Protection from Oxygen Toxicity with Endotoxin

ROLE OF THE ENDOGENOUS ANTIOXIDANT ENZYMES OF THE LUNG

L. FRANK, J. SUMMERVILLE, and D. MASSARO, *The Pulmonary Toxicology Laboratory, Veterans Administration Hospital and the Calvin and Flavia Oak Asthma Research Center, University of Miami School of Medicine, Miami, Florida 33101*

ABSTRACT Endotoxin treatment of adult rats before hyperoxic exposure significantly increases their survival rate in >95% O_2 (*J. Clin. Invest.* **61**: 269, 1978). In this study, we wished to determine: (*a*) whether endotoxin would protect against O_2 toxicity if it were administered after the animals were already in >95% O_2 for 12–48 h; and (*b*) the relationship between the endogenous antioxidant enzymes of the lung and the protective effect of endotoxin treatment.

Our results showed that adult rats given a single 500 μg/kg dose of endotoxin up to 36 h after the onset of O_2 exposure had significantly increased survival rates and decreased lung fluid accumulation compared to untreated animals in O_2 ($P < 0.05$). (Survival, 16/49 [untreated rats]; 18/20 [endotoxin at 12 h after the start of O_2 exposure]; 25/26 [endotoxin-24 h]; 15/20 [endotoxin-36 h].)

Endotoxin-treated animals in O_2 showed increases in pulmonary superoxide dismutase, catalase, and glutathione peroxidase activities before the usual time of onset of measurable pulmonary edema in untreated animals in O_2. When diethyldithiocarbamate was used to block the superoxide dismutase enzyme rise in the endotoxin-treated rats in O_2, the protective action of endotoxin against pulmonary O_2 toxicity was nullified. In endotoxin-treated, O_2-exposed mice, there were no lung antioxidant enzyme increases, and no protective effect from O_2 toxicity was achieved.

We conclude that, in the rat, a single dose of endotoxin given even 36 h after the onset of hyperoxic exposure results in marked protection against O_2-induced lung damage; and the increased lung antioxidant enzyme activity in the endotoxin-treated rats appears to be an essential component of this protective action.

A part of this study was presented at the Annual Meeting of the American Federation for Clinical Research, May 1979, Washington, D. C.

Received for publication 6 August 1979 and in revised form 29 November 1979.

INTRODUCTION

Small doses of bacterial endotoxin markedly increase the survival rate of adult rats exposed to normally lethal periods in hyperoxia, and significantly reduce the usual manifestations of acute pulmonary O_2 toxicity (pulmonary edema, pleural effusion, and lung hemorrhage) (1–3). Unlike other agents reported to protect the lung from O_2-induced injury, endotoxin requires no pretreatment period of several days before exposure to hyperoxia, but is effective even when administered in a single dose just before the onset of high O_2 exposure (2).

We now report that the administration of a single dose of endotoxin at various intervals after the initiation of hyperoxic exposure provides protection against O_2 toxicity. In addition, we have attempted to define the role of the endogenous antioxidant enzymes of the lung in mediating the protective effect against O_2 toxicity in the endotoxin-treated animals. This was done in studies in which (*a*) the time-course for pulmonary fluid accumulation and for changes in pulmonary antioxidant enzyme activities were monitored in endotoxin-treated and untreated adult rats exposed to hyperoxia; (*b*) endotoxin was administered to mice, a species which does not respond to hyperoxic exposure with increased antioxidant enzyme activities; and (*c*) the superoxide dismutase enzyme inhibitor, diethyldithiocarbamate (DDC)[1] was administered to endotoxin-treated rats in O_2.

METHODS

Animals and exposures. For these studies we used Sprague-Dawley albino rats (225–275 g) bred in the Animal Care Facility of the Veterans Administration Hospital, Miami, Fla.; and grey BLD2FIJ strain mice (25–30 g), obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass.

