Experimentally Induced Emphysema

A number of insults, including oxides of nitrogen, cadmium salts, whole cigarette smoke, ozone exposure, and proteolytic enzymes, have been used to induce or augment emphysematous lesions in a variety of experimental animals. The reader interested in a comprehensive review of this topic is referred to the well-documented article by Karlinsky and Snider (1978). The evidence for each of these insults, as they pertain to cigarette smoke, is reviewed below.

Oxides of Nitrogen

Oxides of nitrogen, present in the gas phase of smoke, appear to induce or potentiate emphysema-like lesions in some animals (Karlinsky and Snider 1978).

Nitrogen dioxide (NO_2) exposure causes airway narrowing and an increase in the proteolytic burden within the lungs of experimental animals. Airway narrowing is postulated as a contributing factor in the pathogenesis of pulmonary emphysema (Juhos et al. 1980). Following exposure to NO_2, rats develop bronchiolar stenosis. The nitrogen dioxide exposure also induces an influx of alveolar macrophages (AM) and polymorphonuclear leukocytes (PMNs) (cells known to contain proteolytic enzymes) into the lungs (Juhos et al. 1980). Kleinerman et al. (1982) demonstrated an increased number of alveolar macrophages and PMNs in lung lavages from hamsters exposed to NO_2. Although there is no detectable increase in the elastolytic activity from lung lavages of NO_2-exposed animals, the cell-free culture medium from macrophage cultures of NO_2-exposed animals does show a twofold to fivefold increase in elastolytic activity during the first 2 weeks of exposure (Kleinerman et al. 1982).

Nitrogen dioxide exposure has not been shown to cause alveolar septal disruption, an essential feature of centrilobular emphysema, but it does result in a significant reduction in the internal surface area in the lungs of hamsters exposed for 12 to 14 months (Kleinerman and Niewoehner 1973).

Cadmium Salts

Animals exposed to cadmium, a constituent found in the particulate phase of cigarette smoke, develop a number of histologic and biochemical changes that may lead to emphysematous lesions.

Exposed animals develop pulmonary edema, vascular congestion, intraparenchymal hemorrhages, and a loss of Type I pneumocytes (Palmer et al. 1975; Strauss et al. 1976), and PMNs and mononuclear cells influx into the lungs (Snider et al. 1973). The animals develop acute peribronchial damage followed by the accumulation of granulation tissue near the respiratory bronchioles, thickened alveolar

276

septa, and distortion and distention of neighboring alveoli (Snider et al. 1973). These histologic changes are more suggestive of the scar or paracicatricial form of emphysema than the centrilobular form reported in some industrial workers exposed to $CdCl_2$ (Princi 1947). This disparity in response to $CdCl_2$ could be related to species differences or the interaction of $CdCl_2$ with other factors.

When hamsters are exposed to $CdCl_2$ plus beta-amino proprionitrile (β-APN), an inhibitor of lysyl oxidase, they develop thin-walled subpleural bullae and airspace enlargements resembling panlobular emphysema (Niewoehner and Hoidal 1982). The mean linear distance between alveolar intercepts is significantly increased; pressure–volume studies show overinflation and increased compliance of the lungs (Niewoehner and Hoidal 1982). This study suggests that $CdCl_2$, perhaps in conjunction with some other as yet undetermined agent, may be important in the pathogenesis of pulmonary emphysema. The fact that $CdCl_2$ is a constituent of cigarette smoke (Randi et al. 1969) lends support to this hypothesis.

Cigarette Smoke

Cigarette smoking has been clearly identified as a major causal factor in the development of pulmonary emphysema in humans (Auerbach et al. 1972; 1974; Petty et al. 1967; Andersen et al. 1967; Niewoehner et al. 1974; USDHEW 1979). However, an animal model for the development of emphysema using the inhalation of cigarette smoke alone has not been convincingly demonstrated. Parenchymal disruption resembling human emphysema has been reported in some dogs following prolonged cigarette exposure, but this histologic pattern is not uniformly present (Hernandez et al. 1966; Auerbach et al. 1967a; Zwicker et al. 1978).

This difficulty in developing an animal model for cigarette-induced emphysema may relate to the reluctance of animals to inhale smoke and the relatively long duration of exposure required to produce emphysema in humans. However, it may also result from the need for a combination or sequence of effects to induce emphysematous change. That is, an increased elastase burden might be necessary (secondary to the cellular response to smoke) before the oxidant damage of smoke to α_1AT, or to repair mechanisms, results in emphysema. Hoidal and Niewoehner (1983) examined this question in hamsters exposed to low doses of smoke and elastase. Neither exposure alone resulted in significant emphysematous change, but the combined exposure did cause change. This suggests that an increased elastase burden may be a precondition for smoking-induced emphysematous lung injury, and may also explain the long exposure period required in humans prior to the demonstration of an increased prevalence of emphysema in smokers.

277

Experimental studies have shown that cigarette smoke can induce a number of cellular, biochemical, and metabolic changes within the lungs that may be causally related to the development of emphysema. Macrophages and leukocytes, cells known to contain proteolytic enzymes, are recruited to the lungs of hamsters (Kilburn and McKenzie 1975) and guinea pigs (Flint et al. 1971) following exposure to cigarette smoke, thereby increasing the proteolytic burden of the lungs. Conversely, the a_1AT activity decreases in rats after inhalation of cigarette smoke (Janoff et al. 1979a). The increased proteolytic burden within the lungs coupled with the concomitant diminution in inhibitory capacity tends to create a protease–antiprotease imbalance and a situation whereby unrestrained connective tissue destruction may occur.

The Effects of Smoking on Cellular and Immune Defense Mechanisms

There are important functional differences between macrophages from smokers and those from nonsmokers (Table 1). For example, Warr and Martin (1977) demonstrated that receptors for the third component of complement (C3b) are decreased in number or function on the surface of smokers' alveolar macrophages. The receptors for the Fc portion of IgG, however, are normal on these cells (Warr and Martin 1977). An important function of the C3b receptor is to augment the attachment and phagocytosis of microorganisms and particulates by the macrophages. It is not clear whether this subtle defect in cell function results in a significant alteration in phagocytosis or clearance of particulates or microorganisms by these cells. In this regard, the phagocytosis and killing of a variety of microorganisms by smokers' alveolar macrophages have been shown to be normal by Harris et al. (1970) and Cohen and Cline (1977). One report by Martin and Warr (1977), however, suggests that the capacity of alveolar macrophages to kill bacteria is decreased in smokers.

The observation that human alveolar macrophages from cigarette smokers function normally to kill microorganisms appears to differ, at first glance, from a number of animal studies demonstrating that the capacity of alveolar macrophages to phagocytose and kill bacteria is impaired following exposure to cigarette smoke (Holt and Keast 1973; Rylander 1971, 1973). In these animal studies, there was an initial decrease in the numbers of alveolar macrophages and a decrease in their bactericidal function following exposure to cigarette smoke. With prolonged exposure, however, the number of macrophages increased and their ability to kill microorganisms returned to normal (Rylander 1973, 1974). These observations suggest that cigarette smoke, initially, is toxic to alveolar macro-

phages. However, it is likely that the macrophages, with time, adapt to the presence of cigarette smoke. In addition, a subpopulation of macrophages that are more resistant to cigarette smoke may increase in number in the lung. The macrophages isolated from the lungs of smokers resemble those isolated from animals following prolonged exposure to cigarette smoke. The acute effects of cigarette smoking on the number and functions of alveolar macrophages in man has not been systematically evaluated.

