THE EFFECTS OF CHRONIC CIGARETTE SMOKE EXPOSURE ON LUNG MECHANICS, BRONCHIAL REACTIVITY, AND MUCUS HYPERSECRETION

by

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The pathophysiology of chronic cigarette smoke exposure.

ABSTRACT

A comprehensive canine model of chronic tobacco smoke injury was designed to study various functional - morphological correlates of the lung.

Ten months of smoke exposure caused mucus hypersecretion and hyporesponsiveness to aerosolized but not infused methacholine. Tracheal mucus flux increased and mucus viscoelasticity decreased within 4 months of smoking. The aerosol hyporesponsiveness can most likely be attributed to mucus hypersecretion assuming that infusion reactivity more fairly represents innate airway responsiveness.

Exponential analysis of the expiratory limbs of the quasi-static P-V curves was performed in smokers and non-smokers. The data derived from the P-V curves included PL_{90}, k, and h. All three tests demonstrated a significant loss in elastic recoil with the exponential constant k being the best predictor for the ten month smokers.

Histological examination revealed a significant amount of parenchymal destruction as determined by the destructive index. There was no significant change in either the mean linear intercept (Lm) or the Reid index in the smokers.

RESUMÉ

Nous avons developpé un modèle canin avec lésions chroniques, causées par la fumée de tabac pour étudier différentes corrélations fonctionnelles et morphologiques du poumon.

Dix mois d'exposition à la fumée ont provoqué une hypersécrétion de mucus et une hyporeactivité à la méthacholine en aérosol seulement, et non en infusion.

Le flux de mucus trachéal a augmenté et la viscoélasticité du mucus a diminué durant les quatre premiers mois d'exposition.

L'hyporéactivité à l'aérosol peut être, probablement, attribuée à l'hypersécrétion de mucus, si l'on présume que la réactivité à l'infusion représente bien la réponse innée des voies aeriennes.

Une analyse exponentielle de la courbe P-V quasistatique expiratoire a été effectuée chez les fumeurs et les non-fumeurs, et les données ont été dérivées de la courbe P-V, y compris PL_{90}, k, et h.

Les trois tests ont mis en évidence une perte significative de la rétraction élastique, la constante exponentielle k étant le meilleur indice de prédiction chez les fumeurs.

L'examen histologique a révélé un degré significatif de destruction du parenchyme, déterminé par l'indice de destruction.

Il n'y avait pas de changement significatif de Lm ni de l'indice de Reid chez les fumeurs.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

INTRODUCTION

INTRODUCTION

The relationship between the use of cigarettes and a variety of medical problems has received considerable attention. Responsible scientific opinion can no longer deny that tobacco smoke is implicated in lung cancer, chronic bronchitis, emphysema, cardiovascular diseases, extrapulmonary cancers, and numerous other conditions. The dangers associated with cigarette smoking have been well documented in many reports (1-7). Numerous studies have shown that smokers are at a high risk of premature death due to coronary heart disease (8-11) and that mortality among women smokers using oral contraceptives is significntly higher (12-14). The risk of obtaining cerebrovascular disease is also increased in smokers (15-17). Evidence collected from a study of high school students demonstrated that abnormalities in pulmonary function occurred with as little as five years of smoking experience (18). Gastric ulceration occurs more frequently and has a higher mortality rate than in non-smokers (19). Smoking has also been found to be a causative factor in chronic bronchitis, with the prevalence of cough and sputum production being higher than among non-smokers (20). It is estimated that 360,000 persons die each year in the USA as a consequence of tobacco use. It has also been estimated that one's life is shortened by approximately 14 minutes for each cigarette smoked (21). Thus cigarette smoking poses one of the greatest public health problems and continues to burden society with billions of dollars in excess health costs.

In humans, the pulmonary abnormalities produced by or associated with cigarette smoking are multi-factorial. These include abnormalities

of mucus clearance, airway hyperreactivity, changes in pulmonary vasculature and loss of elastic recoil Such abnormalities are clearly defined in the late stages of the disease but provide no information as to the pathological history of the disease or the inter-relationships of the numerous changes in question. This is not surprising since the vast majority of clinical research has been conducted cross-sectionally rather than longitudinally. Furthermore, the interaction between morphology and physiological function remains unclear. The rationale of the present study was to develop a canine model of tobacco smoke injury that could allow the investigator to describe the effects of varying lengths of smoke exposure on the functional integrity of the lung. The study has also been designed to examine early changes in lung morphology and, if possible, correlate them with any changes in pulmonary function that occur.

In Chapters 1, 2, and 3, I have taken advantage of the option provided by Section 7 of the Guidelines concerning thesis preparation which states that "the candidate has the option, subject to the approval of the Department, of including as part of the thesis the text of an original paper, or papers, suitable for submission to learned journals for publication". The first chapter in the thesis is primarily concerned with the effects of cigarette smoke on bronchial reactivity. Chapter 2 deals with mucus hypersecretion, tracheal mucus clearance and the viscoelastic properties of tracheal mucus in smoking and sham-smoking beagles. Chapter 3 concerns itself with the changes seen in elastic recoil and parenchymal morphology. Of the three papers comprising this thesis I am first author of chapters two and four and co-author of

chapter three. Chapters one through three have been submitted to the American Review of Respiratory Disease for publication. Chapter one has been accepted for publication pending certain revisions. The following portion of the introduction is meant to provide the reader with a brief review of the more relevant work done in these areas of research.

Animal models may be useful to investigate the mechanisms underlying reactivity to nonspecific stimuli and to methacholine. Recent studies in the literature indicate a wide variability both in bronchial sensitivity and reactivity to various agents (22). In healthy human subjects naturally acquired upper respiratory tract infections have been known to cause a transient yet marked increase in bronchial reactivity to methacholine and histamine (23). Various hypotheses have been proposed to support the phenomenon of hyperreactivity. Among these are (a) that airway epithelial damage is related to the development of hyperreactivity and (b) that irritants may play a role in the increasing bronchial reactivity. The information so far provided explains to some degree how airways can become hyperreactive but fails to explain why exposure to irritants has brought about a decrease in bronchial reactivity in certain animal models (24,25). Abnormalities in mucus production and/or clearance may play a role in modifying the response to inhaled bronchoconstrictors.

Most smokers suffer to some degree from chronic bronchitis, which is characterized by an excessive production of mucus by the glands of the trachea and major bronchi (26). Abnormalities of both mucus clearance rate (27-35) and mucus production are the hallmarks of this condition.

Foster et al. demonstrated that while tracheal and large bronchial clearance rates were normal, peripheral mucus clearance was slower in smokers, suggesting that abnormalities of mucus clearance are more prominent in the smaller bronchi of smokers (36). Wanner et al. (34), using a face mask delivery sytem in canines, found a reduction in clearance velocity while other aspects of pulmonary function remained normal. Park et al. (35) also found a reduction of in vivo mucociliary clearance rate in dogs exposed to cigarette smoke over prolonged periods of time. These results suggest that changes in mucociliary clearance velocity are one of the earliest changes seen in smokers. Examinations of bronchiolar epithelium obtained from animals exposed to cigarette smoke (38-41) or sulfur dioxide (38,42-44) revealed changes similar to those found in humans with a history of cigarette smoking. Cosio et al. concluded that prolonged exposure to cigarette smoke could predispose individuals to the development of centrilobular emphysema and that pathologic changes in the small airways may be an important cause of airflow obstruction (45).

Elevations of bronchial reactivity have also been described in studies of human smokers, although in some animal studies in which baboons were exposed to cigarette smoke (24) and dogs to sulfur dioxide gas (25), bronchial reactivity was found to decrease. Various factors contribute to airway reactivity, as defined by the usual criteria describing the response to an inhaled bronchoconstrictive agent. It is important to state that bronchial reactivity is a relative term. Pharmacological bronchial provocation tests induce a measurable amount of

airway obstruction in both normal and abnormal subjects. Abnormal cases respond differently to a given dose (or doses) of a bronchoconstrictive agent when compared to normal subjects. The response may be exaggerated, as in asthmatics, or diminished, as in certain animal models (24,25). In addition to the inherent responsiveness of the smooth muscle, airway reactivity is influenced by the accessibility of the bronchoconstrictive agent to the target site. It was initially our rationale that chronic exposure to cigarette smoke could cause airway hyperresponsiveness by certain mechanisms involving airway epithelial damage and/or inflammatory reactions. Other investigators have suggested that damage to airway epithelium may enhance airway responsiveness to bronchoconstrictor agents (23, 46-47). Boucher et al. demonstrated that tobacco smoke injury can cause damage to the epithelial tight junction, exposing what are thought to be the afferent fibres of irritant receptors (47). However, in a model of chronic bronchitis induced by exposure to sulfur dioxide gas, bronchial reactivity was found to decrease. Drazen et al. suggested that this decrease in reactivity might be due to mucus hypersecretion (25).

The working hypothesis in the present study is that the mucus lining the airways might modify the bronchoconstrictor response. In a study similar to that of Drazen et al. (25), Boileau et al. using a lower dose of sulfur dioxide, found an increase in reactivity to aerosolized methacholine while noting that hypersecretion was not prominent (48). Recent studies have shown an increase in bronchial reactivity in human smokers (49-50). The increase in reactivity in these studies may be due to disorders of autonomic regulation, alteration in smooth muscle,

increased airway caliber, or possibly due to insufficient amount of mucus in the airways. There can be little doubt that in chronic bronchitics glands increase in both size and number and that the ratio of gland to wall thickness is increased with smoking (41,51,52).

Mucus could modify the response to bronchoconstrictors by various means. It could dilute the agent at the target site, impede its passage to the underlying epithelium or in some way inactivate it. The mucus secreted by chronic bronchitis appears to be no different than that of normal subjects from a biochemical point of view (53). Thus, the increase in mucus secretion might serve to act as a defensive mechanism whereby noxious agents are limited in their passage to the underlying epithelium. This might suggest a relationship between mucus gland hyperplasia, epithelial permeability and hyperreactivity whereby an inadequate mucosal barrier or an increase in epithelial permeability may account for the increases in bronchial reactivity found in some human studies (54).

In a study of ozone-induced airway effects, Abraham et al. found an increase in the smooth muscle responsiveness to infused carbachol but could not detect this increase in responsiveness when the sheep were challenged with aerosolized carbachol. They attributed this discrepancy to mucus hypersecretion (55). A recent study by Wallis et al. (56) found that nicotine could alter bronchomotor tone. These authors demonstrated that aerosolized nicotine produced an acute decrease in airway reactivity to methacholine. They further concluded that this decrease was independent of differences in B-adrenergic activity since B-adrenergic

blockade proved to be ineffective because reactivity was found to increase in reactive baboons. To relate the variation of airway responsiveness to methacholine to the quantity of mucus in the airway we looked at the response to infused and aerosolized methacholine. We speculated that the increased quantity of mucus secreted in the airways could blunt the response to methacholine, whereas the infused methacholine would more clearly reflect airway smooth muscle responsiveness. Thus, the main purpose in studying the effect of mucus hypersecretion on bronchial reactivity was two-fold: to study the natural history of this pathological event and to develop an animal model with an elevated yet stable rate of secretion so as to examine its effect on bronchial reactivity.

The effects of chronic exposure to tobacco smoke have been studied in dogs. Auerbach et al. (39,57) found extensive morphological changes in both parenchyma and airways. Frasca et al. (58) noted an increase in fenestration with up to four months of smoking. These alveolar fenestrae are distinguished from the pores of Kohn in that they are larger with irregular shapes and margins. Further examination by scanning electron microscopy demonstrated the destruction and enlargement of alveolar ducts with varying degrees of enlargement of alveolar spaces. Auerbach et al. reported similar findings in humans who had a lengthy history of cigarette smoking (59). Kuhn et al. demonstrated that gross distortion of the internal geometry of the lung may be present without abnormal fenestrations of the alveolar wall in a model of elastase-induced emphysema (60). Auerbach et al. described the changes observed with

emphysema as being due to a rearrangement of the internal geometry of the lung in which alveolar surface area is lost. These observations led Thurlbeck to suggest that destruction be defined qualitatively rather than quantitatively (61).

Many methods have been developed to assess the severity of emphysema. The mean linear intercept (Lm) has been the most widely accepted test in quantitating emphysema. Lm however has been found to be of questionable value in mild emphysema since increased dilatation without destruction can lead to an increased Lm unrelated to classic emphysema. Thurlbeck has shown Lm to increase in an aging lung in the absence of destruction (62). Saetta et al. proposed a new method to quantitate alveolar destruction using a point-count system. This index of destruction (DI) represents the percentage of destroyed space as a fraction of the total alveolar and duct space. DI was found to be a more sensitive and reliable index of destruction when compared to the mean linear intercept (Lm). DI was also found to correlate well with the transpulmonary pressure at 90% TLC while Lm did not. The results of their study also support the idea that in smokers, alveolar walls are first destroyed, then followed by enlargement of air spaces leading to an increase in Lm (63).

The assumption that the loss of elastic recoil in the lung is related directly to the anatomical destruction of pulmonary tissue has been challenged by Thurlbeck (61). A study correlating function with structure has indicated that the relationship between emphysema grade and loss of elastic recoil is not well defined (64). Their study indicates

that changes in elastic recoil may occur in smokers with no apparent evidence of emphysema. Berend et al. also concluded that standard measurements of chord compliance made from pressure-volume curves could be unreliable in the diagnosis of emphysema with a grade of 50 or less. The fractional uptake of CO was found to correlate best with degree of emphysema in their study (64). Thurlbeck proposed an interesting hypothesis whereby emphysema and elastic recoil are related only coincidentally and not as cause and effect. In this theory cigarette smoking could act to alter the scleroprotein framework of the lung resulting in diminished elastic recoil (61). In support of this theory are the results of Hogg et al. where they have shown a decrease in compliance of centrilobular emphysematous spaces relative to the normal surrounding tissue. Interestingly enough, the surrounding tissues had a higher compliance than the emphysematous spaces in one instance (65).

Various approaches have been used to study loss of elastic recoil. One such approach involves the use of a single exponential function. This has been used to describe the static or quasi-static pressure-volume curve of the lung (66-75). The retractive forces of the lungs have been estimated by measuring the slope of the expiratory limb of the pressure-volume curve, i.e., chord compliance, in the tidal range. Utilization of these measurements is complicated by the necessity to standardize for lung volumes and by the curvilinearity of the pressure-volume relationship. Furthermore, measurements of chord compliance involve the use of linear approximations over a comparatively small volume range.

In dealing with these difficulties Salazar and Knowles introduced an exponential mathematical expression to describe the elastic forces of the lung (68). One advantage offered by the use of an exponential function is that it can accommodate volumes over the whole inspiratory capacity without the use of FRC as a reference point. Below FRC, fitted P-V data deviate from the exponential function. This could be due to airway closure (69) or due to compression of the esophageal balloon by the surrounding structures (76,77). Constants derived from the exponential fits have been used to assess the mechanical properties of the lungs (78). The results of Colebatch et al. indicate that if the exponential is fitted from 50% TLC then the variation in the constants for individuals is less than the variation found with duplicate trials (75). Values for the transpulmonary pressure at 90% TLC (PL90) can be derived from the computer-plotted curves, in addition to the bulk elastic constant k, and the half-inflation pressure h. The half-inflation pressure h can be regarded as an index of the stiffness of the lung or of the steepness of the slope.

Paré and co-workers found the bulk elastic constant k to be the best predictor of emphysema in individuals, although it failed to distinguish patients with mild emphysema from those without (79). Silvers et al., using a single exponential function after the method of Salazar and Knowles (68), found no significant difference between two groups of emphysematous patients in which one group had a grade of five or less and the other group a grade of more than five (80). The Silvers group found a significant difference in elastic recoil between patients

with mild emphysema and normals when they expressed the static lung recoil as a percentage of predicted lung volume. Linear correlations made between emphysema grade and PL90 yielded a significant negative correlation indicating a close relation between anatomical emphysema and elastic recoil (80).

Recent studies in aging mice demonstrate a decrease in lung elastin content with age (81). If such findings can be extrapolated to humans, then the decrease in elastin could account in part for the loss of elastic recoil pressure. A study of elastic fibers in normal human lungs and lungs with mild emphysema demonstrated no significant difference in total fiber length or diameter (82). These results indicate that the loss of elastic recoil is not simply due to a decrease in elastic fibers but may be due to changes in scleroproteins or due to a rearrangement in the internal geometry of the lung (61). Thus, it can be seen readily that loss of elastic recoil may involve numerous mechanisms and not simply the anatomical destruction of parenchymal tissue.

REFERENCES

1. Report. Smoking and Health: Report of the Advisory Committee to the Surgeon General of the Public Health Service. Public Health Service Publication No. 1103, U.S. Government Printing Office, Washington, D.C., 1964.

2. Report. The Health Consequences of Smoking: A Public Health Service Review. Public Health Service Publcation No. 1696, U.S. Government Printing Office, Washing, D.C., 1967.

3. Report. Smoking or Health: A Report of the Surgeon General. Washington, D.C., Department of Health, Education and Welfare, 1979.

4. Report. Smoking or Health: A Report of the Royal College of Physicians. London, Pitman Medical, 1977.

5. Report. The Health Consequences of Smoking for Women. A Report of the Surgeon General. Washington, D.C., Department of Health, Education, and Welfare, 1980.

6. Report. The Changing Cigarette: A Report of the Surgeon General. Washington, D.C., Department of Health and Human Services, 1981.

7. Report. The Health Consequences of Smoking-Cancer: A Report of the Surgeon General. Washington, D.C., Department of Health and Human Services, 1982.

8. Levine PN. An acute effect of cigarette smoking on platelet function. A possible link between smoking and arterial thrombosis. Circulation 1973; 48:619-623.

9. Frachi G, Capocaccia R, Verdecchia A, Menoti A, Keys A. Risk factors, changes and coronary heart disease in an observational study. Int J Epidemiol 1981; 10(1):31-40.

10. Friedman GD, Petitti DB, Bawol RD, Siegelaub AB. Mortality in cigarette smokers and quitters. Effects of base-line differences. New Engl J Med 1981; 304(23):1407-1410.

11. Fuller JH, McCartney P, Jarrett RJ, Keen H, Rose G, Shipley MJ, Hamilton PJ. Hyperglycaemia and coronary heart disease: The Whitehall study. J Chronic Dis 1979; 32(11-12):721-728.

12. Rosenberg L, Hennekens CH, Rosner B, Belanger C, Thoman KJ, Speizer FE. Oral contraceptive use in relation to nonfatal myocardial infarction. Am J Epidemiol 1980; 111(1):59-66.

13. Stadel BV. Oral contraceptives and cardiovascular disease. New Engl J Med 1981; 305(11);612-618.

14. Willett WC, Hennekens CH, Bain C, Rosner B, Speizer FE. Cigarette smoking and non-fatal myocardial infarction in women. Am J Epidemiol 1981; 113(5):575-582.

15. Wolf PA, Dawber TR, Kannel WB. Heart disease as a precursor of stroke. Adv Neurol 1978; 19:567-577.

16. Bell BA, Symon L. In: Greenhalgh RM, ed. Smoking and arterial disease. Bath Pitman Press, 1981, pp. 4-8.

17. Bell BA, Symon L. Brit Med J 1979; 1:577.

18. Seely JE, Zuskin E, Bouhuys A. Cigarette smoking: objective evidence for lung damage in teenagers. Science, Wash., 1971; 172:741-743.

19. Konturek SJ, Solomon TE, McCreight WG, Johnson LR, Jacobson ED. Effects of nicotine on gastrointestinal secretions. Gastroenterology 1971; 60:1098-1105.

20. Wanner A. Clinical aspects of mucociliary transport. Am Rev Respir Dis 1977; 116:73-125.

21. Ochsner A. The health menace of tobacco. Am Scient 1971; 59:246-252.

22. Habib MP, Paré PD, Engel LA. Variability of airway responses to inhaled histamine in normal subjects. J Appl Physiol 1979; 47:51-58.

23. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. Am Rev Respir Dis 1976; 113:131-139.

24. Roehrs JD, Rogers WR, Johanson WG Jr. Bronchial reactivity to inhaled methacholine in cigarette-smoking balloons. J Appl Physiol 1981; 50:754-760.

25. Drazen JM, O'Cain CF, Ingram RH Jr. Experimental induction of chronic bronchitis in dogs: Effects on airway obstruction and responsiveness. Am Rev Respir Dis 1982; 126:75-79.

26. Ebert RV, Terracio MJ. The bronchiolar epithelium in cigarette smokers. Am Rev Respir Dis 1975; 111:4-11.

27. Kensler CJ, Battista SP. Chemical and physical factors affecting mammalian ciliary activity. Am Rev Respir Dis 1966; 93:93-102.

28. Kaminski EJ, Fancher DE, Calandra JC. In vivo studies of the ciliostatic effects of tobacco smoke: Absorption of ciliostatic components by wet surfaces. Arch Environ Health 1968; 16:188-193.

29. Albert RE, Lippman M, Briscoe W. The characteristics of bronchial clearance in humans and the effects of cigarette smoking. Arch Environ Health 1969; 18:738-755.

30. Albert RE, Spiegelmann JR, Shatsky S, Lippmann M. The effect of acute exposure to cigarette smoking on bronchial clearance in the miniature donkey. Arch Environ Health 1969; 18:30-41.

31. Albert RE, Alessandro D, Lippmann M, Berger J. Long-term smoking in the donkey: Effect on tracheobronchial particle clearance. Arch Environ Health 1971; 22:12-19.

32. Sanchis J, Dolovich M, Chalmers R, Newhouse M. Quantitation of regional aerosol clearance in the normal human lung. J Appl Physiol 1972; 33:757-762.

33. Pavia D, Thomson ML, Pocock SJ. Evidence for temporary slowing of mucociliary clearance in the lung caused by tobacco smoking. Nature 1971; 231:325-326.

34. Wanner A, Hirsch JA, Greeneltch DE. Tracheal mucous velocity in beagles after chronic exposure to cigarette smoke. Arch Environ Health 1973; 27:370-371.

35. Iravani J, Melville GN. Long-term effect of cigarette smoking on mucociliary function in animals. Respiration 1974; 31:350-357.

