STUDIES OF MUCOCILIARY CLEARANCE, COUGH AND FORCED EXPIRATION IN PATIENTS WITH LUNG DISEASE

BY

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ABSTRACT

Mucociliary clearance is one of the lungs' non-specific host defence mechanisms and together with cough helps to keep the conducting airways clean even when exposed to a polluted atmosphere.

An objective, non-invasive radioaerosol technique was used for the measurement of lung mucociliary clearance. This technique involves inhalation of five micron polystyrene particles labelled with technetium-99m under strictly controlled conditions, followed by deposition and clearance measurements with a sensitive scintillation counter system and - for some studies - with a gamma camera.

Lung mucociliary clearance was adversely affected in patients with pulmonary sarcoidosis. Sarcoid patients in apparent remission and those on oral corticosteroid therapy had better clearance than those on inhaled corticosteroids, but clearance was still reduced compared to healthy control subjects. Mucociliary clearance was also found to be substantially compromised in pigeon fanciers compared to healthy control subjects. The presence or absence of circulating blood precipitins appeared not to be related to the degree of mucociliary clearance impairment.

During sleep lung mucociliary clearance in patients with stable asthma was significantly reduced compared to when the patients were awake. Two weeks' treatment with an oral controlled release beta agonist or slow-release methylxanthine did not enhance lung mucociliary clearance in asthmatic patients while asleep.

The effect of cough and forced expiration technique (FET) on mucus movement within the lungs of patients with airways obstruction was studied by a gamma camera method
giving regional lung data. Unproductive cough and FET compared to control significantly enhanced mucus clearance from all regions of the lungs with the exception of the forced expiration in the outer region. Productive cough and FET significantly enhanced mucus clearance from the tracheal, inner and intermediate regions of the lungs but not from the outer region. Regional and total mucus clearance did not correlate with the amount of sputum expectorated during the assessments nor with the daily sputum production of the patients. Neither peak flow, during the forceful exhalatory manoeuvres, nor viscoelasticity of sputa correlated with regional clearance suggesting that sputum viscoelasticity as well as peak flow provide no guide to clearance efficacy in humans in contrast to the in-vitro studies.
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PUBLICATIONS ARISING FROM WORK PRESENTED IN THIS THESIS

Abstracts


Papers


Supportive Publications

Abstracts


Papers


CHAPTER ONE
GENERAL INTRODUCTION

1.1. Introduction to the Respiratory Tract
The respiratory tract can be divided into upper and lower airways (fig. 1.1). The upper airways (nasopharyngeal structure) not only conduct but also condition (warming and humidifying) the inspired air for the lungs. Additionally many inhaled particles will be filtered from the main airstream in the nose and oropharynx but the efficiency of this filter depends largely on the size of the inhaled particles (Proctor, 1977). The lower airways, including the trachea and lungs, conduct air and perform gas exchange (Bouhuys, 1977). The trachea divides into right and left main bronchi while the lung comprises two distinct zones: the conducting region and the respiratory region. The conducting region is concerned with bulk movement of air. It includes three lobar bronchi on the right and two on the left, which themselves divide into segmental bronchi (containing cartilage in their walls) leading to the bronchioles and terminal bronchioles (no cartilage in their walls). The terminal bronchiole is at the limit of the conducting airways and the structures distal to it constitute the respiratory region (fig. 1.2). The respiratory region includes respiratory bronchioles which divide into alveolar ducts from which open numerous alveolar sacs. Within this region gas exchange of oxygen and carbon dioxide takes place.

1.2. Deposition of Inhaled Particles
Deposition is the process that determines what fraction of the inspired particles will be caught in the respiratory tract and thus will fail to exit with expired air (Brain et al., 1985). All particles that touch a surface are likely to deposit and the site of contact is the site of initial deposition. There are three main mechanisms whereby inhaled particles are deposited in the lung during breathing (Agnew, 1984):
Fig. 1.1. Upper and lower regions of the human respiratory tract (from Anthony and Thibodeau, 1979).
**Fig. 1.2.** Stylised version of Weibel's model of the human lung (from Pavia et al, 1980).
(a) Impaction - When airflow containing particles is deflected, the force exerted on the particles has to overcome the particles' inertia if they are to follow the change in airflow direction. This depends on the size and density of the particles and velocity and angle of deflection of air flow. Therefore the effectiveness of deposition by impaction decreases with depth into the lung as the flow rate lessens.

(b) Sedimentation - Particles travelling in an airstream are subject to an external downward force due to gravity. This depends on the particle settling velocity which in turn depends on the particle size and the duration of time available for particles to settle. Thus the sedimentation of particles increases with depth into the lung as the air becomes stationary where particles may reach their terminal velocity instantaneously.

(c) Diffusion - The random collisions of gas molecules with very small particles pushes these particles about in an irregular fashion called Brownian motion. Therefore a particle in stationary air moves around in a random way even in the absence of gravity. This movement, which promotes deposition on the walls of small airways, becomes more important as the particle size decreases.

Other forces acting to promote deposition such as acoustic, electric, magnetic, thermal and radiational, are normally not significant in the lung. Some of these forces may be used to enhance deposition experimentally.

The effectiveness of the mechanisms responsible for deposition of inhaled particles will depend on various factors many of which can be controlled by the investigator. These factors are (i) the physical properties of the particles, (ii) the mode of inhalation of the particles and (iii) the patency of the airways.

The main physical properties of particles that will affect deposition within the lungs are:

(a) Particle size - The bigger the particle size (diameter = d) the nearer to the mouth it will deposit due to impaction (Lippman & Albert, 1969; Pavia & Thomson, 1976; Pityn
et al., 1989).

(b) Density - For a given particle size (diameter : d) increasing the density (\(f\)) of the material from which the particle is made will result in an effectively bigger particle with an aerodynamic diameter for the particle of \(d_a = f^{1/2} d\) (Stuart, 1973).

(c) Hygroscopicity - A hygroscopic material will absorb water during entry through the respiratory passages and will thus increase in size and deposit nearer to the mouth than the non-hygroscopic material (Cinkotai, 1971; Sinclair et al., 1974; Scherer et al., 1979).

(d) Electric charge - The surface of the respiratory tract is uncharged but electrically conducting. When an electrically charged particle approaches such a surface it induces an image charge of the opposite polarity on the surface and is attracted toward it and thus contributes to deposition behaviour. The effects of electric forces on deposition in the respiratory system are not well known, although charged particles exhibit enhanced deposition (Mercer, 1973; Hashish, 1992).

The main factors during inhalation which can affect deposition of particles in the lungs are:

(a) Inspired volume - As the inspired breath increases more particles will be carried and deposited at the peripheral airways (Booker et al., 1967; Camner et al., 1973; Pavia et al., 1977).

(b) Inspiratory flow rate - As the flow rate increases more particles will be deposited in the proximal airways by impaction due to air turbulence (Goldberg & Lourenco, 1973; Pavia et al., 1977a).

(c) Breath-hold pause - Breath-holding at the end of inspiration will enhance deposition of particles at their furthest point of entry due to sedimentation (Newman et al., 1982).

(d) Lung inflation - Lung volume at the commencement of inhalation will affect the particle site of deposition (Yeates et al., 1975; Newhouse & Ruffin, 1978; Groth & Foster, 1992).
The patency of the airways is important since the efficiency of deposition depends partly on the diameter of the airways. Furthermore, airway anatomy specifies the local linear velocity of the airstream and thus whether the flow is laminar or turbulent. A reduction in airway patency will give rise to a more proximal deposition of the inhaled particles due to impaction (Thomson & Short, 1969; Dolovich et al., 1976; Pavia et al., 1977; Kim et al., 1989; Svartengren et al., 1989; O'Riordan et al., 1993).

1.3. Lung Defence Mechanisms

In the process of inhaling several thousand litres of air daily by each person for oxygen uptake and carbon dioxide elimination, the lung is exposed to a wide variety of foreign substances transported with the inhaled air such as small particulate material, microorganisms and noxious gases. Despite this, the human lung remains sterile from the first bronchial division to terminal lung units.

To protect itself against potentially toxic inhaled material, the lung has a complex protective system that can be divided into a number of different defence mechanisms (Newhouse et al., 1976 and 1976a). Each mechanism appears to have a distinct role, but there is a tremendous degree of interaction and cooperation between them.

The lung protective system can be divided into specific and non-specific defence mechanisms. The specific defence mechanisms are immunological mechanisms in which the lung functions as a lymphoid organ and responds to specific antigen with both cell mediated and humoral antibodies (Green et al., 1977; Holborow & Lessof, 1981). The non-specific defence mechanisms of the lung are aerodynamic filtration, mucociliary clearance, cough and alveolar clearance which will now be considered in detail.
1.3.1. *Aerodynamic Filtration*

Filtration begins in the nose where deposition of inhaled particles is favoured by the shape of the nasal cavities. The change in cross-sectional areas within those sites, coupled with the change in direction of the airflow (turbulence) as it passes through the nose and beyond, is highly efficient in depositing particles by inertial impaction. The efficiency of the nasal filter depends largely on the size of the particles inhaled. The nose traps particles with an aerodynamic diameter in excess of 5-10 μm (Landahl, 1950), while smaller particles bypass the nose and reach the trachea to deposit somewhere in the lung. The nose also acts as a protective filter for highly soluble gases such as ozone, ammonia and sulfur dioxide (Landahl & Herrman, 1964; Speizer & Frank, 1966; Moorman et al., 1973).

Inhaled particles, trapped in the 'nasal filter', are cleared by mucociliary action (Quinlan et al., 1969) sweeping the particles backwards either to be swallowed imperceptibly or to be cleared from the throat whereas particles deposited in the anterior part of the nose may be blown away voluntarily or by sneezing.

1.3.2. *Lung Mucociliary Clearance*

The mucociliary clearance mechanism operates from the level of the terminal bronchioles to the larynx, where the conducting airways are lined with a ciliated mucus-secreting epithelium. This epithelium is pseudostratified and columnar in the trachea and bronchi; as the airways become narrower, the epithelium decreases in height and becomes a single layer of cuboidal cells in the terminal bronchioles (Rhodin, 1966). The epithelium becomes even thinner in the respiratory bronchioles and eventually merges with that of the alveolar duct and alveolus. The epithelial cells have been classified according to the presence of cilia, secretory granules, and their position within the epithelium. Ten cell types have been identified using electron microscopy (Jeffery & Reid, 1977).
1.3.2.1. Cilia

Ciliated cells are present from the level of the trachea to the terminal bronchioles. The total area covered by the ciliated epithelium is about 0.55 m\(^2\) and the total number of ciliated cells per person has been estimated at about 3\(\times10^{12}\) (Afzelius, 1979). The ciliated cells form almost a continuous surface to the epithelium of the larger airways, interspersed with goblet cells and occasional brush cells, but in small airways the proportion of ciliated cells is less. The percentage of ciliated cells decreases from 53 \% in the trachea to 45 \% in the first airway generation, 23 \% in the third and 15 \% in the fifth airway generation (Serafini & Michaelson, 1977) of Weibel's model of the human lung (Weibel, 1963). Each ciliated cell has a diameter of 5 \(\mu\)m or so and carries some 200 cilia, which are closely spaced at about 6 to 8 cilia/\(\mu\)m\(^2\), interspersed with numerous short microvilli about 1-2 \(\mu\)m long (Rhodin, 1966).

The cilia (0.3 \(\mu\)m in diameter) are longer in the proximal (5-7 \(\mu\)m) compared to the peripheral airways (2-3 \(\mu\)m) (Serafini & Michaelson, 1977). These cilia are cellular projections (fig. 1.3) containing fibrils surrounded by cytoplasm and enclosed by the cell membrane (Sleigh, 1977). A cilium consists of three main parts: (i) the ciliary shaft, (ii) the basal body and (iii) the ciliary roots.

The ciliary shaft is composed of longitudinal fibrils called the axoneme which has nine outer microtubules arranged in a ring around two central microtubules. The outer ones each have one complete and one incomplete microtubule attached laterally to each other to form a doublet. They are composed of a contractile protein called tubulin (Mohri, 1968). Dynein arms containing the adenosine triphosphatase (ATPase) protein (Gibbons, 1965) are attached to the peripheral doublets (Afzelius, 1959). The nine doublets are connected by nexin links (Gibbons, 1965). These doublets are also connected to the central tubules through radial spokes (Afzelius, 1959).
Fig. 1.3. Scanning electron micrograph of human bronchial epithelium (from Lopez-Vidriero, 1984).

[Insert diagrams illustrate ciliary structure and mechanism]
At the ciliary base the axoneme continues into the basal body in a modified form. The central microtubules begin to disappear near the level of the cell surface. The peripheral doublets continue into the basal body and below the level of the cell surface each doublet becomes a triplet by the addition of another incomplete microtuble (Sleigh, 1977).

The main anchorage of the axoneme is provided by 'root' fibres attached to the basal body. Microtubules are attached to the basal foot and extend away into the cytoplasm of the cell. The basal foot of the cilium is found at that side of the basal body toward which the cilium bends (Gibbons, 1961) providing a common orientation with adjacent cilia.

The tip of the cilia have a crown of 3-7 projecting claws about 25 nm long that penetrate into the overlying mucus and probably grip the mucus and help propel it toward the epiglottis during the beating action of the cilia (Lee & Forrest, 1991).

The beat cycle of cilia starts with a recovery stroke, during which the cilium moves backward and swings clockwise to carry the shaft of the cilium around fairly close to the cell surface, and finishes with an effective (propulsive) stroke (with a maximal tip speed of 1000 μm/sec) when the main part of the ciliary shaft remains fairly straight and perpendicular to the cell surface. At the end of the effective stroke the cilia come to a rest period, during which the cilium is bent over to one side with its shaft parallel to the cell surface, before starting another cycle. The recovery stroke is twice the period (29 msec) of the effective stroke (15 msec) (Sleigh, 1981). A quarter of each ciliary beat cycle is taken up by the cilia beating 'forward' pushing secretions cephalad and three quarters in bending 'backwards' ready for the next cycle. This ciliary beat cycle is clearly asymmetric with the rapid effective stroke occurring in one plane while the recovery stroke in a different plane (Sleigh et al., 1988).
The bending movements of a cilium is caused by active sliding of the outer microtubule doublets of the axoneme relative to one another (Satir, 1965). This ATP-induced active sliding appears to be due to the dynein arms generating a shear force between adjacent doublets (Afzelius, 1983). The ATP supply available within the cilium is limited (Brokaw, 1965) and continued motility is dependent upon ATP diffusing into the cilium from the cell body. The radial spokes and nexin links do not take part directly in the production of forces of active sliding, but play an important role by limiting and regulating the sliding activities of the microtubules and the propagation of bending along the axoneme.

Cilia do not beat simultaneously, but in a coordinated fashion producing metachronal waves traveling in the opposite direction to the effective stroke (Sleigh, 1983). The formation and propagation of these waves is due to hydrodynamic forces between the moving cilia (Machemer, 1974). The metachronism of cilia that transport mucus is irregular and the degree of coupling between cilia and the metachronism could vary with the frequency of beating (Sleigh, 1984). The ciliary beat frequency ranges from 6 to 16 Hz (Lopez-Vidriero & Clarke, 1982). In humans, the ciliary activity appears to be independent of nervous control and mechanical stimulation. It is influenced by temperature, pH and viscoelastic properties of the mucus.

The observation of Lucas and Douglas (1934) on ciliated epithelium suggested that cilia beat in a watery (sol) layer (periciliary layer) propelling forwards at their tips a viscid (gel) layer (epiphase). This finding was confirmed by the investigations of Sanderson and Sleigh (1981) using scanning electron microscopy.

1.3.2.2. Mucus
The sol layer is soluble and contains mainly proteins and mucous glycoproteins of low molecular weight (Lopez-Vidriero & Reid, 1980).
This periciliary fluid is assumed to be largely an exudate from the epithelial cells (Widdicombe, 1984) and therefore is a low-viscosity watery solution, whose ionic constitution and flux may be regulated by chloride and sodium transport activities of the epithelial cells (Nadel et al., 1979). The depth of the sol layer appears to be maintained at a little less than the ciliary length so that the ciliary tips can penetrate the mucus on top during their effective stroke (Sleigh, 1991). The flow of periciliary fluid in the terminal airways may draw surfactant and macrophages aided by compression and expansion on the surface tension of surfactant during breathing (Litt, 1981).

The gel layer is insoluble and contains mainly mucus glycoproteins of high molecular weight and proteins (Jeffery & Reid, 1977; Lamblin & Roussel, 1993). The mucus is formed within Golgi-derived vesicles of goblet (total volume 1/40 of that of the mucus glands) and mucus gland cells (in the cartilaginous airways) and released by exocytosis in the form of 1 to 2 μm droplets (Jeffery, 1987). These droplets of concentrated glycoproteins rapidly swell by a factor of several hundred-fold over about 3 seconds through absorption of water from serous fluid (Verdugo, 1984). The droplets of mucus spread out under the action of cilia on the epithelial surface to form strands, small flakes, or larger plaques (Iravani & Van As, 1972), which may serve to maintain the depth of the sol layer.

Tracheobronchial mucus is a mixture of secretions from the surface epithelium, submucosal glands and tissue fluid transudate. It comprises of water (95%), glycoproteins (2-3 %), proteoglycans (0.1-0.5 %), lipids (0.3-0.5 %), other proteins and occasionally DNA (Lopez-Vidriero, 1984). The glycoproteins are of prime importance in giving mucus its viscoelastic characteristic. The mucus glycoprotein has a protein core with multiple oligosaccharide side chains cross-linked by disulphide and hydrogen bonds (Roberts, 1978).
Airway mucus is a non-Newtonian pseudoplastic fluid. Its apparent viscosity decreases with increasing shear rate (shear thinning) which is partly recovered after removal of the stress (thixotropic behaviour) (Puchelle et al., 1985). Therefore, airway mucus behaves as a special liquid/solid material where four phases have been identified. It acts as a solid with initial deformation; followed by a viscoelastic behaviour characterised by retarded deformation; this is followed by a period of steady flow (constant rate of deformation) with partial recovery at the end when the force is removed (Litt, 1970; King, 1980).

It is generally accepted that the physical properties of airway mucus, such as apparent viscosity and elasticity, have a major role in mucus transport (Sade et al., 1970; King et al., 1974). An optimum mucus viscosity and elasticity is required for effective mucus transport by cilia. Therefore, any rise or fall below the optimal range will reduce mucus clearance. This has been confirmed by several investigators using various methods of determining mucus viscoelasticity (Shih et al., 1977; Chen & Dulfano, 1978; Meyer & Gelman, 1979; Puchelle et al., 1980). The ideal viscoelastic properties of mucus for efficient transport are high elasticity and low viscosity (Wilson, 1988); but these properties may change during disease (Rubin et al., 1991 and 1992), becoming less elastic during viral infection (Sakakura, 1983) or more viscous during bacterial infection (Burgi, 1973). It has been shown that mucus with high viscosity appears to have little adverse effect on transport in the frog palate preparation, but elasticity correlates well with speed of transport (Dulfano & Adler, 1975). However, some samples of mucus with non-optimal viscoelastic properties were transported well suggesting that there were other rheological factors involved (Puchelle et al., 1981). Indeed Puchelle and associates (1983) demonstrated that the mucus transport was dependent on the spinnability of the mucus which is a measure of the thread-forming properties of mucus. The spinnability of the mucus corresponds to the maximal length of the thread that can be drawn before rupture.
The adhesive or sticky properties of mucus can also influence the flow of mucus (Medici et al., 1973; Puchelle et al., 1987; Girod et al., 1991).

Stimulation of the cholinergic (parasympathetic) efferent nervous supply to the airways increases mucus secretions (Knowles et al., 1982) which could be blocked by cholinergic antagonists (muscarinic) such as atropine (Sturgess & Reid, 1972). The adrenergic (sympathetic) nervous supply may also regulate mucus secretion but to a lesser extent (Phipps et al., 1982). The nonadrenergic noncholinergic nerves may also have a role in regulating mucus secretion; vasoactive intestinal peptide (VIP) is a possible modulator with other neuropeptides (Coles et al., 1981).

In healthy subjects, the quantity of transported mucus per 24 hour is on the order of 10 ml (Toremalm, 1960), but it is possible to collect 200 ml/day of sputum from bronchitic patients during an exacerbation. The flow rate of mucus increases progressively as it travels toward the more proximal airways (Serafini et al., 1976). It has a speed of the order of 2.5 mm/min in the main bronchi and 5 mm/min in the trachea (Foster et al., 1980; Yeates et al., 1981).

1.3.2.3. Mucociliary Clearance

Many studies have been carried out, primarily in human subjects, in order to ascertain the effect of various physiological factors, environmental pollutants, disease and pharmacological agents on lung mucociliary clearance (Wanner, 1977; Pavia et al., 1985; Clarke & Pavia, 1991).

Circadian rhythm (Pavia, 1984), posture (Pavia, 1984; Isawa et al., 1991) and gender (Yeates et al., 1975) do not seem to alter lung mucociliary clearance, while increasing age slows clearance (Goodman et al., 1978; Puchelle et al., 1979; Incalzi et al., 1989).
Sleep in healthy subjects (Bateman et al., 1978) and in asthmatics (Pavia, 1984) reduces clearance whereas exercise on a bicycle ergometer for 30 minutes resulted in an enhancement of lung mucociliary clearance (Wolff et al., 1977). However, pedalling for 15 minutes produced no substantial change (Olsen & Wollmer, 1990).

Sulpher dioxide has been shown to cause a transient acceleration of lung mucociliary clearance (Wolff, 1986) while sulphuric acid reduced this clearance mechanism (Spektor et al., 1989; Schlesinger et al., 1992). However, fog droplets containing sulphuric acid resulted in a rapid tracheal clearance (Laube et al., 1993).

The effect of tobacco smoking on mucociliary transport has been studied extensively by many investigators. The long-term effect of tobacco smoking is an impairment of clearance (Camner et al., 1980) where as studies on the acute effect of smoking have produced conflicting results (Pavia, 1984a). The effect of giving up smoking in asymptomatic smokers is to restore their impaired lung clearance to normality within 3 months of stopping the habit (Camner et al., 1973a). However, giving up smoking in chronic bronchitics has no effect on clearance (Agnew et al., 1982).

Impaired lung mucociliary clearance has been reported in patients with chronic bronchitis (Camner et al., 1973; Puchelle et al., 1980), bronchiectasis (Lourenco et al., 1972; Currie et al., 1987; Isawa et al., 1990), cystic fibrosis (Kollberg et al., 1978), Young's syndrome (Pavia et al., 1981), ciliary dyskinesia including Kartagener's syndrome (Rossman et al., 1981; Pavia et al., 1988; Ruusa et al., 1993) and asthma (Foster et al., 1982; Bateman et al., 1983; Messina et al., 1991) even in remission (Pavia et al., 1985a). Furthermore, patients infected with influenza A virus (Camner et al., 1973b; Garrard et al., 1985) and mycoplasma pneumonia (Jarstrand et al., 1974) have also been found to have a retardate mucociliary clearance whereas patients with rheumatoid disease (Sutton et al., 1982), Sjogren's syndrome (Fairfax et al., 1981) and
emphysema due to alpha-antitrypsin deficiency (Mossberg et al., 1978) have unaltered mucociliary function.

Bronchodilators, such as beta agonists (Mossberg et al., 1976; Foster et al., 1976; Fazio & Lafortuna, 1981; Pavia et al., 1987; Matthys et al., 1987; Isawa et al., 1990a; Mortensen et al., 1993) and theophyllines (Matthys & Kohler, 1980; Sutton et al., 1981; Schmidt et al., 1983; Cotromanes et al., 1985), oral corticosteroids (Agnew et al., 1984a) and sodium cromoglycate (Mezey et al., 1978) have been shown to enhance lung mucociliary clearance in humans while synthetic anticholinergic bronchodilators (Taylor et al., 1986; Wanner, 1986; Pavia et al., 1989) and inhaled corticosteroids (Fazio & Lafortuna, 1986) have no such effect.

Limited evidence of enhancement of mucociliary clearance has been demonstrated following the administration of mucolytics and expectorants (Clarke et al., 1980; Pavia et al., 1985b; Olivieri et al., 1991). The administration of beta antagonists (Matthys et al., 1983; Pavia et al., 1986), aspirin (Gerrity et al., 1983) and sedatives (Hasani et al., 1992) has been reported to retard lung mucociliary transport.

1.3.3. Cough Clearance

Cough is an important defence mechanism of the lungs. It rarely occurs in healthy subjects except in emergency situations, following the inhalation of a foreign body or bronchial irritants. Cough is a reserve mucus clearance mechanism in lung disease (Sutton, 1984), such as chronic bronchitis, bronchiectasis and asthma, where mucociliary clearance is often impaired and the increased amount of secretions cannot be adequately removed (Pavia, 1984a).

1.3.3.1. Physiology of Cough

Coughing disrupts the normal pattern of breathing and replaces it by a highly complex
motor act involving all of the muscles of respiration and of the respiratory tract. Involuntary coughing is entirely a vagal phenomenon and can be initiated only from those structures innervated by the vagus and its branches (Widdicombe, 1989). Mechanical and chemical irritation to the lower part of the oropharynx, the larynx, and the lower respiratory tract are well established as a cause of coughing (Boushey et al., 1980). The carina and points of large bronchial branching are especially sensitive to mechanical stimuli (Korpas & Tomori, 1979).

