Bronchiolar Inflammation and Fibrosis Associated with Smoking

A Morphologic Cross-sectional Population Analysis[1,2]

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SUMMARY The lungs of 42 smokers and 13 nonsmoking males of various ages who died suddenly and unexpectedly were examined grossly using Gough-Wentworth whole-lung sections and by microscopic planimetry to assess the severity and prevalence of emphysema. The bronchioles in representative histologic sections were evaluated for inflammation and epithelial metaplasia as well as for fibrosis and muscular hypertrophy. Postmortem interviews with next of kin established a history of cigarette smoking and excluded possible occupational exposures to toxic or particulate inhalants.

Emphysematous changes were not prominent in members of the study group, but they tended to be more severe in smokers ($p = 0.059$) and increased in severity with age ($p < 0.001$). Inflammatory changes (so-called smoker's bronchiolitis) were evident in smokers of all ages, although they were significantly less prominent in the lungs of older smokers. On the other hand, respiratory and membranous bronchiolar wall fibrosis was increasingly evident in older smokers ($p < 0.05$). Muscular hypertrophy in the bronchiolar walls was significantly greater in smokers, but a change with age was not observed.

These findings strongly suggest that bronchiolar fibrosis is associated with chronic cigarette use. These lesions occur independently of emphysema and may account for some of the subtle physiologic alterations observed in smokers. AM REV RESPIR DIS 1991; 143:144-149

Introduction

The role of cigarette smoking in the development of pulmonary fibrosis is controversial, and conclusions are based largely on radiologic evidence (1, 2). Relatively few critical, epidemiologically oriented, pathologic investigations of this question have been conducted. The postmortem study of Auerbach and coworkers (3, 4) is frequently cited. These workers retrospectively correlated the intensity and duration of cigarette smoking with fibrotic changes in the lungs occurring independently of emphysema. Similar findings were reported by Cosio and colleagues (5) in their effort to associate morphologic changes in the small airways of resected lungs with physiologic parameters of obstruction. Subsequently, a more detailed quantitative analysis of postmortem tissue by Cosio and coworkers (6) failed to demonstrate a significant increase in fibrosis of the bronchiole walls among cigarette smokers. More recently, Wright and coworkers (7) investigated the relationship of pulmonary function to small airways diseases. When FEV_1 proved greater than 80% of predicted, small airways function abnormalities were reflected in pathologic changes of the respiratory bronchioles. These included: airway wall inflammation, intraluminal inflammatory cell infiltration, and fibrosis (7). In each of these studies, inflammatory changes in the airway walls and metaplasia of the mucosa were observed in smokers, regardless of age.

Smoker's bronchiolitis, a chronic, inflammatory lesion of the respiratory bronchioles, is now recognized to be a common feature in the lungs of cigarette smokers (8). It is assumed, but not proved, that this process is a precursor of the destructive changes that occur in the bronchioles and result in centriacinar emphysema. It is reasonable to ask whether this process might also contribute to fibrosis in the walls of the respiratory bronchiole and acinus. The study reported here provides evidence to support this contention.

Critical analysis of this important question poses significant problems for the investigator. Radiologic studies do not suffice inasmuch as early lesions would either not be evident or they would be inconsistently detected, and emphysematous changes compromise the radiologic interpretation of interstitial fibrosis. Of paramount importance, however, is our inability to conduct longitudinal pathologic studies by sequentially examining lung tissue from the same person in a systematic fashion. As a result, cross-sectional pathologic studies of population groups of different ages and with different smoking backgrounds are the only feasible approach to this problem. We report such a study here. It was carried out using the lungs of smoking and nonsmoking residents of the rural state of Vermont who had no known exposure to environmental pollutants.

Methods

Tissue Collection

Over a 2-yr period, the lungs of persons autopsied on weekdays by the Vermont State Medical Examiner were removed by standard protocol. Cases occurring on weekends and holidays were not used because of the unavailability of technicians. The study subjects were of both sexes and ranged from 14 to 84 yr of age. Each had died suddenly and/or unexpectedly of an unexplained cause. The autopsy was carried out to determine cause and manner of death as dictated by state statute. Many were victims of automobile trauma or suicide, but several deaths resulted from one or a combination of acute medical problems.

