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Lung Tissue Responses and Sites of Particle Retention Differ between Rats and Cynomolgus Monkeys Exposed Chronically to Diesel Exhaust and Coal Dust

Kristen J. Nikula, Kelly J. Avila, William C. Griffith, and Joe L. Mauderly

Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, New Mexico 87185

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Several chronic inhalation bioassays of poorly soluble, nonfibrous particles have resulted in an increased incidence of lung tumors in rats, no increase in lung tumors in Syrian hamsters, and inconsistent results in mice. These results have raised concerns that rats may be more prone than other species to develop persistent pulmonary epithelial hyperplasia, metaplasia, and tumors in response to the accumulation of inhaled particles. In addition, particle deposition and the rate of particle clearance from the lung differ between rats and primates, as does the anatomy of the centriacinar region. For these reasons, the usefulness of pulmonary carcinogenicity data from rats exposed to high concentrations of particles for quantitatively predicting lung cancer risk in humans exposed to much lower environmental or occupational concentrations has been questioned. The purpose of this investigation was to directly compare the anatomical patterns of particle retention and the lung tissue responses of rats and monkeys exposed chronically to high occupational concentrations of poorly soluble particles. Lung sections from male cynomolgus monkeys and F344 rats exposed 7 hr/day, 5 days/week for 24 months to filtered ambient air, diesel exhaust (2 mg soot/m^3), coal dust (2 mg respirable particulate material/m^3), or diesel exhaust and coal dust combined (1 mg soot and 1 mg respirable coal dust/m^3) were examined histopathologically. The relative volume density of particulate material and the volume percentage of the total particulate material in defined pulmonary compartments were determined morphometrically to assess the relative amount and the anatomic distribution of retained particulate material. In all groups, relatively more particulate material was retained in monkey than in rat lungs. After adjustment for differences between rat and monkey controls, the coal dust- and the combined diesel exhaust and coal dust-exposed monkeys retained more particulate material than the coal dust- and the combined diesel exhaust and coal dust-exposed rats, respectively. There was no significant difference in the relative amount of retained particulate material between diesel exhaust-exposed monkeys and rats. Within each species, the sites of particle retention and lung tissue responses were the same for diesel soot, coal dust, and the combined material. Rats retained a greater portion of the particulate material in lumens of alveolar ducts and alveoli than monkeys. Conversely, monkeys retained a greater portion of the particulate material in the interstitium than rats. Rats, but not monkeys, had significant alveolar epithelial hyperplastic, inflammatory, and septal fibrotic responses to the retained particles. These results suggest that intrapulmonary particle retention patterns and tissue reactions in rats may not be predictive of retention patterns and tissue responses in primates exposed to poorly soluble particles at concentrations representing high occupational exposures.

Several chronic inhalation bioassays of diesel exhaust in rats, Syrian hamsters, and mice have been conducted (results reviewed by Mauderly, 1995). These bioassays have consistently shown that diesel exhaust, inhaled chronically at high concentrations, causes increased incidences of lung tumors in rats. Exposures resulting in an increased incidence of lung tumors in rats also caused progressive soot accumulation, primarily within alveolar macrophages, alveolar epithelial hyperplasia, chronic–active inflammation, focal fibrosis, and epithelial metaplasia. None of the studies using Syrian hamsters has demonstrated a diesel exhaust-related increase in lung tumors, although soot accumulated in hamster lungs. Mild bronchiolar–alveolar hyperplasia, which was much less severe than in comparably exposed rats, has been observed in hamsters that chronically inhaled diesel exhaust (Heinrich *et al.*, 1986). Inconsistent results have been obtained in studies using mice. Increased incidences of lung tumors occurred in some female groups of Strain A and Sencar mice, strains sensitive to chemical carcinogenesis, but results have been generally negative in other strains. Lung tumors were not increased in CD-1 mice exposed under conditions carcinogenic to rats (Mauderly *et al.*, 1996). These mice progressively accumulated soot, primarily in alveolar macrophages, and septal fibrosis and bronchiolization of alveolar ducts were observed in areas of soot accumulation. However, the amounts of inflammation and epithelial hyperplasia were less in mice than in rats (Henderson *et al.*, 1988; Mauderly *et al.*, 1996).

An interspecies difference in response, with rats but not

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mice showing an increased incidence of lung tumors, has been shown for a number of relatively insoluble particles including talc (NTP, 1993), carbon black (Heinrich *et al.*, 1992), titanium dioxide (Heinrich *et al.*, 1992), silica (reviewed by Saffiotti, 1995), nickel subsulfide (NTP, 1994a), cadmium sulfate (Heinrich *et al.*, 1989; Glasser *et al.*, 1990), cadmium sulfide (Heinrich *et al.*, 1989; Glasser *et al.*, 1990), and cadmium chloride (Heinrich *et al.*, 1989; Glasser *et al.*, 1990). Much of our knowledge of the response of human lungs to heavy particle loading comes from coal miners. They accumulate specific lung burdens of coal dust that are in the range of specific lung burdens associated with particle-induced carcinogenicity in rats (summarized in Mauderly, 1994), yet coal dust exposure alone does not significantly increase the risk for lung cancer in humans (Merchant *et al.*, 1986). These results have raised concerns that rats may be more prone than other species to develop persistent pulmonary epithelial hyperplasia, metaplasia, and tumors in response to inhaled particles.

The rate of particle clearance from the alveolar region differs among species. Rats and mice clear particles from the lung relatively quickly, whereas monkeys and humans clear particles more slowly (Snipes, 1989). Clearance in all these species can be described by two-phase kinetics comprised of a faster and slower component; the half-time for the faster component is 25 to 30 days for all these species. In rats and mice, 90% of the total clearance falls into the faster component, and the half-time of the slower component is approximately 200 to 250 days. In monkeys and humans, only 20 to 30% of the total clearance falls into the faster component, and the half-time of the slower component is 600 to 700 days. These differences in the rate of particle clearance suggest that the mechanisms of clearance and/or the sites of particle retention differ between the faster-clearing and slower-clearing species.

Anatomical differences between the faster-clearing and slower-clearing species could affect particle deposition, retention, and clearance. The functional unit of the lung, the acinus, is composed of the terminal bronchiole and the air spaces it supplies (Schreider and Raabe, 1981). Because mice and rats lack respiratory bronchioles, they have simple acini. Macaque monkeys and humans have similar numbers of generations of respiratory bronchioles between the terminal bronchiole and alveolar ducts (Phalen and Oldham, 1983; Tyler, 1983), and they have larger alveoli and alveolar ducts than rats (Mercer and Crapo, 1988). Therefore, monkeys and humans have more complex, larger acini then rats. The amount of interstitial connective tissue in the lung also differs, with small rodents having less and primates more. A greater portion of the pulmonary parenchyma (composed of air in alveoli and ducts, capillary blood, and septal tissue) is composed of septal tissue in rhesus monkeys and humans than in rats (Pinkerton *et al.*, 1982; Kapanci *et al.*, 1969, 1972; Crapo *et al.*, 1982). Lastly, rats have thin pleura, relatively few pleural lymphatics, and no interlobular connective tissue, while humans have thick pleura, relatively abundant pleural lymphatics, and abundant interlobular connective tissue (McLaughlin *et al.*, 1961, 1966; Leak and Jamuar, 1983). Nonhuman primates generally have pleura that are classified as thin, but which are thicker and have more lymphatics than rat pleura; they have scant interlobular connective tissue (McLaughlin *et al.*, 1961; Tyler and Julian, 1992).

The scientific and regulatory communities are currently debating the usefulness of pulmonary carcinogenicity data from rats exposed to high concentrations of particles for predicting lung cancer risk in humans exposed to much lower environmental or occupational concentrations. For example, Snipes (1996) noted that although substantial pulmonary burdens of dust have been reported for monkeys and humans, altered pulmonary clearance, the defining feature of lung overload in rats, has not been demonstrated in the larger species. He predicted that chronic inhalation of dusts would result in different dust accumulation patterns in the lungs of monkeys or humans than in rats, and that the larger species would not exhibit manifestations of lung overload. Schultz (1996) stressed the importance of distinguishing between nonspecific pathologic effects of dust overload from more dust-specific effects seen at lower lung burdens. He concluded that a minimal overload concentration may be desirable in some inhalation bioassays, but that rat-specific histopathology produced under these conditions would likely have little relevance to humans.

Information relevant to these issues could be gained by comparing the responses of rat and nonhuman primate lungs to particles at exposure concentrations within the range of occupational exposure. Tissues available from the Lewis *et al.* (1989) 2-year bioassay of F344 rats and cynomolgus monkeys exposed at 2 mg respirable particulate material/m^3 to diesel exhaust, coal dust, or a combination of diesel exhaust and coal dust afforded the opportunity to make this comparison. The diesel exhaust exposure concentration was just below that which has proven carcinogenic (3.5 mg/m^3) in rats under similar exposure conditions (Mauderly *et al.*, 1987). The coal dust concentration was at the current permissible airborne concentration in underground coal mines in the U.S. (2 mg respirable particulate/m^3) which has been shown by an extensive database to be noncarcinogenic in miners. Lewis and co-workers (1989) provided excellent documentation of this well-conducted study, and they examined many parameters with an emphasis toward detection of possible synergism between diesel exhaust and coal dust exposure. Interspecies comparisons were not emphasized. The study did not include grading of lesions by the same pathologist using the same terminology and scale in rats and monkeys, nor did it include morphometric analyses of amounts of retained particulate material or sites of particle retention.

