AIRWAY RESPONSE TO INHALED AIR POLLUTANTS - CIGARETTE SMOKE & INDUSTRIAL DUST & FUME

By

SUSAN MARGUERITE KENNEDY

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Department of Pathology

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date 8 March 1984

ABSTRACT

Previous studies which identified inflammation in the peripheral airways of the lung as a potentially reversible early component in the development of chronic airflow obstruction led to the investigations reported here concerning the possible early diagnosis of airway inflammation using gallium-67; the relationship between airway inflammation, increased permeability and hyper-reactivity; and peripheral airway changes seen in persons exposed to mineral dust and fume contrasted with those seen in cigarette smokers.

Aerosolized radiogallium was administered in two studies, first to guinea pigs exposed to cigarette smoke and then to human smokers and the retention and clearance compared to that in non-smoking controls, and in the guinea pigs, to the degree of polymorphonuclear infiltration of the airways. The results revealed no difference in clearance between smokers and non-smokers and prolonged retention of the tracer in the lungs. Autoradiography in guinea pigs and humans suggested that the gallium was taken up by macrophages, not by polymorphonuclear cells. This suggests that the use of radiogallium to mark polymorphonuclear infiltration is not useful.

Increased respiratory epithelial permeability in human smokers was demonstrated by following the disappearance from the lungs of 99m technetium labelled diethylenetriame penta acetate. However, no relationship was seen between increased permeability and airway reactivity as measured by the degree of airflow obstruction following inhalation of increasing doses of histamine. This suggests that

although airway inflammation may result in increased airway permeability, this does not directly induce hyper-reactivity.

Finally, peripheral airway structural and functional changes were compared in two groups of patients undergoing lung resection: one with occupational mineral dust or fume exposure and the other, not exposed to dust or fume, individually matched to the first group for age and smoking history. The results indicated increased airflow obstruction in the exposed group and excess fibrous connective tissue deposition and goblet cell metaplasia in their peripheral airways, particularly in those patients exposed to mineral dust and fume in a non-mining occupation. No increase was seen in degree of inflammatory cell infiltration of the airway walls. These results suggest that the peripheral airway response to mineral dust or fume differs qualitatively from the response to cigarette smoke.

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I. INTRODUCTION

A significant amount of respiratory disease and mortality in our society can be accounted for by the breathing of dirty air. This is true both today and in the past. As early as the sixteenth century, Georgius Agricola recognized mine dust as a cause of much lung disease and death among miners in the Hartz mountains (1). Tobacco smoking became common in the western hemisphere in the late fifteenth century but the health effects only began to be appreciated by the end of the nineteenth century (2). Today, although our recognition and understanding of the problem of inhaled pollutants and how they produce disease has increased greatly, the magnitude of the problem remains as high, if not higher, than in centuries past.

Prevention strategies in place today include smoking education campaigns, which may be effective in reducing the number of tobacco smokers, and legislative controls placed on the extreme occupational exposures previously common in many mines, mills and factories. However, the potential for exposure to harmful agents in the air remains. There are approximately 10,000 new chemicals entering commercial and industrial use every year (3) many of which are respirable. The number of environmental/occupational agents associated with respiratory hypersensitivity or other adverse reactions, is also increasing. In conjunction with this is a tendency on the part of policy-makers to relax environmental standards in

favour of immediate economic concerns. Together, these factors suggest that respiratory disease related to airborne pollutants may not decline in direct proportion to a decrease in tobacco consumption.

The lung, being in constant contact with the environment, has developed an impressive defensive armament by which to maintain its equilibrium, repair damage, and rid itself of inhaled toxic or irritant substances. These defensive mechanisms are generally non-specific but their relative importance as well as their duration, intensity, and ultimate success are dependent both on characteristics of the exposure and on certain host factors.

The introductory sections of this report (Chapters II, III and IV) will review the exposure characteristics which influence lung response, the nature of the host defence mechanisms, and the patterns of reponse which may result in damage to lung tissue.

The specific investigations described in the main body of the report (Chapters V, VI and VII) were directed specifically at the airway response to inhaled pollutants. Much of the work described relates to the effects of tobacco smoke inhalation with some additional data on exposure to mineral dusts or fumes and to inhalation of cedar dust. This work has focussed particularly on the inflammatory response: the determination of early airway inflammation before irreversible tissue damage has occurred, the association between airway inflammation, permeability, and reactivity; and the morphologic identification of inflammation and its sequelae in peripheral airways and its relationship to functional abnormalities.

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II. Deposition of inhaled pollutants - physical determinants

Contaminants in the atmosphere are present in various forms. These can be classified as dusts, mists, fumes, gases, and vapours. The term aerosol is used to refer to airborne particles (solid or liquid) suspended in air, and thus includes both dust and mist. These particles can vary in size from extremely small nuclei ($< 0.001\mu$) to coarse mineral dusts (100 μ or more). Fumes are made up of metal oxides which are created when metals are heated above their melting point and are generally submicronic when formed although they may undergo significant aggregation. Vapours and gases are non-particulate molecular species, the former being normally liquid. The size range of suspended particulates generally encountered in the outdoor environment is from 0.01 μ nuclei to 20 μ aerosols with larger particles generally settling out (1).

Inhalation of atmospheric contaminants does not imply 100% retention. In fact considerable variation exists for the amount and location of deposition which depends on many physical factors. Health effects as a consequence of deposition are then modulated not only by deposition but also by the clearance and defense factors which will be discussed in the following chapter.

1. Inhalation and absorption of gases and vapours

The normal functioning of the respiratory tract requires rapid

and efficient exchange and absorption/desorption of O_2 and CO_2. These processes are governed by physical laws which are generally applicable to the behaviour of any gaseous solutes. These laws govern the pattern of ventilation, or the transport of inspired gas into the various lung components, and diffusion, or the transfer of the gas into the tissue. The factors which influence the normal ventilatory pattern include starting lung volumes and resistance factors which may be related to properties of the gas (eg. density, viscosity) and properties of the lungs (eg. compliance, obstruction). Diffusion through tissue is determined by the available surface area, the tissue thickness, the difference in partial pressure of the gas between the two sides of the tissue, and the gas solubility and molecular weight. These factors have been well described for normal respiration (2,3).

The distribution and uptake of toxic gases and vapours depend on these same factors. However, the site of action and amount of uptake are particularly influenced by solubility, time and surface area available for contact, concentration of the gas in the air, and buffering capacity of the mucus. Theoretical calculation of the amount of absorption would have to include calculations for four transfer processes: mass transport during inhalation, transport through the radial concentration gradient in the airways, mass transfer from gas to liquid, and diffusion through or reaction with liquid (1). Such calculations would have to account for the fact that the liquid lining the airways is heterogeneous and for metabolism of

the gas in the blood.

Solubility of the gas or vapour in the airway fluid is probably the key factor determining the site of absorption. Sulphur dioxide is highly soluble and is almost completely absorbed in the nasopharynx (4,5) whereas ozone is more insoluble and thus penetrates to the alveoli where fractional uptake is about 80% in dogs (6). Chronic ozone exposure has been shown to elicit a fibroblastic response in the terminal bronchiolar and alveolar duct regions (7). Acetone and chlorine are of moderate solubility, thus are absorbed partially by the nose and partially by the deeper airways and alveoli (1,8).

The time available for contact is determined by flow rate and breathing pattern (e.g. breath-holding). Increased duration of contact only increases absorption for less soluble gases and only up to the point at which equilibrium is reached between the partial pressures in the gas and fluid lining (8). Increased flow rates, while decreasing contact time, actually serve to increase the efficiency of absorption of soluble vapours by the nasal passages (9,10) although the fraction which penetrates past the nose also tends to increase. This latter effect was demonstrated in dogs by Frank and his colleagues (10), who found the proportion of SO_2 penetrating beyond the nose increased from 0.1% to 3.2% with a 10 fold increase in flow rate. In the same experiment, but with mouth breathing, the penetration beyond the nose increased from 0.4% to 66%. A similar effect is possible at sites of airway obstruction. An

increase in flow rate would tend to increase the penetration beyond the obstruction (11).

An increase in fractional penetration of SO_2 or other soluble vapours beyond the nose may also result from adsorption of the gas onto inhaled particulate (12) or as a result of saturation of the nasal mucosa if exposure is prolonged (5).

The concentration of the gas or vapour in the inspired air has a variable effect on fractional penetration. SO_2 fractional uptake by the nose, being almost complete, is little affected by concentration (4,5) whereas ozone fractional penetration beyond the nose is increased with increasing concentration (6).

Finally, in vivo alterations in the site of absorption may occur as a result of desorption from mucosal surfaces and subsequent further inhalation deep into the lung or excretion during expiration. Speizer and Frank (4) found that approximately 15% of absorbed SO_2 may be excreted in this manner. Deeper penetration of desorbed gas during the next inspiration could result in continued impairment or damage to tissue after exposure has stopped.

2. Deposition of aerosols

Physical factors which influence deposition of aerosols (dusts and mists) in the lung have been studied much more completely than those relating to toxic gases. These include those which are properties of the aerosol itself (size,density, shape, charge, and

hygroscopicity) and those related to the person inhaling (breathing pattern and airway geometry). As much of the work quoted in the following section was carried out using solid aerosols (ie: dusts), the word particles is generally used; however, the same general principles apply to liquid aerosols (ie: mists).

The physical mechanisms by which deposition can occur have been well described by many authors (12,13,14) and include impaction, sedimentation, diffusion, and interception. Impaction occurs in the nasal passages and at airway bifurcations or any other obstruction where the airstream changes direction and the inertial momentum of the particle (determined by its speed and density) carries it into the airway wall. The angle of change of direction and the velocity of airflow are significant in determining the amount of deposition that will occur by this mechanism. Gravitational settling, or sedimentation, is also dependent on the size and density of the particles which determine its settling velocity. Such deposition is modified by the speed at which air is flowing though the airway (which will tend to counteract settling) and the diameter of the airway. Particles may also deposit as a result of diffusion or movement due to bombardment with gas molecules. This is particularly important for particles $< 0.5\mu$ as at this size, the root mean square displacement due to diffusion equals the settling velocity (14). Particles which are highly irregular in shape (such as asbestos fibres) may deposit in airways by another mechanism not significant for roughly spherical particles, namely, interception. This mechanism comes into play when

the particle size is significantly large in relation to the airway size. Normally this does not occur as large particles deposit by impaction or sedimentation before reaching very small airways; however, fibres may have a low enough density to remain in the airstream longer until airway size becomes important in relation to fibre size. Long, straight fibres are thought to deposit by this mechanism to a greater extent than irregular, curled ones as a result of travelling parallel to the airstream and flipping end over end in a periodic fashion (15). Finally, deposition may occur as a result of electrical forces between the airway wall and the particles. Theoretically, a charged particle will induce an opposite "image charge" in the airway wall and thus be attracted to it. In addition, if an aerosol consists of similarly charged particles, repulsive forces will drive these apart and thus enhance deposition. In practice, however, these effects appear to be insignificant as experimental results indicate little effect on deposition of particles when charges are equilibrated (16).

Particles which are soluble and present in an environment with a relative humidity above a critical level will take on water and increase in size. This can occur in the outdoor environment and within the respiratory tract. However the absorption of water will lower the density of the particle, partially counteracting the effect of increased size. Hounam and Morgan (14) report that growth in size due to such hygroscopicity will be significant for tobacco smoke but not for petroleum exhaust particulate, which are long-chain aggregates

and will condense in humid conditions to form more rounded particles.

These mechanisms by which aerosol deposition may occur are dependent, as indicated, on a wide variety of physical and chemical characteristics of the aerosol. However, the most significant features of any aerosol which influence deposition can be described by defining the frequency distribution of the aerodynamic diameters of the particles (13). The aerodynamic diameter of a particle is the diameter of a unit density sphere with equal settling velocity as the particle. Any aerosol encountered in the environment will contain a distribution of particle sizes, and many of these fit a "log-normal" distribution (14). Determination of the median size (either mass or count median) plus the standard deviation defines the aerosol. When the particles are radioactive or tagged with a radioactive tracer the term activity median aerodynamic diameter is used and this parameter is equal to the mass median aerodynamic diameter (MMAD) only if the radioactivity is distributed evenly throughout the particle.

The ICRP Task Group on Lung Dynamics reviewed studies on aerosol deposition and formulated a deposition model relating deposition to mass median aerodynamic diameter (12). They found that deposition in the various lung compartments was best related to this aerosol parameter and showed surprisingly little variation even with aerosols with a wide range of particle sizes (or a large standard deviation). They calculated deposition in the nasopharyngeal region (extending to the larynx), tracheo-bronchial region (or ciliated airways including terminal bronchioles), and pulmonary region

(including respiratory bronchioles, alveolar ducts and alveoli), for three different tidal volumes respresenting mild to high activity states and a respiratory frequency of 15 cycles per minute.

Total respiratory tract deposition in nose breathing subjects was calculated to be approximately 100% for particles > 5μ, decreasing to a minimum of about 20% for 0.5 μ particles and then increasing again for even smaller particles due to diffusion. More recent investigations (14,17,18) have been in close agreement with the Task Group findings with the exception that total deposition of particles in the range 0.2 - 1μ has been found to be only about 10% and relatively constant throughout this range (14).

The nasopharynx acts as a very effective filter for most large particles with impaction being the major deposition mechanism in this region. Thus, flow rate and directional changes are the most significant contributors to enhanced deposition. The ICRP Task Group calculated that for aerosols > 5 μ MMAD nasopharyngeal deposition would be 70% or greater. More recently, Yu (17) using a trumpet lung model has determined virtually identical deposition in this region. Nasopharyngeal deposition falls off rapidly for smaller sized aerosols to almost zero for 0.1 to 1 μ particles. However deposition by diffusion becomes significant for extremely small particles and molecules. Swift (19) reports a total deposition of 35% for particles 0.02-0.04 μ of which 20-25% was nasopharyngeal, and George and Breslin (20) found 60% deposition in the nasopharynx for radon daughters which were in ionic form.

Particle deposition in the nasopharynx does not appear to be influenced by the hygroscopicity of the particles perhaps because transit time is too short (12). Nasal hairs have been suggested as being important for deposition and clearance (as mucociliary clearance mechanisms are not active in the region containing nasal hairs) (19) although no direct measurements have been made of their contribution. The individual variation found between subjects for inspiratory efficiency of nasal deposition has been found to relate well to the pressure difference across the nose and nasopharynx for flow rates up to 400 cm^3/sec (11).

The efficiency of the nose in preventing penetration of a great proportion of inhaled particles leads on occasion to disease itself. Nasal cancer among wood furniture makers and boot and shoemakers (21) and perforation of the nasal septum in workers exposed to chromate (22) have been attributed to deposition of aerosol particles on the nasal septum.

Particles are deposited much less readily in the tracheobronchial region of the respiratory tract. The ICRP Task Group (12) found a relatively constant deposition of about 8% of inspired dust for particles $> 0.05\ \mu$ which occurs mainly as result of impaction at bifurcations as well as some sedimentation due to the small airway diameters. Deposition for smaller particles increases as total particle deposition increases. Somewhat higher results for small particles ($< .01\mu$) deposition in this region at 750 cm^3 tidal volume have been reported more recently (17). Enhanced

tracheobronchial deposition may occur as result of hygroscopic particles increasing in size as they travel through the airways. Cigarette smoke particles range from 0.2 to 1.5μ diameter (22) and thus would not normally deposit on airway walls to any large extent. However, uptake of water from the airways (which are almost completely saturated) would result in considerable increase in size and consequent deposition on bronchial walls.

Most material which penetrates the larynx is either deposited in the pulmonary compartment or exhaled. Pulmonary deposition increases to about 30% for aerosols with MMAD from 0.5μ to 5μ with a maximum deposition occurring for particles of 2-3 μ (12,13). A sharp increase in deposition occurs for very small particles ($< 0.1\mu$) for which diffusion becomes important as a deposition mechanism. The significant mechanisms responsible for alveolar deposition are sedimentation and diffusion but it has been proposed (23) that mixing of tidal and residual air in the airways is the rate-limiting process.

Pulmonary deposition calculated by Yu (17,18) is considerably lower than the Task Group calculations with a minimum of about 10% deposition for 0.3μ particles at 750 cm^3 tidal volume.

Within the tracheo-bronchial and pulmonary compartments there is considerable heterogeneity of aerosol deposition that is not reflected by calculations or experimentation on deposition in the compartments as a whole. At airway bifurcations, hot spots have been shown to develop with enhanced deposition several times greater than the average (24). An apex to base gradient has also been reported

with increased deposition in apical regions in the dog (25) and the rat (26), regardless of body orientation. On the microscopic level, deposition may also be heterogeneous within the acinus.

Brody and his colleagues (27,28) have shown asbestos fibre deposition in rats throughout the tracheobronchial tree but particularly at bifurcations. They also determined that fibres which penetrate beyond the conducting airways despoit mainly on bifurcations of the proximal alveolar ducts.

It is apparent from the foregoing that aerosol size characteristics are key factors influencing the amount and location of deposition within the respiratory tract. However, a feature common to most experimental investigations of deposition is the wide degree of variation between individual results and even between separate studies on the same person. A large amount of this variation has been shown to be related to breathing pattern.

Inspiration through the mouth results in increased penetration of particles into the tracheobronchial compartment. Foord and colleagues (29) studied deposition of particles from 2.5 to 7.5μ in mouth breathing subjects and found tracheobronchial deposition from 10 to 39% over this range. Mouth breathing typically occurs during a high activity state and is usually accompanied by an increase in tidal volume. Davies has compared a sedentary breathing pattern (in nose, out mouth, with a minute volume of 7.5 1.) to an active breathing pattern (in and out mouth, minute volume 50 1.) for particles from 0.5 to 5μ and calculates a doubling of deposition beyond the larynx for 2μ

particles and tripling for 5μ particles but little change for 0.5 and 1.0 μ particles. The pattern of deposition in the tracheobronchial compared to pulmonary compartment remained about the same (reported as personal communication by Parkes (22)).

An increase in flow velocity results in turbulent flow at deeper levels of the respiratory tract and thus greater mixing of the aerosol with residual gas (13). This has the potential effect of increasing inertial impaction during rapid breathing. This can be offset by other changes in the breathing pattern. Valberg and colleagues (25) studied slow, deep; slow, shallow; and rapid, shallow breathing in dogs and found that total deposition decreased with increasing frequency of breathing and decreased tidal volume. This is in agreement with the predictions of Yu and Taulbee (18) who calculated similar results with varying tidal volume and frequency.

Breath-holding has also been studied and shown to affect deposition in a major fashion. Goldberg and Smith (30) reported that the fraction of aerosol deposited was exponentially related to breath holding time and that this was due to increases in both diffusional and gravitational deposition. Similar results were also found by Palmes and associates (31).

The significance of altered deposition due to breathing pattern is emphasized by a recent study by Higenbottam and colleagues (32) who studied cigarette smokers with varying smoke inhalation patterns. They found consistent airway narrowing when the subjects drew the smoke directly into their lungs through the mouth, but no

consistent response when the inhalation pattern was "normal", consisting of an initial drawing of smoke into the mouth, a pause with some expiration, and then inhalation through nose and mouth.

Finally, deposition may be affected by variations in airway geometry, whether these are inherent or related to the presence of disease. Bronchial cross-sectional areas have been shown to vary considerably among individuals in relation to stature (33). Calculation of the magnitude of a deposition parameter reflecting respiratory airspace dimensions by Palmes and Lippmann (34) indicate a potential for a doubling of tracheobronchial deposition related solely to this factor.

Airway disease also influences deposition with, in most cases, preferential airway over pulmonary deposition. Cigarette smokers tend to have more proximal deposition (35, 36) as do patients with asthma and chronic bronchitis (37). These findings may be the result of several interacting mechanisms. Airway obstruction increases turbulence and therefore promotes mixing and increased inertial impaction. Bronchoconstriction may act similarly and both may also divert flow to other areas resulting in greater heterogeneity of deposition. Excessive secretions have also been implicated in increasing turbulence due to wave motion with little or no change in airway resistance (38). The heterogeneity and increased airway deposition resulting from airway disease may play a role in the pathogenesis of bronchogenic carcinoma.

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III. Lung Clearance Mechanisms

The oxygen requirements of the body must be met by passive diffusion of oxygen across the blood-air barrier in the lung. This means that the area for respiratory exchange in the lung must be immense in order to supply the body's metabolic needs. This surface area of alveolar wall has been estimated at 200 or more square meters, in 300-400 million alveoli, with a thickness of approximately 0.5 microns (1). With an inhaled volume of from 10,000 to 20,000 l. of air per day, this vast expanse of delicate tissue is at obvious risk if the inhaled air contains toxins or irritants. Several defensive structures and mechanisms work to prevent the passage of injurious agents into the lung parenchyma or to remove those that avoid the defensive filter. These have been reviewed extensively recently (2,3,4,5,6) and will only be discussed briefly here.

1. Upper Airway Defences

The nasal air passages represent an extremely important first line of defense against large particles and soluble gases, acting as both a filter for trapping particles and a highly vascular sink for uptake of water soluble gases. The extremely variable vascularity and rich secretory system of the nasal air passages also provides for warming and humidification of inhaled air, an essential element in the

maintenance of the equilibrium of the lower respiratory organ.

With increased flow rates, as encountered in light physical exertion, nasal air passage dimensions are increased slightly (7), decreasing resistance and allowing continued nose breathing. The increase in flow rate allows for greater impaction of inhaled particles in the anterior nares. However with heavier exercise, the resistance of the nasal passages becomes too large to meet the ventilatory demand, and mouth breathing occurs (8). At this point, both the filtering and conditioning functions are bypassed. This is an important consideration in assessment of occupational exposures if the work involves heavy physical exertion. It must also be taken into account in assessment of exposure to pollutants which cause nasal congestion, or in assessment of individual risk in persons with increased nasal resistance or decreased clearance for any reason. Cigarette smoking, of course, also completely bypasses the nasal defenses.

In situations of continued bypass of the nasal defense mechanisms, such as in patients with long-standing tracheostomy, there is never complete compensation for this loss, with the result that those patients suffer from chronic bronchitis, with squamous metaplasia of large airways and impaired mucociliary clearance (2). D.F.Proctor has proposed (2) that persons with minor impairment of nasal clearance and conditioning mechanisms may be putting an undue burden on the smaller airways, predisposing them to disease. He argues that the large conducting airways are unsuited to complete the

air-conditioning process but the small, peripheral airways, may well be able to take up the remainder of the job. However, in order to do this they may resort to the preferential development of goblet cells rather than ciliated cells, leading, if prolonged, to impaired ciliary clearance, altered surface tension properties and ultimately, dysfunction of these small airways. Proctor has further shown (9) that nasal clearance and resistance varies among normal individuals and suggests that investigation of these properties may reveal individuals at greater risk for future lung damage.

2. Tracheo-bronchial defenses

a) Muco-ciliary clearance

The trachea and airways within the lung represent the second line of defense against pollutants which escape the nasal filter. The key mechanism at this level is the trapping and clearance of particles by the muco-ciliary escalator. The success of this system depends on contact of particles with the airway walls; efficient, coordinated ciliary movement; and mucus with biochemical and rheological characteristics conducive to both entrappment of particles and movement by ciliary beating. Particles deposited on the mucous layer are carried upstream as a result of the ciliary beating which propels mucus droplets toward the pharynx.

This "mucous blanket" is believed to be a heterogeneous and

variable lining rather than a continuous layer. It is made up of varying proportions of mucous and serous secretions from specialized submucosal glands and goblet cells, serum and tissue transudates, and inflammatory infiltrates. In the normal healthy person the volume of this fluid amounts to about 10 ml per day (14). Lucas and Douglas (15) initially proposed the concept that the architecture of this fluid layer is that of a watery, serous or sol-phase adjacent to the epithelial cell surfaces and surrounding the cilia and an overlying, mucous or gel-phase. It has since been proposed (16) that the mucous "blanket", or gel-phase, is discontinuous, at least in the more distal airways. Cilia are believed to beat in such a manner that the rapid, effective stroke occurs with the claw-like tip of the cilia extending just to the gel phase and propelling it forward either directly by contact with the mucus droplets or indirectly by sweeping the mucus along with the upper portions of the sol phase. The slow recovery stroke of the cilia takes place in the more watery, peri-ciliary sol-phase.

Extenstive investigation has been carried out in recent years to characterize the biochemical and physical properties of the airway lining fluids, to determine how these properties are influenced by inhaled substances, and to determine whether changes in the fluid lining characteristics lead to or are associated with altered pulmonary function or respiratory disease (17, 18, 19). Analysis of changes in airway secretions is generally carried out by sputum analysis or by special histochemical staining of sections containing

airway walls. It must be noted that production of sputum implies disease and therefore analysis of sputum does not provide "normal" data. Centrifugation of sputum from chronic bronchitics results in separation into sol and gel phases, with the sol phase containing mostly soluble serum glycoproteins and the gel phase, insoluble epithelial glycoproteins (3). Histochemical staining of goblet cells and submucosal glands with Alcian blue - periodic acid Schiff stain separates acid and neutral glycoproteins respectively. Staining at pH 0.5 versus pH 2.5 differentiates sialo- and sulphomucins and sialidase digestion reveals sialidase resistant sialomucins. These techniques show that goblet cells contain mostly acid glycoprotein (both sulpho and sialomucins), mucous cells contain four types of acid glycoproteins, and serous cells contain sialo- and sulphomucins but with different characteristics than those in mucous cells.

Changes in sputum or histochemical staining with various disease states and exposures have been summarized by Lopez-Vidriero, Das and Reid (17). Sputum from chronic bronchitics compared to that produced by normal subjects given $PGF_2\alpha$ to induce expectoration differs in having a higher mannose content suggesting greater serum transudate, increased levels of both epithelial and serum acid glycoprotein, and an increase in sulphated glycoprotein production. This last is also seen in histochemical studies of animals exposed to cigarette smoke or SO_2 (20). SO_2 exposure of canine tracheal pouch preparations has also been shown to cause significant changes in mucus elasticity (by a reflex action, as there was no direct contact

between the gas and the pouch mucosa) (21). What effect these changes have on the defensive action of the lung is less clear. The interaction between ciliary movement and mucus propulsion has been reported to depend on the rheological properties of mucus (viscosity and elasticity) rather than the specific biochemical make-up (22,23). However, the rheological properties of mucus in humans are believed to be determined by the arrangement of the glycoprotein molecules and the presence of cross-linkages, and viscosity has been related to chemical constituents in patients with chronic bronchitis. The thickness of the fluid layer has also been shown to be of importance in clearance, with both too thick or too thin a layer resulting in decreased movement of surface particles (24). The goblet cell metaplasia and gland hypertrophy seen in chronic bronchitics results in increased production of mucus. This is related to significant airway obstruction but the effect that the increased mucus volume itself has on clearance is not evident. In studies on excised chicken tracheas, Hilding (25) has demonstrated that mucus plugs occluding the whole airway lumen can still be transported effectively by ciliary movement.