Three distinct types of nervous receptor have been identified in the larynx (Mathew et al., 1984; Sant'Ambrogio, 1987; Karlsson et al., 1988): pressure, drive, and cold/flow. These receptors are spontaneously active in quiet breathing and therefore it seems unlikely that they are responsible for coughing (Karlsson et al., 1988). The most likely receptors involved in the initiation of coughing are the irritant receptors which are activated by cigarette smoke, distilled water, acid, alkaline, low-chloride solutions, and irritant gases including sulphur dioxide. They are also extremely sensitive to mechanical stimuli such as a fine surface probe or dust. These receptors, when stimulated, produce rather rapidly adapting irregular discharges (Sant'Ambrogio, 1987).

The sensory innervation of the tracheobronchial tree is not as complex as that of the larynx. Five types of receptor have been identified (Coleridge & Coleridge, 1986; Sant'Ambrogio, 1987a). These include slowly adapting and rapidly adapting (irritant) stretch receptors, pulmonary and bronchial C-fiber receptors, and receptors associated with neuroepithelial bodies.

1.3.3.2. Dynamics of Cough
The dynamics of cough have been extensively reviewed (Langlands, 1967; Jones & Clarke, 1970; Irwin et al., 1977).
The cough mechanism can be divided into three phases: inspiratory, compressive, and expulsive. In the inspiratory phase the glottis opens fully and permits the entry of large amounts of air, which is rapidly inhaled through simultaneous operation of the inspiratory muscles and diaphragm. The volume of the inhaled air may vary considerably but does not generally exceed 50% of vital capacity (Lawson & Harris, 1967; Harris & Lawson, 1968); a high volume will allow better contractile efficiency of the expiratory muscles and better elastic recoil of the lung (Mead et al., 1967). During the compressive phase of cough, the glottis closes and the expiratory muscles contract causing a rapid increase in intrathoracic pressure (Macklem et al., 1963). After about 0.2 second of the glottis' closure, it opens up again marking the end of the compressive phase and the beginning of the expulsive phase during which the function of cough is carried out. The trapped intrathoracic air is explosively released at high speed accompanied by the familiar sound of cough due to the vocal cords and parts of the central airways vibration. The expiratory muscles continue to contract after the opening of the glottis ensuring a high pressure gradient between the intrathoracic airways and the mouth and thus maintaining the rapid flow of air (Fry & Hyatt, 1960). The peak flow that is recorded at the mouth immediately after opening of the glottis constitutes the sum of two flows: one coming from the distal airways driven out by the alveolar pressure and the other from the central airways following their dynamic compression (collapse), which is important to maintain the high linear velocity necessary for an effective cough, due to the high intrathoracic pressure (Knudson et al., 1974; Macklem, 1974). After this peak flow, the expiration continues favoured by relaxation of the diaphragm which allows the increased intra-abdominal pressure to be transmitted to the lung. Then the expiratory muscles relax, the pressure falls, the glottis closes and the flow of air stops.

Cough involves the transfer of momentum between the air which is accelerated through the airways and the secretions lining them.
This results in the mucus being sheared and then expelled from the lungs. However, shearing of secretions can only occur when the air linear velocity is in excess of 25 m/sec where "mist flow" is possible (Clarke, 1973) as shown in figure 1.4. When Leith (1968) divided the air flow rate by the cross sectional area of any one airway generation it became apparent that the high linear air velocity essential for an effective cough could only be reached at the sixth or seventh airway generation of Weibel's (1963) model of the human lung. He also suggested that cough is most effective at high expiratory flow rate and therefore at high lung volumes since maximum expiratory flow rates are directly related to lung volumes (Hyatt et al., 1979). However, during high expiratory flows the transmural pressure increases causing dynamic compression (collapse) of the airways. This compression results in a decrease of cross-sectional area giving rise to increased linear velocity of air. Velocities in these regions are very high (240 m/sec) exceeding the minimum velocity required for "mist flow". Macklem and Wilson (1965) found that at 75% of vital capacity this collapse does not extend beyond the third generation (segmental bronchi), whereas at low lung volumes the airway collapse extends distally. It is by this mechanism that cough can still be effective in patients with airway obstruction and thus reduced peak expiratory flow rates. Also from theoretical considerations, Scherer (1981) postulated that under conditions of excess mucus production, the effect of cough can be extended down to the 17th airway generation (respiratory bronchioles), and that its effectiveness is dependent on the viscosity, elasticity, and surface tension of the mucus in a complex model.

1.3.3.3. Physiochemical Properties of Mucus and Cough

The role of mucus viscoelasticity in clearance by cough has been reported by many investigators. Scherer and Burtz (1978) studied the clearance of Newtonian liquids of different viscosities in a section of clear rigid tubing by simulated cough. They found that the efficiency of cough clearance is decreased as the liquid viscosity increased.
**Fig. 1.4.** A schematic diagram of four types of two-phase gas-liquid flow through a tube for different linear gas velocities (from Pavia et al., 1987a).
The importance of elasticity in mucus-airflow interaction was first reported by Clarke and associates (1970) and then confirmed by King and associates (1982), which showed that high elasticity tends to damp down wave formation leading to relatively inefficient clearance. The thickness of the mucus layer is also very important; too thick or too thin a layer will be cleared inefficiently by cough (Clarke et al., 1970).

In an in-vitro model, clearance of mucus simulants (borate cross-linked locust bean gum gels) by a simulated cough machine was investigated by King and associates (1985). For any given depth of mucus, clearance was found to increase with increasing driving pressure which in turn produced an increase in peak flow rate. Clearance was found to increase linearly with increasing mucus depth at constant driving pressure (the equivalent of cough effort). For any given driving pressure and depth of mucus, clearance decreased with increasing mechanical impedance (vectorial sum of viscosity and elasticity) of mucus and at a constant mechanical impedance clearance was increased with increasing loss tangent (ratio of viscosity to elasticity).

Using the same in-vitro model, King and associates (1989) found that for a given viscosity simulated cough clearance is decreased with increasing spinnability and adhesivity of mucus. However, the work of adhesion was shown to be markedly reduced (about 50%) by the addition of a sol phase (Zahm et al., 1989). This reduction of adhesion was associated with a marked enhancement of simulated cough clearance suggesting that the sol phase is essential in mucus clearance by the cough mechanism. Again in an in-vitro study, using the same simulated cough machine, the role of repetitive coughing on the clearance of gel mucus simulants was investigated (Zahm et al., 1991). The repetition of simulated coughing induced significant increase in mucus clearance compared to a single cough. Furthermore, the increase in frequency of the repetitive coughing induced a marked increase in mucus simulant clearance.
It has also been found that airway wall flexibility is directly related to the efficiency of simulated cough clearance (Soland et al., 1987); this may be due to participation of the wall membrane in the wave formation that is necessary for clearance.

1.3.3.4. Radioaerosol Studies of Cough - Whole Lung Clearance

Many studies evaluating the efficacy of cough on the transport of inhaled deposited radioaerosol particles from the human lungs have been reported. Toigo and associates (1963) suggested that cough increased the clearance of labeled carbon particles (40 to 70 μm in diameter) in eight normal subjects and eight patients with chronic lung disease. They observed that after each cough the amount of radiation, measured over the carina, dropped sharply. However, Yeates and associates (1975) studying 42 healthy non-smoking adults reported that coughing did not greatly affect the movement of local concentrations (bolus) of microspheres in the trachea whereas coughing was the major clearance mechanism from the trachea in patients with cystic fibrosis (Yeates et al., 1976). These results were confirmed by Puchelle and associates (1980). The mean percentage of bronchial radioactivity cleared after one hour from inhalation by mucociliary clearance in ten healthy subjects was 30%, which was about twice that eliminated by twenty seven chronic bronchitics (14%). At the end of the one hour period, the healthy subjects and patients were asked to cough and the percentage of retained bronchial radioactivity was measured again. The chronic bronchitics eliminated a further 20% compared to the healthy subjects who cleared only 2.5%. This study demonstrated that in the chronic bronchitics, a high percentage of deposited particles was cleared by coughing while in the healthy subjects this was not so. The effect of cough was also studied in eight patients with respiratory tract disease and six healthy subjects by Camner and associates (1979). The healthy subjects did not produce any sputum and did not clear any test particles on instructed vigorous coughing, while the patients who produced sputum (6 of 8) were able to clear test particles from their lungs by coughing.
It appears that the presence of an increased amount of mucus is an essential prerequisite for cough to be effective as a clearance mechanism. Agnew and associates (1982) reported that tracheobronchial clearance of deposited radioaerosol was faster in chronic bronchitic patients who produced a high volume of sputum and coughed frequently. The same group (Agnew et al., 1983) suggested that cough may also be a very important clearance mechanism in asthma. During the first two hours of their study, the asthmatic patients who coughed frequently cleared more than twice as much radioaerosol as the asthmatic patients who coughed less despite a slightly higher initial radioaerosol penetration.

Voluntary coughing in 12 patients with immotile-cilia syndrome was also found to be an important clearance mechanism (Mossberg et al., 1978a); on average 30% of the deposited particles were removed after coughing. Directed coughing was evaluated in 10 patients with airways obstruction and copious sputum by Sutton and associates (1983). Each patient underwent a 30-minute treatment period where the patients were asked to cough, and another 30 minute as a control period after inhaling radioaerosol particles. The percentage of radioactivity remaining in the lungs after the treatment period was less than the control period (not statistically significant), during which the reduction in radioactivity was due to mucociliary clearance and spontaneous coughing.

Recently the effect of a controlled coughing manoeuvre (10 coughs every 10 minutes for 1 hour) compared to a control period in 12 non-smoking healthy subjects was investigated by Bennett and associates (1990). The controlled cough consisted of forceful exhalation against a closed solenoid valve which automatically opened after a threshold airway pressure was reached. The amount of radiolabeled particles retained after the coughing manoeuvre were significantly less than that retained during the control period.
The same results were observed with rapid inhalations (90 inhalations per hour) rather than exhalations (coughs) when compared to a control period in eight healthy subjects. Therefore, they postulated that the observed enhancement of mucus clearance by controlled coughing may be due to a stimulation of the mucociliary mechanism. However, using the same protocol, ten young asymptomatic smokers were unable to enhance their rate of mucus clearance by coughing or rapid inhalations suggesting a change in the mucociliary transport from normal (Bennett et al., 1992).

1.3.3.5. Radioaerosol Studies of Cough - Regional Lung Clearance

Estimation of regional lung mucus clearance was carried out by three investigators; all of them agree that cough significantly enhances the clearance of mucus from the lungs as a whole and from the central region and that it almost fully compensates for a defective mucociliary clearance in patients with hypersecretion. However, their attempts to study lung mucus clearance from the peripheral region have yielded contradictory results.

Oldenburg and colleagues (1979), studying eight patients with simple and obstructive chronic bronchitis (mean % predicted FEV₁ was 53) and producing sputum volumes ranging from 10 to 120 ml/day, found that coughing produced a very significant effect on whole lung and peripheral lung mucus clearance. Using a similar technique, Bateman and colleagues (1981) studied six patients (three chronic bronchitics and three bronchiectatics) who were more severely obstructed (mean % predicted FEV₁ was 37) and were producing larger sputum volumes (50-300 ml/day). Mucus clearance during cough was significantly increased from the whole lung, central and intermediate regions. However, no significant effect on clearance from the peripheral region was noted and that differs from the study of Oldenburg and colleagues (1979). It is possible in the Oldenburg and colleagues study (1979) that the significant mucus clearance reported from the peripheral region reflected clearance from large conducting...
airways since the proximal boundary of the peripheral region corresponded approximately to 2 to 4 mm airways (i.e. in the peripheral region there must have existed large airways). Furthermore, in the study of Oldenburg and colleagues (1979), the initial radioaerosol deposition was more central than in the Bateman and associates study (1981).

Six patients with cystic fibrosis were studied by Rossman and associates (1982). The mean % predicted FEV$_1$ was 38 and the mean sputum volume was 67 ml/day. The effect of cough on mucus clearance was similar to that reported by Oldenburg and colleagues (1979), where after coughing the mucus transported from the peripheral region of the lungs was increased as well as that from the central region.

1.3.3.6. Airway Dynamic Compression and Cough

In regions where airway dynamic compression (collapse) occurs (Macklem & Wilson, 1965) linear air velocities may be extreme and greatly exceed the required air velocity to shear secretions. Smaldone and associates (1979) postulated that this dynamic compression of the airways (i.e. formation of flow-limiting segments) during cough may locally impair mucociliary clearance. Flow-limiting segments are believed to form in the segmental and sub-segmental bronchi (Smaldone & Smith, 1985). Therefore any effect that flow-limiting segments formation may have on mucociliary clearance would be expected to be manifested most clearly in the central region (Itoh et al., 1981; Smaldone & Messina, 1985; Smaldone et al., 1993).

Flow-limiting segment can be induced in normal humans by coughing, and there is some evidence that repetitive coughing may decrease mucociliary clearance in the central airways (Foster et al., 1988). However, Kamishima and associates (1983) who investigated the adverse effect of coughing (45 coughs over 15 minutes) on mucociliary clearance, in healthy smokers and non-smokers and in patients with chronic obstructive
pulmonary disease, found that cough had no effect on lung mucociliary clearance in the
healthy non-smokers whereas the healthy smokers as well as the asthmatics and chronic
bronchitics had a significant retardation in their total lung and peripheral mucociliary
clearance.

1.3.3.7. Forced Expiration Technique
Forced expiration technique (FET) has been used as an alternative to cough during
chest physiotherapy (Pryor et al., 1979; Gaskell & Webber, 1980; Verboon et al.,
1986). FET was first described by Thompson (1973) comprising a forced expiration
commencing from approximately mid-lung volume to residual volume and followed by
a relaxation period. The transpulmonary pressure gradient is less during FET than
during cough (Langlands, 1967), and therefore airway compression is less, and at least
theoretically, permitting enhanced clearance of mucus compared with a conventional
cough. This forced expiration manoeuvre is performed without closure of the glottis
and the compressive phase that characterises cough.

Sutton and associates (1983) undertook a direct comparison between FET and cough in
clearing radioaerosol particles from the whole lungs of ten patients with airways
obstruction and production of large amounts of sputum (mean 64 ml/day). Their
assessment demonstrated that over 30-minute treatment periods, FET significantly
cleared more radioaerosol particles from the whole lung than instructed coughing.

1.3.4. Two-Phase Gas-Liquid Flow
A third mechanism has been proposed by Kim and associates (1986) for clearance of
secretions from the lungs in addition to mucociliary transport and cough. The mucus
production rate in the normal lung is low and the mucus layer is usually thin.
Therefore, the two-phase flow interaction between the mucus layer and airflow may not
be expected. However, the rate of mucus production is usually high in disease.
This high mucus production rate together with impaired mucociliary clearance could result in accumulation of mucus to a critical level of 5-20% of the airway diameter (Kim et al., 1986a). Once the mucus lining exceeds the critical thickness, the mucus may then be transported during normal tidal breathing.

Periodic airflow appears to be more effective than steady flow in transporting secretions in one direction or another (King et al., 1982). However, for lung secretions to be propelled cephalad it is necessary for the expiratory airflow velocity to be higher than the inspiratory airflow velocity by at least 10% (Kim et al., 1987; Benjamin et al., 1989). The critical airflow rate required for mucus transport decreases rapidly with decreasing airway diameter and the critical conditions can be met in the 8th to 9th airway generations during resting tidal breathing (Sackner & Kim, 1987).

Movement of secretions towards the larynx due to peristaltic waves has been hypothesised as a fourth tracheobronchial clearance mechanism (Pavia et al, 1987a). Movement of mucus by this mechanism would probably be limited to the small (non-cartilaginous) airways where peristaltic waves might be effective. However, any movement of mucus due to peristalsis remains very speculative as does clearance due to respiratory excursions which results in "squeezing" or "milking" the airways of secretions cephalad.

1.3.5. Alveolar Clearance

Terminal airway clearance (Morrow, 1973) is of great importance since up to 50% of inhaled particles (2-5 μm in diameter) may be deposited in this region. Alveolar clearance of inhaled particles which are deposited beyond the terminal bronchioles (i.e. beyond the mucociliary escalator) are achieved by one of two mechanisms (Jones, 1984). Non-absorptive clearance whereby particles are transported from the alveoli to the ciliated airways by surfactant and then removed by the mucociliary clearance.
However, this clearance mechanism involves a very small proportion of particles. The alternative is absorptive clearance mechanism by (a) direct penetration into epithelial cells with subsequent cell death and transport of debris to the mucociliary escalator or the interstitial space, (b) transport through the epithelial wall by transcellular and paracellular pathways, (c) phagocytosis and destruction within the phagocytic system, or (d) transport to lymphatics.

Molecular transport across the epithelial cell of particles of different size and composition may be handled in different ways by the alveolar epithelium. Particles are endocytosed by the alveolar type I cells or are digested by dense secondary lysosomes within the epithelial cells whereas some particles are released into the interstitial space (Green et al., 1977). However, paracellular transport involves the passage of hydrophilic substances through the epithelium (the intracellular tight junction) by passive diffusion.

The permeability (or leakiness) of the epithelial barrier, as measured by diethylenetriamine pentacetic acid (DTPA) may increase considerably in several conditions such as smoking, infections and interstitial lung disease (Jones et al., 1980; Barrowcliffe et al., 1987).

The majority of particles deposited in the alveoli are taken up by the alveolar macrophages which may be transported from the alveolar region to the ciliated airways to be cleared by mucociliary transport directly (Langenback et al., 1990) or the macrophage, having phagocytosed the particle, may migrate through the epithelial wall into the interstitial space and either distributed in the lymphatics or re-enter the airway in the ciliated region (Lauweryns & Baert, 1977).

The time course of alveolar clearance is divided into three phases: an initial fast phase
lasts about 24 hour, an intermediate phase lasts from 3 to 20 days, and a slow phase which may go on for 100 days or more (Bailey et al., 1985).

1.4. Aims of Thesis

The objectives for the studies in this thesis fall into three categories:

Firstly, obtaining information regarding mucociliary clearance in two disease processes namely pulmonary sarcoidosis and pigeon fanciers' lung. The patients with pulmonary sarcoidosis were maintained with different pharmacological therapies and were subdivided into smaller groups depending on concomitant therapy and their lung mucociliary clearance was measured and compared with that of control, healthy subjects. The patients with pigeon fanciers' lung disease were also subdivided according to the presence of circulating precipitins in their blood and their lung mucociliary clearance was ascertained and compared to a control, matched group of healthy subjects. By measuring mucociliary clearance in conditions not previously considered to be ones of impaired clearance, the sensitivity and applicability of the radioaerosol technique, which was used in all the studies of this thesis, was tested.

Secondly, the effect of oral bronchodilators on lung mucociliary clearance was evaluated in patients with asthma during sleep. It is known that clearance rate is reduced in asthma and is depressed even further during sleep. There is strong indirect evidence that abnormally slow mucociliary clearance is undesirable and may worsen airway obstruction. Oral bronchodilators were studied in a double-blind, crossover, placebo controlled fashion as there was evidence that they enhanced mucociliary clearance in awake patients with airways obstruction. Modes of characterising clearance rate for better comparability between drug and placebo assessments were also looked at.
Thirdly, the effect of coughing and forced expiration on tracheobronchial clearance in patients with airways obstruction was assessed by using the radioaerosol technique after modification (the observation period shortened and direct visualisation with a gamma camera was employed). Evaluation of cough and forced expiration on regional clearance basis were carried out in two groups of patients according to whether they produced sputum or not. Within these groups the association between clearance and airflow, sputum rheological properties, and amount of sputum production were examined.

It has been reported that airflow limitation is the most commonest physiological abnormality in sarcoidosis with evidence of large airways narrowing. In the light of such evidence of airway involvement in pulmonary sarcoidosis, it is very likely that the mucociliary clearance mechanism of these airways may be equally compromised. Pigeon fanciers have a high prevalence of chronic bronchitis, large airways involvement and peripheral airways obstruction. Therefore, the efficiency of lung mucociliary clearance in pigeon fanciers could be compromised and might be related to the presence of circulating precipitins in their blood.

Lung mucociliary clearance is impaired in asthmatic patients and reduced even further during sleep. This impairment of mucociliary clearance during sleep may contribute to additional airways obstruction and mucus plugging. Oral slow-release bronchodilators are often used to treat nocturnal asthma and they may have a beneficial effect on mucociliary clearance overnight.

In vitro studies using simulated mucus have suggested that the clearance rate by cough is influenced by the viscoelastic properties of the mucus and depth of mucus layer as well as by high expiratory flow rate. Therefore, it is most likely that the clearance of lung secretions by cough would be affected by the same factors.
CHAPTER TWO
METHOD AND TECHNIQUES

2.1. Introduction

Two approaches have been utilised to test in-vivo the efficiency of the mucociliary transport mechanism (Pavia et al., 1980). In the first, a direct measurement is made of mucus velocity based on the movement of appropriate markers in specified airways. In the second, it is the rate of clearance of tracer particles from the lung, or a major zone of the lung, which is measured. The term lung mucociliary clearance is often used for the second approach although in fact the measurements taken may also reflect transport by cough rather than by the mucociliary mechanism.

2.1.1. Mucus Velocity

This group of methods involves the measurement of the time taken for a marker, placed on the mucus of an anatomically well-defined airway, to move a given distance. There are four variations of this method for measuring the velocity of mucus:

(a) Cinebronchofibrescopic technique (Sackner et al., 1973).
This technique utilises teflon discs which are blown through the inner channel of a fibreoptic bronchoscope onto the tracheal mucosa in a circumferential distribution. The movement of these discs is filmed and measurement of tracheal mucus velocity is obtained.

(b) Roentgenographic technique (Friedman et al., 1977).
This technique is a modification of the cinebronchofibrescopic technique. Teflon tape coated with the radio-opaque substance bismuth trioxide is fed into a specially designed punch which is attached to a fibreoptic bronchoscope.
The punch produces individual discs which are blown into the trachea. The movement of these discs is recorded with a fluoroscopic unit and again a measure of tracheal mucus velocity is obtained.

(c) Radioisotopic technique (Chopra et al., 1977).
A suspension (0.04 ml) of labelled albumin microspheres is deposited (via a fibreoptic bronchoscope) on the mucosal surface at the lower end of the trachea which was predefined. The movement of the microspheres is recorded by a gamma camera and values of tracheal mucus velocity is obtained.

(d) Radioaerosol boli technique (Yeates et al., 1975).
This technique involves the inhalation of an aqueous aerosol containing labelled albumin microspheres. With a suitable inhalation manoeuvre the radioaerosol deposits predominantly in local boli in the large airways. The distance moved by the boli in a given time is recorded by a gamma camera or a multidetector probe and the tracheal mucus velocity is measured.

The techniques used for measuring lung mucus velocity with the exception of the boli technique are invasive and the degree of invasiveness of a technique has been shown to be directly related to the measure of the mucus velocity (Clarke & Pavia, 1981). The use of these techniques is restricted to the large airways and therefore assessment of clearance in small airways, which may be of importance in early lung disease, is not possible. However, these techniques have the advantages of short observation periods thus minimising artefacts due to coughing and permit repeated measurements within a short period of time.

2.1.2. Lung Mucociliary Clearance
These methods involve the inhalation (or insufflation) of insoluble tracer particles and
the monitoring of their subsequent retention in, or clearance from, the lungs. Two specific techniques are important:

(a) Radiographic technique.
This requires insufflation of radio-opaque particles (tantalum powder) through an endotracheal tube and the monitoring of their subsequent transport in the lungs by serial radiographs (Gamsu et al., 1973). This technique is capable of providing a measure of whole lung mucociliary clearance as well as local mucus velocity measurements. However, this technique is invasive and a high dose of radiation from the sequential radiographs is given to the lungs.

(b) Radioaerosol technique.
Albert & Arnett (1955) were the first to use inhaled radioaerosols for the objective measurement of lung mucociliary clearance in humans. This technique involves the oral inhalation of an insoluble aerosol tagged in an unleachable manner with a gamma emitting radioisotope and then monitoring its subsequent retention in or clearance from the lungs using scintillation counters. This technique is non-invasive and can provide a measure of clearance from all the ciliated airways. The amount of tracer radioaerosol inhaled is of the order of a few micrograms. The radiation dose to the lungs is generally a fraction of that received from a single chest x-ray. However, the technique requires long observation periods during which cough may contribute to the radioaerosol movement. This technique was used throughout this thesis for studying mucociliary clearance and the effect of cough on mucus movement within the human lungs.

2.2. Preparation of Radioaerosol Solution
Various radioisotopes have been used, with the radioaerosol technique, to tag aerosol particles (Newman, 1984).
The radioisotope technetium-99m (99mTc) (Lancet, 1968) was used throughout because of its following properties:

(a) Versatile chemistry which enables it to be labelled to a wide variety of materials (Kelly, 1981).

(b) Half-life of 6 hours which permits radioactive counting up to and beyond 24 hours (Harper et al., 1965).

(c) Absence of beta radiation which results in low radiation dose.

(d) Low gamma-ray energy (140 KeV) which is suitable for detection purposes.

The radioisotope 99mTc is the daughter product of molybdenum-99 (99Mo) (Anders, 1960). It is obtained from a 5 gigaBequerel (GBq) AmertecII generator (Amersham) which contains fission-produced 99Mo absorbed on alumina in a sterilised glass column, surrounded by lead shielding and attached to a reservoir of sterile pyrogen-free saline.