[1] *Abbreviations used in this paper*: CAT, catalase; DDC, diethyldithiocarbamate; GP, glutathione peroxidase; PBS, phosphate-buffered saline; SOD, superoxide dismutase.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/80/05/1104/07 $1.00*
Volume 65 May 1980 1104–1110

Exposures to 96–98% O_2 were conducted in 3.5-cu ft exposure chambers constructed from modified clear-plastic nursery isolettes (model 86, Air-Shields Inc., Hatboro, Pa.). Details of the monitored exposure conditions (96–98% O_2; <0.5% CO_2; 22–25°C; 60–80% humidity) have been described (3).

Animal studies. Endotoxin (*Salmonella typhimurium* lipopolysaccharide, phenol-water extraction, Sigma Chemical Co., St. Louis, Mo.) was dissolved in phosphate-buffered saline (PBS) and administered intraperitoneally in a total volume of 0.5 ml/100 g body wt. Control animals in O_2 received equivolume injections of PBS. Air control animals received either similar dosages of endotoxin as the treated test animals in O_2, or equivolume injections of PBS.

For the delayed-treatment studies, endotoxin was administered in a single dose of 500 μg/kg i.p., at either zero time (just before placing the animals in hyperoxia), or at 12, 24, 36, or 48 h after the start of hyperoxic exposure. Survival rate, lung fluid measurements, and antioxidant enzyme activities were determined on the surviving animals after 72 h in hyperoxia.

For the O_2 toxicity, time-course studies in which lung fluid accumulation and antioxidant enzyme activity changes were monitored at intervals during 72 h exposure period, rats received a single injection of endotoxin or PBS at 24 h after the onset of O_2 exposure. Groups of four animals each were then removed at 12-h intervals for lung fluid measurements and for lung enzyme analyses.

For the superoxide dismutase inhibition studies with DDC, rats received a single 500-μg/kg injection of endotoxin at zero time; a subgroup of these animals also received DDC (100 mg/kg i.p., dissolved in PBS) at zero time and after 24 h of O_2 exposure. For the DDC experiments, an O_2 exposure of only 48 h was used because of the high mortality rate after this time.

In the mouse studies, animals received 500-μg/kg doses of endotoxin at zero time, and at 24 and 48 h into the 120-h exposure period. Eight mice each from the treated and untreated groups were removed after 72 and 96 h of exposure time for lung analyses.

Analyses. All animals were killed by exsanguination under pentobarbital anesthesia. Pleural fluid accumulation was measured by absorbing the fluid in the chest cavities with preweighed gauze pads, assuming unit density (1 g = 1 ml). Lung wet weight per body weight measurements were based on the individual animal's body weight at the start of the exposure period. Lung dry weight was determined when a stable lung weight was recorded after drying the lungs for several days in a 60°C oven.

Superoxide dismutase (SOD) (4), catalase (CAT) (5), glutathione peroxidase (GP) (6), protein (7), and DNA (8) were measured in lungs perfused free of blood with cold isotonic buffer (0.1 M potassium phosphate, 0.15 M potassium chloride, pH 7.4) and homogenized in cold hypotonic buffer (0.005 M potassium phosphate, pH 7.8), 1:10 (wt/vol), in a Brinkman Polytron (Brinkmann Instruments, Westbury, N. Y.). CAT and GP enzyme activities were measured on the 15,000 *g* lung supernatant fluid stored frozen overnight. SOD enzyme activity (combined cytosolic or Cu-Zn-SOD and mitochondrial or Mn-SOD, as measured by the cytochrome *c* assay) was determined on fresh lung homogenates.

Statistical analyses. The Student's group *t* test and the Fisher exact nonparametric test were used for statistical analyses (9). Typically, the data from two or three experiments (with $n = 4$ animals per treatment group per experiment for nearly all the studies) were pooled after the validity of data-pooling was established by the chi-square test for homogeneity. A P value of <0.05 was used for assigning statistical significance.