The overall effectiveness of the mucociliary esclator also depends on co-ordinated ciliary beating in a generally cephalad direction. The number of ciliated cells and the rate of ciliary beating increases from peripheral to central airways as does the rate of the mucous transport. The importance of ciliary movement in the prevention of disease is demonstrated by investigation of persons with complete absence of ciliary movement, the "immotile-cilia syndrome",

in whom mucociliary clearance is slow or absent. Patients with this syndrome have been reported (26) to have recurrent upper and lower respiratory tract infections, frequent bronchiectasis, and sometimes, emphysema. Inhaled environmental pollutants can affect ciliary movement, either by paralysis of cilia or by loss of ciliated cells (20). Ciliostasis has been demonstrated in excised animal tracheas (27) and human epithelial cultures (28) after brief exposures to cigarette smoke. Long-term exposure to smoke resulted in decreased ciliary frequency in hamsters but an apparent increase in rats, although there were focal areas of discoordination and absence of ciliary movement (29). SO_2 and NO_2 both produce similar ciliostatic effects with brief exposures. In addition to disturbance of ciliary function,inhalation of these substances is also related to loss of ciliated cells as a result of focal epithelial damage, and squamous and goblet cell metaplasia (20).

The defense of the lung, then, at the level of the tracheo-bronchial tree depends on the combined effect of ciliary beating and mucus production; that is, the interaction of cilia and mucus. This has been measured in humans and animals as "muco-ciliary clearance" by elimination of inhaled aerosols, or "mucus transport" by the movement of markers placed in a known location on the mucosa. Differences in these measurements may be the result of the degree of penetration of the tracer into the surface fluid layer or the surface area over which the tracer is deposited. Measurement of mucociliary clearance or transport rates in human cigarette smokers suggest that

this function may be impaired as a result of smoke exposure but not in all individuals. Camner and Philipson (30) studied 10 pairs of smoking-discordant twins and found that in five pairs, the smoker displayed impaired clearance while in the other five pairs, there was no difference in clearance rates. This study exemplifies the results of many investigations; that is, studies indicating slower clearance in smokers (31) and others (32) which fail to uncover a difference relating to cigarette smoke exposure. In a review of these studies Wanner (22) concludes that the evidence does indicate impairment of mucociliary transport in smokers which may occur after as little as one year of exposure and may precede other respiratory functional abnormalities.

Inhalation of other atmospheric pollutants may produce impaired or augmented clearance. Camner and co-workers studied carbon dust inhalation (33) and found increased clearance in some subjects. Similar results were found with SO_2 and sulphuric acid inhalation in exercising humans, and this was attributed to a reflex increase in secretions in the airways (34,35). Another study (36) noted this increased clearance with sulphuric acid inhalation at low concentrations but found decreased clearance with high concentrations. Animal studies of long-term exposure to SO_2 and NO_2 also indicate impaired mucociliary transport (37,38), although the effects of NO_2 were reversed 7 days after cessation of exposure and no airway pathology was evident in the animals studied. Delehunt and colleagues (39) exposed sheep to a combination of SO_2 and ozone

and found impaired mucus velocity in vivo but no change in ciliary beat frequency in vitro.

This impairment of the coordinated action of mucus and cilia could result in excessive build up of secretions and consequent airway obstruction by mucous plugging; prolonged contact of inhaled toxins or carcinogens with epithelial cells; and alterations in the surface lining characteristics of peripheral airways resulting in airway closure.

b) Reflex airway defenses

In addition to the entrapment and removal of inhaled particles by the mucociliary mechanisms, the airways also provide additional defense against penetration of pollutants into the alveolar space via reflex mechanisms. These airway reflexes and their response to stimuli represent a complex, interactive system of controls on airway calibre, rate and depth of breathing, and specialized response patterns (such as cough and sneeze) which varies with both the site stimulated and the nature of the stimulation.

Generally speaking they represent the initial, immediate response to inhalation of irritant stimuli and can also serve to bring other physiologic defensive responses into play.

In the airways, the "irritant receptors" are myelinated, afferent fibres, located between and beneath epithelial cells (40). Physiologic studies in animals show that these are more concentrated

in central airways (71% within one cm of the hilum in dogs (41) and 54% in cats (42)) and are stimulated by dust, gases, aerosols, and mechanical distortion (42-46). Stimulation of these receptors in the larynx and trachea results in cough, in which a deep inspiration is followed by a forced expiratory effort initially against a closed glottis. The intrathoracic pressure generated is extremely high. The airways are narrowed, both by dynamic compression and reflex bronchoconstriction resulting in increased airflow and greater rigidity of the airways so that they are less likely to collapse with the high pressure generated during the cough (47). The rapid airflow of expiration results in transformation of the liquid within the airway lumen into a mist, which is expelled, taking deposited particles along with it.

The effectiveness of coughing in the elimination of inhaled particulate or excessive secretions is variable among patients. Mossberg and colleagues (26) found that patients with the immotile-cilia syndrome could eliminate test particles by 1-2 minutes of coughing with an efficiency varying from 2 to 48% . Camner and colleagues (48) found that such elimination of test particles was possible only in patients with symptomatic phlegm and expectoration and that healthy subjects could not expel these with voluntary coughing. On the other hand, cough in patients with obstructed airways may be ineffective in removing secretions as the narrowed airways may close as a result of the high compression forces in the chest during coughing.

Cough receptors may be stimulated by various stimuli including inflammation, mechanical or chemical irritation, or thermal stimulation (ie: cold air). The importance of cough as a defensive mechanism is underscored by the potential for lower respiratory tract infection in patients unable to cough.

The physiologic responses resulting from stimulation of "irritant receptors" in the smaller airways are not completely understood. Studies in anaesthetized animals suggest that rapid shallow breathing and bronchoconstriction may result from stimulation of these receptors in the lung (42) but the significance of these to human physiology is not clear. Rapid shallow breathing does not appear to occur in humans exposed to irritants and the role of vagally mediated nervous stimulation in the bronchoconstriction associated with airway hyper-reactivity is controversial.

Such a reflex could, theoretically, serve a defensive function in promoting deposition of particles in larger airways, although its practical value is debatable. Cigarette smoke inhalation in both chronic smokers and non-smokers produces an immediate bronchoconstrictory response (49) but this is not a consistent finding and regular cigarette smokers appear to be able to modify their smoke inhalation to overcome this reaction (50).

c) Removal by blood or lymphatics

Clearance of material deposited in the airways by movement

through the epithelium is lessened by the fact that airway epithelial cells are attached to each other at their apices by a "tight junction" network which normally renders the epithelium impermeable to all but the smallest molecules (51). However several investigators (52,53) have shown that this barrier is broken down and the epithelium becomes much more permeable to macromolecules in the presence of an inflammatory response in the airway. This will be discussed more fully in the following sections. As a clearance route, however, it has been demonstrated that the majority of material which penetrates the airway epithelium as a result of increased permeability, and which is subsequently removed from the lung, is cleared by the bloodstream rather than by lymphatics (54). It is not known whether significant material becomes sequestered in the tissue or in tissue macrophages as a result of this epithelial penetration.

3. Alveolar Clearance

Inhaled substances that reach alveolar spaces may be cleared by one or more of several mechanisms: solubilization, transport and elimination via the bloodstream or lymphatics, or transfer to and removal by the mucociliary escalator.

In vivo solubility of substances within the lung has been studied by comparing the solubility of substances injected into muscle to their rate of disappearance in the lung. Morrow and associates (55) found that retention of a wide variety of metal oxides and other

metallic particles in the lung parenchyma correlated with retention in muscle and with ultrafiltration characteristics in vitro. In addition, others (56,57) have found a correlation between long-term retention of particles and in-vitro solubility. However, while dissolution may be a significant clearance mechanism in some cases, it cannot explain the clearance patterns for highly insoluble particles.

Clearance of inhaled particles by transfer to the lymphatics has been studied by several workers (58-60) who have done serial investigations in animals following instillation or injection of particles including colloidal carbon, ferritin, and iron oxide. These studies indicate that particles cross the alveolar epithelium and appear in the interstitial connective tissue. The mechanism whereby particles traverse the epithelium is not completely known. Some authors have provided morphologic evidence of transport across Type I alveolar epithelial cells, where the particles appear in vesicles (59). Other studies using protein tracers (60) have indicated that a paracellular pathway may be singificant, particularly if the epithelial tight junctions are damaged by irritants such as NO_2 or cigarette smoke. Once across the epithelium, however, the particles then appear in the pulmonary lymphatics where they are seen both within the lymphatic lumena and in vesicles in the lymphatic endothelial cells (59,61). The relative importance of transport of inhaled particulates through the interstitium and into lymphatic vessels (which do not reach as far as the alveoli) is not clear. Total lung lymph flow is very low and the mechanism of transport of

particles through the interstitium is also unknown although tissue fluid movement resulting from negative hydrostatic pressure has been postulated (58). It has been assumed that the amount of inhaled or instilled particles seen in regional lymph nodes represents the amount cleared via lymphatics. This ranges from negligible (62) to about five percent of the lung burden (63) depending on the initial lung burden and the size and composition of the particles. These figures may in fact under-represent the case, as the lymph nodes may not completely filter out all particles carried by the lymph.

The transport of material ingested by alveolar macrophages from the alveolar space into the lymphatics has not been clearly demonstrated. Lauweryns and Baert (58) described the accumulation of both carbon and ferritin particles in macrophages in the interstitium but did not observe actual passage of these cells through the epithelial layer. It has been suggested by Brain and associates (64) that particle-containing phagocytes within the interstitium most likely represent resident macrophages which have ingested free material which passed through the epithelium.

Clearance via the bloodstream appears to be significant only for very small particles which are not taken up by macrophages. Meyer and his colleagues (65) instilled albumin into dog lungs and found a blood/lymph removal ratio of 6.3 to 1 and little evidence of airway clearance. Lauweryns and Baert (58) however, found no evidence of carbon uptake into pulmonary capillaries and only occasional ferritin molecules were seen along endothelial cell junctions and in

micropinocytotic vesicles. Migration of alveolar macrophages into the blood is believed to be very unlikely given the nature of the blood-air barrier and the lack of evidence of blood activity in studies with inhaled radioactive insoluble particles (64).

Another possible route of clearance for inhaled particles ingested by macrophages is transport to the ciliated epithelium of the airways and passage mouthward on the mucociliary escalator. As with the other modes of alveolar clearance, there is considerable debate as to the mechanism and significance of this route.

It is known that most particles deposited in the alveoli are ingested by alveolar macrophages but how these cells migrate toward the bronchiolar surfaces is not known, although several mechanisms have been suggested. These are reviewed by Brain, Godeski and Sorokin (64) and include following a concentration gradient of chemotactic factor, random movement, and passive following of alveolar fluid currents. Kilburn, on the other hand, has proposed (66) that macrophages present in distal alveoli represent a population that does not, except by infrequent random movement, interact with the mucociliary escalator, but rather depends on digestion or solubilization to remove the particles. He postulates a second population of macrophages which sieves through terminal and respiratory airway walls and is cleared by mucous transport. Most investigations, however, suggest that there is some intralumenal translocation of dust laden macrophages from alveoli toward the ciliated epithelium. Sorokin and Brain (59) studied the removal of

iron oxide particles from mouse lungs at intervals up to fourteen months after a single exposure. Macrophages appear in increased numbers on the alveolar surface by one hour after exposure and actively ingest the particles. Both extracellular and cellular mechanisms were believed to be responsible for movement of particles toward bronchioles with a transient retardation of movement at the airway alveolar junction before moving into the bronchiolar lumen. Again, the importance of this route depends on the nature of the inhaled substance. For example, with the inhalation of cytotoxic silica particles, macrophages are damaged and the free particles may penetrate to the interstitium more freely. This concept is supported by studies in which a correlation was found between the amount of particles cleared rapidly via airways and the number of macrophages present (67) and studies in which the clearance of silica was increased when its cytotoxicity was reduced by polyvinylpyridine-N-oxide administration (63).

In general, alveolar clearance of inhaled particles in humans appears to be extremely slow. After an initial rapid clearance phase during the first 24 hours, clearance half-times are reported as long as several months (5). The initial phase is thought to be associated with fluid flow and movement of alveolar macrophages to bronchiolar walls with subsequent mucociliary clearance. Dissolution and removal of particles via interstitial pathways may account for a second phase of clearance. There remains however, a fraction of the inhaled lung burden for which no apparent clearance mechanisms exist. This

fraction is present largely within macrophages which are not cleared by mucociliary action in the airways and could present a concentration phenomenon which may be harmful in that toxic or carcinogenic substances become localized and concentrated. Brain and his colleagues (64) suggest that some of these macrophages which do not clear from the lung become adherent to the airway epithelium and may release their contents with subsequent uptake by subepithelial connective tissue macrophages. These cells are situated such that they are closely associated with cells of the immunologic defense system. Stirling and Patrick (68) also found macrophage-associated epithelial penetration of $BaSO_4$ particles in rat trachea in areas of non-ciliated, cuboidal or squamous epithelial cells which were infiltrated by a conspicuous number of lymphocytes. Increased epithelial permeability may play a role in this kind of trans-epithelial particle movement.

The effectiveness of alveolar clearance mechanisms can be altered by inhalation of various agents. Ferin and Leach (62) found that about 40% of inhaled TiO_2 particles were cleared within 25 days of exposure and that this was mainly due to alveolar macrophage involvement. Exposure to low concentrations of SO_2 and NO_x enhanced this clearance whereas higher concentrations depressed clearance of the TiO_2.

In general it appears that substances which activate and/or recruit macrophages will aid macrophage associated clearance (57). Conversely, inhalation of substances which damage the macrophage such

that its capacity for phagocytosis is diminished render the lung more liable to infection or damage due to retention of an increased burden of particulate. Ozone, high oxygen concentrations, and cigarette smoke have all been shown to decrease the bacteriacidal capacity of macrophages (69). Silica exposure is cytotoxic to macrophages and silica dust is cleared much more slowly than inert particles (63). Long term exposure to silica is also associated with an increased incidence of tuberculosis infection (69). Studies carried out by Camner and associates in rabbits, however, found that in vivo clearance of telflon particles coated with silver, carbon, or beryllium did not differ despite the fact that beryllium coated particles were more toxic to macrophages in vitro (70).

The normally low permeability of the alveolar-capillary membrane prevents significant bloodstream clearance for all but very small particles. This permeability has been shown to be significantly increased after cigarette smoke exposure (71). Whether this has importance for clearance of larger particulates remains to be determined.

In summary, the mechanisms which may be brought into play to defend the very delicate alveoli from inhaled substances are not as well understood as those of the airways. They appear to operate much more slowly and less efficiently than nasal or tracheo-bronchial defenses. Thus the potential for lung injury is increased when the airway defenses are bypassed or overcome.

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IV. The Lung Response and the Inflammatory Process

The preceding chapters have reviewed the factors, both external and internal, which influence deposition and retention of inhaled noxious agents. The lung response, that is, whether or not exposure to these agents results in lung damage or disease, depends only in part on these deposition and retention characteristics. Equally important is the activity of the agent in the lung, the nature of the injury resulting from retention and whether or not the exposure provokes an inflammatory response.

The initial response of the lung tissue to an inhaled toxic aerosol or gas depends largely on the nature or chemical activity of the agent and many diverse types of injury may occur. These can be characterized as injury due to asphyxiation, systemic toxicity, reflex responses, specific or non-specific immunologic mechanisms, or direct mucosal or alveolar damage.

Asphyxiation occurs when the oxygen in alveolar air is replaced by a gas not normally toxic (for example, by CO_2, N_2, or CH_4 as can occur in an underground mining environment). Alternately, tissue asphyxia can occur as a result of inability of a tissue to obtain or utilize O_2, if the O_2 carrying capacity of the blood is decreased (e.g. by CO or HS) or if the respiratory enzyme systems of the cells are poisoned (as with cyanide). This latter example could also be termed damage due to systemic toxicity. Another

example of agents being absorbed via the alveoli and causing systemic disease is believed to occur in "metal fume fever" and "polymer fume fever". In these disorders an influenza-like condition occurs following exposure to the very small pyrolysis products produced during the heating of certain metals or tetrafluoroethylene resins (1).

Certain inhalants may provoke injury by reflex mechanisms associated with airway irritant receptors. Sulphur dioxide gas (2) and particles of charcoal (3) have been reported to directly stimulate airway receptors resulting in airway smooth muscle constriction. Stimulation of irritant receptors has also been implicated in the delayed bronchoconstriction which follows exposure to high concentrations of various toxic gases and aerosols. This is, however, believed to be secondary to an inflammatory response, rather than a direct reflex effect. This will be discussed later in this and subsequent chapters.

Immunologic mechanisms can also result in lung injury. One example of specific antigen directed immune responses is the antigen-induced mediator release from airway mast cells (4) resulting in bronchoconstriction in which specific reaginic antibodies are implicated. Allergens which may be associated with this type of specific response include grain dusts, animal products, insect proteins, B-subtilis enzymes, gum acacia, and castor oil bean (1). Other inhaled allergens associated with fungal spores and avian proteins may provoke a specific immune response in the alveoli resulting in the syndrome of hypersensitivity pneumonitis or extrinsic

allergic alveolitis (1). However this disease has features which suggest the additional involvement of non-immunlogic mechanisms. This is also true of the asthma or bronchoconstriction produced by inhalation of a wide variety of chemicals, metals and wood dusts (5) in which a direct, specific immune response is only partly implicated. Immunologic mechanisms have also been postulated in the progression of the pneumoconioses (6), and a high level of circulating antinuclear antibodies has been found in persons with silicosis (7). Few gases are considered to be antigenic, however vinyl chloride monomer gas inhalation is strongly associated with immune complex aggregation (8,9).

Direct injury or stimulation of cells of lung tissue accounts for a good deal of initial lung injury, either by altering cellular metabolism or producing cell death and tissue necrosis. In the airways, agents such as ammonia and aldehydes (10) have been associated with alterations in the regulation of transport of water and ions by epithelial cells and NO_2 inhalation (11) associated with ciliary alteration. Both these effects may influence mucociliary clearance activity in the airways. Ozone, O_2, and NO_2 may have an oxidant effect damaging membranes of Type I pneumocytes and endothelial cells (12). In low concentrations silica inhalation recruits and activates macrophages (6,13) while in higher doses, in vitro, it is cytotoxic to alveolar macrophages (14) and to Type I and Type II pneumocytes (15).

This catalogue of injurious effects is not intended to be

all-inclusive but rather to indicate the diversity of the initial cellular or tissue injuries which may be associated with inhalation of pollutants.

If exposure is minimal, the tissue response may be such that cells are altered but then return to their normal state when exposure ceases. However, if exposure is prolonged, if the concentration is high, or if the agent is especially toxic, then the injury, whether it is direct or indirect, and regardless of its nature, calls forth an inflammatory response which is not specific to either the agent or the kind of injury. The initial injurious event may be direct or indirect damage to bronchiolar or alveolar epithelial cells, damage or stimulation of immune or inflammatory cells resident in the lung, or antigenic challenge; the common antecedent of all of these types of injury is the recruitment of immune and inflammatory cells from the circulation and the development of an inflammatory response which has the potential for greater tissue damage. Mediators released from injured or activated cells diffuse into the circulation and initiate this response.

The basic features of the acute inflammatory response are non-specific even as to the tissue involved. These include the exudation of fluid from the small vessels in the injured area as well as the emigration of white blood cells, notably polymorphonuclear leukocytes (PMN) from the circulation and their aggregation and activation in the tissue. This acute response may resolve, with the exudative debris being cleared up by a small number of macrophages or

it may persist, becoming chronic. The chronic response is somewhat more dependent on the nature of the injurious agent. If the agent is antigenic there may be a specific lymphocytic and plasma cell response, whereas if the agent is not antigenic the cells will be predominantly macrophages. If the precipitating agent is chemotactic for PMN's itself and if it persists, the resulting histological appearance will include cells typical of both the acute and chronic reponse. The details of these cellular and vascular events involved in the inflammatory response have been reviewed at length recently (16,17,18).

The importance of the inflammatory response and in particular of mediator release from inflammatory cells was recognized as early as 1887 (19) by Mechinkoff, who proposed that "ferments" released from cells in inflamed tissue were responsible for tissue damage. Mediators present in lung which may initiate an inflammatory response are found, in particular, in mast cells and macrophages; thus activation of these cells to release their contents whether directly, reflexively, or during the process of phagocytosis is implicated in the development of inflammation. Mast cells are present in airways in both intra- and sub-epithelial locations (20). As previously pointed out, mediator release may occur via antigenic stimulation or directly, or by a combination of both effects. Cotton bract extract causes histamine release from human lung tissue in vitro (21) presumably by a non-antigenic mechanism, as does western red cedar (22), although immunologic mechanisms are also implicated at least in the latter case

(5). The mediators released from mast cells include histamine, eosinophilic-chemotactic factor (ECF-A), platelet activating factor (PAF), a neutrophil chemotactic factor, prostaglandins, leukotrienes (SRS-A) and a bradykinin-like enzyme (4,18,23,24). The resultant response of the lung is constriction of bronchial smooth muscle, mucosal edema, and the appearance of a PMN infiltrate 4-6 hours after the edema and smooth muscle response (25).

Macrophages are also a potential source of mediators which could provide both positive and negative feedback to the inflammatory process. They contain an impressive array of secretory products including proteases, complement components, enzyme inhibitors, reactive oxygen metabolites, bioactive lipids, and other chemotactic and mitogenic factors for neutrophils, fibroblasts, and lymphocytes (26). However the effective, in vivo, pathways and processes associated with macrophage mediators are not well characterized. Activation of these cells is not a singular event as different activating stimuli induce different functional responses (27,28,29). Asbestos exposure results in release of both neutrophil chemotactic factor and a fibroblast growth factor (27,28); cigarette smoke is reported to induce release of chemotactic factor for neutrophils (29). The potential in vivo significance of release of a neutrophil chemotactic factor is demonstrated by the appearance of acute lung injury following instillation of the supernatant from macrophage preparations incubated with S.aureus or of bronchial lavage fluid harvested after intratracheal injection of this organism (30). Small

amounts of oxidants and connective tissue specific proteases are also released by macrophages in vivo (31), although quantitatively much less than that released by PMN's. Macrophages from cigarette smokers produce more superoxide than those from non-smokers (32) but connective tissue specific protease release does not appear to be influenced by environmental exposures (33). The inter-relationship between macrophages and the immune mechanisms has been partly attributed to the release by macrophages of interluekin-1 (6). This mediator stimulates both T-lymphocytes and fibroblasts and as such has been proposed to be responsible for silica-induced fibrosis and the immunological manifestations seen in silicosis (6,7).

Complement components are present in the lung although their source is not known completely. They may be derived from serum or produced locally by fibroblasts (34) or Type II pneumocytes (35). These components may also be involved in initiation and progression of inflammation. C5a and C5a des arg are highly effective chemotactic agents for neutrophils, as well as for monocytes, eosinophils, and basophils (15). In addition they increase adhesiveness and swelling of PMN's (36) and stimulate degranulation (15). Instillation of C5 fragments into rabbit trachea results in the production of an inflammatory response (37,38).

The chemotactic factors released by mast cells, macrophages, and other cells in the lung all act to recruit large numbers of PMN's from the circulation into the lung tissue, either to airways or alveoli depending on the location of the injury. This leukocyte plays

a key role in mediating the tissue damage associated with inflammation.

Two important mechanisms by which tissue damage has been postulated in association with PMN factors are oxidant injury and connective tissue-specific proteolytic injury. Oxidant injury results either from the action of myeloperoxidase or reactive oxygen species which include superoxide anion, hydroxyl radical, singlet oxygen, and hydrogen peroxide. These oxidative agents, which are effective when directed against invading micro-organisms, are particularly damaging if released into the surrounding tissues (39-41). It has been hypothesized that this release occurs when PMN's attempt to phagocytize tissue which has become coated with either specific antibody or immune complexes (39-42).

In vivo evidence for the destructive activity of oxidants in rat lungs is provided by Johnson and colleagues (43) who instilled enzyme-substrate systems known to produce reactive oxygen metabolites into the trachea and found evidence of acute parenchymal inflammation.

Proteolytic injury to connective tissue structures in the lung has been postulated as implicated in the pathogenesis of emphysema (44). PMN's contain abundant elastase (45) and an influx of these cells would increase the potential elastase load on the lung. However, central to the hypothesis that this may be significant in the pathogenesis of emphysema is the associated de-activation of the major protease inhibitor in the lung ($alpha_1$-antiprotease) by oxidants which can be released both from PMN's (46) and from macrophages (47). Cigarette smoke as well contains a large number of oxidants (48) which

can also damage alpha$_1$-antiprotease. The ability of different brands of cigarettes to inactivate this protease inhibitor has been shown to be directly related to the oxidizing capacity of the aqueous smoke solution and this ability is further enhanced by macrophage-derived peroxidases (49). The anti-elastase activity of alpha$_1$-antiprotease recovered from the lungs of smokers has been reported by Gadek and associates (48) to be only half that from non-smokers; however a recent report by Stone and colleagues (50) has failed to confirm this finding. Similarly, ozone exposure at low concentrations has been reported to decrease the anti-elastase activity in rat lung tissue (51).

PMN elastase has also been implicated in disordering of the repair process following acute lung injury. Fibroblasts, which normally increase their rate of intracellular degradation of newly synthesized collagen when stimulated by beta-agonists, lost this ability when exposed to PMN elastase (52). This raises the possibility that PMN products may be significant in the pathogenesis of fibrosis in the lung. Such progression of acute inflammation to fibrosis was also seen in rat lungs exposed to oxidants (43).

Inflammation can occur in the lung either in alveoli or in airways or both depending on the deposition site of the provoking agent. Inhalation of silica, asbestos, or certain organic dusts associated with hypersensitivity pneumonitis (28,53) evokes an alveolitis as an early change associated with exposure to these agents. However, both silica (54) and asbestos (55,56) exposure as

well as cigarette smoke (57-60) and many inhaled environmental dusts and gases (61-63) also evoke an inflammatory response in the airways, and in particular, in the terminal and respiratory bronchioles. This airway inflammation has been implicated in the pathogenesis of chronic obstructive lung disease in smokers (57,60,64,65) and in workers exposed to pollutants in their occupation (34,56) as well as in the pathogenesis of airways hyper-reactivity (66,67).

The significance of abnormalities in and around these peripheral airways was proposed as early as 1835 by Laennec (68), who suggested that obstruction of peripheral bronchioles may be responsible for the air trapping in emphysema. It has only been in the last two decades, however, that physiologic and pathologic abnormalities in these airways have been fully investigated, and inflammation at this level identified as one of the initial events in the pathogenesis of pulmonary disease related to cigarette smoke exposure (57-60). The development of this concept will be discussed in the following chapter.

