Recent evidence suggests that chronic exposure to high levels of ambient particulate matter (PM) is associated with decreased pulmonary function and the development of chronic airflow obstruction. To investigate the possible role of PM-induced abnormalities in the small airways in these functional changes, we examined histologic sections from the lungs of 20 women from Mexico City, a high PM locale. All subjects were lifelong residents of Mexico City, were never-smokers, had never had occupational dust exposure, and never used biomass fuel for cooking. Twenty never-smoking, non–dust-exposed subjects from Vancouver, British Columbia, Canada, a low PM region, were used as a control. By light microscopy, abnormal small airways with fibrotic walls and excess muscle, many containing visible dust, were present in the Mexico City lungs. Formal grading analysis confirmed the presence of significantly greater amounts of fibrous tissue and muscle in the walls of the airways in the Mexico City compared with the Vancouver lungs. Electron microscopic particle burden measurements on four cases from Mexico City showed that carbonaceous aggregates of ultrafine particles, aggregates likely to be combustion products, were present in the airway mucosa. We conclude that PM penetrates into and is retained in the walls of small airways, and that, even in nonsmokers, long-term exposure to high levels of ambient particulate pollutants is associated with small airway remodeling. This process may produce chronic airflow obstruction. Key words: air pollution, chronic obstructive pulmonary disease, COPD, small airways disease. Environ Health Perspect 111:714–718 (2003). doi:10.1289/ehp.6042 available via http://dx.doi.org/ [Online 19 December 2002]
The age at death (mean ± SD) was 66 ± 9 years for the subjects from Mexico City. Most of the subjects from Mexico City were homemakers. None had a history of asthma or any other chronic lung disease. The causes of death were mostly cardiac failure or complications of myocardial infarction. The mean age at death in the patients from Vancouver was 76 ± 11 years. None had a history of chronic lung disease; some had minor degrees of terminal acute pneumonia. There was a wide variety of causes of death, including complications of Alzheimer disease, myeloma, peritoneal mesothelioma in a patient who had received abdominal radiation for a seminoma, renal failure, cerebrovascular accidents, peritonitis, and bowel infarctions.

Histologic preparations. The Mexico City lung tissue generally consisted of an approximately 6-cm cube of fairly central lung containing both parenchyma and large airways. Two 2 × 1 cm histologic sections were prepared from this tissue for each case. As comparable an area of lung as possible was selected for the lungs from Vancouver, and two histologic sections were again prepared. For grading, the sections were stained with Movat’s Pentachrome stain. Because the lungs were not inflated in a standard fashion, we used a visual grading scheme (Wright et al. 1985) to evaluate airway wall fibrosis and airway wall muscle in the membranous bronchioles (MBs) and respiratory bronchioles (RBs). Using this scheme, the airways from the test slides were compared with a set of standard photographs and graded from 0 to 3, with a grade of 3 indicating severe abnormality. For the Mexico City lungs, 121 RBs and 126s MB were graded; for the Vancouver lungs, 96s RB and 81 MBs were graded. The grading was done in a blinded fashion. MBs and RBs were graded separately; the grades for all airways were then summed and expressed as a proportion of a possible maximum score for each case. Statistical analysis was conducted using the grade (fibrosis or muscle, type of airway) for each case. Because these were autopsy lungs, inflammation may reflect any number of causes, and we did not grade numbers of inflammatory cells.

Particle burden measurements. In order to determine whether PM particles penetrate into the airway walls, we randomly selected four lungs from the 50 Mexico City autopsy samples available to us for airway particle burden analysis. Again, because of random selection, two of the lungs analyzed in this study for airway particle content had been previously analyzed and reported in our study on parenchymal particle retention in Mexico City lungs (Brauer et al. 2001). For each case, airway mucosa, defined as all tissues between the cartilage and the lumen, was microdissected from mainstem, segmental, and subsegmental bronchi, as well as carinas of these airways, and in some cases from MBs. Details of the dissection procedure have been published elsewhere (Churg et al. 1999). The tissues were then dissolved in bleach and the particles were collected and examined in an analytical electron microscope as previously described (Churg et al. 1999). Particles were identified by morphology and chemical composition, and concentrations were expressed as particles per gram dry tissue.
**Results**

On histologic inspection, both the MBs and RBs in the lungs from Mexico City were abnormal, with variably increased amounts of muscle and fibrous tissue compared with the lungs from Vancouver (Figures 1 and 2). The MBs in the Mexico City lungs generally showed no increases or only minimal increases in pigmented dust in the walls, but many of the RBs contained considerable amounts of pigmented dust (Figure 2C,D). No dust or only very small amounts were seen in the RBs of Vancouver lungs (Figure 2A). Many of the airways from Mexico City also showed lumenal distortion (Figure 1C).