RESULTS

Effect of time of administration of endotoxin on survival and lung fluid accumulation. Administration of endotoxin at zero time or at 12 or 24 h after the start of O_2 exposure resulted in nearly 100% survival of the treated animals at the end of the 72 h O_2 challenge period (Fig. 1). 75% of the rats receiving a single dose of endotoxin after 36 h of O_2 exposure survived. All these treatment groups had statistically significant increases in survival rate compared to the 33% survival rate of the untreated O_2 control group ($P < 0.05$). No increase in survival resulted with endotoxin treatment given after 48 h in hyperoxia (35% survival rate).

All the O_2-exposed animals showed evidence of lung and pleural fluid accumulation compared to the air control animals (Table I). However, pleural effusion and lung edema (lung weight per body weight and lung dry weight per wet weight measurements) was significantly less in the animals that received endotoxin at 0–36 h of exposure compared to the untreated O_2-exposed group ($P < 0.05$).

Time-course for development of pulmonary edema and rise in antioxidant enzyme activity. After 36 h of exposure to 96–98% O_2 there was no evidence of pleural effusion or edema in either experimental group (Table II). However, by 48 h of O_2 exposure the untreated rats show some increase in pleural effusion and significant pulmonary edema, as reflected in the decrease in lung dry:wet weight ratio ($P < 0.05$). In

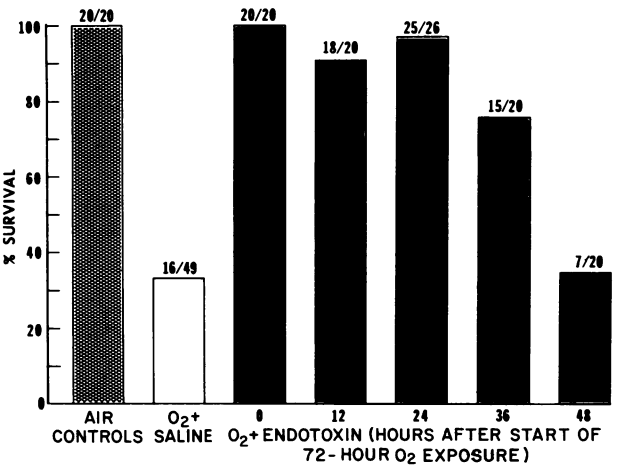

FIGURE 1 Effect of delayed endotoxin treatment on survival of adult rats exposed to hyperoxia (96–98% O_2, 72 h). Animals were treated with a single 500 μg/kg dose of endotoxin, intraperitoneally, either at zero time (just before being placed in hyperoxia) or at 12, 24, 36, or 48 h after the onset of O_2 exposure. O_2 control group received equivolume PBS and air-controls received either endotoxin or equivolume PBS at zero time. Survival rates for air-control group and endotoxin groups 0, 12, 24, 36 h are all significantly greater than O_2-control group survival rate, $P < 0.05$.

TABLE I

*Lung Fluid Accumulation in Adult Rats Exposed to Hyperoxia: Effects of Delayed Treatment with Endotoxin**

Treatment	Pleural fluid	Lung weight per body weight	Lung dry weight per wet weight
	ml	%	
Air-control	0.19±0.03‡	0.505±0.043‡	0.202±0.006‡
O_2 saline	10.80±3.03	0.895±0.151	0.170±0.010
O_2 endotoxin, O h	2.59±1.65‡	0.708±0.021‡	0.181±0.010
O_2 endotoxin, 12 h	1.27±1.61‡	0.700±0.040‡	0.181±0.008
O_2 endotoxin, 24 h	0.27±0.13‡	0.708±0.043‡	0.186±0.006‡
O_2 endotoxin, 36 h	0.21±0.06‡	0.720±0.044‡	0.184±0.010‡
O_2 endotoxin, 48 h	1.44±1.03‡	0.973±0.033	0.169±0.010

* Adult rats exposed to 96–98% O_2 for 72 h. Animals received a single 500 μg/kg dose of endotoxin, intraperitoneally, at either zero time (just before being placed in hyperoxia) or at 12, 24, 36, or 48 h after the onset of O_2 exposure. Air controls received either endotoxin or equivolume PBS. Results expressed as mean value ±SEM for two or three experiments (n = 8–10 animals per treatment group).

