Distribution of injury and microdosimetry of ozone in the ventilatory unit of the rat

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PINKERTON, KENT E., ROBERT R. MERCER, CHARLES G. PLOPPER, AND JAMES D. CRAPO. *Distribution of injury and microdosimetry of ozone in the ventilatory unit of the rat.* J. Appl. Physiol. 73(3): 817–824, 1992.—The distribution of ozone-induced injury across ventilatory units of the lungs was determined and compared with the predicted distribution of ozone dose across the same units to evaluate dose-response relationships. Sprague-Dawley rats were exposed to either 0.98 ppm ozone 8 h/day for 90 days or to filtered air only. En bloc microdissection was used to identify and isolate in longitudinal profile the bronchiole-alveolar duct junction, first pair of alveolar duct generations, and intervening bifurcation ridge. The first alveolar outpocketing along the bronchiolar wall of each isolation was used to identify the center of a series of concentric arcs radiating outward at 100-μm intervals across each ventilatory unit. The intercept lengths of each arc with the tissue of alveolar septal tips (edges) and alveolar walls were measured and expressed as a function of distance into the ventilatory unit. Relative ozone dose across the ventilatory unit was estimated using the geometry of the tracheobronchial tree and the volume and surface area distribution within individual ventilatory units. This mathematical model of ozone dose demonstrated a high degree of correlation to the measured tissue injury response. The findings of this study demonstrate that microdosimetry and microtoxicology can be used to determine dose-response relationships within the ventilatory unit and to assess questions of tissue sensitivity in ozone-induced lung injury.

lung; dose response; bronchiole-alveolar duct junction; en bloc microdissection

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THE VENTILATORY UNIT in the rodent lung has been defined as all alveoli and alveolar ducts distal to each bronchiole-alveolar duct junction (BADJ) (14). This definition encompasses both structural and functional properties of a collection of alveoli involved in gas exchange. A pulmonary acinus is defined as the gas-exchange region arising from a single terminal bronchiole and would be made up of from two to eight ventilatory units. It is well known that the more proximal portions of the alveolar region are primary targets of lung injury after ozone exposure (1, 3, 4). It is not known whether the pattern of attenuation of this injury across the ventilatory unit correlates directly with the pattern of attenuation of dose.

Changes in parenchymal tissues beyond the BADJ are assumed to be due to the relative concentration of ozone in this region. Mathematical models have been used to predict the amount of ozone delivered to the respiratory tract (17, 18). On the basis of these models, the dose of ozone to the nasal cavity, larynx, and tracheobronchial tree is clearly different, and the dose of ozone delivered to proximal vs. distal alveoli within the same ventilatory unit may differ by an order of magnitude. Analysis of the relative ozone dose delivered to the ventilatory unit and correlations with the variability of the biological responses to such exposures have not been investigated. We sought to directly examine in this study the relationship between predicted ozone dose and actual tissue response as a function of distance into the ventilatory units of rodents chronically exposed to high levels of ozone.

To address the question of dose vs. biological response, the design for tissue sampling must be based on an analysis of the ventilatory unit in longitudinal profile. If alveolar tissues can be sampled at known intervals from the origin of the BADJ, changes in response to injury could be measured as a function of distance into the ventilatory unit. Because the identification of original BADJs may be compromised after ozone exposure as a result of the extension of bronchiolar epithelium into alveolar ducts (1), any outpocketings of alveoli along conducting airway walls can be used as criteria for unambiguous identification of these sites.

Respiratory bronchioles are defined as airways with alveolar outpocketings in their walls. These structures, although usually absent in rodents, are occasionally found as short segments of airway between the terminal bronchiole and alveolar duct with one or two alveolar outpocketings close to the BADJ. Should alveolar outpocketings remain easy to identify after exposure to ozone, these structures would serve as reliable landmarks of the original opening to ventilatory units under conditions where bronchiolarization may have pushed the junction of airway columnar epithelium to alveolar squamous epithelium deeper into the ventilatory unit. In this study, the level of the airway that contains the first alveolar outpocketing serves as the reference point from which concentric arcs are drawn to measure tissue characteristics as a function of distance into the ventilatory unit for both control and ozone-exposed animals.

METHODS

Animal exposure. Male Sprague-Dawley rats were obtained from Bantin and Kingman (Fremont, CA). Serology, cultures, and a complete necropsy performed on two

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randomly selected animals revealed no bacterial or viral infections, pulmonary pathogens, or histopathology. At the onset of exposure, all animals were 65 days of age. All rats were maintained in modified 4.2-m^3 Hinners-type exposure chambers. The filtered-air exchange rate in the chambers was 30 times per hour. Temperature was maintained at 24 ± 2°C and relative humidity at 40–50%. Ozone was generated from medical-grade oxygen using silent arc discharge ozonizers (model V, Erwin Sander). A nocturnal exposure was carried out for 8 h/day (9:00 P.M. to 5:00 A.M) to 0.98 ± 0.05 (SD) ppm ozone for a total of 90 days. Ozone concentration was monitored every 8 min by an ultraviolet ozone monitor (model 1003-AH, Dasibi Environmental) calibrated using an absolute ozone photometer (model 1008-PC, Dasibi). Monitoring was controlled and data were recorded by an LSI 1123 computer. Animals had free access to food and water throughout the study.