An elution vial shield containing an empty and sterile pre-evacuated container was introduced to the generator where the eluate was collected as sodium pertechnetate (Na99TcO4). The eluate was transferred to a test tube and its pH was adjusted from 5-7 to 7-9 by adding small quantity of ammonia solution (2 drops); a similar amount of tetraphenylarsonium chloride (5 % aqueous solution) was then added. An approximately equal volume to the eluate of chloroform was added and the mixture thoroughly shaken and passed through a silicon-treated phase separating filter paper (Whatman P1s). The filtrate contained tetraphenylarsonium pertechnetate dissolved in the chloroform. This filtrate is the chelated form of technetium (Few et al., 1970) which was then evaporated to dryness by a gentle flow of compressed air at room temperature.
The residue of tetraphenylarsonium pertechnetate in the form of a thin coating at the bottom of the test tube was dissolved in a 5 millilitre (ml) mixture of xylene and isobutyl methyl ketone (4:1, v/v) containing the required quantity of polystyrene (0.2 % solution). The mixture was thoroughly shaken by ultrasonic bath and was ready for dispersion.

Leaching of labelled polystyrene particles with $^{99m}$Tc is generally very small. About 4 % of the radioactivity is leached from the particles after 3 hours in saline with the loss falling to less than 1 % in 24 hours (Few et al., 1970). Therefore the radionuclide will remain fixed within the polystyrene particles when exposed to the watery fluids of the lungs.

2.3. Generation of Radioaerosol

Aerosol particles can be produced by nebulisers (Mercer, 1981), dry powder and metered dose inhalers (Newman, 1984a), and by a spinning disc generator (Walton & Prewett, 1949). The aerosol particles produced by nebulisation are of varied size and termed heterodisperse while particles produced by a spinning disc generator are of uniform size and termed monodisperse.

Quantitative studies of particle deposition and clearance can be performed in a controlled manner by using monodisperse aerosols (Morrow, 1981). Therefore, a spinning disc generator is the generator of choice for producing radioaerosols for the study of lung mucus clearance.

The prepared radioaerosol solution was injected, from a shielded motor driven glass syringe, through fine tubing at a constant rate of 0.85 ml/min. The solution was dropped from a fine bore needle located above the centre of a stainless steel disc top which was rotated by compressed air (fig. 2.1).
Fig. 2.1. A view from inside a tank of a fine bore needle positioned over the centre of a stainless steel disc where the aerosol solution is dropped to form liquid droplets.
The spinning disc generator (May, 1949) was placed inside a tank. The disc produced liquid droplets from which the solvent rapidly evaporated leaving the required solid spherical polystyrene particles with the label $^{99m}$Tc. In addition to the particles, satellite droplets were also formed at a rate of 4 per main particle and each of a diameter approximately one quarter that of the main particle. The majority of these satellites were drawn inside the generator by a negative pressure created around the rim of the disc top and left the tank via the compressed air outflow.

The size of the aerosol particles depends mainly on the quantity of polystyrene in the solution and the speed of the disc rotation (Fuchs, 1964). Aerosol particles of 5 microns ($\mu$m) in diameter were used throughout and were achieved with a solvent containing 0.2 % polystyrene and with the disc (2.6 cm in diameter) rotating at about 590 revolutions per second (table 2.1). Sizes of solid aerosol particles can be measured by optical or electron microscopy after deposition on a suitable surface (fig. 2.2). The size of the polystyrene particles was checked regularly by using a microscopic glass slide, which was placed inside the tank, and a light microscope with a calibrated graticule eyepiece.

2.4. Radioaerosol Administration System

As the spinning disc (G) rotated, at the required speed [driving air pressure (H) monitored by a mercury manometer (C) was adjusted accordingly] to produce the desired size of particles, the radioaerosol solution was fed (I) on to the disc top for a period of three minutes. This spinning time achieved the maximum concentration of polystyrene particles in the tank (T). Increasing the spinning time would have increased the concentration of the particles but the tank would have become saturated with the solvent vapor preventing more particles from getting dry. Furthermore the loss of particles would increase by settling to the floor of the tank with a settling velocity of 0.7 mm/sec (Hatch & Gross, 1964; Lin & Goodwin, 1976).
Table 2.1.

Aerosol diameter as a function of air pressure driving spinning disc.

<table>
<thead>
<tr>
<th>Driving Air Pressure (mmHg)</th>
<th>Diameter±SD (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>160</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>163</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>165</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>168</td>
<td>4.8 ± 0.4</td>
</tr>
</tbody>
</table>

[The size of polystyrene particles was checked every 2 to 3 months by using a microscopic glass slide which was placed inside the tank. Each slide was divided into three columns and seven rows and five particles were assessed in each section. Therefore the size of 105 particles were assessed and the coefficient of variation was calculated (range between 3-12%).]
Fig. 2.2. Polystyrene particles of 5 μm in diameter as viewed by electron microscope.
Figures 2.3 and 2.4 show respectively a schematic diagram and an overall view of the radioaerosol generation and administration apparatus. The tank, which was placed in a fume cupboard to eliminate any escaped radiolabelled particles and solvent vapor, was made of aluminium and was approximately 56 cm in height, 46 cm in width and 46 cm in depth. A removable perspex window (L; 30 cm x 20 cm) in the front of the tank allowed access to the spinning disc generator for cleaning and maintenance. The window was kept in position by ten screws and a rubber seal round its edge. The operator was able to observe the performance of the disc and the formation of the particles through this window. The tank could be made airtight after producing the aerosol particles by closing the satellite exhaust (E). A water manometer (W) was connected to it to monitor the air tightness.

In order to administer the aerosols under strictly controlled manner a system of solenoid valves was connected to the tank (Thomson & Short, 1969). With valve B shut and valve A open radioaerosol particles could be inhaled through a mouthpiece (M); with A shut and B open fresh air could be inhaled via one-way valves N and O. The one-way valve (O) was connected to a Martindale-MP6 filter (F) to remove the expired aerosols. The dead space between the mouthpiece and the tank was of the order of 50 ml.

The volume of inhaled aerosol was controlled by a Krogh water spirometer (K) connected in series with the tank. This connection was provided with a tap (Q) to either connect the spirometer to the tank while inhaling the aerosols or room air for resetting the spirometer whilst the subject breathed fresh air. The spirometer was fitted with a pre-set microswitch to cut off inhalation through solenoid valve A at the required volume. This was followed by a breath-holding pause which was measured automatically by a timing mechanism which opened solenoid valve B three seconds after the closure of valve A. The purpose of this was to permit deposition by sedimentation of airborne particles particularly in the smaller peripheral airways.

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Fig. 2.3. Schematic diagram of apparatus for generation and administration of radioaerosol particles. A and B = solenoid valves, C = mercury manometer, D = delay unit, E = satellite exhaust, F = filter, G = spinning disc, H = high pressure air supply, I = constant rate syringe injector, J = micromanometer, K = Krogh spirometer, L = perspex window, M = mouthpiece, N and O = one way valves, P = pneumotachygraph, Q = tap, R = ultraviolet light recorder, T = tank, W = water manometer.
Fig. 2.4. General view of radioaerosol generation and administration apparatus.
The flow rate of the inhaled volume of radioaerosol particles was measured by means of a pneumotachygraph (P) interposed between the spirometer and the tank. The pneumotachygraph was composed of two conical shaped brass tubes joined at their widest end with a mesh resistance in the centre. As air flowed through the mesh a pressure difference was produced across it and transformed to an electrical signal by a micromanometer - Hilger I.R.D. (J) which was displayed together with volume on an ultraviolet light recorder - 1706 Visicorder (R).

Prior to the start of each test the system was checked and calibrated for the required volume of inhalation (450 ml). Air was drawn via the inhalation tube of the tank by means of a calibrated syringe and the necessary adjustment was made to the spirometer so that valve A closed at the required volume.

It was possible in every inhalation procedure, by using this radioaerosol administration system, to control and/or record almost all the factors that can affect the site of particle deposition within the human lungs as discussed in chapter one (1.2) and thus achieve similar deposition, at least within the same subject, on different study days.

2.5. Inhalation of Radioaerosol Particles

The subject under investigation was first familiarised with the system by explaining how it works and the noises created by the solenoid valves. The subject was then seated and comfortably attached to the rubber mouthpiece which had a flange fitted between the subject's gums and lips to prevent air leakage. A nose clip was worn and the subject practiced the required breathing pattern with fresh air (valve A closed and B opened).

Inhalation of the radioaerosol particles commenced within one minute after the end of the three minutes spinning of the radioaerosol solution.
The subject was instructed to inhale the radioaerosol particles from the resting level of the lung in a slow and steady manner after the operator had turned the tap (between the spirometer and tank) to tank and activated the two-way valve arrangement (closing valve B and opening valve A). The inhaled flow rate was measured by the pneumotachygraph. After inspiring the preset volume solenoid valve A closed automatically and the subject was forced to hold his breath for three seconds thus allowing time for the particles to settle in the lung. During this time the operator turned the tap to air and reset the spirometer. Thereafter valve B opened automatically and the subject was asked to exhale and any remaining airborne aerosol was trapped in the filter (fig. 2.3). The procedure was repeated for the required number of breaths (usually 8 times). On average the time taken for inhaling the radioaerosol was two minutes.

After completion of the inhalations, the subject was asked to mouthwash and gargle three times with water to remove any particles deposited in the oropharynx. The subject was then asked to drink some water to remove any deposited particles in the oesophagus and thus remove them from the external counting field.

The time that elapsed from the start of the first inhalation to the commencement of the first chest count was on average 4 to 5 minutes.

2.6. Radiation Monitoring System

The radioactivity emanating from the subject's chest after inhaling the radioaerosol particles was monitored externally with two scintillation counters (Camner et al., 1971; Thomson & Pavia, 1974). The counters were placed at midpoint of the sternum anteriorly and posteriorly to the chest (in the same axis) in close contact with the subject's chest (fig. 2.5). They were mounted on a gantry and their vertical position could be adjusted by a hydraulic ram powered by an electric motor.
Fig. 2.5. Dual scintillation counters positioned anteroposteriorly to the subject's chest; each is connected to a scaler-ratemeter.
The horizontal position of each counter could be changed by a hand-controlled lead screw according to the height and size of the subject. The dual scintillation probes have graduated scales in both directions and this allowed repeated measurements to be carried out for the subject at the same position.

Each scintillation counter (I.D.L. type 663C) had a 3.81 cm diameter x 2.54 cm thick NaI(Tl) crystal encased in aluminium and coupled to a 5.08 cm diameter photomultiplier (PM) tube. The signal from the PM tube was fed to a suitable scaler-ratemeter (N.E.L. type SR5) where it was recorded. Each counter was inserted within a heavy lead shield assembly (I.D.L. type 2220) with a short, wide angle collimator 8.89 cm in diameter. The face of the scintillator was set 1.27 cm inside the collimator face (checked before each study) so that the detector should view most of the chest but at the same time virtually exclude the stomach (Thomson & Short, 1969).

A particle deposited at the periphery of the lung would travel toward the axis of the detector altering its radial distance from the centre of the detector. This type of movement has been neglected as being small. However, movement of a particle axially must follow the inverse square law and the error produced is therefore potentially greater than that from circumferential movement (Thomson & Pavia, 1973). Therefore, using opposing detectors would minimize variations in geometrical counting efficiency resulting from the continuous re-distribution of the deposited radioaerosol within the lungs.

Immediately after inhaling the radioaerosols the subject was seated between the opposing scintillation probes and counted (using the scaler) for a preset time of 30 seconds. This was repeated every 30 minutes for up to six hours after the inhalation of the radioaerosol.
The subject returned the following day, approximately 24 hours after inhalation, and a final count was taken over a longer period of time, 300 seconds, due to the physical decay of the radioisotope which by 24 hours leaves only low levels of activity.

2.7. Initial Deposition of Radioaerosol

Since the rate of mucus clearance of deposited radioaerosol depends on its initial site of deposition (penetration) within the lungs as discussed in chapter one (1.3.2.2) it is imperative, when using this technique, to ascertain the initial distribution of the radioaerosol before any interpretation of the clearance is made. This can be achieved by two indices characterising lung aerosol penetration namely "alveolar deposition" and penetration index (Agnew, 1991).

2.7.1. Alveolar Deposition

Clearance of inhaled aerosol particles from the conducting (ciliated) airways in healthy subjects was shown to be completed within 24 hours (Lippman et al., 1971; Agnew et al., 1981; Bennett et al., 1993) while alveolar clearance was much slower taking over several hundred days (Booker et al., 1967; Stahlhofen et al., 1980; Bailey et al., 1985). Thus a measurement of lung retention after 24 hours would indicate the fraction of the inhaled aerosol particles not deposited on the ciliated airways (alveolar deposition) and not available for mucus clearance. However, it has been shown that some patients with airways obstruction demonstrated a clearance element (6 % of the initial deposition) over the second 24 hour period following aerosol deposition (Agnew et al., 1981). This might be due to long term retention of a small fraction of aerosol particles at conducting airways sites causing a slow clearance phase (Agnew et al., 1988; Smaldone et al., 1988). Therefore a 48 hour retention reading could be used as an alternative to the more usual 24 hour reading for estimating alveolar deposition but this would involve a much longer counting time or a higher initial activity (and therefore radiation dose) delivered to the subject.
However, it has been shown that the retention at 24 hour were highly correlated with that at 48 hours in patients with moderately severe asthma (Svantengren et al., 1991). In patients with the "immotile cilia syndrome" there is evidence that tracheobronchial clearance may take 72 hours (Ruusa et al., 1993)-but those are patients whose ciliary function is known to be severely impaired. Throughout this thesis the activity remaining in the subject's chest at 24 hour, which was ascertained by the dual scintillation detectors, was taken to represent "alveolar deposition". A high 24 hour retention value indicates a predominantly peripheral deposition of the radioaerosol within the lungs while a low alveolar deposition suggests a central deposition.

2.7.2. Penetration Index

Measurement of the topographical distribution of radioaerosol within the lungs can be achieved by a gamma camera linked to a computer (Short et al., 1979).

After inhalation of the radioaerosol (within 10-15 minutes) the subject was placed in front of a large field of view gamma camera (Ohio Nuclear-110) and a two-dimensional posterior image (with a total of 40,000 counts) of the deposited particles within the lungs was obtained (fig. 2.6 & fig. 2.7). The subject also had a posterior Krypton \(^{81}\text{Kr}\) ventilation image (200,000 counts) to define the outer boundaries (15 and 30 \% contours) of the lungs (fig. 2.8). The \(^{81}\text{Kr}\) \((T_{1/2}=13 \text{ sec})\) was delivered from a commercial \(^{81}\text{Kr}\) generator (Hammersmith MRC Cyclotron Unit) and administered, through a one way valve connected to a mouth piece, during tidal breathing (Fazio & Jones, 1975) (fig. 2.9). A five minute background reading was recorded either with the subject before inhaling the radioaerosol or with a member of staff seated in front of the camera.
Fig. 2.6. Gamma camera (Ohio Nuclear 110) connected to a computer. After inhalation the subject sits on a low stool with his/her back against the front surface of the collimator. The hexagon shown on the collimator face approximately indicates the field of view of the camera.
Fig. 2.7. Gamma camera image of inhaled radioaerosol in a subject with impaired lung function (% predicted FEV₁ = 63).
Fig. 2.8. A posterior Krypton ($^{81m}$Kr) ventilation image defining the outer boundaries (15 and 30 % contours) of the lungs.
Fig. 2.9. Krypton ($^{81m}$Kr) gas was administered through a one way valve during tidal breathing from a commercial $^{81m}$Kr generator. The $^{81m}$Kr generator (right of picture) is housed within 5 cm thick supplementary lead shielding to ensure that direct radiation from the generator cannot affect the camera images.
All camera images were recorded in 64x64 format in a computer system (Nodecrest V77 600 or Nuclear Diagnostics). Using the computer's display system, the position of the $^{81m}$Kr image was adjusted horizontally and vertically until aligned with the radioaerosol image i.e. visualization of the aerosol image within the outer contours of the lungs (fig. 2.10). When a second aerosol image needed analysis its position was aligned within the $^{81m}$Kr contours and a copy of the background image was shifted horizontally and vertically to the same extent as this second aerosol image. The lung image was divided into tracheal, inner, intermediate and peripheral regions by fitting a 5x8 matrix as closely as possible to the outer contours of the $^{81m}$Kr image (Agnew et al., 1984b) as shown in fig. 2.11. Total counts were determined, in all regions, for the aerosol and the $^{81m}$Kr images as well as for the background image. These counts were then expressed as count rates (using counts per 100 seconds). A penetration index could be then obtained by dividing the outer to inner plus tracheal counts for the aerosol by the outer to inner plus tracheal ratio for the $^{81m}$Kr gas (Agnew et al., 1981a).

This approach is direct and good planar images could be achieved even from low level of lung radioactivity. However, the three dimensional lung was represented by a two dimensional image thus some deposition at small airways sites would be superimposed on the image of the large bronchi. Tomographic imaging would, in principle, offer a much better separation of large from small airways but this would involve higher levels of radioactivity than those now usually used for planar imaging (Agnew, 1991; Chan, 1993).

2.8. **Whole Lung and Tracheobronchial Clearance**

After inhalation of the radioaerosol and assessing its initial site of deposition sequential counts of the lung radioactivity show a progressive fall due to two factors:
Fig. 2.10. Radioaerosol image (posterior view) within the outer contours (15 and 30\% of the lungs.)
**Fig. 2.11.** A 5x8 matrix was fitted as closely as possible to the outer contours of the $^{81m}$Kr image to divide the lung into regions.
(a) physical decay of the radionuclide and (b) biological clearance of the deposited radioaerosol from the lungs. However, after subtracting the background radioactivity from the recorded counts, a correction for the physical decay was made knowing the physical half-life of the radionuclide. Therefore the radioactivity remaining in the lungs, expressed as a percentage of the initial count, was plotted against time after inhalation yielding a whole lung clearance curve (fig. 2.12).

The whole lung clearance curve (as shown in fig. 2.12) consisted of two phases: (i) a fast phase, which is of a few hours' duration and certainly less than one day in healthy subjects (Agnew et al., 1981), due to clearance by cilia and cough in lung disease and (ii) a slow phase, with a biological half-life of several months (Philipson et al., 1985), due to alveolar clearance. By subtracting the estimate of alveolar deposition, which is the radioaerosol remaining in the lungs after 24 hours, from the whole lung clearance curve a tracheobronchial clearance curve was obtained (fig. 2.13) representing the amount of radioaerosol that was cleared from the ciliated airways. Recent lung development work has suggested that in principle, the fast phase (i) can be divided into "bronchial" and "bronchiolar" components (Bair et al., 1991; Bailey et al., 1991). No such division was attempted for whole lung data in the present work but regional analysis (section 2.9) will be influenced by the differing proportion of bronchial and bronchiolar airways within the different regions studied.

Tracheobronchial clearance occurs by mucociliary and cough clearance mechanisms. By careful monitoring of the subject throughout the tracheobronchial clearance observation period it was possible to estimate the amount of radioaerosol cleared from the airways by productive coughing and the amount cleared by mucociliary transport (Fazio & Lafortuna, 1981). Each subject was asked to record the number and type of all coughs (dry or productive) on a detailed 'cough-sheet' during the six hour observation period.
Fig. 2.12. Whole lung clearance curve for deposited radioaerosol. AD (Alveolar deposition) indicates the fraction of inhaled aerosol particles not available for mucus clearance.
Fig. 2.13. Tracheobronchial clearance curve after correction for alveolar deposition.
The subjects were also instructed to collect all sputum samples in special containers during that period which were then weighed and their radioactive content was ascertained. To calculate how much the radioactivity of a single sputum sample would contribute to the loss from the whole chest radioactivity a chest 'phantom' (fig. 2.14) was used (Ruffin et al., 1978). The chest phantom consisted of a plexiglass disc 0.75 cm thick and 30 cm in diameter filled with 1.5 cm of water and 7 cm of air. Samples of a known amount of radioactivity both inside the phantom (simulating lung burden) and outside the phantom (simulating radioactivity in sputum) were placed in the usual measuring positions. The ratio of counts obtained in these two positions, which was 1/20, was used as a scaling factor. Adding the amount of tracheobronchial radioaerosol present in the sputum at the time of expectoration to the clearance curve generated a 'sputum-corrected' curve representing tracheobronchial clearance achieved by the mucociliary mechanism. The tracheobronchial radioaerosol deposition that had been cleared by productive coughing was the difference between the observed tracheobronchial clearance curve and the 'sputum corrected' curve (fig. 2.15).

2.9. Regional Clearance

Regional clearance due to mucociliary action or instructed coughing within the lungs was assessed by means of sequential imaging on a large field-of-view gamma camera linked to a computer system. After delineating the regions of interest (fig. 2.11) for each aerosol image (an image before and after the appropriate manoeuvre) and determining the count rates in each region, by following the method in section 2.7.2, the proportion of radioaerosols deposited in the non-ciliated airways for any one region and the transport of radioaerosols from one region into another could then be measured (Agnew et al., 1984b).

The proportion of alveolar deposition expected in each region was predicted by using the $^{81m}$Kr counts in each region.
Fig. 2.14. A chest phantom.

Footnote
Five samples of technetium-99m (99mTc) of a known amount of radioactivity were used inside and outside the phantom. After background and decay correction the scaling factor (S) was calculated:

\[ S = \frac{\text{Counts outside}}{\text{Counts inside}} \]

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Number of counts in 30 seconds</th>
<th>Scaling factor (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outside phantom</td>
<td>Inside phantom</td>
</tr>
<tr>
<td>1</td>
<td>10801</td>
<td>565</td>
</tr>
<tr>
<td>2</td>
<td>21988</td>
<td>1147</td>
</tr>
<tr>
<td>3</td>
<td>39562</td>
<td>1984</td>
</tr>
<tr>
<td>4</td>
<td>85100</td>
<td>4318</td>
</tr>
<tr>
<td>5</td>
<td>106747</td>
<td>5439</td>
</tr>
</tbody>
</table>

Mean±SD 19.51±0.36
Fig. 2.15. Observed and sputum-corrected tracheobronchial clearance curve.
The $^{81m}$Kr counts in each region was expressed as a percentage of the total in both 5x8 matrices plus tracheal region. The gamma camera estimate of aerosol retention after the appropriate manoeuvre [relative to the initial image (baseline) at 10-15 minutes after inhalation] was obtained from the count rate summed over the two 5x8 matrices plus tracheal region. This was translated into an estimate of 24 hour retention for the gamma camera by multiplying it by the ratio of the probes' 24 hour retention to the after manoeuvre retention values (corrected for background and decay). The estimation of total 24 hour retention was then multiplied by the $^{81m}$Kr predictions of alveolar deposition in each region. This gave estimates for the count rate in each region due to alveolar deposition. Subtracting this alveolar component from the previously calculated whole lung count rates gave values of count rates corresponding to particles deposited on the ciliated airways for each region.

To correct for inter-region transport of particles, the count rates (baseline) for tracheal, inner and intermediate regions were recalculated for the data from the after manoeuvre image as the initial count rate plus the cumulative loss in count rate from the more peripheral regions. Thus, the count rate for the intermediate region was the sum of the initial count rate in the intermediate region plus the count rate lost from the outer region up to the time at which an image was recorded. The count rate for the inner region was the sum of the initial count rate in the inner region plus the amount by which the count rate in the outer plus intermediate regions had decreased. Similarly the count rate for the tracheal region was the sum of the initial count rate in the tracheal region plus the amount by which the count rate from the outer, intermediate and inner regions had decreased. The count rate lost from each region after being corrected for inter-region transport of particles was expressed as a percentage of the total count rate in both 5x8 matrices plus tracheal region.
2.10. **Hazards from Inhaled Particles**

2.10.1. **Radiation Dose**

Radiation dosage to the lungs was estimated by Philipson (1977), for deposited insoluble inhaled particles labelled with $^{99m}$Tc, to be 175 μGy per MBq when clearance from the lungs failed to occur. Assuming that 30% of initial lung burden would be cleared within 5 hours, Short and associates (1979) estimated a radiation dose of 124 μGy per MBq. Therefore the maximum dose per clearance test with an initial lung burden of 1 MBq should be in the range of 124-175 μGy. All these estimates of dose are exceedingly low and should be compared with the average lung dose received during a single chest x-ray which is 400 μGy (Advisory Committee on Biological Effect of Ionizing Radiation, 1977).

It has been shown that in-vitro exposure to radiation will increase ciliary beat frequency (Baldetrop et al., 1976) and could cause ciliary stasis with ultrastructural abnormalities if the radiation level is considerably high (Fujiwara et al., 1972; Baldetrop & Hakansson, 1972; Baldetrop et al., 1977). Furthermore, Ahmed and associates (1979) have shown that in-vivo exposure to radiation, using $^{99m}$Tc labelled albumin microspheres of 70 MBq, caused a temporary increase in tracheal mucus velocity of sheep after 5 minutes of instillation of the radioisotope which returned to base-line after 30 minutes. However, the amount of radioactivity was much larger than that used in the studies of this thesis.

The amounts of radioactivity administered in the studies of this thesis were approved by the Administration of Radioactive Substances Advisory Committee (Department of Health and Social Security) for each of the studies carried out. All studies were also approved by the Ethical Practices Committee of the hospital.
2.10.2. Polystyrene Toxicity

Polystyrene particles are insoluble in body fluids. The actual amount of deposited polystyrene in the lungs used in the studies of this thesis was very small; approximately 50 µg per study. No evidence of toxic effects from polystyrene manufacturers and users have been reported (Black & Walsh, 1970).