Recent experimental work carried out in this field by Boucher (69) and Hulbert (59) who investigated in detail the effects of cigarette smoke-induced airway inflammation in guinea pigs, has shown that acute cigarette smoke exposure produces increased epithelial permeability to the tracer horseradish peroxidase. This increase in permeability is associated temporally with the acute phase of the inflammatory response indicated by a marked increase in the wet/dry ratio of the trachea and massive PMN infiltrate (59). The PMN's are

found in significant numbers in the mucous layer within the airway lumen as well as in the airway wall.

These findings, of increased epithelial permeability together with large numbers of PMN's in the airway lumena, provided additional support for the hypothesis that inflammation may be the crucial early event in the pathogenesis of obstructive airways disease. This increase in permeability was also shown to occur in human smokers (70) and to be reversible upon cessation of smoking (71). This raised the possibility that the initial inflammatory response need not be associated with irreversible damage. Therefore, identification of those patients in whom early airways inflammation is present may allow intervention or at least reduction of exposure to such an extent that irreversible damage might be prevented.

These studies also added support to a second hypothesis: that increased epithelial permeability may be responsible for the hyper-sensitivity of airways which had been reported following exposure to NO_2 and ozone, or following acute viral infection of the upper respiratory tract (72,73). If a similar inflammatory response occurred during these events leading to an increase in epithelial permeability this could allow greater penetration of mediators to the underlying irritant receptors or bronchial smooth muscle (74).

These ideas and hypotheses were followed up in the three separate sets of experiments discussed in this report. The first series was directed towards early identification of airway inflammation by radioactive labelling of the PMN's found within the

airway lumena. The second series followed the hypothesis that the increase in epithelial permeability may result from "unzipping" of epithelial tight junctions or increasing epithelial "pore" size by some mechanism during the inflammatory response (either to cigarette smoke or to antigen challenge) and that this may allow greater penetration of the mediators responsible for airway hyper-reactivity. The third series looked at the possible late consequences of this inflammatory response in persons exposed to the dual onslaught of cigarette smoke and occupational dust and fume. Does the increased permeability allow greater dust penetrance and thus greater or a different pattern of airway disease than that seen among smokers not exposed to dust?

The more specific background information and rationale for each of these series of experiments will be presented at the beginning of each of the following three chapters.

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V. An Investigation of Small Airways Inflammation using Ga-67 as a Marker of Inflammatory Cells

Introduction

Inflammation of peripheral airways

The concept that disease of the small airways is important in the development of airway obstruction was introduced by Hogg and his colleagues in 1968 (1). Using a method for the measurement of airway resistance in experimental animals developed by Macklem and Mead (2), these investigators showed that the resistance of the peripheral airways (< 2mm internal diameter) in a normal human autopsy lungs accounted for only 25% of total airway resistance, but that this proportion rose to 54 to 93% in lungs with emphysema, bronchiectasis, or bronchiolitis. They found this obstruction in peripheral airways due to inflammation, mucous plugging or fibrosis and proposed that this "small airways disease" was comprised of a reversible component of acute inflammation and mucous plugging which may lead to irreversible narrowing due to fibrosis and consequent obliteration of the airways.

Earlier reports of morphologic abnormalities in small airways (3,4,5,6) had also implicated inflammation, mucous plugging and narrowing or obliteration of bronchioles in the early pathogenesis of emphysema.

In 1969, Bignon and co-workers (7) reported a study of patients dying in severe respiratory failure. In the subgroup of these patients with minimal emphysema, they found hyperplastic bronchiolar narrowing; acute, subacute, and chronic inflammation; and fibrosis of membranous bronchioles. A further report of small airway morphology in seven patients with chronic airways obstruction on pulmonary function tests without clinical evidence of emphysema, was presented by Macklem and colleagues in 1971 (8). Inflammation of small bronchi and bronchioles was common to all cases and there was variable mucous plugging, narrowing, and peribronchiolar fibrosis. Additional quantitative studies in patients with chronic airways obstruction (9,10,11) confirm airway narrowing as characteristic of this disorder, although this narrowing was found to be relatively slight in one group of emphysematous patients (11).

The finding by Hogg and associates (1) described above indicated that significant disease may be present in peripheral airways without any affect on total airflow resistance. Anthonisen and colleagues (12) had shown, in 1967, that abnormalities of gas distribution and $\dot{V}/\dot{Q}$ ratios could be present in individuals who had normal routine function tests. Consequently, identification of functional abnormalities of peripheral airways in patients without severe disease became the object of a number of investigations and several new pulmonary function tests were developed to this end (13-16). Young cigarette smokers typically tend to show abnormalities in one or more of these tests without significant change in FEV_1, or

FEV_1/FVC.

Pathological abnormalities in these patients without severe obstruction or limitation to flow were investigated by Matsuba and Thurlbeck (17). They studied patients without evidence of chronic airflow obstruction or emphysema but with mucous hypersecretion and found an excess of very narrow airways (0.2-0.6 mm internal diameter) but no loss of airways and a 3 fold increase in mucus. This was followed in 1974 by Niewoehner and colleagues (18) who compared autopsy lungs from 20 young smokers without evidence of emphysema who died suddenly outside hospital to 19 similarly chosen non-smokers. The smokers' lungs showed accumulations of pigmented macrophages within the lumena of respiratory bronchioles and increases in mural inflammatory cells and denuded epithelium in membranous bronchioles. This lesion has been postulated as a precursor of centrilobular emphysema (19).

In 1978, Cosio and co-workers (20) provided evidence that pathological abnormalities in peripheral airways correlated with abnormalities in those function tests designed to investigate these airways. The airways from lobes or lungs removed surgically were graded using a system similar to that of Niewoehner (18) but taking into account degrees of severity of the variables graded. The pathology variables assessed were degree of occlusion by mucus and cells (corrected for lung inflation), presence or absence of mucosal ulcers, and the presence and severity of goblet and squamous cell metaplasia, inflammatory cell infiltration, connective tissue

deposition, smooth muscle hyperplasia, and deposition of pigment in the wall of membranous airways less than 2 mm internal diameter. The total score for each patient was used to divide the whole group into four subgroups with increasing small airway pathology. The group with the least pathology had no significantly abnormal function tests whereas increasing pathology correlated with abnormal tests of closing capacity, slope of phase III of the N_2 washout curve, and volume of isoflow using helium and air. The specific pathology variables which correlated were inflammatory infiltration, fibrosis, and squamous metaplasia. They concluded that the primary lesion in these patients was an inflammatory one that could be detected at a time when this lesion was still potentially reversible.

Berend and colleagues (21,22) and Petty and colleagues (23) have provided additional evidence that the specialized tests of small airways function correspond to pathological abnormalities of peripheral airways. However, Berend and co-workers (21,22) also point out that inflammation alone does not appear to result in a significant reduction in small airway dimensions. Looking at both membranous and respiratory bronchioles Wright and associates (24) found that in subjects with $FEV_1 > 80\%$ predicted, respiratory bronchioles showed only abnormalities of inflammation, and that inflammation and fibrosis combined correlated with increasing numbers of abnormal small airway function tests. This inflammation was present both within the lumen and the airway wall.

The specific effects of cigarette smoke in peripheral airways

were recently investigated by Cosio and associates (25). Their patients were 25 smokers and 14 lifetime non-smokers all over age 40 (with the non-smokers being somewhat older than the smokers). Again, the smokers showed increased inflammation in membranous and respiratory bronchioles as well as goblet cell metaplasia, increased smooth muscle, and an increase in the number of airways less than 400μ internal diameter. Interestingly, they found considerable overlap between smokers and non-smokers, indicating other factors must also be implicated in the creation of these lesions. Similarly, Wright and colleagues (26) compared current smokers to ex-smokers and non-smokers, finding the smokers to have increased inflammation in respiratory bronchiolar walls and lumena as well as fibrous tissue and pigment while the membranous bronchioles showed goblet cell metaplasia.

Animal models of airway inflammation

The investigations of surgical or autopsy specimens discussed above, appeared to confirm that cigarette smoke exposure results initially in an inflammatory lesion of the peripheral airways, that, in its early stages, is potentially reversible. Experimental evidence from animal investigations provides further confirmation of this relationship, and indicates that exposure to other inhaled environmental agents may provoke a similar response.

Asmundsson and co-workers (27) exposed hamsters to a moderate level of SO_2 gas plus carbon dust and found neutrophil recruitment

into the airway epithelium and lumen 24 hours after exposure. This PMN recruitment was also seen with high SO_2 levels but not when the moderate exposure was given without carbon dust. Similar studies from this same laboratory using exposure to cotton dust and extracts (28) also indicated PMN recruitment through airway walls, although in this case, the peak response occurred 4-6 hours after exposure. These authors attributed the time difference to a more direct mechanism of recruiting leukocytes by the cotton extracts which are soluble in non-polar solvents and thus may react directly with epithelial cell membranes.

Cigarette smoke exposure in this same hamster model produced similar results (29) with PMN recruitment at all levels in the tracheobronchial tree. Hulbert and associates (30) exposed guinea pigs to cigarette smoke and found peak PMN recruitment six hours after exposure.

In all these studies the authors noted that the recruited leukocytes could be found in subepithelial, epithelial, and lumenal locations. In the guinea pig model (30) the lumenal component was particularly large with evidence of massive numbers of PMN's present within the mucous layer.

This finding led to the idea that aerosol administration of a tracer which might bind to PMN's may well be useful as an indicator of this early airway inflammation. To this end, it was proposed to study the radioisotope Gallium -67 to determine its usefulness, in aerosol exposure, as a marker of inflammation.

Use of Radiogallium - Historical Perspective

Radiogallium has been used in nuclear medicine since the late 1940's when it was observed that gallium tended to localize in active osteogenic sites in the body (31). Several unproductive trials were made with Ga-72 to investigate and treat malignancies in bone. The negative results were in part due to the unfavourable decay characteristics of this isotope so investigations started using Ga-67 which had a longer half life (78 hours) and less penetrating gamma radiation (31). It was in the course of these studies in the '50's that significant differences in distribution and excretion patterns were found when using carrier-free gallium verses gallium with added stable gallium. The carrier-free gallium-67 shows a decreased rate of uptake and degree of deposition in bone, increased localization in liver and other soft tissue, slower blood clearance, and a reversal of the previously observed fecal/urinary excretion pattern such that more than 12% of carrier-free gallium-67-citrate is excreted by the kidney in the first 24 hours after which the liver becomes the major route (32).

It was not until the late 1960's however, that it was observed that there was intense localization of gallium-67 in the lymph nodes of patients with Hodgkin's disease (31). At approximately the same time it was noted that gallium-67 deposition was increased in areas of inflammation and in fact there was concern that its tumor localizing properties were really related to the inflammatory

reaction. This is now generally believed not to be the case and it is widely accepted that gallium-67 concentrates in both malignant tissue and in inflammatory tissue.

Localization of Gallium-67

The mechanism of localization of gallium-67 in inflammatory lesions has been investigated in the past decade. Gelrud and associates (33) showed that the radioisotope binds to the polymorphonuclear leukocyte in areas of inflammation; and Swartzendruber and colleagues (34), using light and electron microscopic auto-radiography showed gallium-67 to be associated with lysosomes or related particles in liver, thymus, spleen, and lymph nodes of both leukemic and non-leukemic mice.

However, in vitro studies by Tsan and co-workers (35) demonstrated that at least 50% of the gallium taken up by PMN's is bound to the plasma membrane which serves as a diffusion barrier in normal cells. However heat-killed PMN's show significant increases in uptake of gallium-67. Additional studies by these same workers (35) indicated that 80% of gallium-67 was found in the soluble, non-cellular fraction.

As well, two cases reported by Dhawan and co-workers (36) have shown accumulation of gallium in inflammatory sites in the absence of circulating PMN's. The authors suggested that lymphocytes may have contributed to the accumulation or that a non-cellular

pathway was responsible. (Gallium has been shown to bind to transferrin in plasma and to tissue lactoferrin (36,37)).

These finding lead Tsan and Scheffel (38) to hypothesize in 1979 that gallium-67 accumulated in inflammatory lesions mainly due to leakage of transferrin bound gallium through the more permeable capillaries into the interstitium where it could be bound by non-viable PMN's with a lesser amount attached to the viable PMN's. Investigation by Weiner and associates (39) identified PMN lactoferrin as the major binding protein present in these cells, although binding to this molecule only accounted for 36% of the total activity absorbed by PMN's.

A series of experiments carried out by Hayes and his colleagues (40,41,42) provided additional information regarding the mechanism of uptake of gallium, and lead to a new hypothesis as to the mechanism. They proposed that gallium is present in the plasma in two compartments: free Ga-67, which routes to bone, excreta and tumor; and protein-bound Ga-67 which routes to normal soft tissue and inflammatory lesions. Support for this hypothesis was found in their experiments in which the binding of gallium to plasma proteins was manipulated by various agents. They further hypothesized that endocytosis may be reponsible for the uptake of gallium in inflammatory lesions.

Gallium Accumulation in Lung Disease

Clinical use of injected gallium-67 has shown that

inflammatory lesions can be visualized by scintillation scanning (31,33,42) and that in some cases, the visualization correlates with the activity of the inflammatory process and the response to therapy (33,43,44). False negatives and false positives do occur but with a low frequency (43,44,45).

The use of gallium-67 in the investigation of lung disorders has been shown to be of particular value in selected patients to aid in preoperative evaluation of pulmonary neoplasm, evaluation of pulmonary infiltrates, assessment of the inflammatory activity in interstitial fibrosis, detection of certain diffuse neoplastic and inflammatory processes before radiography is abnormal, detection and evaluation of pneumoconiosis, and for following treatment in sarcoidosis and tuberculosis (31,33-38,44,46-56). Whereas accumulation of gallium-67 in normal lungs after IV administration is usually low, there is substantial accumulation in lungs with focal or diffuse inflammatory or neoplastic disease, and the accumulation has been demonstrated, in some cases, to indicate more extensive diseaes than is apparent on the chest radiograph (47-51,56), although a normal chest x-ray is generally associated with a normal gallium scan.

Studies which have looked at the relationship between the cellularity of broncho-alveolar lavage and gallium scans have found a positive correlation between the amount of the gallium concentration and the % neutrophil and % lymphocytes (particularly T-lymphocytes) but conflicting evidence for correlation with macrophages (44, 53, 54, 57, 58). A recent abstract by Hunninghake and associates (58)

reported that alveolar macrophages and neutrophils from normal individuals increased their in vitro uptake of gallium when stimulated by phagocytosis or exposure to chemotactic factors.

In general, the common feature of pulmonary diseases in which gallium-67 accumulation is seen is the presence of an active process of cellular proliferation; in contrast, scarred and fibrotic processes do not concentrate gallium.

All studies of lung disease to date have been done using injection of gallium-67-citrate. Using this method, scans done on patients with chronic obstructive airways disease are generally negative (46). This is not surprising given the small relative mass of the airways compared to the whole lung and the fact that they are perfused via the bronchial circulation, which normally accounts for 1% of the total cardiac output (59). Aerosol administration of this isotope, however, may overcome these problems and allow selective labelling of airway inflammatory cells.

The following experiments were designed to test the hypothesis that the acute airway inflammation with PMN exudation into airway lumena seen following cigarette smoke exposure may be identified by the enhanced uptake of Ga-67 administered via aerosol.

Methods:

1. Ga-67 retention in guinea pigs exposed to cigarette smoke

Ten Strain 13 guinea pigs (mean weight 436 ± 36 g) were studied. The animals were raised in a contamination-controlled environment with forced air flow and wire mesh bottommed cages over rock salt in order to reduce the incidence of respiratory infections. Five animals were exposed to 200 puffs of whole cigarette smoke over a one hour period in the awake, restrained state using the method described by Simani and associates (60). Five control guinea pigs were exposed to sham smoking by being held in the smoking apparatus but exposed to room air. Twelve hours after the beginning of the smoke or sham exposure, each animal was exposed to an aerosol of Ga-67 citrate (500 μCi in 10 ml saline) generated by a disposable Hudson nebulizer. The aerosol was generated into an enclosed chamber which surrounded the head of the animal. Exposure was continued for 15 minutes at an air flow rate of 8 l/min. The nebulizer was weighed before and after aerosolization and an aliquot of the nebulant was counted for gallium activity in a Beckman Gamma 7000 well scintillation counter and under a Siemens PHO/gamma scintillation camera. From this, calculations were made of the gallium activity nebulized as seen by both the camera and the counter and lung activities were expressed in relation to these calculated amounts nebulized.

Following gallium exposure the animals were carefully and thoroughly washed to remove any radioactivity on the fur or skin, then anaesthetized with sodium pentobarbitol (25 mg/kg IP) and placed prone over the gamma camera which was connected to a PDP/11 computer. Counts were obtained from an area of interest corresponding to the chest of the animal. This imagining took place approximately 1.5 hours after the start of the aerosol exposure. Twenty-four hours after the beginning of cigarette-smoke or sham exposure (12 hr post gallium aerosol) the animals were again anesthetized, the chest was opened and two 1 ml blood samples taken from the heart. These were counted for gallium-67 activity. The lung tissue was fixed in situ as follows: the heart and lungs were exposed with minimal disruption of the surrounding vasculature. An 18 guage needle was inserted into the right ventricle and advanced toward the outlet of the pulmonary artery. The inferior vena cava was cut, then twenty millilitres of normal heparinized saline were infused to clear the pulmonary vasculature. This was followed by infusion of 30 ml. of 4% glutaraldehyde in phosphate buffer. Immediately following this, the lungs were inflated via the trachea, the trachea clamped, then the lungs removed in one piece and immersed for 2 hours in 4% glutaraldehyde and held at $4^{o}C$. Each lung was divided into six roughly equal sections, then each was weighed and counted for gallium activity. Either of the right or left lung (assigned randomly) was dried for wet/dry weight analysis and the other lung processed for light microscopy using methyl methacrylate resin for embedding.

Sections (3.5 μ thick) were taken from each of the six lung segments and stained with ortho-toluidine. Each airway was assessed for the degree of inflammatory infiltrate in both wall and lumen and assigned a grade from 0 to 3 indicating the presence and severity of the infiltrate. Two composite scores were calculated for each tissue section, one for cartilageous airways and one for non-cartilagenous airways, by summing the individual airway scores and expressing the total as a proportion of the maximum possible score. Total scores for each animal were also calculated in the same fashion.

Gallium activity in the lungs when removed from the animal was corrected for background and radioactive decay and expressed per gram dry weight of lung. Gallium retention in the lungs as counted by the gamma camera (1.5 hrs post exposure) and by the well counter (12 hrs post exposure) was compared for smokers and non-smokers using Student's unpaired t-test. Similarly, airway inflammation scores for cartilagenous and non-cartilagenous airways were compared for the two groups using the scores for each tissue section. Correlation between the gallium activity retained and the total airway inflammatory scores (for cartilagenous and non-cartilagenous airways separately and combined) were determined by linear regression analysis.

2. Ga-67 retention in human subjects

Nine smokers and eight non-smokers breathed an aerosol of gallium-67 generated using a DeVilbiss 2.101 ultrasonic nebulizer

containing 2.5 mCi of ^{67}Ga-citrate in 10 ml. saline. This nebulizer has a maximum output of 3 ml/min and generates droplets with a mass mean aerodynamic diameter of 6.3 microns (61). Subjects inhaled from a mouthpiece with nose clips in place, using tidal breathing. The exposure lasted 2 minutes.

Immediately following inhalation, subjects were positioned supine under the gamma camera. Counts were taken over a region of interest corresponding to the lungs, excluding the stomach and the oropharynx, to obtain an assessment of the dose received by the lungs. Repeat counts were obtained in a similar fashion at 24 and 96 hours after Ga-67 exposure. Venous blood samples were taken at 15 minute intervals over the first hour and then again at 24 and 96 hours in several subjects.

Gallium activity recorded over the lungs was corrected for background and radioactive decay and the 24 and 96 hr readings were expressed as a percentage of the initial reading.

3. Ga-67 autoradiography in guinea pigs and man

Six guinea pigs were exposed to cigarette smoke as described above and three were subsequently exposed to the gallium aerosol. The remaining three served as controls for background radioactivity. Tissue fixation was carried out, as in the first series, within one hour after gallium exposure in two pairs of animals. In the third pair (one exposed to gallium, one control) tissue fixation was done 23

hours post gallium expoure. The lungs were divided sagitally into three segments and lungs and trachea counted for ^{67}Ga activity before and after dehydration for embedding purposes. Sections were taken from each lung area (inner, middle and outer slice) and from the trachea, embedded in methyl methacrylate resin and cut 3.5, 5 and 7.5 microns thick. The slides were dipped in Kodak NTB 2 nuclear-type emulsion , and developed after one and two weeks. One control and one gallium exposed animal were processed on each occasion and additional blank slides were also processed as background controls.

One human subject (age 66, smoking history: 2000 cigarette years) about to undergo lung resection for a peripheral coin lesion, was exposed to gallium-67 aerosol as described in series 2. Two tissue blocks were taken randomly from the resected specimen after formalin inflation and fixation (24 hours after gallium inhalation), and processed for autoradiography as described above.

All experimental procedures using human subjects were approved by the human experimentation committee at St. Paul's Hospital and informed, written consent was obtained from all subjects.

Results

1. Ga-67 retention in guinea pigs exposed to cigarette smoke

Gallium activity retained in the lungs of the smoke-exposed and control animals as measured by the gamma camera at 1.5 hr. post exposure and by well counting 12 hrs post exposure (expressed as a fraction of the activity nebulized) is shown in Figure 1 (p 103). The smoke-exposed animals retained significantly more of the isotope at both time periods ($P < .01$ at 1.5 hrs and $p < .02$ at 12 hrs). However the ratio of the activity in control to that in smoke-exposed animals is the same at 12 hours (mean activity in controls/mean activity in smoke-exposed = .38) as at 1.5 hours (.33). That is, there is no difference in the disappearance rate between the two groups from 1.5 to 12 hours post exposure. Blood gallium activity at 12 hours post exposure was also significantly higher in smoke-exposed animals (385 $\pm$ 413 CPM/g) than controls (36 $\pm$ 33 CPM/g) ($p < 0.05$) although it was highly variable in the smoke-exposed group (Table I - p 102).

Airway inflammation scores for cartilagenous and non-cartilagenous airways for both animal groups are shown in Figure 2 (p 104). The values shown represent the mean $\pm$ SD for the scores for each tissue section, thus taking into account variation within each animal as well as between animals. Cartilagenous airways in smoke-exposed animals had significantly more inflammation than controls ($p < 0.05$). Non-cartilagenous airways were remarkably free of inflammation in both groups and showed no difference between

smoke-exposed and control animals. Figure 3 (p 105) indicates the type of inflammation seen in the smoke-exposed animals, showing the extensive PMN infiltrate both in the airway wall and lumen.

Linear regression analysis of the relationship between gallium activity retained in the lungs (at both time periods) and inflammation scores revealed no significant positive correlations. That is, retained gallium activity was not increased in animals with the higher inflammation score. When the lung activity remaining at 12 hours was expressed as a fraction of the activity present at 1.5 hours (to assess the relative rates of clearance of the isotope during that time period), again, increased retention of the tracer did not correspond to a higher inflammation score.

2. Ga-67 retention in human subjects

Individual and mean results for the disappearance of gallium activity from the lung fields are shown in Figure 4 for non-smokers and Figure 5 for smokers (p 107). No difference was seen between the two groups. Gallium activity measured in the blood in six subjects was very low, ranging from 0 to 170 CPM in the first hour and averaging 40 CPM at 24 hours and 10 CPM at 96 hours. There was no difference in blood levels between smokers and non-smokers.

Figures 4 and 5 show that the gallium activity remaining in the lungs at 96 hours was not significantly different than that present at 24 hours post exposure.

3. Ga-67 autoradiography

The three animals exposed to gallium after smoke exposure all showed significant activity in the lungs and lesser amounts in the trachea which was only partially removed during the dehydration process (see Table II - p 108). Control animals showed no Ga-67 activity over background.

Light microscopy on sections from Ga-67 exposed animals developed after 2 weeks, showed occasional accumulations of silver grains deposited preferentially over cells on the surface of the small airways which appear to be macrophages (Figure 6 - p 109). These grain accumulations can be seen over the same cells in serial sections of the tissue. No accumulation of grains were seen over polymorphonuclear leukocytes or any other specific cell type.

The mean ratio of silver grains over these cells per mm^2 to silver grains over background per mm^2 was 67 $\pm$ 19 (n=30).

The tissue from the two animals processed within one hour of aerosol exposure also showed some accumulations of silver grains spread over airway epithelial cells and mucous without apparent cellular specificity. This was not seen in the one animal processed after 23 hours. This animal, however, showed more activity in cells situated on the surface of small airways.

No specific areas of activity were apparent in any cartilagenous airways or in the trachea.

Similar results were seen in the tissue from the one human

patient studied (Figure 7 - p 110). Silver grain accumulations were seen over some cells which appeared to be macrophages adjacent to small airways and in the alveolar spaces. No other cell types indicated accumulation of the isotope. The number of macrophages which showed silver grain accumulations compared to the total number of macrophages present in the tissue was very small.

The ratio of cell counts to background in this case was 95 ± 20 ($n = 10$).

Discussion

The results of this series of experiments indicate that labelling of airway polymorphonuclear leukocytes does not occur with the inhalation of aerosolized gallium-67 and that cigarette smoke exposure does not increase the retention time of this isotope in the lungs.

The guinea pig experiments comparing gallium retention in smoke-exposed versus control animals had several potential methodological sources of error which make interpretation of the results difficult. Although there appeared to be a greater amount of isotope retained by the smoke-exposed animals, the fact that the ratio between smoke-exposed and controls did not change from 1.5 to 12 hours, shows that the only possible difference in retention between the groups must have been related to events occurring before the first imaging at 1.5 hours. This could occur in one or more of three ways: firstly, the control animals could have cleared more of the isotope by mucociliary action during this time period; secondly, there could have been increased transfer of the isotope into the blood in controls, either as a result of more peripheral deposition or less tissue binding; or thirdly, the smoke-exposed animals could have had greater deposition of the isotope in the first place. However when the retention at 1.5 hours was recalculated based on the whole body image instead of the chest only, the activity was approximately double in

both smoke-exposed and controls but the ratio of the two was identical to the ratio based on counting over the chest only. This suggests that faster clearance in the controls (either to the blood or to the gut) was not responsible for the higher activity in the smoke-exposed animals. It is also unlikely that greater clearance could have occurred in controls via urinary excretion as this does not occur rapidly enough to account for the difference seen (32).

Increased deposition in the smoke-exposed animals appears to be the only explanation for the increased activity seen in this group at both time periods. This could occur as a result of airway narrowing either by constriction of bronchial smooth muscle or by increased mucous production. The inflammation seen in the lungs of the smoke-exposed animals was confined to the cartilagenous airways. This indicates that the cigarette smoke acts at this level in these animals and could well result in significant narrowing of these airways.

Another possible mechanism for increased deposition is an increase in minute ventilation in the smoke-exposed animals, perhaps related to their previous uncomfortable experience in the exposure chamber. This was not subjectively apparent to observation during the experiment, as both groups of animals appeared to tolerate the gallium aerosol well. However, no measurements were made of tidal volume or respiratory frequency, to confirm or deny this possibility.

