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Project Summary

Morphometric Studies of the Effects of Ozone on Rodent Lungs

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The degree of lung injury caused by prolonged inhalation of low levels of ozone is relevant because a number of urban environments periodically reach levels of 0.2 - 0.3 ppm ozone. Morphometric methods were used to evaluate the effects of 0.25 ppm ozone on lung tissue of both young adult and juvenile rats. Three regions of the lung were examined, the distal alveolar region, the proximal alveolar region, and the terminal bronchioles. In addition, the effects of inhalation of 0.12 ppm O_3 on the proximal alveolar region of young adult rats were also evaluated.

The results showed that ozone in these low concentrations reacts mainly in the terminal bronchioles and the proximal alveolar region. After 6 weeks of inhaling 0.25 ppm ozone the number of type I epithelial cells in the proximal alveolar region doubled in the animals exposed from 1 day of age. The mean surface area of type I epithelial cells decreased 38% and their mean thickness increased 24%. The number of alveolar type II epithelial cells also increased and the number of alveolar macrophages doubled. Young adult animals exposed to 0.25 ppm ozone showed similar changes in the epithelium of the proximal alveolar region, moreover, these animals showed a doubling of interstitial macrophages indicating a mile inflammatory stimulus in the interstitium. In the terminal bronchioles, exposure to 0.25 ppm ozone produced a significant 14-16% decrease ($p<0.05$) in the average luminal surface area of clara cells. Inhalation of 0.12 ppm ozone for 6 weeks also caused measureable changes in the proximal alveolar region of young adult rats. Type I cell number increased 36% ($p<0.05$) and the mean surface area of both the air side and the basement membrane side of the cell decreased 23%. These results suggest that 0.25 ppm ozone causes epithelial injury in the proximal alveolar region and in the terminal bronchioles of both juvenile and young adult rats. Exposure to 0.12 ppm ozone causes a detectable but less extensive injury to the type I epithelium in the proximal alveolar region.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Ozone is an atmospheric pollutant occurring as a photochemical oxidant in ambient air. The concentration of ozone in ambient air depends on the concentration of the primary pollutants as well as local environmental conditions such as temperature, air stagnation, and sunlight intensity. For several years oxidant concentrations have been measured by state and local control agency monitoring stations. These data are gathered and analyzed by the U.S. Environmental Protection Agency (EPA). Most urban centers have intermittently exceeded the current national air quality standard for ozone (0.12 ppm), and several cities (Los Angeles, Denver, Philadelphia, Houston, and New York) have had high hourly levels in excess of 0.20 to 0.30 ppm. The

chronic effects of such ozone concentrations are unknown.

Currently there is a scientific agreement that ozone concentrations in excess of one ppm are hazardous to human health. Experiments on laboratory animals (rats, mice, guinea pigs, rabbits, and monkeys) at ozone concentrations of 0.2 to 1.0 ppm for short exposures have demonstrated qualitative histopathologic changes in conducting airways and alveolar spaces. Various investigations have described: (1) damage to ciliated cells in the terminal bronchioles with nonciliated progenitor cell division for bronchiolar epithelial cell renewal; (2) hyperplasia and hypertrophy of nonciliated bronchiolar epithelial cells in respiratory bronchioles and intrabronchial accumulation of alveolar macrophages; (3) significant cellular damage at the alveolar duct level; (4) swelling and desquamation of type I alveolar lining cells with subsequent proliferation of type II cells to cover denuded areas; and (5) ultrastructural evidence of damage to endothelial cells and the basement membrane. However, there are currently no qualitative or quantitative morphologic studies of animals exposed to ozone concentrations less than 0.2 ppm. Such experiments are obviously vital to our understanding of possible damage to the human lung in urban areas, where low level exposures occur intermittently in the range of 0.20 ppm or less.

Materials and Methods

Specific pathogen-free male, Fisher 344 rats that were either 1 day or 6 weeks old were exposed to 0.12 ppm or 0.25 ppm ozone. Rats that breathed filtered room air served as controls. After 6 weeks of exposure, rats were sacrificed and their lungs fixed by intratracheal instillation of 2% glutaraldehyde. The procedure used to isolate terminal bronchioles and proximal alveolar tissue from slices of the left lung is illustrated in Figure 1.