36. Foster MW, Langenback EG, Bergofsky EH. Dissociation in the mucociliary function of central and peripheral airways of asymptomatic smokers. Am Rev Respir Dis 1985; 132:633-639.

37. Park SS, Kikkawa Y, Goldring IP, Daly MM, Zelefsky M, Shim C, Spierer M, Morita T. An animal model of cigarette smoking in beagle dogs. Am Rev Respir Dis 1977; 115:971-979.

38. Mawdesley-Thomas LE, Healey P, Barry DH. Experimental bronchitis in animals due to sulfur dioxide and cigarette smoke. An automated quantitative study. In: Walton HW. Inhaled particles III. Gresham Press, Old Woking, Surrey, England 1971, pp 509-525.

39. Auerbach O, Hammond ED, Kirman D, Garfinkel L, Stout AP. Histologic changes in bronchial tubes of cigarette-smoking dogs. Cancer 1967; 20:2055-2066.

40. Lamb D, Reid L. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. Brit Med J 1969; 1:33-35.

41. Hayaski M, Sornberger CG, Huber GL. Morphometric analyses of tracheal gland secretion and hypertrophy in male and female rats after experimental exposure to tobacco smoke. Am Rev Respir Dis 1979; 119:67-73.

42. Reid L. An experimental study of hypersecretion of mucus in the bronchial tree. Brit J Exper Pathol 1963; 437-445.

43. Lamb D, Reid L. Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulfur dioxide. J Pathol Bact 1968; 96:97-111.

44 Angus E, Amyot R, Martin R Morphological changes in beagle dogs following exposures to SO_2 Clin Res 1976; 24:689A (abstract).

45 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.

46. Simani IAS, Inoue S, Hogg JC. Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. Lab Invest 1974; 31:75-87.

47. Boucher RC, Johnson J, Inoue S, Hulbert W, Hogg JC. Effect of cigarette smoke on the permeability of guinea pig airways. Lab Invest 1980; 43:94-100.

48. Boileau R, Delaunois L, Tremblay J, Gauthier J, Martin RR. Enhancement of bronchial responsiveness to methacholine during chronic SO_2 exposure. Fed Proc 1979; 38:1110 (abstract).

49. Buczko GB, Day A, Vanderdoelen JL, Boucher R, Zamel N. Effect of cigarette smoking and short-term smoking cessation on airway responsiveness to inhaled methacholine. Am Rev Respir Dis 1984; 129:12-14.

50. Malo JL, Filiatrault S, Martin RR. Bronchial responsiveness to inhaled methacholine in young asymptomatic smokers. J Appl Physiol 1982; 52:1464-1470.

51. Jeffery PK, Reid LM. The effect of tobacco smoke, with or without phenylmethyloxadiazole (PMO), on rat bronchial epithelium: A light and electron microscopic study. J Pathol 1981; 133:341-359.

52. Reid L. Measurement of the bronchial mucous gland layer; a diagnostic yardstick in chronic bronchitis. Thorax 1960; 15:132.

53. Reid L. Mucus secretion and chronic bronchitis. Med Thorac 1967; 24:40-43.

54. Gerrard JW, Cockcroft DW, Mink JT, Cotton DJ, Poonawala R, Dosman JA. Increased non-specific bronchial reactivity in cigarette smokers with normal lung function. Am Rev Respr Dis 1980; 122:577-581.

55. Abraham W, Champman G, Marchette B. Differences between inhaled and intravenous carbachol in detecting O_3-induced airway effects Environ Res 1984; 35:430-438.

56. Wallis TW, Walter RR, Johanson WG Jr. Effects of acute and chronic exposure to nicotine aerosol on bronchial reactivity to inhaled methacholine. J Appl Physiol 1982; 52:1071-1076.

57. Auerbach O, Hammond ED, Kirman D, Garfinkel L. Emphysema produced in dogs by cigarette smoking. J Am Med Assoc 1967; 199:89-94.

58. Frasca JM, Auerbach O, Carter HW, Parks VR. Morphologic alterations induced by short-term cigarette smoking. Am J Pathol 1983; 111:11-20.

59. Auerbach O, Stout AP, Hammond EC, Garfinkel L. Smoking habits and age in relation to pulmonary changes: Rupture of alveolar septums, fibrosis and thickening of walls of small arteries and arterioles. New Engl J Med 1963; 269:1045-1054.

60. Kuhn C, Tavassoli F. The scanning electron microscopy of elastase-induced emphysema: A comparison with emphysema in man. Lab Invest 1976; 34:2-9.

61. Thurlbeck WM. Aspects of chronic airflow obstruction. Chest 1977; 72:341-349.

62. Thurlbeck WM. The internal surface area of the nonemphysematous lungs. Am Rev Respir Dis 1967; 95:765-773.

63. Saetta M, Shiner RJ, Angus GE, Kim WD, Wang NS, King M, Ghezzo H, Cosio MG. Destructive Index: A measurement of lung parenchymal destruction in smokers. Am Rev Respir Dis 1985; 131:764-769.

64. Berend N, Woolcock AJ, Marlin GE. Correlation between the function and structure of the lung in smokers. Am Rev Respir Dis 1979; 119:695-705.

65. Hogg JC, Nepszy SJ, Macklem PT. Elastic properties of the centrilobular emphysematous space. J Clin Invest 1969; 48:1306-1312.

66. Milic-Emili J, Henderson JAM, Dolovich MB, Trop D, Kaneko K. Regional distribution of inspired gas in the lungs. J Appl Physiol 1966; 21:749-759.

67. Pengelly LD. Curve fitting analysis of pressure-volume characteristics of the lungs. J Appl Physiol 1977; 42:111-116.

68. Salazar E, Knowles JH. An analysis of pressure-volume characteristics of the lungs. J Appl Physiol 1964; 19:97-104.

69. Sutherland PW, Katsura T, Milic-Emili J. Previous volume history of the lungs and regional distribution of gas. J Appl Physiol 1968; 25:566-574.

70. Knudson RJ, Kaltenborn WT. Evaluation of lung elastic recoil by exponential curve analysis. Respiration Physiol 1981; 46:29-42.

71. Gibson GJ, Pride NB, Davis J, Schroter RC. Exponential description of the static pressure-volume curve of normal and diseased lungs. Am Rev Respir Dis 1979; 120:799-811.

72. Greaves IA, Colebatch HJH. Elastic behavior and structure of normal and emphysematous lungs postmortem. Am Rev Respir Dis 1980; 121:127-135.

73. Colebatch HJH, Greaves IA, Ng CKY. Exponential analysis of elastic recoil and aging in healthy males and females. J Appl Physiol Respirat 1979; 47:683-691.

74. Berend N, Skoog C, Thurlbeck WM. Exponential analysis of lobar pressure-volume characteristics. Thorax 1981; 36:452-455.

75. Colebatch HJH, Ng CKY, Nikov N. Use of an exponential function for elastic recoil. J Appl Physiol 1979; 46:387-393.

76. Milic-Emili J, Mead J, Turner JM. Topography of esophageal pressure as a function of posture in man. J Appl Physiol 1964; 19:212-216.

77. Milic-Emili J, Mead J, Turner JM, Glauser EM. Improved technique for estimating pleural pressure from esophageal balloons. J Appl Physiol 1964; 19:207-211.

78. Glaister DG, Schroter RC, Sudlow MF, Milic-Emili J. Bulk elastic properties of excised lungs and the effect of a transpulmonary pressure gradient. Respir Physiol 1973; 17:347-364.

79. Pare PD, Brooks LA, Bates J, Lawson LM, Nelems JMB, Wright JL, Hogg JC. Exponential analysis of the lung pressure-volume curve as a predictor of pulmonary emphysema. Am Rev Respir Dis 1982; 126:54-61.

80. Silvers GW, Petty TL, Stanford RE. Elastic recoil changes in early emphysema. Thorax 1980; 35:490-495.

81. Ranga V, Kleinerman J, Sorensen J. Age-related changes in elastic fibers and elastin of lung. Am Rev Respir Dis 1979; 119:369-376.

82. Niewoehner DE, Kleinerman JR. Morphometric study of elastic fibers in normal and emphysematous human lungs. Am Rev Respir Dis 1977; 115:15-21.

Chapter 2

HYPORESPONSIVENESS TO AEROSOLIZED BUT NOT INFUSED METHACHOLINE IN CIGARETTE-SMOKE-EXPOSED DOGS

ABSTRACT

Nine beagles were exposed via a tracheostomy to smoke from ten cigarettes/day, five days/week for six to ten months; five dogs served as sham-exposed controls. Mucus was collected 2x/week prior to and during the exposure period by resting a cytology brush on the lower trachea for 2-5 minutes. At least once prior to and 3 x during exposure, transpulmonary pressure and flow were monitored under anesthesia to determine resistance R_L. Two airway responses to methacholine were determined: the infusion response Ri, the increase in R_L 4-6 minutes post infusion of 4 mcg/kg/min., and the aerosol response Ra, the increase in R_L at 2 min. Eight of 9 smoking dogs developed persistent mucus hypersecretion. In 5 dogs, tracheal mucus flux increased 5-10 fold; in 3 dogs, the increase was 2-3x control. One of the 5 sham-exposed dogs developed moderate hypersecretion. In smokers (N=6), Ri increased to 3.14x initial value ($\pm$ 1.15 SE); in shams (N=4), Ri increased to 1.36x control $\pm$ 0.49. Neither change was significant. By contrast, Ra at the highest common dose decreased significantly in smokers, to 0.213x control ($\pm$ 0.032 SE, $p < 0.001$); Ra in shams did not change significantly (1.17x $\pm$ 0.47). The difference between aerosol and infusion response change, is an index of methacholine aerosol hyporesponsiveness. For smoking dogs, Rai was -1.92 $\pm$ 0.68 (SE)($p < 0.05$), while for sham-exposed dogs Rai was 0.15 $\pm$ 0.23 (SE)(N=4). Assuming that infusion reactivity more fairly represents innate airway response, the aerosol hyporesponsiveness in smoking dogs can most likely be attributed to the mucus hypersecretion.

Key words: mucus, airway reactivity, smoking, permeability.

INTRODUCTION

Mucus hypersecretion is a well known feature of the chronic bronchitis associated with cigarette smokers and abnormalities of mucus production and clearance are one of the earliest symptoms seen. It is known that long-term exposure of experimental animals to sulfur dioxide (1-4) or tobacco smoke (3,5,6,7) leads to changes in airway morphology similar to those seen in chronic bronchitis; these changes include airway inflammation, damage to ciliated cells, enlargement of mucus glands, and proliferation of goblet cells. It has also been shown that long-term exposure to whole cigarette smoke leads to a reduction of in vivo mucociliary clearance rates in dogs (8,9).

Elevations of bronchial reactivity have also been described in studies of human smokers, although in some animal models of smoking a decrease in reactivity has been reported (10). A number of factors contribute to airway reactivity, as defined by the usual criteria describing the response to an inhaled bronchoconstrictor aerosol. In addition to the inherent responsiveness of the smooth muscle, airway reactivity is influenced by the distribution of the aerosol within the lung and by factors limiting the accessibility of the bronchoconstrictive agent to the target site once it has been deposited in the airway.

In a model of chronic bronchitis induced by chronic exposure to sulfur dioxide gas, Drazen and co-workers demonstrated a decrease in the airway responsiveness to histamine in dogs with clinical mucous hypersecretion (11). They suggested that this might be attributable to the apparent increase in mucus production in these dogs. In the work of

Martin and co-workers, using the same dog model but with a lower dose of SO_2, there was an increased reactivity to aerosolized methacholine (12) but hypersecretion in this case was not very prominent, certainly not in the early stages when the airway hyperreactivity became apparent (13).

Several studies have shown an increase in bronchial reactivity in human smokers (14,15). Although the mechanisms responsible for hyperreactivity remain unknown, four explanations have been postulated to account for this: (1) epithelial damage leading to increased permeability; (2) disorders of autonomic regulation; (3) increased baseline airway caliber; and (4) alteration in smooth muscle responsiveness. Recent studies have suggested that the increase in airway epithelial permeability is due to alterations in the intercellular tight junctions after cigarette smoke exposure (16,17). Hulbert and co-workers (18) demonstrated an increase in permeability in guinea pigs exposed to cigarette smoke and that such changes in permeability were reversible.

Such observations concerning the airway responsiveness to chronic cigarette smoke exposure, mucus hypersecretion and the increase in permeability suggested a possible experimental approach to the relationships between the above factors. In this study we considered the possibility that the mucus lining the airway might modify the bronchoconstrictor response. Mucus could potentially play this modifier role by diluting the pharmaco-active agent or by delaying its passage to the underlying epithelium. These factors could cause a rightward shift in the classical dose response curve. If sufficient mucus was present to

alter baseline airway caliber, it could also alter the aerosol deposition pattern, and hence the response.

To relate the variation of airway responsiveness to methacholine to the quantity of mucus in the airway we chose to compare the response to infused and aeorosolized methacholine chloride. Our hypothesis was that the response to infused methacholine would more clearly reflect airway smooth muscle responsiveness, and that any blunting of the response to aerosolized methacholine would then be related to the quantity of mucus in the airways. The infused and aerosolized doses were chosen to provide equivalent mean changes in total pulmonary resistance prior to the smoking stages of the study. Since cigarette smoke has been found to increase mucosal permeability and that such a mechanism might be of importance with regard to hyperreactivity, all dogs ceased to smoke for three to five days prior to each methacholine challenge. The present study was therefore designed to examine the effects of mucus hypersecretion on bronchial reactivity while allowing for epithelial permeability to return towards normal by allowing for an appropriate period of cessation of smoking.

MATERIAL AND METHODS

Fourteen normal beagles weighing 8 to 16 kg were chosen for the study. Each dog was surgically prepared with a chronic tracheostomy and then left for at least three weeks to recover. None of the dogs had any indication of any respiratory infection throughout the duration of the study. A number of physiological studies were performed to establish control values with regard to lung mechanics and airway responses during a four month initialization period. Cigarette smoke exposure began immediately after this phase of the study.

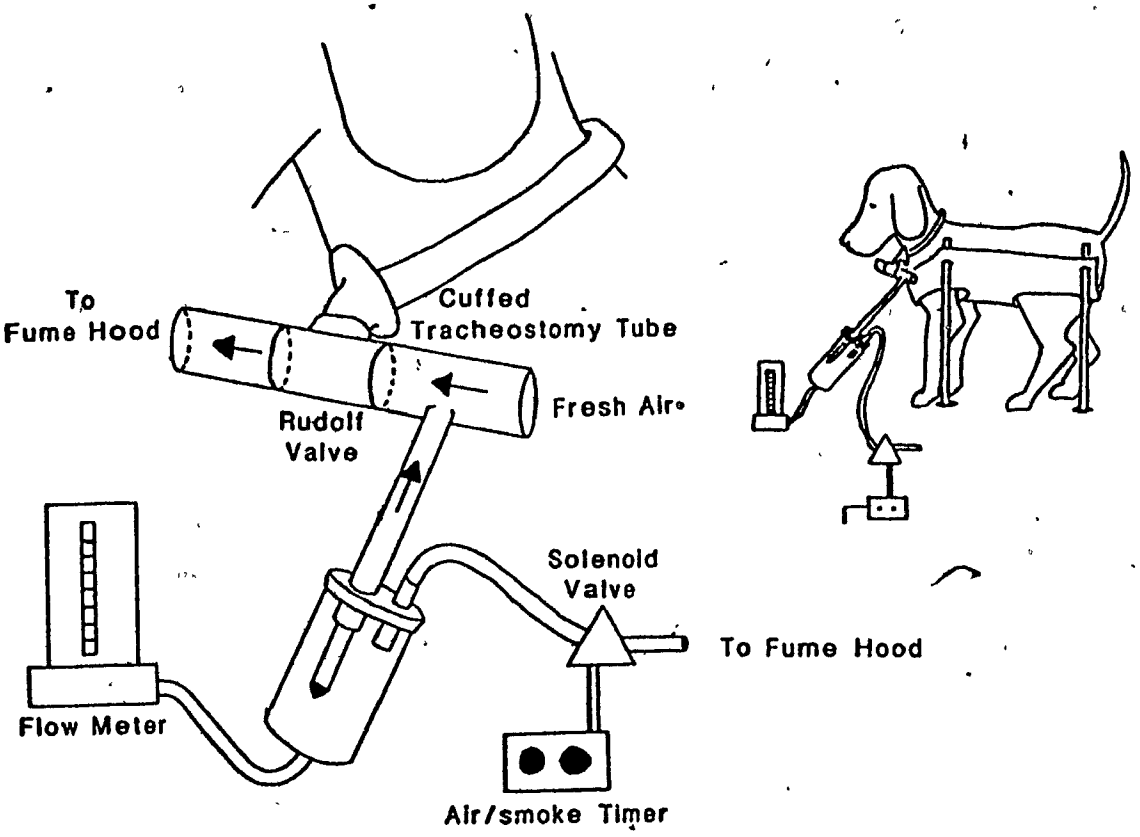
Smoke Exposure Method:

The delivery system employed for the study was similar to others described in the literature (19). The dogs were trained to stand quietly in a harness and accept a cuffed tracheostomy tube (Fig. 2.1). To generate smoke, a positive pressure was applied to a lit cigarette by closing a solenoid valve through which a bypass flow of air normally passed. The solenoid closure period was set by a timer to deliver a 35 ml bolus of smoke to the inspiratory line every 20 seconds until the cigarette was smoked to a standard butt length of 23 mm. After the smoke was fed into the inspiratory line, the dog breathed a mixture of fresh air and smoke via a Rudolf valve with the expirate leading off into a fume hood. The cigarettes that were used were unfiltered 70 mm high tar, high nicotine cigarettes (20 mg tar, 1.2 mg nicotine). The cigarette dose was increased gradually over a run-in period of two weeks up to a maximum of 10 cigarettes per day, delivered over the course of

approximately 2.5 hours, 5 days a week, for a ten-month period of time. All dogs ceased to smoke three to five days prior to the initiation of the airway response experiments.

Figure 2.1

Smoke exposure system. To generate smoke a positive pressure is applied to a lit cigarette by closing a solenoid valve through which a bypass flow of air normally passes. Inset: view of dog in harness with cuffed tracheostomy tube.



Airway resistance measurements:

Each dog was studied under sodium pentobarbital (Nembutal) anesthesia in the supine position. After being intubated with a cuffed endotracheal tube a venous line was established to maintain the level of anesthesia. Measurements were made in a volume displacement body plethysmograph. Volume was determined spirometrically with a linear variable displacement transducer system. Flow at the mouth was measured with a Fleisch No. 2 pneumotachygraph connected to a Validyne MP45 $\pm$ 2 cmH_2O pressure transducer (Validyne Co., Northridge, CA). Transpulmonary pressure was measured with a differential pressure transducer (Validyne MP45 $\pm$ 100 cmH_2O). An esophageal balloon (5 cm long; circumference 1.44 cm) was attached to PE-200 polyethylene tubing with multiple side holes within the balloon. The balloon was passed into the stomach and then withdrawn gradually until a negative pressure was recorded. The balloon was then positioned to record a minimum transpulmonary pressure swing during an occluded breath. Further adjustments were then made to minimize cardiac artifact. Balloon volume was maintained at 0.6 ml and checked periodically throughout the experiment. Pulmonary resistance, R_L was determined by the electrical subtraction method (20).

Methacholine challenges:

Methacholine chloride bronchial challenges were carried out with a Hudson nebulizer at a constant flow rate of 8 l/minute. The same nebulizer was used for each dog. Following an inflation to 20 cmH_2O to

relieve any possible bronchoconstriction, saline was nebulized (particle size 1.6 micrometers) for 1 minute while the dog breathed spontaneously. Two minutes later, measurements of total pulmonary flow resistance were made. Aerosols of doubling concentrations of methacholine chloride diluted in saline were then delivered at 10 minute intervals, starting with 0.5 mg/ml and building up to a maximum concentration of 8 mg/ml for insensitive dogs. Two minutes after each aerosolization R_L was again measured. The aerosol resistance response was defined as the total pulmonary resistance 2 minutes post 2 mg/ml minus the control resistance taken after the saline aerosol. This particular dose was chosen since it was the common dose reached in all dogs in which changes in R_L exceeded baseline resistance by 2 standard deviations.

After the last challenge the dogs were inflated to relieve any bronchoconstriction and allowed to rest for 1 hour. Following this the dog's endotracheal cuff was reinflated and the I.V. line made ready for the infusion response to methacholine chloride. The methacholine chloride was delivered at a constant rate of 4 micrograms/kg/min via a Harvard infusion pump operating at a nominal infusion rate of 0.97 ml/min. Readings of pulmonary resistance were taken every minute and an average was computed over the 4-6 minute range since this time interval corresponded to a plateau response. Following the eight minute mark the infusion was stopped and the dogs were extubated and allowed to recover under supervision. The infusion resistance response was computed as the mean R_L at 4-6 minutes post infusion minus the control resistance immediately prior to infusion.

Mucus collection rates:

Tracheal mucus was collected from the dogs twice weekly by means of the cytology brush technique as described previously (21). The weight of mucus collected on the brush per unit time (mg/min) was used as an index of mucus flux.

