Hyperoxia and Lung Metabolism*

William D. Currie, M.D., Philip C. Pratt, M.D., and Aaron P. Sanders, M.D.

Numerous studies have been conducted on the pathologic changes that occur in the lung due to exposure to hyperbaric oxygen. These studies indicate that the entire lung, from the epithelium of the upper respiratory tract down to the pulmonary capillary endothelium, can be damaged by prolonged exposure to elevated partial pressures of oxygen. Reports of lung tissue changes due to oxygen toxicity include interstitial and intracellular edema, thickening of the oxygen blood barrier, and swelling of mitochondria in the alveolar epithelium. Indirect mechanisms as well as the direct effects of oxygen can be responsible for the damage observed in lung tissue. In vitro studies have shown that many enzymes are inhibited by exposure to hyperbaric oxygen in the absence of hormonal and neurogenic factors. Penrod reported in studies in which only one lung was exposed to the effects of elevated oxygen tensions that damage occurred in the absence of extrapulmonary factors.

Studies on biochemical damage that occurs in the lung due to pulmonary oxygen toxicity are not numerous and little is known about biochemical changes that occur in the lungs of intact animals during exposure to sub-convulsive levels of hyperbaric oxygen. After 15 to 30 minutes of breathing oxygen at 5.0 atmospheres absolute pressure (ATA), dehydrogenase activity and sulphhydryl content of lung tissue are significantly decreased. Other investigators have reported that cats exposed to 100 percent oxygen showed a marked impairment of tracheal mucus flow, which could be reversed by administering epinephrine and adenosine triphosphate (ATP). The authors interpreted the effect of oxygen on mucus flow as an interference by oxygen on carbohydrate metabolism. Proposals as to the mechanism of pulmonary oxygen toxicity include enzyme inhibition, free radical formation, and peroxidation.

Previous pathologic and biochemical studies have shown that significant damage occurs in the lung due to oxygen toxicity. We found biochemical changes occurring in the lung of rats exposed to 1.0 ATA 100 percent O₂ from zero to seven days. Pathologic changes in the lung were monitored, simultaneously. Succinic dehydrogenase activity, basal and ADP-stimulated respiration rates, and ATP concentrations were measured.

**Methods**

Male, Sprague-Dawley rats (150-225 gm) were exposed to 1.0 ATA 100 percent O₂ for one to seven days in a chamber with water flowing to remove urine and fecal material. The exposures were conducted at room temperature, and soda lime was placed in the chamber to absorb CO₂. Oxygen flow was maintained at approximately one liter per minute per animal in the chamber, and the animals were housed two per compartment. Standard laboratory food and water were available at all times. The compartments were constructed from 3" square metal cloth so that there was free flow of gas throughout the chamber. At the end of the exposure period, the animals were decapitated, lungs quickly excised and prepared for assay according to the particular parameter being studied. Respiration and oxidative phosphorylation were measured by the method of Chance and Williams using a Clark oxygen electrode with temperature-controlled vessels to determine basal and ADP-stimulated respiration rates for NAD and FAD-linked substrates. The assays on and homogenization of the lung tissue were performed in a normal air environment after the rats had been exposed to the 100 percent O₂ environment.

ATP concentration of the lung tissue was measured by the firefly luminescence technique of Strehler and Totter. Succinic dehydrogenase activity in lung tissue homogenates was determined by the cytochrome c reductase method of Cooperstein, et al. Results from assays of respiration and oxidative phosphorylation are expressed per milligram of mitochondrial protein, which was isolated according to the method of Reiss.

*From the Division of Radiobiology and Department of Pathology, Duke University Medical Center, Durham, N.C. This work supported in part by NIH Contract 71-2154 between The National Heart and Lung Institute and Duke University and by contract N00014-67-A-0251-02 between the Office of Naval Research and Duke University.

![Figure 1. The succinic dehydrogenase activity in lung homogenates prepared from rats exposed to 100 percent O₂. The assays were performed at room temperature. Other conditions are described in the text.](image-url)
RESULTS

The succinic dehydrogenase activity in lung homogenates isolated from rats exposed to 100 percent O₂ is shown in Figure 1. This plot clearly illustrates the rapid decline of succinic dehydrogenase activity due to the oxygen treatment. Succinic dehydrogenase activity is expressed per mg of mitochondrial protein since it is a mitochondrial enzyme and mitochondrial protein values are less susceptible to change due to oxygen toxicity than total protein within lung cells.

Figure 2 is a plot of the basal and ADP-stimulated respiration rates obtained with sodium succinate and ω-ketoglutarate as substrates versus time of exposure to 100 percent O₂. The basal QO₂ obtained with succinate decreased through four days of exposure to 100 percent O₂ after which the respiration rate increased slightly and remains at that level through day seven. The succinate ADP QO₂ also decreased through the fourth day; however the lowest QO₂ was obtained on day seven. The basal and ADP-stimulated QO₂ obtained with ω-ketoglutarate, an NAD-linked substrate, were dramatically reduced during the first day of exposure to O₂ and remained significantly reduced throughout the seven days of exposure.

