Discussion

Dr. Thurlbeck: Where are the mast cells most commonly found in the lung? Does shock produce the effects you have described or are the changes in mast cells associated findings?

Dr. Wilson: Mast cells are most concentrated in the proximal portions where airways are most concentrated. While I have no final proof, I think shock does cause the degranulation changes described.

Dr. Lauweryns: Do you see intraalveolar edema? Is histamine the only substance released by degranulating mast cells?

Dr. Wilson: No intraalveolar edema was seen; there are many other vasoactive substances released by degenerating mast cells.

Morphologic and Ultrastructural Changes in the Lungs of Animals During Acute Exposure to Ozone*

Edwin S. Boatman, Ph.D., and Robert Frank, M.D.

The morphologic and ultrastructural changes occurring in the respiratory system of animals exposed to low concentrations of ozone (O_3) were followed by light microscopy, scanning electron microscopy and transmission electron microscopy.

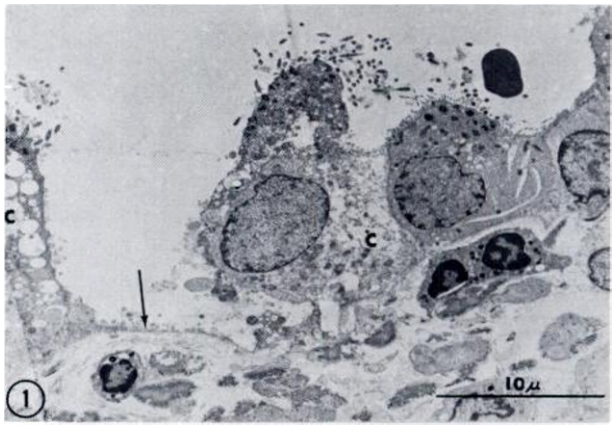
Cats were exposed to either 0.25 ppm of ozone (O_3) 0.5 ppm, or 1.0 ppm for 4.5 hours (single exposure) and rabbits were exposed to 0.5 ppm, 1.0 ppm or multiples of these concentrations for three hours at a time and killed after periods ranging from one hour to six days. All rabbits, except some controls, were subjected to a left pneumothorax prior to O_3 exposure. In case of multiple exposures, pneumothorax was induced prior to each additional exposure (maximum of three). Multiple exposures entailed exposure of the right lung to: (1) 0.5 ppm of O_3 on two consecutive days followed by 1.0 ppm on the third day; or (2) 1.0 ppm on the first day followed by two consecutive days of 0.5 ppm; or (3) 1.0 ppm on three consecutive days.

Functional measurements were made on cats during exposure and on rabbits after death (volume/pressure, V/P). Lungs were removed *in toto* and the lungs degassed and fixed in the expanded state at a distending pressure of 15 cm H_2O for cats and 8 cm H_2O for rabbits with 1.5 percent glutaraldehyde in 0.11 M s-collidine buffer pH 7.4 at 300 mOsm for 15-21 hours, at 22°C. Two adjacent transverse slices of tissue were removed from the trachea, the upper, middle and lower portions of the larger lobes and processed for paraffin and resin embedding, respectively. In some animals, additional slices were processed by the "critical point" method for scanning electron microscopy. Paraffin sections were stained with H&E, PAS and Van Gieson and resin sections were stained with azure-2 methylene blue. Stained resin sections of lung were enlarged photographically x35, mounted on boards and superimposed with a 1 mm square co-ordinate system. Specific areas of lung (1 mm sq) (*eg*, portions of bronchi, bronchioles, etc) were traced back to the resin blocks and were removed with a punch and sectioned for electron microscopy.

All three concentrations of ozone (0.25, 0.5, and 1.0 ppm for 3-4.5 hours) produced considerable desquamation of airway lining cells. The degree of damage was roughly proportional to the concentration. Airways from 2.7 mm to 0.15 mm in diameter showed desquamation.

