparation did not inhibit cytochrome *c* reduction.

Catalase. Catalase was assayed by a previously described method (24). The oxygen produced by catalase upon addition to hydrogen peroxide was measured with a Clark membrane oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio) by using an expanded

scale Varian Recorder (Varian Association, Palo Alto, Calif.). 5 μl of 30% hydrogen peroxide was added to Krebs Ringer phosphate buffer at a final volume of 3.0 ml. The sonicates containing 10–50 μg protein were added, and the linear increase in oxygen concentration was recorded for 1 min. A standard curve was constructed by using purified bovine liver catalase (Sigma Chemical Co.) Catalase activity of the cell sonicate was expressed as units/milligram protein.

Glutathione peroxidase. Glutathione peroxidase was determined by the method of Paglia and Valentine (25) as modified by Holmes et al. (26). The reaction mixture contained 2.48 ml of 0.05 M phosphate buffer, pH 7, containing 5 mM EDTA, 0.1 ml 8.4 mM NADPH, 0.1 ml 0.15 M reduced glutathione, 0.01 ml 1.125 M azide, and 4.6 U glutathione reductase obtained from Sigma Chemical Co. The reaction was initiated by the addition of 0.1 ml 2.2 mM hydrogen peroxide to the reaction mixture containing 500–1,000 μg protein and the change in the optical density was read at 340 nm for 4 min. The data were expressed as nanomoles NADPH oxidized to NADP/milligram protein by using the extinction coefficient of 6.2×10^3 M^{-1} cm^{-1} (27). All data were corrected for the linear NADPH oxidation by hydrogen peroxide alone in the absence of enzyme protein which was always less than 5% of the total reaction.

NAD(P)H cytochrome c reductase. NAD(P)H cytochrome *c* reductases were assayed with the method of Yoshida et al. (28). The reaction mixture consisted of 0.07 mM NAD(P)H, 0.14 mM ferricytochrome *c*, 1 mM potassium cyanide, 50 mM potassium phosphate buffer, pH 7.5, and 500 μg protein sonicate of PMN or AM in a total volume of 3.0 ml. The reaction was initiated by adding NAD(P)H. The reduction of cytochrome *c* was followed for 4 min at 25°C spectrophotometrically at 550 nm. The reaction was linear for 4 min. Activity was expressed as nanomoles reduced cytochrome *c*/milligram protein per minute by using an extinction coefficient of 21 mM^{-1} cm^{-1}. All data were corrected for the spontaneous reduction of cytochrome *c* without NAD(P)H. No reaction was noted between cytochrome *c* and NAD(P)H or potassium cyanide in the absence of protein.

Calculation of total enzyme activity. Total enzyme activity was calculated by adding the total activity of each cell fraction. This was obtained by multiplying specific activity (units/milligram protein/minute) times the total protein of each fraction. All enzymes were determined at 18, 42, and 66 h after the animal was exposed to 85% oxygen. In addition, SOD was assayed at 90 h after oxygen exposure. The control samples for each enzyme were obtained from normal animals kept at room air for the same time periods. At least five experiments were performed for each enzyme at the corresponding time period. Cells were pooled from several animals to obtain sufficient material.

Exposure to superoxide radical. Each of the studied enzymes was exposed to an O_2^- generating system containing 0.05 M potassium phosphate buffer at pH 7.8 with 0.1 mM EDTA, 50 μM xanthine, and varying amounts of xanthine oxidase. After exposure to 11 and 20 nmol O_2^-/5 min, the enzymes were assayed as described above and compared to the controls containing xanthine or xanthine oxidase and to samples containing 100 μg bovine erythrocyte SOD.

RESULTS

Animal survival. In preliminary studies none of the guinea pigs survived 100 h after exposure to 85% oxygen. All animals showed some signs of respiratory