2.10.3. Xylene Vapour Toxicity

The concentration of xylene vapour in the inhaled air was estimated to be more than the maximum permissible concentration for continuous inhalation (Booker et al., 1967), but the subjects inhaled few breaths only so no toxic effects should have arisen. It has also been shown that xylene vapour had no anaesthetic effect on the cilia by using a similar method for inhaling aerosols but without xylene (Booker et al., 1967). The results with subjects who inhaled aerosols generated by both methods showed no differences. The disadvantage of the method without xylene was that the yield of aerosol was very small and thus the measurement of retained activity was not accurate.

2.11. Variability in Tracheobronchial Clearance

It is important to define the variability both within (intra) and between (inter) subjects in tracheobronchial clearance using the radioaerosol technique which was used throughout this thesis.

Variability in the measurements of tracheobronchial clearance using the radioaerosol technique arises from biological variability in the function of the clearance mechanism and also from methodological variability arising from differences in initial distribution of the radioaerosols within the lungs which reflects variability in flow rate and airway patency.
Variability is usually expressed in terms of the coefficient of variation (CoV) which is defined as the ratio of the standard deviation to the mean value as a percentage (SD/mean x 100). Several investigators have reported the inter- and intra-subject variability (Lourenco et al., 1971; Yeates et al., 1981a; Van Hengstum et al., 1986) but in relatively small numbers of subjects and over short observation periods. However, recently Del Donno and associates (1988), from the same laboratory where the work reported in this thesis was carried out, reported the intra and inter-subject variability of the radioaerosol technique, which was used in this thesis, in relatively large numbers of healthy subjects (33 healthy non-smokers and 19 asymptomatic smokers) and in three groups of patients with airways disease of different aetiology (27 chronic bronchitics, 40 asthmatics and 12 bronchiectatics). They found out that the inter-subject/patient CoV of tracheobronchial clearance over 6 hour observation period was 13 % for the healthy non-smokers and 28-39 % for the remaining four groups while the intra-patient CoV was about half of the inter-patient CoV.

2.12. Pulmonary Function Measurement

To assess the degree of airway obstruction for each volunteer subject during each study day, dynamic lung volumes tests (Clarke, 1976) were performed:

1. Large airways function was assessed using a dry bellows spirometer (Vitalograph®) and the Forced Vital Capacity (FVC) as well as the Forced Expiratory Volume in one second (FEV$_1$) were measured. Peak Expiratory Flow Rate (PEFR) was also recorded by a Wright® peak flow meter.

2. Small airways function was assessed using an Ohio® 840 dry spirometer connected to a Bryans® x-y recorder. The flow rates at 25 % of vital capacity ($\dot{V}_{\text{max}25}$) and at 50 % of vital capacity ($\dot{V}_{\text{max}50}$) were measured. The Maximum Mid-expiratory Flow Rate (MMFR$_{25-75}$) was calculated from the forced expiratory spirogram (Vitalograph®).
Each subject whilst sitting and without wearing a nose clip was instructed to take a maximum inspiration and forcibly exhale for as long as possible into the spirometer. The best of three technically acceptable measurements was used for each subject.

The predicted values for FVC, FEV\textsubscript{1}, PEFR and MMFR\textsubscript{25-75} were obtained from Cotes (1979) and the predicted values for \( \dot{V}_{\text{max}25} \) and \( \dot{V}_{\text{max}50} \) were taken from Knudson and associates (1983) which were based on the subjects' sex, age and height.

2.13. Rheology of Mucus

Rheology is defined as the study of deformation and flow properties of matter (Scott Blair, 1969). Thus the mechanical behavior of mucus deformation is referred to as "mucus rheology". Mucus is a non-Newtonian, viscoelastic gel as described in chapter one (1.3.2.2) and therefore the description of its rheological properties must adequately account for these characteristics.

A wide variety of instruments are available to study the rheological properties of mucus. The capillary viscoelastimeter can be used to measure mucus viscosity and elasticity for small samples (Shake et al., 1987). Rotational rheometers have either a coaxial-cylinder, a cone-and-plate, or a parallel-disk sensor system (Davis, 1973; Mitchel-Heggs, 1977; Lopez-Vidriero et al., 1977; Puchelle & Zahm, 1984). These rheometers can be used at a constant speed of rotation or in an oscillatory mode. More recently, a magnetic rheometer has been developed using micron-sized ferromagnetic particles (King & Macklem, 1989a).

A Low-Shear 30 sinus (Contraves®) rheometer (fig. 2.16) was used for measuring the viscous and elastic properties of sputum in the work reported in chapter eight. It is an oscillating rheometer which applies a sinusoidal stress to the sputum sample and the resulting strain is measured.
Fig. 2.16. A Low Shear 30 sinus rheometer (Contraves®).
The temporal relationship of the deformation of the sample is compared to that of the imposed oscillatory force. From the change in magnitude and phase angle of the measured flow, the storage modulus (in phase component) and the loss modulus (out of phase component) can be derived. These dynamic moduli are related to the elastic and viscous properties of the sputum respectively. If the strain is plotted against the stress using an x-y recorder (Bryans®) an ellipse is obtained (fig. 2.17).

The viscoelastic properties are calculated from the following relations:

\[
\begin{align*}
\text{Viscosity } & \eta = \sin \theta \times y/2 \times A \quad \text{(mPa.s).} \\
\text{Elasticity } & G' = \cos \theta \times y/2 \times A \times W \quad \text{(mPa).}
\end{align*}
\]

where \( \theta \) is the phase angle = \( \cos^{-1} a/X \) (fig. 2.17), A and W are constants.

Measurements of apparent viscosity and elasticity were performed with small volumes of sputum (1 ml) which undergoes an extremely low stress causing a minimum destruction to the internal structure of the sputum sample. All sputum samples were tested at the same frequency (0.325 Hz) and temperature (37°C) due to the fact that a decrease in viscosity was observed with increasing temperature (Sturgess et al., 1971).

Three aliquots were analysed for each sample within one hour of being expectorated to avoid evaporation of the water content of sputum and degradation of the bronchial glycoprotein due to enzymes of salivary and bacterial origin present in sputum (Leach, 1967; Lopez-Vidriero & Reid, 1978). Although salivary contamination of sputum can be reduced considerably by placing dental swabs between the gums and the buccal surface (Puchelle et al., 1984), from the practical point it was difficult to use this method because all sputum samples were collected while the patients were instructed to perform coughing or forced expirations and measuring the peak flow achieved during these manoeuvres with a peak flow meter.
Fig. 2.17. Speed versus torque (or strain vs. stress) for viscoelasticity measurement at single frequency. $X, y$ and $a$ are measured in centimetre.
It has been shown that the coefficient of variation in apparent viscosity between three aliquots from the same specimen is below 30 % and between samples from a patient is 40 % while the variation between patients with the same disease is 60 % except for patients with asthma where the coefficient is greater (Charman & Reid, 1972; Charman et al., 1974).

Despite the large intra- and inter-sample variation in apparent sputum viscosity this measure, describing a physical property of sputum, is still in use in the absence of other more suitable and reliable indices of the physical properties of sputa.
CHAPTER THREE

Tracheobronchial Clearance In Patients With Pulmonary Sarcoidosis

3.1. Summary

Lung mucociliary clearance was measured in 13 patients with pulmonary sarcoidosis and 13 "matched", healthy controls. Four of the sarcoid patients had never received any steroid therapy, five were currently on oral corticosteroids and the remaining four were on inhaled corticosteroids only. A statistically significant retardation in tracheobronchial clearance (p<0.02) was observed in the sarcoid patients compared to the controls. The sarcoid patients on inhaled corticosteroid therapy appeared to demonstrate the greatest degree of mucociliary transport impairment. The sarcoid patients in "apparent" remission and those on oral corticosteroid therapy had clearances better than those patients on inhaled corticosteroids, but they were still reduced compared to the controls. This study demonstrates that lung mucociliary clearance is adversely affected in patients with pulmonary sarcoidosis and raises the question of the possible consequences that could follow long term inhaled immuno suppressive therapy on the prime clearance defence mechanism within the human lungs.

3.2. Introduction

Sarcoidosis is a relatively common disease, with worldwide variations in incidence and prevalence. The lung is the most commonly involved organ and the site which is usually associated with severe morbidity (James et al., 1976).

Most patients with pulmonary sarcoidosis are asymptomatic for many years. Constitutional symptoms such as fever, fatigue, malaise, anorexia and weight loss are usually absent or mild. Respiratory symptoms may start insidiously with dry cough, progressive dyspnoea, exercise intolerance and chest pain (Bacharach, 1961).
Physiological studies in patients with sarcoidosis may be influenced by the fact that granulomas may compress or intrude upon the lumen of large and small airways as shown by the histologic study of Longcope & Freiman (1952) and later by the bronchoscopic biopsy study of Friedman and colleagues (1963). Therefore the type and degree of physiological abnormality is related to the location and the extent of the pathology (Udwadia et al., 1990).

The majority of physiological studies in patients with pulmonary sarcoidosis have shown a decrease in diffusion capacity, with a loss in lung volumes (Marshall et al., 1958; Emirgil et al., 1969; Williams, 1983; Athos et al., 1986). Few studies have clearly demonstrated the presence of airway involvement in sarcoidosis even in the absence of clinical symptoms (Holmgren & Svanborg, 1961; Sharma et al., 1966; Miller et al., 1973; Kaneko & Sharma, 1977). Indeed Levinson and associates (1977) observed that abnormal airway function occurs frequently in patients with pulmonary sarcoidosis, involving both large and small airways on occasion. Recently Harrison and colleagues (1991) showed that airflow limitation is in fact the commonest physiological abnormality in sarcoidosis with evidence of large airway narrowing. In the light of such mounting evidence of airway involvement in pulmonary sarcoidosis, it was very likely that the primary clearance mechanism within these airways may be equally compromised.

To date there is no information regarding the efficiency of lung mucociliary clearance in patients with sarcoidosis, thus the purpose of this study was to compare lung mucociliary clearance in patients with pulmonary sarcoidosis at various stages of their disease with that of healthy subjects.

3.3. Subjects & Patients

Thirteen patients with sarcoidosis (S) were recruited for assessment of tracheobronchial
clearance. Four patients were on no treatment (Group A), five patients were on oral corticosteroids (Group B, one patient on 1 mg daily; three patients on 5 mg daily and one patient on 10 mg daily of prednisolone) and the remaining four patients were being treated with inhaled corticosteroids (Group C, three patients on beclomethasone dipropionate and one patient on budesonide).

This group of sarcoid patients were compared with a control group of thirteen healthy subjects chosen from the department's "bank" of data on healthy volunteers. The criteria for selection were that each patient was "matched" as closely as possible with a healthy subject of the same sex and similar age, smoking history and initial radioaerosol distribution within the lungs but with the investigator carrying out the matching "blind" as regards to tracheobronchial clearance.

Tables 3.1-3.4 give the mean±SEM physical characteristics, tobacco consumption and alveolar deposition (AD) for the three groups of patients with sarcoidosis and the corresponding control healthy groups (tables 3.1-3.3) and for the whole group of sarcoid patients with their matched controls (table 3.4).

Informed consent was obtained from each patient prior to the commencement of the study which was approved by the hospital's ethical practices subcommittee.

3.4. Methods

3.4.1. Tracheobronchial Clearance

The radioaerosol technique, which has been described in detail in chapter two (2.1.2), was used to measure lung tracheobronchial clearance. Polystyrene particles (5 μm in diameter), firmly labelled with the radionuclide 99mTc, were generated and inhaled by the volunteer subjects in discrete breaths under strictly controlled conditions.
Table 3.1.
Physical characteristics, tobacco consumption and alveolar deposition (AD) for four patients with sarcoidosis (S) on no treatment (group A) and four matched healthy control subjects (H).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>H</td>
<td>S</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>F</td>
<td>52</td>
<td>25</td>
<td>NS</td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>M</td>
<td>54</td>
<td>77</td>
<td>15*</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>15*</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td></td>
<td>12*</td>
</tr>
</tbody>
</table>

NS : Non-smoker.
* : Ex-smoker.
Table 3.2.
Physical characteristics, tobacco consumption and alveolar deposition (AD) for five patients with sarcoidosis (S) on oral corticosteroid therapy (group B) and five matched healthy control subjects (H).

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>H</td>
<td>S</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>67</td>
<td>58</td>
<td>33*</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>51</td>
<td>47</td>
<td>NS</td>
</tr>
<tr>
<td>F</td>
<td>F</td>
<td>34</td>
<td>69</td>
<td>NS</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>53</td>
<td>31</td>
<td>13*</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>52</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>51</td>
<td>47</td>
<td>23*</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>5</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

NS : Non-smoker.

* : Ex-smoker.
Table 3.3.  
Physical characteristics, tobacco consumption and alveolar deposition (AD) for four patients with sarcoidosis (S) on inhaled corticosteroid therapy (group C) and four matched healthy control subjects (H).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>H</td>
<td>S</td>
<td>H</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>43</td>
<td>31</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>36</td>
<td>47</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>F</td>
<td>F</td>
<td>35</td>
<td>28</td>
</tr>
</tbody>
</table>

Mean: 37  34  3*  7*  28  36  
SE: 2  4  --  --  7  3

NS: Non-smoker.

*: Ex-smoker.
Table 3.4.
Mean±SEM physical characteristics, tobacco consumption and alveolar deposition (AD) for the whole group of patients with sarcoidosis (S; n=13) with their matched healthy control subjects (H; n=13).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>H</td>
<td>S</td>
<td>H</td>
</tr>
<tr>
<td>4F/9M</td>
<td>4F/9M</td>
<td>46±3</td>
<td>40±6</td>
</tr>
</tbody>
</table>

* : Ex-smoker (n=4).
The amount of radioaerosol initially deposited within the lungs was measured and its subsequent clearance was monitored by two collimated, axially opposed scintillation detectors. Counts were taken immediately after inhalation and at half-hourly intervals thereafter for 6 hours with a final count at 24 hours which was taken to be an estimate of AD.

Tracheobronchial clearance was assessed by calculating the area under the tracheobronchial retention curve (AUC) for the first 3 hours, the second 3 hours and the whole 6-hour observation period.

The patients were encouraged to avoid coughing as far as possible. Nevertheless the number of involuntary coughs and wet weight of sputum expectorated throughout the 6-hour observation period were measured and its radioactive content was "added back" to the retention curves in order to take account of the effect of productive coughing using the method described in section 2.8 and illustrated in fig.2.15. At the end of the 6-hour observation period the patients were instructed to cough (3 double coughs per minute for 5 minutes). This was performed in order to ascertain the quantity of radioaerosol which was still available for cough clearance at that time.

3.4.2. Pulmonary Function Tests

Before the inhalation of the radioaerosol each volunteer subject underwent pulmonary function testing as described in chapter two (2.12). Furthermore, 10 out of 13 sarcoid patients were able to attend the pulmonary function laboratory for measurements of their total lung capacity (TLC) and total diffusing capacity for carbon monoxide (TLCO) using the single breath method (P.K.Morgan-Model C). Measurements of $V_{max25}$ and $V_{max50}$, TLC and TLCO were not available for the healthy control subjects.
3.4.3. Statistical Analysis

The data were not normally distributed and statistical analysis was assessed using the non-parametric paired Wilcoxon test and Spearman rank correlation (Siegel, 1956). The level at which the data were considered to be significant was taken at p<0.05.

3.5. Results

3.5.1. Pulmonary Function

Table 3.5 gives the mean±SEM % predicted FEV1, FVC, PEFR as well as the observed % FEV1/FVC for the three groups of patients with sarcoidosis (S) together with those from their respective "matched" control group (H). The mean±SEM % Predicted MMFR25-75, V̇max at 25 and 50 % of VC together with TLC and TLCO for the three groups of patients with sarcoidosis are given in table 3.6. These tables indicate that the pulmonary function indices for group A (no treatment) were similar to those of their control group. However, patients on oral corticosteroids (group B) had reduced pulmonary function indices compared to their control group whereas group C (on inhaled corticosteroids) showed marked deterioration of flow rates in both large and small airways. There were no statistically significant differences in the pulmonary function indices between the three groups of sarcoid patients.

3.5.2. Tracheobronchial Clearance

The mean radioaerosol inspiratory flow rates for the patients with sarcoidosis in group A and C were on average higher than their respective healthy control groups (group A: 52±6 vs. 38±3 l/min; group C: 52±16 vs. 38±7 l/min respectively), in group B the mean inspiratory flow rate was virtually identical (43±4 vs. 42±8 l/min). The mean alveolar depositions were on average lower for the patients with sarcoidosis in all groups (A, B and C) compared to their respective healthy controls (tables 3.1-3.3) despite the attempt made to match for alveolar deposition.
Table 3.5.
Mean±SEM pulmonary function indices for three groups of patients with sarcoidosis (S) and for their respective "matched" healthy controls (H).

<table>
<thead>
<tr>
<th>Pulmonary Function</th>
<th>Group A (n=4)</th>
<th>Group B (n=5)</th>
<th>Group C (n=4)</th>
<th>Total (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Pred.FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>90±17</td>
<td>100±9</td>
<td>109±11</td>
<td>76±8</td>
</tr>
<tr>
<td>% Pred.FVC</td>
<td>84±15</td>
<td>92±6</td>
<td>111±12</td>
<td>75±14</td>
</tr>
<tr>
<td>% FEV&lt;sub&gt;1&lt;/sub&gt;/FVC</td>
<td>85±4</td>
<td>90±2</td>
<td>80±8</td>
<td>79±4</td>
</tr>
<tr>
<td>% Pred.PEFR</td>
<td>104±18</td>
<td>83±2</td>
<td>90±12</td>
<td>99±10</td>
</tr>
</tbody>
</table>

Group B: on oral corticosteroids.
Group C: on inhaled corticosteroids.
Table 3.6.

Mean±SEM  % Pred. MMFR\textsubscript{25-75}, $\dot{V}_{\text{max}}\text{50}$, $\dot{V}_{\text{max}}\text{25}$, TLC and TLCO for three groups of patients with sarcoidosis (group A: no treatment; group B: on oral corticosteroids and group C: on inhaled corticosteroids).

<table>
<thead>
<tr>
<th></th>
<th>Group A (n=4)</th>
<th>Group B (n=5)</th>
<th>Group C (n=4)</th>
<th>Total (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Pred.MMFR\textsubscript{25-75}</td>
<td>85±24(^{(a)})</td>
<td>77±18(^{(b)})</td>
<td>47±19(^{(c)})</td>
<td>70±12(^{(d)})</td>
</tr>
<tr>
<td>% Pred.$\dot{V}_{\text{max}}\text{50}$</td>
<td>95±25</td>
<td>82±22</td>
<td>50±18</td>
<td>76±13</td>
</tr>
<tr>
<td>% Pred.$\dot{V}_{\text{max}}\text{25}$</td>
<td>61±15</td>
<td>60±15</td>
<td>38±13</td>
<td>53±8</td>
</tr>
<tr>
<td>% Pred.TLC</td>
<td>84±11</td>
<td>70±8</td>
<td>71±7</td>
<td>76±5</td>
</tr>
<tr>
<td>% Pred.TLCO</td>
<td>83±10</td>
<td>55±14</td>
<td>74±9</td>
<td>72±8</td>
</tr>
</tbody>
</table>

Control group: 
(a) = 90±25
(b) = 93±15
(c) = 103±19
(d) = 95±10
However, both inspiratory flow rate ($P>0.10$) and alveolar deposition ($0.05<P<0.10$) did not differ statistically between the two whole groups, i.e. 13 sarcoid patients vs. 13 healthy controls.

Eight of the sarcoid patients (3 in group A, 2 in group B and 3 in group C) coughed during the 6-hour observation period (range of number of coughs: 2-31). Of those, 4 subjects produced sputum (2 in group A, 1 in group B and 1 in group C) in each instance containing less than 2% of their initial tracheobronchial deposition save for one subject (group A) whose sputum contained 8% and one other (group C) whose sputum contained 61%. During the instructed coughing only two patients (1 in group A and 1 in group B) produced sputum (wet weight: 1.3 and 3.6 g respectively) containing less than 0.2% of their initial tracheobronchial deposition.

Fig. 3.1 gives the mean±SEM tracheobronchial retention curves (sputum corrected) for the three groups of patients with sarcoidosis as well as those for the whole study group of 13 sarcoid patients vs. the 13 matched healthy control subjects. There were statistically significant differences in tracheobronchial retention at 2, 4 and 6 hours between the 13 sarcoid patients and 13 healthy controls. The tracheobronchial retention curves for the three groups of sarcoid patients indicate a more severe impairment (of tracheobronchial clearance) in the patients on inhaled steroid therapy. There were no statistically significant differences in the area under the tracheobronchial retention curves over the first 3 hours (fig. 3.2) and the second 3 hours (fig. 3.3) of the observation period between the three groups of sarcoid patients.

Table 3.7 gives the mean±SEM area under the tracheobronchial retention curve for the three groups of patients with sarcoidosis and their respective healthy controls over the first 3 hours ($AUC_{0,3}$), the second 3 hours ($AUC_{3,6}$), and the whole 6-hour ($AUC_{0,6}$) observation periods.
Fig. 3.1. Mean tracheobronchial retention curves for four sarcoid patients on no treatment (Group A), five sarcoid patients on oral corticosteroids (Group B), four sarcoid patients on inhaled corticosteroids (Group C), and for all thirteen sarcoid patients compared to thirteen matched healthy control subjects.
Fig. 3.2. Area under the tracheobronchial retention curve over the first three hours ($\text{AUC}_{0-3}$) of the observation period for the sarcoid patients in group A (no treatment), group B (on oral corticosteroids) and group C (on inhaled corticosteroids).
Fig. 3.3. Area under the tracheobronchial retention curve over the second three hours (AUC₃-₆) of the observation period for the sarcoid patients in group A (no treatment), group B (on oral corticosteroids) and group C (on inhaled corticosteroids).
Table 3.7.
Mean±SEM area (%.h) under the tracheobronchial retention curve for the first 3 hours (AUC$_{0-3}$), the second 3 hours (AUC$_{3-6}$) and for the whole 6-hour (AUC$_{0-6}$) observation periods for three groups of patients with sarcoidosis (S) and for their respective matched healthy controls (H).

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC$_{0-3}$</th>
<th>AUC$_{3-6}$</th>
<th>AUC$_{0-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%.h)</td>
<td>(%.h)</td>
<td>(%.h)</td>
</tr>
<tr>
<td>S</td>
<td>H</td>
<td>S</td>
<td>H</td>
</tr>
<tr>
<td>A</td>
<td>142±24</td>
<td>117±12</td>
<td>59±23</td>
</tr>
<tr>
<td>B</td>
<td>139±21</td>
<td>137±9</td>
<td>60±19</td>
</tr>
<tr>
<td>C</td>
<td>217±37</td>
<td>151±13</td>
<td>118±53</td>
</tr>
<tr>
<td>Total</td>
<td>164±18</td>
<td>135±7</td>
<td>78±19</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.10</td>
<td>&lt;0.01</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Group B : on oral corticosteroids.
Group C : on inhaled corticosteroids.
Although, on average, the $\text{AUC}_{0.3}$ for the sarcoid patients was greater (i.e. slower clearance) than their respective healthy controls this was not statistically significantly different ($P< 0.10$). The $\text{AUC}_{3.6}$ was statistically significantly larger for the sarcoid patients compared to their respective healthy controls ($P<0.01$). A statistically significant increase in $\text{AUC}_{0.6}$ for the sarcoid patients was also observed ($P<0.02$) compared to their respective healthy control subjects (fig. 3.4).

No correlation was observed between the various pulmonary function indices and the degree of impairment in lung mucociliary clearance.

3.6. Discussion

Tracheobronchial clearance comprises two main clearance mechanisms, namely mucociliary clearance and cough. Although, the recruited sarcoid patients were asked to avoid coughing during the 6-hour observation period, some did cough.

The tracheobronchial clearance curve was suitably corrected for the productive coughing thereby the curves reflected clearance predominantly due to mucociliary transport (Fazio & Lafortuna, 1981). Unproductive coughing however, results in a proximal shift of secretions within the human lung (chapter six). It is not possible at present to correct for this additive clearance component in the patients. Despite this, the tracheobronchial clearance for the sarcoid patients was statistically significantly slower than that of the healthy subjects; the magnitude of the observed impairment being probably an underestimate.

Instructed coughing at the end of the 6-hour observation period appears to have no effect on the overall tracheobronchial clearance which suggests that either the undue retention of radioaerosol was beyond the reach of productive coughing (Leith, 1968)
Fig. 3.4. Area under the tracheobronchial retention curve between 0 and 6 hours ($AUC_{0.6}$) for thirteen patients with sarcoidosis compared to thirteen matched healthy control subjects.
and/or inadequate hypersecretion which is necessary for productive coughing (Camner et al., 1979).

The rate of removal of inhaled deposited insoluble particles from the human lung depends on the site of their initial deposition. In general, particles deposited proximally in the airways will be cleared faster than those deposited more peripherally (other things being equal) because of the shorter ciliated pathway (Agnew et al., 1981). Every effort in this study was made to ensure similar topographical distribution of the radioaerosol within the lungs of the sarcoid patients and the healthy subjects. Despite this, the alveolar deposition, on average, was greater for the healthy subjects which should have resulted in a slower clearance. The fact that the particles for the healthy subjects were cleared significantly faster compared to the sarcoid patients must once again underestimate the degree of mucociliary clearance impairment observed in the sarcoid patients.