Tissue preparation. Animals were deeply anesthetized within 2 h after the end of the final exposure period by an intraperitoneal injection of pentobarbital sodium (450 mg/kg). Incisions were made in the abdomen and ventral neck, the trachea was cannulated, each hemidiaphragm was ruptured, and a 0.9% glutaraldehyde-0.6% paraformaldehyde solution in 0.065 M cacodylate buffer (330 mosmol, pH 7.4) was instilled intratracheally at a hydrostatic pressure of 30 cm of fixative. This method of fixation was used rather than vascular perfusion as a more reproducible means to evenly inflate the lungs and to ensure the complete unfolding of alveolar walls. After 30 min, the lungs and heart were removed intact from the thorax and stored in fixative for a minimum of 24 h before determination of lung volumes by fluid displacement (26).

Tissues from the left lung were used for analysis. Transverse slices cranial and caudal to the hilar level of the lobe were cut into 2 × 3 × 5-mm blocks, dehydrated in a graded series of alcohol, and transferred stepwise through propylene oxide and araldite resin. All blocks were immersed in fresh araldite resin in 4 × 4 × 6-mm wells and cured at 60°C for 16–24 h. Embedded tissue blocks were softened by prewarming on a hot plate and serially cut with clean razor blades into 0.4- to 0.5-mm-thick slabs. Both surfaces of each slab were sequentially examined under a dissecting microscope to follow airway paths leading to terminal bronchioles. Regions of each slab that contained by random chance in longitudinal profile on or just below the cut surface a terminal bronchiole, a pair of symmetrical alveolar ducts, and the intervening bifurcation ridge were cut from the slab and glued onto a flat beem blank for sectioning (Fig. 1).

A glass knife was used to face each isolated tissue block in a fashion that would further orient the ventilatory unit into the desired longitudinal plane. Sections 0.5 μm thick were examined by light microscopy to identify those that contained a longitudinal cut through the approximate center of a terminal bronchiole and that led into symmetrical alveolar ducts separated by their first bifurcation ridge. This orientation provided an optimal linear profile across the ventilatory unit for analysis (Fig. 1). Sections were rejected if they were not oriented correctly through the central axis of the ventilatory unit. This included

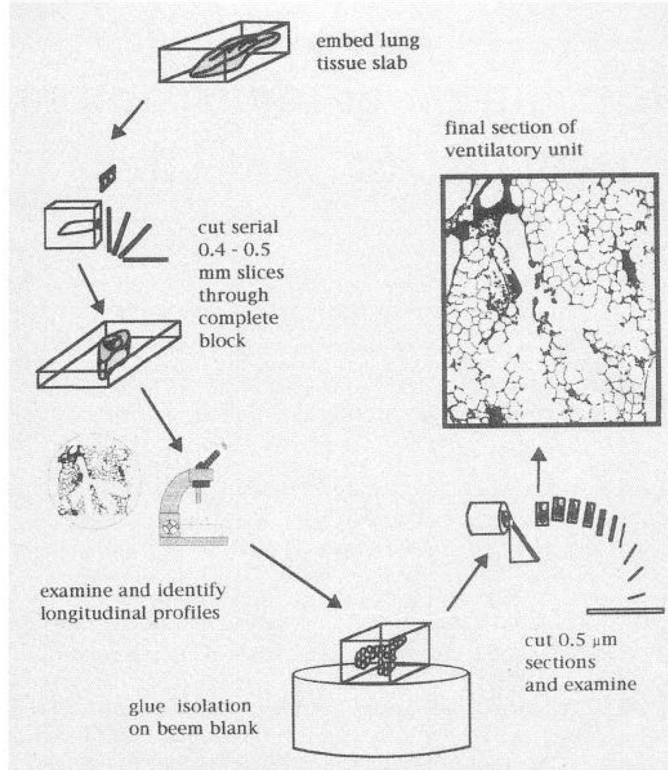

FIG. 1. Steps of ventilatory unit isolation. Several clean sharp razor blades are used to cut serial slabs through a single block. Light reflected from cut surfaces and into each slice facilitates identification of longitudinal profiles through ventilatory unit. Each region is cut from the slice, glued on a beem blank, and sectioned with a glass knife until proper orientation of ventilatory unit is obtained.

sections showing asymmetrical pairs of first alveolar ducts and tissue bifurcation "bridges" immediately beyond the BADJ that interrupted the open air path from the terminal bronchiole to one or both alveolar ducts. Four to seven isolations were prepared per animal in control and ozone treatment groups.

