The Radiolabelling Of Salbutamol With Technetium-99m And Its Applications To The Study Of Salbutamol Deposition In The Human Respiratory Tract.

by

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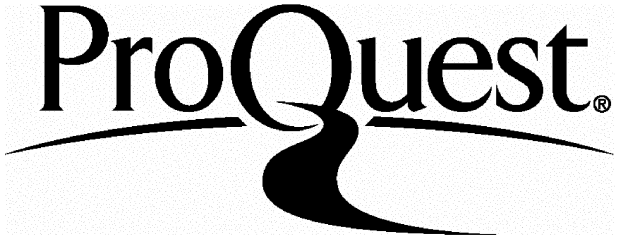
Thesis submitted for the partial
fulfilment of the degree of
Doctor of Philosophy in the
University of London

Department of Medical Physics and Bio-Engineering

March, 1994



Abstract

Radionuclide imaging has the potential to provide a non-invasive and accurate means of obtaining information about the location and movement of an *in-vivo* distribution of labelled drug. Its application to inhaled drugs used for the treatment of diseases of the airways has been limited due to the difficulty in attaching suitable radionuclides. Most information has been obtained by using less accurate indirect methods, which require an inert substitute to be labelled instead, or by measuring concentrations of drug and metabolite in mouth washings, blood and urine.

In 1988 a method was described that permits the direct radiolabelling of $beta_2$-agonists in pressurised metered-dose inhalers (MDI) with the gamma-emitting radionuclide technetium-99m (Köhler *et al*, 1988). However, this method has some criticisms and is not applicable to dry powder inhalers (DPIs).

A new method, based on that of Köhler, has been developed and reported in this thesis, which enables the $beta_2$-agonist salbutamol to be directly radiolabelled with technetium-99m in both MDIs and DPIs. The technique was validated using an Andersen cascade impactor and used to study ten normal volunteers and nineteen patients with asthma. On separate days, subjects inhaled 200 μg of salbutamol from a DPI, an MDI and an MDI via a spacer. The drug was administered under conditions as close as possible to those in which subjects would normally use their inhalers. Inspiratory flow profiles were measured while subjects inhaled from the MDI and DPI.

The proportions of dose depositing in the lungs, throat and stomach were quantified using a dual headed gamma camera. Bronchodilator response was determined by measuring lung function parameters before and after administration. Higher lung drug deposition values were measured than had been reported using indirect labelling techniques. The mean (sd) lung deposition for the normals and asthmatics respectively were 12.4 (3.5)% and 11.4 (5.0)% for the DPI, 21.6 (8.9)% and 18.2 (7.8)% for the MDI and 20.9 (7.8)% and 18.9 (9.1)% for the MDI with spacer.

Acknowledgements

I wish to express my gratitude to Dr M. D. Short for his continuous support and encouragement throughout my period of study in the Department of Medical Physics and Bio-Engineering.

I would also like to thank Mr A. J. Taylor and Dr R. J. Marriott of Glaxo Group Research Ltd for their technical guidance and support.

I am also grateful to Dr S.G. Spiro of the Department of Thoracic Medicine at University College Hospital and I am especially grateful to Dr R Melchor for her assistance during the clinical studies.

Additionally, I would like to express my extreme thanks to my wife Maureen for her support and encouragement throughout and to whom this thesis is dedicated.

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*** Rotahaler, Volumatic, Diskhaler, Ventodisk, Becodisk, Becotide, Becloforte and Ventolin are trademarks of the Glaxo Group of Companies.**

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Chapter 1. Inhaled Drugs In The Lungs

1.1 Introduction

Inhalation is one of the most important drug delivery methods. Therapeutic aerosols are extensively used in the treatment of respiratory disorders and are available to deliver a wide range of drugs. The inhaled route provides a means of delivering the drug directly to the site of action in the lungs where it is effective in small doses. In contrast, other routes of delivery such as the oral route and the intravenous route require the initial uptake of the drug by the systemic circulation with the likely occurrence of side effects. In addition, the onset of action of drugs administered by these routes is usually slower than that obtained by the inhaled route.

Inhaled bronchodilator drugs, delivered from pocket-sized metered dose inhalers (MDI) and dry powder inhalers (DPI), bring rapid relief from the symptoms of severe breathlessness associated with asthma. $Beta_2$-agonists such as salbutamol are a potent and selective sub-group of bronchodilator drugs which are widely prescribed to asthma sufferers. Despite the effectiveness of inhaled drugs only a small portion of the delivered dose reaches the lungs, as the upper respiratory tract acts as an efficient filter to incoming aerosolised drug particles. The largest proportion of the dose is impacted in the throat and swallowed. It enters the digestive system where it is far less effective and may be of no therapeutic value.

In order to improve delivery methods and maximise the therapeutic value of an administered dose of drug it is important to be able to measure accurately its distribution pattern in the respiratory tract. Initial estimates of lung deposition were made by balancing drug, labelled with the radionuclide tritium (^3H), with its metabolites in mouth washings, blood and urine. (Walker *et al*, 1972a; Davies, 1975). Later methods have used radionuclide imaging techniques to visualise and measure the distribution patterns of drugs inhaled into the lungs. Gamma-emitting radionuclides are the most suitable for this purpose since gamma-rays have high tissue penetrating properties and deliver relatively low radiation doses to the patient. Several radionuclides which emit sufficiently energetic gamma-rays to be detected externally and which have no harmful emissions of alpha or beta radiations are readily available. They are used to provide clinical information about the distribution of a wide range of pharmaceutical.

Until recently, however, most of the information about the distribution of inhaled drugs has been obtained from indirect labelling techniques. This is because of the difficulty in attaching gamma-emitting radionuclides to these type of drugs. Instead, an inert substance such as teflon or polystyrene with particle size characteristics similar to the drug in question is used as a substitute for the drug itself (Newman *et al*, 1981a). Alternatively, it can be mixed in with the drug in reconstituted metered dose inhalers (Zainudin *et al*, 1989). The latter technique has the advantage of allowing bronchodilator responses to be measured at the same time as the distribution pattern of the radiolabelled particles.

Use of inert particles provides some information about the site of aerosol deposition in the lungs. However the physical and chemical properties of these particles are likely to be different to those of the active drug. It is therefore difficult to draw conclusions regarding the intrapulmonary distribution of inhaled drugs using these techniques. Recently, a method has been reported for the radiolabelling of $beta_2$-agonists with the radionuclide technetium-99m in metered-dose inhalers without the requirement of substitute particles (Köhler *et al*, 1988).

Besides radiolabelling techniques other experimental designs are currently being used to provide information about the proportion of $beta_2$-agonists delivered to the lungs by inhaler (Borgström and Nilsson, 1990; Hindle and Chrystyn, 1992). These techniques rely on being able to discriminate, in the excreted urine of subjects, the small proportion of drug originally deposited in the lung from the larger swallowed proportion.

In this introductory chapter a review is made of previously published methods including that of Köhler which was used as a basis for the development of the radiolabelling technique employed in this work. The method was validated and applied to the study of the lung deposition of radiolabelled drug in normal volunteers and asthmatic patients. First, the properties of aerosols and their modes of deposition in the human airways are considered, followed by a review of therapeutic inhalers particularly with regard to their use in the treatment of asthma and other related diseases. The properties of salbutamol as a treatment for asthma are considered in detail with reference to relevant publications. Finally the objectives of the work reported in this thesis are outlined.

1.2 Historical Review

The technology of modern drug delivery methods to the human lung dates back only about sixty-four years, when adrenaline was first given by aerosol using a hand held squeeze-bulb nebulizer (Alexander, 1929). However the concept of inhalation therapy has been in existence for thousands of years. Records of inhalation therapy can be found in the writings of ancient cultures in China, India, Greece, Rome and the Middle East (Newman & Clarke, 1992). Most therapies involved the inhalation of either hot aromatic vapours or of smokes derived from burning various types of plant leaf. Hippocrates inhaled the vapours of sulphur and arsenic from a clay pot (Miller, 1973). Some species of tobacco plant were smoked in the sixteenth century as a remedy for lung diseases!

Asthma, in particular, has attracted a great deal of attention over the centuries and many "remedies" for this disease have been devised (Ellul-Micallef, 1976). However, it was not until the mechanisms involved in asthma began to be understood in the 19th century that an effective treatment could be developed. The improvement of drugs for the treatment of respiratory disorders has accelerated in the last sixty years and the benefits of inhalation treatment have been confirmed. Methods of drug delivery have also come a long way during this period. Probably the most significant development in inhalation technology was that of the pressurized MDI in 1956 (Freedman, 1956). Since then the range of drugs available for delivery by MDI has greatly increased and new devices have been introduced.

1.3 The Human Respiratory Tract

The respiratory tract can be divided into the upper airways comprising the nasal cavity, the pharynx and the larynx and the lower airways including the trachea and the lungs (Crofton and Douglas' Respiratory Diseases, 1989). Together they form an intricate system of airways with the primary function of conducting oxygen from the atmosphere to the blood stream to meet the body's metabolic requirements, and to remove carbon dioxide generated by the same metabolic processes (Figure 1.1).

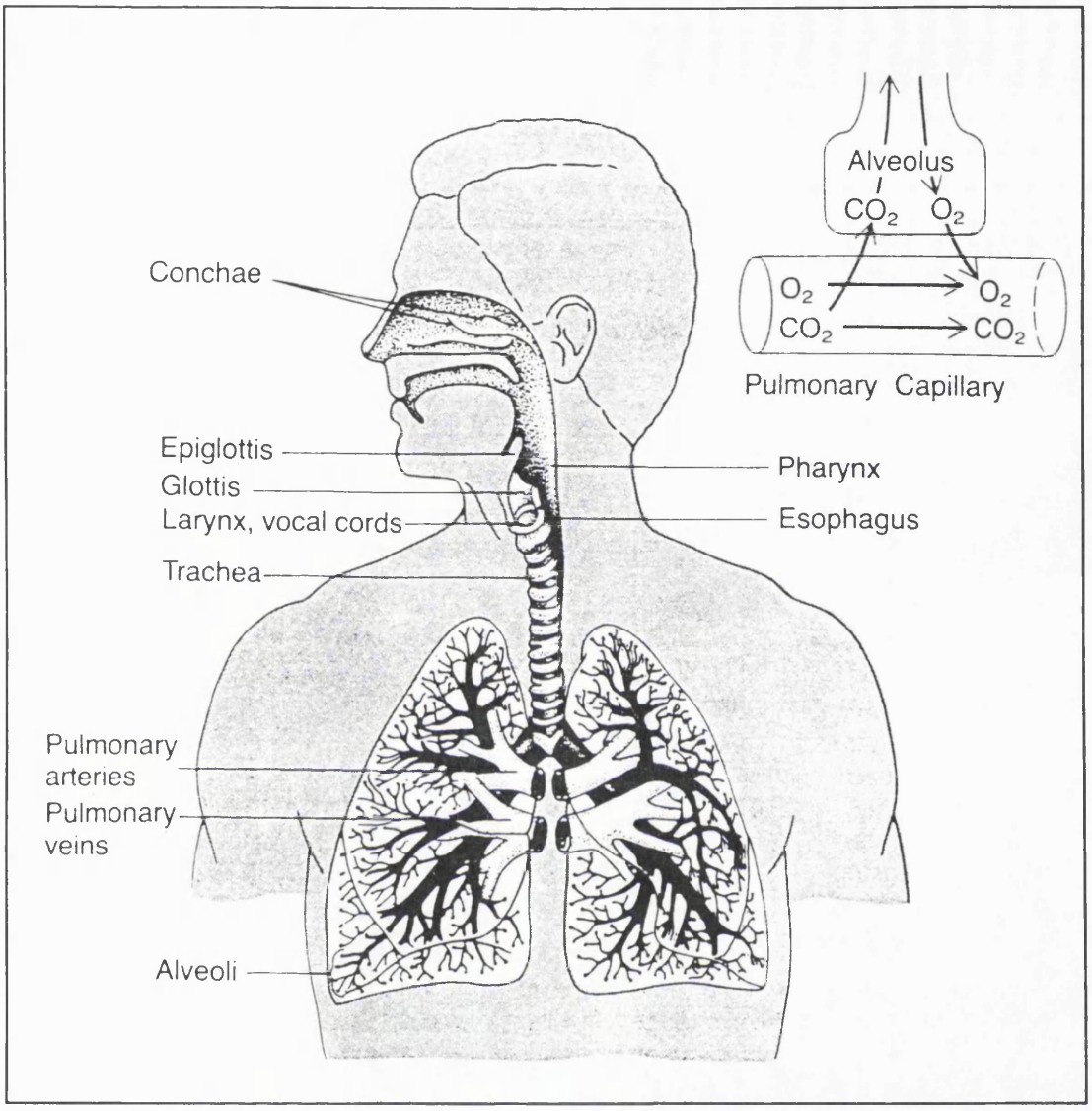


Figure 1.1 ***The Respiratory System Showing the major channels for air and blood. (From Guyton Textbook of Medical Physiology, WB Saunders company. Reproduced with permission.)***

As well as conducting air, the upper airways take part in swallowing, warming and humidifying the air, smell and speech. They also act as a filter for removing many inhaled particles, such as dust, pollens, bacteria, and fungi, which impact in the nose and oropharynx.

The lower end of the trachea divides into the right and left main bronchi. This is the first of a series of divisions (generations) that produces an inverted tree-like arrangement of successively smaller bronchi. The trachea and bronchi are kept open by transverse rings of cartilage, spaced at intervals along the length of the air passages. The narrowest bronchi occur after eight to thirteen generations, and are about 1-3 mm in diameter (Hidy, 1984). They lead to even finer passages called bronchioles, which differ from the bronchi in that they have no cartilage. Branching continues for another ten to fifteen generations, ending in terminal bronchioles approximately 0.6 mm in diameter. The terminal bronchioles divide into three or more respiratory bronchioles which in turn branch into several passages called alveolar ducts, whose walls contain five or six alveolar sacs formed by groups of alveoli. Oxygen, from inhaled air, diffuses across the thin membrane of the alveoli to the pulmonary capillaries, and carbon dioxide passes in the opposite direction. Although each alveolus is very small they are present in such large numbers that efficient gas transfer in respiration is ensured.

The lungs are situated in the thoracic cavity and take up the shape of the available space. They are bounded by the rib cage, the mediastinum and the diaphragm and are roughly cone-shaped. The mediastinum is a block of tissue lying between the lungs separating one side of the thoracic cavity from the other. It contains the trachea, oesophagus and heart with its major blood vessels. The lungs consist of two large spongy organs and contain the airways of the bronchial tree including the alveoli, as well as the pulmonary blood vessels. Each lung is almost completely surrounded by a double layer of membrane called the pleura, which forms a continuous closed sac. The two layers of the pleura are normally in close contact with each other, separated only by a film of fluid which enables them to move over one another with minimal friction. The space between the layers is called the pleural cavity.

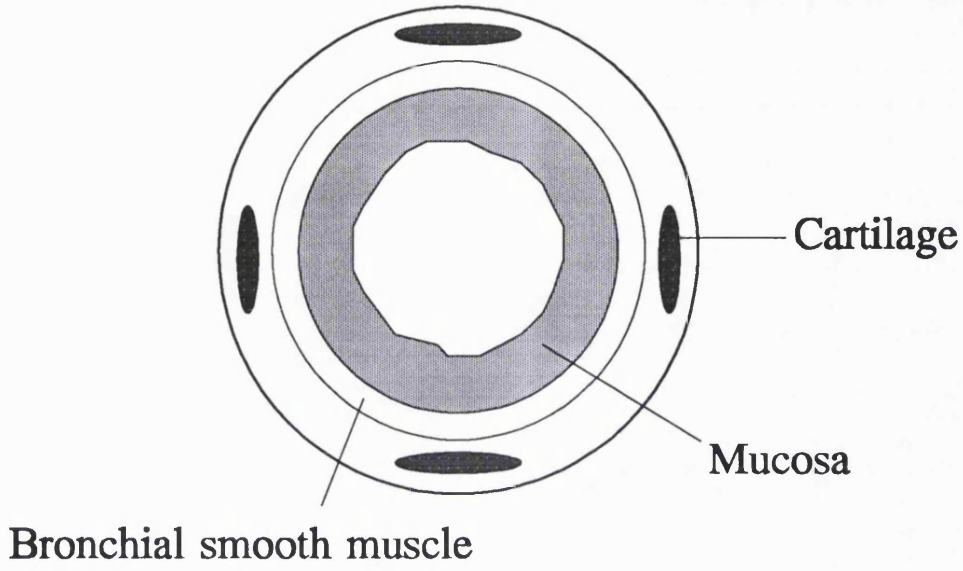
The thoracic cavity acts as an airtight container and as the diaphragm and rib cage move the lungs remain in contact with them. The surface tension between the lungs and the chest wall, and also the air pressure inside the lungs keep them in close contact and prevent the lungs from collapsing. A normal inspiration is accomplished by contracting the diaphragm muscles which pull the diaphragm down. This produces a slight negative pressure in the lungs causing air to flow into them. To expire the diaphragm muscles are relaxed allowing the elastic forces in the lungs to cause the diaphragm to return to its neutral position resulting in air flowing out of the lungs. When greater effort is required in breathing, such as during exercise or during forced expiration, the intercostal muscles within the rib cage assist the diaphragm, to increase the rate and/or depth of breath.

1.3.1 Factors Affecting Airways Calibre

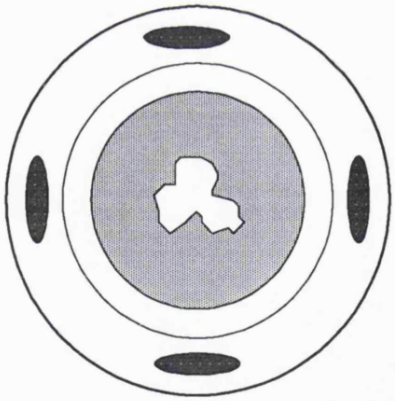
Although the airways consist of a complex branching system they can be simplified to a single tube (Figure 1.2(a)). This allows the factors that determine their calibre and how they may become narrowed by disease to be visualised.

The walls of the airways contain smooth muscle which prevent their collapse during expiratory effort. The size of airways is determined by their elastic properties and smooth muscle tone acting across their walls. Within the wall of the bronchioles the smooth muscle encircles the airways. In normal airways the amount of bronchial motor tone is sufficient to keep a balance between the various forces acting to enlarge or constrict the air passages. However, in abnormal airways excess bronchomotor tone will produce airway narrowing known as bronchoconstriction (Figure 1.2 (b)).

Normal airways are lined by a thin film of mucus which is continually being produced by mucous glands and goblet cells within the bronchial epithelium. It is removed by the beating of microscopic hair-like structures known as cilia which line the major airways. The cilia move together with a wave-like motion which sweep mucus and other particles along it. The main function of this mucociliary "escalator" is clearance of particles which have penetrated the airways. Accumulation of mucus, either because of increased production or impaired cilia transport, may lead to narrowing of larger airways or occlusion of smaller bronchi by mucous plugs.



(a)



(b)

Figure 1.2. ***Cross-sections of (a) normal airway; (b) airway that is undergoing narrowing due to bronchoconstriction. (From G.M. Sterling, Respiratory Disease, Heinemann Medical Books Ltd, London.)***

1.3.2 Obstructive Airways Disease

The three main diseases leading to airways obstruction are chronic bronchitis, asthma and pulmonary emphysema (American Thoracic Society, 1962). Chronic bronchitis is a clinical disorder characterised by excessive mucous secretion in the bronchial tree (Sterling, 1983). Symptoms are recurrent cough and excess sputum production. Airways obstruction may not always be a feature of chronic bronchitis but when present the condition is referred to as chronic obstructive bronchitis. Bronchitic obstruction is usually, but not always, irreversible, whereas asthmatic obstruction is usually, but not always, reversible (Pride, 1992). Pulmonary emphysema is an anatomic alteration of the lung characterised by an abnormal enlargement and destruction of the air spaces distal to the terminal bronchioles. This is accompanied by destructive changes of the alveolar walls (Eriksson, 1991).

Chronic bronchitis and pulmonary emphysema are often both present in the same patient. It is clinically impossible to differentiate between their relative contributions to the patient's symptoms. They are usually classified under the term chronic obstructive pulmonary disease (COPD), with the implication of irreversible and progressive airflow obstruction (American Thoracic Society, 1986; Fletcher *et al*, 1976). COPD most often occurs among males more than 35 years old and is found predominantly in cigarette smokers, although it may be found in non-smokers as well. People living in urban and industrial environments are more often affected than those living in rural areas and it is especially common in cold damp climates (Scadding, 1970).

1.3.2.1 Asthma

Asthma is a very common condition and affects up to 2 million adults (5%) and 1 million children (10%) in the United Kingdom (Pearson, 1993). It is a disease of varying severity and although it is frequently mild and treatable it can sometimes be fatal; in the UK there are about 2000 deaths each year. It may present at any age although it tends to be commoner in children. The main symptoms of asthma are episodic wheezing and breathlessness. Cough with sputum production may be a feature in some patients. Asthma is a complex condition and it is not easily defined (Gross, 1980; Ulrik *et al*, 1992; Pride, 1992). This is because there may be several underlying causes and not enough is known about it to define it uniquely by reference to a single abnormality. One definition states,

" Asthma is a disease characterised by wide variations over short periods of time in resistance to flow in intrapulmonary airways" (Scadding, 1977; Clarke, 1992).

The disease is categorised according to cause and severity and occurs in response to stimuli, which may be external. This leads to widespread narrowing of the airways that changes in severity either spontaneously or as a result of treatment. The three factors which produce airway narrowing in patients with asthma are excess mucus, often with mucus plugs, mucosal oedema (excess fluid in the tissue), and constriction of the bronchial smooth muscle (Sterling, 1983). It has recently been recognised that airway inflammation plays an important role in the development and maintenance of the abnormalities that characterise asthma (Thomson, 1992a).

In susceptible patients various stimuli may trigger the airways obstruction. In a high proportion of patients this is due to an allergic response to certain inhaled particles in the air (allergens). These include animal hair and dander, house dust mite, moulds, certain grass/tree pollens and other substances (Lung defence and immunology, 1989: Crofton and Douglas Respiratory Diseases). In this case the body's normal immunological response to these allergens may be exaggerated or distorted resulting in an abnormal increase of antibodies. In particular, the antibody immunoglobulin E (IgE) (Burney, 1992) has been identified as being particularly important in allergic asthma. It is found in only small quantities in normal subjects but increases in asthmatics who show allergic reaction to inhaled substances. Histamine and other bronchoconstrictor mediators are released from inflammatory cells known as mast cells and basophil cells which have become sensitised by the IgE antibodies during a previous encounter with the allergen. These mediators act on airway smooth muscle receptors, causing the muscle to constrict leading to airway narrowing and the clinical manifestations of asthma (Pepys and Davies, 1977). This mechanism is known as bronchial hyperresponsiveness.

The specific allergen responsible can often be identified by skin-prick tests, where small amounts of common allergen extracts are placed in the superficial layers of the skin by a needle tip. This test is an important means of confirming the patient's sensitivity to suspected substances and may lead to the discovery of others. It is not conclusive proof

of a link between the allergen and the clinical disease and is used together with the patient's clinical history. Patients are then advised to avoid, as much as possible, exposure to specific allergens that have been identified as producing an allergic reaction.

Those who show positive skin-prick tests and increased levels of the antibody IgE are said to be atopic (Pepys and Davies, 1977). However, not all atopic patients develop asthma. These type of reactions known as type I allergic reactions lead to the symptoms of wheezing within minutes of exposure. Other allergic mechanisms (type III) give rise to a late reaction which occurs several hours after exposure.

Allergy also may develop to industrial agents particularly at work (Davies and Pepys, 1977). However, the reaction may be due to irritant rather than allergic causes. A number of irritant stimuli have been shown to produce airways restriction in patients. These include cold air, pollutants such as sulphur dioxide and cigarette smoke. Several other stimuli may be responsible for triggering asthma attacks. The best known are the ingestion of certain food, the ingestion of drugs such as aspirin, upper respiratory tract infections, emotional reactions and exercise. At times, two or more factors occurring together seem to trigger the attack while in other cases the initial stimulus cannot be discovered.

Exercise induced asthmatic attacks are common, particularly in children, and exercise challenge has been used as a diagnostic test for asthma (Anderson *et al*, 1976). Usually several minutes of running, particularly on cold winter mornings, is enough to provoke an attack. The bronchoconstriction usually starts towards the end of the exercise and lasts for 20 - 30 minutes.

1.3.3 Clinical Tests of Lung Function

Most lung diseases affect the mechanical properties of the lung and airways. The main clinical tests of these are based on forced expiratory manoeuvres. Since the main driving pressure for airflow during forced expiration is the elastic recoil of the lungs these tests give a good indication of lung function. Following a full inspiration the patient forcibly expires into a spirometer or a peak flow meter. Spirometry provides information about the speed of airflow as well as the total volume of breath that the patient can take.

During tests of forced expiration the output from the spirometer is either a plot of volume verses time or flow verses volume. From these plots the volume of air expired in the first second (forced expiratory volume in 1 second, or FEV_1) and the total volume expired (forced vital capacity, or FVC) are measured. In addition, the peak expiratory flow (PEF) can also be obtained from the output. FEV_1, FVC and PEF can then be compared with predicted values obtained from data based on normal subjects, the main predictive factors being height, sex and age.

1.4 Properties and Mechanisms of Depositions of Aerosols

An aerosol consists of fine solid or liquid particles suspended in air or other gaseous medium. These particles should be sufficiently large not to diffuse like gas molecules and sufficiently small to remain airborne for some time (Agnew, 1984). Unlike a molecular sized agent such as a gas, diffusion plays only a minor role in the penetration and deposition of aerosol in the human airways.

Aerosols consist of two phases, the solid or liquid particles and the gaseous medium in which they are suspended. The gaseous medium supports the particles against the pull of gravity, restrains random particle motion and sometimes acts as buffer between particles. So as to understand the behaviour of the particles in the aerosol it is first necessary to consider some properties of the gaseous medium in which they are suspended.

1.4.1 Fluid Properties of Gases

Fluid dynamics considers a gas (or liquid) as a continuous medium where all molecules act in harmony with each other. When the medium under consideration is air this is known as aerodynamics. Fluid flow around an obstacle such as an aerosol particle can be characterised by a dimensionless number called the Reynolds number, $\mathbf{R_e}$. If the medium is considered incompressible and neglecting gravity, the main forces present are the inertial force due to the acceleration or deceleration of small fluid masses near the obstacle, and the viscous friction which is due to the viscosity of the medium. The Reynolds number (Equation 1.1) is proportional to the ratio of inertial forces to frictional forces acting on each element of the fluid. It can be expressed in terms of the density of

the fluid medium p_m, the relative velocity between the fluid and the body V, the particle or obstacle diameter d, and the viscosity of the medium η (Hinds, 1982a).

$$R_e = \frac{p_m V d}{\eta} \qquad (1.1)$$

The Reynolds number depends only on the relative velocity between the object and the gas. Therefore, it is aerodynamically equivalent for the gas to flow past a stationary particle or for the particle to settle, at the same relative velocity, in stationary air. The Reynolds number is a very important parameter, and is used in describing the fluid properties associated with an aerosol. At high Reynolds number inertial forces will be much greater than viscous forces and the flow is known as turbulent flow whereas at low Reynolds number ($R_e < 1$) the opposite is true, and the flow is known as laminar flow.

1.4.2 Moving Aerosol Particles

Due to the action of opposing forces such as gravity or electrical force and the resistance of the gas to particle motion, aerosol particles usually come to a constant velocity almost instantly. The resisting force of the gas depends on the relative velocity between the particle and the gas. Stokes' Law (Equation 1.2) describes the resisting force F exerted by a fluid on a moving particle (equivalent to the force exerted by moving fluid on a stationary particle) under the conditions when inertial forces are negligible compared to viscous forces (Low R_e).

$$F = 3\pi\eta V d \qquad (1.2)$$

In addition to a low Reynolds number, the derivation of Stokes' Law (Hinds, 1982a) also assumes that the particle is a rigid sphere with constant motion, and that the fluid is an incompressible and infinite medium with zero fluid velocity at the particle's surface. Most of these assumptions are very close approximations to the real situation and, where

necessary, suitable correction factors can be applied to modify the equation. Because of the low velocities and small particle sizes involved most aerosol motion occurs at low Reynolds number. Stokes' law, therefore, has wide applications to the study of aerosols.

In the case of small particles whose size is close to the mean free path of the gas, the assumption that the relative velocity of the gas at the surface of the particle is zero is not met. Because of "slip" at the surface of the particle, it will settle faster than predicted by Stokes law and a correction factor known as the Cunningham correction factor C_c is necessary in Equation 1.2 (Equation 1.3)

$$F = \frac{3\pi\eta Vd}{C_c} \qquad (1.3)$$

1.4.3 Aerosol Deposition In The Respiratory Tract

The main processes by which aerosol particles in the micrometer size range deposit in the airways are inertial impaction and gravitational sedimentation. Diffusion is also important for small particles. The physical and chemical properties of the aerosol are an important factor in its deposition. These include particle density, particle diameter, hygroscopicity and evaporation of propellants (Heyder *et al*, 1986, Hiller *et al*, 1978). Also important is the mode of inhalation. Depending on the type of inhaler, this includes the inspired volume, inhalation flow rate, breath-holding pause and degree of lung inflation at the beginning of the inhalation manoeuvre (Newman *et al*, 1982a). The patient's lung function is another factor which determines the aerosol penetration and deposition in the airways, with lung and airway abnormalities reducing the penetration of aerosols (Pavia *et al*, 1977).

Therapeutic aerosols tend to be heterodisperse, that is they contain a very wide range of particle sizes. Subsequently, it is difficult to predict the aerodynamic behaviour of these aerosols and how they will deposit in the lungs. Experimental aerosols may be monodisperse and uniform and provide an appropriate means of studying aerosol deposition in the tracheobronchial tree. Studies using monodisperse aerosols containing

unit density spheres of equivalent size range 0.005 - 15 μm (Heyder *et al*, 1986) have indicated that very small particles (< 0.1 μm) behave as gas molecules and Brownian diffusion is the mode of transport into the human airways. For unit density particles with diameters between 0.16 μm and 1 μm, Brownian diffusion is still effective but gravitational sedimentation has also become important. In the size range 2 - 15 μm, particles are deposited by inertial impaction in the upper airways and by gravitational sedimentation in the lower airways. As particle size increases, the site of deposition is shifted from the lower to the upper respiratory tract. Above 15 μm particles are deposited solely by inertial impaction.

1.4.3.1 Brownian Diffusion

Brownian motion is the random motion of particles through uniform, still gas in response to bombardment by gas molecules. Diffusion of aerosol particles is the net transport of these particles in a concentration gradient and is always from a region of higher concentration to a region of lower concentration. The smaller the particles and the longer the aerosol remains in the lungs the larger the diffusion losses in the respiratory tract will be (Heyder, 1981).

1.4.3.2 Inertial Impaction

As aerosol particles enter the respiratory tract, the airflow carrying the particles is deflected as it moves through the upper airways. It must negotiate a series of further direction changes as it reaches the branching lower airway system. At each change in direction the force exerted by the air on the suspended particles must overcome their inertia if they are to follow the airflow. The particles tend to continue a short distance in their original direction and this small displacement from the airstream is sufficient to cause some of the particles near the airway surfaces to deposit there. This process is called inertial deposition.

Stokes' Law can be applied to predict, to a reasonable degree, the particle/air interaction of aerosol entering the respiratory tract under most conditions of importance (Agnew, 1984). In general, for an airflow of velocity U travelling through an airway of radius R, with a change in direction as shown in Figure 1.3, the proportion of particles of diameter

d which will leave the airstream and strike the wall of the airway is a function of a dimensionless number, the Stokes number (***Stk***):

$$Stk = \frac{\rho d^2 U}{18\eta R} \qquad (1.4)$$

(Martonen, 1986)

Where η is the air viscosity and ρ is particle density. The higher the value of ***Stk*** the more easily will particles diverge from the airflow and the more likely they are to deposit by impaction. Due to their relatively large inertia, this effect is greatest for large particles travelling close to the airway walls.

1.4.3.3 Gravitational Sedimentation

The sedimentation of aerosol particles due to gravitational attraction occurs mainly in the lower respiratory tract. This is due to the lower velocity and consequently long period of residence time in this region, especially if breath holding is undertaken after inhalation. An inspired aerosol flows through the upper respiratory tract at high velocity and remains in this region for a much shorter period so that gravitational sedimentation cannot take place. Particle deposition is therefore governed by inertial impaction in this region. Under Stokes' Law conditions, the velocity v at which a particle falls under gravity after time ***t*** is given by:

$$v = \tau g(1-\exp(-t/\tau)) \qquad (1.5)$$

and

$$\tau = \frac{\rho d^2}{18\eta} \qquad (1.6)$$

(Agnew, 1984).

Where ***g*** is the gravitational constant. The particles falling under gravity rapidly acquire a steady settling or terminal velocity (v_g) due to the gravitational force being exactly balanced by air resistance. In the above equation, the exponential term rapidly approaches zero, simplifying the equation to

$$v_g = \tau . g \qquad (1.7)$$

1.4.3.4 The Aerodynamic Diameter

Both inertial impaction and gravitational sedimentation depend on the product ρd^2. The square root of ρd^2 is known as the aerodynamic diameter of a particle. It is often used to describe the effective size of a particle under conditions where inertial impaction and gravitational sedimentation are the main causes of deposition. The aerodynamic diameter may be applied to a particle of any shape, however irregular. It is defined as the diameter of a unit density sphere having the same terminal settling velocity as the particle in question (Agnew, 1984).

1.4.3.5 Other Factor Affecting Deposition

Some therapeutic aerosols are hygroscopic and the inhaled particles absorb water within the humid environment of the respiratory tract, subsequently enlarging in size (Morén, 1982; Newman and Clarke, 1983). In addition, due to the evaporation of propellants, the droplet size of aerosols delivered by MDI will decrease prior to deposition. These two opposing factors make it difficult to predict the aerodynamic behaviour of the aerosol. The influence of humidity on aerosol particle deposition is considered in chapter 3.

Electrical forces may have a significant effect on the deposition of particles in the respiratory tract. Airborne particles can carry an excess of positive or negative charge acquired either in the process of formation or from the atmosphere. The surfaces of the respiratory tract are uncharged, but electrically conducting. When an electrically charged particle approaches this surface, it induces an "image charge" of the opposite polarity in the surface and is attracted towards it. This force becomes stronger as the particle comes closer to the surface and is likely to influence its deposition (Heyder, 1981). Even when a neutral particle approaches a neutral surface, at distances of the order of 1 or 2 μg, electric forces called Van der Waal's forces cause an interaction between the two (Brain and Valberg, 1979).

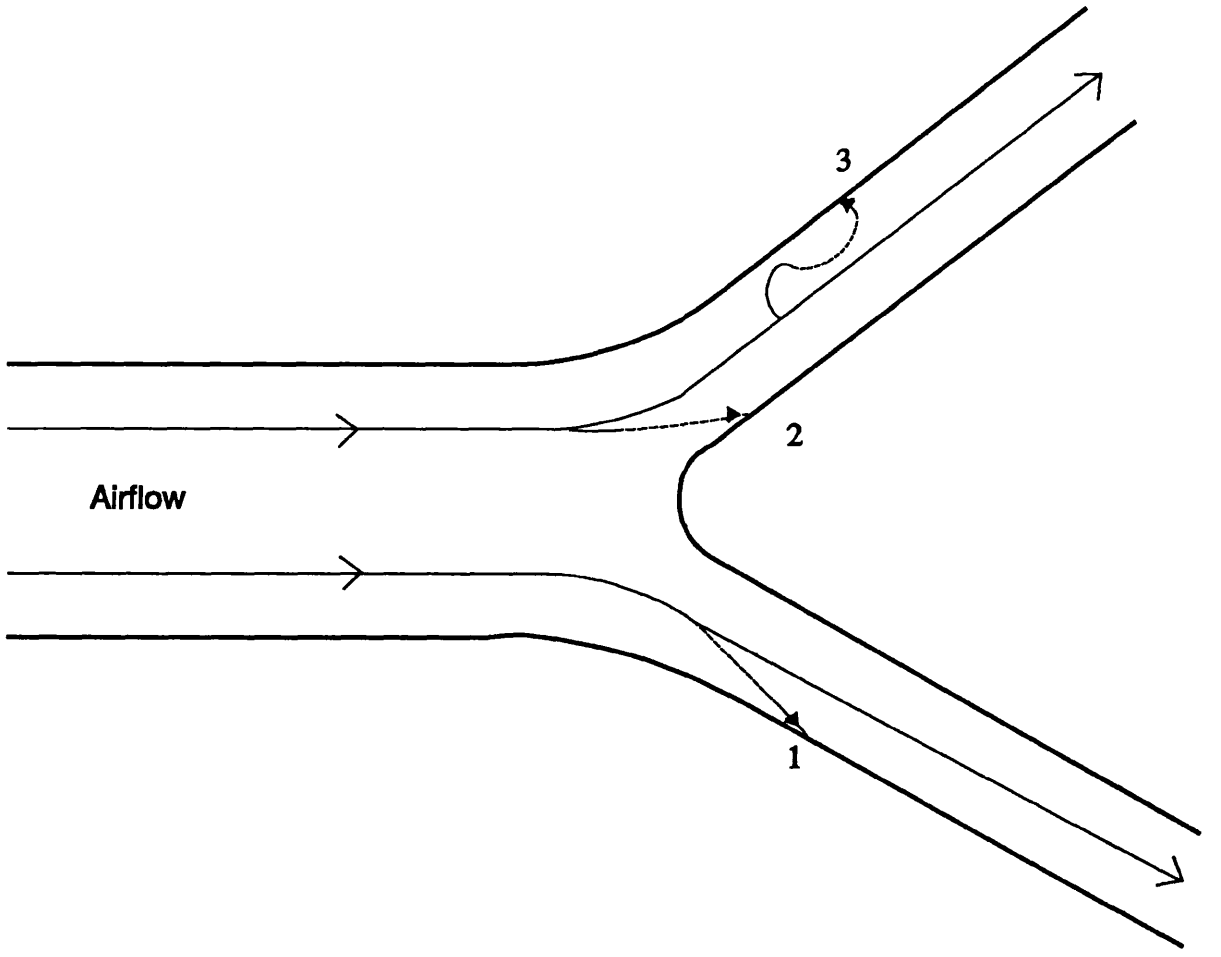


Figure 1.3. *Mechanisms of particle deposition in airways of the lung. The actions of gravitational sedimentation, inertial impaction and diffusion mechanisms are denoted by 1, 2, and 3, respectively.*

1.5 Generation of Therapeutic Aerosols

As the particle size is a major factor influencing the deposition of inhaled aerosols, it is important to be able to generate particles small enough to penetrate into the lung. The ideal size for therapeutic aerosol is not precisely known. However, particles in the 1 to 5 μm range are most likely to penetrate to all parts of the lungs including the alveolated regions (Morrow, 1974; Clarke, 1988). Particles less than 1 μm diameter may not be deposited at all, many being expired like an insoluble gas (Newman & Clarke, 1983). In most circumstances these submicron particles can be neglected since they contribute so little mass (Morrow, 1974).

There are three main types of delivery system for therapeutic aerosols. These are the nebulizer, the pressurized metered dose inhaler and the dry powder inhaler. The nebulizer converts the drug in solution form into a fine mist, either by compressed gas (jet nebulizer), or by high frequency sound waves (ultrasonic nebulizer). In the MDI the drug is suspended or dissolved in chloroflourocarbon propellants contained at high pressure in a canister. The canister contains a metering valve which releases an exact quantity of drug and propellant during each actuation. The DPI contains finely-milled drug together with larger particles of a carrier such as lactose. A unit dose of drug is contained in a gelatin capsule or in "blisters" in a rotating disk device. These have to be broken or pierced to access the drug. Other DPI devices on the market dispense a metered dose from a reservoir containing powdered drug alone. All the DPI devices are activated by the patient's inspiratory breath which produces a turbulent airstream to empty the capsule or chamber.

1.5.1 Metered Dose Inhaler

Metered dose inhalers are the most popular and convenient devices for delivering aerosolised medication. They are compact and portable and may contain several hundred doses. Pressurised canisters contain medication usually in suspension or solution with liquid gaseous propellants under high pressure. The vapour pressure is high enough to keep them in the liquid phase within the canister. Suspension aerosols also contain a surface active agent to prevent agglomeration of the solid drug particles. Each MDI contains a valve with a small metering chamber. The metering chamber contains a

measured dose of drug, propellants and surfactant. The contents are released by applying downward pressure to the stem of the valve. They pass through an orifice in the stem while the propellants undergo a rapid initial vaporisation often called "flashing". This breaks up the liquid stream into large droplets. Their size gradually reduces with distance travelled from the actuator orifice due to the evaporation of the propellants as they acquire heat from the surrounding atmosphere (Newman and Clarke, 1983). The velocity of the propellant droplets may be higher than 30 m per second at the actuator orifice but are subsequently slowed down due to the resistance of the air. The metering chamber refills as the spring-loaded valve stem is released back to its original position. The aerosol canister sits in a specially designed actuator which allows the aerosol to be directed towards the users mouth while allowing pressure to be applied to the valve stem in order to release the dose.

It has been shown that the best results are obtained when the patient actuates the MDI at the beginning of a deep slow inhalation followed by a 10 seconds breath-hold to allow the particles to settle within the lungs (Newman *et al*, 1982a). Other workers have suggested that best results are obtained when the MDI is actuated 4 cm from the wide-open mouth and inhalation is over 5 or 6 seconds with a 10 seconds breath hold (Dolovich *et al*, 1981).

The most frequently reported problem with the MDI is the difficulty many patients have in using it efficiently (Newman and Clarke, 1983; Orehek *et al*, 1976; Paterson and Crompton, 1976). The most important error is poor coordination of the actuation and breath inhalation manoeuvre. This may be due to poor instructions (Guildry *et al*, 1992) or failure to understand the technique (De Blaquiere *et al*, 1989), or in many cases, the patient may not be able to coordinate at all. To help overcome this coordination problem, breath actuated MDIs have been developed in which the patient's inhalation triggers a spring mechanism which fires the inhaler at the correct moment. It has been shown that these devices can benefit those with coordination problems (Newman *et al*, 1991), although they are not widely used at present.

1.5.1.1 Spacers

An important addition to the MDI is the spacer device. This a tube or cone-shaped container placed between the actuator mouthpiece and the patient's mouth. Most spacers act as a holding chamber which slows and contains the aerosol spray (Volumatic® device, Allen and Hanburys Ltd). The large rapidly moving propellant droplets are allowed to evaporate and slow down due to air resistance. After a short delay the patient is then able to inhale the aerosol via a one way valve without the need to synchronise the actuation and the inhalation. It is therefore a very important aid for those patients that have difficulty in coordinating. It has been shown that spacers cut down the oropharyngeal deposition (Newman, 1984a) and raise lung deposition to levels similar to or greater than those obtained from a correctly used MDI (Newman *et al*, 1984a). The large particles that usually impinge on the back of the throat when the MDI alone is used tend to be retained in the spacer. Due to the much reduced throat deposition, spacers are especially helpful in reducing side effects such as hoarseness and fungal infection (candidiasis) frequently associated with the inhalation of corticosteroids.

Some spacer devices are open ended (Newman *et al*, 1989), in which case good coordination is necessary to prevent the loss of the dose. However, when used correctly they retain many of the benefits of the closed spacer such as reduced oropharyngeal deposition and increased lung deposition. They are also more compact and are a useful aid for the teaching of good coordination technique (Newman *et al*, 1989).

1.5.2 Dry Powder Inhalers

The dry powder inhaler is an alternative to the pressurised MDI and dispenses the drug as a powder. The first dry powder inhaler was introduced for sodium cromoglycate (Spinhaler™ inhaler, Fisons Ltd). More recently, inhalers have become available for salbutamol and other $beta_2$-agonists. Dry powder inhalers are breath-actuated and require no coordination. They are therefore particularly useful for patients with difficulty in coordinating an MDI (Hetzel and Clark, 1977; Duncan *et al*, 1977). All that is necessary is for the patient to be able to inhale deeply and rapidly to draw the powder into their airways. Commonly included in dry powder formulations are carrier particles, usually lactose, of much larger size than the drug itself which improve the efficiency of emptying

of the capsule. During inhalation, the drug particles become separated from the carrier and penetrate the airways independently.