Alveolar macrophages of cigarette smokers appear to interact in an abnormal fashion with lymphocytes (Table 1). In this regard, the alveolar macrophages from cigarette smokers function poorly as accessory cells in presenting antigen to autologous lymphocytes (Laughter et al. 1977). This latter defect may be further magnified by the observation that lymphocytes from cigarette smokers also respond poorly to mitogens (Neher 1974; Daniele et al. 1977). Additional evidence for an abnormal interaction of macrophages and lymphocytes in lungs of cigarette smokers is a decreased response of alveolar macrophages to the lymphokine, macrophage migration inhibitory factor (Warr 1979). These observations suggest that cigarette smoking may have broad effects on the ability of the lung to generate a cellular immune response.

In Vitro Effects of Cigarette Smoke on Inflammatory and Immune Effector Cells

The most readily demonstrable effect of cigarette smoke, in vitro; is a decrease in cell viability (Holt et al. 1974; Holt and Keast 1973; Nulsen et al. 1974; Weissbecker et al. 1969). At relatively low concentrations, cigarette smoke and its constituents rapidly kill alveolar and peritoneal macrophages in vitro. Lymphocytes and polymorphonuclear leukocytes are also very susceptible to these agents (Holt et al. 1974; Blue and Janoff 1978).

When sublethal amounts of cigarette smoke are employed, a number of metabolic and functional changes occur in macrophages. Phagocytosis is depressed, as is the function of a number of macrophage enzymes (Vassallo et al. 1973; Green 1968a, b, c, 1969, 1970; Green and Carolin 1966, 1967; Green et al. 1977; Powell and Green 1971; Hurst and Coffin 1971). In addition; protein synthesis is also depressed (Yeager 1969; Low 1974), and stimulatory effects have been noted. While cigarette smoke depresses phagocytosis and intracellular killing, nitrogen dioxide increases the metabolic activity of macrophages (Vassallo et al. 1973). Similar effects have been observed under some conditions with cigarette smoke (Holt and Keast 1973; Leuchtenberger and Leuchtenberger 1971). Results from a number of investigators suggest that the balance between stimulation and inhibition of macrophage activity is determined by dosage, with stimulation occurring at low exposure levels and inhibition at

higher concentrations (Holt and Keast 1973; Lentz and DiLuzio 1974; York et al. 1973). The most potent stimulation occurs after prolonged exposure to low levels of the agent.

These in vitro effects of cigarette smoke are also seen acutely in vivo following exposure to smoke. The immediate effect of exposure to cigarette smoke, and to agents present in the smoke, is a decrease in viability of the pulmonary alveolar macrophages (Holt and Keast 1973a, b; Rylander 1971, 1973; Coffin et al. 1968; Dowell et al. 1970; Holt and Nulsen 1975; Gardner et al. 1969). Although not well studied, it is also likely that cigarette smoke is toxic to polymorphonuclear leukocytes (Blue and Janoff 1978). In support of these observations are studies demonstrating that acute exposure of experimental animals to tobacco smoke, or to components of cigarette smoke, also lowers their resistance to bacterial infection (Rylander 1969, 1971; Acton and Myrick 1972; Gardner et al. 1969; Goldstein et al. 1971; Huber and LaForce 1971; Huber et al. 1971). Short-term exposure to components of cigarette smoke, particularly nitrogen oxide, has also reduced resistance to viral infection, probably by inhibiting interferon production by macrophages (Valand et al. 1970). As noted above, these in vitro and acute in vivo effects of cigarette smoke are not seen following long-term in vivo exposure in animals; very little work has been done on the effects of cigarette smoke in vitro, using human cells. However, several studies have shown that cigarette smoking and nicotine, at levels comparable to those encountered in the circulation of smokers, produce a slight but significant depression of PHA-stimulated DNA synthesis in human peripheral blood lymphocytes (Neher 1974; Silverman et al. 1975; Vos-Brat and Rumke 1969).

The Effect of Cigarette Smoke on Antibody Production

The available data on antibody production in human smokers suggest that cigarette smoking may depress these responses. The production of antibodies was investigated in a large study involving influenza vaccination. Smokers in the population exhibited increased susceptibility to infection during an influenza outbreak (Finklea et al. 1969, 1971). Prior to immunization with influenza vaccine, smokers exhibited significantly lower titers of specific antibodies than did nonsmokers. Immediately following vaccination of both groups, the smokers developed levels of antibodies comparable to those of nonsmokers. However, the antibody titer in the smokers fell below their nonsmoking counterparts within a few weeks, and by a year after vaccination the smokers exhibited markedly depressed levels of circulating antibodies.

The capacity of cigarette smoking to alter antibody production was also studied by evaluating the capacity of a fetus to stimulate lymphocytotoxic antibodies against HLA antigens in the mother

(Nyman 1974). Sera from a large number of pregnant women were tested for the presence of lymphocytotoxic antibodies against a 48-donor panel. The smokers exhibited a significantly lower incidence of these antibodies than did nonsmokers, and the divergence between groups increased with the number of deliveries. Infections during pregnancy were observed significantly more often in the smokers in this trial.

In several animal models, acute exposure to whole cigarette smoke or components of cigarette smoke depressed the numbers of anti-body-forming cells in the spleen and the serum levels of antibodies in animals exposed to a variety of antigens (Miller and Zarkower 1974; Zarkower and Marges 1972; Zarkower et al. 1970). The depression was greatest when the antigen was administered by an aerosol (rather than by systemic inoculation), indicating that smoke appears to exert an effect close to the point of entry. Prolonged exposure ultimately resulted in severe depression both in local and in systemic antibody responses (Esber et al. 1973; Holt et al. 1976; Thomas et al. 1973, 1974a, 1974b, 1975).

Although it is tempting to relate these abnormalities in immune response to the known association between cigarette smoking and increased incidence of upper respiratory infection, it is not clear whether the subtle defects in immune functions can entirely account for the infections present in cigarette smokers. Clearance of bacteria from the respiratory tract is a complex process that involves interplay between a variety of different mechanisms, only some of which include the function of alveolar macrophages and the capacity of the lung to mount cellular and humoral immune responses. Other abnormalities present in cigarette smokers that could account for this increased incidence of infection include a markedly abnormal tracheal bronchial clearance of particulates and an increased adherence of bacteria to airway epithelium (reviewed in USPHS 1971, 1973, 1974).

EFFECTS OF CIGARETTE SMOKE ON AIRWAY MUCOCILIARY FUNCTION

Introduction

There is extensive literature on the effects of cigarette smoke on mucociliary clearance in the airways, with the majority of the reports appearing between 1965 and 1975. Different experimental approaches have been used, including in vivo measurement of mucociliary function in animal models and in human subjects. The results of some of these studies have been contradictory, presumably because of differences in experimental technique or the influence on mucociliary function of factors other than cigarette smoking. For example, tracheal mucociliary transport appears to decline with age in normal subjects (Goodman et al. 1978), an important phenomenon to consider when assessing the effects of long-term cigarette smoking. Another complicating factor is the clearly demonstrated impairment of mucociliary function produced by chronic bronchitis even in nonsmokers, such as in patients with cystic fibrosis (Wood et al. 1975) or immunoglobin deficiency (Mossberg et al. 1982). Therefore, it is difficult to separate the direct effects of cigarette smoke on mucociliary function from those of smoking-associated chronic bronchitis. Finally, in vitro bioassays for ciliotoxicity may not reliably reflect the effects of cigarette smoke on the mucociliary apparatus in the intact airways. Thus, Dalhamn et al. (1967) found that smoke produced by cigarettes containing a high concentration of hydrogen cyanide was more ciliotoxic in vitro than that produced by cigarettes containing a low concentration of hydrogen cyanide, and the two types of cigarettes caused a comparable reduction of mucus transport in vivo.