The purpose of this investigation was to use materials from the Lewis *et al.* study to make direct quantitative comparisons of the patterns of particle retention and the lung tissue responses of rats and monkeys exposed chronically to diesel exhaust, coal dust, or diesel exhaust combined with coal dust. The hypothesis was that particles would be retained differently in lungs of chronically exposed rats and monkeys, and that the tissue responses would also differ.

MATERIALS AND METHODS

Animal exposures, exposure materials, and exposure atmospheres. The lung tissues examined were from the Lewis *et al.* (1989) 2-year bioassay of cynomolgus monkeys and F344 rats exposed to filtered, conditioned, ambient air as controls (C) or to diluted whole diesel exhaust at a target particle concentration of 2 mg/m^3 (DE), to coal dust aerosolized in air at a target respirable particle concentration of 2 mg/m^3 (CD), or to a combination of 1 mg/m^3 diesel soot with the same gaseous or vapor airborne concentrations as in the diesel exhaust atmosphere and 1 mg/m^3 respirable coal dust (DECD). Male cynomolgus (*Macaca fascicularis*) monkeys (4–5 kg when obtained) and male and female F344 rats (8–10 weeks old when exposures were initiated) were exposed in whole-body chambers 7 hr/day, 5 days/week, for periods up to 24 months. The animal care, exposures and exposure atmospheres, and results of the many parameters examined have been described in detail (Lewis *et al.*, 1989).

The diesel exhaust was produced by burning No. 2 diesel fuel containing <0.5% sulfur by mass in a 425 in^3 displacement (7.0 liters) four-cycle, water-cooled, naturally aspirated Caterpillar Model 3304 diesel engine equipped with a water scrubber. The engine was operated by computer to simulate the load–haul–dump operation of diesel-powered trams in coal mining.

Bituminous coal from the Pittsburgh seam was micronized to size specifications of 80% < 10 μm and 50% < 5 μm and aerosolized using a Wright dust feeder (BGI Industries, Waltham, MA).

Detailed methods for determining total and respirable airborne particulate concentrations and particle mass median diameter are reported (Lewis *et al.*, 1989). The mass median diameter of the coal dust in the exposure chamber was 8.6 μm. Mass median diameters of the diesel soot were 0.23 and 0.36 μm by electrical aerosol size analyzer and scanning electron microscopic techniques, respectively. Particles less than 7 μm mass median diameter were defined as respirable. About 40% of the coal dust particulate material and all of the diesel soot were respirable. Total particulate concentrations in the CD and DECD chambers were 4.98 ± 0.82 (mean ± SD) and 3.23 ± 0.60 mg/m^3, respectively. Respirable particle concentrations were 1.95 ± 0.25, 2.00 ± 0.41, and 2.02 ± 0.30 mg/m^3 for the DE, CD, and DECD chambers, respectively (data from Lewis *et al.*, 1989).

Histopathology. Lung sections from rats and monkeys sacrificed 1 day after the end of the 24-month exposure were obtained from the U.S. National Institute of Occupational Safety and Health (NIOSH). Lung sections from 14 C, 14 CD, 15 DE, and 15 DECD monkeys were examined. The lungs had been inflated via intratracheal instillation of buffered formalin at 20–25 cm hydrostatic pressure. After fixation, two sections of each lobe (one from the proximal portion and one from the distal portion) had been embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). The proximal and distal sections from the left apical–cardiac, left diaphragmatic, and right diaphragmatic lobes were examined by light microscopy. These lobes were chosen because they sampled the cranial and caudal lung and the right and left sides and were most consistently available; there were only two cases where one section was missing.

Lungs of eight male rats from each exposure group had been inflated via intratracheal instillation of 2% Karnovsky's fixative in 0.2 M sodium cacodylate buffer at 20 cm hydrostatic pressure. After fixation, a transverse tissue block (approximately 4 mm thick) from the mid-left lobe and from the mid-right diaphragmatic lobe had been embedded in paraffin, sectioned at 5 μm, and stained with H&E. A second slide was generated from each block by cutting a section from the opposite (distal) face and staining with H&E. These four sections were examined by light microscopy. In order to increase the number of rat lungs examined to match the monkeys, lungs from seven additional male rats per exposure group were randomly selected. These lungs had been intratracheally instilled with 10% buffered formalin *in situ* until full inspirational volume was reached. The trachea was clamped and the lungs immersed in formalin. One section each from the left lung, right apical, right cardiac, and right diaphragmatic lobes had been cut and stained with H&E. These sections were examined by light microscopy.

A standard lesion terminology developed previously (Nikula *et al.*, 1995) was used, and histopathologic findings were entered into a computer data base (PathTox; Xybion Medical Systems, Cedar Knolls, NJ). Lesions were scored as present or absent. If present, the severity of each lesion was graded on a scale of slight to marked, indicating the approximate fraction of the lung or structure judged to be involved (slight = 1–2%, minimal = 3–10%, mild = 11–24%, moderate = 25–50%, and marked = 51–100%). A locally intense lesion was scored as slight if only a small portion of the lung was affected. In an effort to be consistent across exposure groups and species, one monkey from each exposure group was examined followed by one rat in each exposure group, then the cycle was repeated. The same lesion terminology and grading scale were used for both species.

Morphometry. Lungs that had been inflated at a constant pressure of 20–25 cm (monkeys) or 20 cm (rats) hydrostatic pressure were used for morphometry. Lungs from eight rats per exposure group were available, and the same four sections used for histopathology from each animal were examined. To examine lungs from an equal number of monkeys, lungs with debris and pigment due to pulmonary acariasis that was scored as mild or above were excluded from selection, and then eight lungs from each exposure group were randomly selected. The same proximal and distal sections from the left apical–cardiac, left diaphragmatic, and right diaphragmatic lobes as used for histopathology were examined. The distal portion of the right diaphragmatic lobe was missing in one case, so the distal portion of the right apical lobe was substituted.

The point-counting method of planimetry (Elias and Hyde, 1983) was used to estimate the relative volume density of particulate material in the lung sections and the volume percentage of the total particulate material in defined anatomic compartments of the lung. These compartments are listed and defined in Table 1. A systematic, random sampling scheme was used to record an average of 102 and 238 lung fields in monkeys and rats, respectively. The relative scarcity of particles in the rat versus monkey lungs necessitated greater sampling of the rat lungs to record a sufficient number of points hitting particulate material for statistical analysis. Digitized images of the lung were captured using a Sony ICX 038AK color camera interfaced to an Olympus BH2-RFCA microscope and a Macintosh Quadra 950 computer. The images were captured using a 40× microscope objective and projected onto the computer monitor screen at a final magnification of 1280×. A 64-point grid (Stereology Toolbox, Davis, CA) was superimposed over each image, and the number of points hitting particulate material, the location of each point, and the number of points hitting the lung section but not hitting particulate material were recorded.

The relative volume density expressed as a percentage is a volume ratio of particulate material to lung and was calculated for each animal from the number of points hitting particulate material divided by the total number of test points × 100%. These data are estimates of the relative amount of retained particulate material in the lung. The volume percentage of the total particulate material in a defined anatomic compartment is a volume ratio of particulate material in a compartment to total particulate material, and was calculated for each animal from the number of points hitting particulate material in a defined compartment divided by the total number of points hitting particulate material × 100%. These data are estimates of the anatomic distribution of the retained particulate material.

TABLE 1
Morphometric Compartments for Particle Counts[a]

Point hitting particle in (1–9):
1. Pleura
   Including associated connective tissue and lymphatics
2. Bronchus-associated lymphoid tissue or intrapulmonary lymph node
3. Bronchovascular interstitium of conducting airways
   Including airway walls, peribronchovascular connective tissue, and associated vessels, including lymphatics
4. Lumen of conducting airway
5. Interstitium of respiratory bronchiole (monkey)
   Including respiratory bronchiolar wall, peribronchiolar and vascular connective tissue, and associated vessels, including lymphatics.
   Not including septa of alveolar outpocketings
6. Lumen of respiratory bronchiole (monkey)
7. Perivascular interstitium
   Perivascular adventitia and lymphatic capillaries surrounding pulmonary arterioles, veins, and venules not associated with conducting airways or respiratory bronchioles. Includes interlobular connective tissue.
8. Septum of alveolar duct or alveolus
   Including septa of alveolar outpocketings of respiratory bronchioles in monkeys
9. Lumen of alveolar duct or alveolus
10. Point hitting lung section but not hitting a particle

[a] For each field, points hitting the lung section (test points) were counted in one of 10 categories. Thus, total points hitting the lung section, total points hitting particles, and points hitting particles in each defined lung compartment could be calculated.

Although the lungs used for morphometry from both species were perfused under constant pressure at similar hydrostatic pressures, slight differences in pressure, shrinkage due to fixing and processing, and sampling strategy could have affected the comparative interspecies results for the relative volume densities of particulate material in the lungs, i.e., volume of particulate material/volume of lung. However, these factors would not have affected the location of the particulate material, i.e., the percentage of total particulate material in defined anatomic compartments; thus, they would not have affected the comparisons of particle location across species or within species by particle type. Also, because the fixing, processing, and sampling factors were the same within each species, they would not have affected the comparisons of relative volume densities by particle type within each species.