There are several types of DPI on the market at the present time. These include the Rotahaler* inhaler (Allen and Hanburys Ltd) which is used to deliver either salbutamol sulphate or beclomethasone dipropionate powders. The drug is contained in a small capsule together with lactose. To operate the device, the patient inserts the capsule into the hole in the end of the device and breaks it by twisting the outer and inner parts of the inhaler barrel. The patient then inhales from the mouthpiece to obtain the drug.

The Diskhaler* inhaler (Allen and Hanburys Ltd) is a recently introduced multidose device in which the drug and lactose are contained in eight single dose blisters formed on a special disk. The Diskhaler is available with either salbutamol sulphate (Ventodisk* blister pack) or beclomethasone dipropionate (Becodisk* blister pack). The disk is supported, within the device, by a rotatable wheel. Each blister is sealed by foil and can be pierced by lifting a flap through 90° forcing a needle through the blister and allowing the powder to drop into a chamber. As the patient begins to inhale from the mouthpiece the chamber is emptied and the drug follows the airstream. The wheel can then be rotated to enable the next blister to be positioned ready for later doses.

Another device is the Turbuhaler™ (Astra) which dispenses the $beta_2$-agonist terbutaline sulphate as well as the corticosteroid budesonide from a complex but easy to use device. It is able to deliver small metered doses of active drug without the need for a carrier and at relatively low inspiratory flow rates. Turbulent airflow in the mouthpiece breaks up aggregates of drug particles yielding fine particles for inhalation.

1.5.3 Measurement Of Aerosol Size Distribution

Due to the heterodisperse nature of therapeutic aerosols their behaviour is best described by means of the Mass Median Aerodynamic Diameter (MMAD) (Newman and Clarke, 1983). Half of the aerosol mass is contained in particles smaller than the MMAD and half of the aerosol mass in particles larger than the MMAD. The usual measure of variance for aerosol particles plotted on a log-normal distribution of cumulative percentage against

particle size is the Geometric Standard Deviation, (GSD), (Morrow, 1981). This is equal to the ratio of the 84.13 percent size to the 50.0 percent size.

The most widely accepted methods currently used to characterize aerosols are optical microscopy, laser light diffraction and cascade impaction for airborne particles. Laser light diffraction is able to determine particle size using the diffraction patterns produced by airborne particles within a laser beam (Ho, 1989). The particles diffract the light according to their size - small particles diffract light through greater angles than large particles. A section of the diffraction pattern is measured with a photomultiplier and the resulting information analysed by computer to produce a particle frequency distribution according to geometrical diameter. Optical or electron microscopy is used to determine the sizes of particles after they have been deposited on a suitable surface.

Cascade impaction classifies particles in terms of their aerodynamic diameter and relies on the same inertial impaction principles that govern aerosol deposition in the respiratory tract. It is particularly useful for the MDI where the entire dose delivered is characterised rather than a portion of it as in optical microscopy. A cascade impactor is made up of a number of classification stages. The aerosol is drawn through a series of plates containing holes or nozzles through which the airflow passes. Air flow is forced to change direction as it passes through each stage. Particles in the aerosol stream having sufficiently large inertia will be unable to follow this change in direction and will impact on collection plates while smaller particles will pass as an aerosol onto the next stage. Progressively finer holes produce higher velocities so that at each stage there is a reduction in the size of the particles that can follow the airflow. Particles too small to be collected in the last stage are usually collected on an after filter. Each stage has been calibrated for the probability of impaction for particles of known aerodynamic size. The Effective Cutoff Diameter (ECD) of each stage is the smallest particle size that deposits there. The particles deposited at each collection plate can then be determined and expressed as a percentage of the total delivered. The cumulative percentage of deposited particles less than each size range is plotted against the ECD for that size range to obtain the MMAD and GSD of the aerosol particles.

1.6 Inhaled Respiratory Drugs for the Treatment of Asthma.

A wide variety of drugs are currently available in inhalers for the treatment of asthma and other respiratory diseases. Drugs used in the management of asthma are classified as prophylactic agents, bronchodilators, and corticosteroids. Prophylactic agents help to prevent the onset of asthma attacks, while bronchodilator drugs reverse the bronchospasms that produce severe breathing difficulty during an attack. Inhaled corticosteroids exert an anti-inflammatory effect on the airways and a long term prophylactic effect when taken regularly.

1.6.1 Bronchodilators

Inhaled bronchodilators are the most widely used form of therapeutic aerosol and to understand their mode of action it is necessary to consider some of the mechanism involved that control airway calibre.

Autonomic nerves regulate many aspects of airway function and influence the tone of airway smooth muscle, airway secretions, blood flow and the release of inflammatory cells (Barnes *et al*, 1992). The main pathways that affect airway function and bronchial smooth muscle tone are the cholinergic and adrenergic nervous systems. These nerves are stimulated by the action of specific substances (agonists) at their receptor sites. The receptor sites of the adrenergic system, adrenoceptors, have been subdivided into α and β (Ahlquist, 1948). In addition, the β-receptors have been further divided into β_1- and β_2-adrenoceptors by Lands *et al* (1967). Beta-adrenergic nerves act to cause bronchodilation of bronchial smooth muscle, whereas cholinergic and α-adrenergic nerves cause bronchoconstriction. Bronchial smooth muscle contain mostly β_2-adrenoceptors, whereas the terminal airways and alveoli contain a mixture of β_1 and β_2 receptors (Carswell and Nahorski, 1983). $Beta_2$-receptors are found additionally in skeletal and vascular smooth muscle (Paterson *et al*, 1979). The heart contains β_1 receptors, and their stimulation causes increased heart beat and cardiovascular effects.

There is considerable evidence that neural control of the airways of asthmatics may be abnormal (Barnes, 1992) and that they are more responsive to cholinergic stimuli than normal subjects. This suggests an imbalance between cholinergic and adrenergic

mechanisms (Paterson *et al*, 1979). However, inflammation also plays an important part in asthma and its interaction with neural control is complex and not fully understood

There are three commonly used types of bronchodilator - β-agonists, anticholinergic and methylxanthines, although methylxanthines are not usually administered to the lungs by inhalation.

1.6.1.1 β-Agonists

Adrenaline was the first reported adrenergic-agonist bronchodilator to be given by inhalation (Matthews, 1910). This was followed by isoprenaline in the early 1960s which soon became established as an important bronchodilator. Both of these drugs are effective bronchodilators. However, adrenaline stimulates α receptors, as well as β receptors in the heart, and isoprenaline stimulates β_1 and β_2 receptors. They both cause undesirable cardiovascular side effects such as tachycardia or arrhythmias (Clarke and Newman, 1984).

Beta-agonists produce bronchodilation in subjects with reversible airflow obstruction through several actions, although the precise contribution of each to the airway response to bronchodilators is uncertain (Hall & Tattersfield, 1992). They oppose the effects (antagonize) of bronchoconstrictor agents on smooth muscles in the airways walls, and produce relaxation of smooth muscle fibres and subsequent bronchodilation. In addition, they also inhibit the release of bronchoconstrictor mediators, such as histamine from sensitized mast and basophil cells (Hall & Tattersfield, 1992; Holgate and Church, 1992). When given regularly they may prevent the onset of airways constriction (Newman, 1984a), particularly for exercise induced asthma (Anderson *et al*, 1976). They also increase mucociliary clearance, probably as a result of increased ciliary beat frequency, and produce dilation of pulmonary blood vessels (Paterson *et al*, 1979).

The most selective group of bronchodilator drugs are the β_2-agonists, which have been introduced over the last 24 years. They act selectively on the β_2 receptors in the bronchial tree in very small doses, while having little effect on cardiac β_1-receptors or on α-receptors (Leifer and Wittig, 1975). The best known β_2-agonists are salbutamol, terbutaline

and fenoterol although there are many other related compounds (Leifer and Wittig, 1975). Beta$_2$-agonists have become the main bronchodilator agents used for the treatment of asthma, and are most commonly administered by inhaler. Salbutamol, terbutaline and fenoterol are equally effective in asthmatic subjects, although fenoterol is less β_2 selective than the other two (Wong *et al*, 1990).

There have been some doubts over the safety of fenoterol and it has been linked to an increase in deaths among asthmatics in New Zealand. However results are inconclusive and there are sharp differences in opinion about the causes (Tattersfield, 1992). In general, side effects with inhaled doses of β_2-agonists are small, although they do increase with increasing doses and with other routes of administration such as intravenous and oral routes. They can also depend on the severity of the asthma and on other drugs being given. The main side effects which may be experienced are compensatory increase in heart rate as a reflex response to fall in blood pressure following dilation of blood vessels, and skeletal muscle tremor (Hall and Tattersfield, 1992).

Recently, longer acting β_2-agonists have been developed and are now available. Salmeterol and formoterol are the two most prominent (Tattersfield, 1992). These have periods of action much longer than other β_2-agonists and need to be taken less frequently (Ullman and Svedmyr, 1988; Wallin *et al*, 1990).

1.6.1.2 Anticholinergic Drugs

Anticholinergic compounds have been known as bronchodilators for some time; the herb Datura stramonium has been used for centuries to treat chest diseases including asthma (Thomson, 1992b). Cholinergic nerves are the dominant neural bronchoconstrictor pathway in human airways and are under the control of the vagus nerve. This mechanism may be exaggerated in asthma (Barnes, 1992; Tattersfield, 1992), although neural control of the airways is complex and this may be only part of the process. Anticholinergic drugs block the effects of cholinergic agonists, such as acetylcholine released by the cholinergic nerves, by competing with them at cholinergic receptor sites in the airways.

Atropine, ipratropium bromide and oxitropium bromide are well known anticholinergic bronchodilators. Atropine has been in use since the 19th century, although its more recent clinical use has been restricted owing to its drying effect on lung secretions and its possible cause of mucus plugging. In addition it also reduces ciliary beat frequency and mucociliary clearance. The synthetic anticholinergic agent ipratropium bromide does not have these adverse side-effects in the normal therapeutic dose (Gross and Skorodin, 1984). Ipratropium bromide has a slower onset of action compared to the selective β_2-agonists, but its duration of action is similar and it is effective in doses as small as 40 μg when administered from an MDI.

Anticholinergic drugs have a relatively limited role in the treatment of asthma, and account for only a small percentage of total asthma prescription. Ipratropium bromide and oxitropium bromide are mainly used as second line bronchodilators usually in combination with inhaled β_2-agonists, which may produce a slightly greater and more prolonged response than either given separately (Thomson, 1992b).

1.6.1.3 Methylxanthines

The naturally occurring substances theophylline and caffeine found in food and drink are among the best known methylxanthines (Weinberger, 1992). Theophylline is the only one that has been extensively used for the treatment of asthma. Synthetic derivatives of theophylline have also been used as bronchodilators. However, they have been shown to be less potent and lack any therapeutic advantage (Weinberger, 1992). Methylxanthines are not usually administered by aerosol and are much less effective than β-agonists when given by the inhaled route (Clarke and Newman, 1984). They are most often given orally or intravenously and theophylline has been marketed in a variety of oral preparations including slow-release tablets. Theophylline is often given in emergency treatment of acute asthma and is used in the long term maintenance of chronic asthma.

1.6.2 Prophylactic Agents

Prophylactic agents are taken before the onset of asthma attacks to prevent them from occurring. They are not bronchodilators, and are therefore not effective at bringing relief during an attack. Sodium cromoglycate was synthesized in 1965 and is the best known

of this group of drugs. It was found to inhibit allergen-induced bronchoconstriction (Thomson, 1992a). Its mode of action is to inhibit the release of mediators from mast cells which have been triggered by the interaction between antigen (allergen) and IgE antibodies. Other inflammatory cells can also be controlled by sodium cromoglycate. Sodium cromoglycate is recommended as first line prophylactic treatment in asthmatic children, although in adults it is often replaced by low doses of inhaled steroids.

Nedocromil sodium is a relatively new prophylactic agent and its effectiveness in asthma compared to other agents has not been fully assessed. However, the available clinical studies have shown that it has significant therapeutic effect in both allergic and non-allergic asthmatics (Pauwels, 1992). Both sodium cromoglycate and nedocromil sodium are available as an MDI and as a nebuliser solution, although sodium cromoglycate was first available as a dry powder inhaler.

1.6.3 Corticosteroids

The recognition that airway inflammation is an important factor in asthma has resulted in an increased emphasis on the use of anti-inflammatory drugs. Although both sodium cromoglycate and nedocromil sodium have anti-inflammatory effects, the most important group of anti-inflammatory drugs are corticosteroids. Their mode of action is to regulate many of the components of the inflammation response that occurs within the airways in asthma. They reduce the number of inflammatory cells (Thomson, 1992a), that are found in increased numbers, and inhibit the release of chemical mediators which cause bronchial hyperresponsiveness. By regular usage they help to reduce the occurrence of asthma attacks and are therefore prophylactic drugs for the treatment of the underlying causes of the disease.

Corticosteroids were first introduced for the treatment of asthma over 40 years ago and it soon became clear that they could successfully control its symptoms. They were administered by oral preparations or by intravenous injection or infusion. However, harmful systemic side effects, such as adrenal suppression and changes in growth rate in children, soon became apparent and the general use of corticosteroids declined.

Since then new corticosteroids have been developed which can be inhaled directly into the lungs from an MDI or DPI. They exert a topical effect directly on the bronchial tree but have low systemic effect as the swallowed portion of the dose is inactivated when absorbed from the gut and does not enter the systemic circulation in an active form (Check & Kaliner, 1990). The doses required are small (400 - 800 μg daily) (Clarke & Newman, 1984), blood plasma levels are low and therefore enable the control of asthma without many of the unwanted side-effects associated with systemic therapy. The first of the topical steroids to be introduced was beclomethasone dipropionate (Becotide* inhaler). This was later reintroduced as a higher dose MDI (Becloforte* inhaler) with each dose containing 250 μg. Since then several others have been developed including budesonide and fluticasone propionate (Thomson, 1992a). The introduction of inhaled corticosteroids have enabled most patients, except those with acute severe cases of asthma, to be switched from systemic doses (oral and intravenous) to inhaled doses (Cochrane, 1992).

Local side-effects in the upper respiratory tract do occur, in some patients who regularly use inhaled corticosteroids, due to much of the dose failing to reach the lungs and depositing there. Oropharyngeal candidiasis (thrush) is a common side-effect as is hoarseness (dysphonia) which may occur independent of thrush (Chung & Clark, 1992). The use of a spacer device to reduce the dose of steroid that deposits in the larynx is an effective way of decreasing this problem.

Inhaled corticosteroids have in the past been prescribed to asthmatics and those suffering from bronchitis when their symptoms cannot be controlled adequately by bronchodilators and non-steroidal prophylactic agents. However, with inflammatory mechanisms now regarded as central to asthma, they are increasingly being used at an early stage of the disease and reduce the use of bronchodilator aerosols to a minimum (British Thoracic Society, 1990).

1.7 Salbutamol

Salbutamol is a selective β_2-adrenoceptor stimulant (β_2-agonist) used widely for the treatment of bronchial asthma and in obstetrics for the prevention of premature labour. It was developed by Allen and Hanburys Ltd and marketed in 1969 as Ventolin* inhaler. It

soon took over from other adrenergic bronchodilators such as isoprenaline and orciprenaline due to its greater selectivity and longer duration of action. In contrast, isoprenaline acts equally on β_1-receptors in the cardiovascular system and β_2-receptors in the bronchial smooth muscle which leads to cardiovascular side-effects. It also has a short duration of action due to rapid metabolism of the drug. Orciprenaline has some β_2-receptor selectivity but has also been shown to have cardiac stimulant effects in man when given by intravenous infusion (Shanks *et al*, 1967).

Salbutamol and other selective β_2 adrenergic drugs were developed in response to the need for bronchodilator drugs that were longer acting and able to provide relaxation of bronchial smooth muscle without direct stimulation of the heart (Warrell *et al*, 1970). They are modifications of the isoprenaline molecule. Differences in the chemical structure of salbutamol compared to isoprenaline are responsible not only for its greater selectivity, but also for its longer duration of bronchodilator action (Hartley *et al*, 1968). The main reason for this longer duration of action is that, unlike isoprenaline, salbutamol is not metabolised by the enzyme catechol-O-methyl transferase (COMT) (Brittain *et al*, 1968). The duration of the bronchodilator action of isoprenaline is limited because of the rapid metabolism by COMT (Davies *et al*, 1975).

1.7.1 Pharmacokinetics of Salbutamol

Salbutamol can be administered by three routes, orally in tablet form, intravenously or inhaled directly into the lungs from an aerosol. The useful pharmacological effect that a given drug exerts may be influenced by the action of enzymes which can modify the structure of its molecules to an inactive metabolite. The extent to which this occurs in bronchodilator drugs is crucial to the effectiveness of the drug and usually varies according to the route of administration. So as to study its mode of action, the profile and relative proportions of free salbutamol and its metabolites have been examined in the blood plasma and excreted urine of animals and humans.

Early studies were performed using tritium-labelled (^{3}H) salbutamol administered orally, intravenously or by inhalation. Blood plasma and urine excreted were assayed for salbutamol and its metabolites using a liquid scintillation spectrometer (Martin *et al*, 1971;

Walker *et al*, 1972a; Evans *et al* 1973; Shenfield *et al*, 1976). More recent studies have used high pressure liquid chromatography (Morgan *et al*, 1986; Hindle and Chrystyn, 1991) and high-performance thin-layer chromatography (Colthup *et al*, 1985).

Preliminary work on the fate of ^{3}H-salbutamol (Martin *et al*, 1971) showed that it was rapidly absorbed from the gastrointestinal tract of rat, rabbit, dog and man and that between 60 and 90 per cent of an oral dose was excreted in the 24-hour urine.

Other studies investigated the pharmacokinetics and metabolism of ^{3}H-salbutamol in asthmatic subjects following oral, aerosol and intravenous administration (Walker *et al*, 1972a; Evans *et* 1973) and in normal subjects (Morgan *et al*, 1986) following intravenous and oral doses of salbutamol. They also found that salbutamol is well absorbed from the gastrointestinal tract and rapidly excreted in the urine. The ratio of unchanged drug to metabolite depends on the route of administration with the metabolite dominating in urine and blood plasma after oral administration, and the unchanged drug predominant after intravenous administration. Following inhalation via pressurised MDI the pattern of excretion is similar to that following oral treatment. Since no metabolism of salbutamol occurs in the lungs itself (Shenfield *et al*, 1974; Shenfield *et al*, 1976), these results suggest that most of the dose from a pressurised MDI is swallowed and metabolised as an oral dose while a much smaller portion reaches the lungs. Comparisons of the patterns of absorption, metabolism and excretion between oral and intravenous doses indicate that metabolism of drug occurs in the gastro-intestinal tract and/or following first pass through the liver.

It was shown that a small dose of inhaled salbutamol produces a measurable improvement in lung function well before it is detected in the blood plasma, with peak effect occurring much earlier than peak plasma concentrations occur (Walker *et al*, 1972a). The inhaled dose was only a fraction of that given orally, and maximum blood plasma levels were correspondingly low. These findings therefore imply that the dose acts locally (topically) on the airways in the bronchial tree, and action does not depend on the blood plasma concentrations.

1.7.2 β_2 Selectivity of Salbutamol

The β_2 selective action of salbutamol has been well demonstrated in animals (Brittain *et al*, 1968; Farmer & Levy, 1969) and later in humans. In isolated guinea-pig hearts, salbutamol was found to be two thousand times less potent than isoprenaline in stimulating β_1 receptors (Cullum *et al*, 1969).

Warrell *et al* (1970) compared cardiorespiratory effects of salbutamol with isoprenaline administered orally, by inhalation and by intravenous infusion in asthmatic patients. They found that salbutamol and isoprenaline were equipotent as bronchodilators but that isoprenaline caused a greater rise in pulse rate and a greater change in blood pressure than the same dose of salbutamol. They also showed that following continuous administration of both drugs, isoprenaline had a greater effect on metabolic rate, pulmonary ventilation, pulmonary gas exchange, cardiac output and heart rate.

Similar drug infusion results were obtained by Paterson *et al* (1971) who compared the effect of equivalent dose of isoprenaline and salbutamol administered intravenously in fifteen asthmatic subjects. In the study, several infusion rates of isoprenaline and then salbutamol were given to the same subjects and FEV_1 and heart rate monitored. They found that with salbutamol heart rate increased up to five beats per minute for a 35.5 per cent increase in FEV_1 whereas with isoprenaline the increase was only 12.5 per cent for the same rise in heart rate. Also, on average, seven times the infusion rate of intravenous salbutamol, as compared with intravenous isoprenaline, was required to produce the same rise in heart rate.

Choo-Kang *et al*, (1969) found that 200 μg of salbutamol, administered by pressurised MDI to twenty-four patients suffering from severe chronic asthma, provided effective bronchodilation for at least three hours without producing detectable cardiac stimulation. They measured FEV_1 and FVC before and after administration and found that the bronchodilator activity of this dose was slightly more prolonged and intense than that of 1,500 μg of orciprenaline, and equal in peak effect to that of 1000 μg of isoprenaline. However, for isoprenaline, the bronchodilator response, though initially more intense, remained effective for less than one hour and was accompanied by tachycardia.

Kennedy and Simpson, (1969) gave 200 μg of salbutamol and 1200 μg of orciprenaline by inhalation (MDI) to twelve asthmatics. They concluded that salbutamol, while having a similar time-response curve (percentage improvement in FVC), was consistently more intense than orciprenaline.

Salbutamol has also been compared to more recently developed β_2-agonist drugs. It is as selective as terbutaline and slightly more selective than fenoterol (Wong *et* al, 1990). These three drugs have a similar duration of action (Gray *et al*, 1982), though this is very much shorter than the newly developed long-acting drugs such as salmeterol (Ullman and Svedmyr, 1988).

1.7.3 Inhaled Salbutamol

Early clinical studies with asthmatic patients by Choo-Kang *et al* (1969) and Kennedy and Simpson (1969) drew attention to the potential of inhaled salbutamol for the effective treatment of asthma.

Kamburoff and Prime (1970) showed that 200 μg of salbutamol administered by MDI to a group of asthmatic patients produced the same peak FEV_1 response as 5 mg given orally to a similar group of asthmatics. However, peak response occurred earlier for the inhaled dose and significant improvement took place within 5 minutes instead of 15 minutes for the oral dose. A 200 μg dose of isoprenaline used as a standard control between the two groups of asthmatics gave a less prolonged and intense effect than either of the other two, although a more rapid initial effect was reported. Very little cardiovascular side effects were observed after either of the two administration methods of salbutamol.

Larsson *et al* (1977) measured cumulative dose-response curves of salbutamol given orally and by inhalation from an MDI. They reported that superior bronchodilating effect on large airways can be achieved with the MDI using lower doses and with fewer side effects. In addition a more rapid onset of action was achieved with the MDI. However the data also suggested that a better effect on small airways is possible with oral treatment. They concluded that, unless special circumstances arise such as the blocking off of parts of the lung by mucous plugging or mucosal edema, or in cases where the patient cannot use an MDI, inhaled salbutamol should be used in preference to oral salbutamol.

Comparisons between doses of inhaled salbutamol and intravenous doses showed that a given dose of salbutamol administered by the aerosol route is more potent than an equal dose given intravenously (Hetzel and Clarke, 1976). It lasts longer and has fewer side effects. However, other studies have shown that intravenous infusion and injection of salbutamol are important in the treatments of severe asthma (May *et al*, 1975; Spiro *et al*, 1975).

1.7.4 Summary

Salbutamol, the first of the selective β_2-agonists to be developed, has been extensively studied and shown to be a very effective bronchodilator in subjects with airflow obstruction. Comparisons have been made between the three methods of administration. These have shown that inhalation provides the most direct means of delivering an effective dose to the β_2-receptor sites in the bronchial smooth muscle. There is a direct local action, enabling rapid bronchodilation of the airways. Inhaled salbutamol also has a more prolonged action than either oral or intravenous doses, with no noticeable side effects for doses that produce effective bronchodilation. In addition, inhaled salbutamol is effective in doses of only 200 μg compared with 4 mg for oral doses. Pharmacology studies have indicated that only a small portion of the inhaled dose reaches the lungs but that it is this fraction that produces the rapid improvement in breathing experienced by the patient.

1.8 Review of Methods for Assessing inhaled Drug Deposition

Several experimental designs have been used to assess the proportion of an inhaled bronchodilator drug reaching the lungs. These designs may be generalised into two main approaches. They are, firstly, methods that use gamma-emitting radionuclides to indicate the position of the drug within the body and, secondly, techniques that measure the level of drug and its metabolites in the mouth washings, blood plasma and excreted urine of the subject.

1.8.1 Radionuclide Imaging of the Lungs

Radionuclide imaging of the lungs is an established clinical technique and is important in the diagnosis, assessment, and management of disorders of lung function (Buxton-Thomas, 1989). To undertake studies involving radioactive compounds in humans, the radionuclide must have a suitable gamma ray energy; high enough for good tissue penetration but easily shielded. To keep the radiation dose low, alpha and beta radiations should be avoided and the half-life of the radionuclide must not greatly exceed the period required for the study.

Technetium-99m (^{99m}Tc) is the most versatile and commonly used radionuclide in clinical use. It has a 140 keV gamma-ray emission and a half-life of 6 hours which make it ideal for imaging (Sharp, 1989). It is readily available from a generator containing its parent molybdenum-99 and is used extensively in nuclear medicine departments since it can be attached to a wide range of compounds suitable for specific organ imaging. In addition, it may be used alone as pertechnetate ($^{99m}TcO^-_4$) to study a number of organs whose cells either accumulate it or excrete it (Smith, 1989). One important clinical application of the radionuclide is perfusion imaging. Albumin macroaggregates or microspheres labelled with ^{99m}Tc are given intravenously. They become trapped in the smallest capillaries in the vascular network of the lung, thereby giving images quantitatively proportional to regional blood flow. Another application of this radionuclide in lung imaging, in some centres, is ^{99m}Tc-Technegas. This is effectively radiolabelled soot and is breathed into the lungs as a ventilation agent.

Radioactive gases such as xenon-133 (^{133}Xe) and krypton-81m (^{81m}Kr) can be breathed directly into the lungs, after dilution with air, and are widely used to produce ventilation images. The diagnosis of pulmonary embolism makes use of both a perfusion scan and a ventilation scan. This condition usually shows up as well defined defects on the perfusion lung image despite a normal looking ventilation image.

With a half-life of only 13 seconds and a gamma-ray energy of 190, keV ^{81m}Kr is an ideal radionuclide for ventilation imaging. Its very short half-life means that within a short time after its application a completely clear background exists thus allowing other studies to

be performed without interference. Krypton-81m is eluted from a generator containing its parent rubidium-81 (^{81}Rb) which is produced in a cyclotron and has a half-life of 4.6 hours.

1.8.2 Radiolabelling of Drugs In Aerosols

Radiolabelling methods have for some time contributed valuable information about the deposition of bronchodilator and other bronchoactive aerosols in the human lungs. In the past the radionuclides tritium (^{3}H) and carbon-14 (^{14}C) have been labelled to a variety of compounds and used to study the clinical pharmacology of inhaled drugs (Walker *et al*, 1972a; Walker *et al*, 1972b; Davies, 1975). Unfortunately, these radionuclides emit beta rays only. Because of their short range in tissue beta rays from internally located sources cannot be detected outside of the body (Newman, 1984b) and cannot, therefore, be used to image the *in-vivo* drug distribution. The labelled drug can be measured *in-vitro* in samples of urine, blood plasma and mouth washings taken from the subject, using a liquid scintillation spectrometer. However, beta rays are more densely ionising than gamma rays giving rise to higher radiation doses.

Most radioaerosol studies now employ gamma-emitting radionuclides. They provide a means of studying the aerosol distribution externally and without discomfort to the subject. A number of such radionuclides have been used to study aerosol particles and the majority emit both gamma and beta rays (Newman, 1984b). However with most, the quantities of beta rays emitted are negligibly low and for practical purposes they may be considered to be pure gamma-emitters.

To study a particular aerosolised bronchodilator drug, ideally the drug should be directly labelled with a suitable radionuclide so that its deposition pattern and bronchodilator responses can be measured simultaneously. However, in the past it has proved difficult to attach gamma-emitting radionuclides to the drug itself and indirect methods of labelling have often been used. An inert substance such as teflon or polystyrene with aerodynamic size distribution similar to the drug itself is labelled.

Suitably sized radiolabelled particles can be produced by a May spinning disk generator (May, 1949). It comprises a rotating disc usually driven by compressed air. Small droplets are generated when the inert material and radionuclide are dissolved in a volatile solvent such as ethanol and fed onto the centre of the rapidly rotating disc. The liquid spreads over the disc surface as a film. At the edge of the disc where the centrifugal force exceeds the retaining force of surface tension, the liquid is flung off tangentially and forms spherical droplets. Droplet size is inversely proportional to the angular velocity of the disk (Newman, 1984b) and so may be varied according to the size of aerosol required. The generator is normally enclosed in a tank which contains the resulting aerosol. It can then be inhaled directly to investigate aerosol lung penetration and clearance (Pavia *et al*, 1977; Short *et al*, 1979; Agnew *et al*, 1984).

In more recent studies the droplets have been allowed to settle on a collecting surface within the tank. The solvent is evaporated leaving solid radioaerosol particles that are added to reconstituted metered dose inhaler canisters together with propellants and surfactant. This can be either in place of the bronchodilator drug (Newman *et al*, 1981a) or in addition to it (Zainudin *et al*, 1989). The deposition patterns of pressurised MDIs are then investigated. In order to simulate the drug it is necessary to generate radioaerosol particles with the same aerodynamic properties (size range and density). *In-vitro* quality control procedures are required to ensure that the radiolabelled particles behave the same as the drug. However, it cannot be certain that the two will have the same physical properties and distribution pattern when inhaled into the thorax. It will therefore always be desirable to use a direct labelling technique if one is available that is suitable and convenient to use.

A direct labelling method for the synthetic anticholinergic bronchodilating drug ipratropium bromide has been available for some time (Short *et al*, 1981). The drug is chemically labelled to the cyclotron generated radionuclide bromine-77 (^{77}Br) which has a physical half-life of 58 hours and principal γ-ray emissions of 239 keV (30 percent abundance) and 521 keV (24 percent abundance). The labelled drug is added to an MDI canister together with cooled liquid propellants and surfactant. This technique provides an accurate and reproducible means of studying lung deposition but is complicated and not readily available since it requires a cyclotron to generate the radionuclide.

Recently, two new methods for the radiolabelling of drugs for delivery by metered dose inhaler have been reported. Vidgren *et al* (1987) have described a method in which a solution of sodium cromoglycate and ^{99m}Tc is spray-dried prior to its incorporation into a metered dose inhaler canister containing surfactant and propellants. A second method has been described by Köhler *et al* (1988) in which the beta$_2$-agonist fenoterol is labelled with ^{99m}Tc within MDI canisters. Both of these methods result in the radiolabelling of the actual drug particles themselves and so provide a more accurate means of tracing these particles directly.

Newman *et al* (1989) have employed similar techniques to Köhler to label sodium cromoglycate and salbutamol (Newman *et al*, 1991) with ^{99m}Tc so as to assess aerosol delivery from a pressurised MDI.

1.8.3 Bioavailability Methods for Evaluating Lung Deposition of Inhaled Drugs

Differences in metabolism after pulmonary and oral absorption can, for some substances, be used to calculate lung deposition. The proportions of drug and metabolite measured in the blood plasma and excreted urine of the subject is used to calculate the pattern of drug deposition. These studies, known as bioavailability studies, require sensitive assay methods to distinguish between the inhaled and swallowed fractions of the dose. Early methods used ^{3}H and ^{14}C to label the drug which was then detected in blood plasma and urine samples using a liquid scintillation spectrometer (Martin *et al.* 1970; Shenfield *et al*, 1976). Later, high pressure chromatographic methods of drug detection were used (Morgan *et al*, 1986). However, because of the low drug plasma concentrations of inhaled drug, detection in the blood is difficult. Concentrations of drug in urine are higher and two recent methods have developed techniques for separating and measuring, in excreted urine, the fraction of drug deposited in the lung and the swallowed fraction.

One of these methods, for evaluating pulmonary deposition of drugs that are not metabolised in the lungs, is to block the gastrointestinal (GI) uptake of swallowed drug with a solution of activated charcoal (Borgström and Nilsson, 1990). Drug detected in the urine is then assumed to have originated in the lungs. As an example, the beta$_2$-agonist terbutaline, inhaled by MDI, has been studied with a group of healthy volunteers

(Borgström and Nilsson, 1990). It was found that a solution of charcoal, swallowed shortly before an oral dose of terbutaline, adsorbs 97% of the drug preventing it from entering the system. It is assumed that this is the case with the swallowed portion of an inhaled dose and that its contribution to the overall systemic drug level is negligible.

In order to determine the absolute bioavailability of drug a reference formulation of deuterium-labelled terbutaline was given intravenously at the same time as the inhaled dose of terbutaline. Urine collected over a 48-hour period was analysed using gas-chromatography plus mass-spectrometry in order to separate and measure the non-labelled and deuterium-labelled drug. The two values were then compared to calculate the percentage absolute drug bioavailability to the lungs.

Another technique, to measure the relative bioavailability of inhaled salbutamol, is dependant on the urinary excretion of the drug in the first 30 minutes after inhalation (Hindle and Chrystyn, 1992). The method is based on experimental observations made when oral and inhaled drug were administered. After oral administration of 4 mg of salbutamol syrup, negligible amounts of both unchanged salbutamol and metabolite were found in urine samples taken in the first 30 minutes. A significantly larger proportion of unmetabolised salbutamol was recovered in the same time interval after inhalation of 400 μg of salbutamol from an MDI. The amount of metabolite was again negligible. However, two hours after administration the pattern of drug and metabolite recovered in urine resembled that recovered for the oral dose, after a similar time interval, with the metabolite predominate. The level of drug collected in the first 30 minutes after inhalation is consistent with results obtained following the placement of salbutamol directly into the lung by bronchoscope (Shenfield *et al*, 1976). In this case the salbutamol was not metabolised in the lungs but rapidly appeared in the blood plasma and urine in the unchanged form. In addition, it has previously been shown that the swallowed portion of an inhaled dose is absorbed and metabolised in the same way as an oral dose, with the metabolite exceeding the free salbutamol, and appearing in the plasma more slowly than the inhaled portion (Walker *et al*, 1972a).

A study to measure optimal inhaler technique for MDIs using the amount of unmetabolised salbutamol excreted in the urine during the first 30 minutes has been reported (Hindle *et al*, 1993). A high performance liquid chromatography method was used to measure concentrations of unchanged salbutamol and its sulphate metabolite in urinary excretion. Several different inhalation procedures were performed. After each inhalation the amount of salbutamol collected in the urine in the first 30 minutes was considered to be representative of the fraction of drug delivered to the lungs. The results were then compared to find the optimal inhalation technique.

1.9 Objectives of the Work Described in this Thesis

The main objective of the work reported in this thesis has been to provide a more complete understanding of the deposition pattern of salbutamol following inhalation in the human respiratory tract. In particular, it has been an ambition to study the deposition of drug delivered by MDI and DPI devices under as typical conditions as possible. Further objectives have been to assess and compare the clinical effect of a given dose and deposition pattern of drug and to simultaneously measure inspiratory flow patterns .

Of the methods currently available for studying the deposition of inhaled drug only radionuclide labelling and imaging methods offer the potential of directly visualising the *in-vivo* drug distribution. One of the advantages of this is that regional lung deposition may be assessed. This potential is only partly realised with indirect radiolabelling methods due to the uncertainty as to whether the substitute particles are deposited in the same way as the drug particles. The main drawback with radiolabelling methods has been the lack of suitable direct labelling techniques. Those that have been available are complicated and require a cyclotron (Short *et al*, 1981). However, methods such as those of Vidgren *et al* (1987) and Köhler *et al*, (1988) are a reliable and less complex alternative to indirect labelling techniques using equipment that is more widely available.

A method to directly radiolabel salbutamol particles with the radionuclide technetium-99m, based on that of Köhler *et al*, (1988), has been developed as the main tool for this investigation. The method has been applied to MDI and DPI salbutamol formulations enabling the fate of the drug delivered from these devices to be studied using a gamma

camera. Validation of the method has been achieved using an eight stage impaction device (Andersen cascade impactor). This showed that the salbutamol and ^{99m}Tc deposited in each stage in similar proportions and indicated that radiolabelling of salbutamol was successful over a wide range of particle sizes. Unlabelled salbutamol was also studied using the Andersen impactor and the results were very similar indicating that the addition of ^{99m}Tc to the salbutamol did not significantly alter its distribution.

Clinical studies were undertaken in which ten normal subjects and nineteen patients with asthma inhaled radiolabelled salbutamol from the DPI, the MDI and the MDI with a Volumatic (Allen & Hanbury's Ltd) spacer attachment. For these studies the DPI that was used was the Diskhaler inhaler (Allen & Hanbury's Ltd) (Section 1.5.2). Inspiratory flow rates were measured and the patient's lung function parameters were recorded before and after receiving the preparation.

Very little previous work has been undertaken to investigate the deposition patterns of salbutamol in the lungs, particularly from a DPI. Also, this study is the first to investigate the deposition of drug inhaled from the Diskhaler inhaler using radiolabelling techniques. No other studies have simultaneously measured inspiratory flow rates, lung deposition and clinical effect.

Chapter 2. The Preparation of Radiolabelled Salbutamol in a Metered Dose Inhaler and a Diskhaler Inhaler.

2.1 Introduction.

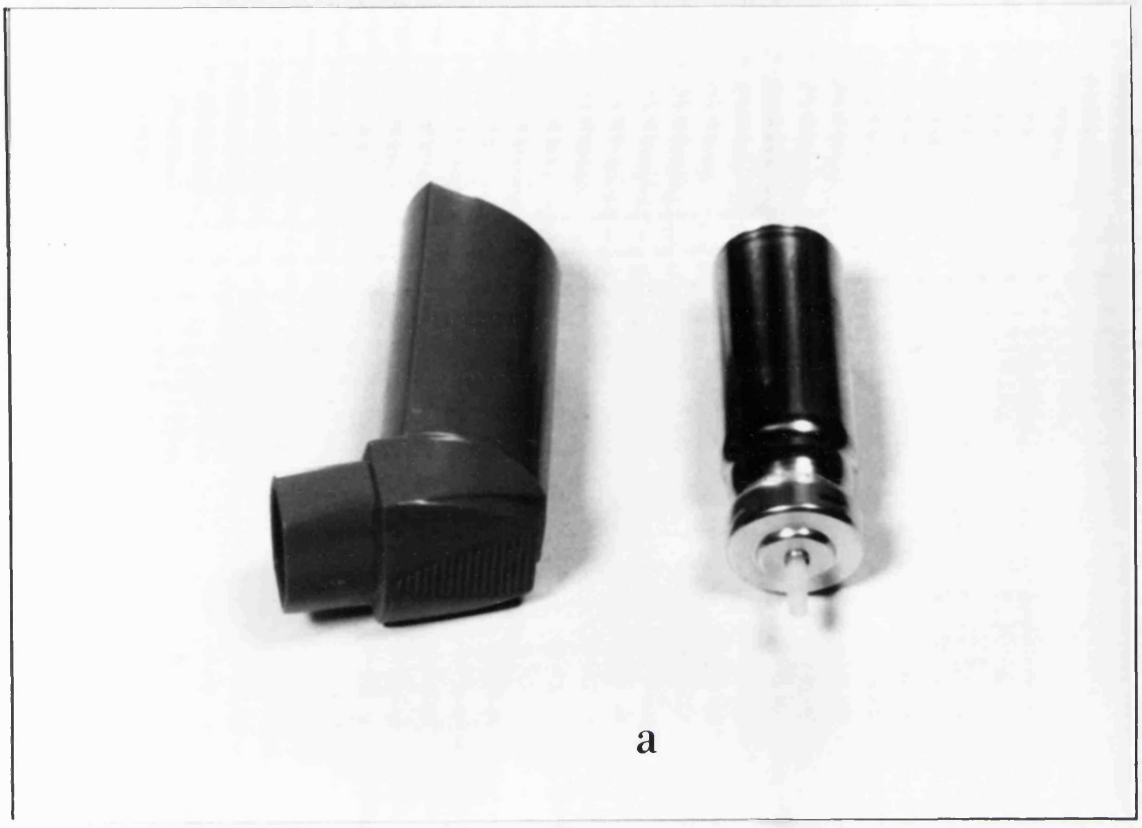
Köhler's method for the radiolabelling of $beta_2$-agonists with the radionuclide Technetium-99m (Köhler *et al*, 1988) has formed the basis for the labelling methods that are described in this thesis. However, it is limited to MDIs and its main weakness is that it requires the addition of extra propellant and surfactant to an already complete inhaler. This means that its formulation is altered.

A largely new method has been developed with the aim of producing good quality MDIs and DPIs from basic ingredients including salbutamol that has been labelled with ^{99m}Tc. Inhalers have been made in accordance with standard inhaler formulations as an alternative to the reconstitution of existing inhalers and without the need to add extra propellant and surface active agent. The metered dose inhaler with its actuator and the Diskhaler inhaler with an 8 dose disk pack are shown in Figure 2.1 (a) and (b) respectively.

2.2 Review of Köhler's Method

Köhler's method is to extract ^{99m}Tc in the pertechnetate form ($^{99m}TcO^-_4$), as eluted from a molybdenum/technetium generator, out of the original water phase with ethyl methyl ketone (Butanone) by mixing them together and allowing the two phases to separate. The butanone phase containing the $^{99m}TcO^-_4$ is collected in a glass beaker and evaporated to dryness. After cooling, Freon 11 with 1% surfactant (sorbitantrioleate) is added and evaporated to a volume of 0.2 ml by continuous stirring with a glass rod. The remaining liquid is aspired with a 1 ml syringe and immediately inverted to prevent its expulsion due to the vapour pressure. A commercial MDI canister containing fenoterol (Berotec) is cooled to -60°C and a hole pierced in its base. The syringe containing the ^{99m}Tc, freon and surfactant is then emptied into the canister through the hole. The hole is sealed with a tin screw and latex seal. The canister is then shaken and rewarmed.

Köhler *et al* (1988) reported that more than 90% of the added ^{99m}Tc is dissolved in the drug in relation to its volume. It is thought that labelling of the drug occurs because of the greater solubility of $^{99m}TcO^-_4$ in micronised $beta_2$-agonist drug than in the propellant with surfactant.



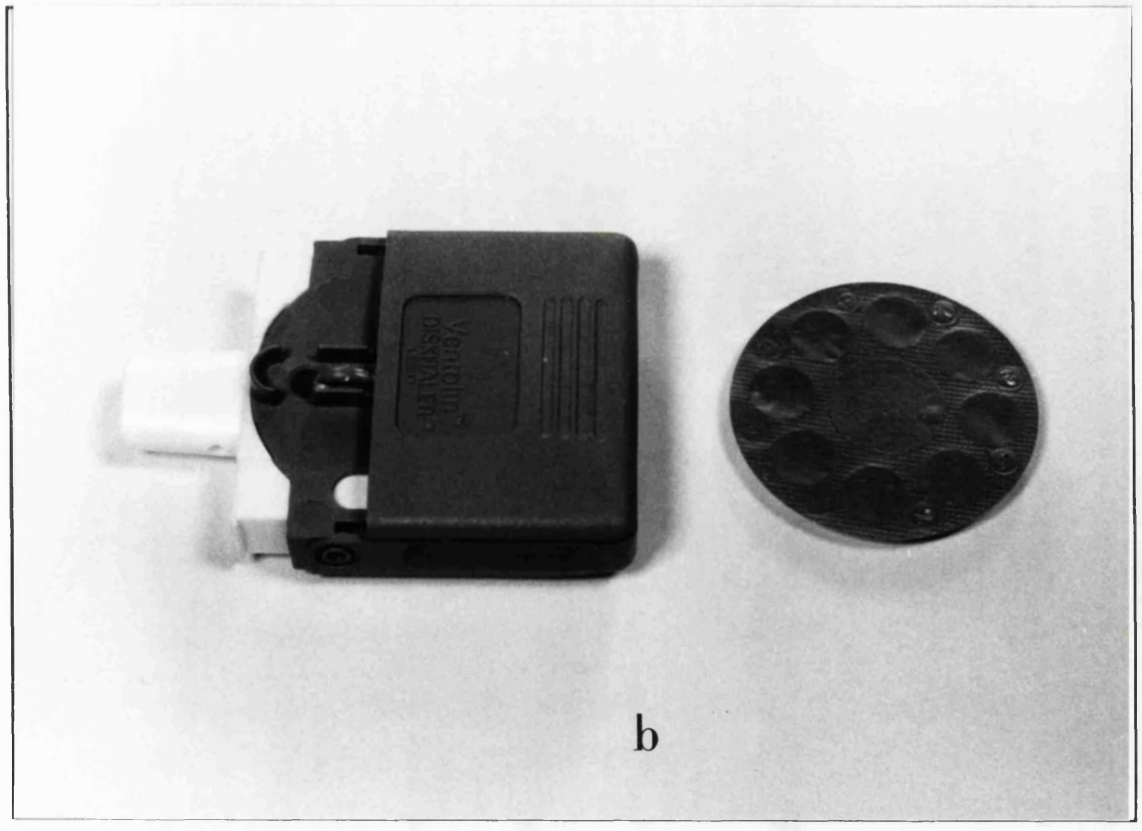


Figure 2.1. (a) ***Metered dose inhaler canister with its actuator*** (b) ***Diskhaler inhaler with an 8 dose disk pack.***

Some preliminary work was undertaken to verify some of the findings of Köhler and co-workers and to investigate the application of their method to the radiolabelling of salbutamol in metered dose inhalers (G.M.Clarke, personal communication, 1988). The ^{99m}Tc pertechnetate was extracted into the butanone as described by Köhler and collected into a glass beaker. Evaporating the butanone left the ^{99m}Tc dried onto the glass. Initially, the method of adding propellant and surfactant alone to the "active" beaker to remove the ^{99m}Tc for later addition to the cooled canister was assessed.

