THE EFFECT OF AGE, SEX AND CIGARETTE SMOKING ON THE AMOUNT AND DISTRIBUTION OF MICROSCOPIC EMPHYSEMA IN MAN: A MORPHOMETRIC STUDY

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This thesis was composed by myself. The work presented was performed by myself, except where otherwise acknowledged. This work has not been submitted to the University in any other form.

ABSTRACT

Emphysema is defined as the increase beyond normal in the size of airspaces distal to the terminal bronchiole. Such increases in airspace size are associated with a reduction in alveolar wall surface area *per* unit volume of lung tissue (AWUV). This study involved the development and assessment of the fast interval processor (FIP) as a new automated technique for measuring AWUV on histological sections of lung tissue. A minimum of 726 individual field AWUV measurements were made from each lung specimen, and frequency distributions of these AWUV values were compiled for each of the 165 specimens in the study. Various aspects of the frequency distributions were then used to establish the normal range of AWUV values with advancing age in non-smokers, and to assess the effects of age, sex and cigarette smoking on the amount and distribution of microscopic emphysema within the lung.

Mean AWUV was found to decrease with advancing age in adult non-smokers, and this decrease was considered to be normal. A range of normal mean AWUV values was established for subjects between the ages of 21 and 93 years. No evidence was found to suggest that senile emphysema exists in non-smokers. Microscopically assessed emphysema (MAE) was defined as the condition where the mean AWUV measurement of a lung was below the lower limit of the normal range. Only 26% of the smokers studied had MAE as defined in this way, suggesting the existence of a susceptible sub-group of smokers. Neither the susceptibility to, nor the severity of, MAE were dose-related to tobacco consumption in the cigarette smokers studied. There were no sex differences in the incidence of MAE. MAE was found to be related to macroscopic panacinar emphysema, but was not related to macroscopic centriacinar emphysema. The 5th and 10th percentile values of the AWUV frequency distribution were found to be related to the presence of centriacinar emphysema. These results indicate that the early (microscopic) lesions in centriacinar emphysema are also focal in their distribution, and do not develop on a background of generalised MAE.

The two most common forms of macroscopic emphysema, centriacinar and panacinar, are the consequence of different pathogenetic mechanisms.

Smokers are not a homogeneous group with regard to the development of microscopic emphysema. Studies of the pathogenesis of emphysema which do not target those smokers who are susceptible to MAE cannot elucidate the mechanisms responsible for the onset of the disease.

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PREFACE

The project described in this thesis involved the study of the amount and distribution of microscopic emphysema in human lungs, in relation to age, sex and cigarette smoking. The thesis comprises 5 chapters:

Chapter 1 contains an overview of the anatomy of the human lung, and its growth and development. The first Chapter also includes descriptions of the terminology and methodology used in studies of the epidemiology and pathogenesis of pulmonary emphysema.

Chapter 2 includes descriptions of the lung specimens used and the methods used in measuring airspace wall surface area *per* unit volume of lung tissue.

Chapter 3 contains an introduction to a new method for measuring airspace wall surface area on histological sections of lung tissue. The development of this technique and its advantages over previously used techniques are discussed.

Chapter 4 consists of a description of all results produced during this study.

In Chapter 5 the methods used and results obtained in the study are discussed in relation to the current literature.

A short introductory paragraph is given at the beginning of each Chapter.

Chapter 1

Introduction

1.1 INTRODUCTION

This chapter consists of an introduction to the study of pulmonary emphysema in man. Before discussing the disease process, the anatomy of the normal adult lung and its growth and development are described. Pulmonary emphysema is defined, and the remainder of the chapter is concerned with an account of the diagnosis, classification, epidemiology and pathogenesis of emphysema. This chapter also includes a section describing the various methods used in the assessment of the incidence and severity of emphysema. Chapter 1 also contains a description of the background to this study and its major aims.

1.2 ANATOMY OF THE ADULT LUNG

1.2.1 Gross Anatomy of the Lung

The adult lung is divided into lobes, each with its own bronchus. The right lung has 3 lobes and the left has 2. Each lobe is sub-divided into bronchopulmonary segments by incomplete fibrous septae, extending inward from the pleural surface. Each segment is supplied by a segmental bronchus. Smaller fibrous septae are present within each segment, and these form the incomplete boundaries of the pulmonary (or secondary) lobules. These are usually pyramidal in shape, with the apex towards the bronchiole supplying the lobule. Each lobule contains 3-5 acinar units, each of which is supplied by a terminal bronchiole (Thurlbeck, 1988).

1.2.2 The Airways

The conducting part of the human respiratory system starts with the trachea. The trachea, a 10-11cm long cartilagenous tube, is lined with ciliated columnar epithelium. Its cartilagenous support consists of 15-20 horseshoe-shaped rings of cartilage which are incomplete posteriorly. The posterior part of the trachea consists largely of connective tissue and smooth muscle. The trachea divides at its end into the 2 main bronchi.

The structure of the main bronchi is similar to that of the trachea. The right main bronchus is slightly shorter than the left and is slightly wider in transverse diameter. The main bronchi divide into lobar then segmental bronchi. Further divisions occur in an uneven dichotomous manner, so that the branches resulting from a division are not necessarily the same size. All bronchi have cartilage in their walls.

Bronchioles are smaller airways than bronchi, and have no cartilage in their walls. They continue to divide in the same manner as the bronchi, with smaller branches becoming thin walled, until the terminal bronchiole is reached. The terminal bronchiole is the last purely conducting airway of the respiratory system.

1.2.3 The Acinar Unit

The respiratory tissue distal to the terminal bronchiole forms the acinus, or acinar unit (Reid, 1958). Bronchioles within the acinus always contain alveoli in their walls, and are called respiratory bronchioles. Each respiratory bronchiole divides into an average of 3 further orders of respiratory bronchioles, and each of these gives rise to increasing numbers of alveoli and alveolar ducts, which are made up completely of alveoli (Figure 1.1).

The alveoli are lined by thin squamous epithelium which consists of 2 main cell types. Type 1 alveolar epithelial cells have long cytoplasmic extensions which form a complete thin layer covering most of the internal surface of the alveoli. Type 2 cells cover only a fraction of the alveolar surface. They occur singly or in small groups between the type 1 cells. These cells are thought to secrete surfactant, which reduces the surface tension of the alveoli. Type 2 cells are proliferative, and some daughter cells are transformed into type 1 cells.

The average diameter of an alveolus is 250μm (Weibel, 1963; Schreider & Raabe, 1981). Around the openings of the alveoli is a supporting network of elastic and collagen fibres. The elastic fibres permit expansion of alveoli during inspiration and recoil on expiration, while the collagen fibres

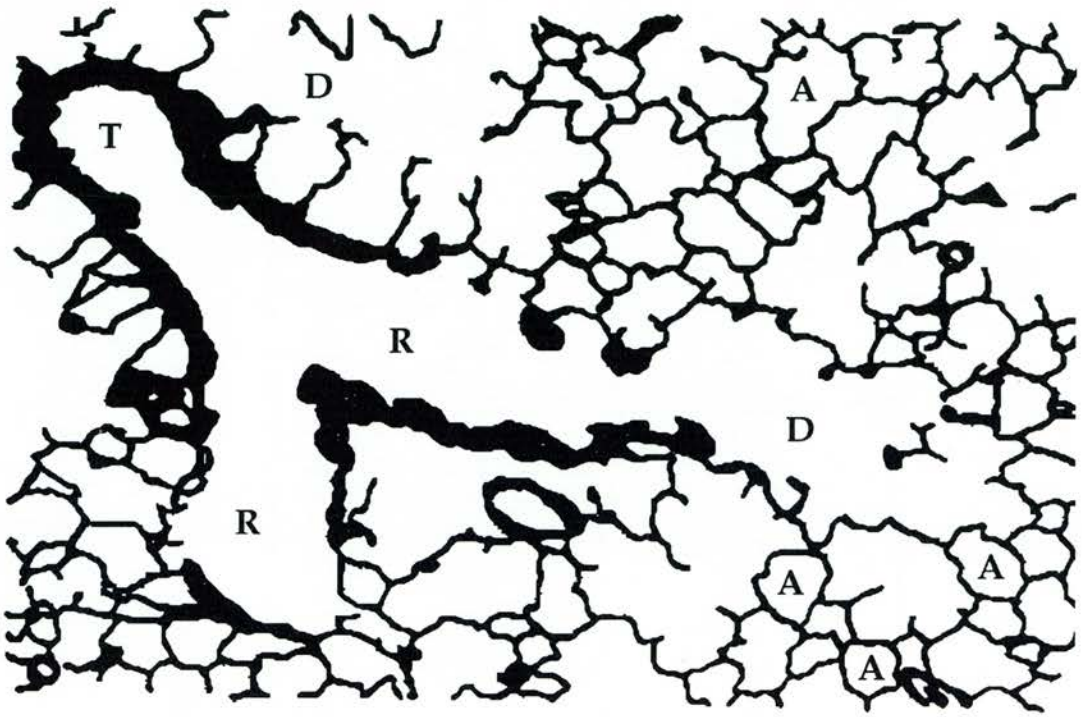


FIGURE 1.1

The acinar unit. This figure shows a binary image of a complete acinar unit, showing a terminal bronchiole (T); respiratory bronchioles (R), with alveoli in the bronchiolar walls; alveolar ducts (D), and several individual alveoli (A).

This image was produced using image processing software (Image 1.41 VDM) on an Apple Macintosh IIfx computer.

prevent overdistension. Adjacent alveoli are separated by the alveolar walls, which contain a network of elastic and collagen fibres.

1.2.4 Gas Exchange in the Lung

The main function of the respiratory system is to permit gas exchange, which involves the uptake of oxygen by the blood, and the elimination of waste gases. Oxygen in dissolved form passes from the alveoli to the capillaries through the blood-air barrier (Figure 1.2), and carbon dioxide passes in the reverse direction. The blood-air barrier consists of:

a) Alveolar epithelium
b) Interstitial space (in many areas, only fused basal laminae of epithelium and endothelium)
c) Capillary endothelium

1.2.5 Pulmonary Blood Supply

Branches of the pulmonary artery carry deoxygenated blood to the lungs. The artery branches repeatedly within the lungs, with branches accompanying the major bronchi, and ends in dense capillary networks. Within the interalveolar septum the network consists of a single layer of capillaries with extremely thin walls.

Oxygenated blood is drained from the capillary network into venules which are derived from the pulmonary vein. Small branches of the pulmonary vein communicate freely with each other and join to form large vessels which ultimately accompany the arteries and bronchi to the hilum. The pulmonary veins open into the left atrium of the heart, delivering oxygenated blood for systemic distribution by the left ventricle (Williams *et al*, 1989).

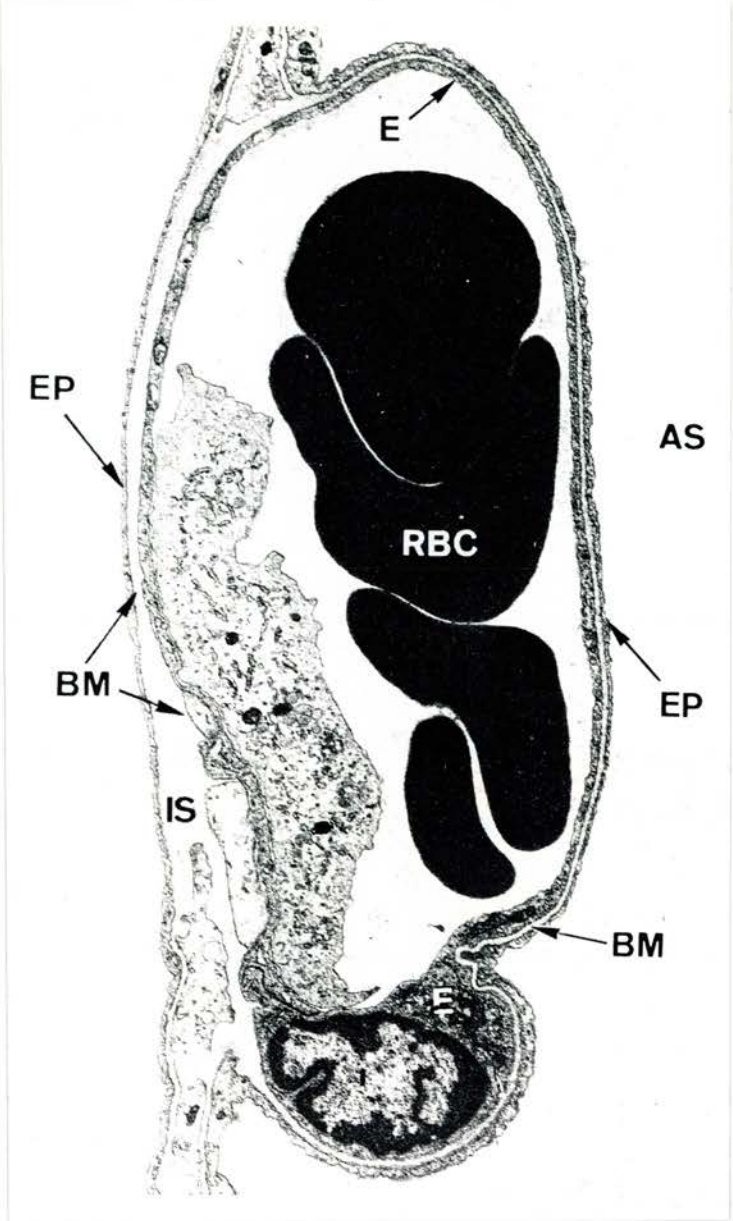


FIGURE 1.2

The blood-air barrier. This plate shows an electron microscope image of a capillary situated within an alveolar wall (*i.e.* alveolar spaces (AS) on either side of the capillary). At its thinnest (to the right of the capillary lumen), the blood-air barrier consists of the fused basement membranes (BM) of the alveolar epithelium (EP) and the capillary endothelium (E). In its thicker areas, the blood-air barrier also contains the interstitial space (IS) between the epithelium and endothelium. RBC = red blood cells within the capillary. Scale: 1mm = 0.125μm.

From Murray (1986).

1.3 GROWTH AND DEVELOPMENT OF THE NORMAL LUNG

The conducting airways in the human lung are all formed before birth (Bucher & Reid, 1961; Reid, 1967a; Thurlbeck, 1975; Murray, 1986). After birth these airways increase in length and diameter until somatic growth stops in adulthood. Lung dimensions are known to be related to body height, and therefore, allowing for biological variation, tall adults have the same number of conducting airways as short adults, but the tall person's airways are larger (Thurlbeck, 1975).

Some alveoli are present at birth, although there are conflicting reports in the literature as to the proportion of the adult number present in the newborn (Dunnill, 1962a; Davies & Reid, 1970; Thurlbeck, 1975; Thurlbeck & Angus, 1975). Nevertheless, it is generally accepted that the alveoli multiply rapidly in the first year after birth (Dunnill, 1962a; Davies & Reid, 1970; Hislop & Reid, 1974; Thurlbeck, 1975). Some of the terminal bronchioles which were present at birth develop alveoli in their walls and thus, by definition, become further generations of respiratory bronchioles (Reid, 1967a; Thurlbeck, 1975; Murray, 1986). This results in fewer, larger acinar units.

The rapid multiplication of the alveoli slows down after the age of 2 or 3 years, but new alveoli continue to be formed after this (Davies & Reid, 1970; Hislop & Reid, 1974; Thurlbeck, 1982). The exact age at which the adult alveolar number is achieved is unclear. Dunnill (1962a) suggested that alveolar multiplication stopped after the age of 8 years, and that from this time until adulthood lung growth involved increases in the dimensions of the alveoli. The results of other workers led them to agree with this suggestion (Reid, 1967a; Davies & Reid, 1970; Hislop & Reid, 1974).

The work of Angus and Thurlbeck (1972) cast some doubt on this opinion when they found a high degree of variation in alveolar number in adult lungs from 32 subjects. Dunnill's theory had been based on the finding that the number of alveoli in the lung of an 8 year-old subject was of the same order of magnitude as the number found in a lung from a 'typical adult male aged 25 years'. He therefore suggested that adult alveolar number was achieved by the age of 8 years. Angus and Thurlbeck suggested the

possibility that due to the variability found in adult lungs, the 8 year-old may have been destined to have more alveoli than the adult value used in Dunnill's study, and that alveolar multiplication may not therefore have been complete.

Lung dimensions are related to height, and male lungs are larger than female lungs for a given body size (Thurlbeck, 1982). There are 2 possibilities regarding the size and number of alveoli in lungs of various sizes:

1. The size of the alveoli varies according to lung size.
2. The number of alveoli varies according to lung size.

Thurlbeck (1967b) found that mean linear intercept measurements (Lm) (*i.e.* the average distance between airspace walls) were not related to height. Therefore the average size of the alveoli is not related to height (Thurlbeck, 1967b; Thurlbeck & Angus, 1975), rather, it would appear that tall people have more alveoli than short people (Thurlbeck, 1982). Since height is influenced by both genetic and environmental factors it seems likely that new alveoli are formed after the age of 8 years (Angus & Thurlbeck, 1972). In a study of alveolar wall surface area *per* unit volume of lung tissue (AWUV), McLean (1987) found that AWUV was not related to height, suggesting that alveolar number rather than size is related to height.

It is now generally accepted that alveolar multiplication occurs after birth and stops some time before somatic growth is complete (Thurlbeck, 1982). The exact age at which formation of new alveoli ceases remains unclear.

1.4 DEFINITIONS OF EMPHYSEMA

The morphological appearance of emphysema in pathology specimens was recognised as early as the 18th century, and the physiological and clinical concepts of the disease were developed extensively in the 19th century (Rosenblatt, 1972; Thurlbeck, 1976). However, until 1959 there was no satisfactory definition of emphysema. With the development of improved techniques for the preparation of inflated lung specimens (Gough & Wentworth, 1949; Heard, 1958), by the 1950s pathologists became able to study the anatomical appearance of pulmonary emphysema (Eriksson, 1991). Reid (1958) identified the respiratory unit of the lung as the acinus, consisting of those structures distal to the terminal bronchiole. The respiratory bronchioles, alveolar ducts and alveoli, which are distal to the terminal bronchiole, are all involved in the gas exchange process.

At the Ciba Symposium on the terminology, definition and classification of chronic pulmonary emphysema and related conditions (Ciba, 1959), it was agreed that emphysema should be defined in terms of the acinus. The proposed definition was:

> 'Emphysema is a condition of the lung characterised by increase beyond the normal in the size of air spaces distal to the terminal bronchiole either from dilatation or from destruction of their walls.'

This original definition did not distinguish between overinflation, which may take place in lungs which are structurally intact, and the disruption of the lung architecture which occurs in emphysema. Various modifications of this definition have since been made, all of which emphasise that a destructive process is associated with emphysema.

The American Thoracic Society suggested that the definition of emphysema should be:

> 'a condition of the lung characterised by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole accompanied by the destruction of their walls.'
>
> (American Thoracic Society, 1962).

This definition was refined by Snider and colleagues (1985) to exclude airspace enlargement with fibrosis:

> 'Emphysema is defined as a condition of the lung characterised by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis.' (Snider *et al*, 1985).

1.5 DIAGNOSIS OF EMPHYSEMA

Pulmonary emphysema is defined in morphological terms. Therefore, although emphysema is clinically associated with airflow obstruction, its diagnosis in the living patient is extremely difficult. Various methods are used, with varying degrees of success, by clinicians, physiologists and radiologists to diagnose emphysema. A brief description of some of these methods is given below.

1.5.1 Clinical Examination

The assessment of physical symptoms alone does not provide sufficient information for the diagnosis of emphysema. Recording the patient's smoking history is important, since non-smokers rarely develop emphysema (unless they have an inherited deficiency of proteinase inhibitor (see section **1.9**)). Physical symptoms such as breathlessness, wheeze, cough and sputum production are often associated with emphysema, but are not specific to this disease. Hyperinflation of the lungs may occur in patients with emphysema, but this is also found in other conditions such as asthma (Flenley, 1991).

1.5.2 Respiratory Function Tests

Several physiological tests of pulmonary function are used to investigate the presence of emphysema. These include measuring the ratio of residual volume to total lung capacity (RV/TLC) and the forced expiratory volume in one second (FEV_1). These are essentially tests of airways obstruction, and do not necessarily relate to emphysema. Studies using diffusing capacity (CO transfer factor) and studies involving analysis of the shape of pressure-volume curves have had limited success in identifying patients with emphysema (Flenley, 1991).

1.5.3 Chest Radiography

Radiological diagnosis of emphysema is made by recognising certain features on chest X-rays, such as arterial deficiency patterns, lung height and width measurements, size of the retrosternal space, heart size and the

position of the diaphragm (Thurlbeck & Simon, 1978). Standard radiological techniques have been found to be particularly unreliable in diagnosing emphysema, and are not generally considered to be acceptable when used alone (Reid & Millard, 1964; Thurlbeck & Simon, 1978; Pugatch, 1983; Bergin *et al*, 1986; Flenley, 1991).

1.5.4 Computed Tomography

There has been much interest in recent years in the use of computed tomography scanning as a diagnostic tool in the study of emphysema. The computed tomography (CT) scan has been found to be a more sensitive and specific indicator of the presence and severity of emphysema than pulmonary function tests (Bergin *et al*, 1986). CT scans can be used to measure regional lung density and can locate emphysematous regions in human lungs in life (Gould *et al*, 1988). Unfortunately, localised lesions which are found in certain forms of emphysema (see section **1.6** below) are not detected by this method. Until a range of normal CT densities relating to age is established, the diagnosis of early emphysema using CT scanning alone is not possible.

1.5.5 Diagnosis of Emphysema by Examining Lung Tissue

As described above, emphysema is defined in morphological terms. Therefore, the accurate diagnosis of emphysema requires the examination of lungs after their removal from the thoracic cavity, either during surgery, or at post-mortem examination. The various methods used in the assessment of emphysema are described in section **1.7** below.

1.6 CLASSIFICATION OF EMPHYSEMA

Although emphysema is often thought of as a single entity, the disease has been recognised as occurring in various forms, each of which may be the result of different pathogenetic mechanisms. Some forms of emphysema, such as infantile lobar emphysema and Swyer-James Syndrome (Reid, 1967b) are not smoking-related, and will not be considered in this thesis.

Emphysema can be recognised by examining slices of fixed inflated lung specimens with the naked eye, or using a hand lens or dissecting microscope (macroscopic emphysema); or by examining tissue sections at a microscopic level (microscopic emphysema). The various forms of emphysema are described below.

1.6.1 Macroscopic Emphysema

A number of patterns of distribution of emphysema have been identified in the lung, and macroscopic emphysema has been classified into 4 main types according to these distribution patterns. The 4 main types of emphysema are:

1. Centriacinar
2. Panacinar
3. Paracicatricial
4. Paraseptal

Each of these forms will be described below.

1.6.1.1 *Centriacinar Emphysema*

Centriacinar emphysema (also called centrilobular or proximal acinar emphysema) is the most common form of emphysema. It involves the enlargement of the airspaces around the respiratory bronchiole *i.e.* the proximal acinus (Figure 1.3). This form of emphysema is often associated with areas of black pigmentation (Figure 1.4). It tends to occur most commonly in the upper lobes of the lung, and tends to be more severe in the upper lobes (Thurlbeck, 1963a). Centriacinar emphysema is almost exclusively associated with cigarette smoking (Weissler, 1987) and this may account for the finding that it is more common in men than in women

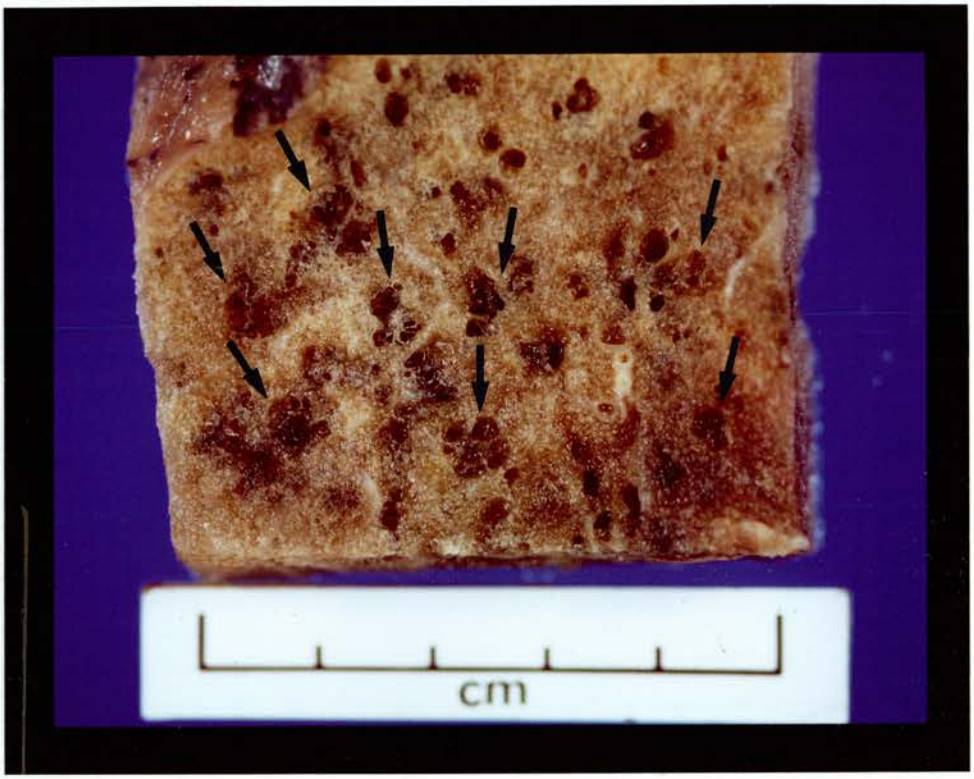


FIGURE 1.3

A photograph of an area of inflated lung tissue showing centriacinar emphysema. The emphysematous lesions are marked by the arrows.

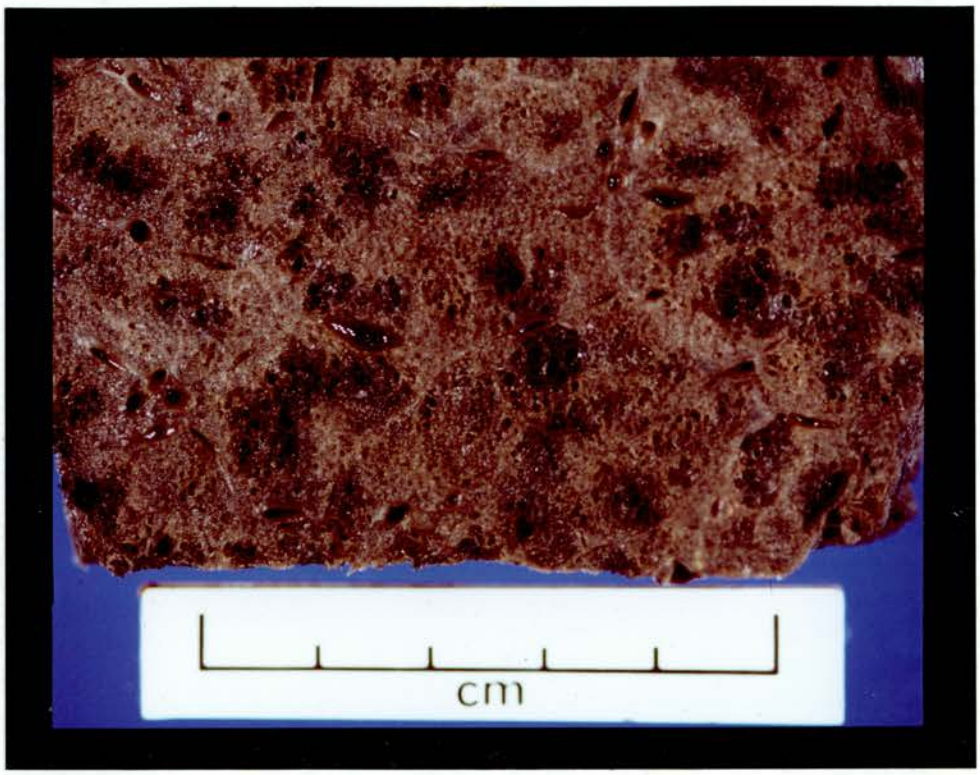


FIGURE 1.4

This photograph shows an area of lung with centriacinar emphysema. This specimen shows centriacinar pigmentation.

(Snider, 1983). It is also most commonly found in people living in the polluted atmosphere of large cities. Centriacinar emphysema is a common condition, found in more than 50% of autopsy lungs (Thurlbeck, 1963a).

1.6.1.2 *Panacinar Emphysema*

Panacinar emphysema (also called panlobular emphysema) is characterised by progressive enlargement of all the alveoli in the acinus so that the distinction between alveolar ducts and alveoli is lost (Figure 1.5). Airspaces become progressively enlarged and gross simplification of lung structure occurs (Weissler, 1987). Panacinar emphysema can be widespread throughout the lung, but in severe forms it is commonly more predominant in the lower lobes (Thurlbeck, 1963a). The severity of this form of emphysema may vary from involving only small areas of the lung in the mildest cases, to almost complete destruction of the alveolar tissue of the lung.

Although centriacinar and panacinar emphysema have been described as separate entities, they often occur in the same lung (Dunnill, 1987). As centriacinar emphysema progresses and becomes widespread and severe, it may be difficult to differentiate centriacinar from panacinar emphysema (Snider, 1983). Some workers classify severe emphysema as centriacinar in type on the basis of predominant involvement of the upper lung fields (Pratt & Kilburn, 1970; Snider, 1983). However, once centriacinar lesions have become confluent, the whole of the acinus becomes involved (Figure 1.6) and some workers believe that by definition, this type of emphysema should be called panacinar (Snider, 1983; Thurlbeck, 1976; Lamb, personal communication).

There is some debate over whether centriacinar and panacinar emphysema are different diseases at all (Mitchell *et al*, 1970; Anderson & Foraker, 1973). It has been suggested that panacinar emphysema is a natural progression of centriacinar emphysema (McLean, 1957; Crofton & Douglas, 1981). However, the fact that widespread centriacinar emphysema can exist with no evidence of panacinar emphysema suggests that there are differences between these 2 types, and it is possible that the different patterns of involvement of these 2 forms of emphysema reflect different pathogenetic mechanisms (Weissler, 1987).

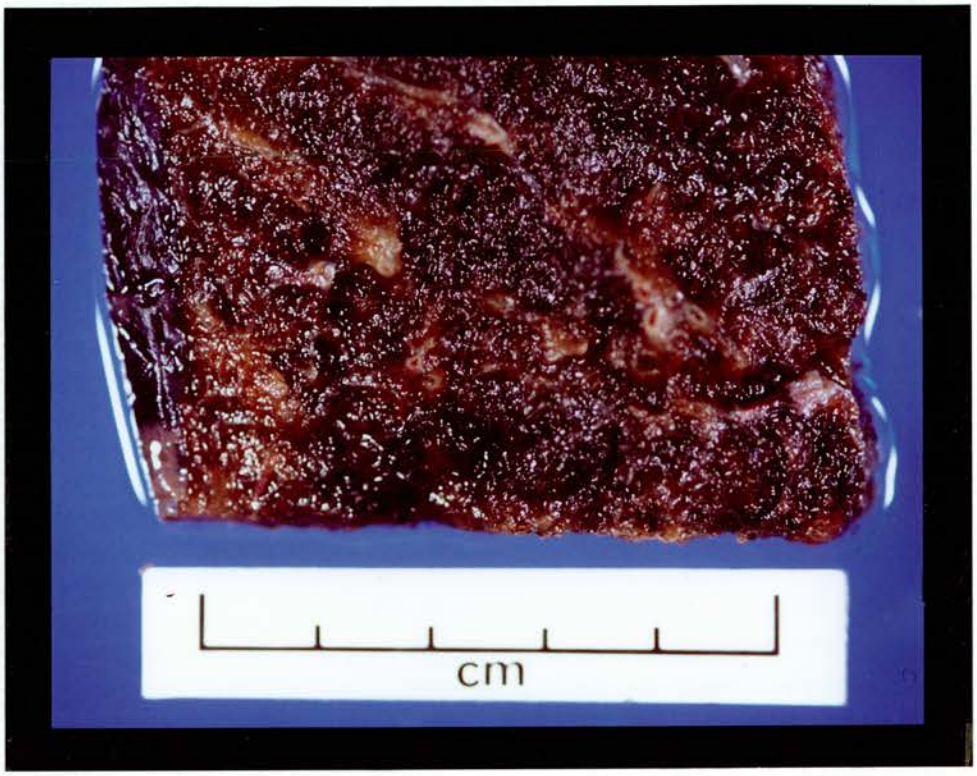


FIGURE 1.5

A lung specimen showing widespread macroscopic panacinar emphysema. Note the gross pigmentation, and complete disruption of the parenchymal structure.

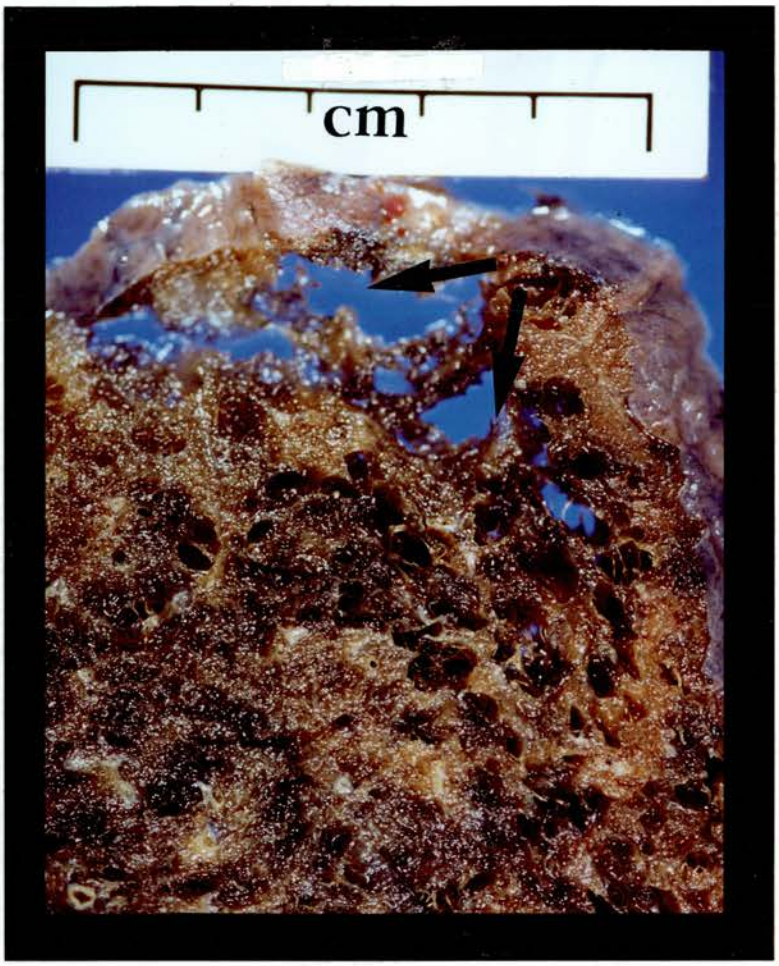


FIGURE 1.6

This photograph shows the appearance of confluent centriacinar emphysema (indicated by the arrows) at the apex of the upper lobe. Note that the acinar structure has been completely destroyed in the areas of confluence.

1.6.1.3 *Paracicatricial Emphysema*

Paracicatricial or scar emphysema (Figure 1.7) occurs when scars are formed within the lung parenchyma, either as a result of industrial lung disease, or following non-resolution of an inflammatory process. Abnormally enlarged airspaces which surround a scar lack any special distribution within the acinus (Dunnill, 1987).

1.6.1.4 *Paraseptal Emphysema*

Paraseptal emphysema is predominantly sub-pleural in location (Figure 1.8) and tends to be more severe in the upper lung zones. Its aetiology is not understood - it may be the result of a congenital defect (Weissler, 1987). When paraseptal emphysema occurs sub-pleurally it can result in spontaneous pneumothorax in young adults.

It must be remembered that, while pure forms of each of the above conditions may occur, it is more usual to find more than one type of emphysema in any given lung (Dunnill, 1987).

1.6.2 Microscopic Emphysema

Until recently, the classification of different forms of microscopically recognised emphysema was not common practice. However, various patterns of microscopic tissue destruction have been recognised.

The loss of peribronchiolar alveolar attachments has been found to relate to loss of airway support and consequent airflow obstruction (Linhartova *et al*, 1971; 1977; Petty *et al*, 1986). It is thought that the loss of these alveolar walls occurs in emphysema (Saetta *et al*, 1985a; Petty *et al*, 1986; Nagai *et al*, 1991), but there is some evidence to suggest that the attachments are not lost in all emphysematous lungs (McLean *et al*, 1987), and this may help to explain the variations in the decline of pulmonary function in patients with emphysema.

Kim and co-workers (1991) have noted the distinction between microscopic centrilobular and panlobular emphysema, assessed using non-quantitative criteria, in smokers. Their results have indicated that these 2 forms of

microscopic emphysema have different functional implications, and the findings suggest that different pathogenetic mechanisms may be involved.

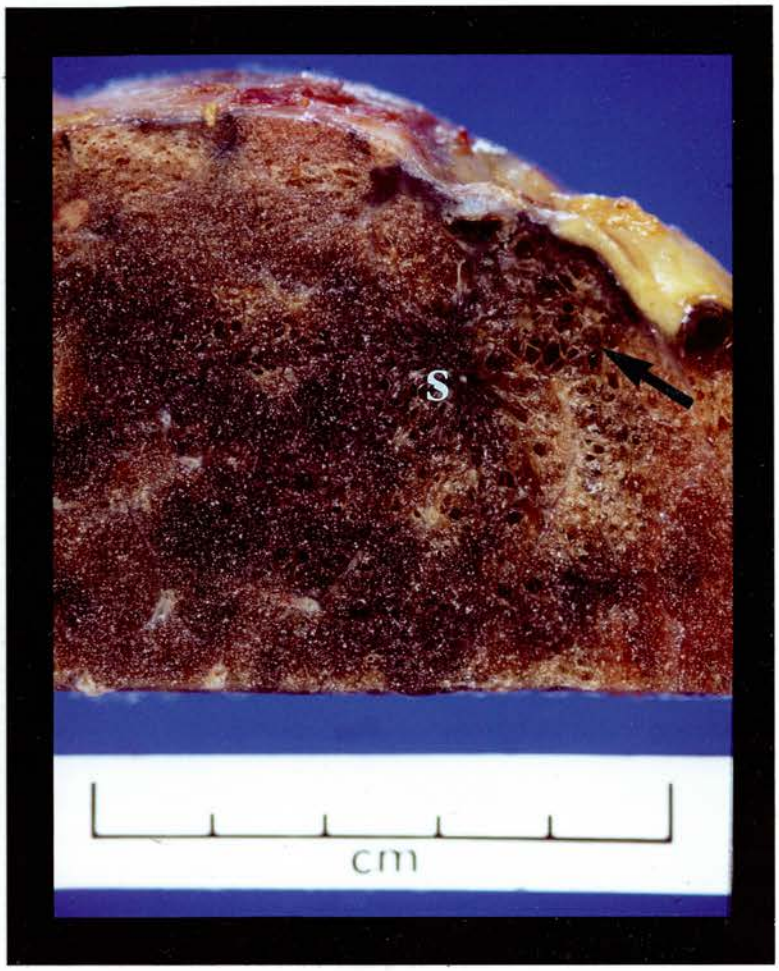


FIGURE 1.7

A lung specimen showing paracicatricial emphysema (arrow) next to an area of scarring (S).

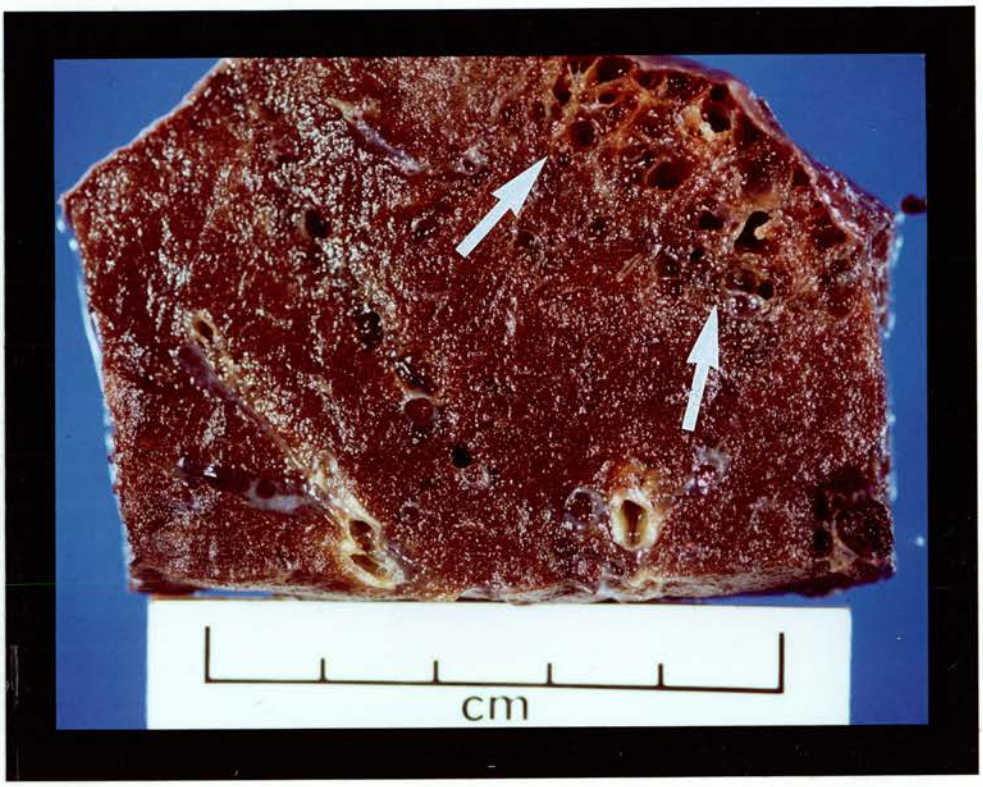


FIGURE 1.8

This photograph shows an example of paraseptal emphysema. The lesion is situated sub-pleurally, and the surrounding parenchyma appears normal.

1.7 METHODS USED IN THE ASSESSMENT OF EMPHYSEMA SEVERITY

This section contains a brief description of some of the most commonly used methods for assessing the severity of pulmonary emphysema. Some of the advantages and disadvantages of the various methods are mentioned.

1.7.1 Macroscopic Assessments of Emphysema

Several techniques are used in the macroscopic assessment of emphysema. In some of the techniques, the preparation of Gough-Wentworth paper-mounted thin sections of whole lungs is recommended (Gough & Wentworth, 1949). This is a laborious technique, and whole lung slices of approximately 1cm thickness are often used instead.

1.7.1.1 *The Ciba Method*

The method proposed by the Ciba Guest Symposium in 1958 represented an attempt to standardise the assessment of emphysema (Ciba, 1959). Using this method, the average severity of centriacinar, panacinar or focal emphysema is assessed for each lobe. Illustrations are provided as examples of mild, moderate and severe emphysema. Using these standards and the following guidelines, each lobe is graded as follows:-

Mild emphysema - less than 25% involvement of respiratory tissue
Moderate emphysema - 25%-50% involvement of respiratory tissue
Severe emphysema - more than 50% involvement of respiratory tissue

1.7.1.2 *Ryder's Grid Technique* (Ryder *et al*, 1969)

A grid consisting of 5 equidistant radiating lines is drawn on a transparent sheet of plastic, and this is superimposed on each paper-mounted lung section, dividing the lung section into 10 equal regions. Each region is examined and given an emphysema score from 0 - 3, where

0 = absent
1 = mild
2 = moderate
3 = severe

The total emphysema score for each section is then calculated. This method was designed for use with paper-mounted lung sections, but it can be used on slices of fixed lung. Several variations on this method have been described, using various numbers of zones (Heard & Izukawa, 1964; Thurlbeck, 1967a) but they are all based on the same principle.

1.7.1.3 *Thurlbeck's Panel Grading* (Thurlbeck *et al*, 1969)

Paper-mounted sections of lung are graded according to the extent of emphysema using a series of standard 10" x 8" photographs of paper-mounted sections. Grades range from 0 to 100 and were chosen as follows:-

0 - normal lung (*i.e.* no visible emphysema)
20 - a good example of mild emphysema
50 - an example of moderate emphysema
80 -severe emphysema
100 - the worst case of emphysema encountered in that series of cases
Intervening standards at intervals of 10 were selected from a collection of around 500 paper-mounted lung sections.

This grading system was updated in 1970 when the 5 original 'milestone' standards were kept, but intervening standards were set at intervals of 5 between 0 and 50, and at intervals of 10 from 60 to 100 (Thurlbeck *et al*, 1970a).

The object of this method is to assign a grade to each lung section according to the standard photograph which most closely resembles it. If the extent of emphysema appears to fall between 2 standards, then an intermediate grade should be given.