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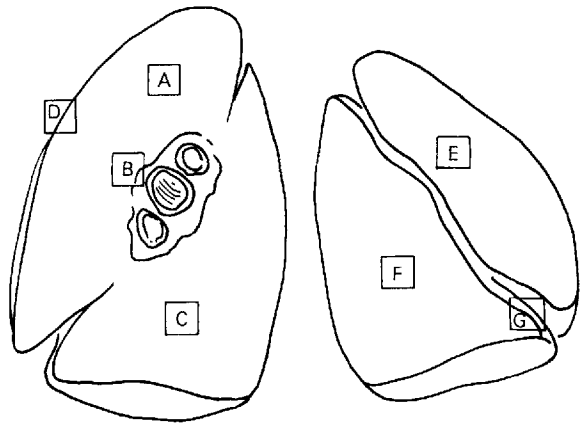


Fig. 1. Schematic diagram of the bisected left lung showing the lateral and medial segments from which tissue blocks for histologic sections were routinely prepared.

Tissue Preparation

The left lung from each of 160 subjects was prepared for morphologic studies. The tissue from several subjects was not processed because tears in the pleura and contusions made perfusion impossible. The left lung was cannulated through the primary bronchus, and 10% neutral formalin was instilled at a pressure of 25 cm H_2O for 2 h from a constant-pressure gravity infusion apparatus. The bronchus and blood vessels were then tied, and the lung was fixed in a bath of formalin for 7 days. Sections of lung approximately 1.5 cm thick were then cut. A midsection of lung was embedded in a gelatin solution, and after the gelatin had solidified, the block was frozen at -20° C. Thin sections were then prepared at a thickness of approximately 20 μm, using a rotating blade meat cutter. Individual whole lung sections were mounted on opaque paper and laminated after drying as previously described (9, 10). Specimens were excluded from analysis if the whole lung slices were incomplete or exhibited major defects.

Seven tissue blocks were prepared for histologic examination from representative preselected areas of the lateral and medial sections of the left lung (figure 1). Histologic sections 5 μm thick were stained with hematoxylin-eosin and Masson's trichrome, and by the periodic acid-Schiff reaction. The trichrome stain was used to evaluate fibrosis and the muscle in the walls of the bronchioles. The periodic acid-Schiff reaction demonstrated metaplastic goblet cells in the bronchioles.

Analysis of Tissue

This work was done without prior knowledge of the subject's background. Whole lung slices were visually evaluated independently by two of the investigators (AA and JC) using whole-lung section photographic standards prepared by Thurlbeck and coworkers (11) and the average of the two scores used in the analysis. Our scores agreed within 5 points in 87% of cases, and within 10 points in 98% of cases.

Microscopically, the membranous (MB) and respiratory (RB) bronchioles were systematically assessed by one of us (AA) for inflammatory cell infiltration of the wall and lumen (for RB). Fibrosis, muscular hypertrophy, goblet cell and squamous cell metaplasia (the latter two in the MB only) were also evaluated. These individual parameters were scored on a zero to 3 scale using standard reference pictures supplied by the University of British Columbia Pulmonary Research Laboratory (12). The subtotal pathology score for each of the upper and lower lobes and the total pathology score of the lung as a whole was taken as a sum of the scores for each of the parameters in each of the MB and RB assessed in sections of each lobe. The scores are expressed as percentages of the maximal possible score for each lobe or the whole lung, respectively.

Smoking and Environmental Exposure History

Postmortem medical and occupational histories were obtained by a trained nurse epidemiologist using a standard questionnaire. About 3 months after death, an individualized letter was sent to the next of kin, who was usually identified through the assistance of morticians, the medical examiner, or published obituaries. The letter explained the general research purpose of the study, but did not focus on respiratory problems. A home visit was requested. About 2 wk later, the next of kin was telephoned, and an interview was requested. When there was neither a telephone listing nor a response to repeated calls, a second letter was sent requesting a response using an enclosed, stamped envelope and form. If the next of kin again failed to respond, a home visit was attempted, and if the residence could not be found, inquiries were made in the town of residence of the deceased by contacting neighbors, storekeepers, the town clerk, and police in an attempt to locate the next of kin.

Attempts to establish contact with the next of kin were successful in 64.4% of the 160 subjects. Several contacts either overtly refused permission for an interview or expressed such ambivalence that an interview was not scheduled. As a result, satisfactory postmortem histories were obtained in only 58.3% of the male and 57.5% of the female subjects. When a history was not obtained, the subject was excluded from analysis even though satisfactory lung sections were available.

When permission for an interview was granted, the nurse epidemiologist visited the home and talked with the spouse or closest relative. In practice, one or more additional members of the family often participated in the interview. Written informed consent was obtained in all cases, and participants were accorded the right to refuse to answer specific questions or to participate. A number of questions regarding diet, family, and general health matters such as common respiratory symptoms were then explored. A detailed smoking, occupational, and residence history was obtained. Confirmatory questions were incorporated in the questionnaire. Finally, the reliability of the data was evaluated by the interviewer.