As a comparison, a well blended suspension of salbutamol and trichlorofluoromethane (propellant 11) together with surfactant (oleic acid), in solution with further propellant 11, was added to a similarly produced "active" beaker. It was demonstrated that the P11/surfactant solution alone did not solubilise the sodium pertechnetate adequately; instead a suspension of crystals formed in the propellant 11. The second method gave a much improved uptake of ^{99m}Tc from the glass beaker. This technique is also more adaptable since it is possible to add the radionuclide to the drug independently, allowing it to be further treated or studied prior to its use in the manufacture of inhalers.

The second method is more suitable for developing into a labelling technique which can be applied to the production of inhalers containing labelled drug particles. The emphasis of the initial stages of this investigation was therefore to develop an efficient and reproducible technique for producing inhalers of a high standard which can be used to study aerosol drug deposition in the lung.

2.3 Assessment of Inhaler Performance with the Twin Impinger.

The twin impinger apparatus (Apparatus A Appendix XVII C, page 204, British Pharmacopoeia, 1988) is a two stage instrument which can be used for the routine assessment of aerosol quality. It is a convenient and reliable means of distinguishing good and poor aerosols (Hallworth and Westmoreland, 1987). An aerosol entering the instrument is fractionated by passing through a simulated throat and then through an impinger stage of defined aerodynamic particle size cutoff characteristics.

Stage 1 of the apparatus has an effective aerodynamic particle cutoff size of 6.4 μm at an airflow rate of 60 l/min. Droplets/particles with an aerodynamic diameter of 6.4 μg have a 50% probability of progressing beyond the first stage and depositing in the second stage. Droplets/particles larger than this size have progressively less probability of reaching stage two and particles larger than 10 μm have virtually no probability of reaching it.

In order to ensure that individual inhalers manufactured were consistent in their performance and of suitable quality, the twin impinger was used during the development of the radiolabelled aerosols and as a means of ensuring the quality of each inhaler used in the clinical studies. The instrument was assembled as shown in Figures 2.2 and 2.3 and consisted of a throat with a moulded rubber mouthpiece adaptor, an upper stage (stage 1), a lower stage (stage 2) and a vacuum pump. Methanol to the volumes of 7ml and 30ml were introduced into stages 1 and 2 respectively. Air was then drawn through the instrument at a flow rate of 60 l/ min.

Each MDI was shaken vigorously and the first ten actuations were discharged to waste in order to prime the metering chamber. The canister was then weighed using a Mettler PE300 balance. The actuator, with canister in place, was inserted into the moulded rubber mouthpiece adaptor, the pump was turned on and ten actuations were discharged into the apparatus. The canister was re-weighed to calculate mean shot weight. The twin impinger was dismantled and methanol used to wash each stage in order to remove the salbutamol. This was collected into two volumatic flasks and the radioactivity and drug content were measured for each. The actuator and valve stem were washed in the same way and the methanol was collected into a third flask.

The performance of each Diskhaler inhaler was similarly assessed using the twin impinger. In this case, the device was inserted into its mouthpiece adaptor, the blister pierced and the pump turned on for 2 seconds, to empty each of the eight unit dose blisters in turn. The apparatus and inhaler were again washed with methanol and the radioactivity and drug content of each collection flask measured. The mouthpiece adaptor used for the Diskhaler inhaler is slightly different to that used for the MDI as its mouthpiece is a different size and shape.

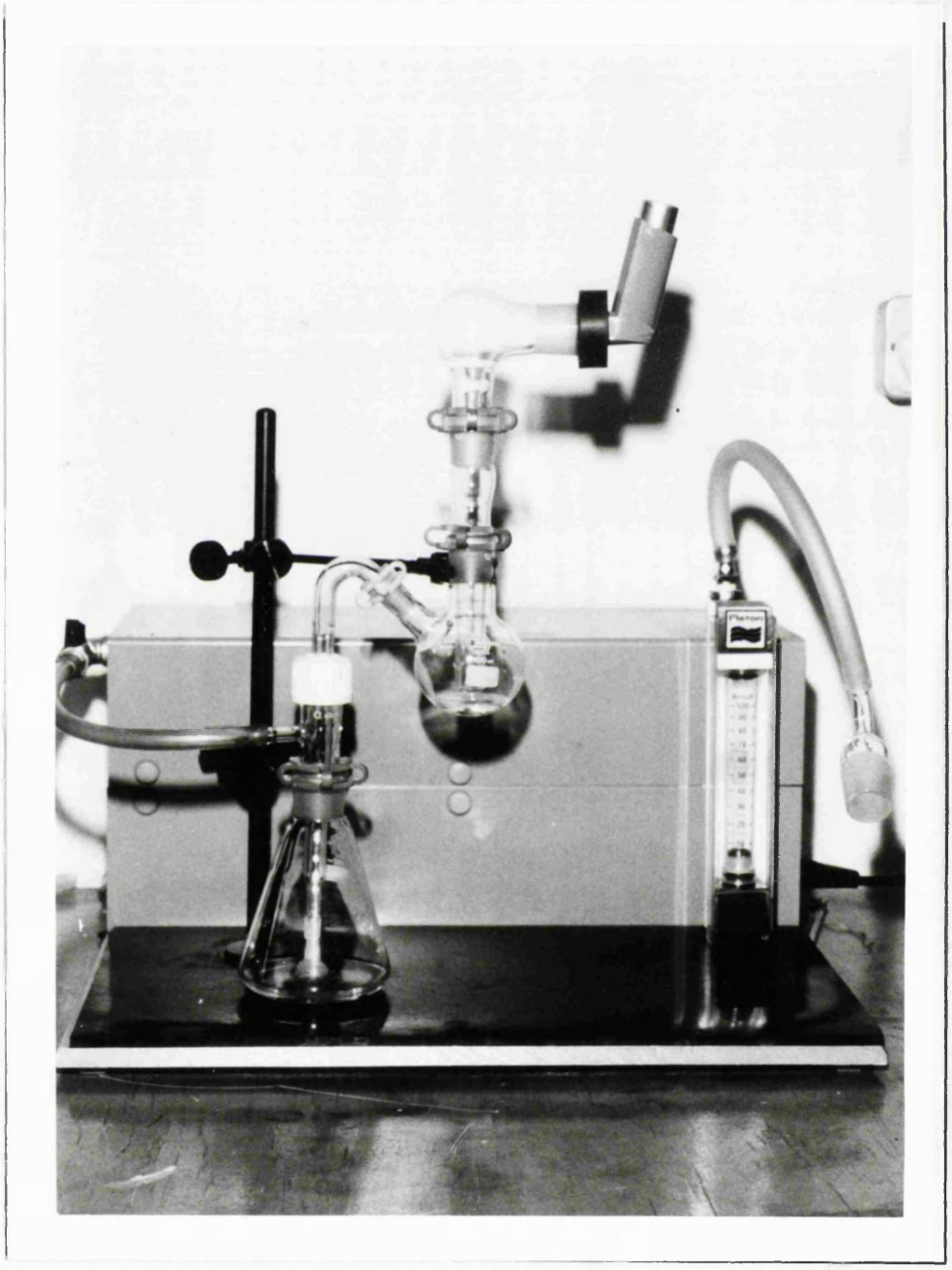


Figure 2.2. ***Twin impinger with MDI inserted into the mouthpiece***

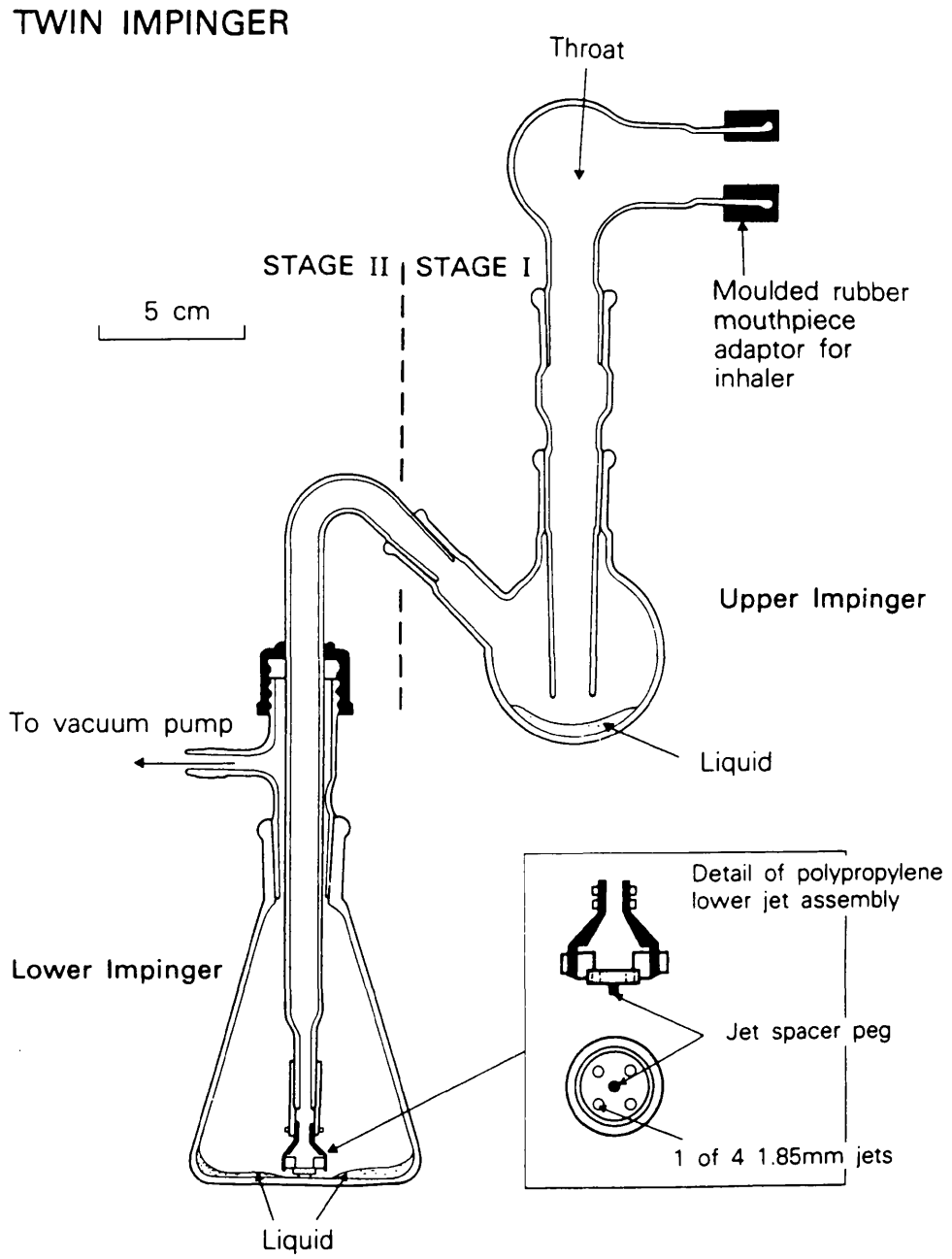


Figure 2.3. ***Schematic diagram of twin impinger.***

The following quality control criteria were applied in order to assess the performance of each inhaler manufactured.

2.3.1 Metered Dose Inhaler Quality Control Criteria.

The MDI was considered satisfactory if:

(i) the weight per actuation was between 80 and 90 mg;

(ii) the total amount of salbutamol per actuation was between 80 and 120 μg;

(iii) the fraction of salbutamol deposited in stage 2 of the apparatus was greater than 40% of the total amount of salbutamol delivered;

(iv) the ratio of radioactivity and drug in each stage of the apparatus was between 90 and 110%

2.3.2 Diskhaler Blister Packs Quality Control Criteria.

The contents of the Diskhaler blisters were considered satisfactory if:

(i) the total amount of salbutamol per blister was between 160 and 240 μg;

(ii) the fraction of salbutamol deposited in stage 2 of the apparatus was greater than 10% of the total amount of salbutamol delivered;

(iii) the ratio of radioactivity and drug in each stage of the apparatus was between 90 and 110%

2.4 Radiolabelling Methods

The success of any radiolabelling technique depends on the degree of radionuclide uptake by the compound being investigated. It must also be evenly distributed amongst the drug particles to enable meaningful conclusions to be made about the behaviour of the drug itself. The first stage of this investigation was to assess the available data. This was related to MDIs only.

The process by which the ^{99m}Tc was transferred to the salbutamol particles can be summarised into two main steps:

(1) The separation and extraction of the ^{99m}Tc pertechnetate from the original sodium chloride into butanone followed by its transfer to a glass beaker which was heated to allow the evaporation of the butanone;

(2) The transfer of the ^{99m}Tc from the glass beaker to the salbutamol - *either* (i) by initial up-take by propellant and surfactant followed by its addition to cooled inhaler (Köhler *et al*, 1988) *OR* (ii) by its transference directly from the glass beaker to the drug (G M Clarke, personal communication, 1988);

Step (2) (ii) was performed by adding salbutamol, propellant 11 and a solution of oleic acid and propellant 11 to the beaker and blending using a Silverson high shear mixer for 20 minutes.

2.4.1 Experimental Procedures for Large Batches of Suspension

Sufficient salbutamol/propellant 11 suspension was made for thirty inhalers of 240 doses each, according to the formulation given in section 2.5. A small volume of eluate from a technetium-99m generator (Elumatic; CIS,France), containing a mean level of activity of 230 MBq ^{99m}Tc as sodium pertechnetate in normal saline, was made up to 2 ml in a syringe with sterile water. The solution was added to a 20 ml separating funnel together with an additional 3 ml of sterile water and 5 ml of butanone. The separating funnel was then shaken for 3 minutes. During this process most of the activity was extracted into the butanone phase. The lower aqueous phase was discarded and the butanone phase was collected in a 250 ml beaker which was then placed on a hot plate (100^{0}C) and evaporated to dryness.

After the beaker had cooled 10g of a previously prepared solution containing oleic acid (79.2 mg) and propellant 11 was weighed into the beaker together with a similar quantity of propellant 11 and 792 mg of salbutamol. More propellant 11 was weighed into the beaker to give approximately 80% of the required final weight of 171 g. The resulting

suspension was blended using a Silverson high shear mixer for 20 minutes. The homogeneous suspension was made up to 171 g with further propellant 11, and transferred to a 250 ml separating funnel, before being dispensed from there into empty MDI canisters.

Propellant 12 was initially dispensed under pressure, using a Pamasol gaser, following crimp-sealing of the canister with a metering valve. However, it proved difficult to accurately dispense the small quantities required for each aerosol and instead propellant 12 was discharged from a pressurised cylinder into a vacuum flask. It was then poured as a cold liquid directly into cooled canisters containing the propellant 11/salbutamol suspension. A metering valve was then crimped rapidly onto each canister, which after rewarming was ready to use.

2.4.2 Distribution of Radioactivity in Centrifuged Drug Suspensions

A comparatively simple experiment was performed on the suspensions to investigate the relationship between the ^{99m}Tc and the micronised salbutamol in suspension. Samples from suspensions were divided into two equal portions which were placed into two test tubes and centrifuged at 2000 r.p.m. for 20 minutes. Each test tube was then imaged close to the collimator of one of the heads of a Siemens dual-headed gamma camera (Chapter 5). Data were acquired for 60 seconds. Three regions of interest were drawn over the acquired image using a light pen, dividing it into top, middle and bottom regions.

In the initial experiments, a large solid pellet of activity, with a density greater than either that of the salbutamol or propellant 11 was sometimes present. This was assumed to be a technetium salt insoluble, or poorly soluble, in the propellant 11. However, this problem which was thought to be related to the sodium chloride content of the initial solution as eluted from the generator, was resolved by using a smaller volume of higher specific activity ^{99m}Tc eluate and diluting it to 5 ml with sterile water. Subsequent results from seven batches of suspensions gave a mean value of 85% of the radioactivity in the top layer of the test tube. This corresponded to the salbutamol which was seen in a layer on the surface with the body of the test tube almost completely clear. An example of the gamma camera image obtained in one experiment is shown in Figure 2.4(a).

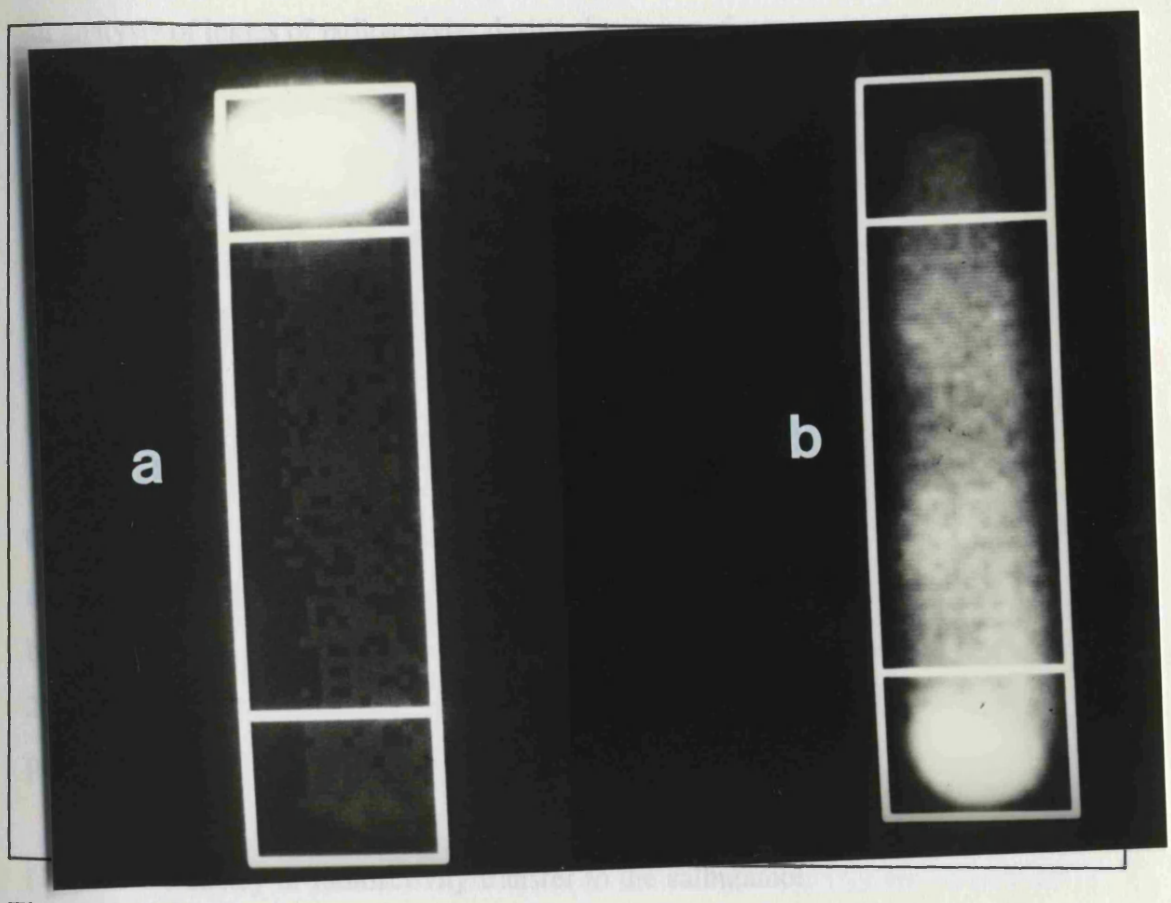


Figure 2.4. (a) ***Gamma camera image of a sample of salbutamol labelled with ^{99m}Tc suspended in propellant 11 and oleic acid in a test tube and centrifuged at 2000 r.p.m. for 20 minutes. Regions of interest indicate radioactivity in the top, middle and bottom of the test tube.*** (b) ***Gamma camera image of a test tube containing propellant 11, oleic acid and ^{99m}Tc only. No salbutamol was present. Every other condition was the same as for*** (a).

A control experiment was performed where no salbutamol was added to the preparation but every other step in the process was the same as above. The propellant 11/oleic acid solution was transferred to two test tubes and centrifuged again at 2000 r.p.m. They were imaged as before and the activity measured. The distribution of the radioactivity was quite different to that when salbutamol was present with much of the activity found in the bottom of the test tube (Figure 2.4(b)). In addition, a much lower amount of radioactivity was present in the test tubes compared to when salbutamol was present. These experiments were repeated, subsequent to the refinement of the radiolabelling process, and an analysis of losses of radioactivity during the stages of preparation shown in Table 2.3.

2.4.3 Efficiency of ^{99m}Tc Transfer and Reliability of MDI Aerosols

Preliminary MDI twin impinger analysis using large batches of suspension were promising with regard to stage 2 results (Table 2.2); there was a good match between the drug and radionuclide and they were both above 40%. However it was necessary to reduce the batch size from thirty to six in order to increase the radioactivity that was available to each inhaler.

One consequence of reducing the amount of suspension produced was that the Silverson mixer could no longer be used in the blending process as it was necessary for its head to be completely covered by suspension. An alternative blending technique was to place the 250 ml beaker in an ultrasonic bath for 5 minutes. However, the inhalers that were produced in this way were characterised by two main problems.

(1) poor efficiency of radioactivity transfer to the salbutamol.
(2) poor reliability with which inhalers were made as indicated by poor twin impinger performance.

The main reason for (1) was relatively poor and irregular uptake of ^{99m}Tc from the glass beaker; as much as 80% of the ^{99m}Tc was sometimes transferred to the salbutamol, although it was often much less than this. Inevitably, due to the large surface area of the 250 ml beaker, much of the salbutamol and ^{99m}Tc were left dried to the glass and evaporation of the propellant was a problem with the small amounts of suspension. This

meant that, even though the salbutamol and propellants were initially accurately weighed and dispensed, at the end of the blending process there were significant losses which led to (2) poor reliability of aerosols and poor twin impinger stage 2 deposition and weight per actuation.

The blending process is crucial to the successful production of the inhalers since it is essential for the salbutamol suspension to be homogeneously mixed, and it is during this process that the ^{99m}Tc is transferred to the salbutamol.

2.4.3.1 Experimental Procedures

The efficiency of the labelling process as a whole was examined by recording the radioactivity that was retained at each stage, including the amount reaching the inhaler canisters. A series of experiments were performed in which the size and shape of the blending vessel were varied as well as the blending time, batch size and dose per can.

In place of the 250 ml beaker a 20 ml conical beaker, a 25 ml beaker and a 20 ml screw top glass vial (scintillation vial) were used. The blending time was increased from 5 to 20 minutes and the batch size reduced from 1/5th of the original size (6 cans each of 240 doses) to 1/10 of the original size (6 cans of 120 doses *or* 3 cans of 240 doses).

The twin impinger was used to assess the performance of canisters manufactured by the modified processes in order to see how their performance was affected.

The efficiency of butanone extraction and the labelling efficiency are defined as:

$$\textbf{Efficiency of Butanone Extraction} = \frac{\textbf{Activity transferred to dried beaker}}{\textbf{Activity initially in 20 ml sep. funnel}} \times 100\%$$

$$\textbf{Labelling Efficiency} = \frac{\textbf{Beaker activity before P11/surfactant added} - \textbf{Beaker activity after P11/surfactant removed}}{\textbf{Beaker activity before P11/surfactant added}} \times 100\%$$

2.4.3.2 Results

Table 2.1 shows the mean labelling efficiency and the mean activity in the canisters for six experiments (A - F) in which the blending process and the batch size were varied. The extraction process was exactly the same for each experiment and the mean value and SD of the efficiency of butanone extraction was 61.0 (8.8)%. Table 2.2 shows the twin impinger stage 2 deposition and drug recovery per actuation for MDIs produced using five of the techniques shown in Table 2.1.

It can be seen from Table 2.1 and Table 2.2 that labelling method F, in which three canisters of 240 doses each were made and the suspension was blended in the 20 ml screw top glass vial, was the most efficient and gave the most reliable and acceptable twin impinger results. The mean labelling efficiency was 88.6 (SD 8.6)% with a mean of 18.2 (SD 1.7)% of the initial radioactivity reaching each canister compared to 70.2 (SD 10.4)% and 2.1 (SD 0.7)% for the original thirty inhaler method. The twin impinger analysis indicated that a mean of 43.9 (SD 3.9)% of the radioactivity and 47.9 (SD 4.3)% of the salbutamol reached the second stage and the mean weight of salbutamol per actuation was 87.0 (SD 11.3) μg.

The screw top vial was sealed during the blending process to prevent the escape of suspension and the evaporation of propellant; both a problem with previous methods. Also the small volume and relatively low surface area meant more ^{99m}Tc was removed and less salbutamol remained dried to the glass.

In an attempt to further increase the amount of radioactivity that was available to the inhaler and to reduce losses, one additional experiment was performed in which the butanone containing the ^{99m}Tc was collected in the empty canister instead of the glass vessel. The canister was heated (100^{0}C) and the butanone evaporated using warm air. The canister was then cooled and a pre-blended suspension of unlabelled salbutamol/P11/surfactant in the correct proportions was added. The propellant 12 was added as before and a metering valve crimped into place. The canister was then shaken using a mechanical shaker for 30 minutes. After allowing the canister to stand it was imaged using the gamma camera. This showed that virtually all of the radioactivity was fixed to the base of the canister and had not labelled the salbutamol.

Batch Size:	A	B	C	D	E	F
Doses per can Number of Cans	240 30	240 6	120 6	120 6	240 3	240 3
	n = 3	n = 3	n = 4	n = 5	n = 2	n = 3
Mean % Labelling Efficiency (SD)	70.2 (10.4)	43.8 (10.1)	53.7 (9.9)	56.5 (24.8)	65.7 (26.9)	88.6 (8.6)
Mean % Can Activity (SD)	2.1 (0.7)	5.0 (0.5)	6.0 (1.4)	5.3 (2.6)	15.3 (6.0)	18.2 (1.7)
Blending Process	Silverson Blender 20 mins 250 ml beaker	U/S bath 5 mins 250 ml beaker	U/S bath 5 mins 250 ml beaker	U/S bath 5 mins 20 ml conical	U/S bath 5 mins 25 ml beaker	U/S bath 20 mins 20 ml vial with lid

Table 2.1 ***Results from investigations to improve efficiency of transfer of ^{99m}Tc from glass vessel to salbutamol suspension during the manufacture of MDIs. Batch size, size and shape of vessel and blending time have been varied. Also shown are the percentage of initial activity measured in each canister and number of repeat experiments, n.***

Batch Size	A	B	C	E	F
Doses per can	240	240	120	240	240
Number of cans	30	6	6	3	3
	n=7	n=26	n=8	n=9	n=10
Twin Impinger Stage 2					
Activity % (SD)	41.6 (10.0)	38.6 (7.1)	34.2 (2.6)	37.6 (3.2)	43.9 (3.9)
Salbutamol % (SD)	43.6 (6.1)	39.4 (5.9)	38.4 (3.5)	38.2 (6.8)	47.9 (4.3)
Drug Deposition Recovery (Dose per actuation, μg)	85.0 (16.8)	92.1 (11.2)	125.4 (28)	85.1 (14.3)	87.0 (11.3)
Blending process	Silverson bath 20 mins. 250 ml beaker	U/S bath 5 mins 250 ml beaker	U/S bath 5 mins 250 ml beaker	U/S bath 5 mins 25 ml beaker	U/S bath 20 mins. 20 ml vial with lid

Table 2.2 *MDI Twin impinger stage 2 deposition, for radioactivity and salbutamol, for 5 of the methods of Table 2.1. Also given are the mean dose of salbutamol per actuation. Number of repeat experiments = n.*

Activity in Apparatus	Salbutamol Present %	No Salbutamol %
Left in syringe	0	0
In aqueous waste	39.1	29.4
Left in empty vial	6.7	61.0
Left in 20 ml separating funnel	1.1	1.0
Left in 250 ml separating funnel	1.3	0
In test tube A	26.6	4.4
In test tube B	27.3	4.4
Recovered	102.1	100.2

Table 2.3 ***Comparison of percentage radioactivity at each stage of the radiolabelling process, for repeat centrifuge experiments, when salbutamol was added and when no salbutamol was added.***

2.4.4 Repeat of Centrifuge Experiments

The centrifuge experiments of Section 2.4.2 were repeated following refinement of the radiolabelling process according to method F. The patterns of radioactivity and salbutamol distributions were the same as before. In addition, the quantities of radioactivity that were retained at each stage of the process were measured and are shown as percentages of initial radioactivity in Table 2.3. Only 12.6% of the ^{99m}Tc that was available in the glass vial during the preparation was transferred to the propellant/surfactant alone. This is in contrast to a labelling efficiency of 89% when salbutamol was present and indicates that the radionuclide binds mainly to salbutamol and not to propellant 11 or surfactant.

2.5 Protocol for Labelling of Salbutamol in the MDI

Metered dose inhalers were produced according to the following formulation supplied by Glaxo Group Research Ltd:

Materials	per 85 mg	per can	per batch of 30 inhalers
Salbutamol	110 μg	26.4 mg	792 mg
Oleic Acid	11 μg	2.64 mg	79.2 mg
Propellant 11	to 23.75 mg	to 5.7 g	to 171 g
Propellant 12	61.25 mg	14.7 g	441 g

Table 2.4 ***Formulation of MDI.***

Inhalers should conform to the following specifications:

Nominal shot weight = 85 mg
Number of shots per can = 240
Nominal weight of salbutamol per shot = 100 μg

Each inhaler was prepared and documented according to pre-written and approved batch sheets which were adhered to throughout the investigation period. On each study day, three 240 dose canisters containing labelled salbutamol and propellants were prepared for use. A well shielded radionuclide work area within a fume cupboard was used throughout the preparation and lead equivalent gloves were used where necessary. A Mettler PE300 balance and a Sartorius 1201 microbalance were used to measure the weight of materials and suspensions.

Firstly, a solution containing 158.4 mg of oleic acid and made up to 100 g with propellant 11 was prepared and stored in a refrigerator until required. A volume of no more than 1 ml of sodium pertechnetate, eluted from a Molybdenum-99/Technetium generator, containing 11000 MBq of radioactivity was drawn up into a shielded syringe. It was transferred to a 20 ml separating funnel together with 4 ml of sterile water (to dilute the saline content) and 5 ml of Analar grade butanone.

With the lid in place, the funnel was shaken for 3 minutes so that its contents were well mixed. During this process approximately 60% of the $^{99m}TcO^-_4$ was transferred to the butanone phase. After allowing the two phases to separate, the lower aqueous phase was discarded and the organic phase collected into a 20 ml screw top glass vial.

The open vial was then placed on a hot plate at 100°C and evaporated to dryness by passing warm air over it, leaving the $^{99m}TcO^-_4$ dried onto the base. After allowing it to cool, the vial was placed on a tarred balance and 5 g of the previously prepared oleic acid/propellant 11 solution was added to the vial together with 79.2 mg of micronised salbutamol (the ratio of oleic acid to drug was 10%). Further propellant 11 alone was added until the weight of the suspension was 17.1 g. The lid was firmly screwed onto the glass vial which was then placed in an ultra-sonic bath for 20 minutes. During this process the TcO^-_4 was adsorbed onto the salbutamol. After removing from the ultrasonic bath the vial was weighed again to ensure that no losses of propellant due to evaporation had occurred. Any such losses were immediately replaced with propellant 11.

The suspension was transferred to a 250 ml separating funnel which had a fine aperture to enable accurate pouring. Next, 5.7 g of this suspension was carefully weighed into each, cooled, empty MDI canister. Immediately, 14.7 g of cold liquid propellant 12 was dispensed from a vacuum flask into the canister. Finally, a metering valve was crimped on to each canister. It was necessary for these last steps to be performed as quickly as possible to minimise the formation of water condensation within the canisters. Each canister was placed in the ultrasonic bath for 5 minutes before use.

2.6 Protocol for Labelling Of Salbutamol in the Diskhaler Blister Packs.

The drug component of the dry powder inhalers was salbutamol sulphate instead of salbutamol base. Experiments were performed to see if the radiolabelling process used to treat the salbutamol base in MDIs could be applied to the salbutamol sulphate. A suspension of propellant 11 and salbutamol sulphate, this time without surfactant, was made up. The suspension was blended in a 20 ml glass vial containing the ^{99m}Tc dried to the base, as before. It was then necessary to remove the propellant, by evaporation, and mix the resulting powder with lactose in the correct proportions.

The labelling efficiency of transfer of radionuclide from the vial to the powder was 41.8 (SD 6.1)% This was lower than for the MDI because of the difficulty in recovering all of the powder which was dried to the glass. A percentage of 28.3 (SD 4.7) of the initial radioactivity was in the final powder blend. Batches of dry powder for the Diskhaler blister packs were manufactured according to the formulation given in Table 2.5.

Ingredients	Quantity per 25 mg	Batch Quantity per 10g
Salbutamol Sulphate (micronised)	0.241 mg	96.4 mg
Lactose	24.76 mg	9.904 g

Table 2.5 ***Formulation of Diskhaler blister packs.***

Base equivalent weight = 1.205.

The initial stages of preparation involving the extraction of the $^{99m}TcO^-_4$ into the butanone and transfer to glass vial were exactly the same as in the MDI preparation (Section.2.5).

After allowing the vial to cool, approximately 10 g of propellant 11 was added together with approximately 60 mg of salbutamol sulphate. The lid was placed firmly onto the vial which was then placed in the ultra-sonic bath for 20 minutes.

Following this, the lid was removed from the vial and the propellant 11 was allowed to evaporate with the assistance of warm air. The dried labelled salbutamol was then recovered from the vial with a spatula and carefully weighed. It was then blended with lactose carrier in the ratio of 1:102.7. The salbutamol sulphate was placed into a mortar and the lactose gradually added while thoroughly mixing with a pestle. In addition to mixing the salbutamol sulphate and lactose, this action served to break up any agglomerates of particles that had formed.

The well blended powder was then carefully weighed using a microbalance and was placed into individual blisters of a preformed Diskhaler pack base. Each of the eight blisters was filled with 25 ± 5 mg of powder which contained 200 ± 4 μg of salbutamol (base equivalent weight = 1.205). Foil was then placed over the Diskhaler base and all the blisters covered. Finally, a hot iron was used to heat the foil and seal the blisters. Excess foil was cut away and the preparation was then ready to use.

2.7 SEM of Labelled and Unlabelled Particles

A scanning electron microscope (SEM) was used to examine the treated and untreated salbutamol and salbutamol sulphate in more detail. A suspension of radiolabelled salbutamol base was prepared in the manner described earlier. However instead of pouring it into an MDI canister the propellant 11 was allowed to evaporate from the open vial, leaving a dry powder. This was removed with a spatula. A further sample of salbutamol base was prepared in the same way except that ^{99m}Tc was not used. Samples of labelled and unlabelled salbutamol sulphate were similarly prepared. The salbutamol sulphate was not mixed with lactose but a sample of lactose particles was examined separately. Samples of untreated salbutamol base and salbutamol sulphate were also examined.

Powder from each of the seven samples was sprinkled onto SEM stubs and coated with gold\palladium using a Polaron E5200 SEM autocoating unit. They were then placed in the SEM (Cambridge S200) and scanned at several magnifications. Photographs of the SEM images for the seven powder samples are shown in Figure 2.5 (a) - (g). The first six pictures were made at the highest magnification possible before image quality and sharpness deteriorated.

Very little difference can be seen between the unlabelled and labelled salbutamol base shown in Figures 2.5(b) and 2.5(c) respectively. The particles of untreated salbutamol base shown in Figure 2.5(a) are very irregular in shape while those from the treated samples are slightly more spherical. The particles of salbutamol sulphate that were treated but unlabelled (Figure 2.5(e)) and those that were radiolabelled (Figure 2.5(f)) appear very similar in size and shape to the untreated particles (Figure 2.5(d)). All of the drug particles shown here are below 5 μm although some slightly larger particles were measured on other pictures. However they were far smaller than the lactose particles with which the salbutamol sulphate would normally be mixed. The large particle of lactose shown in Figure 2.5 (g) is nearly 200 μm across.

The SEM has provided information about the appearance and size of the solid drug particles. However the most important information about the characteristics of the airborne particles was provided with a cascade impactor. This was used to measure the size distributions of both drug and radionuclide and validate the radiolabelling process prior to its use with volunteers. The details are given in the following chapter.

Figure 2.5 (a)

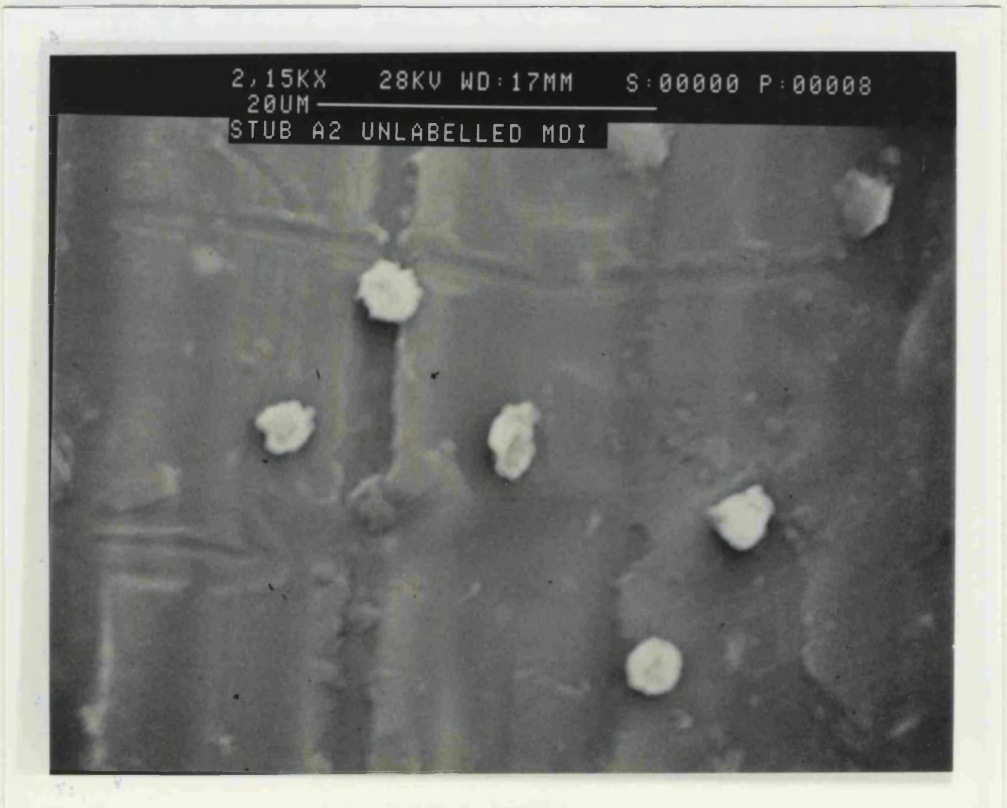


Figure 2.5 (b)

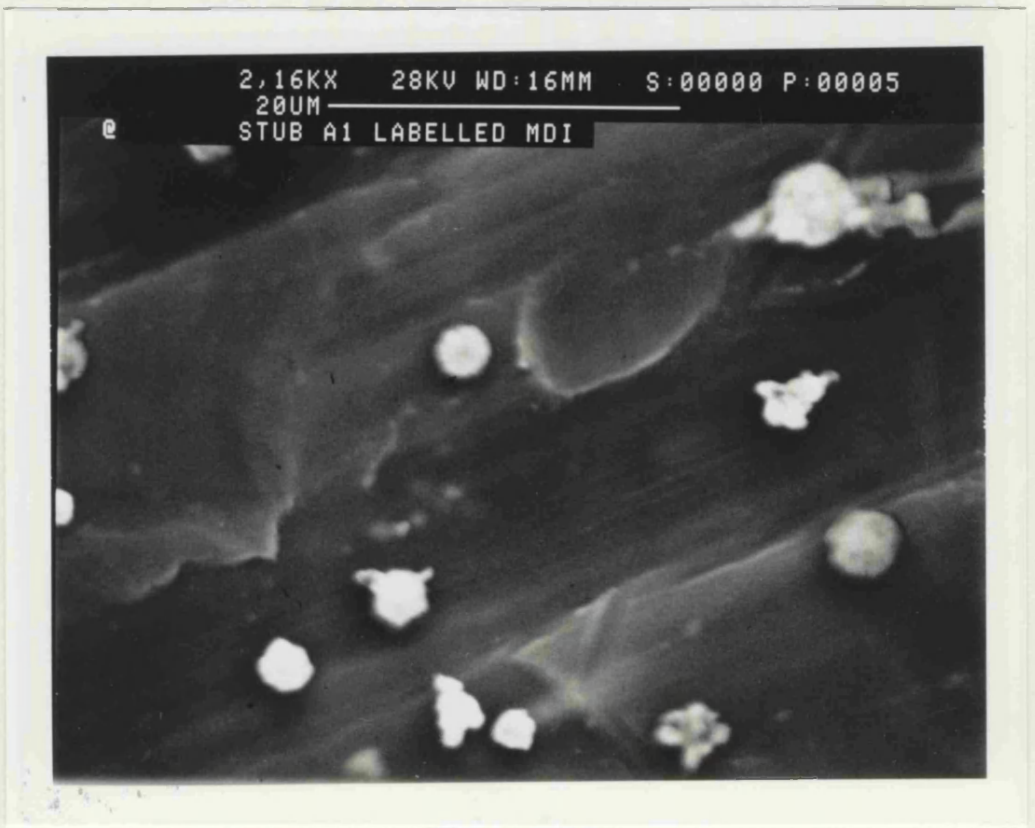


Figure 2.5 (c)

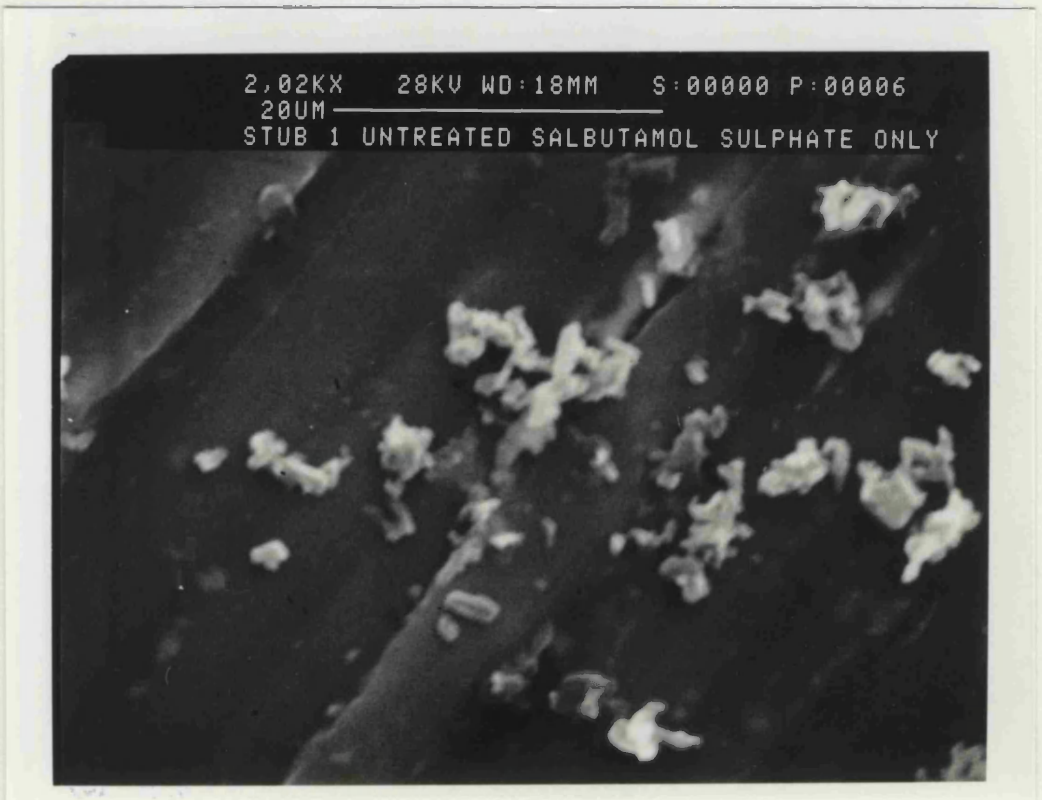


Figure 2.5 (d)

Figure 2.5 (e)

Figure 2.5 (f)

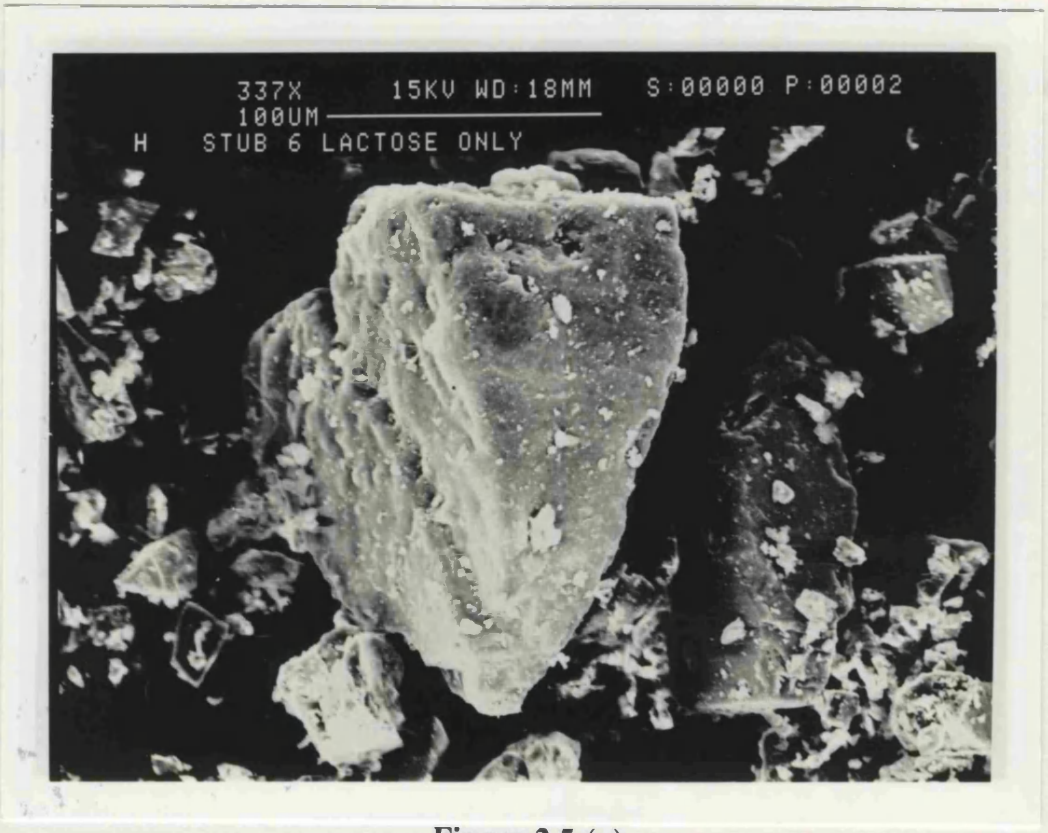


Figure 2.5 (g)

Chapter 3. *In-Vitro* Validation of Inhalers Using the Andersen Cascade Impactor.