TABLE I
SOD in PMN

	U mg protein^{-1} minute^{-1} (mean±SEM)			
Hours of exposure to 85% O_2	16,000 *g* pellet	100,000 *g* pellet	100,000 *g* supernate	Total activity*
0	16.20±1.2	1.20±0.12	0.177±0.009 0.030±0.006‡	8.1±1
18	36.60±2.7 ($P < 0.001$)	3.18±0.12 ($P < 0.001$)	0.219±0.018 (NS) 0.060±0.015‡ ($P < 0.01$)	18.8±2 ($P < 0.001$)
42	31.89±1.8 ($P < 0.001$)	3.75±0.45 ($P < 0.001$)	0.255±0.021 ($P < 0.02$) 0.030±0.018‡ (NS)	17.0±1 ($P < 0.001$)
66	42.87±2.7 ($P < 0.001$)	5.64±0.60 ($P < 0.001$)	0.288±0.030 ($P < 0.001$) 0.069±0.015‡ ($P < 0.01$)	22.0±1 ($P < 0.02$)
90	24.39±3.6 ($P < 0.02$)	3.45±0.30 ($P < 0.001$)	0.273±0.015 ($P < 0.001$) 0.081±0.009‡ ($P < 0.01$)	12.2±1 ($P < 0.02$)
SOD in PMN of actinomycin-D treated guinea pigs				
18	16.74±1.2 (NS)	4.39±0.33 ($P < 0.001$)	0.234±0.021 ($P < 0.01$)	8.8±0.6 (NS)
42	31.08±0.3 ($P < 0.001$)	1.71±0.21 (NS)	0.150±0.012 (NS)	15.7±0.5 ($P < 0.02$)
66	37.50±2.7 ($P < 0.001$)	1.98±0.12 (NS)	0.186±0.015 (NS)	18.6±1.4 ($P < 0.001$)

P values of student *t* test; enzyme activity compared to control at 0 time.
* See text for details of calculation.
‡ Cyanide sensitive SOD.

distress by 42 h, and after 66 h the mortality was 15%. At 72 h animals ceased drinking and eating. At this time the additional intraperitoneal injection of 12% casein increased the mortality; thus, by 90 h only 60% of the animals survived. The actinomycin D treatment did not alter the mortality of guinea pigs exposed to a FI O_2 of 85%.

During the 90-h of exposure to hyperoxia, the protein distribution was similar in the different cell fractions of PMN and AM. As noted previously, in both cell types 48±3% of the total recovered protein was found in the 16,000-*g* pellet containing membranes, granules, and mitocondria 12±2% was in the 100,000-*g* microsomal pellet; and 40±4% was in the supernate (15). Although the distribution of protein did not differ between the phagocytes, the AM contained 2.6 times more protein per cell than PMN. It was not the intent to carry out true subcellular distributions of the enzymes under study but rather to insure total recovery of the enzymes from cells completely disrupted by sonication.

PMN

SOD. In PMN, the 16,000-*g* pellet contained 95% of the total recovered SOD activity; the 100,000-*g* pellet and the 100,000-*g* supernate contained 3.4±0.7% and 1.6±0.3% of total recovered SOD activity, respectively.

As noted in Table 1, by 18 h of oxygen exposure, SOD activity increased twofold in the 16,000-*g* pellet and threefold in the 100,000-*g* pellet. There was no significant increase in SOD activity in the 100,000-*g* supernate. The increased SOD activity in the 16,000-*g* pellet persisted throughout the period of exposure to an FI O_2 of 85% whereas the SOD activity in the 100,000-*g* microsomal pellet progressively increased and reached a maximum by 66 h. At 66 h an increase of SOD activity was also observed in the 100,000-*g* supernate. Cyanide sensitive SOD was present only in the supernate. Both the cyanide sensitive and insensitive SOD activities progressively increased during the 90-h of exposure to high oxygen. But, after 66 h total SOD activity in all three cell fractions progressively decreased.

To investigate whether the increase in SOD activity in the various fractions arose from new enzyme synthesis and not from an alteration of the active site of the enzyme molecule, DNA dependent messenger RNA was blocked by daily intracardiac injections of 50 mg/kg body weight of actinomycin D. The treatment was started 24 h before the exposure to an Fi O_2 of 85%. At 18 h only the 16,000-*g* pellet failed to increase the SOD activity. By 42 h, however, in the 100,000-*g* microsomal pellet and the supernate SOD activity did not rise significantly compared to actinomysin D treated animals exposed to room air