Ultrathin sections of terminal bronchioles and proximal alveolar regions were obtained and EM micrographs taken from them. Morphometric analysis was applied to study structural changes caused by inhalation of ozone. Randomly selected blocks of distal alveolar tissue were also studied.

Results

Effects of Inhalation of 0.25 ppm O_3 on the Distal Alveolar Region of Juvenile and Young Adult Rats

Morphometric methods were used to evaluate the effects of 0.25 ppm ozone on lung tissue randomly selected from the alveolar region of mature and neonatal rats. For this study this area is defined as the distal alveolar region and it includes all alveoli beyond the terminal bronchioles. The results indicate that few structural changes occur in the distal alveolar region as a consequence of this level of O_3 exposure. There were reductions in the body weight gained by mature animals exposed to ozone for 6 weeks ($p=0.05$) and by neonates exposed for 1 week ($p=0.06$). Previous investigators have reported this difference in rats exposed to 1 ppm or less of ozone. The arithmetic mean thicknesses of the epithelium and endothelium increased 10% ($p=0.08$) and 16% ($p=0.08$), respectively, in mature rats. The volume density of air in the alveolar region was significantly smaller in neo-

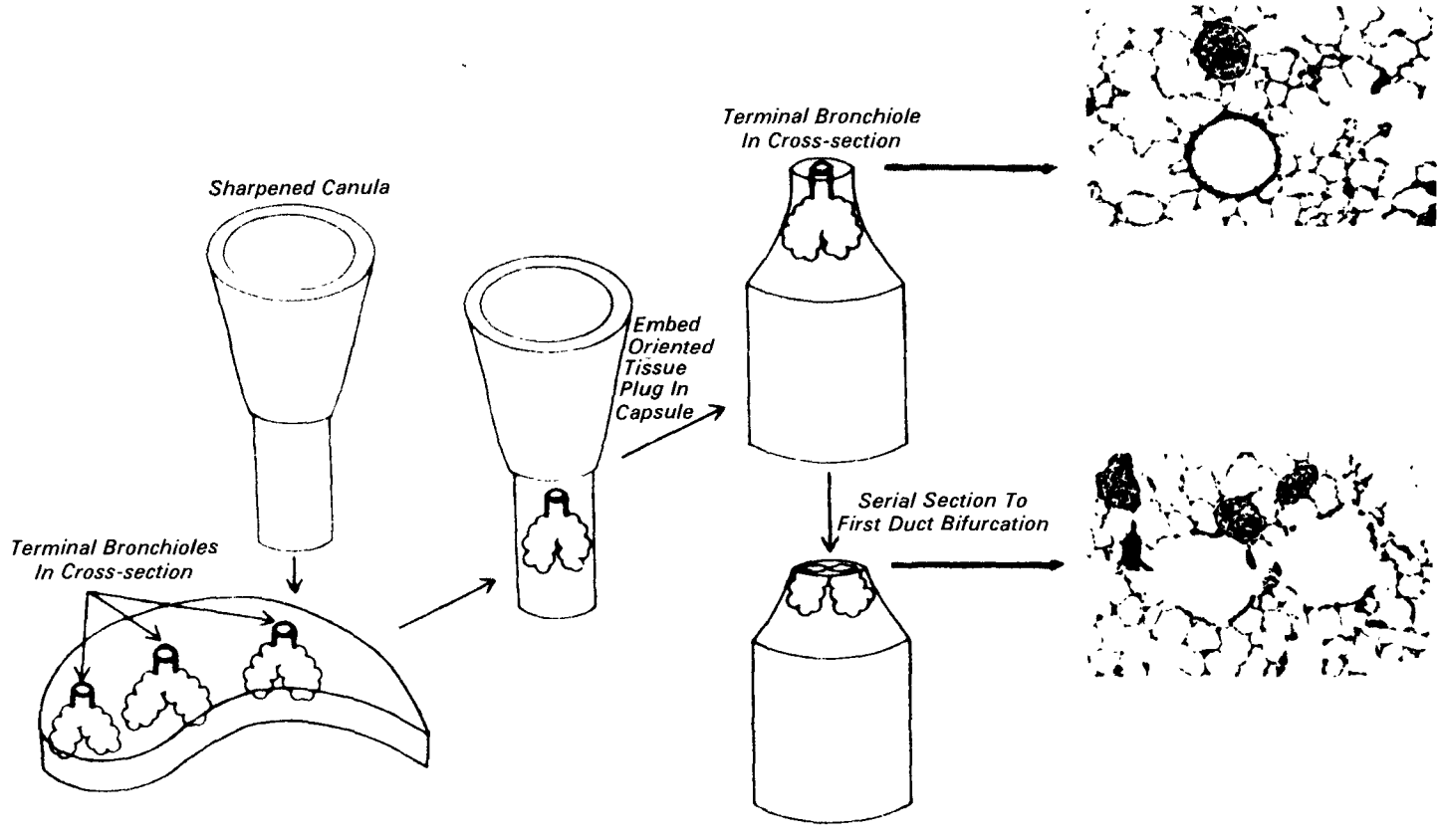

Figure 1. Schematic of the microdissection procedure used to isolate lung tissue containing terminal bronchiole and proximal alveolar region.

natal rats exposed to ozone for 1 week, but this change may have been caused by variations in fixed lung volumes of these small rat lungs. In neonatal rats exposed to ozone for 6 weeks, the average basement membrane surface area covered by type I cells decreased 20% (p=0.06). There were no significant increases in the arithmetic mean thickness of epithelium or endothelium. No other significant changes were found in the morphometric characteristics of the distal alveolar region of the adult or neonatal rats.