This review is divided into three parts. The first part summarizes the normal structure and function of the mucociliary system in the airways. The second part deals with the direct effects of short-term and long-term cigarette smoke exposure on mucociliary function, and the third part discusses mucociliary function in chronic bronchitis.

Normal Mucociliary Function

The principal function of the airway mucociliary system is its contribution to host defenses. This is accomplished by physical removal of inhaled foreign material from the ciliated airways by mucous transport and by biochemical and immunological processes that protect against invasion of the mucosa by infectious agents. Normal mucociliary clearance depends upon an optimal interaction between cilia and mucus.

Cilia

The respiratory mucosa from the proximal trachea to the terminal bronchioles consists of a pseudostratified epithelium with cilia protruding from the luminal surface of columnar cells (Figure 1). The larynx contains a mucus-secreting squamous epithelium over most of its surface, and cilia are present only in the posterior commissure (Wanner 1977). The major cell types in the respiratory mucosa are basal cells, intermediate cells, nonciliated columnar cells, ciliated columnar cells, and goblet cells. In the larger airways, the major part of the epithelial surface is ciliated. The ratio of ciliated columnar cells to goblet cells is approximately 5:1 in the trachea, with a relative decrease in the number of both cell types toward the peripheral airways. The surface of each ciliated columnar cell contains approximately 200 cilia with an average length of 6 μm and diameter of 0.2 μm. Both ciliated and nonciliated columnar cells are characterized by microvilli on their luminal surface. These measure 0.3 μm in length and 0.1 μm in diameter. The ultrastructure of cilia in lower animals and mammals is remarkably similar. Each cilium contains longitudinal microtubules that appear to represent contractile elements. Two single microtubules form a central core, and nine microtubules with a doublet structure are arranged in a circular fashion in the periphery of the cilium. A basal body in the apex of the cell corresponds to each cilium. Circular and radial bridges have been demonstrated between the peripheral microtubules and between the peripheral and central microtubules. These bridges (dynein arms, nexin links, radial spokes) appear to be crucial for ciliary bending.

Mucus

Respiratory secretions consist of mucus produced by submucosal glands and goblet cells and tissue fluid. The total volume of all mucus-producing structures has been estimated at approximately 4 ml in human lungs; submucosal glands make up most of this volume (Wanner 1977). The submucosal glands are under parasympathetic nervous control, with an estimated daily volume of respiratory secretions between 10 and 100 ml. Human respiratory secretions contain approximately 95 percent water. The rest consists of micromolecules (electrolytes and amino acids) and macromolecules (lipids, carbohydrates, nucleic acid, mucins, immunoglobulins, enzymes, and albumin). In situ, the respiratory secretions take the form of two layers, i.e., periciliary fluid (sol phase), and mucus (gel phase), as shown in Figure 1. Mucus has been clearly identified as the product of submucosal glands and goblet cells; the origin of the periciliary fluid has not been definitely established, although transepithelial water transport appears to be the most likely source. In central airways, the mucus layer is 5 to 10 μm deep and may be
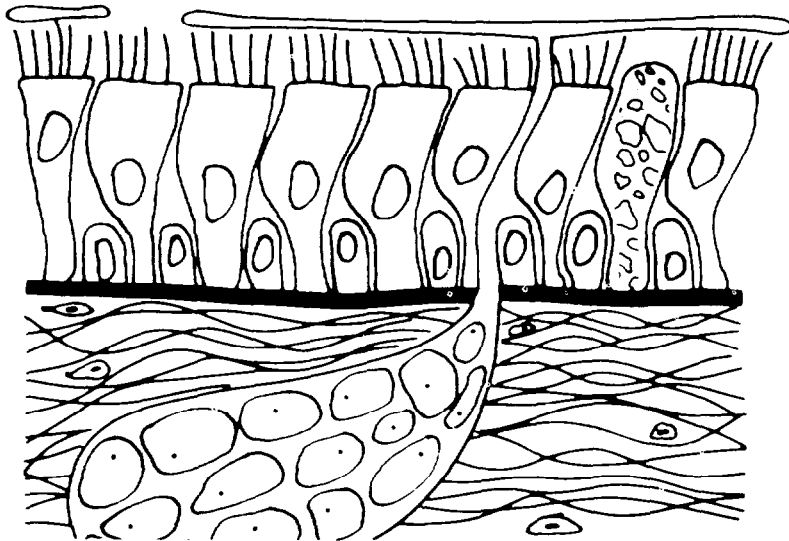
**FIGURE 1.—Schematic representation of normal mucosa
(central airway) with the components of the
mucociliary apparatus**

NOTE: From top (airway lumen) to bottom, note mucus layer, periciliary fluid layer, epithelium with a predominance of ciliated columnar cells and an interspersed goblet cell, basement membrane, and submucosal gland.

SOURCE: Wanner (1979).

discontinuous. In peripheral airways where submucosal glands are absent and goblet cells are rare, mucus is either absent or present only in small quantities. Regeneration of injured ciliated respiratory epithelium takes approximately 2 weeks in animals; the exact regeneration time of damaged human tracheobronchial ciliated epithelium is not known (Wanner 1977). It appears that regeneration does not begin until 2 to 3 days following mechanical injury.

Mucociliary Interaction

Mucociliary interaction depends on ciliary activity, the rheologic properties and depth of the mucus layer, and the depth of the periciliary fluid layer. The viscoelastic properties of mucus are determined by its biochemical characteristics (disulfide cross-linking and hydrogen bonding between glycoprotein molecules and water) (Wanner 1977). Cilia beat in one plane, with a fast effective stroke (power stroke) in the cephalad direction and a recovery stroke that is two to three times slower. Adenosine triphosphate has been identified as the energy source for ciliary bending. In mammals, the average normal ciliary beat frequency is approximately 1,000 beats per minute, with coordinated motion in adjacent cilia on an individual cell and in cilia of adjacent cells. This interciliated

285

TABLE 2.—Measurement of airway mucociliary function in humans

Method	Reference
Clearance of inhaled radioactive aerosols from the lungs	Morrow et al. (1967)
Transport of discrete markers	Friedman et al. (1977) Sackner et al. (1973)
Central airway clearance of inhaled boli of radioactive microspheres	Yeates et al. (1975)

pattern of motion by which adjacent cilia beat one after another to generate a wave of ciliary motion is called metachronism.

The "normal" thickness of the periciliary fluid layer is less than, or at best equal to, the length of the ciliar shafts, which measure approximately 6 µm in the central airways. The luminal surface of the mucus layer appears smooth, whereas the surface in contact with the cilia is irregular, and the mucus penetrates between the ciliary shafts. This penetration and the claw-like projections at the cilia tips may further facilitate the mechanical interaction between cilia and mucus. In the trachea, the "normal" surface mucus transport velocity is between 5 and 20 mm per minute depending upon the method of measurement. Mucous transport velocity decreases toward the peripheral airways.