Although the number of sarcoid patients in each of the three groups was small, it is nevertheless of interest to note that the sarcoid patients in "apparent clinical remission" (group A) who had not required any medication during the course of their illness appeared to have the smallest degree of mucociliary transport impairment. This was equally true of those patients who were receiving oral corticosteroids. The results from both these groups were however in sharp contrast to those patients on inhaled corticosteroid therapy (group C). These patients appeared to have the greatest impairment of mucus transport. It must be stressed that patients in both group B and group C were clinically worse at presentation of their disease than those in group A, and as part of another study had received either inhaled or oral corticosteroids for their symptoms (Spiteri et al., 1989).

The lack of an association in the present study between pulmonary function indices and
impairment in lung mucociliary clearance is consistent with the finding reported in chronic bronchitics (Pavia, 1984a). On the other hand, a positive correlation has been reported in cystic fibrosis between MMFR_{25-75} and tracheal mucus velocity (Yeates et al., 1976). The mechanisms which may inter-relate lung function impairment and mucociliary defence impairment thus merit further investigation.

Lung mucociliary clearance has been reported to be impaired in other lung pathology even when apparently in clinical remission (Pavia et al., 1985a), e.g. chronic bronchitis (Agnew et al., 1982), bronchiectasis (Currie et al., 1987), asthma (Bateman et al., 1983). It would thus seem that mucociliary defence of the lung can readily be impaired prior to any demonstration of overt symptoms. The question then arising is how much this impaired clearance places the patient at risk of still further impairment from respiratory infection or a worsening of the underlying condition.

The short-term administration of inhaled corticosteroids in healthy subjects and chronic bronchitics has indicated no effect on mucociliary transport (Fazio & Lafortuna, 1986). Furthermore the administration of beclomethasone dipropionate in sheep has been shown not to alter the flow of mucus in the trachea (Sackner et al., 1977). Conversely, the long-term administration of oral corticosteroids in asthmatics has been shown to result in an enhancement of tracheobronchial clearance (Bateman et al., 1980) and in particular of mucus clearance from the peripheral airways (Agnew et al., 1984a).

It is therefore postulated that the improved mucus clearance in group B relative to group C may be attributed to the oral corticosteroid therapy which, systemic side effects apart, appears to be at least more effective as far as mucociliary transport is concerned than inhaled corticosteroid therapy. This may possibly be because of the larger doses of the drug being delivered systemically and ultimately reaching the lungs whereas via the inhaled route only some 10% of the metered dose is likely to be deposited in the lungs.
(Newman et al., 1981). Furthermore, since these patients have evidence of airways narrowing then this will have the tendency to reduce penetrance of the drug to the distal airways (Pavia et al., 1977a).

This is the first study to report that one of the primary host defence mechanisms of the human lung operating in the conducting airways, namely mucociliary clearance, is adversely affected in patients with sarcoidosis and the possible consequences of therapy upon it. This clearance defect represents an important aspect of airways involvement in this disease and thus adds to the growing impression reviewed above (section 3.2) that airways involvement in sarcoidosis is clinically significant.
CHAPTER FOUR

Tracheobronchial Clearance In Pigeon Fanciers

4.1. Summary

Lung mucociliary clearance was measured in fifteen pigeon fanciers - who were subdivided into two groups: group A (n=10) with circulating blood precipitins and group B (n=5) without circulating precipitins. The data for both groups A and B were compared with those of matched control groups of healthy subjects. The mean area under the tracheobronchial retention curves (AUC₀₋₆) over the six hour observation period was statistically significantly increased (clearance significantly retarded) for the pigeon fanciers compared with their respective healthy control groups in both groups A (257±27 vs. 177±16 %·h; p=0.02) and B (282±34 vs. 150±15 %·h; p=0.02). The presence or absence of circulating blood precipitins appears not to be related to the degree of mucociliary clearance impairment. The work presented in this chapter demonstrates that the hobby or "occupation" of pigeon fancying functionally affects the conducting airways and indeed compromises a major defence mechanism of these airways.

4.2. Introduction

Inhalation of organic material derived from pigeons is known to provoke hypersensitivity reactions (extrinsic allergic alveolitis, EAA) in the lungs of pigeon fanciers (Fink et al., 1968; Schatz & Patterson, 1983).

Circulating antibodies against a wide range of pigeon-derived material, including extract from serum, droppings, feathers and bloom have been demonstrated in the serum of pigeon fanciers (Edwards et al., 1970; Fredericks, 1978; Banham et al., 1982). Antibody response was more pronounced in pigeon fanciers with symptoms than in symptomless fanciers (Fink et al., 1968a; Banham et al., 1986; Reynolds et al., 1991).
Pathological studies and animal models of EAA suggest that inflammation is not confined to
the alveoli but also involves the bronchi and smaller airways (Seal et al., 1968; Schuyler et
al., 1983). Pigeon fanciers have a high prevalence of chronic bronchitis (Bourke et al.,
1989; Carrillo et al., 1990), large airways involvement and peripheral airways obstruction
(Allen et al., 1976; Bourke et al., 1989a) in addition to the well recognised restrictive
reported that 8.4% of the pigeon fanciers surveyed had chronic bronchitis as their only
manifestation of pigeon related symptomatology. The classical symptoms of affected pigeon
fanciers are shortness of breath, cough, malaise and bronchitis (Fink et al., 1972; Boyd et
al., 1982).

There is no information regarding the efficiency of lung mucociliary clearance in pigeon
fanciers and specifically whether this important host defence mechanism of the lung is in any
way compromised. The purpose of this study was to compare lung mucociliary clearance in
pigeon fanciers with and without circulating blood precipitins, with that of healthy subjects
tested under the same conditions.

4.3. Subjects
At a national meeting for pigeon racing, fifteen pigeon fanciers (PF) volunteered to
participate in the study; ten PF subjects (group A) had circulating blood precipitins and five
(group B) had no circulating precipitins.

For comparison purposes the data for both groups were compared with those of two
historical, control groups of healthy subjects. These two control groups (ten and five
subjects respectively) were selected from the department's "bank" of control data on the
basis of having similar physical characteristics, smoking habits and alveolar deposition (AD)
of inhaled radioaerosol as those of the two study groups but blind as to their
tracheobronchial clearance (i.e. by the same method of "matching" as in the previous
chapter).
The method of assessment of tracheobronchial clearance used for the controls was identical to that used for assessing tracheobronchial clearance in the PF subjects. The importance of matching as closely as possible for AD was to try to ensure that initial test particle distribution in the controls was closely similar to that in the PF subjects.

The mean±SEM physical characteristics, tobacco consumption and AD for PF and control subjects in groups A and B and for the whole group are shown in tables 4.1-4.3.

Nine of the ten PF subjects in group A had two or more of the following chest symptoms: cough, sputum, shortness of breath and wheeze. However only two subjects were on medication. One was taking inhaled salbutamol and the other inhaled salbutamol, ipratropium bromide and beclomethasone dipropionate. The dose of medication was taken at least 2.5 hours prior to the measurement of lung mucociliary clearance. In group B one subject reported shortness of breath and one other wheeze and none were on medication.

Informed consent was obtained from each subject prior to the commencement of the study which was approved by the hospital's ethical practices subcommittee.

4.4. Methods

4.4.1. Pulmonary Function Tests

The pulmonary function of each subject was assessed prior to the inhalation of the radioaerosol as described in chapter two (2.12). Furthermore, nine of the ten PF subjects with chest symptoms and circulating precipitins were able to attend the pulmonary function laboratory for measurement of their total lung capacity (TLC) and total diffusing capacity for carbon monoxide (TLCO) using the single breath method (P.K. Morgan-Model C). Measurements of $\dot{V}_{\text{max}}^{25}$, $\dot{V}_{\text{max}}^{50}$, TLC and TLCO were not available for the healthy control subjects.
Table 4.1.
Physical characteristics, tobacco consumption and alveolar deposition (AD) for ten pigeon fanciers (PF) with circulating precipitins (group A) and ten matched healthy control subjects (C).

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF</td>
<td>C</td>
<td>PF C</td>
<td>PF C</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>67</td>
<td>58</td>
<td>60*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>44*</td>
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<td></td>
<td>22</td>
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<tr>
<td>F</td>
<td>F</td>
<td>61</td>
<td>44</td>
<td>Ns</td>
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<td>Ns</td>
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<td>46</td>
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<tr>
<td>M</td>
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<td>4*</td>
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<td>7*</td>
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<td>49</td>
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<tr>
<td>M</td>
<td>M</td>
<td>33</td>
<td>30</td>
<td>4*</td>
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<td>48</td>
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<td></td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32</td>
</tr>
</tbody>
</table>

Mean 45 40 14* 9* 39 45
SE 5 3 9 7 5 3

Ns : Non-smoker.
* : Ex-smoker.
** : Current smoker.
Table 4.2.
Physical characteristics, tobacco consumption and alveolar deposition (AD) for five pigeon fanciers (PF) with no circulating precipitins (group B) and five matched healthy control subjects.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>C</td>
<td>PF</td>
<td>PF</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>51</td>
<td>37</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Ns : Non-smoker.
* : Current smoker.

Table 4.3.
Mean±SEM physical characteristics, tobacco consumption and alveolar deposition (AD) for the whole group of pigeon fanciers (n=15) with their matched healthy control (C) subjects (n=15).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tobacco Consumption (Pack-Year)</th>
<th>AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>C</td>
<td>PF</td>
<td>PF</td>
</tr>
<tr>
<td>14M/1F</td>
<td>45±4</td>
<td>39±3</td>
<td>14±6*</td>
</tr>
</tbody>
</table>

* : Smokers and Ex-Smokers (PF; n=10, C; n=7).
4.4.2. *Tracheobronchial Clearance*

Tracheobronchial clearance was measured using the radioaerosol technique which has been described in detail in chapter two (2.1.2).

Monodisperse polystyrene particles 5 μm in diameter, firmly labelled with technetium-99m, were inhaled by the pigeon fanciers in single breaths under strictly controlled conditions.

The initial whole lung burden was measured immediately after inhalation using two collimated scintillation detectors. Subsequently counts were made at half-hourly intervals for a total of 6 hours with a final count at 24 hour which was used to estimate AD. An index of the individual efficiency of tracheobronchial clearance was derived for each subject by measuring the area under the tracheobronchial retention curve (AUC0,6) between 0 and 6 hours.

During the 6 hour observation period all coughs were recorded and any sputum produced was collected and its radioactive content was assessed and added "back" to the retention curves in order to correct for the effect of productive coughing (as described in section 2.8). At the end of the observation period the pigeon fanciers were instructed to cough (3 double coughs per minute for 5 minute) in order to assess the quantity of the remaining radioaerosol which the cough action could clear at that time.

4.4.3. *Statistical Analysis*

Due to the small number of subjects, statistical analysis was undertaken using the two sample Wilcoxon test for unpaired data (Siegel, 1956). The level of significance to demonstrate effect was taken at p<0.05.

4.5. *Results*

Pulmonary function indices for the PF and control subjects in both groups A and B are shown in table 4.4.
Table 4.4.
Mean±SEM pulmonary function indices of two groups of pigeon fanciers (PF) with (A) and without (B) circulating precipitins with their respective healthy control groups (C).

<table>
<thead>
<tr>
<th>Pulmonary Function Indices</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF</td>
<td>C p</td>
</tr>
<tr>
<td>% Pred. FEV₁</td>
<td>86±7</td>
<td>113±3</td>
</tr>
<tr>
<td>% Pred. FVC</td>
<td>97±7</td>
<td>117±4</td>
</tr>
<tr>
<td>% Pred. MMFR₂₅-₇₅</td>
<td>71±11</td>
<td>101±10</td>
</tr>
<tr>
<td>% Pred. Vₘₐₓ₅₀</td>
<td>63±10</td>
<td>*</td>
</tr>
<tr>
<td>% Pred. Vₘₐₓ₂₅</td>
<td>45±8</td>
<td>*</td>
</tr>
<tr>
<td>% Pred. TLC</td>
<td>106±4</td>
<td>*</td>
</tr>
<tr>
<td>% Pred. TLCO</td>
<td>84±12</td>
<td>*</td>
</tr>
</tbody>
</table>

* : Measurements were not available.
NS: Non-Significant.
The table shows that subjects in group A had a tendency (but not one achieving statistical significance) towards reduced pulmonary function indices compared to their controls, whereas subjects in group B, without symptoms, had a similar lung function compared to their controls.

The mean alveolar depositions were similar for the PF and healthy subjects in group A (table 4.1), in group B the mean alveolar depositions were virtually identical (table 4.2).

The AUC$_{0-6}$ was statistically significantly larger for the PF subjects in both group A (p=0.02) and group B (p=0.02) compared to their respective healthy control groups (fig. 4.1). The mean±SEM AUC$_{0-6}$ for the PF subjects in group A was 257±27 %.h compared with 177±16 %.h for its control group; that for group B was 282±34 %.h compared to 150±15 %.h for its control group.

Figures 4.2 and 4.3 give the mean tracheobronchial retention curves for the PF subjects in both groups A and B compared to their respective healthy control groups. The mean tracheobronchial retention curves for the whole study group of fifteen PF subjects vs. the fifteen matched, healthy subjects used as control are shown in figure 4.4. Tracheobronchial clearance for the PF subjects was statistically significantly reduced (p<0.01) compared to the control group.

Mean±SEM AUC$_{0-6}$ was 263±24 vs. 170±10 %.h (p=0.02) for the five non-smoking PF subjects compared to their respective controls. Furthermore, the mean±SEM AUC$_{0-6}$ was 323±40 vs. 169±39 %.h (p>0.10) for the four current smoking PF subjects compared to their respective controls (fig.4.5). Within the PF group there were no significant differences in AUC$_{0-6}$ between the smoking categories.

Nine of the fifteen PF subjects (6 in group A) coughed during the six hour observation period. The range of the number of coughs per subject was 2-21.
Fig. 4.1. Area under the tracheobronchial retention curve between 0 and 6 hours (AUC<sub>0-6</sub>) for ten pigeon fanciers (PF) with circulating precipitins and five pigeon fanciers without circulating precipitins compared to matched healthy control subjects (C).
Fig. 4.2. Mean±SEM tracheobronchial retention curves for 10 pigeon fanciers (PF; group A) with circulating precipitins and 10 matched healthy control subjects (C).
Fig. 4.3. Mean±SEM tracheobronchial retention curves for 5 pigeon fanciers (PF; group B) without circulating precipitins and 5 matched healthy control subjects (C).
Fig. 4.4. Mean±SEM tracheobronchial retention curves for all 15 pigeon fanciers (PF) and all 15 matched healthy control subject (C). [significance of differences between curves was tested at 2, 4 and 6 hours].
Fig. 4.5. Area under the tracheobronchial retention curves between 0 and 6 hours (AUC\(_{0-6}\)) for 5 pigeon fanciers (non-smokers) and 4 pigeon fanciers (smokers) compared to matched healthy control subjects.
Of these nine subjects, five (4 in group A) produced sputum (range of wet weight: 0.2-1.9 g) containing % of initial T-B radioactive deposition in the range of 0.3-7.8.

At the end of the 6 hour observation period only two PF subjects, both in group A, produced sputum under instructed coughing (wet weight: 0.7 and 0.9 g) containing % of initial T-B radioactive deposition of 0.1 and 1.2 respectively. The mean±SEM tracheobronchial retention for the fifteen PF subjects before and after instructed coughing at 6 hours were similar (20±4 vs. 21±4 %).

4.6. Discussion

Although both FEV$_1$ and FVC for PF subjects in group A were within the "normal" range they were, nevertheless, significantly reduced compared to the matched control group. This observation together with the reduced flow rates for the tests of small airways function (MMFR$_{25-75}$, $\dot{V}_{max,50}$ and $\dot{V}_{max,25}$) may well reflect the early stages of onset of chronic bronchitis which has been reported to be present in a proportion of pigeon fanciers (Bourke et al., 1989). Since both total lung capacity and total diffusing capacity were within the normal range for PF subjects in group A then it is unlikely that restrictive defect, a well known phenomenon in pigeon fanciers, was present in this study population. PF subjects in group B, with absence of circulating precipitins and minimal chest symptoms, demonstrated normal lung function tests with the exception of $\dot{V}_{max,25}$ which was on average only 54 % of predicted.

The clearance of radioaerosol from the lungs depends on its initial site of deposition within the airways (Agnew et al., 1981). In general radioaerosol deposited proximally will be cleared more rapidly from the lungs than radioaerosol deposited peripherally within the lungs. In this study, it was possible to ensure that the radioaerosol deposition within the lungs (i.e. alveolar deposition) of the PF subjects was similar to that of the healthy control subjects in both groups A and B.
The use of historical controls is not ideal. However, it is not feasible to study a control group prospectively since it is impossible to ensure similar radioaerosol deposition within the lungs for the healthy subjects as for the PF subjects. Historical controls were therefore selected from a bank of data for a group of healthy subjects who inhaled radioaerosol at different flow rates to achieve a range of alveolar depositions.

Tracheobronchial clearance of the PF subjects in both groups were found to be statistically significantly reduced compared to their respective healthy control groups (figures 4.1-4.3). This finding indicates that the presence or absence of circulating precipitins is not related to defective mucociliary clearance. The presence of circulating precipitins was correlated with exposure and there was a progressive tendency towards sensitisation (Fink et al., 1972; Banham et al., 1986). However, the findings in this study population would suggest that level of exposure is immaterial to the degree of impairment in tracheobronchial clearance. Taking all PF subjects as a whole group (fig. 4.4), similar retardation was seen in tracheobronchial clearance at a higher level of statistical significance because of the increased number of subjects.

It is unlikely that cigarette smoking may have confounded the interpretation of the observed data since tracheobronchial clearance in the non-smoking PF subjects was slower (significantly) than the corresponding control group. It is of interest to note that the on average retardation in tracheobronchial clearance in the current smoking PF subjects compared to their controls was greater in magnitude than in the non-smoking group (154 vs. 93 %). Although the number of subjects in each group is low it is possible that smoking and exposure to pigeons may have a cumulative adverse effect on tracheobronchial clearance. The apparent lack of a difference in tracheobronchial clearance between smoking groups within the PF subjects can be attributed to differences in physical characteristics and alveolar deposition (e.g. AD for the non-smokers 42±6 % vs. 33±5 % for the smokers).
Cough appears to have played a small part in the overall tracheobronchial clearance with only five PF subjects producing sputum during the 6 hour observation period. This finding together with any help in tracheobronchial clearance due to unproductive coughing (chapter six) would result in an underestimate of the degree of reported impairment in lung mucociliary clearance. The lack of any significant enhancement in tracheobronchial clearance at 6 hours following instructed coughing would suggest that the undue retention of the radioaerosol was beyond the reach of productive coughing (Leith, 1968) i.e. probably beyond the seventh generation on Weibel's model of the human lung (Weibel, 1963) and/or due to lack of appropriate hypersecretion which is necessary for productive coughing (Camner et al., 1979; Puchelle et al., 1980).

It is of interest to note that the degree of lung mucociliary clearance impairment in PF subjects within group B was comparable to that seen for those subjects in group A with circulating precipitins, reduced lung function and increased symptomatology. This is consistent with the reported apparent lack of relationship between degree of impairment of lung mucociliary clearance and pulmonary function indices in patients with chronic bronchitis (Pavia, 1984a).

This study has demonstrated that lung mucociliary clearance is compromised in pigeon fanciers. This is further evidence of involvement of the large airways in these groups of subjects. Any reduction of lung mucociliary clearance will tend to increase the residence time of any inhaled antigen. Inhaled antigen has been shown to result in reduced lung mucociliary clearance (Ahmed et al., 1981) and undue retention of lung secretions has been reported to result in atelectasis thereby giving rise to an increased incidence of chest infections (Gamsu et al., 1976).

Avian precipitins have been shown to have a role, as a non-invasive test, in the diagnosis of bird breeder's disease (Reynaud et al., 1990).
This study using an objective, non-invasive technique has demonstrated that lung mucociliary clearance is compromised in pigeon fanciers irrespective of the presence or absence of avian precipitins. The data, albeit on a small number of subjects, appears to demonstrate that mucociliary clearance may have at best a limited relationship to immune activation in PF subjects.

The detection of an abnormal clearance in PF subjects with absence of avian precipitins may be akin to the observation by Lourenco and associates (1971) of abnormal mucociliary transport in asymptomatic smokers. Smokers who develop chronic bronchitis, where chest symptoms are present, are known to have impaired mucociliary clearance (Agnew et al., 1982). As such, abnormal mucus transport may be an early indicator of the disease process. Delayed clearance may represent one aspect of a vicious cycle (Cole, 1984) with prolonged retention at airway sites of inhaled antigen itself promoting a further decline in the efficacy of the mucociliary mechanism. This in turn could cause undue retention of lung secretions and thereby an increased incidence of chest infections (Pavia, 1987b).

The clearance deficit demonstrated in this study provides further evidence that pigeon fanciers suffer lung abnormalities involving the conducting airways. It is worthy of note that this degree of clearance dysfunction was detected in pigeon fanciers who had agreed to volunteer while at a meeting to do with their interest - and not when seeking medical attention.
CHAPTER FIVE

Effect Of Oral Bronchodilator Therapy on Lung Mucociliary Clearance During Sleep In Asthmatic Patients

5.1. Summary
Nine asthmatics (mean±SEM age: 65±5 year, % pred. FEV₁: 61±9) participated in a double-blind, placebo controlled, within subject crossover study to assess the effect of two weeks' treatment with salbutamol (Volmax®; 8 mg b.d.) or theophylline (Phyllocontin®; 350 mg b.d.) on lung mucociliary clearance during sleep. The observation period for radioaerosol clearance was approximately 0.3 hour pre-sleep, 6.0 hours during sleep and 0.6 hour post-sleep. Statistically the means of the highest morning and evening peak expiratory flow rates (l/min) were significantly greater after Phyllocontin® (morning: 259 vs. 236, p<0.01; evening: 275 vs. 260, p<0.02) and Volmax® (morning: 270 vs. 236, p<0.05; evening: 290 vs. 260, p<0.05) compared to placebo. Mean mucociliary clearance rates for Phyllocontin®, placebo and Volmax® for the pre-sleep period were: 39, 39 and 32 %/hr respectively; during sleep: 11, 10 and 9 %/hr respectively and post-sleep: 39, 32 and 35 %/hr respectively. The study showed that during sleep lung mucociliary clearance in stable asthma is reduced and that controlled/slow release oral bronchodilator therapy has no effect on sleep-associated slowing of clearance.

5.2. Introduction
Lung mucociliary clearance is impaired in patients with asthma (Agnew et al., 1983; Bateman et al., 1983). Furthermore, lung mucociliary clearance has been shown to be reduced during sleep in healthy subjects (Bateman et al., 1978) and in asthmatics (Pavia, 1984). This may contribute to additional airways obstruction, early morning cough and wheeze in asthmatics. It is also possible that this reduced mucus clearance during sleep may contribute to the mucus plugging known to be present in the small airways (Dunnill, 1971).
Beta-2 adrenergic drugs and methylxanthines in addition to their bronchodilating properties have been shown to enhance tracheobronchial clearance (Matthys & Kohler, 1980; Cotromanes et al., 1985). Indeed Sutton and colleagues (1981) showed that oral administration of sustained release aminophylline caused a significant increase in tracheobronchial clearance in patients with obstructive lung disease. A significant increase in mucociliary clearance rate was also observed by Fazio and Lafortuna (1981) in patients with chronic bronchitis after inhaling a high dose of salbutamol. Oral slow-release bronchodilators are often prescribed to patients who are not able to co-ordinate satisfactorily the use of inhaled bronchodilators. They are also used to treat nocturnal asthma.

This study compared the effects, in asthmatic patients, of controlled release salbutamol tablets (Volmax®, a β-2 agonist), Phyllocontin® Forte Continus tablets (a methylxanthine) and placebo on overnight changes in lung mucociliary clearance and on nocturnal control of respiratory symptoms.

5.3. Patients

Twelve (6 male) asthmatic patients volunteered for the study of whom three (2 female) were withdrawn. Two of these were withdrawn because of an exacerbation of their asthma; one during the run-in period and the other during the Volmax® period. The third patient was withdrawn because he was unable to manage, during the run-in period, without an oral bronchodilator.

The physical characteristics and tobacco consumption for the nine patients who completed the study are given in table 5.1. Their pulmonary function indices (when first seen) are given in table 5.2. Seven patients were on inhaled beta-2 agonists of whom three were also taking inhaled anticholinergic therapy. These seven patients were also taking inhaled corticosteroids and one other was on inhaled sodium cromoglycate only. The ninth patient was on no medication.
Table 5.1.

Physical characteristics and tobacco consumption for the nine asthmatics who completed the study.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>Sex (M/F)</th>
<th>Age (year)</th>
<th>Tobacco Consumption (Pack-Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>68</td>
<td>15.0*</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>61</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>68</td>
<td>4.6*</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>72</td>
<td>19.5*</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>74</td>
<td>2.5*</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>77</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>62</td>
<td>0.1*</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>26</td>
<td>1.5*</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>79</td>
<td>40.0*</td>
</tr>
</tbody>
</table>

Mean: 5M/4F
SD: 12.0*

NS: Non-smoker (n=2).
* : Ex-smoker (n=7).
Table 5.2.