‡ Significant difference from the O_2 saline (untreated) group, $P < 0.05$.

contrast, the endotoxin-treated animals show no pleural effusion at 48 h of exposure time and a nonsignificant change in lung dry per wet weight measurements compared to the air control group. In addition, there is no further lung fluid accumulation during the remaining 24 h in 96–98% O_2 in the endotoxin-treated group, in contrast to the progressive fluid accumulation that occurs in the untreated animals. Table III shows there is already an increase in lung antioxidant enzyme activity at 36 h in the O_2-exposed, endotoxin-treated rats. This increase precedes by 12 h the first evidence of an increase in pleural fluid or lung water (Table II). SOD, CAT, and GP enzyme activities are significantly elevated above control levels at 60 h of exposure. The untreated O_2-exposed rats demonstrate no significant changes in lung enzyme activity compared to the air control animals (except for an increase in CAT activity at the 60-h time point).

Endotoxin treatment in mice. The lung antioxidant enzyme activities in the endotoxin-treated mice (unlike in the endotoxin-treated rats) did not increase during hyperoxic exposure (Table IV). Endotoxin treatment, in the scheduled dosage, had no effect on the survival rate of mice exposed to prolonged hyperoxia or on the accumulation of lung fluid. Enzyme activities measured at 72 h of exposure time were also unchanged in the endotoxin-treated and untreated groups, and lungs from both groups were equally edematous (not shown).

The effect of SOD inhibitor (DDC) treatment. Treatment with DDC reversed the protective effect of endotoxin (48 h survival, 40 vs. 100% with endotoxin

TABLE II

*Time-Course Study: Lung Fluid Accumulation in Adult Rats Exposed to Hyperoxia**

h	36	48	60	72
			ml	
Pleural fluid				
Air control	0.13±0.03	0.17±0.05	0.15±0.04‡	0.17±0.04‡
O_2 saline	0.14±0.03	0.47±0.57	6.53±3.05	8.65±1.64
O_2 endotoxin	0.09±0.05	0.15±0.07	0.41±0.62‡	0.51±0.53‡
Lung dry weight per wet weight				
Air control	0.200±0.006	0.200±0.006‡	0.199±0.006‡	0.200±0.007‡
O_2 saline	0.201±0.004	0.175±0.008	0.162±0.005	0.175±0.006
O_2 endotoxin	0.198±0.006	0.186±0.006	0.185±0.004‡	0.185±0.004‡

* Adult rats exposed to 96–98% O_2 for 36, 48, 60, or 72 h. Treated animals received a single 500 μg/kg dose of endotoxin, intraperitoneally, at 24 h after the onset of O_2 exposure. Air controls received either endotoxin or equivolume PBS. Results expressed as mean value±SEM for two experiments (n = 8 animals per treatment group).

‡ Significant difference from O_2 saline (untreated) group, $P < 0.05$.

TABLE III

*Time-Course Study: Lung Antioxidant Enzyme Activity in Adult Rats Exposed to Hyperoxia**

Treatment	SOD	CAT	GP
	36 h Exposure		
	U/lung	*IU/lung*	*μM NADPH oxid/min/lung*
Air control	691±104	7,540±1,828	42.6±3.2
O_2 saline	655±139	6,566±2,455	42.6±8.7
O_2 endotoxin	786±93 (+14%)	8,104±1,057 (+7%)	51.0±7.0 (+20%)
	60 h Exposure		
Air control	631±63	6,190±976	36.9±6.6
O_2 saline	596±41	7,630±1,332‡	43.2±8.4
O_2 endotoxin	958±96‡ (+52%)	9,490±1,890‡ (+53%)	66.3±6.0‡ (+80%)

* Adult rats exposed to 96–98% O_2 for 36 or 60 h. Treated animals received a single 500 μg/kg dose of endotoxin, intraperitoneally, at 24 h after the onset of O_2 exposure. Air controls received either endotoxin or equivolume phosphate-buffered saline. Results expressed as mean±SEM for two experiments (n = 8 animals per treatment group). Values in parentheses are the percent increase in enzyme activity compared to air control values.