The three principal methods for the measurement of tracheobronchial mucociliary function in humans are listed in Table 2. All of these have been used in studies of the effects of cigarette smoke on mucociliary function.

Theoretically, mucociliary dysfunction can result from alterations in ciliary beat frequency and coordination, the quantity and viscoelastic properties of mucus, and the thickness of the periciliary fluid layer. In addition, focal destruction of the respiratory epithelium, producing areas without cilia or mucus, is also associated with impaired or absent mucociliary transport.

Effects of Cigarette Smoke on Mucociliary Function

The irritant effects of cigarette smoke on mucociliary clearance were recognized by Mendenhall and Shreeve (1937). They observed a decrease in the transport rate of carmine particles on the mucosa of excised cat tracheas after bringing them in contact with cigarette smoke, either directly or by dissolving it in the solution in which the tracheas were immersed (Mendenhall and Shreeve 1937, 1940). These early findings were confirmed by Hilding (1956) and Dalhamn

286

(1959) approximately 25 years ago. Since then, investigations to assess the effects of cigarette smoke on ciliary activity and mucociliary transport have proliferated. Because cigarette smoke can impair mucociliary transport by interfering with ciliary activity or mucus secretion, the studies relating to these two component functions are discussed separately and are complemented by a review of experiments involving mucociliary transport, the ultimate expression of mucociliary function.

The effects of cigarette smoke on mucociliary function have been extensively studied in vitro, in intact animal models, and in human subjects. Comparison among the results of these experimental approaches is difficult, as there are major differences in inhalation patterns even between animal models and human subjects. Cigarette smoke is modified during its passage through the upper airways, and this may vary depending upon the mode of inhalation. By using smoke produced by radio-tracer spiked cigarettes, it has been shown that mice, who are obligatory nose breathers, retain 50 percent of the inhaled radioactivity in the nasal passages, 20 percent in the lungs, and the rest in the esophagus, stomach, and other organs (Page et al. 1973). Using artificial airways to bypass the nasopharynx in experimental animals eliminates the problem of nasal cigarette smoke absorption, but also prevents the oral modification of cigarette smoke that is a typical feature of human smoking. In subjects inhaling cigarette smoke from a smoke dosage apparatus that delivers standard puffs, 86 to 99 percent of most components of gas and particulate phases of cigarette smoke are retained, with the exception of carbon monoxide (CO), of which only 54 percent is retained (Dalhamn et al. 1968). Much of the smoke appears to be retained in the mouth. In human subjects who hold cigarette smoke in their mouth for 2 seconds, 60 percent of the water-soluble components of the gas phase, 20 percent of the water-insoluble components of the gas phase, and 16 percent of particulate matter are absorbed or retained in the upper airways (Frances et al. 1970; Stupfel et al. 1974). This marked modification of cigarette smoke might decrease its ciliotoxic effect in the lower airways. Passing unfiltered cigarette smoke through a chamber with wet surfaces before bringing it in contact with ciliated epithelium decreased the cilioinhibitory capacity of the cigarette smoke (Kaminski et al. 1968). When filtered cigarette smoke was used, the wet surfaces had no additional effect on ciliotoxicity, indicating that the mucosa of the upper airway may serve as a filter (Kaminski et al. 1969). Similar observations have been made when cigarette smoke was passed through a water trap (Albert et al. 1969).

Because of the differences in inhalation pattern between humans and animals, it might be argued that nasal mucociliary function should be measured to assess the effects of inhaled cigarette smoke

TABLE 3.—Effects of cigarette smoke on airway mucociliary system

Exposure	Ciliary dysfunction	Mucus hypersecretion	Impaired mucociliary clearance
Short term	Yes	?	?
Long term	Yes	Yes	Yes

in animal models. However, it has been clearly shown that nasal mucociliary transport is not a good marker of tracheobronchial mucociliary transport because of differential responses at the two sites. For example, exposure to the whole cigarette smoke of up to 30 cigarettes does not impair nasal mucociliary transport in donkeys (Frances et al. 1970), whereas the same number of cigarettes clearly alters tracheobronchial deposition and clearance of radioactive aerosols (Albert et al, 1969). Likewise, Hilding (1965) has concluded from his studies that the nose is not an acceptable organ for the study of the effects of cigarette smoke on mucociliary transport.

Realizing the problems with experimental models of ciliary function in response to cigarette smoke inhalation, Dalhamn (1969) postulated that a proper experimental design should fulfill the following requirements: (1) the exposure pattern and level of cigarette smoke inhalation should simulate that of natural smoking in human subjects, (2) cigarette smoke should be delivered in air and not as an aqueous solution, (3) the components of inhaled cigarette smoke should be analyzed, and (4) exposure should be of long duration. Although these criteria are obviously not met by many of the studies quoted in this review, understanding these principles allows a more critical assessment of the reported results as shown in Table 3.

Short-Term Exposure

The effects of short-term cigarette smoke exposure on the morphology of the respiratory mucosa have not been investigated in man. Cigarette smoke residue has been shown to cause ciliary damage in cultured rabbit tracheal epithelium with a contact-time-dependent effect (Kennedy and Allen 1979). The most consistent abnormalities were cellular desquamation and alterations in mitochrondria, cilia, and microvilli, some of which occurred as early as 1 hour after exposure commenced.

Cytotoxicity has also been observed after short-term exposure of ciliated epithelium to aqueous extracts of cigarette smoke condensate in vitro (Donnelly 1969), but it is difficult to extrapolate data from these in vitro studies to the in vivo conditions that occur during cigarette smoking.

288

Cilia

With a few exceptions, e.g., Proetz (1939), most investigators have demonstrated an irritant effect of smoke on ciliated epithelium, usually characterized by ciliostasis. Residues of cigarette smoke passed through an aqueous medium have been shown to produce ciliostasis in protozoa (Weiss and Weiss 1964; Wang 1963) and in fragments of human respiratory epithelium (Ballenger 1960). In fragments of rat trachea, brief exposure to whole cigarette smoke appears to elicit a biphasic response, with a short period of stimulation during 1 to 2 minutes followed by a marked decrease in ciliary beat frequency (Guillerm et al. 1961, 1972). In an excised rabbit trachea model, 71 1-ml puffs or 35 10-ml puffs of whole cigarette smoke were necessary to produce ciliostasis; similar relationships were demonstrated in the tracheas of living cats (Dalhamn 1970; Dalhamn et al. 1968). Several investigators have established a stimulus–response relationship between dilutions of aqueous cigarette smoke extract and the time of exposure required for total stoppage of ciliary beat frequency in different experimental models (Donnelly 1969, 1972; Das et al. 1970; Donnelly et al. 1981). The mechanism by which cigarette smoke acutely depresses ciliary function is not clearly known, but may involve enzyme inhibition of adenylate kinase, thereby reducing adenosine triphosphate (ATP), the energy source for ciliary bending (Mattenheimer and Mohr 1975; Schabort 1967). Ciliary function in response to short-term cigarette smoke inhalation has not been studied in man.