However, the increased level of gallium seen in the smoke-exposed animals is clearly not related to the degree of

inflammation in the airways. This is borne out by the failure to find a significant correlation between gallium activity at either time period and airway inflammation scores. Even if the gallium activity present at 1.5 hours is assumed to reflect the dose received by the animal, then the ratio of the activity at 12 hours to that at 1.5 hours would represent the clearance profile during that time period. If the original hypothesis was correct, then a high ratio (or slow clearance) would correspond to a high inflammation score. Such a relationship was not found in these animals.

The results show clearly, then, that the presence of inflammatory cells in the airways as a result of cigarette smoke exposure does not result in increased gallium retention.

This is further confirmed by the human study. The interesting finding in this experiment, in addition to confirming the lack of increased retention in smokers, was the remarkably slow clearance from 24 to 96 hours in all subjects. This indicates that more than half of the inhaled dose became bound to tissue or cells in the lungs. The extremely low blood levels at all time periods seen in these subjects, both smokers and non-smokers, is further evidence that the gallium activity is resident in the lung tissue.

The studies using light microscopic autoradiography provide the information which allows further interpretation of these experimental results. The guinea pigs exposed to cigarette smoke had a polymorphonuclear infiltrate in the cartilagenous airways but these cells showed no uptake of the gallium. Only macrophages present on

the surface of peripheral airways showed evidence of gallium accumulation. This and the similar finding in the human tissue suggest that the gallium penetrates to the small airways and alveoli where it becomes attached to or ingested by macrophages. This would account for the very slow clearance in the human subjects, after the initial drop in the first 24 hours. This initial decrease is likely due to mucociliary clearance of the isotope which may be deposited in mucous and perhaps binds to lactoferrin present in the mucous (64).

Cigarette smokers have been shown to have an excess of macrophages in respiratory bronchioles (18). If this is the case, and the gallium accumulates in these cells, why was there not any increase in retention in the smokers? The answer to this may again lie in the autoradiographic studies. The dose of isotope given to the human subjects was extremely low, in keeping with the experimental nature of the study. The tissue from the one human subject which was processed for autoradiography shows that only a very small proportion of the macrophages present had accumulated any significant quantity of the isotope. In order to see any difference between smokers and non-smokers a much higher dose would have to be given. This is, in fact, the case with injected gallium studies. The dose injected is approximately equal to the activity present in the nebulizer in this study (of which less than 5% is deposited), and the results of these scans do correlate positively with the presence of alveolitis.

Only very few studies have been reported in the literature which specifically implicate macrophages in gallium accumulation.

Hayes and his colleagues (42) have recently hypothesized that phagocytosis of protein-bound gallium-67 by macrophages was responsible for inflammatory accumulation and he pointed for evidence to an earlier autoradiographic study by Swartzendruber and Idoyaga-Vargas (65) in which gallium was seen to accumulate preferentially in macrophages over granulocytes, despite equal phagocytosis of latex particles by both cell types. In a previous study on mice, Swartzendruber and associates (34) had demonstrated gallium accumulation notably in macrophages of the lymphoreticular system, in thymic epithelial reticular cells, Kupffer cells, hepatic cells, and kidney cells of the proximal convoluted tubules. In an abstract, in 1981, Hunninghake and his colleagues (58) reported correlation between bronchoalveolar lavage cellularity and positive gallium scans in patients with interstitial lung disease. In eight patients with idiopathic pulmonary fibrosis, 66% of the activity in the lavage was associated with macrophages and 34% with neutrophils. In 10 sarcoid patients, 95% was associated with macrophages and 5% with T-lymphocytes.

Clearly, the previously held notion that gallium accumulation in areas of inflammation is due to attachment to polymorphonuclear leukocytes needs re-evaluation in light of these reports and the results of this study. Accumulation by activated inflammatory and immune effector cells, particularly macrophages as postulated by Hayes (42), appears to be the most likely mechanism.

In summary, this series of experiments supports the

hypothesis that gallium-67 accumulates in macrophages. However, the potential for using aerosolized gallium inhalation to mark the acute polymorphonuclear leukocyte response to cigarette smoke has been shown to be minimal, as the gallium does not appear to bind to PMN's. However, the binding to macrophages may have potential value in studying the activity of this cell in other lung disorders.

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TABLE I

Ga-67 Blood Levels @ 12 Hours Post Exposure

(CPM/G. Wet Wt.)

Control	Smoke Exposed
38	30
6	440
64	1076
0	208
74	172
36.4 ± 33.3	385 ± 413.3

$P < 0.05$

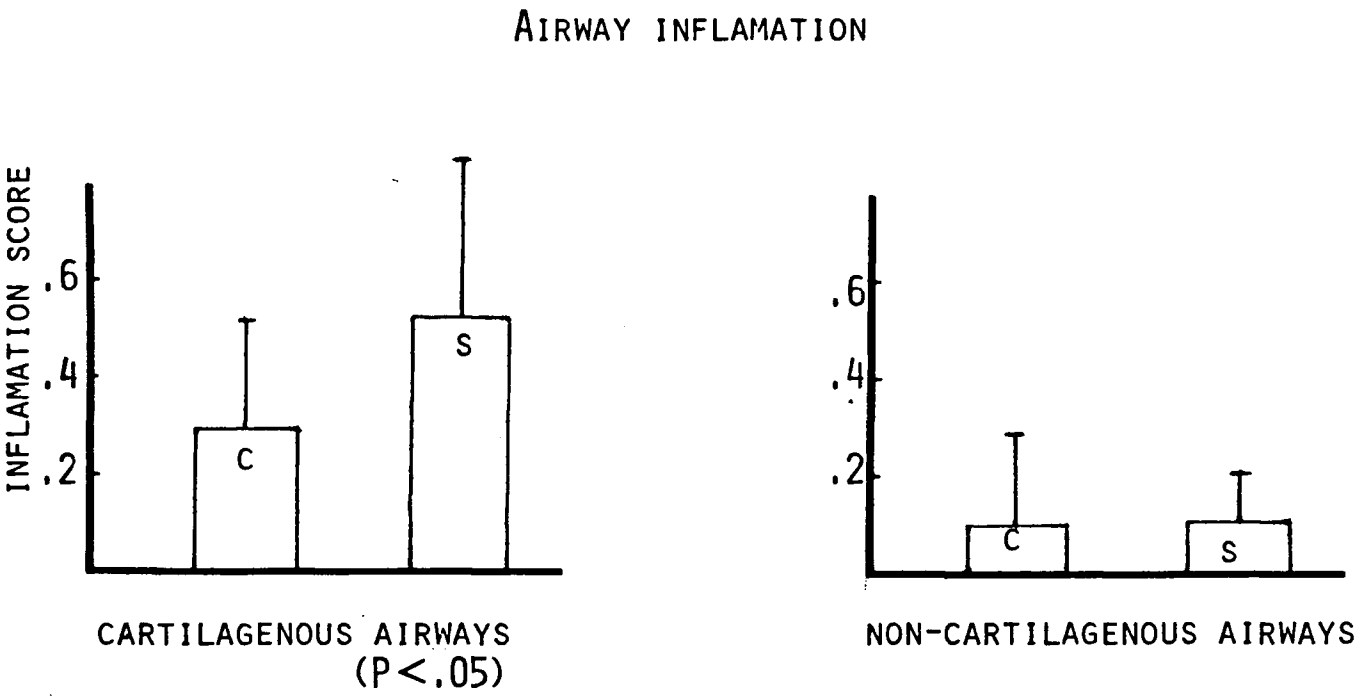

Figure 1 Gamma camera measurement of ^{67}-Gallium activity in lungs of control (C) and smoke-exposed (S) animals at 1.5 and 12 hours after inhalation, expressed as a fraction of the activity in the amount of gallium nebulized, showing much greater retention in the smoke-exposed animals. (n = 5 for each group)

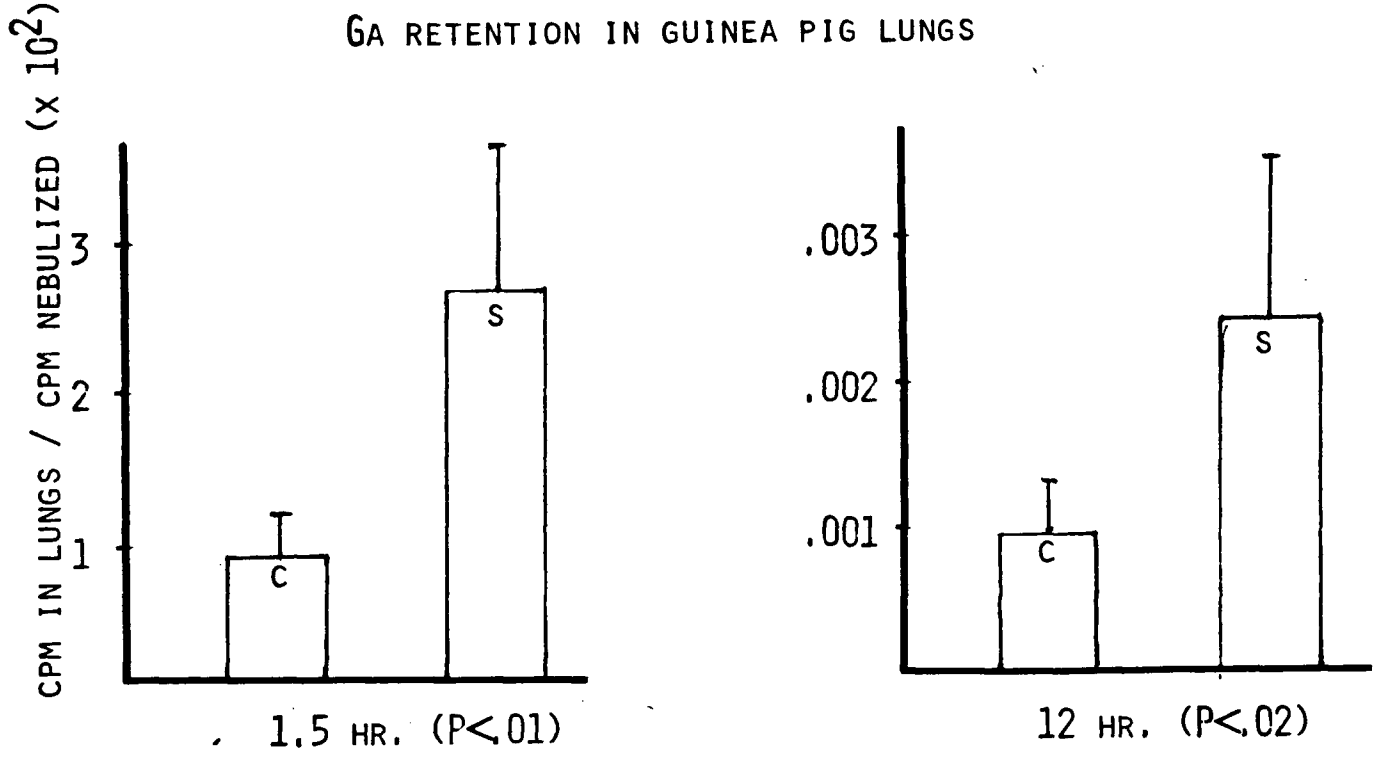

Figure 2 Inflammation scores for cartilagenous and non-cartilagenous airways (see text for description) for control (C) and smoke-exposed (S) animals, n=35 sections, controls; n=33 sections, smoke-exposed. Only cartilagenous airways in the smoke-exposed group had significantly more inflammatory infiltrate.

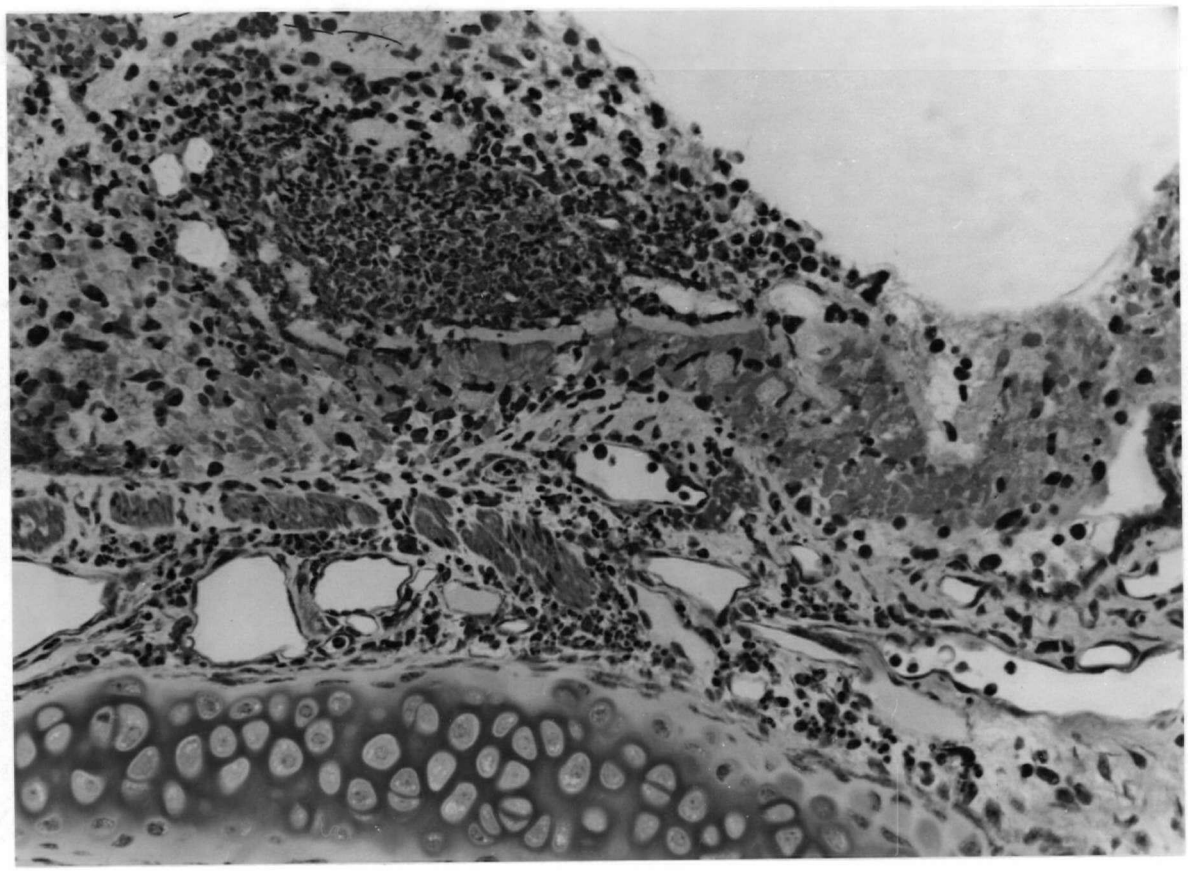

Figure 3 Photomicrograph of a portion of a cartilagenous airway from a smoke-exposed guinea pig 12 hours after smoke-exposure, showing extensive polymorphonuclear leukocyte (PMN) infiltration both in the wall and lumen.
(Original magnification: 160x)

Figures 4 and 5 ^{67}Gallium activity in human subjects as a percentage of the initial activity inhaled, showing initial decrease in activity between 0 and 24 hours and no further change 96 hours after exposure. Note no difference in retention between (a) non-smokers and (b) smokers.

Figure 4 NON-SMOKERS

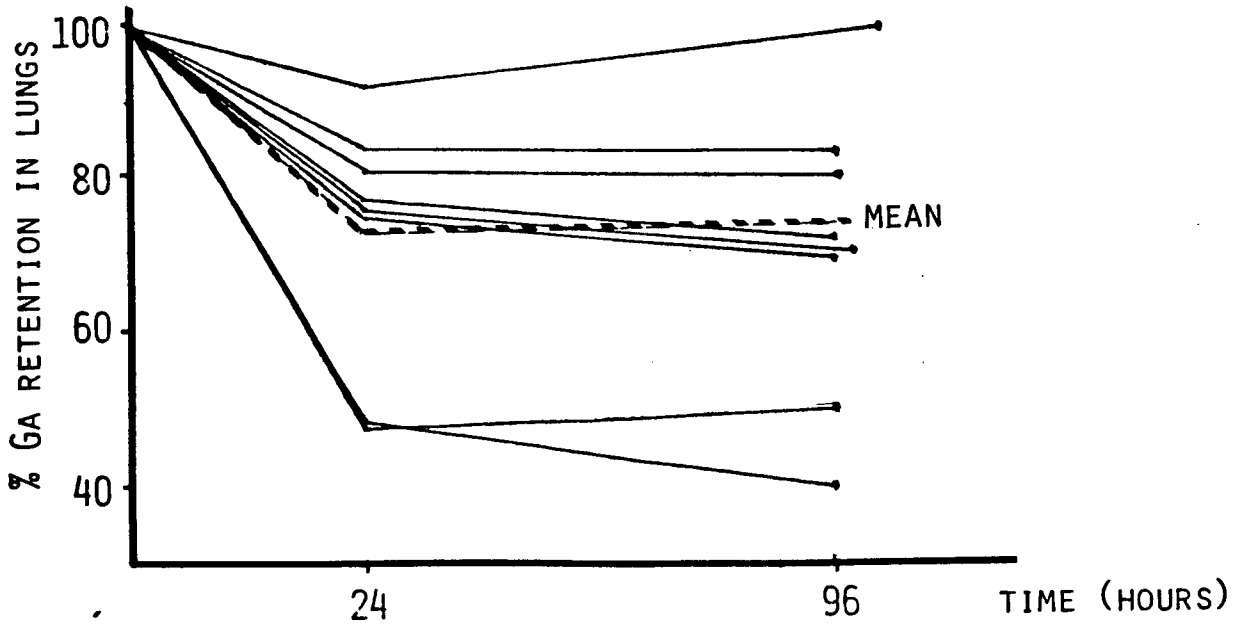

Figure 5 SMOKERS

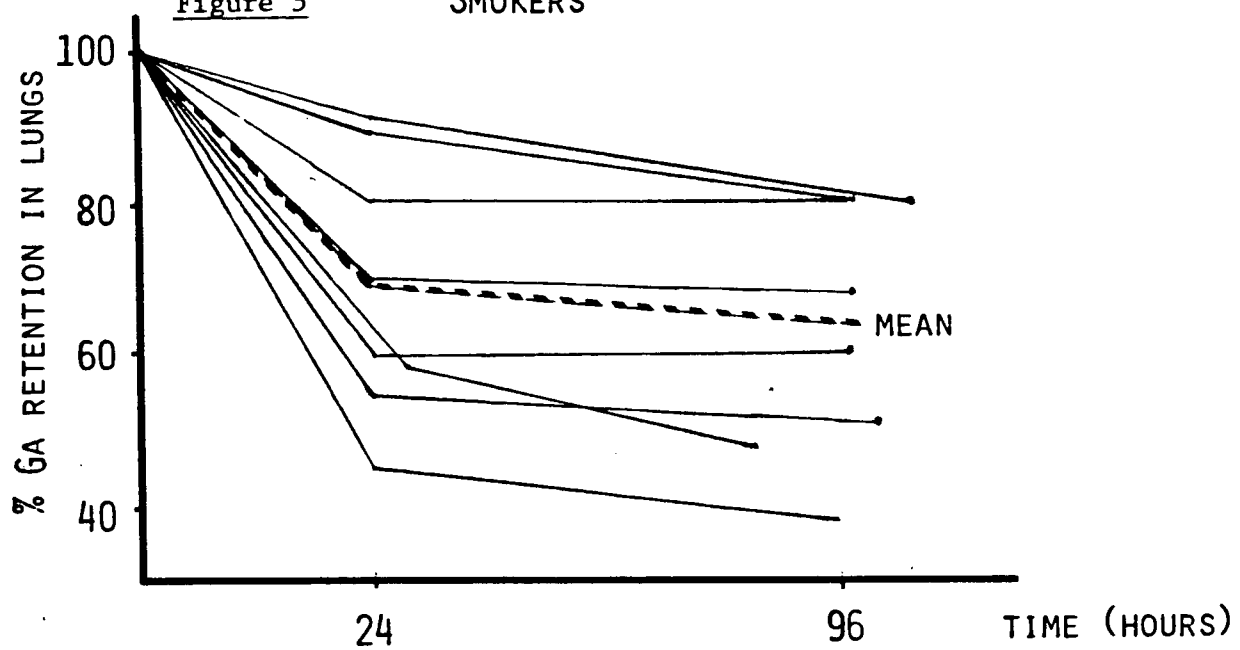

TABLE II

Ga-67 Activity of Lungs and Trachea of Ga-67-exposed Guinea Pigs

	Animal 1	Animal 2			Animal 3		
	(CPM/g.wet wt.)	before dehydr.	after dehydr.	%	before dehydr.	after dehydr.	%
Trachea	4448	10,028	7401	74	2599	1223	47
Inner segment lung	24,084	53,107	35,572	67	35,926	31,227	87
Mild segment lung	21,227	26,247	16,625	63	51,213	44,694	87
Outer segment lung	21,244	69,498	40,609	58	43,572	37,715	87

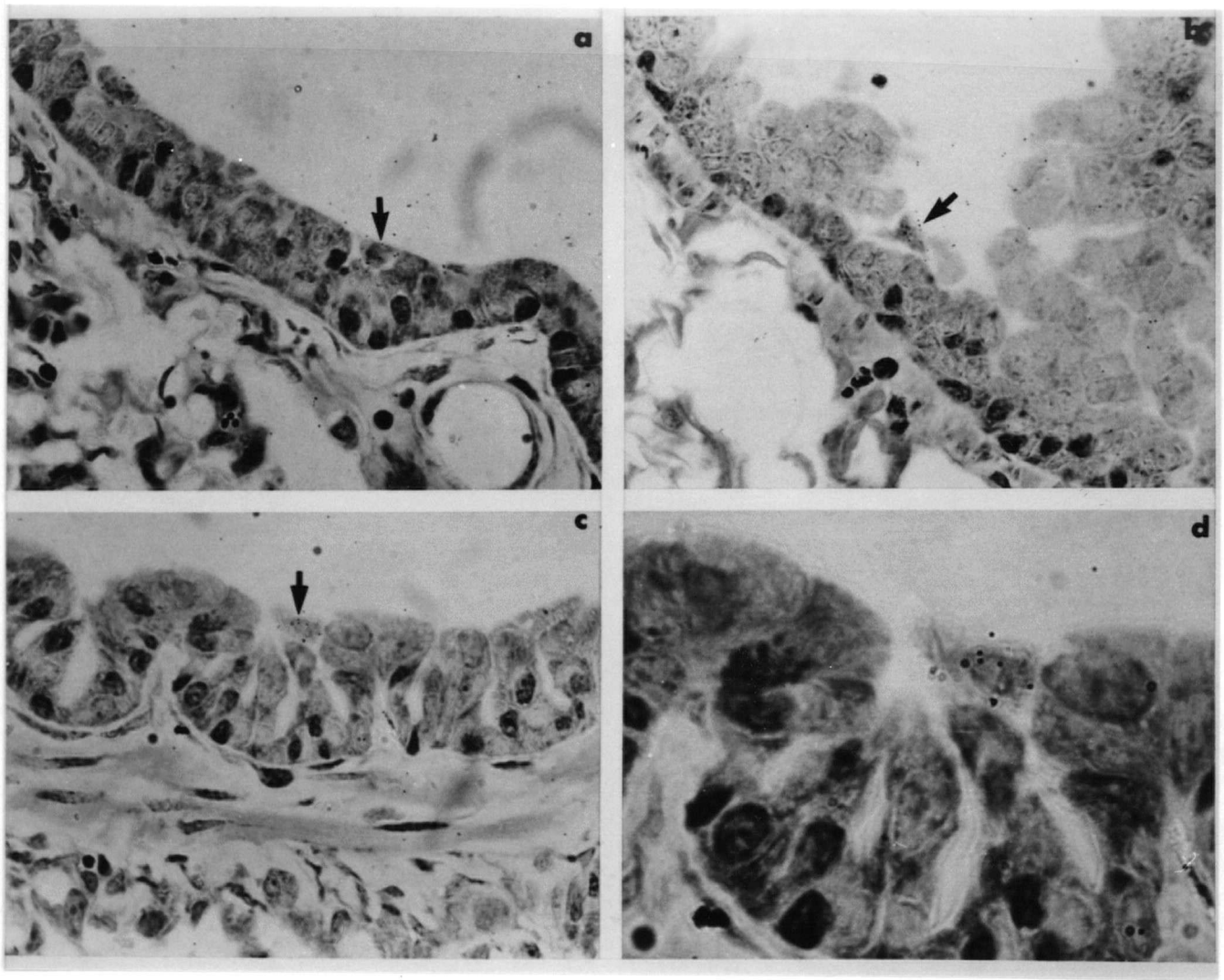

Figure 6 Autoradiographs from lung sections taken from three guinea pigs exposed to cigarette smoke.

(a,b,c): Silver grain accumulations (➡) over cells on the surface of small airways which appear to be macrophages (original magnification 160x).

(d): Higher power view of cell indicated in (c).