Effects of Inhalation of 0.25 ppm O_3 on the Proximal Alveolar Region of Juvenile and Young Adult Rats

Since the alveolar region most adjacent to terminal bronchioles has been reported as one of the major sites of injury due to ozone inhalation, this specific lung region was studied using ultrastructural morphometric analysis. After 6 weeks of exposure to 0.25 ppm O_3, qualitative examination of the tissue revealed no observable damage to the terminal bronchioles or the adjacent proximal alveolar tissues. However, by morphometric analysis, significant changes occurred in the alveolar epithelium of the proximal alveolar region. In the animals exposed from 1 day of age (juvenile animals) the number of type I epithelial cells doubled, their mean surface area decreased 38%, and their mean thickness increased 24%. The number of alveolar type II epithelial cells increased and the number of alveolar macrophages doubled. Young adult animals exposed to ozone showed similar changes in the epithelium of the proximal alveolar reigon. The changes in type I and type II cells are illustrated in Figures 2 and 3 respectively. Compared to the juvenile animals, the young adult, ozone exposed animals showed more interstitial cell reaction with a doubling of interstitial-macrophages suggesting a mile inflammatory stimulus in the interstitium. The change in number and size of type I cells is consistent with an increased cell turnover rate due to prolonged ozone inhalation. These results suggest that 0.25 ppm ozone causes a chronic epithelial injury in the proximal alveolar region of both juvenile and young adult rats.

Effects of Inhalation of 0.12 ppm O_3 on the Proximal Alveolar Region of Young Adult Rats