% predicted pulmonary function indices for the nine asthmatics when first seen.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>FEV₁</th>
<th>FVC</th>
<th>FEV₁/FVC (observed)</th>
<th>PEFR</th>
<th>MMFR₂₅-₇₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>44</td>
<td>59</td>
<td>35</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>67</td>
<td>57</td>
<td>48</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>98</td>
<td>47</td>
<td>75</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>82</td>
<td>34</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>119</td>
<td>119</td>
<td>76</td>
<td>106</td>
<td>127</td>
</tr>
<tr>
<td>8</td>
<td>58</td>
<td>66</td>
<td>68</td>
<td>61</td>
<td>43</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>80</td>
<td>56</td>
<td>48</td>
<td>23</td>
</tr>
<tr>
<td>11</td>
<td>86</td>
<td>108</td>
<td>67</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>73</td>
<td>50</td>
<td>42</td>
<td>35</td>
</tr>
</tbody>
</table>

Mean | 61 | 82 | 57 | 58 | 38 |
SD   | 27 | 23 | 13 | 24 | 35 |
Five patients were also on oral bronchodilators (4 on methylxanthines and one on a beta-2 agonist) which were discontinued for the one-week run-in period and the duration of the trial.

All nine patients had demonstrated a 15% or greater increase of FEV\textsubscript{1} following the inhalation of 200\,µg of salbutamol and/or a 20% or greater difference between the maximum and the minimum peak expiratory flow rates (PEFR) obtained for that day on at least two days where PEFR had been measured on seven consecutive days.

5.4. Methods

5.4.1. Study Design

The study was a double blind, randomised, crossover (within patient) design of seven weeks' duration. Volmax\textsuperscript{®} tablets (8\,mg) b.d., Phyllocontin\textsuperscript{®} tablets (350\,mg) b.d. and placebo tablets (identical to the Volmax\textsuperscript{®} but not to the Phyllocontin\textsuperscript{®} tablets) were compared creating a three treatment study. The tablets were kept in opaque containers so that the investigator did not see the medications and the patients were told that they were going to try three different medications.

Patients underwent a one week run-in period when any oral bronchodilator therapy was discontinued. This was followed by three two-weekly treatment periods which were not separated by wash-out periods. Although the study design was that the three treatment periods followed one another immediately, in practice this was not possible always because of previous commitments by the patients and/or exacerbation of their asthma. Those patients who had an exacerbation did not proceed into the next period of the study until one month after the end of symptoms and/or cessation of any oral corticosteroid therapy.

The patients were allowed to take their regular treatment throughout the study, recording on diary cards the number of puffs taken daily of their usual beta-2 agonists and any other medication, as well as the usage of the test medication, during each two-week treatment
period. While receiving each treatment, the patients also recorded their PEFR on rising and before retiring at night (prior to using their inhaled bronchodilator). The diary cards were returned along with the medication containers and any unused tablets. The compliance of the patients regarding test medication was assessed by issuing a known amount of tablets to each patient and counting the number returned at the end of the trial. The last tablet was taken after the inhalation of radioaerosol, prior to sleep, under supervision, with the investigator "blind" as to the type of tablet.

Informed written consent was obtained from each patient. The study was approved by the hospital's ethical practices subcommittee.

5.4.2. Tracheobronchial Clearance

The tracheobronchial clearance was measured using the radioaerosol technique which has been described in detail in chapter two (2.1.2). Polystyrene particles (5 μm in diameter) labelled with $^{99}$Tc were inhaled by the patients. Mean inspiratory flow rate was recorded by a pneumotachygraph and recorded on a U.V. recorder.

The initial deposition of the tracer radioaerosol was measured using two scintillation detectors. A count was made immediately after inhalation and approximately at 0.3, 6 and 36 hours thereafter. All radioactivity counts were corrected for background and radioactive decay and expressed as a percentage of the initial count.

Sequential counts of lung activity were recorded over a period of typically 5 or 10 to 30 minutes past inhalation period to sleep. Similar counts were then recorded over a time period of some 40 minutes following the sleep period. For the pre- and post-sleep periods a least squares fit to the count versus time data was used to estimate clearance rate. For the sleep period clearance was calculated from the difference between mean readings at the beginning and end of that period. In each instance clearance was expressed in relationship to the lung radioactive content at the start of the period in question.
The initial topographical distribution of the radioaerosol within the lungs was measured by a gamma camera linked to a computer. The radioaerosol distribution was quantitatively expressed in terms of a penetration index (PI) as outlined in chapter two (2.7.2). The amount of radioaerosol present in the lungs at 36 hours was taken to be an estimate of alveolar deposition (AD).

During the observation period any sputum samples produced were collected, weighed and their radioactive content were ascertained.

5.4.3. Pulmonary Function Tests
The pulmonary function of each patient was measured, as described in chapter two (2.12), when first seen and during the half an hour which preceded the inhalation of the radioaerosol on each study night.

5.4.4. Statistical Analysis
The data were analysed with the nonparametric methods due to the small number of patients. The Wilcoxon signed rank sum test for matched pairs were used (Siegel, 1956). The level of significance was taken at p<0.05.

5.5. Results
5.5.1. Pulmonary Function
The mean of the highest morning and evening PEFR recorded in the diary cards for the nine patients who completed the study for the three treatment periods are shown in fig. 5.1. The highest morning PEFRs (l/min) were statistically significantly greater after Phyllocontin® (259 vs. 236; p<0.01) and Volmax® (270 vs. 236; p<0.05) compared to placebo as were the highest evening PEFRs (l/min) for Phyllocontin® (275 vs. 260; p<0.02) and Volmax® (290 vs. 260; p<0.05) compared to placebo.
Fig. 5.1. Mean of highest morning (A) and evening (B) peak expiratory flow rates (PEFR) for nine asthmatics during two weeks' treatment with Phyllocontin®, placebo and Volmax®.
The % predicted pulmonary function indices for the nine patients measured at the laboratory after two weeks on each treatment but prior to inhaling the test radioaerosol are given in table 5.3. On average, pulmonary function indices were greater following oral bronchodilator therapy of either kind compared to placebo although statistical significance was only attained between placebo and Volmax® for FEV₁ (p<0.05).

The mean±SEM of the daily number of puffs of inhaled bronchodilators were similar for the three treatment periods placebo, Phyllocontin® and Volmax®: 5.4±1.6, 4.8±1.6 and 5.3±1.6 puffs respectively. Usage of other medication remained unchanged during the three periods.

The mean±SEM compliance of trial medication for the study group was 99±2, 98±1 and 98±3 % for the placebo, Phyllocontin® and Volmax® respectively.

5.5.2. Tracheobronchial Clearance

Table 5.4 gives the mean±SEM radioaerosol inspiratory flow rate, alveolar deposition and penetration indices for the nine patients following the three treatments. The radioaerosol inspiratory flow rates were similar between the three treatments. Although on average the alveolar deposition and penetration indices were greater after Phyllocontin® and Volmax® compared to placebo the differences did not attain statistical significance.

The mean±SEM time for the pre-sleep observation period following radioaerosol inhalation for the placebo, Phyllocontin® and Volmax® study days were 23±5, 18±3 and 22±3 minutes respectively; the corresponding times during the sleep period were 350±9, 342±8 and 357±8 minutes respectively whilst the corresponding times for the three treatments post-sleep period were 38±6, 36±7 and 38±8 minutes respectively.

Fig. 5.2 gives the mean±SEM rate of clearance in %/hr pre-, during and post-sleep periods for the nine asthmatics following the three treatments.
Table 5.3.
Mean±SEM % predicted pulmonary function indices for the nine asthmatics following treatment.

<table>
<thead>
<tr>
<th>Pulmonary Function Indices</th>
<th>Placebo</th>
<th>Phyllocontin®</th>
<th>Volmax®</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>53±7</td>
<td>55±8</td>
<td>60±9*</td>
</tr>
<tr>
<td>FVC</td>
<td>75±8</td>
<td>77±9</td>
<td>79±9</td>
</tr>
<tr>
<td>FEV1/FVC (obs.)</td>
<td>55±4</td>
<td>55±4</td>
<td>57±5</td>
</tr>
<tr>
<td>PEFR</td>
<td>50±7</td>
<td>55±7</td>
<td>52±7</td>
</tr>
<tr>
<td>MMFR25-75</td>
<td>27±7</td>
<td>27±7</td>
<td>32±8</td>
</tr>
<tr>
<td>( \dot{V}_{max}50 )</td>
<td>18±4</td>
<td>26±7</td>
<td>21±6</td>
</tr>
<tr>
<td>( \dot{V}_{max}25 )</td>
<td>21±6</td>
<td>23±5</td>
<td>22±5</td>
</tr>
</tbody>
</table>

* \( P = <0.05 \) (compared to placebo).
Table 5.4.
Mean±SEM radioaerosol inspiratory flow rate, alveolar deposition and penetration indices for the nine asthmatics for each of the three treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insp. Flow Rate (l/min)</th>
<th>A.D. (%)</th>
<th>P.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>36±4</td>
<td>18±4</td>
<td>0.29±0.06</td>
</tr>
<tr>
<td>Phyllocontin®</td>
<td>30±2</td>
<td>26±6</td>
<td>0.38±0.10</td>
</tr>
<tr>
<td>Volmax®</td>
<td>30±4</td>
<td>27±6</td>
<td>0.42±0.12</td>
</tr>
</tbody>
</table>

P NS NS NS

NS : Not significant.
Fig. 5.2. Mean±SEM tracheobronchial clearance rates for nine asthmatics pre-, during and post-sleep periods following treatment with Phyllocontin®, placebo and Volmax®.
There were no statistically significant differences in the rates of radioaerosol clearance from the lungs between the three treatments for the pre-, during and post-sleep periods. Within each of the three treatments the rate of clearance during sleep was statistically significantly reduced (p<0.01) compared with pre- and post-sleep clearance.

Only one subject produced sputum during the observation period. The amount of sputum produced in the placebo run was 3.6 g and it contained 15 % of the initial tracheobronchial deposition. During the Phyllocontin® run the same subject produced 2.0 g of sputum containing 31 % of the initial tracheobronchial deposition.

5.6. Discussion

The morning and evening highest PEFR before use of inhaled bronchodilators were statistically significantly greater after either kind of oral bronchodilator therapy than the corresponding value for placebo.

The pulmonary function tests performed in the laboratory prior to inhaling the radioaerosol were on average greater following either kind of oral bronchodilator therapy compared to placebo, and significantly so for Volmax® as judged from the FEV₁ data. However, as free use of inhaled bronchodilators was permitted prior to attending the laboratory, the pulmonary function measurements could not reliably be expected to demonstrate differences between treatments. The concomitant use of inhaled bronchodilator therapy did however remain similar between the three treatment periods.

As already discussed in preceding chapters, the rate of tracheobronchial clearance of deposited radioaerosol depends on its site of deposition within the lungs. In this study both indices of topographical distribution of inhaled radioaerosol, namely AD and PI, were for both oral bronchodilators on average greater than placebo, although not by a significant margin, indicating possibly deeper penetration into the lungs following oral bronchodilator therapy.
The deeper the penetration of the radioaerosol into the lungs the slower will be its subsequent clearance because of the longer transit path (Agnew et al., 1983a; Langenback et al., 1990a). A possible explanation for the greater penetration might be the enhanced airway patency following bronchodilator therapy (Pavia et al., 1977a).

Tracheobronchial clearance comprises mucociliary clearance and cough. However, only one subject expectorated sputum on two of the three assessments. Because of this small contribution from productive coughing in this study, tracheobronchial clearance is likely to reflect mucociliary clearance per se.

Lung mucociliary clearance is (as has been reviewed in earlier chapters) impaired in various lung diseases such as chronic bronchitis, asthma and bronchiectasis. Enhancement of a depressed lung mucociliary clearance should be of benefit by reducing the residence time of bronchial secretions and thereby lessening the risk of chest infections (Pavia, 1987b). Mucolytic and expectorant drugs aim to enhance clearance of lung secretions but with varying degrees of clinical success (Richardson & Phipps, 1978; Clarke et al., 1980; Pavia et al., 1983; Olivieri et al., 1991). By contrast a bronchodilator is primarily administered for the relief of bronchospasm. If it also enhances clearance of secretions then this should be considered as an additional beneficial effect of the drug. Salbutamol (Fazio & Lafortuna, 1981) has been shown to enhance lung mucociliary clearance in chronic bronchitis when given topically from a metered dose inhaler, albeit at 2.5 times the normal recommended dose. Methylxanthines have been shown to enhance lung mucociliary clearance (Matthys et al., 1983; Schmidt et al., 1983) and in a study on patients with reversible airways obstruction the administration of 450 mg aminophylline b.d. (for one week) resulted in an enhancement of mucus clearance in 75% of the study population (Sutton et al., 1981).

During sleep, lung mucociliary clearance has been shown to be reduced in healthy subjects (Bateman et al., 1978) and in patients with asthma (Pavia, 1984).
A pilot double blind, cross over within subjects study on four asthmatics treated for one week with slow release aminophylline (450 mg b.d.) showed that three of the four asthmatics had a faster clearance during sleep whilst on aminophylline compared to placebo (Pavia et al., 1985).

This study provides strong evidence that during sleep lung mucociliary clearance in asthmatics is statistically significantly reduced compared to when the patients are awake. This is in agreement with previous observations from this laboratory (Pavia, 1984 & 1985). This retardation has been shown in normal subjects to be associated with sleep per se and not to circadian rhythm (Bateman et al., 1978). Reduction of clearance of lung secretions during sleep in addition to the already impaired lung mucociliary transport in asthmatics (Agnew et al., 1983; Bateman et al., 1983) may well contribute to the early morning wheeze, cough and dip in pulmonary function reported by these patients.

The study has however also demonstrated that two weeks' treatment with an oral controlled-release beta agonist or slow-release methylxanthine did not enhance lung mucociliary clearance during sleep in asthma. This finding contrasts with the observations reported with both these types of drugs in healthy subjects and patients when awake (Cotromanes et al., 1985; Fazio & LaFortuna, 1981; Matthys et al., 1983; Schmidt et al., 1983) and must therefore be regarded as disappointing.

The dose of methylxanthine in this study was lower to that used by Sutton and associates (1981) and Pavia and associates (1985) and could possibly explain the lack of effect. It was not possible to do blood theophylline levels to ascertain whether the dose of methylxanthine given to patients was within the therapeutic window (10-20 µg/ml).

Mucus secretion rates may decrease during sleep, thus lessening the load applied to the mucociliary escalator. This data suggest that the asthmatic patients who fall asleep with partly blocked airways are very unlikely to clear those airways during the night.
The long night-time residence of secretions containing infective or inflammatory agents could well contribute significantly to progression of symptoms.

A clear case has been established by this investigation for further studies on mucociliary clearance during sleep. Such studies should look for the harmful effects of secretions retained overnight. They should also investigate whether other pharmacological agents could influence night-time clearance.
CHAPTER SIX

Effect Of Unproductive Cough And Forced Expiration Technique On Regional Mucus Transport In Patients With Airways Obstruction

6.1. Summary

This study investigates the regional mucus transport in a group of subjects with airways obstruction who failed to expectorate following instructed cough and forced expiration technique. Fourteen patients (mean±SEM age: 68±2 years) with airways obstruction (mean±SEM % predicted FEV₁: 54±5; daily wet weight sputum: 9.1±2.0 g) took part in the study which was a controlled, randomised, three-way crossover within-patient design. Each patient underwent three treatment manoeuvres: control, cough and forced expiration. There was no correlation between the radioaerosol clearance from all regions and (a) mean 24 hour sputum production, (b) % reversibility in FEV₁. There were no differences in regional radioaerosol clearance between cough and forced expiration. However, both cough and forced expiration resulted in significant clearance compared to control for all regions with the exception of the forced expiration in the outer region. This study is the first to demonstrate that unproductive cough and forced expiration results in movement of secretions proximally from all regions of the lung.

6.2. Introduction

Cough is a back-up mechanism for mucus clearance. It comes into action in health during emergency situations, and in lung disease where often the mucociliary clearance mechanism is compromised (chapter one, 1.3.2.3).

It has been argued, from theoretical models, that cough is effective in clearing secretions from the trachea down to the 7th or 8th airway generation in Weibel's model of the human lung (Leith, 1968). Scherer (1981) has concluded from an in-vitro study that in the presence of excess secretions the efficacy of cough in clearing secretions could be extended down to the respiratory bronchioles, i.e. the 17th airway generation.
The forced expiration technique (FET) or "huffing" was introduced by physiotherapists as an alternative to coughing for the removal of excess lung secretions (Thompson, 1973). FET is claimed to reduce transpulmonary pressure compared to cough so that airway compression and closure are less (Langlands, 1967). It has been claimed that FET results in an enhancement of mucus clearance compared to cough (Pryor et al., 1979).

Non-invasive, radioaerosol techniques have been used to evaluate the efficacy of cough and FET in clearing excess secretions from the human lung (chapter one, 1.3.3.4, 5). These studies have either examined the effect of cough/FET on regional mucus clearance (Oldenburg et al., 1979; Bateman et al., 1981; Rossman et al., 1982) or on whole lung clearance (Yeates et al., 1975; Puchelle et al., 1980; Mossberg et al., 1981; Sutton et al., 1983). For effective clearance of inhaled radioaerosol particles it has been found that the presence of excess secretions is essential (Camner et al., 1979).

This study examines the regional mucus movement following cough and FET compared to control in a group of patients with airways obstruction who did not expectorate under instructed coughing and FET conditions.

6.3. Patients

Fourteen subjects were studied; six had the diagnosis of chronic bronchitis, four had asthma, three had a mixture of chronic bronchitis and asthma and one had bronchiectasis. The physical characteristics and tobacco consumption of the group are shown in table 6.1.

Two chronic bronchitic patients and the one with bronchiectasis were on no medication. Of the remaining eleven patients, all were taking inhaled bronchodilators, five were also taking methylxanthines. Ten of these eleven patients were on inhaled steroids, two of whom were also taking oral corticosteroids.
Table 6.1.
Physical characteristics and tobacco consumption of the patients.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>Sex (M/F)</th>
<th>Age (year)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>Tobacco Consumption (pack-year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>76</td>
<td>1.76</td>
<td>64</td>
<td>62.5*</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>75</td>
<td>1.63</td>
<td>72</td>
<td>51.0*</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>65</td>
<td>1.71</td>
<td>88</td>
<td>165.0*</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>62</td>
<td>1.94</td>
<td>73</td>
<td>NS</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>76</td>
<td>1.73</td>
<td>67</td>
<td>112.5*</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>67</td>
<td>1.90</td>
<td>86</td>
<td>15.0**</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>53</td>
<td>1.58</td>
<td>51</td>
<td>25.5**</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>71</td>
<td>1.70</td>
<td>56</td>
<td>62.5**</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>77</td>
<td>1.71</td>
<td>64</td>
<td>67.5*</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>71</td>
<td>1.70</td>
<td>91</td>
<td>10.0*</td>
</tr>
<tr>
<td>30</td>
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<td>50</td>
<td>1.67</td>
<td>75</td>
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</tr>
<tr>
<td>37</td>
<td>F</td>
<td>72</td>
<td>1.61</td>
<td>46</td>
<td>50.0*</td>
</tr>
<tr>
<td>41</td>
<td>M</td>
<td>68</td>
<td>1.72</td>
<td>85</td>
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</tr>
<tr>
<td>42</td>
<td>M</td>
<td>70</td>
<td>1.81</td>
<td>67</td>
<td>1.5*</td>
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</tbody>
</table>

Mean: 12M/2F 68 1.73 70 58.9*
SD: 8 0.10 14 52.9*

NS: Non-smoker (n=2).
*: Ex-smoker (n=9).
**: Smoker (n=3).
The percent predicted (based on sex, age and height) pulmonary function indices of the group when first seen are shown in table 6.2. The degree of airways reversibility (% change in FEV₁ above baseline) following two puffs (100 µg each) of salbutamol from a metered dose inhaler and the mean daily wet weight of sputum for the study group are given in table 6.3.

Informed written consent was obtained from all patients and the study was approved by the hospital’s ethics practices subcommittee.

6.4. Methods

6.4.1. Study Design

The study was a controlled, randomised, three-way crossover within patients design. Each patient attended on three separate occasions at least three days apart and underwent an identical experimental procedure with the exception of the three treatment manoeuvres. These manoeuvres were control, cough and PET. During the cough manoeuvre the patients were instructed to perform six coughs per minute for five minutes with one minute rest period every six coughs. During the PET manoeuvre the patients were instructed to perform six forced expirations (with the glottis open) per minute starting from approximately mid-lung volume and ending the exhalation close to residual volume for five minutes with one minute rest period after every six PETs. During the control run the patients rested and were asked to refrain from coughing. All three treatment manoeuvres were carried out with the patients sitting in the upright position.

All patients were asked to refrain from taking any inhaled bronchodilators for at least two hours prior to each of the three visits to the laboratory. They also were instructed to collect all sputum over a 24 hour period prior to each visit.
Table 6.2.

Pulmonary function indices expressed as % predicted values (based on sex, age and height) of the group when first seen in the laboratory.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>FEV₁</th>
<th>FVC</th>
<th>PEFR</th>
<th>MMFR₂₅-₇₅</th>
<th>( \dot{V}_{\text{max}50} )</th>
<th>( \dot{V}_{\text{max}25} )</th>
</tr>
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<td>20</td>
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<td>14</td>
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Table 6.3.

Degree of airways reversibility and mean daily wet weight of sputum for the study group.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>Reversibility of airways obstruction; change in FEV₁ (%)</th>
<th>Mean daily sputum* wet weight (g)</th>
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<td>2.8</td>
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<tr>
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<td>0.5</td>
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<tr>
<td>Mean</td>
<td>14</td>
<td>9.0</td>
</tr>
<tr>
<td>SD</td>
<td>9</td>
<td>7.6</td>
</tr>
</tbody>
</table>

* : Mean of three 24 hour assessments.
The mean wet sputum weight for the three collections was used to describe the daily sputum production for each patient.

6.4.2. Radioaerosol Technique

The radioaerosol technique, which has been outlined in chapter two (2.1.2) was used to monitor mucus movement within the lungs of patients. Polystyrene particles 5 μm in diameter were firmly tagged with the radionuclide ⁹⁹ᵐTc. The radioaerosols were inhaled, under strictly controlled conditions, through the mouth. Discrete breaths of 0.45 litre were taken from the resting level of the lungs by each patient and followed by a 3 second breathhold in order to enhance the peripheral deposition of the radioaerosol within the lungs.

Immediately after inhalation and 24 hours later the amount of radioactivity present in the lungs was measured by two axially opposed scintillation detectors. The 24 hour radioaerosol retention, corrected for background radiation and physical decay of the radionuclide, was expressed as a percentage of the initial value and was taken to represent alveolar deposition (AD).

6.4.3. Regional Tracheobronchial Clearance

The initial topographical distribution and subsequent clearance of the radioaerosol from the lungs was measured using a large field-of-view gamma camera linked to a computer as outlined in chapter two (2.7.2). This distribution was expressed quantitatively in terms of a penetration index (PI).

Interpretation from the gamma camera images of the radioaerosol clearance was based on dividing the lung into four arbitrary regions (fig. 6.1): tracheal region (comprising mainly the trachea), an inner region (comprising mainly large airways), an intermediate region and an outer region (comprising mainly small airways). The division of the lung image into the four regions was based on fitting a 5x8 matrix as closely as possible to the outer contours of a ⁸¹mKr image.
Fig. 6.1. Definition of four regions (Tra: Tracheal region; Inn: Inner region; Int: Intermediate region; Out: Outer region) relative to contours (15% and 30%) of $^{81m}$Kr ventilation image.
The regional clearance of the radioaerosol was corrected for those particles which were deposited in the alveolated regions and for inter-regional movement of particles.

Each of the three treatment manoeuvres commenced within 10-15 minutes post-inhalation of the radioaerosol. A final image of the distribution of the radioaerosol within the lungs was taken immediately after the completion of each manoeuvre.

All patients were provided with sputum containers and were encouraged to expectorate any sputum during the cough and FET manoeuvres.

6.4.4. Pulmonary Function Tests
The patients had their pulmonary function assessed as described in chapter two (2.12). In order not to disturb secretions within the lungs the pulmonary function tests were conducted on each of the three study days after the radioaerosol assessments were completed.

6.4.5. Statistical Analysis
The data were not assumed to be normally distributed and the following non-parametric statistical techniques were used in the analysis: paired Wilcoxon test and Spearman rank correlation coefficient (Siegel, 1956). The value of p<0.05 was taken to represent the level of significance of the data.

6.5. Results
The mean±SEM pulmonary function indices for the group for the three study days are given in table 6.4. There were no statistically significant differences in any of the pulmonary function indices.

The mean±SEM inspiratory flow rate, alveolar deposition and penetration index for the control, cough and FET runs were shown in table 6.5. There were no differences in these three parameters between any two of the study days.
Table 6.4.
Mean±SEM pulmonary function indices of the group for the three study days.