The volume fraction of the lung occupied by the various compartments, as represented in these lung sections, was determined so that values obtained from these lung sections could be compared with normal values to assess the lung inflation and sampling. A systematic, random sampling scheme was used to record an average of 36 fields in the monkeys and rats. Using the same imaging system described above, images were captured using a 10× objective and projected onto the screen at a final magnification of 320×. A 42-point grid was superimposed over each image, and the number of points hitting each anatomic compartment of the lung section was recorded. The volume fraction of the lung occupied by specific compartments was calculated for each animal from the number of points hitting the compartment divided by the total number of test points.

Because of the anatomic difference between rat and monkey lungs and because the diesel and coal particles differ morphologically, it was not possible to evaluate the sections while blinded to species and treatment group. However, because grid points were evaluated, and these were chosen randomly, the morphometry should be free from bias. In addition, the morphometric and histopathologic evaluations were conducted by different people, thus providing another guard against bias.

Statistical analyses. The criterion for statistical significance was set at $p < 0.05$ for all analyses. The pathology severity scores were analyzed using polychotomous logistic regression to estimate the prevalence of lesions with a particular score. This is a technique for analyzing ordered category data with more than two outcomes, and the spacing between the categories does not have to be equal. In this technique, the cumulative distribution probabilities of the scores are modeled, i.e., the probability of a score and all lower scores. A linear model of the logit of the cumulative probabilities was estimated. Other explanatory variables were then tested in the analysis to determine if they were significantly related to the prevalence of the lesions being scored. The simplest model of parallel regressions for scores was used (McCullagh and Nelder, 1983).

Using this analysis, it was possible to determine whether exposure to particles or a type of particle was a significant explanatory variable and whether there were any significant differences between species. An odds ratio and its 95% confidence interval were estimated for each factor. The odds ratio summarizes the effect of each factor for all severity scores. Odds ratios greater than 1 indicate an increased response above the comparison group, values less than 1 indicate a decreased response, and a value of 1 indicates no change from the comparison group. The statistical significance of the odds ratio is at the $p \leq 0.05$ level when the 95% confidence interval does not contain the value of 1. In some cases, it was not possible to estimate an odds ratio because all or almost all of the controls had no response, and all of the exposed animals had at least a slight response. In these cases, because the controls were the denominator and had 0 response (or nearly 0), the odds ratio could not be estimated.

Because the relative volume density of particles was estimated based upon counting the number of grid points falling on particles, the data were analyzed using Poisson regression for which it is assumed that the variability in the number of particles counted follows a Poisson distribution. In this study there were additional sources of variation among animals, especially monkeys, beyond those due to sampling. This was accounted for in the analysis by using a scaling factor, greater than 1, to multiply the sampling variance. Also, because slightly different numbers of grid points were examined for each animal, an offset based upon the log of the number of grid points was included in the Poisson regression (McCullagh and Nelder, 1983).

The estimates of the volume percentages of the total particulate material in the lumens of alveolar ducts and alveoli and in interstitial compartments were based upon the number of points hitting particles in these compartments divided by the total number of points hitting particles for each animal. These data were assumed to be binomially distributed, and logistic regression was used for the statistical analysis. Again, there were additional sources of variation among animals beyond those due to sampling, and this was accounted for in the analysis by using a scaling factor, greater than 1, to multiply the sampling variance (McCullagh and Nelder, 1983).

RESULTS

Histopathology

General Descriptive Overview

The lungs of diesel exhaust-exposed (DE), coal dust-exposed (CD), and diesel exhaust combined with coal dust-exposed (DECD) rats exhibited the same histopathology. The incidences and severity of some specific components of the pulmonary response to particles differed only slightly. Overall, the particles were observed mainly within multifocal collections of alveolar macrophages. Most commonly

these macrophage aggregates were located within centriacinar alveoli (Fig. 1A), but the alveoli immediately adjacent to the pleura was another common location (Fig. 1B). A lesser portion of the retained particles was located in the interstitium. The characteristic tissue response to densely aggregated alveolar macrophages was alveolar epithelial hyperplasia. Other responses, in descending order of incidence, were particle-associated inflammation, a local septal fibrotic reaction, and alveolar proteinosis. The frequency of these lesions was directly correlated with the amount of particulate material observed within aggregated alveolar macrophages. The DE rats, which appeared to have the most aggregates of heavily particle-laden alveolar macrophages, had slightly greater incidences of these associated responses.

Most of the control monkey lungs contained particulate material, and similar nonsoot, noncoal-dust, particulate material was present along with the soot and coal dust in exposed monkey lungs. The nonsoot, noncoal-dust, particulate material, which was generally lighter colored, gray to golden, and more loosely packed than the diesel soot or coal dust in exposed monkeys, consisted of endogenous pigments, materials inhaled and retained over the lifetime of the animal, and, in some of the monkeys, debris from pulmonary mites. The incidences (one-third of the monkeys were affected) and severity of pulmonary acariasis and the associated focal chronic eosinophilic bronchiolitis were the same across all exposure groups. This bronchiolitis, which affected respiratory bronchioles and alveolar ducts, was characterized by eosinophilic, chronic granulomatous inflammation, presence of mites or mite debris, and frequently concomitant thickening of bronchiolar or ductular walls and adjacent septa due to smooth muscle hyperplasia, increased collagen, and slight alveolar epithelial hyperplasia. When these characteristic mite-induced findings occurred together, the single diagnostic term "chronic eosinophilic granulomatous bronchiolitis" was used instead of scoring each subpart of the lesion separately.

At low magnification, particulate material was more apparent in the monkey lungs than in the rat lungs. Unlike the rats, the DE monkey lungs appeared to contain less particulate material than the CD monkeys. The predominant sites of particle retention and the characteristic tissue responses differed between the rats and monkeys, although they were similar in DE, CD, and DECD monkeys. The retained particles had a multifocal distribution in the monkey lungs, but, in contrast to the rat, more of the particulate material was located in the interstitium than in the alveoli. The most common locations of particle-laden macrophages were (1) within the lumens of alveoli at the level of first- and second-generation alveolar ducts (Fig. 1C), (2) within the alveolar septa (Figs. 1C and 1D), (3) within the interstitium of respiratory bronchioles (Fig. 1D), (4) within the adventitia and lymphatic capillaries surrounding arterioles and veins within the pulmonary parenchyma, and (5) in the pleura. The interstitial particulate material did not seem to elicit a tissue response. The portion of the particulate material within intralumenal collections of alveolar macrophages was smaller than that in rats, and the aggregates of particle-laden macrophages elicited much less of a tissue response in the monkeys than in the rats.

Lesions, Severity Scores, and Statistical Analyses

Tables 2 and 3 show the incidences and average severity scores of each category of lesion. These lesions, the comparative histopathological findings, and results of the statistical analyses are described below.

Alveolar macrophage hyperplasia. Alveolar macrophage hyperplasia in particle-exposed rats consisted of increased numbers of alveolar macrophages that contained diesel soot or coal dust (Fig. 1A). The macrophages were multifocally aggregated within small groups of alveoli, rather than evenly distributed among the alveoli. The most commonly affected alveoli were along the first-generation alveolar ducts. The macrophages in these aggregates were heavily particle-laden, and the lumens of alveoli were sometimes obliterated by the macrophages. Peripheral to the focal accumulations of particle-laden macrophages, the number of macrophages per alveolus was markedly decreased. Widely scattered macrophages with particles were disseminated throughout the lungs. The scattered macrophages were smaller and contained less particulate material than those in the focal accumulations.

Alveolar macrophage hyperplasia in particle-exposed monkeys also consisted of increased numbers of alveolar macrophages that contained particulate material (Fig. 1C). Particle-laden macrophages were most commonly aggregated in alveoli adjacent to first- and second-generation alveolar ducts. In monkeys, macrophages rarely obliterated alveoli, which are larger in monkeys than in rats. As in the rats, scattered, smaller macrophages containing less particulate material were disseminated throughout the lungs.