3.1 Introduction

Particle size is one of the most important factors determining the deposition of inhaled substances in the lung. The human respiratory tract acts as an aerodynamic classifying system for incoming aerosols, with large particles more likely to be impacted in the upper airways (Section 1.4.3). The natural filtering action of the upper respiratory tract, and branching system of airways contribute to an important pulmonary defence mechanism preventing the penetration of unwanted large particles into the lungs. However, this same mechanism makes it relatively difficult to achieve penetration of therapeutic aerosols into the lungs.

Particles in the 1 to 5 μm range are considered to be the optimum size for penetration to all parts of the lungs including the large airways, the small airways and alveoli (Morrow, 1974; Clarke, 1988). However, a wide range of particle sizes is usually present with therapeutic aerosols making it difficult to predict the deposition pattern of inhaled drug particles. An important process in the assessment of aerosol penetration into the human respiratory tract is to measure the distribution of particle sizes present. Of the methods available for the measurement of aerosol particle size, cascade impaction is the most widely used and convenient technique available. It is also the only one to assess the whole of an emitted aerosol cloud thus allowing the measurement of the total mass distribution. In addition, cascade impactors characterise aerosol particles directly in terms of their aerodynamic diameter, the parameter most appropriate for describing aerosol particle behaviour (Hiller *et al*, 1978). The aerodynamic diameter takes into account the affect of all relevant physical factors, including size, shape, density and surface characteristics, that influence the behaviour of aerosol particles (Section 1.4.3.4). A further advantage of inertial impaction is that it is able to take into account the dynamic nature of the aerosol cloud emitted from an MDI; the sizes of the particles are rapidly changing due to the evaporation of propellants.

Previously, cascade impactors and multistage liquid impingers have been used to determine the particle size distribution of therapeutic aerosols (May, 1966; Polli *et al*, 1969; Hallworth and Andrews, 1976). Other, more empirical, devices have been developed to simulate the human respiratory tract in order to predict the distribution pattern within

the lungs without actually measuring particle size (Kirk, 1972; Davies *et al*, 1976). Some workers have gone further by taking into account the high relative humidity and temperature of the lung environment (Davis & Bubb, 1978; Martin *et al*, 1988).

As an essential validation process in this work, an Andersen MkII cascade impactor (Andersen Samplers, Inc) was used to evaluate the aerosol delivered from MDIs and Diskhaler inhalers, produced in the laboratory and containing ^{99m}Tc labelled salbutamol. The distribution of radionuclide and salbutamol delivered by the inhalers were compared and the extent to which the radionuclide distribution resembled that of the drug measured over a wide range of aerodynamic sizes. These measurements were performed initially under ambient conditions and later repeated under conditions approaching the high relative humidity and temperature of the lungs.

3.2 Theory of Impactor Operation

The principle of operation of a simple single stage inertial impactor is shown in Figure 3.1. An aerosol within an airflow is passed through a nozzle or jet and the output stream is directed against a flat plate. This plate, known as an impaction plate, deflects the airflow forming an abrupt 90° bend. Particles with sufficient inertia are unable to follow the airflow and impact on the flat plate. Smaller particles are able to follow the airflow without hitting the impaction plate. A single impactor separates an aerosol into two size ranges; particles larger than a certain aerodynamic size are removed from the airflow, and those smaller than this size remain airborne.

As in the case of aerosols entering the respiratory tract, the Stokes number ***Stk*** indicates the collection efficiency of the impactor. For an impactor this is defined as the ratio of the stopping distance of a particle, of diameter ***d***, at the average nozzle exit velocity ***U*** to the nozzle (jet) diameter, ***Dj*** (Hinds, 1982b).

$$Stk = \frac{\rho d^2 U C_c}{9 \eta D_j} \qquad (3.1)$$

C_c is the Cunningham slip correction factor, η is the air viscosity, and ρ is the particle density. The square root of Stokes number, $\sqrt{Stk}$, is thus directly proportional to particle

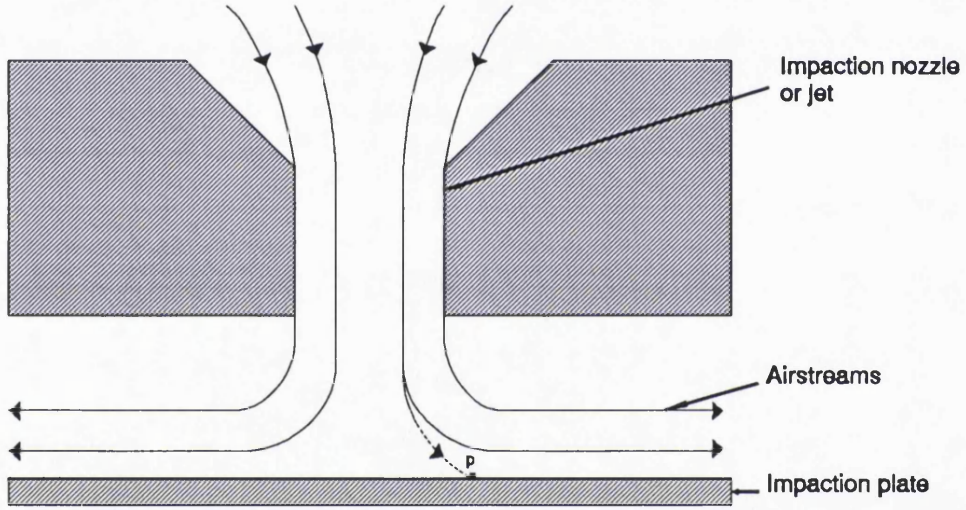


Figure 3.1. Cross-sectional view of a simple impactor showing airflow. p represents those particles with sufficient inertia to leave the airstream and hit the impaction plate.

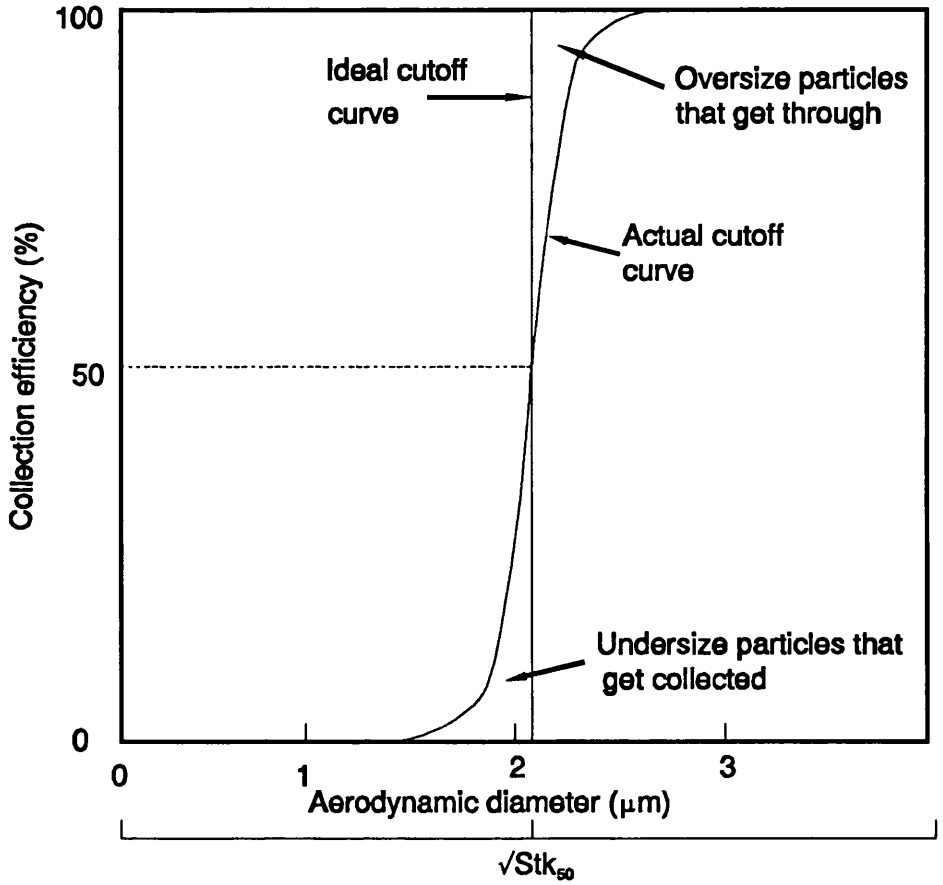


Figure 3.2 *Typical impactor efficiency curve.*

size. A sharp cutoff aerodynamic diameter is desirable, above which all particles are collected and below which all particles pass through.

In practice the collection efficiency curve is usually as shown in Figure 3.2, with some oversize particles getting through and some undersize particles being collected. However, most well designed impactors can be assumed to be ideal and their efficiency curves characterised by a single number Stk_{50} which is the Stokes number that gives 50% collection efficiency (Hinds, 1982b).

3.3 The Andersen Cascade Impactor

Cascade impactors comprise several impactors in series. Each impactor, usually containing multiple nozzles, is called an impactor stage. The stages are arranged in order of cutoff size with the largest cutoff size first. The cutoff size is reduced in each stage by decreasing the nozzle size and varying the number of nozzles in the stage. Reducing the orifice diameter, while maintaining constant flow rate through the instrument, increases the air velocity U and therefore increases the collection efficiency of the stage so that smaller particles are impacted.

The Andersen cascade impactor (Figure 3.3) is an eight stage instrument modelled to the particle collecting characteristics of the human respiratory system. Both solid and liquid airborne particles can be classified according to their aerodynamic size distribution by the amount of impaction measured at each of the stages.

In order to use the instrument with inhalers and direct the aerosol plume into the upper impaction stage a twin impinger glass throat is inserted into the sampler head. The inhaler is supported and interfaced to the throat by the same tight-fitting rubber adaptor that is used with the twin impinger. A pump provides a constant flow rate of air through the instrument allowing the same volume to pass through each stage. The particle size cutoffs of each stage are initially defined for a flow rate of 28.3 l/minute at the input. However, the instrument may also be operated at other flow rates provided the cutoff points have been recalculated accordingly. The diameter of the orifices becomes progressively smaller from stage 0 (top) to stage 7 (bottom) and this produces increasingly

Figure 3.3 ***The Andersen Cascade Impactor.***

higher airflow velocities with each successive stage collecting smaller particles. Submicrometre particles are collected by a backup filter in the base of the instrument so that clean air passes into the pump.

3.4 Measurement of Aerosol Size Distribution Under Ambient Conditions

3.4.1 Metered Dose Inhaler

On each of two investigation days, two MDI canisters containing ^{99m}Tc labelled salbutamol and two canisters containing unlabelled salbutamol were prepared. The inhalers containing radiolabelled salbutamol were prepared according to the method described in Chapter 2. For the inhalers containing unlabelled drug, the only difference in the preparation was that the ^{99m}Tc was not present during the blending process.

Each MDI canister was actuated thirty times into the Andersen cascade impactor while air was drawn through the device at the standard flow rate of 28.3 l/minute as measured at the inlet. After dismantling the instrument, the throat, actuator and the eight stages were washed with HPLC grade methanol to dissolve and collect the salbutamol into separate samples. For samples containing radiolabelled salbutamol, radioactive counts were measured by placing the sample between the two heads of a double headed gamma camera (Chapter 5) and acquiring for a measured period. This process was performed for each of the canisters containing labelled drug.

The dose of salbutamol in each sample from the eight canisters were determined using ultraviolet spectrophotometry (Perkin-Elmer 554) at a wavelength of 246 nm. In the case of the radioactive samples, this was performed several days later to allow the radioactivity to decay.

3.4.2 Diskhaler Inhaler

The contents of three Diskhaler blister packs, each comprising eight doses of ^{99m}Tc labelled salbutamol sulphate, were separately drawn into the Andersen cascade impactor at a flow rate of 60 l/minute as measured at the inlet of the device. The configuration was different to that used for the MDIs and included a preseparator stage between the throat and the upper impaction stage for collecting the large lactose particles. The particle size

cutoff at 60 l/minute for each stage of the impactor was calculated using equation 3.1 and rearranged to give equation 3.2. It is assumed that the Stokes number for each stage of the impactor is constant for the two different flow rates,

$$d_2 = \sqrt{\frac{d^2{}_1 U_1}{U_2}} \qquad (3.2)$$

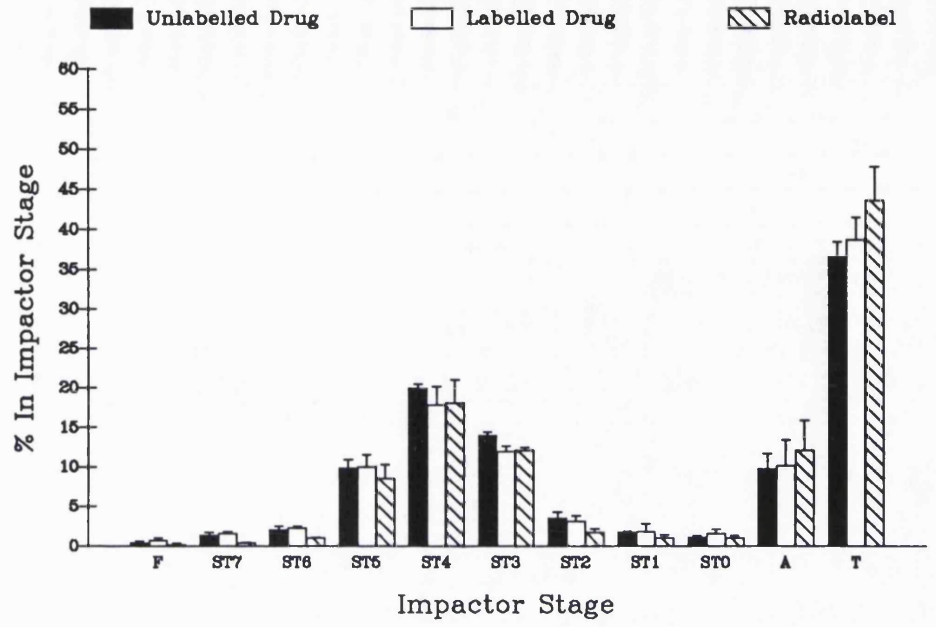
where d_1 is the cutoff diameter at the original velocity U_1 and d_2 is the cut off diameter for the stage at velocity U_2 .

The salbutamol deposited at each stage was collected, using methanol, and the radioactive counts in each sample were again measured with the gamma camera. The drug contents were measured using high pressure liquid chromatography (HPLC), following radioactive decay of the radionuclide.

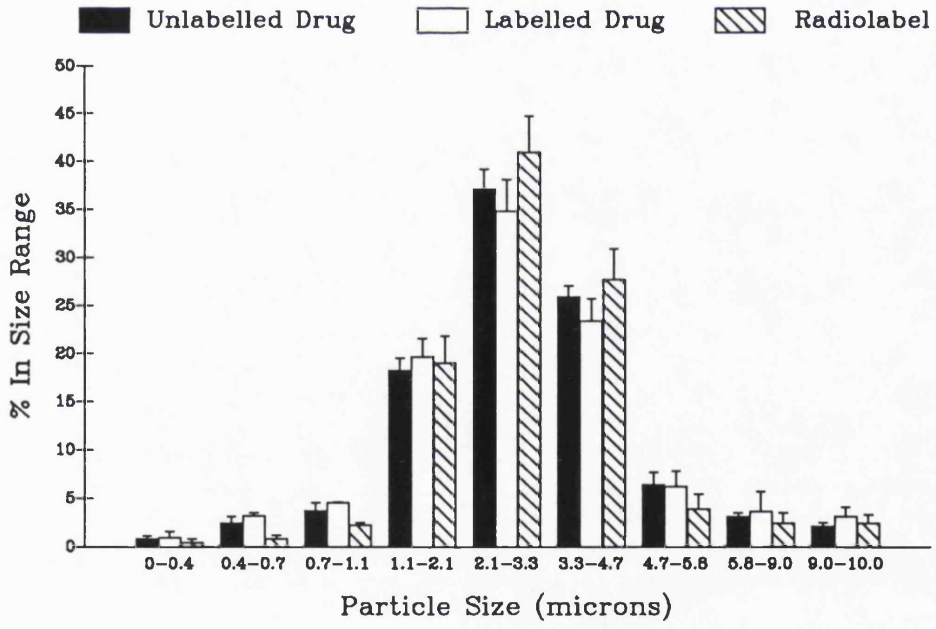
Additionally, three disks containing unlabelled drug were prepared as before, except for the absence of the radionuclide in the early stages of preparation. The contents of these three disks were sampled by the impactor as above. The drug deposited at each stage was again measured using HPLC.

3.4.3 Results

Figure 3.4(a) shows the mean quantities of dose, delivered from the four radioactive MDIs, of labelled salbutamol and radiolabel in the throat, actuator and each of the eight stages and filter of the Andersen impactor expressed as a percentages of the total from all of these collection sites. The percentages of unlabelled salbutamol impacted at the collection sites from the other four MDIs are also shown. Figure 3.4(b) shows the quantities of unlabelled drug, labelled drug and radiolabel, expressed as percentages within the specified size range, of the amount collected at the impactor stages only. The distribution of each assessment is a skewed normal curve typical of the distribution of heterodisperse aerosols. The collection ranges for each impactor stage are shown in micrometres. The ECDs are the lower limits of each range. The cumulative percentage dose less than each size range were plotted against each ECD in order to measure the

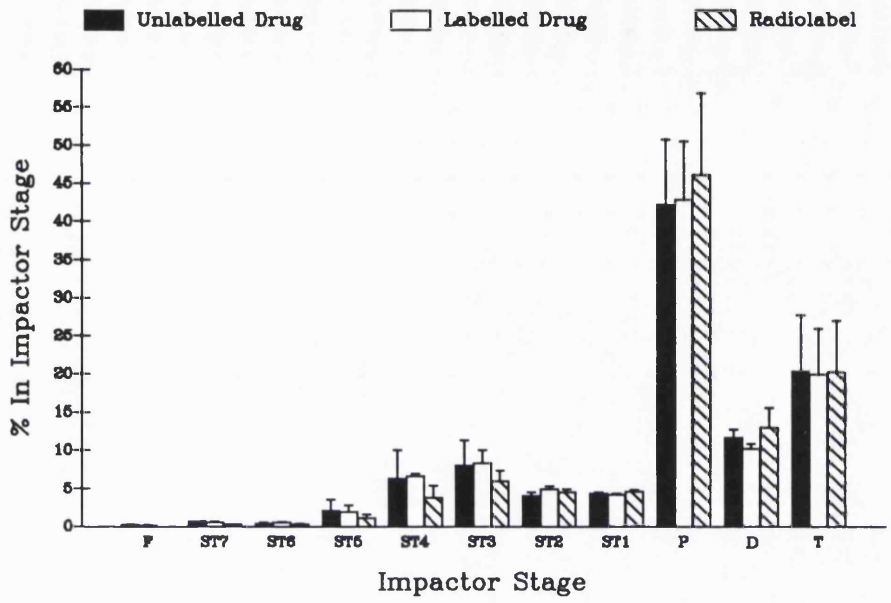


(a)

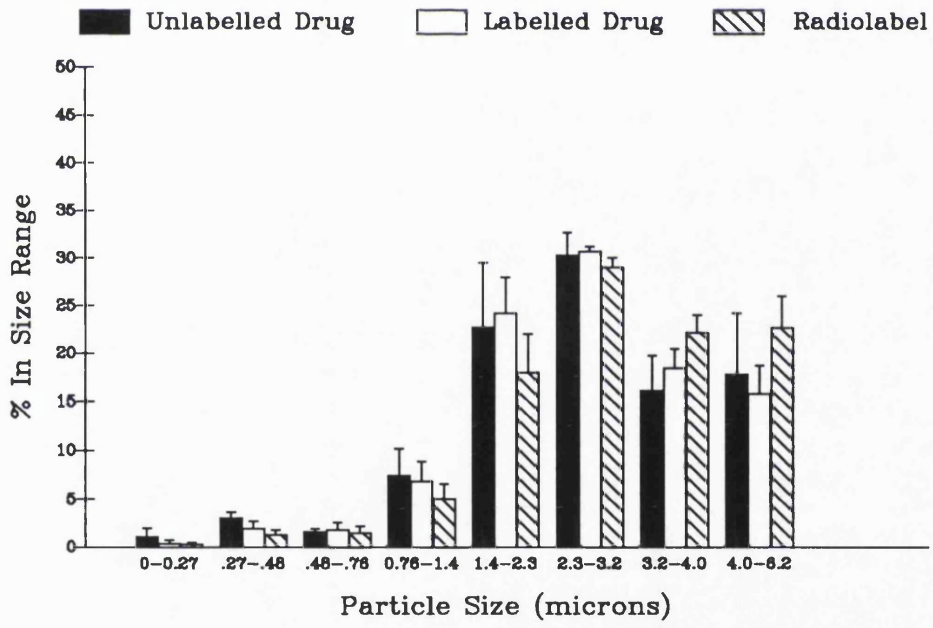


(b)

Figure 3.4. *The distributions of labelled and unlabelled salbutamol delivered to the Andersen cascade impactor by MDI. (a) F = filter; ST0 - ST7 = impactor stages; A = actuator; T = throat. (b) Shows deposition in stages 0 to 7 and filter as a percentage of deposition in these stages together with the size ranges in micrometres. Error bars indicate standard deviation.*



(a)



(b)

Figure 3.5. ***The distributions of labelled and unlabelled salbutamol delivered to the Andersen cascade impactor by Diskhaler inhaler. (a) F = filter; ST1 - ST7 = impactor stages; P = preseparator stage; D = Device; T = throat. (b) Shows deposition in stages 1 to 7 and filter as a percentage of deposition in these stages together with the size ranges in micrometres. Error bars indicate standard deviation.***

MMADs and GSDs of the aerosol assessments (Section 1.5.3). Figures 3.5(a) and (b) show the corresponding information for the three radiolabelled and three unlabelled Diskhaler doses. The preseparator stage and stage 0 have been combined.

These results show a close similarity between the radioactivity and the drug level for each stage, and indicate that the two are closely associated over a wide range of particle sizes. The distributions of unlabelled and labelled drug levels are also virtually the same and indicate that the distribution pattern of the drug has not been significantly altered by the addition of the radionuclide. The major sites of both drug and radiolabel recovery were in the inhaler and in the throat, and for the Diskhaler inhaler a substantial amount was deposited in the preseparator stage.

The MMAD (GSD) for the unlabelled and labelled drug delivered from the MDIs were 2.9 (1.5) μm and 2.8 (1.6) μm respectively while the MMAD for the radioactivity assessment was 2.8 (1.5) μm. In the case of the Diskhaler inhaler, the MMAD (GSD) for the unlabelled drug was 2.7 (1.6) μm whilst the MMADs for the labelled drug and radiolabel assessment were 2.7 (1.5) μm and 3.0 (1.5) μm respectively.

3.5 Aerosol Size Distribution Under Humid Conditions

The warm humid environment of the lungs has an important effect on the deposition of aerosol droplets. The relative humidity within the lungs is reported to be 99.5% (Porstendörfer, 1971). Under such conditions hygroscopic aerosol droplets and particles will grow in size, due to the accumulation of moisture, until they reach an equilibrium size when the vapour pressure exerted by the droplets equals the vapour pressure of water in the lung (Davis and Bubb, 1978).

Initial particles generated by MDIs are encased in chlorofluorocarbon propellants, which evaporate rapidly and reduce the particle size. Although the surface coating of propellant is continually disappearing, it is likely to provide some protection from hygroscopic growth. These two opposing effects make it difficult to accurately estimate the size of MDI-generated aerosols in the human airway.

As part of this investigation the affect of humidity on the aerosol particles generated by MDI and Diskhaler inhaler were studied. Their distributions in the stages of the Andersen cascade impactor were measured as before with the obvious addition of a high relative humidity and temperature environment. Another very important aspect of this investigation was to address the question; would the salbutamol and radiolabel behave the same under these conditions or would the presence of air with high water content cause the two to become separated before they are deposited? This has obvious implications for the validity of applying the radiolabelling techniques to the study of drug deposition in the high humidity environment of the respiratory tract.

3.5.1 Metered Dose Inhaler.

The experimental arrangement of the apparatus used to provide warm humid air to the Andersen cascade impactor while sampling aerosol particles is shown in Figure 3.6. The Andersen cascade impactor was placed in a large sealable perspex tank of dimensions 48cm x 48cm x 60 cm. Humid air was generated by bubbling air from a pressurised cylinder through hot water contained in a humidifier and was piped into the tank. The relative humidity and temperature of the air within the tank was indicated by a humidity probe (Rotronic Hygroskop DT) placed in close proximity to the cascade impactor.

The relative humidity and temperature of the air within the tank was allowed to build up until the output of the probe indicated that they were close to 99% and 37°C. A 240 W light served as a heat source enabling the air temperature to reach 37°C. In practice it was extremely difficult to achieve both these values at the same time but it was possible to get sufficiently close and generate a good approximation to the conditions of the lungs.

Access to the impactor was through a circular opening in the front of the tank. While the humidity was building up this was sealed by a removable door which was screwed firmly into place. When the humidity was suitable the MDI was shaken, the door was removed and the actuator and canister placed into the impactor adapter. The pump was turned on and thirty doses discharged into the impactor as before. A polythene cover, with two openings through which to insert the hands of the operator, ensured that the humid air was not diluted with ambient air whilst operating the inhaler inside the tank. The aerosols

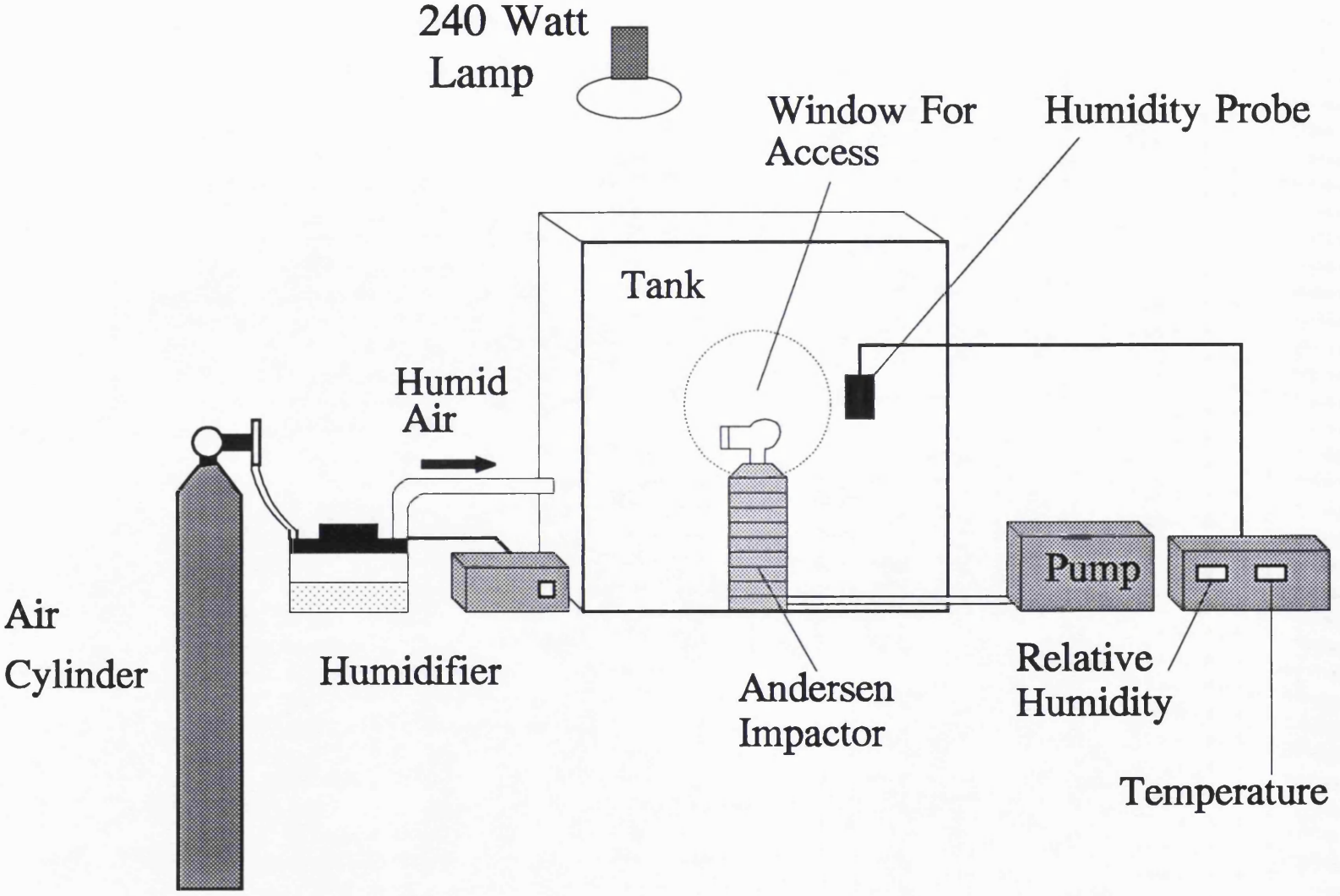


Figure 3.6. ***Experimental arrangement of apparatus for drawing warm humid air through the Andersen cascade impactor.***

delivered from four MDIs containing radiolabelled salbutamol and four containing unlabelled salbutamol were individually sampled according to the above procedure.

The ideal environment for this work is a temperature and humidity controlled climatic cabinet (Martin *et al*, 1988). Such apparatus was not available at the time of the experiment. However, the relative humidity and temperature of the above apparatus changed very little during the process (Table 3.1) and very humid warm air was generated using this arrangement.

3.5.2 Diskhaler Inhaler.

The same apparatus was initially used in order to investigate the effect of humidity on the dry powder preparation delivered from the Diskhaler inhaler. Following build up of temperature and humidity, each blister was pierced and the device inserted into the rubber adaptor of the Andersen impactor within the tank. The door was sealed and the pump switched on. This was repeated for a further fifteen blisters (two disks in total). Afterwards the inhaler was removed and the impactor dismantled as before. Initial inspection of the collection plates and inhaler suggested that most of the powder had remained in the blister and the inhaler device. This was confirmed by measurement of the radiolabel distribution which showed that only a fraction of the dose had penetrated into the impactor.

The main problem was that as soon as the humid air came into contact with the powder the drug and lactose began to absorb water and bind together forming a mass which could not then be aerosolised by the airstream. In order to perform this experiment effectively it is necessary for the powder to be first aerosolised by a relatively dry air stream before it comes into contact with the humid air. Martin *et al*, (1988) managed to avoid this problem by designing their apparatus such that the humid air was re-routed at the last minute through the inhaler.

To separate the dry air from the humid air the apparatus shown in Figure 3.7 was used. The warm humid air was generated using the humidifier as before. Air at ambient temperature and humidity was initially drawn into a 1 litre container, R, from the surroundings via valve V. This acted as a reservoir of dry air which was separated from

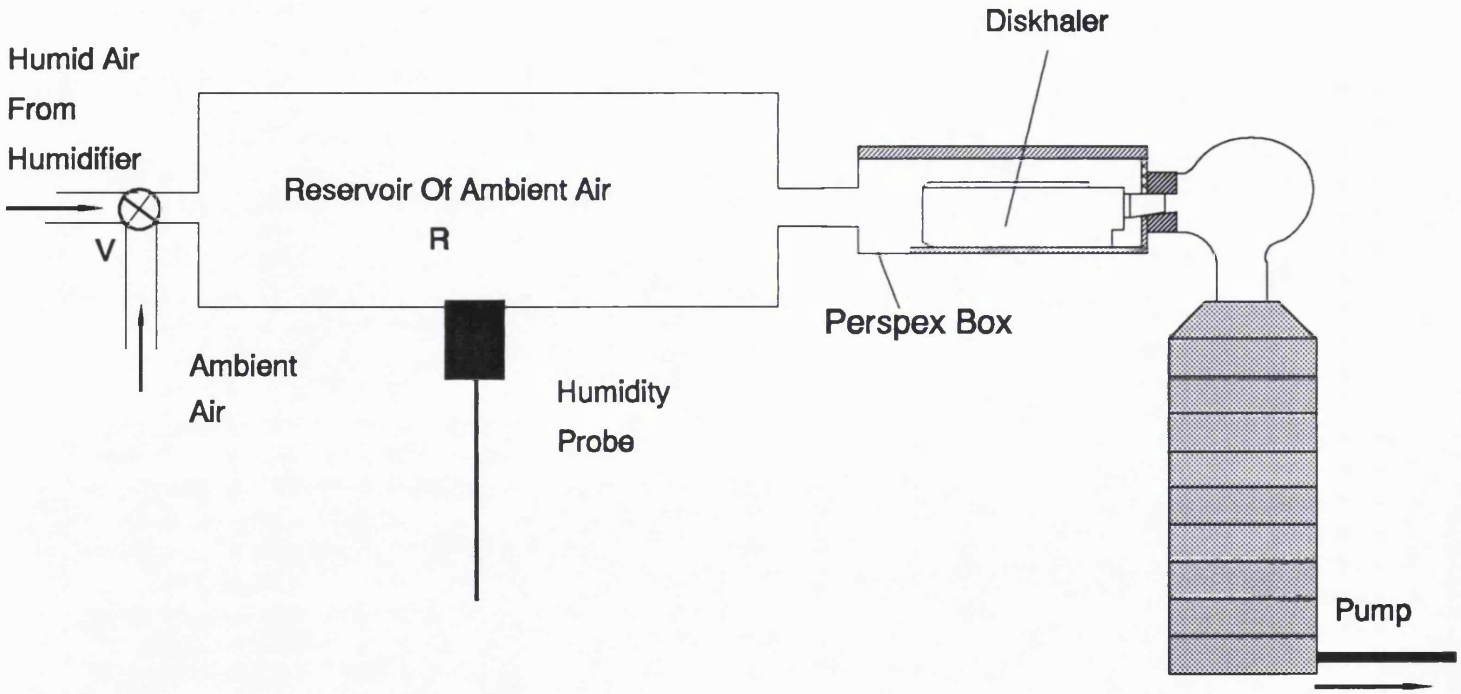


Figure 3.7. ***Apparatus for Andersen humidity experiment with the Diskhaler inhaler***

the humid air by the valve. The Diskhaler inhaler was contained in a specially made perspex box (Section 4.2.2.2) with its mouthpiece protruding through a tight fitting aperture. This was fitted into the rubber adapter of the cascade impactor as before. A second aperture at the rear of the box connected it with reservoir R.

Humid air was initially passed through the impactor before it was connected as shown in the diagram. The Diskhaler blister was then pierced, the pump was turned on and the valve V switched to allow the humid air to pass through the apparatus. In theory the dry air would initially be drawn through the inhaler, aerosolising the powder, followed by the humid air. The two should then mix and pass through the impactor stages carrying the drug particles with them. The temperature and humidity of the air flow was monitored by the humidity probe contained in R. Sixteen such doses of powder were used in order to provide enough salbutamol for later detection by HPLC.

In practice this arrangement performed little better than the previous one. The dose of powder from the first blister was almost completely cleared from the inhaler. However subsequent doses were not, due to the difficulty in separating further amounts of dry air. The powder was extremely sensitive to moisture, and if a small amount remained in the container from the previous run it was impossible to aerosolise the dose.

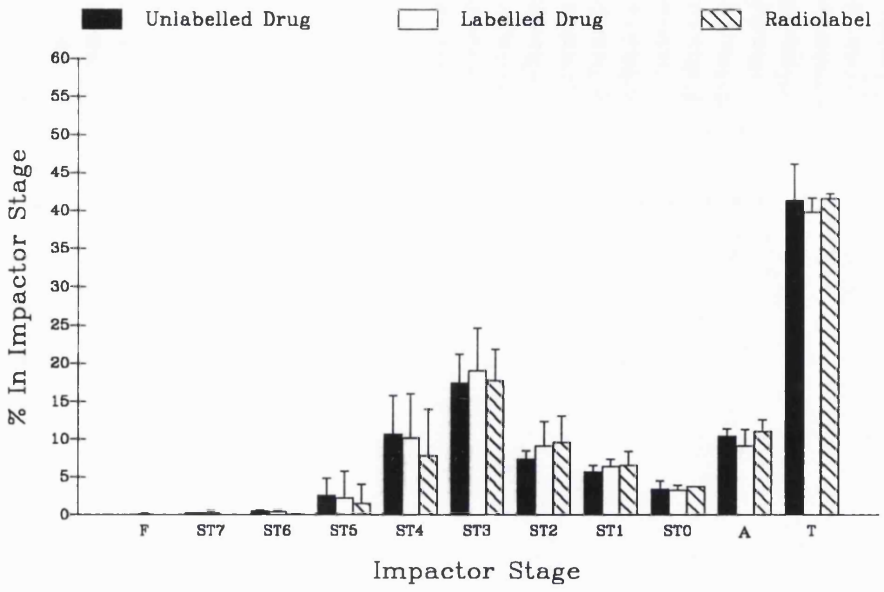
3.5.3 Results

Table 3.1 shows the relative humidity and temperature of the air in the tank at the start and finish of the thirty actuations of each MDI canister. The mean (SD) relative humidity was 96.7 (0.7)% and the mean (SD) temperature was 35.9 (1.1)°C. During the course of the experiment the average variation in relative humidity was less than 0.5% and the average variation in temperature was 0.7% This low variation in relative humidity and temperature confirms the suitability of the apparatus used.

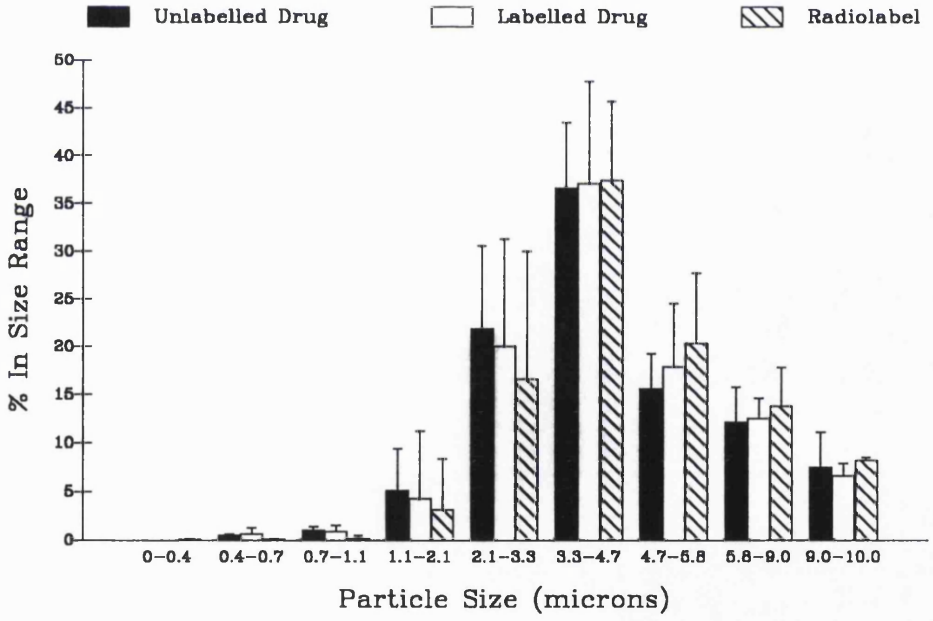
Figure 3.8 shows the mean proportions of dose, delivered from the four labelled and four unlabelled MDIs under humid conditions. Figure 3.8 (a) shows the quantities of unlabelled and labelled salbutamol and radiolabel in the throat, actuator and each of the eight stages and filter of the Andersen impactor expressed as a percentages of the total dose collected.