Statistical treatment of data:

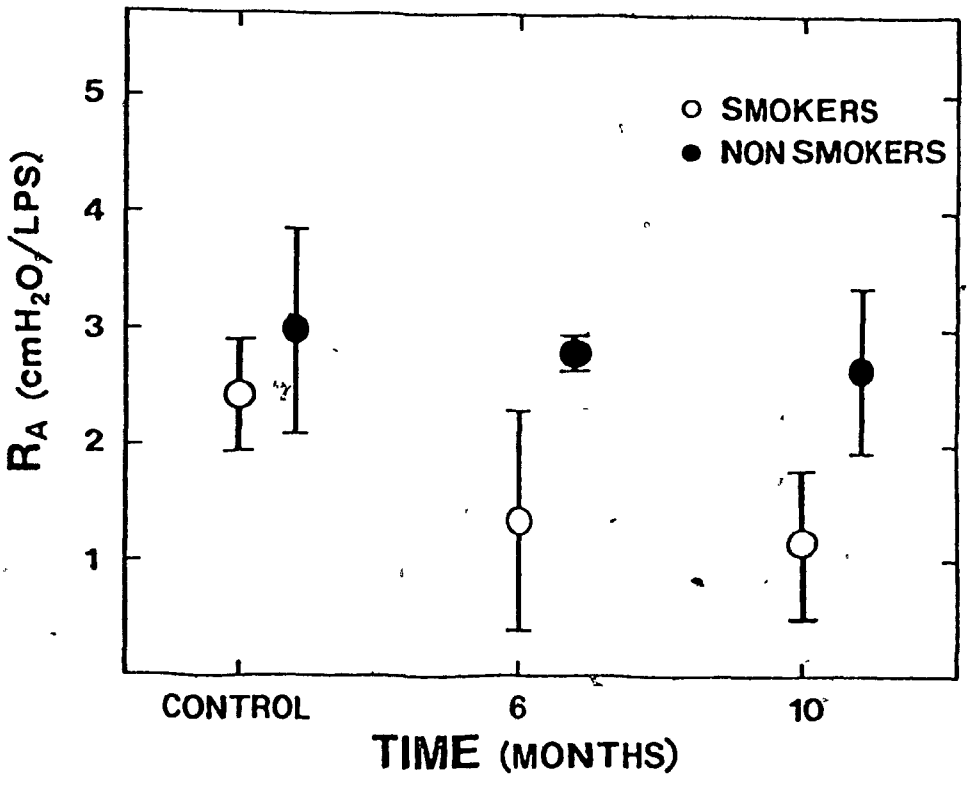
Comparisons of responses were made for both the aerosol and infusion responses within smokers and non-smokers and between both groups using two paired t-tests. A p value less than 0.05 was considered significant.

RESULTS

The variation in Ra, the methacholine aerosol response, over the course of 10 months of cigarette-smoke or sham exposure is illustrated in Fig. 2.2. The aerosol response in smokers decreased significantly by paired t-test ($p < 0.05$) while that in the non-smokers remained unaltered.

Figure 2.2

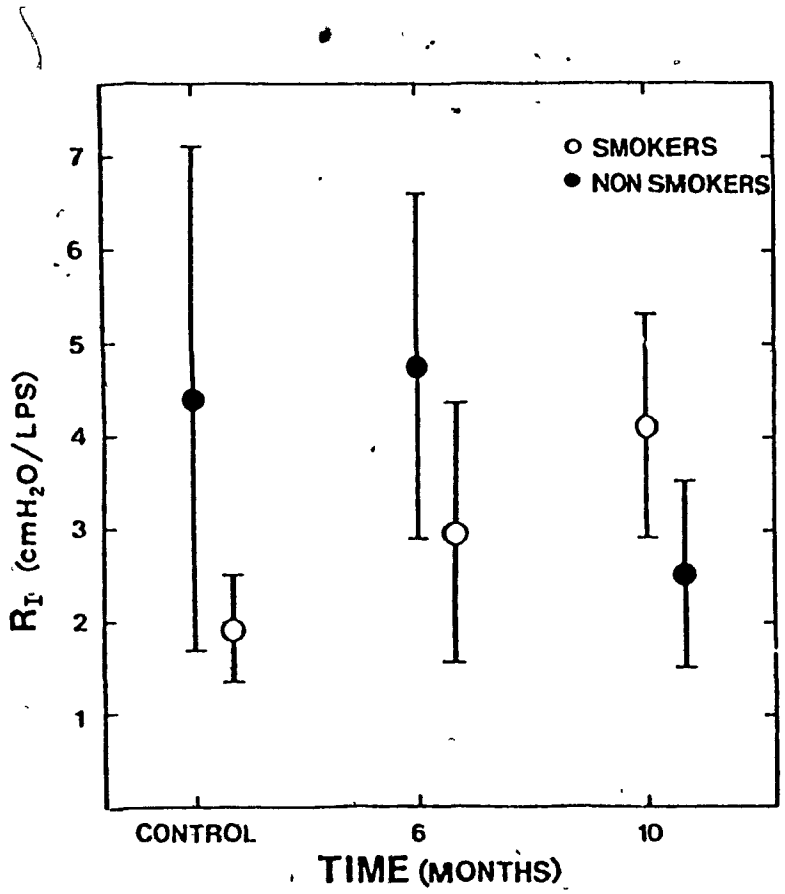
This figure shows the aerosol response to methacholine (2 mg/ml) at control, 6 and 10 months of smoke exposure or sham exposure. Smokers became progressively hyporesponsive over the 10 month period of exposure. Standard error bars are indicated.



By contrast, Ri, the methacholine infusion response, was progressively enhanced over the 10 months of smoking, although the increase did not achieve statistical significance ($0.05 < p < 0.10$). This is illustrated in Fig. 2.3. The mean-initial pre-exposure responses, Ra and Ri, were not significantly different between smokers and non-smokers.

Figure 2.3

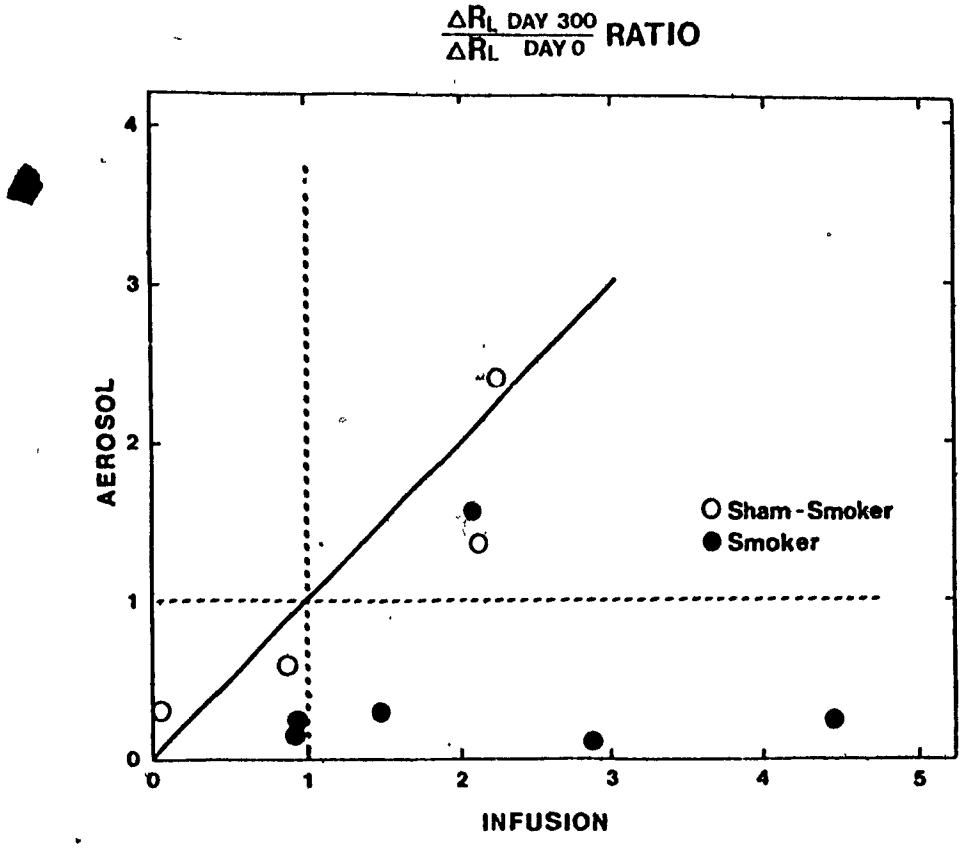
Responses of the methacholine infusion (4 ug/kg/min) as a function of time at control, 6 and 10 months of smoke exposure or sham exposure. Smokers showed an increased responsiveness to the infused methacholine over the smoke exposure period though this did not reach statistical significance. Standard error bars are indicated.



The relative change in aerosol and infusion response at 10 months was defined as $Rai_{10} = Ra_{10}/Rac - Ri_{10}/Ric$, a negative quantity indicating a hyporesponsiveness to aerosol vis-à-vis infusion. The data in the 6 smokers and 4 non-smokers with both infusion and aerosol challenge are illustrated in Fig. 2.4.

Figure 2.4

Change in aerosol response at 300 days versus change in infusion response at 300 days in smokers and sham-smokers. The solid line is the line of identity and the dashed lines divide the figure into four quadrants.



(Individual data points are given in Table 2.1.) This figure indicates that in non-smokers, the changes in infusion response are reasonably tracked by the changes in aerosol response, since the points are scattered about the identity line. In the smokers, however, all the points lie to the right of the identity line, indicating that the changes in infusion response are not matched by equivalent changes in aerosol response. In fact, three of the six smokers showed a substantial decrease in aerosol response in the face of an actual increase in infusion response. The development of relative hyporesponsiveness to aerosolized methacholine vis-à-vis infused methacholine in smoking dogs but not in sham-exposed control was statistically significant ($p < 0.01$).

TABLE 2.1. Response to infused and aerosolized methacholine.

Smokers			Non-smokers		
Dog	*Ral0/Rac	Ril0/Ric	Dog	Ral0/Rac	Ril0/Ric
9ACI	0.179	0.933	9AC4	2.424	2.246
OMO1	0.230	0.951	OMO3	1.368	2.110
OMO2	0.112	2.858	9BP1	0.570	0.867
2JD1	0.303	1.472	2DU3	0.319	0.047
1082	0.241	4.450			
2HL1	1.557+	2.084			
9CQ2	-	8.152			

+ Excluded as an outlier.

* $p < 0.001$.

The baseline resistance level, determined prior to the initial methacholine exposure in each experiment, was found to increase in the smoking dogs ($p < 0.02$), but not in the non-smokers. This increase occurred between 6 and 10 months of exposure, as indicated in Table 2.2. Considering that the aerosol and infusion responses were defined as the absolute changes in R_L with methacholine administration, if the responses had been defined as the fractional change in R_L instead, the mean change in infusion responsiveness at 10 months would have been very slight (140% of control), while the decrease in aerosol responsiveness at the same point would have appeared even more exaggerated, reaching a mean value of only 20% of control.

Table 2.2. Baseline pulmonary resistance (cmH_2O/LPS).

	Control	6 months	10 months
(N=7) Smokers	0.98 ± 0.40[+]	0.69 ± 0.15	1.70 ± 0.47*
(N=4) Non-smokers	1.10 ± 0.30	-	1.46 ± 1.13

* $p < 0.02$

[+] Standard deviation

DISCUSSION

Our data suggest that smokers have decreased nonspecific airway responsiveness to aerosolized methacholine when compared to non-smokers. These differences in reactivity may be accounted for by the chronic mucus hypersecretion which developed over the period in which the dogs smoked. In a model of chronic bronchitis Drazen et al. (11) demonstrated a decrease in the responsiveness to aerosolized histamine in dogs with chronic mucus hypersecretion. In the work of Martin and co-workers, using the same dog model but with a lower dose of SO_2, there was an increased reactivity to aerosolized methacholine (12) but hypersecretion in this case was not very prominent, certainly not in the early stages when the airway hyperreactivity became apparent (13). The findings of Roehrs et al. (10) support our own whereby bronchial reactivity to aerosolized methacholine decreased in cigarette smoking baboons. Although previous studies have reported increases in goblet cells after tobacco smoke exposure they have not focused on the immediate changes. Histologically, Reid et al. (22) reported in rats a decrease in goblet cells after 2 weeks of smoke exposure which was followed by an increase after 4 weeks of exposure. This suggests that a minimum period of smoke exposure is needed before goblet cell proliferation and secretion might occur. Blunting of a response to an aerosol by excess airway mucus is most likely attributed to delayed or restricted access of the pharmaco-active agent to the target site. This could occur by direct dilution or by reduced permeability through the mucus blanket. All of these possibilities could produce a similar effect of reducing the peak

concentration of the agent at the active site when it is given in step-wise increasing concentrations.

The secretory role of the goblet cells may play an increasingly important role in the mechanisms governing hyperreactivity. In a study of airway epithelial permeability in guinea pigs Hulbert et al. (18) found a sharp reduction in the number of goblet cells immediately after the initial cigarette smoke exposure. Interestingly enough mucosal permeability was found to increase as the goblet cell count decreased. Simani and co-workers (16) suggested that the increase in permeability of respiratory epithelium after exposure to tobacco smoke was due to alterations around tight junctions. It has since been shown that the altered permeability was associated with structural damage leading to leaky tight junctions and that this damage was dose-related (17). In the absence of a mucosal barrier, irritant receptors found below the tight junctions could be exposed to pharmaco-active agents such that the individual would appear to have hyperreactive airways.

This suggests a relationship between the observed mucus gland hyperplasia, the epithelial permeability and hyperreactivity. An inadequate mucosal barrier or an increase in epithelial permeability by prior tobacco smoke exposure may account in part for the increase in bronchial reactivity found in some human studies (14,15,23).

Since the increase in permeability has been shown to be reversible to some extent given an adequate period of cessation of smoking (18,24), it is possible that the increases in bronchial reactivity reported in these former studies may in fact reflect only the acute effects of

cigarette smoke. Paré et al. demonstrated in ascaris-allergic rhesus monkeys that with prior antigen inhalation there is a marked increase in airway resistance in response to a given dose of histamine. It was also demonstrated that the increase in airway resistance produced by inhalation of antigen was associated with changes in bronchial permeability (25). This increase in permeability might facilitate passage of pharmaco-active agents to underlying irritant receptors.

Sellicke and co-workers were able to demonstrate an increase in the discharge rate of action potentials from single fiber recordings following smoke exposure in rabbits. They speculated that this might be due to stimulation of irritant receptors found in the epithelium (23). It is unlikely that such permeability changes might have played a role in modifying reactivity in our study since all dogs ceased to smoke 3-5 days prior to the experiments. The human studies reflecting an increase in bronchial reactivity can be criticized on the grounds that in two of the studies smokers refrained from smoking at least two hours prior to the challenges (14,15) and in the third only one hour before the tests (20). These time intervals would not be sufficiently long to exclude the possible effects of the acute permeability changes found with smoke exposure. These studies would also rely heavily on the cooperation of smokers taking part in the study introducing an inherent degree of uncertainty in their results.

In the second part of the study we compared the responses to aerosolized and infused methacholine. As earlier stated our hypothesis was that the response to infused methacholine would more clearly reflect

airway smooth muscle responsiveness, and that any blunting of the response to aerosolized methacholine would then be related to a quantity of mucus found in the airways. Since the infused dose was carefully matched to provide an equivalent mean change in pulmonary resistance similar to that of the highest common aerosolized dose amongst all dogs one could monitor the response to the two over a 10 month period of time. In dogs with elevated levels of airway secretion, for a given response to an infusion of methacholine, the relative response to an aerosol of methacholine was reduced with respect to what it would have been if there were little mucus in the airways. If airway hyperreactivity to an aerosolized bronchoconstrictor agent can be caused by increased permeability to that agent and/or increased responsiveness of the smooth muscle to the inhaled agent, then, if infusion reactivity represents the true or inherent airway reactivity, the reactivity to inhaled aerosol may thus underestimate the true value when mucus hypersecretion occurs. In a study of O_3-induced airway effects Abraham and co-workers reported a significant increase in the smooth muscle responsiveness to infused carbachol in sheep (27). This increase in the smooth muscle responsiveness was not detectable by inhalation challenge due to a concomittant decrease in epithelial permeability. They attributed this decrease in epithelial permeability to mucus hypersecretion. In Fig. 2.3 the smokers exhibited similar results in which the methacholine infusion response was progressively enhanced throughout the smoke exposure period. This increase did not, however, achieve statistical significance in our study but might have, given a

longer exposure to tobacco smoke. It has been reported that the narrowing of airways by mucus can enhance inertial deposition by increasing the linear velocity of flow hence causing more central deposition. This increase in bronchial deposition has been reported in patients with chronic bronchitis (28). If such was the case in those dogs that were hypersecretors one would predict an enhancement in the response to methacholine and not the diminution seen in our study. In fact, following the arguments of Strohl et al. (29) a central shift in the aerosol deposition should if anything increase R_L because of the increased concentration of methacholine in the airways contributing most strongly to resistance. Kim and co-workers (30) reported that increased mucus depth would enhance aerosol deposition more than resistance. The increase in central deposition may in fact have been offset by the barrier effect of the mucus blanket.

In conclusion this study indicates that chronic cigarette smoke exposure reduced bronchial reactivity as indicated by the aerosol response. It is interesting to speculate that the development of mucus hypersecretion and the concomitant diminution of the aerosol response could be intimately related to one another and that this mucus barrier could serve to dilute or impede the passage of noxious agents to the underlying epithelium.

REFERENCES

1. Reid L. An experimental study of hypersecretion of mucus in the bronchial tree. Brit J Exper Pathol 1963; 44:437-445.

2. Lamb D, Reid L. Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulfur dioxide. J Pathol Bact 1968; 96:97-111.

3. Mawdesley-Thomas LE, Healey P, Barry DH. Experimental bronchitis in animals due to sulfur dioxide and cigarette smoke. An automated quantitative study. In: Walton HW. Inhaled Particles III. Gresham Press, Old Woking, Surrey, England, 1971, pp 509-525.

4. Angus E, Amyot R, Martin R. Morphological changes in beagle dogs following exposures to SO_2. Clin Res 1976; 24:689A (abstract).

5. Auerbach O, Hammond ED, Kirman D, Garfinkel L, Stout AP. Histologic changes in bronchial tubes of cigarette-smoking dogs. Cancer 1967; 20:2055-2066.

6. Lamb D, Reid L. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. Brit Med J 1969; 1:33-35.

7. Hayaski M, Sornberger CG, Huber GL. Morphometric analyses of tracheal gland secretion and hypertrophy in male and female rats after experimental exposure to tobacco smoke. Am Rev Respir Dis 1979; 119:67-73.

8. Wanner A, Hirsch JA, Greeneltch DE. Tracheal mucous velocity in beagles after chronic exposure to cigarette smoke. Arch Environ Health 1973; 27:370-371.

9. Park SS, Kikkawa Y, Goldring IP, Daly MM, Zelefsky M, Shim C, Spierer M, Morita T. An animal model of cigarette smoking in beagle dogs. Am Res Respir Dis 1977; 115:971-979.

10. Roehrs JD, Rogers WR, Johanson WG Jr. Bronchial reactivity to inhaled methacholine in cigarette-smoking baboons. J Appl Physiol 1981; 50:754-760.

11. Drazen JM, O'Cain CF, Ingram RH Jr. Experimental induction of chronic bronchitis in dogs: Effects on airway obstruction and responsiveness. Am Rev Respir Dis 1982; 126:75-79.

12. Boileau R, Delaunois L, Tremblay J, Gauthier J, Martin RR. Enhancement of bronchial responsiveness to methacholine during chronic SO_2 exposure. Fed Proc 1979; 38:1110 (abstract).

13. King M, Boileau R, Delaunois L, Martin RR. Alteration in tracheal mucus viscoelasticity with chronic sulfur dioxide exposure. Am Rev Respir Dis 1980; 121(2):243 (abstract).

14. Buczko GB, Day A, Vanderdoelen JL, Boucher R, Zamel N. Effects of cigarette smoking and short-term smoking cessation on airway responsiveness to inhaled methacholine. Am Rev Respir Dis 1984; 129:12-14.

15. Malo JL, Filiatrault S, Martin RR. Bronchial responsiveness to inhaled methacholine in young asymptomatic smokers. J Appl Physiol 1982; 52:1464-1470.

16. Simani IAS, Inoue S, Hogg JC. Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. Lab Invest 1974; 31:75-87.

17. Boucher RC, Johnston J, Inoue S, Hulbert W, Hogg JC. The effect of cigarette smoke on the permeability of guinea pig airways. Lab Invest 1980; 43:94-100.

18. Hulbert WC, Walker DC, Jackson A, Hogg JC. Airway permeability to horseradish peroxidase in guinea pigs: The repair phase after injury by cigarette smoke. Am Rev Respir Dis 1981; 123:320-326.

19. Battista S, Guerin M, Gori G, Kensler C. A new system for quantitatively exposing laboratory animals by direct inhalation: Delivery of cigarette smoke. Arch Environ Health 1973; 27:376-382.

20. Mead J, Whittenberger J. Physical properties of human lungs measured during spontaneous respiration. J Appl Physiol 1953; 5:779-796.

21. King M. Interrelation between mechanical properties of mucus and mucociliary transport: Effect of pharmacologic interventions. Biorheology 1979; 16:57-68.

22. Reid L. Bronchial mucus production in health and disease. In: Liebow AA, Smith DE, eds. The Lung. International Academy of Pathology Monograph No. 8. New York, The Williams and Wilkins Co., 1978; 8:87-108.

23. Gerrard JW, Cockcroft DW, Mink TT, Cotton DJ, Poonawala R, Dosman JA. Increased non-specific bronchial reactivity in cigarette smokers with normal lung function. Am Rev Respir Dis 1980; 122:577-581.

24. Jones JG, jordan C, Minty BM. Rapid improvement in abnormal pulmonary epithelial permeability after stopping cigarettes. Brit Med J 1981; 282:1183-1186.

25. Boucher RC, Pare PD, Hogg JC. Relationship between airway hyperreactivity and hyperpermeability in ascaris-sensitive monkeys. J Allergy Clin Immunol 1979; 64:197-201.

26. Sellicke H, Widdicombe J. Stimulation of lung irritant receptors by cigarette smoke, carbon dust and histamine aerosol. J Appl Physiol 1971; 31:15-19.

27. Abraham W, Champman G, Marchette B. Differences between inhaled and intravenous carbachol in detecting O_3-induced airway effect. Environ Res 1984; 35:430-438.

28. Lippman M, Albert RE, Peterson HT Jr. The regional deposition of inhaled aerosols in man. In: Walton WH, ed. Inhaled particles III, Vol. I. Sussex, England. Union Brothers 1971:105-120.

29. Strohl KP, O'Cain CF, Ingram Jr, Yarka MA, Kaplan WD, McFadden ER Jr. Inhalation patterns and predominant site of bronchoconstriction in healthy subjects. J Appl Physiol 1981; 50:575-579.