Morphometric analysis was carried out on lung tissue of the proximal alveolar

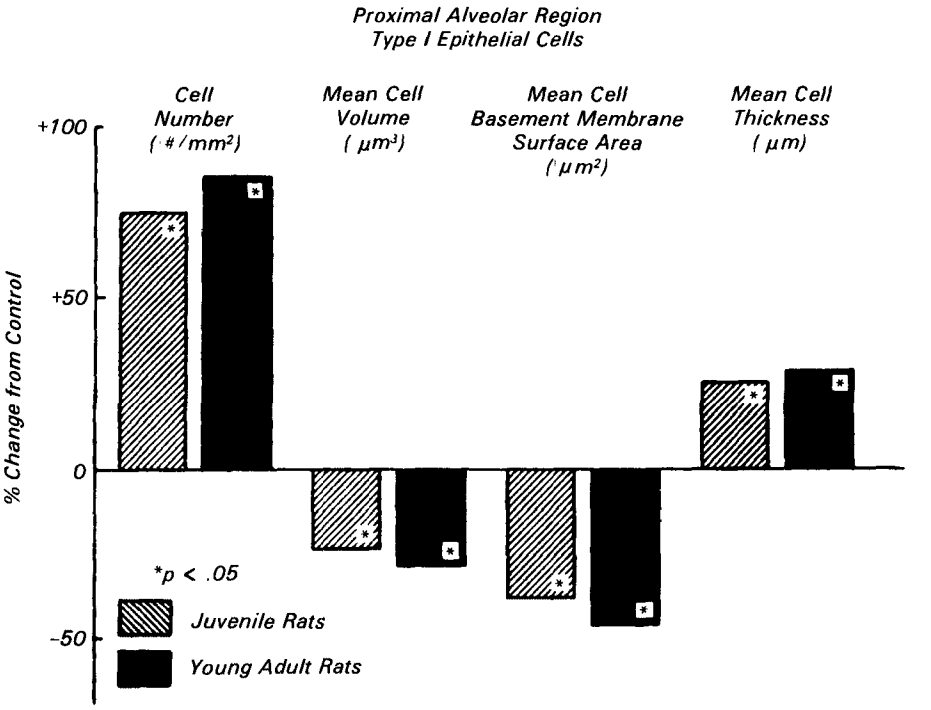

Figure 2. *Effects of inhalation of 0.25 ppm ozone on the characteristics of type I epithelial cells in the proximal alveolar region of juvenile and young adult rats.*

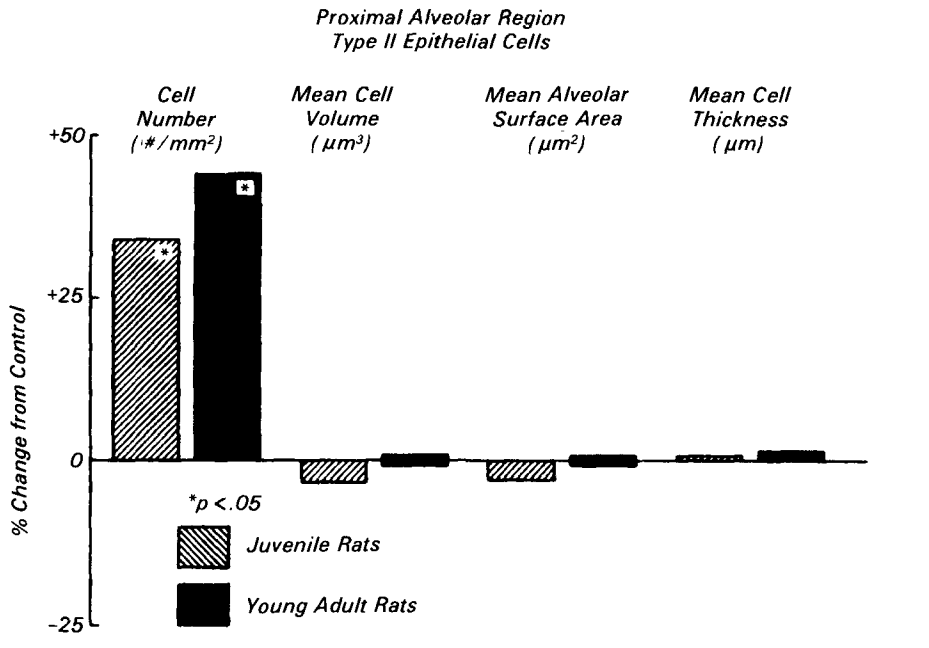

Figure 3. *Effects of inhalation of 0.25 ppm ozone on the characteristics of type II epithelial cells in the proximal alveolar region of juvenile and young adult rats*