	Relative Humidity (%) (Start - Finish)	Temperature °C (Start - Finish)
MDIs Containing Radiolabelled Salbutamol		
Can 1	97.4 - 97.2	36.6 - 36.4
Can 2	96.6 - 96.0	38.4 - 38.1
Can 3	97.8 - 97.7	35.6 - 35.8
Can 4	96.5 - 95.4	36.5 - 36.3
MDIs Containing Unlabelled Salbutamol		
Can 1	96.7 - 95.2	34.7 - 34.7
Can 2	96.0 - 96.0	34.7 -34.6
Can 3	97.4 - 97.0	35.1 - 35.0
Can 4	97.1 - 97.1	35.8 - 35.8

Table 3.1 ***Relative humidity and temperature of air at start and finish of Andersen impactor investigations for labelled and unlabelled MDIs.***



(a)



(b)

Figure 3.8 *The distributions of labelled and unlabelled salbutamol delivered to the Andersen cascade impactor by MDI under conditions approaching the temperature and humidity of the lungs. (a) Deposition in actuator, throat, 8 impactor stages and filter (b) Deposition in stages 0 to 7 and filter with the size ranges of each stage in micrometres. Error bars indicate standard deviation. (The Conversion from deposition per stage to particle size range has not been corrected for the effects of temperature and humidity).*

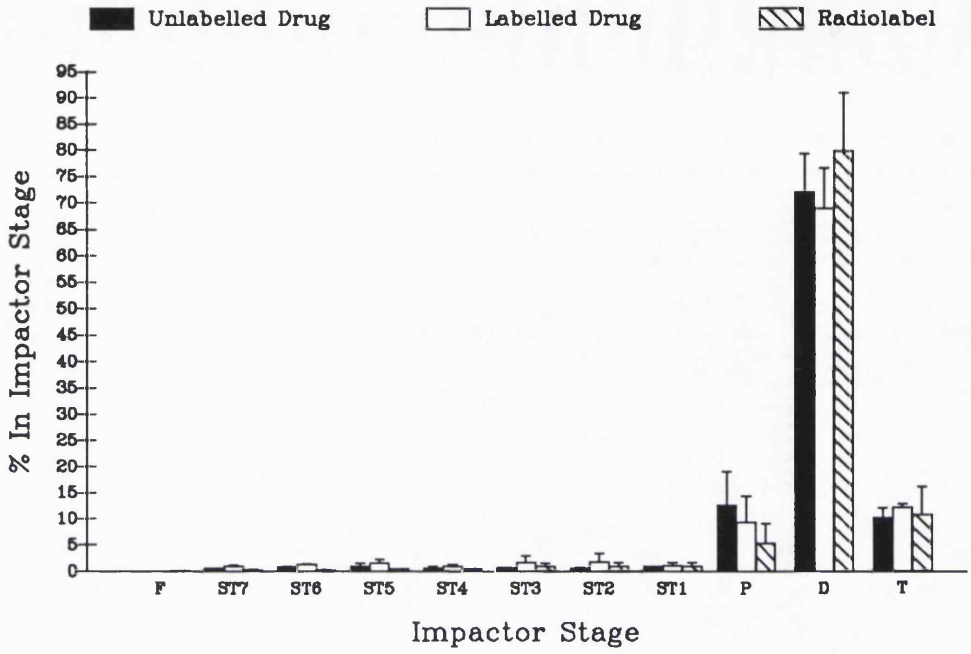


Figure 3.9 ***The distributions of labelled and unlabelled salbutamol delivered to the Andersen cascade impactor by Diskhaler inhaler under conditions of high humidity.***

	MMAD (GSD) μm			
	Ambient Conditions		Humid Conditions	
	Diskhaler	MDI	Diskhaler	MDI
Radiolabel	3.0 (1.5)	2.8 (1.5)	------	4.4 (1.5)
Labelled Drug	2.7 (1.5)	2.8 (1.6)	------	4.2 (1.5)
Unlabelled Drug	2.7 (1.6)	2.9 (1.5)	------	4.1 (1.5)

Table 3.2. ***Summary of MMADs and GSDs for aerosols generated by MDIs and Diskhaler inhalers using the Andersen cascade impactor.***

Figure 3.8(b) shows the quantities of unlabelled drug, labelled drug and radiolabel expressed as percentages within each size range of the amount collected at the impactor stages only.

Again these results show a close similarity between the radioactivity and the drug level for each stage, and indicate that the two have remained together throughout the range of particle sizes measured. The distribution of unlabelled and labelled drug levels are also virtually the same. The MMAD (GSD) for the unlabelled and labelled drug delivered from the MDIs were 4.1 (1.5) μm and 4.2 (1.5) μm respectively while the MMAD for the radioactivity assessment was 4.4 (1.5) μm (Table 3.2).

Figure 3.9 shows the cascade impactor distribution for the drug and radiolabel from a Diskhaler inhaler under conditions of high temperature and humidity. The majority of the dose has remained in the device. A small percentage has penetrated into the first two stages of impactor.

3.6 Percentage of Particles within the Respirable Range

Particles in the size range 1 - 10 μm are often referred to as the 'respirable range' (Morrow, 1974). However, it is particles in the size range 1 - 5 μm which are most suitable for penetrating to all parts of the lungs including the alveolated regions. When classifying particles using the Andersen impactor the respirable portion of the dose may be taken as those particles depositing at stage 2 and below (particles of size 5.8 μm and below) when operated at a flow rate of 28.3 l/min. This convention was used here in order to compare the proportion of particles from the MDI depositing within this range under ambient conditions with those deposited under conditions of simulated lung temperature and humidity. For the Diskhaler inhaler, since the Andersen impactor was calibrated at a flow rate of 60 l/min, the impaction stage cutoffs do not correspond exactly with the stages calibrated at 28.3 l/min. In this case particles depositing at stage 1 and below (6.2 μm and below) were taken as the respirable range.

Figure 3.10 shows the distribution of labelled salbutamol under ambient and humid conditions. It can be seen that while the deposition in the throat and actuator remained

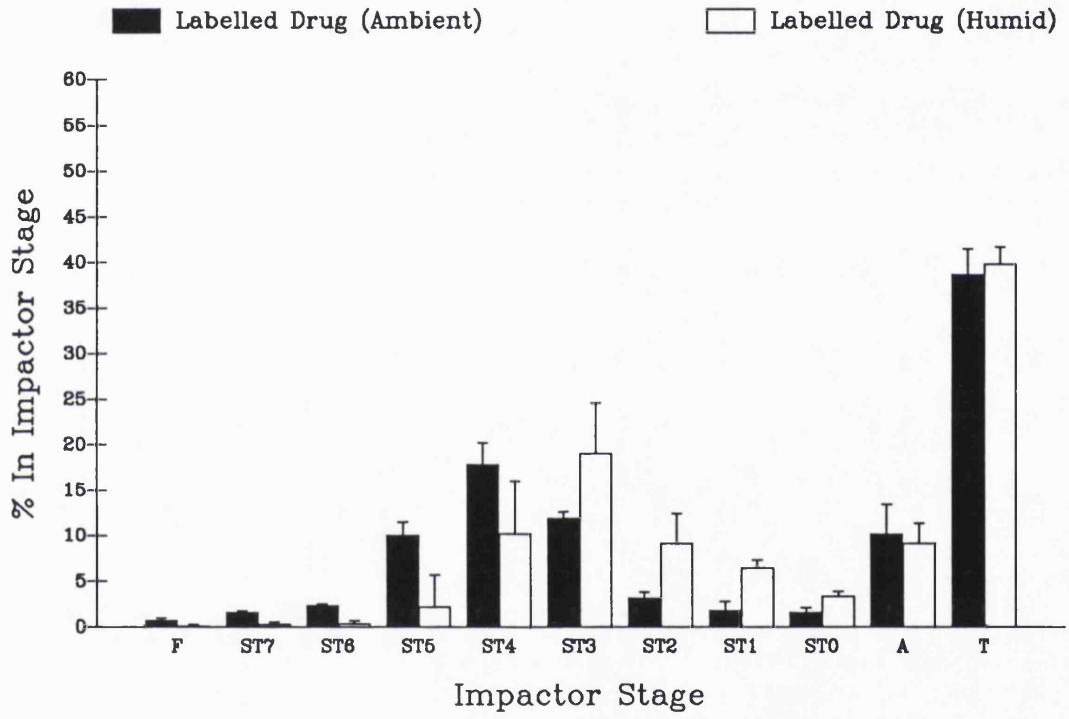


Figure 3.10 ***The effect of humidity upon the distribution of labelled salbutamol, delivered from the MDI, in the Andersen cascade impactor.***

unaltered under humid conditions the distribution was shifted towards the upper part of the impactor. This implies that a higher proportion of larger particles were collected in the upper stages and a lower proportion of small particles penetrated towards the lower stages. Maximum deposition, excluding throat, occurred at stage 3 (3.3 - 4.7 μg) instead of stage 4 (2.1 - 3.3 μg) for ambient conditions. A comparison of Figures 3.4(a) and 3.8(a) shows that this was the case for labelled and unlabelled drug and radiolabel.

The proportions of the dose from both MDI and Diskhaler inhaler deposited in the respirable range are shown in Table 3.3. The percentage of labelled drug deposited in particles of size 5.8 μg and smaller decreased from 47.6 (SD 3.4)% to 41.3 (SD 1.9)%. These two means were significantly different from each other ($t = 3.2$, $p < 0.02$) when compared using an independent t-test .

Mean (SD) %	Unlabelled Drug	Labelled Drug	Radiolabel
MDI (Ambient)	50.8 (2.8)	47.6 (3.4)	42.0 (3.9)
MDI (Humid)	38.7 (7.1)	41.3 (1.9)	36.7 (2.1)
Diskhaler (Ambient)	26.0 (9.0)	27.0 (5.2)	20.7 (3.8)

Table 3.3 ***showing the percentage of salbutamol contained in particles of size 5.8 μm and below for the MDI and 6.2 μm and below for the Diskhaler inhaler. For the MDI, measurements were made under ambient conditions and conditions of high humidity and temperature***

3.7 Nature of Particle Labelling and Distribution

As already discussed, cascade impaction measures the mass distribution of an aerosol. Other techniques such as laser light scattering measure the numerical or frequency distribution of the aerosol particles within a given size range. This can then be converted to the mass distribution if required. For heterodisperse aerosols the mass distribution is usually very different from the frequency distribution. The mass distribution arises mainly from the larger particles in the aerosol (Brain and Valberg, 1979). Morrow (1974) showed that in a typical aerosol distribution more than 50 percent of the mass is distributed in

particles larger than 4.2 μm diameter, whereas in the frequency distribution, particles of this size constitute only about 2 per cent of the total population. This is because the volume or mass of a particle is proportional to the cube of its radius and even though more numerous the submicron particles carry far less mass than the larger particles. For example, the mass of a 3 μm particle is 27 times greater than the mass of a 1 μm particle. In the case of soluble drug particles the mass distribution is therefore an important determinant of the dose delivered. The distribution pattern of deposited particle mass in the lungs may therefore strongly depend on the fate of the relatively few particles carrying a high proportion of the total dose.

When radiolabelling aerosol particles the distribution of radiolabel may be proportional to the surface of the particle or the volume of the particle or intermediate to these. If the radionuclide is homogeneously located throughout the mass of particles the distribution will be identical to that of mass or volume. It is characteristic of heterodisperse aerosols that the volume or mass distribution is usually very different from both the frequency and the surface distributions but the GSDs of their log-normal plots are all the same (Morrow, 1974, Agnew, 1984). Figure 3.11 shows the frequency, surface and volume distributions of a typical heterodisperse aerosol. For the reasons explained above, the frequency distribution is usually biased towards the smaller particles while the mass distribution is shifted towards the larger particles and the surface distribution is intermediate to these two.

The distribution of radiolabel in this investigation appears to be proportional to mass since it is the same as that of the salbutamol drug (Figures 3.4, 3.5 and 3.8). Köhler *et al*, (1988) concluded this to be the case for their labelling method. Alternatively, the drug may actually be surface-labelled due to adsorption rather than the radionuclide being homogeneously distributed throughout the particle volume. If this is the case, the "mass" distribution of radiolabel seen here may be explained by the shape of the drug particles which are not actually spherical but are very irregular. They may be indented and behave in the same way as a sponge to such an extent that the radiolabel, though on the surface, is also within the volume of the drug particles. This second explanation is plausible since the particles were already pre-formed when labelled and did not dissolve and lose their original form at any time during the labelling process.

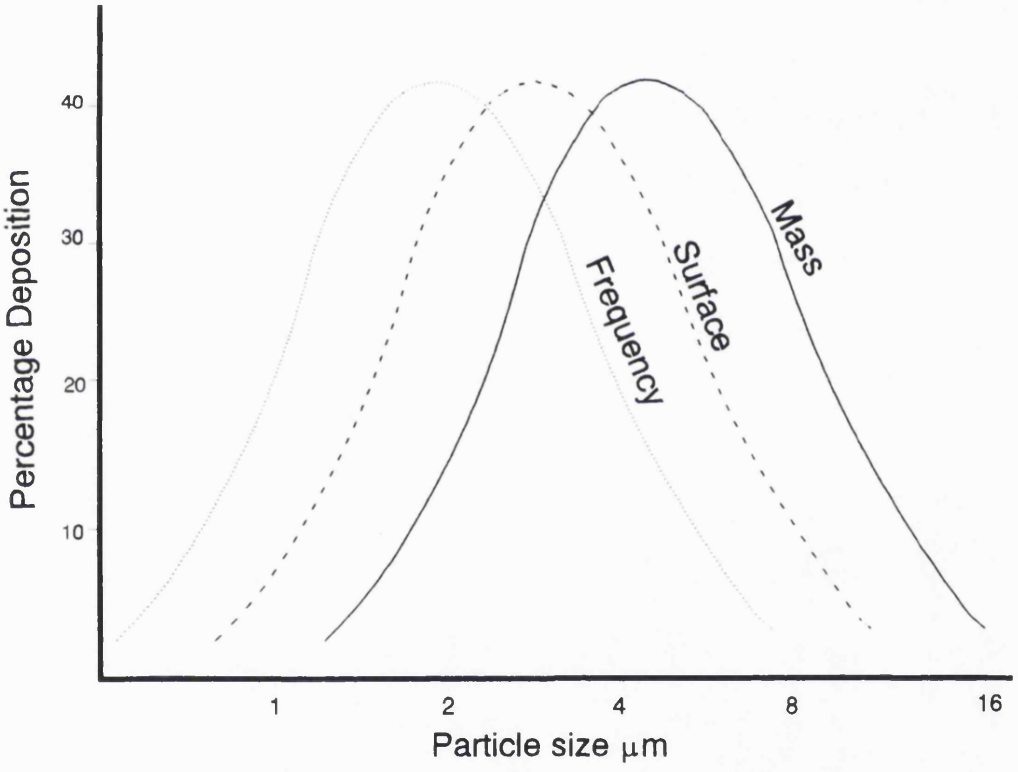


Figure 3.11. *Log-normal plot of mass, surface and frequency distributions of typical heterodisperse aerosol particles.*

3.8 Discussion

The Andersen cascade impactor was used to validate the radiolabelling processes over a wide range of particle sizes. The results showed a close similarity between the properties of the radionuclide and the drug for aerosols generated by both MDI and Diskhaler inhaler; the percentage of radionuclide and salbutamol deposited on each stage of the Andersen impactor were closely matched. This is strong evidence that the two are attached since, on their own, the TcO^-_4 molecules and salbutamol particles are very different in size and density and would therefore be expected to have totally different aerodynamic properties. The results also showed that the labelled and unlabelled salbutamol both had almost identical distributions indicating that the presence of the radionuclide did not alter the aerodynamic properties of the drug.

The percentage of the dose deposited within the 'respirable range' was much higher for the MDI than for the Diskhaler inhaler. This indicated that, compared to the MDI, the Diskhaler inhaler is inefficient at delivering drug in the size range most suitable for inhaling into the lungs. However, despite the lower proportion of the initial dose reaching the collecting plates of the impactor, the MMADs and GSDs of this portion under ambient conditions were almost exactly the same for both the MDI and the Diskhaler inhaler.

Under conditions of simulated lung humidity and temperature the MMAD of the aerosol particles penetrating into the impactor increased. This was also reflected in the significant decrease in the respirable portion of the dose. The significance of these results is that in humid conditions the aerosol droplets collect water condensation and become larger. The aerosol distribution shifts towards larger particles and this affects their efficiency of penetration into the impactor. The implication to the delivery of aerosol particles to the human respiratory tract is that the high relative humidity found there will tend to increase particle size and reduce efficiency of penetration into the lungs.

Previous data have shown a strong dependence between the percentage of the dose from an MDI depositing in the respirable portion of the Andersen impactor and ambient temperature (Van Oort *et al*, Personal communication). The respirable portion of the dose increased with increasing temperature, corresponding to an increase in the rate of

evaporation of the propellant droplets making up the aerosol cloud and a resulting decrease in the effective particle size. However, the data from the present investigation have shown that, despite a large increase in air temperature, the effect of increasing relative humidity to around 97% predominated thus resulting in an overall decrease in the respirable portion of the dose.

The data presented here are very similar to those of Martin *et al*, (1988). They reported that under conditions of low relative humidity (30%) the percentage of deposited dose of size 5.8 μm and below were 45.4 (4.0)% for a salbutamol MDI and 25.4 (3.0)% for a salbutamol sulphate DPI (Rotahaler inhaler). Under conditions of high relative humidity (97.5%) these proportions decreased to 38.0 (3.9)% for the MDI and 18.0 (3.1)% for the DPI.

Other studies that have simulated humidity and temperature in order to assess the effect of these factors on aerosol deposition within the lung (Davis and Bub, 1978; Hiller *et al*, 1980) have also concluded that MDI and DPI aerosols increase in mass when exposed to high humidity.

Apart from the influence on aerosol particle size the other objective of studying drug delivery under conditions of high relative humidity and temperature was to see if the radionuclide and drug became totally or partially separated. The results obtained and shown in Figure 38(a) indicate that separation has not happened for radiolabelled salbutamol delivered by MDI and that drug and radiolabel are distributed, as before, in the same proportion throughout the various stages of the Andersen impactor. Despite failure to obtain a meaningful impactor distribution for the Diskhaler inhaler under humid conditions the data does indicate that the drug and radiolabel were deposited in the same proportion in the upper part of the impactor as well as that retained in the inhaler.

It can be concluded from this investigation that the radionuclide ^{99m}Tc acts as a reliable marker for the presence of salbutamol over a wide rage of aerodynamic sizes under ambient conditions as well as conditions of simulated lung temperature and humidity. It can also be concluded that the presence of the radiolabel does not affect the size

distribution of the drug itself. The size distribution and MMADs of the aerosol particles are comparable to other published data using commercially produced salbutamol inhalers (Martin *et al*, 1988; Hiller *et al*, 1978; Hiller *et al*, 1980) and are of a suitable size for inhaling to all parts of the lung. The inhalers produced in the laboratory are therefore appropriate for the study of salbutamol deposition in the respiratory tract of human volunteers.

Chapter 4. Specification of Normal and Asthmatic Subjects and Physiological Measurement and Drug Administration Procedures.

4.1 Introduction

Ten normal subjects and nineteen asthmatic subjects took part in studies to investigate the deposition of inhaled salbutamol in the respiratory tract. Each subject inhaled ^{99m}Tc labelled doses of salbutamol from a Diskhaler inhaler, an MDI and an MDI with Volumatic spacer (Allen and Hanburys Ltd). Their inspiratory flow curves were measured while inhaling from the Diskhaler inhaler and the MDI. The lung function of each asthmatic subject was measured before and after administration of the salbutamol. Photographs of the MDI and Diskhaler are shown in Figure 2.1 while the spacer is shown in Figure 4.1.

The main objectives of these studies were (a) to administer the radiolabelled salbutamol from the three inhalers under as typical conditions as possible; (b) to assess and compare the resulting drug distribution patterns for each technique with particular regard to the proportion reaching the lungs; (c) to measure inspiratory flow rates and compare these with the drug deposition patterns; (d) to compare data from the asthmatic subjects with those of the normal subjects; (e) to measure improvements in lung function, in the asthmatic subjects, and compare these with the drug deposition patterns.

4.2 Methods

4.2.1 Measurement of Lung Function.

Lung function measurements were performed using a portable spirometer (Flowmate, Jaeger). The Flowmate is an automated spirometer which combines a pneumotachograph, a micro-computer and a small printer. This enabled pre-programmed tests to be performed and the results output via the printer.

Subjects performed forced vital capacity flow-volume loops. They were instructed to make a full and rapid expiration, through the mouthpiece, before making a full and rapid inspiration. From the resulting flow-volume loop FVC, FEV_1 and PEF were measured. For each study, tests were performed at 15 minutes and immediately before administration and at 15 and 60 minutes following administration. These measurements gave baseline and post values, and allowed baseline variability to be avoided and maximum improvement to be seen.

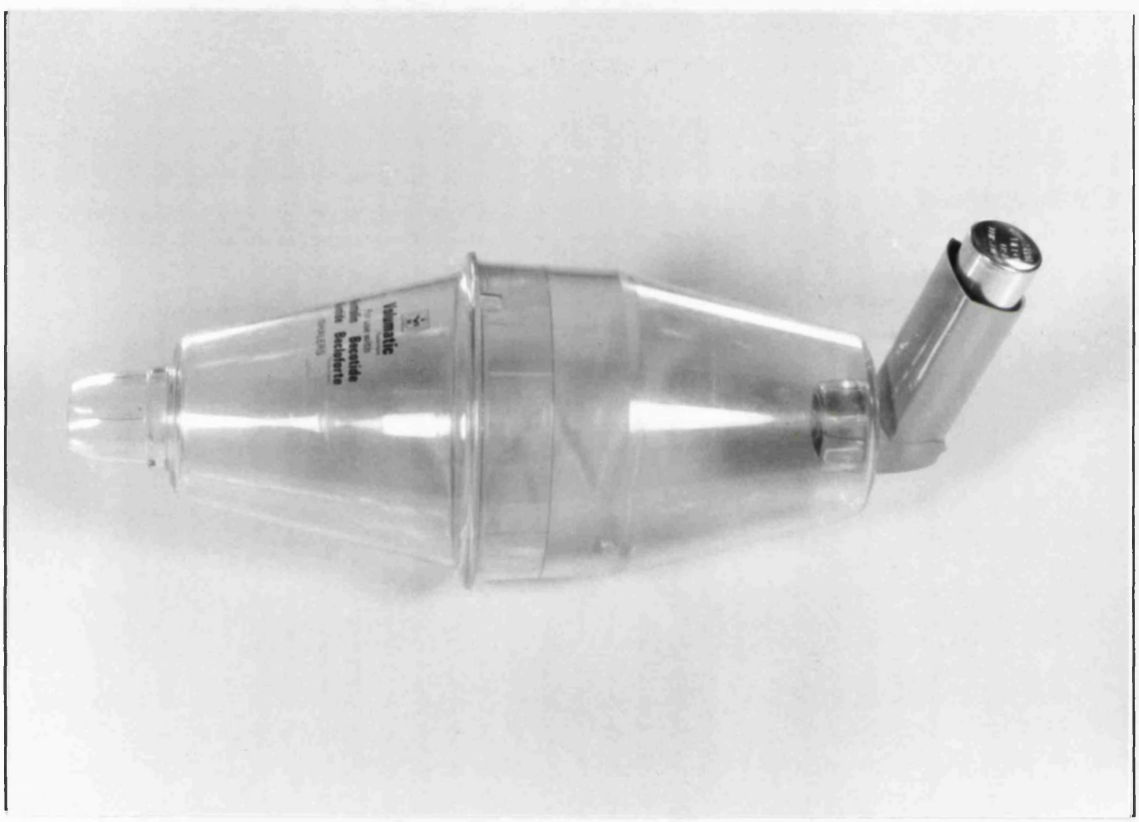


Figure 4.1. ***Volumatic spacer with MDI.***

The value of lung function tests are that they provide a means of measuring the ventilation capability and breathing mechanisms of patients with obstructive airways disease. In clinical diagnosis, they enable the identification of lung impairment and provide a baseline from which the progression of a disease or its response to treatment can be assessed.

Obstructive airways diseases, such as asthma, are characterised by an increased resistance to airflow. This resistance makes it difficult for the sufferer to move air rapidly out of their lungs and reduces the volume that they can exhale in the first second of the forced vital capacity test. Tests of forced expiration, particularly FEV_1, therefore provide a suitable index with which to assess the severity of airflow resistance.

4.2.2 Measurement of Inspiratory Flow Rates

The maximum and average inspiratory flow rate of each subject were measured whilst they inhaled salbutamol from the Diskhaler inhaler and the MDI. The duration of inhalation and volume inhaled were also measured. This was possible by the use of specially designed adaptors into which the inhalers were placed. They enabled flow measurement to be determined without inhibiting the subjects operation and use of the inhaler. The adaptors, which were designed such that the inhalers themselves were unmodified and the airflow unaffected, were connected to a Fleisch flow transducer head and Godart Pneumotachograph .

4.2.2.1 MDI Adaptor

The MDI adaptor, Figure 4.2, was constructed from cylindrical perspex with a removable plastic lid. The MDI canister in its actuator was inserted into a tight fitting aperture at the base of the adaptor with a further opening in the lid through which the base of the inverted canister extended. In order to seal this opening a piece of thin plastic film was placed over the top of the adaptor and canister base and held in place by the lid. This enabled unrestricted operation of the MDI by the subject whilst ensuring an air-tight seal. Inspired gases could then be drawn through the adaptor via the Fleisch Flow Transducer head into an opening on the side of the adaptor.

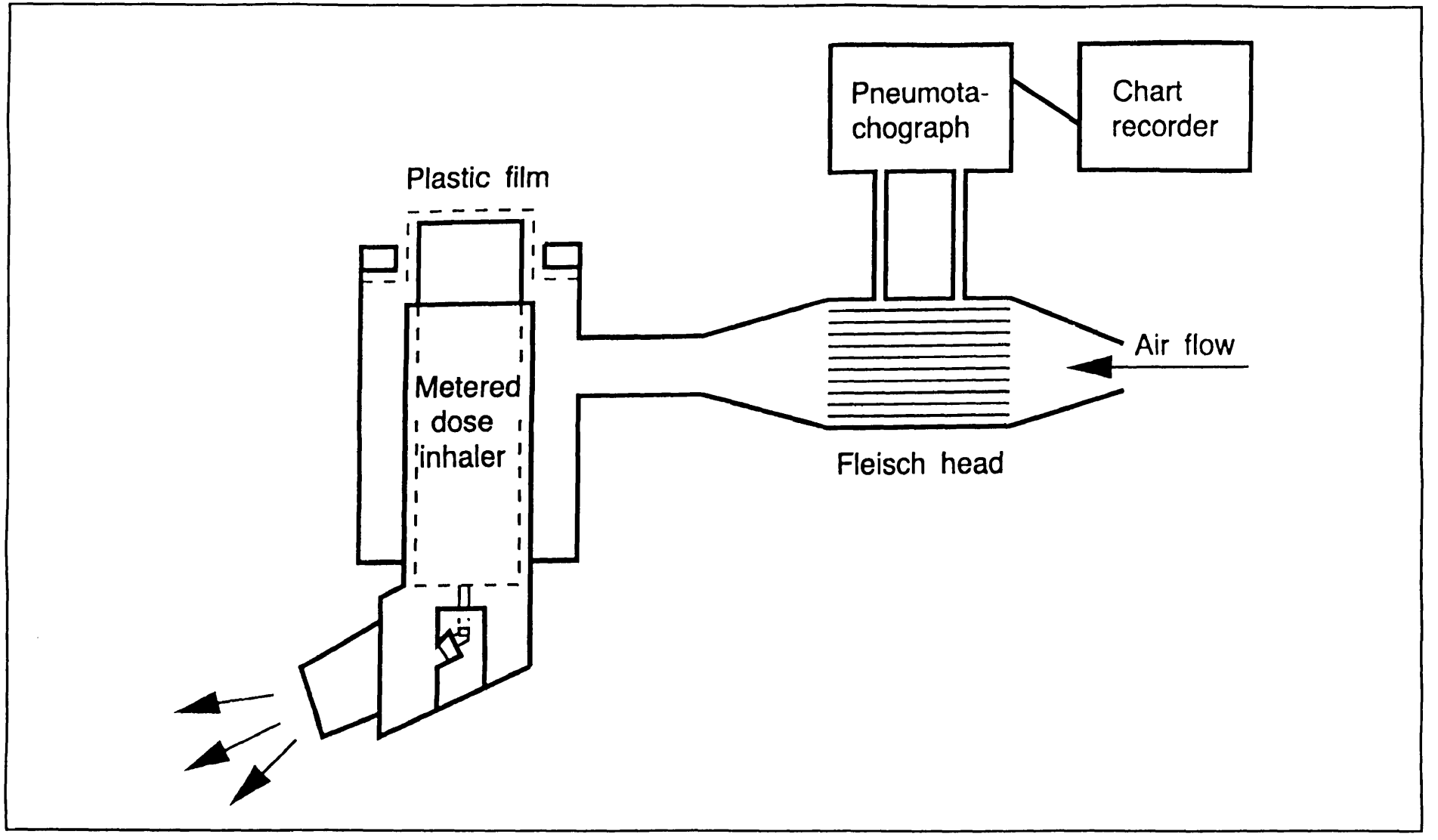


Figure 4.2. ***Diagram of the MDI adaptor and the equipment used for the measurement of inspiratory flow during studies.***

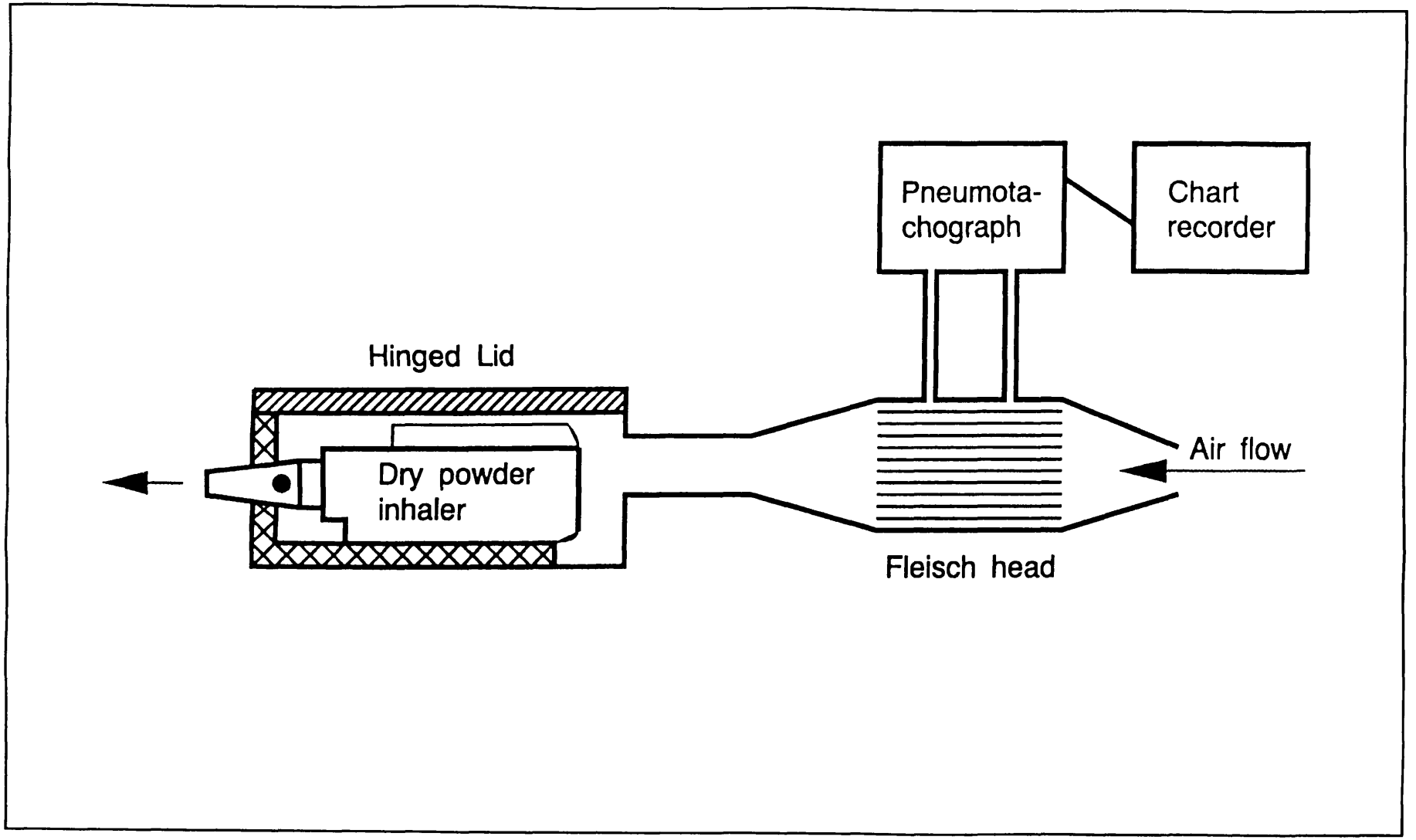


Figure 4.3. ***Diagram of the Diskhaler inhaler adaptor and the equipment used for the measurement of inspiratory flow during studies.***

4.2.2.2 Diskhaler Inhaler Adaptor

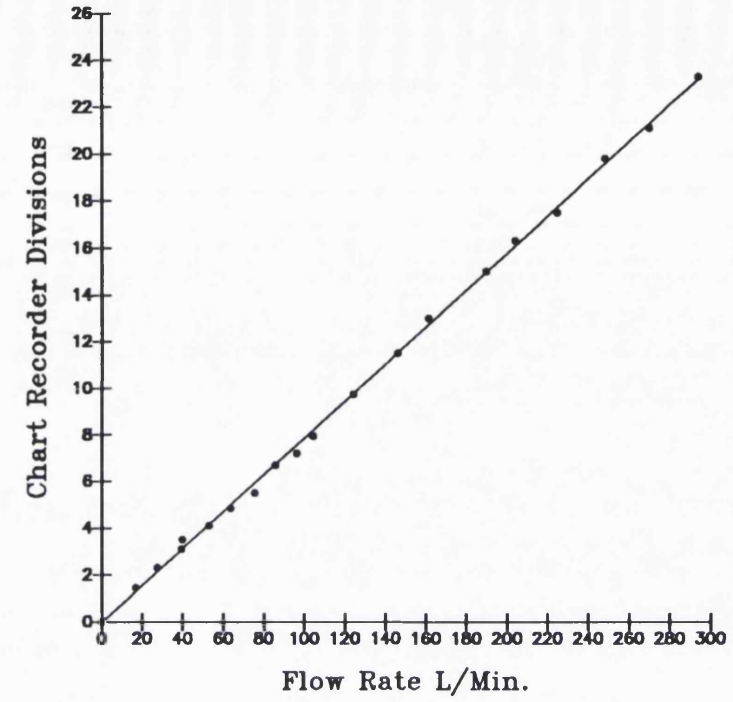
The Diskhaler inhaler adaptor, Figure 4.3, comprises a clear perspex box with hinged lid. The inhaler was placed in the box with the mouthpiece extended through a tightly fitting aperture. The lid was closed and sealed by two clasps. As the subject began to inhale, air was drawn through the distal end of the box via a second opening which was connected to the Fleisch Head.

The output of the pneumotachograph was linked to a pen tracer (Bryans model BS314) which produced a record of the subjects flow-time profile as they inhaled. The maximum and average inspiratory flow rates as well as the total volume inhaled and the duration of the manoeuvre were all derived from the output trace.

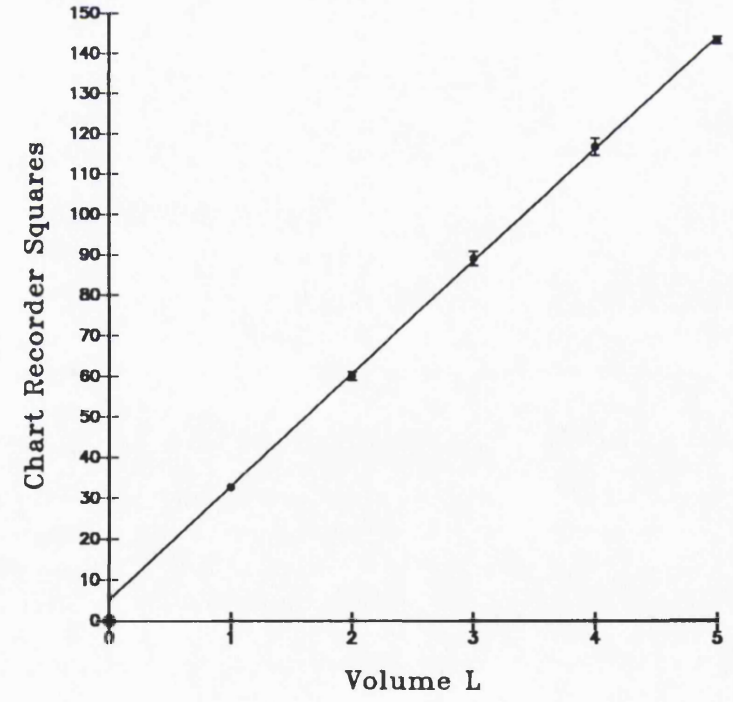
4.2.2.3 Calibration

The pneumotachograph and chart recorder output were calibrated for both flow rate and volume using a Velocicalc Air Velocity Meter (TSI Incorporated). The air velocity meter is a hand-held, battery operated meter that measures both air velocity and air temperature. The instrument uses constant-temperature hot-wire anemometry in which the sensor is held at a constant temperature by a control circuit. As the speed of the air flowing past the sensor increases, more electrical power is required to maintain the sensor's temperature. The power supplied to the sensor is directly related to the air velocity and is indicated by a digital read-out. The instrument automatically compensates for variations in ambient temperature.

The probe was housed in a perspex tube of known cross-sectional area through which the air stream passed. Air velocity was then converted to flow rate by multiplying by the cross-sectional area of the tube. The air flow was provided by a vacuum pump with controllable output. Each inhaler was placed in its flow adaptor and coupled to the Fleisch flow transducer head as for a study. The Velocicalc probe was placed between the adaptor and the Fleisch head. Air was passed through the Fleisch head, and inhaler in its adaptor, and the actual air velocity was measured by the air velocity meter. This was converted to flow rate and plotted against chart recorder output for a range of flow values. Calibration



(a)



(b)

Figure 4.4. ***Calibration of chart recorder for (a) flow rate and (b) volume of air.***

factors were then computed using linear regression and these were used during studies to convert chart recorder output to inspiratory flow rate. The resulting curve is shown in Figure 4.4(a).

The volume of air inhaled by the subject during an inspiration is proportional to the area under the flow-time curve produced by the chart recorder (Figure 4.4 (b)). This area was integrated by counting the squares under the curve and comparing the area to that given by passing a known volume of air through the Fleisch head. This was provided by accurately calibrated 1 and 5 litre cylinders. The chart recorder also gave a direct indication of volume by way of a second pen trace. In all cases the two methods of calculating volume were in good agreement.

4.2.3 Measurement of Blood Pressure and Pulse Rate.

The blood pressure and pulse rate of each subject were monitored at regular intervals throughout each study.

4.3 Subjects

The normal subjects comprised seven males and three females, with a mean age of 34.3 years (range 23 - 49), of which nine were non-smokers and one smoked one cigar per day. None had a history of any respiratory illness. Subjects were considered normal if their FEV_1 and FVC were > 80% predicted for age sex and height and these values did not increase by more than 14% after receiving salbutamol. They were largely recruited from medical and research personnel and lung function technicians based at the hospital.

The asthmatic patients comprised thirteen males and six females with a mean age of 52 years (range 22 - 71). One was a smoker. They were selected from the outpatients clinics at University College Hospital or the Royal Brompton Hospital. All had a documented history of reversible airflow obstruction in response to bronchodilators. Patients were asked to stop their inhaled bronchodilators at least 6 hours before the study and the oral ones at least 12 hours before. They were allowed to continue taking their oral and inhaled steroids. Subjects were only considered if they were over 16 years of age, and for females, not of child bearing potential or with any possibility of being pregnant.

4.3.1 Normal Volunteers

Details of the age, sex and smoking habits of each of the ten normal volunteers are given in Table 4.1 below.

Subject	Age	Sex	Smoker
1	27	Male	No
2	48	Male	No
3	35	Female	No
4	33	Male	No
5	23	Male	No
6	46	Female	No
7	27	Male	No
8	49	Male	Yes (Cigars)
9	31	Male	No
10	24	Female	No

Table 4.1. ***Characteristics of Normal Volunteers.***

4.3.2 Asthmatic Patients

The following information was obtained from the records of the patients:

Patient 1 - A 65 year old male who had been asthmatic for 5 years with no concurrent disease.

Patient 2 - A 65 year old male who had been asthmatic for 11 years with a skin prick test positive to house dust mite in 1979. There was no concurrent disease.

Patient 3 - A 52 year old male who had been asthmatic for 22 years with a skin test positive to house dust mite in 1969. Symptoms were variable but usually well controlled and there was no concurrent disease.

Patient 4 - A 48 year old female who had had asthma since the age of 2 and suffered with bad episodes of breathlessness.

Patient 5 - A 47 year old female who had had asthma for 33 years.

Patient 6 - A 53 year old female who had been asthmatic for 10 years, triggered by cold and stress. There were no other illnesses.

Patient 7 - A 60 year old male who had a 38 year history of asthma, mainly at night.

Patient 8 - A 70 year old female who had had asthma since childhood. Symptoms were reasonably stable and no other disease was present.

Patient 9 - A 54 year old male who had had asthma for 34 years and was allergic to housedust mite. No other illnesses were present.

Patient 10 - 46 year old male who has had asthma since childhood.

Patient 11 - A 46 year old male who had had asthma for 15 years.

Patient 12 - A 52 year old male who had had asthma since childhood and was a smoker. He had no other disease.

Patient 13 - A 42 year old male who had had asthma for 27 years.

Patient 14 - A 71 year old male who had a 7 year history of asthma and had a duodenal ulcer.

Patient 15 - A 55 year old male who had had asthma since childhood. He had also been diagnosed as having allergic bronchialpulmonary asperigillosis.

Patient 16 - A 67 year old female who had had asthma for 6 years with no other disease.

Patient 17 - A 41 year old female who had a 27 year history of asthma but had no other disease.

Patient 18 - A 22 year old male who had had asthma for 6 years but had no other disease.

Patient 19 - A 32 year old male who had had life-long asthma and eczema but no other diseases.

4.3.3 Ethical and ARSAC Approval

Before the commencement of the studies, each patient gave informed consent in writing. The study was approved by the joint University College Hospital and Middlesex School of Medicine Committee on the Ethics of Clinical Investigation. The study was also approved by the Administration of Radioactive Substances Advisory Committee (ARSAC) with regard to the radiation exposure to normal volunteers and patients.

4.4 Administration Procedures

The study of each group of subjects was in three parts with both groups inhaling radiolabelled salbutamol from a Diskhaler inhaler, an MDI and an MDI with spacer. Subjects inhaled from each device on a separate day and in random order. Flow measurements were not made for the MDI with spacer. Each subject was given simple instructions on how to use the inhaler, and allowed some practice runs with placebo inhalers. No attempt was made to regiment the subject's inhalation technique other than the use of these simple guidelines.

Assistance during the administration procedures was provided by Dr R. Melchor of the Thoracic Medicine Department at University College Hospital who also performed the lung function tests and measured the blood pressure and pulse rate of each subject. Dr Melchor was also responsible for recruiting the subjects.

4.4.1 Diskhaler Inhaler

Subjects inhaled 200 μg of salbutamol from a single unit dose Diskhaler blister. The normal subjects inhaled twice from the same blister while the patients inhaled only once. All subjects breathed out to residual volume, placed the mouthpiece in their mouth, and then rapidly inhaled. They then held their breath for 10 seconds before gently exhaling into a bag. This last step permitted an assessment of the proportion of aerosol particles exhaled.

4.4.2 Metered Dose Inhaler

Subjects inhaled 200 μg of salbutamol as two separate 100 μg doses. They inhaled from the MDI after breathing out to residual volume followed by a steady deep inhalation actuating the MDI themselves immediately after the start of the manoeuvre. At the end of the inhalation, they held their breath for 10 seconds before gently exhaling into a bag. The second dose of salbutamol was then inhaled in exactly the same fashion.

4.4.3 Meter Dose Inhaler with Spacer

Subjects inhaled 200 μg of salbutamol as two separate 100 μg doses from an MDI via the spacer. Subjects were again asked to breath out, but this time not to residual volume. They

placed the spacer mouthpiece into their mouth, actuated the MDI into the spacer and performed a slow gentle inhalation, similar to a large tidal breath. They held their breath for ten seconds and then exhaled into the collecting bag. A second inhalation was carried out in the same way without actuating the MDI. Following a 10-second breath hold the subject again exhaled into the collecting bag. The MDI was then actuated into the spacer for the second time followed by two inhalations as before.