(Original magnification: 400x)

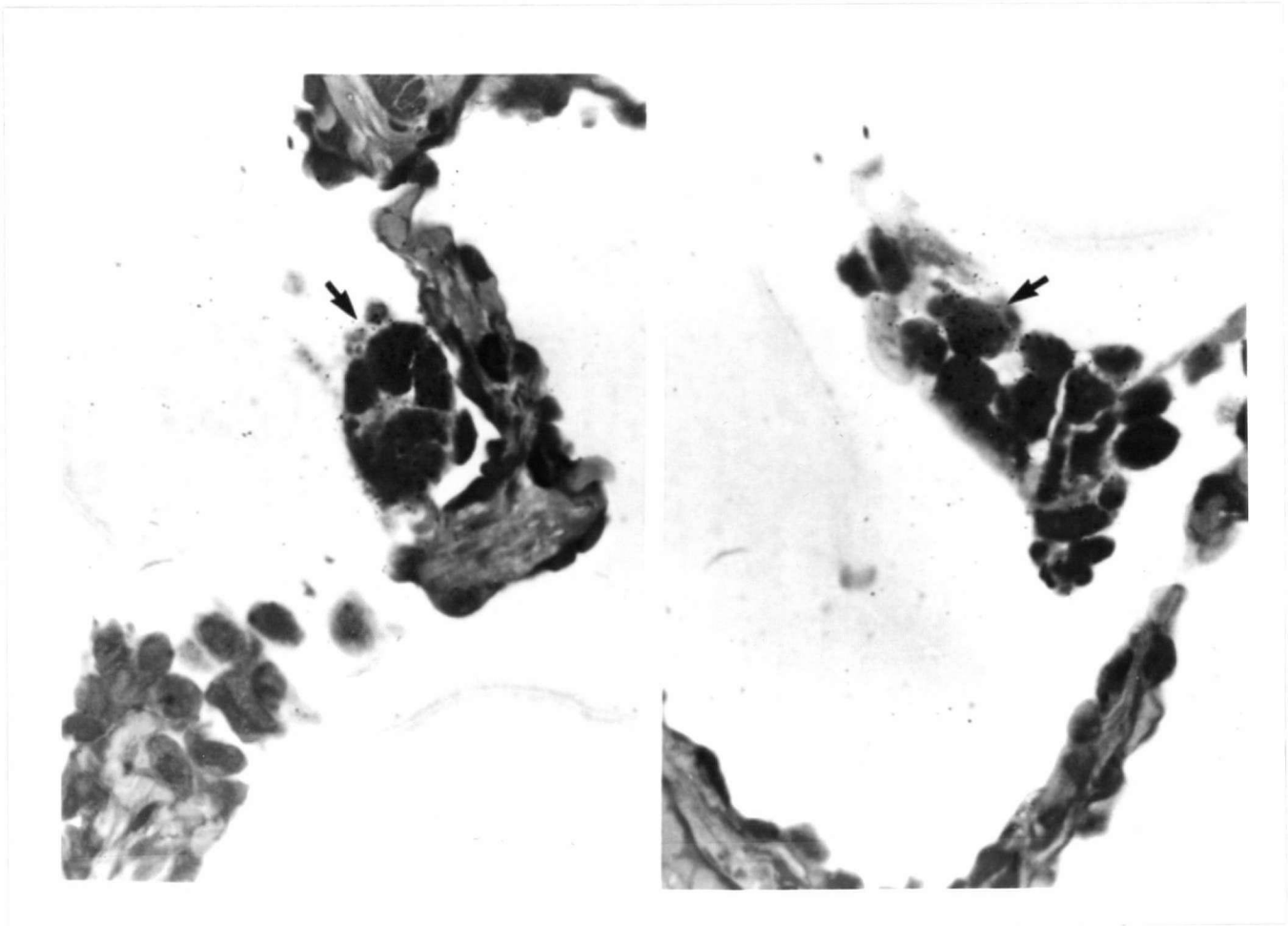

Figure 7 Autoradiographs of lung sections taken from the one human subject, showing silver grain accumulations (➡) over alveolar macrophages. Notice relative absence of grains elsewhere over tissue or background.
(Original magnification: 400x)

VI. Airway Mucosal Permability and Reactivity

Introduction:

A relationship between airway mucosal damage and the increased airway reactivity commonly seen among individuals after upper respiratory tract viral infection or exposure to pollutants was first proposed by Nadel and his colleagues (1,2,3). They studied normal non-atopic individuals after infection (1) and after exposure to low levels of ozone (1,3,4) and found a transient hyper-reactivity to both histamine, citric acid, and methacholine. Repeated ozone exposure (3) resulted in adaptation such that the hyper-reactivity to histamine disappeared even when ozone exposure was continued. Atropine pretreatment blocked the reactivity increase (1), suggesting involvement of cholinergic irritant receptors. The fact that the subjects were previously healthy and had normal spirometry, even after the infection or exposure, ruled out alterations in baseline airway calibre, hypertrophy or hyperplasia of airway smooth muscle, and chronic allergic disease as implicated in the response. These investigators proposed that the epithelial damage (desquamation and degeneration) which has been identified after viral infection or exposure to airborne irritants results in "sensitization" or increased accessibility of the rapidly adapting epithelial receptors.

The fact that reactivity increased in response to

methacholine challenge in the subjects exposed to ozone (2) suggested increased post-ganglionic cholinergic sensitivity as well as sensitization of irritant receptors. The authors hypothesized that this could result from an increase in the permeability of the airway epithelium, allowing more agonist to reach both irritant receptors and the bronchial smooth muscle.

Similar increases in non-specific reactivity to inhaled pharmacologic agents are also seen in atopic subjects following specific antigen challenge (5,6), chronic antigen exposure (7,8), and exposure to other environmental agents (9); and in some non-atopic subjects following the development of occupational asthma (8,10,11).

In addition to the hypothesis that this increased reactivity is associated with altered epithelial permeability, other mechanisms have been proposed. Some investigators have postulated that a reduction in the starting calibre of the airways could account for the hyper-reactivity as resistance to airflow varies inversely with the fourth power of the airway radius (12,13), thus a similar degree of narrowing may cause differing decreases in airflow if the starting radius is different. However studies in normal subjects such as those described above (3,4) have demonstrated that hyper-reactivity develops in subjects in which there is no apparent change in airway calibre.

Abnormalities or alterations in the airway smooth muscle have also been proposed to account for enhanced non-specific reactivity. Increased "sensitivity" of the muscle as a result of exposure to toxins has been hypothesized as has conversion of airway smooth muscle

from a multi-unit to a single unit or scyncytium; however, no real evidence has been presented to support these concepts (14). Alterations in the control of airway smooth muscle may also account for the hyper-reactivity. Szentivanyi (15) formulated the beta-adrenergic blockade theory of asthma in which he postulated that decreased responsiveness of beta-adrenergic receptors responsible for airway relaxation allowed unopposed alpha-adrenergic stimulation of airway smooth muscle. Several workers have investigated the effects of beta-adrenergic antagonists and found that while asthmatics respond significantly to propranolol with bronchoconstriction (16), normal subjects respond only slightly (17) or not at all (18, 19). In certain studies however, of smokers (17,20) or workers exposed to occupational antigens (21), propranolol treatment has resulted in significantly enchanced reactivity.

A non-adrenergic inhibitory nervous system has been demonstrated in vitro (22) and proposed to be the major system for inhibition of bronchial smooth muscle constriction. A defect or alteration in this system could account for airway hyper-reactivity. Conversely, an abnormality in the parasympathetic nervous system could result in increased stimulation of bronchial smooth muscle. Vagal mediation of the effects of various stimuli which induce hyper-reactivity has been demonstrated by the reduction in reactivity after atropine administration (1,23,24). However in the study of ozone exposure described above (4), Holtzman and colleagues found that the response to methacholine was not altered in the presence of the

ganglionic blocking agent hexamethonium, suggesting a direct rather than reflex action on bronchial smooth muscle. In addition, although ozone exposure results in enhanced reactivity in vivo, in vitro investigation of dog vagal afferent fibres after ozone exposure indicated decreased sensitivity to histamine (25). These contradictory results have suggested that increased access to, rather than increased sensitivity of, irritant receptors may be the pathogenetic event in hyper-reactivity. Thus, mucosal inflammation or damage which results in such an increase in accessibility emerges again as potentially important in the development of non-specific bronchial reactivity.

Mucosal inflammation has also been proposed to account for the delayed asthmatic response as well as the increased non-specific reactivity seen in many individuals after exposure to certain airborne dust, vapours, or fumes in the workplace (5,6,8,10,11). This occupational asthma generally develops over time with a symptom-free period followed by sensitization. The provoking agents are chemically heterogeneous and often, not allergens (26). Laboratory challenge with the provoking agent induces either an immediate asthmatic response and/or a delayed response. Associated with the asthmatic obstruction, but persisting even when the airway obstruction has disappeared (6,10,11) is the increase in non-specific airway responsiveness. Lam and co-workers (8) reported this increase in all patients exposed to Western Red Cedar regardless of whether or not they had an immediate or a delayed reaction, whereas Cockcroft and

colleagues (5) found it only in association with the delayed asthmatic response. The hyper-reactivity eventually returns to normal after cessation of exposure in some subjects but persists in others (8). Cartier and colleagues (6) have provided additional evidence of a relationship between the hyper-reactivity and the delayed asthmatic response, showing that the magnitude of the asthmatic response correlates positively with the magnitude and duration of the increase in reactivity to histamine, suggesting that the hyper-reactivity may be induced by the same mechanisms which cause the delayed response, namely, mucosal inflammation with increased epithelial permeability.

The concept that altered mucosal permeability may be related to the pathogenesis of asthma was first proposed in 1929 by Cohen and co-workers (27) who measured the time required after antigen inhalation for reaction with specific antibody injected into the skin. Asthmatics showed a delayed reaction, indicating decreased rate of transfer from lungs to blood. Similar results have been reported more recently by Braley and associates (28) who found reduced antigen absorption in the isolated lungs of immunized rabbits compared to non-immunized.

However, Salvaggio and Leskowitz and their colleagues (29,30) have suggested that patients with atopic rhinitis may have a defect in the nasal mucosa which allows inhaled antigens greater access to immunocompetent cells. Buckle and co-workers (31) demonstrated more rapid absorption of labelled albumin from the nasal mucosa in patients with allergic rhinitis, although this did not occur in patients with

extrinsic asthma. They suggested that persons with atopic rhinitis may have enhanced permeability in the nasal mucosa while asthmatics may have enhanced bronchial permeability.

Evidence that enchanced permeability may play a role in acquired non-specific reactivity was provided by Matsumura and colleagues (32) who found increased rate of absorption of albumin in guinea pig lungs after exposure to ozone. These animals were also shown to have enhanced reactivity to inhaled antigen after the ozone exposure (33). More recent investigation of the effects of ozone in guinea pig lungs by Davis and co-workers (34) has demonstrated increased airway epithelial permeability to horseradish peroxidase (HRP).

Specific investigation of the relationship between epithelial permeability and hyper-reactivity was carried out by Boucher and his colleagues (35) who studied naturally ascaris-sensitive monkeys. They found no difference in baseline permeability between the allergic and non-allergic monkeys; however, antigen challenge lead to increased permeability to albumin in the allergic animals which persisted well after the respiratory mechanics had returned to normal. Further study (36) indicated that antigen challenge in the monkeys was followed by enhanced reactivity to histamine and increased permeability to tritium-labelled histamine. Similarly, in the guinea pig, histamine and methacholine challenge resulted in increased epithelial permeability to horseradish peroxidase (HRP) (37).

Cigarette smoke exposure was earlier identified by Simani and

his colleagues (38) as causing increased tracheal epithelial permeability to HRP and Boucher and co-workers (39) provided additional freeze fracture evidence to support this and to suggest that loss of integrity of the epithelial tight junctions may be the mechanism for the increased permeability. Hulbert and associates (40) showed that this increase in permeability in the guinea pig is coincident with the exudative phase of the acute inflammatory reaction that follows smoke exposure. Reactivity in these animals has also been shown to be increased following cigarette smoke exposure, but only in the presence of beta-sympathetic and parasympathetic blockade (41). This increase in reactivity occurs at the same time as does the permeability increase.

Human studies of epithelial permeability and reactivity have also indicated no difference in permeability between stable asthmatics and non-asthmatics (42) despite widely differing reactivities, although permeability was enhanced in non-asthmatics following the inhalation of histamine. Epithelial permeability has been investigated in cigarette smokers by Jones and his colleagues (43) and shown to be elevated in current smokers but returning toward normal with the cessation of smoking (44,45). However, studies of reactivity differences between smokers and non-smokers report conflicting results (46,47,48,49,50). Whereas Gerrard and associates found symptomatic smokers to be more reactive than non-smokers (47), Cockcroft and colleagues (46) have recently reported asymptomatic young smokers to be less reactive than non-smokers. They suggested that this may be

due to a nicotine-induced increase in circulating catecholamines in the smokers which results in a greater degree of beta-adrenergic relaxation of the airways, thus counteracting a possible increase in reactivity via other mechanisms.

Introduction to experiments

The studies reported in this section were designed to investigate further the hypothesis that airway hyper-reactivity may be related to enchanced mucosal permeability. First, permeability was studied in a group of smokers and non-smokers and compared to reactivity to aerosolized histamine with and without prior beta-blockade to eliminate a possible nicotine-associated effect on beta-adrenergic airway relaxtion. Second, permeability was studied in a group of patients suspected of having occupationally induced asthma as a result of Western Red Cedar exposure. In this group, permeability was assessed before and after challenge with plicatic acid, the antigen identified as responsible for this form of asthmatic response (51), and this was related to reactivity to inhaled methacholine before and after antigen challenge.

The technique used to measure epithelial permeability in these studies was to follow the disappearance from the lung and the appearance in the blood of the inhaled tracer, 99m-Technetium Diethylentriaminepentacetic acid (^{99m}Tc-DTPA). This tracer has been used extensively in recent years to study lung epithelial permeability

in humans and animals (42,43,44,45,52-56). Being soluble and of low molecular weight, it is believed to be cleared from the lungs mainly by diffusion across the epithelium into the bloodstream (53,54). Once in the blood, it rapidly diffuses into the extravascular extracellular fluid space and its chief route of elimination is via the kidneys (57). Sixty-nine percent of an injected dose was reported by McAfee and colleagues (57) to be excreted by the kidneys with a half-life of 1.73 hours and a further 27% with a t1/2 of 9.23 hours, with only 4% remaining at 24 hours.

Chopra and co-workers (53) studied the clearance of soluble aerosols of different molecular weights in patients with systemic sclerosis and found increased permeability compared to normal subjects for both TcO_4(MW-163) and Tc-DTPA (MW - 492). The faster clearance in these patients of the smaller molecular weight aerosol was believed to be consistent with the hypothesis that the clearance of aqueous solutes through the epithelium is dependent on molecular size in relation to the size of pores between the epithelial cells. Tc-DTPA permeability in patients with interstitial lung disease was studied by Rinderknecht and associates (54) and found to be increased, but they found no change in five patients with chronic obstructive lung disease. As discussed above, several studies have now been reported in which Tc-DTPA has been used to assess increased permeability in smokers (43,44,45).

Morphological and functional evidence that the principle barrier to clearance of aqueous solutes through the alveolar/capillary

membrane is the alveolar epithelium, has been provided by studies (58-62) in which the interepithelial junctions have been shown to be continuous zonulae occludentes, composed of several strands, whereas the endothelial junctions usually contain only a single strand with intermittent breaks. Schneeberger (62) has estimated the endothelial pore radii to be 40-58 A and that of the epithelium, 6-10 A, and concluded that for water soluble molecules up to a molecular weight of 40,000, the epithelium represents the chief diffusion barrier.

The investigations of Chopra (53), Rinderknecht (54), and Mason (45) have all reported increased clearance from the upper zones of the lung in normal subjects and in smokers. They have proposed that this is due to the increased alveolar inflation in the upper lobes resulting either in more permeable interepithelial junctions, due to stretch, or an increase in the ratio of surface area to the volume of fluid containing the tracer. Similar increases in solute permeability in experimental animals have been seen when the lung is inflated with fluid (63) or with air (64). These findings, together with the observation that regional permeability differences are lost when positive end-expiratory pressure (PEEP) is applied after the inhalation of the tracer (to eliminate regional differences in alveolar inflation) (54) lend support to the concept that the greater inflation in the upper lobes is responsible for the increased clearance.

In summary, it is generally believed that the disappearance of ^{99M}Tc-DTPA from the lung after inhalation is an indicator of the

permeability of the pulmonary epithelium. Although most studies have focussed on alveolar clearance, Elwood and colleagues (42) and Oberdorster and co-workers (55) have used it successfully to study airway epithelial permeability as well.

Methods:

Study 1: Permeability and Reactivity in Smokers and Non-Smokers

Subjects

Ten asymptomatic smokers, (mean age 32.4 ± 6.4 years) who smoked at least 20 cigarettes/day (mean pack years 17.2 ± 7.6) were studied. One subject was atopic. Eight asymptomatic, non-smoking, non-atopic medical and laboratory personnel served as controls (mean age 31.1 ± 7.3 years). All subjects denied recent upper respiratory tract infection and provided written consent after the aims and procedures of the study had been explained to them.

Pulmonary Function Tests

Pulmonary function testing was performed on each subject using a volume displacement body plethysmograph. Volume was measured with a Krogh spirometer equipped with a Shaevitz linear displacement transducer; flow was measured with a Fleisch No.3 pneumotachograph coupled to a Sanborn 270 pressure transducer, and pressure was measured with a Validyne 45-MP differential pressure transducer.

Functional residual capacity (FRC) was measured by Boyle's Law technique (65) and residual volume (RV) and total lung capacity (TLC) were calculated. Maximum expiratory maneuvers were performed and flow and volume signals were digitized to an accuracy of 0.4% and fed to an Apple II computer, programmed to calculate volume in the first second of forced expiration (FEV_1) and maximum flow at 50% forced vital capacity ($\dot{V}_{50}$). Nitrogen concentration of expired air, following a single maximal inspiration of O_2, was measured by a N_2 analyzer and the signal was digitized and displayed against volume on the Apple computer for calculation of the slope of phase III of the nitrogen washout curve ($\Delta N_2/l$), closing volume (CV) and closing capacity (CC).

FEV_1 and $\dot{V}_{50}$ were expressed as percent predicted based on the prediction formulas of Morris et al (66). $\Delta N_2/l$ and CC/TLC were expressed as percent predicted based on the prediction formulas of Buist and Ross (67, 68).

Determination of Permeability

Airway epithelial permeability was assessed using an aerosol of technetium-labelled diethylenetriamine pentacetic acid (^{99m}Tc-DTPA) as a tracer. The aerosol was generated using a DeVilbiss 2.101 ultrasonic nebulizer containing 2.5 mCi ^{99m}Tc-DTPA in 10 ml normal saline. This nebulizer has a maximum output of 3 ml/min and generates droplets with a mean mass aerodynamic diameter of 6.3 microns (69). Subjects inhaled from a mouthpiece with nose clips in place.

Immediately after a 2 minute inhalation of the tracer, subjects were positioned under a Siemens PHO/gamma LFOV scintillation camera linked to a PDP/11 computer. Continuous counts were taken over the chest and recorded every 30 seconds for thirty minutes and a region of interest corresponding to the lungs and trachea was subsequently analysed. Corrections were made for decay, background radioactivity, and radioactivity in the heart and pulmonary vasculature by the method described by Elwood and associates (42).

The disappearance of tracer from the lung fields fit the exponential equation $y = Ae^{-Bx}$ (mean r = .96, non smokers; .98, smokers) in which y = counts/min. in the lung, x = time, A = CPM at time 0, and B is a constant which describes the shape of the curve and is used to calculate the rate of decrease of lung activity over time. For each subject, a lung half-life was determined by extrapolation from the disappearance curve as the point at which 50% of initial activity would remain in the lungs (i.e. y = .5A), and the decrease in lung activity per minute was calculated according to the formula $1-e^{B}$.

Serial 2 ml. venous blood samples were taken via a heparinized indwelling #21 butterfly at 7, 10, 15, 20, 25, 30, 40, 50 and 60 minutes after the start of aerosol delivery. These were counted in a Beckman Gamma 7000 gamma counter.

A permeability index was calculated for each time period in which blood was drawn, according to the following formula:

$$\text{Permeability Index} = \frac{\text{CPM in 1 ml. blood X body wt. (kg.)}}{\text{camera CPM over lung fields at time zero}}$$

To assess the relative distribution of the label for each subject, average gamma camera counts for the first 2 minutes following aerosol delivery were calculated for equal sized regions of interest corresponding to central airways and lung periphery and the ratio of central to peripheral deposition was determined.

Assessment of Bronchial Reactivity

Bronchial reactivity was determined in 17 of the 18 subjects using a modification of the method of Cockcroft and associates (8,70). Histamine dihydrochloride at doubling doses from 4 mg/ml (of the salt) to a maximum of 64 mg/ml was delivered using a Bennett-Twin nebulizer (output 0.25 ml/min) at an oxygen flow rate of 5 l./min. for 2 min. On a separate occasion, a second assessment of reactivity was obtained for 16 of the 17 subjects. On this occasion histamine exposure was preceded by aerosol administration of propranolol at a concentration of 15 mg/ml nebulized for 4 minutes. Histamine dose-response curves were constructed for each individual by plotting the percentage decrease in FEV_1 from pre-histamine levels against the log of the histamine dose. For the FEV_1 response after beta-blockade, the control FEV_1 used was that obtained after the propranolol aerosol but before histamine.

Following both histamine challenge sessions, eleven subjects were given aerosol salbutamol (albuterol) and FEV_1 was recorded at 10 minutes post bronchodilator. By 10 minutes all subjects were at or very near pre-histamine levels for FEV_1 when no propranolol was given. However, when histamine was preceded by propranolol, the FEV_1, recorded at 10 minutes post bronchodilator, showed only slight or moderate improvement, indicating

persistant beta-adrenergic blockade.

For both histamine challenges, the dose required to cause a 20% decrease in FEV_1 (PC_{20}) was calculated by linear interpolation between the last two points on the histamine dose response curve. This calculation was not possible for all subjects since several failed to show a 20% decrease in FEV_1. Therefore, in addition to the calculation of PC_{20}, a reactivity index (RI) was also calculated as follows:

$$\frac{\text{maximum percentage decrease in } FEV_1}{\log [\text{histamine}] \text{ associated with this } FEV_1 \text{ decrease}}$$

This calculation provided a numerical assessment of the high end of the histamine dose-response curve and made it possible to compare smokers to non-smokers, and to compare individual subjects before and after beta-blockade. The difference between the RI without propranolol and that with propranolol, we termed the ΔRI.

Data Analysis

Mean values for pulmonary function variables, lung half-life of ^{99m}Tc-DTPA, and the reactivity index (with and without beta-blockade), were compared for smokers and non-smokers using Students unpaired t-test. Permeability indices for all time periods were compared for smokers and non-smokers using 2-way analysis of variance. Paired t-tests were used to compare the reactivity index without beta-blockade to that after beta-blockade within the two subjects groups. Possible relationships between permeability, reactivity and pulmonary function variables in the two groups together and separately were determined by linear regression analysis.

Study 2: Permeability and Reactivity before and after plicatic acid challenge.

Subjects

Nine patients referred to the Vancouver General Hospital Occupational Respiratory Disease Unit for assessment of possible Western Red Cedar asthma were studied. Their ages ranged from 22 to 63 years (mean 47.0±15.6). Five were lifetime non-smokers and the other four were ex-smokers. Informed,written consent was obtained from all patients.

Permeability

Permeability was assessed, as described above for the smoking study, on the morning of day 1, and then repeated on the morning of day 3, approximately 24 hours after plicatic acid challenge. Prior to the second permeability study on day 3, control blood samples were taken and scanning performed over the lung fields to insure that there was no residual ^{99m}Tc activity in the lungs or blood.

Pulmonary Function, Methacholine Challenge, Antigen Challenge

On the afternoon of day 1, pulmonary function testing and methacholine challenge for assessment of non-specific airways reactivity were carried out by the staff of the Occupational Respiratory Disease Unit at VGH. A PC_{20} for methacholine was determined in a similar manner to that of Cockcroft and co-workers (70). Briefly this involved aerosol inhalation of graded doses of methacholine (up to a maximum of 16 mg/ml) and measurement of the subsequent change in FEV_1 in the same fashion as described above for the histamine challenge.

Plicatic acid inhalation challenge was performed by the VGH group on the morning of day 2 and the response was monitored throughout the day and during the evening by means of spirographic measurements of FEV_1 and measurement of peak flow with a Wright's peak flow meter. The details of these procedures have been described previously (8).

Following the second permeability assessment on day 3, a repeat methacholine challenge was performed.

Data Analysis

Student's t-test was used to compare FEV_1 and t1/2 values between Study 1 and Study 2 subjects, and t1/2 before and after antigen challenge. Two-way analysis of variance was used to assess possible differences in the permeability indices at all time points, before and after challenge.

Results

Study 1: Smokers vs Non-smokers

Individual subject data and pulmonary function test results are shown in Table III (p.161). The FEV_1/FVC ratio was the same for smokers and non-smokers but there were significant differences between the two groups for $\Delta N_2/1$, closing capacity, and the maximum flow rate at 50% vital capacity ($\dot{V}_{50}$) ($p < 0.05$), with the smokers having compromised lung function.

Permeability

The pattern of aerosol deposition is shown in Figure 8 (p.156). The mean ratio of CPM in central to peripheral regions was identical for the two groups (non-smokers: 1.18 $\pm$ 0.17; smokers: 1.19 $\pm$ 0.15). Figure 9 (p.157) shows the disappearance of the ^{99m}Tc-DTPA tracer from the lung fields over time. The lung half-life and disappearance rate for each subject is shown in Table IV (p.162). The non-smoking group had a mean half-life in the lung of 110.0 $\pm$ 62.7 min and a mean disappearance rate of 0.90 $\pm$ 0.54 percent per minute. The smoking group had a significantly lower mean half-life of 42.4 $\pm$ 16.8 min and a faster mean disappearance rate of 2.12 $\pm$ 0.84 percent per minute ($p < 0.005$).

Figure 10 (p.158) shows the appearance of the tracer in the blood over time. The appearance rate is increased in smokers compared to non-smokers. Individual permeability indices at 10, 25 and 60 minutes are shown in Table IV (p.162). Mean values at all time periods were significantly higher for smokers than for non-smokers ($p<0.001$).

Reactivity

Histamine dose response curves for each subject, with and without prior propranolol treatment, are shown in Figure 11 (p.159). Individual results for PC_{20} and for the reactivity index (with and without propranolol) are shown in Table IV (p.162).

The mean reactivity index without beta-blockade, was 11.8 $\pm$ 5.3 for non-smokers and 12.0 $\pm$ 8.0 for smokers. After beta-blockade, reactivity was again similar between the two groups, but had increased, resulting in a reactivity index of 17.6 $\pm$ 7.0% for non-smokers and 19.4 $\pm$ 13.9% for smokers. Ten subjects (5 non-smokers and 5 smokers) showed a clear increase in reactivity and a decrease occurred in only one case. This increase was significant ($p<0.001$) but highly variable, and of the same magnitude in both groups.

There was no significant correlation between the reactivity index, with or without beta-blockade, and lung half-life of ^{99m}Tc-DTPA, permeability indices, or any of the pulmonary function tests performed.

Study 2: Before and after antigen challenge

Table V (p.163) shows the mean values for age, baseline FEV_1, and PC_{20} to methacholine before and after antigen challenge, in the four subjects who responded to plicatic acid challenge and in the five who did not respond. (A positive response to plicatic acid was determined from the measurements of FEV_1 and peak flow after challenge).

The mean pre-challenge FEV_1 for the nine subjects was 79.9% predicted which was significantly lower than that of the subjects in the first study. In three of the four subjects who responded to the plicatic acid challenge the response was both immediate and late, and in the fourth, only a late response was seen. The PC_{20} to methacholine following antigen challenge decreased in three of the four plicatic acid responders and in one of the non-responders.

Permeability data before and after antigen challenge is shown in Table VI (p 163). Comparison of antigen responders to non-responders revealed no significant difference between those groups for the permeability variables either before or after plicatic acid challenge. The t1/2 and PI_{25} for responders before antigen challenge were 59.4 min and 10.6 respectively, compared to 55.1 min and 14.3 for non-responders. The corresponding figures after challenge were 71.0 min and 8.4 for responders and 72.0 min and 11.8 for non-responders. These data do, however, reveal a significant increase in t1/2 from the before challenge to after challenge permeability studies for the non-responders and for the group as a whole (56.9 min. before challenge compared to 71.5 min after challenge) ($p < .01$) and a trend toward the same in

the responders. No concommitant decrease in the permeability indices was seen in either group, however.