It should be stressed that the grades in this method represent 'arbitrary intuitive milestones in the spectrum of severity of emphysema' (Thurlbeck *et al*, 1969). The values do <u>not</u> represent percentages.

Although all of the above methods of assessing macroscopic emphysema are quick and fairly simple to use, they are all subjective and semi-quantitative. The results obtained using these methods are therefore likely to vary according to the opinions and experience of the observer.

1.7.1.4 *Dunnill's Point Counting Technique* (Dunnill, 1962b)

Fixed inflated lungs are cut into 1cm parasagittal slices. A grid consisting of a transparent plastic sheet with equidistant points on it is placed over each slice. The lung tissue under each point is examined and classified as:-

1. Non-parenchyma (bronchi & blood vessels > 2mm in diameter)
2. Normal parenchyma
3. Abnormal airspaces

The number of points which lie on abnormal airspaces (*i.e.* >1mm in diameter) is divided by the total number of points on parenchyma, and this is expressed as a percentage.
This method is more objective than those described above, but it does have a subjective element, since the classification of each point is based on the judgement of the observer.

As Thurlbeck (1967a) noted, a major disadvantage of Dunnill's technique is that it measures the extent of involvement of a lung by emphysema, but it does not necessarily measure the severity of emphysema. No distinction is made between 100% involvement with mild panacinar emphysema and 100% involvement with severe panacinar emphysema. In addition, no distinction is made between types of macroscopic emphysema. Therefore, using this technique, widespread panacinar emphysema would be given a high score, while localised severe centriacinar emphysema would be given a low score. For these reasons, the predominant type of macroscopic emphysema should be noted when using this method.

1.7.1.5 *Objective Methods for Assessing Macroscopic Emphysema*

In the 1960s two automated techniques were described for the objective assessment of macroscopic emphysema on lung sections. These were based on the principle that an emphysematous lung would be less dense than a normal lung. The devices described by Kory *et al* (1966) and Longfield & Hentel (1966) both measured the amount of light transmitted through thin sections of whole lungs. Longfield and Hentel experimented with the use of sound and beta-radiation energy sources for transmission through the lung, but found that light transmission gave the closest correlation to conventional macroscopic assessments.

The disadvantage of these methods was that they could only detect gross changes in lung structure, and the measurements would be influenced by variations in the thickness of the whole lung sections. Also, the degree of pigmentation in the lungs - caused by blood or anthracitic deposits would affect the light transmission through the lung slices and hence affect the tissue density measurements.

1.7.1.6 *Advantages and Disadvantages of Macroscopic Techniques*

All of the macroscopic assessment techniques above have their advantages and disadvantages. A major advantage of examining whole lung slices is that it allows a quick assessment of the type and extent of macroscopic emphysema present. This does not involve selective sampling of small areas of lung tissue. However, there are several important criticisms which may be made regarding the available macroscopic techniques. With the exception of the automated techniques described in section **1.7.1.5**, none of the macroscopic techniques described above are truly quantitative. They allow lung specimens to be ranked according to greater or lesser degrees of severity, but the grading involved is not on a linear scale, and therefore the intervals between grades are not necessarily constant. It is therefore inappropriate to use parametric statistics, including mean grades of emphysema severity, in the comparison of various groups of subjects. Such parametric analyses are frequently quoted in the literature, and are not statistically valid.

The type of macroscopic emphysema detected in the lungs is often not specified when using the popular assessment techniques. It should be noted that if a mixture of emphysema types is present, the severity of each type should be assessed separately.

Another major criticism of macroscopic techniques is that they are insensitive to early emphysema. Macroscopic assessments are performed using the naked eye, or, at most, a dissecting microscope, and airspaces of 1mm in diameter or larger are considered to be emphysematous. However, the average diameter of a normal airspace has been shown to be in the region of 250μm (Weibel, 1963; Schreider & Raabe, 1981). Therefore, by the time the average airspace diameter has increased to 1mm, the airspace wall

surface area available for gas exchange will have been reduced by as much as 75%, leading to a significant reduction in respiratory efficiency (Lamb, 1990). Hence, macroscopic techniques assess only gross changes in lung structure, and are representative of end-stage disease. In order to study the changes involved in the onset of emphysema, it is necessary to assess the parenchymal tissue at a microscopic level.

1.7.2 Microscopic Assessments of Emphysema

Various methods have been used in the microscopic assessment of emphysema. These fall into 2 categories - subjective and objective techniques. Some of the most well-known of these are described below.

1.7.2.1 *The Destructive Index* (Saetta *et al*,1985b)

Using this technique, histological sections of lung tissue are examined using an eyepiece graticule (Weibel No.2). Airspaces lying under each point on the graticule are classified as normal (N) or destroyed (D), according to several criteria (Saetta *et al*, 1985b). The destructive index (DI) is calculated using the formula:-

$$DI = D / (D+N) \times 100$$

Although much of the definition of the DI seems to be objective, Saito and colleagues, in a study of the DI (Saito *et al*, 1989) found that the most influential criterion for classifying an airspace as 'destroyed' was the 'classical emphysematous lesion', the definition of which is completely subjective. Saito *et al* found that the DI was an extremely time-consuming technique which was no better than conventional macroscopic assessments of emphysema.

1.7.2.2 *Picture Matching Technique*

Nagai and co-workers (1989) described a technique which involves the comparison of 3cm x 2cm x 5µm histological sections of lung tissue with standard photographs. Each section is given a grade from **0 - 10** where **0** = normal lung and **10** = almost complete destruction of lung parenchyma. Six sections are examined from each lung, and the mean of their scores is used as the score for the entire lung.

This method is similar in design to Thurlbeck's panel grading method, and has the same disadvantages due to its subjectivity. The scale of grades is not linear, and mean scores are therefore inappropriate. This is not a truly quantitative method.

1.7.2.3 *Classification of Two Forms of Microscopic Emphysema*

Kim and co-workers (1991) used published descriptions of the features of 2 types of macroscopically recognisable emphysema (panlobular and centrilobular) to develop a technique for assessing the presence of these 2 types on histological sections. The diagnosis of panlobular emphysema (PLE) is made when the enlargement of airspaces involves the whole acinar unit, and the distinction between alveoli and alveolar ducts is lost. Microscopic centrilobular emphysema (CLE) is considered to be present when sharply demarcated emphysematous spaces, separated from the acinar periphery by intact alveolar ducts and sacs of normal size, are observed.

This method is essentially a means of classifying the microscopic patterns of emphysema observed. These workers did not describe the relationship of their microscopic classification of emphysema to the macroscopic patterns of emphysema present in their sample. This method is non-quantitative in nature, and the grading of the severity of the 2 forms of microscopic emphysema is not involved.

1.7.2.4 *The Mean Linear Intercept*

The respiratory surface of emphysematous lungs is reduced due to the destruction of alveolar walls. The mean linear intercept technique, and the other objective techniques described below, involve the measurement of this destruction in one form or another, in order to assess the extent of microscopic emphysema.

The mean linear intercept is the most commonly used index of alveolar destruction. The mean linear intercept (Lm) is essentially the mean distance between alveolar walls. Lm is measured on histological sections using an eyepiece graticule. Intercepts between alveolar walls and the cross-hairs of the graticule (test-lines) are counted. Providing the lengths of the test-lines are known, then

$$\mathbf{Lm = NxT / I}$$

where N = number of fields measured
T = test-line length
I = total number of intercepts

The Lm is used as an index of emphysema, where Lm is increased with increasing emphysema. Alternatively, the surface area of the alveolar walls can be calculated using a standard formula

$$SA = 2V / Lm$$

where V is the volume of tissue (*e.g.* lung volume or unit volume) (Aherne & Dunnill, 1982). The proof of this formula is given by Underwood (1970).

Measuring Lm is very time-consuming, as it involves counting intercepts on a large number of histological fields to ensure a large enough sample size to give a reasonable estimate of the true average distance between alveolar walls.

1.7.2.5 *Image Analysis Techniques*

The introduction of image analysis systems into the field of morphometry has taken much of the effort out of surface area measurements. These systems are either semi-automatic or fully automatic.

1.7.2.5.1 *Semi-Automated Techniques*

McCartney *et al* (1988) described a semi-automatic technique which was based on the Lm principle. A series of parallel lines was drawn on a sheet of transparent plastic, and superimposed on a projected image of each histological field. An electronic pen was used to measure the length of lines which fell across the airspaces, giving an indication of the increase in airspace size associated with inherited emphysema in the Blotchy mouse. This technique, although less labour-intensive than the Lm technique, was relatively slow, taking 30 minutes to measure 5 fields.

It is possible to measure alveolar wall surface area using a digitising tablet. A camera lucida attachment allows the perimeter of the alveolar walls to be traced on a computer-linked digitising tablet using an electronic cursor. Perimeter length can be converted to surface area using a standard morphometric formula:-

$$SA = P \times 4/\pi \quad \text{(Williams, 1977)}$$

Some of McLean's early airspace wall surface area measurements were made using this method (McLean, 1987) and the same principle was later used with an interactive automated image analysis technique (section **1.7.2.5.2**).

This technique, as with all microscopic morphometric techniques, involves a sampling procedure. Sufficient fields must be measured to produce values which are representative of the lung. This method is consequently very slow.

1.7.2.5.2 *Fully Automated Techniques*

In 1964, Duguid and co-workers described an automated scanning system which measured sections of lung tissue mounted on cine films. More recently, McLean (1987) and Gould and colleagues (1988) used the IBAS automatic image analysis system to measure the total airspace wall perimeter in random fields of tissue sections.

The IBAS (Kontron Ltd., Watford, England) is a computerised system which involves analysis of stored images of histological fields from tissue sections. The system consists of a microscope equipped with a video camera. An image of each selected histological field is captured using the camera and transmitted to the image processing system. The IBAS stores images in a digitised form, where digital images consist of arrays (512 x 512) of square picture elements, or 'pixels'. The IBAS constructs 8-bit digitised images, where 256 grey levels are recognised, ranging from 0 which represents white and 255 representing black. A series of image processing adjustments can be used to enhance the contrast of the image, which can then be edited by the user to exclude unwanted details from the image. (*e.g.* Non-parenchymal structures can be excluded when only the alveolar walls need to be included in the measurement). Total airspace wall perimeter *per* unit area (mm/mm^2) is then quantified automatically. The number of histological fields measured must be large enough to produce a stable running mean. This ensures that the sample is representative of each lung specimen.

Although this type of system produces very accurate measurements from single histological fields, the interactive editing and enhancement of the

digitised images is extremely time-consuming. Therefore, the number of fields which can be measured is limited by the time it takes to measure a single field.

1.7.2.6 *Advantages and Disadvantages of Microscopic Techniques*

The major advantages of microscopic techniques for assessing emphysema severity are that they are sensitive to early emphysematous changes (involving enlargement of airspaces which are still invisible to the naked eye), and that they are often quantitative.

Unfortunately, by necessity all microscopic techniques involve some form of tissue sampling, which, while providing sufficient information for an overview of the lung or lobe, results in limited information if intra-lung comparisons of individual measurements are required. The accuracy of all sampling techniques depends on the method used for the selection of fields. Traditionally-used manual methods such as Lm are extremely tedious and time-consuming to perform, and the automated and semi-automated techniques which have been available to date have all been slow enough to limit the sample size which can feasibly be studied.

It was therefore recognised that there was a need to develop a new automated technique for assessing airspace size in the objective diagnosis of microscopic emphysema on tissue sections. The technique developed in this study incorporated the Lm principle in a computerised scanning device, the fast interval processor (FIP). The development and assessment of the FIP are described and discussed in Chapter 3 of this thesis.

1.8 THE EPIDEMIOLOGY OF EMPHYSEMA

This project involved not only the development of a new method for assessing airspace size in human lung tissue, but also the study of the effects of age, sex and cigarette smoking on airspace size. The study group in this project was selected to show a range of ages and smoking histories in men and women, and may not be truly representative of the Scottish population. Nevertheless, a study of this type is related to epidemiological factors, and as an introduction to this aspect of the study, the following section is a review of the current evidence of the epidemiology of emphysema.

Epidemiological studies of pulmonary emphysema have generally been based on observations of the incidence and severity of macroscopically recognisable emphysema in lungs from autopsy specimens. These studies have shown that emphysema, in particular the centriacinar form, is linked to tobacco smoking. This conclusion has been based on the consistent finding that emphysema occurs more frequently, and with increased severity in smokers than in non-smokers (Anderson, Hernandez *et al*, 1964; 1966; Ishikawa *et al*, 1969; Ryder *et al*, 1971; Alli, 1972; Spain *et al*, 1973; Thurlbeck *et al*, 1974; Bignon *et al*, 1980).

The incidence and severity of macroscopic emphysema increase with age in smokers (Anderson *et al*, 1966; Burgess & Burgess, 1966; Hernandez *et al*, 1966; Petty *et al*, 1967; Ryder *et al*, 1971; Anderson *et al*, 1972; Auerbach *et al*, 1972; Spain *et al*, 1973; Thurlbeck *et al*, 1974; Sutinen *et al*, 1978; Sobonya & Burrows, 1983; Dijkman, 1986; Snider, 1989).

Increases in the incidence of macroscopic emphysema with advancing age have also been reported in non-smokers (Thurlbeck *et al*, 1974; Dijkman, 1986). However, there is substantial evidence that many of the changes occurring with age in the lungs of non-smokers are normal, and should not be confused with disease processes; in particular, the term 'senile emphysema' should be avoided (Burgess & Burgess, 1966; Thurlbeck, 1990).

Macroscopic emphysema has been found more frequently in men than in women, and is generally more severe in men (Alli, 1972; Anderson *et al*, 1972; Thurlbeck *et al*, 1974; Snider, 1983; 1989). This may be due to

differences in smoking habit between the sexes (Anderson *et al*, 1966; Snider, 1989), but it has been suggested that there are fundamental sex differences in the susceptibility to emphysema (Anderson *et al*, 1972; Thurlbeck *et al*, 1974; Bignon *et al*, 1980; Dijkman, 1986).

Emphysema has been found to occur more frequently in patients with a genetic deficiency of the protease inhibitor, alpha-1-antiprotease, especially if they are smokers (Laurell & Eriksson, 1963; Eriksson, 1964). The possibility that other genetic factors may influence the incidence of emphysema has been investigated but, with the exception of alpha-1-antiprotease deficiency, no single genetic influence has been identified (Faling, 1983; Redline & Weiss, 1989).

Occupations such as mining, with high level exposure to fine particulate dust, are high risk occupations with regard to lung diseases including emphysema, and individuals are particularly at risk if they smoke (Alli, 1972; Garshick & Schenker, 1989). Unfortunately it is often difficult to separate the effects of smoking and occupational hazard in studies of this type (Snider, 1989). The extent of the influence of air pollution on the susceptibility to emphysema is thought to be a minor one (Snider, 1989), although there is some evidence to suggest that the incidence of emphysema is higher in highly polluted, industrial areas as opposed to rural communities (Ishikawa *et al*, 1969; Alli, 1972; Thurlbeck *et al*, 1974; Higgins, 1991). Obviously, this may be related to occupational exposure to pollutants.

The techniques used in studies of the prevalence of microscopic changes in the lungs due to emphysema have been non-quantitative in nature (*e.g.* Auerbach *et al*, 1963; 1974). This may be due to the fact that the quantitative techniques available have been tedious to use. For the same reason, the limits of normal airspace size have not been defined, and therefore the information required to meet the criteria for diagnosing microscopic emphysema has not been available.

Clinical epidemiological studies of emphysema are difficult to perform because, as discussed earlier, clinical diagnoses of emphysema are extremely unreliable. Recent evidence has shown that CT scan density measurements

relate to alveolar wall surface area measurements (Gould *et al*, 1988; Flenley, 1990; 1991; MacNee *et al*, 1991), and it is hoped that CT scanning may be used in clinical epidemiological studies in the future, but the baseline data on the range of normal CT density values with age have still to be obtained.

1.9 THE PATHOGENESIS OF EMPHYSEMA

The information obtained from studies of early, microscopic emphysema may shed some light on factors relating to the pathogenesis of emphysema. It is therefore important to include an account of some of the theories of pathogenesis in this thesis. The subject of the pathogenesis of emphysema has received much attention in recent years, mostly relating to the protease-antiprotease theory, the basis of which will be described. It is outwith the scope of this thesis to give a detailed literature review of this subject, and much of the information included here has been obtained from review articles.

Several theories have been proposed to explain the mechanisms responsible for the onset of pulmonary emphysema. One of the earliest theories to be considered was that emphysema was due to the mechanical overinflation of the lungs (Rosenblatt, 1972; Dunnill, 1987; Eriksson, 1991). This theory was first proposed by Laennec in 1834. Laennec suggested that emphysema was due to partial bronchial obstruction leading to a pressure increase in the lung distal to the obstruction, which eventually led to tissue destruction (Eriksson, 1991). This view was generally held for over 100 years.

However, normal alveolar walls have been shown to withstand extreme overdistension without rupture, possibly due to the collateral ventilation which occurs through the pores of Kohn (Anderson & Foraker, 1962). In support of this, experimental attempts to produce emphysema by obstruction of the bronchi have been unsuccessful (Eriksson, 1991). Also, asthmatic patients do not develop destructive emphysema despite having continuous or intermittent airflow obstruction (Pratt & Klugh, 1967). The experimental results and findings in patients with asthma suggest that the mechanical overdistension hypothesis is unlikely to be accurate.

It has been suggested that the onset of emphysema is related to inflammation of the bronchi and bronchioles leading to weakening and destruction of the alveolar walls (Gough, 1952; Reid, 1954; Leopold & Gough, 1957; Anderson & Foraker, 1962; Anderson, Azcuy *et al*, 1964). Although this theory was widely accepted for many years, the mechanisms responsible for the alveolar wall destruction remained unknown.

Two major breakthroughs in the study of the pathogenesis of emphysema occurred in the early 1960s. In 1963, Laurell and Eriksson reported on the high incidence of emphysema in patients with an inherited deficiency of the protease inhibitor alpha-1-antiprotease (also called alpha-1-antitrypsin). In 1964 Gross and colleagues produced an experimental form of emphysema in rats by exposing them to the plant-derived proteolytic enzyme papain.

These two important studies and the many which followed led to the development of a theory that unrestrained proteolytic activity (in particular elastolytic activity) in the lung was the major pathogenetic mechanism responsible for the development of pulmonary emphysema (Weissler, 1987). This hypothesis has become known as the protease-antiprotease (elastase-antielastase, enzyme-inhibitor) hypothesis.

Several mechanisms are thought to be involved in creating the protease-antiprotease imbalance in the lungs. In patients with inherited deficiency of alpha-1-antiprotease (the most abundant protease inhibitor in the circulation), normal levels of protease activity are thought to be sufficient to overload the inhibitory capacity of the antiprotease, and this may lead to tissue destruction.

In patients with normal circulating alpha-1-antiprotease levels, a localised enzyme-inhibitor imbalance is thought to be related to tobacco smoking. The introduction of tobacco smoke into the lungs is thought to initiate a number of responses.

Smokers have been found to have an increased number of macrophages in bronchoalveolar lavage fluid compared with non-smokers. It is thought that macrophages migrate to the alveoli during the inflammatory response to the presence of tobacco smoke (Janoff, 1983; Fels & Cohn, 1986). Macrophages release chemotactic factors which attract neutrophils to the alveoli, and induce the neutrophils to secrete elastase (Hunninghake *et al*, 1980; Janoff, 1985; Dunnill, 1987; Weissler, 1987). In experimental studies it is the destruction of elastin molecules which leads to emphysema, and therefore excess neutrophil elastase secretion may be linked to the onset of human emphysema (Weissler, 1987).

Cigarette smoke releases oxidants which are thought to be directly related to tissue destruction. The oxidants in tobacco smoke may stimulate neutrophils to release more elastase. Therefore, in addition to greater numbers of neutrophils being present, each of these may also produce larger than normal levels of elastase (Janoff, 1983; Gadek & Pacht, 1990). Oxidants are thought to inactivate protease inhibitors, including alpha-1-antiprotease, which inhibits trypsin and collagenase as well as elastase (Janoff, 1985; Smith *et al*, 1986; Weissler, 1987). As described above, the uninhibited action of proteases is thought to lead to destruction of the alveolar walls. In addition to these destructive effects, the oxidising agents released by tobacco smoke are thought to inhibit the action of the enzyme lysl oxidase, which forms cross-links during elastin synthesis. This mechanism may directly inhibit connective tissue repair by inhibiting elastin resynthesis following injury by protease-antiprotease imbalance (Weissler, 1987; Gadek & Pacht, 1990).

It must be emphasized that the evidence supporting the protease-antiprotease hypothesis is largely indirect. Much of the supporting evidence has been obtained from experimental studies involving inducing emphysema in laboratory animals. However, experimental emphysema is invariably panacinar in type (Weissler, 1987), while the type most commonly found in smokers is centriacinar (Thurlbeck, 1963a).

Some authors have attempted to use the protease-antiprotease hypothesis to explain the variations in the mechanisms responsible for the onset of centriacinar and panacinar emphysema. These workers have concentrated on the ventilation/perfusion relationships within the lung (Cockcroft & Horne, 1982; Dunnill, 1987) and have hypothesized that centriacinar emphysema is the result of a localised imbalance, and panacinar emphysema is the result of a systemic protease-antiprotease imbalance. These theories are interesting and have received some recent support (Kim *et al*, 1991).

The protease-antiprotease hypothesis is now widely accepted, and a vast amount of experimental work is currently being performed on the assumption that it is accurate. However, in accepting the hypothesis, a point which is often overlooked is that while it is generally accepted that

every smoker responds to the insult of tobacco smoke in the same way, not every smoker develops emphysema (Sobonya & Burrows, 1983). As Wewers and Gadek (1987) noted, there is a need for a precise definition of the cellular and genetic basis for the considerable differences in susceptibility of individual smokers to the adverse effects of cigarette use.

1.10 BACKGROUND TO THIS STUDY

The various definitions of emphysema all include reference to the abnormal enlargement of airspaces distal to the terminal bronchiole (Ciba, 1959; American Thoracic Society, 1962; Reid, 1967b; Snider *et al*, 1985). Detailed studies of human lungs have shown that there is variation in airspace size, but the average diameter in the adult is around 250μm (Weibel, 1963; Schreider & Raabe, 1981). The lung is a dynamic organ, and its structure changes with advancing age in adulthood (Azcuy *et al*, 1962; Thurlbeck, 1990); therefore, normal airspace size is likely to become altered with age.

The need to establish the normal range of airspace size with age and sex was recognised at the Ciba Guest Symposium (Ciba, 1959) but unfortunately the limits of normal airspace size remain undefined.

As described earlier in this chapter, most of the studies of emphysema have been based on macroscopic observations, with airspaces larger than 1mm in diameter considered to be emphysematous. Macroscopic techniques are therefore insensitive to early emphysema, and most of the techniques which have been available for assessing microscopic emphysema have been tedious to use. In the course of this study, a new automated technique has been developed for the accurate measurement of airspace wall surface area *per* unit volume of lung tissue.

1.11 AIMS OF THIS STUDY

The aims of this study were as follows:

1. To develop and assess a new automated technique for measuring airspace wall surface area on histological sections.

2. To measure airspace wall surface area *per* unit volume of lung tissue (AWUV) in a group of non-smokers' lungs; to investigate the relationship between AWUV and age, and to establish a range of normal AWUV values.

3. To determine whether the AWUV/age relationship is the same in men and women.

4. To examine the effect of smoking on AWUV, by observing the AWUV/age relationship in a group of smokers; and by using the information obtained in the study of the non-smokers as the basis for an anatomical diagnosis of microscopic emphysema.

5. To measure AWUV in a series of whole lung specimens to assess the variation in AWUV measurements from the apex to the base of the lung.

6. To assess the extent of macroscopic emphysema in the study sample; to study the relationship between the distribution of macroscopic emphysema and the distribution of AWUV measurements within the lung; and to study the relationship between macroscopic and microscopic emphysema.

Chapter 2

Materials and Methods

This Chapter contains details of the tissue sample collected, and descriptions of the methods used in preparing the lung specimens for histology, assessment of macroscopic emphysema, a brief summary of the morphometric technique used (details of this technique are discussed in Chapter 3), and details of the methods used in data handling and statistical analysis.

2.1 COLLECTION OF LUNG SPECIMENS

At the beginning of this project I intended to use a series of autopsy lung specimens which were being collected by staff at the Institute of Occupational Medicine in Edinburgh. These specimens were to be used as controls in a study of the effects of dust exposure on the lungs. The lungs came from accidental or sudden death victims, where death was not due to respiratory disease. Full details of occupational history and smoking history were obtained from interviews with relatives. Where occupational and smoking history could not be obtained the specimens were excluded from the study. None of these cases had any occupational dust exposure. The aim was to collect a total of approximately 160 lungs representing male and female smokers and non-smokers of a wide age range.

Unfortunately, funding for the Occupational Medicine project ended before the collection of these specimens was complete. In addition, many of the lungs were not fixed in inflation and were therefore unsuitable for this study. As a result, only 30 of the specimens were included, and an alternative source of lung specimens had to be found. Fourteen lungs were collected from routine autopsies. Smoking histories were documented for 13 of these individuals. The remainder of the sample consisted of surgical lung specimens. A previous study in the Pathology Department involved collecting lobes or lungs which had been removed surgically as treatment for a peripheral tumour. 40 of these specimens were included in this study, and the collection of surgical specimens was continued. Smoking histories and simple respiratory function data were obtained from clinical records for all the surgical specimens.

The final sample studied in this project consisted of 165 lung specimens. Of these, 44 were lungs obtained at autopsy and 121 were lungs or lobes obtained by surgical resection. Of this sample 125 individuals were smokers, 39 were non-smokers, and there was 1 individual whose smoking history was unknown. The age range of the sample was 21 to 93 years (mean age 59.9 years). The age of one of the non-smokers was unknown, and this case was excluded from all analyses involving age. The non-smoking group included 16 males and 23 females, and the smoking group consisted of 95 males and 30 females. A detailed description of the numbers, sex and smoking history of all the individuals in this study is given in Table 2.1.

Individuals were included in the non-smoking group only if they were documented as 'lifelong non-smokers'. In the smoking group, length of smoking history and the number of cigarettes smoked each day were recorded when this information was available. These individuals were divided into 3 sub-groups based on the extent of tobacco consumption as follows:

1. Less than 20 cigarettes each day
2. Between 20 and 29 cigarettes each day
3. 30 or more cigarettes each day.

In order to study airspace surface area in relation to macroscopic emphysema, the sample was subdivided into 2 groups based on the presence or absence of macroscopically visible airspaces (*i.e.* larger than 1mm in diameter) in the mid-sagittal slice. The sub-group with evidence of macroscopic emphysema was then further sub-divided. Firstly the sub-group was split into 4 groups according to the type of macroscopic emphysema, and then the severity of each type was assessed. The 4 groups based on type of emphysema were as follows:

1. Centriacinar emphysema only
2. Panacinar emphysema only
3. Panacinar and centriacinar emphysema
4. Other types of macroscopic emphysema

Section **2.9** details the method by which macroscopic emphysema was assessed.

TABLE 2.1 A summary of the study sample, showing the number of male and female subjects in each of the sub-groups assigned according to smoking history.

	Non-smokers			Smokers			Unknown smoking history			
	A	**B**	**Sub-total**	**A**	**B**	**Sub-total**	**A**	**B**	**Sub-total**	**TOTAL**
Male	3	13	16	18	77	95	0	0	0	112
Female	14	9	23	8	22	30	1	0	1	53
TOTAL	17	22	39	26	99	125	1	0	1	165

A = autopsy specimen
B = biopsy specimen

2.2 INFLATION AND FIXATION

All the specimens were inflated intra-bronchially with neutral buffered formalin at a pressure of 25cm H_2O using the 'natural contour' inflation technique (American Thoracic Society, 1959). Buffered formalin was introduced into the lung intra-bronchially via a plastic tube connected to a reservoir of fixative situated 25cm above the specimen. Formalin was allowed to flow into the bronchi until the pleural surface was smooth and firm to the touch, and the natural contours of the pleura were established. When the lungs were fully inflated, they were floated in buffered formalin in a covered container for a minimum of 24 hours until fixation was complete.

2.2.1 Preparation of Neutral Buffered Formalin (Kiernan, 1990)

4L 40% technical grade formaldehyde
260g Anhydrous di-sodium hydrogen orthophosphate (Na_2HPO_4)
160g Sodium di-hydrogen orthophosphate ($NaH_2PO_4.H_2O$)
Cold water

The buffer salts were dissolved in warm water and the solution allowed to cool. Formaldehyde was added, and the mixture was made up to 40 litres with cold water.

2.3 SPECIMEN TRIMMING

The lungs were cut into 1cm parasagittal slices (usually 4 or 5 for each lung). This was done by placing each lung in turn on a trimming board with 1cm raised sides, with the hilar surface of the lung upwards. The lungs were then sliced using a disposable stainless steel trimming knife with a 12" blade.

2.4 TISSUE SAMPLING

Tissue blocks were cut from the fixed lungs using a 2cm x 2cm plastic template. The block thickness was restricted to a maximum of 0.5cm, to facilitate tissue processing. Different sampling techniques were used for the lobectomy and whole lung specimens.

2.4.1 Single Lobe Specimens

Tissue blocks were cut from the lateral 2 parasagittal slices of the lobes. This was in keeping with the sampling technique of a previous study of lung function and structure. The lateral 2 slices were used because they contained fewer large airways and vessels, and the non-respiratory bronchioles were more likely to be transversely sectioned in these slices. Sections from 40 of the lobes in the structure/function study were re-analysed in this study. A minimum of 6 2cm x 2cm tissue blocks were cut at random from the lateral 2 slices of each lobe. These blocks were sampled by overlaying the lateral 2 parasagittal slices with a grid of 2cm x 2cm squares on a transparent sheet (Figure 2.1). Six blocks were cut from each slice, using a table of random numbers to provide the co-ordinates of each block. Abnormal areas of tissue were excluded from the sample.

2.4.2 Whole Lung Specimens

The whole lung specimens were used to study the variation in airspace surface area from apex to base of the lung. The sampling technique used was

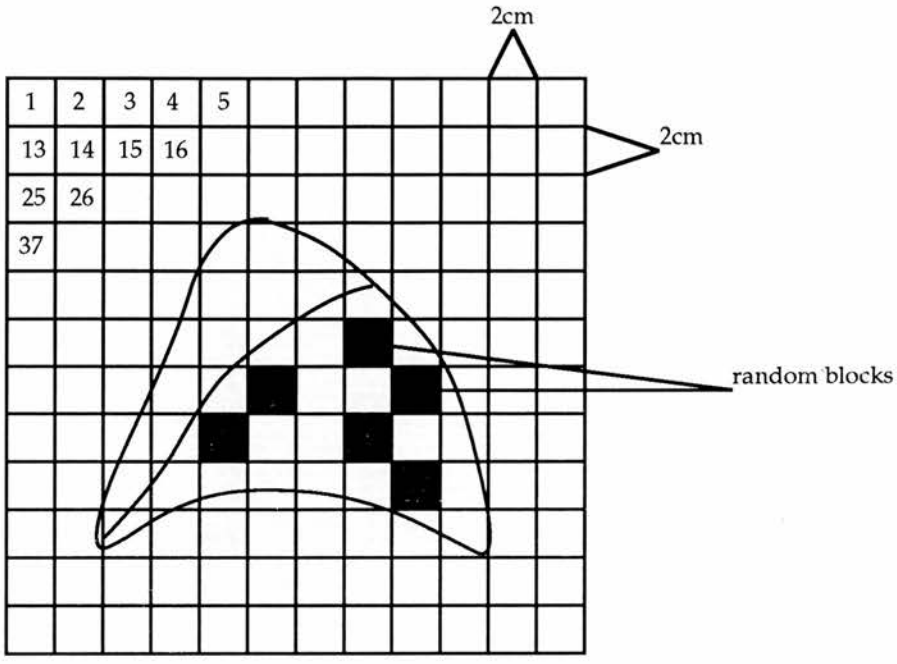


FIGURE 2.1

The sampling technique used for selecting tissue blocks from single lobe specimens. The lateral 2 parasagittal slices of each specimen were overlaid by a transparent sheet marked in 2cm x 2cm squares. Each square on the sheet was numbered, and the position of the blocks was chosen by selecting numbers from a random number table. Where the grid number fell over an area of the tumour, another random number was selected, so that abnormal areas of tissue were excluded from the sample. Six random blocks were cut from each specimen in this way.

as follows. Tissue blocks were cut from the mid-sagittal slice of each lung. The mid-sagittal slice was chosen because of its size, representing the full apex to base height of the lung. The mid-sagittal slice was divided into 6 zones, 3 in the upper lobe and 3 in the lower lobe as follows: The apex to base height of each lobe was measured and divided by 3 to give the height of each zone. The upper and lower lobes were then divided accordingly (Figure 2.2). For simplicity, where the specimen was a right lung, the middle lobe was considered to be part of the upper lobe. Two random 2cm x 2cm blocks were cut from each zone (a total of 12 blocks from each lung).

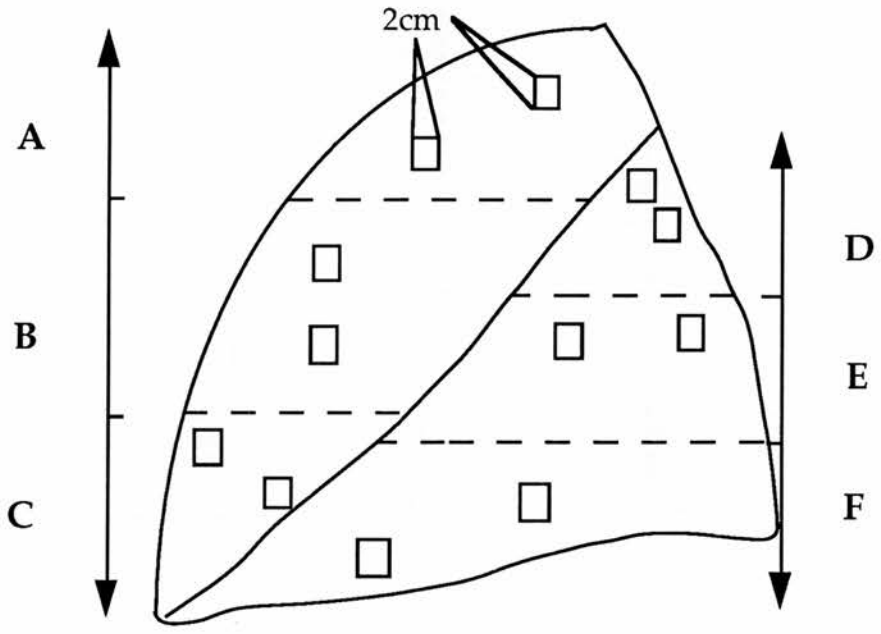


FIGURE 2.2

A diagrammatic representation of the sampling design used with the whole lung specimens. The mid-sagittal slice of each lung was divided into 6 zones based on the apex to base height of the upper and lower lobes (*i.e.* zones A, B, C in the upper lobe; and zones D, E, F in the lower lobe). Two random 2cm x 2cm tissue blocks were cut using a template from each zone, to produce a sample size of 12 tissue blocks from each whole lung specimen.

2.5 TISSUE PROCESSING

The formalin-fixed blocks were processed and embedded in glycol methacrylate resin (GMA) using the procedure described below.

The tissue blocks were dehydrated through a series of alcohols, O.P spirit and acetone, each for 2 hours at the concentrations shown below.

10% ethanol
20% ethanol
30% ethanol
50% ethanol
80% ethanol
64 O.P. spirit
64 O.P. spirit
74 O.P. spirit
74 O.P. spirit
acetone
absolute alcohol

The constituents of the GMA resin were as follows:

A. *Infiltrating Solution*

2 Hydroxyethyl methacrylate	400ml
2 Butoxyethanol	40ml
Benzoyl peroxide	4g

(Benzoyl peroxide was added last and the mixture stirred automatically for 2 hours.)

B. *Promoter Solution*

Polyethylene glycol 400	8ml
N.N. Dimethylaniline	1ml

The alcohol within the tissue was gradually replaced with infiltrating solution over two to three days with fresh solution being added every 24 hours. The final solution of GMA was obtained by adding 1ml of the promoter solution to 42ml of the infiltrating solution. The solutions were then rotated for 5 minutes to ensure thorough mixing (Sims, 1974).

When the final GMA mixture was prepared, plastic moulds (Park Scientific, Northampton) were filled with the mixture, and the tissue blocks were oriented within the GMA. The addition of the promoter solution caused an exothermic polymerisation reaction, and to slow this reaction and prevent bubbles forming in the GMA, the moulds were placed on crushed ice for an hour. The moulds were then peeled off and the blocks hardened in an oven at 60 degrees centigrade. Excess resin was then trimmed from the blocks using a band saw and the blocks glued to wooden chucks prior to sectioning.

2.6 TISSUE SECTIONING

Tissue sections were cut to 3μm using a Reichart Jung Autocut with a tungsten carbide knife. One section was taken to represent each block.

2.7 TISSUE STAINING

Sections were mounted on microscope slides and stained by the Haematoxylin & Eosin method which gave a good contrast between the tissue and the background, which is a prerequisite for image analysis.

Bullard's Haematoxylin and Eosin:
Bullard's Haematoxylin:
8g haematoxylin
16ml glacial acetic acid
144ml 50% ethanol
20g aluminium ammonium sulphate (ammonia alum)
250ml distilled water
8g red mercuric oxide
275ml 95% ethanol
330ml glycerol
18ml glacial acetic acid
40g aluminium ammonium sulphate

Preparation:
The haematoxylin was dissolved in 144ml 50% ethanol. 16ml glacial acetic acid and a heated solution of 20g ammonia alum in 250ml distilled water were added. The mixture was heated to boiling and 8g red mercuric oxide were added. The solution was then cooled rapidly and filtered. 275ml 95% ethanol, 330ml glycerol, 18ml glacial acetic acid, and finally 40g ammonia alum were added. The resulting solution was mixed thoroughly and stored at room temperature.

Method:

Sections were stained in Bullard's haematoxylin at room temperature for 10 minutes, and washed in water for 2-3 minutes. The sections were then differentiated in 1% acid alcohol, washed for 5 minutes in running tap water, and counterstained in 1% eosin for 5 minutes. They were then rinsed, dehydrated through graded alcohols and cleared in xylene prior to mounting in DPX (adapted from Drury & Wallington, 1980).

2.8 CHEMICALS

The chemicals used in this project were supplied by BDH Ltd, Poole, Dorset, and Sigma Chemicals Ltd, Poole, Dorset. A full list of all chemicals used is shown below.

BDH Ltd:-

Absolute alcohol
Aluminium ammonium sulphate
Anhydrous di-sodium hydrogen orthophosphate
Benzoyl peroxide
DPX
Eosin
Ethanol
40% Formaldehyde
Glacial acetic acid
Glycerol
Haematoxylin
O.P. spirit
Polyethylene glycol 400
Red mercuric oxide
Sodium di-hydrogen orthophosphate
Xylene

Sigma Chemicals Ltd:-

2 Butoxyethanol
2 Hydroxyethyl methacrylate
N N dimethylaniline

2.9 MACROSCOPIC ASSESSMENT OF EMPHYSEMA

The mid-sagittal slice of each lung specimen was examined by an experienced pathologist (DL), and the extent and types of macroscopic emphysema present were assessed in the following manner: Each lung slice was placed in a shallow tray containing enough water to just cover the specimen. The cut surface of each slice was then examined carefully. The types of emphysema observed in each lobe were recorded, and the extent of each type described, *i.e.* where the specimen was a whole lung, the type and extent of macroscopic emphysema in each of the lobes were recorded separately. Any centriacinar lesions were counted. Where panacinar, paraseptal, paracicatricial or bullous emphysema were present, the percentage area of the slice involved was measured by tracing the outline of the mid-sagittal slice onto a transparent sheet. (A bullous lesion was defined as an emphysematous space of more than 1cm in diameter (Figure 2.3)). The emphysematous areas were then traced onto this sheet, the areas of the tracings measured using graph paper, and the percentage of the lobe area showing macroscopic emphysema was calculated.

The type of macroscopic emphysema in each lobe was recorded as follows:

1. Centriacinar emphysema only
2. Panacinar emphysema only
3. Both centriacinar and panacinar emphysema
4. Other types of macroscopic emphysema

The severity of each type of macroscopic emphysema was then graded for each lobe as follows:

1. Centriacinar emphysema

Mild	<10 lesions
Moderate	10 - 20 lesions
Severe	>20 lesions

2. Panacinar, paraseptal, paracicatricial or bullous emphysema

Mild	<10% area of mid-sagittal slice involved
Moderate	involving 10% - 40% of mid-sagittal slice area
Severe	>40% area of mid-sagittal slice involved

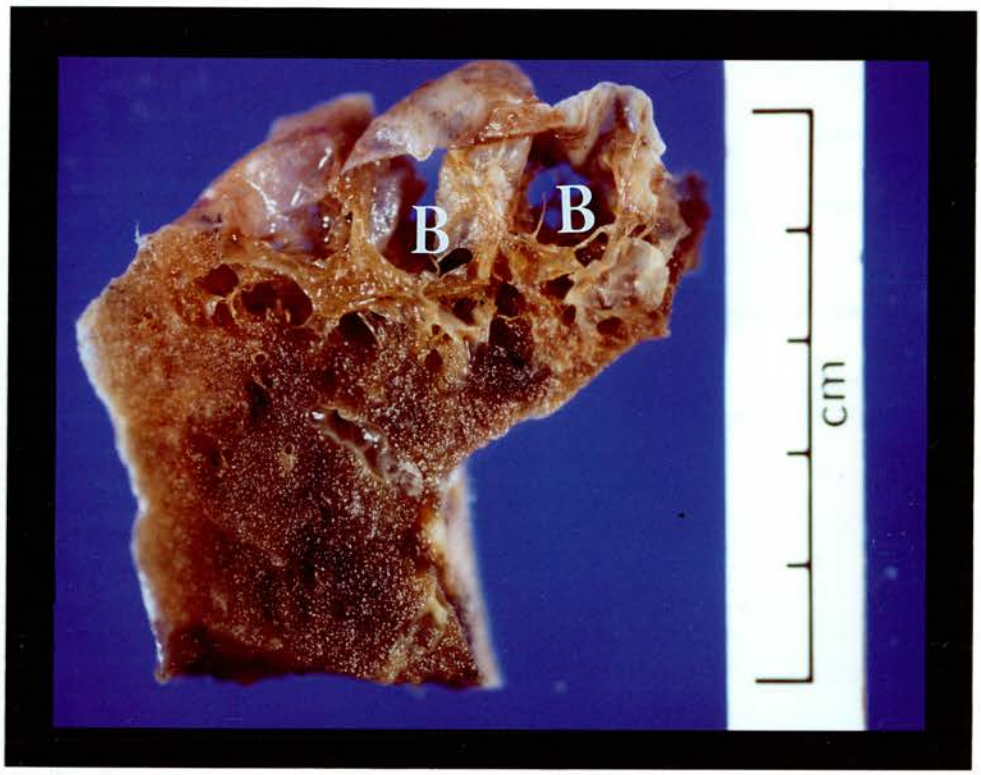


FIGURE 2.3

This photograph shows a piece of lung tissue cut from the apex of the upper lobe of a lung specimen showing bullous lesions (B). Note that the diameter of these lesions is at least 1cm.

2.10 THE FAST INTERVAL PROCESSOR (FIP)

The FIP is a fully automated scanning system which was used to measure airspace wall surface area in this study. The FIP uses the same approach to measuring surface area as the mean linear intercept (Lm) technique, whereby the number of intercepts with a test-line is counted, and this figure is used to calculate the average distance between intercepts. A value for tissue surface area can be derived from Lm (Aherne & Dunnill, 1982).

A detailed description of the FIP, and a discussion of its development and assessment are given in Chapter 3.

2.11 THE DEFINITION OF AWUV

Airspace wall surface area measurements can be used to quantify the loss of respiratory tissue due to emphysema. In this study airspace wall surface area was measured and expressed as mm^2/mm^3 of lung volume, *i.e.* the surface area of **A**irspace **W**alls contained in a **U**nit of lung **V**olume (**AWUV**) (Lamb *et al*, 1986; Gould *et al*, 1988).

AWUV can be calculated in several ways using standard morphometric formulae. It is possible to measure airspace wall perimeter in a unit of lung area, and to convert this to AWUV using the formula:

Surface Area = Perimeter x $4/\pi$ (Lamb *et al*, 1986).

Alternatively, AWUV can be derived from a linear intercept measurement using the formula:

Surface Area = 2V/Lm (Aherne & Dunnill, 1982).

As described in detail in Chapter 3, this is the method which has been used to calculate AWUV in this study. AWUV is expressed in mm^2/mm^3, *i.e.* **V** = $1mm^3$. The formula thus becomes:

AWUV = 2 / Lm (mm^2/mm^3).