In both species, alveolar macrophage hyperplasia had scores of none to mild, and there was no significant difference between species ($p = 0.5$). In both species, alveolar macrophage hyperplasia was significantly greater in particle-exposed than in control animals ($p < 0.001$). However, it was not possible to estimate the odds ratio because most of the controls did not exhibit alveolar macrophage hyperplasia, and all of the exposed animals had at least slight alveolar macrophage hyperplasia. DECD rats had a slightly lower response compared to DE and CD rats ($p = 0.04$) with an odds ratio (and 95% confidence interval) of 0.24 (0.055, 0.99), but there was no statistical difference in the response when the DE or the CD rats were compared separately to the DECD rats. There was no significant difference in alveolar

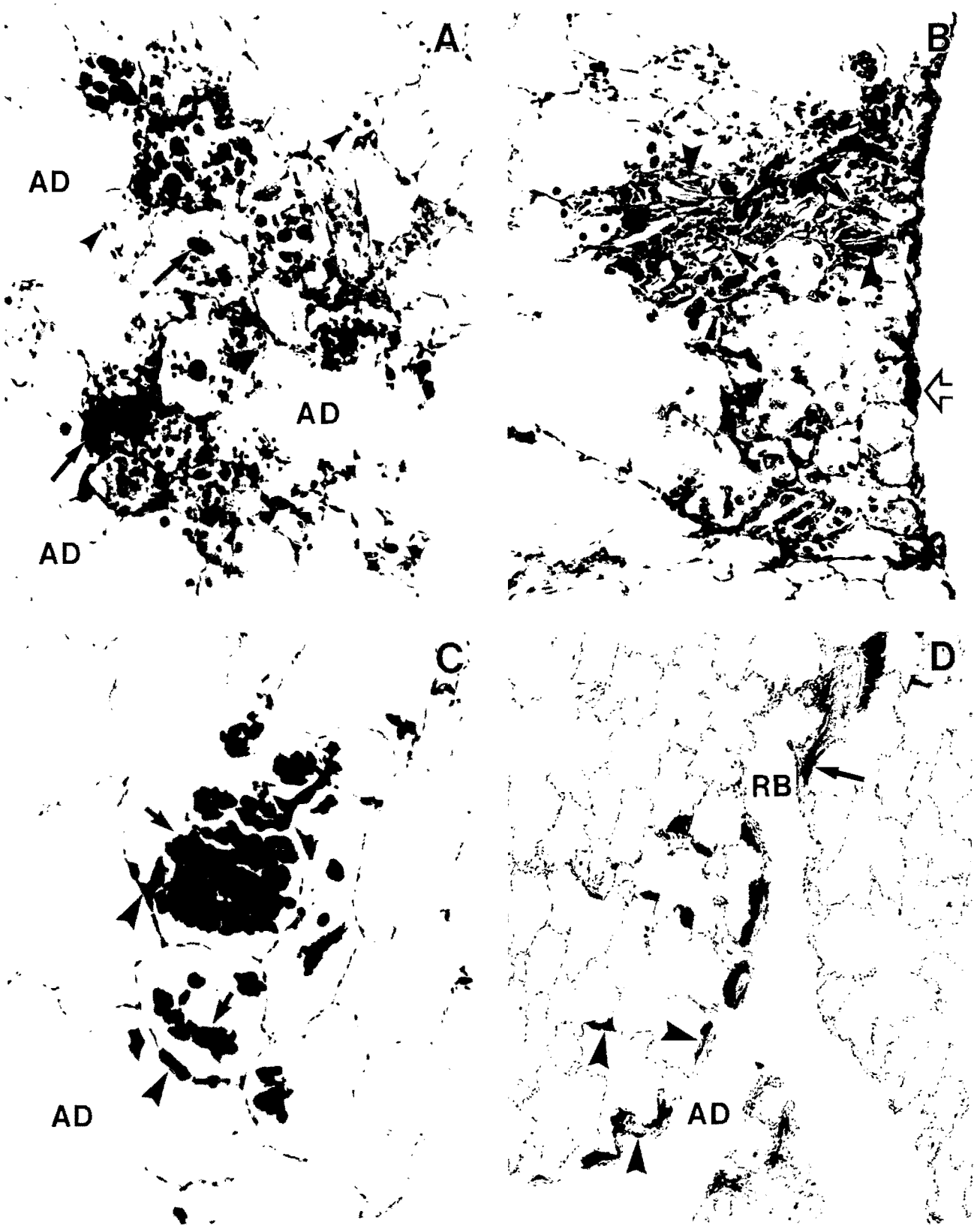

FIG. 1. Overview of particulate material in exposed rats and monkeys. (A) DECD rat. The particulate material is primarily within intralumenal macrophages in centriacinar alveoli. Arrowheads, small macrophages with few particles; arrows, large macrophages or macrophage aggregates with many particles; AD, lumen of alveolar duct. ×200. (B) DE rat. Soot primarily within intralumenal macrophages in alveoli adjacent to the pleura. Soot also located within the pleura in this region (open arrow). Chronic inflammation with mixed neutrophilic and mononuclear cells (arrows) and cholesterol clefts in multinucleated giant cells (arrowheads). ×150. (C) DE monkey. Soot within macrophages in alveolar lumens (arrows) and alveolar septa (arrowheads). AD, lumen of alveolar duct. ×200. (D) DECD monkey. Particulate material in the interstitium of a respiratory bronchiole (RB, arrow) and the alveolar septa (arrowheads). AD, lumen of alveolar duct. ×75.

TABLE 2

Incidences and Average Severity Scores of Particle-Associated or Possibly Particle-Associated[a] Histopathologic Findings in Rats

Diagnosis	Exposure group[b]			
	C	DE	CD	DECD
Number of rats examined	15	15	15	15
Alveolar macrophage hyperplasia	1 (1)[c]	15 (1.7)	15 (1.5)	15 (1.2)
Alveolar epithelial hyperplasia	1 (1)	15 (1.7)	14 (1.5)	15 (1.2)
Particle-associated inflammation	0 (—)	10 (1.1)	7 (1.0)	7 (1.0)
Septal fibrotic reaction	0 (—)	7 (1.1)	4 (1.0)	4 (1.0)
Alveolar proteinosis	0 (—)	4 (1.0)	2 (1.0)	3 (1.0)
Particles in lumens of alveolar ducts and alveoli	0 (—)	15 (1.7)	15 (1.3)	15 (1.2)
Particles in insterstitium	0 (—)	15 (1.1)	15 (1.1)	15 (1.1)

[a] Histopathologic findings whose incidence or severity suggested a possible particle association in any exposure group in either rats or monkeys.

[b] Rats were exposed to filtered air as controls (C), diesel exhaust (DE), coal dust (CD), or a combination of diesel exhaust and coal dust (DECD) at target concentrations of 2 mg respirable particulate material/m^3.

[c] First number is the number of rats with the findings. Number in parenthesis is the average severity score calculated as $\frac{\text{sum of the severity scores}}{\text{number of rats with the finding}}$. Lesions were scored as 1, slight; 2, minimal; 3, mild; 4, moderate; and 5, marked.

macrophage hyperplasia among the DE, CD, or DECD monkeys ($p = 0.7$).

Epithelial hyperplasia. Alveolar epithelial hyperplasia was observed in the lungs of all particle-exposed rats, except one CD rat. Epithelial hyperplasia was multifocal and consisted of an increased number of hypertrophic, cuboidal, alveolar epithelial cells lining alveolar septa. Affected alveoli contained particle-laden macrophages; most often, the alveolar lumens were densely packed with macrophages (Fig. 2A). Usually, the severity of the alveolar epithelial hyperplasia was proportional to the aggregation of particle-laden alveolar macrophages. Sometimes, the amount of alveolar epithelial hyperplasia was disproportional to the amount of particulate material (Fig. 2B). One control rat exhibited a single focus of alveolar epithelial hyperplasia of unknown etiology; no particles or other alterations were associated with the lesion.

Alveolar epithelial hyperplasia was less common in particle-exposed monkeys than in rats. In one C and one DECD monkey with this lesion, the alveolar epithelial hyperplasia was focal and consisted of an increased number of hypertrophic, cuboidal to columnar epithelial cells lining subpleural,

TABLE 3

Incidences and Average Severity Scores of Particle-Associated or Possibly Particle-Associated[a] Histopathologic Findings in Monkeys

Diagnosis	Exposure group[b]			
	C	DE	CD	DECD
Number of monkeys examined	14	15	14	15
Alveolar macrophage hyperplasia	2 (1.0)[c]	15 (1.2)	14 (1.4)	15 (1.5)
Alveolar epithelial hyperplasia	2 (1.5)	4 (1.5)	3 (1.0)	4 (2.0)
Particle-associated inflammation	1 (1.0)	3 (1.0)	4 (1.0)	4 (1.2)
Septal fibrotic reaction	3 (1.3)	0 (—)	0 (—)	1 (1.0)
Alveolar proteinosis	0 (—)	0 (—)	0 (—)	0 (—)
Particles in lumens of alveolar ducts and alveoli	1 (1.0)	15 (1.3)	14 (1.4)	15 (1.5)
Particles in interstitium	10 (1.3)	15 (1.9)	14 (2.0)	15 (2.1)

[a] Histopathologic findings whose evidence or severity suggested a possible particle association in any exposure group in either rats or monkeys.

[b] Monkeys were exposed to filtered air as controls (C), diesel exhaust (dE), coal dust (CD), or a combination of diesel exhaust and coal dust (DECD) at target concentrations of 2 mg respirable particulate material/m^3.

[c] First number is the number of monkeys with the findings. Number in parenthesis is the average severity score calculated as $\frac{\text{sum of the severity scores}}{\text{number of monkeys with the finding.}}$ Lesions were scored as 1, slight; 2, minimal; 3, mild; 4, moderate; and 5, marked.