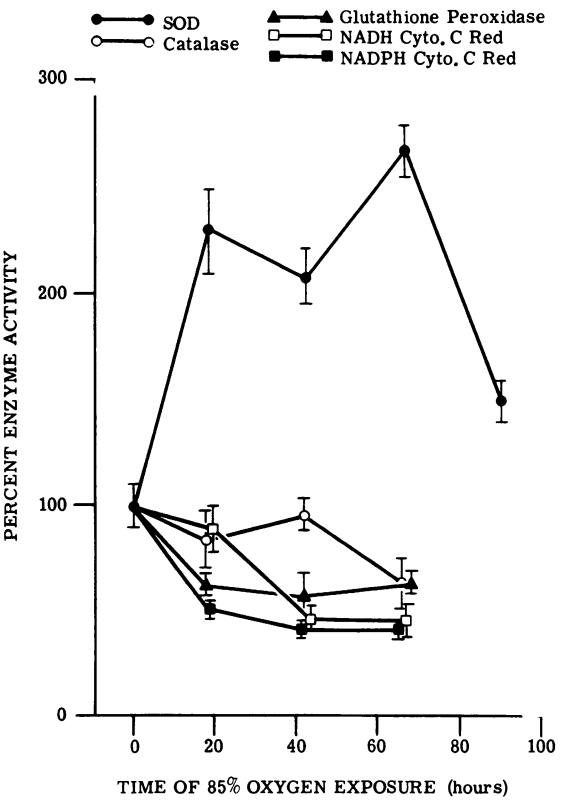

FIGURE 1 Percent of change in total SOD, catalase, glutathione peroxidase, and NAD(P)H cytochrome *c* reductase activities of PMN during animal exposure to 85% oxygen. 100% represents enzyme activity of control animals. (Mean ±SEM)

whose SOD activity did not change from untreated control animals. (Table I, Fig. 1).

Catalase. Catalase was found in each cell fraction. The 16,000-*g* pellet, the 100,000-*g* pellet, and the 100,000-*g* supernate contained 5±1%, 12±2%, and 83±3%, respectively of total catalase activity. After exposure of the guinea pig to hyperoxia, no change was noted in catalase activity in either type of cell pellet, but by 66 h catalase activity had decreased to 57% of its original value in the 100,000-*g* supernate. (Table II, Fig. I).

Glutathione peroxidase. Glutathione peroxidase was found in each cell fraction of the PMN. The 16,000-*g* pellet, the 100,000-*g* pellet, and the 100,000-*g* supernate contained 13±1%, 19±1%, and 68±6%, respectively, of total enzyme activity. After 18 h of exposure to an FI O_2 of 85% the glutathione peroxidase activity decreased in all three cell fractions, leading to a loss of 38% of its original total activity. Over the entire period of the study, glutathione peroxidase activity remained diminished (Table III, Fig. I).

NAD(P)H cytochrome c reductases. Both pyridine nucleotide dependent cytochrome *c* reductases were mainly located in the 16,000-*g* pellet which contained 80±2% of total enzyme activity; the 100,000-*g*

TABLE II
Catalase in PMN and AM

Hours of exposure to 85% O_2	16,000 *g* pellet	100,000 *g* pellet	100,000 *g* supernate	Total activity*
	U mg protein^{-1} *minute*$^{-1}$ *(mean±SEM)*			
I. PMN				
0	31±5	338±70	526±80	260±10
18	45±20 NS	265±110 NS	423±70 NS	218±40 NS
42	55±30 NS	214±40 NS	502±90 NS	250±20 NS
66	25±2 NS	304±80 NS	298±60 $P < 0.02$	162±30 $P < 0.02$
II. AM				
0	65±12	288±56	874±97	410±50
18	106±20 NS	230±87 NS	986±100 NS	470±70 NS
42	93±38 NS	233±76 NS	295±30 $P < 0.02$	190±20 $P < 0.02$
66	103±37 NS	238±46 NS	428±60 $P < 0.02$	250±40 $P < 0.02$

P values of student *t* test; enzyme activity compared to control at 0 time.
* See text for details of calculation.

pellet and the 100,000-*g* supernate contained only 15±2% and 5±1%, respectively, of total enzyme activities. After 18 h exposure to an FI O_2 of 85%, no change was noted in the NADH cytochrome *c* reductase activity in the 16,000-*g* pellet and 100,000-*g* supernate, but in the 100,000-*g* pellet a significant decrease to 52±2% of the original activity occurred. By 42 h the 16,000-*g* and 100,000-*g* pellet showed decreased enzyme activity (Table IV, Fig. I).

NADPH cytochrome *c* reductase activity decreased significantly in both pellets by 18 h and remained low over the entire exposure time. No change in NAD(P)H cytochrome *c* reductases was noted in the 100,000-*g* supernate.