Finally the t1/2 for the whole group before challenge (56.9 $\pm$ 18.6 min) was significantly lower than that of the non-smokers in Study 1 (110.0 $\pm$ 62.7 min) and higher than that of the smokers (42.4 $\pm$ 16.8 min) ($p < 0.5$), although there was no difference in permeability indices between those nine subjects and the non-smokers in the first study.

Subjective assessment of the gamma camera images after ^{99m}Tc-DTPA inhalation revealed more variability in the deposition pattern in these subjects compared to those in Study 1, with deposition being more central in all but one of these subjects. Comparison of pre- and post-challenge deposition of the tracer revealed no change in any of the non-responders and slightly more peripheral penetration of the tracer after challenge in three of the four responders with the opposite pattern in the remaining responder (see Figure 12 p.160).

DISCUSSION

This study was designed to test the hypothesis that hyperreactivity of the airways may be related to an increase in airways permeability as a result of mucosal damage either by cigarette smoke or antigen challenge. Although a significant difference in permeability between smokers and non-smokers was found, there was no correlation between this and reactivity. Furthermore, despite elevated reactivity in both smokers and non-smokers following propranolol aerosol, these groups were still identical with respect to reactivity and this increased reactivity bore no relation to permeability.

Finally, permeability in the three patients who responded to plicatic acid challenge did not increase after antigen challenge, despite the fact that non-specific reactivity was elevated in the patients after the antigen exposure.

Smokers versus Non-smokers

The observed permeability difference between the smokers and non-smokers in the first part of this study most likely reflects an alteration in the epithelium of the smokers such that the tracer can diffuse more readily from the lungs into the blood (39,40). These results correspond with those of Jones and his colleagues (43) who have reported increased alveolar permeability to Tc^{99m}-DTPA in smokers compared to non-smokers.

There are, however, other factors in this study which could affect the disappearance of the tracer from the lungs or its appearance in the blood, and could conceivably account for the difference seen between smokers and non-smokers. These are: 1) differing muco-ciliary clearance rates; 2)

variations in the area of initial deposition of the tracer; 3) altered perfusion of the lungs; and 4) differences in the volume of distribution or rate of removal of the tracer once it reaches the bloodstream.

Muco-ciliary clearance of the tracer solution would result in a measurable disappearance of the tracer from the lung and confound the disappearance directly into the blood stream. However, the rate of disappearance seen in this study is much faster than could be accounted for by muco-ciliary clearance alone. Sanchis and associates (71) studied the clearance of an aerosol of human serum albumin with droplets of mass median aerodynamic diameter (MMAD) of 3μ and found the half-life for the whole lung to be 23.0 hr. The aerosol used in this study had a MMAD of 6.3μ, however, this difference in MMAD would, according to the Task Group on Lung Dynamics of the ICRP (72), result in a greater degree of nasopharyngeal deposition but not a greater difference in the relative deposition in pulmonary tracheo-bronchial regions of the lung. In addition the long length and angled shape of the tubing used in the aerosol delivery system would tend to reduce the number of larger particles in the aerosol stream by impaction on the walls of the tubing and by evaporation. This could account for the observed deposition pattern in the first study (Figure 8) which appears to be throughout the tracheo-bronchial tree and in the lung periphery. Tracer deposited at this level in the lungs would not be solely cleared by the muco-ciliary escalator with the half-life observed in this study. Diffusion into the blood stream must be the main route for the disappearance of the tracer from the lungs. This is further borne out by the positive correlation seen between the lung half-life and the blood levels of the tracer as measured by the permeability

indices (R^2 = .57).

Muco-ciliary clearance from the larger airways does, however, occur rapidly and could conceivably account for the altered disappearance seen in the smokers if muco-ciliary clearance was increased in the smoking group. Studies in which muco-ciliary transport rates have been compared between smokers and non-smokers, however, show either no effect (73) or impaired clearance (74) in smokers, and the data shows no increase in central deposition in the smokers.

More peripheral deposition of the tracer in the smokers, and therefore a greater surface area available for diffusion, could also result in increased transfer across the epithelium. Jones and colleagues (43), who used techniques to achieve smaller particle size and consequently deposited the aerosol mainly in alveoli, found a mean half-life of the tracer in non-smokers of 50 min and in smokers of 20 min. This is approximately twice the rate seen in this study. Similarly, Rinderknecht and associates (54) who also had predominantly peripheral deposition, found a higher rate of disappearance in their normal subjects (mean 1.56%/min compared to 0.9%/min), and Oberdorster and his colleagues (55) showed a half-life of 72 minutes when techniques were used to deposit the aerosol mainly in conducting airways and 43 minutes when deposition was mainly alveolar. However, it has been suggested that smokers would, if anything, tend to have more central deposition than non-smokers (75) and, in this study, the relative ratios of central to peripheral deposition in smokers and non-smokers were identical. Thus, it is unlikely that differences in surface area of deposition are responsible for the elevated rate of transfer of the tracer into the blood in smokers.

Greater perfusion in the lungs of the smokers is an unlikely cause of an elevated disappearance rate because it has been shown that perfusion-limitation is only significant for gases that are highly soluble in the blood (76). This has recently been confirmed by Rizk and his colleagues (77), studying permeability to Tc-DTPA in dogs, who diverted the whole of the cardiac output through one lung, and found no increase in permeability.

Finally, it is possible that the measured appearance of the Tc-DTPA in the blood is elevated in the smokers as a result of alterations in the volume of distribution or delayed removal of the tracer from the blood. The actual volume of distribution could have been measured in each case by injecting DTPA tagged with a different label, as did Huchon et al (52), but this was not done because of the increased radiation involved. Instead, it was decided to partly control for variations in individual volume of distribution by including body weight in the calculation of the permeability index (although ideal body weight may theoretically be more proportional to body water than is actual body weight, no significant difference was found when the permeability index was calculated using ideal weight). However, the clearance of the tracer from the blood was not controlled. Tc-DTPA is cleared from the blood mainly by the kidneys (57); therefore, it is conceivable that if renal clearance were impaired in the smokers, elevated blood counts would be seen. The positive correlation found between the lung half-life and the blood levels as measured by the permeability index suggests, though, that this index is a reasonable reflection of the transfer of the tracer into the blood, although clearly other factors must be involved or the correlation would be stronger. These factors may well be related to the behaviour of the tracer in

equilibration with body water and its elimination from the blood.

The evidence supports the concept of a decrease in the effectiveness of the diffusion barrier presented by the epithelium, as a result of cigarette smoke exposure. This could result from areas of denuded epithelium, an increase in the effective pore size between cells, or metabolic damage to the epithelium cell membranes such that they are no longer a barrier to diffusion of the tracer. Several investigators using animal models have suggested that changes in the interepithelial junctions are a likely cause of increased effective pore size. Damage to junctional complexes was suggested by Simani and his associates (38) and Boucher and co-workers (39) were able to show qualitative changes in the junctional strands using freeze fracture techniques. Similarly, Davis and colleagues (34) showed junctional complex discontinuities after ozone exposure using freeze fracture. However, Walker and his associates (78), using quantitative techniques, could find no changes in junctional complexes along lateral cell surfaces in guinea pigs exposed to cigarette smoke. Evidence from their study suggested that changes in the tricellular junctions at the corners of the epithelial cells may be the site of the increased permeability.

Boucher (14) has proposed three potential mechanisms whereby junctional damage may occur. These include damage by reactive oxidant species either direct (eg: from cigarette smoke) or from PMN's recruited to the area, enzymatic cleavage of the junctions as a result of the increased effective proteolytic activity, and metabolic changes to the cells themselves. It is possible that these mechanisms or others could result in damage not only to the junctional complexes but also to the cell membranes.

It is of interest that in both animal and human studies, the epithelial permeability returns to normal during the repair phase of the injury (40,44,45). The animal data show that the normal epithelium is partially replaced by a reparative basal cell layer during the repair phase. This suggests that the return to normal may be based on these proliferating basal cells which are large and flat with fewer corners per unit of surface area than normal epithelium. In a study of rat tracheal epithelial regeneration, Gordon and co-workers (79) found that intercellular junctions were present between these basal cells six hours after injury. This could be an important method of restoring the barrier. Whether the apparently normal permeability seen in the patients in the second study as well as in patients with stable asthma (42) is based on a repairing epithelium is an interesting possibility. Such a mechanism might also explain the data of Rinderknecht and associates (54) who accounted for the observed increase in alveolar permeability seen in patients with interstitial lung disease by the increased numbers of Type II alveolar epithelial cells. These small cuboidal cells would also have increased numbers of pores per unit surface area when compared to the normal Type I cell. Smokers, however, are subject to repeated acute injury with continual epithelial damage and increased permeability.

Mason and his colleagues (56) have provided evidence that the increase in effective pore size in smokers is related to an increase in "pore dimensions" rather than rise in the number of accessible pathways. He studied the proportionate clearance of two indicators of different molecular size and found that clearance of the larger indicator, ^{99m}Tc-DTPA, increased significantly more in smokers than that of the smaller indicator,

$^{99m}TcO_4^-$, suggesting a widening of the pathway for diffusion.

It was hypothesized that such an increase in the permeability of the pulmonary epithelium may leave the underlying structures more accessible to agonists and thus result in hyperreactivity of the airways (2,80). However, although this data and others (43,45) show a clear difference between smokers and non-smokers with respect to permeability, no parallel reactivity differences between these groups were seen in this study. This is consistent with the results of Brown and associates (48) who studied young, light smokers and found no reactivity difference between these subjects and non-smokers. Others have found smokers to be less reactive (46) or more reactive (10,47) than controls with no significant change in reactivity on cessaton of smoking (49). Baboons taught to smoke (and in whom self-selection can have no role), were less reactive than baboons not exposed to cigarette smoke (81).

It has been suggested (46,81) that one reason for the decreased reactivity in smokers in some studies may be an attenuation of the airway response to histamine by beta-adrenergic relaxation of airway smooth muscle as a result of nicotine-induced elevation in circulating catecholamines (82). Beta-blockade has been used by several workers prior to studying reactivity, again with varied results. Habib and colleagues (19) found that injected propranalol had no effect on reactivity in normal subjects. On the other hand, beta-blockade does result in increased reactivity in asthmatics (16) and in non-asthmatics suffering from hay fever (18). Similar increased reactivity has also been shown in moderate to heavy smokers by Zuskin and colleagues (20) who found no decrease in flow rates immediately after smoking two cigarettes

but significant reduction in flow rates when the cigarettes were preceded by beta-blockade. Ploy-Song-Sang and co-workers (17) found that normal subjects given IV histamine alone or IV propranalol alone showed no change in pulmonary function but when the two agents were given together an increase in resistance, a decrease in maximum flow rates at 50% lung capacity and a decrease in density dependence during partial flow volume tests occurred. Studies of normal subjects and of workers exposed to hemp, by Bouhuys et al (21, 83), also showed increased responsiveness to histamine and to hemp fibre with propranalol pre-treatment.

In this study both smokers and non-smokers showed increased reactivity to histamine following beta-blockade via propranolol aerosol. This suggests that beta-adrenergic mechanisms do play a role in reactivity to histamine but that this role is similar in both groups and not specific to a possible nicotine-induced effect in smokers only. An alternate hypothesis is that the histamine exposure of the test itself results in an increase in circulating catecholamines (84) which tend to counteract the effect of histamine on airway calibre. With beta-blockade, this counterbalancing effect may be reduced thus resulting in an apparent increase in reactivity.

The lack of correlation between permeability and reactivity in this study may relate to a differing physical <u>locus</u> for these responses. The smokers in this study have physiological evidence of abnormalities in the peripheral airways (Table III) and this has been shown by others (85,86) to be associated with inflammatory changes. Thus the site of altered permeability, if it is associated with inflammation, may be in the peripheral airways, whereas altered reactivity is most likely a central airways phenomenon, given

the predominantly central distribution of the irritant receptors (87).

Pre-and post-antigen challenge

The results of the second part of this study provide further evidence that increased reactivity is not the direct result of increased epithelial permeability. Although only four of the subjects studied responded to the antigen challenge, there was an increase in non-specific reactivity following the challenge in three of these four subjects. However, there was no concommitant rise in permeability after the plicatic acid challenge by which to explain this increased reactivity.

The low PC_{20}'s to methacholine before challenge and the low baseline FEV_1's in all but one subject (the same subject who showed a deposition pattern of the tracer identical to those in Study 1) suggest that even the non-responders in this study cannot be considered "normal" subjects and that differences seen in the group as a whole compared to the subjects in Study 1 may be indicative of changes due to an asthma-like condition.

As in the first part of the study, factors relating to deposition of the aerosol, if different from one assessment of permeability to another, could confound the results. In these nine subjects, there was, on the average, greater central deposition than in the first study. This probably reflects the fact that there was greater baseline airway obstruction in these patients, as evidenced by the lower mean FEV_1, which is not surprising given their referral to the clinic for occupational asthma investigation. The disappearance half-life of the tracer in all these patients was shorter than that of the non-smokers in the first study, suggesting an increase in permeability. The blood levels of the tracer, however, were not elevated.

This suggests that clearance from the lungs is enhanced due to increased muco-ciliary activity rather than increased transfer directly into the blood across the lung mucosal epithelium. This is consistent with the more central deposition pattern and may also reflect an increase in mucous production in these subjects.

However, as discussed above, disappearance of the tracer in other studies is enhanced by more peripheral, alveolar deposition rather than vice versa. It may be that a peak in lung retention time is reached when deposition is primarily in small airways where mucociliary and alveolar clearances are limited and retention time decreases when deposition becomes predominantly alveolar or predominantly in large airways or if mucous production is increased.

The primary question being investigated in these patients was the difference in permeability before and after challenge; thus a change in the pattern of deposition after antigen exposure could alter the apparent permeability and mask an increase in actual permeability of the epithelium.

Although no measurements were made of the distribution of ventilation before and after challenge, the subjective assessment of the gamma camera images indicated no obvious changes in the aerosol deposition pattern after the plicatic acid exposure in the non-responders and only slight and inconsistent changes in the responders. It is not possible to assess the affect of altered distribution in these subjects as it is not apparent whether the changes seen would lead to enhanced clearance due to more alveolar deposition or retarded clearance due to deposition in peripheral airways with slower muco-ciliary clearance. The t1/2 results indicate that clearance of

the tracer was prolonged in all subjects (responders and non-responders) after antigen challenge, but the lack of parallel change in the blood appearance levels suggests that this may also be related to muco-ciliary activity. It can be speculated that there is a non-specific alteration in mucous production or clearance dynamics in all these subjects relating to exposure to plicatic acid as an irritant, with bronchoconstriction occurring only in the four "responders".

The increase in reactivity that occurred in four subjects (3 responders and one non-responder) was not reflected by any consistent change in permeability, again suggesting, as in the first study, no cause and effect relationship between these two parameters.

These results correspond with those of Elwood and colleagues (42) who found no difference in mucosal permeability between stable asthmatics and non-asthmatics when permeability was assessed with the Tc-DTPA tracer deposited more centrally. In fact the t1/2 for the normal subjects in that study was 66 $\pm$ 19 min., very similar to that seen in this study. However, Boucher and his associates (35) did find increased permeability in allergic monkeys after antigen challenge to ^{99m}Tc-albumin and enhanced reactivity to histamine and increased permeability to tritium-labelled histamine (36) after antigen challenge. Similarly, Elwood and his colleagues (42) showed enhanced permeability in normal human subjects immediately following inhalation of histamine. They proposed that histamine release by mast cells as a result of antigen-antibody interaction may alter bronchial-mucosal permeability thus amplifying the allergic response.

This study differs from these previous investigations in that the second

measurement of permeability was performed several hours after antigen challenge. It is possible that a transient increase in permeability may have occurred immediately after the challenge, perhaps related to histamine release from mast cells, but no prolonged alteration in permeability was seen, despite the continued increase in non-specific reactivity following the antigen challenge.

In summary, these studies show that although bronchial epithelial permeability is elevated by cigarette smoke, this is not followed by an increase in airway reactivity regardless of the presence or absence of beta-blockade; and that although antigen challenge can result in an enhancement of non-allergenic reactivity, no parallel increase in mucosal permeability occurs. Clearly, there is no specific cause and effect relationship between permeability and reactivity. More likely these are both associated with the inflammatory reaction but result from the activation of different physiological pathways. Recent investigation by Holtzman and his colleagues (88) has provided additional evidence for the association between inflammation and hyper-reactivity. They showed that enhanced reactivity to acetylcholine in dogs following ozone exposure correlates positively with the number of epithelial and subepithelial neutrophils in the trachea. Particularly interesting in their study is the fact that four of the ten dogs did not show enhanced reactivity and there was no increase in neutrophil number in these animals. They also found a positive correlation between the level of reactivity and the increase in neutrophil number. These authors

suggest that this relationship may be due to the release of inflammatory mediators from the PMN's which alter the bronchial smooth muscle directly or affect its innervation such that the muscle is more responsive. They also suggest that subtle changes in epithelial cells during the inflammation, which increase permeability, may play a role in enhanced reactivity by allowing more effective chemotaxis of neutrophils. O'Byrne and his associates (89) have also reported recently that ozone induced hyper-responsiveness in dogs is eliminated by neutrophil depletion. The studies in guinea pigs by Hulbert and co-workers (41), however, indicate that reactivity is increased immediately following smoke-exposure and returns toward normal by the time the PMN numbers peak. Clearly, this question remains unresolved at this time.

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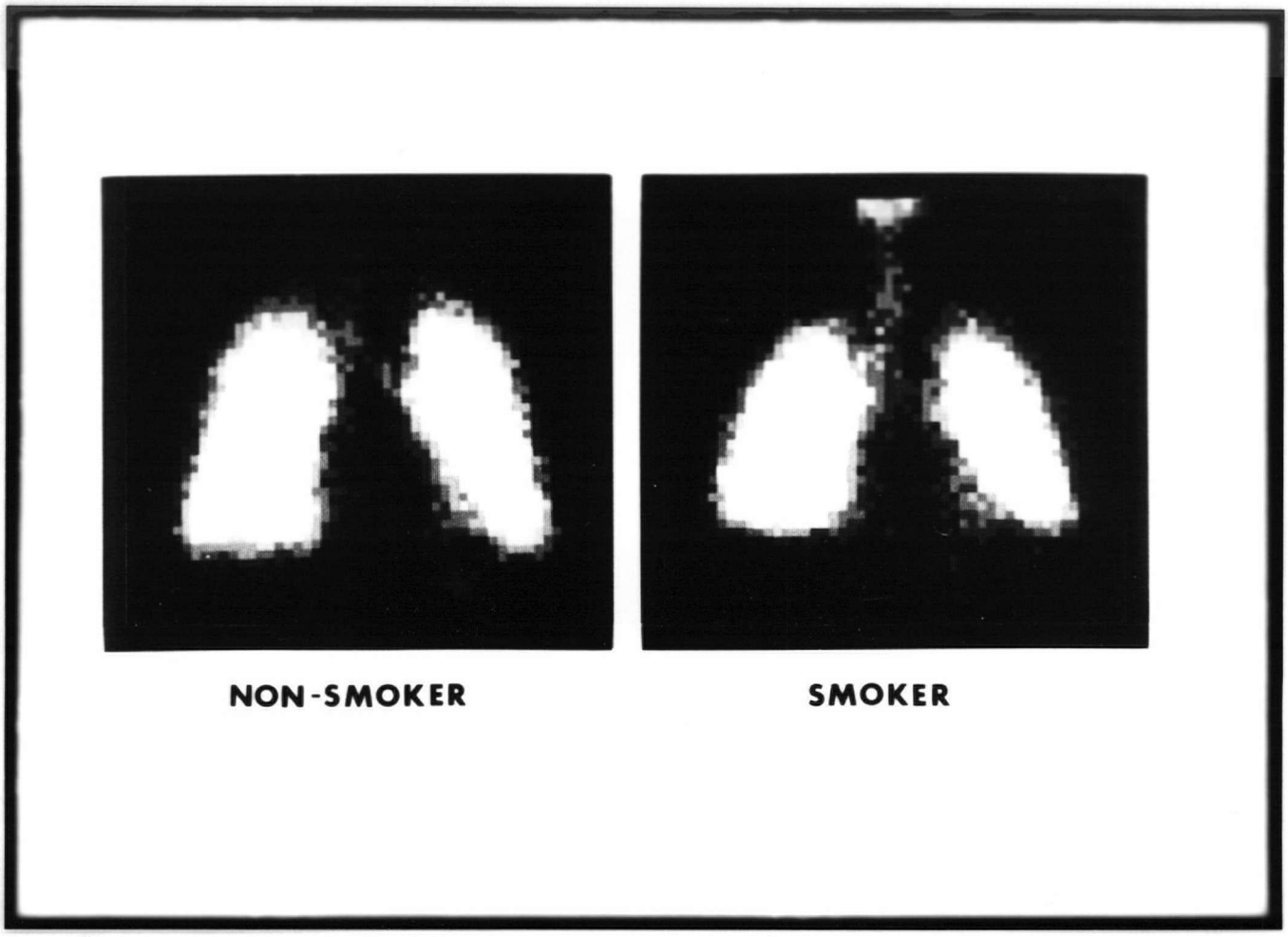

Figure 8 Deposition pattern of aerosolized Tc-DTPA in lungs - gamma camera image of two typical subjects, 1 non-smoker and 1 smoker showing diffuse deposition of the tracer in both cases.

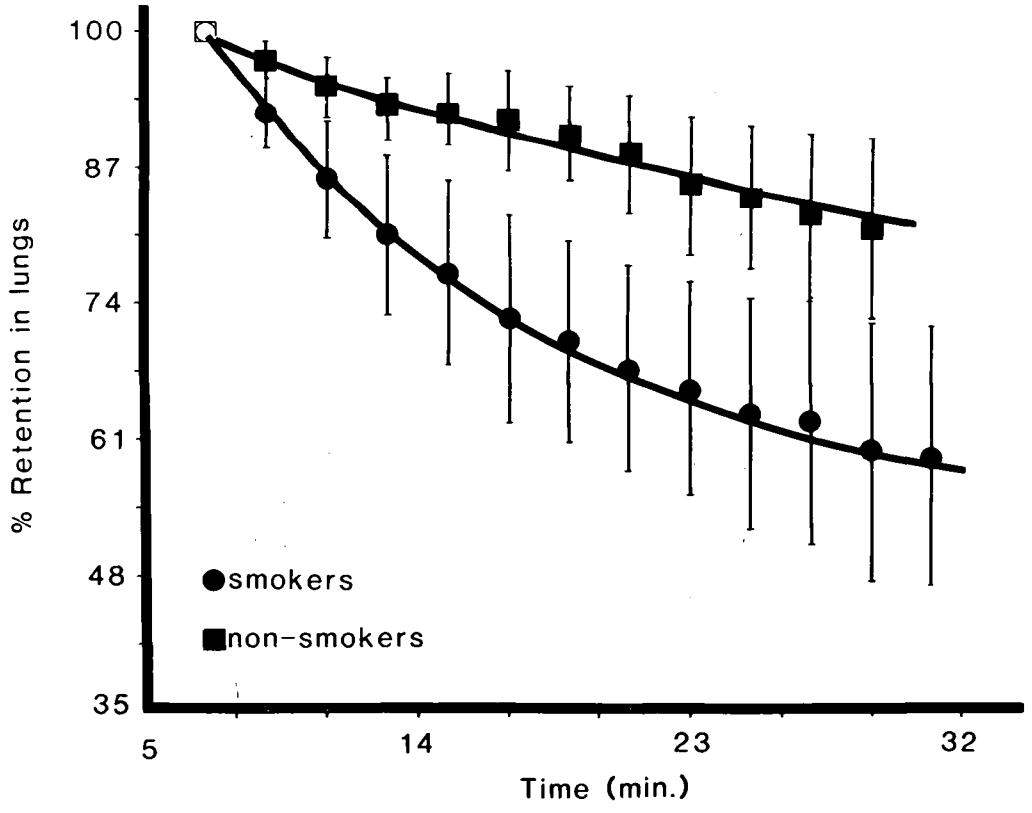

Figure 9 Disappearance of Tc-DTPA from lungs - gamma camera counts per minute (CPM) remaining in lungs as % of CPM at time zero (7 minutes after beginning of aeroslization of tracer) vs. time; mean ± S.D

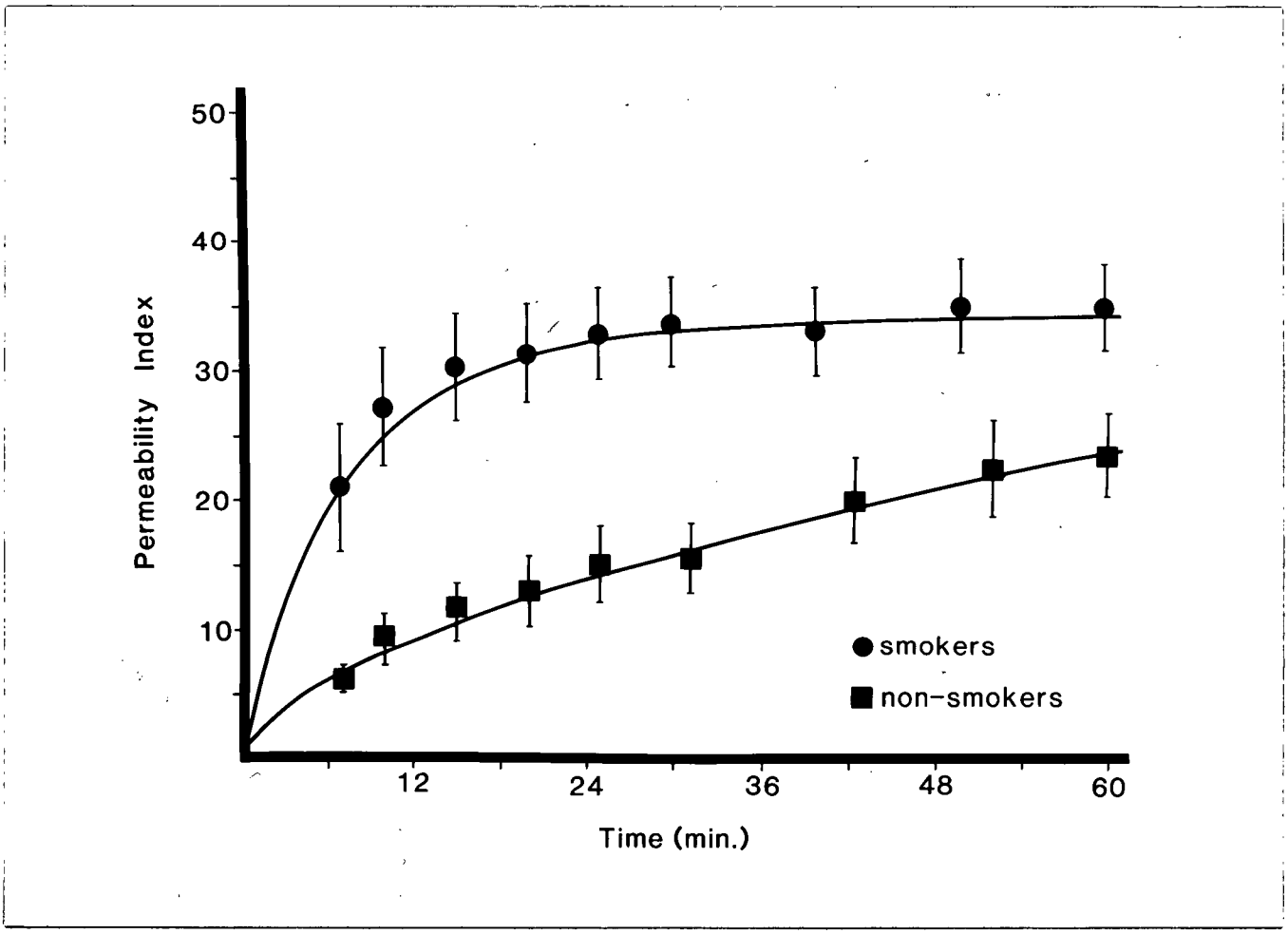

Figure 10 Appearance of Tc-DTPA in blood - permeability index vs. time; mean ± S.D. (see text for definition of permeability index).