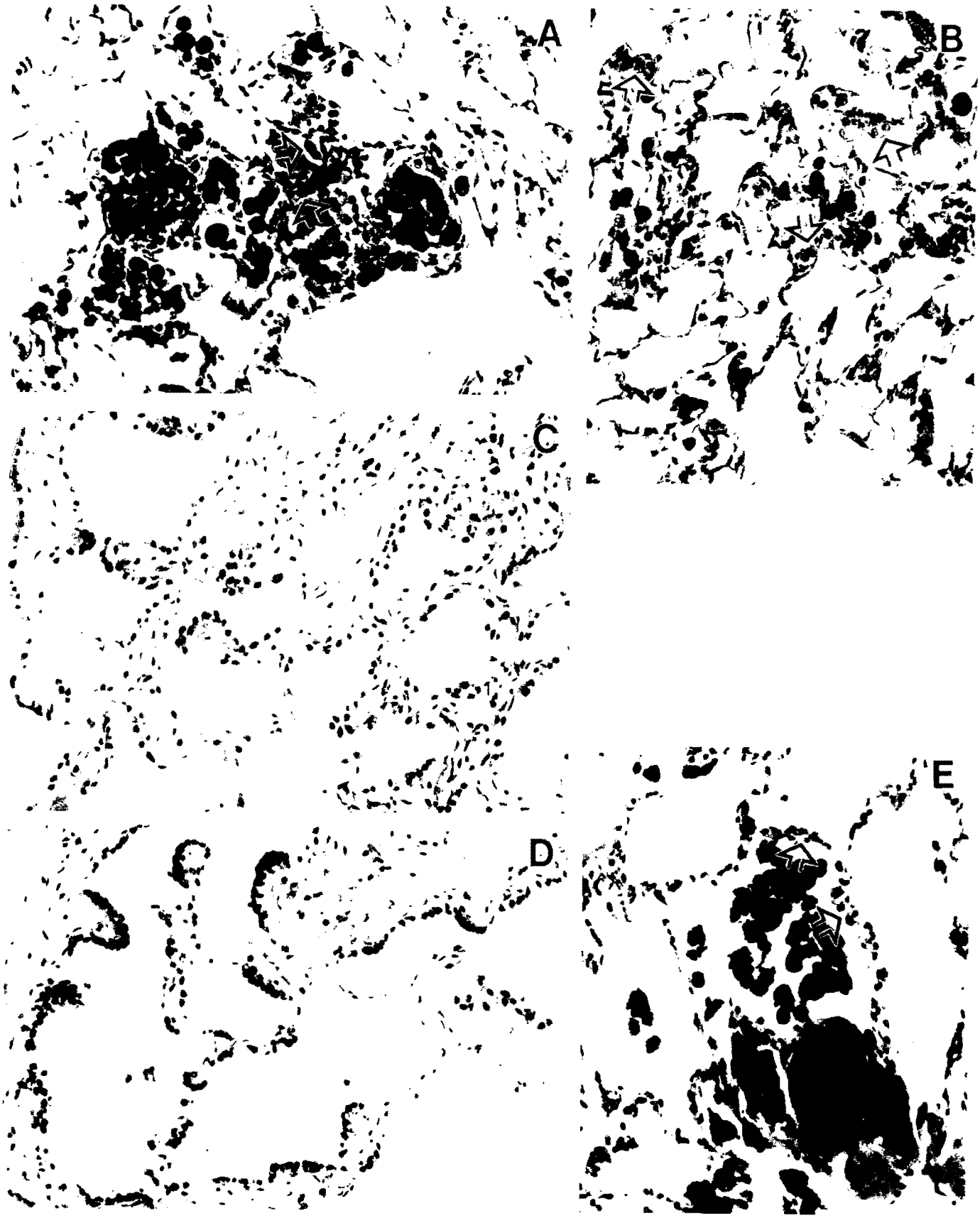
A
B
C
D
E

TABLE 4
Distribution of Histopathologic Severity Scores for Alveolar Epithelial Hyperplasia among Groups of Rats and Monkeys

Exposure group[b]	Severity score[a]									
	Rats					Monkeys				
	0	1	2	3	4	0	1	2	3	4
C	14[c]	1	0	0	0	12	1	1	0	0
DE	0	7	6	1	1	12	2	2	0	0
CD	1	11	0	2	1	11	3	0	0	0
DECD	0	12	3	0	0	11	2	0	2	0

[a] Severity scores: 0, lesion not observed; 1, slight; 2, minimal; 3, mild, and 4, moderate.

[b] Animals were exposed to filtered air as controls (C) or to diesel exhaust (DE), coal dust (CD), or a combination of diesel exhaust and coal dust (DECD) as described earlier. 15 rats examined in each group. 14 C, 15 DE, 14 CD, and 15 DECD monkeys were examined.

[c] Number of animals with each severity score.

fibrotic alveolar septa (Figs. 2C and 2D). In one C, one DE, and two CD monkeys, slightly increased numbers of alveolar epithelial cells were seen in alveoli with a concomitant mixed inflammatory infiltrate including eosinophils or in alveoli with nearby, but not contiguous, lesions of chronic eosinophilic bronchiolitis. Although the lesions in these alveoli were not pathognomonic for pulmonary acariasis, it is likely that a reaction to mites caused or contributed to the lesions. In one DE and two DECD monkeys, small foci of alveolar epithelial hyperplasia occurred in alveolar ducts with smooth muscle hyperplasia or in subpleural alveoli in the absence of other lesions. In two DE, one CD, and one DECD monkey, foci of alveolar epithelial hyperplasia occurred in association with acute or chronic inflammation that was not eosinophilic. In these last four cases, particle-laden macrophages were located in the areas of the lesions. The epithelial hyperplasia consisted of a slight increase in the number of type II cells, but, unlike the rats, most of the surface of the affected alveolus was still lined by type I cells (Fig. 2E).

Alveolar epithelial hyperplasia had scores of none to moderate in rats and none to mild in monkeys. Table 4 shows the distribution of severity scores among groups in both species. Rats had a significantly greater response to particle exposure than monkeys ($p < 0.001$) with an odds ratio (95% confidence interval) of 14 (5.5, 39). Alveolar epithelial hyperplasia was significantly greater in particle-exposed than control rats ($p < 0.001$) with an odds ratio of 620 (36, 11,000), but there was no difference in the hyperplastic response due to type of particle exposure ($p = 0.3$). The monkeys did not have a significant alveolar epithelial hyperplastic response to particle exposure ($p = 0.4$).

Inflammation. Particle-associated inflammation was not common in rats. Most foci of aggregated particle-laden macrophages did not exhibit inflammation, and only 10, 7, and 7 DE, CD, and DECD rats, respectively, exhibited this lesion. Particle-associated inflammation in rats ranged from low numbers of neutrophils in alveolar lumens and septa concomitant with aggregation of particle-laden macrophages to small foci of chronic inflammation, which involved alveoli adjacent to the pleura and were characterized by degenerating macrophages, cholesterol clefts, fibrosis, and low numbers of neutrophils or mononuclear inflammatory cells (Fig. 1B).

In monkeys, possibly particle-associated inflammation was much less common than in rats. In one DE, one CD, and one DECD monkey, the inflammation appeared to be related more to pulmonary acariasis than inhaled particles based on the presence of eosinophils. In the remaining nine cases, including one control, where some foci of inflammation appeared to be associated with particulate material, the inflammation was predominantly observed as increased septal neutrophils and less often as a few neutrophils in alveolar lumens or as chronic interstitial inflammation. Focal lesions with cholesterol clefts, fibrosis, and inflammation like those in the particle-exposed rats did not occur in the monkeys.

Particle-associated inflammation had scores of none to minimal in both species. Rats had a significantly greater inflammatory response to particles than monkeys ($p = 0.02$) with an odds ratio (95% confidence interval) of 2.8 (1.2, 6.7). Particle-exposed rats had significantly greater inflammation than C rats ($p < 0.001$), but it was not possible to estimate an odds ratio because none of the controls had inflammation. There was no significant difference in the inflammatory response due to the type of particle exposure in rats ($p = 0.5$). In monkeys, there was no significant effect of particle exposure ($p = 0.1$).

Septal fibrotic reaction. The septal fibrotic reaction in rats occurred as increased collagen within alveolar septa in

FIG. 2. Comparison of alveolar epithelial hyperplasia in rats and monkeys. (A) DE rat. Hyperplastic, cuboidal alveolar epithelium (open arrows) lining septa of alveoli containing aggregates of soot-laden macrophages. ×240. (B) DE rat. Hyperplastic, hypertrophic alveolar epithelial cells (open arrows) in an area with less aggregation of particle-laden macrophages than in 2A. ×240. Compare the amounts of particulate material and alveolar epithelial hyperplasia in these rats with the typical reaction to alveolar particulate material in monkeys illustrated in Fig. 1C. (C and D) Control monkey (C) and DECD monkey (D). Hyperplastic alveolar epithelium lining fibrotic alveolar septa near the pleura. Note the lack of active inflammation and particulate material. Both C and D, ×240. (E) CD monkey. The number of alveolar type II epithelial cells (open arrows) is slightly increased surrounding the aggregated particle-laden macrophages. Note that the degree of hyperplasia is much less than that in A or B. ×240.