Because the men from Vancouver might, despite the screening process, have had more dusty occupations or be otherwise different from women, a statistical comparison of the histologic grades between the Vancouver men and women was carried out. No statistical differences were found, and thus all 20 Vancouver cases were used for comparison with the Mexico City cases.

Table 1 shows the mean grades of all cases per location for muscle and fibrous tissue by airway type. Significantly greater amounts of both components were seen in the Mexico City lungs for both MBs and RBs. The differences are presented graphically as box plots showing the value for each case in Figure 3.

The electron microscopic analysis revealed the presence of chained carbonaceous or carbon/sulfur aggregates of spherical ultrafine particles in the airway mucosa in all four cases from Mexico City. We have previously shown (Brauer et al. 2001) that these particle aggregates are similar to particle aggregates sampled from ambient air in Mexico City. Particles of this type were found in 20 of the 25 airway samples examined and in all locations including the mainstem bronchi, segmental and subsegmental bronchi, and the MBs; concentrations of particles ranged from 10 to >1,000 × 10^6/g dry tissue (Figure 4). The geometric mean size of the individual particles making up the aggregates varied from 0.040 to 0.067 µm, and of the aggregates themselves from 0.34 to 0.54 µm (Table 2). These are similar in size to the carbonaceous aggregates found in the parenchyma in the Mexico City lungs in our previous study (Brauer et al. 2001).

**Discussion**

Although there is epidemiologic evidence indicating that chronic exposure to PM is associated with chronic airflow obstruction, the anatomic basis of this effect is unknown. The traditional view of the effects of ambient PM on lung structure can be found in standard texts such as Spencer’s *Pathology of the Lung* (Spencer 1985), which states that anthracosis (i.e., black particles inhaled from the air and retained in the lung) is not associated with lung pathology. However, more recent studies suggest that, in fact, ambient PM does produce airway disease. Souza et al. (1998) evaluated forensic (violent death) autopsy lungs from 34 residents of low PM regions of Brazil and 50 residents of São Paulo, a high PM region (mean annual PM₁₀ approximately 80–100 µg/m³ between 1991 and 1995). Histories were obtained from relatives. About 90% of subjects were male. The overall mean age at death was approximately 28 years, and 61% were smokers (average pack-years about 7). Small airways were graded visually for four parameters (anthracosis, inflammation, wall thickness, and mucus hypersecretion); in addition, the gland/wall ratio in the large airways was determined. No differences were seen between high-PM and low-PM subjects in gland/wall ratio, regardless of smoking status. The other four measures were generally larger.
in smokers and somewhat larger comparing high-PM to low-PM subjects in either the smoking or nonsmoking groups. There was a clear correlation between anthracosis score, used as a surrogate of PM exposure, and either airway wall inflammation or airway wall thickness.

The findings of Souza et al. (1998) would suggest that long-term residence in high PM regions leads to chronic structural changes in the airways, changes that, in cigarette smokers, have been shown to correlate with clinical airflow obstruction (Wright et al. 1992). However, in that study more than half the subjects were smokers and more than half had occupational dust exposures. Although the statistical analysis still indicated an effect of PM levels after adjustment for smoking and occupational dust exposure, the authors’ graphical representations of their data suggest that the PM effects, apart from anthracosis, were small.

Pinkerton et al. (2000) examined the autopsy lungs of 42 Hispanic males from the Fresno County Coroner’s Office; this region of California has high PM (during the time the samples were collected, mean PM$_{10}$ levels in Fresno were 43.5 µg/m$^3$ and mean PM$_{2.5}$ levels were 22 µg/m$^3$). The median age of the subjects was 33 years, and approximately half were smokers. Most had worked in local farming operations or in blue-collar occupations. Lungs were inflated with fixative and carefully midribs dissected along two paths into the left upper lobe. Histologic samples were taken from airway generations 2, 4, 6, 9, and distal parenchyma containing terminal bronchioles and RBs.

Pinkerton et al. (2000) found histologic evidence of cigarette smoke injury (chronic bronchitis and small airways disease) in about half the subjects. Few abnormalities were noted in the larger airways, but the small airways showed both carbonaceous dust and birefringent particles in most cases. Particle loads were highest in the walls of generation 1 RBs and in MBs. The most intriguing observation was that the amount of dust (pigment) correlated with the degree of fibrosis in these airways. The study of Pinkerton et al. (2000) was very carefully designed and undertaken and was intended to show that exposure to PM caused abnormalities in the small airways, but the inclusion of a high percentage of subjects who were smokers or had occupational dust exposure (farming is known to be a very dusty process in this region) may confound the results. Smoking produces lesions similar in location and appearance [pigmentation, inflammation, and fibrosis of the walls of the membranous and to a certain extent the RB (Wright et al. 1992)] to those caused by dusts, although smoke-induced lesions tend to be more proximal in the bronchioles and dust-induced lesions more distal. Synergistic interactions between smoke and dust may amplify dust effects as indicated in animal models (Tron et al. 1987); thus, determining what effects really are chronic ambient PM effects is not straightforward.