Complete studies were accomplished on a total of 55 males including 42 smokers (age range, 14 to 84 yr; mean, 41.4 yr), and 13 nonsmokers (age range, 18 to 65 yr; mean, 40.8 yr) (figure 2) who were documented lifetime residents of the state of Vermont. Mean ages and variance for age did not differ significantly ($p = 0.922$, $p = 0.738$, respectively) between the two groups. After evaluating the nurse epidemiologist's overall assessment of the reliability of the data on cigarette use, we chose not to consider, for the purpose of this analysis, quantitative estimates (by the next of kin) of the number of cigarettes consumed. Because a relatively small number of women were autopsied during the study period and historical information was obtained on only half, women were not included in this study.

Statistical Analysis

Statistical evaluation of microscopic information was performed using SAS (13). Analysis of covariance (ANCOVA) methods (14) were used to investigate the relationships of lung measurements with age (the covariate) and smoking (the grouping variable: smokers versus nonsmokers). Outcome variables were tested for normality and homogeneity of smoking group variances (assumptions of the ANCOVA model), using the Shapiro and Wilk test (15) and the Levene test (16), respectively. For respiratory bronchiole inflamma-

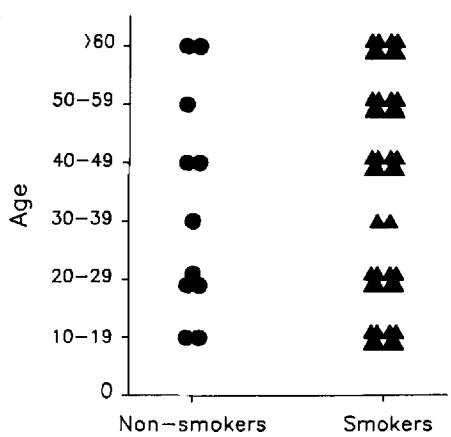


Fig. 2. Age distribution of members of the smoking and nonsmoking study groups.

tory measures, variances of smokers and nonsmokers differed significantly; however, this was corrected by a log transformation, i.e., the natural logarithm of the measurement (plus 1) was analyzed instead of the original measurement.

Results

Lung tissue from 42 male smokers and 13 male nonsmokers was available for analysis after excluding subjects who were not lifetime residents of Vermont and those for whom a complete postmortem history had not been obtained. None of the members of this study group proved to have a history of exposure to recognized environmental or occupational pollutants, although a few had worked on dairy farms. As can be seen in figure 2, members of both groups were stratified by age. Emphysematous changes in the whole lung slices of both smokers and nonsmokers usually were not striking. Analysis of the tissue by the Thurlbeck visual grading system documented a gradual increase in scores with advancing age in both groups of subjects (figure 3). However, the effect associated with smoking was overshadowed by the gradual increase in air-space size that accompanies aging in the nonsmoking population. After adjusting for age-related trends, smokers had a significantly higher emphysema score than did nonsmokers in the whole-lung section analysis (table 1).

Inflammatory changes associated with metaplasia of the MB mucosal epithelium and intraluminal macrophage accumulation were commonly observed in the lungs of smokers (figure 4A). The changes were particularly prominent in younger subjects and became less evident in members of the older age groups. MB and RB wall inflammation correlated negatively with age (figure 4B). Intraluminal macrophages were evident in the lungs of smokers of all ages, but they were seen infrequently in nonsmokers. The number tended to decrease with age, but the correlation was only marginally significant ($p = 0.074$) (figure 4C). The severity of MB and RB inflammation in the upper and lower lobes was comparable ($p = 0.588$ for MB, $p = 0.323$ for RB, using paired t tests). This observation fails to confirm earlier reports by Cosio and colleagues (6).

Fibrosis of the walls of both the MB and RB of smokers increased with age (figure 5); the mean values of the smokers and nonsmokers differed significantly. Muscular wall hypertrophy (RB and MB) did not correlate with age, but there was a significant difference between the means for smokers and nonsmokers (table 1).

In addition, structural changes of varying severity indicative of alveolar septal wall rupture with the formation of boutons were noted in the region of the RB in some older smokers. This change defied quantification. Goblet cell metaplasia, but not squamous metaplasia, was prominent in the MB of smokers.