Lung ATP concentration is shown as a percentage of control in Figure 3. After the first day of treatment with 100 percent O₂, the ATP level decreased to approximately 80 percent of control levels. At the end of seven days of exposure, the ATP level was reduced to approximately 75 percent of control values.

Pathologic studies made concurrently with the biochemical studies indicated the amount of histologic reaction began to increase after 24 hours of exposure to 100 percent O₂ and reached a maximum at 96 hours, after which it fluctuates at about that level. Lung weight increased due to edema after 72 hours and continued to increase through day six.

DISCUSSION

Two days of exposure to 100 percent O₂ resulted in the rats eating less than normal amounts of food and in a weight loss. The results obtained on the lung of animals exposed to 100 percent oxygen indicated that the biochemical changes preceded the observed pathologic changes, although the amount of histologic reaction was found to begin and continue to increase after 24 hours of exposure to O₂. However, the lung weight increased after 72 hours due to edema. Prior studies made at 1.5 and 2.0 ATA O₂ demonstrated that decreased succinic dehydrogenase activity, respiration and oxidative phosphorylation, and ATP concentration of the lung preceded the earliest observed pathologic changes. It was proposed that the pathologic changes (perivascular and alveolar edema) could be caused by a lowered ATP concentration which would render the lining of the vasculature less capable of maintaining its integrity and the cells less able to maintain the sodium pump at normal levels. This would effect the transport of sodium, accompanied by water, across cell membranes and result in edema.

Mitochondrial respiratory activity is necessary for the synthesis of ATP within the mitochondria; thus the
decrease in this activity is consonant with the lowered ATP concentration found in lung after exposure of the rats to 1.0 ATA O₂. These observations led to the conclusion that interference with ATP production is a major site of damage in pulmonary oxygen toxicity. This conclusion is strongly supported by studies conducted at 1.5 and 2.0 ATA O₂ in which a decrease in ATP concentration was shown to be accompanied by a decrease in nonstimulated and Mg²⁺-stimulated ATPase activity.¹⁴ Thus, ATP levels were shown to be lowered after exposure to hyperbaric oxygen which decreased the cellular capability of synthesizing ATP and did not increase the ATPase activity.

Succinic dehydrogenase activity and respiration with succinate, an FAD-linked substrate, were reduced by exposure of the animals to 100 percent O₂. These results coupled with decreased respiratory activity using α-ketoglutarate, and NAD-linked substrate, can account for the lowered ATP levels found in lung tissue. Respiration via the mitochondrial electron transport chain is responsible for the major portion of ATP synthesized within cells. A reduction in the synthesis of ATP is considered a prime factor in the genesis of pulmonary oxygen toxicity observed in the lungs of rats exposed to 100 percent oxygen.

REFERENCES

DISCUSSION
Dr. Mustafa: I see that the general trend of your data, for mitochondrial activity as a function of time, is to reach a plateau. With our ozone exposure, mitochondrial activity decreased progressively as a function of exposure time and also ozone concentrations, that is, dose-related changes were observed.

Release of B Glucuronidase and Elastase from Alveolar Mononuclear Cells*
N. R. Ackerman, Ph.D., and J. R. Beebe, B.A.
Since the original report by Laurell and Erickson¹ in 1963 linking the deficiency of α₁-antitrypsin (α₁-AT) and pulmonary emphysema, evidence has been accumulating in support of the hypothesis that proteolysis of the lung parenchyma is responsible for the pathogenesis of this disease. Most important is the finding that certain enzymes administered directly to the lung cause emphysemalike changes in rats² and dogs.³ The α₁-AT has the capacity to inhibit many of these enzymes including collagenase,⁴ neutral leukocyte proteases, and elastase.⁵ A question remains as to the cellular origin of these enzymes in vivo, and most evidence implicates the polymorphonuclear (PMN) leukocyte. These cells containing large amounts of the proteolytic, or lysosomal, enzymes, are sequestered in the lung bases,⁶ the primary site of panlobular emphysema, and release a significant portion of their proteolytic enzyme load during phagocytosis in vitro⁶ Galdston et al⁷ have recently demonstrated an inverse relationship between the severity of clinical symptoms in patients with α₁-AT deficiency and the PMN concentration of an elastaselike esterase; that is, patients with low α₁-AT and low PMN elastase activity have a more favorable clinical course than those with low α₁-AT and a normal or high PMN elastase activity.

Alveolar macrophages have also been implicated in the pathogenesis of emphysema: they are present in large amounts in both infectious and noninfectious states, contain greater concentrations of certain lysosomal enzymes than the PMN,⁸ and have an elastaselike esterase which is not inhibited by α₁-AT.⁹ At present, these cells have not been shown to release lysosomal enzymes during phagocytosis; should this occur, potentially injurious material would be deposited on the al-

*From the Medical Research Laboratories, Pfizer, Inc., Groton, Connecticut.