region isolated from young adult rats that were exposed to 0.12 ppm, the current air quality standard for ozone. This investigation is therefore of fundamental importance in evaluating the effectiveness of present air quality control standards. The results of morphometric analysis indicated that after 6 weeks of ozone inhalation, significant changes occurred in the type I epithelium of the proximal alveolar region. Type I cell number increased 36% ($p < 0.05$) and both their mean surface area and mean basement membrane surface area decreased 23% ($p < 0.05$). While the average type I cell volume decreased 11%, this change was not statistically significant. The volume of type I epithelium increased 13% and the thickness of type II epithelium increased 29%. No significant changes were found in the mean cell volume or surface area of type II cells. The changes observed with the inhalation of 0.12 ppm ozone were found to be similar to, but of lesser magnitude than, those occurring with exposure to 0.25 ppm ozone (see Figure 4).

The higher concentration of ozone produced both a greater and a broader degree of injury. In addition to increases in type I epithelium, the volumes of type II epithelium, alveolar macrophage, and cellular interstitium were found to increase after 6 weeks of exposure to 0.25 ppm ozone. This study demonstrates that the exposures to ozone concentrations near current air quality standard of 0.12 ppm ozone can cause measurable structural changes in the alveolar epithelium. The degree of tissue injury caused by ozone can be rigorously quantified and increased with exposure to higher concentrations of ozone.

Effects of Inhalation of 0.25 ppm O_3 on the Terminal Bronchioles of Juvenile and Young Adult Rats

Terminal bronchioles comprise the final portion of the conducting airway in the lung. It has been shown to be particularly sensitive to the effects of ozone exposure. Morphometric methods were developed to evaluate the effects of inhalation of 0.25 ppm ozone on the terminal bronchioles of rats. This concentration of ozone is near the current air quality standard of 0.12 ppm and is below the level at which changes can be reliably documented qualitatively. The results of the morphometric analysis indicated that ciliated cells composed 48% (juvenile) or 53% (young adult) of the total population of terminal bronchiolar cells and clara cells were 33% (juvenile) or 29% (young adult). The average terminal bronchiolar diameter (210 μm) and the average thickness of the bronchiolar epithelium (7.5 μm) was not significantly changed by the exposure to ozone. Exposure to 0.25 ppm ozone for 6 weeks produced a significant 14 - 16% decrease ($p < 0.05$) in the average luminal surface area of clara cells in the terminal bronchioles of both juvenile and young adult rats. No changes occurred in mean clara cell volume or in average clara cell basement membrane surface area. The changes found in ciliated cells are illustrated in Figure 5.

The effect of ozone on terminal bronchiolar cells are subtle, especially when compared with changes induced in the epithelium of the proximal alveolar region after exposure to the same concentrations of ozone. These differences may be due to the terminal bronchiolar epithelial cells being less susceptible to this concentration of ozone than are the cells of the alveolar epithelium. Alternatively, it may be due to the fact that the epithelium

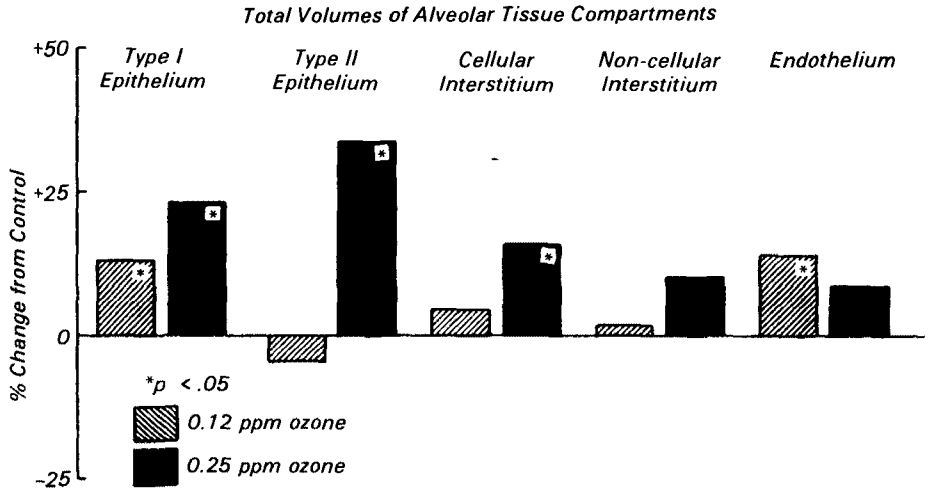

Figure 4. *Effects of inhalation of 0.12 ppm or 0.25 ozone on the total tissue volumes of the various tissue compartments in the proximal alveolar region of young adult rats.*