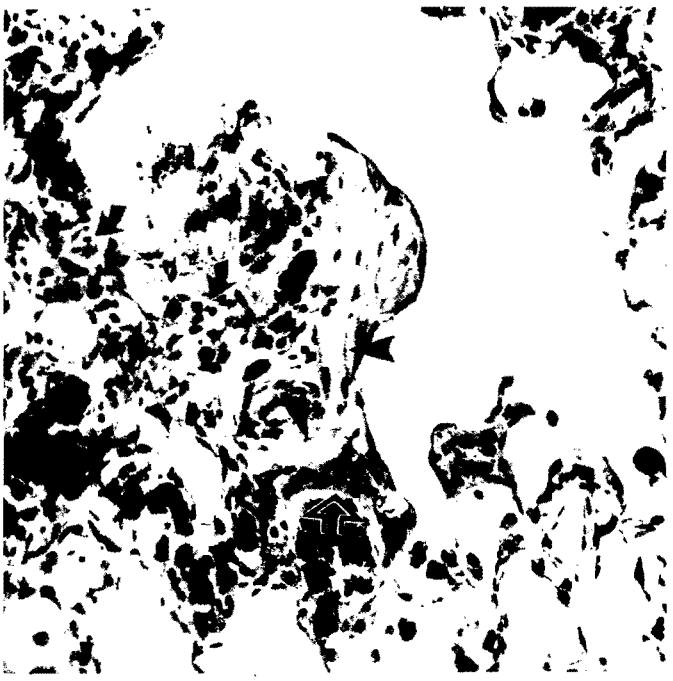

FIG. 3. Septal fibrotic reaction in a DE rat. Arrowheads indicate increased collagen. Note the concomitant aggregation of particle-laden macrophages, epithelial hyperplasia (open arrow), and inflammatory cell infiltrate (curved arrows). ×240.

foci of alveolar macrophage aggregation, alveolar epithelial hyperplasia, and particle-associated inflammation (Fig. 3). The fibrotic reaction occurred only in the presence of all three of these other lesions.

In monkeys, a septal fibrotic reaction was not associated with diesel soot or coal dust particles. In one C monkey, the reaction occurred near an area with chronic eosinophilic bronchiolitis. In the other three cases, two C and one DECD, it occurred as small scars or fibrotic sequelae of chronic inflammation of unknown etiology (Figs. 2C and 2D).

Rats had a significantly greater septal fibrotic reaction to particles than monkeys ($p = 0.006$). In rats, this reaction was significantly greater in particle-exposed than control rats ($p = 0.001$), but it was not possible to estimate an odds ratio because none of the controls had a septal fibrotic reaction. There was no significant difference in the septal fibrotic reaction due to the type of particle exposure in rats ($p = 0.15$). There was less septal fibrotic reaction in particle-exposed than control monkeys ($p = 0.02$) with an odds ratio (95% confidence interval) of 0.083 (0.008, 0.9). However, the incidence of this lesion (three controls and one exposed affected) was low.

Alveolar proteinosis. Alveolar proteinosis occurred only in a small portion of particle-exposed rats and in single foci where the combined particle-associated lesions described above were most severe. There was no significant effect of exposure to particles ($p = 0.5$) and no difference between exposure groups ($p = 0.8$) in rats. Alveolar proteinosis did not occur in the monkeys.

Site of particle retention. The relative distribution of the retained particles between the alveolar spaces and the interstitium was estimated during the histopathologic examination by scoring the amount of observed particulate material in these compartments. Low magnification (40×) was used to scan the slides and assess the amount of particulate material; higher magnification (200 to 400×) was used as needed to determine particle location. For this scoring, particles in lymphatics and those in the connective tissue beneath the pleural mesothelium were grouped with those in the other interstitial tissues of the lung.

In the rats, more of the material appeared to be in the alveolar lumens than in the interstitium (Figs. 1A and 2A). The material in the lumens was primarily within aggregated alveolar macrophages as described above. The material in the interstitium was primarily in the septa of alveoli containing aggregated macrophages (Fig. 4A). Other common sites for interstitial particles in rats were the connective tissue and lymphatics surrounding postcapillary venules and small veins in the parenchyma contiguous with foci of alveolar macrophage aggregation (Fig. 4A) and in the pleura adjacent to foci of alveolar macrophage aggregation (Fig. 4B).

In monkeys, the retained particulate material was primarily in the interstitium. Retained particles were most commonly observed in the septa of alveolar ducts (Figs. 1D and 5A), the pleura (Fig. 5B), the walls of respiratory bronchioles (Fig. 5C), and the connective tissue and lymphatics surrounding veins and arterioles in the pulmonary parenchyma (Fig. 5D). Unlike the rats, material was frequently located in interstitial tissues in the absence of particles within adjacent alveolar lumens.

Particulate material in lumens of alveolar ducts and alveoli had scores of none to mild in both rats and monkeys, and there was no significant difference between species ($p = 0.7$). In both species, particles in lumens of alveolar ducts and alveoli increased significantly due to particle exposure ($p < 0.001$), but it was not possible to estimate an odds ratio because none of the controls had alveolar lumenal particles scored, except one C monkey. There was significantly less particulate material in lumens of alveolar ducts and alveoli in DECD rats than in DE rats ($p = 0.007$) with an odds ratio of 0.12 (0.022, 0.63) and nearly significantly less in the CD rats than in the DE rats ($p = 0.0505$) with an odds ratio of 0.23 (0.05, 1.1). There was no significant difference due to the type of particle exposure in monkeys ($p = 0.4$).

Particulate material in the interstitium had scores of none to minimal in rats and none to mild in monkeys (Table 5). Particulate material in the interstitium was significantly greater in monkeys than rats ($p < 0.001$) with an odds ratio of 210 (42, 1100). In rats, there was significantly more interstitial particulate material in particle-exposed than in C rats

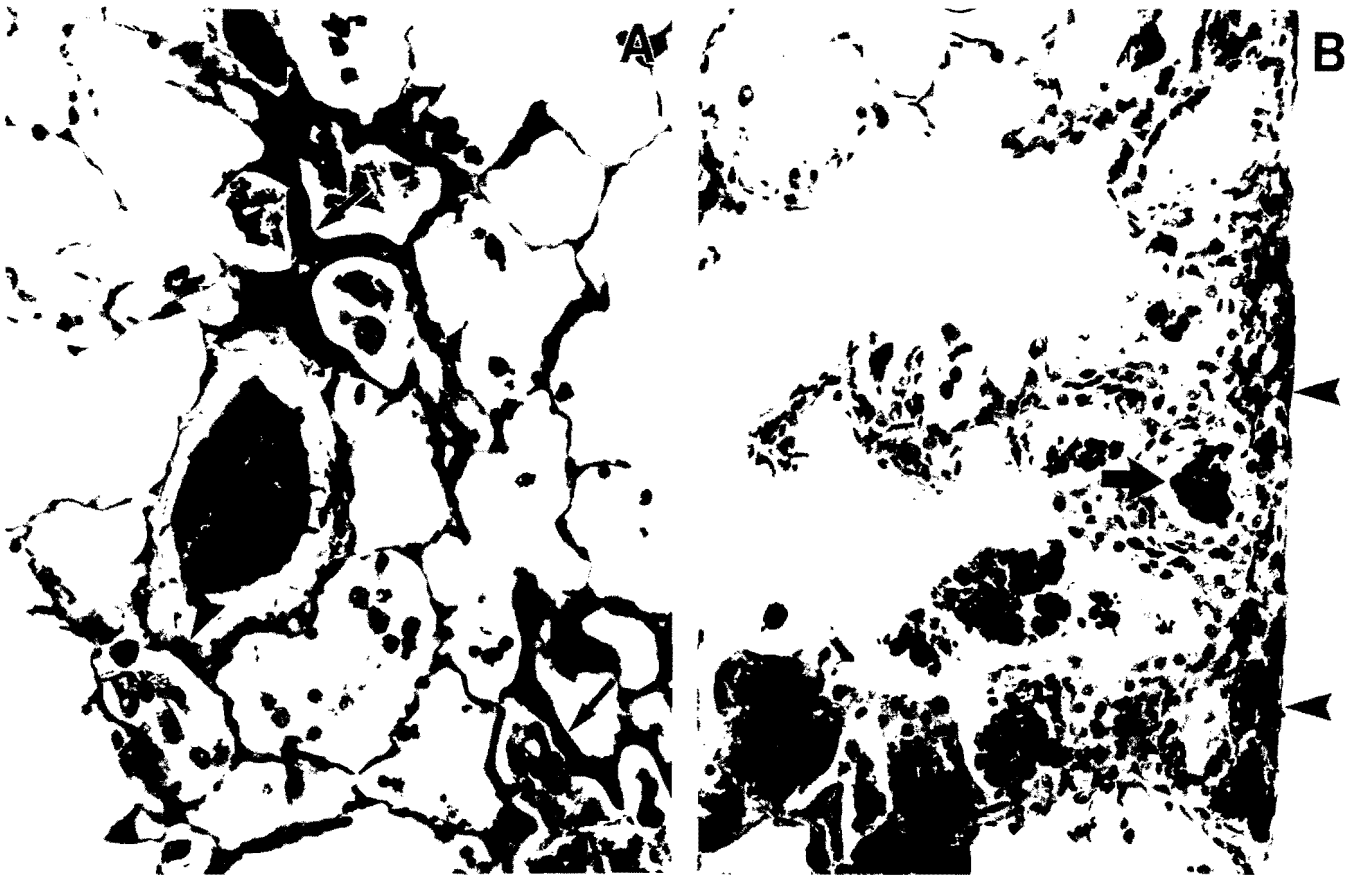

FIG. 4. Sites of particle retention and tissue responses in rats. (A) DE rat. Soot within macrophages in alveolar septa (arrows) and a small amount in the perivascular interstitium (arrowheads). In rats, particulate material was found in these interstitial compartments only in conjunction with particulate material in alveoli. ×200. (B) CD rat. Particulate material within macrophages in the pleura (arrowheads). In rats, particulate material was located in the pleura in foci where particle-laden macrophages were located in adjacent alveolar lumens (arrow). Note the locally intense alveolar epithelial hyperplasia in conjunction with the macrophage aggregation. ×240.