2.12 DATA HANDLING

The FIP was controlled by a Plessey MIPROC computer. As the FIP scan proceeded, intercepts with lung tissue were recorded (as described in Chapter 3), and the intercept total for each $1mm^2$ field was stored on the MIPROC. This created a large volume of data, and unfortunately the storage capacity of the MIPROC was limited. All FIP results were therefore transferred to the Edinburgh University Mainframe computer (Castle) for storage and data manipulation. The data were transferred using the Kermit communication package (Da Cruz, 1987).

When the data were transferred to the mainframe they were contained in files which were in an inappropriate format for the statistical package used in this study (Statistical Package for the Social Sciences (SPSS)). Each data file was edited on Castle using the line editor 'EDIT' and the screen editor 'MICROEMACS'. (All data files were kept for long term storage on the Castle archive system.)

SPSS was then used to convert each intercept total from the data files into an AWUV value, using the formulae described in section **2.11** above, and to compute mean AWUV, mode AWUV, and 5th, 10th, 90th and 95th percentile AWUV values.

2.13 ILLUSTRATION OF AWUV PATTERNS

As described above, the AWUV measurements from each individual 1mm^2 field scanned in this study were stored on the Castle mainframe computer. Since a list of numerical values would be difficult to interpret, an attempt was made to use these values to illustrate the patterns of airspace sizes within tissue sections.

An Apple Macintosh IIfx was used to construct several grids which were designed to represent the pattern of AWUV values in individual tissue sections.

A blank grid, consisting of 121 small squares of equal size, was drawn using Claris Macdraw II (Figure 2.4). The grid was intended to represent the 121 1mm^2 fields scanned by the FIP on a single tissue section (See Chapter 3 for details). This grid was then imported into an image analysis package (Image 1.41 VDM), and each square in the grid was filled in with a grey shade chosen to represent the AWUV for a single field. Image 1.41 recognises 256 grey shades on stored images. These shades range from 0 - 255, where 0 represents white and 255 represents black. It was therefore simple to allocate grey shades to squares representing AWUV measurements of 0 - 25.5 mm^2/mm^3, simply by multiplying the AWUV value by 10. AWUVs higher than 25.5 mm^2/mm^3 were all given the maximum grey level value of 255.

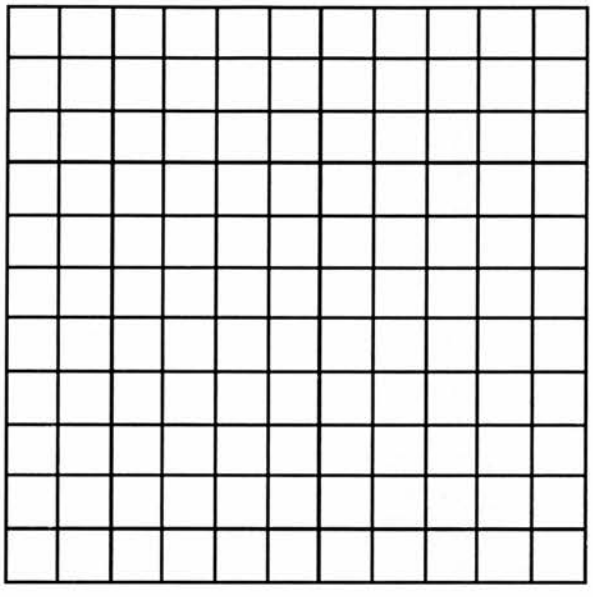


FIGURE 2.4

This figure shows a grid consisting of 121 squares of equal size. The grid was created using Claris Macdraw on an Apple Macintosh IIfx. Each square is intended to represent a single $1mm^2$ field on the area scanned using the FIP. To create a 'grey level' grid to represent the pattern of AWUV values on a single histological section, each square in the grid was filled with a grey level corresponding to the AWUV measurement, using Image 1.41 VDM image processing software. In this way, fields with high AWUV values were represented by darker grey shades than those fields with low AWUV values.

2.14 STATISTICAL ANALYSIS

The statistical tests used in this study are listed below.

The adequacy of the sample size of 6 tissue blocks *per* lobe was assessed by calculating the mean AWUV and the standard error of the mean for the 6 blocks (726 histological fields). The 95% confidence limits of the mean were then calculated using the formula **CI = mean +/- 1.96(SEM)**, where CI is the confidence interval, 1.96 is the value of the 't' distribution for large sample sizes (from statistical tables), and SEM is the standard error of the mean. The sample size was deemed to be representative if the confidence intervals were within +/- 10% of the mean AWUV (Weibel, 1963).

The Spearman correlation coefficient (Siegel & Castellan, 1988) was used to assess the relationship between AWUV and age in the smokers and non-smokers. Regression analysis was used to describe further the AWUV/age relationship in the non-smokers. Multivariate regression analysis was used to assess the influence of sex on the AWUV/age relationship.

The apex to base distribution of AWUV measurements within the lung was assessed using the Friedman 2-way analysis of variance (Siegel & Castellan, 1988), and the upper and lower lobe AWUV values were compared using the Wilcoxon Signed Ranks test (Siegel & Castellan, 1988).

In general, no assumptions were made about the normality of the data, and nonparametric statistics were preferred. This was particularly important in the analysis of the apex to base variation in AWUV because, especially in cases with low mean AWUV, the frequency distribution of AWUV values within the lung was not always normal.

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., 1988). The 95% prediction limits for the various regression equations were computed using the Minitab package (Ryan *et al*, 1985).

2.15 GRAPHICS

All the figures and tables in this thesis were constructed using the following packages on an Apple Macintosh IIfx computer:

Claris Macdraw II 1.1	Claris Corporation Mountain View California USA
Cricketgraph 1.2	Cricket Software Malvern Pennsylvania USA
Image 1.41 VDM	Image Processing and Analysis National Institutes of Health Research Services Branch NIMH USA
Microsoft Word 4.0	Microsoft Corporation Redmond Washington USA

Chapter 3

The Development and Assessment of a New Technique

3.1 INTRODUCTION

As described in Chapter 1, most of the methods used for assessing emphysema are subjective, non-quantitative and insensitive to early disease. The measurement of airspace wall surface area *per* unit volume of lung tissue (AWUV) has been found to be an efficient method for assessing parenchymal tissue density. The increases in airspace size associated with pulmonary emphysema can be expressed as decreases in AWUV values (McLean *et al*, 1992).

The term 'AWUV' was introduced by Lamb and colleagues in 1986. Their technique for assessing AWUV involved measuring airspace wall perimeter in a unit area of lung tissue, and converting this to AWUV using the formula:

Surface Area *per* Unit Volume = Perimeter *per* Unit Area x $4/\pi$
(Williams, 1977).

Airspace wall perimeter was measured in $1mm^2$ fields using a digitising tablet, or an automated image analysis system (IBAS, Kontron Ltd, Watford). Both of these techniques were labour-intensive and time-consuming. (Although the IBAS was an automated system, the digital image of each histological field had to be edited carefully to eliminate unwanted objects from the measurement). Perimeter measurements were made on sufficient fields to produce a stable running mean - usually 25-30 $1mm^2$ fields from each lung specimen. The average time to measure the total perimeter in a single $1mm^2$ field was 5-10 minutes.

One of the aims of this project was to develop a new technique for measuring AWUV, which would be faster and less labour-intensive than the methods which had previously been used. The remainder of this Chapter contains an account of the technique used, including a description of the fast interval processor (FIP), its development and operation; an account of the FIP's reproducibility; and an assessment of the compatibility of FIP measurements with those made using an established morphometric technique.

Section **3.4** on the assessment of the FIP technique is divided into 3 sub-sections with the standard headings '**Introduction**', '**Methods**', '**Results**' and '**Discussion**'. These headings have not been used in their strictest sense in this Chapter, and in particular, the 'Discussion' sub-section contains some results which seemed to be more appropriately placed as part of the discussion of the differences between the FIP and an established image analysis system.

3.2 DEVELOPMENT OF A NEW TECHNIQUE FOR MEASURING AWUV

The fast interval processor (FIP) (Figure 3.1) is a fully automated scanning system which was developed by staff at the MRC Human Genetics Unit in Edinburgh. The machine was originally designed as a pre-screening device for cervical cytology specimens (Shippey *et al*, 1981; Tucker & Shippey, 1983). It was adapted for use with lung tissue using software written by ASJ Farrow.

The FIP consists of a computer-linked Nikon inverted microscope with a 6.3x objective. The microscope is equipped with a motorised stage and a charge-coupled device (CCD) linear image sensor. The sensor consists of a stationary array of photosensitive units which recognise the optical density pattern of histological specimens.

AWUV measurement using the FIP is accomplished using the same method as the mean linear intercept (Lm) technique, whereby the number of intercepts between a tissue component and a test-line is counted, and the average distance between intercepts can be calculated, provided the length of the test-line is known. A value for tissue surface area can be derived from Lm using a standard morphometric formula:

Surface Area = 4V / Lm (Aherne & Dunnill, 1982).

The FIP scans tissue sections in the following manner. Each microscope slide is mounted on the motorised stage. The stage is programmed to move the section in the x-axis, past the stationary image sensor, which scans the section electronically in the y-axis at 10µm intervals. A digitised 'image' is formed by the electronic scan. This image consists of a grid of picture elements or 'pixels', each of which measures 2µm x 2µm. A user-defined threshold level determines which pixels are recognised as stained tissue and which are unstained background pixels.

Contiguous groups of thresholded pixels detected by each electronic scan are treated together as 'intervals'. A size filter ensures that intervals of less than 3 pixels (*i.e.* 6µm) in diameter are ignored by the image sensor. This

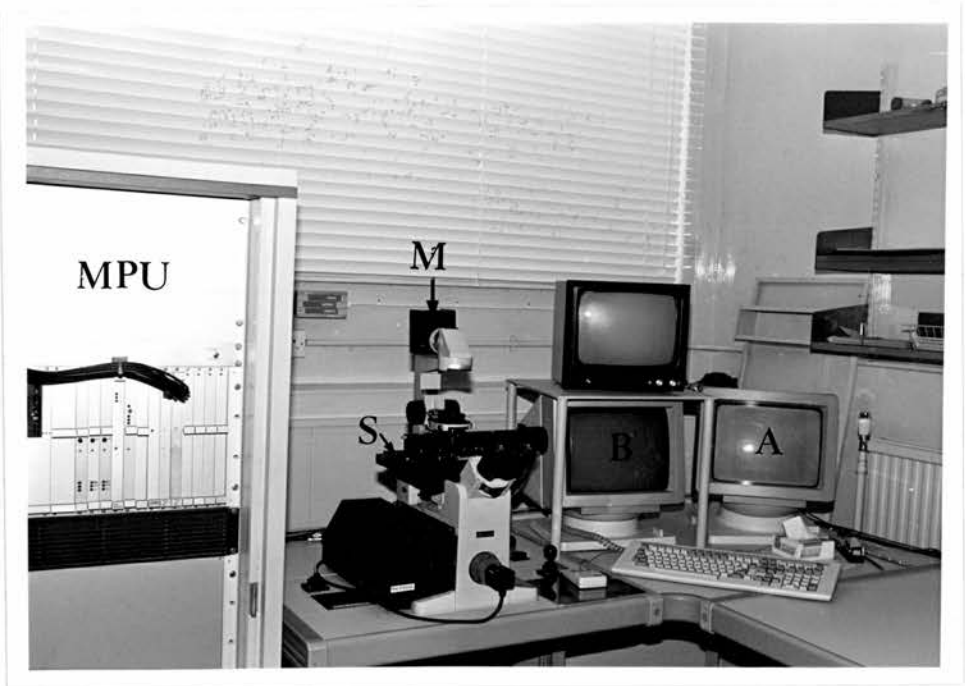


FIGURE 3.1

A photograph of the fast interval processor (FIP). The FIP consists of a Nikon inverted microscope (M), controlled by a Miproc microprocessing unit (MPU). Each histological section is mounted on the motorised stage (S), and a digitised image of each scan is shown on the monitor marked 'A'. Monitor 'B' displays the Miproc commands which are keyed in by the user.

filters out most of the small cells or dust particles which have been thresholded, but are unwanted in the AWUV measurement.
An electronic signal is recorded by the computer each time the boundary between tissue pixels and background pixels is detected by the image sensor. Therefore 2 'intercepts' are counted for each interval (Figure 3.2). This is equivalent to counting the number of intercepts between a test-line on a graticule and the boundaries between tissue and background using the conventional Lm technique. Two intercepts are counted for each alveolar wall because both sides of the wall are involved in the gas exchange process (Aherne & Dunnill, 1982). For ease of calculation, the intercept totals from each $1mm^2$ (*i.e.* unit area) are stored by the computer.

Each electronic scan creates a 'test-line' 1mm in length for each $1mm^2$ field, and, as fields are scanned at 10μm intervals, the total test-line length is 100mm for each field (100 electronic scans 1mm in length in each field). The mean linear intercept can thus be calculated:

Lm = Total test-line length / Total number of intercepts

The morphometric formula for calculating tissue surface area is SA = 4V /Lm (Aherne & Dunnill, 1982). However, in this case, 2 intercepts have been counted for each airspace wall.
The formula thus becomes **SA = 2V / Lm**.

The FIP has been programmed to recognise the edges of each $1mm^2$ field in the y-axis. The image sensor can recognise if the first intercept at the top of the field is a 'start' or 'end' intercept for the interval. The same is true at the bottom of each field (Figure 3.3). This ensures that there is no overlap in intercept counts between adjacent fields.

During the FIP scan, the mechanical stage is capable of moving at speeds of up to 2mm *per* second. However, an important feature of the 'lung' program was that a binary image of the thresholded and non-thresholded pixels should be produced while the tissue section was being scanned. This feature has been included to enable the user to see the thresholded areas detected by the image sensor, and to reject sections which contain large areas of thresholded non-parenchymal tissue. The display of the binary image

cannot be accomplished at speeds of greater than 1mm *per* second. Therefore the scan speed has been limited accordingly.

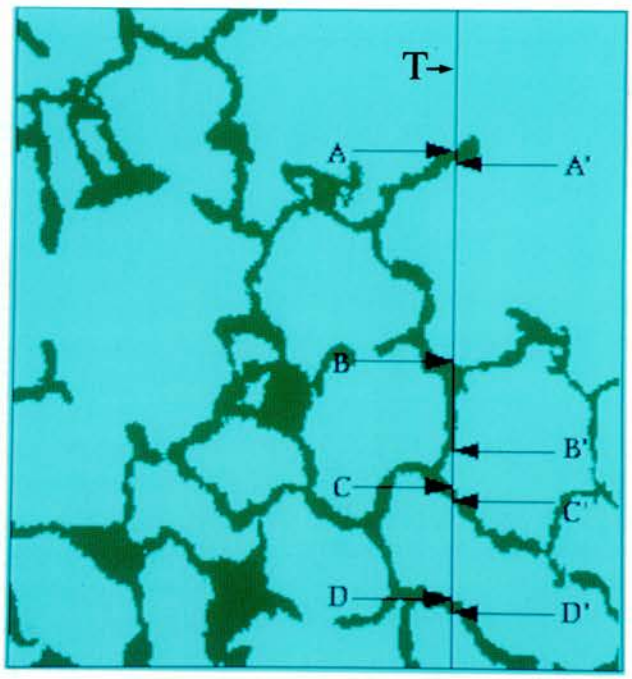


FIGURE 3.2

This colour plate shows a binary image of lung tissue, created using Image 1.41VDM image processing software on the Apple Macintosh IIfx computer. The black border surrounding the binary image represents the boundaries of a single 1mm^2 histological field. The black vertical line (T) represents the electronic scan creating a test-line at 10μm intervals across the field. An intercept is counted when the boundary between tissue and background density levels is encountered. There are therefore 2 intercepts for each alveolar wall (one counted on entry of the test line into the wall, and the second on its exit). Thus, arrows A, B, C and D represent the first intercept for each alveolar wall and arrows A', B', C' and D' represent the second intercept counted for each wall.

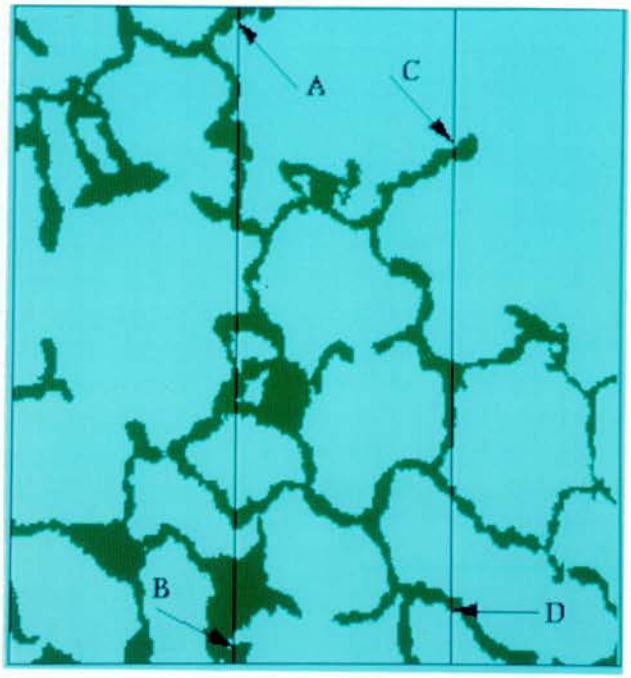


FIGURE 3.3

As in Figure 3.2, this colour plate is a digitised image of a histological field from a section of lung tissue. Again, the black border represents the boundaries of a single $1mm^2$ field, and the black vertical lines represent the test lines created by electronic scans of the section by the FIP. Arrow A indicates an intercept which is the first encountered by the test line on a scan, and is recognised as the exit from tissue to background. The last intercept on this scan is an entry into an alveolar wall, and this is shown by arrow B. Arrows C and D indicate the first and last intercepts on a later scan of the same field, but this time the first intercept occurs on the entry of the test line into the alveolar wall, and the last intercept occurs on the exit from another alveolar wall. These key intercepts are recognised as such by the FIP, so that there is no overlap in intercept counts from one field to the next.

Each mechanical scan, or 'swathe' has been programmed to be 11mm long, and 11 swathes are completed on each section from left to right, then back from right to left repeatedly until the scan is complete (Figure 3.4). This results in an area of $121mm^2$ being scanned on each tissue section. Experimentation with scans of various sizes showed that an 11mm x 11mm scan was suitable.

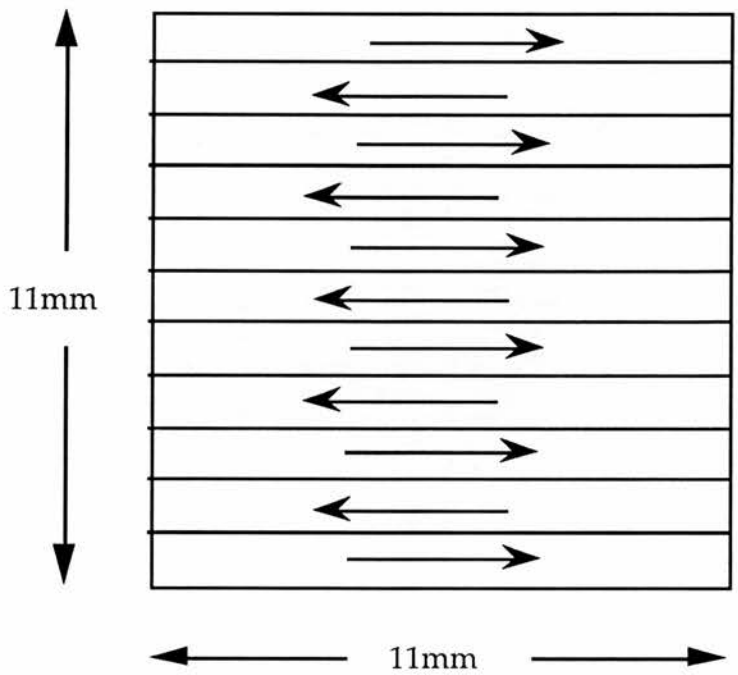


FIGURE 3.4

A diagrammatic representation of the 11 mechanical scans performed on each histological section during FIP operation. Each section is scanned from left to right, then right to left in 11 'swathes' over an area of 121mm^2. On completion of measurements, the central field on the tissue section is located automatically.

3.3 FIP OPERATION

The methods used in the operation of the FIP are described below. User input has been kept to a minimum, and with practice the initial steps can be accomplished in a few minutes.

The microscope slide, on which the tissue section is mounted, is placed on the microscope stage. The center of the tissue section is located using the computer-driven motorised stage. This is achieved by measuring the horizontal and vertical dimensions of each histological section, and positioning the objective in the center. The co-ordinates of the central $1mm^2$ field are recorded for future reference using an England Finder graticule (Graticules Ltd., Tonbridge, Kent).

The next step is to select an appropriate threshold level to enable the image sensor to discriminate between the stained airspace walls and the unstained airspaces. The threshold levels recognised by the sensor have been given arbitrary numerical values ranging from 0-35, where low numerical values enable the sensor to recognise very pale pixels, and high threshold values are required for densely stained pixels.

When a threshold level has been selected, the central $1mm^2$ field is scanned, and the binary image produced by this scan appears on a monitor. Thresholded pixels appear white and background pixels black. By comparing the binary image on the monitor with the 'live' image which is observed through the microscope eyepieces, the user can decide whether the chosen threshold level is representative. If unexpected breaks appear in the airspace walls in the binary image, the threshold has been set too high and should be lowered. Conversely, if background pixels have been detected by the sensor, the threshold level is too low and should be raised. The central $1mm^2$ field can be scanned repeatedly until the user is satisfied that the threshold level is appropriate.

When the threshold level has been set, the user enters the command which initiates the fully automated scan of the tissue section. The central field of each section is relocated automatically at the end of each scan. The intercept

totals from each $1mm^2$ field are stored on the computer at the end of the scan.

3.4 ASSESSMENT OF THE FIP

3.4.1 Introduction

After several months of test-runs and adjustments, the 'lung' program for the FIP was finalised. Before using the FIP as a routine method for measuring AWUV, it was first necessary to ensure that the measurements were reproducible and accurate. Intra- and inter-observer reproducibility were assessed, and the FIP results were compared with those obtained using the IBAS image analysis system, which has previously been used for measuring AWUV (McLean, 1987; Gould *et al*, 1988; McLean *et al*, 1992).

3.4.2 Methods

3.4.2.1 *Intra-Observer Reproducibility*

Before beginning each measuring session on the FIP, a 'test section' was scanned to assess the reproducibility of the machine. This section was selected at random from the sample pool at the beginning of the project, and retained for use as the test section for the remainder of the study. The test section was scanned and its mean AWUV recorded each time the FIP was used.

To examine the intra-observer reproducibility of the FIP measurements, 20 AWUV values from the test section, obtained over a period of 6 months, were analysed. The coefficient of variation of these values was calculated. The coefficient of variation is defined as the standard deviation divided by the mean value for a series of measurements. It gives an indication of the extent of variation within that series, and is usually expressed as a percentage value (SPSS Inc., 1988).

3.4.2.2 *Inter-Observer Reproducibility*

Ten cases were selected at random from the sample pool. AWUV was measured using the FIP on a total of 100 histological sections from these cases by a second observer (MRL). The second observer had no access to the AWUV results from the original FIP scans. The AWUV measurements

obtained by the 2 observers were compared using the Mann-Whitney U test (Siegel & Castellan, 1988).

3.4.2.3 *Comparison with an Established Technique*

AWUV was measured on tissue sections from 40 lung specimens using the FIP and the IBAS image analysis system. The relationship between the results using the 2 methods was assessed by examining the degree of correlation of the results and the linearity of this relationship was assessed using regression analysis.

3.4.3 Results

3.4.3.1 *Intra-Observer Reproducibility*

The AWUV measurements from the test section ranged between 20.21 and 20.81 mm^2/mm^3, with a mean value of 20.58 mm^2/mm^3 and a standard deviation of 0.15. The coefficient of variation for this sample of 20 AWUV measurements was 0.72%, indicating that the variation in the measurements was minimal.

3.4.3.2 *Inter-Observer Reproducibility*

There were no significant differences between the AWUV measurements obtained by the 2 observers ($W = 9881.5$, $p = 0.68$). The correlation coefficient for the 2 sets of results was 0.986 ($p < 0.001$).

3.4.3.3 *Comparison with an Established Technique*

There was a high degree of correlation between the AWUV measurements using the 2 techniques ($r = 0.882$, $p < 0.001$) (Figure 3.5). The results from the FIP and IBAS were linearly related. However, some differences between the two techniques were observed, with the FIP tending to give higher AWUV than IBAS at low mean AWUV levels, and lower results than IBAS in cases where the mean AWUV was high (FIP = 4.94 + 0.63 IBAS).

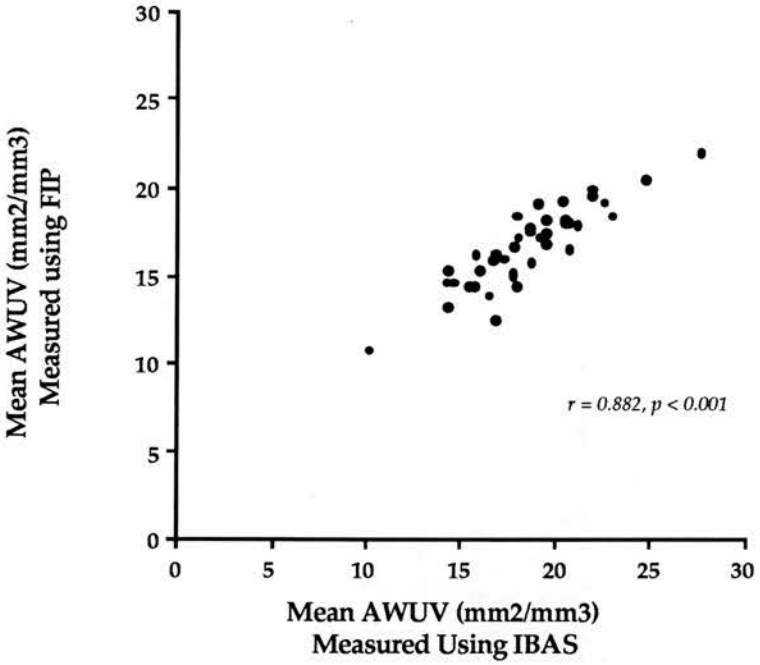


FIGURE 3.5

This graph shows the mean AWUV values from 40 lung specimens measured using the FIP plotted against the mean AWUV measurements obtained on the same specimens using the IBAS system. The correlation coefficient describing the association between the results of the two techniques is also shown on this Figure.

3.4.4 Discussion

3.4.4.1 *Reproducibility*

The FIP was found to be a reliable machine which enabled measurements with a high degree of intra- and inter-observer reproducibility to be made. This was largely due to the extent of automation of this technique. However, this level of automation has some disadvantages. There is no facility for interactive editing of individual fields. Therefore, objects such as some macrophages and dust particles, which are dark enough to be thresholded and too large to be excluded by the size filter, cannot be excluded from the measurement. Also, areas containing bronchioles and blood vessels are likely to be included in the measurement. These situations are likely to introduce errors. However, these errors will occur in all measurements, and will affect all the FIP results to a similar degree. Also, because the number of fields scanned by the FIP is so large, these variations in AWUV tend to stabilise in most cases.

A degree of editing is possible when using the FIP. Unlike the IBAS, where interactive editing of the image can take place, using the FIP, the results produced by each scan can be edited so that measurements from fields containing a large proportion of non-parenchyma may be excluded from the results. This is done by identifying the unsuitable fields using their England Finder co-ordinates, locating the results from these fields on the computer and deleting them from the results file. Alternatively, sections which contain particularly large areas of non-parenchyma can be excluded from the scan altogether.

These editing measures are very useful in most situations. However, due to the lack of interactive editing of individual fields, sections from oedematous lungs, or sections of poor quality are unsuitable for FIP analysis. This is especially a problem when dealing with lungs obtained at autopsy, where fluid and cellular infiltrate are often found in the alveoli.

The advantages of the FIP's automation are that it enables measurements to be made at high speed on a large number of fields *per* section, and ensures that the measurements are always made objectively.

3.4.4.2 *Comparison with an Established Technique*

There was a close correlation between the results of the 2 methods used to measure AWUV. However, the results were not identical because of the fundamental differences between the 2 techniques.

The IBAS system was designed to produce highly accurate measurements on individual 1mm^2 fields. (See section **1.7.2.5.2** for more details of IBAS). The FIP scan involves obtaining an estimate of the distance between airspace walls on many 1mm^2 fields (Figure 3.6A). However, although each IBAS measurement is highly accurate, the number of fields measured from each lung specimen is limited (Figure 3.6B) because of the time taken to complete each measurement (it takes 5-10 minutes to measure each 1mm^2 field). This means that the sample of lung tissue measured has less chance of being representative of the entire lung. The principal of 'do more less well', is fundamental to the success of sampling techniques because the precision of an estimate is affected more by the number of sample images measured than by the precision with which each single image is measured (Gundersen & Osterby, 1981). Therefore the sampling technique employed by the FIP, which scans 121mm^2 in 2 minutes, should result in mean AWUV measurements which are more representative of the structure of the lung as a whole.

As well as producing a representative mean AWUV value for each lung specimen, the size of the FIP sample produces AWUV measurements from many individual histological fields (a minimum of 726 fields from a lung with 6 sections). This enables more detailed assessment of lung architecture if required.

Another important difference between the FIP and the IBAS is that an interactive editing facility is available when using the IBAS, while the FIP scan is fully automated with no such facility. As mentioned above, unsuitable sections can be excluded from the FIP scan, and the results can be edited, but there is still a tendency for errors to occur. Negative errors lead to the underestimation of AWUV. These errors are usually caused by the presence of bronchioles and their accompanying blood vessels in the tissue section. An artificially low AWUV is measured in the fields which include these structures. Positive errors lead to the overestimation of AWUV.

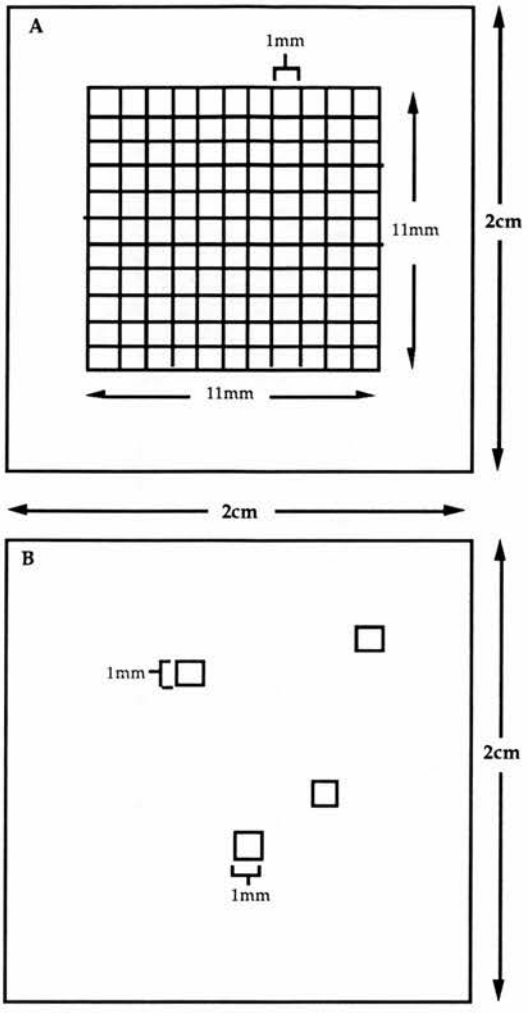


FIGURE 3.6

These diagrams demonstrate the contrast in the sampling used with the FIP and IBAS techniques for measuring AWUV.

Figure 3.6A represents the area scanned on each tissue section using the FIP. A total of 121 1mm^2 fields are scanned and this allows detailed frequency distributions of individual field AWUV measurements to be compiled.

Figure 3.6B shows that only a few 1mm^2 fields are measured on each tissue section using the IBAS system. The number of fields measured must be adequate to provide a stable running mean of AWUV values, and this usually involves measuring AWUV on 3 or 4 randomly selected 1mm^2 fields on each section.

These are caused by thresholded inflammatory cells and debris on the tissue section which are large enough to be measured.

These unavoidable errors do not create major problems in most cases, because, as described above, they tend to cancel each other out. However, in situations where the mean AWUV is particularly low or high, the effects of these errors become more obvious. In lungs where the average AWUV is very low, positive errors lead to a tendency for the FIP AWUV measurements to be higher than IBAS measurements. In contrast, when the mean AWUV is extremely high, the effect of negative errors becomes more obvious, and FIP values have a tendency to be lower than IBAS values.

In an effort to assess the extent of the differences between the FIP and the IBAS due to editing differences, 10 cases were selected at random from the 40 in the comparison study. Up to 247 results fields were excluded from the results of the FIP scan. These were from histological fields which were identified as containing structures such as bronchioles and blood vessels, and would have been rejected from sampling using the IBAS system. The mean AWUV values obtained after excluding these fields varied from the unedited results by not more than +/- 4% (Table 3.1). This form of editing did not affect the relationship between the FIP and IBAS techniques (Figure 3.7).

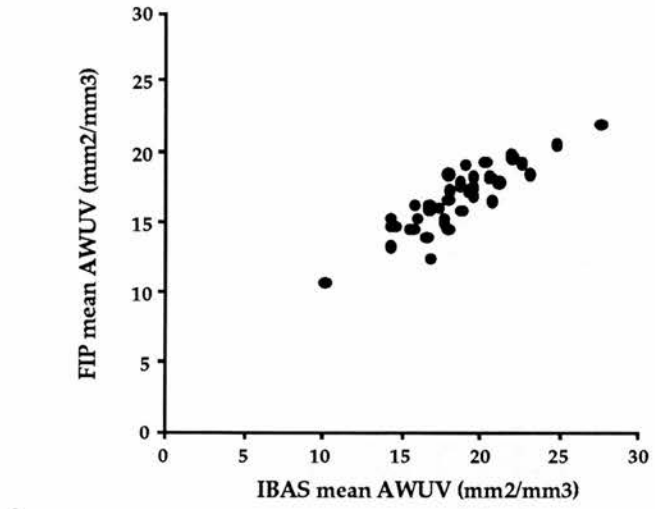
It therefore appears that there are fundamental differences between the 2 techniques which cannot be explained by differences in the editing procedure, and that these differences lead to differing absolute values of AWUV being produced. However, the changes in AWUV with age and the differences between normal and emphysematous lungs are likely to be similar using these 2 techniques.

The editing out of individual field results from the FIP scans was not continued for the remainder of the study. However, as discussed above, sections which were not suitable due to large areas of non-parenchyma, or the presence of inflammatory infiltrate or oedema, were not scanned.

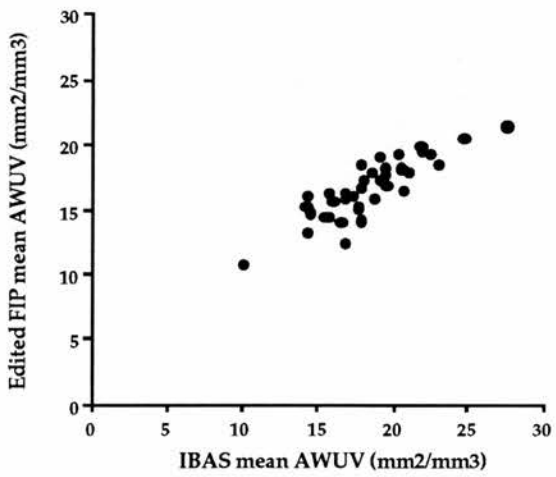
TABLE 3.1

Mean AWUV values measured on 10 specimens using the IBAS and FIP techniques. AWUV values are expressed in mm^2/mm^3. Edited FIP values are those obtained after editing the FIP results files. Figures in the 'Difference' column are the percentage differences between unedited and edited versions of the FIP AWUV measurements for the same specimens.

IBAS	FIP	Edited FIP	Difference
14.36	15.32	15.99	+4.0%
23.04	18.38	18.42	+0.2%
27.59	21.98	21.42	- 3.0%
17.94	14.42	14.10	- 2.0%
14.29	14.72	15.25	+4.0%
24.74	20.53	20.64	+0.5%
19.47	17.42	17.61	+1.0%
18.70	17.54	17.88	+2.0%
22.58	19.15	19.26	+0.6%
16.01	15.30	15.53	+2.0%



a



b

FIGURE 3.7

The relationship between FIP AWUV measurements and those made on the same specimens using the IBAS.
Figure 3.7a shows the same graph as in figure 3.5, with the FIP measurements unedited (correlation coefficient, $r = 0.882$, $p < 0.001$).
Figure 3.7b shows FIP AWUV values after editing to exclude up to 247 fields from the results files, plotted against IBAS AWUV measurements. The degree of association between these variables is no greater than that found between the unedited FIP results and IBAS results (correlation coefficient, $r = 0.862$, $p < 0.001$).

If data from the FIP are to be compared with IBAS results in the future, it is possible to convert FIP values to IBAS equivalents, and vice-versa, using the regression equation from the comparison of these techniques:

FIP AWUV = 4.94 + 0.63 IBAS AWUV

As Thurlbeck (1976) noted, although all fully automated systems are subject to errors, the same errors are introduced in all measurements, and differences between normal and emphysematous lung tissue should still be apparent.

The FIP has 2 major advantages over the IBAS. An extensive area is scanned on each tissue section. This means that it is possible to examine the patterns of AWUV values which exist within a lobe or a lung. The limited sampling technique employed when using IBAS would make this type of assessment difficult.

The other major advantage of the FIP is its speed. The scan rate used in this study was $1mm^2$ *per* second. This enables AWUV measurements to be made on a larger number of lung specimens than would have been feasible using a slower method.

3.5 CONCLUSIONS

The FIP scanning system is an efficient reliable technique which is easy to use. Its major advantages over conventional morphometric techniques are its speed and ability to scan large areas of tissue sections. These properties make the FIP a useful device for measuring AWUV on large numbers of lung specimens, and for assessing the AWUV patterns within histological sections.

Chapter 4

Results

This Chapter contains the results of the studies of AWUV in relation to age, sex and smoking; the variation in AWUV from the apex to the base of the lung; and the relationship between AWUV and macroscopic emphysema. The section describing the results of the study of age, sex and smoking in relation to AWUV appears first in this Chapter, as it was felt necessary to describe the changes in AWUV occurring with age before describing the distribution of AWUV values within the lung.

Each section in the Chapter begins with an introductory paragraph relating to that particular aspect of the study. The lung specimens used in each section are described, but Table 2.1 in Chapter 2 gives a comprehensive summary of the complete sample used in this study.

4.1 THE EFFECTS OF AGE, SEX AND SMOKING ON AWUV

4.1.1 Introduction

The measurement of airspace wall surface area *per* unit volume of lung tissue (AWUV) was used in this study to assess the effects of age, sex and smoking on parenchymal structure. The mean of all the AWUV measurements for a lung specimen was used as a general measure of the alveolar wall surface area within that lung. A more detailed study of the patterns of tissue loss due to age and smoking took the form of analysis of the frequency distributions of individual AWUV measurements. This analysis was based on the fact that in the normal young adult lung, the left of the AWUV frequency distribution represents the least alveolated portion of the acinus, *i.e.* the alveolar ducts and areas around the respiratory bronchioles, and the right of the distribution represents the most alveolated portion, *i.e.* the distal acinus (Figure 4.1).

163 cases were included in the age/sex/smoking analysis, and a frequency distribution of the AWUV measurements from each histological field was available for every case. To assess the changes in the shape of the AWUV distribution due to age and smoking, the following aspects of the distribution were analysed for each case:-

FIGURE 4.1

Figure 4.1a is a binary image of a lung tissue section showing the components of the acinar unit. The square boxes labelled 'P' and 'D' represent 1mm^2 fields in the proximal and distal regions of the acinus, respectively. The structures represented on this diagram are a terminal bronchiole (T), 2 respiratory bronchioles (R), acinar ducts (AD), and single alveoli (A).

Figure 4.1b is the frequency distribution of AWUV measurements from the FIP, made on tissue sections from a 21 year old male non-smoker's lung. AWUV values from fields in the proximal region of the acinus are represented by the lowest AWUV values (which can be expressed as the 5th percentile value of the distribution). The highest AWUV values are found in the distal region of the acinus, occupied mainly by alveoli, and this can be represented using the 95th percentile value of the distribution. The 5th and 95th percentile values are indicated by the vertical lines on this Figure (5th percentile AWUV = 14.65 mm^2/mm^3; 95th percentile AWUV = 29.13 mm^2/mm^3).

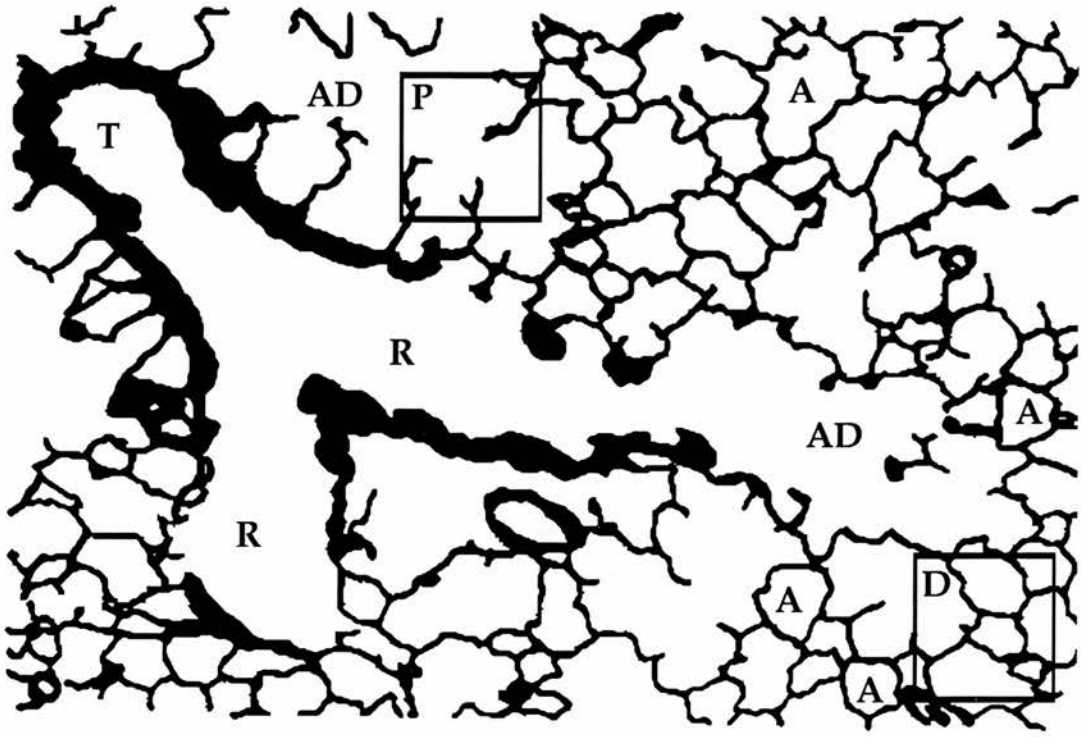


FIGURE 4.1a

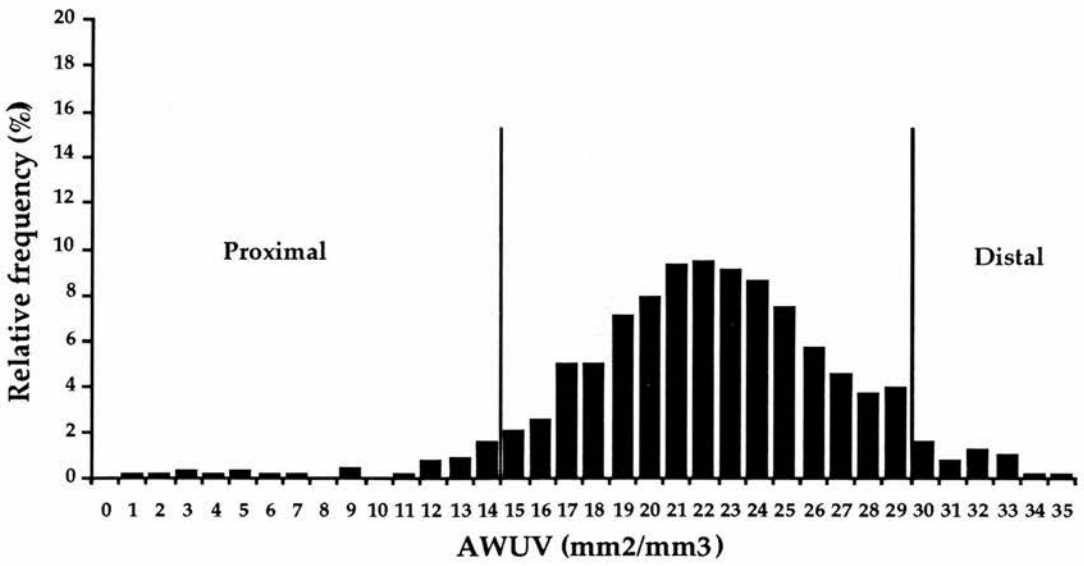


FIGURE 4.1b

a) Mean AWUV
b) Mode AWUV
c) 5th percentile AWUV
d) 10th percentile AWUV
e) 90th percentile AWUV
f) 95th percentile AWUV

The percentile AWUV values are the values below which a noted percentage of all the AWUV measurements in the distribution fall. Therefore, the 5th and 10th percentile AWUV values represent the left of the distribution, and the 90th and 95th percentiles represent the right.