4.5 Statistical Analysis

Statistical analysis was performed on various parameters to determine whether there were any significant differences between the three inhalation methods within each group of subjects. The data were also analysed to determine if there were significant differences between normal and asthmatic subjects. Data were first tested for normality using the Wilk-shapiro test of normality available on the RS/1 statistical package. All further statistical tests were carried out with the SPSS/PC package (SPSS Inc.) using a desk top computer.

The normal volunteers and the asthmatic patients were two distinct groups of subjects and within each group all individuals inhaled from the three different inhalers. Comparisons made within each group were made on the same individuals after inhaling the same dose of salbutamol from each of three inhalers. Any differences between the corresponding mean values should therefore be due only to the mode of inhalation. Variables could only be compared between the two groups of subjects as independent variables since they were obtained from two separate sets of subjects of unequal size.

When comparing the mean values of two data sets the Student's t-test may be used to determine if they are significantly different. The null hypothesis determines the probability p of obtaining a t value at least as large as the one observed if the two sets of data are equal, and it asserts that there is no real difference between the two. If the observed significance level is judged small enough, the null hypothesis can be rejected and the two samples can be considered to be from separate populations. The larger the size of t, the less likely it is that the result is due to chance, and the smaller the p value the more significant is the result.

The t-test is used for small samples (n < 25) and requires that the population distributions are normal but it is robust against moderate departures from this assumption (Kirkwood, 1992a). When comparing the means of two samples, the validity of the t-test also depends on the equality of the two population standard deviations. In many situations it is reasonable to assume this equality. However, if the sample standard deviations are very different an alternative test must be used. The number of degrees of freedom is calculated from the sample size in the two groups and is used together with the t value in establishing the observed significance level.

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{S_1^2/N_1 + S_2^2/N_2}} \qquad (4.1)$$

where $\overline{X}_1$ and $\overline{X}_2$ are the sample means of group 1 and group 2 respectively, S^2_1 and S^2_2 are their variances and N_1 and N_2 are the two sample sizes.

The pooled-variance t-test may be calculated by replacing the individual group variance in Equation 4.1 by the pooled estimate of the variance S^2_p. It is assumed that the population variances in the two groups are equal and this is tested by the ratio of the larger sample variance to the smaller, known as the F value. The degrees of freedom for this statistic is equal to the sum of the sample sizes in the two groups minus 2. The two-sample t-test was used to compare equivalent data sets obtained from normals and asthmatics when inhaling from the same device. A p value of < 0.05 was taken as significant.

The paired t-test is used to test the difference between a pair of variables measured on each individual, such as the response to two separate treatments. Interpreting the significance results from paired experiments follows the same basic steps as for independent samples, except that the t value is calculated by a different computation. The sampling distribution of t is Student's t with N-1 degrees of freedom, where N is the number of pairs.

When there are more than two sets of data to compare, it is possible to take the samples two at a time and perform separate t-tests. With several samples this can not only be tedious but theoretically unsound. Carrying out a large number of t-tests increases the risk of obtaining a statistically significant result by chance alone. The t-test is generalised for more than two group comparisons by means of the analysis of variance. The basis of the test is to pool all the data to get a single, much better estimate of variance, with a larger number of degrees of freedom. There are both between-groups and within-groups degrees of freedom.

A two-way analysis of variance was used to compare the deposition parameters resulting from each inhaler within each group. Data was classified in two ways; by inhaler type and by individual, with one observation for each technique for each subject. The two-way analysis of variance for a balanced design without replication is an extension of the one-sample paired t-test, comparing the values of more than two variables measured on each individual (Kirkwood, 1992b). The null hypothesis was used to test that the means recorded were different samples from the same population, as for the t-test. The size of the variance, between each inhaler, was compared to the overall variance to test the null hypothesis. In this case the test statistic was the F test. The F test or variance ratio, is defined for analysis of variance as the ratio of the between group mean squares (MS) to the within group mean squares. In the case of two-way analysis of variance for a balanced design without replication, F is calculated from the ratio of the MS of the main effect (inhaler technique) with the MS of the interaction between observations.

If the null hypothesis is true, no further analysis is necessary. However, if the null hypothesis is rejected, the conclusion is that there is a real difference between the inhaler techniques or, more precisely, at least two of the techniques differ significantly from each other. The data must be analysed further to reveal exactly which of the techniques differ. In this case pairs of data were tested using the method of linear contrasts (Armitage and Berry, 1987). This is a common method for subdividing data in order to examine the differences contributing to a significant effect in more detail. A t-value and its significance level are given for each comparison made. For these studies a p value of < 0.05 was taken as significant.

For analysis of variance, as for the t-test, it is assumed that the samples are taken from populations whose data are normally distributed and that the standard deviations of the two means being compared are the same. The analysis of variance is again robust against moderate departures from normality but large differences in standard deviation are serious enough to require the data to be transformed.

The standard deviations of the deposition data for the three inhaler methods were not all the same. One possible remedy to this problem is to transform the data before performing the analysis of variance. This may be achieved by taking logarithms to base ten of each data element (Kirkwood, 1992c). This operation has the effect of equalising the standard deviations as well as removing any skewness in the data. However, this had little or no effect on the standard deviations of the data sets. An alternative was to use the non-parametric Friedman two-way analysis of variance test. Non-parametric tests are a less powerful method of comparing means than their parametric equivalent but their advantage is that they make no assumptions about the underlying distribution of the data and are therefore more accurate in cases of non-normality or unequal standard deviations. When performing the Friedman test the variables to be compared are first ranked in order for each case, and the mean ranks for the variables are calculated and compared, resulting in a test statistic with approximately a chi-square distribution (SPSS/PC Statistics 4.0 manual, 1990).

The Spearman rank correlation coefficient was used to establish association between the flow parameters (maximum and mean inspiratory flow rates, duration, and volume inhaled) and lung deposition, and also between lung deposition and improvement in lung function parameters (FEV_1, FVC and PEF). The correlation coefficient is a dimensionless quantity ranging from -1 to +1 and indicates the strength of association between two variables which are linearly related. A positive correlation coefficient is one in which both the variables increase together. A negative correlation is one in which one variable increases as the other decreases. When variables are exactly linearly related the correlation coefficient either equals +1 or -1. The Spearman rank correlation is a non-parametric measure of association between two numerical variables based on the ranks of the data. The spearman rank correlation coefficient r_s is given by equation 4.2

$$r_s = 1 - \frac{6\Sigma d^2}{N(N^2-1)} \qquad (4.2)$$

Where d is the difference between each pair of ranks and N is the number of subjects. To test if r_s is significantly different from zero **t** is calculated with N-2 degrees of freedom.

$$t = r_s \sqrt{\frac{(N-2)}{(1-r_s^2)}} \qquad (4.3)$$

When the correlation coefficient is based on the original observations it is known as the Pearson correlation coefficient **r**.

To determine whether the correlation coefficient is an appropriate measure of association, a plot of the data is first made. The correlation coefficient should not be used if the relationship is non-linear, even if there is a perfect association between the two variables (Campbell and Machin, 1990). A correlation between two variables shows that they are associated but does not necessary imply a 'cause and effect' relationship.

Chapter 5. Radionuclide Imaging System and Calibration Procedures

5.1 Introduction

In order to visualise an in-vivo distribution of gamma emitting radiopharmaceutical compound, the principle instrument in clinical use is the gamma camera. It produces an image of the compound's distribution from the emitted radiation detected at the surface of the subject being studied. The final image is formed either on a photographic film or is digitised and stored on computer for further processing.

In these studies, all radionuclide images were obtained using a Siemens ROTA Camera interfaced to a DPS-3300 Nuclear Medicine Computer System (ADAC Laboratories). This is a dual-headed gamma camera capable of acquiring anterior and posterior views simultaneously using two opposed 37-tube ZLC detectors, each having a 40 cm field of view. The system is also capable of single photon emission computed tomography (SPECT), although it was not used in this mode during these studies.

5.2 The Imaging System

The ROTA system (Figure 5.1 (a)) comprises three major components: a rotating gantry assembly supporting two detector heads; a patient couch; and a control console. Each detector head assembly is steel and lead shielded and contains a scintillation crystal, light guide, photomultiplier (PM) tubes, and electronic circuits which detect and locate the gamma radiation being emitted by the radionuclide. Interchangeable collimators can be fitted to each detector and are supported on storage carts when not in use. The patient couch is connected to the gantry and comprises a cantilevered pallet (bed) to support the patient. For this study it was not used since the subjects were seated in an up-right position on a chair during imaging (Figure 5.1(b)). The control console comprises timers and counters to determine the length of the exposure, radionuclide analyser windows, system controls and two display scopes from which hard copy images on photographic film may be obtained.

5.2.1 Collimators

As gamma rays are emitted isotropically, a gamma camera image cannot be produced without using a collimator. The function of the collimator is to transmit the gamma rays from a radionuclide distribution within a subject to the scintillation crystal and provide

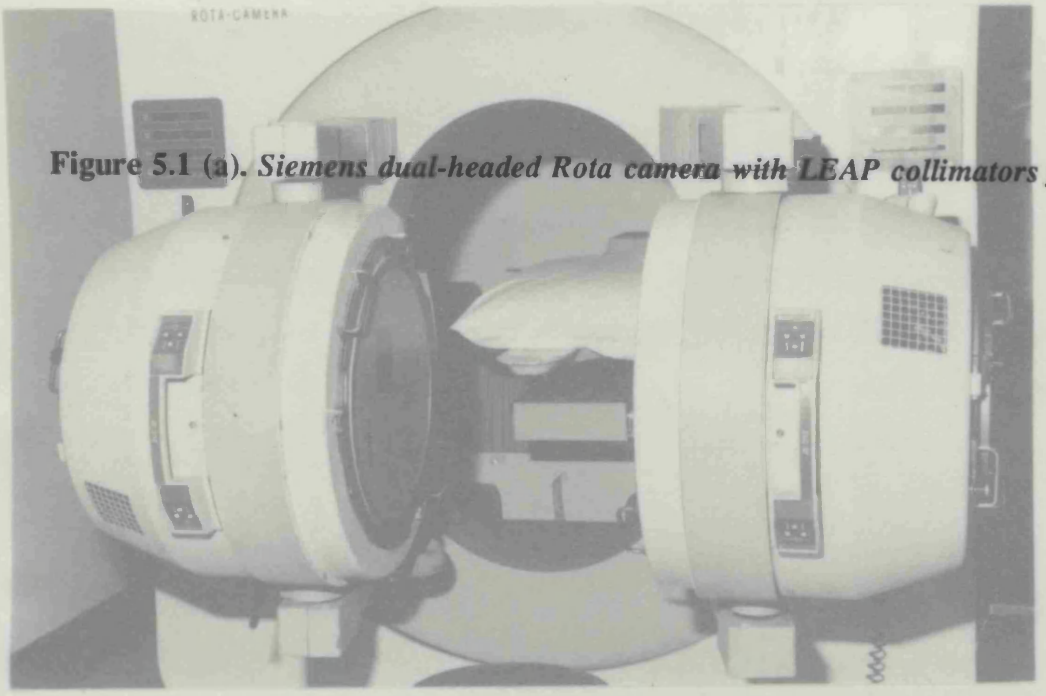


Figure 5.1 (a). ***Siemens dual-headed Rota camera with LEAP collimators fitted***

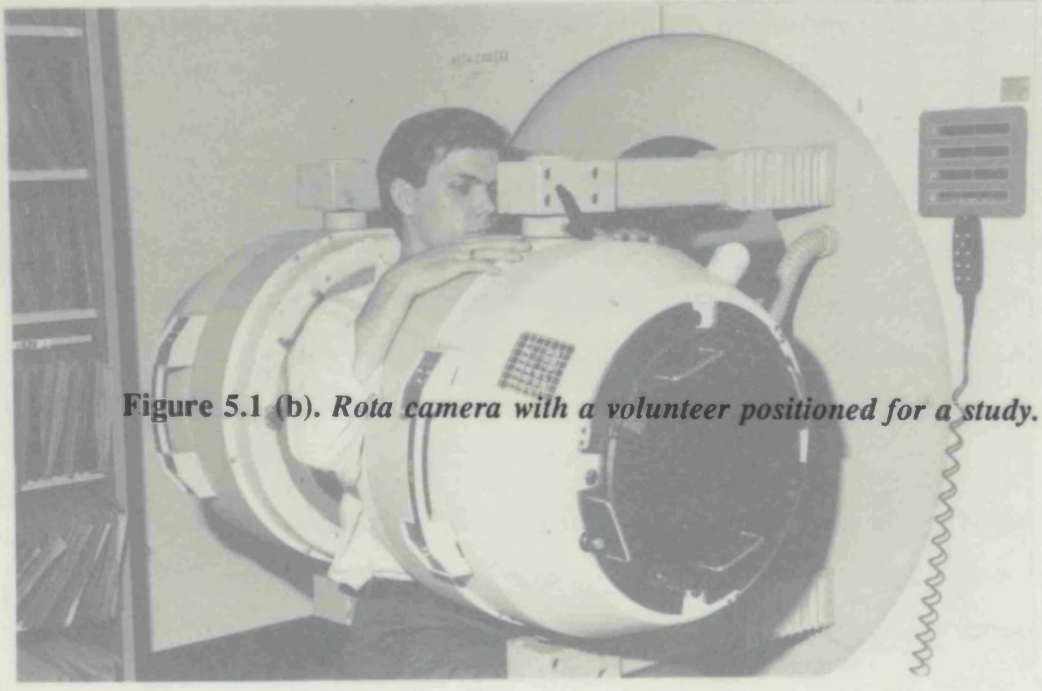
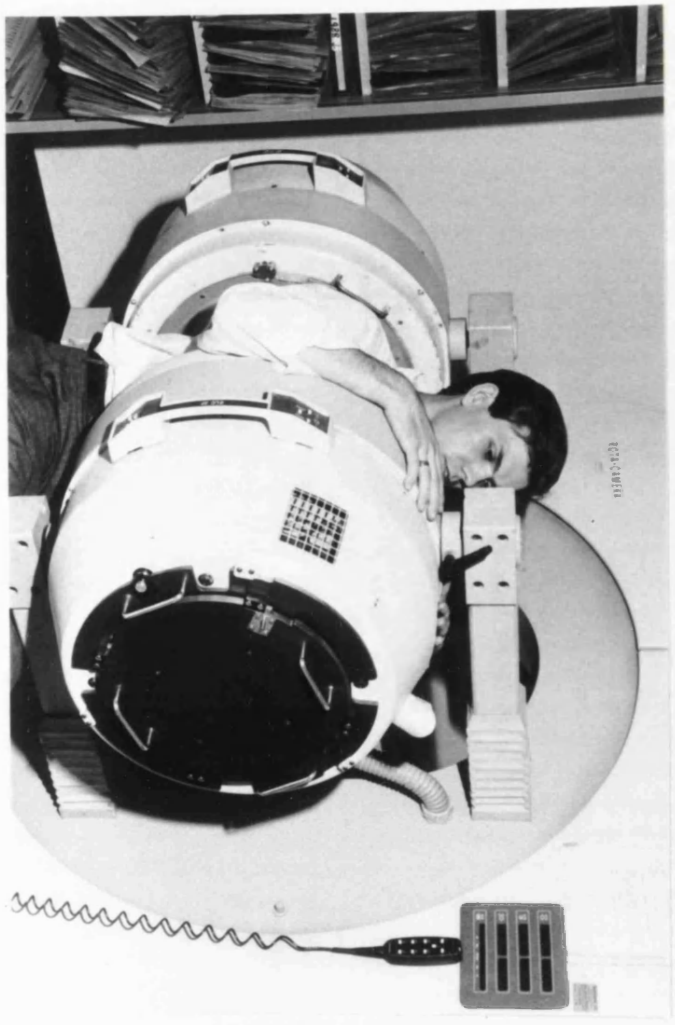
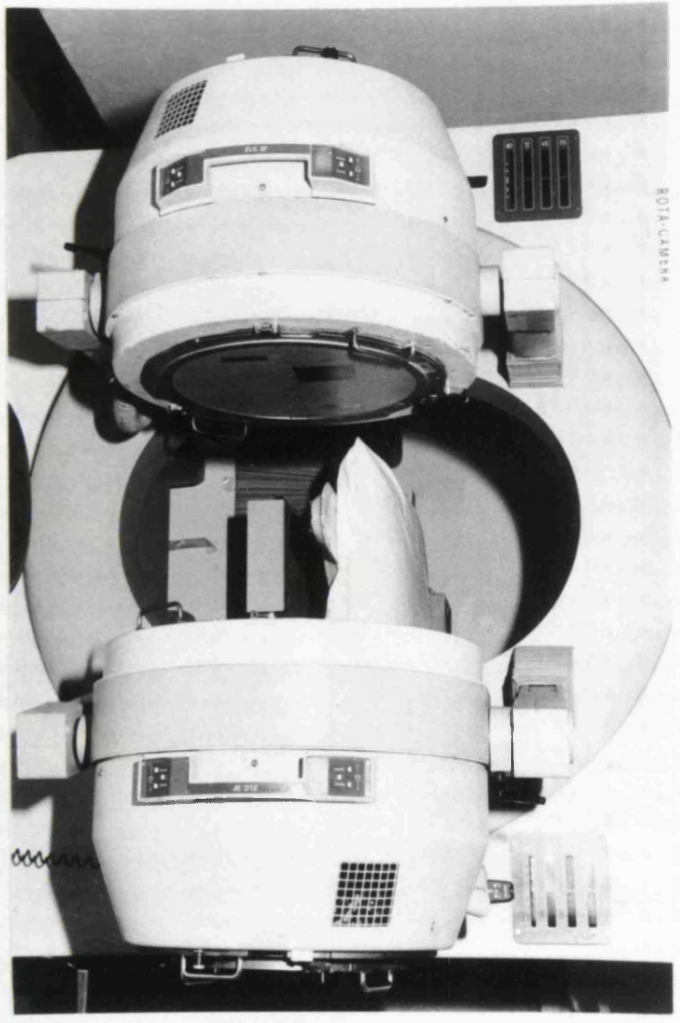


Figure 5.1 (b). ***Rota camera with a volunteer positioned for a study.***




ROTA-CAMERA

an accurate relationship between the position at which they hit the detector and that from which they were emitted. Although several designs of collimator are available, the parallel hole collimator can be used for most applications. With this type of collimator, only those gamma rays which are travelling in a direction approximately perpendicular to its face are transmitted through the holes.

It is necessary to select a collimator appropriate for the energy of the gamma radiation being used otherwise excessive septal penetration or poor sensitivity will degrade the image. For these studies, both heads were fitted with a low energy all purpose collimator (LEAP) when imaging gamma rays of energy 140 keV from ^{99m}Tc and with a medium energy collimator when imaging the more energetic (190 keV) gamma radiation emitted from Krypton-81m (^{81m}Kr) gas.

5.2.2 Theory of Operation

The gamma radiation from the area being imaged strikes the face of the collimator which is mounted on the detector head (Figure 5.2). The gamma rays which are transmitted strike a sodium iodine scintillation crystal which has been activated by the addition of a small quantity of thallium, NaI(Tl). The scintillation crystal changes the energy of each gamma ray which strikes it into light energy in direct proportion to the amount of energy dissipated within the crystal. The light photons are transmitted through a clear plastic light guide to an array of 37 PM tubes. Each tube converts the light energy into an electrical pulse whose amplitude is directly proportional to the light energy striking the tube. The position of the scintillation in the crystal is translated by the PM tube array and the electronic positioning circuits to a corresponding position on the display. The output of each PM tube in the array depends on its proximity to the scintillation and on the energy of the event.

Analysing and positioning circuits receive the output from the array, separate out the energy component (Z), and produce a positioning matrix such that each detected event can be accurately assigned an X and Y coordinate. Energy threshold circuits allow only those signals above a preset minimum level to be registered and form the image. An energy window is set by the operator according to the radionuclide being used in order to select

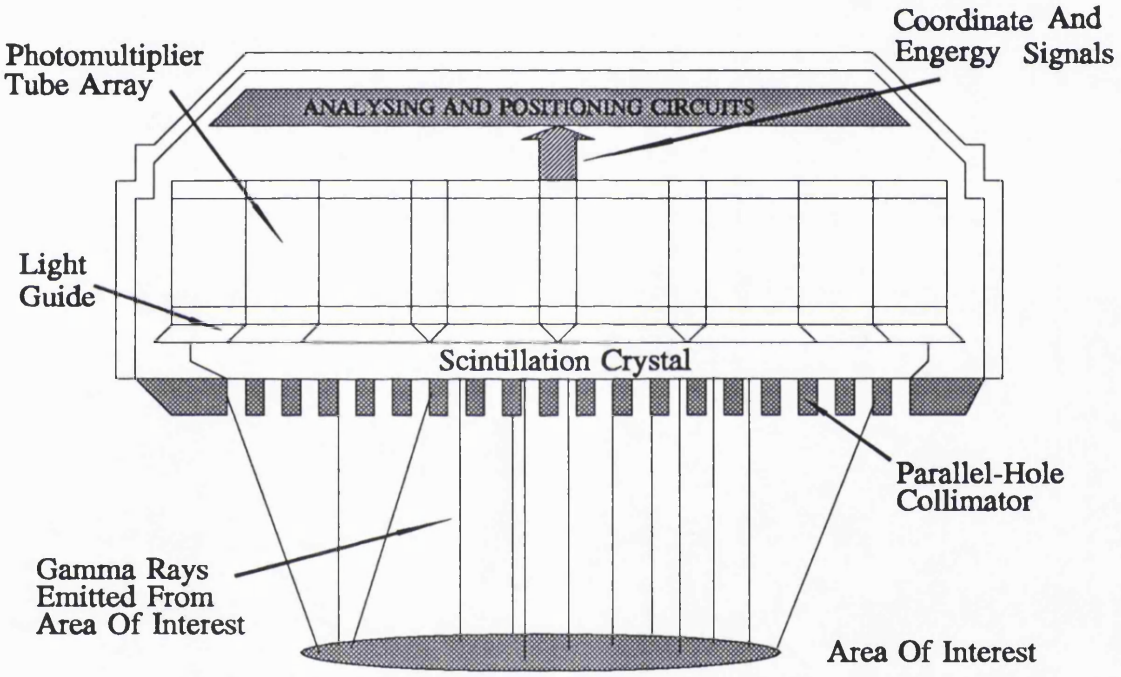


Figure 5.2 *Diagram of a ROTA Camera detector head.*

only those pulses resulting from photoelectric interactions within the crystal (Photopeak). This prevents low energy signals such as those due to Compton scatter and signals from PM tubes that are distant from the scintillation from degrading the image. Pulse pile-up rejection circuits improve image contrast and energy resolution by rejecting two or more signals occurring very close together when sources of high count rate are imaged. Two low level signals could otherwise be added together to generate a " false" event and produce an artifact on the display.

The system produces an analogue X and Y output. This is converted to a digital signal, suitable for computer input, by an analogue-to-digital converter (ADC) located within the DPS-3300 computer cabinet.

5.2.3 The Computer System

The DPS-3300 is a minicomputer system comprising a cental processing unit (CPU), an array processor, RAM and video memory, two console screens, a light pen, keyboard, one floppy disc drive, two 20 MByte Winchester hard disc drives, and a magnetic tape drive. An NEC spinwriter printer is linked to the system for providing output listings. The two console screens enable the separate display of software control menus and acquired images. The software provided with the system permits a wide range of nuclear medicine applications and enables the collection and reconstruction of data as static, dynamic, cyclic gated, and tomographic images. The same system also permits the processing of image data.

5.2.4 Gamma Camera Performance

Since these studies involve extensive quantification of drug deposition images, it was necessary to periodically check the performance stability of the Rota camera using a suitable quality control package available in the department. Parameters such as energy resolution, spatial resolution, spatial distortion and count rate performance were measured 6-monthly and at other times as appropriate (e.g. system failure). Uniformity of response and total system sensitivity were checked weekly and committed to a written log.

5.2.4.1 Uniformity of Response

Ideally the response to a uniform flux of radiation should not vary significantly from one part of the crystal to another. Any variation is referred to as non-uniformity and is demonstrated by imaging a uniform distribution of radioactivity such as a flood source. The system non-uniformity (collimator fitted) is the most important in clinical use, and regular measurement with a flood source will show up any deterioration of the camera performance. For the quality control measurements carried out during these studies a ^{57}Co flood source (200 MBq) was used to obtain a 64 X 64 pixel quantitative image containing 30 million counts. Analysis of data yielded integral and differential non-uniformity and pixel populations within defined percentages of the mean pixel value.

5.2.4.2 Sensitivity

Sensitivity can be regarded as the overall detection efficiency of the camera which is primarily affected by the collimator. It is also related to the conversion efficiency of the PM tubes and occasionally it is necessary for them to be tuned by adjusting their high voltage in order to maintain stability of output.

The sensitivity of each head, for a given parallel hole collimator, was measured at the same time as the ^{57}Co flood source acquisition. A record of counts per second per MBq was obtained which permitted a comparison between values obtained over a period of time and after allowing for radionuclide decay.

5.2.5 Advantages of the Dual Headed System.

An important advantage of this system over single headed cameras is that dynamic acquisitions can be undertaken, to follow the movement of rapidly changing radionuclide distributions, without the need to reposition the subject during the process and without subsequent loss of data and inconvenience to the subject. As both anterior and posterior images are obtained at the same time the subject maintains the same position for both.

Simultaneous acquisition ensures more accurate quantification of the radionuclide distribution within the body by enabling the geometric mean (GM) of the anterior and posterior counts to be calculated. The GM (net anterior count X net posterior count)$^{1/2}$, gives a value that is effectively independent of the changing source depth within a fixed thickness of tissue (Fleming, 1979).

5.3 Imaging Procedures and Dose Quantification

Prior to the start of each study the energy window for each detector head was set (peaked) for Technetium-99m. This was achieved by placing a low level ^{99m}Tc source between the two heads, and activating the pre-calibrated ^{99m}Tc window from the console control panel. The spectrum and window were both displayed to ensure that the two coincided. For ^{99m}Tc, the photopeak energy was selected at 140 keV and the window width 20%.

Following administration of the inhaler preparation, as described in Chapter 4, subjects were immediately seated between the heads of the camera with their lungs at the centre of the field of view as shown in Figure 5.1 (b). Images were acquired using a 64 x 64 pixel matrix (16-bit depth), and data collection was divided into five 60 second time frames so that movement of the radiolabel could be followed as well as the initial deposition assessed. Images were later summed to increase the number of counts per pixel. Immediately afterwards the subject was repositioned to obtain images of the radionuclide distribution in the throat and in the stomach. These images were acquired separately for 120 seconds each. Afterwards subjects were asked to gargle with water in order to remove the radioactivity that had collected in the throat.

Radioactivity retained in the MDI valve and actuator was collected into a flask by washing with methanol. This solution was then counted for 100 seconds using the gamma camera. Similarly the proportion of dose remaining in the Diskhaler device after inhalation was removed by methanol, into a flask, and again measured with the gamma camera. The activity in the spacer was measured by placing it between the camera heads and imaging for 100 seconds. The counts from the exhalation bag were similarly measured.

The potential activity delivered from two actuations of the MDI was calculated by actuating five times into an adapted collection bag (Figure 5.3) and then obtaining the GM counts from the radioactivity collected. This result was divided by a factor of 2.5 to give the mean activity from two actuations. For the Diskhaler inhaler, the potential activity was measured by counting over a single dry powder unit dose blister before administration.

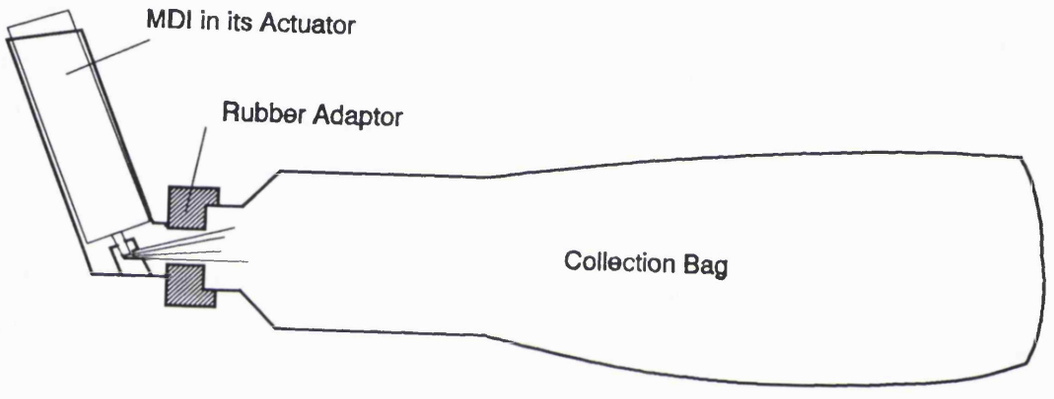


Figure 5.3 ***MDI collection bag.***

In addition to the three radioaerosol studies, subjects underwent a Krypton (^{81m}Kr) scan in order to provide a clear outline of their lungs to be used in the data analysis. The krypton gas was obtained from a ^{81}Rb generator (MRC Cyclotron Unit) by eluting with water saturated air from a compressed air cylinder. The krypton/air dilution was piped directly to the subject via a face mask. The subject inhaled the gas by tidal breathing and anterior and posterior images were acquired. Since the ^{81m}Kr gas has a half life of only 13 seconds, this study was usually performed on the same day, and shortly before, one of the radioaerosol studies.

5.4 Data Analyses

All data analyses were carried out using the software resident on the ADAC computer system. Using a light pen, regions of interest (ROI) were drawn on the images of the lung, throat and stomach in order to delineate the counts attributable to each separate region of the image. Each subject's krypton image was used as a template enabling an outline of their lungs to be drawn with the light pen. The computer software permitted the transfer and alignment of these outlines to the corresponding radioaerosol deposition images. The counts from radioactivity located between the two lung fields, corresponding to that part of the dose within the oesophagus and the lower part of the trachea, were attributed to the mediastinum ROI.

Since it was difficult to prevent subjects from swallowing, during the 5 minutes of imaging time, there was usually a relatively high level of radioactivity accumulated in the stomach, due to the fraction of the dose that was initially impacted in the throat being swallowed. This was not the case when subjects inhaled from the MDI with a spacer device since very little was impacted in the throat. In order to prevent stomach counts being included in the lung ROIs and thus being incorrectly attributed to lung deposition, it was necessary in some cases to modify the lung ROI. However, in most cases, any overlap was small. Counts within each ROI were then calculated by the computer.

For each image, the counts due to background radiation were estimated by initially taking a small ROI away from the main image fields and determining the number of counts per pixel within it. These were then multiplied by the number of pixels contained within each ROI boundary and then subtracted from the ROI count.

Following correction for background counts, the anterior and posterior images were combined by calculating the geometric mean (GM) of each ROI. This was corrected for the radionuclide decay, which had occurred between imaging of the various views, and attenuation due to the body tissue.

The percentage of radionuclide deposited in the lungs, throat, mediastinum and stomach, as well as that exhaled and that remaining in the inhaler and spacer, were calculated by comparing the counts determined for each site with those obtained from the potential dose administered during the study. The potential dose was taken as 100% and the percentage deposited in each location determined accordingly.

5.4.1 Attenuation Correction

Gamma-ray photons emitted by a ^{99m}Tc source located within the body are attenuated by body tissues before reaching the gamma camera detectors. This means that the counts detected are less than would be observed if the source were in air alone. A method to correct for this attenuation using gamma ray-transmission, and based on the principles outlined by Tothill and Galt (1971) for quantitative profile scanning, has been adopted.

A small ^{99m}Tc source of known radioactivity was sandwiched between layers of tissue equivalent perspex placed between the two heads of the gamma camera. The GM of the counts obtained was not dependent on the depth of the source within the perspex but only on the thickness of perspex that lay between the two heads (Tothill & Galt, 1971). This meant that for a constant total perspex thickness the source could be moved to several positions relative to the two detector heads and almost identical GM counts obtained. The GM counts for several total thicknesses of perspex were obtained and then divided by the GM counts obtained when the source was in air alone to give relative response factors for each thickness of perspex. The relative response factors were then plotted as a function of perspex thickness (Figure 5.4).

For ^{99m}Tc deposited in the abdomen or pelvis, these factors are very similar to the actual attenuation of the tissue overlying the source and can be directly applied to correct for this attenuation by measuring the patient with callipers. However, the thorax has a much lower

and less uniform density and these factors over-estimate the amount of ^{99m}Tc within the lungs. It was therefore necessay to determine "effective" tissue thickness for the thorax and throat regions.

To achieve this, the percentages of gamma-ray photons transmitted through known thicknesses of perspex were determined. Different thicknesses of perspex were placed between a flood source of 99m-technetium and one of the heads of the gamma camera. The percentage transmission for each thickness of perspex was obtained by comparing the counts acquired for a given thickness with those obtained when no perspex was present. The percentage transmission was plotted as a function of perspex thickness (Figure 5.5). The two plots were then combined to obtain a third plot of relative response as a function of percentage transmission (Figure 5.6).

A transmission image of the chest, throat and abdomen of each subject was obtained using the 99-technetium flood source. For convenience this was usually obtained whilst the subject was present for one of the inhaler studies, it being carried out prior to the commencement of this study. The subject was seated between the flood source and the gamma camera head that was used to acquire the image. Regions of interest were drawn over the lungs, throat and stomach, and the counts they contained determined. The image was then compared to the " in air " image of the flood source alone to derive the percentage transmission for each region. This was then used to determine the modified relative response factors to correct for attenuation in each separate region.

In order to assess the accuracy of this method, a phantom (Alderson Research Laboratories Incorporated) of the head and torso was used. Water was placed in the chest/head compartment and a transmission scan was obtained. Relative response factors were derived as explained above. Small amounts of radioactivity comparable with that expected in the lungs during a study were counted first in air and then in the lung compartment of the phantom. An average discrepancy between the in air counts and the corrected phantom counts of 3% was obtained from four such experiments.

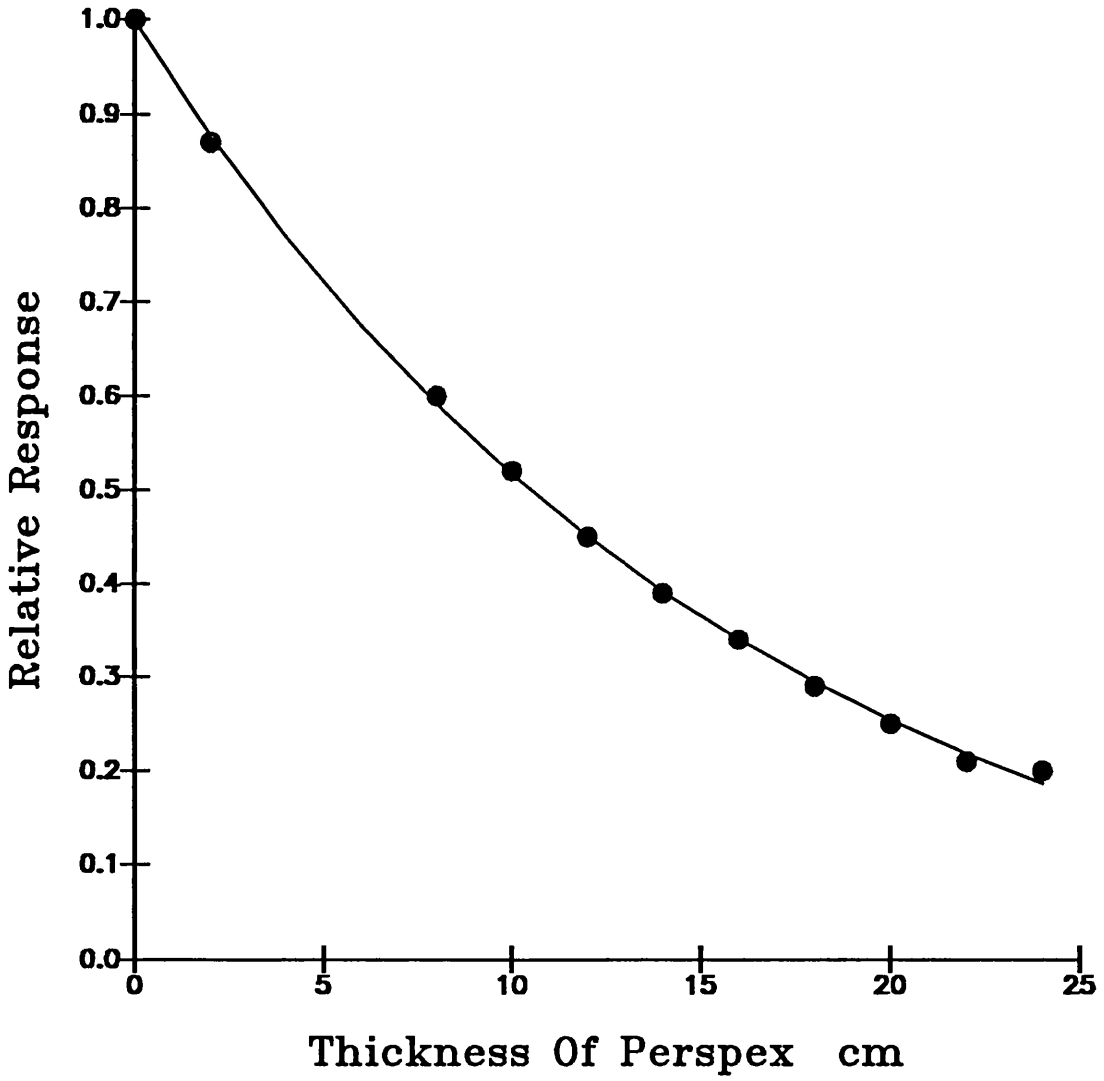


Figure 5.4 *The geometric mean response from the two heads of the gamma camera to a ^{99m}Tc point source between layers of perspex expressed relative to the same source in air.*

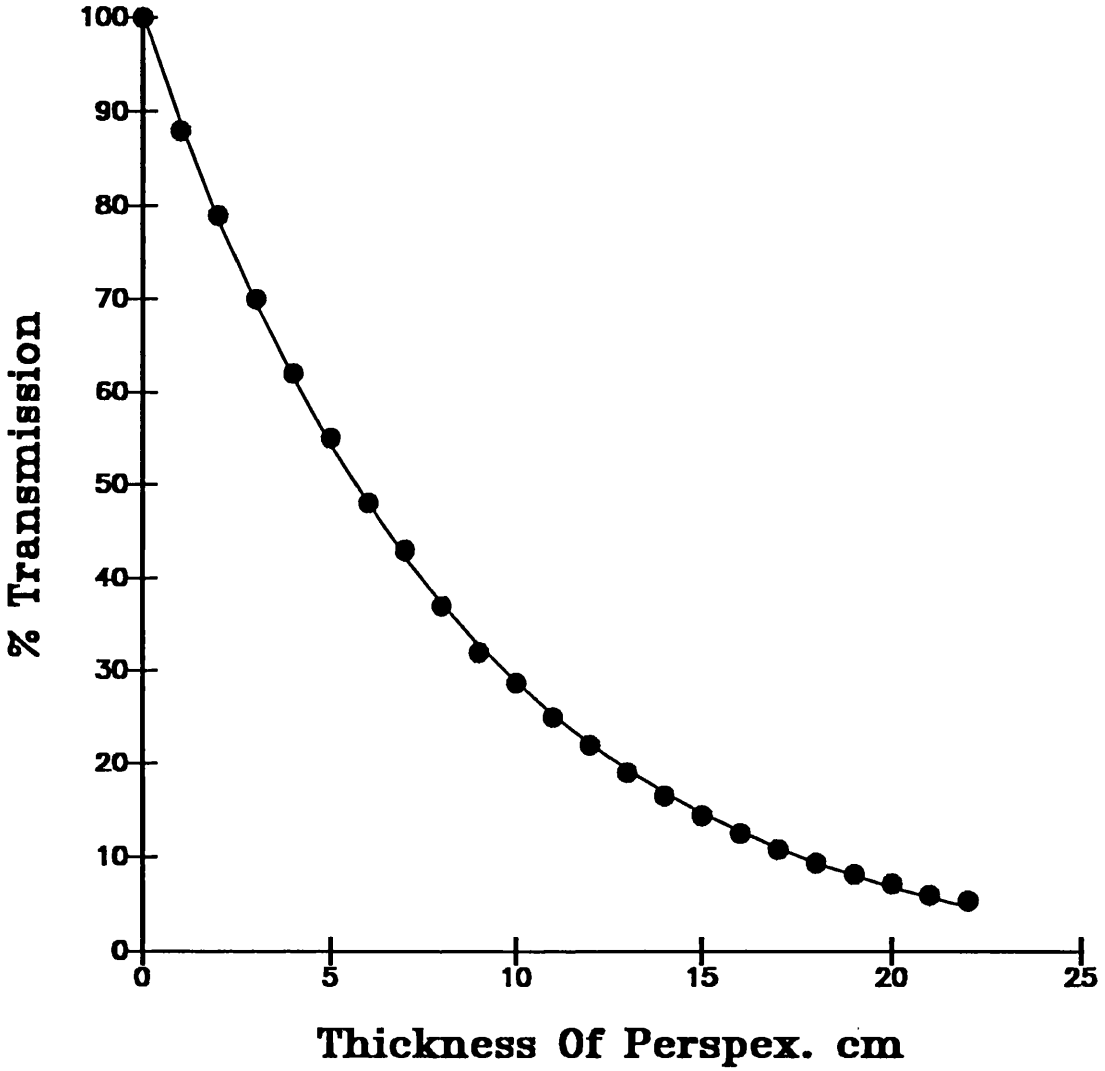


Figure 5.5 *Transmission of ^{99m}Tc gamma-rays through layers of perspex.*

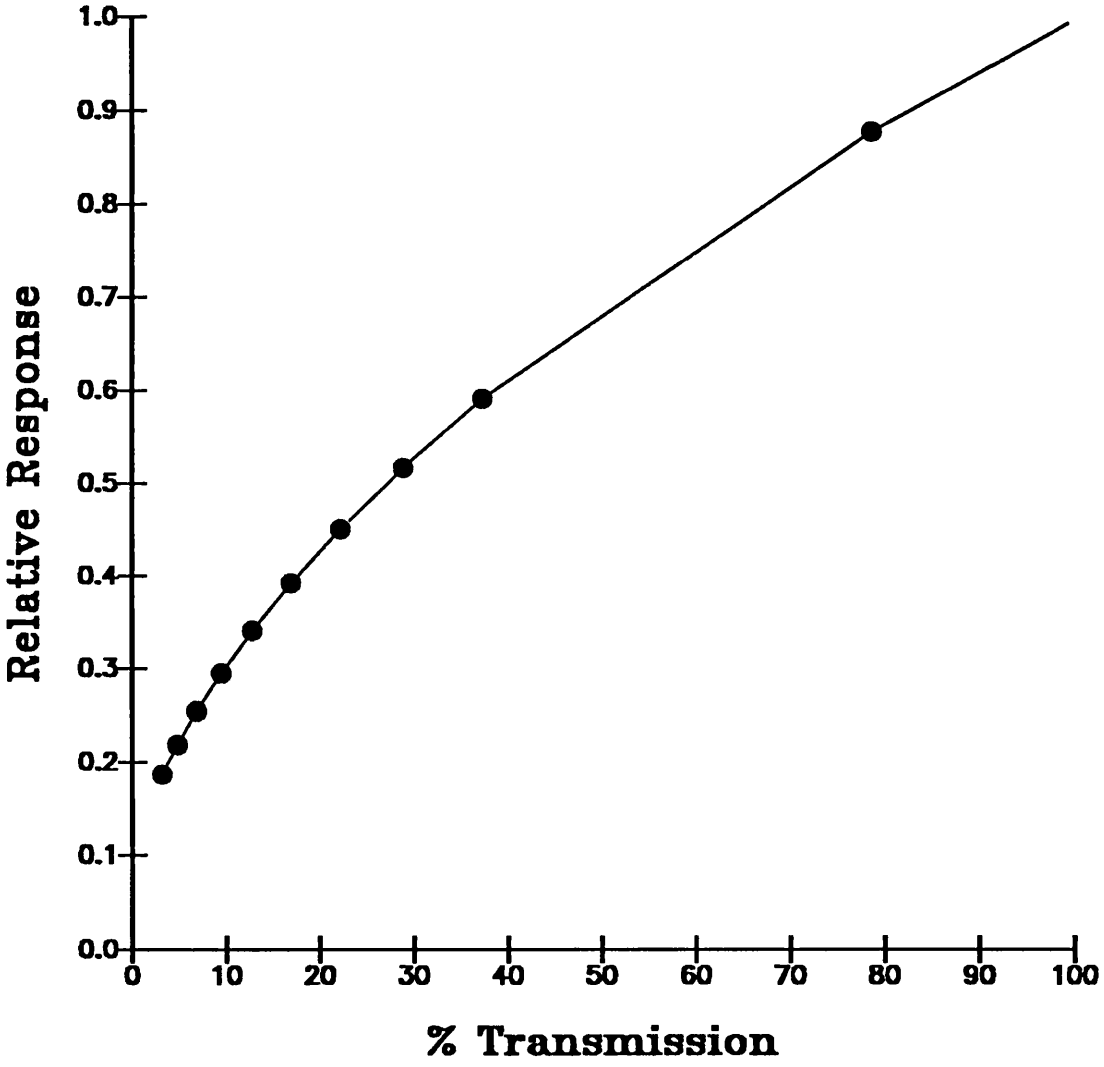


Figure 5.6 *Relative response to a ^{99m}Tc source between layers of perspex and in air, plotted as a function of percentage gamma-ray transmission through perspex.*

5.5 Measurement of Regional Lung Deposition

The exact site to which therapeutic aerosols should be delivered in the lungs is not fully understood (Newman, 1984a). In the case of β_2-agonists, most evidence points to a direct action of the drug on the β_2-receptor sites located in the bronchial smooth muscle (Chapter 1). Since beta receptors are located in both peripheral and central airways (Newhouse and Ruffin, 1978; Carstairs *et al*, 1985), with greatest abundance at or beyond the terminal bronchioles (Kuitert, 1992), it is important for the drug particles to penetrate to all parts of the lungs including the very small airways of the peripheral region.