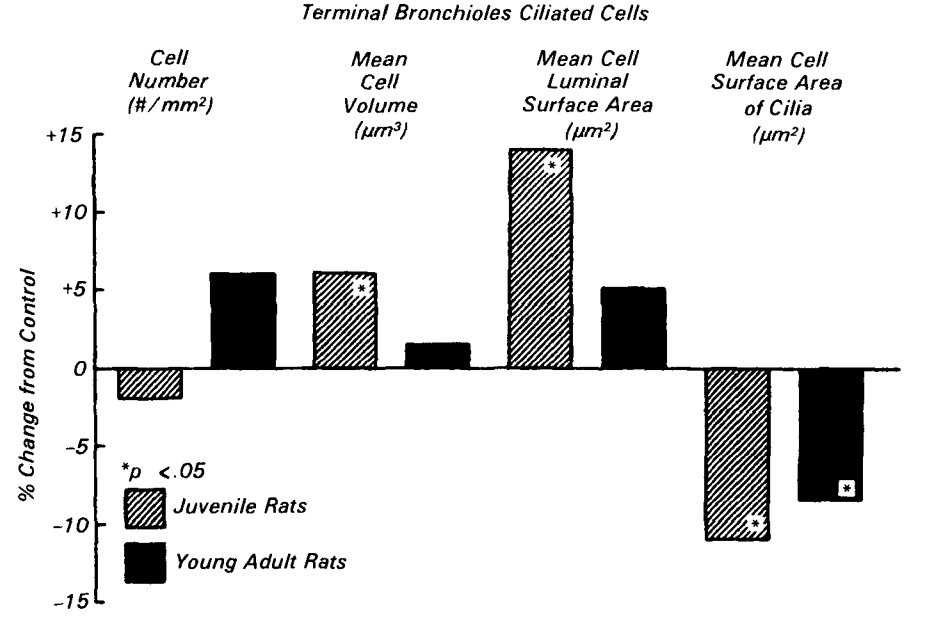

Figure 5. *Effects of inhalation of 0.25 ppm ozone on the characteristics of ciliated cells in the terminal bronchioles of juvenile and young adult rats.*

in the terminal bronchiole is covered by a mucous/serous coat which may react with the low concentrations of O_3 and thereby prevent the O_3 from reaching the underlying cells.

Conclusions and Recommendations

EM morphometry is extremely sensitive and particularly suited as a tool to evaluate health effects of air pollutants such as ozone at low concentrations. By the combined use of microdissection and EM morphometry, we demonstrated in this study that subchronic exposure to 0.25 ppm ozone produced significant structural changes in the cells of the terminal bronchioles and their adjacent alveolar tissues. In addition, inhalation of 0.12 ppm O_3, the current ambient air quality standard, caused structural changes to occur in the alveolar epithelium immediately peripheral to the terminal bronchioles.

A wide array of effects have previously been reported as a result of subchronic exposure to ozone. These investigations, although mostly at higher concentrations of O_3, were essentially in agreement with the results of the present study in the location and nature of the injury invoked by ozone. The major impact of the current study is that by using quantitative techniques subchronic exposure to ozone at extremely low levels can be shown to cause structural changes in lung cells which are suggestive of injury. These changes can be detected at the current air quality standard of 0.12 ppm O_3. Because the long-term effects of lung cell injury caused by low levels of O_3 are not known, chronic exposure to O_3 at near ambient levels should be performed and adverse health effects carefully defined.

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***Jean Wiester** is the EPA Project Officer (see below).*

The complete report, entitled "Morphometric Studies of the Effects of Ozone on Rodent Lungs," (Order No. PB 85-207 470/AS; Cost: $8.50, subject to change) will be available only from:

National Technical Information Service
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