Ultrastructural Findings

Ciliated cells sustained more damage as a result of exposure to O_3 than either goblet cells, mucus secreting cells or cuboidal cells, and many ciliated cells were found free in the airway luminae. Desquamation of such cells frequently exposed the underlying basal cells and



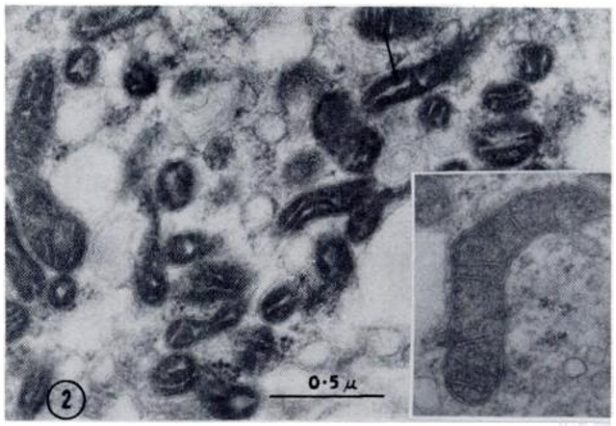


FIGURE 1. Portion of a large airway (rabbit, right lung, 2.5 mm diameter) exposed to 1.0 ppm, O_3 for 3 hours. Note exposure of basement membrane (*arrow*) and cytoplasmic vacuolization of ciliated cells (c). FIGURE 2. Example of ultrastructural changes in ciliated cell mitochondria after exposure to 0.25 ppm, O_3, 3 hours. Note swollen cristae and dense mitochondrial matrix.

*From The Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle.

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sometimes the basement membrane of the lamina propria (Fig 1). This was particularly true of the larger airways. Two major abnormalities were seen in the ciliated cells: (1) marked vacuolization of the cytoplasm, often with large vacuoles situated peripherally to the nucleus; and (2) morphologically altered mitochondria, generally appearing smaller and more electron dense than normal and with swollen longitudinally aligned cristae (Fig 2). This type of mitochondrial change was observed in the ciliated cells of both animal species and was also seen in an occasional basal cell, capillary endothelial cell and alveolar macrophage. Although membranous whorls were seen in ciliated cells of normal airway lining epithelium, their numbers were increased in O_3-exposed lungs. Basal cells were generally of normal ultrastructure as were the intermediate undifferentiated cells.

In rabbits, the extent of cell desquamation in the large airways was most clearly appreciated when compared with control airways after examination by scanning electron microscopy. In O_3-exposed lungs, longitudinal slices of airways at low magnification showed that desquamation was focal and sometimes appeared more intense at a bifurcation. Multiple exposures to O_3 (0.5/0.5/1.0) produced changes of no greater severity than those observed after a single exposure to 1.0 ppm of the gas.

At the alveolar level in cats and rabbits, changes seen by light microscopy were focal areas of intra-alveolar hemorrhage and accumulations of inflammatory cells. Only by electron microscopy could changes to the type 1 surface lining epithelium and capillary endothelium be detected. After exposure to 1.0 ppm O_3, these changes ranged from denudation of type 1 epithelium with exposure of the basement membrane to focal swelling of the cytoplasm. Endothelial cells were ruptured or showed marked swelling. In some capillaries both cell linings were damaged, in others, only one. Areas were also found which showed no damage. At 0.5 ppm and 0.26 ppm of O_3, type 1 cells were usually intact but showed focal areas of swelling of the cytoplasm; the endothelial cells were rather more frequently damaged.