It is recognised that the presence of airways obstruction is a major factor in reducing aerosol penetration into the peripheral regions of the lungs (Pavia *et al* ,1977; Dolovich et al, 1976; Short *et al* 1979). In asthma this is usually due to bronchial smooth muscle constriction, or increase in the airways mucus coating. Bronchial constriction can occur well into the bronchial tree where the smooth muscle encircles the bronchioles (Hidy, 1984). When airways obstruction develops, changes in the distribution of flow in individual airways alters the deposition pattern of the normal airways and obstruction in the large airways increases central impaction compared with the normal lung (Agnew *et al*, 1981).

In the above studies, subjects with varying degrees of airways obstruction inhaled, by steady tidal breathing, either nebulised aerosol or aerosol from a spinning disk generator. Some investigators have indicated that aerosol lung deposition and penetration from an MDI is less affected by airways obstruction (Newman *et al*, 1983; Spiro *et al* ,1984) than aerosol inhaled by steady breathing. It was therefore important in the present studies to compare drug deposition in the lungs of normal subjects and asthmatic patients in order to ascertain if there were any differences in the deposition patterns of aerosol delivered, by the three devices, between these two groups of subjects.

Frequently used assessments of aerosol lung penetration are aerosol penetrance (Dolovich *et al*, 1976), the penetration index (Agnew *et al*, 1981, Hannan *et al*, 1982) and the alveolar deposition (Sanchis *et al*, 1972). Penetration index is a measure of the penetration of aerosol particles relative to the penetration of an inhaled radioactive gas

such as ^{81m}Kr. It is obtained by separating the lung field into inner, intermediate and outer regions and dividing the ratio of counts measured in the outer to inner regions for the aerosol by the same ratio measured for the gas.

Alveolar deposition is determined by measuring the proportion of radioaerosol particles retained in the lungs 24 hour after inhalation. This assessment assumes that particles deposited on the ciliated airways are cleared relatively quickly from the lungs with complete removal within 24 hours. Those deposited in the nonciliated respiratory bronchioles and alveoli are assumed to undergo negligible clearance in that time.

To measure aerosol penetrance the lung is separated into inner, intermediate and outer regions. It is determined by measuring the ratio of the amount of radioactivity penetrating to the nonciliated peripheral airways, measured by 24 hour retention in the outer region, to the amount of radioactivity deposited in ciliated airways, measured by the initial aerosol deposition in the inner region, and corrected for lung volume using a radioactive gas. The main difference between this measurement and penetration index is that outer region alveolar deposition is used instead of outer region total deposition.

To evaluate the regional lung deposition of the salbutamol particles a simpler method was used. Each lung field was divided into central and peripheral regions. The regions were proportionally equal between subjects and depended on the dimensions of the lung field. For both anterior and posterior lung images, the vertical edge of the central region was drawn with the light pen to bisect the lung field. The height of this region was 2/3 of the maximum lung height (Figure 5.7). The remaining portion of lung was defined as the peripheral region.

The peripheral region represents, approximately, the small conducting airways and alveoli of the lung, while the central region represents the larger bronchi. The central region here corresponds to the 'central' and 'intermediate' regions defined in some other studies that have divided the lung into three regions for the purpose of studying aerosol penetration and differential clearance rates (Sanchis *et al*, 1972; Short *et al*, 1979). Since the lung counts were already low dividing the lungs into three regions would have yielded very

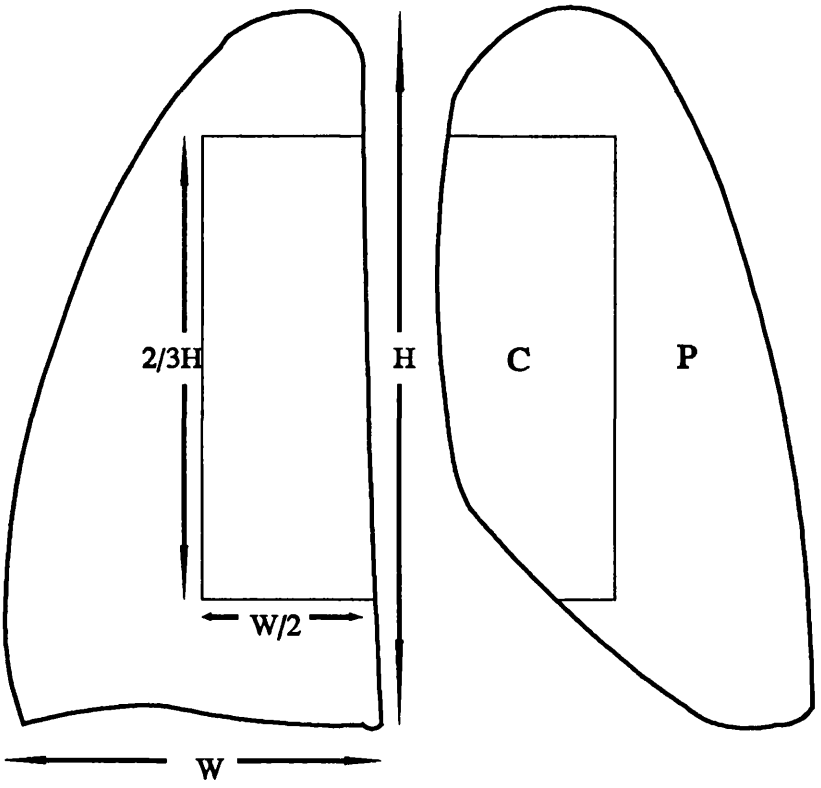


Figure 5.7. ***Division of the lung fields into peripheral (P) and central (C) regions.***

little extra information and would have reduced counts in the central region considerably. The proportions of aerosol lung dose deposited in the central and peripheral regions were determined by obtaining the GM counts for each region.

Any method for dividing the lung into central and peripheral regions based on a conventional two-dimensional radionuclide image will always be an approximation since the lungs are three dimensional organs. The image therefore represents the radionuclide distribution within the lung volume projected onto a plane. It is not possible to obtain information about the relative depth of each event from these images since all are superimposed as if they originated from the same plane. This results in a loss of contrast in any one plane due to the presence of overlying and underlying image counts from other planes.

Single photon emission computed tomography (SPECT) is a widely used technique for separating the image counts originating in one section through a patient or individual organ. It achieves this by collecting conventional plane images of the patient viewed from different directions and, by mathematical reconstruction of these images, produces a set of sections through the patient. The ROTA Camera is capable of SPECT imaging but the technique requires a very stable distribution of radionuclide. It would not be practical to use this technique for these aerosol studies since in order to build up sufficient counts several hours of imaging would be necessary. Also, for reasons described in Chapter 8, the lung image counts decay far too rapidly for meaningful SPECT data to be obtained.

Chapter 6. Results from the *In-Vivo* Study of Radiolabelled Salbutamol Deposition

6.1 Introduction

The data resulting from the radioaerosol studies in human subjects are presented and analysed in this chapter and in Chapter 7. Those relating to the deposition of the labelled salbutamol are introduced and initially analysed here, while those relating to inspiratory flow and clinical efficacy are introduced and analysed in Chapter 7. All data were also analysed with the intention of identifying relationships between flow pattern, drug deposition, and clinical effect.

6.2 Radionuclide Images

A representative sample of the gamma camera images of the thorax obtained from the studies are shown in Figures 6.1 - 6.8. The images shown were obtained for one normal subject and one asthmatic patient (normal number 2 and patient number 3). The Figures show corresponding images from the Diskhaler inhaler, MDI, MDI with spacer and krypton studies. Each inhaler image was formed from the summation of three 1 minute time frames. The main sites of deposition were the lungs, throat, mediastinum, stomach and inhaler device. The mean number of counts per image for the inhaler studies was 37,000. The krypton images were acquired to a count limit of 300,000. Also shown are the ROIs used to divide the lung fields into peripheral and central regions.

These images illustrate the main features of the deposition patterns obtained for the three inhalation methods, and for the two groups of subjects. In the normal subject the salbutamol is, in general, more peripherally and uniformly distributed in the lungs than in the patient. The images also illustrate the striking reduction in throat and stomach deposition when the spacer was used compared to those obtained when the MDI was used on its own.

6.3 Deposition Results

Tables 6.1 - 6.6 summarise the deposition of radiolabelled salbutamol, expressed as percentage of total dose available, in normal and asthmatic subjects using the Diskhaler inhaler, MDI and MDI with spacer. The activity deposited in the mouth, throat, mediastinum and stomach have been added under the collective name oropharynx. This is appropriate since it may be assumed that most of the activity detected in the stomach

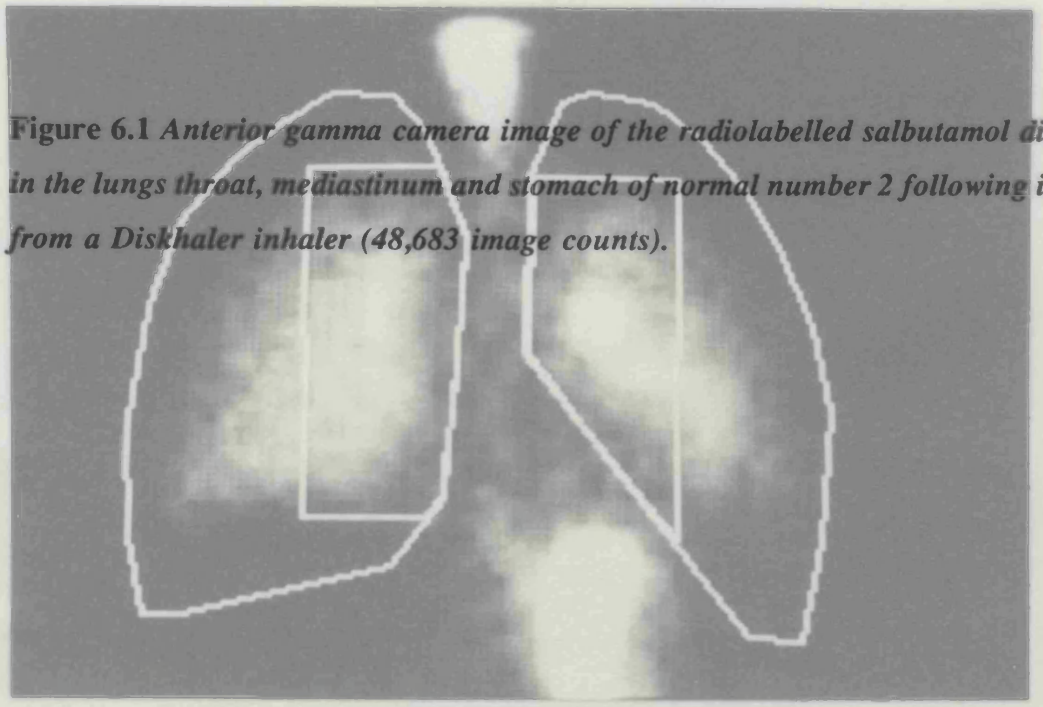


Figure 6.1 ***Anterior gamma camera image of the radiolabelled salbutamol distribution in the lungs throat, mediastinum and stomach of normal number 2 following inhalation from a Diskhaler inhaler (48,683 image counts).***

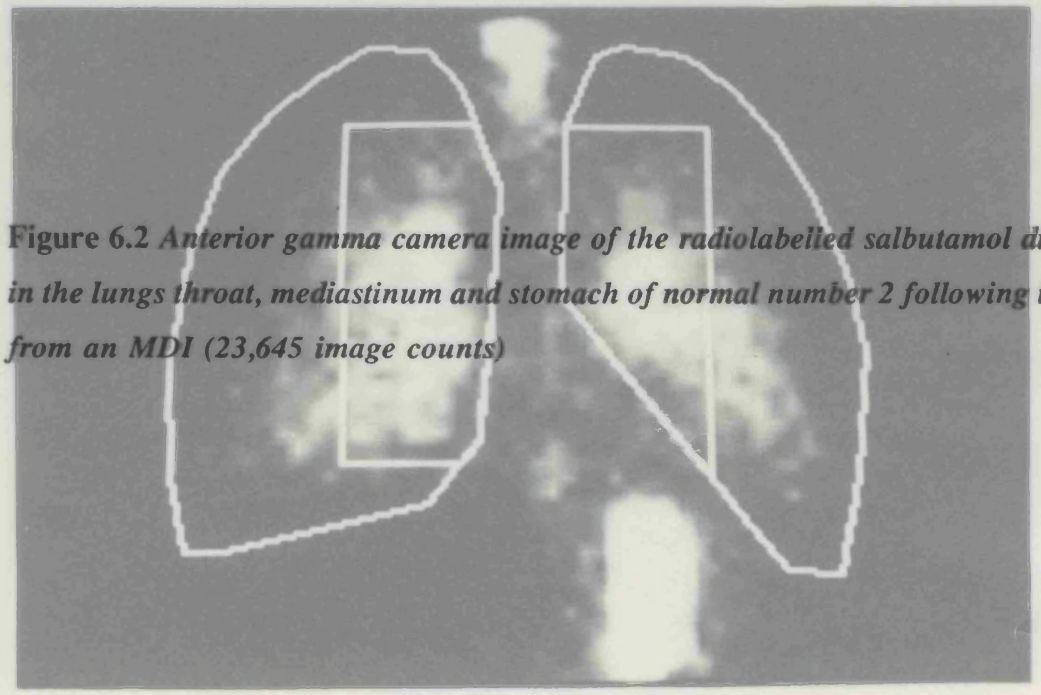
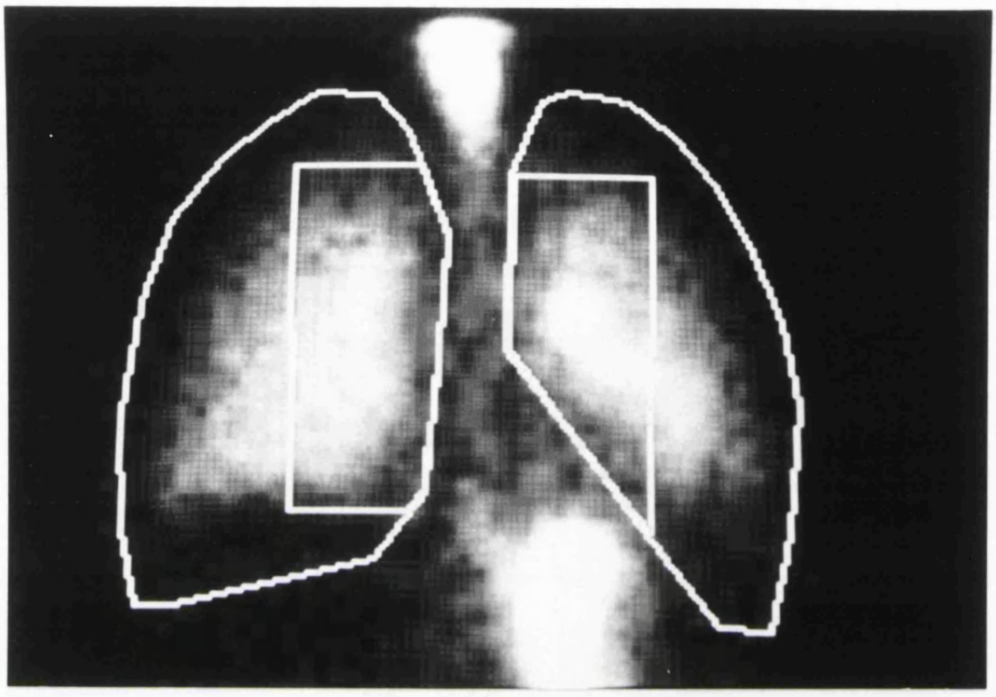
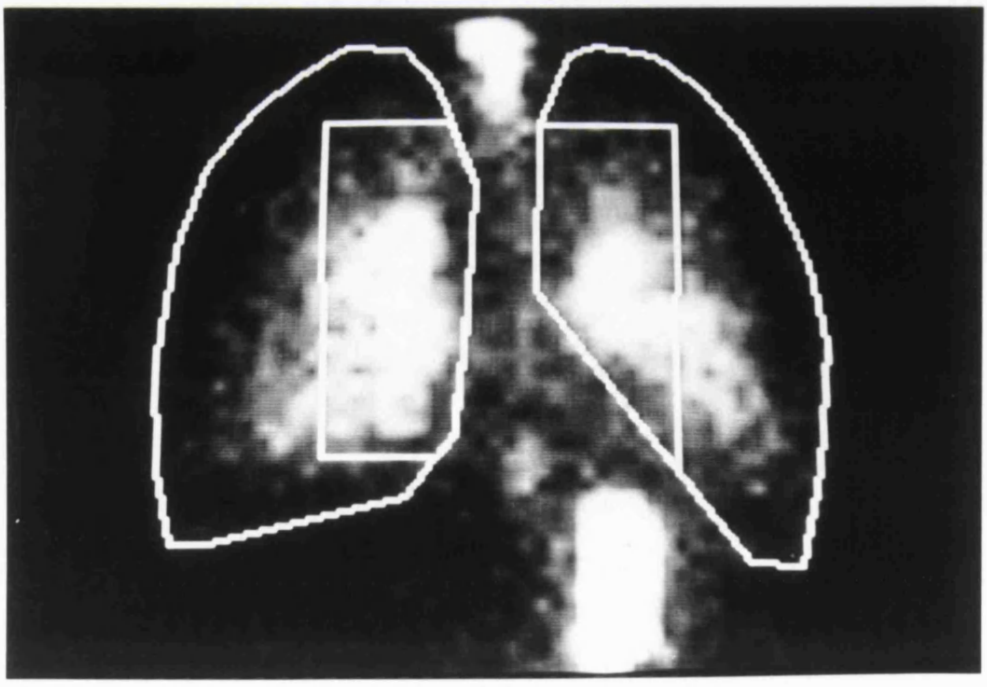


Figure 6.2 ***Anterior gamma camera image of the radiolabelled salbutamol distribution in the lungs throat, mediastinum and stomach of normal number 2 following inhalation from an MDI (23,645 image counts)***





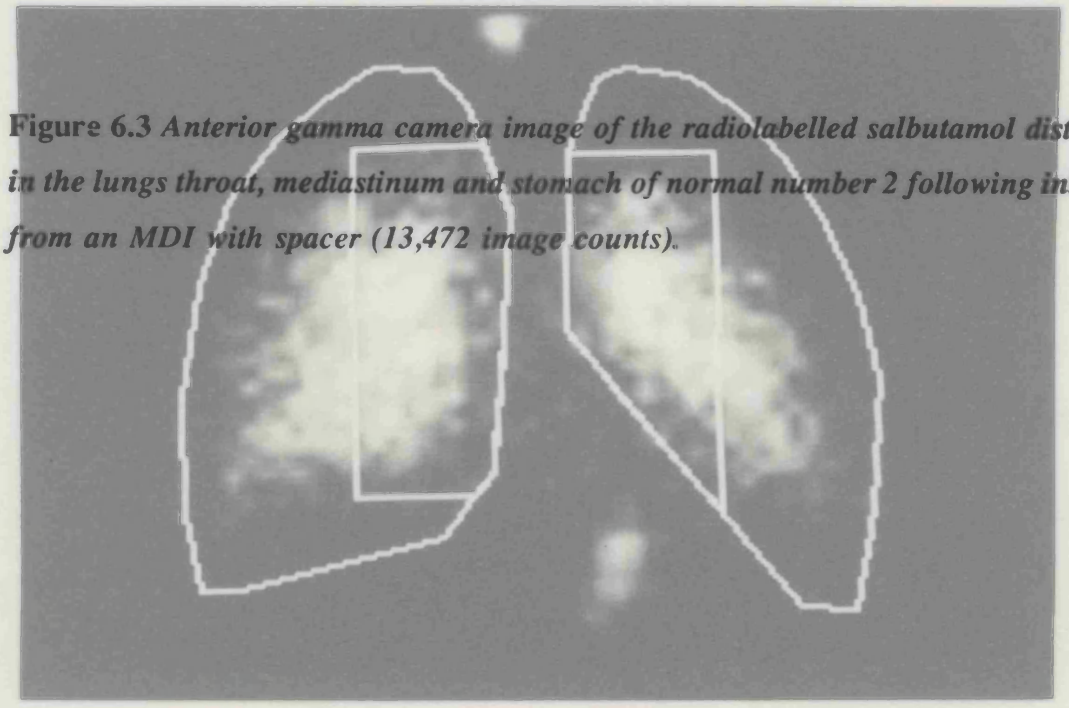


Figure 6.3 ***Anterior gamma camera image of the radiolabelled salbutamol distribution in the lungs throat, mediastinum and stomach of normal number 2 following inhalation from an MDI with spacer (13,472 image counts).***

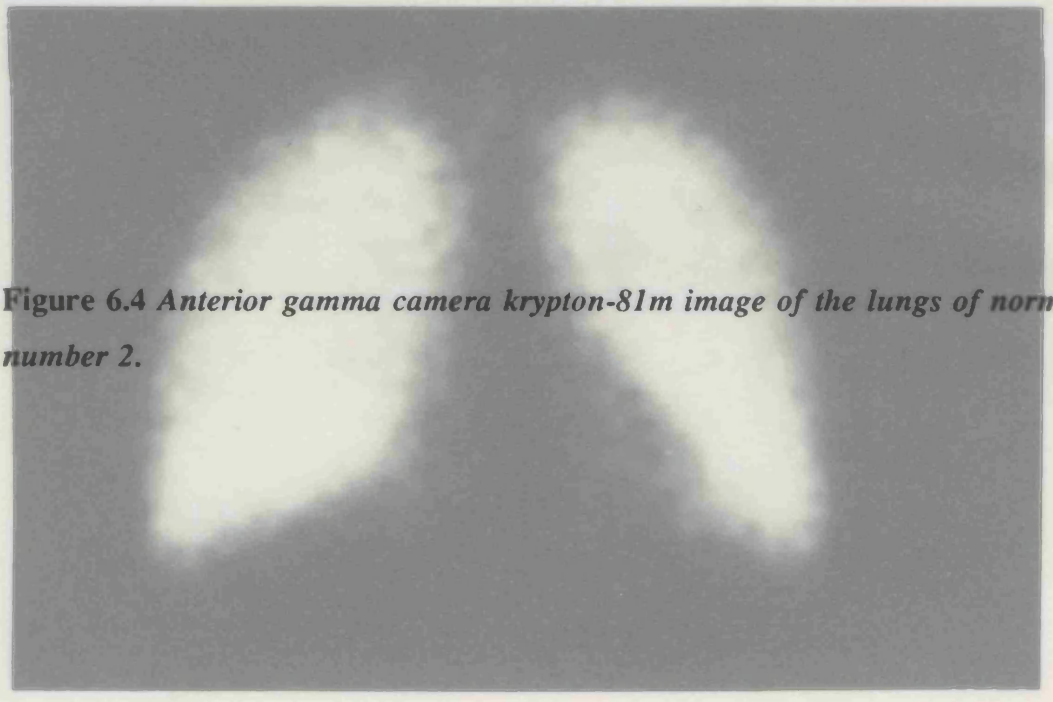
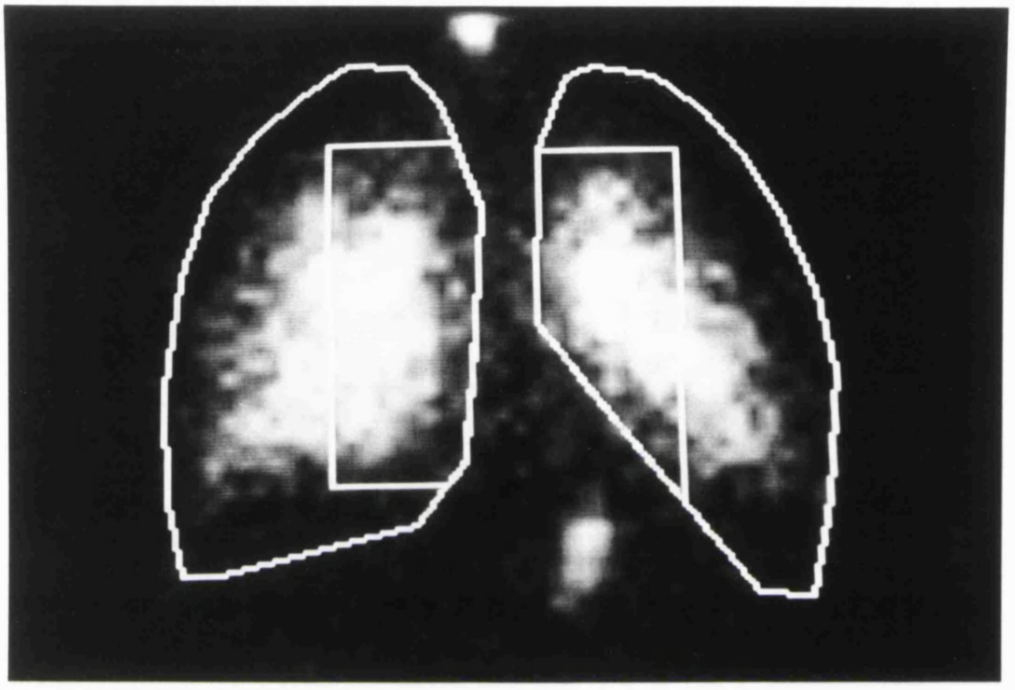
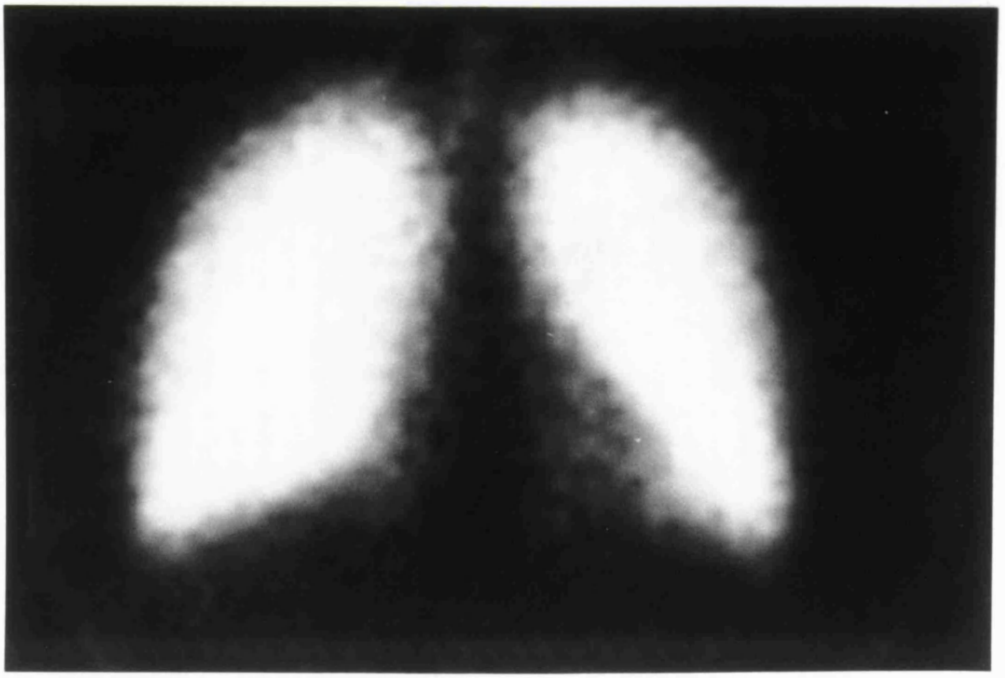


Figure 6.4 ***Anterior gamma camera krypton-81m image of the lungs of normal number 2.***





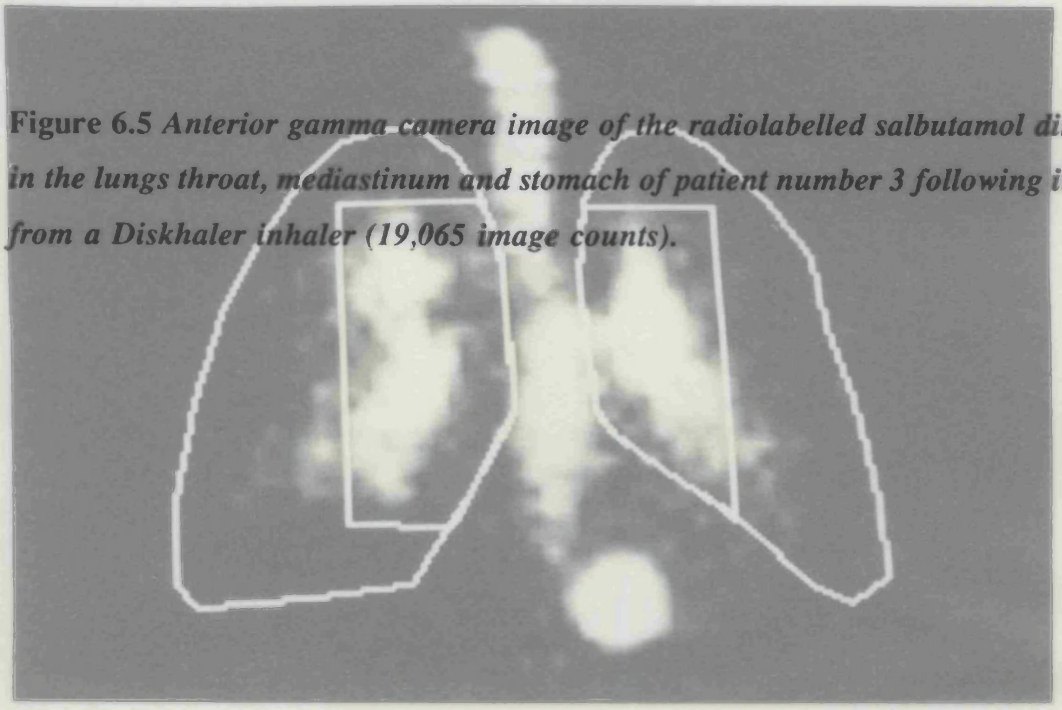


Figure 6.5 ***Anterior gamma-camera image of the radiolabelled salbutamol distribution in the lungs throat, mediastinum and stomach of patient number 3 following inhalation from a Diskhaler inhaler (19,065 image counts).***

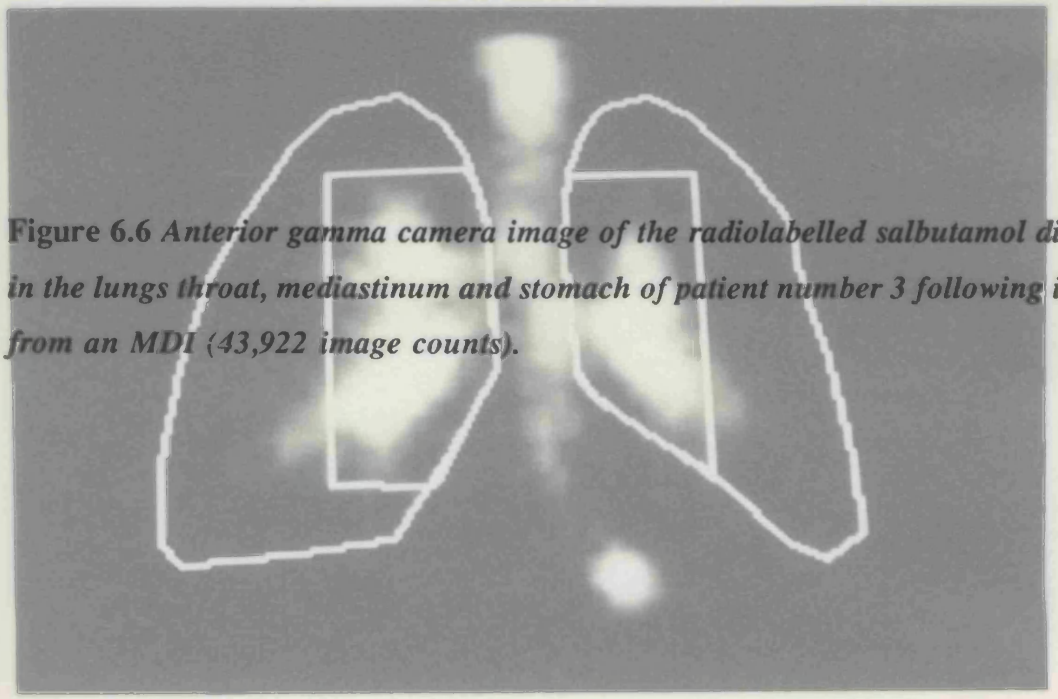
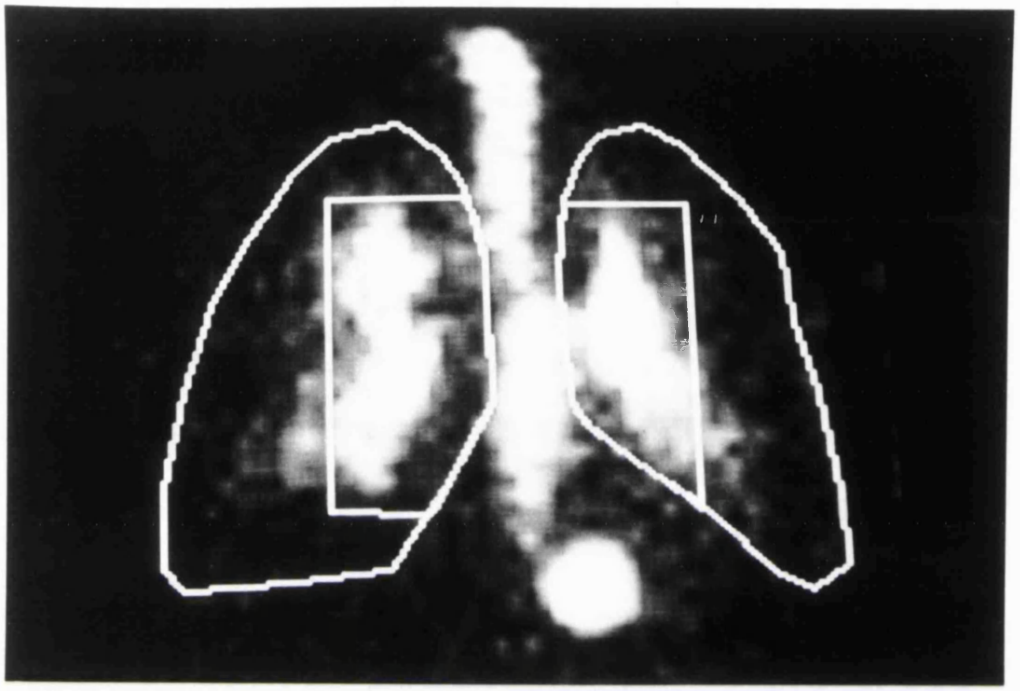
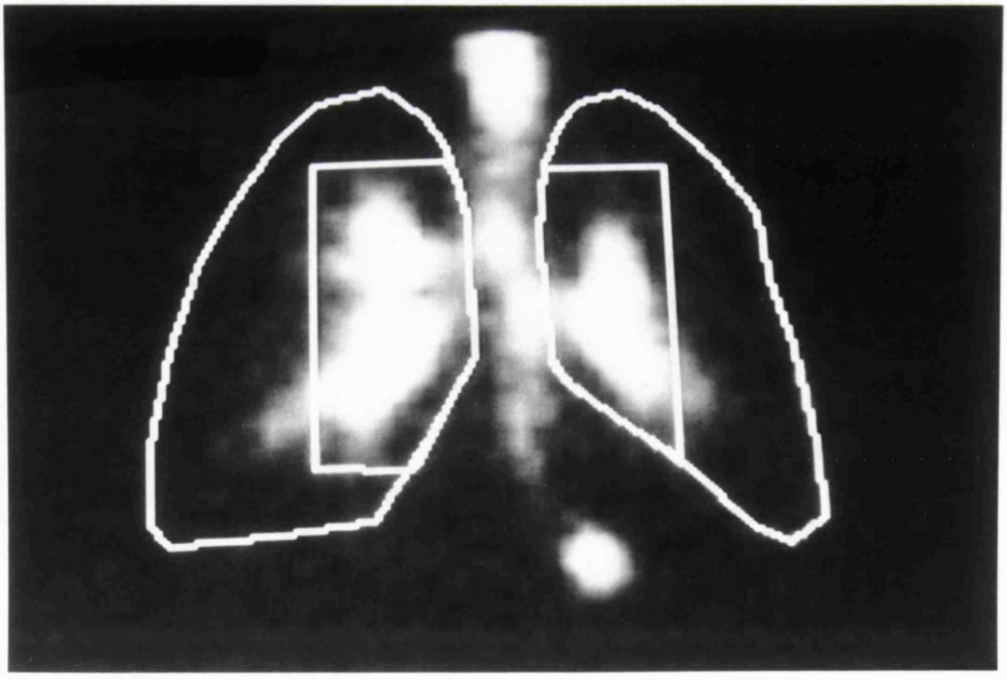


Figure 6.6 ***Anterior gamma camera image of the radiolabelled salbutamol distribution in the lungs throat, mediastinum and stomach of patient number 3 following inhalation from an MDI (43,922 image counts).***





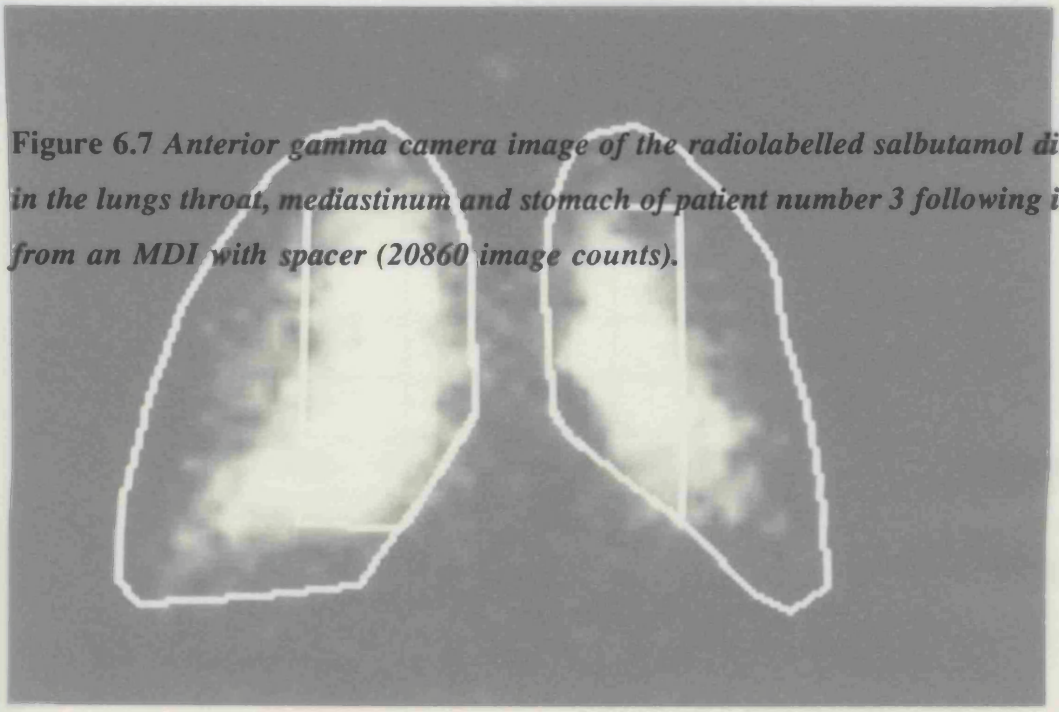


Figure 6.7 ***Anterior gamma camera image of the radiolabelled salbutamol distribution in the lungs throat, mediastinum and stomach of patient number 3 following inhalation from an MDI with spacer (20860 image counts).***