The age-associated slopes (figure 4A and 5A) for inflammatory and fibrotic indices in the lungs for smokers tended to diverge from those of nonsmokers, but this trend did not prove to be statistically significant. This could reflect the confounding influences of individual variability and the limited statistical power of an analysis based on relatively small numbers of cases (figure 6).

TABLE 1

PULMONARY STRUCTURAL CORRELATES ON STUDY GROUP

	Age (yr)		Smoking†		
Measurement	Coefficient*	p Value	Yes	No	p Value
"Thurlbeck" (11)	0.29	< 0.001	8.73	3.87	0.059
Inflammation					
Log (RB wall)	−0.002	0.024	0.93	0.71	< 0.001
Log (RB lumen)	−0.004	0.074	0.83	0.11	< 0.001
MB	−0.008	0.009	1.92	1.44	< 0.001
Fibrosis					
RB	0.014	< 0.001	0.87	0.40	< 0.001
MB	0.005	0.046	1.08	0.68	0.002
Muscular hypertrophy					
RB	0.000	0.983	1.51	1.09	0.002
MB	−0.001	0.716	1.39	0.86	0.001
Goblet cells					
MB	0.007	0.205	1.74	0.43	< 0.001

Definition of abbreviations: RB = respiratory bronchioles; MB = membranous bronchioles.

* Change in outcome value per additional year of age (+ = increase; − = decrease).

† Mean value adjusted for age. Results of analyses for smokers and nonsmokers are combined since the coefficient for each of the two groups did not differ significantly.

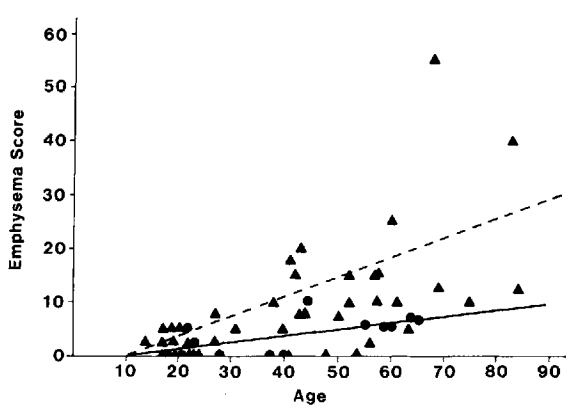


Fig. 3. Emphysema scores (Thurlbeck method [11]) by age in smokers (broken lines, closed triangles) and in nonsmokers (solid line, closed circles).

Discussion

Previous pathologic studies of the lungs of smokers have employed either surgical specimens or autopsy material from hospitalized patients or the lungs of forensic cases, generally victims of accidents and suicide in major urban areas (3–8, 17–19). In one of these studies, whole-lung sections were used exclusively (18), whereas in three others the analysis was based on lung tissue obtained at surgery (5, 7, 19).

In designing the investigation reported here, an effort was made to address many of the potential shortcomings of a cross-sectional postmortem analysis that compares groups of individuals of different ages. In doing so, we attempted to exclude environmental and occupational effects on the respiratory tract by selecting for study subjects who had no occupational exposure to potentially toxic respiratory inhalants, including dusts, and were lifelong residents of this pre-