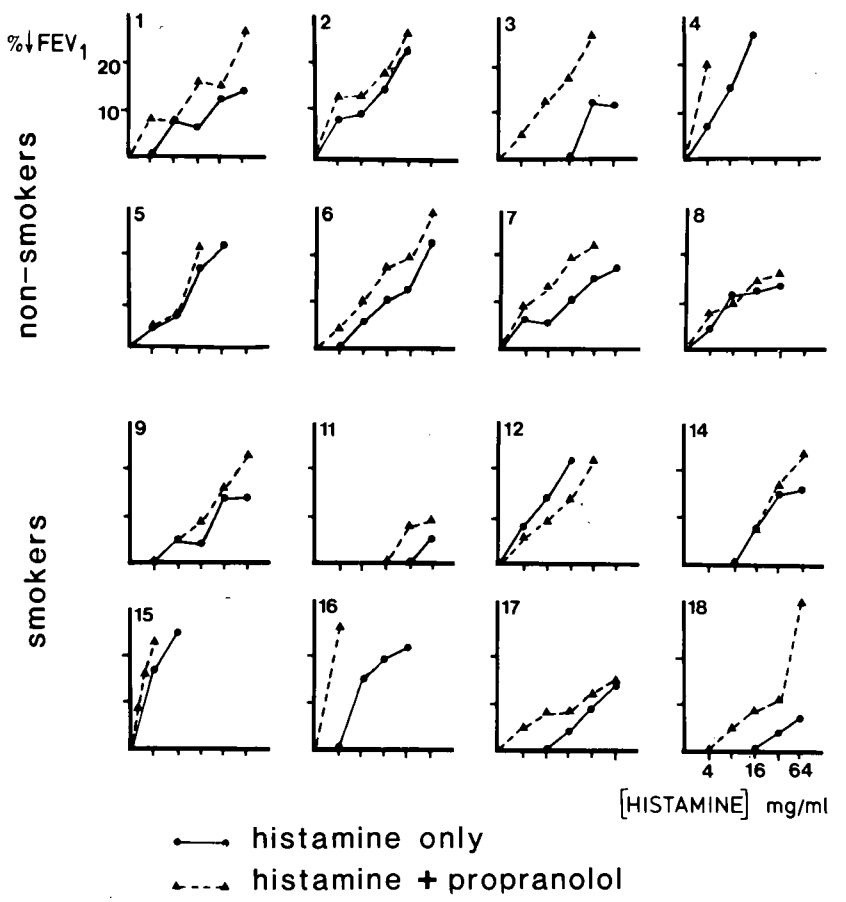

Figure 11 Individual histamine dose-response curves - % decrease in FEV_1 from pre-histamine levels following histamine dose given; —— response following aerosolized histamine alone, ---- response following aerosolized propranolol plus histamine; subject number indicated in upper left of each graph.

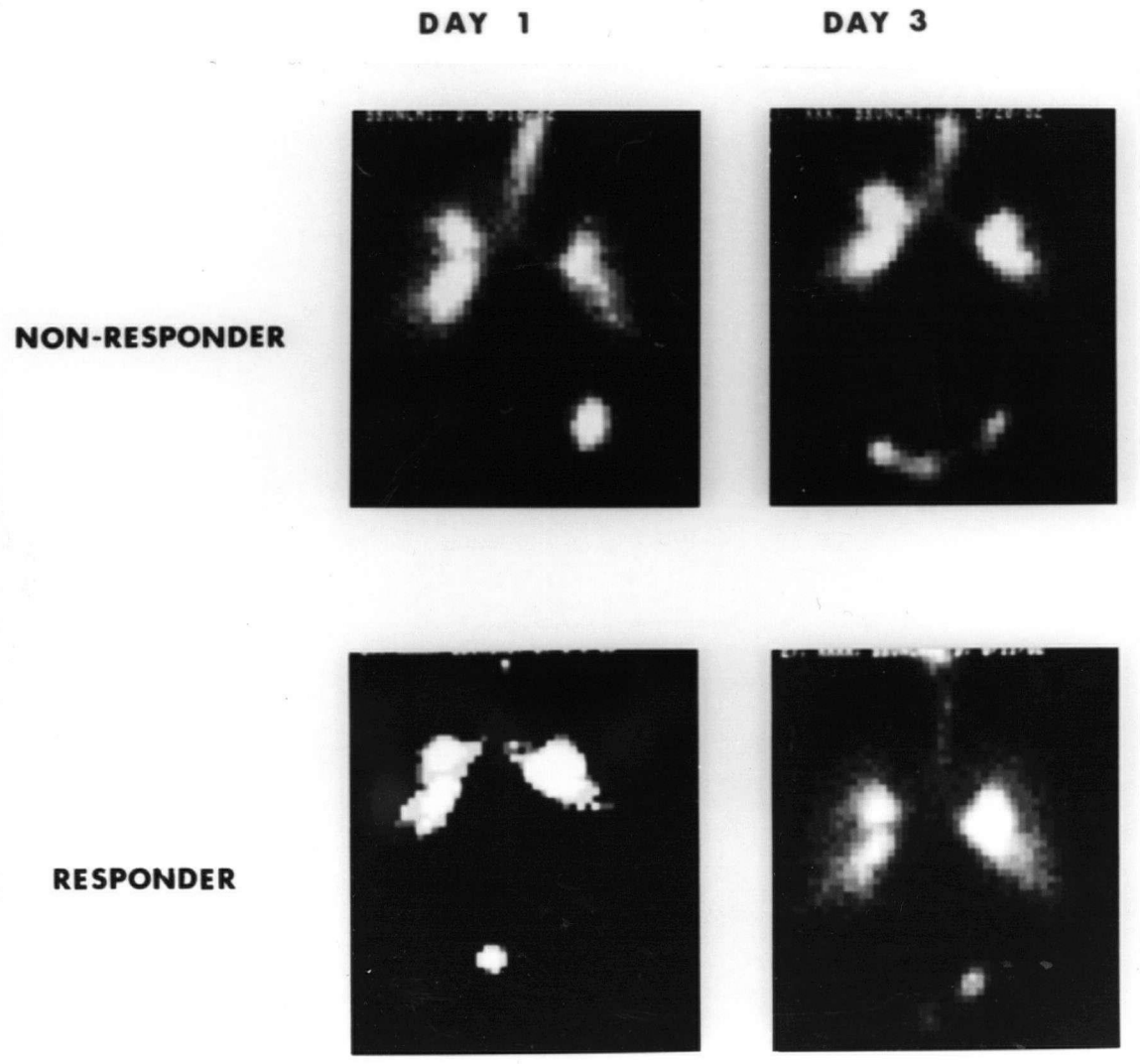

Figure 12 Deposition pattern of aerosolized Tc-DTPA in patients before (Day 1) and after (Day 3) antigen challenge - gamma camera image of two typical subjects, 1 responder and 1 non-responder to plicatic acid, showing more central deposition in both cases, compared to Study 1 subjects (see Figure 8). Deposition is not changed after antigen challenge in the non-responder but is slightly more peripheral after challenge in the responder.

TABLE III

Subject Characeteristics and Pulmonary Function - Study 1

					←---------% Predicted----→			
Subject	Age	Sex	Pack Yrs. Smoked	$\frac{FEV_1}{FVC}$	FEV_1	$\Delta N_2/1$	$\frac{CC}{TLC}$	$\dot{V}_{50}$
Non-Smokers								
1	30	F	0	80	119	99	87	97
2	28	M	0	76	102	70	98	102
3	23	F	0	85	106	61	60	74
4	28	M	0	77	114	130	105	98
5	35	M	0	84	131	64	75	141
6	25	M	0	84	121	82	97	123
7	46	M	0	83	108	82	127	114
8	34	M	0	83	124	79	95	106
$\bar{x}$	31			82	116	83	93	107
SD	7			3	10	22	20	20
Smokers								
9	25	M	10	86	105	63	162	106
10	33	M	20	88	98	87	111	84
11	33	M	18	84	118	128	111	112
12	37	M	11	72	106	139	80	60
13	31	M	13	74	111	98	113	71
14	28	F	9	86	111	104	62	106
15	41	F	24	77	103	160	145	83
16	22	F	16	83	91	124	141	74
17	32	M	17	78	103	91	114	82
18	42	M	34	77	103	183	124	87
$\bar{x}$	32		17	80	105	118	116	86
SD	6		8	6	7	36	30	17
p value	N.S			N.S.	N.S.	$<.05$	$<.05$	$<.05$

TABLE IV

Permeability and Reactivity Data - Study 1

Subj.	T1/2 (min)	%↓/min.	PI_{10}	PI_{25}	PI_{60}	PC_{20} (mg/ml) (no β-block)	RI	PC_{20} (mg/ml) (with β-block)	RI	Δ RI
Non-Smokers										
1	52.7	1.50	10.4	18.7	29.4	64	7.8	47.9	15.0	7.2
2	157.0	0.45	5.9	8.0	25.9	25.1	15.3	18.8	17.3	2.0
3	53.0	1.56	5.1	11.1	18.4	64	6.1	20.0	17.3	11.2
4	217.6	0.15	3.0	5.4	13.9	11.6	21.7	4.0	33.3	11.6
5	166.0	0.42	21.2	30.6	39.2	26.9	14.7	16.0	18.3	3.6
6	57.0	1.38	12.5	21.0	23.8	53.7	12.2	41.7	16.1	3.9
7	75.6	1.00	9.2	15.2	22.0	64	9.4	26.9	14.7	7.3
8	101.0	0.72	7.4	10.2	11.4	64	7.2	64	8.9	1.7
$\bar{x}$	110.0	0.90	9.3	15.0	23.0		11.8		17.6	6.1
SD	62.7	0.54	5.7	8.2	8.9		5.3		7.0	3.9
Smokers										
9	42.9	1.79	16.5	23.7	27.0	64	7.8	47.8	12.8	5.0
10	29.6	2.45	58.0	48.2	34.7	-	-	-	-	-
11	34.4	2.47	25.0	30.0	32.4	64	2.8	64	5.0	2.2
12	48.3	1.59	18.6	21.4	19.9	14.7	18.3	26.0	16.0	-2.3
13	77.4	0.90	20.7	24.9	26.9	25.6	16.0	-	-	-
14	28.7	3.01	27.1	37.0	39.3	56.2	8.9	42.7	13.3	4.4
15	32.0	2.52	38.7	49.2	52.6	5.4	27.8	2.4	38.3	10.5
16	61.0	1.24	20.3	30.0	44.0	21.3	14.7	3.0	43.3	28.6
17	47.0	1.62	20.8	28.1	32.5	64	7.9	64	8.3	0.4
18	22.8	3.59	47.9	41.6	33.5	64	3.9	36.3	17.8	13.9
$\bar{x}$	42.4	2.12	29.4	33.4	34.5		12.0*		19.4*	7.8
SD	16.8	0.84	14.1	10.1	9.3		8.0		13.9	9.9
p value (smokers vs. non-smokers)	<.005	<.005		<.001			N.S.		N.S.	N.S.

*comparison of RI with and without beta-blockade: non-smokers: $p<.01$
smokers: $p<.05$
all subjects: $p<.001$

Table V

Subject Characteristics and Antigen Response - Study 2

	Age	Baseline FEV_1 (%pred)	Pre-Challenge PC_{20} (mg/ml)	Post-Challenge PC_{20} (mg/ml)
Responders (n=4)				
$\bar{x}$	49.2	86.8	1.25	0.71
SD	19.2	9.2	1.06	0.26
Non-responders (n=5)				
$\bar{x}$	45.2	74.4	5.32	9.80
SD	14.1	23.9	6.11	8.49
Total group				
$\bar{x}$	47.0	79.9*	3.51	5.76
SD	15.6	19.0	4.87	7.68

* FEV_1↓compared to subjects in study 1 ($p < .001$)

Table VI

Permeability Data - Study 2

	Before Challenge				After Challenge			
	$t_{1/2}$	PI_{10}	PI_{25}	PI_{45}	$t_{1/2}$	PI_{10}	PI_{25}	PI_{45}
Responders (n=4)								
$\bar{x}$	59.4	7.0	10.6	14.7	71.0	4.3	8.4	12.1
SD	11.4	7.4	8.6	8.6	12.8	2.7	3.6	4.3
Non-responders (n=5)								
$\bar{x}$	55.1	9.3	14.3	22.0	72.0*	7.3	11.6	15.1
SD	18.6	4.9	8.3	9.6	13.2	3.1	4.2	5.7
Total group								
$\bar{x}$	56.9+	8.3	12.7	18.6	71.5*	6.0	10.3	13.6
SD	15.1	5.8	8.1	9.5	12.2	3.2	4.1	5.0

*t1/2 (total and t1/2 (non-resp) after challenge>before challenge ($p < .01$)

+ { t1/2 (total) Study 2 subjects < Study 1 non-smokers
  { t1/2 (total) Study 2 subjects > Study 1 smokers

VII Small Airway Structure and Function in Persons Exposed to Mineral Dust and Fume - Compared to Cigarette Smokers

Introduction

Studies carried out by Cosio and his colleagues (1) and other investigators (2,3) have indicated that inflammation in the peripheral airways is an early event in the pathogenesis of chronic obstructive pulmonary disease in cigarette smokers. These investigations have been discussed in preceding chapters. Airway pathological abnormalities have also been reported in persons exposed to industrial dusts and fumes (4-7) although in most cases these people are also cigarette smokers and the particular effects of the environmental pollutants are difficult to separate from the effects of the cigarette smoke.

The response of the lung interstitium to certain occupational pollutants such as coal, silica, asbestos, and other dusts which cause pulmonary fibrosis have been well described (8) although the specific cellular events responsible for the fibrotic response to these agents are not completely understood. Many earlier accounts of the pathology of these pneumoconioses described the interstitial fibrotic response which results in a restrictive functional impairment but held the airways to be mainly untouched. Other studies have demonstrated airway obstruction and pathological changes in the small airways with exposure to various industrial dusts which may or may not also result in pneumoconiosis.

As early as 1935 Simson (4) described the pathology of the early silicotic lesion using 3-dimensional reconstruction techniques. He showed that the inhalation of injurious dust from the Rand gold mines resulted in accumulation of dust cells and free pigment in the walls of respiratory bronchioles and alveolar ducts before any sign of the whorled fibrosis appeared, and that silicotic nodules were usually situated in the angle formed by two dividing respiratory bronchioles.

Epidemiologic studies of silicosis in South African gold mines (9,10) have revealed evidence of obstructive airways abnormalities although the role played by cigarette smoking was found to be greater than that of the mine dust in the development of these abnormalities (11).

Coal dust exposure also results in abnormalities in the vicinity of peripheral airways. The coat dust macule which is characteristic of simple coal workers pneumoconiosis consists mainly of carbonaceous deposits around respiratory bronchioles which may be dilated and show some centrilobular emphysema. A clear relationship has been shown between exposure to respirable coal dust and decline in FEV_1 (12).

Airway lesions in asbestos exposure have recently been sufficiently well characterized to be included in the definition of asbestosis put forward by the Pneumoconiosis Committee of the College of American Pathologists (13). This committee suggests that fibrosis of respiratory bronchioles along with the presence of asbestos bodies indicates the early form of asbestosis. Pathologic evidence of this

lesion in asbestos-exposed workers has been presented by Wright and Churg (5). However, these investigators have also noted the same lesion in workers exposed to non-asbestos mineral dust and have suggested that it is not specific to asbestos exposure but rather a non-specific reaction to mineral dust (6). Several studies of functional abnormalities in asbestos exposed individuals have indicated increasing small airway abnormalities correlating with increasing dust exposure (14-16), although these studies are also complicated by cigarette smoking among most of the subjects.

Evidence of airway obstruction has also been reported among workers exposed to occupational environment pollutants which are not associated with the development of pneumoconiosis. A 12 year followup study of workers in eleven Paris factories (17) demonstrated that the rate of decline in FEV_1 correlated positively with exposure to mineral dust (even excluding exposure to silica), grain dust, gases and heat.

However, Parkes points out, in his book Occupational Lung Disorders (8), that it is important to distinguish between the presence of chronic cough and sputum which is clearly associated with the inhalation of dusts and fumes and airflow obstruction with respiratory disability which he reports as principally associated with cigarette smoking and social factors, although a synergistic effect between dust and cigarette smoke is suggested. Similarly, Morgan (18) has found that non-smoking miners have functional indications of large airway narrowing while smoking miners have small airway obstruction as well.

Seaton (19) suggests that the matter is not quite so clear-cut and discusses several studies among coal miners which indicate an additional effect of dust exposure on peripheral airways.

A possible synergistic effect between smoke and industrial dust exposure could be related to the increase in epithelial permeability in cigarette smokers which was demonstrated in the preceding chapter. This change could result in enhanced absorption of dust particles. Conversely, dust particles in inspired air may act as carriers for adsorbed toxic gases from the cigarette smoke or other sources.

Although studies such as those described above suggest that industrial dust exposure may affect the airways, the nature of this effect is not known. It is unclear whether the airway response is similar to that produced by smoke or whether the pathology can be distinguished. It is possible that the small airway inflammatory response demonstrated in cigarette smokers may be altered or enhanced as a result of increased epithelial permeability which may reduce the effectiveness of the epithelial barrier to dusts or other irritants.

The study described below was designed to test this hypothesis, to determine if the small airway pathological changes seen in cigarette smokers were modified or increased by additional exposure to mineral dust or fume. This involved a comparison of pulmonary function, small airway pathology, and chest radiographs in subjects with mineral dust or fume exposure and those without dust exposure, individually matched for age and smoking history.

Methods:

1. Subjects:

Subjects were selected from all patients admitted to St. Paul's Hospital from October 1978 to December, 1982 for surgical resection of a lobe or lung due to, in most cases, lung cancer. Any patient with evidence of obstructive pneumonitis or whose lesion was found to obstruct a segmental or larger airway was excluded. Occupational histories were obtained from all patients before surgery. Every patient who reported ten or more years as a miner or with exposure to mineral dust or fume in a non-mining occupation was selected for the study. Altogether there were 19 such patients. Control subjects were chosen from among those patients who reported no occupational exposure to dust or fume of any kind. One control subject was chosen for each exposed subject, matched for age $\pm$ 1 year and smoking history. The smoking history match took into account both current smoking status and lifetime exposure (based on cigarettes per day x years = cigarette years).

2. Pulmonary Function:

Pulmonary function tests were performed within one week before surgery using a volume displacement body plethysmograph as described in

the preceding chapter. In addition to determination of the subdivisions of lung volume, maximum expiratory flow volume curves and N_2 washout curves as described above, steady-state CO diffusing capacity and fractional uptake of CO was calculated using the prediction formulae of Bates and colleagues (20).

3. Small Airway Pathology:

Surgically resected specimens were fixed by inflation through the bronchus using 10% formalin or 3% glutaraldehyde at 25 cmH_2O pressure and subsequent immersion in fixative for 24 hours. Histological specimens were prepared from 3-6 stratified random blocks from the medial and lateral slices taken from areas away from any obstruction. Paraffin sections (5 μ thick) were stained with hematoxylin and eosin, Masson's trichome, and periodic acid-Schiff stains.

Every non-cartilagenous airway less than 2mm internal diameter was graded in a single blind fashion using the pictorial grading system described by Cosio and his colleagues (1) for membranous bronchioles and Wright and associates (3) for respiratory bronchioles. A separate grade was determined from 0-3 for each airway depending on the presence and severity of each of the following pathological variables: mural inflammation, fibrosis, smooth muscle hypertrophy, and pigment, and squamous and goblet cell metaplasia in membranous bronchioles; and mural inflammation, fibrosis, smooth muscle hypertrophy, and pigment

and intralumenal macrophages in respiratory bronchioles. A single score was then determined for each variable for each subject by summing the individual airway score and expressing this total as a percentage of the maximum possible score. Respiratory and membranous bronchioles were assessed on separate occasions so that the scores for one were not influenced by the scores for the other.

4. Radiology and pathologic/radiographic correlation:

Pre-operative chest radiographs were read in a single-blind fashion by a separate investigator, experienced in the assessment of radiographic pneumoconiosis, and assigned a pneumoconiosis grade according to the 1971 ILO-U/C classification (21).

In order to determine the histologic correlate of the radiographic densities seen, slices were taken from the whole lung or lobe specimen (1/4 inch thick) and dried in a microwave oven at the lowest voltage setting for several days. These dried slices were then radiographed and the precise location of any densities seen on the film were located on the slice. This area of tissue was then removed from the lung slice, reimmersed in formalin for 24 hours, and processed for the preparation of paraffin sections. This was done because the randomly selected tissue specimens did not reveal any obvious silicotic lesions nor other evidence of pneumoconiosis.

5. Data analysis:

Pulmonary function test values and pathology variable scores were compared for exposed and non-exposed subjects using student's paired t-test. When function data was missing for any subject, both that subject and its corresponding match were eliminated from the analysis for that test.

Miners exposed to dust were compared to non-miners exposed to dust or fume by computing the difference between exposed and matched controls in each group, then applying student's unpaired t-test to compare these differences.

Results:

Exposure histories of the 19 mineral dust or fume exposed subjects are shown in Table VII (p 187). Ten were miners, with all but one of these being hard rock miners. Of the nine non-miners, a majority were exposed mainly to fume rather than dust alone, being welders and smelter workers.

As indicated in Table VIII (p 188) the nineteen exposed workers had a mean age of 62.3 $\pm$ 10.3 years and cigarette exposure of 981.3 $\pm$ 520.7 cigarette years. Of these, five were ex-smokers and the remainder current smokers or had only stopped smoking within 6 months. The controls were 62.4 $\pm$ 10.6 years old and had a smoke exposure of 1020.2 $\pm$ 488.2 cigarette years with the same proportion of ex- and current smokers.

Pulmonary Function:

Functional differences between the dust and fume exposed workers and matched controls are shown in Table VIII (p 188). These include significantly lower flow rates (FEV_1, $\dot{V}_{50}$ and $\dot{V}_{25}$) and increased $\Delta N_2/1$ indicating more airway obstruction and peripheral airways abnormalities in the exposed subjects. When the exposed group was divided according to occupation into a mining group and a non-mining, dust or fume exposed group and each group compared to their corresponding controls, the results indicated functional differences from controls in both occupational groups to approximately the same extent (Tables IX p 189 and X p 190). Although in these subgroups,

only the flow rates at low lung volumes are significantly different from controls there is a trend to more airway obstruction in both groups. When the heaviest smokers among the miners are singled out (Table IX p 189) these functional differences become more pronounced, suggesting a synergistic effect between smoke and dust.

Direct comparison of miners and non-miners exposed to dust and fume could not be carried out as the miners are significantly older (66.4 years compared to 57.8 years) and have smoked more (1154 vs 889 cigarette years) than the non-miners. Comparison of the differences between each subject and the matched control however revealed no significant differences between these two groups for any of the pulmonary function variables.

Small Airway Pathology:

Table XI (p 191) shows the small airway pathology variables for the exposed and control subjects. There was significantly more mural fibrosis in both membranous and respiratory bronchioles and more goblet cell metaplasia of membranous bronchioles in the lungs of the exposed workers. Examples of these lesions are shown in Figure 13 (p 195).

Table XII (p 192) shows these variables broken down by occupational group. The results in this table indicate that most of the increased small airway pathology previously noted in the exposed group was attributable to changes in the small airways of the non-miners exposed to dust or fume, and there were no differences observed in small airway pathology between miners and their matched

controls.

Again, comparison of miners and non-miners by investigation of the differences between the subjects and controls, confirmed that the non-miners had significantly more fibrosis in both respiratory and membranous bronchioles ($p < .05$) and there were no differences between the two exposed groups for any other pathology variables.

Radiographs:

The radiographic assessments are shown in Table XIII (p 193). Seven of the 9 miners for whom radiographs were available were graded as positive for pneumoconiosis. Three of the other dust/fume exposed group had changes suggesting possible dust exposure and one control subject had similar changes.

Radiographic/pathologic correlation:

Dried lung slices were obtained from all of the exposed subjects and from three of the controls, chosen randomly. Densities similar to those seen on the pre-operative chest radiograph (and classified as pneumoconiotic lesions) were identified in only three cases, each being one of the miners previously graded for positive pneumoconiosis. Histologic examination of the tissue responsible for the densities revealed the classic whorled fibrotic nodules characteristic of silicosis (see Figure 14 p 197). In addition, subpleural calcification was identified both radiologically and histologically in a fourth case.

Discussion:

The results of this study suggest that both functional and structural changes occur in response to mineral dust or fume in excess of those attributable to cigarette smoking. There are however, certain features of this study that must be taken into consideration when evaluating the results. Occupational histories of all subjects were obtained pre-operatively by questionaire without reference to any study which may make use of this information. This has the desirable aspect of minimizing interviewer bias but unfortunately no detailed information was obtained about actual exposure conditions for any of the subjects. Consequently, no specific conclusions can be drawn relating the findings to particular exposures. It does seem reasonable, however, to assess the workers exposed in mining occupations separately from those exposed in non-mining jobs. The differences observed in lung structure (both in histology and radiography) between these two groups support this separation.

A second important feature of this study is the concentration on pathology in the small airways. No attempt was made to assess all the histological changes which may have occurred in the lung tissue. Thus, functional or radiographic changes may be found which do not correlate with the pathology as assessed in this study, but there may in fact be correlations with large airway or lung interstitial changes.

Thirdly, the patients studied all had a lung or lobe removed because of possible carcinoma. Although the sections studied for

pathological analysis were taken as far away as possible from the tumor or other lesion, an assumption is implied that the lesion does not adversely affect the rest of the lung. Also, it is assumed that the lobe or lung removed is representative of the lung as a whole. The fact that both the control group and the exposed group were drawn from the same population of tumor patients undergoing resection, minimizes this problem as far as comparison between these groups is concerned; but the problem remains in drawing conclusions relating structure and function in any one group. This is particularly evident in the results stemming from the analysis of radiographs and the subsequent search for the pathological correlate of the radiograph densities in the dried lung slices.