30. Kim CS, Brown LK, Sackner MA. Relationship between aerosol deposition and flow resistance in partially obstructed airway model. Fed Proc 1982; 41:1692 (abstract).

Chapter 3

MUCUS HYPERSECRETION IN CIGARETTE-SMOKING DOGS

ABSTRACT

Tracheostomies were fashioned in 14 adult beagle dogs. Mucus was collected twice weekly without drugs by resting a cytology brush on the lower trachea for periods of 2-5 minutes. The mucus was weighed, and the galactose content determined by the phenosulfuric acid assay. The mucus collection rate served as an index of tracheal mucus flux, the galactose assay as a marker of mucous glycoprotein content. The tracheal mucus clearance rate TMCR was determined periodically under xylazine analgesia by observing charcoal particle transport bronchoscopically. After an initial 4 month control period, the cigarette smoke exposures were started. The dogs were trained to stand quietly in a harness, and smoke was delivered via a cuffed tracheostomy tube. A 35 cc bolus was introduced to the inspiratory line each 20 seconds, using unfiltered 70 mm cigarettes (20 mg tar, 1.2 mg nicotine). Each dog smoked 10 cigs/day over 2.5 hrs., 5 days/wk. Two dogs were exposed for 6 months incl. a 2 wk run-in period; 7 dogs were exposed for 10 months with a 2 month run-in period. The other 5 dogs served as controls. Eight of 9 smoking dogs developed persistent mucus hypersecretion. In 5 dogs, tracheal mucus flux increased 5-10 fold; in 3 dogs, the increase was 2-3x control. One of the 5 sham-exposed dogs developed moderate hypersecretion. The pattern was variable, the maximal increase occurring from 2 months to 10 months. In the first 2-4 months of smoking, the galactose content of the mucus fell, consistent with a decrease in elasticity. However, after 6 months, the elasticity rose back towards control levels, while the galactose content remained low, suggesting an alteration in the nature of the mucous glycoprotein. The TMCR did not change significantly. The alterations in mucus were chronic rather than acute, in that there were no significant differences in quantity or galactose content on Monday vs Friday, nor after one cigarette vs before.

Key words: mucus, smoking, hypersecretion.

INTRODUCTION

Studies in human smokers suggest that abnormalities in mucociliary clearance are one of the earliest functional alterations associated with cigarette smoking. In one study, tracheal mucus velocity was found to be lower in young smokers than in non-smokers (1). In another study (2), however, while tracheal and large bronchial clearance rates were normal, peripheral mucus clearance was slower in smokers, suggesting that the smaller bronchi are more suspectible to the development of clearance abnormalities. Our own studies (3) have delineated a structural basis for early functional impairment in smokers, residing in early inflammatory change and epithelial abnormalities in the small airways.

A number of investigators have demonstrated, with histological and morphometric techniques, that mucus hypersecretion develops in animals exposed to cigarette smoke for extended periods of time (4-7). Auerbach et al. (4) examined the effects of smoke exposure in tracheostomized dogs and found extensive morphological changes in both parenchyma and airways. The airway changes included epithelial hyperplasia, decreased numbers of ciliated cells, and areas of squamous metaplasia. Wanner et al. (8) measured tracheal mucus velocity in beagles exposed to cigarette smoke through a face mask. They found a reduction in clearance velocity, while other aspects of pulmonary function remained normal, suggesting that changes in mucus clearance are an early result of smoking; unfortunately their study did not include histology. Park et al. (9), with a similar face mask delivery, also found a reduction in mucus clearance rate after long-term smoke exposure. Morphological changes in

the central airways were noted, including goblet cell hyperplasia, but not parenchymal changes. Since ciliary function appeared unaltered, they attributed the changes in mucus clearance to alterations in mucus viscoelasticity, although this was not tested.

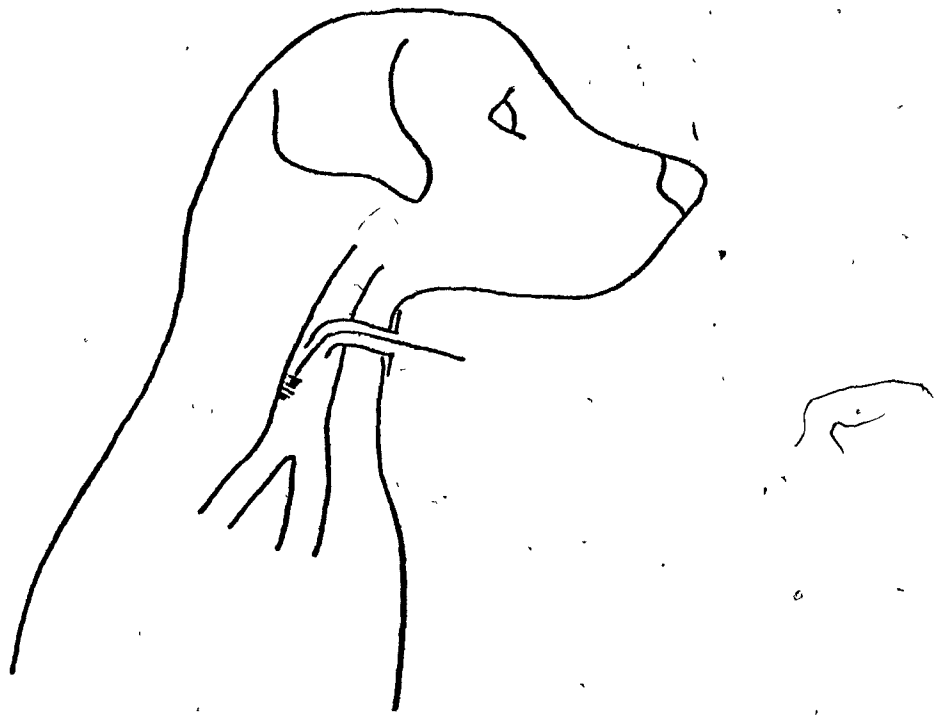
In the study described here, we examined the development of mucus hypersecretion in a group of beagle dogs exposed for several months to moderately high doses of cigarette smoke. Our main purposes were to develop an animal model with an elevated and relatively stable secretion level in order to examine the inter-relationship between mucus hypersecretion, viscoelasticity, and clearance, and to see how mucus hypersecretion related to other aspects of pulmonary pathology.

MATERIAL AND METHODS

We fashioned permanent tracheostomies in fourteen adult beagle dogs (Fig. 3.1). Mucus was collected without drugs by opening the stoma with an uncuffed tracheostomy tube, inserting a cytology brush, and resting it on the lower tracheal mucosa for a period of 2 to 5 minutes. Mucus collected on the brush by mucociliary flow. At the end of the collection period, the brush was withdrawn and the mucus removed by

Figure 3.1

Collection of mucus from a dog with a permanent tracheostomy. The tracheostomy is fashioned by excising an oval segment of anterior trachea and suturing the outer skin directly onto the remaining trachea. The dog is then left for 2-3 weeks to recover.

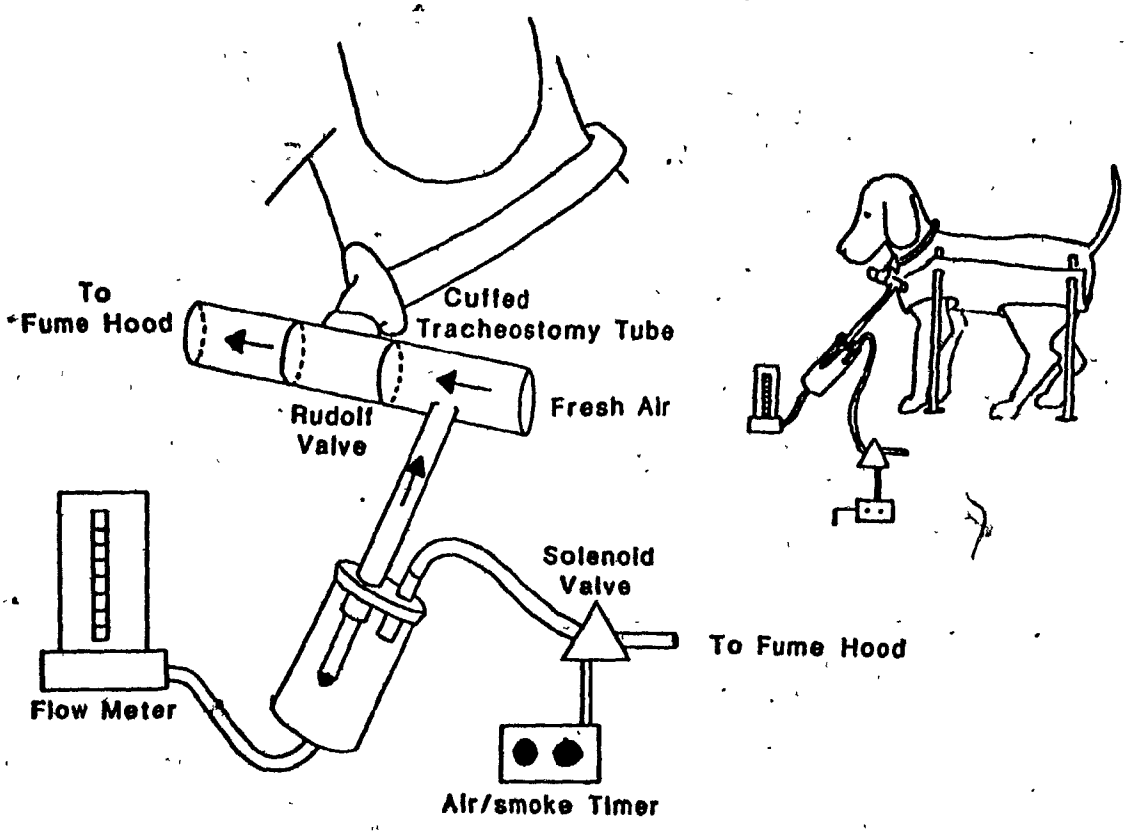


gently scraping the brush with a dull scalpel blade. Each sample of mucus was weighed. The collection rate was computed by dividing the weight of mucus by the tracheal contact time; this defined an index of mucus flux (10). In this study, we also routinely analyzed each sample for its content of neutral hexose, using galactose as the standard (11); this provided an index of mucus glycoprotein content. The majority of samples were also analyzed rheologically, using the magnetic rheometer technique (12), which gives viscosity and elasticity as a function of frequency.

Mucus was normally collected twice weekly before and throughout the smoke exposure period. After a three to four month initialization period, during which time a number of physiological studies were performed, the smoke exposures were begun. The dogs were trained to stand quietly in a harness and accept a cuffed tracheostomy tube (Fig. 3.2). To generate smoke, positive pressure was applied to a lighted cigarette by closing a solenoid valve through which a bypass flow of air normally passed. The solenoid closure period was set by a timer to provide an average bolus of 35 cc of smoke; it was activated every 20 seconds until the cigarette was smoked down to a standard butt length of 23 mm. The smoke was fed into the inspiratory line; the dog breathed this mixutre via a Rudolf valve, the expiratory line leading off to a fume hood. The exposure method is comparable to that described by Battista et al. (13). The cigarettes that we used were unfiltered 70 mm cigarettes that were formulated to give a high tar and nicotine output (20 mg tar, 1.2 mg nicotine per cigarette).

Figure 3.2

Smoke exposure method. Inset: The dog is trained to stand quietly in a restraining harness and accept the insertion of a cuffed tracheostomy tube. Smoke is generated when the bypass flow of air is blocked by temporarily closing the solenoid valve. The smoke feeds into the inspiratory line via a T connector and then to the dog via a Rudolf valve. The solenoid closure interval is set to give a mean bolus volume of 35 cc; the time between closures is set at 20 seconds, so that a cigarette takes 8-10 minutes to complete. The cigarettes used are 70 mm unfiltered cigarettes of a formulation that produces 19.8 mg tar, 1.19 mg nicotine, and 15.8 mg CO per cigarette when smoked to a standard butt length of 23 mm.



The cigarette dose was increased gradually over a run-in period, up to a maximum of 10 cigarettes a day, delivered over the course of about 2.5 hours. The first two dogs studied (series I) were exposed for a total of 6 months, including a 2-week run-in period; the other seven (4 in series II and 3 in series III) were exposed for a total of 10 months each, including a 2-month run-in period. Five dogs were kept as controls, one in series I, three in series II and one in series III.

On one occasion prior to smoking and on one or two occasions during the smoke exposure period (after 6 or 10 months), the tracheal mucus clearance rate, TMCR, was observed by a direct observation method (14). The dogs were administered xylazine, an anesthetic 2 mg/kg i.m., and placed in the lateral decubitus position. A flexible fiberoptic bronchoscope was inserted through the tracheal stoma, and 5-10 ul of a suspension of finely divided charcoal was deposited on the dependent wall of the lower tracheal mucosa. The progression of the leading edge of the charcoal deposit was observed for a period of 5-10 minutes, and TMCR was computed as the cephalad displacement divided by the time elapsed.

On several occasions, venous blood was drawn from the dogs into a heparinized syringe before the daily smoke exposure and immediately after the tenth cigarette of the day. The total hemoglobin content, as well as the percent carboxyhemoglobin, was determined with an IL 282 Co-oximeter equipped for the analysis of canine blood.

At the end of the smoke exposure period, the dogs were sacrificed following a final series of lung mechanics experiments. The lungs were excised and fixed by inflating in buffered formalin. Segments of the

lower trachea of 6 smokers and 4 controls were processed for the measurement of Reid index, which was determined according to the procedure described by Thurlbeck and Angus (15).

RESULTS

Persistent mucus hypersecretion, lasting for a least the last two months of exposure, developed in 8 of 9 smoking dogs. The pattern, however, was quite variable. Figure 3.3 illustrates the various patterns of mucus collection rate that developed in the 9 dogs. Each point represents the mean collection rate for 8 sampling periods over a 4-week interval. The pattern was extremely variable. Three dogs illustrated in Figure 3.3 showed a rapid increase in mucus flux, followed by a long and relatively stable period of hypersecretion. Two other dogs showed an apparent latent period of about four months before the onset of hypersecretion.

In contrast, the other four dogs showed only modest levels of hypersecretion over the 10-month exposure period. One dog in particular (OM01) showed an early and a late phase of elevated secretion level, but a long intermediate phase of hyposecretion. Another dog (2JD1) developed hypersecretion early in the smoke exposure period, but this had vanished by about 8 months.

In Figure 3.4 we summarize the observations in the nine smoke-exposed dogs. The data presented are the mucus collection rates over the 3-week period prior to smoking and the last 4-week period of

Figure 3.3

Mucus collection rates in the nine smoking dogs prior to and during 6-10 months of smoke exposure. Each data point is the mean of up to 16 values determined over a 2-month interval.

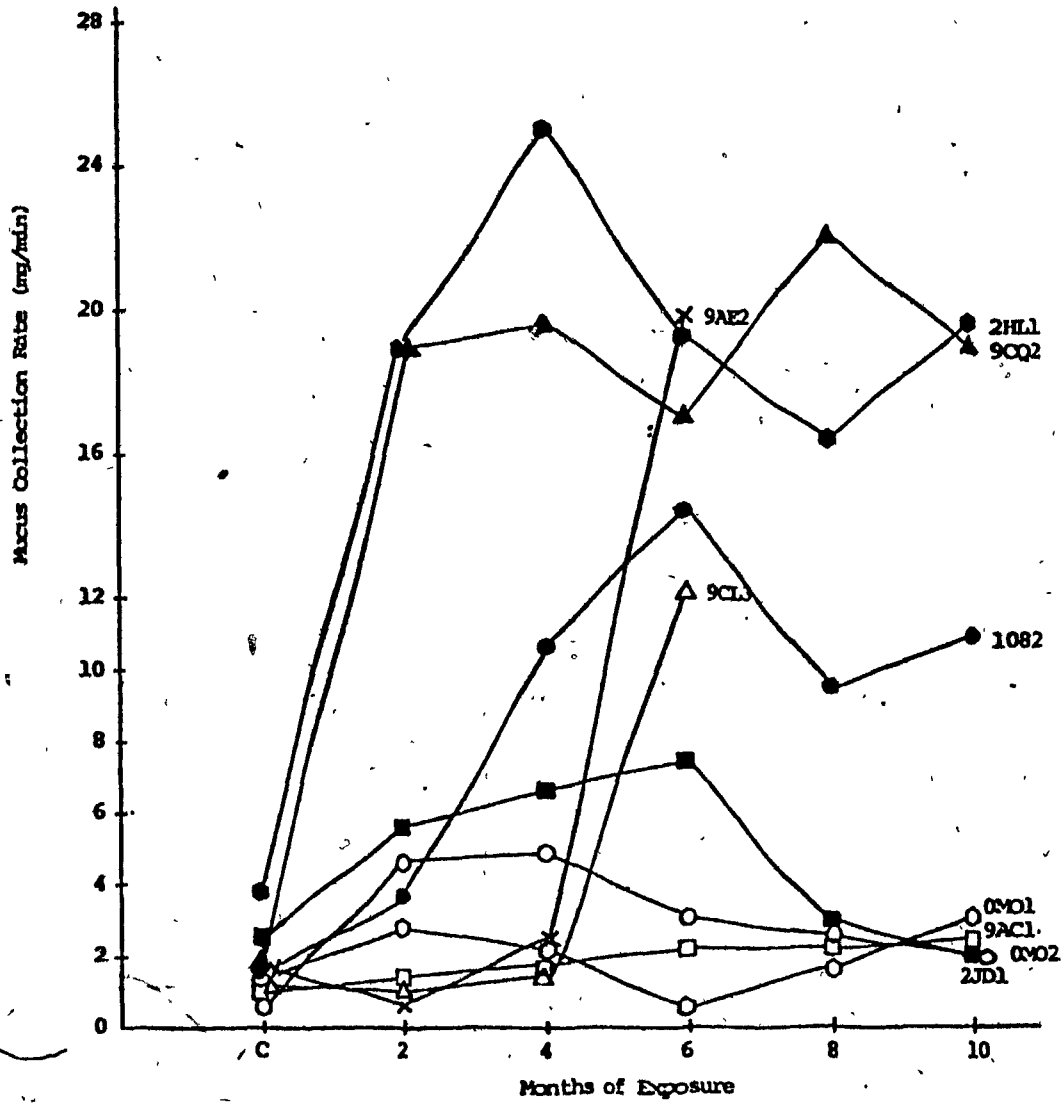
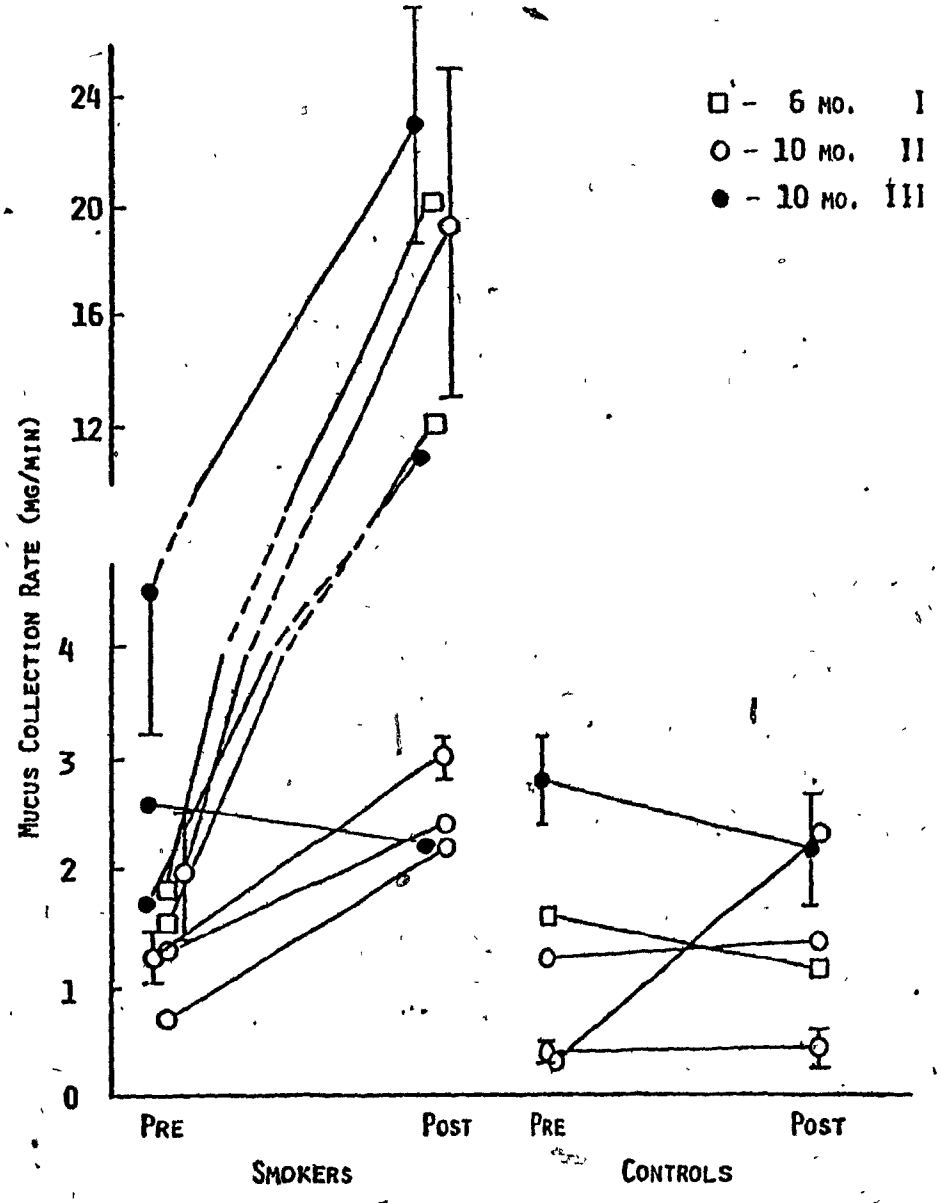


Figure 3.4

Mucus collection rates in nine smoke-exposed dogs and five control dogs prior to and during the last month of exposure. Representative standard errors are indicated.



smoke exposure (except for phase I dogs, where the data pertain to the 2-week period after cessation of smoking). Each of the dogs, except one, showed an elevation over its original level by the end of the smoke exposure period; in five cases this was quite marked, and in the other three it was less so. One of the five controls developed a level of hypersecretion similar to that seen in the less susceptible smokers.