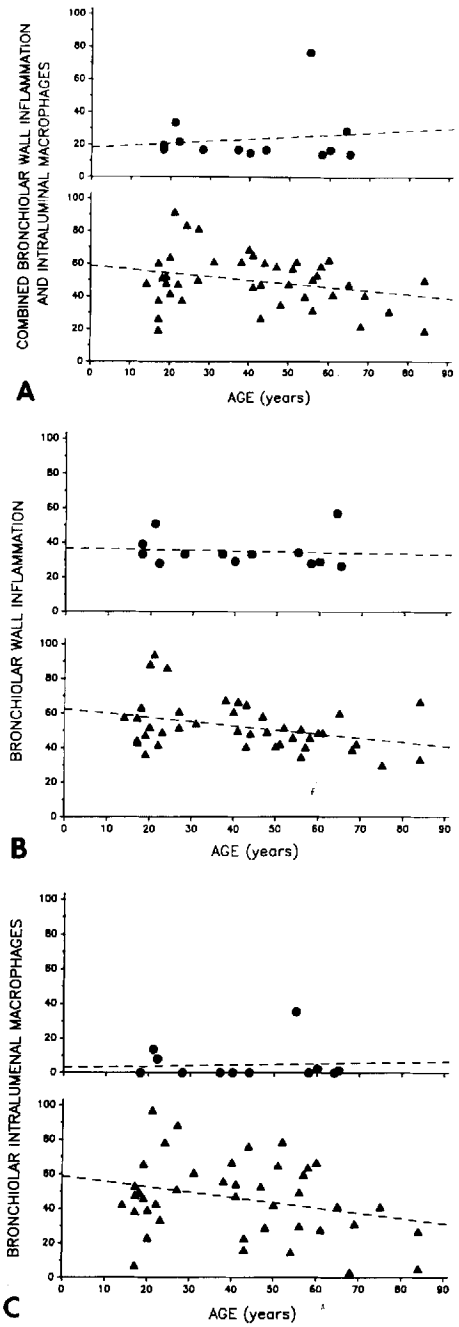


Fig. 4. Comparison of combined bronchiolar wall and intraluminal inflammatory cell infiltration (A), bronchiolar wall inflammation (B), and intraluminal macrophage accumulations (C) in the respiratory bronchioles by age in smokers (bottom panels) and in nonsmokers (top panels).

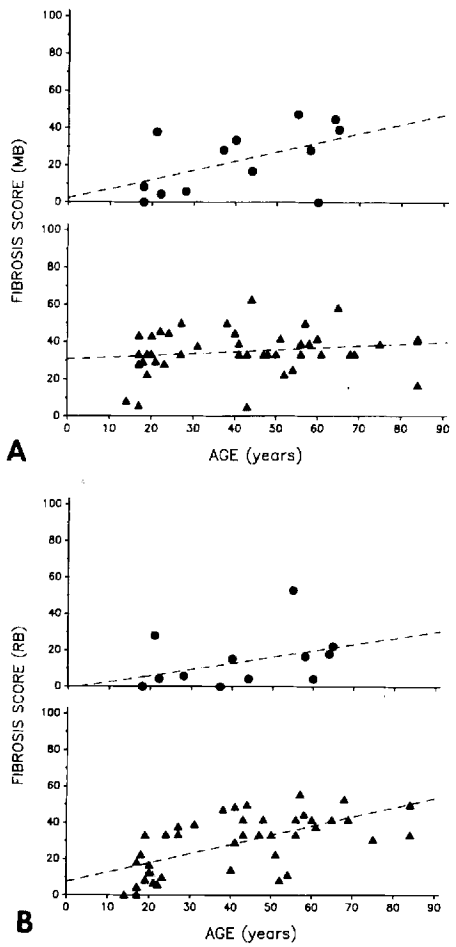


Fig. 5. Fibrosis in respiratory bronchioles (A) and membranous bronchioles (B) by age in smokers (bottom panels) and in nonsmokers (top panels).

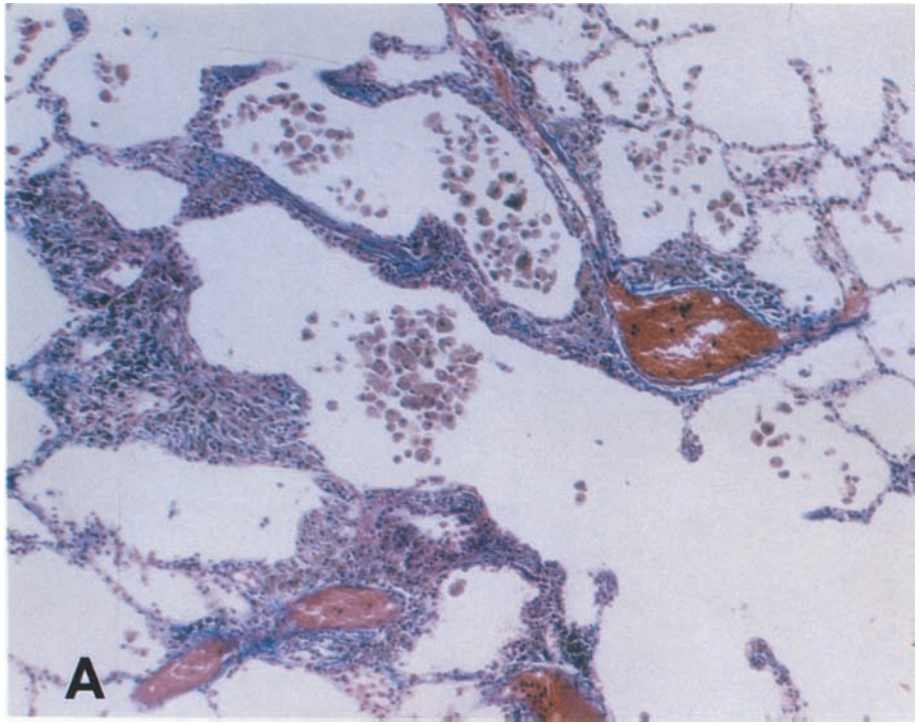
dominantly rural state. Histories on these subjects were obtained by a trained surveyor, although it was necessary to request information through secondary sources after death. Tissues from those who died suddenly and unexpectedly were used to exclude from consideration in the pathologic analysis agonal changes in the lungs resulting from illness or hospitalization. Throughout our investigation sampling of tissue was systematically carried out by investigators who had no knowledge of the smoking history of the subject.