TABLE III
Glutathione Peroxidase in PMN and AM

Hours of exposure to 85% O_2	16,000 *g* pellet	100,000 *g* pellet	100,000 *g* supernate	Total activity*
	nmoles NADPH mg protein^{-1} *minute*$^{-1}$ *(mean±SEM)*			
I. PMN				
0	18.1±4.29	15.09±2.61	55.62±6.39	32.7±3.0
18	10.0±1.50 ($P < 0.05$)	5.35±1.2 ($P < 0.001$)	37.80±2.10 ($P < 0.001$)	20.4±1.2 ($P < 0.01$)
42	6.3±0.60 ($P < 0.05$)	8.40±1.2 ($P < 0.01$)	42.00±2.40 ($P < 0.01$)	19.2±2.7 ($P < 0.01$)
66	8.7±0.30 ($P < 0.05$)	6.00±1.2 ($P < 0.01$)	40.20±2.10 ($P < 0.01$)	21.0±1.2 ($P < 0.01$)
II. AM				
0	31.00±6.21	117.50±2.4	139.56±16.47	82.8±12.0
18	27.60±1.50 (NS)	98.70±1.5 (NS)	38.70±6.30 ($P < 0.01$)	39.0±3.0 ($P < 0.02$)
42	26.40±2.7 (NS)	41.70±2.1 ($P < 0.001$)	88.80±19.50 ($P < 0.02$)	52.8±7.5 ($P < 0.02$)
66	33.90±2.1 (NS)	53.40±6.0 ($P < 0.001$)	35.10±3.60 ($P < 0.001$)	36.3±4.2 ($P < 0.001$)

P values of student *t* test; enzyme activity compared to control at 0 time.
* See text for details of calculations.

TABLE IV
NADH-Cytochrome C Reductase in PMN

Hours of exposure to 85% O_2	16,000 *g* pellet	100,000 *g* pellet	100,000 *g* supernate	Total activity*
	nmol Ferricytochrome c mg protein^{-1} minute^{-1} (mean ±SEM)			
0	1.50±0.25	1.87±0.380	0.066±0.010	0.95±0.11
18	1.54±0.22 (NS)	0.39±0.063 ($P < 0.02$)	0.078±0.012 (NS)	0.84±0.09 (NS)
42	0.69±0.10 ($P < 0.02$)	0.76±0.270 ($P < 0.02$)	0.087±0.003 (NS)	0.46±0.05 ($P < 0.02$)
66	0.56±0.18 ($P < 0.01$)	1.26±0.130 ($P < 0.001$)	0.111±0.018 (NS)	0.45±0.10 ($P < 0.01$)
NADPH-Cytochrome *c* reductase PMN				
0	1.60±0.29	1.68±0.420	0.105±0.020	1.00±0.20
18	0.87±0.15 ($P < 0.02$)	0.58±0.180 ($P < 0.05$)	0.072±0.020 (NS)	0.52±0.02 ($P < 0.02$)
42	0.61±0.13 ($P < 0.02$)	0.79±0.220 ($P < 0.05$)	0.110±0.010 (NS)	0.43±0.09 ($P < 0.02$)
66	0.58±0.22 ($P < 0.01$)	1.00±0.090 ($P < 0.05$)	0.090±0.020 (NS)	0.43±0.12 ($P < 0.02$)

P values of student *t* test; enzyme activity compared to control at 0 time.
* See text for details of calculations.

AM

SOD. The distribution of SOD differed between PMN and AM. The 16,000-*g* pellet, 100,000-*g* pellet, and 100,000-*g* supernate of AM of control animals contained 48%, 8%, and 44%, respectively, of total SOD activity. The same fractions of animals exposed to an FI O_2 of 85% represented 42, 28, and 30% of total enzyme activity. This change in the enzyme distribution was observed already by 18 h of exposure to hyperoxia and persisted during the course of the study. An increase of SOD activity was found by 18 h of exposure to hyperoxia in all three cell fractions. At this time, the 100,000-*g* pellet contained almost three times more SOD activity than the respective fraction of control animals and rose to fivefold by 42 h. Thereafter, SOD activity progressively decreased to threefold at 90 h. In the 16,000-*g* pellet SOD activity was increased twofold by 66 h and fell to initial levels at 90 h. A twofold increase in SOD activity occurred in the supernate over 42 h but then progressively decreased to control values by 66 h (Fig. 2). The increase in SOD activity in all cell fractions was mainly limited to the cyanide insensitive enzyme (Table V). After the administration of actinomycin D the SOD activity in AM rose with the exception of the 100,000-*g* supernate at 66 h. Although significantly different from the control animals at 0 time, the increase noted at 42 h in the 100,000-*g* supernate and at 66 h in the 100,000-*g* pellet was also significantly different ($P < 0.02$) and $P < 0.05$) from untreated animals exposed to hyperoxia (Fig. 3). SOD activity of actinomycin D treated animals exposed to air did not differ from untreated animals.