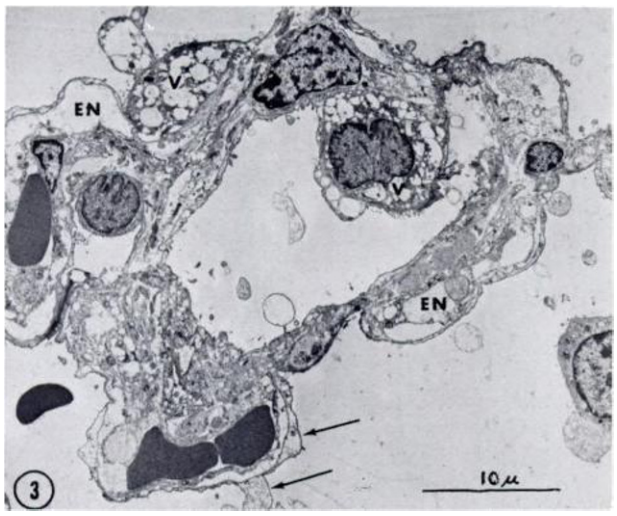

FIGURE 3. Alveolar area from right lung of rabbit exposed to 1.0 ppm, O_3, for 3 hours, *day* 2. Type 1 epithelium shows focal swelling (arrows), capillary endothelium is disrupted (EN), interstitial edema is seen and type 2 cells (V) show cytoplasmic changes.

Table 1—*Summary of damage to alveolar areas from lungs of rabbits exposed to O_3 and observed at different time intervals*

Ozone exposure concentration:	time	Observed at	Alveoli: degree of damage and cell type right lung	left lung
0.5 ppm	3 hrs.	Day 1	±EP, +EN	———
1.0 ppm	3 hrs.	Day 1	++EP, EN	———
1.0 ppm	3 hrs.	Day 2	+++EP, EN, TII	+EP, EN, TII
0.5/0.5/1.0	3 hrs. ea.	Day 4	++EP, EN, TII	++EP, EN, TII
1.0 ppm	3 hrs. both lungs	Day 1	+EN	+EN (no pneumothorax)

EP = surface lining epithelium (Type 1) TII = Type II epithelial cell
EN = capillary endothelium + = relative magnitude of change

Type 2 epithelial cells were generally normal morphologically on the day of exposure, but in lungs from rabbits examined one or more days after exposure to 1.0 ppm of O_3 showed marked cytoplasmic vacuolization. At this concentration of gas cellular changes were more severe on the second day (Fig 3) than on the day of exposure, and there was interstitial edema.

In rabbits alveolar areas from the left lungs (collapsed during exposure) exposed to 1.0 ppm of O_3 for three hours, although normal on the day of exposure became abnormal the following day and showed changes similar to the right lung but not as severe.

Lungs exposed on three consecutive occasions and observed the day after (day four) showed alveolar damage to be similar in both left and right lungs and that all three cell types were involved.

A summary of the damage found in alveolar areas from rabbit lungs exposed to ozone and observed at different intervals is shown in Table 1.

Rabbits exposed to 1.0 ppm of O_3 for three hours, and allowed to recover for six days showed minor vesiculation in both cytoplasm of ciliated epithelial cells lining the airways and in the cytoplasm of type 1 alveolar cells.

The V/P behavior of the exposed right lungs following exposure to 1.0 ppm O_3 for three hours was significantly depressed (less volume per unit of distending pressure) than the control (pneumothorax) left lung. The degree of depression reached its peak after four to seven days and was found to persist in some animals for as long as two weeks.

At exposures of 0.5 ppm (single) morphologic damage was less severe and V/P behavior showed minimal depression. In triple-exposed lungs, morphologic changes were similar in both left and right sides and there was no difference in V/P behavior.

On the second day interstitial edema was detected in the alveolar areas of the right lungs and thus could account, in part, for the observed increase in wet weight of these lungs. At present, it is not known what percentage of alveoli show such changes but it is known that some alveoli appear less affected than others and that animals exposed to this concentration of ozone survive the insult. An additional factor in the observed abnormal V/P behavior was the significant decrease in the diameters of the small airways <0.2 mm diameter measured 24 hours after exposure to 1.0 ppm of O_3 for

three hours.

Other studies of the effects of ozone on the ultrastructure of the lungs were mainly components of the blood-air barrier, specifically the type 1 and capillary endothelial surface lining cells, the type 2 cell and the alveolar macrophage.[1,2,4-6] Our experience would suggest that the surface lining epithelium of the airways is affected at least as much.