<table>
<thead>
<tr>
<th>Pulmonary Function Indices</th>
<th>Control</th>
<th>Cough</th>
<th>FET</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Pred. FEV₁</td>
<td>52±5</td>
<td>55±4</td>
<td>55±5</td>
</tr>
<tr>
<td>% Pred. FVC</td>
<td>74±3</td>
<td>76±3</td>
<td>76±3</td>
</tr>
<tr>
<td>% Pred. PEFR</td>
<td>55±5</td>
<td>53±5</td>
<td>54±5</td>
</tr>
<tr>
<td>% Pred. MMFR₂₅₋₇₅</td>
<td>30±6</td>
<td>29±5</td>
<td>29±5</td>
</tr>
<tr>
<td>% Pred. ( \dot{V}_{\text{max}50} )</td>
<td>22±6</td>
<td>19±5</td>
<td>24±7</td>
</tr>
<tr>
<td>% Pred. ( \dot{V}_{\text{max}25} )</td>
<td>20±4</td>
<td>17±3</td>
<td>20±4</td>
</tr>
</tbody>
</table>

Table 6.5.
Mean±SEM inspiratory flow Rate (\( \dot{V}_{\text{Insp}} \)), alveolar deposition (AD) and penetration index (PI) of the patients for the three treatment manoeuvres.

<table>
<thead>
<tr>
<th>Treatment Manoeuvre</th>
<th>( \dot{V}_{\text{Insp}} ) (l/min)</th>
<th>AD (%)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40±4</td>
<td>27±3</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>Cough</td>
<td>40±3</td>
<td>25±4</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>FET</td>
<td>37±3</td>
<td>26±3</td>
<td>0.42±0.05</td>
</tr>
</tbody>
</table>
Figure 6.2 gives the mean±SEM initial tracheobronchial distribution of the radioaerosol within the four regions of the lungs for the three treatment manoeuvres. There were no statistically significant differences between any two of the three study days for any one region.

The amount of tracheobronchial radioaerosol clearance from the lungs expressed as a percentage of initial deposition during the three study days was (mean±SEM) 5±1, 16±3 and 14±3 % for the control, cough and FET treatments respectively. Both cough and FET cleared significantly (p<0.01) more radioaerosol than control; there was no difference in clearance however between cough and FET.

The mean±SEM radioaerosol clearance from the outer, intermediate, inner and tracheal regions of the lungs are shown in figure 6.3. There were no differences in regional clearance between cough and FET. However both cough and FET resulted in significant differences in clearance compared to control for all regions save for the FET which failed to attain statistical significance in the outer region only (p>0.01).

There was no correlation between the radioaerosol tracheobronchial clearance from all regions and (a) mean 24 hour sputum production by the patients and (b) % reversibility in FEV₁ following 200 μg of salbutamol.

6.6. Discussion

Although the patients were instructed to collect sputum during the treatment manoeuvres, they were unable to produce any in either the cough and FET runs. These patients therefore provide a unique population of subjects in whom to examine whether cough or FET can produce any cephalad movement of radioaerosol within the lungs despite the absence of expectoration. These patients did have modest hypersecretion as indicated by the three 24 hour sputum collections although only in five did the daily amount of sputum collected exceed 10 g.
Fig. 6.2. Mean±SEM initial tracheobronchial deposition of the radioaerosol within the four regions of the lungs during the three treatment manoeuvres.
Fig. 6.3. Mean±SEM tracheobronchial clearance of the radioaerosol from the four regions of the lungs during the three treatment manoeuvres.
Studies of the effects of various physiotherapy manoeuvres on the clearance of lung secretions using the radioaerosol technique have in general focussed on patients with daily sputum production in excess of 30 g (Bateman et al., 1979; Sutton et al., 1983). The data of this study should re-open the debate on who can benefit from chest physiotherapy. Production of appreciable sputum amounts has been presented as a yardstick of success or failure for chest physiotherapy (Murray, 1979). Such an approach has been widely accepted in analysing the efficacy of percussion, vibration and postural drainage. The ketchup bottle must contain appreciable ketchup if it is to be emptied (Murray, 1979); evidence of its emptying will then become visible. Perhaps FET can work more quietly. The results of this study suggest that long term FET, a relatively undemanding form of therapy, might help to avoid prolonged retention of airway secretions even in the patients who overtly produce little sputum.

The patients remained stable throughout the period of their three assessments; this was supported by the similarity of the pulmonary function tests on the three study days as well as in the initial topographical distribution of the tracer radioaerosol as assessed by the PI and AD.

It has previously been reported that unproductive coughing in both healthy subjects and patients did not result in any significant loss of radioaerosol particles from the lungs (Yeates et al., 1975; Camner et al., 1979; Puchelle et al., 1980). More recently Bennett and associates showed an improvement of whole lung clearance after instructed coughing in healthy subjects (1990) but not in a group of asymptomatic smokers (1992).

This study is the first to demonstrate that in mild hypersecretion apparently unproductive cough/FET results in movement of secretions proximally from all regions of the lungs. As such, unproductive coughing must be aiding the well documented compromised mucociliary transport in such patients. It is therefore of importance that due allowance be made for unproductive coughing when interpreting lung mucociliary clearance curves.
Mucus clearance during the control run reflects transportation of lung secretions due to the mucociliary mechanism alone. Cough and FET appear to be equally effective in enhancing movement of secretions proximally from all regions. The observation that cough significantly enhanced clearance from the outer region of the lungs implies that cough is effective in distal ciliated airways as few large airways are present in the outer region analysed.

These data cannot indicate which specific airway generations are represented in the outer region in which cough is effective. Taking together modelling studies on aerosol deposition (Gerrity et al., 1979; Agnew et al., 1982) and the dimension of the outer region, it is reasonable to suggest that both cough and FET were in those patients probably at least effective down to airways equivalent to generations 10 or 12 of the Weibel model of the lung (Weibel, 1963). The mean outer region clearance was considerably higher with both cough and FET for at least some of the patients, it is therefore possible that cough and FET may - in accord with the in-vitro data of Scherer (1981) - have been effective down to the most distal ciliated airways.

Recently, it has been speculated that clearance of radioaerosol particles achieved during cough in the lungs of healthy subjects may be due to stimulation of the mucociliary mechanism by the high-velocity expired air rather than via a two-phase gas-liquid flow mechanism (Bennett et al., 1990). Thus the enhancement of clearance by unproductive cough from the outer region of the lungs might be explained by this stimulation.

The study has shown no apparent association of mucus clearance from any region with the mean daily sputum production and the degree of airways reversibility following the administration of bronchodilator.
CHAPTER SEVEN
Regional Lung Clearance During Productive Cough
And Forced Expiration Technique

7.1. Summary
Regional mucus movement within the lungs was investigated in a group of patients with
airways obstruction who expectorated after instructed cough and forced expiration technique
(FET). Nineteen patients (11M/8F) participated in this controlled, randomised and
crossover study. Their mean±SEM age, % predicted FEV₁ and daily sputum wet weight
were 64±2 years, 52±6 % and 37.5±7.9 g respectively. Each patient underwent three
assessments: control, cough and FET. Compared to the control run (mean±SEM clearance:
16±3 %), there was a statistically significant (p<0.01) increase in clearance from the whole
lung during cough (44±5 %) and FET (42±5 %). Both significantly (p<0.01) enhanced
clearance of inhaled, deposited radioaerosol from the trachea, inner and intermediate regions
of interest of the lungs but not from the outer region. There were however no differences in
regional clearance between cough and FET. Neither regional nor total clearance correlated
with the amount of sputum expectorated during the assessments and the daily sputum
production of the patients prior to the study.

7.2. Introduction
Cough is a reserve defence mechanism for removal of lung secretions. It is particularly
important as a back-up mechanism in airway diseases where often the primary host defence
mechanism for mucus clearance, mucociliary transport, is impaired (Chapter one, 1.3).

Several studies have addressed the question of cough efficacy in clearing inhaled, deposited
radioaerosols from the lungs of healthy non-smokers (Camner et al., 1979; Puchelle et al.,
1980; Bennett et al., 1990), asymptomatic smokers (Bennett et al., 1992) and patients with
lung disease (Camner et al., 1979; Oldenburg et al., 1979; Puchelle et al., 1980; Bateman et
al., 1981; Rossman et al., 1982; Agnew et al., 1982; Sutton et al., 1983; Agnew et al.,
Only three of these studies examined the effectiveness of cough in clearing lung secretions on a regional basis from the lungs (Oldenburg et al., 1979; Bateman et al., 1981; Rossman et al., 1982). The other studies considered effectiveness only for mucus clearance from the lungs as a whole. The regional clearance studies have given conflicting evidence as to cough effectiveness in peripheral regions of the lungs.

The forced expiratory technique (FET) or "huffing" was introduced as an alternative to coughing for the removal of excess lung secretions (Thompson, 1973). Its introduction was based on the claim that it reduces transpulmonary pressure compared to cough, thereby resulting in less airway compression and closure (Langlands, 1967). Its effect on mucus clearance from the lungs as a whole using radioaerosols has been studied in patients with hypersecretion (Sutton et al., 1983).

This study investigates the effect of productive cough and FET on regional mucus clearance in a group of patients with airways disease. This study also looks at the relationship between cough/FET and a measure of these patients' hypersecretion.

7.3. Patients

Nineteen patients participated in the study. Their physical characteristics and tobacco consumption are shown in table 7.1.

Twelve patients (6 female) had chronic obstructive pulmonary disease (COPD) and seven (2 female) bronchiectasis (B). Fifteen patients were on inhaled bronchodilators of whom eight were also taking oral bronchodilator therapy and twelve were maintained with inhaled corticosteroid therapy (one of them was also taking oral corticosteroids). One patient was on home nebuliser bronchodilator therapy and four patients (2 COPD and 2 B) were on no medication.
Table 7.1.
Physical characteristics and tobacco consumption of the study group.

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<th>Weight (kg)</th>
<th>Tobacco Consumption (pack-year)</th>
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<tbody>
<tr>
<td>11M/8F</td>
<td>64</td>
<td>1.63</td>
<td>63</td>
<td>31.4*</td>
</tr>
<tr>
<td>SD</td>
<td>9</td>
<td>0.09</td>
<td>14</td>
<td>15.4*</td>
</tr>
</tbody>
</table>

NS : Non-smoker (n=4); * : Ex-smoker (n=13); ** : Smoker (n=2).
The pulmonary function indices of the group when first seen are shown in table 7.2. The degree of airways reversibility (% change in FEV₁ above baseline) following two puffs (100 μg each) of salbutamol and the daily sputum wet weight production for the group are given in table 7.3.

7.4. Methods

7.4.1. Study Design

The study was designed in a randomised, three-way and crossover (within patients) manner. Each patient attended the laboratories on three occasions separated by at least three days. During each visit the patient underwent an identical experimental procedure but performed one of three treatment manoeuvres (similar to those of chapter six). These manoeuvres were cough, FET and control which were carried out with the patient sitting in the upright position. During the cough manoeuvre the patient was instructed to perform six coughs per minute, repeated five times, with a rest period of one minute every six coughs. During the FET manoeuvre the patient was instructed to perform six forced expirations per minute with the glottis open, starting from approximately mid-lung volume and ending the exhalation close to residual volume, for five minutes with one minute rest period after every six forced expirations. During the control manoeuvre the patient just rested with no coughs or forced expirations.

The patients were provided with sputum containers and were encouraged to expectorate any sputum during the cough and FET manoeuvres. They were also instructed to collect all sputum produced over a 24 hour period prior to each visit. The patients were asked to refrain from taking any inhaled bronchodilators for at least two hours prior to each visit to the laboratories.

Informed written consent was obtained from all the patients and the study was approved by the hospital's ethical practices sub committee.
Table 7.2.

Pulmonary function indices (expressed as % predicted values) of the group when first seen.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>FEV₁</th>
<th>FVC</th>
<th>PEFR</th>
<th>MMFR₂₅-₇₅</th>
<th>Vₘₐₓ50</th>
<th>Vₘₐₓ25</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>26</td>
<td>44</td>
<td>29</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>76</td>
<td>39</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>79</td>
<td>63</td>
<td>38</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>108</td>
<td>108</td>
<td>84</td>
<td>85</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td>14</td>
<td>84</td>
<td>84</td>
<td>105</td>
<td>79</td>
<td>61</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>59</td>
<td>61</td>
<td>73</td>
<td>48</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td>19</td>
<td>42</td>
<td>56</td>
<td>37</td>
<td>21</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>41</td>
<td>86</td>
<td>60</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>21</td>
<td>21</td>
<td>31</td>
<td>32</td>
<td>8</td>
<td>19</td>
<td>37</td>
</tr>
<tr>
<td>23</td>
<td>68</td>
<td>93</td>
<td>48</td>
<td>38</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>91</td>
<td>66</td>
<td>37</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>26</td>
<td>53</td>
<td>68</td>
<td>68</td>
<td>22</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td>27</td>
<td>25</td>
<td>61</td>
<td>22</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>29</td>
<td>38</td>
<td>66</td>
<td>34</td>
<td>17</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>31</td>
<td>39</td>
<td>59</td>
<td>35</td>
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<td>8</td>
<td>13</td>
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<tr>
<td>33</td>
<td>33</td>
<td>45</td>
<td>41</td>
<td>14</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>35</td>
<td>83</td>
<td>82</td>
<td>79</td>
<td>54</td>
<td>52</td>
<td>70</td>
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<td>39</td>
<td>29</td>
<td>55</td>
<td>29</td>
<td>13</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>40</td>
<td>65</td>
<td>71</td>
<td>63</td>
<td>27</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Mean</td>
<td>52</td>
<td>69</td>
<td>53</td>
<td>29</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>SD</td>
<td>24</td>
<td>19</td>
<td>23</td>
<td>23</td>
<td>18</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 7.3.
Pre- and post-bronchodilator FEV₁ (absolute values), degree of airways reversibility, and mean daily sputum wet weight for the study group.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>FEV₁ (pre; litre)</th>
<th>FEV₁ (post; litre)</th>
<th>Reversibility (change in FEV₁; %)</th>
<th>Mean daily sputum* (wet weight; g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.83</td>
<td>0.88</td>
<td>6</td>
<td>50.9</td>
</tr>
<tr>
<td>7</td>
<td>1.27</td>
<td>1.35</td>
<td>6</td>
<td>34.0</td>
</tr>
<tr>
<td>8</td>
<td>1.77</td>
<td>1.99</td>
<td>12</td>
<td>28.2</td>
</tr>
<tr>
<td>10</td>
<td>3.91</td>
<td>4.19</td>
<td>7</td>
<td>35.9</td>
</tr>
<tr>
<td>14</td>
<td>1.81</td>
<td>1.81</td>
<td>0</td>
<td>18.5</td>
</tr>
<tr>
<td>15</td>
<td>1.71</td>
<td>1.70</td>
<td>0</td>
<td>145.0</td>
</tr>
<tr>
<td>19</td>
<td>0.90</td>
<td>0.90</td>
<td>0</td>
<td>25.1</td>
</tr>
<tr>
<td>20</td>
<td>1.47</td>
<td>1.59</td>
<td>8</td>
<td>28.1</td>
</tr>
<tr>
<td>21</td>
<td>0.20</td>
<td>0.27</td>
<td>35</td>
<td>8.5</td>
</tr>
<tr>
<td>23</td>
<td>1.28</td>
<td>1.79</td>
<td>40</td>
<td>6.5</td>
</tr>
<tr>
<td>25</td>
<td>1.39</td>
<td>1.38</td>
<td>0</td>
<td>75.5</td>
</tr>
<tr>
<td>26</td>
<td>1.06</td>
<td>1.06</td>
<td>0</td>
<td>78.1</td>
</tr>
<tr>
<td>27</td>
<td>0.86</td>
<td>0.86</td>
<td>0</td>
<td>33.0</td>
</tr>
<tr>
<td>29</td>
<td>0.82</td>
<td>0.92</td>
<td>12</td>
<td>36.2</td>
</tr>
<tr>
<td>31</td>
<td>0.86</td>
<td>0.86</td>
<td>0</td>
<td>21.9</td>
</tr>
<tr>
<td>33</td>
<td>0.79</td>
<td>0.79</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>35</td>
<td>1.16</td>
<td>1.70</td>
<td>6</td>
<td>10.4</td>
</tr>
<tr>
<td>39</td>
<td>0.76</td>
<td>0.84</td>
<td>11</td>
<td>67.4</td>
</tr>
<tr>
<td>40</td>
<td>1.15</td>
<td>1.47</td>
<td>28</td>
<td>8.3</td>
</tr>
</tbody>
</table>

| Mean          | 1.26              | 1.39               | 9                                 | 37.5                             |
| SD            | 0.76              | 0.82               | 12                                | 34.5                             |

* : Mean of three 24 hour assessments.
7.4.2. Radioaerosol Technique

To assess movement of mucus within the patient's lungs the radioaerosol technique, which has been described in detail in chapter two (2.1.2), was used. 5 μm polystyrene particles labelled with $^{99m}\text{Tc}$ were inhaled under controlled conditions through the mouth. Each patient inhaled discrete breaths of 0.45 litre which was followed by a breath-hold pause (3 seconds).

Two collimated scintillation detectors located anteroposteriorly to the chest were used to ascertain the radioactivity present in the lungs immediately after inhaling the radioaerosol and 24 hours later. The 24 hour particle retention was taken to represent alveolar deposition (AD) within the lungs. The scintillation detectors were also used before and after pulmonary function testing.

7.4.3. Regional Clearance

A large field-of-view gamma camera linked to a computer was used to assess the initial topographical distribution, which was expressed as a penetration index (PI), and subsequent clearance of the radioaerosol particles from the lungs as described in chapter two (2.7.2).

The measurement of the radioaerosol clearance was based on dividing the gamma camera images of the lung into four arbitrary regions as outlined in chapter six (6.4.3.; fig.6.1). A 5x8 matrix was fitted as closely as possible to the outer contours of a $^{81m}\text{Kr}$ ventilation image, which was taken for each patient, to define the four regions of the lung. The regional clearance of the radioaerosol particles was corrected for alveolar deposition and inter-regional movement.

Images of the distribution of the radioaerosol particles within the lungs were taken immediately before and after each of the three treatment manoeuvres which were commenced within 10-15 minutes after the radioaerosol inhalation.
7.4.4. *Pulmonary Function Tests*

The pulmonary function for each patient was assessed as outlined in chapter two (2.12). The tests to ascertain the lung function were carried out on each of the three visits after the radioaerosol assessments were completed in order not to disturb the lung secretions.

7.4.5. *Statistical Analysis*

The data were analysed using the following non-parametric statistical methods: paired Wilcoxon and Spearman rank correlation (Siegel, 1956). The level of significance for effect was taken at p<0.05.

7.5. *Results*

The mean±SEM pulmonary function indices for the group during the three study days are given in table 7.4. The patients' pulmonary function remained unchanged between the visits.

The initial topographical distribution of the radioaerosol within the lungs was similar between the three study days. The mean±SEM alveolar deposition and penetration index as well as the inspiratory flow rate for the control, cough and FET runs are shown in table 7.5.

Figure 7.1 shows the mean±SEM percentage of initial tracheobronchial deposition of the radioaerosol in the four regions of interest of the lungs for the three assessments. The radioaerosol distribution for any one region between the three manoeuvres was similar.

Tracheobronchial radioaerosol clearance (mean±SEM) from the lungs as a whole and measured with the scintillation detectors showed that clearance was significantly enhanced (p<0.01) following cough (44±5 %) and FET (42±5 %) compared to control (16±3 %).
Table 7.4.
Mean±SEM pulmonary function indices of the group for the three study days.

<table>
<thead>
<tr>
<th>Pulmonary Function Indices</th>
<th>Control</th>
<th>Cough</th>
<th>FET</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Pred. FEV₁</td>
<td>50±5</td>
<td>53±5</td>
<td>52±6</td>
</tr>
<tr>
<td>% Pred. FVC</td>
<td>69±5</td>
<td>73±4</td>
<td>71±5</td>
</tr>
<tr>
<td>% Pred. PEFR</td>
<td>50±5</td>
<td>53±5</td>
<td>52±5</td>
</tr>
<tr>
<td>% Pred. MMFR₂₅₋₇₅</td>
<td>28±5</td>
<td>29±6</td>
<td>28±5</td>
</tr>
<tr>
<td>% Pred. Vₘ₉₅₀</td>
<td>22±4</td>
<td>21±4</td>
<td>23±4</td>
</tr>
<tr>
<td>% Pred. Vₘ₉₂₅</td>
<td>24±5</td>
<td>24±4</td>
<td>25±4</td>
</tr>
</tbody>
</table>

Table 7.5.
Mean±SEM inspiratory flow rate ($\dot{V}_{\text{insp}}$), alveolar deposition (AD) and penetration index (PI) of the patients for the three treatment manoeuvres.

<table>
<thead>
<tr>
<th>Treatment Manoeuvre</th>
<th>$\dot{V}_{\text{insp}}$ (l/min)</th>
<th>AD (%)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36±3</td>
<td>24±2</td>
<td>0.38±0.05</td>
</tr>
<tr>
<td>Cough</td>
<td>40±5</td>
<td>25±2</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>FET</td>
<td>38±5</td>
<td>24±2</td>
<td>0.39±0.04</td>
</tr>
</tbody>
</table>
Fig. 7.1. Mean±SEM initial tracheobronchial deposition of the radioaerosol within the four regions of the lungs for the three study days.
Following the pulmonary function tests (which themselves involved 9 forceful exhalatory manoeuvres) the mean±SEM tracheobronchial clearance was significantly faster (p<0.05) for the control run (22±4 %) compared to both cough (10±1 %) and FET (9±2 %).

Figure 7.2 illustrates the regional tracheobronchial clearance for the manoeuvres. Cough and FET significantly enhanced clearance of radioaerosol from the tracheal, inner and intermediate regions of interest of the lungs but not from the outer region. There were however no differences in regional clearance between cough and FET.

The amount of sputum produced under instruction was identical between the cough and FET manoeuvres (mean±SEM: 3.4±0.5 g).

There was no correlation between the tracheobronchial clearance from all regions and the amount of sputum expectorated during the manoeuvres (fig. 7.3) as well as the daily sputum production of the patients prior to the study (fig. 7.4).

7.6. Discussion
The observations of an enhancement of whole lung radioaerosol clearance following instructed coughing in this study confirm previous observations reported by other workers on smaller number of patients (Camner et al., 1979; Puchelle et al., 1980; Sutton et al., 1983). Furthermore the enhancement of whole lung radioaerosol clearance following FET is in keeping with the observations of Sutton and associates (1983).

On theoretical grounds and using Weibel's model of the human lung Leith (1968) postulated that cough may be effective in clearing secretions from the trachea down to the 7th or 8th airway generation. Scherer (1980), however, as already noted, suggested that cough in the presence of hypersecretion may be effective in clearing secretions from the airways as far as the respiratory bronchioles.
Fig. 7.2. Mean±SEM tracheobronchial radioaerosol clearance from the four regions of the lungs for the three assessments.
Fig. 7.3. Regional clearance of radioaerosol from the lungs compared to amount of sputum expectorated during cough and FET manoeuvres for the study group.
Fig. 7.4. Regional clearance of radioaerosol from the lungs compared to mean daily sputum production of the study group.
The data in this study on regional clearance shows cough to be effective in clearing secretions from the trachea, inner and intermediate regions in keeping with observations from other published data. However, clearance of secretions from the outer region due to cough failed to attain statistical significance. This is in accord with much earlier cough studies by Bateman and associates (1981) but contrasts with those reported by Oldenburg and colleagues (1979) and Rossman and associates (1982). The apparent discrepancy may have arisen because of differences in (a) selection of regions of interest between those studies and (b) higher deposition of radiotracer particles (20% approximately) in the outer region in this study and the study by Bateman and colleagues (1981) compared to the other two studies (12.5 % approximately).

The present study shows that FET on its own is effective in clearing secretions from the tracheal, inner and intermediate regions of the lungs compared to the control run. As such its role in physiotherapy for the clearance of lung secretions with reduced peak flows must be advocated as an alternative to cough (Pryor et al., 1979).

Early studies have indicated that for cough to be effective in clearing inhaled radioaerosol from the lungs the presence of hypersecretion was essential (Camner et al., 1979; Puchelle et al., 1980). More recently Bennett and associates showed a small improvement in whole lung clearance following 60 coughs over a one hour period in healthy subjects (1990) but not in a group of asymptomatic smokers (1992). The number of coughs used by Bennett and associates (1990,1992) were far in excess of those used by other investigators.

Mucus clearance by cough/FET in this study was not correlated with the amount of sputum produced during the forceful exhalatory manoeuvres nor with the daily sputum production of the patients which was taken to be a rough guide of their hypersecretory state.

The significantly enhanced clearance during the pulmonary function testing, which required nine forceful exhalatory manoeuvres, in the control run compared to the other two runs was
predictable. These exhalatory manoeuvres enhanced the clearance of the excess secretions which were still present in the airways during the control run whereas in the other two runs instructed cough and FET had already helped to expel the excess secretions.
CHAPTER EIGHT

Effect Of Flow Rate And Sputum Viscoelasticity On Regional Lung Clearance During Cough And Forced Expiration Technique

8.1. Summary
In vitro studies have suggested that both the viscoelastic properties of lung secretions and the peak flow attained during simulated cough influence clearance. This study examines the possible association of these parameters with measured effectiveness of mucus clearance induced by instructed cough and forced expiration technique (FET) in patients with airways obstruction. This chapter brings together data from the two patient groups separately analysed in, respectively, chapter six and seven, namely those who did not expectorate during instructed cough or FET procedures and those who did produce sputum during these procedures. This chapter therefore analyses data from thirty three patients (23M/10F) whose mean±SEM age and % predicted FEV₁ were 66±2 years and 53±4 % respectively. Each underwent the three manoeuvres (control, cough and FET) during which peak expiratory flow rate was measured at the mouth. Sputum apparent viscosity and elasticity were measured by viscometer. Cough and FET compared to control significantly (p<0.01) enhanced clearance of inhaled, deposited radioaerosol from all regions of the lung. Neither peak flow nor viscoelasticity correlated with regional clearance. The results confirm that cough and FET both promote effective clearance but suggest that sputum viscoelasticity as well as peak flow provide no guide to clearance efficacy in humans in contrast to the in vitro studies.