‡ Significant difference from air control enzyme value, $P < 0.05$.

treatment alone) (Table V). The DDC plus endotoxin-treated animals manifested the usual pulmonary signs of acute experimental O_2 toxicity including pulmonary edema, pleural effusion, and areas of lung hemorrhage. No such findings were evident in animals treated with DDC alone or with endotoxin plus DDC while being maintained in room air (survival, 17/17) (Table V).

Whereas the animals given endotoxin alone did show

TABLE IV

*Survival, Pulmonary Edema, and Lung Antioxidant Enzyme Activity in Adult Mice Exposed to Hyperoxia**

Treatment	Survival‡	Body wt	Lung wt	Lung wt per body wt	Lung dry wt per wet wt
	%	*g*	*g*	%	
Air control	24/24 (100)	27.74	0.147	0.532	0.221
O_2 saline	29/41 (71)	21.97§	0.322§	1.470§	0.145§
O_2 endotoxin	29/40 (73)	21.54§	0.376§	1.729§	0.132§

Treatment	SOD	CAT	GP
	U/lung	*IU/lung*	*uM NADPH oxid/min/lung*
Air control	109±6	1,922±387	5.67±0.67
O_2 saline	97±9	1,393±410	5.15±1.16
O_2 endotoxin	96±11	1,435±380	5.92±0.74

* Adult mice exposed to 96–98% O_2 for 96 h. Treated animals received 500 μg/kg of endotoxin, intraperitoneally, at zero time (just before being placed in hyperoxia) and at 24 and 48 h after the onset of O_2 exposure. Air controls received endotoxin or equivolume PBS. Results for edema measurements are mean values for a single experiment with n = 4 animals per treatment group (standard variations omitted to avoid over-cluttering of table). Results for enzymes are mean values±SD for a single experiment with n = 4 animals per treatment group.

‡ Survival at 120 h: O_2 saline, 1/29 (3%) and O_2 endotoxin, 2/29 (6%).

§ Significant difference from air control value, $P < 0.05$.

TABLE V
*Survival and Lung Fluid Accumulation in Adult Rats Exposed to Hyperoxia: Effects of Endotoxin Treatment ±DDC**

Treatment	Survival	Pleural fluid	Lung dry wt per wet wt
	%	*ml*	
Air saline	15/15 (100)	0.20±0.02	0.194±0.002
Air endotoxin + DDC	17/17 (100)	0.13±0.06	0.195±0.004
O_2 endotoxin	17/17 (100)	0.15±0.07	0.179±0.007
O_2 endotoxin + DDC	14/35 (40)‡	4.68±2.49‡	0.161±0.003‡

* Adult rats exposed to 96–98% O_2 for 48 h. Animals received a single 500 μg/kg dose of endotoxin, intraperitoneally, at zero time (just before being placed in O_2) ±DDC, 100 mg/kg i.p., at zero time and at 24 h after the onset of O_2 exposure. Results expressed as mean values±SEM for two experiments (n = 8 animals per treatment group).
‡ Significant difference from all other treatment groups, $P < 0.05$.

significantly increased pulmonary SOD activity after 48 h in O_2 (Table VI), those given endotoxin plus DDC showed no increase in lung SOD activity. CAT and GP activities were increased in both groups of animals, and the changes were greater in the endotoxin-treated group than in the group receiving combined drug treatment. (The enzyme results in Table VI have been expressed as per milligram DNA instead of on a per lung basis. This is because whole lungs were not used for enzyme analyses in these experiments and because of the hemorrhagic changes in the lungs of endotoxin plus DDC animals in O_2, which would seriously influence the enzyme values expressed on a per lung basis due to erythrocyte enzyme contamination.)