($p < 0.001$), but it was not possible to estimate an odds ratio because none of the controls had particles scored in the interstitium. There were no significant differences due to type of particle exposure in rats ($p = 1.0$). In monkeys, there was significantly more interstitial particulate material in particle-exposed than in C monkeys ($p < 0.001$) with an odds ratio of 84 (12, 570). There were no significant differences due to type of particle exposure in monkeys ($p = 0.3$).

Morphometry

In the rats, the mean volume fraction of alveolar parenchyma (alveolar air and alveolar septa including capillaries) was 89% of the total lung, the mean volume fraction of air was 79% of the parenchyma, and there were no differences among controls or any of the exposure groups. These values are similar to those reported for F344 rats of 82 and 85% for the volume fractions of alveolar parenchyma in the lung and air in the parenchyma, respectively (Pinkerton *et al.*, 1982). Although these results suggest that there was slightly greater sampling of the parenchyma than the hilus, and that the lungs were slightly less inflated than the lungs examined by Pinkerton and co-workers (1982), the differences were small. Because most of the particulate material is located in the parenchyma of rats, the sampling and inflation of these lung sections would tend to increase the value for the volume density of particulate material (volume of particulate material/volume of lung). However, any error would be small, and it would be uniform across all exposure groups.

In monkeys, the mean volume fraction of alveolar parenchyma was 81% of the total lung, the mean volume fraction of air was 75% of the parenchyma, and there were no differences among control or any of the exposure groups. Volume fraction of alveolar parenchyma has not been reported for cynomolgus or other macaque monkeys, but the volume fraction of alveolar parenchyma is consistently 80–90% of the total lung fixed under physiologic conditions for a large range of mammals including rats (82%), dogs (85%), pigs (86%), baboons (82%), and humans (85–90%) (reviewed


A
B
C
D
AD
RB

TABLE 5
Distribution of Histopathologic Severity Scores for Particles in the Interstitium among Groups of Rats and Monkeys

Exposure group[b]	Severity score[a]							
	Rats				Monkeys			
	0	1	2	3	0	1	2	3
C	15[c]	0	0	0	4	7	3	0
DE	0	14	1	0	0	1	14	0
CD	0	14	1	0	0	1	12	1
DECD	0	14	1	0	0	0	13	2

[a] Severity scores: 0, lesion not observed; 1, slight; 2, minimal; and 3, mild.

[b] Animals were exposed to filtered air as controls (C) or to diesel exhaust (DE), coal dust (CD), or a combination of diesel exhaust and coal dust (DECD) as described earlier. 15 rats examined in each group. 14 C, 15 DE, 14 CD, and 15 DECD monkeys were examined.

[c] Number of animals with each severity score.

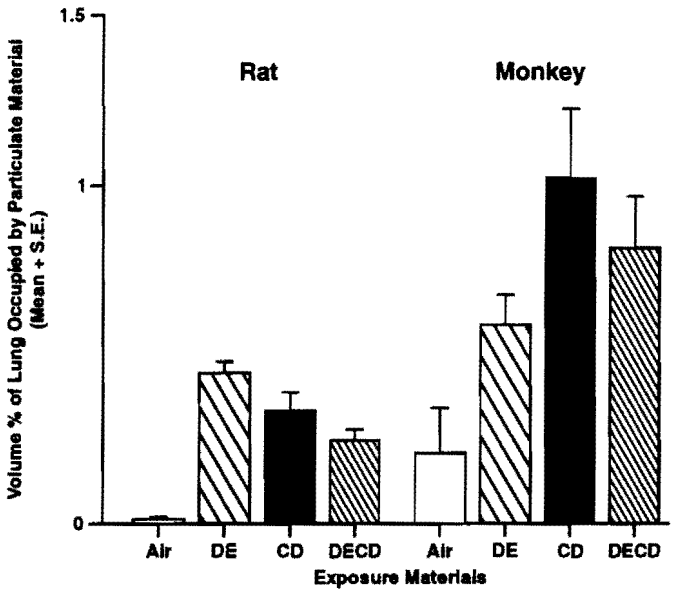

FIG. 6. Relative volume densities of particulate material in rat and monkey lungs as the mean volume percentage (+SE) for animals exposed to air, diesel exhaust (DE), coal dust (CD), or diesel exhaust and coal dust combined (DECD).

by Pinkerton *et al.*, 1992). The volume fraction of air in the parenchyma is 78% in rhesus macaques (Kapanci *et al.*, 1969). These results suggest that the sampling was representative of the total monkey lung. The inflation may have been slightly less than that of the rhesus monkeys examined by Kapanci and co-workers (1969), but that the difference was minimal. Overall, these results suggest that the values for volume density of particulate material in the monkey lungs can be accepted with confidence.

The relative volume density of particulate material was greater in the DE than in CD and DECD rats (Fig. 6). These differences in relative volume density were statistically significant between the DE and DECD rats ($p = 0.003$), but were not statistically significant between the DE and CD rats ($p = 0.065$) or the CD and DECD rats ($p = 0.16$). In the monkeys, the relative volume density of particles was less in the DE than the CD and DECD monkeys (Fig. 6). These differences in relative volume density were statistically significant between the DE and CD monkeys ($p = 0.026$), but not between the DE and DECD monkeys ($p = 0.29$), or the CD and DECD monkeys ($p = 0.19$). The relative volume density was also compared between rats and monkeys for each type of exposure after adjustment for differences between controls for each species. The CD and DECD monkeys had significantly greater relative volume densities of particulate material than the CD and DECD rats, respectively ($p < 0.001$). There was no significant difference in the relative volume density of particulate material between DE monkeys and rats ($p = 0.024$). As explained earlier, interspecies comparisons of the relative volume density of particulate material must be interpreted cautiously. However, data for parenchymal and air volume fractions suggest that sampling and fixation were adequate; if errors were introduced, they would be small and would slightly increase the values for volume density of particulate material in rat lungs.

These data correspond to the histopathologic observations that (1) the monkey lungs contained more total particulate material than the rat lungs, and (2) DE rat lungs seemed to contain more particulate material, while DE monkey lungs contained less particulate material, than the other exposure groups within each species. The particulate material in the control monkeys consisted of endogenous pigments, mite debris in some monkeys, and miscellaneous particles. The rare particulate material in control rats, which was seen at the 1280× magnification used for morphometry but not in

FIG. 5. Sites of particle retention and tissue responses in monkeys. (A) DECD monkey. Particulate material in alveolar septa (arrowheads). Contrast the scarcity of particulate material in alveolar lumens and the lack of epithelial hyperplasia or other tissue responses with the photomicrographs from rats. AD, lumen of alveolar duct. ×200. (B) DE monkey. Soot in alveolar macrophages in the pleural connective tissue and lymphatics. Note the scarcity of particulate material in adjacent alveoli and the lack of tissue reaction in contrast with 4B. ×240. (C) DECD monkey. Particulate material in the interstitium of a respiratory bronchiole (RB). Macrophages containing particulate material are aggregated at bronchiolar branching sites (arrows). ×60. (D) DE monkey. Soot in the perivascular adventitia and lymphatics surrounding a pulmonary vein. Note the lack of soot in the surrounding alveoli in contrast with Fig. 4A. ×170.

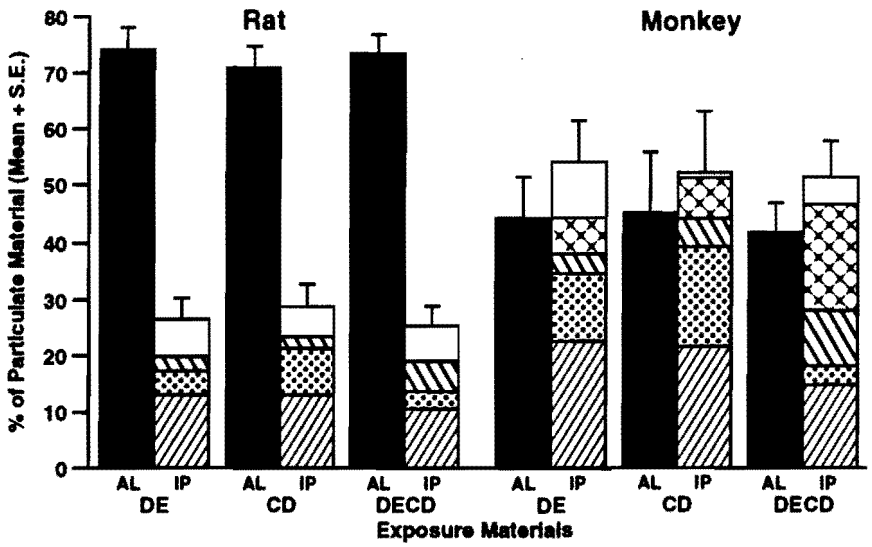

FIG. 7. Volume percentages of particulate material in lumens of alveolar ducts and alveoli (AL) versus interstitium and pleura (IP) as the mean percentages of the total particulate material (+SE) for animals exposed to diesel exhaust (DE), coal dust (CD), or diesel exhaust and coal dust combined (DECD). The interstitial compartments with the greatest portion of the retained particulate material in each species are shown. Key to interstitial compartments: ▩ alveolar septa, ⊞ pleura, ▧ perivascular interstitium, ▦ interstitium of respiratory bronchioles (monkeys only), □ other interstitial compartments.

the histopathologic examination, consisted of endogenous pigments.