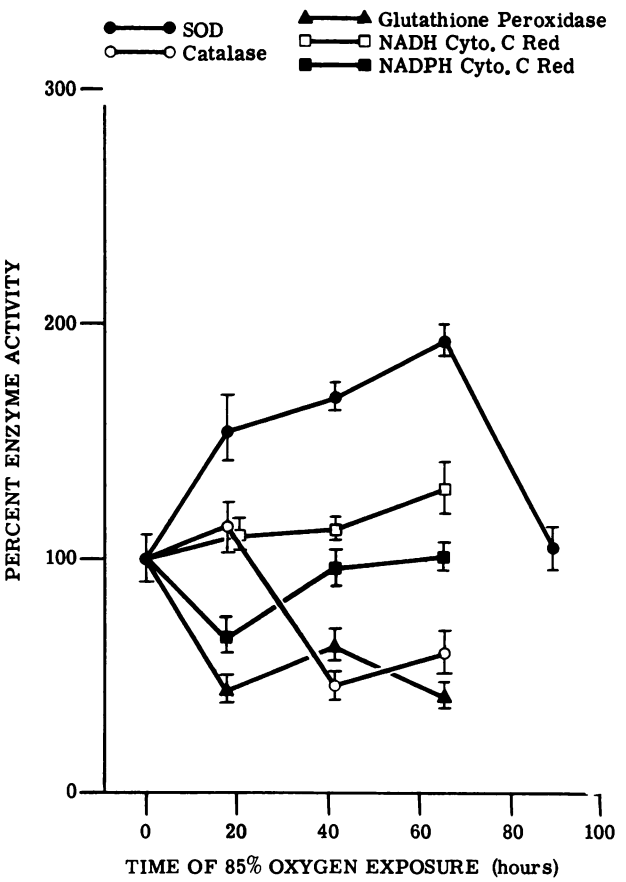

FIGURE 2 Percent of change in total SOD, catalase, glutathione peroxidase, and NAD(P)H cytochrome *c* reductase activities of AM during animal exposure to 85% oxygen. (Mean±SEM)

TABLE V
SOD in AM

Hours of exposure to 85% O_2	16,000 *g* pellet	100,000 *g* pellet	100,000 *g* supernate	Total activity*
	U mg protein^{-1} minute^{-1} (mean ±SEM)			
0	21.90±1.2	4.65±0.75	13.44±0.69 4.86±0.60‡	17.0±1.0
18	28.56±3.9 ($P < 0.01$)	15.36±1.59 ($P < 0.01$)	27.00±0.30 ($P < 0.001$) 3.00±0.30‡ (NS)	26.6±2.0 ($P < 0.01$)
42	27.30±1.5 ($P < 0.001$)	27.00±2.40 ($P < 0.01$)	31.26±0.30 ($P < 0.001$) 8.19±0.30‡ ($P < 0.01$)	28.8±1.0 ($P < 0.001$)
66	48.36±0.3 ($P < 0.001$)	22.74±2.70 ($P < 0.01$)	16.05±1.80 (NS) 3.00±0.60‡ (NS)	33.0±1.0 ($P < 0.01$)
90	21.30±1.5 (NS)	12.54±2.10 ($P < 0.05$)	15.30±2.10 (NS) 3.60±0.60‡ (NS)	18.0±2.0 (NS)
SOD in AM of actinomycin-D treated guinea pigs				
18	30.00±0.3 ($P < 0.01$)	35.10±3.00 ($P < 0.01$)	13.71±1.20 (NS)	24.0±2.0 ($P < 0.02$)
42	25.86±1.5 ($P < 0.02$)	25.41±3.00 ($P < 0.01$)	23.79±2.40 ($P < 0.02$)	25.0±2.0 ($P < 0.02$)
66	32.94±1.2 ($P < 0.02$)	13.50±1.20 ($P < 0.02$)	16.47±1.80 (NS)	24.0±1.5 ($P < 0.02$)

P values of student *t* test; enzyme activity compared to control at 0 time.
* See text for details of calculation.
‡ Cyanide sensitive SOD.