DISCUSSION

Comparison between endotoxin and other treatment modalities which protect against hyperoxia. Agents such as alpha naphthylthiourea, oleic acid, vitamin E, and the "tolerance" to 100% O_2 induced by first preexposing rats to sublethal concentrations of O_2, all require several days of pretreatment to achieve a protective effect against O_2-induced lung injury and lethality (2, 10–12). Also, whereas each of these other protectants may result in increased survival rates in hyperoxia, they do not prevent substantial lung damage from occurring, as assessed by histological examination after O_2 exposures (10–12). In contrast, endotoxin provides protection without requiring any pretreatment interval before exposure to hyperoxia, and the endotoxin-treated animals show minimal lung alterations after exposure to similar hyperoxic challenge (1–3).

Furthermore, a single small dose of endotoxin (500 μg/kg, equivalent to ~1/40th of the median lethal dose for the species), can provide significant protection to hyperoxic-exposed animals even when it is administered as late as 24–36 h after the onset of high O_2 exposure (Fig. 1, Table I). We are unaware of any other experimental agent that is able to provide such

TABLE VI
*Lung Antioxidant Enzyme Activity in Adult Rats Exposed to Hyperoxia: Effects of Endotoxin Treatment ±DDC**

Treatment	SOD	CAT	GP
	U/mg DNA	*IU/mg DNA*	*uM NADPH oxid/min/mg DNA*
Air saline	53.5±8.3	504±65	5.21±0.56
Air endotoxin + DDC	53.1±5.7	564±89	5.26±0.69
O_2 endotoxin	76.3±10.8‡	860±124‡	7.73±0.83‡
O_2 endotoxin + DDC	51.7±5.2	765±76‡	6.79±0.46‡

* Adult rats exposed to 96–98% O_2 for 48 hours. Animals received a single 500 μg/kg dose of endotoxin, intraperitoneally, at zero time (just before being placed in O_2) ±DDC, 100 mg/kg i.p., at zero time and at 24 h after the onset of O_2 exposure. Results expressed as mean values±SEM for two experiments (n = 6–8 animals per treatment group).
‡ Significant difference from both air control groups, $P < 0.05$.

protection when administered after the exposure period has begun.

Evidence that the protective effect of endotoxin is dependent upon increased pulmonary antioxidant enzyme activity. The mechanism of action of endotoxin in the hyperoxic setting is not known. In untreated neonatal animals, protection from O_2-induced severe lung damage, lung fluid accumulation, and lethality is correlated with the ability of the neonatal animal to mount a rapid lung antioxidant enzyme response to O_2 challenge (13–15). Untreated adult rats exposed directly to 95–100% O_2 usually succumb within 60–72 h, and no change in lung SOD, CAT, and GP enzyme activities are found (13, 14). Similar findings have been reported for adult primates exposed to 95% O_2 (16). Adult rats preexposed for several days to 85% O_2 show increased lung SOD activity, and on subsequent exposure to 100% O_2 demonstrate prolonged survival (10, 12, 17). Endotoxin-treated adult rats have lung biochemical responses to O_2 exposure which are very similar to the adaptive responses of neonatal animals, i.e., significant rapid increases in lung SOD, CAT, and GP have been consistently observed in response to hyperoxic challenge (1).

We consider the following as important evidence that the endogenous antioxidant enzyme system response to hyperoxia in the endotoxin-treated animals is essential to the protective action of this agent: (*a*) Increases in lung SOD, CAT, and GP activities occur in endotoxin-treated animals 12 h before the onset of progressive pulmonary edema in untreated animals in O_2; (*b*) DDC interferes with the expected increase in SOD enzyme activity in endotoxin-treated rats in response to hyperoxia and nullifies the usual protective action of endotoxin; (*c*) In the dosage regimen we used, endotoxin treatment concomitantly fails to elicit an increase in lung antioxidant enzymes in mice and fails to protect mice against O_2-induced lung edema and lethality.