BADJ identification. Analytic approaches to measure the distribution of tissue changes in ventilatory units of animals exposed to ozone require a strategy that ensures the proper identification of the BADJ. If these junctions are not easily identified, inappropriate comparisons of mismatched alveolar duct generations may occur between control and treated animals. This problem arises after exposure to high levels of ozone due to the extension of bronchiolar epithelium from the terminal bronchiole into the proximal alveolar regions of the ventilatory unit (1, 4). Such a change potentiates the possibility of inadvertant comparisons of first alveolar duct generations in control animals to second- and third-order generations of alveolar ducts in the lungs of animals exposed to ozone. However, if in the process of bronchiolarization no alveoli are obliterated, the first alveolar outpocketing along the airway path may serve as a constant landmark for the identification of the original BADJ. To determine if alveoli near the BADJ are preserved after exposure conditions used in this study, tissues from the right middle lobe of each animal were critical point dried and airways followed by microdissection (25) out to the level of the ventilatory unit. Prepared specimens were

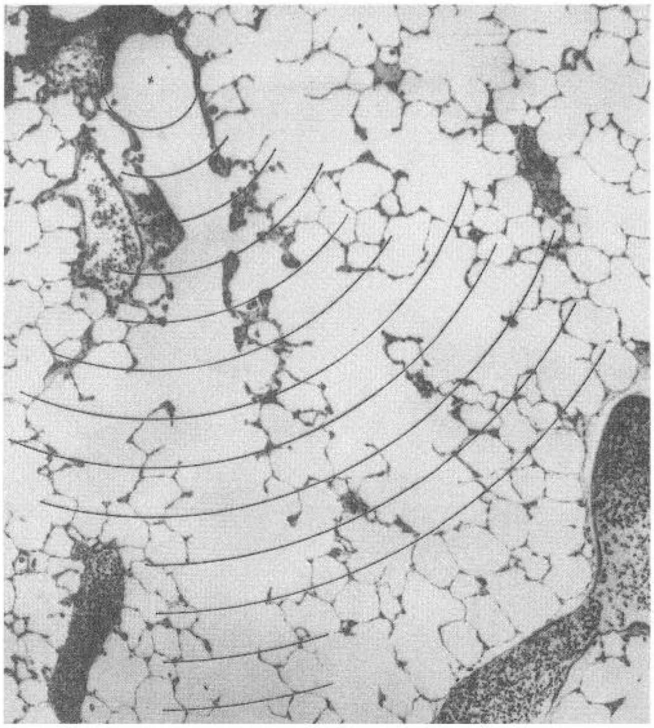

FIG. 2. Ventilatory unit isolation from lung of rat exposed to 0.98 ppm ozone 8 h/day for 90 days. At level of first alveolar outpocketing along airway wall a reference point (×) is identified from which concentric arcs at 100-μm intervals have been drawn into ventilatory unit.

sputtered coated with gold and examined with a scanning electron microscope.

Serial section analysis of terminal bronchioles and ventilatory units was also used to determine whether the most proximal alveoli within these regions were altered or destroyed as a result of the exposure. The right cranial lobe from each animal was dehydrated and embedded in paraffin. Serial 6-μm-thick sections were cut through the entire lobe. By random selection, BADJs were identified on single sections and followed through the series to study the complete BADJ in three dimensions. Identification of alveoli was based on location, size, composition, and surface contour. Briefly, both approaches confirmed that no alveoli were lost under the exposure conditions used. Complete details will be presented in RESULTS.

Morphometric analysis. Each ventilatory unit isolation was photographed and printed on 11 × 14-in. paper. From a single reference point placed at the level of the first alveolar outpocketing, concentric arcs were drawn at 100-μm intervals (Fig. 2). Each arc denoted the symmetrical "front" of a reactive gas moving toward the alveolar epithelial surfaces from the BADJ. Prints were made of each isolation to serve as a guide or map to identify arc intercepts made with tissues along the ducts of each ventilatory unit. Only those arc intercepts with tissues along open duct paths within a 30° angle incident to either side of a line bisecting the ventilatory unit profile were measured. The span of each concentric arc drawn in Fig. 2 illustrates this general rule followed that also served to enhance the accuracy of the actual distance from the reference point of each tissue intercept. Tissue intercept lengths were measured using a Zeiss optical microscope, a cursor with a digitizing pinpoint light source, and tablet interfaced to an IBM PS/2 computer. A microscope photoextension tube facilitated measurements with the light cursor while directly viewing the tissue under the microscope. An ×40 objective was used to increase the resolution of tissues compared with that seen on the arc map to permit more accurate visualization of tissue surfaces and distinction of alveolar macrophages from septal tissues. Each intercept measurement followed the precise path of the arc traversing over tissue as seen on the "arc map." Exceptions to this rule were intercepts of tissue with the first 100-μm arc. Because of the significant curvature of this arc, a line perpendicular to the surface at the point where the curve intercepted tissue was measured rather than the actual path of the arc. All measurements were taken through the complete tissue wall including the capillary space. Intercepts that passed through a blood vessel (arterioles and venules) >30 μm in diameter were excluded from measurement. Of note is the observation that septal capillary diameter rarely exceeded 15 μm in rats.