The differences in pulmonary function observed between the exposed and non-exposed groups indicate slightly more airflow obstruction in the exposed group rather than a restrictive lung disease as might be expected if the exposed group had extensive interstitial involvement with fibrosis or fibrotic nodules. This absence of restriction is in keeping with the radiographic evidence which indicated only early pneumoconiosis in some cases. The additional airway obstruction is consistent with many other reports of airway involvement in miners and other industrial workers exposed to dust. The similar functional decrements in both miners and dust/fume exposed non-miners suggests that these functional abnormalities are not specific to any single agent. Similar results were reported recently by Manfreda and his colleagues (22) who found the pulmonary function

parameters derived from maximal expiratory maneuvers and the single-breath N_2 test to be worse in over 1000 miners and smelter workers than in a general population sample. Interestingly, among the current smokers, the smelter workers had more functional abnormalities than did the underground miners.

The striking increase in functional differences between exposed and non-exposed among the heaviest smokers in this study lends support to the concept of synergism between cigarette smoke exposure and mineral dust. Unfortunately the small number of heavy smokers among the non-mining exposed group made it impossible to determine if this effect was similar in both exposure categories.

Relating the pulmonary function differences seen between the exposed and non-exposed workers to the airway pathology is difficult in these subjects, particularly because the significant changes seen in the airway pathology were observed only among the non-miners, whereas the functional differences were found equally in both exposed groups.

The considerable fibrosis observed around the peripheral airways in the non-mining dust or fume exposed subjects is very similar to that reported recently by Churg and Wright (5,6) in both asbestos and non-asbestos mineral dust exposures. The absence of increased airway fibrosis (compared to non-exposed smokers) in the miners (Table XII), however, remains to be explained.

Some factors which may be important in determining the nature and progression of airway lesions in these groups include the length of time spent underground or at the ore face, and other exposure conditions such as the presence of diesel exhaust, and wet or dry mining techniques. It is possible that the airway fibrosis seen in the non-miners and the radiographic pneumoconiosis seen in the miners reflect differing deposition patterns of the inhaled dusts or fumes in these workers. Evidence for the importance of deposition in determining the nature of the fibrotic response is found in the animal model of bleomycin-induced fibrosis. Parenteral administration of bleomycin in baboons produces interstitial fibrosis while intratracheal instillation produces airway fibrosis particularly in the walls of respiratory bronchioles (24). PMN's or macrophages may be present in both the airways or alveoli and may be activated by particulate phagocytosis or by exposure or oxidents and release factors which initiate the fibrogenesis.

This explanation may also account in part for the excess goblet cell metaplasia seen in the non-miners but not in the miners. An increase in mucous production in relation to dust exposure has been observed in several studies (7,22,25) and Morgan (18) has suggested that this is a large airway response to dust. Perhaps the mineral fume to which the non-miners were exposed penetrates beyond the large airways by adsorption on smoke particles and provokes a greater peripheral mucous hypersecretion (in this case evidenced by an excess of goblet cells) than that induced by the smoke alone. Examination of

the larger, cartilagenous airways of these workers may provide additional information about mucous production in relation to dust exposure. It is important to note that no other significant differences appeared in the other small airway pathology variables assessed. In particular, there was no increase in pigment deposition in the airway walls as might be expected in the dust exposed workers. This corresponds with previous reports in which the amount of dust seen in histological specimens bore no relation to the severity of fibrosis, the degree of functional impairment, or the extent of the radiographic changes (7,8,26).

Cosio and his colleagues (1) studied small airway pathology in a similar series of 36 resected specimens, divided into groups according to severity of the lesions found. They reported that inflammation and fibrosis of airway walls and squamous metaplasia were the earliest pathological changes to occur in the development of disease in the small airways and that the amount of inflammatory infiltrate did not change as the disease progressed. Fibrosis, muscle hypertrophy and pigment deposition increased progressively, while goblet cell metaplasia became significant only in the most severe cases. Wright and co-workers (3), studying the same large group of patients from which these dust exposed subjects were taken, examined small airway pathology differences in smokers, ex-smokers, and non-smokers. Their results indicated that membranous bronchioles in smokers and ex-smokers had increased goblet cell metaplasia compared to non-smokers and that significant increases in inflammation, fibrosis,

pigment, and intralumenal macrophages were found in the respiratory bronchioles of smokers and ex-smokers.

Comparison of the results of this study with those of Cosio (1) and Wright (3) and their colleagues suggest that the response to mineral dust and fume exposure differs from the response to cigarette smoke alone in that fibrosis and goblet cell metaplasia predominate while the other variables are unaltered from the level attributable to the smoke exposure. This predominance of fibrous tissue deposition which is irreversible, over inflammation which may be reversible, could account for the observation reported by Wiles and Faure (27) that chronic obstructive bronchitis in ex-gold miners is irreversible while ex-smokers tend to show improved function, on the average, over current smokers (3).

Although the only objective analysis of the histological specimens from these patients was that of small airway pathology, a subjective assessment was undertaken of the lung interstitium and revealed no obvious differences in exposed and non-exposed subjects or in miners and non-miners. Specifically no silicotic nodules were seen, nor was any excessive fibrotic scarring apparent in the exposed workers, despite the radiographic appearance of simple pneumoconiosis in many of the miners. This subjective finding led to the subsequent drying and re-analysis of lung slices to determine the histological correlate of the radiographic densities. The sharp reduction in the number of cases in which nodules or other densities were seen when single dried slices were examined (although in these cases, the nodules

were identified as silicotic-type lesions), and the failure to locate any such lesions in the stratified random blocks originally analysed, indicate the extreme heterogeneity of the interstitial process in pneumoconiosis and suggest that extensive sampling is necessary in order to discover the true extent of these lesions in suspected cases.

The specific structural abnormalities responsible for the additional pulmonary function decrements observed in the exposed workers cannot be determined from this study. The excess small airway changes in the non-miners may well account for the increase in airflow obstruction in this group but the same cannot be said for the miners. It is unlikely that the parenchymal abnormalities indicated by radiography are responsible for the functional changes in this group as these were minimal in most cases and the correlation between radiographic pneumoconiosis and pulmonary function is generally found to be poor (26,28,29). Abnormalities in large airways or changes in the lung interstitium (whether emphysematous or fibrotic) not detected in this investigation may also contribute to the observed functional changes. In their series of 52 cases of atypical pneumoconiosis, Gaensler (26) and his colleagues found that the most important histological determinant of lung function was the integrity of the lung tissue between the large, obvious fibrotic foci, with the severity of interstitial pneumonia correlating closely to physiological impairment. However, in their patients, pulmonary function was characteristically restrictive rather than obstructive.

Exposure to inhaled pollutants other than mineral dust or fume

could also play a role in the development of both the functional and airway structural manifestations in these workers. These could include diesel emissions, various gases such as ozone and nitrogen oxides, and organic vapours which may be present in the workplace and have been implicated in the production of respiratory abnormalities (8,17,30,31). It is clear, however, that occupational exposure to airborne pollutants present in mining or metal processing shops, in combination with cigarette smoking, results in additional abnormalities of small airway structure and function and that the presence of excessive fibrosis and goblet cell metaplasia in histological specimens may signal such exposure in certain cases, although the absence of these features does not rule out exposure. Finally, this study confirms that functional impairment greater than that accounted for by cigarette smoking, can occur in these workers in the absence of radiological evidence of pneumoconiosis.

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TABLE VII

Exposure Histories

Miners

1.	Copper	>10 years
2.	Gold, Coal, Asbestos	18 years
3.	Hard rock	30 years
4.	Gold, Lead/Zinc, Uranium, Tungsten	32 years
5.	Gold	>10 years
6.	Coal, Gold	17 years
7.	Quartz	14 years
8.	Gold, Radium	>10 years
9.	Lead/Zinc, Gold, Copper	>10 years
10.	Hard Rock Mining	11 years
	Quarrying	22 years

Other Mineral Dust/Fume Exposure

1.	Iron Worker/Gas Welder	>10 years
2.	Lead/Zinc Smelter (Asbestos Exp.)	35 years
3.	Grinder	>10 years
4.	Gas Welder (Asbestos Exp.)	20 years
5.	Iron Blast Furnace Operator	25 years
6.	Welder/Steel Fabricator	36 years
7.	Iron Blast Furnace/Foundry	>10 years
8.	Welder	>10 years
9.	Machinist	34 years

Table VIII

PULMONARY FUNCTION

Exposed Workers Compared to Controls

	Exposed	Controls
Age	62.3 ± 10.3	62.4 ± 10.6
Cigarette years	981.3 ± 520.7	1020.2 ± 488.2
n	19	19
Pulmonary Function (% Pred.)		
TLC	110.9 ± 17.1	108.4 ± 14.2
FRC	134.1 ± 32.0	124.1 ± 18.8
RV	151.0 ± 45.7	134.9 ± 30.6
FEV_1	86.4 ± 19.6*	97.0 ± 21.3
FEV_1/FVC (actual)	66.6 ± 10.5	72.1 ± 8.1
$\dot{V}_{50}$	45.4 ± 29.9**	65.3 ± 37.0
$\dot{V}_{25}$	32.3 ± 17.7**	48.7 ± 27.3
$\Delta N_2/L$	446.9 ± 331.7*	231.0 ± 152.4
Fr. uptake-CO	83.6 ± 19.0	90.7 ± 20.4
Diff.cap.-CO	80.4 ± 34.8	88.7 ± 30.9

(Mean ± S.D.)

* $p < 0.05$
** $p < 0.01$

Table IX

MINERS COMPARED TO CONTROLS ($\bar{X} \pm SD$)

	Total Group		Heavy Smokers Only (> 800 Cig. Yrs.)	
	Miners	Controls	Miners	Controls
Age	66.4 ± 7.1	66.7 ± 5.8	65.7 ± 5.7	66.2 ± 5.4
Cigarette years	1095 ± 618	1154 ± 600	1403 ± 519	1469 ± 578
n	10	10	6	6
Pulmonary Function (% predicted)				
TLC	116.2 ± 15.6	110.5 ± 16.1	124.6 ± 11.4	106.0 ± 18.5
FRC	138.9 ± 33.0	125.9 ± 20.2	155.0 ± 26.2	122.9 ± 25.6**
RV	166.3 ± 47.4	138.3 ± 29.2	188.6 ± 41.6	134.1 ± 35.3*
FEV_1	87.1 ± 23.5	98.3 ± 20.4	80.3 ± 26.1	92.2 ± 13.1
FEV_1/FVC (actual)	65.3 ± 8.7	70.7 ± 8.2	61.3 ± 7.8	71.6 ± 8.2*
$\dot{V}_{50}$	41.9 ± 21.0	59.6 ± 26.0	31.6 ± 17.2	58.6 ± 25.6*
$\dot{V}_{25}$	31.5 ± 12.6	47.6 ± 24.0*	26.2 ± 9.8	44.7 ± 22.0*
$\Delta N_2/L$	395.6 ± 240.3	190.8 ± 81.2	481.1 ± 252.1	162.8 ± 86.7*
DL_{CO}	83.3 ± 19.6	99.1 ± 36.3	78.3 ± 46.0	81.9 ± 11.0
FU_{CO}	84.2 ± 19.6	99.6 ± 18.6	76.7 ± 17.4	94.8 ± 9.8*

*$p < .05$
**$p < .01$

Table X

DUST/FUME compared to CONTROLS ($\bar{X}$ + SD)

	Dust/Fume		Controls
Age	57.8 ± 11.8		57.6 ± 12.9
Cigarette years	889 ± 419		849 ± 283
n	9		9
Pulmonary Function (% Predicted)			
TLC	104.2 ± 17.5		105.7 ± 12.0
FRC	127.8 ± 32.1		121.8 ± 18.1
RV	131.5 ± 37.9		130.6 ± 34.2
FEV_1	85.5 ± 15.0		95.3 ± 23.9
FEV_1/FVC (actual)	68.5 ± 13.1		73.8 ± 8.2
$\dot{V}_{50}$	49.9 ± 40.0	*	72.7 ± 49.1
$\dot{V}_{25}$	32.2 ± 23.9	*	49.9 ± 33.1
$\Delta N_2/L$	524.0 ± 452		291.0 ± 217
DL_{CO}	76.7 ± 20.0		75.3 ± 16.1
FU_{CO}	82.8 ± 19.8		79.2 ± 17.6

*$p < .05$

Table XI

Small Airway Pathology - Exposed vs. Controls

($\bar{X} \pm SD$)

	Exposed	Controls
n	19	19
Membranous Bronchioles		
Inflammation	32.5 ± 14.7	32.4 ± 25.1
Fibrosis	48.3 ± 21.5	29.5 ± 14.2*
Muscle	35.2 ± 20.2	31.1 ± 15.8
Pigment	18.6 ± 11.7	18.0 ± 10.6
Goblet Cell Metaplasia	37.7 ± 21.8	24.8 ± 20.7*
Squamous Metaplasia	13.6 ± 12.6	14.2 ± 10.6
Total Pathology Score	185.9 ± 55.1	151.1 ± 42.5*
Respiratory Bronchioles		
Inflammation	30.5 ± 13.9	25.7 ± 11.3
Fibrosis	37.7 ± 12.9	30.1 ± 9.2*
Muscle	13.8 ± 9.8	17.6 ± 13.7
Pigment	32.9 ± 18.7	28.5 ± 9.3
Intralumenal Macrophages	21.8 ± 16.8	22.2 ± 13.1
Total Pathology Score	136.6 ± 44.2	124.6 ± 27.0

* $p < 0.05$

Table XII

Small Airway Pathology

	Fibrosis (Membr. Br.)	Fibrosis (Resp. Br.)	Goblet Cell Metaplasia
Miners	37.5 ± 21.3	32.1 ± 12.4	33.4 ± 25.2
Other Dust/Fume	60.3 ± 14.8**+	44.0 ± 10.8*+	38.0 ± 22.3*
Controls for miners	33.3 ± 17.4	32.1 ± 7.4	28.5 ± 22.2
Controls for others	30.8 ± 11.6	27.9 ± 10.8	20.7 ± 19.3

Dust/Fume > Controls * $p < .05$

** $p < .005$

Dust/Fume > Miners + $p < .05$
(by comparison of differnces from respective controls)

Table XIII

Radiology

	Pneumoconiosis	Possible Dust Change	No Dust Change
Miners	7	0	2
Other Dust/Fume	0	3	5
Controls	0	1	15

Pneumoconiosis Grade	Type of Work
P 1/1, S 2/2	Copper Mining
P 1/1	Gold Mining
P 1/0, S 1/0	Coal and Gold Mining
P 1/0. Ho	Quartz Mining
P 1/0	Gold and Radium Mining
P 1/1, S 1/1	Lead/Zinc, Gold, Copper Mining
P 1/1	Hard Rock Mining & Quarrying

Early Dust Changes	Iron Worker/Gas Welder
Interstitial Fibrosis	Grinder
Few Nodules	Lead/zinc smelter
Fibrosis - Ant. RUL	Management

Figure 13 (a) Portion of a membranous bronchiole from the lung of a blast furnace operator showing marked distortion of the architecture with fibrous connective tissue (FCT) thickening of the airway wall as well as inflammatory cell infiltration (I). (Hematoxylin and eosin; bar=100μ).

(b) Respiratory bronchiole from the lung of a smelter worker with dense fibrous connective tissue (FCT) thickening and distortion of the wall. (Hematoxylin and eosin; bar=100μ).

(c) Higher magnification of an adjacent portion of the airway shown in (a), indicating numerous fibroblasts (FB) and excess fibrous connective tissue. (Hematoxylin and eosin; bar=10μ)

(d) Section of a membranous bronchiole from the lung of a lead/zinc smelter worker stained with Periodic acid - Schiff stain (PAS) indicating goblet cell metaplasia. The PAS positive goblet cells (which appear dark in the photograph) are extremely numerous and are seen around the whole circumference of the airway (bar=100μ).

Inset: Higher magnification of the box indicated in (d), clearly showing the PAS positive goblet cells (bar=10μ).

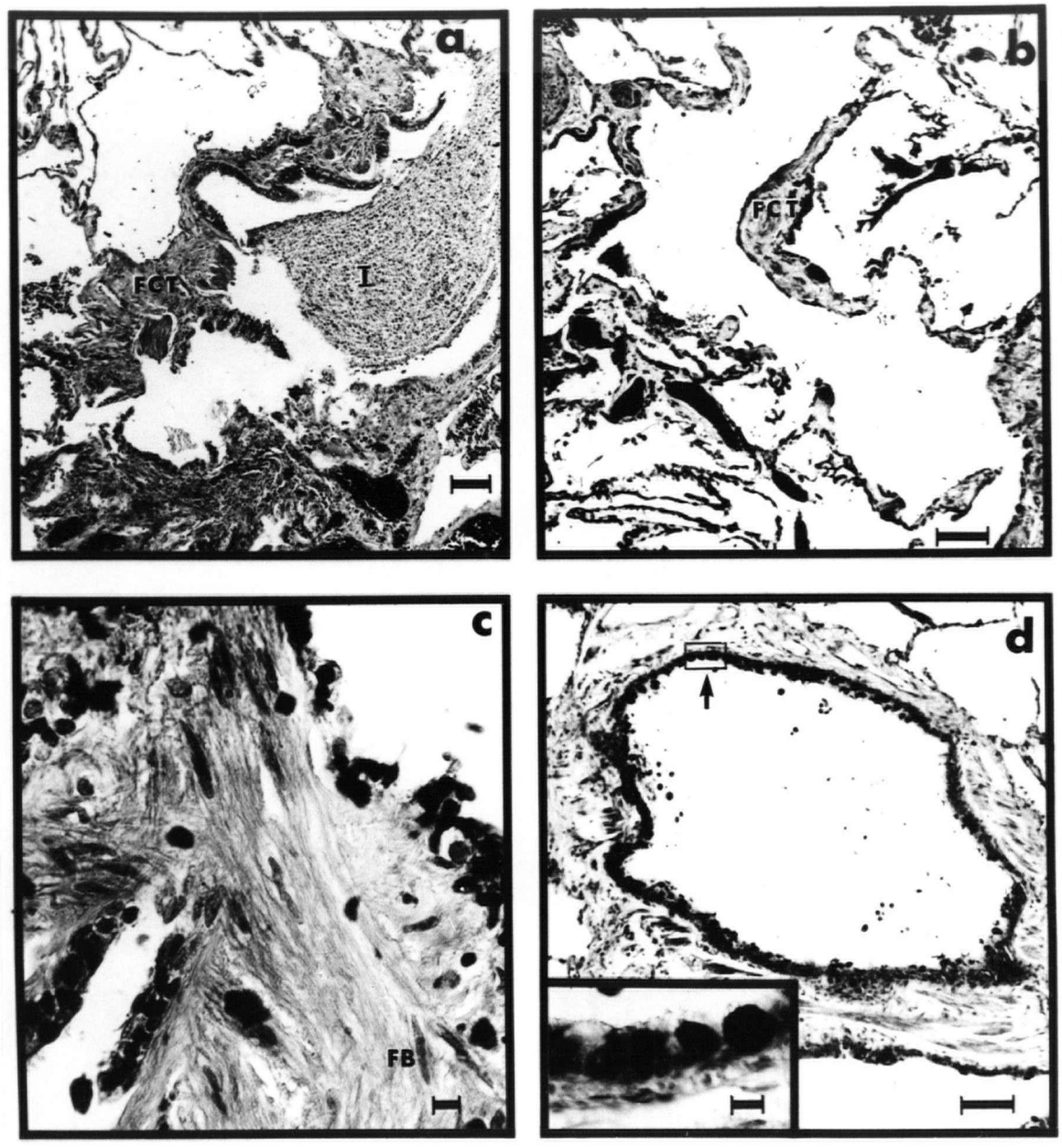
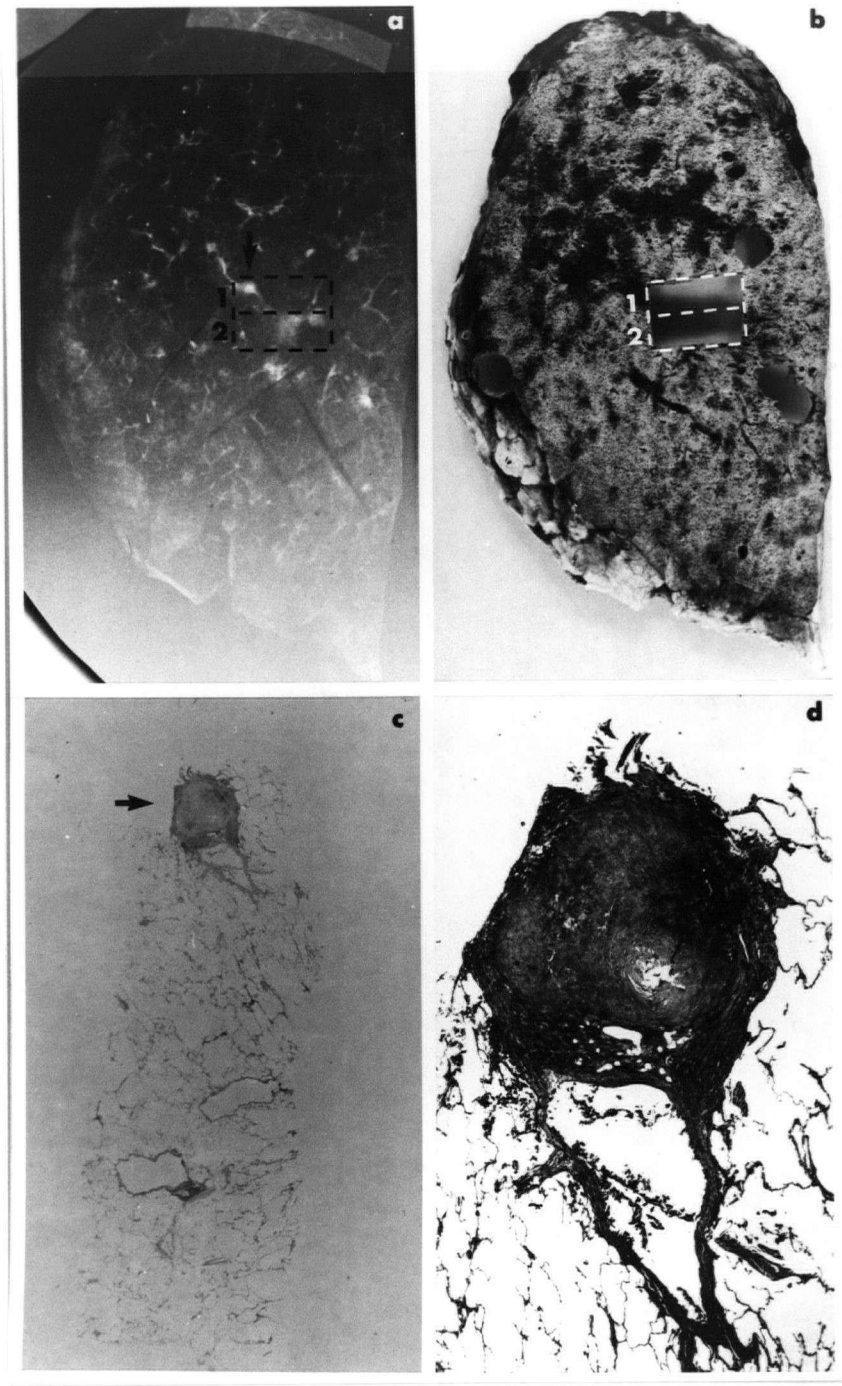

a
FCT
I
b
FCT
c
FB
d

Figure 14 (a) Radiograph of dried slice from lung of a miner whose pre-operative chest radiograph indicated grade P 1/1, S 1/1 pneumoconiosis indicating areas where two tissue blocks will be removed (1,2) and nodular density in block one (➡).

(b) Photograph of dried slice indicating blocks one and two removed for histological assessment.

(c)Photograph of histological slide from block 1 from (a) after re-immersion in fixative and sectioning, indicating nodule corresponding to radiographic density.

(d) Low power micrograph of nodule shown in (c) indicating whorled fibrosis pattern typical of silicosis; (Hematoxylin and eosin; bar=1mm).


a
1
2
b
1
2
c
d

Summary and Concluding Comments

The studies described in the preceding chapters were designed to investigate the nature of the inflammatory process in the airways. In particular, questions were asked relating to its early identification and its structural and functional manifestations and implications in cigarette smokers and those exposed to wood and mineral dust.

In summary, the results of the investigations carried out include the following. First, the use of radiogallium to "label" the polymorphonuclear infiltrate seen in the airways following cigarette smoke exposure does not appear to be a useful technique for identification of airway inflammation, probably because this isotope binds preferentially to macrophages rather than polymorphonuclear leukocytes. A potential relationship between epithelial disruption following cigarette smoke or occupational antigen exposure and enhanced airway reactivity was not ruled out but no direct correlation was seen between increased epithelial permeability and non-specific airway reactivity, both measured in human subjects by non-invasive techniques. Finally the structure-function correlation study indicated that the progression of the inflammatory process in persons exposed to industrial pollutants in addition to cigarette smoke differs from that in cigarette smokers without dust exposure and results in additional functional and

structural abnormalities.

In addition to the conclusions and speculations put forward in the specific discussions of the three sets of experimental investigations, some general comments can also be made arising from this body of work.

The inflammatory process in guinea pig airways following smoke inhalation is identifiable morphologically by a PMN infiltrate which includes PMN's in the airway lumena. This is associated with an increase in permeability. Increased airway epithelial permeability in humans is easily demonstrated using radioactive tracers but if polymorphs are present on the airway surfaces in these subjects, labelling of these by inhalation methods will require a tracer which specifically marks these cells alone.

Previous research has identified airway inflammation as the early event in the progression toward significant small airway pathology. This inflammation is likely also associated with increased airway permeability and this may be a factor which allows enhanced penetration of injurious or irritant substances through the airway epithelium. This could result in the enhanced structural and functional changes seen in the dust-exposed workers.

Epithelial inflammation may also play a role in the enhancement of airway reactivity but this cannot be demonstrated by association with increased permeability, either in people with known increased permeability or in those with antigen-associated increased reactivity. Measurement of non-specific airway reactivity in future

structure-function correlation studies may provide additional information about its relationship to airway inflammation. In addition, the further investigation of airway epithelial permeability changes during exposure to inhaled irritants may reveal changes which cannot be shown when permeability and reactivity are measured on separate occasions.

Finally, this work clearly reveals the potential for the development of functional and structural abnormalities in the airways which may arise from airway inflammation, and underscores the importance of early detection of the inflammatory process in these airways in both cigarette smokers and in persons exposed to substances in their environment which may provoke or augment airway inflammation.

Susan M. Kennedy

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