DDC treatment alone has not been associated with evidence of lung toxicity (15, 18; Table V). It should be kept in mind, however, that DDC is not a specific inhibitor for SOD, but also inhibits other copper-containing enzymes such as aldehyde dehydrogenase, xanthine oxidase, dopamine β-hydroxylase, and cytochrome *c* oxidase (15, 19). None of these other enzymes is believed to be directly involved in the O_2 toxicity process, but cytochrome *c* oxidase is the only one that has been systematically studied. The activity of this enzyme in the lungs appears to be unaffected by hyperoxic exposure (15, 20).

It is possible that the endotoxin dosage selected in the mouse study (comparable to the rat dose of ~1/40–1/50th of the median lethal dose) was insufficient to provide protection in this species. There is known to be a great difference in the sensitivity of different species to the biological actions of endotoxin, however, the mouse and the rat sensitivities are reported to be very similar (21).

Unanswered questions. The mechanism by which endotoxin treatment results in a rise in antioxidant enzymes in rats exposed to hyperoxia is unclear. Endotoxin is a mitogen for certain cells including reticuloendothelial cells, B lymphocytes, and also liver cells (22, 23). Studies in our laboratory (with Dr. Ming-Jen Chiang) have shown significant increases in lung DNA content within 24 h of endotoxin treatment. Furthermore, islands of cuboidal alveolar lining cells resembling type II cells have been observed in the lungs of endotoxin-treated, O_2-exposed rats (1–3). Thus, the type II cells, which seem to have innate resistance to hyperoxia, may be stimulated to proliferate by endotoxin and may represent the cell source for the increased antioxidant enzyme activities in treated adult rats. This notion is supported by the demonstration that hyperoxic exposure of isolated type II cells produces increased SOD activity in these cells (24, 25).

In addition to its apparent mitogenic effect on the lung, endotoxin treatment of rats exposed to hyperoxia results in an increase in the ratio of RNA to DNA in the lung.[2] This suggests the possibility that in addition to acting as a mitogen, endotoxin in some manner "activates" lung cells and facilitates a biosynthetic response to the increase in O_2^- substrate which is present under hyperoxic conditions and which is reported to be the direct biological stimulus for SOD induction (26, 27). Currently, we are investigating this proposed mechanism of action of endotoxin on the adaptive response of the lung to O_2 challenge.

ACKNOWLEDGMENTS

The authors express their appreciation to Teresa Rosario and Ondina Garcia-Pons for their assistance in the preparation of this manuscript.

This work was supported in part by National Institutes of Health grants HL07283, HL20366 and Veterans Administration funds.

REFERENCES

1. Frank, L., J. Yam, and R. J. Roberts. 1978. The role of endotoxin in protection of adult rats from high oxygen lung toxicity. *J. Clin. Invest.* **61**: 269–275.
2. Frank, L. and R. J. Roberts. 1979. Endotoxin protection against oxygen-induced acute and chronic lung injury. *J. Appl. Physiol.* **47**: 577–581.
3. Frank, L., and R. J. Roberts. 1979. Oxygen toxicity: protection of the lung by bacterial lipopolysaccharide (endotoxin). *Toxicol. Appl. Pharmacol.* **50**: 371–380.
4. McCord, J. M., and I. Fridovich. 1969. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244**: 6049–6055.

[2] Unreported data from Dr. Ming-Jen Chiang.