Tissue measurements were separated into septal edge (tip) and alveolar wall categories. Distinction of alveolar septal tips from alveolar walls was made by drawing a line to separate alveolar air spaces from the alveolar duct lumen (Figs. 3 and 4). All tissues that touched this line were classified as a part of the alveolar septal tip that forms the mouth opening or crest of each alveolus into the duct. Tissues not touching this line were considered to be the wall of the alveolus. This method provided a simple way of separating these two parameters without any subjective bias on the part of the observer. The actual path of the arc intercept with each tissue structure was used as a measure of septal edge and alveolar wall thickness. When the path of an arc passed through the septal tip and down the alveolar wall parallel to the tissue air surface, only that length of the arc that intercepted the septal tip was measured. This approach helped to eliminate inappropriately long measurements passing through septal tissue walls parallel to the alveolar surface.

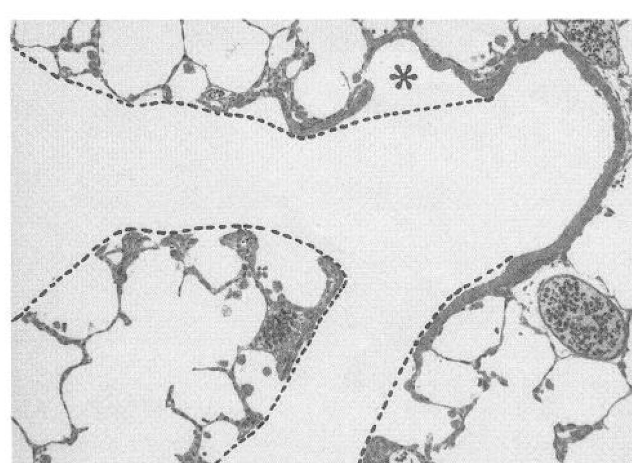

FIG. 3. Proximal alveolar region from lung of rat exposed to 0.98 ppm ozone 8 h/day for 90 days. Outpocketing of alveolus (*) is present within wall of this isolation lined with cuboidal and bronchiolar epithelial cells. Dashed lines that separate alveoli from alveolar duct lumina define alveolar tips (edges) for analysis in this study. Magnification of micrograph is ×115.

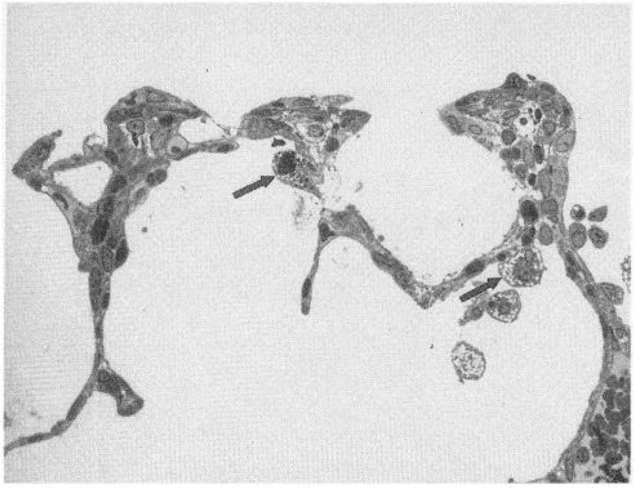

FIG. 4. Enlargement of alveolar septal tips and alveolar walls in proximal alveolar region of Fig. 3. Thickened epithelial cells line septal tips of each alveolus, while alveolar wall appears to be relatively unaffected. Increased numbers of interstitial cells are present within these septal tips. Alveolar macrophages within alveolar air spaces are also prominent (arrows). Magnification of micrograph is ×390.

Lung modeling. Modeling of the uptake of a reactive gas inspired into the lungs was used to predict the amount of ozone delivered to the ventilatory unit as a function of distance from the BADJ (13). For the modeling, structural parameters affecting gas uptake such as volume and gas-exchange surface area were determined along with factors influencing dead space volume contributed by the proximal tracheobronchial tree. These structural parameters were obtained by serial section analysis methods previously described (13, 14). Average ventilatory unit volume and surface area obtained from these measurements were 0.53 ± 0.03 mm^3 and 18.1 ± 0.7 mm^2, respectively. Previously reported dimensions of the tracheobronchial tree (15) were used to describe the dead space volume and surface area of airways proximal to the BADJ defining entry into the ventilatory unit.