Mucus

Very little is known about the quantity and rheologic properties of airway secretions after short-term cigarette smoke exposure. A brief exposure of slugs to cigarette smoke has been reported to stimulate the production of mucus containing an increased number of acid glycoprotein fibers (Wilde 1981). The significance of this observation with respect to the human respiratory tract is not clear, except that an increased number of acid glycoprotein fibers has also been demonstrated in sputum obtained from cigarette smokers.

Mucociliary Interaction

Using a variety of different techniques in animal experiments, ciliary dysfunction and impairment of mucociliary transport by short-term exposure to cigarette smoke have been demonstrated in rats (Iravani 1972; Dalhamn 1964; Ferin et al. 1966), rabbits (Dalhamn 1964; Holma 1969), cats (Carson et al. 1966; Dalhamn 1964, 1969; Kaminski et al. 1968), dogs (Guillerm et al. 1972; Sakakura and Proctor 1972; Isawa et al. 1980), donkeys (Albert et al. 1974, 1969), chickens, and sheep (Stupfel et al. 1974). A few reports

have not demonstrated that short-term exposure to smoke depresses mucociliary function in animal models (La Belle et al. 1966; Bair and Dilley 1967). The reasons for the discrepancy between these and the previously listed studies are not clear, but may be related to methodology and dose of exposure. Stimulus response curves between dose of cigarette smoke and the degree of mucociliary inhibition have been shown in airways of chickens and dogs (Battista and Kensler 1970b; Sakakura and Proctor 1972; Isawa et al. 1980). In one study, for example, 9 puffs of nonfiltered cigarette smoke had variable effects on tracheal mucociliary transport in intact dogs, but tracheal mucociliary transport was consistently inhibited by 12 puffs (Sakakura and Proctor 1972). Likewise, the number of 4-second exposures to cigarette smoke (separated by 1 minute) required to reduce mucus transport in intact chicken tracheas by more than 90 percent has been shown to increase with increasing dilutions (from 50 to 3 percent) of smoke in air (Battista and Kensler 1970b).

Measurements of mucociliary clearance in man immediately after smoking one or more cigarettes have shown conflicting results, with either increased (Albert et al. 1973; Camner et al. 1971; Albert et al. 1975; Camner and Philipson 1971, 1974), inconsistent, or unchanged rates (Yeates et al. 1975; Pavia et al. 1971; Goodman et al. 1978) or decreased (Nakhosteen et al. 1982) rates. Transient effects on mucociliary clearance have been reported in both smokers and nonsmokers (Hilding 1956; Pavia et al. 1971). Such differences between human subjects may reflect a difference in the dose and inhalation pattern of cigarette smoke.

Long-Term Exposure

In dogs inhaling cigarette smoke through a tracheostomy, histologic changes have been observed in the bronchi after 229 to 421 days of exposure (Auerbach et al. 1967b). These changes consisted of epithelial hyperplasia, decreased number of ciliated cells, and areas of squamous metaplasia. This may be criticized as a poor model of cigarette smoking in humans because the upper airway, which absorbs part of the smoke and decreases its toxicity, is bypassed. The irritant effect of cigarette smoke on the tracheobronchial mucosa could be enhanced in this model. However, inflammatory changes in the airways have also been observed in animals that inhaled cigarette smoke via their upper airways (Leuchtenberger et al. 1958; Rylander 1974; Mattenheimer and Mohr 1975; Park et al. 1977; Basrur and Basrur 1976; Jones et al. 1973; Iravani 1973). Various types of lesions have been observed, including tracheal and bronchial epithelial hyperplasia (Park et al. 1977; Frasca et al. 1974), goblet cell proliferation and submucosal gland hypertrophy (Jones et al. 1973; Park et al. 1977), bronchiolar metaplasia of mucus-secreting cells (Basrur and Basrur 1976), increased quantities of airway mucus

290

that appear to be adherent to submucosal gland openings (Iravani 1973), and a decreased number of ciliated epithelial cells (Basrur and Harada 1979). One study suggested a dose-dependence of the mucosal lesions when comparing hamsters exposed to either four cigarettes per day or eight cigarettes per day for 2 weeks (Basrur and Basrur 1976). The pathologic changes produced by long-term cigarette smoke exposure appear to be reversible if the exposure time is not excessive. Thus, inflammatory changes in the airways of hamsters exposed to cigarette smoke for 4 weeks showed marked reversibility with a recovery time of several weeks (Basrur and Harada 1979).

The histologic changes in the airways of cigarette smokers are similar to those produced by cigarette smoke in animal models, and consist of varying degrees of denudation of the ciliated epithelium, an increase in the number of goblet cells, submucosal gland hypertrophy, and squamous metaplasia (Regland et al. 1976; Jones 1981). Morphometric studies have demonstrated an increased quantity of mucus in the airway lumen without histologic evidence of coexistent emphysema or a history of obstructive lung disease, whereas this is not observed in the lungs of healthy nonsmokers (Niewoehner et al. 1974; Matsuba and Thurlbeck 1971). Electron microscopic examination of ciliated epithelium in surgical lung specimens obtained from cigarette smokers has revealed ciliary abnormalities consisting of compound cilia, single axoneme, intracyctoplasmic microtubular doublets, and cilia within periciliary sheaths (McDowell et al. 1976). If bronchial biopsy material is used to detect ciliary abnormality in cigarette smokers, the results must be interpreted with caution, for a single biopsy may be misleading owing to the focal nature of the lesions (Fox et al. 1981).

The morphologic changes of the respiratory mucosa in animals exposed to cigarette smoke for prolonged periods and in human cigarette smokers strongly suggests the presence of mucociliary dysfunction. This has been clearly demonstrated, particularly with respect to the production and clearance of mucus.

Cilia

Ciliary function after long-term cigarette smoke exposure has not been extensively studied. Iravani and Melville (1974) demonstrated a decrease in ciliary beat frequency in the airways of hamsters exposed to cigarette smoke for 1 year; however, in rats also exposed for 1 year under almost identical conditions, ciliary frequency was generally increased, although there were zones of ciliary inactivity or discoordination. A sustained inhibition of adenylate kinase activity in ciliated tracheal cells of hamsters exposed to cigarette smoke for up to 9 months has also been reported (Mattenheimer and Mohr 1975). Because inhibition of this enzyme leads to a decreased generation of adenosine triphosphate, the energy source of ciliary

bending, a decreased ciliary activity might be expected (Mattenheimer and Mohr 1975).

Mucus

Mucus hypersecretion has been clearly demonstrated in the airways of several animal species exposed to cigarette smoke for prolonged periods of time (Battista and Kensler 1970a; Iravani and Melville 1974). Rheologic measurements of airway mucus have not been reported in such animal experiments, but biochemical analysis has revealed the presence of serum proteins that might have cilioinhibitory effect (Dalhamn and Pira 1979; Battista 1980). Mucus hypersecretion may occur as early as 1 month after beginning a smoke inhalation equivalent to as little as one cigarette per day (Battista and Kensler 1970a). Rheologic and biochemical examinations of airway secretions in healthy smokers have not been carried out, primarily because these subjects do not have a productive cough. Once a smoker develops chronic productive cough, he or she is no longer considered healthy, but by definition, has chronic bronchitis.