In the first 2-4 months of the study, the mechanical impedance ("viscosity plus elasticity") of the mucus fell in all the smoking dogs, irrespective of whether they developed hypersecretion or not. This is illustrated in Figure 3.5 for the seven dogs of series II and III. The mechanical impedance of tracheal mucus from four control dogs did not change significantly over the course of the study. By six months of exposure the viscoelastic properties of the mucus had returned to control levels.

In each of the smoking dogs, the galactose content of the mucus fell over the course of the study. Figure 3.6 shows the data for the seven dogs in second series II and III. A fall in the galactose assay could indicate a reduction in overall glycoprotein content, which could be consistent with a fall in mucus elasticity. This appeared to hold in the first two dogs exposed (series I), where we found a single relationship between mucus elasticity and galactose content (Figure 3.7). In terms of mucociliary clearance, the mucus produced would be moved slightly more easily by ciliary action, as predicted by frog palate model studies (16). However, in series II and III dogs, which were exposed for a longer period of time, the elasticity eventually returned

to control levels, while the galactose content remained low. Thus for longer exposures (6-10 months) the single relationship between elasticity and galactose content did not hold. This suggests that in the longer term exposures there was a change in the nature of the mucus - either a lower fraction of glycoprotein assaying as neutral sugar, or a change in molecular weight or in the nature of the crosslinking.

There was no significant change in TMCR over the course of the smoke exposure period, nor was any difference observed between exposed and non-exposed dogs (Figure 3.8). There was a trend ($0.05 < p < 0.10$) to an increase in TMCR in exposed dogs, but this was matched by a comparable trend in the non-exposed group.

Figure 3.5

Mechanical impedance (G* at 100 rad/s) in seven smoke-exposed dogs over the course of the exposure period. Each data point is the mean of 10-16 values determined over a 2-month interval. Representative standard errors are shown for dog 2JD1. Mean values of G* (± 1SD) for four control dogs are also shown.

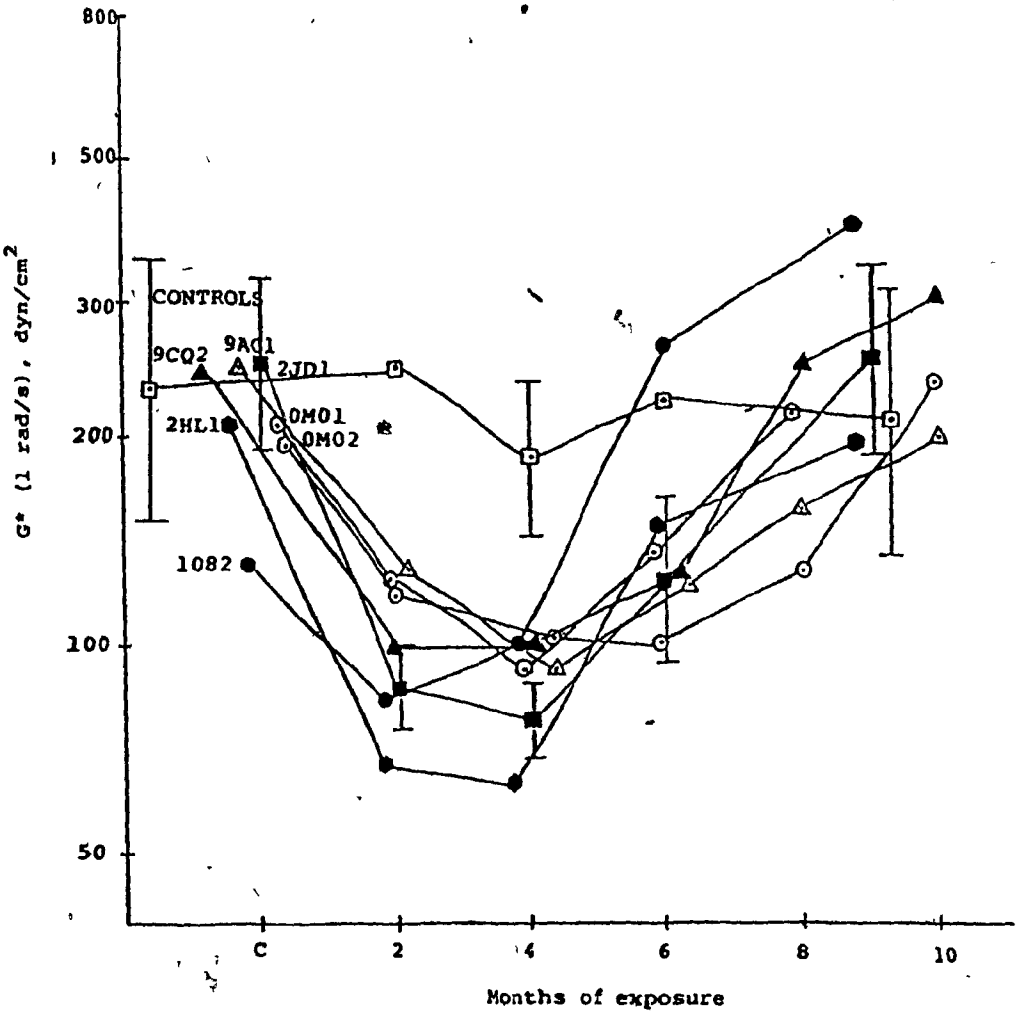
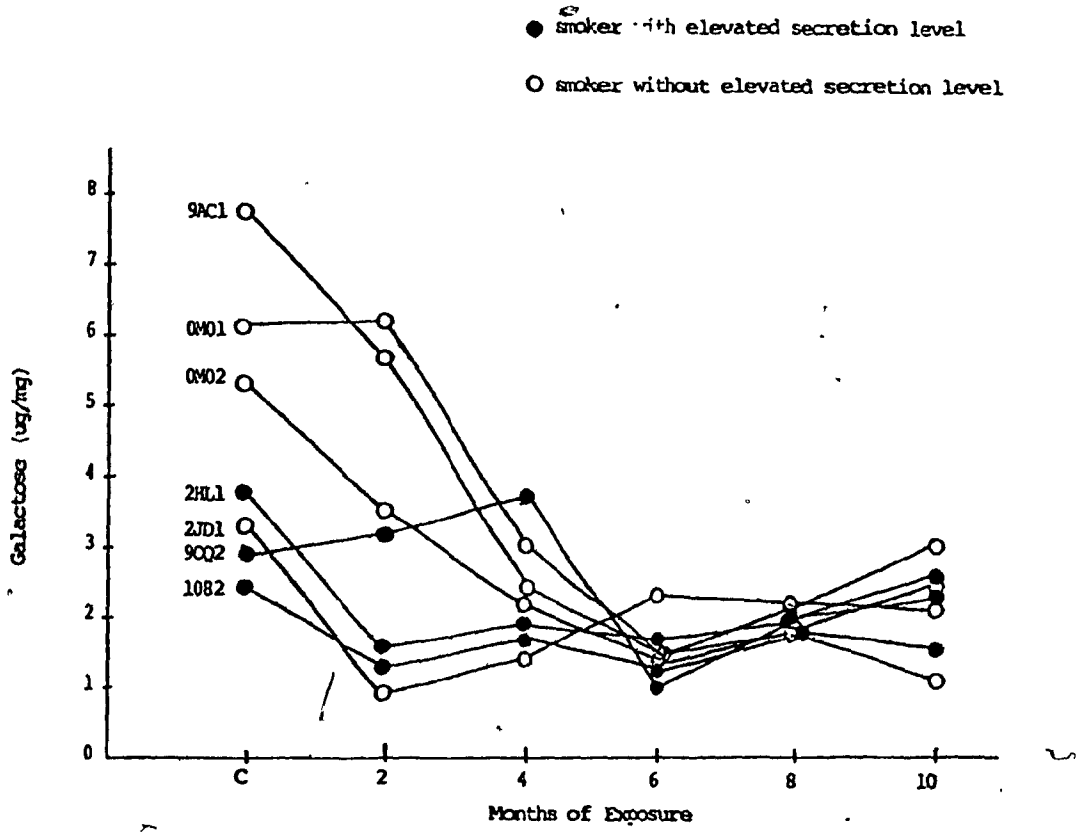


Figure 3.6

Galactose content of tracheal mucus in 7 smoke-exposed dogs over the course of the smoke exposure period. Each data point is the mean of up to 16 values determined over a 2-month interval.



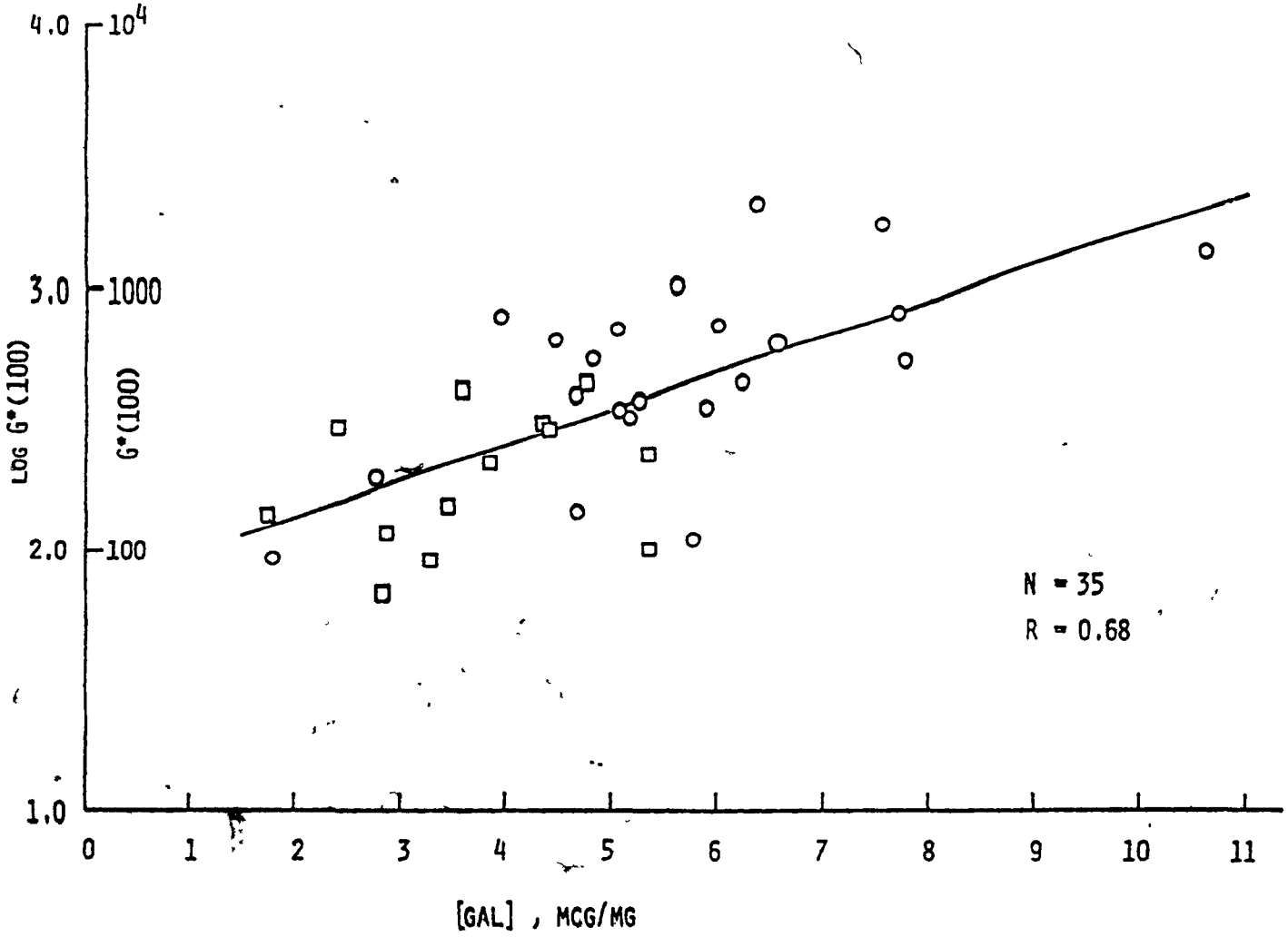
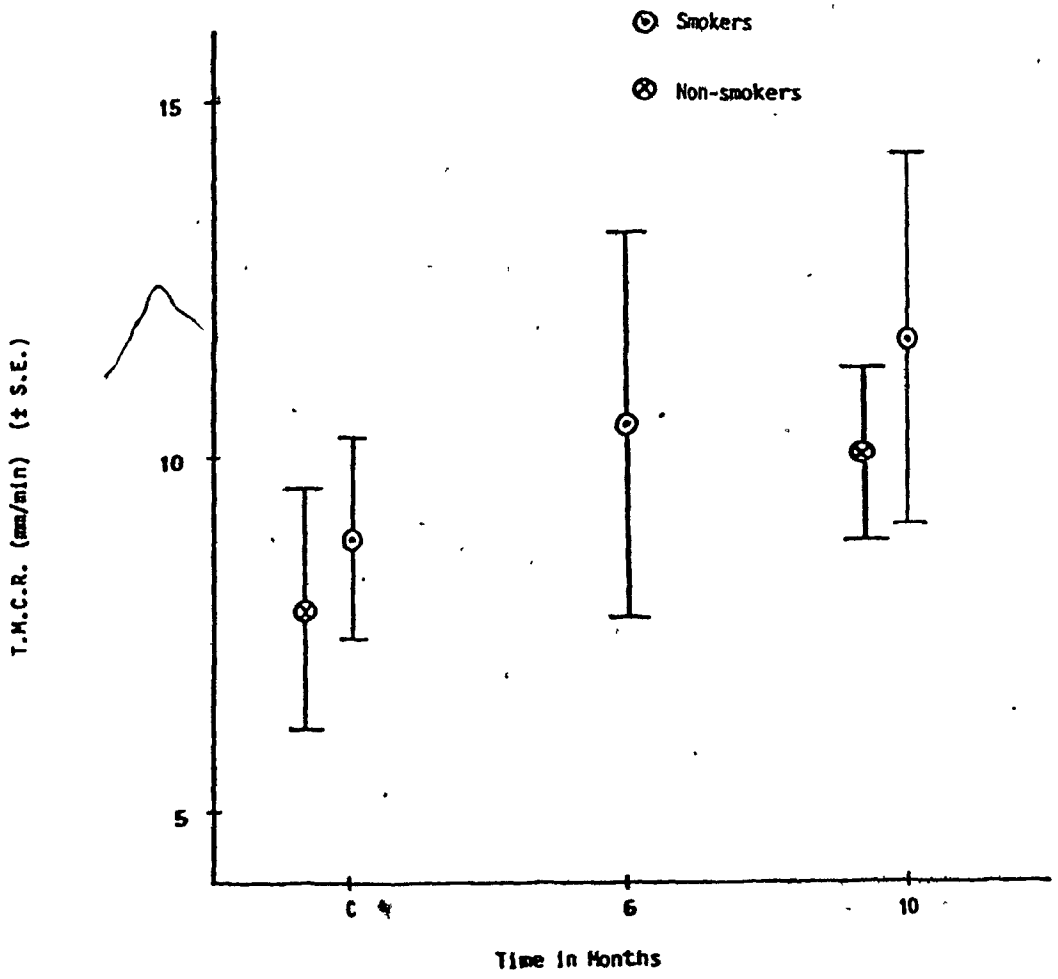


Figure 3.7
Tracheal mucus elasticity (G* at 100 rad/s) versus galactose content.
□-data from two smoke-exposed dogs; ○-data from three non-exposed dogs.

Figure 3.8

Mucus collection rate prior to and after one cigarette in two dogs at various times over the course of 10 months of exposure.



The values of Reid index are tabulated in the following table (Table 3.1). The mean value of Reid index in smokers was higher than in non-smokers, although there was considerable overlap and the difference was not statistically significant.

TABLE 3.1. Tracheal Reid index in smoking and non-smoking beagles.

Smokers with greater than 5 x secretion rate	Reid index
9AE2	0.32
9CL3	0.39
9CQ2	0.45
Smokers with 2-3x secretion rate	
9AC1	0.29
OMO1	0.42
OMO2	0.28
Non-smokers	
KG71	0.36
9BP1	0.24
OMO3	0.43
9AC4	0.30

The carboxyhemoglobin levels attained after the tenth cigarette in the smoking dogs were quite high - 14.5% ± 3.2% (SD)(N=26). This compares with 1.2 ± 0.5% (N=6) in non-exposed dogs and 1.1% ± 0.3% (N=10) in smokers prior to the day's exposure. No systematic differences were noted in COHb level after 10 cigarettes between dogs, nor within dogs between 4 and 9 months of exposure. There were also no significant differences in total Hb content between smokers and non-smokers (15.8 ± 1.1 g/dl for smokers between 4 months and 9 months of exposure versus 16.5 ± 1.7 g/dl for non-smokers).

It should be emphasized that these alterations in secretion level are chronic rather than acute. In the dogs in series II, we compared the mucus collection rates before and immediately after the first cigarette of the week on a regular basis, with the assumption that the cigarette would act as a standard acute irritant. However, except perhaps for the first few exposures, there was no significant difference in quantity of mucus collected after the first cigarette versus the amount prior to it. Figure 3.9 illustrates the data for two dogs; the data for the other two were comparable. There were also no significant differences in the galactose assays of these mucus samples. Furthermore, there were no differences in either the collection rate or galactose content of mucus obtained on Fridays, after 4 days of smoking, versus that obtained on Mondays, after 2 days of non-exposure. Finally, the mucus collection rate in the two weeks following the end of the exposure period was comparable to that in the last four weeks of smoking.

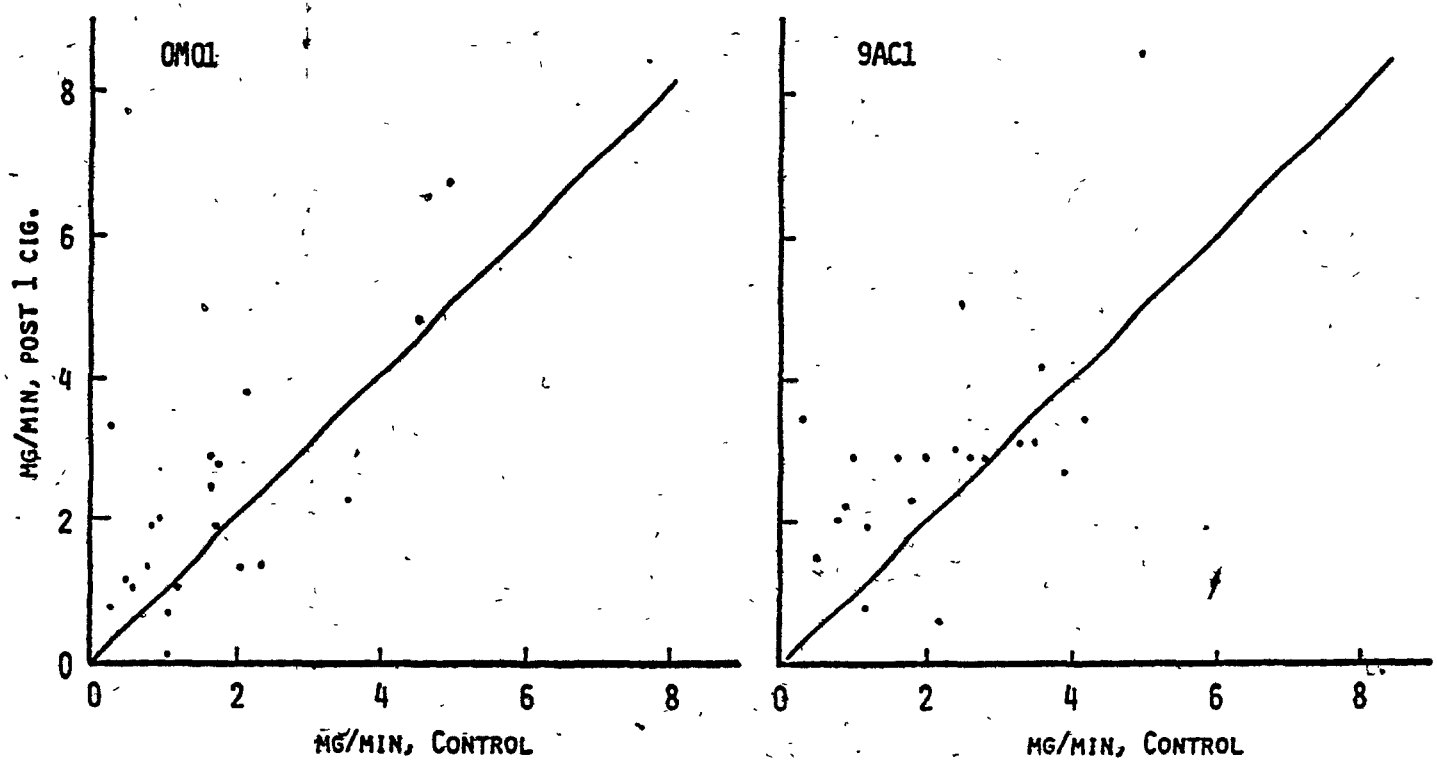


Figure 3.9
Mucus collection prior to and after one cigarette in two dogs at various times over the course of ten months of exposure.

DISCUSSION

The lack of change in TMCR with chronic smoke exposure (in fact a slight increase) is at variance with the observations of Wanner et al. (8), who reported a significant decrease in TMCR after 12 months of exposure to 20 cigarettes per day via a face mask. The difference in findings could be due to the higher nominal cigarette dose used in the latter study, although it had been assumed that 10 cigarettes per day via a tracheostomy would have a greater effect because of the lack of filtering action of the upper airway. In any case, the lack of change in TMCR is consistent with a number of factors associated with the present study, including the favorable alteration in mucus rheology, implying that the cilia, although more loaded, were presented with mucus that was more easily cleared. In fact, the way in which our dogs responded to the insult by alterations in both quantity and quality of mucus might be interpreted as evidence for a protective mechanism preventing major damage to the airway epithelium or the parenchyma. With higher doses of smoke or more prolonged exposure, the mucus overload might become too great, leading to a cycle of reduced clearance rate, infection, and damage.