Statistical analysis. Changes in the thickness of alveolar septal tips and walls as a function of distance into each ventilatory unit were compared. To increase the power of analysis, measurements for each 200-μm interval into the ventilatory unit were combined to perform one-way analysis of variance and Duncan's multiple range comparison test (6). All comparisons with $P \leq 0.05$ were considered to be statistically significant.

RESULTS

The three-dimensional anatomy of BADJs in the lungs of rats was easily visualized using scanning electron microscopy (Fig. 5). The majority of terminal bronchioles ended abruptly with a rapid transition from airway walls to ducts lined by alveoli. In a few instances, one or two alveolar outpocketings were present in terminal bronchioles proximal to the BADJ. These alveoli were found near the end of the bronchiole in close proximity to alveoli forming the first alveolar duct just beyond the junction (Fig. 5*A*). In the lungs of animals exposed to ozone, original BADJs were more difficult to identify because bronchiolarization of the proximal alveolar duct walls caused the distinct transitional region to be less evident. Alveolar outpocketings were visible in terminal bronchioles, but, in most instances, the alveolar mouth openings were markedly narrowed (Fig. 5*B*). A marked change was the extension of bronchiolar epithelium from each airway into the ventilatory unit along the septal mouth edges of the most proximal alveoli.

Serial section analysis of randomly selected BADJs from the lungs of animals exposed to ozone and to filtered air provided greater resolution of alveolar structures near airway terminations. As noted with scanning electron microscopy, the most prominent ozone-induced changes near the BADJs were epithelial cell changes associated with alveolar septal tips in the proximal regions of the ventilatory unit. Many of these septal tips were lined with cuboidal and bronchiolar epithelial cells. A general thickening of the interstitial compartment was also noted in septal edges and to a lesser degree in alveolar walls. Alveolar outpocketings were preserved within terminal bronchioles, but the air space volume of these alveoli was reduced as a result of the replacement of type

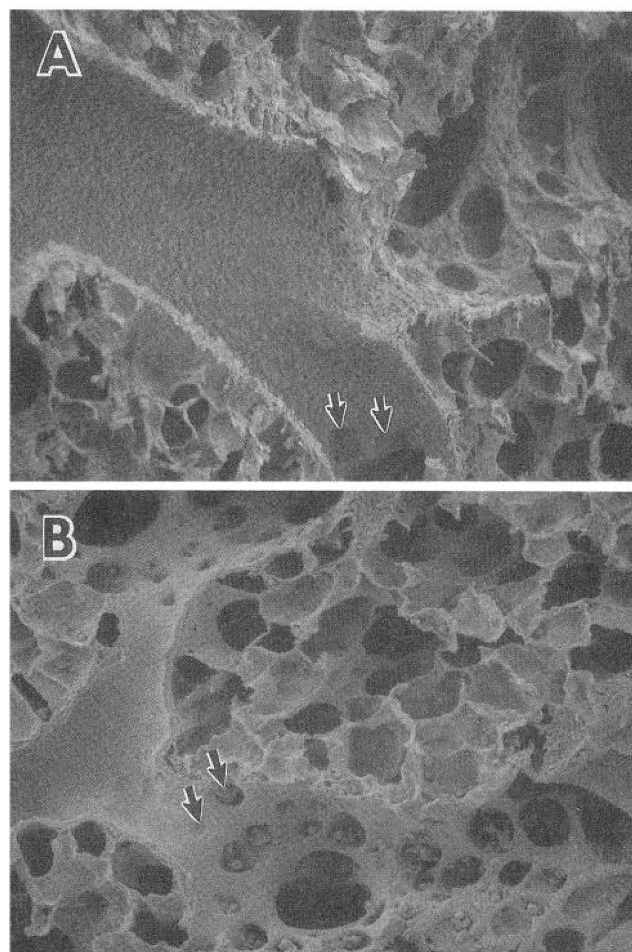

FIG. 5. Scanning electron micrograph of the bronchiole-alveolar duct junction in lungs of a control (*A*) and ozone-exposed (*B*) animal. *A*: 2 alveolar outpocketings (arrowheads) are seen in wall of bronchiole near bronchiole-alveolar duct junction to form short respiratory bronchiole. *B*: extension of bronchiolar epithelium and thickening of septal edges (tips) forming mouth openings around each alveolus. Alveolar outpocketings (arrowheads) are maintained within walls of original bronchiole. Numerous inflammatory cells can be seen within air spaces of this region. Magnification of each micrograph is ×125.

I and type II epithelial cells with cuboidal-to-columnar epithelium completely lining these surfaces. The original contour of each alveolus appeared to be maintained. These observations suggested that no alveoli were lost with exposure to ozone.