In this study we have attempted to overcome these limitations by selecting a group of subjects who were never-smokers, did not have a history of occupational dust exposure, and, for the women from Mexico City, did not cook with biomass fuels. Cooking with biomass fuels produces very high PM exposures (Brauer et al. 1996; Smith 1993), has been associated with chronic airways disease (Dennis et al. 1996; Perez-Padilla et al. 1996), and can even produce a form of pneumoconiosis when cooking occurs in enclosed spaces.

It is possible that other confounders such as differences in exposure to environmental tobacco smoke might exist between the Vancouver and Mexico City subjects. Given that airway abnormalities in cigarette smokers are, in a broad sense, proportional to the amount of smoking, it is extremely unlikely that such exposures could produce the types of airway lesions that were observed. Nevertheless, the available data did not allow us to accurately characterize environmental tobacco smoke exposure or other potential confounders such as genetic differences, and this must be recognized as a limitation of the available data.

Because of difficulties in obtaining autopsies and interviews for our Vancouver control group, we were forced to use both men and women and to use a population that was, on average, 10 years older than the test group. Although this may be considered a limitation of our analysis, it is most likely that this age difference would decrease the ability to detect differences between the Mexico City and Vancouver groups, as dust and airway abnormalities tend to accumulate with age. An additional issue is that the autopsy lungs from the two sites were not saved in their entirety, and we could not exactly match sample sites. However, given the marked differences between the Mexico City and Vancouver lungs, it is unlikely that sampling variations, variations that are effectively random, could explain the differences observed.

PM is not the only air pollutant present in higher concentrations in Mexico City compared with Vancouver. The concentrations of ozone, nitrogen dioxide, sulfur dioxide, and carbon monoxide are also considerably higher (Brauer M. Unpublished data). Thus, one could argue that our findings might relate to a pollutant other than PM. However, because essentially identical types of airway lesions are found in workers occupationally exposed to mineral dusts in the absence of other pollutants (Wright et al. 1992), we believe that PM is the primary pollutant responsible for these effects. It is possible that synergisms between PM and other pollutants might play a role in augmenting these lesions, particularly since experimental data suggest that combinations of particles plus ozone lead to greater particle uptake by epithelial cells and greater inflammatory responses compared with particles alone (Adamson et al. 1999; Churg et al. 1996). It is also possible the other pollutants that adhere to particles may play a role in the observed differences in airway lesions.

The present results suggest, therefore, that chronic exposure to high levels of PM produces distinctly visible small airway lesions, in particular, increases in fibrous tissue, muscle, and, in the RBs, pigmented dust. The morphologic changes seen in the lungs from Mexico City are very similar to those found in cigarette smokers and in workers with high-level occupational exposure to many kinds of dusts (Wright et al. 1992), and in both of these groups, airway remodeling of this type appears to be associated with chronic airflow obstruction. Our findings thus provide an anatomic basis for the functional abnormalities detected in persons with chronic exposures to elevated PM concentrations.

In our previous analysis of bulk lung samples from our Mexico City test population, we found numerous chain aggregates of ultrahfine carbon or carbon plus sulfur particles (Brauer et al. 2001); the same particles were found in Mexico City air and are very similar in terms of morphology, size, and composition to published descriptions of diesel exhaust particles (Harrison et al. 1999). We have now shown that exactly the same particles are present in the airways walls. Because the current approach is a bulk analysis of airway tissue, we cannot determine exactly where in the airway walls these particles reside, but the light microscopic images of black pigment in the walls of the airways, particularly the RBs, suggest that they are in the connective tissue beneath the basement membrane, rather than in the epithelium. Thus, these electron microscopic data confirm for the first time that this visible pigment is PM that has entered and been retained in airway walls.

Tissue penetration appears to require high local PM levels, as we have never observed these particles in extensive electron microscopic examinations of the airways of lung samples from Vancouver residents (Churg et al. 1999). Our intention here was only to demonstrate that PM particles enter and are retained in the airway walls, and hence we did not sample a larger number of lungs nor pursue in detail the question of particle concentration by airway location and size.

Depending on particle type, entry of particles into the airway wall may be associated with a subsequent fibrogenic response. We have previously shown in a tracheal explant...
system using a model air pollution particle that this is a direct effect of the particle and does not require added inflammatory cells (Dai et al. 2002). We suggest, therefore, that PM particles are fibrogenic in individuals exposed to high levels for long periods, and that the resulting airway wall remodeling may be associated with chronic airflow obstruction.

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