The carboxyhemoglobin level, as in index of smoke exposure, was considerably more elevated than that reported by Park et al. (9) and indicates that the dogs were exposed to regular periods of hypoxia. Despite this fact, they did not show evidence of the polycythemia reported by Auerbach et al. (4). The reasons for these discrepancies of response are not apparent.

The relative independence of the variables relating to mucus secretion and clearance is interesting. The alterations in mucus viscoelasticity and the galactose content in the first four months of exposure are consistent with the secretion of a more dilute glycoprotein matrix. For the dogs that did not develop major hypersecretion, the total glycoprotein output was probably not much changed, i.e., most of the excess volume of mucus could be attributed to increased water secretion. In the dogs that developed major hypersecretion (> 5x control), the fall in galactose content was far less than the rise in secretion volume, indicating that in these dogs, total glycoprotein output probably increased.

The dissociation between the galactose assay and viscoelasticity in more prolonged exposure suggests an alteration in the biochemical nature of the mucus secreted. This could certainly be consistent with the secretion of a more acid glycoprotein, as has been reported for smoke or SO_2 exposure (5,6). This aspect is worthy of further study.

The fact that TMCR changed very little if at all over the course of the smoke exposure period, while the mucus collection rate varied widely, indicates that mucus collection rate, in this series, is a reasonable indicator of secretion volume. It also showed that the ciliary system remained intact, or at least functional, and suggests that the cilia are capable of handling a wide range of loads before mucociliary dysfunction occurs.

The lack of any correlation between the mucus hypersecretion and the Reid index is somewhat surprising. It might suggest that the

increased mucus production is due to goblet cell hyperplasia rather than glandular hypertrophy. Mucus occluding the lumens of small airways was observed frequently in smokers but not in non-smokers, but an examination of two extreme cases - a smoker with considerable hypersecretion and a non-smoker with no change in production failed to reveal any apparent excess of goblet cell numbers in the small airways. This might suggest that the mucus hypersecretion was due to structures intermediate between the small airways and the trachea. Another possibility is that the excess mucus production was due mainly to an increase in production rate rather than an increase in production capacity. Finally, the alteration in mucus quantity could arise from a difference in mucous gland cell type, with those with a higher volume output being favoured.

In summary, we have shown that chronic mucus hypersecretion can be developed in dogs with 6 to 10 months' exposure to whole cigarette smoke via a tracheostomy. The hypersecretion is highly variable but relatively stable, lasting for at least two months, so that smoke-exposed dogs can be used in studies of pulmonary function requiring animals with elevated secretion levels.

REFERENCES

1. Goodman RM, Yergin BM, Landa JF, Golinvaux MH, Sackner MA. Relationship of smoking history and pulmonary function tests to tracheal mucus velocity in non-smokers, young smokers, ex-smokers, and patients with chronic bronchitis. Am Rev Respir Dis 1978; 117:205-214.

2. Foster WM, Langenback E, Bergofsky EG. Disassociation in the mucociliary function of central and peripheral airways of asymptomatic smokers. Am Rev Respir Dis 1985; 132:633-639.

3. Cosio MG, Hale KA, Niewoehner DE. Morphologic and morphometric effects of prolonged cigarette smoking on the small airways. Am Rev Respir Dis 1980; 122:265-271.

4. Auerbach O, Hammond EC, Kirman D, Garfinkel L, Stout AP. Histologic changes in bronchial tubes of cigarette-smoking dogs. Cancer 1967; 20:2055-2066.

5. Lamb D, Reid L. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. Brit Med J 1969; 1:33-35.

6. Mawdesley-Thomas LE, Healey P, Barry DH. Experimental bronchitis in animals due to sulphur dioxide and cigarette smoke: An automated quantitative study. In: "Inhaled Particles III". WH Walton ed., Gresham Press, Old Woking, Surrey, England. 1971, pp. 509-525.

7. Hayashi M, Sornberger GC, Huber GL. Morphometric analyses of tracheal gland secretion and hypertrophy in male and female rats after experimental exposure to tobacco smoke. Am Rev Respir Dis 1979; 119:67-73.

8. Wanner A, Hirsch JA, Greeneltch DE. Tracheal mucous velocity in beagles after chronic exposure to cigarette smoke. Arch Environ Health 1973; 27:370-371.

9. Park SS, Kikkawa Y, Goldring IP, Daly MM, Zelefsky M, Shim C, Spierer M, Morita T. An animal model of cigarette smoking in beagle dogs. Am Rev Respir Dis 1977; 115:971-979.

10. King M, Engel LA, Macklem PT. Effect of pentobarbital anesthesia on rheology and transport of canine tracheal mucus. J Appl Physiol 1979; 46:504-509.

11. Dubois H, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 23:350-356.

12. King M, Macklem PT. Rheological properties of microliter quantities of normal mucus. J Appl Physiol 1977; 42:797-802.

13. Battista SP, Guerin MR, Gori GB, Kensler CJ. A new system for quantitatively exposing laboratory animals by direct inhalation. Arch Environ Health 1973; 27:376-382.

14. King M, Phillips DM, Zidulka A, Chang HK. Tracheal mucus clearance in high frequency oscillation II: Chest wall versus mouth oscillation. Am Rev Respir Dis 1984; 130:703-706.

15. Thurlbeck WM, Angus GE. The variations of Reid index measurements within the major bronchial tree. Am Rev Respir Dis 1967; 95:551-555.

16. King M. Interrelation between mechanical properties of mucus and mucociliary transport: Effect of pharmacologic interventions. Biorheology 1979; 16:57-68.

Chapter 4

A STUDY OF ELASTIC RECOIL AND PARENCHYMAL MORPHOLOGY IN CIGARETTE SMOKING BEAGLES

ABSTRACT

Ten tracheostomized beagles were exposed to cigarette smoke for periods of time ranging from two to ten months. After an initial run-in period, each dog smoked 10 cigarettes (20 mg tar, 1.2 mg nicotine) a day over 2.5 hr., 5 days a week. Using a point count system, an index of parenchymal destruction D.I. was obtained and compared to a more widely used morphometric index Lm. Quasi-static P-V curves were obtained in the prone position from smokers and sham smokers at 2, 6, and 10 months of exposure. P-V data between TLC and FRC were then fitted to an exponential function of the form $V = A\text{-}Be^{-kp}$, using an r^2 optimization procedure, and from the computer fitted curves the half-inflation pressure h, the exponential constant k, and elastic recoil pressure at 90% TLC were derived. Although Lm did not differ significantly in the 10 month smokers and sham smokers, significant differences between the DI of the two 10 month groups ($p < 0.05$) were found. PL_{90}, h, and k all demonstrated ($p < 0.05$) significant differences in elastic recoil in the 10 month smokers and sham smokers though neither of the three correlated with Lm or DI. Of the three tests k was found to be the best predictor of loss of elastic recoil. These findings suggest that 10 months of exposure to tobacco smoke is sufficient to demonstrate a significant loss of elastic recoil. Our results also suggest that DI is a more sensitive index than Lm in detecting and quantitating early parenchymal changes associated with cigarette smoking.

Key words: cigarette smoke, elastic recoil, parenchymal destruction, and exponential analysis.

INTRODUCTION

The pulmonary abnormalities produced by or associated with cigarette smoking are multifactorial. These include abnormalities of mucus clearance (1-4), alterations in pulmonary epithelial structure (5,6), airway reactivity (7,8), and changes in pulmonary vasculature (9-11). These changes are clearly recognizable when the disease is already well established but the order of development and the interaction between them is not fully understood. It is thus important to develop an animal model of tobacco smoke injury that enables the investigator to study the natural progression of the disease and allows to study morphological-function correlates in the early stages of the disease.

The effects of chronic exposure to tobacco smoke in dogs have been described. Auerbach et al. (12,13) examined the effects of smoke exposure in tracheostomized dogs and found extensive morphological changes in both parenchyma and airways. Frasca et al. (14) found extensive changes in parenchyma, in particular an increase in the number and size of fenestrae. Park et al. (15) using a face mask delivery system found modest changes in FRC and pulmonary resistance after one year of smoking. No parenchymal changes were found however in the canines.

Various attempts have been made to study pulmonary function in human smokers. These studies however are for the most part cross sectional in nature and not longitudinal. Investigators have looked at such indices as elastic recoil pressures at standard volumes, forced expiratory flows, and the pressure-volume relationship of the lung.

Pulmonary compliance has been used to analyze the recoil properties of the lungs by measuring the slope of the deflation limb of the P-V curve in the tidal range. The compliance expresses the volume change per unit of pressure change in L/cmH_2O. Problems, however, arise by the use of chord compliance when comparing different individuals and when comparing healthy versus diseased subjects. The first problem arises from the direct comparison of recoil properties of individuals of different sex, height, weight, and lung volume. Compliance measurements are further complicated by the curvilinearity of the pressure-volume relationship and the use of linear approximations over a comparatively small volume range. Predicted lung volumes that are based on anthropometric data are subject to some variability hence are of questionable value in making corrections.

In order to bypass these difficulties Salazar and Knowles introduced an exponential mathematical expression describing the elastic recoil forces of the lung (16). They found the pressure-volume characteristics of the lungs could be described by the following exponential function:

$$V = Vo\ (1 - e^{-kP}), \qquad (1)$$

where V and P are the actual volume and pressure data and Vo is the maximal pulmonary volume measured from the resting position. The above expression can accommodate volumes over the whole inspiratory capacity and does not require the use of FRC as a reference point. Due to the curvilinearity of the P-V relationship the curve can be visualized as having an infinite number of different slopes. The volume change per

unit pressure change can therefore be expressed as its derivative with dV/dP tending to zero as the volume approaches the maximal pulmonary volume Vo. dV/dP is clearly then a function of the difference (Vo - V). Salazar and Knowles expresses this by the following equation:

$$dV/dP = K\ (Vo - V), \qquad (2)$$

where K = constant. If one assumes that V = 0 when P = 0, the solution to the differential equation becomes

$$V = Vo\ (1 - e^{-kP}),$$

which is identical to that given in (1). Vo represents the volume at infinite pressure whereby dV/dP = 0, and where Vo is greater than the inspiratory capacity. As previously mentioned the P-V curve can be fitted over the whole range of volumes above FRC. Below FRC, the data deviate from the exponential function, possibly due to airway closure as well as artifact in the esophageal balloon measurements produced by the mediastinal contents.

By virtue of the exponential character of the equation, Salazar introduced the concept of the half-inflation pressure h, which can be described as follows;

$$e^{-kh} = 1/2 \text{ or } h = \ln 2/k \qquad (3)$$

Since the half-inflation pressure h is independent of lung volume, it is of practical importance. Salazar and Knowles (16) demonstrated this from data taken from Nissel et al. (17) whereby the half-inflation pressure h remained unchanged despite changes in absolute lung volume. Salazar found no correlation between h and such indices as FRC, height, VC, and TLC. Furthermore they found h to be constant throughout the IC range.

Consequently h can be regarded as an index of the stiffness of the lung or more simply as an index of the steepness of the slope. Thus with a smaller half-inflation pressure h, one would expect a steeper slope or in other words less transpulmonary pressure necessary to reach a designated volume.

Exponential analysis has been used to describe the effect of aging (18,19) and to assess the loss of elastic recoil found in emphysema patients (20-22). Paré and co-workers concluded that the exponential constant k was the best predictor of emphysema in individual subjects, although k failed to distinguish patients with mild emphysema from those without emphysema (22). Greaves et al. demonstrated a good correlation between emphysema grade and the exponential constant k on post-mortem lungs (21). Berend et al. found a similar correlation between k and emphysema grade in excised lungs from patients with isolated tumors. However, tests of elastic recoil (either k or chord compliance) were ineffective in distinguishing patients with mild emphysema (score 50) from those without, while diffusing capacity, particularly the fractional uptake of CO correlated better with the degree of emphysema in this range (23). Gibson et al. using a similar analysis, found abnormally high values of k in patients with a clinical diagnosis of emphysema when compared to controls (20).

The effect of smoking on pulmonary mechanics in this study was assessed by serially fitting the quasi-static pressure-volume deflation curves to the single exponential function of Salazar and co-workers (16). This method allows the computation of a shape factor (k),

interpreted as the bulk elastic constant of the lungs, which may be compared for each dog before and after the smoking or control intervention. In addition, necropsy histological examination of the lungs allowed the quantitative assessment of the effect of smoking by comparing the physiological data with the destructive index (D.I.), a morphometric index introduced by Saetta et al. (24), which uses the abnormal or destructive fenestration of alveolar walls as an early marker of emphysematous change. Some of the dog lungs were also micro-dissected and prepared for scanning electron microscope studies.

The principal objective of this study was to attempt to define and characterize both morphological and functional changes in a chronic canine model of tobacco smoke injury. The exponential constant k, half-inflation pressure h, and PL_{90} were all used to assess any possible loss of elastic recoil.

MATERIAL AND METHODS

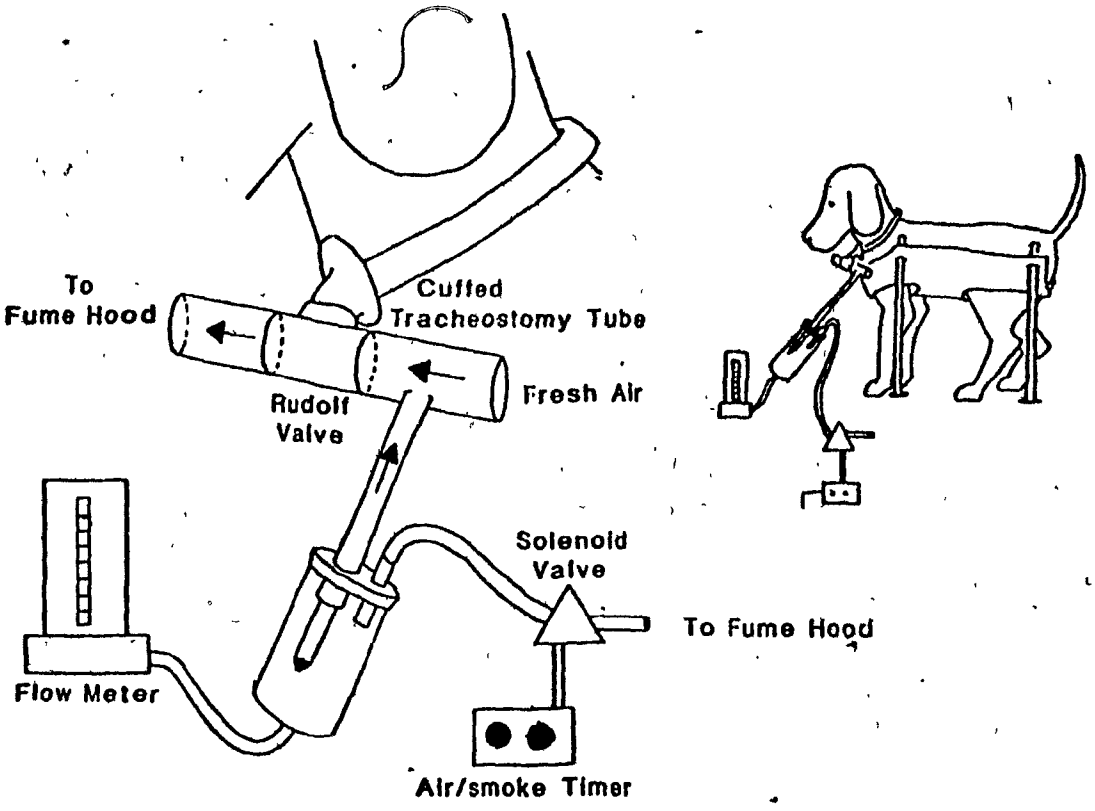
Seventeen normal beagles weighing 8 to 16 kg were selected for the study. Each dog was surgically prepared with a chronic tracheostomy and then left for at least three weeks to recover. None of the dogs had any indication of any respiratory infection throughout the duration of the study. A number of physiological studies were performed to establish control values during a four month initialization period. Cigarette smoke exposure began immediately after this phase of the study.

Smoke exposure method

The delivery system employed for the the study was similar to others described in the literature (25). The dogs were trained to stand quietly in a harness and accept a cuffed tracheostomy tube (Fig. 4.1). To generate smoke, a positive pressure was applied to a lit cigarette by closing a solenoid valve through which a bypass flow of air normally passed. The solenoid closure period was set by a timer to deliver a 35 ml bolus of smoke to the inspiratory line every 20 seconds until the

Figure 4.1.

Smoke exposure system. To generate smoke a positive pressure is applied to a lit cigarette by closing a solenoid valve through which a bypass flow of air normally passes. Inset: View of dog in harness with cuffed tracheostomy tube.



cigarette was smoked to a standard butt length of 23 mm. After the smoke was fed into the inspiratory line, the dog breathed a mixture of fresh air and smoke via a Rudolf valve with the expirate leading off into a fume hood. The cigarettes that were used were unfiltered 70 mm high tar, high nicotine cigarettes (20 mg tar, 1.2 mg nicotine). The cigarette dose was increased gradually over a run-in period of two weeks up to a maximum of 10 cigarettes per day, delivered over the course of approximately 2.5 hrs, 5 days a week, for a ten-month period of time. All dogs ceased to smoke three to five days prior to the initiation of the experimental procedures.

Pressure-volume curves

Each dog was studied under sodium pentobarbital (Nembutal) anesthesia in the prone position. After being intubated with a cuffed endotracheal tube a venous line was established to maintain the level of anesthesia. Measurements were made in a volume displacement body plethysmograph. Excursions in volume were obtained from the plethysmograph by the movement of the wedge spirometer which displaces the core of a linear variable differential transducer (LVDT). Flow at the mouth was measured with a Fleisch No. 2 pneumotachygraph connected to a Validyne (MP 45 $\pm$ 2 cmH_2O; Validyne Co., Northridge, CA) pressure transducer. Transpulmonary pressure was obtained by the electrical subtraction of Pes from Ptr and was measured with a differential pressure transducer (Validyne MP 45 $\pm$ 100 cmH_2O). An esophageal balloon (5 cm long; circumference, 1.44 cm) was attached to PE-200 polyethylene tubing

with multiple side holes within the balloon. The balloon was passed into the stomach and then withdrawn gradually until a negative pressure was recorded. The balloon was then positioned to record a minimum transpulmonary pressure swing during an occluded breath. Further adjustments were then made to minimize cardiac artifact. Balloon volume was maintained at 0.6 ml and checked periodically throughout the experiment. A second catheter (PE-200) with its end sealed, but with numerous side holes in its terminal end was placed into the tracheal cannula to measure changes in tracheal pressure. All signals were recorded on a Hewlett Packard 8-channel recorder. Functional residual capacity (FRC), defined as the lung volume at end expiration during spontaneous breathing, was determined by the method of Dubois et al. (26). To measure quasi-static lung pressure-volume curves a system of electronically controlled solenoid valves was connected to a flow meter and vacuum reservoir. This was used to inflate and deflate the lungs at a constant flow. After a volume history was obtained the dogs were inflated to TLC which was defined as the lung volume at Ptp = 30 cmH_2O. From TLC the dogs lungs were deflated at a constant flow rate of 50 ml/min to RV and then reinflated to 15 cmH_2O to relieve any bronchoconstriction.

Quasi-static pressure-volume curves obtained as described above were plotted on 25 x 38 cm graph paper and digitized manually, obtaining at least 30 points to span the range of lung volumes from TLC to FRC.

All P-V points above FRC were fitted to a single mono exponential equation:

$$V = Vmax - (Vmax - Vo)\, e^{-kP}$$

using an interactive least-squares technique on the original scale (non-transformed) data. Adequacy of fit was tested by computing r^2, the residual variance, and in selected cases by the runs test. This method of analysis provided a satisfactory fit in the majority of th PV curves. Values for the transpulmonary pressure at 90% of measured TLC (PL_{90}) were derived from the computer plotted curves along with h, the half-inflation pressure and k, the exponential constant describing the shape of the curve. All data was subjected to statistical analysis using paired and unpaired two tailed t-tests. A value of $p < 0.05$ was considered significant.

Morphology

All specimens were fixed with 10% buffered formalin by intrabronchial infusion for at least 48 hours at a constant distending pressure of 20 cmH_2O. The lungs were then sectioned sagitally and random templates obtained for light microscopy and scanning electron microscopy. The parenchymal destructive index (DI) was calculated for the two groups by light microscopy, using a point count method to quantitate the percentage of destroyed space as a fraction of the total alveolar and duct space (Saetta et al. 24). The mean linear intercept (Lm) was also determined for the smokers and non-smokers. Pieces of lung

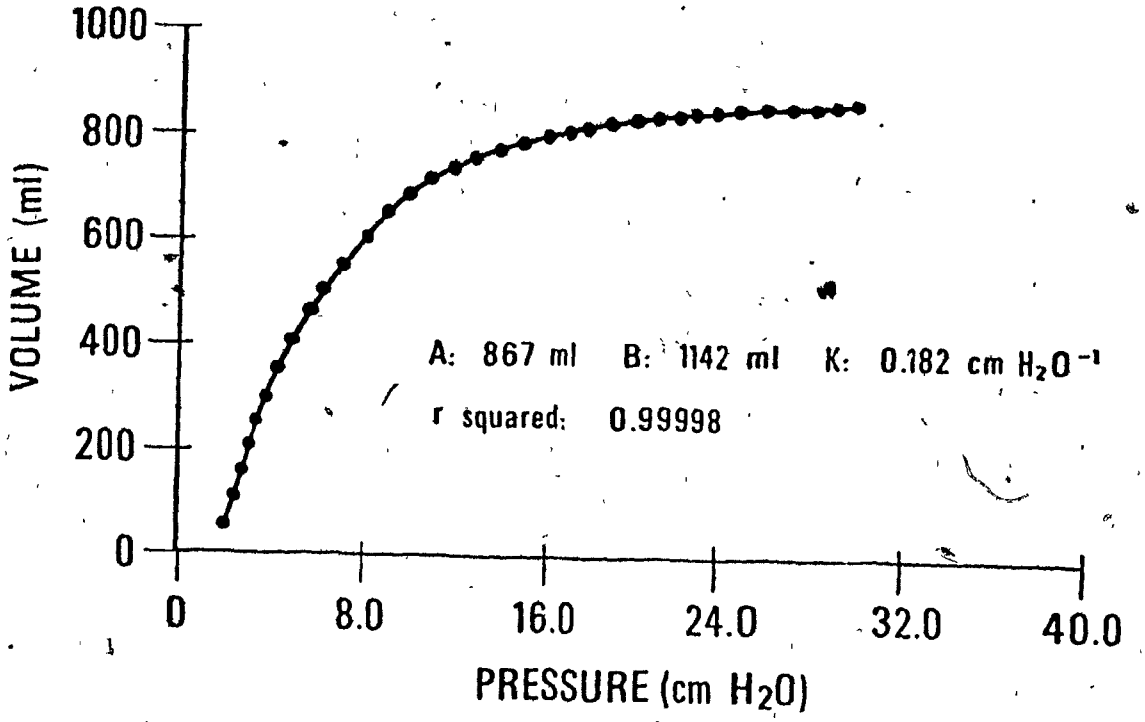
parenchyma adjacent to those taken for light microscopy were further dissected to expose the terminal bronchiole and distal alveoli. The specimens were then fixed in gluteraldehyde and then dehydrated with graded acetone solutions. After dehydration specimens were dried by the critical point method of Wang and Wei (27) using liquid CO_2 and then mounted on aluminum blocks for sputtering with a gold-palladium mixture.