It is possible that the mitochondrial changes seen in the ciliated cells may constitute one of the initial responses to O_3 in the upper airways. If so, this would be of importance because mitochondria are the prime sources of energy for the cell and probably ciliary activity through the process of oxidative phosphorylation. Freebairn[3] found ozone to inhibit the oxygen uptake *in vitro* of mitochondrial preparations from spinach and cabbage, an effect which could be reversed by the addition of reducing agents.

Under experimental conditions, concentrations of ozone encountered in urban and industrial environments for short periods have an immediate effect on the cells lining the airways and alveoli and although cellular recovery is almost complete within one week the functional behavior of the lung remains abnormal. Whether changes in surface tension forces or altered tissue elasticity contribute to the abnormal V/P behavior has not yet been determined.

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A Comprehensive Ultrastructural Study of Pulmonary Injury and Repair in the Rat Resulting From Exposures to Less Than One PPM Ozone*

R. J. Stephens, M.D.; M. J. Evans, Ph.D.; M. F. Sloan, B.S.; and G. Freeman, M.D.

The purpose of this research is to describe the initial processes of injury and repair following exposure to levels of ozone (O_3) that often exist in the polluted air of major cities. Previous results from these laboratories have shown that pulmonary tissue injury caused by NO_2 is most severe in the anatomic unit composed of the terminal bronchiole, proximal portion of the alveolar ducts, and proximal alveoli.[1] Initial response of the tissue to O_3 was similar but not identical to that described for nitrogen dioxide (NO_2). Longterm exposure to O_3 has yet to be fully examined.

Young male rats were exposed continuously in chambers to concentrations of 0.54 ± 0.08 or 0.88 ± 0.08 ppm of O_3 for up to 48 hours prior to death. Other animals were exposed for 2, 4, 8, and 12 hours and were then allowed to recover until a total of 48 hours had elapsed before they were killed. Still others were continually exposed to 0.54 ± 0.08 ppm O_3 for as long as six months and killed at various intervals beginning with 72 hours. Specimens from these experiments were prepared for both scanning and transmission electron microscopy and for light microscopy.[2]

Preliminary studies made it obvious that the terminal bronchiole and proximal alveoli were dramatically (affected during the early exposure (compare Fig 1 with 2, 3). Many ciliated cells in the terminal bronchiole became necrotic (Fig 2) and exfoliated (Fig 3) within the first six to ten hours and most of the remaining ciliated cells lost their cilia. In the proximal alveoli, many type 1 cells were seriously damaged (cf Fig 4, 5) and sloughed away from the basement lamina (Fig 6, 7), leaving significant areas without an epithelial covering after as little as two hours of exposure to 0.5 ppm. The type 1 cells in the distal alveoli were not destroyed. In contrast, type 2 cells in the proximal alveoli were strikingly resistant to O_3 (Fig 6) as were the nonciliated cells of the terminal bronchiole (Fig 2,3). The injurious phase reached its height before 24 hours of continuous exposure, and the tissue was repaired during the next 24-30 hours through proliferation of the type 2 cells in the alveoli[1,3-7] and of the nonciliated cells in the terminal bronchioles.[5,6] This process resulted in an increased cellularity of this anatomic unit.[1,3] The same general response was noted when animals were exposed relatively briefly to O_3 followed by 48 hours of recovery.[3,4] The extent of the damage was proportionately reduced in exposures of six hours or less. However, eight hours of exposure, followed by 40 hours of recovery, produced a tissue response similar in extent and appearance to that resulting from continuous exposure for the full 48 hours.[3,4] The endothelium was affected minimally under these conditions, but at higher levels it also became involved. Moreover, a similar response was noted in rats exposed intermittently to cigarette smoke for 48 hours.[4]

Beyond 48 hours the epithelial response became stable and its appearance tended to become more normal, although readily distinguishable from the control. The

*From The Life Sciences Division, Stanford Research Institute, Menlo Park, California.