Mucociliary Interaction

Long-term effects of cigarette smoke on airway mucociliary transport have been studied in different animal species. In purebred beagle dogs exposed to cigarette smoke (100 cigarettes per week) for 13.5 months via a mask that administered cigarette smoke through both the mouth and the nose for 1.5 hours twice daily, tracheal mucus transport rate was decreased to approximately 30 percent of that observed in control animals (Wanner et al. 1973). Pulmonary function did not differ significantly between the two groups. It has subsequently been shown that the abnormality in mucociliary transport in beagles may already be present after 6 months of cigarette smoke exposure (Park et al. 1977). An impairment of mucociliary clearance with long-term cigarette smoke exposure has also been demonstrated in rabbits, guinea pigs, rats, and chickens (Okajima 1971; Rylander 1971b; Iravani and Melville 1974; Battista and Kensler 1970a). In some of those experiments, impaired mucociliary clearance was already observed 4 weeks after the beginning of exposure.

The long-term effects of cigarette smoking on mucociliary function in human subjects has been investigated by aerosol clearance techniques and discrete marker transport techniques. Some of the investigators using radioactive aerosols demonstrated no abnormality of overall clearance in habitual cigarette smokers, particularly in those who already may have had symptoms of chronic bronchitis (Sanchiz et al. 1972; Yeates et al. 1975; Pavia et al. 1970; Pavia and Thomson 1970). However, the deposition of the inhaled radioactive

292

aerosol is more central in normal smokers and in patients with chronic bronchitis than in nonsmokers (Lippman et al. 1970). Because clearance is faster in central airways than in peripheral airways, this centralization of aerosol deposition may compensate for the overall decrease in mucociliary clearance. Investigations that have related mucociliary clearance to deposition pattern have generally found an impairment of mucociliary clearance in cigarette smokers (Lourenco et al. 1971; Camner et al. 1973a; Camner and Philipson 1972, 1974). Camner and Philipson (1972), in a study of 10 pairs of twins discordant for cigarette smoking, showed a significantly lower average clearance rate in smokers compared with nonsmokers; in 5 pairs clearance was slower in the smoker than in the nonsmoker, whereas in the remaining 5 pairs there was no difference. Analysis of regional clearance has produced further evidence that overall clearance of inhaled radioactive aerosols may fail to detect an abnormality in mucociliary clearance. Thus, Bohning and co-workers (1975) studied the deposition and clearance of 7 μm diameter particles in the tracheobronchial tree of six pairs of monozygotic twins, four of whom were discordant for cigarette smoking. They found comparable overall mucociliary clearance in the smoking and nonsmoking pairs, but more central deposition and slower central clearance in the smokers. Others have reported an impairment of peripheral mucociliary clearance and alveolar clearance as well (Matthys et al. 1983; Cohn et al. 1979).

Discrete particle techniques involving either bronchoscopy or radiography have been used to assess mucus transport in central airways, notably the trachea. Most investigators have reported a decrease of tracheal mucus velocity in healthy smokers, with values ranging between 20 percent and 80 percent of those of nonsmoking controls (Goodman et al. 1978; Toomes et al. 1981; Nakhosteen et al. 1982).

The bulk of the evidence indicates that long-term cigarette smoking alters mucociliary transport mechanisms and that these changes can occur as early as 1 year after smoking onset. Partial recovery of mucociliary transport has been observed in cigarette smokers after cessation for 3 months or more, but not after 1 week of cessation (Camner et al. 1973). These observations have also been supported by animal experiments (Albert et al. 1971).

Fractionation and Filtering of Cigarette Smoke

Whole cigarette smoke is composed of volatile elements and particulate matter, and it has become customary to distinguish between the gas phase and the particulate phase. The gas phase, by definition, consists of the components that remain after cigarette smoke has been "effectively" filtered by passing it through appropriate filters (Dalhamn 1966; Kensler and Battista 1963; Falk et al.

1959). The major constituents of the particulate phase are nicotine, phenols, hydrocarbons, aldehydes and ketones, organic acids, and alcohols. Although 95 percent of the gas phase (approximately 300 ml per cigarette) consists of combustion products and admixed air (nitrogen, oxygen, carbon dioxide, carbon monoxide) in concentrations that do not affect mucociliary transport, some trace gases are important (Battista et al. 1962). These include nitrogen dioxide, ammonia, cyanides, aldehydes, ketones, acrolein, and acids. As is shown below, some controversies still remain about whether the gas phase or the particulate phase of cigarette smoke is primarily responsible for its depressant effect on mucociliary activity. This problem is relevant when comparing the effects on mucociliary clearance of low tar versus high tar cigarettes, low nicotine versus high nicotine cigarettes, and filtered versus nonfiltered cigarettes.

Dalhamn (1966) reviewed the controversy over the separate effects of the gas and the particulate phases of cigarette smoke on mucociliary function. In protozoa, both phases of cigarette smoke have been shown to possess ciliotoxic properties (Kennedy and Elliott 1970). Falk and associates (1959) reported that exposure to whole cigarette smoke for 30 seconds resulted in a biphasic response, with an initial stimulation, followed by depression with a minimum value at about 15 minutes and a tendency toward recovery 45 minutes after exposure. Removal of the particulate matter in cigarette smoke by passing it through filters decreased its depressant effect on mucus transport, indicating that the major effect on mucociliary clearance was related to the particulate phase. Similar observations have been made by others (Rylander 1970; Falk et al. 1959). In contrast, Kensler and Battista (1963) incriminated the gas phase of cigarette smoke; they exposed strips of rabbit trachea to smoke from different cigarettes for 12 seconds and identified various gas phase constituents as having a depressant effect on mucus transport. These findings have also been confirmed by others in in vitro and in in vivo animal experiments (Kensler and Battista 1963; Hee and Guillerm 1973; Dalhamn 1956; Albert et al. 1974; Carson et al. 1966).

The most comprehensive study of individual gas and semivolatile constituents of cigarette smoke has been conducted by Petterson et al. (1982). Using chicken tracheal organ cultures, they showed that at a 5 mm concentration, 36 percent of 316 different compounds caused ciliostasis after 15 seconds of exposure, but 50 percent were without effect after an exposure time of 60 seconds. On the basis of this criterion of separation, either alkylated phenylethers, benzonitriles, benzaldehydes, benzenes, napthalenes and indoles, or α-saturated, β-unsaturated ketones and aldehydes, or aliphatic alcohols, aldehydes, acids, and nitrates were found to be ciliotoxic. Inactive compounds included benzoic acids, esters, polyaromatic hydrocar-

294

bons, amines, and N-heterocycles (except indoles). With respect to aldehydes, the time to ciliostasis on tissues of rabbit trachea has been reported shortest for formaldehyde, followed by acetaldehyde, acrolein, crotonaldehyde, and methacrolein. The ciliotoxic effects of aldehydes have been confirmed by others using different experimental approaches (Guillerm et al. 1968; Hee and Guillerm 1973; Kensler and Battista 1963). It has also been shown that acute acrolein inhalation causes denudation of ciliated cells, goblet cell discharge, exfoliation of surface epithelial cells, and infiltration of inflammatory cells in the lower airways of several different mammals (Dahlgren et al. 1972). Another volatile constituent of cigarette smoke with marked cilioinhibitory effects is hydrogen cyanide (Wynder et al. 1965a).