5. Holmes, R. S., and C. J. Masters. 1970. Epigenetic interconversion of the multiple forms of mouse liver catalase. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **11:** 45–48.
6. Paglia, D. E., and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70:** 158–169.
7. Schacterle, G. R., and R. L. Pollack. 1973. A simplified method for the quantitative assay of small amounts of protein in biological material. *Anal. Biochem.* **51:** 654–655.
8. Richards, G. M. 1974. Modifications of the diphenylamine reactions giving increased sensitivity and simplicity in the estimation of DNA. *Anal. Biochem.* **57:** 369–376.
9. Steel, R. G., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York.
10. Tierney, D. F., L. Ayers, and R. S. Kasuyama. 1977. Altered sensitivity to oxygen toxicity. *Am. Rev. Resp. Dis.* **115:** 59–65.
11. Smith, G., P. M. Winter, and R. F. Wheelis. 1973. Increased normobaric oxygen tolerance of rabbits following oleic acid-induced lung damage. *J. Appl. Physiol.* **35:** 395–400.
12. Crapo, J. D., and D. F. Tierney. 1974. Superoxide dismutase and pulmonary oxygen toxicity. *Am. J. Physiol.* **226:** 1401–1407.
13. Yam, J., L. Frank, and R. J. Roberts. 1978. Oxygen toxicity: comparison of lung biochemical response in neonatal and adult rats. *Pediatr. Res.* **12:** 115–119.
14. Frank, L., J. R. Bucher, and R. J. Roberts. 1979. Oxygen toxicity in neonatal and adult animals of various species. *J. Appl. Physiol.* **45:** 699–704.
15. Frank, L., D. Wood, and R. J. Roberts. 1978. The effect of diethyldithiocarbamate (DDC) on oxygen toxicity and lung enzyme activity in immature and adult rats. *Biochem. Pharmacol.* **27:** 251–254.
16. Robinson, L. A., W. G. Wolfe, and M. L. Salin. 1978. Alterations in cellular enzymes and tissue metabolism in the oxygen toxic primate lung. *J. Surg. Res.* **24:** 359–365.
17. Crapo, J. D., and J. M. McCord. 1976. Oxygen-induced changes in pulmonary superoxide dismutase assayed by antibody titrations. *Am. J. Physiol.* **231:** 1196–1203.
18. Deneke, S. J., S. P. Bernstein, and B. L. Fanburg. 1979. Enhancement by disulfram (Antabuse) of toxic effect of 95 to 97% O_2 on the rat lung. *J. Pharmacol. Exp. Ther.* **208:** 377–380.
19. Heikkila, R. E., F. S. Cabbat, and G. Cohen. 1976. In vivo inhibition of superoxide dismutase in mice by diethyldithiocarbamate. *J. Biol. Chem.* **251:** 2182–2185.
20. Stevens, J., and A. P. Autor. 1977. Induction of superoxide dismutase by oxygen in neonatal rat lung. *J. Biol. Chem.* **252:** 3509–3514.
21. Zweifach, B. W. 1961. Aspects of comparative physiology of laboratory animals relative to the problem of experimental shock. *Fed. Proc.* **20:** 18–29.
22. Berry, L. J. 1977. Bacterial toxins. *CRC Crit. Rev. Toxicol.* **5:** 239–318.
23. Shtasel, T. F., and L. J. Berry. 1969. Effects of endotoxin and cortisone on synthesis of ribonucleic acid and protein in livers of mice. *J. Bacteriol.* **97:** 1018–1025.
24. Raffin, T. A., W. Douglas, L. M. Simon, J. Theodore, and E. D. Robin. 1977. Effects of hyperoxia on ultrastructure and superoxide dismutase (SOD) activity in cultured type II pneumocytes. *Clin. Res.* **25:** 422A. (Abstr.)
25. Forman, H. J., H. Berman, L. Furia, and A. Fisher. 1979. Increased mitochondrial superoxide dismutase in lung type II epithelial cells from oxygen exposed rats. *Fed. Proc.* **38:** 1436. (Abstr.)
26. Hassan, H. M., and I. Fridovich. 1977. Regulation of the synthesis of superoxide dismutase in *Escherichia coli*: the glucose effects. *J. Bacteriol.* **132:** 505–510.
27. Hassan, H. M., and I. Fridovich. 1977. Regulation of the synthesis of superoxide dismutase in *Escherichia coli*: induction by methyl viologen. *J. Biol. Chem.* **252:** 7667–7672.