The modal value of the frequency distribution represents the peak of the curve. Therefore a shift in the mode of the distribution to the left indicates that the most frequently occurring AWUV value has decreased, and a shift in the mode accompanied by a shift in the 5th and 10th percentile values results in an increase in the skewness of the distribution.

4.1.2 The Use of Single Lobes in the Analysis of AWUV

115 of the 121 surgical specimens used in this study were single lobes. The analysis of the AWUV values from 42 whole lung specimens showed that using a careful sampling technique, the mean AWUV from a single lobe was representative of the mean AWUV for a whole lung, with the exception of lungs showing extensive macroscopic emphysema. However, even in these lungs, although the AWUV values of the upper and lower lobes differed, when the mean AWUV for the lung was abnormal, the AWUV values from both lobes were abnormal, although to differing degrees. Therefore, for the purposes of this analysis, a single lobe has been accepted as presenting an adequate representation of each lung. A detailed description of the justification for accepting a single lobe as representative is given in section **4.2** below.

4.1.3 Sampling Technique

The 95% confidence intervals around the mean AWUV were calculated for 20 specimens. In each case, where 6 blocks were selected from each lobe (726 fields), the 95% confidence interval was no more than +/- 5.6% of the mean

(Table 4.1). The mean AWUV values of these specimens ranged from 8.09 - 21.98mm^2/mm^3 (age range 21-72 years). This indicates that 6 blocks constitute an adequate sample for AWUV measurements to ensure that the mean AWUV is representative of the lobe.

4.1.4 The Relationship Between AWUV and Age in Non-smokers

There was a negative relationship between mean AWUV and age in the 38 non-smokers studied ($r = -0.78$, $p < 0.001$). This negative relationship was linear and resulted in a reduction in mean AWUV of approximately 30% between the ages of 20 and 90 years (Figure 4.2). Table 4.2 contains details of the age, sex and mean AWUV values for each of the non-smokers.

The modal AWUV value, and the percentile AWUV values all showed a rate of decline with age which was similar to that found for the mean AWUV (Figures 4.3, 4.4, 4.5).

The 95% prediction limits of the regression lines for each of the AWUV values with age were calculated. The 95% prediction limits for values of mean AWUV are shown in Figure 4.6. These lines were used as the limits of normality of AWUV values for individuals between the ages of 21 and 93 years. Mean AWUV values which fell below the lower 95% prediction limit were described using the term Microscopically Assessed Emphysema (**MAE**). Examples of the lower 95% prediction limit of mean AWUV for a variety of ages are given in Table 4.3.

These results indicate that there is a normal loss of airspace wall surface area with age in adult non-smokers. This tissue loss results in a shift of the AWUV frequency distribution to the left (Figure 4.7). This implies that the loss of parenchymal tissue associated with age in non-smokers is a generalised loss involving the whole acinar unit. The generalised nature of this tissue loss is illustrated in Figure 4.8. Figure 4.8a represents the AWUV measurements from a single histological section of a 21 year-old non-smoker. Figure 8b represents the scanned area on a section of lung from a 93 year-old non-smoker. Note that the decrease in tissue density in the elderly individual has affected the whole of the 121mm^2 area measured.

TABLE 4.1 The information used to justify accepting 6 blocks as an adequate sample size for AWUV measurements on single lobes. This table shows the smoking history, mean AWUV value (mm^2/mm^3), and the standard error of the mean AWUV (SEM) measured on 6 tissue blocks from 20 subjects selected at random from the sample pool. Also shown are the 95% confidence interval of the mean AWUV (95% CI), and this figure expressed as a percentage of the mean AWUV. In all 20 specimens, the 95% confidence intervals of the mean AWUV were within +/- 5.6% of the mean value.

Case	Smoking history	Mean AWUV (mm^2/mm^3)	SEM	95% CI	95% CI (% of mean)
1	s	19.88	0.15	0.29	1.5
2	s	15.91	0.16	0.31	1.9
3	ns	15.99	0.11	0.22	1.4
4	ns	18.24	0.16	0.31	1.7
5	ns	21.90	0.18	0.35	1.6
6	ns	21.75	0.18	0.35	1.6
7	s	16.75	0.16	0.31	1.9
8	s	21.98	0.13	0.25	1.1
9	s	17.57	0.15	0.29	1.7
10	s	15.38	0.15	0.29	1.9
11	s	10.79	0.20	0.39	3.6
12	s	16.54	0.15	0.29	1.8
13	s	12.50	0.14	0.27	2.2
14	s	14.99	0.18	0.35	2.3
15	s	13.44	0.20	0.39	2.9
16	s	8.09	0.23	0.45	5.6
17	s	13.77	0.14	0.27	2.0
18	s	16.99	0.17	0.33	1.9
19	s	15.09	0.18	0.35	2.3
20	s	16.89	0.16	0.31	1.8

s = smoker, ns = non-smoker
SEM = standard error of the mean
95% CI = 95% confidence interval of the mean

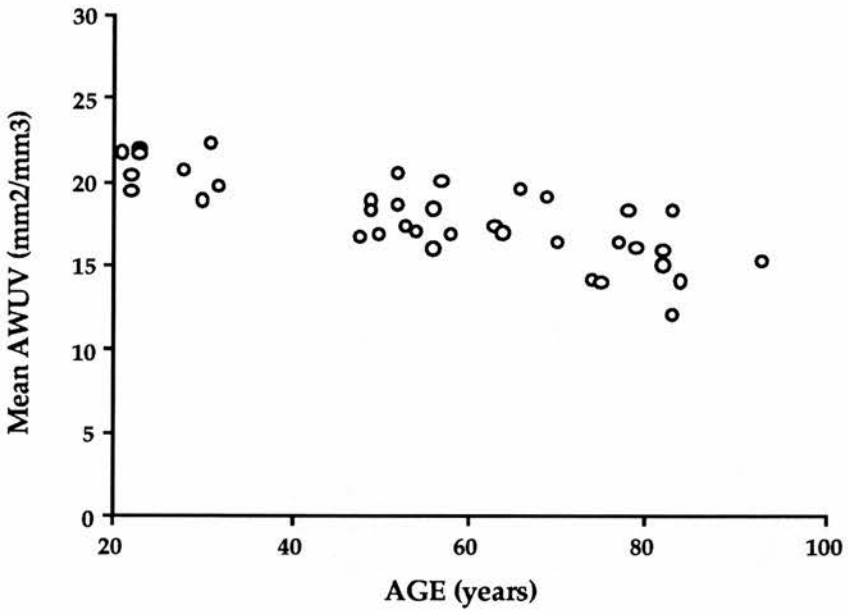


FIGURE 4.2

Mean AWUV plotted against age for the 38 non-smoking subjects. The relationship between mean AWUV and age was negative ($r = -0.78$, $p < 0.001$) and linear (AWUV = 23.1 - 0.09AGE). Mean AWUV was reduced by approximately 30% between the ages of 20 and 90 years.

TABLE 4.2 Age, sex and mean AWUV values for the 38 non-smoking specimens

AUTOPSY SPECIMENS

CASE	SEX	AGE (mm^2/mm^3)	AWUV
1	F	22	20.38
2	F	22	19.43
3	F	23	21.90
4	F	52	18.62
5	F	57	20.13
6	F	70	16.32
7	M	74	14.27
8	F	75	14.02
9	M	77	16.33
10	F	79	16.09
11	F	82	15.04
12	F	82	15.88
13	F	83	18.24
14	F	83	12.03
15	F	84	14.10
16	F	93	15.33

Cases are listed in order of age
Age range 21-93 years, mean 55.6 years
AWUV range 12.03 - 22.34 mm^2/mm^3
M = male, F = female

BIOPSY SPECIMENS

CASE	SEX	AGE (mm^2/mm^3)	AWUV
1	M	21	21.75
2	M	23	21.98
3	M	23	21.58
4	M	28	20.69
5	F	30	18.86
6	M	31	22.34
7	M	32	19.73
8	F	48	16.68
9	F	49	18.90
10	F	49	18.27
11	F	50	16.87
12	M	52	20.51
13	M	53	17.38
14	M	54	17.06
15	M	56	18.38
16	M	56	15.99
17	M	58	16.90
18	F	63	17.30
19	F	64	17.03
20	F	66	19.55
21	F	69	19.15
22	M	78	18.30

Figures 4.3, 4.4 and 4.5 show the relationships between age and the mode, 5th and 10th, and 90th and 95th percentile AWUV values in the 38 non-smoking subjects. These 3 figures illustrate the similarities in the rate of decline with age of these aspects of the AWUV frequency distribution.

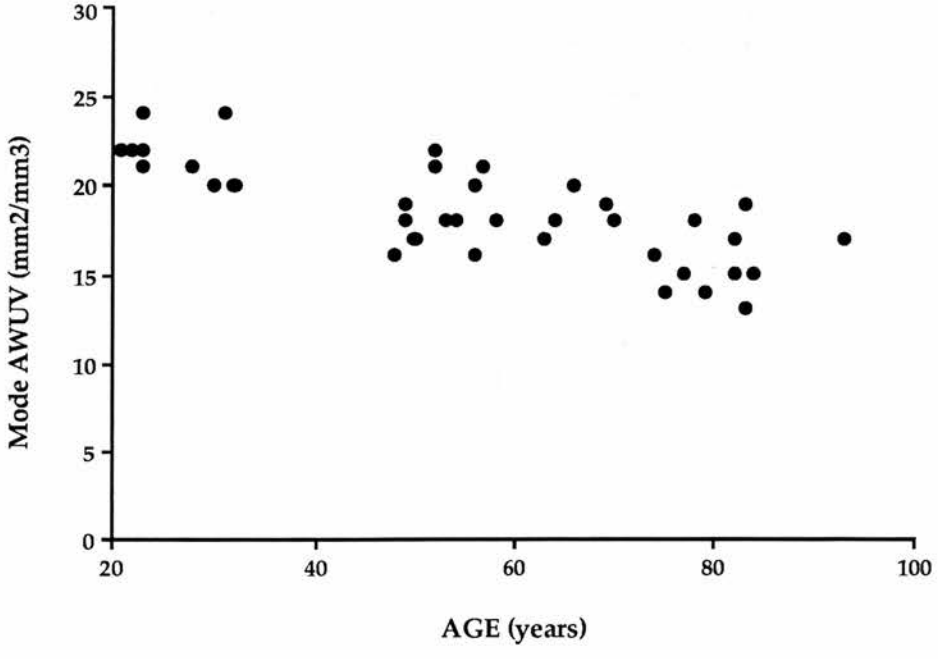


FIGURE 4.3

The relationship between modal AWUV and age.

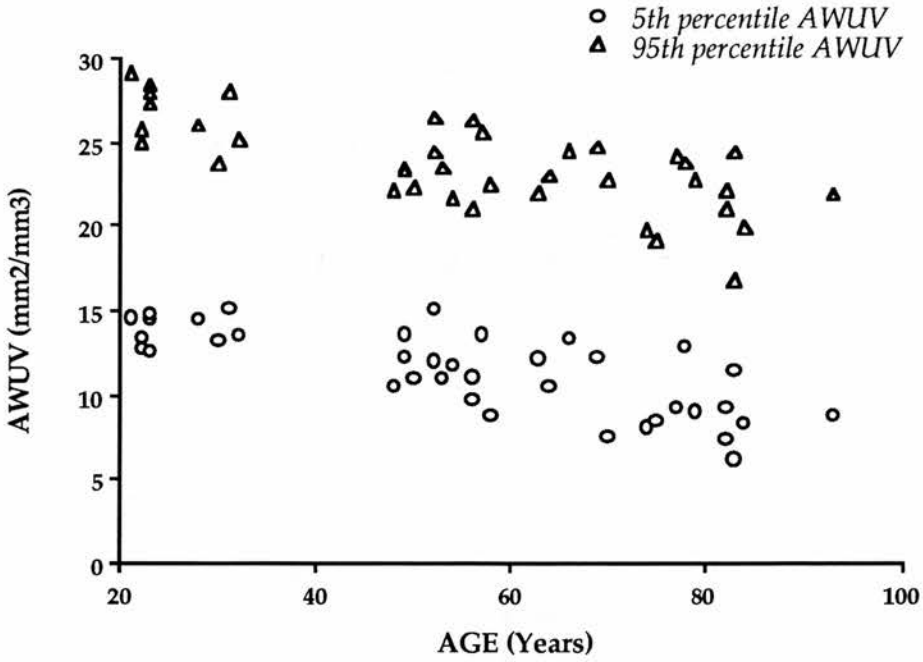


FIGURE 4.4

The relationship between the 5th and 95th percentile values and age.

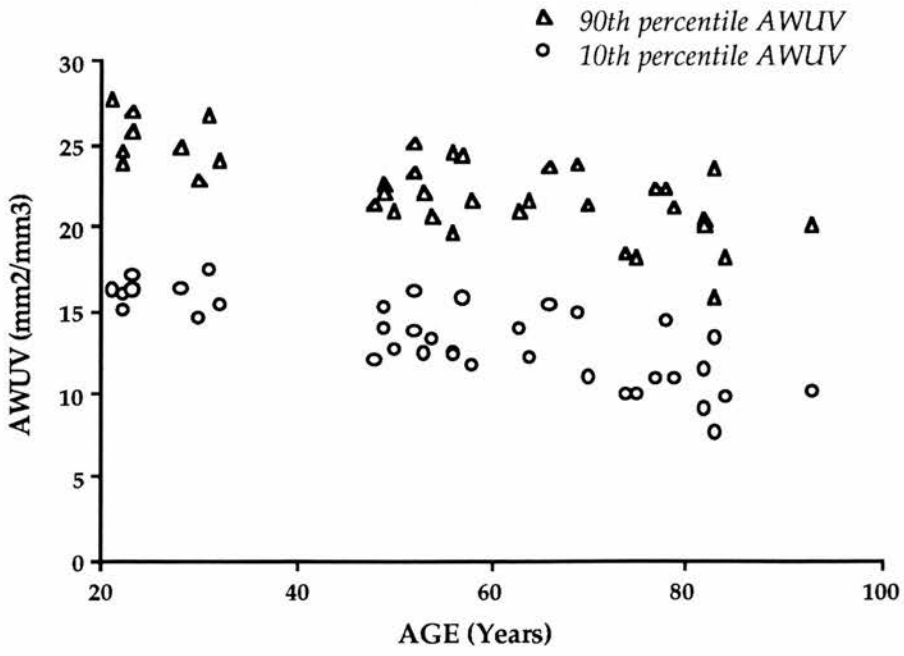


FIGURE 4.5

The relationship between age and the 10th and 90th percentiles.

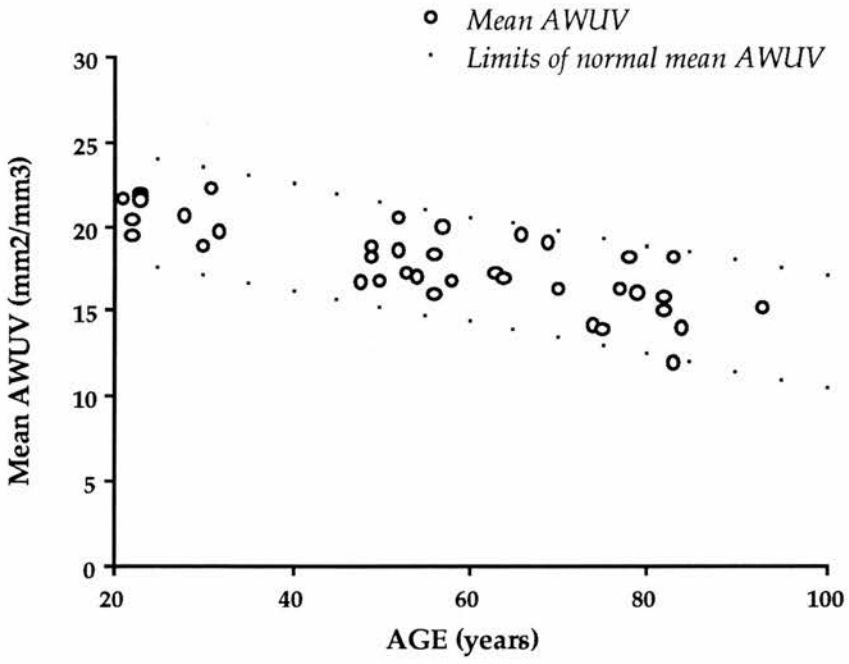
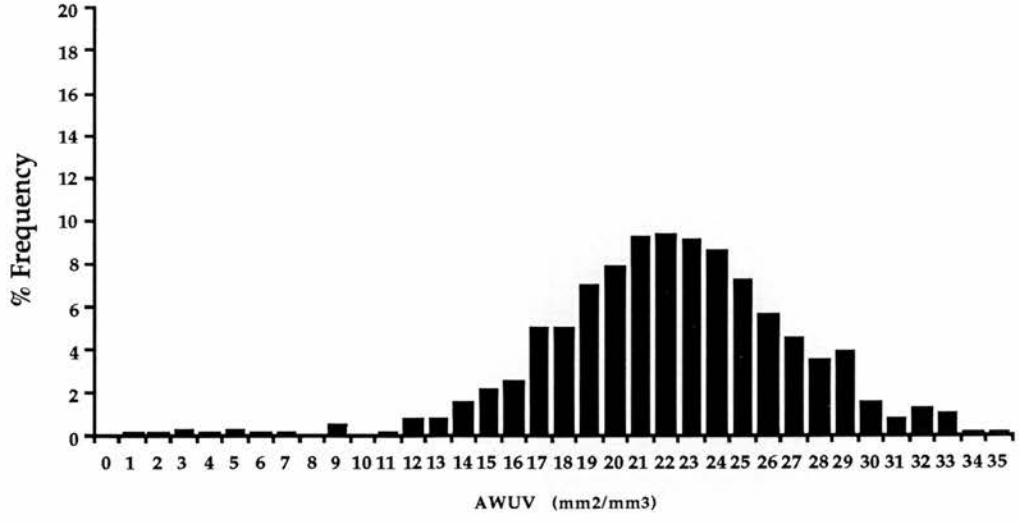


FIGURE 4.6

This graph shows the relationship between mean AWUV and age for the 38 non-smokers, with the 95% prediction limits of this relationship indicated. The 95% prediction limits were used as the limits of normal mean AWUV for subjects aged between 21 and 93 years.

TABLE 4.3 Some examples of the lower 95% prediction limit of the mean AWUV/age relationship in non-smokers (*i.e.* the lower limit of normal AWUV for a given age). Age is shown in years, and AWUV values are expressed in mm^2/mm^3.

AGE	Lower limit of normal mean AWUV
20	17.99
25	17.56
30	17.12
35	16.68
40	16.24
45	15.79
50	15.33
55	14.87
60	14.41
65	13.94
70	13.47
75	13.00
80	12.52
85	12.03
90	11.54
95	11.05



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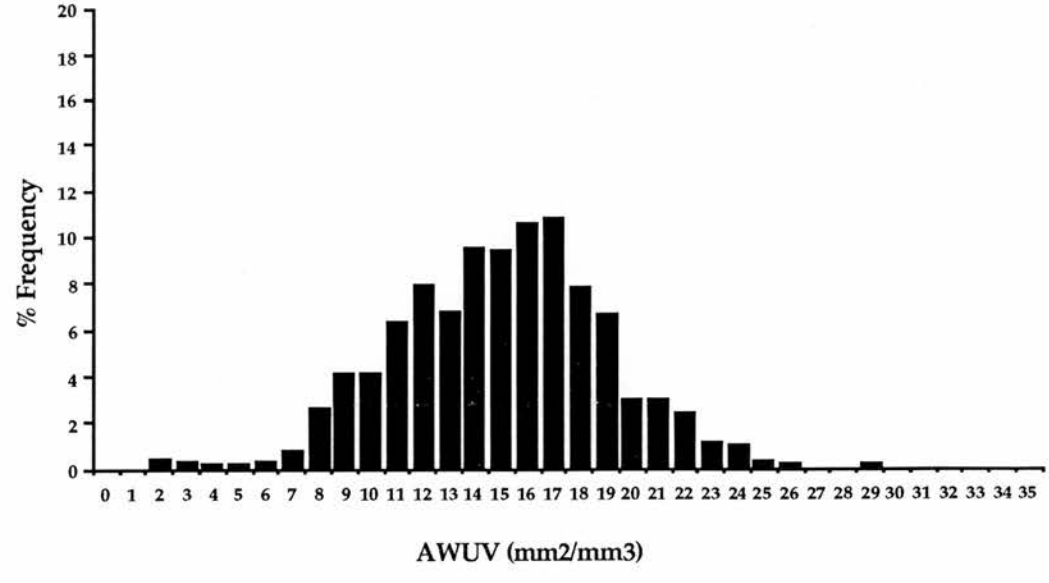
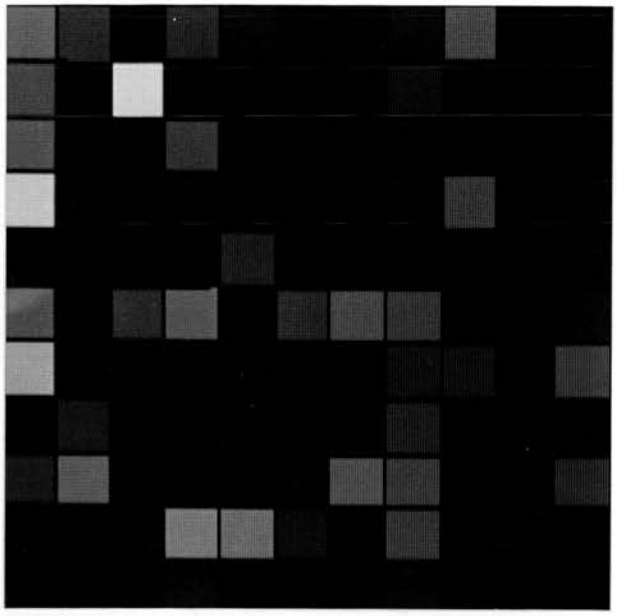


b

FIGURE 4.7

Histograms of the AWUV frequency distribution for 2 non-smoking subjects. Figure **4.7a** is from a 21 year-old (mean 21.75mm^2/mm^3, mode 22mm^2/mm^3, 5th percentile 14.65mm^2/mm^3, 95th percentile 29.13mm^2/mm^3), and **4.7b** is from a 93 year-old individual (mean 15.33mm^2/mm^3, mode 17mm^2/mm^3, 5th percentile 8.83mm^2/mm^3, 95th percentile 21.96mm^2/mm^3). The mean, mode and percentile values are lower in the 93 year-old, and this has resulted in a shift of the frequency distribution to the left.

a



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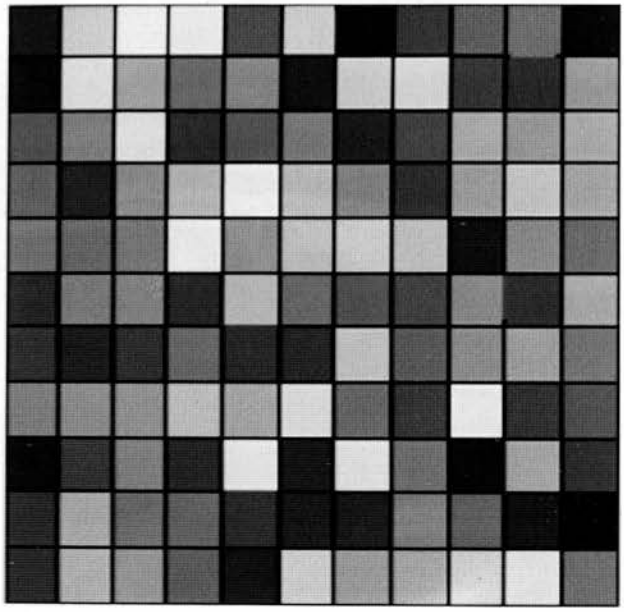

FIGURE 4.8

The plates in Figures 4.8a and 4.8b show grey level grids representing the AWUV patterns on scanned areas of histological sections from the 2 subjects described in Figure 4.7. Figure 4.8a represents a 21 year-old non-smoker, and Figure 4.8b a 93 year-old non-smoker. The density of grey shades in the small squares shown in these plates are intended to reflect the alveolar wall density in each $1mm^2$ histological field, so that a dark grey shade reflects a high AWUV value, and a low AWUV is represented by a pale grey shade. Therefore by comparing the grids from these 2 non-smokers, it is possible to visualise the generalised nature of the alteration in tissue density occurring in the lungs as part of the aging process.

4.1.5 The Relationship Between AWUV and Age in Smokers

There was a decrease in mean AWUV with advancing age in the 125 smokers studied ($r = -0.37$, $p < 0.001$). This relationship is shown in Figure 4.9. The mode and percentile AWUV values were also negatively related to age in the smoking group.

4.1.5.1 *Mean AWUV*

Some smokers (26%) had mean AWUV values which were below the normal limits for their age (*i.e.* these smokers had microscopically assessed emphysema, or MAE as defined above). However most (74%) of the smoking group had mean AWUV values which were within the normal range (Figure 4.10). This indicates that within the smoking group a minority of individuals developed MAE.

4.1.5.2 *5th and 10th Percentile AWUV*

37% of the smokers had 5th percentile AWUV values which were abnormally low, and 34% had abnormal 10th percentile values. Since only 26% of smokers had abnormally low values for mean AWUV, some individuals without MAE had abnormal 5th and 10th percentile values. This suggests enlargement of the airspaces at the proximal part of the acinus, and may represent widening of the alveolar ducts. Figure 4.11a shows an AWUV distribution from a 59 year-old individual with a normal mean AWUV value whose 5th percentile value was abnormal. Figure 4.11b represents the scanned area on a single tissue section from the same individual.

These results suggest that the 5th and 10th percentile AWUV values may be indicators of the earliest changes in parenchymal structure associated with the onset of MAE, or focal lesions which may represent localised areas of panacinar emphysema or centriacinar lesions.

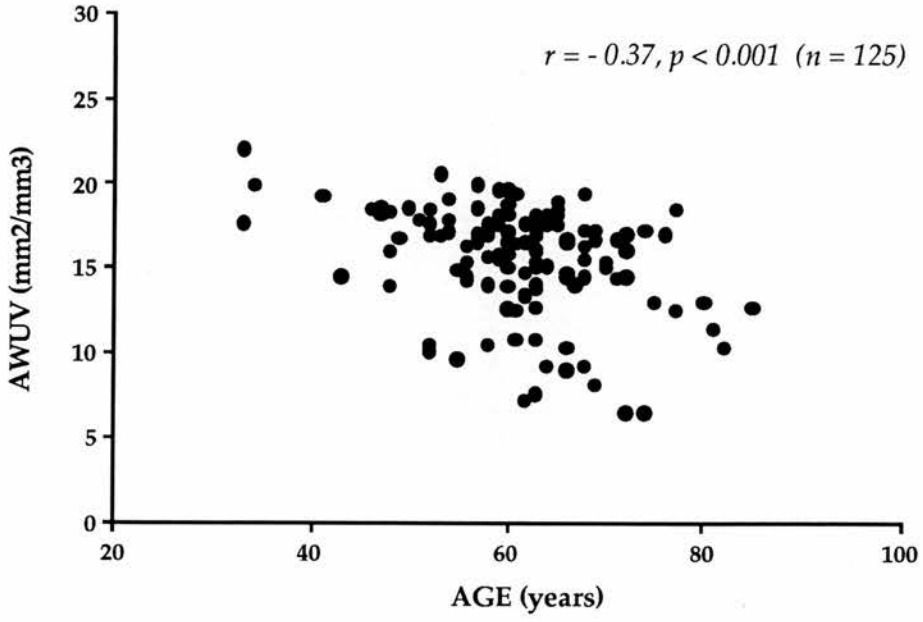

FIGURE 4.9

Mean AWUV plotted against age in 125 smokers. This relationship was negative, and the association between mean AWUV and age is indicated on the graph by the correlation coefficient, r.

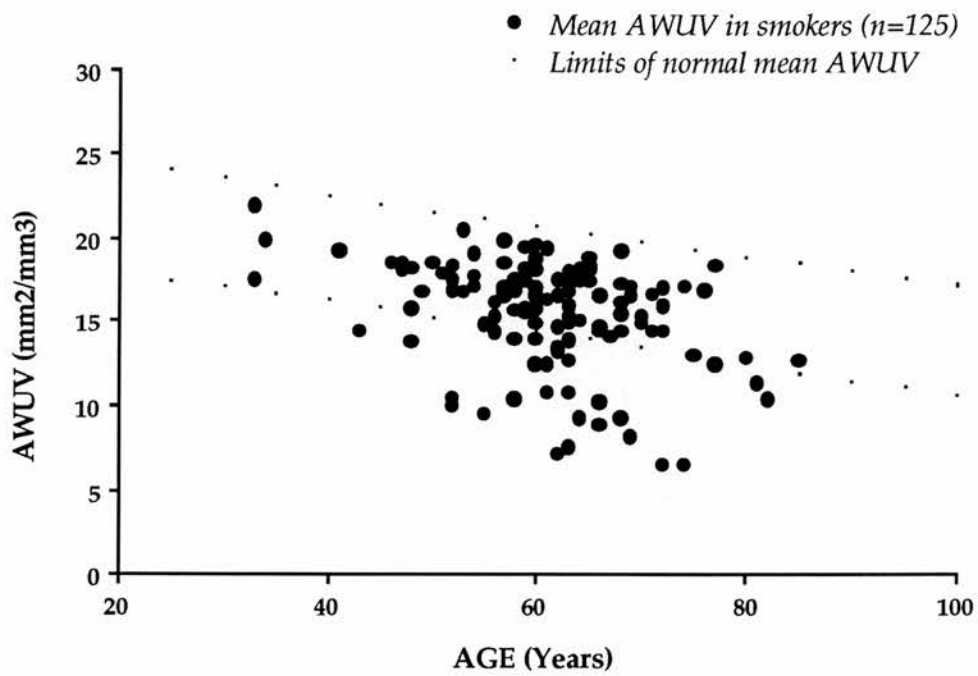

FIGURE 4.10

Mean AWUV is plotted against age for the 125 smokers in the study sample. Also shown on the graph are the limits of normal mean AWUV (*i.e.* the 95% prediction limits of the AWUV/age relationship in the non-smoking group). Mean AWUV values which are below the normal limits indicate the presence of microscopically assessed emphysema (MAE) and this figure illustrates that only 26% of the smoking group had MAE.

FIGURE 4.11

The diagrams in Figure 4.11 were created using AWUV data from a 59 year-old individual with normal mean AWUV, but whose 5th percentile value was abnormally low. In Figure 4.11a the frequency distribution of AWUV measurements from this subject shows that the relative frequency of AWUV values to the extreme left of the histogram is higher than that found in the young non-smoker represented in Figure 4.7. However, rather than the shift in the distribution seen in the 93 year-old non-smoker in Figure 4.7, the shape of the distribution in this individual has altered. The plate shown in Figure 4.11b illustrates the focal distribution of low density areas on the histological section, and these reflect focal airspace enlargement.

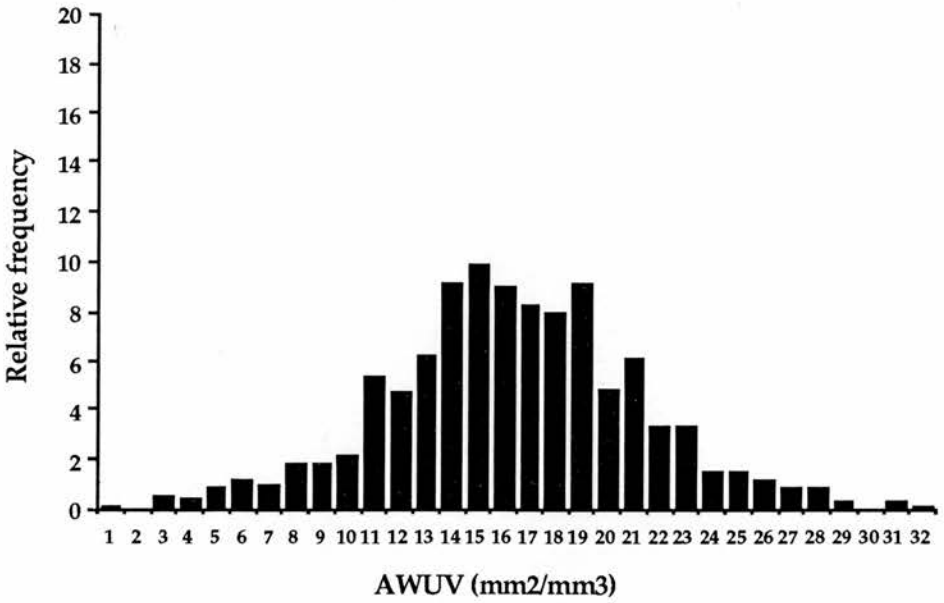

FIGURE 4.11a

The AWUV frequency distribution from a subject with normal mean AWUV ($15.53mm^2/mm^{3)}$ and abnormal 5th percentile AWUV value ($7.41mm^2/mm^3$).

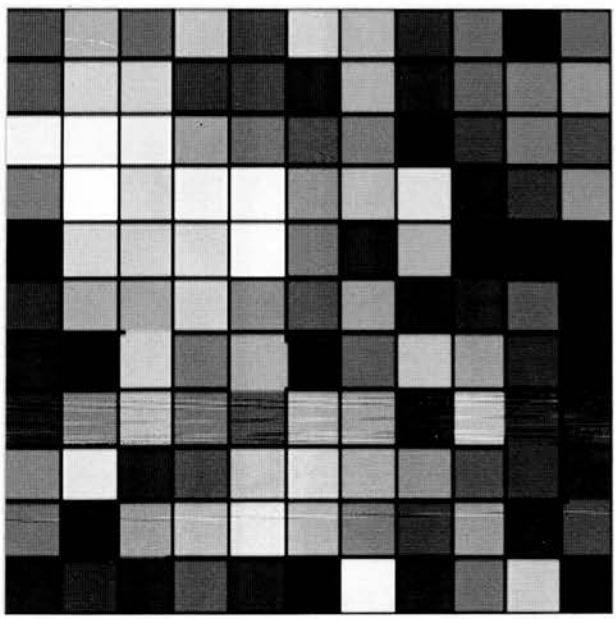

FIGURE 4.11b

A plate showing the grey shade grid representing the AWUV pattern observed on a scanned area from a single histological section from the same subject as is represented in Figure 11a.

4.1.5.3 *Modal AWUV*

In 19% of cases, the modal AWUV value was below normal, indicating that the 'peak' of the AWUV distribution in these cases was shifted to the left. All the cases with abnormally low modal AWUV values also had abnormal mean AWUV values (*i.e.* MAE). In all but 1 case when the mode was abnormal the 5th and 10th percentiles were also abnormal, leading to skewness in the AWUV distribution. Figure 4.12 shows the AWUV distribution from a 66 year-old, with abnormal mean and modal AWUV values. Note the increase in the skewness of this distribution, indicating widespread airspace enlargement.

4.1.5.4 *90th and 95th Percentile AWUV*

9% of the smokers had abnormally low values for the 90th percentile AWUV, and only 6% had abnormal 95th percentile AWUV values. In all the cases with abnormal 90th and 95th percentile AWUV values, the mean AWUV was abnormal, and in all but 1 case the mode was also abnormal. Therefore these results represent individuals with widespread enlargement of the airspaces across the acinar unit, *i.e.* extensive panacinar emphysema. Figure 4.13 shows the AWUV distribution and a grid representing the area scanned on a tissue section from a specimen with abnormal 90th and 95th percentiles (the subject was aged 74 years).

Of the 33 smokers with MAE, 21 (64%) had normal 90th and 95th percentile AWUV values, suggesting that some normal airspaces were retained within these lungs. However, it is possible that lung inflation may have been irregular in some of the lungs.

4.1.6 The Influence of Smoking Habit on AWUV

Having found that only 26% of the smoking group had abnormally low mean AWUV values, the aim of this part of the study was to assess whether these individuals were heavier smokers than those with normal mean AWUV.

In the group of 125 smoking individuals, there were 26 subjects for whom details of daily tobacco consumption could not be obtained. Of the 99 smokers with detailed smoking histories, 2 subjects smoked pipe or cigars

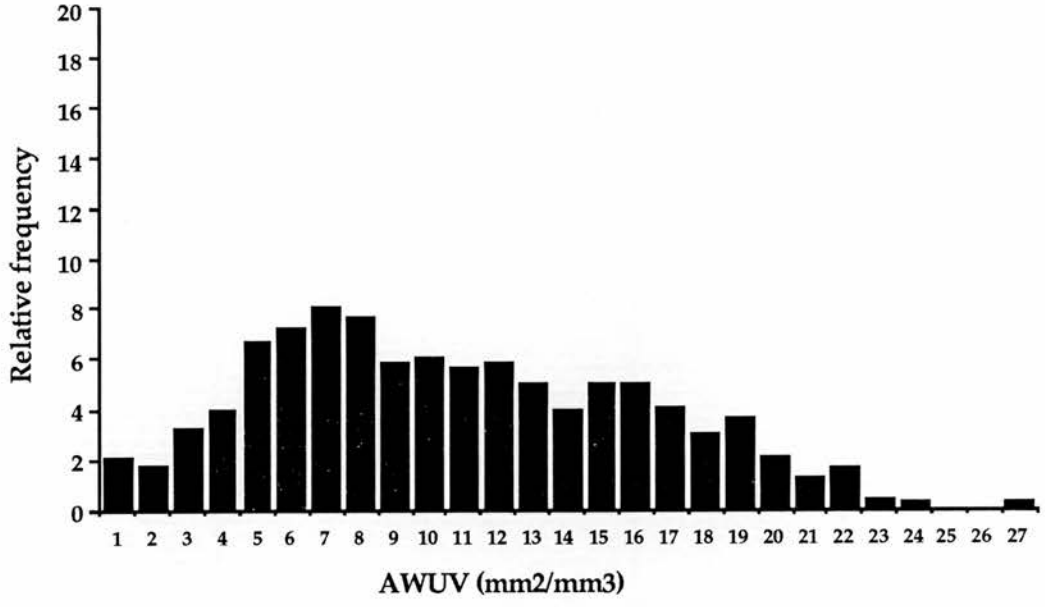

FIGURE 4.12

The frequency distribution of AWUV values from a 66 year-old subject with abnormal mean (10.22mm^2/mm^3) and modal (7.00mm^2/mm^3) AWUV values.

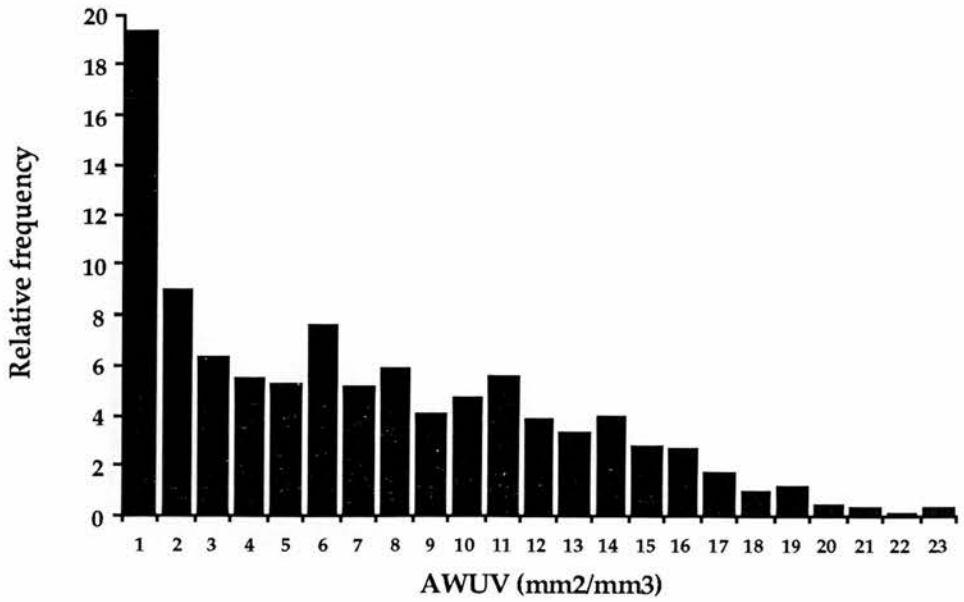

FIGURE 4.13a

Frequency distribution of AWUV measurements from a 74 year-old subject with abnormal mean, mode, and percentile AWUV values (mean 6.50mm^2/mm^3, mode 1.00mm^2/mm^3, 5th percentile 0.16mm^2/mm^3, 95th percentile 16.07mm^2/mm^3). This histogram shows that the most frequent AWUV measurements in this specimen were less than or equal to 1mm^2/mm^3.

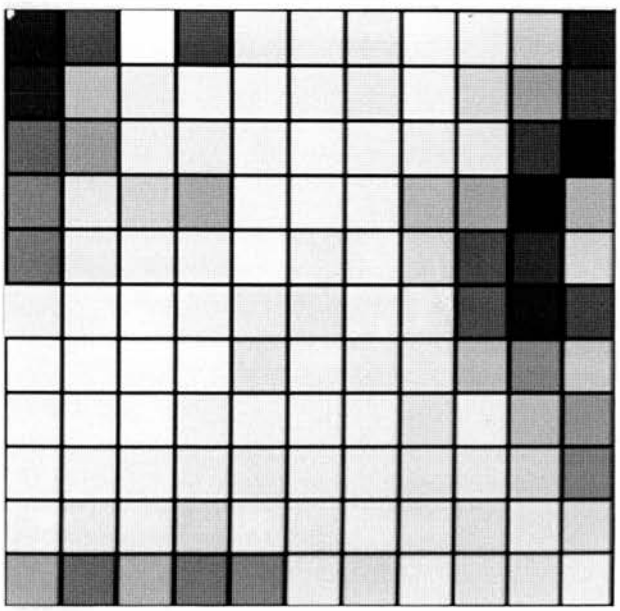

FIGURE 4.13b

A grid showing grey shades representing the AWUV pattern on the scanned area of a single tissue section from the same subject as described in Figure 4.13a above. The widespread airspace enlargement found in this specimen is demonstrated by the uniformly pale grey shades in this plate.

only. The remaining 97 were cigarette smokers, and details of the number of cigarettes smoked each day were obtained. These smokers were divided into 3 sub-groups as described in Chapter 2 (section **2.1**). The numbers in each of the 3 smoking groups are shown in Table 4.4. The age ranges in the 3 sub-groups were similar (Table 4.5).

One of the pipe/cigar smokers had MAE. No conclusions on the effect of pipe or cigar smoking on the development of MAE can be drawn from this result.

Twenty-four of the 97 cigarette smokers (25%) had mean AWUV values which were below the normal range for their age. The individuals with abnormal mean AWUV values came from all 3 smoking sub-groups (Figure 4.14). This indicates that individuals who developed MAE were not necessarily heavier smokers than those who did not.

Table 4.6 shows the percentages of each smoking sub-group with abnormally low AWUV values. Although more of the subjects in the sub-group of heaviest smokers (30+ cigarettes *per* day) had MAE, the differences in incidence between the sub-groups were not significant (χ^2 = 1.14, ns; Table 4.7). Percentile AWUV values tended to be abnormal in a higher percentage of the heaviest smoking group than in the 2 other groups (Table 4.6). In particular, 44% of the group who smoked at least 30 cigarettes *per* day had abnormally low 5th and 10th percentile AWUV values. Section **4.3.4.1** includes results which show that the 5th and 10th percentile values are related to centriacinar emphysema. The results shown here therefore indicate that centriacinar emphysema was related to cigarette consumption in this group of smokers.

The severity of MAE was assessed by expressing the mean AWUV as a percentage of the value of the lower limit of normal AWUV for the subject's age. Therefore, a percentage value of 100 or more indicated an AWUV at or above the lower limit for the subject's age, and lungs with MAE had AWUV values which were less than 100% of the lower limit.

TABLE 4.4 The number of subjects in each of the 3 sub-groups of cigarette smokers.

	MALE	FEMALE	TOTAL
Group 1	17	9	26
Group 2	32	7	39
Group 3	28	4	32
TOTAL	77	20	97

Group 1 = 1-19 cigarettes *per* day
Group 2 = 20-29 cigarettes *per* day
Group 3 = 30 or more cigarettes *per* day

TABLE 4.5 The range of ages and AWUV values in each of the 3 sub-groups of cigarette smokers.