Weissbecker et al. (1971) used a different approach to assess the effects of several volatile cigarette smoke constituents on mucociliary transport in the cat trachea. The addition of individual volatile cigarette smoke components (isoprene, nitric oxide, and nitrogen dioxide) to carbon-filtered cigarette smoke either aggravated the impairment of tracheal mucus velocity produced by the filtered smoke or abolished the protection afforded by the carbon filter. When these constituents were added to whole cigarette smoke, no further impairment of mucus transport velocity was observed, indicating a saturation by whole cigarette smoke of receptors responsible for mucociliary depression.

A direct relation has been reported between tar content and the ciliotoxic effect of cigarette smoke (Dalhamn and Rylander 1967; Falk et al. 1959). However, Falk and associates (1959) found no difference between low tar and high tar cigarette residues with regard to in vitro mucociliary transport. The effects of nicotine on mucociliary transport are also controversial, although more investigators have demonstrated a lack of effect (Falk et al. 1959; Guillerm et al. 1972; Rakieten et al. 1952; Donnelly 1972) than a depression of mucociliary transport (Carson et al. 1966). Indeed, a biphasic dose-dependence has been suggested, with stimulation at lower concentrations and depression at higher concentrations (Tsuchiya and Kensler 1959). The stimulation of mucociliary function may be related to stimulation of nicotinic ganglionic receptors causing cholinergic ciliostimulation. This is based on the observation that the stimulating effect of nicotine-containing cigarettes on the metachronal wave frequency in the maxillary sinus of anesthetized rabbits is blocked by atropine and hexamethonium (Hybbinette 1982).

Among the different cigarette tobacco additives, menthol does not interfere with mucociliary transport (Rakieten et al. 1952). With respect to phenols, one investigator has reported that the ciliotoxicity of cigarette smoke produced by freeze-dried tobacco is the same as that produced by conventionally cured tobacco although the former

contains less phenol (Enzell et al. 1971). On the other hand, phenols have been shown to impair mucociliary activity and mucus transport both in vitro (Dalhamn and Lagerstedt 1966; Bernfeld et al. 1964; Dalhamn 1968) and in vivo (Dalhamn 1968). Dalhamn and associates (Dalhamn and Lagerstedt 1966; Dalhamn 1968) have even attempted to relate the toxicity of various phenols to their boiling points. Addition of the anti-inflammatory agents phenylvinyloxadiozole and phenylmethyloxadiozole to tobacco has been shown to reduce the ciliotoxicity of tobacco smoke (Dalhamn and Rylander 1971; Rylander 1971b; Dalhamn 1969), and treatment of rats undergoing long-term exposure to tobacco smoke with phenylmethyloxadiozole has been shown to protect the animals against the cigarette-smoke-induced increase in the number of goblet cells in the respiratory mucosa (Jones et al. 1973).

It can be concluded from these studies that both the particulate phase and the gaseous phase of cigarette smoke impair mucociliary function, that a large number of volatile components are ciliotoxic, that nicotine may or may not contribute to ciliotoxicity, and that the additive phenol is ciliotoxic, but the anti-inflammatory agents phenylmethyloxadiozole and phenylvinyloxadiozole afford partial protection against the deleterious effects of cigarette smoke. The mechanisms by which the various constituents of cigarette smoke interfere with mucociliary transport are unknown. On the basis of experiments in the fresh water mussel, it has been suggested that ciliotoxicity depends on their pH in solution (Wynder et al. 1963). It should be noted, however, that such in vitro experiments requiring an aqueous medium do not necessarily reflect the type of exposure occurring in smokers in whom contact between cigarette smoke and the ciliated epithelium is made by impingement or bypass.

Effects of Filters

Because the toxic effect of cigarette smoke on mucociliary transport mechanisms seems to reside both in the gas phase and in the particulate phase, the filtering of cigarette smoke before inhalation may be protective. It has been clearly shown that a longer exposure time is needed for ciliostasis to occur with smoke from filtered cigarettes than from unfiltered cigarettes, with respect to both ciliary activity in vitro and mucociliary transport in vivo (Dalhamn and Rylander 1964; Dalhamn 1964).

Four major types of filters have been evaluated: cellulose acetate (Cambridge filter), charcoal, glass fiber, and aqua. The histologic changes in the airways of guinea pigs exposed to unfiltered cigarette smoke for 4 to 8 weeks were not seen when cigarette smoke was passed through a Cambridge filter (Rylander 1974). Likewise, Kaminski and coworkers (1968) have shown that cellulose-acetate filters provide protection for the mucociliary activity in the cat

trachea. Similar results have been obtained in other experiments involving in vitro and in vivo systems (Dalhamn and Rylander 1968; Donnelly 1972; Wynder et al. 1965b), and cellulose-acetate filters have been found to reduce the inhibitory effect of cigarette smoke on tracheal epithelial adenylate kinase activity in hamsters exposed for 1 to 5 days (Mattenheimer and Mohr 1975). Charcoal filters are also capable of reducing the ciliotoxicity of cigarette smoke (Kaminski et al. 1968; Kensler and Battista 1963; Battista and Kensler 1970a, b). In one study involving cat tracheas, short-term exposure to a standardized dose of cigarette smoke decreased particle transport rates by 50 percent when unfiltered smoke was used, by 40 percent when the cigarette smoke was passed through a cellulose-acetate filter, and by 20 percent when a carbon-cellulose filter was used (Carson et al. 1966). In another comparison of different studies, a charcoal filter was more effective than a cellulose-acetate filter in reducing the metachronal wave frequency and mucus transport of the eulamellibranch gill in vitro (Wynder et al. 1965b). Glass-fiber and aqua filters were generally less effective (Isawa et al. 1980; Wynder et al. 1965b).

As expected, better protection might be provided by combined filters because they remove components of the particulate and the gaseous phase of cigarette smoke more effectively. Thus, a combination of cellulose-acetate and charcoal filter has been found to be more effective than either filter alone (Dalhamn 1966; Wynder et al. 1965b).

Mucociliary Function in Chronic Bronchitis

Since chronic bronchitis is defined clinically as chronic productive cough rather than by clearly defined morphologic or functional abnormalities (American Thoracic Society 1962), some of the previously reviewed studies of mucociliary function in cigarette smokers may have included patients with chronic bronchitis as well. Conversely, most patients with chronic bronchitis are cigarette smokers or have been cigarette smokers in the past. Although it is very difficult to separate the direct effects of cigarette smoke on mucociliary transport from those related to the pathophysiologic changes of chronic bronchitis, the discussion herein is limited to mucociliary function in chronic bronchitis without considering the direct effects of cigarette smoke on the mucosa.

The histologic changes of the mucociliary apparatus in chronic bronchitis include hypertrophy and hyperplasia of the submucosal glands, an increase in the number and distribution of goblet cells, and goblet cell metaplasia in smaller airways (Reid 1967). In addition, atrophy of the columnar epithelium (Wright and Stuart 1965) and spotty squamous metaplasia (Kleinerman and Boren 1974)

297

have been reported. A decrease in both the number of ciliated cells and the mean ciliary length has been noted in the larger airways in patients with chronic bronchitis (Wanner 1977), and electron microscopic examinations of the airway epithelium show subtle abnormalities in bronchial biopsy material (Miskovitz et al. 1974). Auerbach and associates (1962), in a large post-mortem study of cigarette smokers, reported epithelial lesions with loss of cilia in up to 30 percent of random sections, compared with approximately 15 percent of sections from nonsmokers. These ultrastructural changes consisted of swelling and serration of the epithelium with transformation of the goblet cell granules. The capsule surrounding the cilia was irregular, with areas of breakage and outward projections; some cilia showed fibrillar degeneration or were fused to form compound cilia.