Isolation of ventilatory units by the en bloc microdissection approach (Fig. 1) worked well and yielded three to six isolations per block for analysis. Although not all isolations could be used, a large number could be reoriented in the process of cutting 0.5-μm-thick sections to improve the longitudinal profile for the placement of the reference point from which concentric arcs were drawn at 100-μm intervals. The pattern of reference points and radiating concentric arcs was highly consistent from one isolation to the next. The tissue bifurcation ridge formed by the branching of alveolar ducts distal to the BADJ was consistently found between the 300- and 500-μm concentric arcs (Fig. 2). Measurements of tissue thickness were based strictly on the random intercepts of arcs with tissues and excluded any subjective bias in the selection of where measurements along the ventilatory unit path would be taken. For some isolations, measurements extended along duct paths as deep as 1,700 μm into the ventilatory unit. The average linear depth into a ventilatory unit from the reference point was 1,000 μm.

Distinction of alveolar septal tips from alveolar walls using a line to separate alveolar air spaces from the lumen of alveolar ducts (Fig. 3) provided a simple way of separating alveolar duct walls from septal tip edges in a consistent manner. The majority of measurements were easy to classify, while only a few fell into a category of crossing through both the septal tip and the alveolar wall. In these instances, only that length of the intercept passing through the septal tip was measured. Tissue thicknesses at intercept sites with septal edges and alveolar walls were measured and averaged for all ventilatory unit isolations for each animal and subsequently for each treatment group. Table 1 summarizes the average tissue thickness as measured radially outward across the ventilatory unit. The thickness of septal edges and alveolar walls was averaged for each 200-μm interval to increase the power of analysis at each distance interval. The predicted dose of ozone for each progressively deeper level of the ventilatory unit based on the mathematical modeling of the gas moving outward into a greater volume and reacting with an increasing surface area within the ventilatory unit is also given in Table 1. The predicted uptake per unit surface area in the proximal portions of the ventilatory unit was significantly greater than that of the more distal portions of the ventilatory unit. Increases were significant for septal tip and alveolar wall thickness measurements in the first 0.6 mm of the ventilatory unit. Septal edge thickness in the first 400 μm averaged 20 μm compared with 12 μm in controls. The peak difference in septal edge thickness was an 80% increase above control value at 200–400 μm deep into the ventilatory unit. The greatest difference in alveolar wall thickness was a 143% increase compared with control value within the first 200 μm of the ventilatory unit. Changes in septal tissue from the proximal to distal portions of the ventilatory unit correlated well to the predicted ozone dose derived by the mathematical model of ozone distribution and uptake into the ventilatory unit (Table 1, Predicted Dose). Figures 6–8 graphically illustrate changes in septal edge, alveolar wall, and predicted dose measurements as a function of distance, respectively.

TABLE 1. *Microdosimetry analysis*

Distance, mm	Alveolar Wall, μm		Septal Tip, μm		Predicted Dose, %
	Control	Exposed	Control	Exposed	
0–0.2	5.6±1.1	13.6±2.6*	11.6±1.6	19.6±2.0*	100.0
0.2–0.4	5.9±1.1	10.9±2.7*	12.3±1.8	22.2±1.9*	42.0
0.4–0.6	5.1±0.3	7.1±0.7*	8.6±1.2	18.1±1.5*	14.0
0.6–0.8	5.1±0.5	6.0±0.4	8.3±0.9	10.8±1.8	5.6
0.8–1.0	5.2±0.5	5.5±0.6	8.8±1.0	9.6±1.0	0.2

Values are means ± SE for 4 rats per group. Predicted dose is expressed as percentage of maximal uptake (μm/mm² of epithelial surface per m³ inhaled) in ventilatory unit. * Statistically different from control, $P < 0.05$ (Duncan's multiple comparison test).