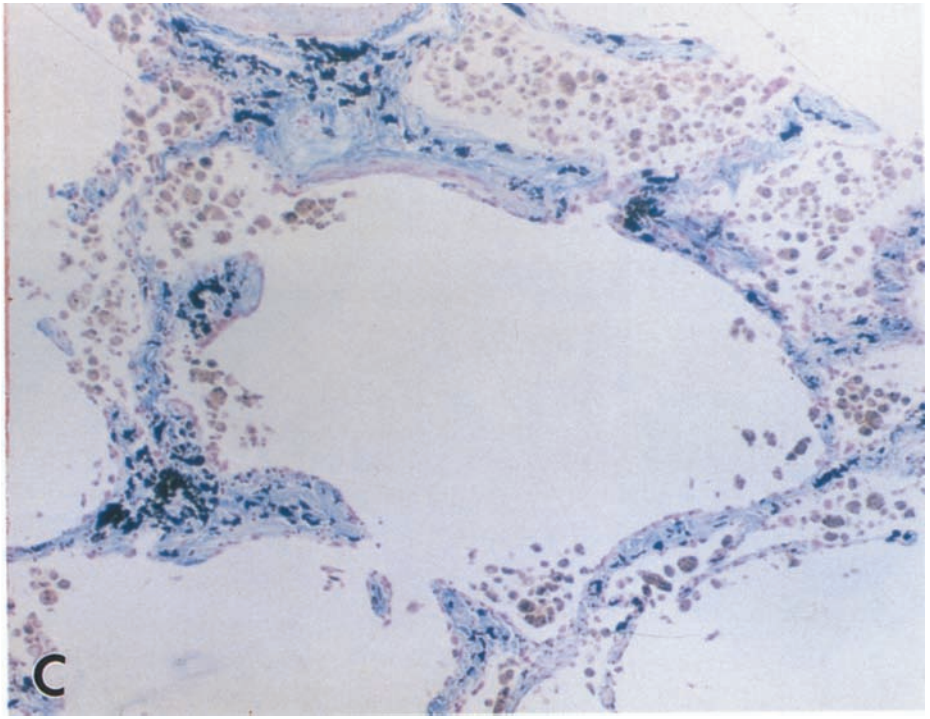
Inevitable weaknesses exist in a study of this type. Our reliance on postmortem interviews with the next of kin to obtain smoking histories precluded a critical evaluation of the duration and intensity of cigarette use since, overall, we judged the quantitative information provided by the informants to be of uncertain reliability. This limitation may account in part for the scatter in the data obtained for smokers (figures 3 and 4) although individual differences in responsivity to cigarette smoke is a likely alternate explanation. Particularly disappointing was our inability to contact many next of kin. This substantially reduced the size of the study group, but there is no evidence to suggest that it introduced bias into the evaluation. Finally, the use of forensic material precluded an evaluation of the pulmonary status of the subject prior to death. Because a substantial proportion of the subjects died of trauma, it is possible the study group is not a representative sample of the general population.

Accepting these limitations, we hypothesized that fibrosis of the walls of the respiratory bronchioles is a pathologic change associated with, and most presumably consequent to, cigarette smoking. The data reported here clearly support this conclusion. The fibrotic changes often were accompanied by subtle destructive alterations in the walls of air spaces, a microscopic feature of the early, small airways lesions of the emphysematous lung. However, the traditional changes of emphysema observed by the unaided eye usually were not found in this series of autopsy cases. The demonstration of a difference between smokers and nonsmokers in the fibrotic index of the small airways contrasts with the findings of Cosio and colleagues (6). In their report, the smokers and the control, nonsmoking group were not well matched for age, whereas in our study, the age distribution of smokers and nonsmokers proved comparable, and the study group was substantially larger.