The presence of visible respiratory secretions is a frequent endoscopic finding in patients with chronic bronchitis, and increased amount of bronchial secretions can be seen on pathologic sections of the lung (Kleinerman and Boren 1974; Hogg et al. 1968). Thus, the morphologic changes of chronic bronchitis involve both the ciliary apparatus and the mucus-producing structures.

Cilia

In vitro examination of ciliated lower airway epithelial cells obtained from chronic bronchitis patients by brushing has failed to reveal an abnormality in beat frequency (Yager et al. 1980). However, in vitro study of ciliary function is of limited informative value since the ciliated cells are suspended in an artificial medium and are not exposed to their natural milieu. This may explain the discrepancy between this study and one reported by Iravani and Van As (1972), in which ciliary motion was observed in vivo with an incident light technique. In the carefully dissected tracheobronchial tree of rats with experimental chronic bronchitis, the ciliary system showed discoordination and zonal akinesia. In addition, reversals of transport direction, whirlpool formations, and inactive zones without ciliary motion as large as 2 mm by several hundred μm were seen.

Mucus

The distribution, amount, and rheologic properties of mucus within the airways have not been studied in chronic bronchitis, but extensive literature exists on the biochemistry (Boat and Mathews 1973) and rheology of expectorated sputum from patients with chronic bronchitis. These results must be interpreted with caution, partly because of contamination with saliva and the rapid physical alteration of expectorated sputum, and partly because normal respiratory secretions for comparison are virtually impossible to obtain. Mucoid sputum of patients with chronic bronchitis is

298

biochemically similar to sputum of normal subjects induced by hypertonic saline aerosol, with the exception of a slightly higher fucose and neuraminic acid content in the former (Lopata et al. 1974). In purulent sputum from these patients, biochemical changes typical of inflammatory conditions (increases in the dry weight and deoxyribonucleic acid content and increased cross-linking by hydrogen bonding) were observed. Reid (1968) showed that the neuraminic acid content of sputum is increased in chronic bronchitis, suggesting augmented secretion by the mucus-producing structures. This finding is supported by histochemical studies indicating distended acini of the submucosal glands in patients with chronic bronchitis compared with normal subjects, along with an increase in the volume of both the acid and the neutral mucopolysaccharide-producing acini (Reid 1968).

Impaired mucus transport in chronic bronchitis may, in part, be related to the rheologic abnormalities of respiratory secretions. Deviation from the ideal ratio between viscosity and elasticity may prevent an optimal interaction between cilia and mucus, thereby decreasing mucus transport rates (Dulfano and Adler 1975; Adler and Dulfano 1976). Higher values of sputum viscosity and lower values of sputum elasticity have been observed during exacerbations of chronic bronchitis than during clinical stability (Dulfano et al. 1971). In addition, purulent sputum has a higher viscosity than mucoid sputum (Charman and Reid 1972; Mitchell-Heggs et al. 1974), suggesting a relationship between the concentration of certain mucus constituents and mucus rheology. Indeed, examination of sputum obtained from patients with chronic bronchitis has shown positive correlations between protein content (particularly IgA) and mucus glycoprotein content on the one hand and viscosity on the other (Harbitz et al. 1980; Lopez-Vidriero and Reid 1978). That altered rheologic properties of airway secretions play a role in abnormal mucociliary clearance has been suggested by an observed relationship between in vivo mucociliary clearance, in vitro transportability of expectorated sputum (using the frog palate), and the viscoelastic properties of sputum (Puchelle et al. 1980).

Mucociliary Interaction

Mucus transport has been studied either by directly or indirectly observing the motion of discrete particles placed on the tracheal mucosa (Santa Cruz et al. 1974; Goodman et al. 1978) or by the deposition pattern and clearance rates of inhaled radioactive aerosols (Lourenco 1970; Camner et al. 1973a, b; Luchsinger et al. 1968; Patrick and Stirling 1977; Dulfano et al. 1971). In one study, a marked slacking of tracheal mucus velocity was found in 15 patients with chronic bronchitis who were between 57 and 71 years of age (Santa Cruz et al. 1974). Clinical examination and pulmonary
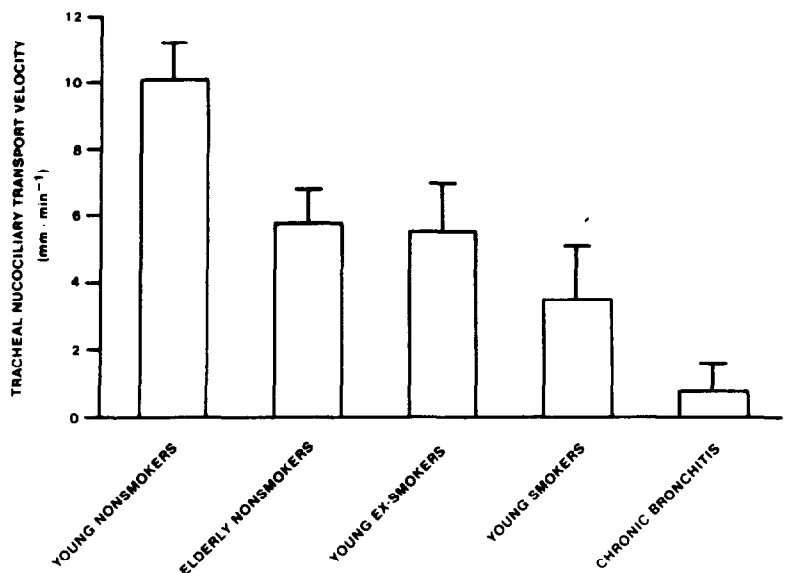
FIGURE 2.—Comparison of mean (S.E. in bracket) tracheal mucociliary transport velocity among young nonsmokers (n=10), elderly nonsmokers (n=7), healthy young ex-smokers (n=9), healthy young smokers (n=15), and patients with chronic bronchitis (n=14)

function tests diagnosed these patients as having both chronic bronchitis and emphysema. Mucociliary clearance of inhaled aerosols is also altered in patients with chronic bronchitis. The clearance of inhaled particles from the lung is influenced by the deposition pattern, which in turn depends on particle size and flow regime in the airways. Clearance rates, therefore, can be interpreted only if particle deposition is carefully monitored (Pircher et al. 1965; Lopez-Vidriero 1973). Coughing, which is difficult to control in such patients, may also contribute to the clearance of particles (Toigo et al. 1963). For these reasons, it is not surprising that mucociliary clearance has been reported to be increased (Muller et al. 1975; Luchsinger et al. 1968), normal (Thomson and Short 1969), or decreased (Lourenco 1970; Camner et al. 1973a, b; Tiogo et al. 1963; Mossberg and Camner 1980; Agnew et al. 1982) in patients with chronic bronchitis.

Once a subject has developed chronic bronchitis, cessation of smoking does not reverse the effect on mucociliary function, and a similar impairment of mucociliary transport has been reported in smokers and ex-smokers with this disorder (Agnew et al. 1982; Santa Cruz et al. 1974; Goodman et al. 1978). Persistence of mucociliary

300