	AGE (years)	AWUV (mm^2/mm^3)
Group 1	33-74 (59.7)	6.49-21.98 (15.79)
Group 2	34-77 (59.6)	7.12-19.88 (15.73)
Group 3	48-82 (62.7)	9.21-20.53 (15.19)

The 3 smoking sub-groups are based on the daily cigarette consumption, as described in Table 4.4 above. Figures in brackets are the mean values for each group.

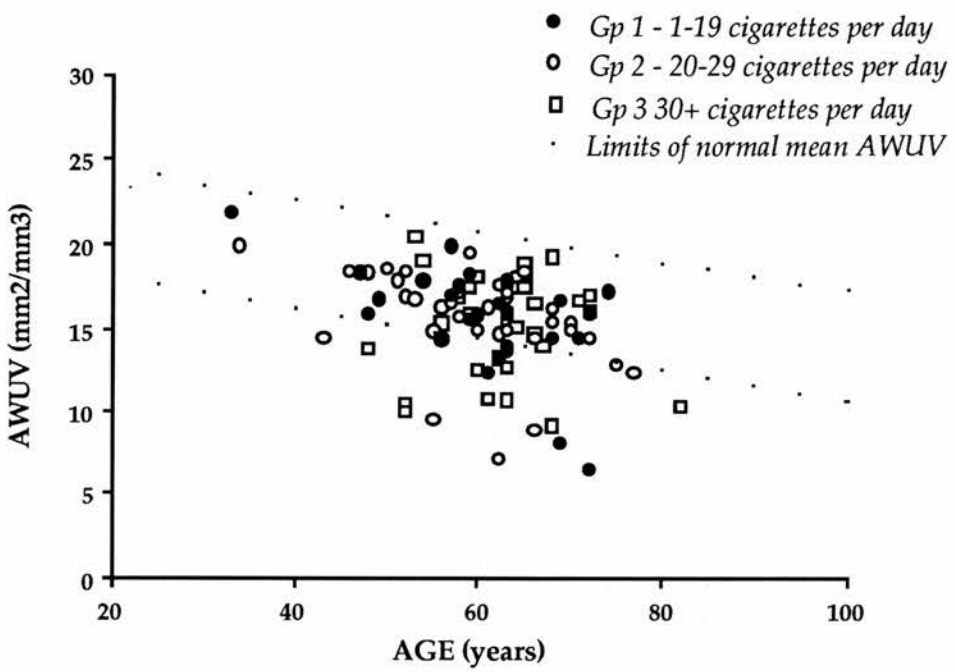

FIGURE 4.14

Mean AWUV plotted against age for the 97 cigarette smokers with available information on the number of cigarettes smoked each day. These smokers have been divided into 3 sub-groups based on daily cigarette consumption, and each sub-group is represented on the graph as a different symbol. This Figure shows that the individuals with MAE came from all 3 sub-groups, indicating that subjects who developed MAE were not necessarily heavier smokers than those who did not.

TABLE 4.6 The number of subjects in each smoking sub-group with abnormal AWUV values. The figures in brackets represent the percentage of each sub-group with abnormal values for each value of the AWUV frequency distribution.

			Percentiles:			
	Mean	Mode	5th	10th	90th	95th
Group 1	6 (23%)	4 (15%)	8 (31%)	7 (27%)	2 (8%)	1 (4%)
Group 2	8 (21%)	6 (16%)	11 (28%)	10 (26%)	2 (5%)	2 (5%)
Group 3	10 (31%)	7 (22%)	14 (44%)	14 (44%)	4 (13%)	3 (9%)
TOTAL	24 (25%)	17 (18%)	33 (34%)	31 (32%)	8 (8%)	6 (6%)

TABLE 4.7 The number of subjects in each of the sub-groups of cigarette smokers showing normal and abnormal mean AWUV values.

	Normal mean AWUV	MAE	TOTAL
Group 1	20	6	26
Group 2	31	8	39
Group 3	22	10	32
TOTAL	73	24	97

The proportions of the three sub-groups with MAE were similar ($\chi 2 = 1.14$, ns), indicating that daily cigarette consumption was not the primary factor in susceptibility to MAE.

Group 1 = 1-19 cigarettes *per* day
Group 2 = 20-29 cigarettes *per* day
Group 3 = 30 or more cigarettes *per* day

When these percentage values were examined, similar ranges of values were found in the 3 sub-groups of smokers, indicating that the severity of MAE did not increase with daily cigarette consumption (Figure 4.15).

These results indicate that although heavy smokers were not necessarily more likely to develop microscopic emphysema affecting the whole lung than lighter smokers, they were more likely to have evidence of early or focal tissue destruction, in particular centriacinar lesions (see section **4.3.4.1**).

4.1.7 Sex Differences in the Relationship Between Mean AWUV and Age

There were no sex differences in the relationship between mean AWUV and age, either in the non-smokers (Figure 4.16) or in the smokers (Figure 4.17). Multivariate regression analysis confirmed that sex was not an influential factor in the AWUV/age relationship. 27% of the male smokers studied and 23% of the female smokers had MAE. This implies that neither sex was more likely to develop MAE than the other.

4.1.8 Summary

In 76 of the smokers studied (61%) the changes in the shape of the AWUV distribution with age were similar to those found in the non-smoking group.

26% of the smokers had abnormally low mean AWUV values (*i.e.* microscopically assessed emphysema (MAE)), while 37% had abnormal 5th percentiles and 34% had abnormal 10th percentiles. Therefore, the 5th and 10th percentile values are more sensitive indicators of early or focal tissue destruction than the mean AWUV.

Less than 10% of the smokers had abnormal 90th and 95th percentile values. The abnormality of these AWUV values is likely to represent panacinar emphysema involving the whole lobe or lung.

The likelihood of developing MAE did not appear to be simply related to the number of cigarettes smoked each day, and the severity of MAE did not appear to increase with increased daily cigarette consumption.

There were no sex differences in the AWUV/age relationship, either in the smokers or in the non-smokers, implying that neither sex was more likely to develop MAE than the other.

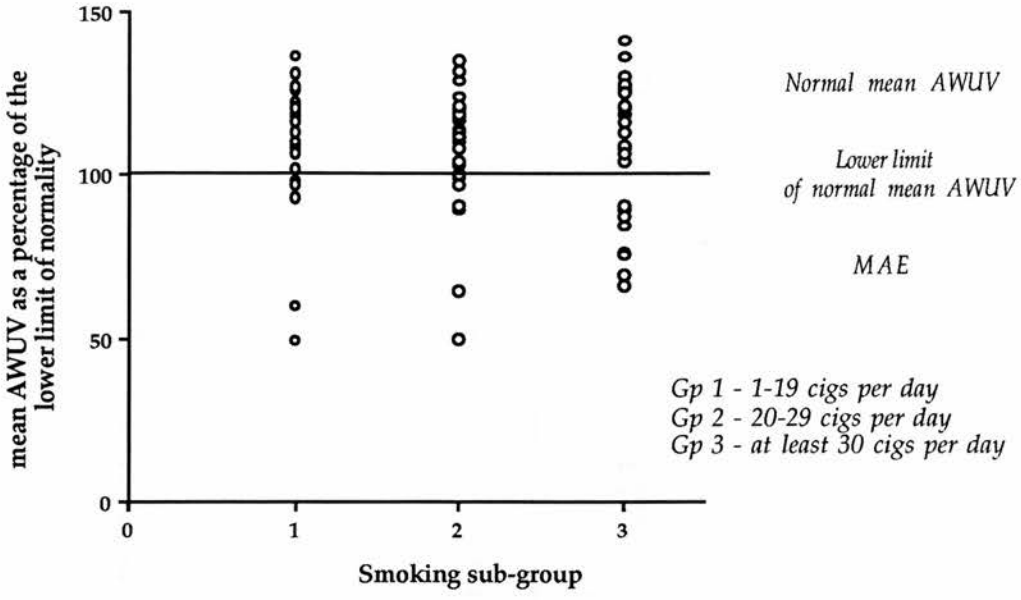

FIGURE 4.15

The severity of MAE in each of the 3 sub-groups of cigarette smokers. Mean AWUV has been expressed as a percentage of the lower limit of normal mean AWUV to indicate the severity of MAE in susceptible individuals. This graph shows that the severity of MAE was not increased with increased daily cigarette consumption.

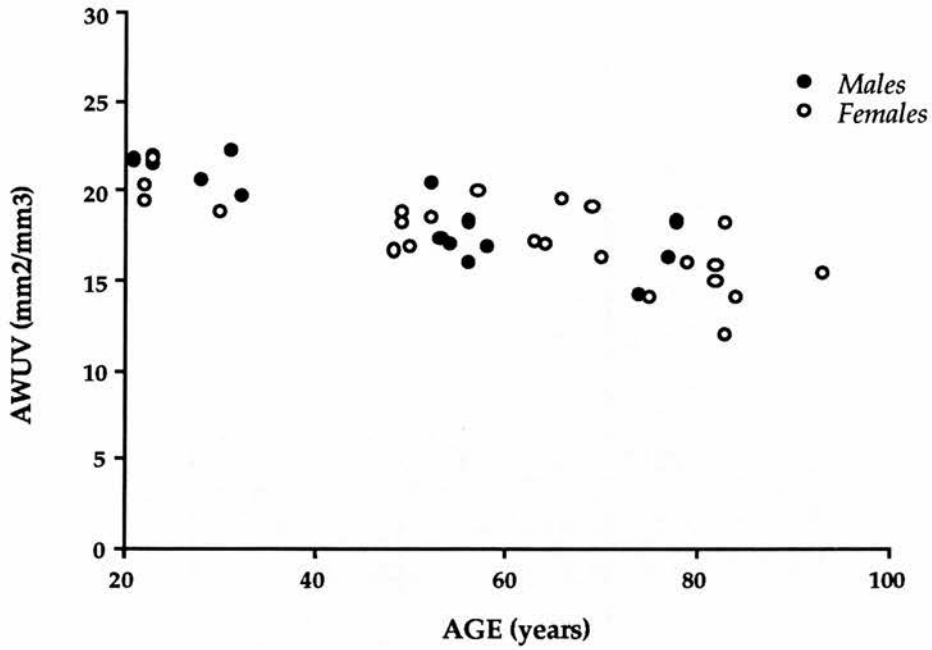

FIGURE 4.16

Mean AWUV plotted against age in the non-smokers, with males and females identified separately. This graph shows that there were no sex differences in the AWUV/age relationship in the non-smokers.

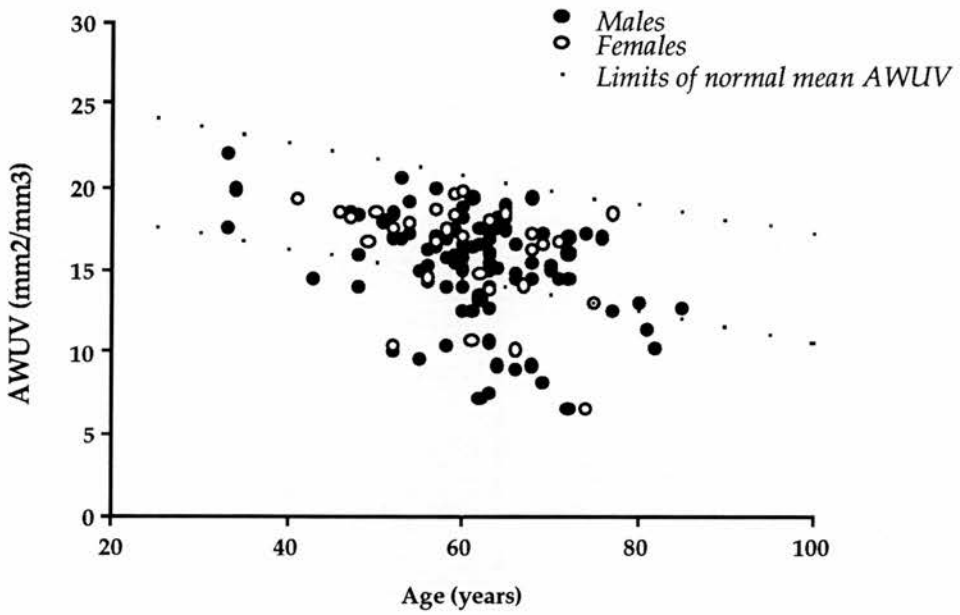

FIGURE 4.17

The relationship between mean AWUV and age in the smoking subjects. This graph shows the similarity of the AWUV/age relationships in the male and female smokers, and illustrates that similar proportions of the male and female sub-groups had MAE.

4.2 THE DISTRIBUTION OF AWUV WITHIN THE LUNG

The aims of this part of the study were to assess whether the mean AWUV from a single lobe could reasonably be used to represent the mean AWUV for a whole lung, and to examine the relationship between the distribution of AWUV values within the lung and the presence of macroscopic emphysema. As discussed above, although the justification for using single lobes was required for the study of AWUV in relation to age, sex and smoking, the variation in AWUV within the lung should be discussed in relation to the effect of age on AWUV. For this reason, the AWUV/age/sex/smoking results were presented first.

4.2.1 Comparison of the Upper and Lower Lobes

The mean AWUV values from the upper and lower lobes of 42 whole lung specimens were obtained. The age range of this group was 22-93 years (mean age 66 years). Twenty-four of the whole lung specimens were from male subjects, and 18 from females. There were 12 non-smokers and 30 smokers in this group. The source of 34 lung specimens was autopsy material, and 8 were pneumonectomy specimens.

The ratio of upper lobe mean AWUV to lower lobe mean AWUV was calculated for all 42 specimens, and the frequency distribution of the ratios was plotted (Figure 4.18). 81% of these cases had a ratio between 0.85 and 1.15 (*i.e.* the difference between the upper and lower lobe AWUV was not more than 15%).

Eight lungs were found to have upper/lower lobe differences of more than 15%. In 7 of these cases, the upper lobe AWUV was lower than the lower lobe AWUV. All but 1 of these 7 lungs had macroscopic panacinar emphysema which occupied at least 40% of the area of the mid-sagittal slice of the upper lobe, and at least 20% of this slice in the lower lobe. Re-examination of the tissue sections from the lung without macroscopic emphysema showed that the lower lobe had not been inflated to the same degree as the upper lobe, and this had resulted in the difference in AWUV between the 2 lobes. This lung had already been included in the other parts

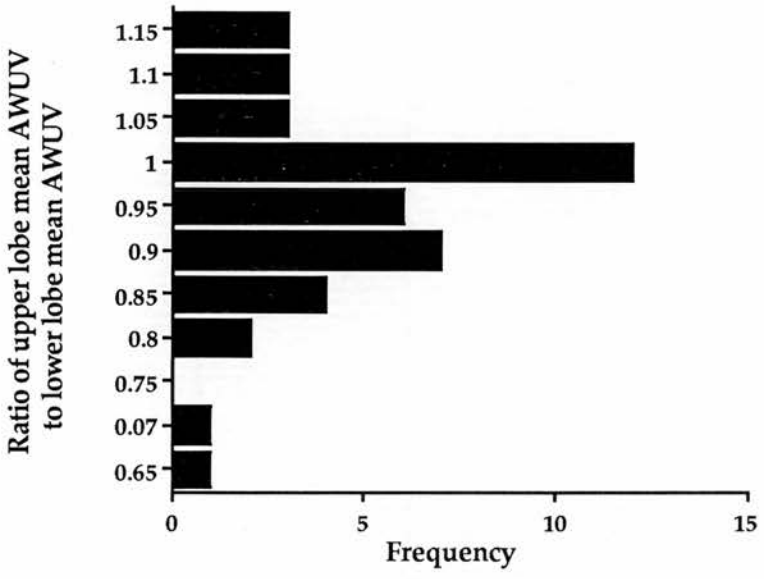

FIGURE 4.18

A frequency distribution of the ratios between upper lobe and lower lobe AWUV values. This histogram shows that the majority (81%) of the whole lungs studied had AWUV values in the upper and lower lobes which differed by less than 15%.

of the study (*i.e.* AWUV in relation to age, sex and smoking; AWUV in relation to macroscopic emphysema; and the relationship between macroscopic and microscopic emphysema). Since the results from this single lung were unlikely to affect the overall findings to any significant extent, the results from this lung were not excluded from any of the analyses described in this thesis.

The comparison of AWUV values from the upper and lower lobes indicated that the differences in AWUV values between the upper and lower lobes were related to the presence of moderate to severe macroscopic panacinar emphysema, some of which may represent confluent centriacinar lesions. Macroscopic centriacinar emphysema on its own (*i.e.* with no panacinar involvement) was not related to upper/lower lobe AWUV differences.

Forty-one of the 42 whole lungs studied had documented ages. It was therefore possible to assess whether the upper and lower lobe AWUV values were normal or abnormal, given the age of the subject. 30 of these lungs had normal mean AWUV values in both the upper and lower lobes, 7 had abnormal mean AWUV measurements in both the upper and lower lobes, 3 showed an abnormal mean AWUV in the upper lobe only, and 1 lung had an abnormal mean AWUV in the lower lobe only. One of the specimens with an abnormal mean AWUV in the upper lobe only was the specimen mentioned earlier, where inflation of the lower lobe was inadequate. The mean AWUV for the whole lung was abnormal, and this seems likely to be an accurate appraisal of the specimen as a whole.

Therefore, in 90% of these lungs, if MAE was present in one lobe, it was present in the other lobe also. These results suggest that in diagnosing microscopically assessed emphysema (MAE), a single lobe adequately represents the whole lung.

4.2.2 The Distribution of AWUV Within the Lobes

Analysis of variance of the AWUV measurements from the 3 zones in each lobe was used to assess the apex to base variation in AWUV within the lobe.

There was no apex to base variation within the upper or lower lobes in lungs without macroscopic emphysema, or where the emphysema was of the pure centriacinar type (Table 4.8). In cases with moderate or severe panacinar emphysema there was a tendency for AWUV to increase from the upper to lower zones of the upper lobe (Table 4.9). No such trend was observed within the lower lobes of these lungs.

These results indicate that in order to obtain a representative sample from a lung specimen, the upper lobe should be sampled widely from apex to base, particularly when panacinar emphysema occupies 10% or more of the lobe area.

TABLE 4.8 Probability values describing the within-lobe apex to base variation in AWUV values. P values are quoted to show the significance of any variation. These results are based on the Friedman 2-way analysis of variance.

	NO MACRO	CAE	PAE	MIXED
UPPER LOBE	p = 0.85	p = 0.45	p = 0.01*	p = 0.02*
LOWER LOBE	p = 0.27	p = 0.11	p = 0.42	p = 0.75

Macro = macroscopic emphysema; **CAE** = pure centriacinar macroscopic emphysema; **PAE** = pure panacinar macroscopic emphysema; Mixed = mixed types of macroscopic emphysema.

* = $p < 0.05$ (*i.e.* significant at the 5% level)

TABLE 4.9 Probability values showing the within-lobe variation in AWUV values in relation to severity of panacinar emphysema. Included in this table are results from all specimens with any panacinar emphysema (*i.e.* pure panacinar emphysema and mixed macroscopic emphysema).

	MILD	MODERATE	SEVERE
UPPER LOBE	p = 0.22	p = 0.04*	p = 0.02*
LOWER LOBE	p = 0.61	p = 0.75	p = 0.51

Mild = less than 10% of the mid-sagittal area of the specimen showing panacinar emphysema; **Moderate** = 10% - 40% area with panacinar emphysema; **Severe** = more than 40% of the mid-sagittal area of the specimen occupied by panacinar emphysema.

* = $p < 0.05$

4.2.3 The AWUV Variation Within the Lung in Relation to Age and Sex

Apex to base variation in AWUV measurements was not related to age in this sample. The age ranges were similar in the various sub-groups with and without macroscopic emphysema (Table 4.10). There were no sex differences in the distribution of AWUV within the lung (Table 4.11).

4.2.4 Summary

The mean AWUV values from the upper and lower lobes of 34 (81%) of the 42 whole lung specimens studied differed by not more than 15%. Differences between AWUV measurements in the upper and lower lobes appeared to be related to the presence of panacinar emphysema in the upper lobe, some of which may have been due to the confluence of severe centriacinar lesions.

Apex to base variation in AWUV measurements within the lung was not found to be related to age or sex.

The mean AWUV from a single lobe was found to be representative of the AWUV from the whole lung, provided an adequate sampling technique was used, particularly in lobes with panacinar emphysema.

It was concluded that where the diagnosis of MAE is required, a single lobe is representative of the whole lung.

TABLE 4.10 The range of ages in the sub-groups of whole lung specimens with and without macroscopic emphysema.

	Age Range	Mean Age
No macroscopic emphysema (n=20)	22 - 93	66.1
Macroscopic emphysema (n=22)	47 - 85	66.0

Ages are noted in years.

TABLE 4.11 Probability values describing the between-lobe differences and within-lobe variation in AWUV values in the whole lung specimens. These values show the probability of there being no differences between the upper and lower lobe AWUV values, or between the AWUV values of the 3 zones within each lobe. P values below 0.05 indicate significant differences between lobes, or within individual lobes.

	NO MACRO		MACRO	
	MALE	**FEMALE**	**MALE**	**FEMALE**
Differences between upper and lower lobes	p = 0.25	p = 0.27	p = 0.15	p = 0.14
Variation within upper lobe	p = 0.10	p = 0.78	p = 0.02*	p = 0.02*
Variation within lower lobe	p = 0.45	p = 0.53	p = 0.11	p = 0.78

Macro = macroscopic emphysema

* = below the 5% level of significance

Between lobe differences were assessed using the Wilcoxon Signed Ranks test. Within-lobe variation in AWUV was assessed using the Friedman 2-way Analysis of Variance.

4.3 MACROSCOPIC EMPHYSEMA

Evidence of macroscopic emphysema, defined as the presence of airspaces larger than 1mm in diameter, was assessed on the mid-sagittal slice of each of the 165 lung specimens as described in section **2.9**. The incidence of macroscopic emphysema in the study sample, and the relationships between macroscopic and microscopic emphysema are described in this section.

4.3.1 The Incidence of Macroscopic Emphysema

In 83 of the 165 lung specimens in this study (50.3%) there was no evidence of macroscopic emphysema. Table 4.12 shows the sex and smoking histories of these cases. 54% were males and 46% were females. The age range in this group was 21 to 93 years, with a mean age of 57.3 years.

Three (4%) of the cases without macroscopic emphysema had abnormally low mean AWUV values (Figure 4.19), 5% had low 5th percentiles, 4% low 10th percentiles, and 1 case (1.2%) had low 90th and 95th percentile AWUV values. All other lungs had normal values in all aspects of the AWUV distribution.

Evidence of macroscopic emphysema was found in 82 lung specimens. The age range in this group was 34 to 85 years. Table 4.13 shows the sex and smoking histories of the subjects with macroscopic emphysema and the incidence of the various types of emphysema in these subjects.

4.3.2 Macroscopic Emphysema in the Non-smokers

Three of the subjects with macroscopic emphysema were non-smokers. These were an 84 year-old woman with 9 centriacinar lesions in the upper lobe; a 75 year-old woman with 6 centriacinar lesions in the upper lobe; and a 77 year-old man with mild panacinar emphysema occupying 10% of the upper lobe. The mean AWUV was normal in each of these 3 cases.

TABLE 4.12 The number of male and female subjects with no evidence of macroscopic emphysema.

	Male	Female	TOTAL
Non-smokers	15	21	36
Smokers	30	16	46
TOTAL	45	37	82

In addition to the subjects listed in the table, one female subject with no documented smoking history was found to have no evidence of macroscopic emphysema. (This subject was included in the sample because the specimen was a whole lung, and smoking history was not required for the analysis of the variation in AWUV values from the apex to the base of the lung). Therefore, the total number of subjects with no macroscopic emphysema was 83.

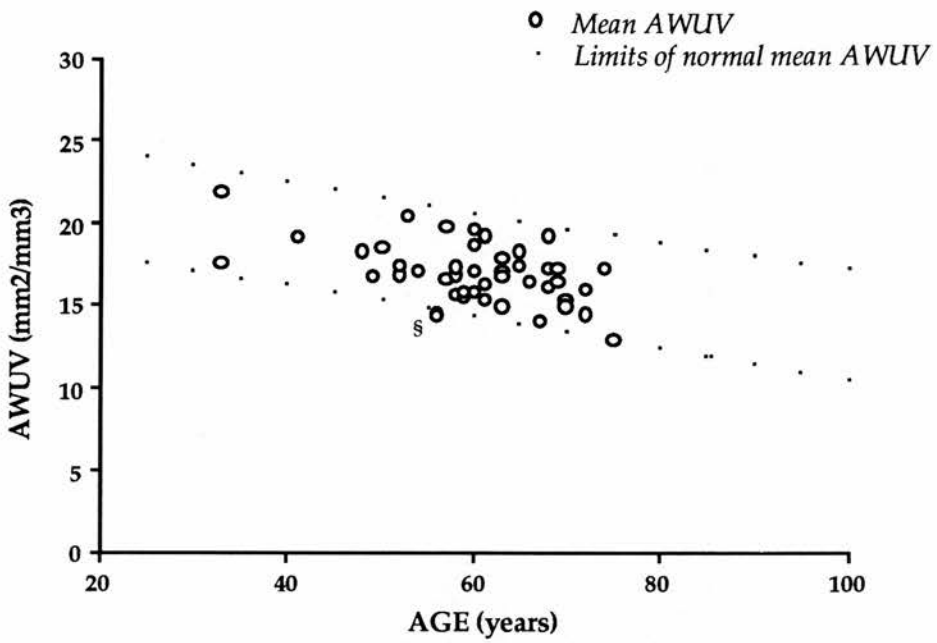

FIGURE 4.19

Mean AWUV plotted against age in the smoking subjects with no evidence of macroscopic emphysema. Only 3 subjects (4%) had MAE. The symbols representing 2 of these subjects overlap on this graph, this is indicated by the § symbol.

TABLE 4.13 The number of male and female subjects in each of the sub-groups with macroscopic emphysema.

	CAE		PAE		MIXED		OTHER		
	NS	S	NS	S	NS	S	NS	S	TOTAL
MALE	0	25	1	14	0	24	0	2	66
FEMALE	2	9	0	2	0	2	0	1	16
TOTAL	2	34	1	16	0	26	0	3	82

CAE = centriacinar emphysema, **PAE** = panacinar emphysema, **MIXED** = mixed types of macroscopic emphysema, **OTHER** = paraseptal or bullous emphysema.

NS = non-smokers, **S** = smokers.

4.3.3 Sex Differences in the Incidence of Macroscopic Emphysema

Sixty-six of the 82 cases with macroscopic emphysema (80%) were male. When broken down into the various types of macroscopic emphysema, the numbers of females in each group became too small for statistical analysis. However, the sex differences in the incidence of centriacinar emphysema appeared to increase with the severity of emphysema (Table 4.14).

The sex differences found were not due to differences in the smoking habit between the sexes, since all but 3 of the subjects with macroscopic emphysema were smokers. It would appear from these results that the males in this sample were more likely to develop macroscopic emphysema than the females, and when macroscopic emphysema was present it was more likely to be severe in males.

4.3.4 The Relationship Between Macroscopic Emphysema and AWUV in the Smokers

4.3.4.1 *Pure Centriacinar Emphysema*

Pure centriacinar emphysema (*i.e.* with no evidence of any other types of macroscopic emphysema) was found in 34 smokers' lung specimens. Its severity was mild in 14 lungs, moderate in 13 and severe in 7 lungs (Table 4.14).

AWUV values were plotted against age for the 34 smoking subjects with pure centriacinar emphysema (Figures 4.20 - 4.22). The limits of normal AWUV are also shown on these graphs.

The relationship between mean AWUV and age in the subjects with centriacinar emphysema was remarkably similar to the mean AWUV/age relationship in the non-smokers. Only 15% of those smokers with centriacinar emphysema had MAE.

Figure 4.21 shows that none of the cases with pure centriacinar emphysema had abnormally low 90th or 95th percentile AWUV values. The 5th and 10th percentile AWUV values were abnormal in increasing proportions of

TABLE 4.14 The incidence of pure centriacinar emphysema. Two of the female subjects with mild centriacinar emphysema were non-smokers; the remainder of the subjects listed in this table were smokers.

	Male	Female	TOTAL
Mild	10	6*	16
Moderate	8	5	13
Severe	7	0	7
TOTAL	25	11	36

* 4 smokers, 2 non-smokers

Mild = fewer than 10 CAE lesions in the mid-sagittal slice
Moderate = 10 - 20 CAE lesions
Severe = more than 20 CAE lesions

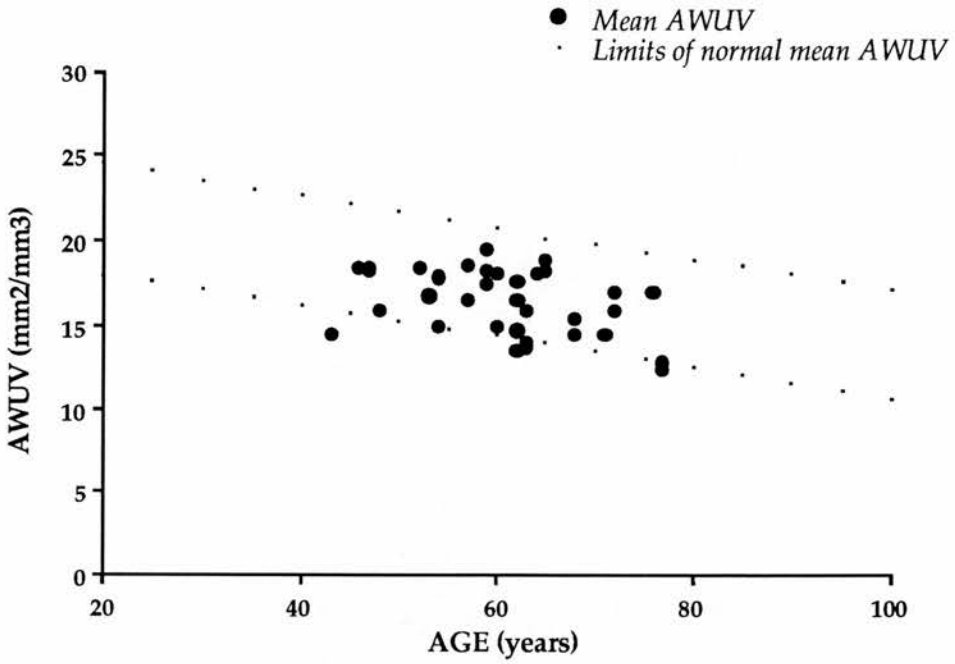

FIGURE 4.20

Mean AWUV plotted against age for the subjects with pure centriacinar emphysema. The limits of normal mean AWUV are also shown, and this graph illustrates that 85% of these individuals had normal mean AWUV values.

FIGURE 4.21 shows that all 34 subjects with pure centriacinar macroscopic emphysema had 90th and 95th percentile values above the lower limit of normality.

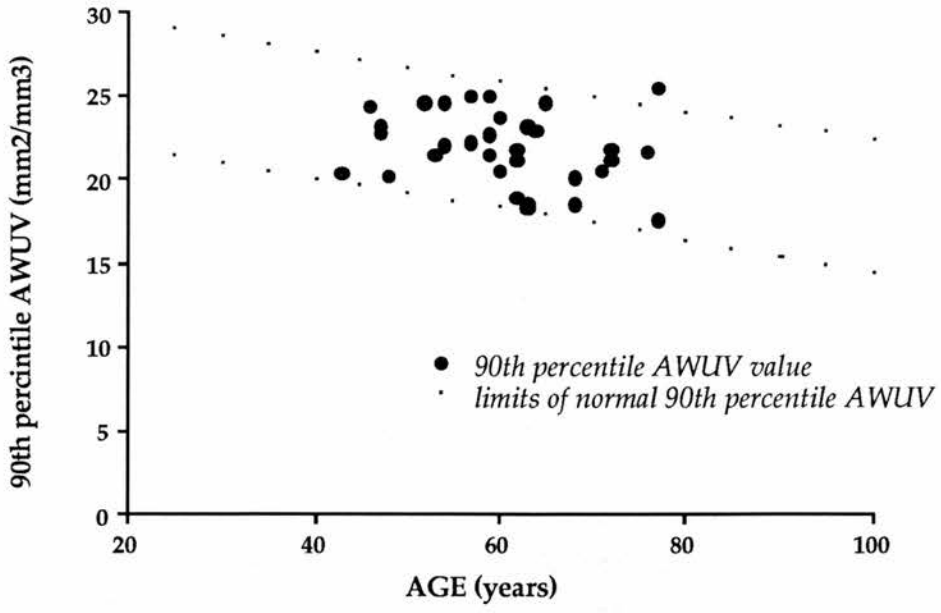

FIGURE 4.21a
The 90th percentile AWUV value plotted against age for the 24 subjects with pure centriacinar emphysema.

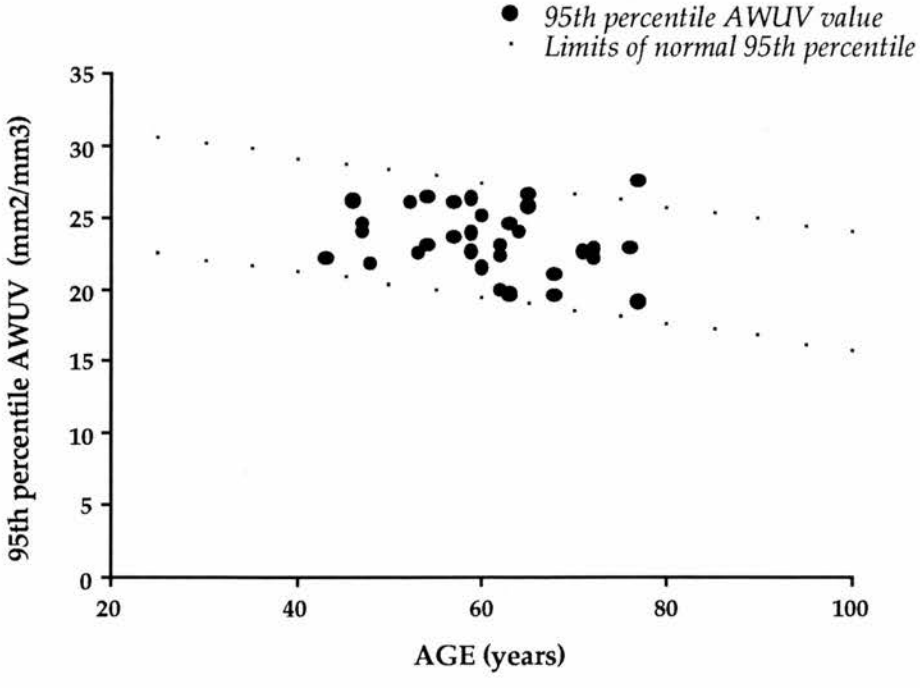

FIGURE 4.21b
The 95th percentile AWUV plotted against age for cases with pure centriacinar emphysema.

FIGURE 4.22 shows the relationships between age and the 5th and 10th percentile AWUV values in the subjects with pure centriacinar macroscopic emphysema. The severity of centriacinar emphysema is indicated on both of these graphs, with mild, moderate and severe centriacinar emphysema shown as different symbols. These graphs show that the incidence of abnormally low 5th and 10th percentile AWUV values was higher in the sub-groups with moderate and severe centriacinar emphysema.

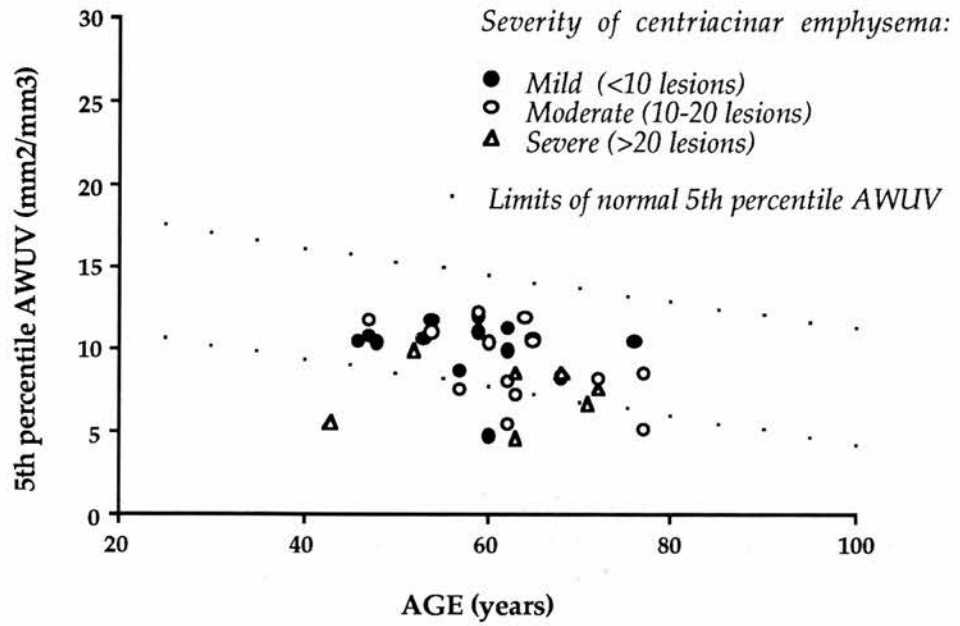

FIGURE 4.22a
5th percentile AWUV plotted against age for those smokers with pure centriacinar emphysema.

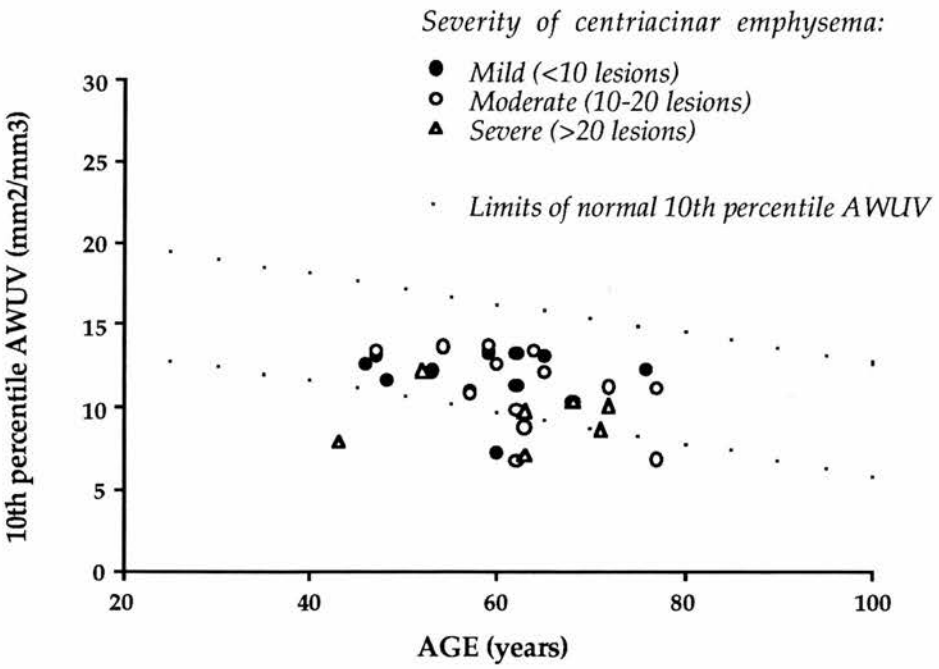

FIGURE 4.22b
10th percentile AWUV values plotted against age for the subjects with centriacinar emphysema.

the groups with moderate and severe centriacinar emphysema (as defined in Chapter 2 - section **2.9**) (Figure 4.22).

These results suggest that MAE, described in terms of mean AWUV, is not related to centriacinar emphysema. The results also indicate that centriacinar lesions are surrounded by normal parenchymal tissue, and that the 5th and 10th percentile AWUV values are more sensitive indicators of the presence of focal emphysematous lesions than the mean AWUV.

4.3.4.2 *Pure Panacinar Emphysema*

The graph of mean AWUV against age for the 16 smoking subjects with pure panacinar emphysema shows that 63% had MAE, (Figure 4.23). The incidence of MAE increased with the severity of panacinar emphysema. 76% of all cases with MAE had some evidence of macroscopic panacinar emphysema. Figure 4.24 shows that the severity of MAE was related to the severity of panacinar emphysema.

Abnormal values of 90th and 95th percentile AWUV occurred more frequently with increased severity of panacinar macroscopic emphysema (Figure 4.25).

These results indicate that the incidence and severity of MAE are associated with the incidence and severity of macroscopic panacinar emphysema, and that abnormally low 90th and 95th percentile AWUV values occur in lungs with macroscopic panacinar emphysema.

4.3.4.3 *Mixed Macroscopic Emphysema*

The AWUV/age relationships in the 26 lungs with evidence of both centriacinar and panacinar types of macroscopic emphysema show that 58% had abnormally low mean AWUV, 85% had abnormal 5th and 80% abnormal 10th percentile AWUV values; and 12% had low 90th and 8% low 95th percentiles (Figures 4.26-4.28).

Panacinar emphysema occupied more than 10% of the mid-sagittal slice in 65% of the specimens with mixed macroscopic emphysema. Given that centriacinar emphysema on its own does not appear to be related to MAE, it seems likely that the panacinar component of the macroscopically visible

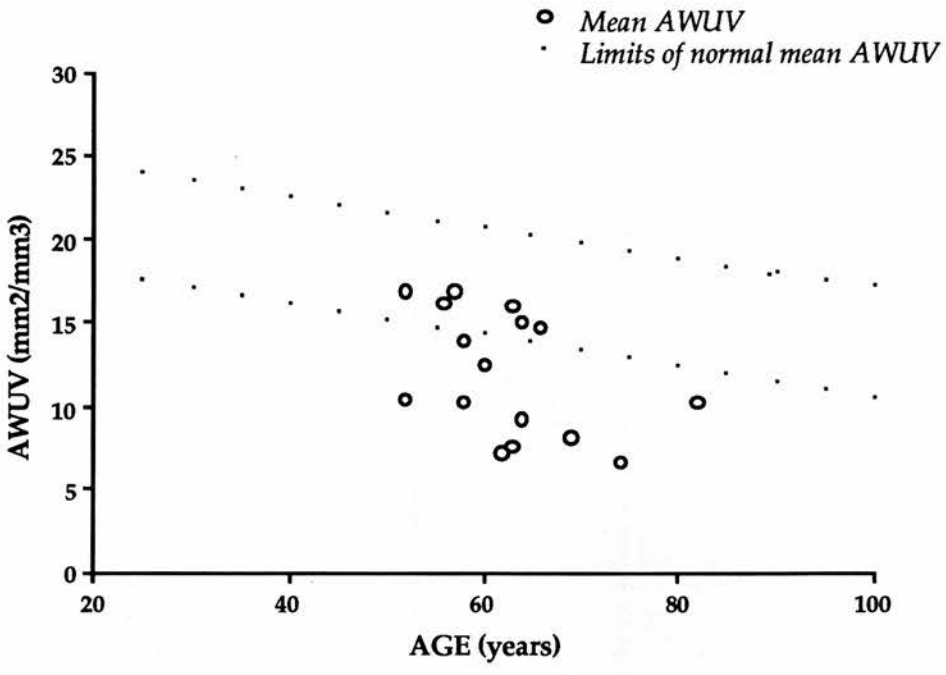

FIGURE 4.23

Mean AWUV plotted against age for the 16 smokers with pure panacinar macroscopic emphysema. 63% of this group had MAE.

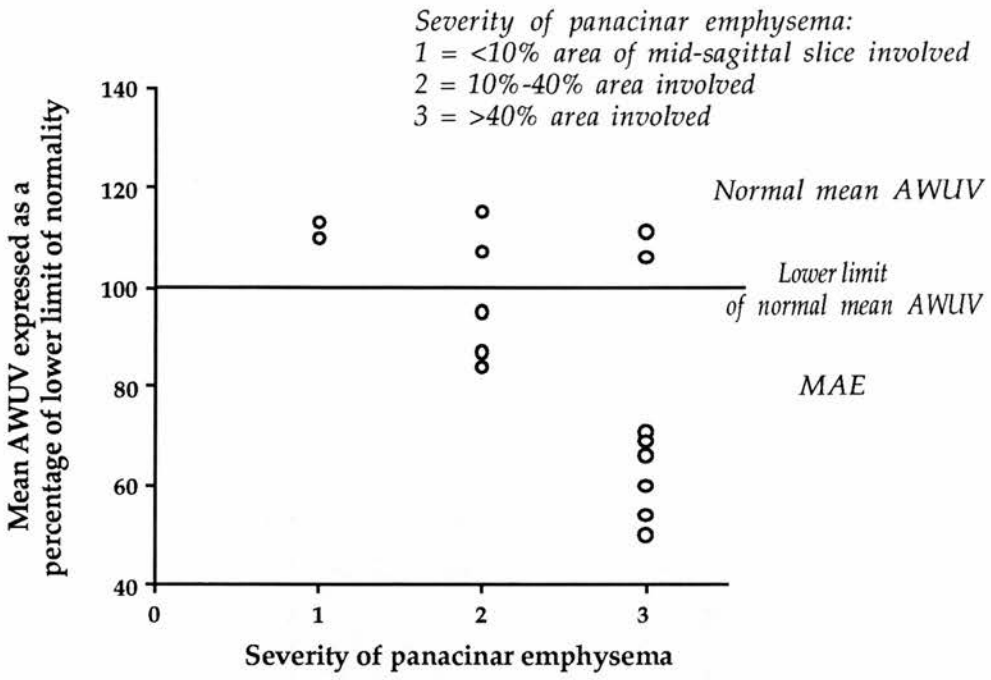

FIGURE 4.24

The severity of MAE expressed in relation to the severity of macroscopic panacinar emphysema in those subjects with pure panacinar emphysema. The severity of MAE has been shown by expressing the mean AWUV for each case as a percentage of the lower limit of normal mean AWUV. The severity of panacinar emphysema was expressed in terms of the area of the mid-sagittal slice of each specimen showing panacinar emphysema.