Abundant experimental and pathologic evidence supports the notion that inflammation of the bronchioles is a major factor in the pathogenesis of chronic obstructive pulmonary disease. As was initially demonstrated by Neiwoehner and coworkers (8), inflammation proved to be a prominent feature of the bronchioles in the relatively young members of our study group. This histologic finding correlates well with the results of functional studies on young smokers by others (17, 20, 21). In addition, an increase in fibrosis of the respiratory bronchioles in smokers was associated with abnormalities of small airways function in an older population studied by Wright and colleagues (7). These studies documented the early appearance of so-called small airways disease characterized by obstructive pulmonary function and its persistence into later life. We conclude that the inflammatory response contributes to the well-recognized destructive effects of cigarette smoking in the bronchioles, as well as the fibrotic alterations that accompany this lesion. Inflammation of the bronchioles in older smokers in the study group was overshadowed by the fibrotic changes, and differences in its intensity between the younger and older groups of subjects were statistically significant.

In cross-sectional studies, fewer than half of a population of smokers exhibit-





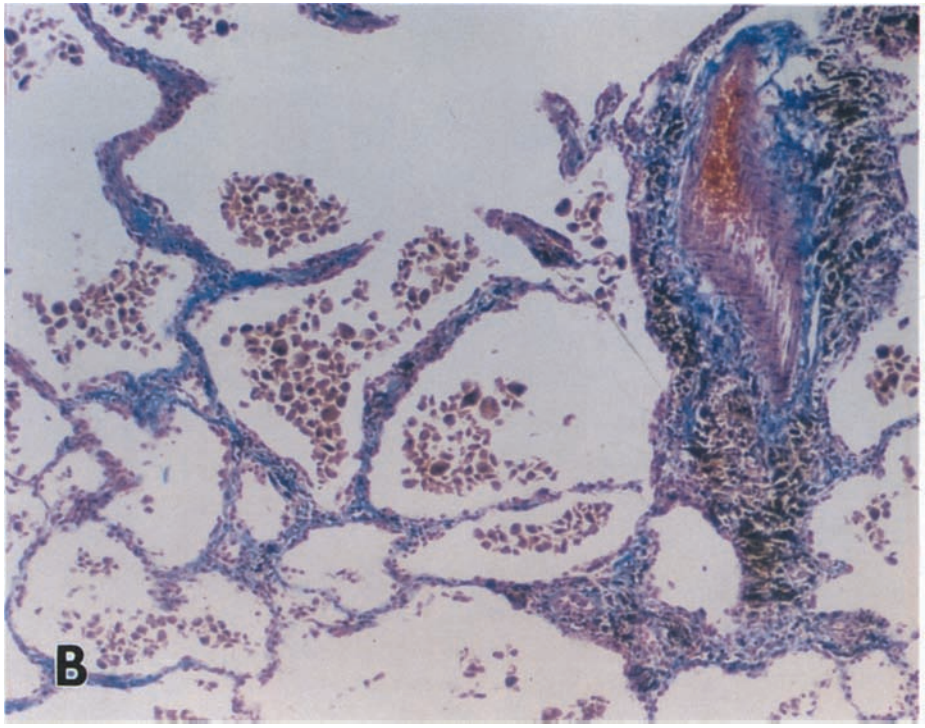


Fig. 6. Histologic sections of the lungs of a 24-yr-old smoker (A: ×250) showing prominent inflammatory mononuclear cell infiltration and intraluminal macrophages. Compare with tissue from 38-yr-old (B: ×250) and 57-yr-old (C: ×250) smokers showing increased fibrous tissue (blue stain) in the walls of the respiratory bronchioles with age. Note the presence of macrophages in air spaces in persons of all ages (Masson's Trichrome stain).

ed physiologic evidence of chronic airway obstruction (20). Could the differences in the severity of bronchiolar disease observed in our study account for variability in the population response?

Use of controlled substances was not explored in postmortem inquiries in this study. One might ask whether or not the histologic pulmonary changes could be attributable, partially or wholly, to marijuana smoking. Tashkin and coworkers (22) noted a reduction in airway conductance and resistance (primarily measures of large airways function) and Gong and colleagues (23) described squamous metaplasia in bronchial biopsies from young, habitual marijuana users. An increase in populations of alveolar macrophages in bronchoalveolar lavages from users of marijuana has been noted (24). Reports conflict as to the effects on diffusion capacity (25). In a 1987 survey of Vermont high school seniors, only 5% reported using marijuana once or twice per week (26). In view of the uncommonly low consumption of marijuana among the young people of this state and the likely major irritating effect of marijuana smoke on large airways, we consider it unlikely that this substance was a common contributing factor in the development of small airways lesions in our study population.