Catalase. There was no difference in the cellular distribution of this enzyme between AM and PMN. During hyperoxia the enzyme activity of the 16,000-*g* and 100,000-*g* pellets of AM did not change. In contrast to the PMN catalase activity decreased in the 100,000-*g* supernate by 42 h of hyperoxia and persisted for the length of the study (Table II, Fig. 2).

Glutathione peroxidase. There was no difference in the distribution of glutathione peroxidase between the two cell types. The enzyme activity in the 16,000-*g* pellet was not affected by the high oxygen exposure, but the 100,000-*g* pellet and especially the 100,000-*g* supernate showed a progressive decrease of enzyme activity during the entire course of the study. Glutathione peroxidase was decreased fourfold by 18 h in the cytoplasmic fraction (Table III, Fig. 2).

NAD(P)H cytochrome c reductases. In contrast to the cytoplasmic enzymes the distribution of the pyridine nucleotide dependent cytochrome *c* reductases differed between AM and peritoneal PMN. The 16,000-*g* pellet, the 100,000-*g* pellet, and the 100,000-*g* supernate contained 59±8, 15±2, and 26±5%, respectively, of total enzyme activity. There was also a difference in alteration of activity of these enzymes between PMN and AM. No change in NAD(P)H cytochrome *c* reductases occurred in any of the cell fractions during 66 h of oxygen exposure (Table VI, Fig. 2).

In vitro exposure of catalase, glutathione peroxidase, and NAD(P)H cytochrome c reductases to superoxide anion. Since catalase, glutathione peroxidase, and the pyridine nucleotide-dependent cytochrome *c* reductases decreased in some cell fractions, the effect of O_2^- on these enzymes was investigated. Purified catalase, the 100,000-*g* supernate containing glutathione peroxidase, and the 16,000-*g* pellet containing NAD(P)H cytochrome *c* reductases of PMN were exposed to 11 and 20 nmol O_2^- generated during 5 min. After an exposure to 11 nmol O_2^-/5 min catalase, glutathione peroxidase, and NAD(P)H cytochrome *c* reductase activities did not change. Upon exposure to 20 nmol O_2^-/5 min catalase, glutathione peroxidase, NADH, and NADPH cytochrome *c* reductases fell to 66±4, 72±4, 52±8, and 40±9%, respectively, of their original activity. The addition of 0.100 mg of bovine erythrocyte SOD to the various reaction mixtures prevented the diminution in enzyme activity. However, no inactivation occurred with xanthine or xanthine oxidase alone (Table VII).

DISCUSSION

SOD has been induced by exposing yeast and bacteria to increased concentrations of oxygen (11,

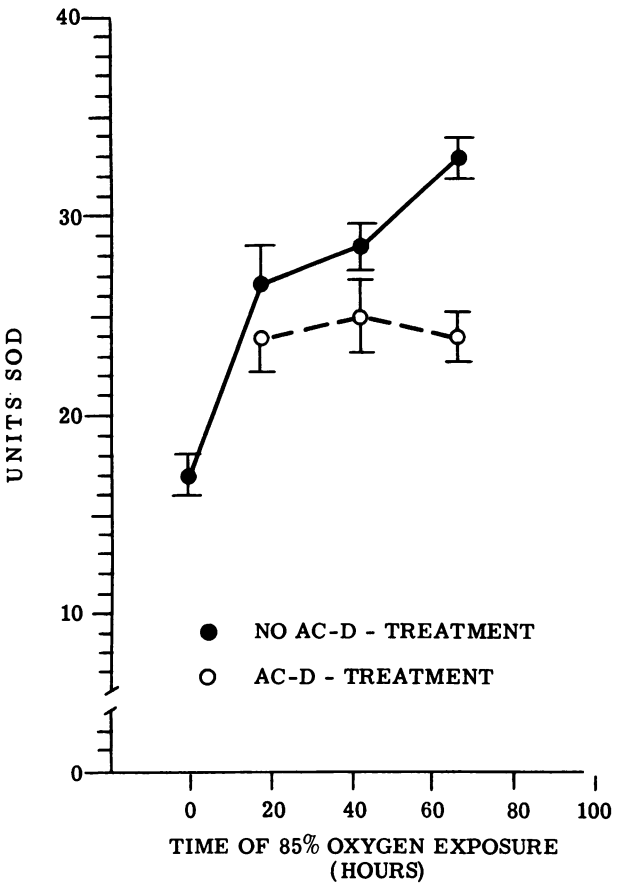

FIGURE 3 Total SOD activity per milligram AM protein of actinomycin D (AC-D) treated and untreated animals during exposure to 85% oxygen. (Mean±SEM)

12, 29). In mammalian species an FI O_2 above 80% induced SOD activity in the lung of rats and neonatal rabbits (13, 30). Our study extends these observations and shows that SOD can be induced in PMN as well as isolated AM of guinea pigs exposed to an FI O_2 of 85%. The increased SOD activity in both phagocytes was attributed mainly to the cyanide insensitive mitochondrial type enzyme which is in accordance with the results of others (30).