This graph illustrates the finding that in subjects with pure panacinar emphysema, the severity of MAE increased as the severity of panacinar emphysema increased.

FIGURE 4.25 shows the relationships between age and the 90th and 95th percentile AWUV values for the 16 smoking subjects with pure panacinar macroscopic emphysema. The severity of panacinar emphysema is indicated on the graphs as mild, moderate or severe. Mild refers to panacinar emphysema which occupied less than 10% of the mid-sagittal slice of the lung specimen, moderate panacinar emphysema occupied between 10% and 40% of the mid-sagittal area, and severe panacinar emphysema affected more than 40% of the mid-sagittal area of the specimen.

The graphs in Figures 4.25a and 4.25b illustrate the finding that abnormal values of the 90th and 95th percentiles of the AWUV frequency distribution occurred more frequently with increasing severity of panacinar macroscopic emphysema.

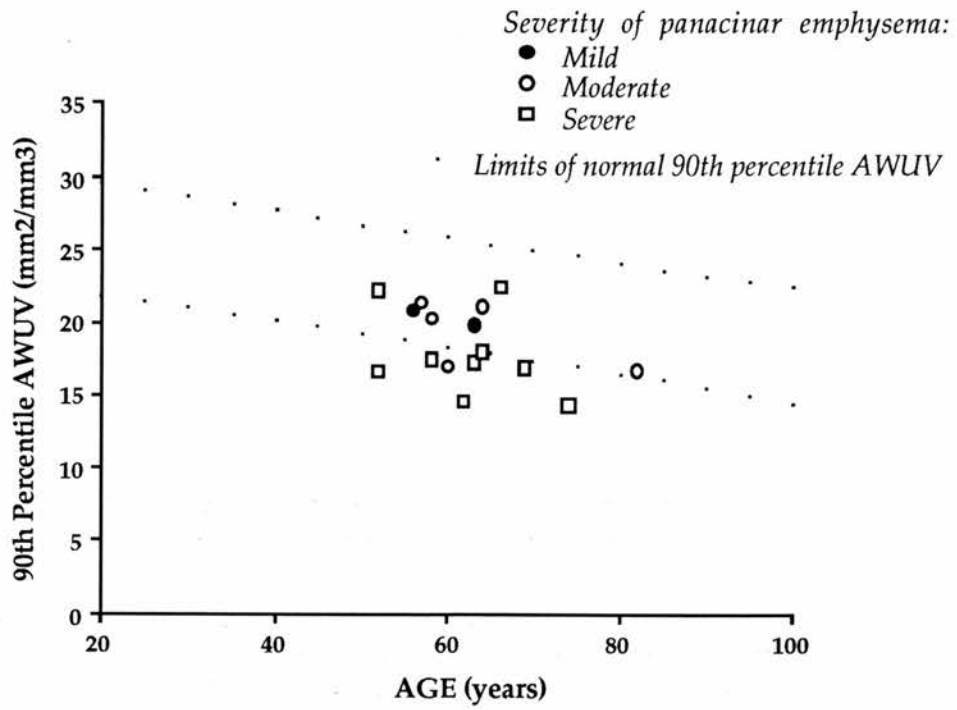

FIGURE 4.25a
The 90th percentile AWUV plotted against age for the subjects with pure panacinar emphysema.

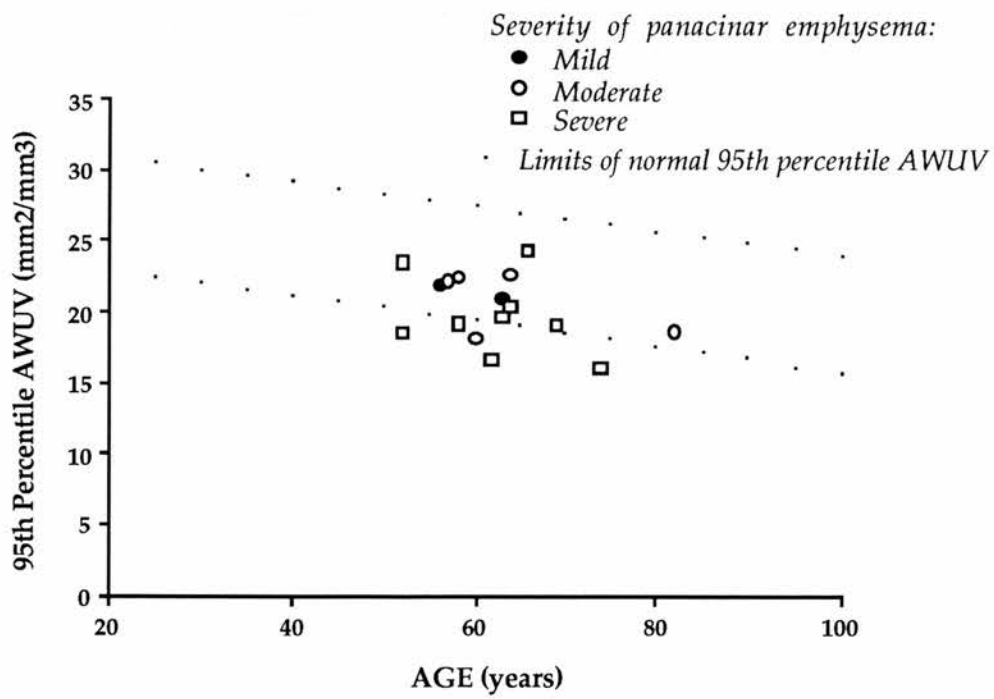

FIGURE 4.25b
The 95th percentile AWUV value plotted against age for subjects with pure panacinar emphysema.

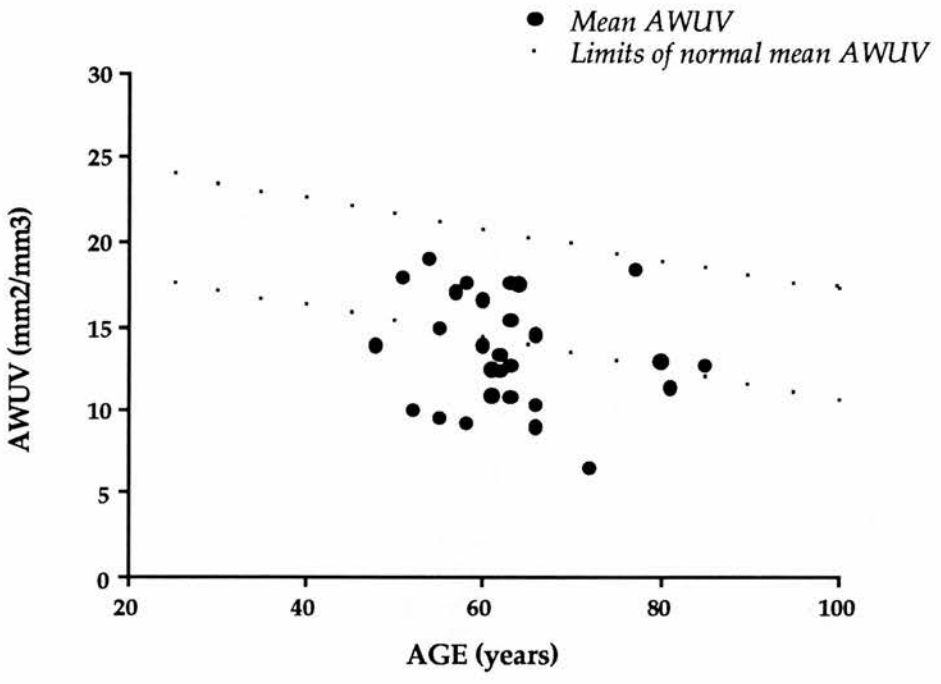

FIGURE 4.26

Mean AWUV plotted against age for the 26 lungs with a mixture of panacinar and centriacinar macroscopic emphysema. 58% of these subjects had MAE.

FIGURE 4.27

The 5th and 10th percentile AWUV values plotted against age in cases where the macroscopic emphysema present was mixed in type. Figure 4.27a shows that 85% of these individuals had abnormally low 5th percentile values, and Figure 4.27b shows that 80% had abnormally low 10th percentile values.

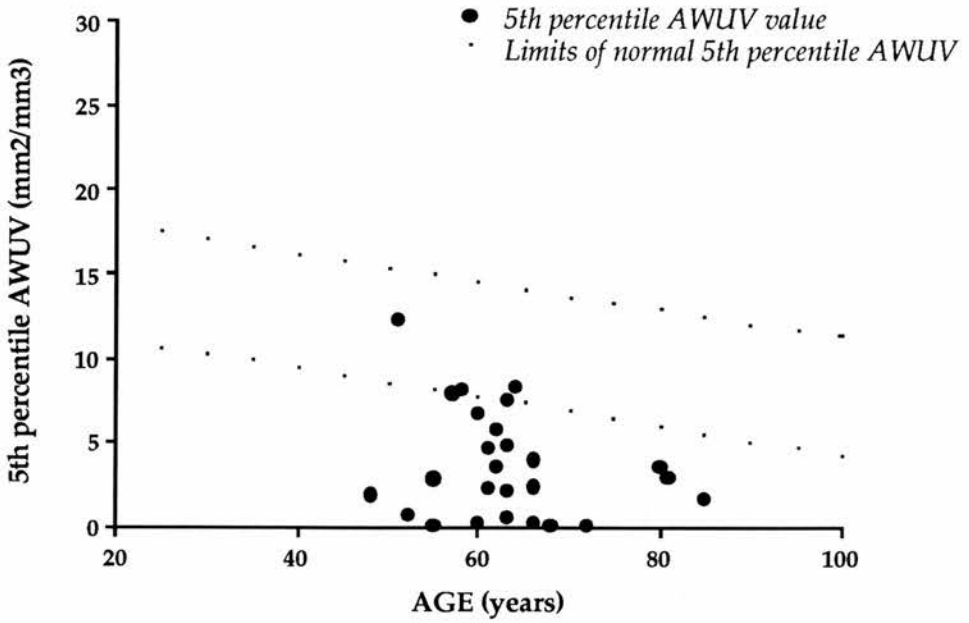

FIGURE 4.27a
The 5th percentile AWUV plotted against age for those specimens with mixed types of macroscopic emphysema.

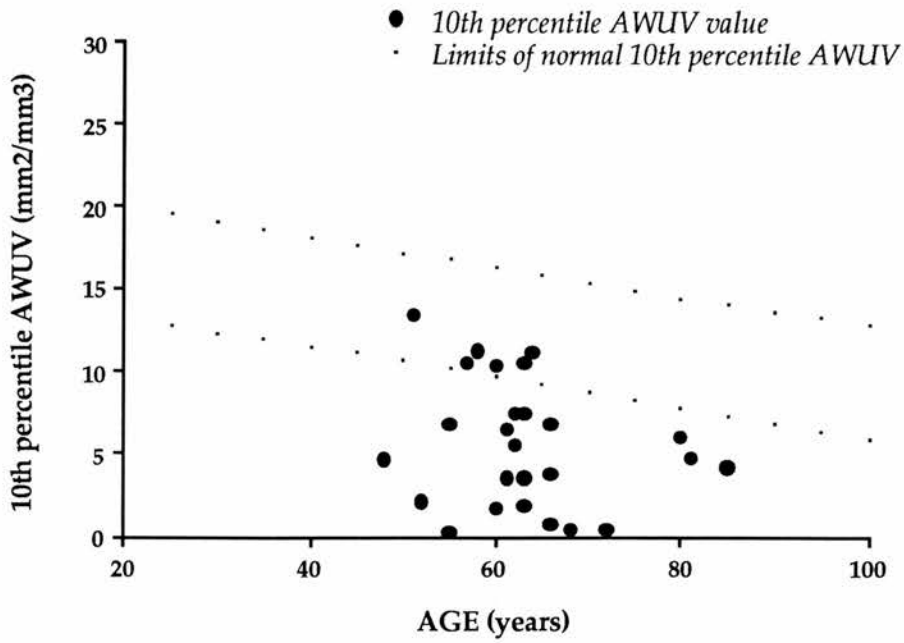

FIGURE 4.27b
10th percentile AWUV values plotted against age for the cases with mixed macroscopic emphysema.

FIGURE 4.28

The 90th and 95th percentile AWUV values plotted against age for the 26 specimens with mixed macroscopic emphysema. These graphs illustrate the finding that 12% had abnormally low 90th percentile AWUV values, and 8% had 95th percentiles below the limits of normality.

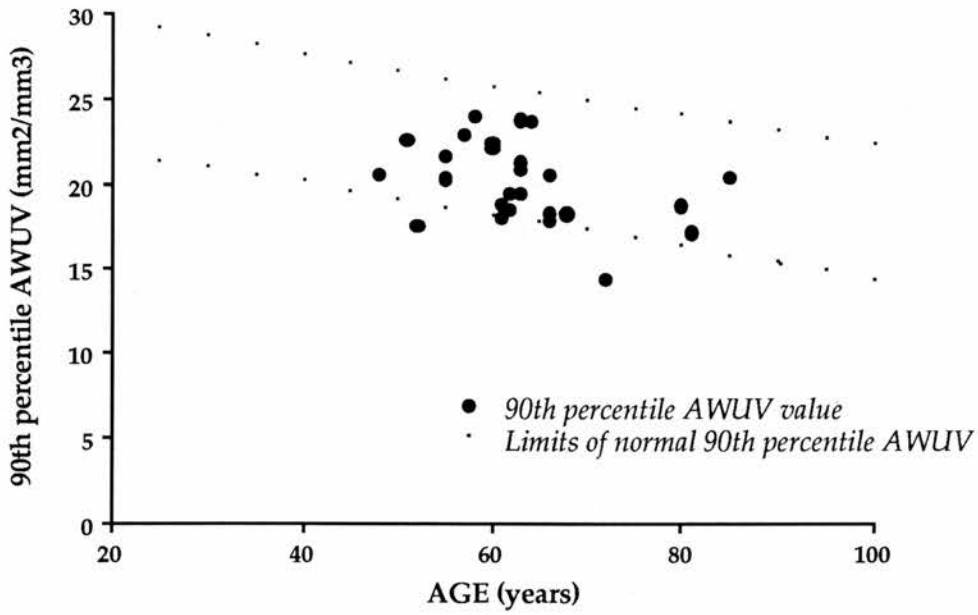

FIGURE 4.28a
The 90th percentile AWUV plotted against age in cases with mixed macroscopic emphysema.

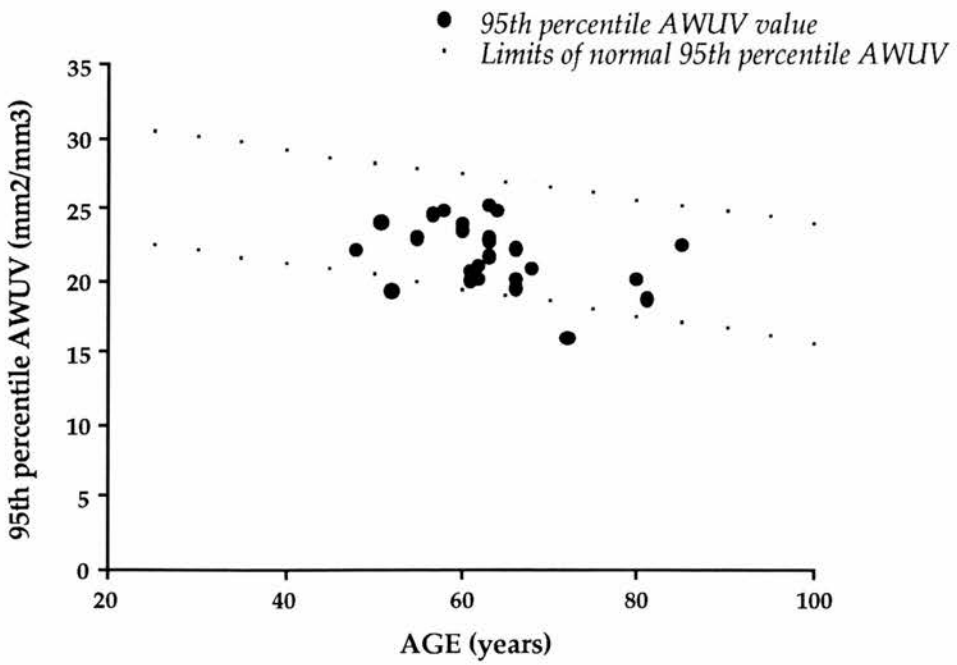

FIGURE 4.28b
95th percentile AWUV values plotted against age in the 26 subjects with mixed types of macroscopic emphysema.

emphysema is linked to the presence of MAE. In 6 of the lungs with both centriacinar and panacinar types of macroscopic emphysema, some of the areas of panacinar emphysema were due to confluent centriacinar lesions. 5 of these specimens (83%) had MAE. In these 5 lungs, the AWUV values from tissue blocks in areas avoiding the confluent lesions were found to be abnormally low. This indicates that MAE was present elsewhere in these lungs.

4.3.4.4 *Other Types of Macroscopic Emphysema*

Two smokers in this study had paraseptal emphysema, and 1 had apical bullous lesions (defined as emphysematous spaces more than 1cm in diameter; Ciba, 1959). In each of these cases the emphysematous lesions occupied less than 10% of the area of the lobe's mid-sagittal slice. The mean and percentile AWUV values were normal for all 3 of these cases.

4.3.4.5 *Summary*

Mean AWUV values were not affected by centriacinar emphysema or by small areas of panacinar or paraseptal emphysema or bullous lesions (*i.e.* occupying <10% of the lobe area). Mean AWUV was abnormal in increasing numbers of lungs with increased severity of panacinar emphysema.

The 5th and 10th percentile AWUV values became abnormally low with increasing severity of centriacinar emphysema, but the 90th and 95th percentiles were not affected by centriacinar emphysema.

The 5th, 10th, 90th and 95th percentile AWUV values all became abnormal in increasing numbers of lungs with increasing severity of panacinar emphysema.

These results indicate that in lungs with centriacinar emphysema, the lung tissue between lesions may be normal, and therefore the mean AWUV is not a sensitive indicator of centriacinar emphysema. In general, microscopically assessed emphysema, measured in terms of mean AWUV, is more closely related to panacinar emphysema.

4.4 SMOKING HABIT IN RELATION TO MACROSCOPIC EMPHYSEMA

Of the 97 subjects with documented daily cigarette consumption, only 38 had pure forms of macroscopic emphysema (9 cases of panacinar emphysema, and 29 cases of centriacinar emphysema). Unfortunately there were too few of these subjects with pure panacinar macroscopic emphysema for detailed analysis of the relationship between its severity and the severity of smoking habit to be performed.

Twenty-nine of the subjects with pure centriacinar emphysema had documented daily cigarette consumption. These subjects were sub-divided into 3 groups based on the severity of emphysema as described in detail in section **2.9**. The percentage of subjects smoking at least 20 cigarettes each day was found to increase with increased severity of centriacinar emphysema (Table 4.15). This result suggests that the severity of centriacinar emphysema was related to the daily dose of tobacco.

TABLE 4.15 The incidence of centriacinar emphysema in relation to daily cigarette consumption.

	<20	>20	Unknown	TOTAL
Mild	7 (44%)	7 (44%)	2 (12%)	16
Moderate	3 (23%)	7 (54%)	3 (23%)	13
Severe	2 (29%)	5 (71%)	0	7
TOTAL	12	19	5	36

<20 = smoked less than 20 cigarettes per day
>20 = smoked more than 20 cigarettes per day
Unknown = daily cigarette consumption not documented

4.5 SUMMARY OF FINDINGS

Tissue Sampling.

1. A sample size of 6 tissue blocks from each lobe was found to be adequate for representative AWUV measurements.

The relationship between AWUV and age in non-smokers.

1. There was a negative linear relationship between AWUV and age in 38 non-smokers, implying a normal increase in airspace size with advancing age in adult lungs.

2. The limits of normality of AWUV between the ages of 21 and 93 years were defined as the 95% prediction limits of the AWUV/age relationship.

3. The loss of airspace wall surface area with age in the non-smokers was found to involve the whole acinar unit.

4. Microscopically Assessed Emphysema (**MAE**) was defined as a useful term for describing the condition of the lung where the mean AWUV value was below the 95% prediction limits.

The relationship between AWUV and age in smokers.

1. A negative relationship was found between AWUV and age in 125 smokers' lungs, but only 26% of the smoking group had abnormally low mean AWUV (*i.e.* MAE).

2. 37% of the smokers had abnormally low 5th percentile AWUV values, and 34% had abnormal 10th percentiles, indicating that these percentile values may be more sensitive indicators of early or focal tissue destruction than the mean AWUV.

3. Less than 10% of the smokers had abnormal 90th and 95th percentile AWUV values.

4. Severity of smoking habit was not found to influence the incidence or severity of MAE.

5. The percentile AWUV values were found to be abnormal in more heavy smokers than light smokers. This indicates that heavy smokers are more likely to have early or focal tissue destruction, which may be centriacinar emphysema - see **24** below; and in those cases with MAE, the whole acinar unit may be involved.

Differences between the sexes in the AWUV/age relationship.

There were no sex differences in the relationship between AWUV and age in the smokers and non-smokers. MAE was found in 26% of the male smokers and 23% of the female smokers. This implies that neither sex is more likely to develop MAE.

The apex to base variation in AWUV.

1. Centriacinar macroscopic emphysema was not related to differences in mean AWUV between the upper and lower lobes.

2. Differences in mean AWUV values between the upper and lower lobe were found in lungs with panacinar emphysema occupying at least 40% of the mid-sagittal area of the upper lobe, and at least 20% of the mid-sagittal area of the lower lobe.

3. The incidence of MAE was found to be similar in the upper and lower lobes of the same lungs, suggesting that the mean AWUV from a single lobe is adequate for the diagnosis of MAE.

4. There was no apex to base variation in AWUV within lobes with no macroscopic emphysema, or in lobes with pure centriacinar emphysema.

5. AWUV was found to increase from the upper to lower zones of the upper lobe in some lungs with more than 10% of the mid-sagittal area of the lobe showing panacinar (possibly confluent centriacinar) emphysema. This indicates that to obtain a mean AWUV which is representative of a lung, the upper lobe should be sampled widely from apex to base, particularly when panacinar emphysema is present.

6. Apex to base variation in AWUV measurements was not related to age or sex.

The incidence of macroscopic emphysema.

1. 83 of the 165 lung specimens in this study had no evidence of any macroscopic emphysema.

2. 80% of the lungs with macroscopic emphysema came from male subjects.

3. The sex differences in the incidence of centriacinar emphysema increased with the severity of the disease.

4. Small areas of macroscopic emphysema were found in lungs from 3 non-smokers. The AWUV values (mean and percentiles) were normal in all 3 cases.

The relationship between centriacinar emphysema and AWUV in smokers.

1. Pure centriacinar emphysema was found in 34 lung specimens in the smoking group.

2. Mean AWUV values were normal in 85% of the smokers with pure centriacinar emphysema.

3. The 5th and 10th percentile AWUV values were abnormal in increasing numbers of lungs with increasing severity of centriacinar emphysema. This indicates that the 5th and 10th percentiles may be more sensitive indicators of focal lesions than the mean AWUV.

4. The 90th and 95th percentile AWUV values were normal in 100% of the lungs with pure centriacinar emphysema. This indicates that the parenchymal tissue surrounding centriacinar lesions is normal.

The relationship between panacinar emphysema and AWUV in smokers.

1. Pure panacinar emphysema was found in 16 smokers' lung specimens.

2. The mean and percentile AWUV values were found to be normal in all lungs where pure panacinar emphysema occupied less than 10% of the area of the mid-sagittal slice of a lobe.

3. The number of cases with normal mean and percentile AWUV values was reduced with increasing severity of panacinar emphysema.

4. The 90th and 95th percentile AWUV values were abnormal in increasing numbers of lungs in subjects with moderate and severe panacinar emphysema. This result suggests that decreases in these percentile values are related to panacinar and not centriacinar emphysema.

The relationship between AWUV and mixed types of macroscopic emphysema.

1. Twenty-six lungs were found to have a mixture of centriacinar and panacinar macroscopic emphysema. All 26 subjects were smokers. The panacinar component of the macroscopic emphysema appeared to have more influence on the AWUV values in these lungs than the centriacinar component.

2. Confluent centriacinar lesions were found in the upper lobe of 6 lungs with a mixed pattern of macroscopic emphysema. In 83% of these specimens, MAE was present in the lung tissue outwith the area of confluence.

The relationship between AWUV and other types of macroscopic emphysema.

Two lung specimens were found to have paraseptal emphysema, and one had apical bullous lesions. These lesions occupied less than 10% of the mid-sagittal area of each lobe, and the mean and percentile AWUV values were normal in all 3 cases.

The relationship between MAE and macroscopic emphysema.

1. MAE was not related to focal (centriacinar) lesions or to small areas of macroscopic emphysema.

2. In general, the presence of MAE appeared to be more closely related to panacinar than to centriacinar emphysema.

Chapter 5

Discussion

5.1 INTRODUCTION

The 'Discussion' Chapter falls into 3 major parts:
1. A discussion of the use of the term 'AWUV' in measuring airspace wall surface area (section **5.2**).
2. Discussion of the study group and the methodology used in the study (sections **5.3** and **5.4**)
3. Discussion of the results of the study and their implications (sections **5.5** - **5.8**).

The most important findings of the study are listed in section **5.9**, publications arising from the project are listed in section **5.10**, and an outline of some proposals for future work is presented at the end of the Chapter in section **5.11**.

The use of the FIP as a new technique in the study of emphysema formed a vital part of this project. A discussion of the development and assessment of this technique has been presented in Chapter 3, and therefore a detailed discussion of the FIP is not repeated in this Chapter.

5.2 THE USE OF AWUV IN THE STUDY OF MICROSCOPIC EMPHYSEMA

This thesis has been based on measurements of Airspace Wall surface area *per* Unit Volume of lung tissue (**AWUV**). It therefore seems appropriate to discuss the choice of this unit of measurement in assessing the effect of age, sex and cigarette smoking on the amount and distribution of microscopic emphysema in a group of human lungs.

The term 'AWUV' was introduced by Lamb and colleagues in 1986, and has been used by D Lamb's research group ever since. The initials **A W U V** are perhaps an unfortunate choice, since it is not immediately obvious that they are the initials of a phrase describing a surface area measurement. However, the term is now established in the literature (Gould *et al*, 1988; Wilkinson, 1989; McLean *et al*, 1992), and to introduce another term at this stage would only have caused confusion.

As described above, AWUV is the surface area measurement of the alveolar walls contained in a unit of lung volume ($1mm^3$). It is possible to extrapolate from this type of measurement an estimate of the total alveolar wall surface area in the whole lung. Estimates of this kind have been made by several authors (Campbell & Tomkeieff, 1952; Weibel, 1963; Dunnill, 1964; Duguid *et al*, 1964; Thurlbeck, 1967a; 1967b; 1967c; Bignon *et al*, 1969). Such estimates of total lung surface area depend on the assessment of an accurate lung volume for each subject, or on the normalisation of the measurements to correspond to a standard lung volume (Thurlbeck, 1967b; 1967c). Normalisation of surface area measurements was performed because, as discussed in section **1.3**, until recently it was thought that alveolar size may have been related to lung size, and therefore that alveolar surface area *per* unit lung volume would vary according to the size of the lungs (Weibel, 1963; Hislop & Reid, 1974). However, the results of several studies (Thurlbeck, 1967b; 1975; 1982; Matsuba & Thurlbeck, 1971; Schreider & Raabe, 1981; McLean, 1987) have shown that alveolar size is relatively constant, regardless of lung size. For this reason, AWUV can be used to compare the structure of many lungs without correcting for lung dimensions.

The mean linear intercept can also be used to compare the tissue density of lungs of various sizes, since it is also independent of total lung volume. However, the concept of the mean linear intercept, that of estimating the average distance between alveolar walls, is more difficult to grasp than the actual measurement of tissue destruction which is represented by decreases in AWUV. This is because AWUV is a direct measurement of the airspace walls.

Another advantage of expressing AWUV *per* mm^3 of lung tissue is that accurate AWUV measurements for each $1mm^2$ measured are available, and this permits the detailed assessment of microscopic changes associated with age and smoking. In particular, this form of expression of the results allowed the patterns of emphysema within the acinar unit to be analysed. Such detailed analyses of the exact location of microscopic emphysematous lesions would not be possible using more traditional methods of lung surface area measurement.

A useful property of AWUV measurements is that they can be compared with the results of biochemical and morphometric studies of the connective tissue component of the alveolar walls. This use of AWUV is presently being incorporated in a collaborative study involving the Pathology and Biochemistry Departments of Edinburgh University. It is hoped that this type of study will be helpful in identifying some of the specific mechanisms relating to microscopic emphysema.

Although for this study it was most appropriate to express AWUV in terms of mm^2/mm^3, if the results presented in this Thesis are to be compared with mean linear intercept estimates or total lung surface area measurements made by other research groups, AWUV can easily be converted to either.

5.3 THE STUDY GROUP

The lung specimens used in this study came from 2 sources - autopsy and surgical material. As a result, various selection factors were introduced in the sampling of these specimens. The advantages and disadvantages of a mixed study group such as this are discussed below.

There were several reasons for including surgical specimens in this study. Firstly, an earlier study in the Pathology Department involved comparing morphometric analysis of lung structure with pulmonary function measurements made on the same lungs, and therefore only surgical specimens were appropriate (McLean, 1987). As described in section **2.1**, 40 of these surgical specimens formed part of the group of surgical specimens in the present study. However, since a different morphometric technique was used in this study, each of the tissue sections from these specimens was re-analysed.

Tissue sections from freshly fixed surgical material are usually of better quality than those obtained from autopsy material, since they do not contain desquamated alveolar lining cells, and there is less likelihood of the presence of inflammatory infiltrate. Fortunately, surgical lung specimens are readily available in this Pathology Department. The availability of high quality tissue sections was particularly important in this study, since the FIP was fully automated, and interactive editing of $1mm^2$ fields prior to their measurement was impossible (see Chapter 3 for details).

Another advantage of using surgical specimens is that smoking histories are usually readily available for patients undergoing surgery, and this is not always the case with autopsy material. An additional reason for selecting surgical material was the availability of pulmonary function data, which will allow future analysis of the structure/function relationships in these patients.

Several selection factors were influential in obtaining the 121 surgical lung specimens. The subjects had to be fit enough to undergo major surgery, and this led to the exclusion of many elderly patients (only 3 were over 75 years old), and those patients with severely impaired pulmonary function,

including many cases of advanced emphysema. The majority of the surgical specimens came from patients who were undergoing surgical resection as treatment of carcinoma, and therefore most (82%) were smokers. All of the surgical specimens had peripheral tumours, and care was taken to cut the sample blocks from tissue away from the tumour. Although the area of the tumour was always avoided, it is possible that since lung cancer and emphysema are both associated with tobacco smoking, the susceptibility factors responsible for these conditions may be related, and this could have affected the observed incidence of microscopic emphysema. The selection factors involved in obtaining surgical specimens from non-smokers were different from those relating to the smoking subjects, in that most of the lesions were metastatic deposits or benign tumours (See Appendix).

Autopsy specimens are likely to be free of such selection factors, but there were other disadvantages in using them in this morphometric study. None of the autopsy subjects had died as a result of respiratory disease, and the subjects were chosen to represent as wide an age range as possible. Therefore, these subjects did not represent a random sample of the autopsy population.

Technical problems were associated with the tissue sections from the autopsy specimens. The presence on the sections of desquamated alveolar lining cells in the alveoli, and evidence of terminal infection caused problems when using the FIP due to the lack of interactive editing available. Consequently, some tissue sections, and in certain cases some lungs, were unsuitable for AWUV measurements.

The smoking histories of the autopsy cases were obtained from interviews with relatives at the time of death, or from the clinical records of those patients who died in hospital. For obvious reasons, smoking histories of deceased individuals are difficult to substantiate, and this is a disadvantage, particularly as it limits the number of certain lifelong non-smokers which may be included in a study such as this.

The major reasons for selecting autopsy material were that tissue from older subjects (especially those older than 75 years of age) could be obtained, and

whole lungs could be collected for the study of the distribution of AWUV measurements within the lung.

The 38 non-smokers included in this study (surgical and autopsy specimens) were documented as being lifelong non-smokers, and this represents a larger group of known non-smokers than has been reported previously in studies of microscopic emphysema (Saetta *et al*, 1985b; Gould *et al*, 1988).

The group of smokers in the present study contained 125 subjects. Several other workers have used various methods to study the incidence of microscopic emphysema in smokers, but their study groups have invariably been smaller than the one described here (Saetta *et al*, 1985b: n=23; Gould *et al*, 1988: n=43; Nagai *et al*, 1989: n=41; Eidelman *et al*, 1990: n=23; Kim *et al*, 1991: n=34).

5.4 METHODOLOGY

The methods used in any study, particularly a morphometric study, have a significant influence on the results obtained. The influential features of the methodology used in the present study are discussed below.

5.4.1 Lung Inflation and Fixation

In studies of pulmonary emphysema, it is essential to examine the lung in its inflated state (Ciba, 1959; Heard, 1960; Thurlbeck, 1964; Dunnill, 1970; Thurlbeck *et al*, 1970b; Sutinen *et al*, 1982; Dunnill, 1987). Several methods of preparing inflated lung specimens are available, and these have been described in detail by Silverton (1965) and Dailey (1973).

In the present study a standardised technique was required which was capable of inflating and fixing surgical and autopsy specimens to a similar degree. The major problem in inflating lung specimens for morphometry is standardising the degree of inflation achieved. Several authors have described the 'constant pressure' inflation/fixation technique (Heard, 1958; Dunnill, 1987; Thurlbeck, 1988). This technique involves applying an arbitrary constant pressure of fixative throughout the inflation and fixation process (Thurlbeck, 1976). For a constant pressure of fixative to be applied during the fixation process, a tube conveying the fixative must be attached to the bronchus. For this reason, the constant pressure technique is not suitable for inflating lobectomy specimens, since the segmental or sub-segmental bronchi are cut during surgery, leaving a stump too short to be attached to the fixation apparatus (D Lamb, personal communication).

The method used for lung inflation and fixation in this study was the 'smooth contour' method (section **2.2**). This is the simplest, quickest and least expensive method of lung inflation, and using this method lungs are fixed following inflation by submerging them in formalin solution until fixation is complete (Gough & Wentworth, 1949; Wentworth, 1950; American Thoracic Society, 1959; Heppleston & Leopold, 1961; Thurlbeck, 1976; Dunnill, 1987). This is a commonly used method for preparing lung tissue for histological examination, and was appropriate for the present

study since it enabled lobectomy, pneumonectomy and autopsy specimens to be inflated to a similar degree.

A problem associated with fixing tissue in formalin, regardless of the technique used, is tissue shrinkage. Further shrinkage usually occurs during tissue processing, and this is discussed in section **5.4.2** below. The inflation and fixation techniques described in this Thesis were standardised as far as possible. However, it must be stressed that AWUV values obtained using other inflation/fixation techniques are likely to differ from the values quoted here.

5.4.2 Tissue Processing

Shrinkage of tissue components occurs during histological processing of blocks of tissue. In particular, sections which have been embedded in paraffin wax tend to shrink, and this is a major problem in morphometric studies. Measurements made on paraffin sections should be adjusted to correct for shrinkage (Weibel, 1963; Dunnill, 1964; Thurlbeck, 1967a; 1967b; 1967c). An additional problem with paraffin embedding is that the sections become compressed to a variable extent during cutting (Leeson *et al*, 1985). Unfortunately, lung tissue is particularly affected by these problems.

The processing technique used in this study involved embedding the tissue blocks in glycol methacrylate resin. This processing technique produces tissue sections with excellent preservation of morphology. A comparison of the artefacts produced by paraffin and glycol methacrylate embedding was performed in this laboratory by McLean & Lamb in 1983. They found that while shrinkage and compression resulted in a decrease in the area of paraffin embedded tissue sections by as much as 40%, shrinkage during processing and compression due to section cutting were negligible when glycol methacrylate resin was used as the embedding medium (McLean & Lamb, 1983).

5.4.3 Morphometric Techniques

The development and assessment of the FIP as a technique for measuring AWUV has already been discussed in Chapter 3. The purpose of this section is to discuss tissue sampling in relation to the FIP and to summarise briefly some important points in the use of the FIP.

5.4.3.1 *Tissue Sampling Procedure*

As with any morphometric technique involving the use of histological tissue sections, AWUV measurements require tissue sampling. A representative sample for AWUV measurements must be large enough to give a precise estimate of the true mean AWUV value for each lung. Of course, the actual AWUV of the whole lung is unknown, but the precision of the estimate can be assessed by calculating the 95% confidence intervals around the mean. The true mean has a 95% probability of lying in the range: Sample Mean +/- (1.96xSEM), where SEM is the standard error of the sample mean (Williams, 1977). A tissue sample can be considered to be representative of the organ if the 95% confidence intervals of the mean are within +/- 10% of the mean (Weibel, 1963).

An efficient sampling design involves obtaining a representative sample with the minimum of effort (Mayhew, 1983). Using the FIP as the method of measuring AWUV ensured that an adequate number of fields was scanned on each section. However, it was important to make sure that the minimum number of blocks sampled from each lung constituted a representative sample of that lung.

Forty of the surgical specimens included in this study were originally part of a previous study of the structure/function relationships in the human lung (McLean, 1987). Twelve tissue blocks were selected at random from the lateral 2 sub-pleural slices of the fixed specimens, and this was found to be a representative sample from each lung (McLean, 1987). The tissue processing technique used in this and McLean's study involved embedding the lung tissue blocks in glycol methacrylate resin, a process which is extremely time-consuming and results in the production of an average of only 36 histological sections *per* week. The present study was designed to include AWUV measurements on a large number of lung specimens, and in the case of the surgical specimens, a representative mean value for the whole

specimen was the major requirement. The sample size was therefore reviewed. An attempt was made to reduce the number of blocks cut from each lung as far as possible, while still ensuring that the sample was representative of the specimen.

The 95% confidence intervals around the mean AWUV from 6 blocks of 20 specimens were found to be within +/- 5.6% of the mean (section **4.1.3**). This indicates that a sample of 6 blocks is adequate to produce a representative AWUV value from a single lobe using the FIP. Weibel (1963) suggested that 5-10 tissue blocks should be an adequate sample from a lung, and other workers have used similar sample sizes in their studies of lung structure (Thurlbeck, 1976; Langston *et al*, 1979; Saetta *et al*, 1985b; Nagai *et al*, 1989; Saito *et al*, 1989).

The block size used in the present study was 2cm x 2cm. Blocks of this size were used because the tissue sections cut from these blocks are an ideal size to fit on a standard microscope slide. This size of block is particularly useful in studying lung structure, since components of several acinar units are found on each section. Similar sized blocks have been used by several other groups in their microscopic studies of lung structure (Dunnill, 1964; Thurlbeck, 1967b; 1967c; Saetta *et al*, 1985b; Gould *et al*, 1988; Nagai *et al*, 1989; Eidelman *et al*, 1990; 1991; Kim *et al*, 1991)

5.4.3.2 *The FIP as a Technique for Measuring AWUV*

As discussed in Chapter 3, the FIP has proved to be an excellent method for measuring AWUV. It is much faster than previously used techniques, and the reproducibility of its measurements is excellent - largely due to the fact that it is fully automated. The high degree of automation of the system may lead to some errors being introduced into the measurements, but as Thurlbeck noted (Thurlbeck, 1976) these errors are likely to affect measurements on normal and abnormal tissue alike. Therefore, the relationships between AWUV and age, sex and smoking quoted in this thesis are valid.

The comparison of the FIP results with those produced using the IBAS system (see section **3.4.4.2**) has drawn attention to the fact that, as with tissue fixation and processing, the use of different techniques is likely to result in

different values for AWUV. This point must be taken into consideration when comparing the results of this study with those obtained in other laboratories.

5.5 THE EFFECTS OF AGE, SEX AND SMOKING ON AWUV

Three of the original aims of this study were related to assessing the effects of age, sex and smoking on AWUV. These aims were approached firstly by studying a group of non-smokers and observing the changes in AWUV with age in men and women; and secondly by studying a group of male and female smokers, and comparing the results from this group with the results from the non-smokers to isolate the effects of smoking on AWUV. The results of these aspects of the study are discussed below.

5.5.1 The Relationship Between AWUV and Age in Non-smokers

The various definitions of emphysema which have been proposed all include reference to the abnormal enlargement of airspaces distal to the terminal bronchiole. To apply these definitions, quantitative data must be obtained on the limits of normal airspace size.

In this study, mean AWUV was found to decrease with age in 38 non-smokers, and this decrease was found to be linear. The 95% prediction limits were plotted to indicate the range of AWUV values which could be expected in non-smokers with advancing age. The 95% prediction limits represent the AWUV range within which 95% of non-smokers of a particular age are likely to fall.

It is not appropriate to consider elderly patients with AWUV values within the 95% prediction limits for their age as having senile emphysema, even though their mean AWUV values are significantly lower than those of subjects in early adult life. This view is in keeping with the approach to tests of pulmonary function, where the progressive decline in lung function from young adulthood with advancing age is accepted as normal (Cotes, 1979).

The non-smokers included in this study came from both urban and rural areas of the country. Their only known pulmonary abnormality was the small peripheral lesion for which resection was performed in the surgical cases. It is reasonable to accept that these individuals represent a population with normal lung structure, and to use the 95% prediction limits of mean

AWUV in relation to age as the limits of normal mean AWUV between the ages of 21 and 93 years. Hence, if only those patients with mean AWUV values below the 95% prediction limits of AWUV for non-smokers are abnormal, the diagnosis and assessment of microscopic emphysema in quantitative terms is made possible.

The entity of 'senile emphysema' has been described as the barely visible macroscopic abnormality of the lung in the elderly (Reid, 1967b). The rate of decline of AWUV in the present study group might therefore have been expected to increase with advancing age corresponding to the onset of senile emphysema. However, as stated above, the decline in AWUV with advancing age was linear and the AWUV measurements from all 38 non-smokers fell within the 95% prediction limits of the regression line. There was therefore no evidence to suggest that a sub-group of these non-smokers might develop senile emphysema.

The Ciba report in 1959 recommended that the term 'senile emphysema' should not be used until the normal range of size of airspaces in the lung at different ages was established. Thurlbeck (1970; 1976; 1991) has also stated that the changes in airspace size with age were a normal consequence of aging, and that this should not be confused with the presence of emphysema. Thurlbeck also found a linear change in airspace size with age in his study of mean linear intercept measurements in macroscopically non-emphysematous lungs from 25 subjects (Thurlbeck, 1967b). Unfortunately however, the smoking histories of his subjects were not recorded.

Using the limits of normal mean AWUV as described above, microscopic emphysema may be described as a condition of the lung in which the mean AWUV measurement is below the lower limit of normality. An appropriate term to describe this condition is microscopically assessed emphysema (MAE). This conforms to the definition of emphysema as the abnormal enlargement of airspaces distal to the terminal bronchiole.

Gould and colleagues (1988) reported on a structure/function study of the lung which included the comparison of computed tomography (CT) scan density measurements with AWUV measurements made on the same

lungs. These workers found a linear relationship between the density of lung tissue measured by CT scanning and AWUV measurements, suggesting that it may be possible to use CT scanning to recognise early emphysema in life. The results of the present study indicate that normal lung density decreases with advancing age. Therefore, if CT scanning is to be used in diagnosing emphysema in the patient during life, the range of normal CT densities according to age should be established. In fact, all clinical and pathological studies must take the age-related change in normal values for mean AWUV into account when identifying emphysema.

All 38 non-smokers in this study had mean AWUV values within the normal range for their age, and the relationship between mean AWUV and age was similar in men and women. Given that men have larger lungs than women, even when height is taken into consideration (Thurlbeck, 1982), this result indicates that alveolar size is not related to lung size, and therefore implies that larger lungs contain additional generations of alveoli compared with small lungs. This conforms to the suggestion that alveolar multiplication may continue throughout childhood (Emery & Wilcock, 1966; Nakamura *et al*, 1967; Angus & Thurlbeck, 1972), although obviously the results of a study of adult lungs such as this can give no information on the timing of completion of alveolar multiplication.