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References

1. Weiss W. Cigarette smoking asbestosis, and small irregular opacities. Am Rev Respir Dis 1984; 130:293–301.
2. Kilburn KH. Cigarette smoking does not produce or enhance the radiologic appearance of pulmonary fibrosis. Am J Ind Med 1981; 2:305–8.
3. Auerbach O, Stout AP, Hammond EC, Garfinkel L. Smoking habits and age in relation to pulmonary changes. N Engl J Med 1974; 269: 1045–54.
4. Auerbach O, Garfinkel L, Hammond EC. Relation of smoking and age to findings in lung parenchyma: a microscopic study. Chest 1974; 65: 29–35.
5. Cosio MG, Ghezzo H, Hogg JC, *et al.* The relations between structural changes in small airways and pulmonary function tests. N Engl J Med 1977; 298:1277–81.
6. Cosio MG, Hale KA, Niewoehner DE. Morphologic and morphometric effects of prolonged cigarette smoking on the small airways. Am Rev Respir Dis 1980; 122: 265–71.
7. Wright JL, Lawson LM, Pare PD, Kennedy S, Wiggs B, Hogg JC. The detection of small airways disease. Am Rev Respir Dis 1984; 129:989–94.
8. Niewoehner DE, Kleinerman J, Rice DB. Pathologic changes in the peripheral airways of young cigarette smokers. N Engl J Med 1974; 291:755.
9. Gough J, Wentworth JE. Thin sections of entire organs mounted on paper. Harvey Lectures. Vol 53. New York: Academic Press, 1958; 171.
10. Whimster WF. Rapid giant paper sections of lungs. Thorax 1969; 24:737–41.
11. Thurlbeck WM, Dunnill MS, Hartung W, Heard BE, Heppleston AG, Ryder RC. A comparison of three methods of measuring emphysema. Hum Pathol 1970; 1:215–6.
12. Wright JL, Cosio M, Wriggs B, Hogg JC. A morphologic grading scheme for membranous and respiratory bronchioles. Arch Pathol Lab Med 1985; 109:163–5.
13. SAS users guide. Statistics. Version 5 ed. Cary, NC: SAS Institute, 1985.
14. Dunn OJ, Clark VA. Applied statistics: analysis of variance and regression. 2nd ed. New York: John Wiley and Sons, 1987.
15. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika 1965; 52:591–611.
16. Levene H. Robust tests for equality of variance. In: Olkin I, ed. Contributions to probability and statistics. Palo Alto: Stanford University Press, 1960; 278–92.
17. Bignon J, Khoury F, Even P, Andre J, Brouet G. Morphometric study in chronic obstructive bronchopulmonary disease. Am Rev Respir Dis 1969; 5:669–95.
18. Thurlbeck WM, Ryder RC, Sternby N. A comparative study of the severity of emphysema in necropsy populations in three different countries. Am Rev Respir Dis 1974; 109:239–48.
19. Berend N, Wright JL, Thurlbeck WM, Marlin GE, Woolcock AJ. Small airways disease: reproducibility of measurements and correlation with lung function. Chest 1981; 79:263–8.
20. Fletcher G, Pet R. The natural history of chronic airflow obstruction. Br Med J 1977; 1:1645–8.
21. Spain DM, Siegel H, Bradess VA. Emphysema in apparently healthy adults. Smoking, age and sex. JAMA 1973; 224:322–5.
22. Tashkin DP, Coulson AH, Clark VA, *et al.* Respiratory symptoms and lung function in habitual heavy smokers of marijuana alone, smokers of marijuana and tobacco, smokers of tobacco alone, and nonsmokers. Am Rev Respir Dis 1987; 135: 209–16.
23. Gong H, Fligiel S, Tashkin DP, Barbers RG. Tracheobronchial changes in habitual, heavy smokers of marijuana with and without tobacco. Am Rev Respir Dis 1987; 136:142–9.
24. Barbers RG, Gong H, Tashkin DP, Oishi J, Wallace JM. Differential examination of bronchoalveolar lavage cells in tobacco cigarette and marijuana smokers. Am Rev Respir Dis 1987; 135:1271–5.
25. Tilles DS, Goldenheim PD, Johnson DC, Mendelson JH, Mello NK, Hales CA. Marijuana smoking as cause of reduction in single-breath carbon monoxide diffusing capacity. Am J Med 1986; 80:601–6.
26. Swisher JD, Gorman JS. Report to Office of Alcohol and Drug Abuse Programs, Agency of Human Services, May 1987. Washington, DC: US Government Printing Office.