Approximately 73% of the particulate material in exposed rats was in the lumens of alveolar ducts and alveoli (Fig. 7). A much lower portion, approximately 43%, of the particulate material was in the lumens of alveolar ducts and alveoli of exposed monkeys (Fig. 7). Approximately 27 and 52% of the particulate material in exposed rats and monkeys, respectively, was in the interstitium. As shown in the figure, particles in the pleural lymphatics and connective tissue were grouped with the interstitial particles for this analysis. Interspecies comparison showed a significantly greater volume percentage of the total particulate material in the lumens of alveolar ducts and alveoli in the exposed rats than in the exposed monkeys ($p < 0.001$). Conversely, a significantly greater volume percentage of the total particulate material was in the interstitium of exposed monkeys than in exposed rats ($p < 0.001$). Within each species, there were no statistical differences between DE, CD, and DECD animals for the volume percentage of the total particulate material in lumens of alveolar ducts and alveoli or for the volume percentage of the total particulate material in the interstitium. These data correspond well to the histopathologic observations of the relative distribution of the retained particles.

In the rats, the interstitial compartments with the greatest portion of the retained particulate material, in descending order of amount, were the alveolar and alveolar duct septa, the pleura, and the perivascular interstitium (Figs. 4 and 7). These compartments comprise 21% of the volume fraction of the lung and contained 21% of the particulate material in exposed rats. In monkeys, the interstitial compartments with the greatest portion of the retained particulate material, in descending order of amount, were the alveolar and alveolar duct septa, the pleura, the interstitium of respiratory bronchioles, and the perivascular interstitium (Figs. 5 and 7). These compartments comprised 27% of the volume fraction of the lung and contained 47% of the particulate material in exposed monkeys.

DISCUSSION

Both the histopathologic examination and the morphometric determination of the relative volume density of particulate material suggested that a slightly, though not significantly, greater amount of diesel soot than coal dust was retained in the rat lungs. Although it was not possible to directly compare the amount of material visible by light microscopy to the amount of material determined by chemical analyses, these results are consistent with particle type differences in the lung burden data of Lewis *et al.* (1989). They determined that the particulate content of rat lungs, expressed as a percentage of lung dry weight and corrected for analytic efficiency of recovery, was 1.13 and 0.92% for diesel soot and coal dust, respectively, at 24 months of exposure. In the monkeys, the histopathologic examination and the morphometry suggested that less diesel soot than coal dust was retained. Lewis *et al.* (1989) did not report lung burden data for the monkeys. Overall, both the histopathologic and morphometric data suggested that relatively more particulate material, regardless of type of exposure, was retained in

the monkey lungs. The difference between the DE rats and monkeys in amount of particulate material visible by light microscopy was small and not statistically significant after correcting for the particulate material in controls. The larger interspecies difference in the CD and DECD animals was statistically significant after correcting for particulate material in controls.

Both the histopathologic evaluation and the morphometry showed clear differences between the rats and monkeys in the predominant sites of particle retention. In rats, almost three-fourths of the particulate material was retained in the lumens of alveolar ducts and alveoli. In monkeys, the particulate material was almost equally divided between the lumens of alveolar ducts and alveoli, and the interstitium, but more of the material tended to be in the interstitium, and the amount in the interstitium was disproportionate to the size of the interstitial compartment. Within each species, the predominant site of particle retention did not vary by exposure material. The tendency for monkeys to retain particles in the interstitium may be related to structural features of the primate lung. For example, monkeys have respiratory bronchioles, and particulate material collected in the interstitium of the respiratory bronchioles. In primates, lymphatic vessels exist at the alveolar level adjacent to the respiratory bronchioles (Leak, 1977; Lauweryns and Baert, 1974). Blockage of these lymphatics has been proposed as the primary event in formation of coal macules in coal workers' lungs (Heppleston, 1954). In rats, which lack respiratory bronchioles, lymphatic vessels are located adjacent to the terminal bronchioles, but they do not exist at the alveolar level. The present findings, combined with the known anatomical differences and data showing that primates clear deposited particles more slowly than rats (Snipes, 1989, 1996), might suggest that a greater proportion of particles or particle-laden macrophages penetrate the airway epithelium and enter the interstitium in primates than in rats. Alternatively, during chronic exposure, particle-laden macrophages aggregated within alveoli of respiratory bronchioles may become incorporated into the interstitium as these alveoli are obliterated (Heppleston, 1989; Green and Laquer, 1980). The particles in the interstitium may be cleared more slowly than those cleared via mucociliary action. If the presence of respiratory bronchioles, the amount of interstitial and pleural tissue, and the thickness of the alveolar septa are important determinants of the sites of particle retention, the differences between rats and humans might be even greater than the differences between rats and monkeys because human lungs have more extensive interlobular septa, thicker pleura, and wider interstitial spaces in the alveolar septa than monkeys (McLaughlin *et al.*, 1966; Weibel, 1970, 1979).

The response to particles, including alveolar epithelial hyperplasia, inflammation, and focal septal fibrosis, was significantly greater in rats than monkeys. This difference was due to two factors: (1) the particles in the alveoli elicited much more of a response in the rats than monkeys, and (2) the particles in the interstitium, the retention site of over 50% of the particulate material in the monkeys, did not elicit proliferative or inflammatory responses in the monkeys at this exposure concentration and resultant lung burden. Thus, at equivalent exposure concentrations, both diesel soot and coal dust elicited less response in monkeys than in rats, despite the greater relative volume density of particulate material, especially coal dust, in the monkeys. This finding suggests that at equivalent lung burdens of coal dust, the greater response of rats than of monkeys would have been even more striking.

This is not the only case where chronic inhalation of particulate material resulted in different histopathology in rats and monkeys. Sprague–Dawley rats exposed to aerosols of petroleum coke dust developed accumulations of pigmented macrophages, chronic pulmonary inflammation, bronchiolization and adenomatous hyperplasia, sclerosis, squamous metaplasia of alveolar epithelium, and keratin cysts. Identically exposed cynomolgus monkeys accumulated coke within pulmonary macrophages, but they did not develop the other lesions (Klonne *et al.*, 1987). MacFarland and coworkers (1982) identically exposed F344 rats and cynomolgus monkeys to raw or processed shale dusts. All of the rats developed proliferative bronchiolitis and alveolitis (i.e., inflammation with epithelial hyperplasia), and most developed chronic inflammation with nonprogressive fibrosis, cholesterol clefts, and microgranulomas. The monkeys accumulated pigment-laden macrophages in the bronchiolar and alveolar walls more than in the alveolar lumens. The majority of monkeys had no reaction to the accumulated material; a minority had occasional foci of subacute inflammation. None of the monkeys developed epithelial hyperplasia or a fibrotic reaction. Squirrel monkeys and two strains of rats (Charles River-Caesarean derived and Greenacres Controlled Flora) were identically exposed to bertrandite or beryl ore by Wagner and co-workers (1969). Alveolar epithelial hyperplasia and chronic inflammation with granulomas were present in rats exposed to both materials, and the incidence of neoplasms increased in the beryl-exposed rats. No lesions other than accumulations of particle-containing macrophages and mononuclear cells around respiratory bronchioles and blood vessels were present in monkeys.

Epithelial hyperplasia concomitant with the aggregation of particle-laden macrophages in alveolar lumens is a characteristic response to many poorly soluble particles in the rat lung, both at exposure concentrations that result in lung tumors (Nikula *et al.*, 1995; NTP, 1993, 1994a,b) and at exposure concentrations below those resulting in lung tumors (Mauderly *et al.*, 1987; NTP, 1993, 1994a,b). Hyperplasia of the surrounding epithelium in response to accumu-

lation of particulate material in focal aggregates of alveolar macrophages was not characteristic of the response to diesel soot or coal dust in monkeys in this investigation, nor is it characteristic of coal workers' pneumoconiosis (Green and Laqueur, 1980; Merchant *et al.,* 1986; Kleinerman *et al.,* 1979), silicosis (Peters, 1986) or talc pneumoconiosis (Gamble, 1986) in humans.

If human lungs respond to particles more like monkey lungs than rat lungs, this investigation suggests that the pulmonary response of rats to particles may not be predictive of the response in human lungs at concentrations representing high occupational exposures. Epidemiological studies suggest that diesel exhaust may increase lung cancer risk in heavily exposed humans (reviewed in Mauderly, 1992; California EPA, 1994; Health Effects Institute, 1995), and it is assumed that diesel soot-associated organic carcinogens may be important to this response. However, it has been shown that diesel-soot-associated organic carcinogens play little role in the carcinogenicity of diesel soot in rats (Heinrich *et al.,* 1992; Nikula *et al.,* 1995). Therefore, the mechanism of carcinogenicity in rats exposed at high concentrations may differ from the potential mechanism in humans exposed at lower concentrations. The present findings suggest that perhaps the carcinogenicity data from rats exposed to high concentrations of diesel exhaust, which greatly exceed expected human exposure concentrations, should not be used to quantitatively predict responses in humans exposed at lower rates because the inflammatory and epithelial proliferative responses that seem critical to the rat response to high concentrations of particles may not occur in primate lungs exposed at environmental or occupational concentrations.

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