The increased SOD activity in AM cannot be explained by an inflammatory process or lung infection. Histologic and microbiologic studies of the lung by 18 h exhibited no difference between animals exposed to air or to an FI O_2 of 85%.

Ultrastructural studies have revealed that hyperoxia alters the morphology of mitochondria from type II alveolar living cells ("great alveolar cells") obtained from rats (31). The mitochondria elongate and became cupshaped leading to an increase of their overall volume (32). In addition, hyperoxia leads to an increase in free ribosomes and dilatation of the cisternae of the endoplasmic reticulum (33). Our biochemical findings of increased cyanide insensitive, mitochondrial-type SOD are consistent with these morphological observations.

The partial inhibition of SOD induction by actinomycin D verifies that new enzyme synthesis was responsible, in part, for the increased SOD activity in PMN and AM during hyperoxia. Others have shown that the dosage of actinomycin D employed in our study is sufficient in vivo to inhibit selectively the induction of an enzyme system but does not alter protein synthesis in general (34, 35). Three daily

TABLE VI
NADH-Cytochrome c Reductase in AM

nmol Ferricytochrome c mg protein^{-1} minute^{-1} (mean±SEM)				
Hours of exposure to 85% O_2	16,000 *g* pellet	100,000 *g* pellet	100,000 *g* supernate	Total activity*
0	3.09±1.02	2.64±0.93	1.46±0.27	2.40±0.45
18	3.78±0.09 (NS)	2.73±0.90 (NS)	1.23±0.18 (NS)	2.66±0.21 (NS)
42	3.54±0.43 (NS)	2.82±1.50 (NS)	1.76±0.33 (NS)	2.75±0.10 (NS)
66	5.01±1.00 (NS)	2.31±0.63 (NS)	1.31±0.06 (NS)	3.24±0.60 (NS)
NADPH-Cytochrome *c* reductase in AM				
0	6.39±1.38	3.96±1.11	2.16±0.66	4.47±1.10
18	3.60±0.30 (NS)	5.40±0.60 (NS)	1.65±0.45 (NS)	3.00±0.39 (NS)
42	6.30±1.20 (NS)	4.53±0.30 (NS)	1.76±0.18 (NS)	4.32±0.69 (NS)
66	6.78±0.30 (NS)	4.29±0.15 (NS)	1.86±0.27 (NS)	4.56±0.30 (NS)

P values of student *t* test; enzyme activity compared to control at 0 time.
* See text for details of calculation.

TABLE VII
In Vitro Exposure to O_2^- Generating System

Enzyme	Control activity	20 nmol O_2^- 5 min
Catalase* (purified Sigma)	3.00	1.90±0.100
Glutathione peroxidase‡ (PMN 100,000 *g* supernate)	55.62	40.00±2.000
NADPH-cytochrome *c* reductase§ (PMN 16,000 *g* pellet)	1.48	0.71±0.010
NADPH-cytochrome *c* reductase§ (PMN 16,000 *g* pellet)	1.60	0.96±0.050

* Units.
‡ Nanomoles oxidized NADPH.
§ Nanomole reduced Cytochrome *c*.

injections of actinomycin D did not alter SOD activity in PMN and AM of guinea pigs exposed to air. In AM the induction of SOD activity was inhibited in all cell fractions whereas it was inhibited only in the 100,000-*g* microsomal pellet of PMN. The difference in the ability of actinomycin D to inhibit the SOD induction in AM compared to the PMN may be related to lower protein synthesis in PMN.

In our study hyperoxia induced SOD selectively in PMN and AM whereas in bacteria and yeast catalase could also be induced by hyperoxia (12). Rather than an increase in catalase there was diminution after the exposure to an FI O_2 of 85%. Impaired activity was also observed in glutathione peroxidase of PMN and AM and NAD(P)H cytochrome *c* reductases of PMN. On the other hand, NAD(P)H cytochrome *c* reductases of AM were unaffected by hyperoxia.