It thus appears unlikely that the published sex differences in the incidence of macroscopic emphysema (Azcuy *et al*, 1962; Anderson *et al*, 1972; Auerbach *et al*, 1974; Thurlbeck *et al*, 1974; Sutinen *et al*, 1978; Sobonya & Burrows, 1983; Dijkman, 1986; Dunnill, 1987; Snider, 1989) are due to differences between the sexes in normal airspace size.

Other aspects of the distribution of AWUV values within a specimen can be useful in examining age changes in lung structure. As described in section **4.1.1**, in the normal lung, the 5th and 10th percentile AWUV values represent fields in the proximal acinus and the 90th and 95th percentiles represent the distal acinus, with the mean giving a general impression of the density of tissue across the lung. The modal AWUV value is useful in assessing changes in the shape of the AWUV distribution. Frequency distributions of AWUV measurements could be compiled for each subject in this study because the FIP measured AWUV in a minimum of 726 $1mm^2$

fields from each specimen. Analysis of the various aspects of the AWUV distribution from individual lungs was performed in order to study whether the loss in AWUV with age was localised to any one area of the acinus, or whether the age change was generalised.

In the 38 lifelong non-smokers, the mode and percentile values of AWUV were related to age in the same way as the mean AWUV, *i.e.* the rate of decline of mean, mode and percentile AWUV values with age were similar. These results indicate that there was an even decrease in AWUV across the acinar unit. This is in contrast to the published opinion that in the aging lung, the decrease in tissue density occurs specifically in the region of the alveolar ducts, *i.e.* in the proximal acinus (Thurlbeck, 1976). However, macroscopically recognisable panacinar increases in airspace size in the elderly have previously been described (Azcuy *et al*, 1962; Reid, 1967b; Thurlbeck, 1991), although as discussed above, it is inappropriate to describe this change as emphysema.

5.5.2 The Effect of Smoking on Mean AWUV

Having established the limits of normal mean AWUV in the study of the non-smokers, the next step was to assess the relationship between mean AWUV and age in the smokers.

The mean AWUV/age relationship in the smokers was negative, but only 26% had mean AWUV values which were below the normal limit for their age (*i.e.* these smokers had MAE as described in section **5.5.1**). This result indicates that susceptibility differences may have existed within the group of 125 smokers. Differences in the susceptibility to macroscopic emphysema have frequently been reported in the literature, as reviewed by Sobonya & Burrows (1983).

In this study, the mean AWUV/age relationship was similar in men and women, and the percentages of these 2 groups who developed microscopic emphysema were similar. These results are interesting, since macroscopic emphysema has consistently been found to be more common in men (Thurlbeck, 1963a; 1963b; Thurlbeck *et al*, 1974; Sutinen *et al*, 1978; Sobonya & Burrows, 1983; Snider, 1989), and it has even been suggested that females

may have some protective factor against developing the disease (Sutinen *et al*, 1978; Bignon *et al*, 1980). The results presented here suggest that this is not the case with microscopic emphysema. These results also reinforce the suggestion that airspace size is similar in men and women, and sex differences in the incidence of macroscopic emphysema are therefore unlikely to stem from inherent differences in the lungs of men and women.

The apparent lack of sex differences in the susceptibility to MAE suggests that the pathogenetic mechanisms responsible for MAE may be different from those relating to the most common form of macroscopic emphysema, centriacinar emphysema. This point is discussed further in section **5.7**.

Given that only a minority of the smoking group had MAE, as defined in terms of mean AWUV, the most obvious question to ask was 'were these subjects simply heavier smokers than those who did not develop MAE?', in other words, was susceptibility dose-related with respect to tobacco consumption? Several workers have found that the relative incidence of macroscopic emphysema was higher with increased tobacco consumption (Anderson *et al*, 1966; Auerbach *et al*, 1972; Spain *et al*, 1973; Thurlbeck *et al*, 1974; Sobonya & Burrows, 1983).

In this study the presence of MAE did not appear to be due to the extent of smoking habit. In addition, the severity of MAE was not related to daily cigarette consumption.

The method chosen to record tobacco consumption was the number of cigarettes smoked each day. This method was more appropriate for the analysis of AWUV in smokers than pack years data, because both pack years and AWUV are related to age, and thus the true relationship between the degree of tobacco consumption and AWUV would be masked by the age effect. It is accepted that smoking histories (including pack years estimates) obtained by interviews with patients are subject to inaccuracies. When questioned, many patients underestimate their tobacco consumption (Viegi *et al*, 1991). Nevertheless, the 3 broad sub-groups of smoking habit selected for the present study represent a range of tobacco consumption from light to severe.

Given that inaccuracies in smoking habit information do occur, it might be expected that the subjects in all 3 sub-groups will have underestimated the extent of their actual smoking habit, and yet even in the sub-group of heaviest smokers, 69% of subjects had normal mean AWUV values for their age. It would therefore appear that while tobacco smoking is undoubtedly related to the onset of MAE (since MAE did not occur in any of the non-smokers), the severity of the smoking habit is not the major factor in determining an individual's susceptibility to MAE, or the severity of MAE which susceptible individuals will develop. A similar opinion, relating to macroscopically assessed emphysema, was expressed by Anderson and colleagues (1966), who commented that since large numbers of individuals with heavy tobacco consumption did not develop macroscopic emphysema, the variations in susceptibility to the disease were not accounted for by smoking habit alone. Pratt (1988) noted differences in the susceptibility to centriacinar emphysema amongst heavy smokers. Pride (1983) stated that the wide differences in susceptibility to airflow obstruction in smokers, which may be related to emphysema, were not due to the amount of tobacco smoked.

5.6 THE DISTRIBUTION OF AWUV WITHIN THE LUNG

The various forms of macroscopic emphysema are not distributed uniformly throughout the lung. In particular, centriacinar emphysema occurs more frequently and is usually more severe in the upper lobes, and in severe forms panacinar emphysema may be more predominant in the lower lobes (section **1.6.1**). When a sample includes single lobes obtained at surgery, it is important to ensure that a single lobe is representative of the whole lung. The descriptions of the general distribution of macroscopic emphysema suggest that in specimens containing macroscopic emphysema, a single lobe may not present a pattern of emphysema which is representative of the whole lung. However, Wright and colleagues (1986) noted that the upper and lower lobes of their series of pneumonectomy specimens had similar grades of macroscopic emphysema, as assessed by Thurlbeck's panel grading system.

5.6.1 AWUV Distribution in Relation to Macroscopic Emphysema

The results of the present study showed that macroscopic centriacinar emphysema was not related to differences between the mean AWUV values of the upper and lower lobes. Where differences in AWUV between the upper and lower lobes occurred, the mean AWUV from the upper lobe tended to be lower than that from the lower lobe. Contrary to expectations based on the published trends in its distribution, these differences usually occurred when panacinar emphysema occupied more than 10% of each lobe. The most likely explanation for this is that some of the panacinar emphysema present in the upper lobe was the result of the confluence of severe centriacinar lesions, and this phenomenon is more likely to occur in the upper lobe and in the apical region of the lower lobe.

In 41 of the 42 whole lungs studied it was possible to assess whether the mean AWUV from each of the upper and lower lobes was normal or abnormal. In 90% of these lungs, the diagnosis of MAE was the same in both lobes (*i.e.* MAE was either present in both lobes or absent from both lobes). Hence, with regard to MAE, in general, a single lobe was found to give a representative diagnosis for the whole lung. This is in general agreement with the results of Wright and colleagues (1986), and of

Auerbach and co-workers (1963), who found that rupture of the alveolar walls occurred to a similar degree in right and left lungs, and in the upper and lower lobes of these lungs.

Lichros and colleagues (1991) found that the occurrence of microscopic panlobular emphysema was similar in the upper and lower lobes of 41 lungs. This corresponds with the finding in the present study that the incidence of MAE was similar in the upper and lower lobes.

When they examined the distribution of microscopic centriacinar emphysema, Lichros and co-workers found that it occurred more frequently in the upper lobes. It was suggested in section **4.1** that the 5th and 10th percentile AWUV values may be more sensitive indicators of focal microscopic lesions than the mean AWUV (this point will be discussed further in section **5.7**). If this is the case, it would be interesting to note whether these percentile AWUV values differ between the upper and lower lobes. This is an area which requires some further work.

The study of the apex to base variation in AWUV values within individual lobes showed that in the absence of macroscopic emphysema, or where the macroscopic emphysema was of the pure centriacinar type, there was no evidence of significant variation in AWUV from the apex to the base of either the upper or the lower lobe. It has been suggested that in the upright lung there is a gradient of alveolar size from the apex to the base (Glazier *et al*, 1966). However, further work by the same group indicated that the potential size of all the alveoli in the lung was the same, since the apex to base gradient in alveolar size disappeared in the supine lung (Glazier *et al*, 1967). The results of the present study of whole lungs inflated under standardised conditions, suggest that in anatomical terms, there is no apex to base gradient in airspace size. This indicates that the gradient observed in the above mentioned study by Glazier and colleagues was due to the physiological influences on the upright lung.

In the present study, the distribution of AWUV measurements within individual lobes was not related to the incidence of centriacinar emphysema, but when panacinar emphysema occupied more than 10% of the mid-sagittal area of the upper lobe, there was a tendency for an apex to

base gradient of AWUV to exist. As discussed above, this may be related to the presence of confluent severe centriacinar lesions. When the lungs with mixed types of macroscopic emphysema were examined it was found that panacinar emphysema was likely to occur elsewhere in the lung when centriacinar lesions were large enough to become confluent.

Saetta and colleagues (1990), and Kim and colleagues (1991) have described centrilobular and panlobular microscopic emphysema, but neither group has commented on the distribution of these types of emphysema within the lung. Lichros and co-workers (1991) commented on the incidence of microscopic centrilobular and panlobular emphysema in the upper and lower lobes. They did not comment on the upper/lower lobe differences in incidence of mixed types of microscopic emphysema, but they did note that the panlobular type was always predominant in such specimens. These authors did not describe the distribution of the different types of microscopic emphysema within individual lobes.

5.6.2 The Distribution of AWUV in Relation to Age and Sex

There were no sex differences in the apex to base AWUV distribution within the 42 whole lungs studied. This indicates that there were no sex differences in the spatial distribution of MAE in this study group. This result contradicts the common finding that there are sex differences in the incidence of macroscopic centriacinar emphysema, and implies that MAE incidence does not follow the same pattern of incidence as macroscopic centriacinar emphysema.

The distribution of AWUV within the lung was not related to age. Therefore, the tendency for some subjects to have upper/lower lobe differences in AWUV values was not due to advancing age.

5.7 THE INCIDENCE OF MACROSCOPIC EMPHYSEMA

Macroscopic emphysema is identified by the presence of airspaces at least 1mm in diameter (Ryder *et al*, 1971). The classification of macroscopic emphysema into 4 main types (as described in section **1.6**) is accepted, but there is little available information on the microscopic changes underlying these macroscopic lesions. It is logical to assume that macroscopically visible emphysema develops on a background of microscopic emphysema, but an important point to consider is whether microscopic lesions are present in the same patterns as their macroscopic counterparts. It is possible that all macroscopic lesions, regardless of their type, develop on a background of generalised (panacinar) microscopic emphysema. The studies by Saetta, Kim, Lichros and colleagues (Saetta *et al*, 1990; Kim *et al*, 1991; Lichros *et al*, 1991) lead to the suggestion that various forms of microscopic emphysema do exist which correspond to the documented patterns of macroscopic emphysema. However, the assessments of microscopic emphysema performed by these groups have been non-quantitative, and they have not reported on the types of macroscopic lesions identified in their lung specimens.

Various forms of macroscopic emphysema were found in 3 of the non-smokers and in 79 of the smokers in this study group. In this section, the incidence of macroscopic emphysema is described, and the relationships between macroscopic emphysema, AWUV and microscopic emphysema are discussed.

5.7.1 Macroscopic Emphysema in the Non-Smoking Group

Only 3 of the non-smoking subjects in this study had any evidence of macroscopic emphysema. All 3 subjects were over 70 years of age, and the macroscopic emphysema was focal or localised in nature. The mean AWUV in all 3 specimens was within the normal range. This result shows that small areas of panacinar emphysema, or focal lesions of the centriacinar type, which represent abnormally large airspaces in small areas of the lung, may not affect the overall mean AWUV values. This suggests that any underlying microscopic emphysema is also focal in its distribution. These types of lesions are best described as focal or localised macroscopic

emphysema. This illustrates that the term 'emphysema' should not be used unqualified, but rather the type and severity of the observed lesions should be described. For a patient or a lung as a whole to be considered as having 'emphysema' the term 'microscopically assessed emphysema' (MAE) is useful, as it represents a mean AWUV value outwith the 95% prediction limits of AWUV for the population, thus taking into account the age-related changes in airspace size discussed in section **5.5.1**.

5.7.2 Macroscopic Emphysema in the Smoking Group

Macroscopic emphysema is well documented as being more commonly found in men than in women (Azcuy *et al*, 1962; Anderson *et al*, 1972; Auerbach *et al*, 1974; Thurlbeck *et al*, 1974; Sutinen *et al*, 1978; Sobonya & Burrows, 1983; Dijkman, 1986; Dunnill, 1987; Snider, 1989). The results of this part of the study were in agreement with this finding, as the majority of smokers with macroscopic emphysema were males, and this was true for all types of macroscopic emphysema observed, with the exception of the single female subject with a bullous lesion.

This result is interesting, particularly in view of the finding that there were no sex differences in the incidence of MAE in the same study group (section **5.5.2**). It may have been expected that the lack of sex differences were a feature peculiar to this sample, since these smokers may not represent the general smoking population. However, the sex differences in the incidence of macroscopic emphysema indicate that this is not the case, rather, these results indicate that there are fundamental differences between the susceptibility to microscopic and macroscopic emphysema. It is possible that although women are just as likely as men to develop microscopic emphysema, they have some protective factor against developing macroscopic emphysema. Alternatively, it may be some inherent feature in smoking habits which makes women less likely to have macroscopic emphysema (*e.g.* differences in brand preferred, smoking technique, etc ; Anderson *et al*, 1972).

The incidence of macroscopic emphysema has previously been found to increase with increased tobacco consumption (Auerbach *et al*, 1972; Spain *et al*, 1973; Thurlbeck *et al*, 1974). In this study, details of daily cigarette

consumption were available for 97 smokers, and of these only 38 had pure forms of macroscopic emphysema, which limited the extent of the analysis which could be performed with the results from these specimens. However, it did appear that the severity of centriacinar emphysema was related to daily cigarette consumption.

Although MAE, defined in terms of mean AWUV, was not directly influenced by daily cigarette consumption, the 5th and 10th percentile values of AWUV were. The incidence of abnormally low 5th and 10th percentiles increased with increased daily cigarette consumption. These results provide support for the suggestion that low 5th and 10th percentile values represent the focal microscopic lesions which lead to macroscopic centriacinar emphysema (section **4.3.4.1**). This suggestion is discussed further in section **5.7.3**.

5.7.3 The Relationship Between Focal Macroscopic Emphysema and AWUV

The results in section **4.3.4** indicate that mean AWUV is not usually reduced below normal levels in subjects with pure centriacinar emphysema. This suggests that the underlying microscopic lesions are also focal in their distribution.

It may be suggested that the sampling technique used here resulted in the omission of emphysematous areas of lung tissue. Careful examination of the lung specimens showed that this was not the case. The sampling procedure in this study was designed to produce a representative sample of the lungs' structure (section **5.4**), and areas containing macroscopic centriacinar lesions were not avoided. Specimens with up to 80 centriacinar lesions in a single lobe slice were found to have normal mean AWUV values. It would appear therefore that the mean AWUV is not a sensitive indicator of focal emphysema.

The lack of association between focal emphysematous lesions and mean AWUV was also evident in the 3 lung specimens with paraseptal and bullous lesions. In each of these subjects, the focal lesions occupied less

than 10% of the lobe area, and the mean AWUV values were normal in each case.

Macroscopic centriacinar emphysema is known to be related to tobacco smoking, and has been found to be more severe with increasing dose of tobacco consumption. As discussed above, this was also found to be the case in those subjects in this study for whom detailed smoking habits were documented (section **5.7.2**). Interestingly, the frequency of abnormal 5th and 10th percentile AWUV values was increased with increased daily cigarette consumption. Also, the frequency of abnormal 5th and 10th percentiles increased with increasing severity of macroscopic centriacinar emphysema. These findings provide further evidence for the suggestion that abnormally low 5th and 10th percentile AWUV values represent the presence of centriacinar emphysema in lungs with normal mean AWUV values.

The fact that the mean AWUV was not usually reduced in cases with pure centriacinar emphysema, and the finding that the 90th and 95th percentile AWUV values were normal in every case of pure centriacinar emphysema, indicate that the parenchymal tissue surrounding centriacinar lesions is normal, and hence that the underlying microscopic lesions are also focal. This is in agreement with Sweet and co-workers (1961) who stated that even in cases where centriacinar emphysema was advanced, a 'rim' of normal tissue was to be found.

The results of this part of the study give further evidence that the pathogenesis of centriacinar emphysema and MAE may be linked to different mechanisms, and this evidence has been revealed by using one of the most useful features of the FIP, the availability of individual AWUV values from each $1mm^2$ field. These values have been used to provide more specific information on the nature of emphysema than a single mean value for a lung, which is produced by most other morphometric techniques, could have supplied.

Kim and colleagues have used a different analysis, but have also utilised the information from individual field measurements (Kim *et al*, 1991). They found that the coefficient of variation in mean linear intercept measurements was higher in lungs with subjectively identified microscopic

centrilobular emphysema than in lungs with microscopic panlobular emphysema. These results indicated that the tissue distribution around the area of the lesions was heterogeneous (*i.e.* focal microscopic emphysema). Kim and colleagues noted that the tissue surrounding these lesions 'appeared' normal. However, this observation was not substantiated by quantitative data, and the subject's age was not taken into consideration.

5.7.4 The Relationship Between Macroscopic Panacinar Emphysema and AWUV

If the mean value of AWUV is an indicator of generalised loss of airspace walls throughout the lung, then it is reasonable to expect that it may be related to macroscopic panacinar emphysema, which affects the whole of the acinar unit (Reid, 1967b; Dunnill, 1987) and is said to be found throughout the lung (Thurlbeck, 1963a). In this study, 76% of the smoking subjects with MAE (indicated by mean AWUV values below the lower limit of normality for their age) also had macroscopic panacinar emphysema. In addition, the incidence and severity of MAE were increased with increasing severity of panacinar emphysema. As discussed in section **5.5.2**, the relationship between daily cigarette consumption and MAE was not clear. The association between daily cigarette consumption and macroscopic panacinar emphysema is also thought to be complex (Anderson *et al*, 1964; Thurlbeck, 1976). Therefore, MAE and macroscopic panacinar emphysema represent a single disease process with varying degrees of severity.

Every subject with abnormally low 90th and 95th percentile AWUV values had macroscopic panacinar emphysema (in contrast, these values were normal in every case of macroscopic centriacinar emphysema). The frequency of abnormal 90th and 95th percentiles increased with increasing severity of macroscopic panacinar emphysema. These results suggest that abnormal values of the 90th and 95th percentile AWUV are associated with widespread macroscopic panacinar emphysema.

It seems reasonable to assume that the 5th and 10th percentile AWUV values will be reduced in most cases where the mean AWUV is lower than normal. It may therefore be suggested that in some circumstances, where the mean AWUV is normal, abnormally low 5th and 10th percentiles are

associated with early panacinar emphysema. In a cross-sectional study such as this it is impossible to predict which of the young individuals with abnormal 5th and 10th percentiles and normal mean AWUV will eventually develop MAE or macroscopic panacinar emphysema, and which will develop macroscopic centriacinar emphysema. Therefore, abnormally low 5th and 10th percentile values of AWUV cannot be used in isolation to predict the type of macroscopic emphysema which is likely to develop. However, when the mean AWUV is normal, they usually indicate the presence of microscopic centriacinar emphysema rather than panacinar emphysema.

The results discussed above suggest strongly that MAE is associated with the presence of panacinar emphysema, and that MAE may represent an early form of panacinar emphysema.

5.7.5 The Relationship Between Mixed Macroscopic Emphysema and AWUV

Although several types of macroscopic emphysema have been described (section **1.6**), two or more of these types often co-exist in a single lung (Dunnill, 1987). The distinction between panacinar and centriacinar emphysema becomes particularly difficult when severe centriacinar lesions become confluent in the upper lobe. There are conflicting opinions on how to classify emphysema in such circumstances, and these opinions relate to the authors' assumptions concerning the mechanisms responsible for the development of confluent centriacinar emphysema.

Increasing severity of macroscopic centriacinar emphysema may take the form of the appearance of new lesions, or of an increase in the size of existing lesions (Pratt, 1988). Pratt has commented that the increase in size of centriacinar lesions continues in some cases to confluence, and that this should always be described as centriacinar emphysema, regardless of the extent of destruction of the acinar unit (Pratt, 1988). If this is the case, it is difficult to understand what the differences are between patients who have severe centriacinar emphysema in which all the lesions are discrete, and those who have confluence of the centriacinar lesions in the upper lobes.

An alternative opinion is that confluent emphysematous lesions must be classified as panacinar, regardless of their origin (Thurlbeck, 1976; D Lamb, personal communication). Confluent centriacinar emphysema usually occurs when macroscopic emphysema is widespread (Thurlbeck, 1976), and in such cases, panacinar emphysema is often present elsewhere in the lung (Sweet *et al*, 1961; Thurlbeck, 1963a; 1963b; 1976; 1991; Mitchell *et al*, 1970; Spencer, 1985; Dunnill, 1987; Lamb, 1990).

In the present study, lung specimens from 26 smokers had evidence of both macroscopic centriacinar and macroscopic panacinar emphysema, and 6 of these specimens had severe confluent centriacinar emphysema in the upper lobe. All of these had macroscopic panacinar emphysema to a varying degree elsewhere in the lung, and 5 of the 6 (83%) had MAE. Of the 5 subjects with MAE, the AWUV values of the majority of tissue blocks from the lung specimen, including those blocks away from the confluent centriacinar lesions, were below the normal range. Three specimens with MAE and confluent centriacinar emphysema were whole lungs. In each of these specimens, the upper lobe and lower lobe mean AWUV values were abnormal. These results show that MAE was present in the tissue outwith the area of confluent centriacinar emphysema. 'Confluent centriacinar emphysema' thus appears to be the consequence of the coincidence of 2 processes - centriacinar emphysema and MAE.

5.8 THE CONCEPT OF 'EMPHYSEMA'

The discussion of the results presented in this thesis raises the problem of defining what 'emphysema' is. As stated in section **1.4** above, emphysema is defined as:

> 'a condition of the lung characterised by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis' (Snider *et al*, 1985).

However, as described in section **1.6**, and discussed in section **5.7**, emphysema exists in several forms, and can be considered to exist on the basis of various criteria. Emphysema is therefore not a single entity, but rather the term is a description of one or several patterns of tissue destruction in a lung, each of which may have different pathogenetic mechanisms and different causes.

Should emphysema be described in microscopic or macroscopic terms? The definition suggests that normal airspace size should be defined, and once this size is exceeded, emphysema exists. How far should the limits of normality be exceeded before emphysema is recognised? What is the functional significance of each of the microscopic and macroscopic patterns of emphysema?

The functional significance of microscopic emphysema is still unclear. It is possible that MAE may affect lung function more than small areas of macroscopically visible emphysema, and may therefore be more important.

This study has involved the identification of a range of normal AWUV values, and the description of microscopically assessed emphysema. The identification of a sub-group of smokers who are susceptible to MAE will be useful in studying the pathogenetic mechanisms responsible for this process.

Most studies of the pathogenesis of emphysema fail to consider the various types of emphysema as separate entities, with possibly different pathogenetic mechanisms. An additional failing of such studies is that smokers are

usually considered to be a homogeneous group. The results presented in Chapter 4 of this thesis and discussed in this Chapter indicate that differences in susceptibility within the smoking population must be recognised before the pathogenesis of the emphysemas can be fully understood.

5.9 IMPORTANT FINDINGS OF THIS STUDY

A new method for assessing airspace wall surface area *per* unit volume of lung tissue (AWUV) was developed and tested in the course of this study. It was found to be reliable, reproducible and efficient.

The results of AWUV measurements in lung specimens from 38 lifelong non-smokers indicated that airspace size increased with advancing age and it was suggested that this increase is normal.

The limits of normal airspace size were established for subjects within the age range 21 - 93 years, allowing the diagnosis of emphysema according to its definitions.

No evidence was found to support the existence of senile emphysema.

Microscopically assessed emphysema (MAE) was suggested as a useful term to describe the condition of the lung when the mean AWUV measurement was below the normal limits.

MAE was found to exist in a minority of smokers, suggesting the existence of a susceptible sub-group of smokers.

The susceptibility to MAE was found to be related to smoking, but neither susceptibility to MAE nor severity of MAE appeared to be dose-related to daily cigarette consumption.

There were no sex differences in the relationship between AWUV and age, either in the non-smokers or in the smokers, and the susceptibility to MAE was not found to be more common in either sex.

The tissue surrounding macroscopic centriacinar emphysematous lesions was found to be normal, and this suggests that the early lesions in centriacinar emphysema are also focal in their distribution.

The 5th and 10th percentile AWUV values were found to be more sensitive indicators of focal macroscopic emphysema than the mean AWUV.

MAE, as described in terms of mean AWUV, was found to be related to macroscopic panacinar emphysema, but was not related to macroscopic centriacinar emphysema.

The results of this study suggest that different pathogenetic mechanisms are responsible for macroscopic panacinar and centriacinar emphysema.

It should be noted that the results reported in this thesis are not intended to represent definitive statements on the incidence of emphysema in the general Scottish population. However, it is hoped that the study sample described here is large enough for the results to make a useful contribution to the study of the epidemiology of microscopic emphysema.

5.10 PUBLICATIONS ARISING FROM THIS STUDY

Published papers

1. Gillooly M, Lamb D, Farrow ASJ. New automated technique for measuring emphysema on histological sections. *J Clin Pathol* 1991;**44:**1007-1011.

2. Gillooly M, Lamb D. Airspace size in non-smokers' lungs: The effects of age and sex. *Thorax* 1992; *in press.*

Papers submitted for publication

1. Gillooly M, Lamb D. Microscopic emphysema in relation to age and smoking habit. Submitted to *Thorax.*

Presentations with published abstracts

1. Gillooly M, Farrow ASJ, Lamb D. New automated technique for the assessment of emphysema on tissue sections. *Thorax* 1990;**45**:326P.

2. Gillooly M, Lamb D. Microscopic emphysema - its distribution within the lung and its relation to age and smoking. *Am Rev Respir Dis* 1991;**143**(Suppl):A670.

3. Gillooly M, Lamb D. Microscopic emphysema in relation to age and smoking. *Thorax* 1991;**46**:301P.

4. Gillooly M, Lamb D. Microscopic emphysema in relation to age and smoking habit. *Am Rev Respir Dis* 1992;**145** (Suppl):A762.

5. Lamb D, Gillooly M, Farrow ASJ. Microscopic emphysema and its variations with age, smoking and site within the lungs. *Ann NY Acad Sci* 1991;**624**:339-341.

Abstracts submitted for presentation

1. Gillooly M, Lamb D. Cigarette smoking and the susceptibility to microscopic emphysema. British Thoracic Society, Winter Meeting, London, December 1992.

2. Gillooly M, Lamb D. Relationship between the centriacinar and microscopic forms of emphysema. British Thoracic Society, Winter Meeting, London, December 1992.

5.11 PROPOSALS FOR FUTURE WORK

The FIP has proved to be a useful technique for measuring AWUV in a large group of lung specimens. In its ability to provide accurate measurements on many individual $1mm^2$ fields it is a valuable system for assessing the distribution of tissue density within the lung, and also for examining patterns of tissue loss due to microscopic tissue destruction. It is hoped that the FIP will now be used to provide more data on AWUV measurements in a larger sample from the Scottish population. In particular, AWUV will be measured in lungs from patients with end-stage obstructive airways disease. Also, a larger number of young adult and elderly smokers and non-smokers will be included to improve the age distribution in the study sample.

Study of the distribution of 5th and 10th percentile AWUV values within the upper and lower lobes of whole lung specimens should provide information on the location of microscopic focal emphysematous lesions. It will be interesting to discover whether the spatial distribution of these focal lesions is similar to the distribution of macroscopic lesions, and this will help to clarify whether in fact the microscopic lesions are necessarily precursors of the macroscopic lesions. In addition, study of the 5th and 10th percentile AWUV values within lungs with macroscopic confluent centriacinar lesions should be performed to discover whether focal microscopic lesions exist in the tissue elsewhere in these lungs.

An important area to consider is the relationship between AWUV and lung function. McLean and colleagues (1992) found that carbon monoxide gas transfer was related to AWUV. However, the study group contained only 2 non-smokers, and the necessary information on the normal limits of AWUV was not available to assess whether the AWUV values in the smoking group were normal or abnormal. It will be useful to re-analyse McLean's data in view of the information presented in this thesis on the normal AWUV range.

Carbon monoxide transfer factor measurements are available for several of the surgical specimens included in this thesis, and FEV_1 measurements are available for all of the surgical specimens, including McLean's study group.

Therefore, it is now possible to assess the relationships between lung structure and function in non-smokers in relation to age, and in smokers with and without MAE.

The alveolar walls attached to the outer aspects of the bronchioles are important in the function of the peripheral airways. Destruction of these peribronchiolar alveolar walls leads to premature bronchiolar collapse during the expiratory phase of ventilation (Linhartova *et al*, 1971; 1973; Petty *et al*, 1986; McLean, 1987). The loss of peribronchiolar alveolar walls can be assessed by measuring the average distance between the alveolar wall attachments to the bronchiolar wall; the inter-alveolar attachment distance, or IAAD (McLean, 1987). A preliminary study by McLean (1987) has shown that increases in IAAD were not directly related to decreasing AWUV measurements. However, as stated above, McLean's analyses using AWUV were performed before the limits of normal AWUV with advancing age were defined. In light of this, IAAD should be measured in a group of non-smokers to establish the relationships, if any, between IAAD and age; and between IAAD and AWUV. When these relationships have been established, IAAD measurements should be made in lungs from smokers with normal mean AWUV values and in smokers with MAE. Using the results of these studies, the following questions should be answered:

Is there a change in IAAD with age?
Is there a relationship between IAAD and AWUV in non-smokers?
Is there any apex to base variation in IAAD measurements within whole lung specimens?
Is alteration in mean IAAD associated with smoking history?
Is alteration in mean IAAD independent of MAE?
Is IAAD related to alterations in the 5th or 10th percentile AWUV value?
Is IAAD related to macroscopic centriacinar or macroscopic panacinar emphysema?
Is there a pattern of attachment loss which is unrelated to other forms of microscopic or macroscopic emphysema?

The results of the present study permit the isolation of specific groups of smokers, and this should be used in studying the pathogenesis of the various types of emphysema.

Macroscopic centriacinar emphysema has been associated with bronchiolar inflammation (Thurlbeck, 1976; Cockcroft & Horne, 1982; Kim *et al*, 1991). A study of the inflammatory cell population within the bronchiolar walls has been planned, and this will involve immunocytochemical techniques, and possibly interactive colour image analysis. The aim of such a study is to investigate the relationship between the numbers of inflammatory cells and microscopic measures of emphysema including mean AWUV, 5th and 10th percentile AWUV values, and IAAD.

It has been suggested that the pathogenesis of panacinar emphysema may be related to a systemic process (Thurlbeck, 1976; Cockcroft & Horne, 1982; Kim *et al*, 1991). Using the information obtained in the present study, it is possible to identify sub-groups of smokers showing susceptibility to the various forms of emphysema. Preliminary work is underway in the Pathology Department to use tissue from smokers' lungs to investigate some of the systemic factors which may be related to susceptibility to MAE and macroscopic panacinar emphysema.

References

Aherne WA, Dunnill MS. *Morphometry*. London: Edward Arnold 1982.

Alli AF. Pulmonary emphysema in Ibadan: a pathological study of unselected necropsy lungs. *Trop Geogr Med* 1972;**24**:28-38.

American Thoracic Society. Report of committee on preparation of human lungs for macroscopic and microscopic emphysema. *Am Rev Respir Dis* 1959;**80**(suppl):114-117.

American Thoracic Society. Chronic bronchitis, asthma and pulmonary emphysema. A statement by the committee on diagnostic standards for nontuberculous respiratory diseases. *Am Rev Respir Dis* 1962;**85**:762-768.

Anderson AE Jr., Azcuy A, Batchelder TL, Foraker AG. Experimental analysis in dogs of the relationship between pulmonary emphysema, alveolitis and hyperinflation. *Thorax* 1964;**19**:420-432.

Anderson AE Jr., Foraker AG. Relative dimensions of bronchioles and parenchymal spaces in lungs from normal subjects and emphysematous patients. *Am J Med* 1962;**32**:218-228.

Anderson AE Jr., Foraker AG. Centrilobular and panlobular emphysema: two different diseases. *Thorax* 1973;**28**:547-550.

Anderson AE Jr., Hernandez JA, Eckert P, Foraker AG. Emphysema in lung macrosections correlated with smoking habits. *Science* 1964;**144**:1025-1026.

Anderson AE Jr., Hernandez JA, Holmes WL, Foraker AG. Pulmonary emphysema: prevalence, severity and anatomical patterns in macrosections with respect to smoking habits. *Arch Environ Health* 1966;**12**:569-577.

Anderson JA, Dunnill MS, Ryder RC. Dependence of the incidence of emphysema on smoking history, age and sex. *Thorax* 1972;**27**:547-551.

Angus GE, Thurlbeck WM. Number of alveoli in the human lung. *J Appl Physiol* 1972;**12**:483-485.

Auerbach O, Garfinkel L, Hammond EC. Relation of smoking and age to findings in lung parenchyma: a microscopic study. *Chest* 1974;**65**:29-35.

Auerbach O, Hammond EC, Garfinkel L, Bananta C. Relation of smoking and age to emphysema. Whole lung section study. *N Engl J Med* 1972;**286**:853-857.

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

Azcuy A, Anderson AE Jr., Foraker AG. The morphological spectrum of aging and emphysematous lungs. *Ann Intern Med* 1962;**57**:1-17.

Bergin C, Muller N, Nichols DM, Lillington G, Hogg JC, Mullen B, Grymaloski MR, Osborne S, Pare PD. The diagnosis of emphysema. A CT-pathological correlation. *Am Rev Respir Dis* 1986;**133**:541-546.

Bignon J, Khoury F, Even P, Andre J, Brouet G. Morphometric study in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1969;**99:**669-695.

Bignon J, Lenfant C, Scarpa GL. Emphysema: past, present and future. *Bull Eur Physiopathol Respir* 1980;**16**(Suppl 4):423-428.

Bucher U, Reid L. Development of the intrasegmental bronchial tree: the pattern of branching and development of cartilage at various stages of intra-uterine life. *Thorax* 1961;**16**:207-218.

Burgess AM, Burgess SB. Chronic obstructive pulmonary emphysema. A review, with special reference to its relation to cigarette smoking. *R I Med J* 1966;**49**:461-465.

Campbell H, Tomkeieff SI. Calculation of the internal surface of a lung. *Nature* 1952;**170**:117.

Ciba. Terminology, definitions and classification of chronic pulmonary emphysema and related conditions. Report of the conclusions of a Ciba Guest Symposium. *Thorax* 1959;**14**:286-299.

Cockcroft DW, Horne SL. Localisation of emphysema within the lung. *Chest* 1982;**82**:483-487.

Cotes JE. *Lung function: assessment and application in medicine.* Fourth Edition. Oxford: Blackwell Scientific Publication, 1979.

Crofton J, Douglas A. *Respiratory Diseases.* Third Edition. Oxford: Blackwell Scientific Publications, 1981:346-389.

Da Cruz F. *Kermit, a file transfer protocol.* Bedford, Mass: Digital Press, 1987.

Dailey ET. Preparation of inflated lung specimens. In: Heitzman ER, ed., *The Lung: Radiologic-pathologic correlations.* Saint Louis: The CV Mosby Company, 1973:4-12.

Davies G, Reid L. Growth of the alveoli and pulmonary arteries in childhood. *Thorax* 1970;**25**:669-681.

Dijkman JH. Morphological aspects, classification and epidemiology of emphysema. *Bull Eur Physiopathol Respir* 1986;**22**:241s-243s.

Drury RAB, Wallington EA. *Carleton's histological technique.* Fifth Edition. Oxford: Oxford University Press, 1980.

Duguid JR, Young A, Cauna D, Lambert MW. The internal surface area of the lung in emphysema. *J Path Bact* 1964;**88**:405-421.

Dunnill MS. Postnatal growth of the lung. *Thorax* 1962a;**17**:329-333.

Dunnill MS. Quantitative methods in the study of pulmonary pathology. *Thorax* 1962b;**17**:320-329.

Dunnill MS. Evaluation of a simple method of sampling the lung for quantitative histological analysis. *Thorax* 1964;**19**:443-448.

Dunnill MS. The recognition and measurement of pulmonary emphysema. *Pathol Microbiol* (Basel) 1970;**35:**138-145.

Dunnill MS. *Pulmonary pathology.* Second edition. Edinburgh, New York: Churchill Livingstone, 1987;97-132.

Eidelman DH, Saetta MP, Ghezzo H, Wang NS, Hoidal JR, King M, Cosio MG. Cellularity of the alveolar walls in smokers and its relation to alveolar destruction. *Am Rev Respir Dis* 1990;**141:**1547-1552.

Eidelman DH, Ghezzo H, Kim WD, Cosio MG. The destructive index and early lung destruction in smokers. *Am Rev Respir Dis* 1991;**144**:156-159.

Emery JL, Wilcock PF. The post-natal development of the lung. *Acta Anat* 1966;**65**:10-29.

Eriksson S. Pulmonary emphysema and alpha-1-antitrypsin deficiency. *Acta Med Scand* 1964;**175**:197-205.

Eriksson S. Emphysema before and after 1963. Historical perspectives. *Ann NY Acad Sci* 1991;**624**:1-6.

Faling LJ. Genetic influences in the development of emphysema in persons with normal serum proteins. *Clin Chest Med* 1983;**4**:377-387.

Fels AOS, Cohn ZA. The alveolar macrophage. *J Appl Physiol* 1986;**60:**353-369.

Flenley DC. Diagnosis and follow-up of emphysema. *Eur Respir J* 1990;**3**(Suppl 9):5s-8s.

Flenley DC. The diagnosis of emphysema. In: Cherniak NS, (ed). *Chronic obstructive pulmonary disease.* Philadelphia: WB Saunders Company, 1991;351-357.

Gadek JE, Pacht ER. The protease-antiprotease balance within the human lung: implications for the pathogenesis of emphysema. *Lung* 1990;**168**(Suppl):552-564.

Garshick E, Schenker MB. Occupation and chronic airflow limitation. In: Hensley MJ, Saunders NA (eds). *Chronic obstructive pulmonary disease.* New York: Marcel Dekker Inc., 1989:227-258.

Glazier JB, Hughes JMB, Maloney JE, Pain MCF, West JB. Decreasing alveolar size from apex to base in the upright lung. *Lancet* 1966;**2**:203-204.

Glazier JB, Hughes JMB, Maloney JE, West JB. Vertical gradient of alveolar size in lungs of dogs frozen intact. *J Appl Physiol* 1967;**23**:694-705.

Gough J. Discussion of the diagnosis of pulmonary emphysema. *Proc Roy Soc Med* 1952;**45**:576-577.

Gough J, Wentworth JE. The use of thin sections of entire organs in morbid anatomical studies. *J Roy Microsc Soc* 1949;**69**:231-235.

Gould GA, MacNee W, McLean A, Warren PM, Redpath A, Best JJK, Lamb D, Flenley DC. CT measurements of lung density in life can quantitate distal airspace enlargement - an essential defining feature of human emphysema. *Am Rev Respir Dis* 1988;**137**:380-392.

Gross P, Babyak MA, Tolker E, Kaschak M. Enzymatically produced emphysema: a preliminary report. *J Occup Med* 1964;**6**:481-484.

Gundersen HJG, Osterby R. Optimising sampling efficiency of stereological studies in biology, or 'Do more less well!'. *J Microsc* 1981;**121**:65-73.

Heard BE. A pathological study of emphysema of the lungs with chronic bronchitis. *Thorax* 1958;**13**:136-149.

Heard BE. Pathology of pulmonary emphysema. Methods of study. *Am Rev Respir Dis* 1960;**82**:792-799.

Heard BE, Izukawa T. Pulmonary emphysema in 50 consecutive male necropsies in London. *J Path Bact* 1964;**88**:423-431.

Heppleston AG, Leopold JG. Chronic pulmonary emphysema: anatomy and pathogenesis. *Am J Med* 1961;**31**:279-291.

Hernandez JA, Anderson AE Jr., Holmes WL, Foraker AG. Macroscopic relations in emphysematous and aging lungs. *Geriatrics* 1966;**21**:155-166.

Higgins M. Risk factors associated with chronic obstructive pulmonary disease. *Ann NY Acad Sci* 1991;**624:**7-17.

Hislop A, Reid L. Development of the acinus in the human lung. *Thorax* 1974;**29**:90-94.

Hunninghake G, Gadek J, Crystal R. Human alveolar macrophage neutrophil chemotactic factor: Stimuli and partial characterisation. *J Clin Invest* 1980;**66**:473-483.

Ishikawa S, Bowden DH, Fisher V, Wyatt JP. The 'emphysema profile' in two midwestern cities in North America. *Arch Environ Health* 1969;**18**:660-666.

Janoff A. Biochemical links between cigarette smoking and pulmonary emphysema. *J Appl Physiol : Respirat Environ Exercise Physiol* 1983;**55**:285-293.

Janoff A. Elastases and emphysema: Current assessment of the protease-antiprotease hypothesis. *Am Rev Respir Dis* 1985;**132**:417-433.

Kiernan JA. *Histological and histochemical methods: theory and practice.* Second edition. Oxford: Pergamon Press, 1990.

Kim WD, Eidelman DH, Izquierdo JL, Ghezzo H, Saetta MP, Cosio MG. Centrilobular and panlobular emphysema in smokers. Two distinct morphological and functional entities. *Am Rev Respir Dis* 1991;**144**:1385-1390.

Kory RC, Runterkus LT, Korthy AL, Cote RA. Quantitative estimation of pulmonary emphysema in lung macrosections by photoelectric measurement of transmitted light. *Am Rev Respir Dis* 1966;**39**:758-768.

Laennec RTH. *A treatise on the diseases of the chest and on mediate auscultation.* Translated by J Forbes. Fourth Edition. London: Longmans 1834.

Lamb D. Chronic obstructive pulmonary disease (COPD) - pathology. In: Brewis RAL, Gibson GJ, Geddes DM (eds), *Respiratory medicine.* London: Balliere Tindall, 1990;497-506.

Lamb D, McLean A, Flenley DC. A new technique for measuring alveolar surface and the assessment of microscopic emphysema. *Thorax* 1986;**41**:716.

Langston C, Waszkiewicz E, Thurlbeck WM. A simple method for the representative sampling of lungs of diverse size. *Thorax* 1979;**34**:527-530.

Laurell CB, Eriksson S. The electrophoretic alpha-1-globulin pattern of serum in alpha-1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963;**15**:132-140.

Leeson CR, Leeson TS, Paparo AA. *Textbook of histology.* Fifth edition. Philadelphia: WB Saunders Company, 1985.

Leopold JG, Gough J. The centrilobular form of hypertrophic emphysema and its relation to chronic bronchitis. *Thorax* 1957;**12**:219-235.

Lichros I, Kim WD, Saetta M, Ghezzo H, Eidelman DH, Cosio MG. Distribution of microscopic emphysema in whole lungs of smokers. *Am Rev Respir Dis* 1991;**143** (Suppl):A536.

Linhartova A, Anderson AE Jr., Foraker AG. Radial traction and bronchiolar obstruction in pulmonary emphysema. *Arch Pathol* 1971;**92**:384-391.

Linhartova A, Anderson AE Jr., Foraker AG. Non-respiratory bronchiolar deformities. Graphic assessment in normal and emphysematous lungs. *Arch Pathol Lab Med* 1973;**95**:45-47.

Linhartova A, Anderson AE Jr., Foraker AG. Further observations on luminal deformity and stenosis of nonrespiratory bronchioles in pulmonary emphysema. *Thorax* 1977;**32**:53-59.

Longfield AN, Hentel W. Lung destruction measured by energy transmission through fume-fixed lungs. *Dis Chest* 1966;**50**:225-231.

MacNee W, Gould G, Lamb D. Quantifying emphysema by CT scanning. Clinicopathologic correlates. *Ann NY Acad Sci* 1991;**624**:179-194.

McCartney AC, Fox B, Partridge TA, MacRae KD, Tetley TD, Phillips GJ, Guz A. Emphysema in the blotchy mouse: a morphometric study. *J Pathol* 1988;**156**:77-81.