RESULTS

The quality of fit for most dogs was very good (Fig. 4.2) over the volume range used, both with regard to the reduction of original variance (r^2) as suggested by Salazar and Knowles (16) and on visual inspection. At least three P-V curves were obtained from each dog at control, two, six, and ten months of study. With few exceptions

Figure 4.2

An example of P-V relationship derived by fitting the original non-transformed data to an exponential function (dog 9CQ2, control). Data points between TLC and FRC were fitted to the curve.



individual P-V curves were highly reproducible in each dog. The P-V relationships derived by fitting the original nontransformed data to the monoexponential function demonstrated a significant loss of elastic recoil in the smoking group at 10 months of exposure. All of the tests used (PL_{90}, h, k, and DI), except Lm, separated the 10-month non-smokers from the smokers. The individual data for PL_{90}, DI, Lm, h, and k are summarized for the two groups at 10 months in Table 4.1. The

Table 4.1

Definition of abbreviations: DI - destructive index; k - bulk elastic constant; h - half-inflation pressure; PL_{90} - transpulmonary pressure at 90% of measured TLC.

	DATA AT TEN MONTHS OF STUDY				
10 MONTH SMOKERS (N=7)	PL90	H	K	DI	Lm
9AC1 #	9.25	2.98	.233	8.6	.158
0M01	9.24	2.82	.246	6.6	.124
0M02	10.42	3.33	.200	9.8	.144
9CQ2	10.97	3.66	.190	16.6	.164
1082	9.07	3.30	.210	20.6	----
2JD1	12.25	4.59	.151	9.5	----
2HL1	7.95	3.00	.224	8.0	----
	9.88±1.43	3.40±.59	.209±.03	11.39±5.2	.148±.017
10 MONTH NONSMOKERS (N=4)					
9BP1	10.39	3.84	.181	3.0	.132
0M03	12.85	4.38	.159	6.1	.155
9AC4	15.44	4.85	.143	5.8	.153
2DU3	10.92	4.00	.174	7.5	----
	12.4±2.29	4.27±.45	.164±.02	5.6±1.89	.146±.012

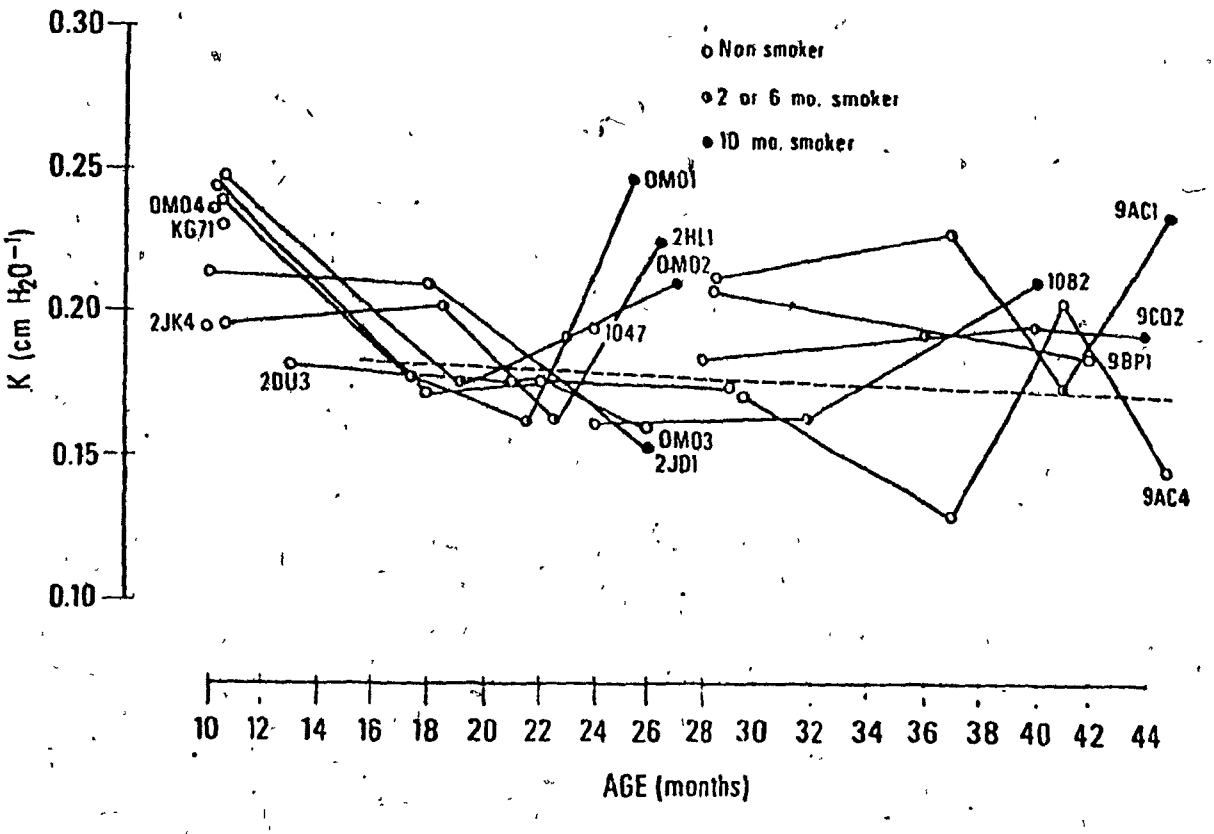
mean-initial pre-exposure values for PL_{90}, k, and h were not significantly different between smokers and non-smokers. The half-inflation pressure h, which represents the transpulmonary pressure needed to inflate the lung to 50% of the maximal pulmonary volume from the resting volume Vr, and was significantly smaller ($p < 0.05$) in the 10 month smokers when compared to the non-smokers. The mean half-inflation pressure h for the smokers was 3.40 $\pm$ 0.59 cmH_2O and 4.27 $\pm$ 0.45 cmH_2O for the non-smokers at 10 months. The exponential constant k, in the 10 month smokers was significantly higher ($p < 0.05$) than the non-smokers. The mean value of k for the smokers was 0.209 $\pm$ 0.031 cmH_2O^{-1} and 0.164 $\pm$ 0.017 cmH_2O^{-1} for the non-smokers at 10 months. The mean values of k for the non-smokers are higher than the values given by Haber et al. (28) in excised dog lungs filled with air. PL_{90} was significantly lower at ten months ($p < 0.05$) when compared to the non-smokers. Of the three physiological parameters used to assess loss of elastic recoil, k was the most sensitive and resulted in the best separation of the two groups. The mean value of r^2 was 0.99 and most of the P-V relationships passed the runs test. There was no significant difference in the mean r^2 values between smokers and non-smokers and futhermore h, PL_{90}, and k were all highly reproducible in both groups. The exponential constant k correlated well with both PL_{90} ($r = 0.843$, $p < 0.01$) and h ($r = 0.981$, $p < 0.001$) respectively.

Several of the dogs that were studied were immature at the time of first study (10-10 1/2 months), and it was apparent that their P-V curves were of different shape than older control beagles. When we analyzed the

P-V curve data in terms of absolute age, we found the relationship illustrated in Fig. 4.3. The mean k values for the seven dogs studied at age 10 or 10 1/2 months was significaltly higher than mean k value for beagles over 1 year in age on first study (0.225 $\pm$ 0.021 versus 0.186 $\pm$ 0.019, $p < 0.005$). The control data for (first study and sham smoking)

Figure 4.3

Bulk elastic constant k versus absolute age. Open symbols refer to non-smoking or sham smoking dogs. Filled or partially-filled symbols refer to smoking dogs. Solid lines inicate serial data in individual dogs. The dashed line is the line of best fit for all control points greater than 12 months.



described a nearly horizontal line with a slight downward slope (r = -0.18, slope = -0.00044). Six of the seven data points for k at 10 months of smoking lay above the line of best fit. Furthermore, in six of the seven smoking dogs, the line of best fit (ignoring data for less than 1 year) showed a positive slope. All four non-smoking shams showed a slight negative slope over the same time frame. The difference is significant.

DISCUSSION

It is generally believed that cigarette smoking can lead to the development of emphysema in certain people. Previous investigators have attempted to correlate lung function and emphysema by making either in vivo measurements of pulmonary function in patients with a clinical diagnosis of emphysema or by the analysis of postmortem excised lungs or lobes. The anatomical destruction of lung parenchyma in emphysema has been related to the functional loss of elastic recoil pressure. Our study tested the ability of 3 tests (PL_{90}, k, h) to assess any early loss of elastic recoil pressure following ten months of cigarette smoke exposure and to relate these changes to two morphometric indices of parenchymal destruction (DI and Lm). Investigators have already demonstrated a decrease in lung elastic recoil with advanced anatomical emphysema (29,30). In this study, we investigated the changes in lung elastic recoil associated with cigarette smoking in an equivalent model of a seven pack year smoker.

The first attempt to describe the P-V curve by a mathematical function was done by Salazar and Knowles (16). Niewoehner and co-workers on the other hand proposed a different exponential model with two independent constants describing the effects of elastic behavior and size on the P-V curve (31). Paiva and associates used a sigmoid mathematical model to describe the P-V curve and found it resulted in a better fit than the currently used monoexponential function used in this study and first proposed by Salazar and Knowles. For the expiratory curves the model of Paiva provided a significantly better fit than that of Salazar in 20 young subjects (32).

In our study, the Salazar equation provided an accurate description of the deflation limbs of the P-V relationship over the range of volumes used. This equation provided a better fit than did the model of Paiva and associates (32) as was determined both by visual inspection and by the reduction in variance afforded by the theoretical P-V curves (Fig. 4.2). Numerous studies have demonstrated a loss of elastic recoil with age (18,19,33-36). A decrease in elastic recoil has also been demonstrated in excised lungs with a mild grade of emphysema less than or equal to 5 (36). Our results indicate that significant changes occurred in elastic recoil in the 10 month smokers when compared to the non-smokers even though the smokers had no indication of emphysema in the classical sense, i.e., airspace enlargement, since Lm was normal.

Greaves et al. found that the exponential constant k was related to the mean linear intercept (Lm) which attempts to define mean alveolar

diameter in the maximally inflated, liquid filled lung (21). The mean linear intercept of four smokers and four non-smokers (Table 4.1) showed no significant difference between the 10 month smokers and non-smokers. A major limitation in the usefulness of the Lm in quantitating emphysema is that increased dilation in the absence of destruction leads to an increase in Lm unrelated to classic emphysema. One also tends to find an increase in Lm in an aging lung with non apparent parenchymal destruction (21). In this study we compared DI with Lm in a series of lungs of smokers and non-smokers, and in addition made a comparison between DI and the three parameters studied (h, k, and PL_{90}). The destructive index (DI) separated the 10 month smokers from the controls (Table 4.1). DI however was not found to correlate significantly with either the half inflation pressure h, the exponential constant k, or PL_{90}, perhaps due to the small range of DI values determined. Saetta et al. found DI to correlate best with PL_{90} and found it to be a far more sensitive test in distinguishing the smokers and non-smokers when compared to Lm (24).

The exponential constant k is beneficial in that it is independent of lung volume and uses a much greater range of volumes than do other conventional means like chord compliance. This study confirms the observations of Paré et al. (22) that k the exponential constant was the best predictor of loss of elastic recoil in the smokers when compared to either h or PL_{90}. Berend et al. (23) found the use of k did not improve the discriminatory power of the P-V curve to detect emphysema. In fact they found measurements of diffusion such as Fco to correlate best with the grade of emphysema.

The values of h obtained here for our control dogs are smaller than those calculated from the figures presented by Wohl and co-workers (37). It must be emphasized that these authors did static P-V curves instead of quasi-static P-V curves and may have employed a different technique of fitting the data. The half-inflation pressure h, separated out the 10 month smokers from the non-smokers ($p < 0.05$) but failed to correlate with DI. In an unpaired t-test for the individual 10 month data PL_{90} was sufficiently sensitive to separate out the smokers from the non-smokers ($p < 0.05$). Saetta et al. (24) found DI to correlate best with PL_{90} ($r = -0.61$, $p < 0.05$) in a study of 23 human smokers whereas in our own study in beagles it did not ($r = 0.096$).

Numerous investigators have suggested other methods to correlate pulmonary function with postmortem or post-surgery morphometric measurements of emphysema. Burrows et al. (38) found measurements of diffusing capacity correlated well with emphysema grade whereas the studies of Boushy et al. (39) and Park et al. (29) concluded that the best predictors of emphysema were diffusing capacity and static recoil pressures combined together.

Frasca et al. (14) produced pulmonary fibrosis and emphysema in a study in which tracheostomized beagles were exposed to cigarette smoke of higher tar content than our own for 7 days a week, at a peak dose of seven cigarettes a day for a maximum period of 4 months. Examination of the lungs by scanning electron microscopy revealed subpleural enlargement of air spaces and widespread air space enlargement with scattered tissue destruction in all lobes. In view of these results we might have

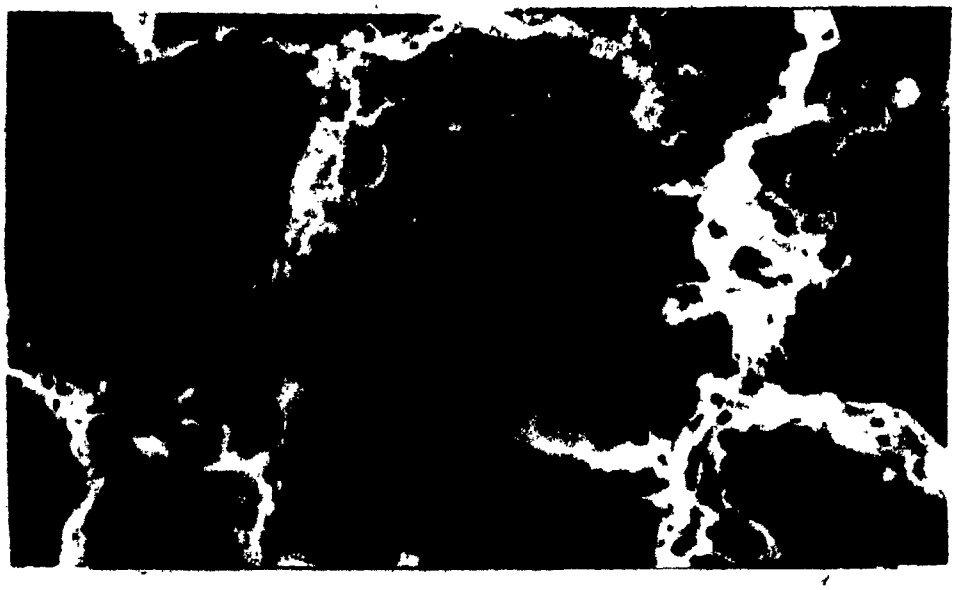
expected comparable changes at the very least in view of the fact that our dogs smoked for a longer period of time. Examination of the electron micrographs (SEM) provided by Frasca et al. (14) demonstrate in a rather striking way that despite a slight increase in the number and size of fenestrae in our 10 month smokers they appear to be no more affected than the controls in the Frasca study (Fig. 4.4).

In conclusion, our results are consistent with the idea that, with 10 months of cigarette smoke exposure, the exponential constant k, the half inflation pressure h, and PL_{90} were all able to demonstrate a loss of elastic recoil pressure in the 10 month smokers when compared to the 10 months controls. K was the best predictor of loss of elastic recoil but did not correlate with a morphometric index of parenchymal destruction, DI. The mean linear intercept (Lm) failed to distinguish smokers from non-smokers at ten months due to considerable overlap in the data. Examination of the data of Frasca et al. (14) reveal that considerable parenchymal damage was produced in their beagles whereas in our own study results of the scanning electron micrographs demonstrate only slight alterations in a model where the canines smoked for a longer period of time (10 months as opposed to 4 months). The Salazar equation proved to be an effective means of assessing early loss of elastic recoil pressure in our chronic model of cigarette smoke exposure.

Figure 4.4
Electron micrographs taken from smokers and sham-smokers.

(a) Electronmicrograph of alveoli of a sham-smoker, showing numerous fenestrae. (x 200) (from Frasca et al.)

(b) Electronmicrograph of alveoli of a 4 month smoker. Alveoli appear distorted with numerous fenestrae bridged by delicate strands of tissue. (x 200) (from Frasca et al.)



(c) Electronmicrograph of alveoli of a ten month smoker from the present study (dog 9CQ2). The fenestrae do not appear to differ significantly in number or size from the sham-smokers of Frasca et al. (x 300)

REFERENCES

1. Goodman RM, Yergin BM, et al. Tracheal mucous velocity in non-smokers, young smokers, ex-smokers, and patients with chronic bronchitis. Am Rev Respir Dis 1978; 117:205-214.

2. Foster WM, Langenbach E, and Bergofsky EG. Study of the early mucus membrane abnormality in smokers. Am Rev Respir Dis 1980; 121(2):134 (abstract).

3. Wanner A, Hirsch JA, Greeneltch DE. Tracheal mucus velocity in beagles after chronic exposure to cigarette smoke. Arach Environ Health 1973; 27:370-371.

4. Park SS, Kikkawa Y, Goldring IP, Daly MM, Zelefsky M, Shim C, Spierer M, Morita T. An animal model of cigarette smoking in beagle dogs. Am Rev Respir Dis 1977; 115:971-979.

5. Simani IAS, Inoue S, Hogg JC. Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. Lab Invest 1974; 31:75-87.

6. Boucher RC, Johnston J, Inoue S, Hulbert W, Hogg JC. The effect of cigarette smoke on the permeability of guinea pig airways. Lab Invest 1980; 43:94-100.

7. Roehrs JD, Rogers WR, Johanson WG Jr. Bronchial reactivity to inhaled methacholine in cigarette smoking baboons. J Appl Physiol 1981; 50:754-760.

8. De Sanctis GT, Saetta MP, Wight A, Kelly SM, Shiner RJ, Stril JL, Phillips DM, Hakim TS, King M. Cigarette smoke exposure in dogs causes mucus hypersecretion and hyporesponsiveness to aerosolized but not infused methacholine. Fed Proc 1985; 4 :614 (abstract).

9. Auberbach O, Stout AP, Hammond EC, Garfinkel L. Smoking habits and age in relation to pulmonary changes: Rupture of alveolar systems, fibrosis and thickening of walls of small arteries and arterioles. N Engl J Med 1963; 269:1045-1054.

10. Hale KA, Niewoehner DE, Cosio MG. Morphologic changes in the muscular pulmonary arteries: Relationship to cigarette smoking, airway disease and emphysema. Am Rev Respir Dis 1980; 122:273-278.

11. Hakim TS, King M, Wang CG, Cosio M. Effect of chronic cigarette smoke exposure on pulmonary vasomotion in beagle dogs. J Appl Physiol 1985 (in press).

12. Auberbach O, Hammond EC, Kirman D, Garfinkel L. Emphysema produced in dogs by cigarette smoking. J Am Med Assoc 1967; 199:89-94.

13. Auberbach O, Hammond EC, et al. Histologic changes in bronchial tubes of cigarette smoking dogs. Cancer 1967; 20:2055-2066.

14. Fransca JM, Auerbach O, Carter HW, Parks VR. Morphologic alterations induced by short-term cigarette smoking. Am J Pathol 1983; 111:11-20.

15. Park SS, Kikkawa Y, et al. An animal model of cigarette smoking in beagle dogs. Am Rev Respir Dis 1977; 115:971-979.

16. Salazar E, Knowles JH. An analysis of pressure-volume characteristics of the lungs. J Appl Physiol 1964; 19:97-104.

17. Nisell OI, Dubois AB. Relationship between compliance and FRC of the lungs in cats and measurement of resistance to breathing. Am J Physiol 1954; 178:206-210.

18. Colebatch HJH, Greaves IA, Ng CKY. Exponential analysis of elastic recoil and aging in healthy males and females. J Appl Physiol 1979; 47:683-691.

19. Knudson RJ, Kaltenborn WT. Evaluation of lung elastic recoil by exponential curve analysis. Respir Physiol 1981; 46:29-42.

20. Gibson GJ, Pride NB, Davis J, Schroter RC. Exponential description of the static pressure-volume curve of normal and diseased lungs. Am Rev Respir Dis 1979; 120:799-811.

21. Greaves IA, Colebatch HJH. Elastic behavior and structure of normal and emphysematous lungs postmortem. Am Rev Respir Dis 1980; 121:127-136.

22. Pare PD, Brooks LA, Bates J, Lawson LM, Nelems JMB, Wright JL, Hogg JC. Exponential analysis of the lung pressure-volume curve as a predictor of pulmonary emphysema. Am Rev Respir Dis 1982; 126:54-61.