8.2. Introduction
The effectiveness of cough for clearance of lung secretions in healthy subjects is believed to be of limited importance (Yeates et al., 1975; Camner et al., 1979; Puchelle et al., 1980). However, in airway diseases such as asthma and chronic bronchitis the normal mucociliary clearance mechanism is often impaired (Wanner, 1977; Pavia, 1984a).
The cough action then becomes a vital mechanism for clearance. Theoretical considerations indicate that cough is most effective at high lung volumes where high expiratory flows can be reached but, as previously discussed, effect more peripherally may be possible.

Some in vitro studies on the clearance of liquids, with various viscosities, by simulated cough indicate that the efficiency of clearance increased as the liquid viscosity decreased (Scherer & Burtz, 1978). Furthermore, Clarke and associates (1970) showed that the elastic component of the liquid is also important in determining the effectiveness of cough.

This study examines the association of regional mucus clearance from the lungs during instructed cough and FET with peak flow attained during these manoeuvres and viscoelasticity of the expectorated secretions in patients with airway diseases.

8.3. Patients

Thirty three patients (23 male, 10 female) took part in the study. The study group comprises the two sub-groups described in chapters six (6.3) and seven (7.3).

Twenty five patients had chronic obstructive airway disease (COAD) and eight bronchiectasis (B). The mean±SD age, height and weight of the group were 66±9 yr, 1.67±0.10 m and 66±14 kg respectively. The group comprised six non-smokers, five current smokers with a mean±SD of 43±23 pack-years and twenty two ex-smokers with a mean±SD of 43±37 pack-years.

Seven patients (4 COAD and 3 B) were on no medication. Of the remaining twenty six, all were taking inhaled bronchodilators, thirteen were also taking oral bronchodilators and one patient was on home nebuliser bronchodilator therapy. Twenty two patients were maintained with inhaled corticosteroid therapy, three of whom were also taking oral corticosteroids. The mean±SEM pulmonary function indices of the group when first seen are shown in table 8.1.
Table 8.1.
Pulmonary function indices expressed as percentage of predicted values (based on sex, age and height) for all thirty three patients when first seen.

<table>
<thead>
<tr>
<th>Pulmonary Function Indices</th>
<th>Mean±SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$FEV_1$</td>
<td>53±21</td>
</tr>
<tr>
<td>FVC</td>
<td>72±17</td>
</tr>
<tr>
<td>PEFR</td>
<td>54±21</td>
</tr>
<tr>
<td>MMFR$_{25-75}$</td>
<td>29±22</td>
</tr>
<tr>
<td>$\dot{V}_{\text{max}50}$</td>
<td>22±20</td>
</tr>
<tr>
<td>$\dot{V}_{\text{max}25}$</td>
<td>23±17</td>
</tr>
</tbody>
</table>
8.4. Methods

8.4.1. Study Design

The study design and treatment manoeuvres were described in chapters six (6.4.1) and seven (7.4.1). During the cough and FET manoeuvres the peak expiratory flow rate (PEFR) was measured at the mouth using a spirometer (Vitalograph® compact). The mean value of the PEFRs measured for the thirty coughs/FETs was calculated for each patient.

8.4.2. Radioaerosol Technique

Mucus movement within the lungs of patients was ascertained by the radioaerosol technique outlined in detail in chapter two (2.1.2) and the sets of measurements described in chapters six (6.4.2) and seven (7.4.2).

8.4.3. Regional Clearance

The initial topographical distribution, which was expressed quantitatively as a penetration index (PI), and subsequent clearance of the radioaerosol from the lungs was measured [chapter six (6.4.3) and seven (7.4.3)] using a gamma camera as described in chapter two (2.7.2). The four arbitrary regions of the lungs which were chosen has been outlined in chapter six (6.4.3. & fig. 6.1).

8.4.4. Pulmonary Function Tests

The pulmonary function for each patient was assessed as outlined in chapter two (2.12) and is described in chapters six (6.4.4) and seven (7.4.4).

8.4.5. Rheological Properties of Sputum

All sputum samples expectorated during the cough and FET manoeuvres were collected and the wet weight was ascertained. The viscoelastic properties of these samples were measured as described in chapter two (2.13). Whenever possible, three samples of 1 ml in volume were tested within one hour of production for each patient and the results were expressed as the mean.
8.4.6. **Statistical Analysis**

The data were analysed using the paired Wilcoxon test and Spearman rank correlation. The level of significance for effect was taken at p<0.05.

8.5. **Results**

The mean±SEM pulmonary function indices of the group for the three study days are given in table 8.2. The patients' pulmonary function remained unchanged between the three study days.

The mean±SEM inspiratory flow rate, alveolar deposition and penetration index for the control cough and FET manoeuvres are shown in table 8.3. The initial topographical distribution and alveolar deposition of the radioaerosol within the lungs are similar between the three treatment manoeuvres.

Figure 8.1 gives the mean±SEM initial tracheobronchial deposition of the radioaerosol within the four regions of the lungs for the three assessments. The distribution of the radioaerosol for any one region between the three assessments was similar.

The mean±SEM tracheobronchial radioaerosol clearance from the tracheal, inner, intermediate and outer regions of the lungs is shown in figure 8.2. Cough and FET resulted in significant (p<0.01) enhancement of clearance of radioaerosol from all regions of the lungs. There were however no differences in regional clearance between cough and FET.

The peak expiratory flows achieved during cough and FET for the study group are given in table 8.4. The peak flow recorded during the manoeuvres for cough was significantly (p<0.01) higher than that measured during the FET manoeuvres.
Table 8.2.
Mean±SEM % predicted pulmonary function indices for the study group for the three assessment days.

<table>
<thead>
<tr>
<th>Pulmonary Function Indices</th>
<th>Control</th>
<th>Cough</th>
<th>FET</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td>51±4</td>
<td>53±3</td>
<td>53±4</td>
</tr>
<tr>
<td>FVC</td>
<td>71±3</td>
<td>74±3</td>
<td>73±3</td>
</tr>
<tr>
<td>PEFR</td>
<td>53±4</td>
<td>53±3</td>
<td>53±4</td>
</tr>
<tr>
<td>MMFR₂₅-₇₅</td>
<td>29±4</td>
<td>29±4</td>
<td>28±3</td>
</tr>
<tr>
<td>( \dot{V}_\text{max} )₅₀</td>
<td>22±3</td>
<td>20±3</td>
<td>23±4</td>
</tr>
<tr>
<td>( \dot{V}_\text{max} )₂₅</td>
<td>23±3</td>
<td>21±3</td>
<td>23±3</td>
</tr>
</tbody>
</table>

Table 8.3.
Mean±SEM inspiratory flow rate (\( \dot{V}_{\text{insp}} \)), alveolar deposition (AD) and penetration index (PI) of the patients for the three assessment days.

<table>
<thead>
<tr>
<th>Treatment Manoeuvre</th>
<th>( \dot{V}_{\text{insp}} ) (l/min)</th>
<th>AD (%)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37±3</td>
<td>25±2</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>Cough</td>
<td>40±3</td>
<td>25±2</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>FET</td>
<td>38±3</td>
<td>25±2</td>
<td>0.40±0.03</td>
</tr>
</tbody>
</table>
Fig. 8.1. Mean±SEM initial tracheobronchial deposition of the radioaerosol within the four regions of the lungs during the three assessment days.
Fig. 8.2. Mean±SEM tracheobronchial radioaerosol clearance from regions of the lungs for the three treatment manoeuvres.
Table 8.4.
Mean±SD peak expiratory flow rate (PEFR) achieved during instructed cough and FET for
the study group.

<table>
<thead>
<tr>
<th>Patient's no.</th>
<th>PEFR during cough (l/min)</th>
<th>PEFR during FET (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>270±28</td>
<td>125±18</td>
</tr>
<tr>
<td>2</td>
<td>241±28</td>
<td>173±15</td>
</tr>
<tr>
<td>3</td>
<td>350±34</td>
<td>233±34</td>
</tr>
<tr>
<td>5</td>
<td>199±43</td>
<td>107±19</td>
</tr>
<tr>
<td>6</td>
<td>528±61</td>
<td>543±30</td>
</tr>
<tr>
<td>7</td>
<td>361±49</td>
<td>281±23</td>
</tr>
<tr>
<td>8</td>
<td>431±44</td>
<td>359±33</td>
</tr>
<tr>
<td>9</td>
<td>369±31</td>
<td>273±23</td>
</tr>
<tr>
<td>10</td>
<td>622±35</td>
<td>518±81</td>
</tr>
<tr>
<td>11</td>
<td>530±39</td>
<td>297±46</td>
</tr>
<tr>
<td>13</td>
<td>172±35</td>
<td>89±19</td>
</tr>
<tr>
<td>14</td>
<td>374±35</td>
<td>280±26</td>
</tr>
<tr>
<td>15</td>
<td>449±56</td>
<td>267±52</td>
</tr>
<tr>
<td>18</td>
<td>274±31</td>
<td>249±15</td>
</tr>
<tr>
<td>19</td>
<td>260±35</td>
<td>258±16</td>
</tr>
<tr>
<td>20</td>
<td>231±52</td>
<td>150±23</td>
</tr>
<tr>
<td>21</td>
<td>55±25</td>
<td>64±25</td>
</tr>
<tr>
<td>22</td>
<td>221±24</td>
<td>283±17</td>
</tr>
<tr>
<td>23</td>
<td>204±38</td>
<td>183±27</td>
</tr>
<tr>
<td>24</td>
<td>293±40</td>
<td>290±35</td>
</tr>
<tr>
<td>25</td>
<td>227±14</td>
<td>190±21</td>
</tr>
<tr>
<td>26</td>
<td>277±15</td>
<td>257±15</td>
</tr>
<tr>
<td>27</td>
<td>225±22</td>
<td>140±13</td>
</tr>
<tr>
<td>29</td>
<td>260±25</td>
<td>210±21</td>
</tr>
<tr>
<td>30</td>
<td>646±36</td>
<td>475±53</td>
</tr>
<tr>
<td>31</td>
<td>158±29</td>
<td>66±15</td>
</tr>
<tr>
<td>33</td>
<td>216±19</td>
<td>128±16</td>
</tr>
<tr>
<td>35</td>
<td>399±46</td>
<td>169±39</td>
</tr>
<tr>
<td>37</td>
<td>286±24</td>
<td>174±32</td>
</tr>
<tr>
<td>39</td>
<td>213±29</td>
<td>120±16</td>
</tr>
<tr>
<td>40</td>
<td>304±20</td>
<td>112±20</td>
</tr>
<tr>
<td>41</td>
<td>473±39</td>
<td>399±26</td>
</tr>
<tr>
<td>42</td>
<td>373±26</td>
<td>211±30</td>
</tr>
</tbody>
</table>

Mean±SD        318±135  233±122
Only nineteen patients produced sputum during the cough and FET manoeuvres as described in chapter seven while the other fourteen patients were unable to produce any sputum despite the instruction and encouragement to do so as outlined in chapter six. Four out of the nineteen patients (no. 10, 14, 33, 35) produced small amounts of sputa during the cough and FET manoeuvres which were insufficient for assessment of their viscoelastic properties. The apparent viscosity values for the sputa produced during the cough compared with FET assessments were similar as was the elasticity (table 8.5).

There was no correlation between the radioaerosol tracheobronchial clearance from all regions of the lungs and the mean peak flow attained during the cough and FET manoeuvres (fig.8.3) as well as the apparent viscosity (fig.8.4) and elasticity (fig.8.5) of sputa.

8.6. Discussion

The data presented in this study relates to two subgroups of patients, which were described in chapters six and seven, the aim of which was to examine the regional mucus clearance from the lungs during instructed cough and FET.

In this study the observation that cough and FET enhanced clearance from all regions of the lungs implies that both cough and FET are effective in peripheral airways.

It has been suggested that the mucus clearance rate by cough is influenced by both the viscoelastic properties of the mucus and depth of mucus layer (Clarke, 1973). This was confirmed by the in vitro work of King and associates (1985) who demonstrated that a definite inverse relationship between viscoelasticity and clearance rate exists.

In vitro studies (King, 1987; King et al., 1989) using simulated mucus with apparent viscosity in the range of 1-77 Pa.s have indicated that cough is more effective in less viscous material.
Table 8.5.
Apparent viscosity and elasticity of the sputa produced during instructed cough and FET for the study group.

<table>
<thead>
<tr>
<th>Patients no.</th>
<th>Apparent Viscosity (mPa.s)</th>
<th>Elasticity (mPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cough</td>
<td>FET</td>
</tr>
<tr>
<td>5</td>
<td>393</td>
<td>407</td>
</tr>
<tr>
<td>7</td>
<td>925</td>
<td>1133</td>
</tr>
<tr>
<td>8</td>
<td>678</td>
<td>355</td>
</tr>
<tr>
<td>15</td>
<td>710</td>
<td>1022</td>
</tr>
<tr>
<td>19</td>
<td>677</td>
<td>602</td>
</tr>
<tr>
<td>20</td>
<td>336</td>
<td>364</td>
</tr>
<tr>
<td>21</td>
<td>499</td>
<td>369</td>
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<tr>
<td>23</td>
<td>618</td>
<td>1689</td>
</tr>
<tr>
<td>25</td>
<td>602</td>
<td>452</td>
</tr>
<tr>
<td>26</td>
<td>662</td>
<td>542</td>
</tr>
<tr>
<td>27</td>
<td>1299</td>
<td>1089</td>
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<tr>
<td>29</td>
<td>445</td>
<td>256</td>
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<td>31</td>
<td>1259</td>
<td>970</td>
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<tr>
<td>39</td>
<td>338</td>
<td>663</td>
</tr>
<tr>
<td>40</td>
<td>955</td>
<td>343</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Viscosity</td>
<td>693</td>
<td>301</td>
</tr>
<tr>
<td>Elasticity</td>
<td>2979</td>
<td>1947</td>
</tr>
</tbody>
</table>
Fig. 8.3. Regional clearance of radioaerosol from the lungs compared to mean peak flow rate during cough and FET for the study group.
Fig. 8.4. Regional clearance of radioaerosol from the lungs of fifteen patients compared to mean apparent viscosity of expectorated sputum during cough and FET manoeuvres.
Fig. 8.5. Regional clearance of radioaerosol from the lungs compared to mean elasticity of expectorated sputum from fifteen patients during cough and FET manoeuvres.
The lack of a correlation between cough/FET mucus clearance and viscoelasticity of expectorated secretions in this study may possibly be explained by the fact that the viscosity of the patients' sputa ranged over a much narrower 'window' (0.3-1.7 Pa.s) and as such the in vitro studies may wrongfully predict what actually happens in vivo. The lack of a correlation between cough/FET mucus clearance and elasticity of expectorated secretions in this study also contrast with published in vitro studies (King et al., 1985; King, 1987) which suggest that elastic forces in the mucus gel impede cough clearability.

This lack of correlation between viscoelasticity of expectorated secretions and cough/FET mucus clearance could be attributed to other physical variables such as spinnability (i.e. the capacity of the mucus to form threads) and adhesivity. It has been suggested that an increase in these two parameters could cause a further decrease in cough clearance (King et al., 1989).

Again in vitro studies (King et al., 1985, King, 1987) using mucus simulants have indicated that cough clearance is significantly enhanced with increases in peak flow rate. This study in patients does not confirm this and indeed shows that FET and cough result in similar radioaerosol clearances from the lungs despite a statistically significant reduction (mean: 30%) in peak flow rate during the former manoeuvre. This may be clinically important in so far as the patient does not have to try excessively hard to achieve valuable enhancement of clearance of excess lung secretions.

This study indicates the possible risks in extrapolating data from in vitro studies to what actually happens in clinical practice.
CHAPTER NINE
GENERAL DISCUSSION AND CONCLUSIONS

Mucociliary clearance is one of the lungs' non-specific host defence mechanisms. It results from beating cilia propelling cephalad the overlying secretions, carrying both trapped inhaled material and locally produced biological debris out of the lungs. An efficient mucociliary clearance mechanism relies on the intricate inter-relationship between the integrity of the ciliated epithelium, the ciliary beat and coordination, the composition, amount and rheological properties of the sol and gel layers. Mucociliary clearance can be altered by: physiological factors, environmental pollutants, pharmacological agents and disease. Abnormal mucociliary clearance may be an early indicator of a disease process. Delayed clearance may represent one aspect of a vicious cycle with prolonged retention at airways sites of inhaled bacteria or antigen itself promoting a further decline in the efficiency of the mucociliary mechanism. This in turn may cause undue retention of lung secretions and thereby an increased incidence of chest infections.

Mucociliary transport is impaired in a wide range of clinical conditions. Chapters three and four illustrate marked clearance problem in two categories of people or patients in whom the extent of clearance impairment had not previously been documented. Earlier work had importantly indicated how physiological factors may also affect clearance in particular how sleep leads to slowing down of mucus clearance. Chapter five demonstrate how very marked this effect can be in a group of subjects suffering from a common condition, namely asthma, well-established as a cause of impaired clearance. Chapters six, seven and eight employ the radioaerosol approach to test the efficacy of forced expiration techniques - specifically cough and FET - in helping patients with impaired mucociliary clearance to rid their lungs of retained mucus.
The mucociliary clearance mechanism is adversely affected in patients with pulmonary sarcoidosis. The magnitude of the observed impairment is probably an underestimate due to the fact that the initial distribution of the radioaerosol within the lungs of the sarcoid patients was more proximal than the healthy control subjects and some of the patients also had unproductive coughing during the observation period. This clearance defect represents an important aspect of airways involvement in this disease. The sarcoid patients in "apparent clinical remission", who had not required any medication during the course of their illness, were found to have the smallest degree of mucociliary clearance impairment. This is equally true of those patients who were receiving oral corticosteroids. This observation is however in sharp contrast to that seen in patients on inhaled corticosteroid therapy. These patients demonstrate the greatest impairment of mucociliary transport. It is therefore postulated that the improved mucociliary clearance in the sarcoid patients who were receiving oral corticosteroids may be attributed to their treatment which appears to be more effective as far as mucociliary clearance is concerned than inhaled corticosteroid therapy. This may possibly be due to the larger doses of the drug being delivered systemically and ultimately reaching the lungs.

Mucociliary clearance of patients with pigeon fanciers' lung disease with and without blood circulating precipitins is significantly reduced compared to matched healthy control groups. This finding indicates that the presence or absence of circulating precipitins is not related to defective mucociliary transport and also suggests that level of exposure is immaterial to the degree of impairment in tracheobronchial clearance. The degree of lung mucociliary clearance impairment in the pigeon fanciers without blood circulating precipitins and minimal chest symptoms is comparable to that seen for pigeon fanciers with circulating precipitins, increased symptomatology, and reduced lung function. Avian precipitins have been shown to have a role, as a non-invasive test, in the diagnosis of pigeon fanciers' disease.
The use of an objective, non-invasive radioaerosol technique demonstrates that lung mucociliary clearance is compromised in pigeon fanciers irrespective of the presence or absence of avian precipitins and as such it might lend itself as a more sensitive screening test.

Lung mucociliary clearance in patients with asthma is significantly reduced during sleep compared to when the patients are awake suggesting that the asthmatic patients who fall asleep with partly blocked airways are very unlikely to clear those airways during the night. The long night time residence of secretions containing infective inflammatory agents could well contribute significantly to progression of symptoms. Reduction of clearance of lung secretions during sleep in addition to the already impaired lung mucociliary transport in asthmatics may well contribute to the early morning wheeze, cough and dip in pulmonary function reported by these patients. Two weeks' treatment with an oral controlled release beta agonist or slow release methylxanthine does not enhance lung mucociliary clearance during sleep in asthma. This finding contrasts with the observations reported with both these types of drugs in healthy subjects and patients when awake. However, the morning and evening highest peak flow rates before use of inhaled bronchodilators are significantly greater after either kind of oral bronchodilator therapy than the corresponding value for placebo.

Cough is one of the lungs' mucus clearance mechanisms and together with mucociliary clearance helps to keep the conducting airways clean. The observation of a significant enhancement of whole lung radioaerosol clearance in patients with airways obstruction following instructed coughing and forced expirations confirms previous observations with smaller number of patients. Regional clearance shows productive coughing is significantly effective in clearing secretions from the tracheal, inner, and intermediate regions in keeping with previous observations. However, clearance of secretions from the outer region due to productive cough failed to reach a significant level.
Productive forced expirations on their own are effective in clearing secretions from the tracheal, inner, and intermediate regions of the lungs. As such the role of the forced expiration technique in physiotherapy must be advocated as an alternative to cough. There is no apparent association of mucus clearance from any lung region with the amount of sputum produced during the forceful exhalatory manoeuvres or the daily sputum production.

It has previously been reported that unproductive coughing in both healthy subjects and patients did not result in any significant loss of radioaerosol particles from the lungs. More recently however, an improvement of whole lung clearance after controlled coughing was observed in healthy subjects but not in asymptomatic smokers. In patients with airways obstruction and mild hypersecretion unproductive cough/FET significantly enhanced movement of secretions proximally from all regions of the lungs. As such, unproductive coughing must be aiding the well documented compromised mucociliary transport in such patients. Mucus clearance by unproductive cough/FET is not correlated with the daily sputum production of the patients.

Apparent viscosity and elasticity of the patients' sputa produced during productive cough/FET are not correlated with the mucus clearance achieved during these manoeuvres. This lack of correlation contrast with published in-vitro studies which suggest that an inverse relationship exists between viscoelasticity and cough clearability. The lack of correlation between viscoelasticity of expectorated secretions and cough/FET mucus clearance may be attributed to other physical variables such as spinnability and adhesivity which could cause a further decrease in cough clearance.

Previous work from other centres has strongly suggested that clearance by cough depends on the peak flow rate achieved. Results reported in the present thesis contradict this view. Patients were able to achieve worthwhile clearance by FET with
peak flow rates much lower than those they achieved in the cough manoeuvre. Indeed clearance by FET, with those lower flow rates, was (within measurement limits) equally as good as that achieved by cough. It may even have been as effective in clearing mucus from the distal small airways (there is certainly no significant FET:cough difference for outer region clearance). This finding is of clinical importance. Patients can be counselled to adopt FET rather than cough. Compared to forceful coughing, they should achieve equal benefit but with a much lower risk of damage to the ciliated epithelium.

Previously published work on regional mucus clearance has generated uncertainty as to whether cough can be effective at enhancing mucus transport within peripheral airways. In the present work, when patients with productive and unproductive cough are taken together, there is clear evidence of a very significant effect on outer region clearance for both cough and FET manoeuvres. The present results thus strongly suggest that forceful expiration techniques do have an effect deep within the peripheral airways and deeper than previously imagined. It is difficult to speculate what individual factors may influence the effect of cough or FET within these airways. Mucus properties and the extent of mucus hypersecretion must, as in the large airways, play some role. But average linear airflow velocities are obviously very much lower. This is why theoretical calculations based on the anatomy of the Weibel model of the lung have suggested cough might be ineffective at augmenting clearance within the more distal bronchioles. The practical efficacy actually demonstrated may therefore partly reflect local irregularities of linear flow rate (with perhaps localised regions of relatively high flow) and it may partly reflect clearance by repetitive squeezing of the airways. This practical efficacy is of sufficient magnitude to be relevant to the clinical management and care of patients with impaired mucociliary transport - perhaps particularly so for asthmatics where mucus plugging of peripheral airways is of serious concern. The relatively gentle FET manoeuvre was on average able to increase outer region clearance
by about 50% - a highly significant amount.

Using historical controls may be a limitation of the methodology but this has to be so until such time as our knowledge and methodology has improved as to be able to administer radioaerosols in subjects and achieve a pre-defined topographical distribution pattern within their lungs.

The planar gamma camera imaging which was employed in this thesis can readily be sub-divided into regions of interest representing relatively central or relatively peripheral parts of the lung. Inevitably these regions cannot precisely reflect lung anatomy. Some deposition of inhaled particles at alveolar or small airways sites will generally be superimposed on the image of the larger bronchi. However, this approach is direct and good images can generally be obtained even from low levels of lung radioactivity. Tomographic imaging would in principle offer a much better separation of central from peripheral structures. Such imaging has however required administration of higher levels of radioactivity than those now usually adopted for planar imaging. Tomographic imaging may thus play a limited, highly specialised role at a few centres due to the lack of tomographic equipment which are more costly than the conventional imaging and more sophisticated in both the technical and computing aspects. The extra time, effort, complexity and cost required by tomographic technique must be balanced against the extra information that they offer over and above that of planar imaging.

The methodology lends itself for the prospective, non-invasive, evaluation of the effect of the various emerging non-steroidal anti-inflammatory drugs, particularly for asthma with regards to their ability to reduce inflammation in the conducting airways and thus improve the integrity of the ciliated epithelium.
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