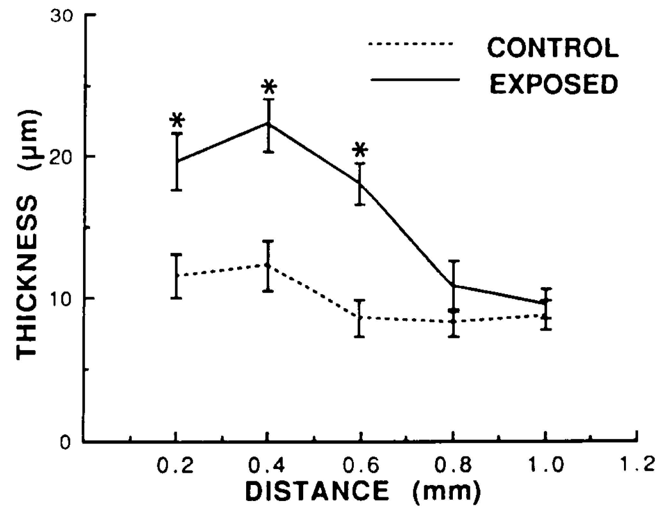

FIG. 6. Changes in thickness of tip or septal edge of alveolus that form walls of alveolar ducts as function of distance from bronchiole-alveolar duct junction (means ± SE, $n = 4$ for each group; * $P < 0.05$). This subcompartment of lung parenchyma was chosen for selective study because it is representative of degree of interstitial remodeling because of high density of connective tissue fibers and interstitial matrix present in this site in the normal animal (16). Unlike alveolar wall thickness measurements that decreased exponentially with distance, thickness of septal edge was significantly elevated at a nearly constant level out to 600 μm. This change corresponded to significant connective tissue hypertrophy that was observed in this region. Results demonstrate that first 600 μm of ventilatory unit have a significant response to ozone exposure. Although not shown, measurements beyond 1.0 mm were not significantly different between control and ozone-exposed animals.

DISCUSSION

Exposure to ozone, a major component of environmental air pollution, constitutes a potential health risk in many metropolitan areas of the world today. In 1986, the United States Environmental Protection Agency estimated that seventy-five million people were living in areas where ozone concentrations exceeded the National Air Quality Standard (30). Air quality settings for ozone are based on both concentration and duration of exposure. To exceed these limits may result in adverse biological responses in the form of functional and/or structural

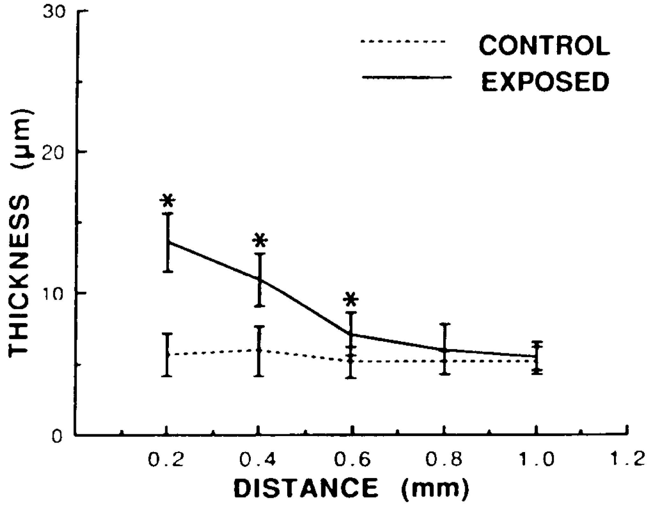

FIG. 7. Changes in thickness of alveolar septal wall as a function of distance from bronchiole-alveolar duct junction. Thickness of alveolar wall in first 200 μm from bronchiole-alveolar duct junction of exposed animals (solid line) was increased by 140% of corresponding control value (dashed line). This thickening was due predominantly to replacement of squamous type I epithelial cells with a mixture of cuboidal epithelial cells and bronchiolar epithelial cells extending from airway into proximal alveolar regions of ventilatory unit (means ± SE, $n = 4$ for each group; * $P < 0.05$).

changes within the respiratory system. If such changes are directly related to dose, then the schema proposed in this study could provide a sensitive means to establish dose-response relationships for ozone in the lungs. Numerous studies support this concept, but few have directly compared the dose of ozone to biological change at specific target sites in the lungs. This is the first study to compare tissue sensitivity and injury in which a local relative dose-response curve for ozone exposure is appropriately modeled. The results reflect a simple approach to compare tissue microtoxicology with ozone microdosimetry within the entire ventilatory unit.

The effects of ozone are not limited to a single region or specific cell type of the respiratory system (1–5, 7–11, 19–22, 24, 28–31). The diversity of cell populations in the lungs have an important role in the type and degree of injury manifested by exposure to environmental pollutants. Cell injury may be present within all levels of the respiratory system, but the degree and extent of injury can be highly variable. This heterogeneity is particularly true within the gas-exchange regions of the lungs (1, 3, 4). Ozone injury in this region is highly focal, centering around the BADJ while sparing the more distal alveolar tissues. Because of these variations in ozone response, sampling of the lung parenchyma to determine the extent and nature of these changes requires an unbiased sampling strategy. One approach is to optimize for the sampling of the basic structural and functional unit involved in gas exchange. Isolation of ventilatory units in longitudinal profile permits a simple morphometric approach for the evaluation of these changes. The method is uncomplicated and straightforward yet powerful in demonstrating parenchymal changes as a function of distance in well-defined structural and functional units of the lungs.

The remodeling of distal airways, the BADJ, and proximal alveolar regions in the lungs of rats chronically exposed to ozone have been studied extensively. In the literature, this region collectively has been referred to as the centriacinar region (1, 4). The recent work of Barr and colleagues (1) describes thickening of the terminal bronchiole wall and formation of respiratory bronchioles within the proximal alveolar ducts after identical exposure conditions as used in our study. The formation of respiratory bronchioles was the result of cuboidal epithelial cells replacing type I and type II epithelial cells along alveolar septal tips of the most proximal alveoli. Beyond these respiratory bronchioles a further remodeling localized to the septal tips of alveoli was also described (1).