23. Berend NC, Woolcock AJ, Martin GE. Correlation between the function and structure of the lung in smokers. Am Rev Respir Dis 1979; 119:695-705.

24. Saetta M, Shiner RJ, Angus GE, Kim WD, Wang NS, King M, Ghezzo H, Cosio MG. Destructive index: A measurement of lung parenchymal destruction in smokers. Am Rev Respir Dis 1985; 131:764-769.

25. Battista S, Guerin M, Gori G, Kensler C. A new system for quantitatively exposing laboratory animals by direct inhalation: Delivery of cigarette smoke. Arch Environ Health 1973; 27:376-382.

26. Dubois AB, Bottello SY, Bedell GW, Marshall R, Comroe JH. A rigid-plethysmographic method for measuring functional residual capacity in normal subjects. J Clin Invest 1956; 35:322-326.

27. Wang NS, Wei F. Scanning electron microscopy of capillary and elastic fibre networks in alveoli. In: Scanning Electron Microscopy. Johari O, Corvin I (eds). ITT Research Institute, Chicago, Ill., 1976, pp. 233.

28. Haber PS, Colebatch HJH, Ng CKY, Greaves IA. Alveolar size as a determinant of pulmonary distensibility in mammalian lungs. J Appl Physiol 1983; 54:837-845.

29. Park SS, Janis M, Shim CS, Williams MH Jr. Relationship of bronchitis and emphysema to altered pulmonary function. Am Rev Respir Dis 1970; 102:927-936.

30. Finucane KE, Colebatch HJH. Elastic behavior of the lung in patients with airway obstruction. J Appl Physiol 19691 26:330-338.

31. Niewoehner DE, Kleinerman J, Liotta L. Elastic behavior of postmortem human lungs: Effects of aging and mild emphysema. J Appl Physiol 1975; 39:943-949.

32. Paiva M, Yernault JC, Van Eerdeweweghe P, Englert M. A sigmoid model of the static volume-pressure curve of the human lung. Respiration Physiol 1975; 23:317-323.

33. Knudson RJ, Clark DF, Kennedy TC, Knudson DE. Effect of aging alone on mechanical properties of the normal adult human lung. J Appl Physiol: Respir Environ Exercise Physiol 1977; 43:1054-1062.

34. Bode FR, Dosman J, Martin RR, Ghezzo H, Macklem PT. Age and sex differences in lung elasticity, and in closing capacity in non-smokers. J Appl Physiol 1976; 41:129-135.

35. Turner JM, Mead J, Wohl ME. Elasticity of human lungs in relation to age. J Appl Physiol 1968; 25:664-671.

36. Silvers GW, Petty TL, Stanford RE. Elastic recoil changes in early emphysema. Thorax 1980; 35:490-495.

37. Wohl MEB, Turner J, Mead J. Static volume-pressure curves of dog lungs in vivo and in vitro. J Appl Physiol 1968; 24:348-354.

38. Burrows B, Fletcher CM, Heard BE, Jones NL, Wottlif JC. The emphysematous and bronchial types of chronic airways obstruction: A clinicopathological study of patients in London and Chicago. Lancet 1966; 1:830-834.

39. Boushy SF, Aboumrad MH, North LB, Helgason AH. Lung recoil pressure, airway resistance, and forced flows related to morphologic emphysema. Am Rev Respir Dis 1971; 104:551-561.

Chapter 5

CONCLUSION

Our results indicate that in an equivalent model of a seven-pack year smoker, there is a significant loss of elastic recoil as determined by three indices of pulmonary function. The transpulmonary pressure at 90% TLC (PL 90), the half-inflation pressure (h) and the bulk elastic constant (k) were all used to assess loss of elastic recoil. We related these changes to two morphometric indices of parenchymal destruction (DI and Lm) and tried to establish a correlation between the loss of functional elastic recoil and parenchymal destruction.

Pathological studies on lungs obtained from human smokers demonstrate a clear relationship between the number of cigarettes smoked and the duration of tobacco exposure to the degree of emphysema and pulmonary fibrosis (1,2). Short-term exposure to cigarettes has been found to produce certain morphological alterations often seen in models of experimental emphysema. The enlargement of interalveolar pores into fenestrae has been described in the literature (3). Examination of electron micrographs taken from a ten-month smoker (dog 9CQ2) show that the fenestrae do not appear to differ significantly in number or size from the sham smokers of this study and from the sham smokers of Frasca et al. (3). Pulmonary fibrosis and emphysema were produced in beagle dogs by direct inhalation of cigarette smoke over a four month period of time in the latter study. Our failure to find equivalent morphological evidence of emphysema and pulmonary fibrosis in our findings leads us to believe that our dogs were less vulnerable to the smoke exposure than Frasca's dogs. Since the doses were comparable we would have expected similar results otherwise. Since fenestrae have been shown to coalesce

together (4,5) possibly leading to the dilatation of alveolar ducts and respiratory bronchioles it was important to observe if these results were found in our model and, if found, to see if the dilatation could in turn result in the shortening of interalveolar septa and the enlargement of alveoli as has been already described in the literature (4).

Close examination of electron micrographs taken from the smokers and sham smokers from the present study support the hypothesis of Saetta et al. that alveolar walls are first destroyed and then there is enlargement of air spaces (6).

On this basis one could expect to measure abnormal values of DI when Lm was still within normal limits. Our findings support the above hypothesis in that there were significant differences in the destructive index (DI) at ten months of smoke exposure when the values of Lm were very similar for both groups. One important variable in the methodology of Lm deals with the degree of inflation at the time of fixation. If the lung is too emphysematous it will tend to overinflate and if it is slightly fibrotic it may underinflate. Despite numerous attempts to deal with this problem no methodology is at present available to insure perfect inflation. Because the DI is a ratio and therefore dimensionless, it is less affected by minor differences in inflation (6). In the study of Saetta et al. DI was found to correlate best with the PL 90, whereas in our study it did not correlate significantly with either PL 90, K, or h, perhaps due to the small range of DI values determined. Regardless of the lack of correlation it is interesting to

note that Berend et al. demonstrated that changes in elastic recoil may occur in the absence of anatomical emphysema in smokers (7).

Thus, it would appear from our results that subtle changes in the DI may in part have brought about a functional loss of elastic recoil. In view of the lack of correlation between DI and the three parameters (K, PL 90, h) we acknowledge the possibility that loss of elasticity and anatomical destruction may progress along non-parallel pathways and may be related only coincidentally, as has been suggested by Thurlbeck (8).

Single exponential functions have been used to improve the quantitative representation of elastic recoil in static or quasi-static P-V curves. Since the volume reached at any give PL is dependent on the retractive properties of the lungs, fitting an exponential to the data permits one to express the measurements in a form that is independent of lung volume. The overall pressure-volume curve, and the exponential function fitted to it permits quantification of the continuous change in slope of the P-V curve in units of cm H_2O^{-1}. Glaister et al. concluded that the use of chord compliance was unsatisfactory due to the non-linearity of the P-V curve (9). One distinct advantage is that the bulk elastic constant K describes the behaviour of the P-V curve over the entire inspiratory capacity range. We found no correlation between K and either DI or Lm in the smokers at ten months intervention but did find K to be a better predictor of loss of elastic recoil than either PL 90 or h. The half-inflation pressure (h) separated out the ten-month smokers from the sham smokers but failed to correlate with any of the morphometric indices.

In our study, the Salazar equation provided accurate and highly reproducible fits on the original nontransformed data. The quality of fit was good overall, as determined either by visual inspection or by the residual of variances (r^2). Our initial attempts at using logarithmic transformations produced poor results, with the exponential fit deviating systematically at lower lung volumes. Those of a sigmoidal function also proved to be problematic, with the computer-fitted curves deviating at lower lung volumes as well.

A value of K above the normal range is considered to be indicative of loss of elastic recoil. However, it could also indicate incomplete maturation. When K was plotted as a function of absolute age in the seven smoking beagles prior to smoking, four sham controls, and four other controls, the exponential constant in these non-smokers was significantly higher in the young dogs (less than one year) than in the older dogs (over 1 year old at the time of first study). Therefore data for dogs less than one year old were excluded from further analysis. Based on the remaining data, of the seven smoking dogs, six showed an upward trend in K, and the values after 10 months of smoking ended up above the best fit line of the non-smokers. Thus, once the P-V curve data were analyzed according to the absolute age of the dogs, it became apparent that there was a significant difference in K between the ten-month smokers and age-matched sham smokers.

The above results indicate that significant changes occurred in elastic recoil in the ten-month smokers when compared to the sham smokers. This loss of elastic recoil occurred in the absence of air

space enlargement, since the mean linear intercept was normal. Since DI is derived by point-counting alveoli and alveolar duct spaces surrounded by destroyed tissue as a percentage of total space, it is reasonable to conclude that changes in DI will precede changes in Lm. The range of DI (8.0-20.6) is well below the range reported in human smokers (6).

In summary, we have demonstrated that ten months of cigarette smoke exposure in tracheostomized beagles is enough to cause alterations in the DI but not the Lm. Ten months of exposure is also sufficient to lead to a significant loss of elastic recoil as measured by K, PL 90, and h. Since the loss of elastic recoil is out of proportion to the damage seen we conclude that there might be other underlying mechanisms involved to account for the loss of elastic recoil.

Changes in tracheal mucus clearance rates have been studied in humans and in animal models. Experiments conducted in animal models and human studies have often yielded conflicting results and conclusions. Recent studies have demonstrated either a reduction in mucus transport in smokers (10,11), an increase in clearance (12,13), or no change in bronchial clearance rates (14,15). Variations in experimental technique and exposure period make it difficult to compare the results and conclusions from these studies.

In our study the data obtained from the smokers demonstrate no difference in clearance rates between the smokers and non-smokers. This is in sharp contrast to the findings of Wanner et al. (16), who reported a significant decrease in tracheal mucus clearance rates. In their study beagles were exposed via a face mask for a period of twelve months. As

far as the tracheal mucus clearance rates are concerned we did not observe any stasis or stagnation of pooling of mucus in the large airways. Since the beagles in the present study smoked via a tracheostomy, thus avoiding the filtration action of the upper respiratory tract, we can only speculate that the differences in tracheal mucus clearance rates between our study and that of the latter one lie in experimental technique or more probably are due to alterations in mucus rheology.

It appears from the results of the present study that tracheal mucus clearance can be affected by both the quantity and quality of secreted mucus. Our results indicate that an increase in mucus production may not be detrimental providing the mucociliary transport system is intact. Dulfano et al. observed a decrease in viscosity and an increase in elastic recoil values during periods of clinical stability in chronic bronchitics (17). The results of the present study demonstrate that in the first two to four months of the study the viscosity fell in the smokers. This is of benefit to the smokers since recent rheological studies demonstrate that mucus of high elasticity and low viscosity is best suited for optimal clearance (18-19).

Despite persistent mucus hypersecretion in eight of nine smokers, there were no significant change in tracheal mucus clearance rates in the smokers of the dogs that developed major mucus hypersecretion the fall in galactose content was in comparison, far less than the rise in secretion volume. The decrease in elasticity observed in the first two to four months was later found to rise back to the control values at six months

of studying the smokers. We conclude that the alterations in mucus viscoelasticity and galactose content during the first four months are consistent with our observations of the secretion of a more dilute glycoprotein matrix. Thus, the cilia, although more loaded were capable of clearing the more dilute glycoprotein matrix in the smokers studied.

The observed alterations in both quantity and quality of mucus may be a protective mechanism triggered by the chronic insult. This protective mechanism may also explain the near-absence of parenchymal damage and the lack of change in Reid index in the smokers. With a higher dose of tobacco smoke or a more prolonged exposure, the rate of bronchial clearance could be compromised by an excess of mucus production. Abnormalities in tracheal clearance rates may then in turn lead to mucus plugging, denudation of epithelium and a decrease in both the number of ciliated cells and the mean ciliary length in the large airways.

With short-term exposure to cigarette smoke there was a decrease in both mucus elasticity and overall galactose content, whereas with longer exposure periods (more than six months) there was a disassociation between the mucus elasticity and galactose content. A rise in mucus elasticity would be consistent with a rise in overall glycoprotein content. This however was not the case in that after six months of study, the galactose content remained low, whereas the elasticity rose back to the control values in the smokers. We suggest that this dissociation between rheology and galactose content is due to a change in the chemistry of the mucin secreted.

Although eight of the nine developed mucus hypersecretion, there was no significant difference in the Reid index between smokers and non-smokers. This discrepancy may be due to goblet cell hyperplasia rather than the glandular hypertrophy as seen in human patients. We might further attribute the hypersecretion in the smokers to an increase in production rate rather than to an increase in production capacity. Reid found an increase in goblet cells in rats exposed to tobacco smoke for a six week period. The increase in goblet cells was dose dependent and was most prominent in the proximal intrapulmonary airways (20). We cannot explain the absence of this effect in the smoking dogs.

In conclusion we have developed a reliable and reproducible model of mucus hypersecretion with six to ten months of cigarette smoke exposure. Although highly variable at times, the hypersecretion was for the most part stable throughout the present study.

The effect of smoking on airway responsiveness has not been clearly elucidated in the literature. Our data suggest that smokers have decreased airway responsiveness when compared to sham smokers. Recent studies suggest that increased airway responsiveness may be due to airway epithelial damage (21) and that smoking causes an increase in epithelial permeability in animals (22) and humans (23). Although epithelial permeability was not measured in the present study, our hypothesis was that with the development of mucus hypersecretion there would be a loss of airway responsiveness rather than an increase. Our findings in fact confirm this hypothesis and are in agreement with the studies of Drazen et al. (24), Roehrs et al. (25), and Abraham et al. (26).

Although our results indicate a decrease in airway responsiveness, other studies have shown either no difference or an increase in airway responsiveness in human smokers. The effects of chronic smoking on bronchial reactivity are difficult to interpret because one's inherent reactivity may very well influence one's decision to smoke or not. Animal studies may be easier to interpret since one removes this self-selection bias and since one can study the entire history of the animal from start to finish.

The results of the present study indicate that in the smokers with mucus hypersecretion, for a given response to the intravenous challenge, the relative response to the aerosolized bronchoconstrictor is diminished with respect to what it would have been given no mucus hypersecretion. We do not think the hyporesponsiveness was due to technical problems since the doses were calculated with extreme care and administered with the same nebulizer to all dogs studied. The fact that chronic smoke exposure slightly enhanced the airway responsiveness to intravenous methacholine most likely suggests an alteration in the sensitivity of the airway smooth muscle.

Interestingly enough, nicotine aerosols have been found to blunt bronchial reactivity to methacholine in highly reactive baboons. Wallis et al. suggested that this effect was due to the neuromuscular blocking effect of nicotine (27). This may offer an alternate hypothesis for the observed hyporesponsivenss in the smokers. We excluded this possibility on account of the fact that the dogs ceased to smoke three to five days before the bronchial provocation tests. This would have excluded any possible acute effects of nicotine.

It has been reported that the narrowing of airways by mucus could enhance inertial deposition by increasing the linear flow velocities. This would in turn result in more central particle deposition as has been observed in chronic bronchitics (28). If such was the case one would predict an increase in responsiveness due to the more central deposition of methacholine in the airways contributing most strongly to airway resistance. This was not the case since the mucus hypersecretors demonstrated a decrease and not an increase in responsiveness.

The secretory role of the goblet cells may play an important role in the lung's defense against chronic noxious insults. To avoid any possibility that these acute permeability changes might influence the bronchial provocation tests all dogs ceased to smoke three to five days prior to the challenges. Despite the increased permeability in smokers, a recent study by Kennedy et al. found no evidence of increased reactivity between smokers and non-smokers (29).

Of the sham smokers one dog developed spontaneous mucus hypersecretion. The reason for this is not known at present but the effect of the hypersecretion on methacholine aerosol responsiveness was the same, i.e., a decrease for the dogs that developed mucus hypersecretion where all of them showed a decrease in airway responsiveness vis-à-vis their infusion response.

It is interesting to speculate that there might be an intimate relationship between mucus hypersecretion, epithelial permeability, and bronchial reactivity. Ideally one might conduct a study in which a secretagogue is used to produce mucus hypersecretion but has no effect on

smooth muscle activity. This would allow the investigator to study airway responsiveness more clearly.

The importance of these studies is clearly substantiated when one considers the large numbers of smokers suffering from chronic bronchitis and/or bronchial hyperreactivity. The underlying mechanisms of bronchial reactivity and the changes in mucus rheology associated with cigarette smoking may help clarify these disease processes. It is felt that more biochemical work should be done in the future to determine whether alterations at the molecular level could account for the observed changes in mucous rheology. Permeability studies would also be useful to determine by both smoke exposure in the absence and in the presence of a mucus barrier. Lastly, more structure-function type studies are needed to study how changes in lung parenchyma may contribute to loss of elastic recoil. Such studies should include morphometric work using scanning electron microscopy to investigate the pathological history of parenchymal damage. Certain transitional changes in the parenchyma may shed light on how elastic recoil may be reduced.

REFERENCES

1. Auerbach O, Hammond EC, Kirman D, Benante C. Relation of smoking and age to emphysema: Whole lung section study. N Engl J Med 1972; 286:853-857.

2. Auerbach O, Garfinkel L, Hammond EC. Relation of smoking and age to findings in lung parenchyma: A microscopic study. Chest 1974; 65:24-35.

3. Frasca JM, Auerbach O, Carter HW, Parks VR. Morphologic alterations induced by short-term cigarette smoking. Am J Pathol 1983; 111:11-20.

4. Port CD, Ketels KV, Coffin DL, Kane P. A comparative study of experimental and spontaneous emphysema. J Toxicol Environ Health 1977; 2:589-604.

5. Pratt PC, Kilburn KG. A modern concept of the emphysemas based on correlations of structure and function. Hum Pathol 1970; 1:443-463.

6. Saetta M, Shiner RJ, Angus GE, Kim WD, Wang NS, King M, Ghezzo H, Cosio G. Destructive index: A measurement of lung parenchymal destruction in smokers. Am Rev Respir Dis 1985; 131:764-769.

7. Berend N, Woolcock AJ, Marlin GE. correlation between the function and structure of the lung in smokers. Am Rev Respir Dis 1979; 119:695-705.

8. Thurlbeck WM, Henderson JA, Fraser RG, Bates DV. Chronic obstructive lung disease: a comparison between clinical, roentgenologic, functional and morphologic criteria in chronic bronchitis, emphysema, asthma and bronchiectasis. Medicine 1970; 49:81-145.

9. Glaister DG, Schroter RC, Sudlow MF, Milic-Emili J. Bulk elastic properties of excised lungs and the effect of a transpulmonary pressure gradient. Respir Physiol 1973; 17:347-364.

10. Battista SP, Kensler CJ. Use of the non-immersed in vitro chicken tracheal preparation for the study of ciliary transport activity. Arch Environ Health 1970; 20:318-325.

11. Hilding AC. On cigarette smoking, bronchial carcinoma and ciliary action. New Engl J Med 1956; 254:1155.

12. Albert RE, Lippman M, Peterson HT Jr, Berger J, Sanborn K, Bohning D. Bronchial deposition and clearance of aerosols. Arch Intern Med 1973; 131:115-127.

13. Camner P, Philipson K, Arvidsson T. Cigarette smoking in man. Arch Environ Health 1971; 23:421-426.

14. Yeates DB, Aspin N, Levison H, Jones MT, Bryan AC. Mucociliary tracheal transport rates in man. J Appl Physiol 1975; 39:487-495.

15. Luchsinger PC, Lagarde B, Kilfeather JE. Particle clearance from the human tracheobronchial tree. Am Rev Respir Dis 1968; 97:1046-1050.

16. Wanner A, Hirsch JA, Greeneltch DE. Tracheal mucous velocity in beagles after chronic exposure to cigarette smoke. Arch Environ Health 1973; 27:370-371.

17. Dulfano MJ, Adler K, Philippoff W. Sputum viscoelasticity in chronic bronchitis. Am Rev Respir Dis 1971; 104:88.

18. Adler K, Dulfano MJ. The rheological factor in mucocilary clearance. J Lab Clin Med 1976; 88:22.

19. Dulfano MJ, Adler KB. Physical properties of sputum. Am Rev Respir Dis 1975; 112:341.

20. Lamb D, Reid L. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. Brit Med J 1969; 1:33-35.

21. Pare P. Bronchial reactivity in animals and its relationship to bronchial permeability. In: Hargrave F, ed., Airway reactivity. Mechanisms and clinical relevance. Mississauga, Ontario: ASTRA 1980:49-53.

22. Simani AS, Inoue S, Hogg JC. Penetrations of the respiratory epithelium of guinea pigs following expsoure to cigarette smoke. Lab Invest 1974; 31:75-84.

23. Jones JG, Lawler P, Crawley JCW, Minty BD, Hulands G, Veall N. Increased alveolar epithelial permeability to cigarette smokers. Lancet 1980; 1:66-68.

24. Drazen JM, O'Cain CF, Ingram H Jr. Experimental induction of chronic bronchitis in dogs: Effects on airway obstruction and responsiveness. Am Rev Respir Dis 1982; 126:75-79.

25. Roehrs JD, Rogers WR, Johanson WG Jr. Bronchial reactivity to inhaled methacholine in cigarette-smoking baboons. J Appl Physiol 1981; 50:754-760.

26. Abraham W, Chapman GA, Marchette B. Differences between inhaled and intravenous carbachol in detecting O_3-induced airway effects. Environ Res 1984; 35:430-438.

27. Wallis TW, Rogers WR, Johanson WG Jr. Effects of acute and chronic exposure to nicotine aerosol on bronchial reactivity to inhaled methacholine. J Appl Physiol 1982; 52:1071-1076.

28. Lippman M, Albert RE, Peterson HT Jr. The regional deposition of inhaled aerosols in man. In: Walton WH, ed. Inhaled particles III, Volume 1. Sussex, England, union Brothers. 1971; 105-120.

29. Kennedy SM, Elwood RK, Wiggs BJR, Paré PD, Hogg JC. Increased airway mucosal permeability of smokers: Relationship to airway reactivity. Am Rev Respir Dis 1984; 129:143-148.