A high oxygen environment damages pulmonary tissue as well as erythrocytes (31, 36–41). Since high oxygen concentrations increase the flux of O_2^- (9, 10), it is possible that the enzyme alterations are caused by this toxic and highly reactive radical. Indeed, we demonstrated that an O_2^- generating system inactivates a purified enzyme such as catalase as well as crude enzyme extracts. The addition of SOD to this system did prevent the deleterious effects of O_2^-. The similar distribution of SOD and NAD(P)H cytochrome *c* reductases in AM may provide an explanation for maintaining NAD(P)H cytochrome *c* reductases activity observed in AM during hyperoxia in vivo.

Based upon these observations we developed a model for the mechanism of oxygen toxicity in PMN and AM. Superoxide anion is generated during the univalent reduction of oxygen and is dismutated by SOD to hydrogen peroxide and oxygen (4). Hydrogen peroxide is mainly detoxified by catalase and glutathione peroxidase (3, 42, 43).

After an exposure to an FI O_2 of 85% the formation of O_2^- may increase in vivo similar to that found in vitro (9, 10). In response to the increasing amounts of substrate O_2^-, SOD is induced (11, 12, 29). Since SOD generates hydrogen peroxide, the intracellular increase of SOD leads to elevated hydrogen peroxide concentrations. Phagocytized latex particles coated with SOD also increase hydrogen peroxide concentrations in PMN (44). Others have shown that increasing the partial pressure of oxygen will raise hydrogen peroxide in isolated rat liver and rat liver mitochondria (45–47). In addition, in vivo studies with mice exposed to hyperoxia showed accumulated hydrogen peroxide in their erthrocytes (48). Another mechanism for the accumulation of hydrogen peroxide may be the inactivation of hydrogen peroxide detoxifying enzyme systems such as catalase and glutathione peroxidase. At 85% oxygen the SOD induction may be saturated, and O_2^- is in such excess that it alters hydrogen peroxide decomposing enzymes leading to low catalase and glutathione peroxidase activity as demonstrated in our study. Less catalase and glutathione peroxidase activity may increase hydrogen peroxide to concentrations that inactivate SOD (49, 50) (Fig. 4). It has been demonstrated that sufficient catalase is able to prevent an inactivation of SOD by its reaction product, hydrogen peroxide (51). This mechanism of inactivation of SOD by hydrogen peroxide can explain the decrease in SOD activity noted after 66 h of oxygen exposure since no decrease in the PO_2 of the animals exposed to an FI O_2 of 85% was noted (Fig. 1 and 2). The loss of SOD activity finally compromises the first defense mechanism of the cell against oxygen and its reduction product, O_2^-. This model is supported by the observation that catalase and SOD together, but not one of these enzymes alone, protected erythrocytes against lipid peroxidation induced by oxygen and dialuric acid (41). In addition, this model may provide a method to quantitate oxygen toxicity in PMN and AM.

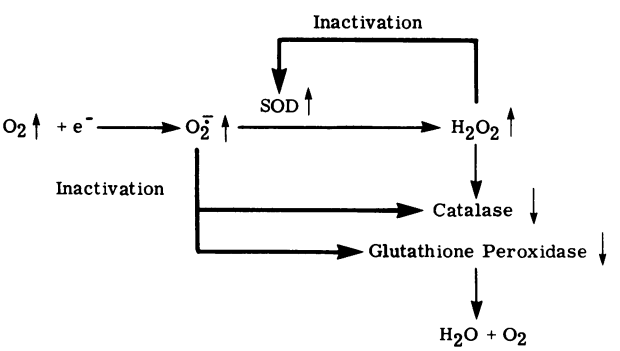

FIGURE 4 Model of interaction between superoxide anion and hydrogen peroxide and SOD, catalase, and glutathione peroxidase in AM, and PMN. See details in text.

Since glutathione peroxidase activity decreased after 18 h of 85% oxygen exposure followed by a loss of catalase activity, both enzymes could be used as a biochemical marker to quantitate oxygen toxicity in PMN and AM.

Clinical and morphology studies showed that the human organism is also susceptible to oxygen toxicity (52, 53). Although recent studies of human PMN and AM showed SOD distribution and activity similar to guinea pig cells (54), the application of our model to humans must await further studies.

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