A similar pattern of change was also noted in our study. Prominent epithelial cell changes occurred within both the terminal bronchioles and the proximal alveolar regions. An important finding not previously described was the frequent extension of ciliated and nonciliated epithelium into alveolar outpocketings of terminal bronchioles and to a limited degree within alveoli of the first 200 μm of the original BADJ. These epithelial changes were typically located between the BADJ and first alveolar duct bifurcation ridge. Interstitial changes were more prominent in the more distal portions significantly affected by ozone. In this instance, the pattern of reaction was more marked in alveolar septal tips and edges than in the alveolar wall. However, it is noteworthy that significant changes in alveolar septal thickness extended to the same level for both septal tips and alveolar walls. Therefore, the reaction of alveolar duct walls to ozone cannot be argued to be more severe than that found in alveolar septal walls. It is obvious from Table 1 and Figs. 6 and 7 that under normal conditions alveolar septal edges are naturally greater in thickness than are alveolar walls, but each proportionately responded in a similar manner. Therefore, alveolar duct walls and alveolar septal walls are similarly affected by the same relative concentration of ozone.

The mathematical model to predict ozone dose deliv-

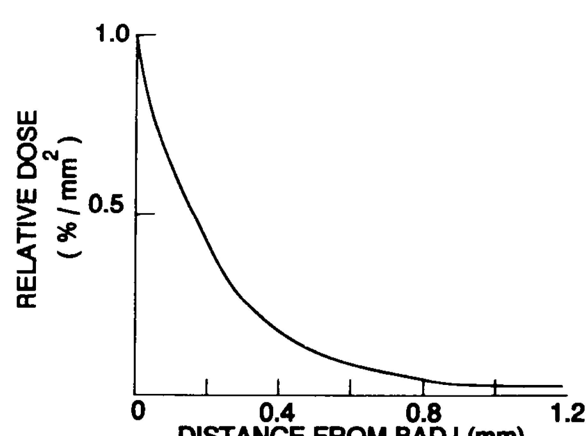

FIG. 8. Mathematical model used to predict uptake of ozone in ventilatory unit. More than 90% of relative effective dose of ozone initially present at bronchiole-alveolar duct junction (BADJ) has been taken up into tissues ~0.5 mm into ventilatory unit. Rapid uptake with increasing distance from BADJ is a reflection of a tremendous increase in alveolar surface area on which gas can be adsorbed.

ered to increasingly deeper levels of the ventilatory unit is based in part on the models described by Miller and associates (17, 18). The relative dose of ozone available rapidly dissipates as a function of dead space air volume and alveolar surface area. The high reactivity of a gas such as ozone with liquid lining films and membranes will result in a rapid drop in concentration as these surfaces are encountered down a diffusion path. Thus the model is useful in predicting focal injury sites that occur from low levels of inhaled pollutants. Because the magnitude of change in the thickness of the septal tissue seen in this study was small relative to the cross-sectional area of the bronchiole and alveolar ducts (~200 μm), it is unlikely that gas distribution would be dramatically affected within the ventilatory units of the lungs. However, there is a possibility that some effect may occur such as a change in compliance if the lesion involves a significant connective tissue response.

The changes in predicted dose of ozone correlate well with the observed changes in tissue thickness as a function of distance into the ventilatory unit and strongly suggest that injury to the lung parenchyma is dose dependent. Measures of septal edge and alveolar wall thickness serve well as sensitive markers of the biological response of these tissues to the relative dose encountered. The method of analysis is consistent and completely objective, with little variability between isolations. The technique is easy and fast and does not require the more laborious task of tissue preparation for electron microscopy to obtain high-resolution images for analysis. Light-microscopic observation of tissues is actually more efficient than electron microscopy to resolve differences in tissue values throughout the complete ventilatory unit path. In essence, this approach constitutes the first study to correlate microdosimetry and microtoxicology by analyzing specific target sites and changes occurring in the lung parenchyma as a function of distance into the gas-exchange unit in response to a highly reactive gas such as ozone.

In summary, this technique could easily be extended to other species, including those higher-order mammals possessing true respiratory bronchioles. Respiratory bronchioles contain a diverse population of cells with the presence of both bronchiolar and alveolar epithelial cells. If a differential response to ozone occurs for each of these cell types (5, 9, 19, 29) and is dependent on ozone concentration, site-specific acinar and ventilatory unit measures could prove useful in the elucidation of dose-response effects. Both structural and functional properties of the gas-exchange regions of the lungs in different species will surely have a significant role in the pattern of injury observed. The methods used in this study when applied to a variety of species will serve to further our understanding of the relative risks associated with exposure to oxidant pollutants and to more accurately define dose-response relationships at the cellular level.

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