McLean A. *Morphometry of human lung with physiological correlations.* PhD Thesis, University of Edinburgh, 1987.

McLean A, Lamb D. Morphometry of small airways in man. *J Pathol* 1983;**141**:520.

McLean A, Lamb D, Gould G, Warren PM, Flenley DC. Morphometric factors associated with airflow limitation in early COAD. *Thorax* 1987;**42**:210.

McLean A, Warren PM, Gillooly M, MacNee W, Lamb D. Microscopic and macroscopic measurements of emphysema: relation to carbon monoxide gas transfer. *Thorax* 1992;**47**:144-149.

McLean KH. The histology of generalised pulmonary emphysema. 1. The genesis of the early centrilobular lesion: focal emphysema. *Aust Ann Med* 1957;**6**:124-140.

Matsuba K, Thurlbeck WM. The number and dimensions of small airways in non-emphysematous lungs. *Am Rev Respir Dis* 1971;**104**:516-524.

Mayhew T. Stereology: progress in quantitative microscopical anatomy. In: Navaratnam V, Harrison RJ. eds. *Progress in Anatomy.* Volume 3. Cambridge: Cambridge University Press 1983:81-103.

Mitchell RS, Silvers GW, Goodman N, Dart G, Maisel JC. Are centrilobular and panlobular emphysema two different diseases? *Hum Pathol* 1970;**1**:433-441.

Murray JF. *The normal lung.* The basis for diagnosis and treatment of pulmonary disease. Second Edition. Philadelphia: WB Saunders Company, 1986.

Nagai A, Yamawaki I, Takizawa T, Thurlbeck WM. Alveolar attachments in emphysema of human lungs. *Am Rev Respir Dis* 1991;**144**:888-891.

Nagai A, Yamawaki I, Thurlbeck WM, Takizawa T. Assessment of lung parenchymal destruction by using routine histologic tissue sections. *Am Rev Respir Dis* 1989;**139**:313-319.

Nakamura T, Takizawa T, Morone T. Anatomic changes in lung parenchyma due to aging process. *Dis Chest* 1967;**52**:518-524.

Petty TL, Ryan SF, Mitchell RS. Cigarette smoking and the lungs. Relation to postmortem evidence of emphysema, chronic bronchitis and black lung pigmentation. *Arch Environ Health* 1967;**14**:172-177.

Petty TL, Silvers GW, Stanford RE. Radial traction and small airways disease in excised human lungs. *Am Rev Respir Dis* 1986;**133**:132-135.

Pratt PC. Emphysema and chronic airways disease. In: Dail DH, Hammer SP (eds), *Pulmonary pathology.* New York: Springer-Verlag, 1988: 651-669.

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

Pratt PC, Klugh GA. Chronic expiratory air flow obstruction: Cause or effect of centrilobular emphysema? *Dis Chest* 1967;**52**:342-349.

Pride NB. Which smokers develop progressive airflow obstruction? *Eur J Respir Dis* 1983;**64** (Suppl 126):79-83.

Pugatch RD. The radiology of emphysema. *Clin Chest Med* 1983;**4**:433-443.

Redline S, Weiss ST. Genetic and perinatal risk factors for the development of chronic obstructive pulmonary disease. In: Hensley MJ, Saunders NA (eds). *Clinical epidemiology of chronic obstructive pulmonary disease.* New York: Marcel Dekker Inc., 1989:139-168.

Reid L. Pathology of chronic bronchitis. *Lancet* 1954;**1**:275-278.

Reid L. The secondary lobule in the adult human lung, with special reference to its appearance in bronchograms. *Thorax* 1958;**13**:110-115.

Reid L. Embryology of the lung. In: De Reuck AVS, Porter R (eds), *Development of the lung: a Ciba Foundation symposium.* London: Churchill Livingstone, 1967a: 109-124.

Reid L. *The pathology of emphysema.* London: Lloyd-Luke Ltd., 1967b.

Reid L, Millard FJC. Correlation between radiological diagnosis and structural lung changes in emphysema. *Clin Radiol* 1964;**15**:307-311.

Rosenblatt MG. Emphysema: Historical perspective. *Bull N Y Acad Med* 1972;**48**:823-841.

Ryan BF, Joiner BL, Ryan TA. *Minitab Handbook.* Second Edition. Boston: Duxbury Press, 1985;218-235.

Ryder R, Dunnill MS, Anderson JA. A quantitative study of bronchial mucous gland volume, emphysema and smoking in a necropsy population. *J Pathol* 1971;**104**:59-71.

Ryder RC, Thurlbeck WM, Gough J. A study of interobserver variation in the assessment of the amount of pulmonary emphysema in paper-mounted whole lung sections. *Am Rev Respir Dis* 1969;**99**:354-364.

Saetta M, Izquierdo JL, Kim WD, Cosio MG. Centrilobular and panacinar emphysema in smokers. Two different diseases. *Am Rev Respir Dis* 1990;**141** (Suppl):A713.

Saetta M, Ghezzo H, Kim WD, King M, Angus GE, Wang NS, Cosio MG. Loss of alveolar attachments in smokers. A morphometric correlate of lung function impairment. *Am Rev Respir Dis* 1985a;**132**:894-900.

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

Saito K, Cagle P, Berend N, Thurlbeck WM. The 'destructive index' in nonemphysematous and emphysematous lungs. *Am Rev Respir Dis* 1989;**139**:308-312.

Schreider JP, Raabe OG. Structure of the human respiratory acinus. *Am J Anat* 1981;**162**:221-232.

Shippey G, Bayley R, Farrow S, Lutz R, Rutovitz D. A fast interval processor (FIP) for cervical prescreening. *Anal Quant Cytol* 1981;**3**:9-16.

Siegel S, Castellan NJ. *Nonparametric statistics for the behavioural sciences.* Second Edition. New York: McGraw-Hill, 1988.

Silverton RE. Gross fixation methods used in the study of pulmonary emphysema. *Thorax* 1965;**20**:289-297.

Sims B. A simple method of preparing 1-2 micron sections of large tissue blocks using glycol methacrylate. *J Microsc* 1974;**101**:223-227.

Smith SF, Guz A, Cooke NT, Winning AJ, Foxall J, Tetley T. Effects of cigarette smoke on antiproteases at the human lung surface. *Eur J Respir Dis* 1986;**69** (Suppl 6):139-143.

Snider GL. A perspective on emphysema. *Clin Chest Med* 1983;**4**:329-336.

Snider GL. Chronic obstructive pulmonary disease: Risk factors, pathophysiology and pathogenesis. *Ann Rev Med* 1989;**40**:411-429.

Snider GL, Kleinerman J, Thurlbeck WM. The definition of emphysema. Report of a National Heart, Lung and Blood Institute, Division of Lung Diseases, Workshop. *Am Rev Respir Dis* 1985;**132**:182-185.

Sobonya RE, Burrows B. The epidemiology of emphysema. *Clin Chest Med* 1983;**4**:351-358.

Spain DM, Siegel H, Bradess VA. Emphysema in apparently healthy adults. Smoking, age and sex. *JAMA* 1973;**224**:322-325.

Spencer H. *Pathology of the lung.* Volume 1. Fourth Edition. Oxford: Pergamon Press, 1985: 557-594.

SPSS Inc. *SPSS-X Users' guide.* Third Edition. Chicago: SPSS Inc., 1988;849-870.

Sutinen S, Lohela P, Paakko P, Lahti R. Accuracy of postmortem radiography of excised air-inflated human lungs in assessment of pulmonary emphysema. *Thorax* 1982;**37**:906-912.

Sutinen S, Vaajalahti P, Paakko P. Prevalence, severity and types of pulmonary emphysema in a population of deaths in a Finnish city. Correlation with age, sex and smoking. *Scand J Respir Dis* 1978;**59**:101-115.

Sweet HC, Wyatt JP, Fritsch AJ, Kinsella PW. Panlobular and centrilobular emphysema: correlation of clinical findings with pathological patterns. *Ann Intern Med* 1961;**55**:565-581.

Thurlbeck WM. The incidence of pulmonary emphysema, with observations on the relative incidence and spatial distribution of various types of emphysema. *Am Rev Respir Dis* 1963a;**87**:206-215.

Thurlbeck WM. Pulmonary emphysema. *Am J Med Sci* 1963b;**246**:332-353.

Thurlbeck WM. The diagnosis of emphysema. *Thorax* 1964;**19:**571-574.

Thurlbeck WM. Measurement of pulmonary emphysema. *Am Rev Respir Dis* 1967a;**95**:752-764.

Thurlbeck WM. The internal surface area of non-emphysematous lungs. *Am Rev Respir Dis* 1967b;**95**:765-773.

Thurlbeck WM. Internal surface area and other measurements in emphysema. *Thorax* 1967c;**22**:483-496.

Thurlbeck WM. Present concepts of the pathology and pathogenesis of pulmonary emphysema. *Pathol Microbiol* 1970;**35**:130-133.

Thurlbeck WM. Postnatal growth and development of the lung. *Am Rev Respir Dis* 1975;**111**:803-844.

Thurlbeck WM. *Chronic airflow obstruction in lung disease.* Philadelphia: WB Saunders Company, 1976.

Thurlbeck WM. Postnatal human lung growth. *Thorax* 1982;**37**:564-571.

Thurlbeck WM. Chronic airflow obstruction. In: Thurlbeck WM, (ed), *Pathology of the lung.* New York: Thieme Medical Publishers Inc., 1988:538-575.

Thurlbeck WM. Pathology of chronic airflow obstruction. *Chest* 1990;**97** (Suppl 2):6s-10s.

Thurlbeck WM. Pathology of chronic airflow obstruction. In: Cherniak N (ed), *Chronic obstructive pulmonary disease.* Philadelphia: WB Saunders Company, 1991:3-20.

Thurlbeck WM, Angus GE. Growth and aging of the normal human lung. *Chest* 1975;**67**(Suppl):3s-6s.

Thurlbeck WM, Dunnill MS, Hartung W, Heard BE, Heppleston AG, Ryder RC. A comparison of three methods of measuring emphysema. *Hum Pathol* 1970a;**1**:215-226.

Thurlbeck WM, Henderson JA, Fraser RG, Bates DV. A comparison between clinical, roentgenologic, functional and morphologic criteria in chronic bronchitis, emphysema, asthma and bronchiectasis. *Medicine* 1970b;**49**:81-145.

Thurlbeck WM, Horowitz I, Siemiatycki J, Dunnill MS, Maisel JC, Pratt P, Ryder R. Intra- and inter-observer variations in the assessment of emphysema. *Arch Environ Health* 1969;**18**:646-659.

Thurlbeck WM, Ryder RC, Sternby N. A comparative study of the severity of emphysema in necropsy populations in three different countries. *Am Rev Respir Dis* 1974;**109**:239-248.

Thurlbeck WM, Simon G. Radiographic appearance of the chest in emphysema. *Am J Roentgenol* 1978;**130**:429-440.

Tucker JH, Shippey G. Basic performance tests on the CERVIFIP linear array prescreener. *Anal Quant Cytol* 1983;**5**:129-137.

Underwood EE. *Quantitative stereology.* Reading, Mass: Addison-Wesley Publishing Company, 1970.

Viegi G, Paoletti P, Vellutini M, Carrozzi L, Di Pede F, Baldacci S, Modena P, Pedreschi M, Di Pede C, Guitini C. Effects of daily cigarette consumption on respiratory symptoms and lung function in a general population sample of North Italian men. *Respiration* 1991;**58**:282-286.

Weibel ER. *Morphometry of the human lung.* Berlin: Springer-Verlag, 1963.

Weissler JC. Pulmonary emphysema: current concepts of pathogenesis. (Southwestern internal medicine conference). *Am J Med Sci* 1987;**293**:125-138.

Wentworth JE. Thin slices of whole organs: The development of a new technique. *Lab J* 1950;**8**:323-328.

Wewers MD, Gadek JE. The protease theory of emphysema. *Ann Int Med* 1987;**107**:761-763.

Wilkinson M. Emphysema in the blotchy mouse: a morphometric study (letter). *J Pathol* 1989;**157**:155-156.

Williams MA. *Quantitative methods in biology.* Amsterdam: North-Holland Publishing Company, 1977:5-85.

Williams PL, Warwick R, Dyson M, Bannister LH. *Gray's Anatomy.* Thirty-seventh Edition. Edinburgh: Churchill Livingstone, 1989.

Wright JL, Wiggs B, Pare PD, Hogg JC. Ranking the severity of emphysema on whole lung slices. Concordance of upper lobe, lower lobe and entire lung ranks. *Am Rev Respir Dis* 1986;**133**:930-931.

Appendix

This appendix contains a summary of specimen details for the study sample, including the sex and age in years of each subject. The specimen description, cause of death for the autopsy specimens and diagnosis of the lesion in the surgical specimens are also listed.

Abbreviations:

f - female

m - male

R - right

L - left

NON-SMOKERS

Case No.	Sex	Age	Specimen Description	**Cause of death** (autopsy specimens)
1	f	79	L lung	Coronary thrombosis
2	f	82	L lung	Coronary thrombosis
3	f	57	L lung	Acute myocardial infarction
4	f	93	L lung	Myocardial infarction
5	f	83	L lung	Congestive cardiac failure
6	f	52	L lung	Myocardial insufficiency
7	f	23	L lung	Cardiac failure
8	f	75	L lung	Cardiac failure
9	f	22	L lung	Myocardial infarction
10	f	84	L lung	Coronary thrombosis
11	f	22	L lung	Cardiac arrest
12	f	82	L lung	Coronary thrombosis
13	f	70	L lung	Cardiac failure
14	f	83	L lung	Coronary occlusion
15	m	77	L lung	Coronary occlusion
16	m	74	L lung	Cardiac failure
17	m	-	L lung	Cerebrovascular accident

NON-SMOKERS (continued)

Case No.	Sex	Age	Specimen Description	**Diagnosis** (surgical specimens)
18	f	69	R upper lobe	Carcinoid tumour
19	f	66	L lower lobe	Cyst
20	f	30	L lower lobe	Cyst
21	f	49	L lower lobe	Malignant fibrous histiocytoma
22	f	48	R lower lobe	Carcinoid tumour
23	f	64	L lower lobe	Squamous carcinoma
24	f	63	L upper lobe	Atypical carcinoid tumour
25	f	49	L upper lobe	Cyst
26	f	50	R lower lobe	Leiomyoma
27	m	23	L upper lobe	Metastatic tumour
28	m	56	R upper lobe	Chondromatous hamartoma
29	m	53	R upper lobe	Bronchiectasis
30	m	52	R upper lobe	Metastatic tumour
31	m	21	R upper lobe	Metastatic tumour
32	m	58	L lower lobe	Squamous carcinoma
33	m	32	R upper lobe	Mixed differentiation carcinoma
34	m	78	R upper lobe	Carcinoid tumour
35	m	31	L lower lobe	Benign teratoma
36	m	56	R upper lobe	Bronchiectasis
37	m	54	R lower lobe	Squamous carcinoma
38	m	28	L lower lobe	Cyst
39	m	23	L lower lobe	Metastatic tumour

SMOKERS

Case No.	Sex	Age	Specimen Description	**Cause of death** (autopsy specimens)
1	f	60	L lung	Acute and chronic haemorrhage
2	f	77	L lung	Ischaemic heart disease
3	f	69	L lung	Pulmonary embolism
4	f	57	L lung	Subarachnoid haemorrhage
5	f	57	L lung	Myocardial insufficiency
6	f	52	L lung	Coronary thrombosis
7	f	41	L lung	Acute cardiac failure
8	f	74	L lung	Coronary thrombosis
9	m	62	L lung	Myocardial insufficiency
10	m	56	L lung	Cardiac failure
11	m	60	L lung	Coronary thrombosis
12	m	80	L lung	Coronary thrombosis
13	m	66	L lung	Coronary occlusion
14	m	76	L lung	Coronary thrombosis
15	m	52	L lung	Cardiac failure
16	m	63	L lung	Coronary occlusion
17	m	81	L lung	Coronary thrombosis
18	m	62	L lung	Coronary occlusion
19	m	63	L lung	Coronary thrombosis
20	m	85	L lung	Shock and haemorrhage
21	m	72	L lung	Chronic obstructive airways disease
22	m	58	L lung	Coronary artery disease
23	m	64	L lung	Cerebrovascular accident

SMOKERS (continued)

Case No.	Sex	Age	Specimen Description	**Cause of death** (autopsy specimens)
24	m	72	L lung	Cardiac failure
25	m	57	L lung	Acute myocardial infarction
				Diagnosis (surgical specimens)
26	f	46	R middle lobe	Adenocarcinoma
27	f	71	R upper lobe	Adenocarcinoma
28	f	56	R upper lobe	Adenocarcinoma
29	f	62	L lower lobe	Papillary adenocarcinoma
30	f	54	L upper lobe	Squamous carcinoma
31	f	59	R upper lobe	Large cell carcinoma
32	f	59	R upper lobe	Adenocarcinoma
33	f	67	R upper lobe	Squamous carcinoma
34	f	68	L upper lobe	Metastatic tumour
35	f	75	L lower lobe	Squamous carcinoma
36	f	52	L lung	Large cell carcinoma
37	f	50	L lower lobe	Follicular bronchiectasis
38	f	65	R upper lobe	Metastatic tumour
39	f	66	R upper lobe	Small cell carcinoma
40	f	49	L upper lobe	Adenocarcinoma
41	f	58	L lung	Carcinoid tumour
42	f	60	L lower lobe	Pulmonary infarct

SMOKERS (continued)

Case No.	Sex	Age	Specimen Description	**Diagnosis** (surgical specimens)
43	f	63	L lower lobe	Adenocarcinoma
44	f	61	L upper lobe	Squamous carcinoma
45	f	63	L upper lobe	Squamous carcinoma
46	f	47	L upper lobe	Wegener's granulomatosis
47	f	68	R upper lobe	Squamous carcinoma
48	m	33	R lower lobe	Adenocarcinoma
49	m	65	R lower lobe	Squamous carcinoma
50	m	54	R upper lobe	Metastatic tumour
51	m	58	R upper lobe	Squamous carcinoma
52	m	66	R lower lobe	Adenocarcinoma
53	m	56	L upper lobe	Adenocarcinoma
54	m	63	R upper lobe	Cyst
55	m	63	R upper lobe	Adenosquamous carcinoma
56	m	70	R upper lobe	Squamous carcinoma
57	m	62	L upper lobe	Squamous carcinoma
58	m	66	L upper lobe	Squamous carcinoma
59	m	57	L upper lobe	Adenocarcinoma
60	m	51	R lower lobe	Adenocarcinoma
61	m	69	L lower lobe	Undifferentiated carcinoma
62	m	63	R upper lobe	Adenosquamous carcinoma
63	m	53	R lower lobe	Squamous carcinoma
64	m	59	R lower lobe	Squamous carcinoma
65	m	58	R lower lobe	Adenocarcinoma

SMOKERS (continued)

Case No.	Sex	Age	Specimen Description	**Diagnosis** (surgical specimens)
66	m	48	L lower lobe	Small scarred area - no tumour
67	m	64	R lower lobe	Adenocarcinoma
68	m	56	R middle lobe	Hamartoma (adenochondroma)
69	m	48	L upper lobe	Old fibrocaseous tuberculosis
70	m	61	R upper lobe	Adenocarcinoma
71	m	74	L upper lobe	Adenocarcinoma
72	m	62	R upper lobe	Squamous carcinoma
73	m	70	R upper lobe	Adenocarcinoma
74	m	68	R lower lobe	Squamous carcinoma
75	m	63	L lower lobe	Squamous carcinoma
76	m	63	L upper lobe	Large cell carcinoma
77	m	61	L upper lobe	Adenocarcinoma
78	m	71	R upper lobe	Squamous carcinoma
79	m	59	L upper lobe	Squamous carcinoma
80	m	66	L upper lobe	Adenosquamous carcinoma
81	m	61	L lower lobe	Poorly differentiated carcinoma
82	m	60	R lower lobe	Small cell carcinoma
83	m	68	L lower lobe	Adenocarcinoma
84	m	58	R upper lobe	Atypical carcinoid tumour
85	m	58	R upper lobe	Poorly differentiated carcinoma
86	m	60	R upper lobe	Pulmonary hamartoma
87	m	53	L lung	Squamous carcinoma
88	m	43	L upper lobe	Squamous carcinoma

SMOKERS (continued)

Case No.	Sex	Age	Specimen Description	**Diagnosis** (surgical specimens)
89	m	56	R lung	Small cell carcinoma
90	m	65	R lung	Squamous carcinoma
91	m	64	L lower lobe	Large cell carcinoma
92	m	47	R lung	Squamous carcinoma
93	m	34	R upper lobe	Squamous carcinoma
94	m	48	R lung	Mixed differentiation carcinoma
95	m	55	L upper lobe	Adenocarcinoma
96	m	62	R lower lobe	Squamous carcinoma
97	m	52	R upper lobe	Fibrocaseous tuberculosis
98	m	33	R upper lobe	Metastatic tumour
99	m	65	R upper lobe	Adenocarcinoma
100	m	82	L lung	Squamous carcinoma
101	m	54	L lower lobe	Metastatic tumour
102	m	60	L lower lobe	Squamous carcinoma
103	m	68	R upper lobe	Squamous carcinoma
104	m	59	R upper lobe	Tuberculosis
105	m	52	L lower lobe	Adenocarcinoma
106	m	58	R upper lobe	Squamous carcinoma
107	m	60	L upper lobe	Squamous carcinoma
108	m	57	R upper lobe	Squamous carcinoma
109	m	55	R upper lobe	Squamous carcinoma
110	m	68	L upper lobe	Malignant neuroendocrine tumour
111	m	66	R lower lobe	Small cell carcinoma

SMOKERS (continued)

Case No.	Sex	Age	Specimen Description	**Diagnosis** (surgical specimens)
112	m	72	L lower lobe	Adenocarcinoma
113	m	62	L lower lobe	Squamous carcinoma
114	m	63	L upper lobe	Adenocarcinoma
115	m	77	R upper lobe	Squamous carcinoma
116	m	52	R upper lobe	Squamous carcinoma
117	m	60	R upper lobe	Undifferentiated carcinoma
118	m	63	L upper lobe	Squamous carcinoma
119	m	63	R lower lobe	Squamous carcinoma
120	m	64	L upper lobe	Small cell carcinoma
121	m	72	L lower lobe	Squamous carcinoma
122	m	62	R lower lobe	Small cell carcinoma
123	m	72	R upper lobe	Squamous carcinoma
124	m	63	L lower lobe	Small cell carcinoma
125	m	69	R upper lobe	Adenocarcinoma

SMOKING HISTORY UNKNOWN

Case No.	Sex	Age	Specimen Description	**Cause of death**
1	f	83	L lung	Intra-abdominal non-Hodgkin's lymphoma

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New automated technique for assessing emphysema on histological sections

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Abstract

The assessment of emphysema in human lungs has traditionally been based on observations made on whole lung slices. These methods are inappropriate for the study of early emphysema, because as much as 75% of the alveolar wall surface area may have been lost by the time airspaces are visible to the naked eye. A new, automated image analysis system, the Fast Interval Processor (FIP), was used to measure airspace wall surface area per unit volume of lung tissue (AWUV). AWUV was measured on histological sections of lung tissue and expressed in mm^2/mm^3. The study sample consisted of resection specimens from 40 patients (32 men and 8 women whose ages ranged from 23-74 years). Histological sections from the inflated specimens were scanned using the FIP, and a mean AWUV value was calculated for each. The intra- and interobserver reproducibility of this method of measuring AWUV were examined. The results obtained using the FIP were also compared with those from an established image analysis system.

The FIP is a fast, efficient technique which gave highly reproducible results comparable with those obtained with an established and much more time consuming measuring technique.

Emphysema is defined as "a condition of the lung characterised by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis".[1]

Emphysema has traditionally been assessed by examining whole lung slices for the presence of macroscopically recognisable emphysema.[2-7] By the time emphysematous spaces are visible to the naked eye, however, as much as 75% of the alveolar surface area may have been lost.[8] Macroscopic assessments are therefore inappropriate for the study of early emphysema.

Various attempts have been made to assess the extent of early emphysema on histological sections, but the methods used have tended to be subjective and non-quantitative.[9-11]

The mean linear intercept (Lm) has become the standard technique for measuring alveolar surface area on tissue sections, an approach intended to reflect the loss of respiratory tissue due to emphysema.[2 5 12-19] As Nagai *et al* reported, however,[11] in addition to being labour intensive, this is also a time consuming technique, taking an average of 45 minutes for one section.

Semiautomatic and automatic image analysis systems take much of the effort out of microscopic measurements of emphysema. These systems give accurate measurements on individual fields. The sampling procedure, however, is limited by the time taken to complete each measurement, usually around five minutes for a single histological field.[20 21]

Methods

THE TECHNIQUE

The Fast Interval Processor (FIP) (fig 1) is a rapid scanning device which was developed by staff at the Medical Research Council Human Genetics Unit in Edinburgh. It is a prototype version of a scanner which is now commercially available as the "Cytoscan" (Image Recognition Systems, Warrington, Cheshire, England). The machine was originally designed as a prescreening device for cervical cytology specimens[22 23] and has been adapted for use with lung tissue. The FIP uses the same approach as the mean linear intercept (Lm) technique, whereby the number of intercepts of tissue with a test line is counted, and this figure is used to calculate the average distance between intercepts. A value for tissue surface area can be derived from Lm.[24]

The FIP consists of a computer-linked Nikon inverted microscope equipped with a motorised stage and a Fairchild CCD linear image sensor. The sensor consists of a stationary array of photosensitive units which recognise the optical density pattern of the specimen. Each histological section is scanned electronically in the y-axis by the sensor at 10 μm intervals. Sections are scanned mechanically in the x-axis by moving the stage in 1 μm steps. The scanning rate is 2000 1 μm steps per second, so the stage moves continuously.

In total, an area of 121mm^2 is scanned on each histological section. As the stage is moved the section passes, and is scanned by, the stationary linear image sensor. The image obtained from the scan consists of a grid of picture elements or "pixels". A user-defined threshold limit determines which pixels are recognised as tissue pixels and which are background pixels. A size filter ensures that groups of thresholded pixels less than 6 μm in diameter are ignored. This gets rid of most of the cells, debris, and background "noise" which may be thresholded. Contiguous groups of thresh-

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Figure 1 The Fast Interval Processor (FIP).

olded pixels in one scan line are treated together as "intervals". Intercepts with the electronic "test-line" and the start and end of each interval—that is, two intercepts for each interval—are recorded (fig 2).

For ease of calculation, intercept totals from each 1 mm^2 field (unit area) are stored by the computer. Each electronic scan creates a "test-line" 1 mm in length for each field, and as fields are scanned at 10 μm intervals the total test-line length is 100 mm for each field (100 electronic scans 1 mm in length in each field). The mean linear intercept can thus be calculated:

Lm = total test-line length/total number of intercepts

As two intercepts have been counted for each airspace wall (interval) the formula for surface area is:

$$SA = 2V/Lm$$ [24]

Airspace wall surface area per unit volume (AWUV) is expressed in mm^2/mm^3 ($V = 1 mm^3$). The formula thus becomes:

$$AWUV = 2/Lm\ (mm^2/mm^3)$$

Histological sections from 40 lungs were scanned using the FIP. The central coordinates of these sections were recorded using an England Finder Graticule (Graticules Ltd, Tonbridge, Kent, England) so that each 1 mm^2 field could be relocated.

SAMPLING TECHNIQUE

Lungs or lobes were obtained from 40 surgica resections for peripheral tumours. Of these, 3 patients were male and eight were female, wit ages ranging from 23 to 74 years. The resecte lung or lobe (hereafter referred to as lobe, as i most cases the complete lung was not availabl for study) was immediately inflated with formo saline at 25 cm H_2O and fixed in formol salin for 24 hours, and then cut into 1 cm thic parasaggital slices. The lateral two slices wer overlaid by a grid of 2 cm × 2 cm squares on transparent sheet, and six 2 cm × 2 cm block were cut from each slice, using a table o random numbers to provide the coordinates o each block (fig 3). The blocks were then embed ded in glycol methacrylate before being cut int 3 μm thick sections and stained with haema toxylin and eosin.

TISSUE PROCESSING

The sections used in this study were initiall prepared as part of a study of the dimensions o small airways. It was therefore important t ensure that the distortion of the tissue due t processing was kept to a minimum. For thi reason, glycol methacrylate (GMA) was used a the embedding medium, as it had been show to produce negligible shrinkage or compres sion.[25] As this processing method is extremel time consuming, however, (only two cases ar processed in a week), and because small airwa morphology was not a concern of this study, th possibility of using paraffin wax embedde tissue was examined.

Tissue sections from 12 of the 40 cases wer available for studying the differences in result produced by embedding in paraffin wax o GMA. Each section was scanned routinel using the FIP, and two mean AWUV value calculated for each case—a GMA mean AWU and a paraffin wax mean AWUV. The result from the paraffin wax sections were correcte for shrinkage as follows. The tissue block are was measured before processing and the area o the cut section measured after processing. Th ratio of these measurements was used to com pute the area shrinkage, and the square root o this gave the linear shrinkage. The reciprocal o this figure was used as the correction factor. A AWUV results from the paraffin wax embedde blocks were multiplied by the correction facto

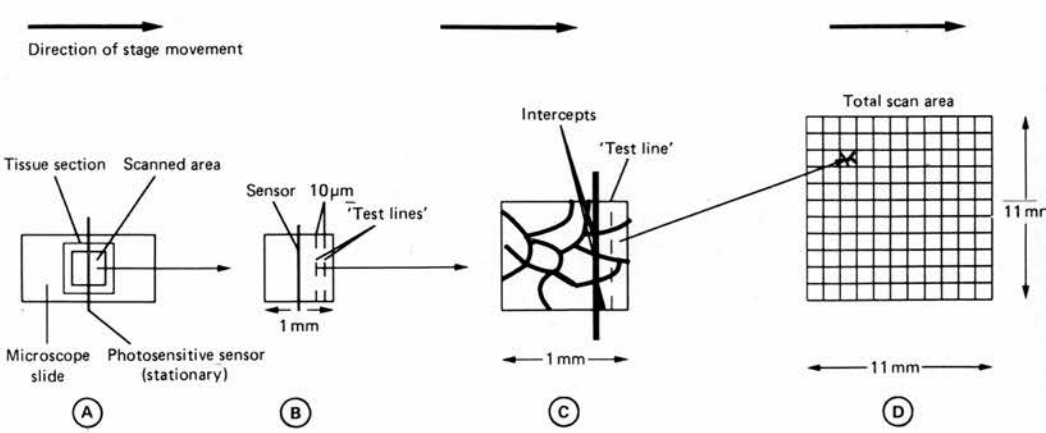

Figure 2 A diagrammatic representation of an FIP scan. (A) Sections are scanned mechanically in the x-axis using the motorised stage and electronically in the y-axis with the stationary linear image sensor. (B) The sensor scans the section at 10 μm intervals, each electronic scan producing a test-line 1 mm in length. (C) Intercepts with the test-line and tissue borders are recorded. (D) A total of 121 1 mm^2 fields are scanned on each tissue section. The arrows indicate the direction of the mechanical scan.

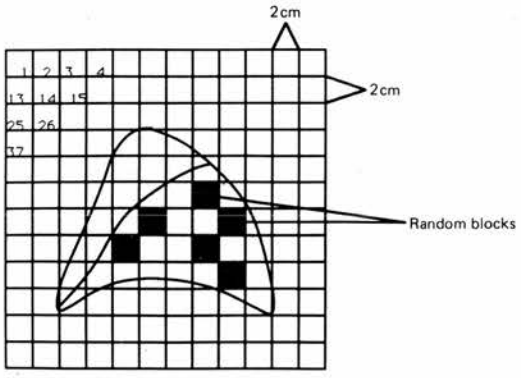

Figure 3 The method of random sampling of blocks from the lung slices. A 2 cm × 2 cm square grid was overlaid on the lateral two parasagittal slices cut from the fixed inflated specimen after resection. A table of random numbers was used to give the co-ordinates of the six blocks to be taken from each slice.

prior to comparison with the AWUVs from the GMA blocks. The correlation between the results using these two embedding media was then assessed.

COMPARISON WITH AN ESTABLISHED METHOD

To test whether the AWUVs obtained using the FIP were comparable with the results of previous studies, the mean AWUV values of the 40 lobes were compared with the results for the same lobes using the IBAS2 semiautomatic image analysis system (Kontron Elektronik Ltd, Watford, Hertfordshire, England). This system has been used to measure AWUV in the past, and has been shown to give highly accurate and reproducible results.[21 26 27]

INTRAOBSERVER REPRODUCIBILITY

Histological sections from 10 lobes were selected at random from the sample. These were scanned a second time, and the mean AWUV for each lobe compared with the mean AWUV from the first scan.

INTEROBSERVER REPRODUCIBILITY

Ten lobes were chosen at random from the total of 40. These were scanned by a second observer, and the mean AWUV values obtained were compared with the results of the first observer. (The 10 lobes used for this comparison were not necessarily the same lobes which were used in the intraobserver reproducibility trial).

All statistical tests were carried out using the Minitab package.

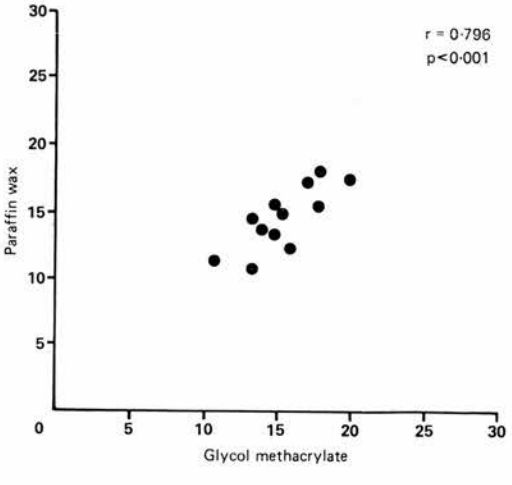

Figure 4 Mean AWUV measured on paraffin wax embedded blocks plotted against mean AWUV measured on glycol methacrylate blocks from the same lungs. The correlation coefficient for these points is also shown.

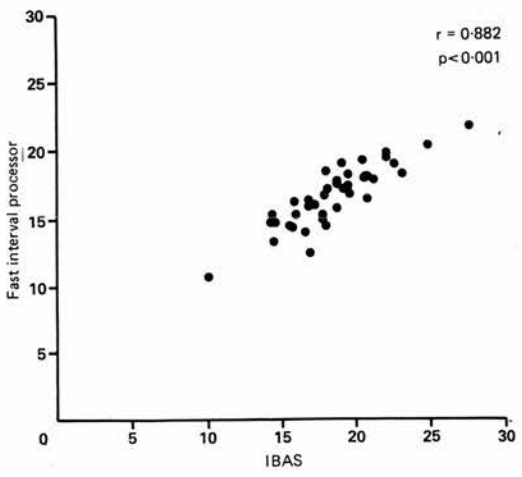

Figure 5 The mean AWUV from each lobe from the FIP is plotted against the mean AWUV obtained using the IBAS2. The correlation coefficient for these points is also shown.

Results

There was a strong correlation between the mean AWUVs from the paraffin wax and GMA embedded blocks ($r = 0{\cdot}796$; $p < 0{\cdot}001$) (fig 4).

There was a good correlation between the FIP results and the IBAS2 results ($r = 0{\cdot}882$; $p < 0{\cdot}001$). The mean AWUV values obtained by the two methods are plotted in fig 5.

A high degree of correlation was found between the results of the first and second scans by the same observer ($r = 0{\cdot}995$; $p < 0{\cdot}001$) and between the results of the FIP scans by two different observers ($r = 0{\cdot}984$; $p < 0{\cdot}001$).

Discussion

The FIP gave comparable results using paraffin wax and GMA embedded tissue taken from the same cases when paraffin wax results were corrected for shrinkage. This result indicates that it is feasible to use paraffin wax embedded sections to measure the mean AWUV for a lung. Therefore, if the time available for tissue processing is limited, and if the measurement required is a single AWUV value for a lung, then tissue sections processed in paraffin wax are of adequate quality for use with the FIP, provided that shrinkage of the block is taken into consideration in the calculation of AWUV.

A high degree of correlation was found between the AWUV results from the FIP and those from the IBAS2, but the results from the two systems were not identical for two major reasons.

First, the two systems work on fundamentally different principles. IBAS2 results are based on very accurate measurements from small areas of lung. FIP results are based on morphometric estimates from wider areas of lung. Such estimates are likely to be more representative of the overall state of the lung, according to the principle of "do more less well", because the precision of an estimate is affected more by the number of sample images measured than by the precision with which the single image is measured.[28]

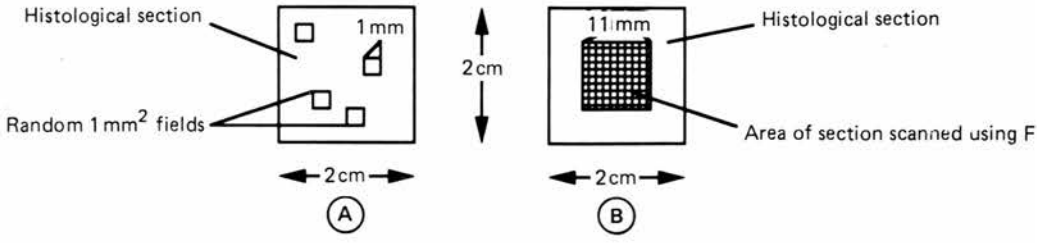

Figure 6 A diagrammatic representation of the differences in sampling technique between the IBAS2 and the FIP. (A) Using the IBAS2, three or four single 1 mm² fields are selected at random from each section. (B) Using the FIP, 121 1 mm² fields are scanned on each section.

Second, an interactive editing function is available on the IBAS2, while the FIP is fully automated. This means that any non-parenchymatous tissue which is thresholded will be measured using the FIP, but not using the IBAS2. In general, errors caused in this way do not account for a large part of the FIP result, because the sample size is so large that positive errors caused by, for example, macrophages in the alveolar spaces (if they are too large to be excluded by the size filter), will be cancelled out by negative errors, such as lumina of bronchioles or arterioles. Areas of non-parenchyma which are likely to affect the measurement to a large extent can be excluded from the analysis by editing the results as described below.

Although there were differences between the methods, the correlation between the results was considered close enough for FIP AWUVs to be acceptable as representative of the alveolar surface area of the histological sections measured. For comparisons with previous studies FIP results can be converted to IBAS2 equivalents using the regression equation for the line which best fits the points in fig 5. A similar conversion may also be required if the FIP results are to be compared with surface area measurements using other image analysis systems.

The FIP produced negligible intra- and interobserver variation. The reproducibility is largely due to the high degree of automation of the system. This has its advantages and disadvantages. While it ensures the objectivity of the measurements, it also means that it is impossible to edit the image presented to the system. Because of this, sections from oedematous lungs or sections of poor quality are unsuitable for FIP analysis. This is especially a problem when dealing with lungs obtained at necropsy, where fluid and cellular infiltrate are often found in the alveoli. A degree of editing is possible, however, whereby intercept totals from fields which contain a large amount of cells or debris may be excluded from the results. This is done by identifying individual fields using their England-Finder coordinates, locating the results from these fields on the computer, and deleting them from the results file. Alternatively, sections which contain a large proportion of non-parenchyma may be excluded from the scan altogether.

It is feasible to edit out intercept totals from the results in this way because the area scanned in each section is so large. An area of 121 mm² was scanned on each section in this study, leading to at least 1000 1 mm² fields for each lobe, as opposed to an average of 30 fields using the IBAS2 (fig 6). The scanning of such a large area on each section is one of the majo advantages of the FIP. It is possible to rela the AWUV values for each individual field the histological section and study the AWU patterns, and hence the patterns of microscop emphysema within a lobe or lung. (It should b noted that the size of the FIP scan can b altered according to the requirements of th operator. The area of 121 mm² was adequa for the purpose of this study).

The other major advantage of the FIP is i speed. Scanning at a rate of 2 mm² per secon 12 histological sections can be scanned i around 30 minutes, and much of this time involved in positioning the microscope slid and setting threshold limits (the manual input It is therefore possible to assess emphysema i a much larger number of cases than would hav been feasible using a slower method.

We conclude that the FIP is a rapid scannin system which can be used to assess alveolar wa surface area in histological sections. It is a use friendly device giving highly reproducib results which are comparable with thos produced using an established image analys system. The combination of its speed an the large number of fields analysed make th FIP a valuable method for assessing earl emphysema in groups of subjects, and fo investigating the patterns of early emphysem within the lung itself.

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1 Report of the National Heart, Lung and Blood Institute. Th definition of emphysema. *Am Rev Respir Dis* 198 **132**:182-5.
2 Dunnill MS. Quantitative methods in the study of pulmo ary pathology. *Thorax* 1962;**17**:320-8.
3 Dunnill MS. The recognition and measurement of pulmo ary emphysema. *Pathol Microbiol (Basel)* 1970;**35**:13 45.
4 Ryder RC, Thurlbeck WM, Gough J. A study of inte observer variation in the assessment of the amount pulmonary emphysema in paper-mounted whole lu sections. *Am Rev Respir Dis* 1969;**99**:354-64.
5 Thurlbeck WM. Measurement of pulmonary emphysem *Am Rev Respir Dis* 1967(a);**95**:752-64.
6 Thurlbeck WM, Anderson AE, Janis M, *et al.* A c operative study of certain measurements of emphysem *Am Rev Respir Dis* 1968;**98**:217-28.
7 Thurlbeck WM, Dunnill MS, Hartung W, Heard B Heppleston AG, Ryder RC. A comparison of thr methods of measuring emphysema. *Human Pathol* 197 1:215-26.
8 Lamb D. Chronic obstructive pulmonary disease (COPD) Pathology. In: Brewis RAL, Gibson GJ, Geddes DM eds. *Respiratory Medicine*. London: Balliere Tinda 1990:497-506.
9 Saetta M, Shiner RJ, Angus GE, *et al.* Destructive Index: measurement of lung parenchymal destruction in smo ers. *Am Rev Respir Dis* 1985;**131**:764-9.
10 Saito K, Cagle P, Berend N, Thurlbeck WM. T "Destructive Index" in nonemphysematous and emph sematous lungs. *Am Rev Respir Dis* 1989;**139**:308-12.
11 Nagai A, Yamawaki I, Thurlbeck WM, Takizawa T. Asses ment of lung parenchymal destruction by using routi histologic tissue sections. *Am Rev Respir Dis* 1989;13 313-19.

12 Bignon J, Khoury F, Even P, Andre J, Brouet G. Morphometric study in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1969;**99**:669-95.
13 Depierre A, Bignon J, Lebeau A, Brouet G. A quantitative study of parenchyma and small conductive airways in chronic nonspecific lung disease. *Chest* 1972;**62**:699-708.
14 Duguid JR, Young A, Cauna D, Lambert MW. The internal surface area of the lung in emphysema. *J Clin Pathol* 1964;**88**:405-21.
15 Dunnill MS. Evaluation of a simple method of sampling the lung for quantitative histological analysis. *Thorax* 1964:**19**:443-8.
16 Hansen JE, Ampaya EP. Human air space shapes, sizes, areas and volumes. *J Appl Physiol* 1975;**38**:990-5.
17 Hasleton PS. The internal surface area of the adult human lung. *J Anat* 1972;**112**:391-400.
18 Thurlbeck WM. The internal surface area of non-emphysematous lungs. *Am Rev Respir Dis* 1967(b);**95**: 765-73.
19 Thurlbeck WM. Internal surface area and other measurements in emphysema. *Thorax* 1967(c);**22**:483-96.
20 McCartney AC, Fox B, Partridge TA, *et al*. Emphysema in the blotchy mouse: a morphometric study. *J Pathol* 1988;**156**:77-81.
21 Gould GA, MacNee W, McLean A, *et al*. CT measurements of lung density in life can quantitate distal airspace enlargement—an essential defining feature of human emphysema. *Am Rev Respir Dis* 1988;**137**:380-92.
22 Shippey G, Bayley R, Farrow S, Lutz R, Rutovitz D. A fast interval processor (FIP) for cervical prescreening. *Anal Quant Cytol* 1981;**3**:9-16.
23 Tucker JH, Shippey G. Basic performance tests on the CERVIFIP linear array prescreener. *Anal Quant Cytol* 1983;**5**:129-37.
24 Aherne WA, Dunnill MS. *Morphometry*. London: Edward Arnold, 1982:46-59.
25 McLean A, Lamb D. Morphometry of small airways in man. *J Pathol* 1983;**141**:520.
26 Lamb D, McLean A Flenley DC. A new technique for measuring alveolar surface area and the assessment of microscopic emphysema. *Thorax* 1986;**41**:716.
27 McLean A, Lamb D, Gould G, Warren P, Flenley DC. Morphometric factors associated with airflow limitation in early COAD. *Thorax* 1987;**42**:210.
28 Gunderson HJG, Osterby R. Optimising sampling efficiency of stereological studies in biology. *J Microsc* 1981;**121**:65-73.