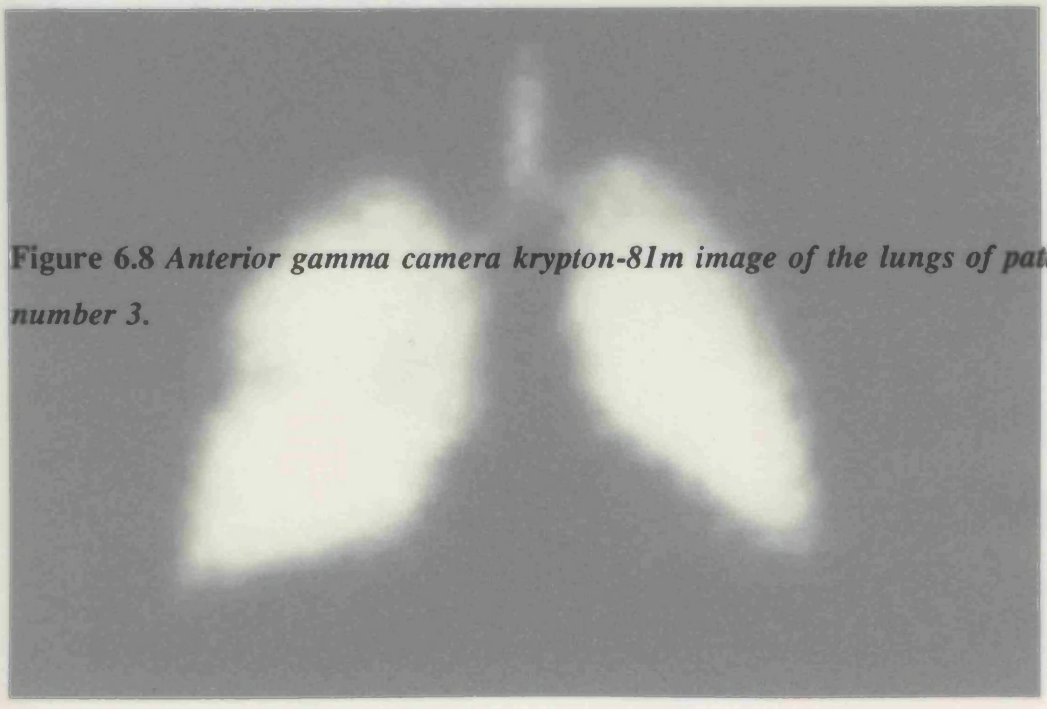
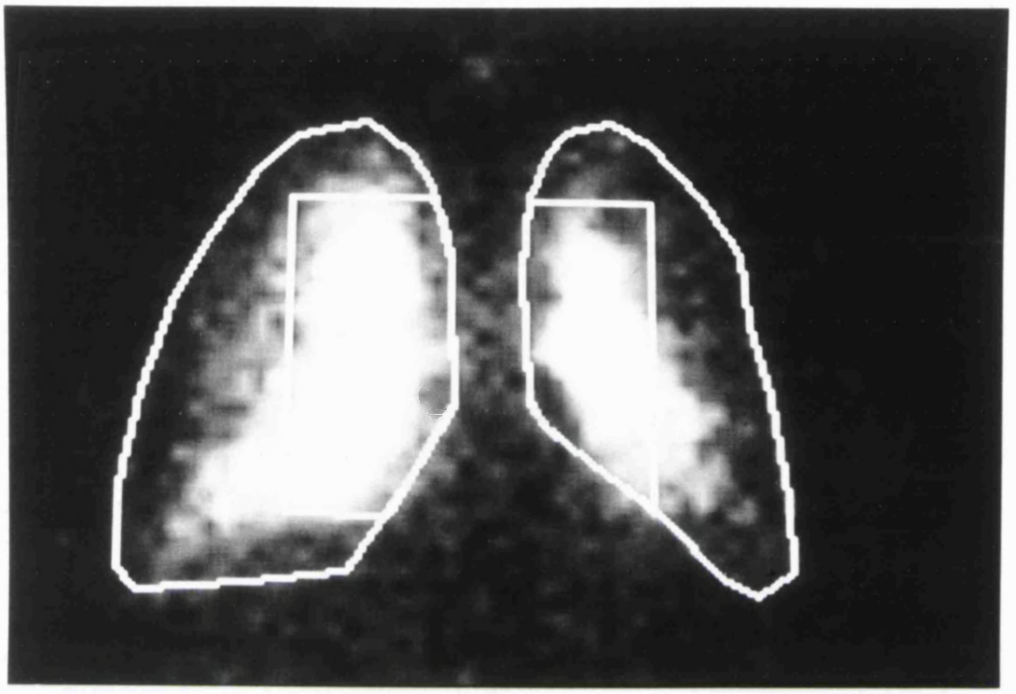
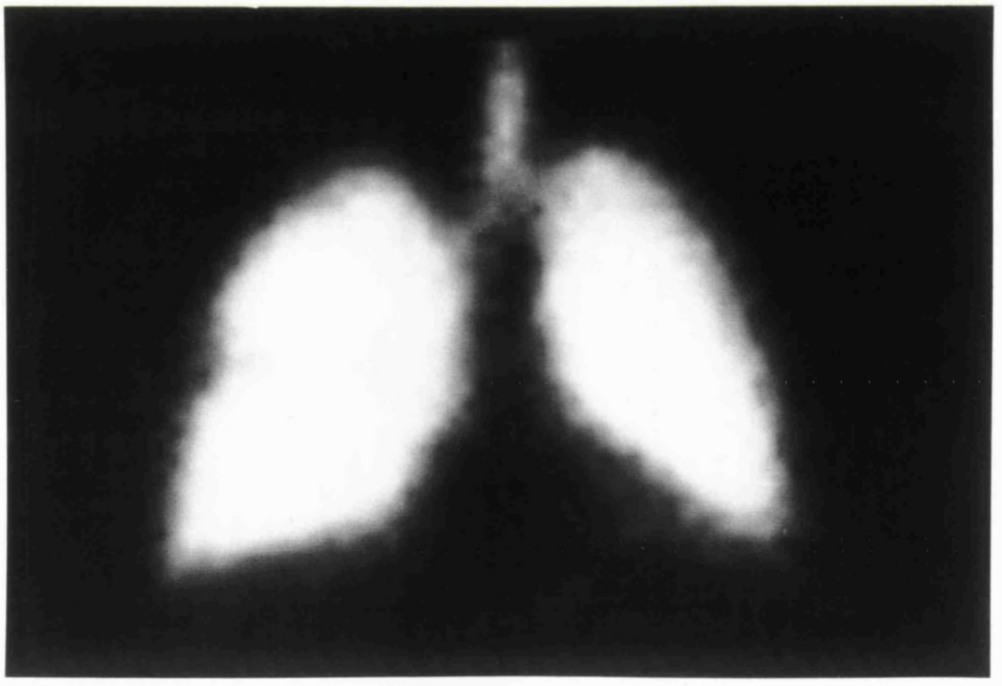


Figure 6.8 ***Anterior gamma camera krypton-81m image of the lungs of patient number 3.***





Subject	Lungs	Oropharynx.	Device	Exhaled	Recovered
1	8.5	67.0	7.3	0	82.8
2	10.8	62.2	9.0	0.1	82.1
3	14.9	66.4	3.7	0	85.0
4	8.2	54.7	17.4	0	80.3
5	19.6	55.1	4.5	0	79.2
6	14.0	52.6	11.7	0	78.3
7	9.7	63.7	13.4	0	86.8
8	10.8	52.5	28.0	0	91.3
9	13.8	48.0	12.1	0.7	74.6
10	13.5	69.4	7.8	0	90.7
Mean S.D. Range	12.4 3.5 8.2 - 19.6	59.2 7.4 48.0 - 69.4	11.5 7.1 3.7 - 28.0	0.1 0.2 0 - 0.7	83.1 5.4 74.6 - 91.3

Table 6.1. ***Percentage deposition of radiolabelled salbutamol for 10 normal volunteers, following inhalation from a Diskhaler inhaler.***

Subject	Lungs	Oropharynx.	Actuator	Exhaled	Recovered
1	21.4	37.1	27.7	0	86.2
2	17.0	45.2	14.3	0.7	77.2
3	15.2	64.9	21.5	1.5	103.1
4	20.1	40.0	12.2	0.3	72.6
5	23.2	30.0	18.2	0.1	71.5
6	15.0	31.6	17.7	0.7	65.0
7	31.6	48.2	13.5	0	93.3
8	40.5	50.6	16.1	0.5	107.7
9	22.6	58.1	15.4	1.1	97.2
10	9.8	64.2	22.9	0.2	97.1
Mean S.D. Range	21.6 8.9 9.8 - 40.5	47.0 12.6 30.0 - 64.9	17.9 4.8 12.2 - 27.7	0.5 0.5 0 - 1.5	87.1 14.8 65.0 - 107.7

Table 6.2. ***Percentage deposition of radiolabelled salbutamol for 10 normal volunteers, following inhalation from a metered dose inhaler.***

Subject	Lungs	Oropharynx.	Actuator	Spacer	Exhaled	Recovered
1	15.3	6.4	16.5	21.6	0.7	60.5
2	22.5	6.3	15.8	53.2	0.5	98.3
3	25.5	8.4	11.5	24.4	1.3	71.1
4	12.6	1.7	18.5	49.3	0	82.1
5	37.9	18.6	11.2	31.9	0.4	100.0
6	21.1	17.6	19.7	51.2	1.5	111.1
7	25.7	6.9	12.0	20.0	0.6	65.2
8	20.1	2.8	15.6	58.1	0.3	96.9
9	10.7	0.3	13.8	45.4	0	70.2
10	18.0	8.1	13.2	23.8	0	63.1
Mean S.D. Range	20.9 7.8 10.7 - 37.9	7.7 6.1 0.3 - 18.6	14.8 2.9 11.2 - 19.7	37.9 14.9 20.0 - 58.1	0.5 0.5 0 - 1.5	81.8 18.3 60.5 - 111.1

Table 6.3. ***Percentage deposition of radiolabelled salbutamol for 10 normal volunteers, following inhalation from a metered dose inhaler with spacer.***

Subject	Lungs	Oropharynx.	Device	Exhaled	Recovered
1	22.5	74.3	10.7	0.1	107.6
2	11.1	69.4	13.6	0	94.1
3	15.9	61.8	8.8	0	86.5
4	7.9	89.0	9.3	0.1	106.3
5	12.6	62.3	17.9	0	92.8
6	7.6	71.2	15.1	0	93.9
7	5.0	62.3	24.2	0.1	91.6
8	7.8	56.2	26.0	0.5	90.5
9	9.4	65.2	15.5	0	90.1
10	6.1	59.4	21.1	0	86.6
11	8.9	58.5	18.6	0	86.0
12	6.6	63.0	33.6	0	103.2
13	12.6	67.4	19.5	0.3	99.8
14	19.0	54.9	15.3	0.3	89.5
15	15.1	60.1	10.2	0	85.4
16	6.0	48.0	38.4	0.2	92.6
17	13.3	49.1	35.3	0	97.7
18	10.5	75.5	13.9	2.0	101.9
19	18.5	67.4	12.0	0	97.9
Mean	11.4	63.9	18.9	0.2	94.4
S.D.	5.0	9.6	8.7	0.4	6.9
Range	5.0 - 22.5	48.0 - 89.0	8.8 - 38.4	0 - 2.0	85.4 - 107.6

Table 6.4. ***Percentage deposition of radiolabelled salbutamol for 19 asthmatic patients, following inhalation from a Diskhaler inhaler.***

Subject	Lungs	Oropharynx.	Actuator	Exhaled	Recovered
1	16.6	27.0	50.4	2.0	96.0
2	15.9	55.7	16.0	0	87.6
3	34.0	47.4	18.5	0.2	100.1
4	24.4	36.6	17.1	0.5	78.6
5	6.2	76.7	24.2	0.3	107.4
6	25.5	42.5	58.3	0.4	126.7
7	15.3	63.4	17.7	0.2	96.6
8	23.0	36.2	17.9	0.1	77.2
9	19.3	39.6	16.2	0.2	75.3
10	20.8	43.5	16.9	0.3	81.5
11	0.5	45.8	23.4	1.3	71.0
12	19.7	65.9	30.7	1.1	117.4
13	7.3	47.7	13.0	0.1	68.1
14	26.0	30.2	13.5	0.4	70.1
15	22.8	50.7	17.8	0	91.3
16	13.5	52.8	63.0	0	129.3
17	20.9	58.0	45.0	0	123.9
18	15.6	56.4	13.5	0.2	85.7
19	19.6	76.0	23.4	0	119.0
Mean **S.D.** **Range**	18.2 7.8 0.5 - 34.0	50.1 13.9 27.0 - 76.7	26.1 15.8 13.0 - 63.0	0.4 0.5 0 - 2.0	94.9 20.4 68.1 - 129.3

Table 6.5. ***Percentage deposition of radiolabelled salbutamol for 19 asthmatic patients, following inhalation from a metered dose inhaler.***

Subject	Lungs	Oropharynx.	Actuator	Spacer	Exhaled	Recovered
1	16.6	3.4	55.8	25.4	0.7	101.9
2	28.4	6.9	22.6	49.5	0.3	107.7
3	21.9	2.0	20.2	54.4	0.6	99.1
4	20.4	6.3	14.1	38.5	0.7	80.0
5	19.8	6.6	20.3	46.4	0.3	93.4
6	34.0	12.8	13.7	20.3	0.5	81.3
7	35.8	12.5	18.8	34.8	0.7	102.6
8	6.8	1.7	13.2	73.3	0.4	95.3
9	25.5	8.3	15.3	40.4	0.2	89.7
10	15.5	2.9	3.8	40.3	0.3	62.8
11	11.8	5.8	16.0	52.0	0	85.6
12	22.7	8.8	13.8	29.2	0.4	74.9
13	11.3	1.3	27.0	59.5	0	99.1
14	11.7	9.0	19.6	50.7	1.0	92.0
15	7.4	3.2	24.3	78.0	0	112.9
16	15.9	8.4	15.8	63.0	0	103.1
17	31.1	4.9	9.6	23.4	0	69.0
18	7.8	2.3	20.2	40.7	0.2	71.2
19	15.8	12.5	14.8	30.9	0.2	74.2
Mean **S.D.** **Range**	18.9 9.1 6.8 - 35.8	6.3 3.7 1.3 - 12.8	18.9 10.4 3.8 - 55.8	44.8 16.2 20.3 - 78.0	0.3 0.3 0 - 1.0	89.2 14.4 62.8 - 112.9

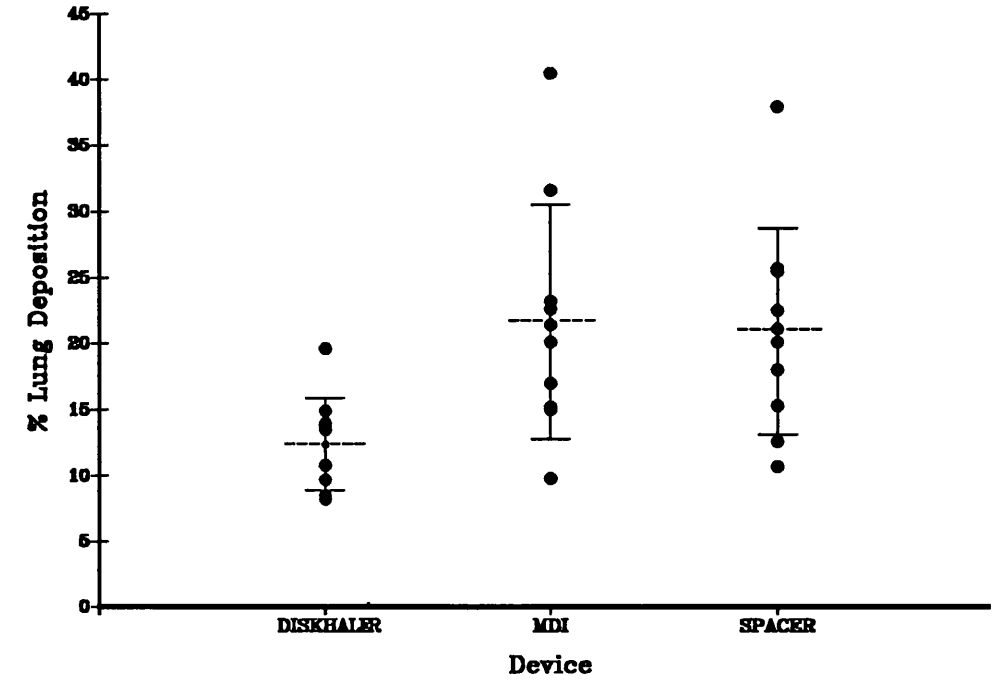
Table 6.6. ***Percentage deposition of radiolabelled salbutamol for 19 asthmatic patients, following inhalation from a metered dose inhaler with spacer.***

Subject	Diskhaler Inhaler	Metered Dose Inhaler	Metered Dose Inhaler + Spacer
1	29.9	41.3	50.7
2	44.9	35.6	44.3
3	36.9	44.5	49.0
4	47.0	54.3	55.1
5	24.0	25.2	34.2
6	51.0	50.8	54.0
7	46.2	48.5	52.5
8	37.9	57.0	50.8
9	43.8	49.1	52.3
10	32.9	35.0	51.4
Mean S.D. Range	39.4 8.6 24.0 - 47.0	44.1 9.9 25.2 - 57.0	49.4 6.1 34.2 - 55.1

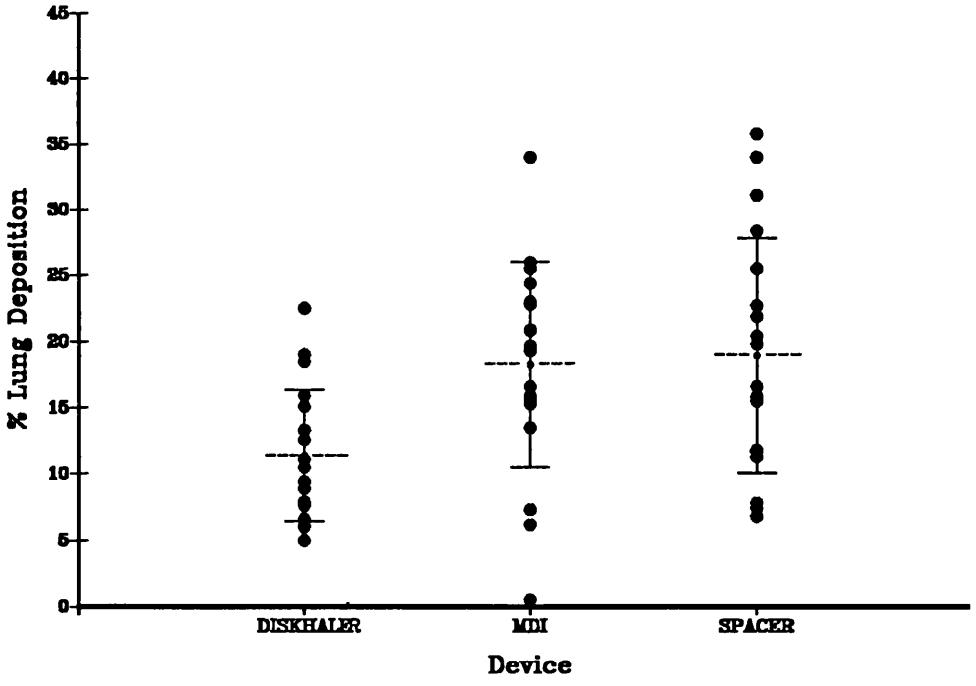
Table 6.7. ***Percentage of total lung deposition of radiolabelled salbutamol deposited in the peripheral region, for 10 normal volunteers.***

Patient	Diskhaler Inhaler	Metered Dose Inhaler	Metered Dose Inhaler + Spacer
1	18.4	32.7	43.1
2	14.0	20.4	25.7
3	25.2	15.4	29.4
4	22.1	29.6	51.7
5	34.5	33.4	44.2
6	41.0	35.2	50.2
7	24.0	34.7	42.2
8	26.4	17.9	36.3
9	26.0	32.1	34.1
10	28.1	32.9	44.0
11	40.7	52.8	43.9
12	37.1	34.9	45.5
13	14.6	22.0	16.8
14	41.2	36.7	53.3
15	24.6	36.8	40.0
16	39.5	36.8	47.6
17	12.7	12.4	22.6
18	24.8	30.0	18.4
19	39.1	31.2	43.5
Mean S.D. Range	28.1 9.6 12.7 - 41.2	30.4 9.4 12.4 - 52.8	38.5 11.1 16.8 - 53.3

Table 6.8. ***Percentage of total lung deposition of radiolabelled salbutamol deposited in the peripheral region, for 19 asthmatic patients.***



(a)



(b)

Figure 6.9 ***Percentage lung deposition of radiolabelled salbutamol for (a) normal subjects and (b) asthmatic patients, following inhalation from the three inhaler devices.***

and mediastinum regions resulted from the swallowing of activity originally deposited in the mouth and throat. Within the mediastinum region, however, it was not possible to differentiate the small part of the dose within the trachea from the part within the oesophagus.

The amount of salbutamol deposited in the lungs was of most interest since it is from this proportion that the main therapeutic effect is derived (Ruffin *et al*, 1978). Figure 6.9 shows the range of lung deposition values obtained for the normal and asthmatic subjects. The studies in which subjects inhaled from an MDI with and without a spacer have almost identical means. However, there is a wide spread of values suggesting that some subjects had more difficulty in using these two devices than others. The spread of values obtained from the Diskhaler inhaler studies was much less. Tables 6.7 and 6.8 show the percentage deposition in the peripheral lung regions of the normal and asthmatic subjects respectively.

6.4 Studies with Normal Subjects

6.4.1 Deposition in the Lung

Mean (SD) percentage total lung deposition for the Diskhaler inhaler, MDI and MDI with spacer was 12.4 (3.5)%, 21.6 (8.9)% and 20.9 (7.8)% respectively. Statistical analysis of these mean values (Table 6.9) indicated that percentage lung deposition achieved in the Diskhaler study was significantly less than for the other two studies ($p < 0.01$ for Diskhaler to MDI and $p < 0.05$ for Diskhaler to MDI with spacer). The difference between total lung deposition with the MDI on its own compared to the MDI with spacer was not significant.

Mean (SD) peripheral lung deposition, expressed as percentage of total lung deposition, for the Diskhaler inhaler, MDI and MDI with spacer were 39.4 (8.6)%, 44.1 (9.9)% and 49.4 (6.1)% respectively. Analysis of this data (Table 6.10) indicated that peripheral lung deposition resulting from use of the MDI with spacer was significantly higher than that obtained with the MDI alone ($p < 0.05$). Both inhalers gave significantly greater peripheral deposition than the Diskhaler ($p < 0.05$ for Diskhaler to MDI and $p < 0.001$ for Diskhaler to MDI with spacer).

Friedman Two-way Analysis of Variance	Chi-Square	D.F.	Significance
	9.60	2	0.008
Study Comparisons	**t-value**		**Significance**
Diskhaler to MDI	3.08		0.006
Diskhaler to MDI/Spacer	2.85		0.011
MDI to MDI/Spacer	-0.23		0.818

Table 6.9. ***Comparison of mean total lung deposition in 10 normal subjects following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

Two-way Analysis of Variance	F	D.F.	Significance
	11.00	2	0.001
Study Comparisons	**t-value**		**Significance**
Diskhaler to MDI	2.20		0.041
Diskhaler to MDI/Spacer	4.69		0.000
MDI to MDI/Spacer	2.49		0.023

Table 6.10. ***Comparison of mean peripheral lung deposition in 10 normal subjects following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

6.4.2 Deposition in the Oropharynx

Mean (SD) percentage activity measured in the oropharynx for the Diskhaler inhaler, MDI and MDI with spacer were 59.2 (7.4)%, 47.0 (12.6)% and (7.7) (6.1)% respectively. Inhalation from both the Diskhaler inhaler and the MDI resulted in a high proportion of the dose being initially deposited in the throat. Table 6.11 shows that the MDI deposited significantly less dose in the oropharynx than the Diskhaler inhaler ($p < 0.01$). However, the use of the spacer with the MDI resulted in a considerable reduction in throat deposition and subsequently the activity detected in the mediastinum and stomach ($p < 0.001$).

Friedman Two-way Analysis of Variance	**Chi-Square** 18.20	**D.F.** 2	**Significance** 0.000
Study Comparisons	**t-value**		**Significance**
Diskhaler to MDI	-2.93		0.009
Diskhaler to MDI/Spacer	-12.41		0.000
MDI to MDI/Spacer	-9.47		0.000

Table 6.11. ***Comparison of mean deposition in the oropharynx in 10 normal subjects following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

6.5 Studies with Asthmatic Patients

6.5.1 Deposition in the Lungs

Mean (SD) percentage total lung deposition for the Diskhaler inhaler, MDI and MDI with spacer in the asthmatic group was 11.4 (5.0)%, 18.2 (7.8)% and 18.9 (9.1)% respectively. These values were slightly less than the corresponding data for the normal group, although the differences were not statistically significant. Table 6.12 shows that the Diskhaler inhaler gave significantly less total lung deposition than the other two studies ($p < 0.01$ for both comparisons). However, as in the normal group, total lung deposition due to the MDI was not improved by using the spacer.

Mean (SD) peripheral lung deposition, as expressed as a percentage of total lung deposition, for the Diskhaler inhaler, MDI and MDI with spacer were 28.1 (9.6)%, 30.4 (9.4)% and 38.5 (11.1)% respectively. As with the normal group, Table 6.13 shows that there was a significant improvement in peripheral lung deposition when the spacer was used compared with the MDI alone ($p < 0.001$). The MDI with spacer also gave significantly more peripheral deposition than the Diskhaler ($p < 0.001$). In all three studies, although there was no significant difference in mean total lung deposition between the two groups of subjects (Table 6.14), peripheral lung deposition was significantly less ($p < 0.01$) in the patients than in the normal group (Table 6.15). Figure 6.10 shows the proportions of dose in the peripheral lung region for all three inhalation methods and for both groups of subjects.

6.5.2 Deposition in the Oropharynx

Mean (SD) percentage activity measured in the oropharynx for the Diskhaler inhaler, MDI and MDI with spacer were 63.9 (9.6)%, 50.1 (13.9)% and 6.3 (3.7)% respectively. As in the normal group, inhalation from the Diskhaler inhaler and the MDI resulted in a high proportion of activity being detected in these regions. As before, the MDI deposited significantly less ($p < 0.001$) of the dose in the oropharynx than the Diskhaler (Table 6.16). The deposition in the oropharynx was again considerably reduced by using the spacer. The deposition values in each study were not significantly different from the corresponding values in the normal group (Table 6.17).

6.6 Discussion of Results

It is generally acknowledged in the literature that only a small proportion of the dose released from MDIs and DPIs reaches the lungs. However, there is quite a broad range of estimates for this proportion. A number of studies have reported that little more than 10% of an MDI dose reaches the lungs even when a good inhaler technique is employed. Davies (1975) first estimated the lung deposition of isoprenaline delivered by pressurised MDI using bioavailability studies. By balancing the proportion of tritium (^3H) labelled drug and metabolite in mouth washings, blood and urine he calculated that approximately

Two-way Analysis of Variance	F 6.31	D.F. 2	Significance 0.004
Study Comparisons	**t-value**		**Significance**
Diskhaler to MDI	2.92		0.006
Diskhaler to MDI/Spacer	3.21		0.003
MDI to MDI/Spacer	0.30		0.768

Table 6.12. ***Comparison of mean total lung deposition in 19 asthmatics following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

Two-way Analysis of Variance	F 16.82	D.F. 2	Significance 0.000
Study Comparisons	**t-value**		**Significance**
Diskhaler to MDI	1.22		0.230
Diskhaler to MDI/Spacer	5.52		0.000
MDI to MDI/Spacer	4.30		0.000

Table 6.13. ***Comparison of mean peripheral lung deposition in 19 asthmatics following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

Two-Sample T-Test	Pooled Variance Estimate				
	F	Prob.	D.F.	t-value	Probability
Diskhaler	2.03	0.277	27	0.56	0.580
MDI	1.30	0.603	27	1.06	0.298
MDI/Spacer	1.29	0.715	27	0.59	0.557

Table 6.14. ***Comparison of mean total lung deposition between the two groups of subjects following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

Two-Sample T-Test	Pooled Variance Estimate				
	F	Prob.	D.F.	t-value	Probability
Diskhaler	1.26	0.751	27	3.12	0.004
MDI	1.11	0.808	27	3.67	0.001
MDI/Spacer	3.29	0.073	27	2.86	0.008

Table 6.15. ***Comparison of mean peripheral lung deposition between the two groups of subjects following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

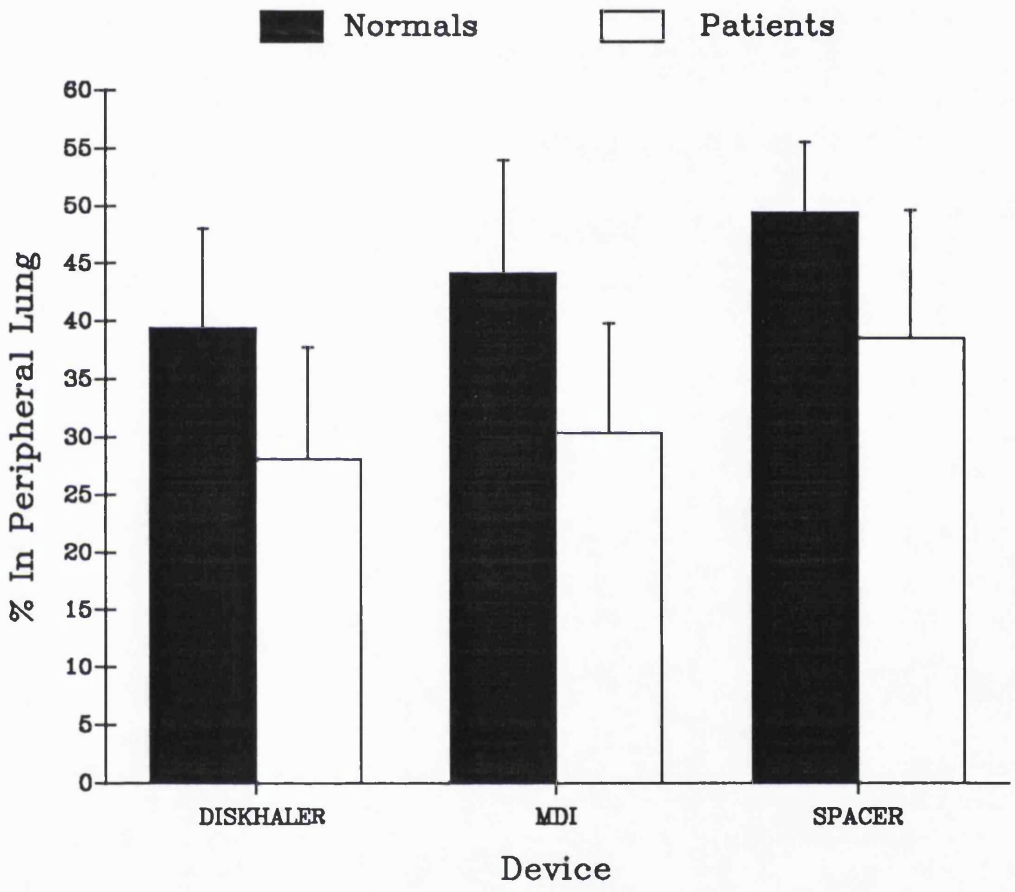


Figure 6.10. ***Peripheral lung deposition of radiolabelled salbutamol for normal volunteers and asthmatic patients following administration from the Diskhaler inhaler, the MDI and the MDI with Spacer***

Friedman Two-way Analysis of Variance	Chi-Square	D.F.	Significance
	29.79	2	0.000

Study Comparisons	t-value	Significance
Diskhaler to MDI	-4.24	0.000
Diskhaler to MDI/Spacer	-17.68	0.000
MDI to MDI/Spacer	-13.44	0.000

Table 6.16. ***Comparison of mean deposition in the Oropharynx in 19 asthmatic patients following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

Two-Sample T-Test	Pooled Variance Estimate				
	F	Prob.	D.F.	t-value	Probability
Diskhaler	1.68	0.430	27	-1.37	0.183
MDI	1.22	0.786	27	-0,59	0.559
MDI/Spacer	2.65	0.075	27	0.77	0.445

Table 6.17. ***Comparison of mean deposition in the Oropharynx between the two groups of subjects following inhalation from the Diskhaler inhaler, MDI and MDI with spacer.***

10% of the drug was deposited in the lung. Most of the drug (80%) was deposited in the mouth and stomach. Using similar techniques it had earlier been estimated that less than 4% of the dose of sodium cromoglycate, delivered as a dry powder (Spinhaler inhaler), reaches the lungs (Walker *et al*, 1972b).

In several studies radiolabelled teflon has been used in inhalers to simulate the drug particles. Newman *et al* (1981a) studied the lung deposition patterns in patients with obstructive airways disease using radiolabelled teflon particles incorporated into MDIs, and estimated that 8.8% of the dose was deposited there. They later showed that under optimum inhalation conditions a 14% lung deposition can be achieved (Newman *et al*, 1982a). Zainudin *et al* (1990), using similar teflon labelling techniques, estimated that 11.2% of an MDI dose and 9.1% of a DPI dose (Rotahaler inhaler) was deposited in the lungs. Dolovich *et al* (1981) calculated a maximum of 12.4% lung deposition from an MDI. More recently, values of 9 - 11% have been obtained in studies using radiolabelled sodium cromoglycate delivered by pressurised MDI (Vidgren *et al*, 1987; Newman *et al*, 1989).

The mean values for MDI lung deposition obtained in this study are nearly twice those given above. They are, however, only slightly higher than the mean of 16% obtained from the study of directly labelled ipratropium bromide (Short *et al*, 1981) which gave a range of 13 - 29%. They are similar to results obtained by Köhler and co-workers (Köhler *et al*, 1988), who recorded a mean lung deposition of 18.7% for fenoterol in healthy volunteers inhaling from residual volume. They are also in line with results reported recently by Newman *et al* (1991), using directly labelled salbutamol. In that study they measured a mean total deposition of 22.8% in the lungs of asthmatic patients using their inhalers in the correct fashion, but only 7.2% when inhaler technique was faulty. Even higher lung deposition has been reported by Matthys *et al*, (1988) who administered radiolabelled salbutamol to normal subjects and obtained a mean lung deposition of 26% of the dose.

Several factors may be responsible for the higher lung deposition results of this study compared to some former studies. It can be argued that while the teflon particles employed in the indirect labelling methods have nearly the same aerodynamic diameters

as actual drug particles used in inhaler therapy, the chemical and physical properties of the two substances are very different from each other. They might therefore be expected to behave differently when inhaled into the lungs. It is also difficult to predict the effect of added teflon particles to the inhaler performance.

It may not be appropriate to compare the results from this study with those from the sodium cromoglycate studies since different drug products are being considered. Also, the dose of sodium cromoglycate delivered per actuation was ten times larger than the dose of salbutamol delivered in these studies. It is possible that high drug concentrations may lead to high particle agglomeration. In addition, inhaler formulation is a major factor in determining particle characteristics (Morén, 1978). There is evidence to show that, within the particle range 3.2 - 6.4 μm, inhaler formulation is more important than changes in drug particle size in determining the site of deposition within the respiratory tract (Newman et al, 1984b). They reported that there was no difference in distribution pattern between particles of MMAD 3.2 μm and particles of MMAD 6.4 μm. This is in contrast with results obtained when dry particles were inhaled by steady breathing (Heyder *et al*, 1986), where an increase in size resulted in a shift of particle deposition from the lower to the upper respiratory tract. It has also been demonstrated that changes in both propellant vapour pressure and metered-volume size alter the pattern of pressurised aerosol deposition in the respiratory tract (Newman et al, 1982b). This is presumably because changes in the formulation alter the size of the propellant droplets within which the drug crystals are enclosed (Polli et al, 1969). It is the size of these droplets, rather than the size of the solid drug particles, that governs deposition in the respiratory tract.

The wide range of percentage deposition within the lungs in both normal subjects and patients was to be expected since no attempt was made to control inspiratory flow rates or volume inhaled. Subjects were allowed to use the inhalers in their own fashion, after some basic tuition according to typical instructions of inhaler manufacturers. The data therefore represent the range of deposition that would be seen in normal clinical practice.

Comparisons between the mean dose delivered from the Diskhaler inhaler and those delivered by the other two inhalers suggests that it is the least efficient of the three at

delivering drug to the lungs. It delivers just over half as much drug there as the MDI both with and without the spacer and deposited significantly more of the dose in the throat. This is consistent with the *in-vitro* data, reported in Chapter 3 which showed that the Diskhaler inhaler deposited only half the dose of the MDI in the "respirable portion" of the Andersen cascade impactor (Table 3.3). It is also consistent with recommendations that twice the dose of salbutamol should be taken from a DPI compared to that taken by an MDI (Newman and Clarke, 1992).

When considering the data from individual subjects, the Diskhaler inhaler was not always the least efficient technique and in two patients it was the most efficient. However, the lung dose reached 20% in only one of the twenty-nine subjects. This compares with fourteen for the MDI and fourteen for the MDI with spacer. In four of the patients and one normal the Diskhaler inhaler deposited more drug in the lungs than the MDI. In all but one, this was due to the MDI deposition being well below average. This was particularly true of patient 11 who only achieved 0.5% in the lungs with the MDI because he failed to inhale deeply and fast enough. The ranges of lung deposition values were far smaller with the Diskhaler inhaler (8.2 - 19.6% for the normals and 5.0 - 22.5% for the patients) compared to the ranges for the MDI (9.8 - 40.5% for the normals and 0.5 - 34% for the patients). Despite the lower mean lung deposition, these observations confirm the value of Diskhaler inhalers in enhancing the lung dose of those subjects who have difficulty in using an MDI efficiently. It must also be considered whether the higher proportions of dose deposited in the lungs with the MDI lead to significantly greater bronchodilation in asthmatic sufferers. The relative potencies of the doses delivered by the three inhalers are considered in Chapter 7.

The results obtained by using the MDI with the addition of the spacer device showed a significant improvement in peripheral lung deposition in both groups of subjects. This increase was expected since the spacer modifies the MDI aerosol spray by 'filtering' out the large particles, reducing the aerosol velocity and giving the propellants more time to evaporate. This process leads to smaller and slower moving particles that are more suitable for inhaling to all parts of the lung. However, it was expected that this effect would also lead to greater total lung deposition, similar to that reported in some studies

(Newman et al, 1984a; Matthys *et al*, 1988; Ashworth *et al*, 1991). This result may be explained by examining the data in more detail. The mean deposition in the oropharynx, for the MDI study, can be compared with the combined deposition in the spacer and oropharynx, for the MDI with spacer study. This shows that the two values are almost exactly the same with identical standard deviations. It was the case for both normal and asthmatic subjects and implies that the spacer collected the large particles that would otherwise have impacted in the throat and mouth, and that no extra proportion of dose reached the lungs.

The data show that both the Diskhaler inhaler and the MDI deposited large proportions of the dose in the oropharynx. However, it also confirms the dramatic reduction in the dose to the oropharynx when a spacer is used, as reported by other investigators (Newman *et al*, 1984a; Matthys *et al*, 1988; Ashworth *et al*, 1991). The ability to remove particles that would otherwise have impacted in the throat is extremely advantageous clinically and is of particular relevance in reducing the ingested dosage of drug. This is especially beneficial when corticosteroids are inhaled, and reduces the incidence of local side effects in the throat (Toogood *et al,* 1984). For bronchodilator drugs, this ingested dose is far too small to cause significant bronchodilation (Ruffin *et al*, 1978) and no loss of potency should be experienced due to its removal.

Mean drug deposition for the whole lung was slightly less in the patient group than in the normal group for each corresponding inhaler study. However, none of these differences reached statistical significance. These findings agree with the results of Spiro *et al*, (1984) who studied the deposition patterns of directly radiolabelled ipratropium bromide, delivered by MDI, in the lungs of normal subjects and those with airways obstruction. However, unlike the results presented here, they also reported that there were no differences in the distribution of central and peripheral lung regions between the two groups of subjects. This was also the conclusion of Newman *et al*, (1983) who reported that the lung deposition of aerosol from an MDI was unaffected by FEV_1.

The results from the present studies show peripheral lung deposition to be significantly greater for the normal subjects compared to the asthmatic subjects. These findings are more consistent with the results from studies based on the tidal breathing of radiolabelled

aerosol particles, generated by nebuliser or spinning disk generator (Dolovich *et al*, 1976; Pavia et al, 1977; Short *et al* 1979). They compared aerosol penetration in the lungs of normal subjects with those suffering from airways obstruction. The results showed that the presence of airways obstruction is a major factor in reducing aerosol penetration into the peripheral regions of the lungs.

The results from these studies indicate that airways obstruction does affect the degree of penetration of aerosol into the lungs, for all three modes of delivery. However, airways obstruction has a much smaller effect on whole lung deposition. For the Diskhaler inhaler, comparisons must be made with caution since the normal subjects inhaled twice from the same blister while the patients inhaled only once.

In all three studies significantly more of the dose remained in the device/actuator for the patients compared to the normals. In the Diskhaler inhaler studies, the improved emptying may be due in part to the extra inhalation taken by the normals.

A variable portion of the radioactivity remained unaccounted for in the data from several deposition studies, while for others more than 100% was recovered. The low values may be partly explained by a dissolution problem resulting in the rapid clearance of the radionuclide deposited in the lungs and its subsequent redistribution into the systemic circulation. This effect may also take place in the stomach although it would be difficult to detect since it would be masked by the gradual build up of stomach counts as the subject swallows some of the dose initially deposited in the throat. There is also evidence that in some cases, particularly with the MDI, the subject failed to inhale efficiently causing dose to be lost to the surrounding air. This is particularly evident in patient 11 who only managed to achieve 0.5% of the dose in the lungs with a total recovery of 71%. In cases where it was obvious that the subject had failed to coordinate correctly, despite instructions and practice with the placebo inhaler, they were not asked to repeat the study. This was because of the objective of investigating the lung deposition achieved under conditions as close to reality as possible. The dissolution of radionuclide from the lungs and an assessment of the reasons for the high recovery values are considered in more detail in Chapter 8.

Chapter 7. Results of the Study of Inspiratory Flow and Clinical Effect.

7.1 Introduction

The inspiratory flow data, measured while the subjects inhaled from the Diskhaler inhaler and the MDI, have been analysed in order to see how they relate to the salbutamol deposition patterns determined by radionuclide imaging. Of particular interest were the correlations between deposition sites and maximum and average flow rates, volume of air inhaled and duration of inhalation.

The simultaneous measurement of salbutamol deposition and clinical effect has provided the opportunity of seeing how the two are related in the asthmatic patients. The data were initially inspected for each patient in turn to investigate whether there were any obvious correlation between the quantities of drug reaching the lungs and the improvement in lung function. To measure the 'closeness of association' between the chosen variables, they were plotted on scatter graphs and analysed using Spearman rank correlation coefficients.

7.2 Inspiratory Flow Results.

Table 7.1 summarises the maximum and average inspiratory flow rates, volume inhaled and duration of inhalation, as measured during the Diskhaler inhaler and MDI studies. The mean and standard deviations for both groups of subjects are given. The full set of data obtained for each individual subject is given in Tables 7.2 - 7.5.

Subjects	Inhaler	Inspiratory Flow (L/Min.) Maximum	Average	Volume (L)	Duration (seconds)
Normals	Diskhaler	154.2 (46.4)	90.0 (26.6)	3.9 (0.9)	2.7 (0.7)
	MDI	178.8 (73.9)	78.7 (31.3)	3.0 (1.1)	2.4 (0.9)
Patients	Diskhaler	125.5 (29.4)	54.3 (18.0)	2.4 (0.9)	2.8 (1.0)
	MDI	153.8 (46.7)	53.7 (21.5)	2.1 (0.9)	2.5 (1.0)

Table 7.1. ***Summary of mean (SD) inspiratory flow parameters for normal and asthmatic subjects.***

Normal	Inspiratory Flow (L/Min.) Maximum	Average	Volume (L)	Duration (Secs.)
1	182.1	120.5	3.8	1.9
2	122.2	68.1	2.9	2.6
3	195.0	112.2	3.7	2.0
4	143.2	102.8	4.6	2.7
5	241.5	133.9	5.8	2.6
6	114.7	75.8	3.3	2.6
7	161.8	81.6	4.1	3.0
8	85.7	57.3	4.2	4.4
9	117.8	57.4	2.4	2.6
10	178.1	90.6	4.2	2.8

Table 7.2. ***Maximum and average inspiratory flow, volume inhaled and duration of inhalation, for 10 normal volunteers inhaling from a Diskhaler inhaler. Each value is the mean for two sequential inhalations from one dose.***

Normal	Inspiratory Flow (L/Min.) Maximum	Average	Volume (L)	Duration (Secs.)
1	161.7	79.9	2.0	1.5
2	166.8	60.9	3.2	3.2
3	176.4	75.0	1.4	1.0
4	141.0	77.5	3.2	2.5
5	340.5	126.2	5.1	2.4
6	149.2	48.4	1.9	2.4
7	124.1	69.1	3.7	3.2
8	117.8	57.3	3.6	3.8
9	127.2	50.8	2.2	2.6
10	283.1	141.9	3.6	1.5

Table 7.3. ***Maximum and average inspiratory flow, volume inhaled and duration of inhalation for 10 normal volunteers inhaling from an MDI. Each value is the mean obtained from the inhalation of two sequential doses.***

Patient	Inspiratory Flow (L/Min.) Maximum	Average	Volume (L)	Duration (Secs.)
1	102.1	42.6	1.5	2.2
2	120.9	51.8	2.6	3.0
3	127.2	35.8	3.7	6.2
4	133.5	32.4	1.9	3.5
5	80.7	37.2	1.3	2.1
6	77.0	48.0	2.6	3.3
7	139.8	62.1	3.5	3.4
8	118.4	51.1	1.5	1.7
9	170.0	75.7	3.4	2.7
10	110.9	29.2	1.1	2.2
11	59.4	22.1	1.4	3.9
12	144.8	71.5	3.0	2.5
13	131.0	65.3	3.2	3.0
14	167.4	69.8	2.5	2.2
15	122.2	43.4	1.3	1.8
16	151.1	77.7	1.9	1.5
17	146.1	70.2	2.3	2.0
18	144.8	77.2	4.1	3.2
19	138.5	69.6	2.7	2.3

Table 7.4. ***Maximum and average inspiratory flow, volume inhaled and duration of inhalation for 19 asthmatic patients inhaling from a Diskhaler inhaler (single inhalation).***

Patient	Inspiratory Flow (L/Min.) Maximum	Average	Volume (L)	Duration (Secs.)
1	115.5	41.7	1.7	2.5
2	127.2	40.0	3.1	4.7
3	234.0	72.3	3.8	3.2
4	139.8	26.4	1.7	4.0
5	184.4	69.9	1.4	1.2
6	143.5	64.2	2.3	2.2
7	164.3	66.5	3.0	2.7
8	177.5	70.5	1.4	1.2
9	230.3	94.0	3.2	2.0
10	107.1	31.5	0.6	1.2
11	53.7	14.5	0.5	2.0
12	156.8	61.3	1.5	1.5
13	112.8	33.9	2.3	4.2
14	215.8	84.3	2.4	1.7
15	108.4	26.5	1.6	3.6
16	156.8	44.4	1.5	2.0
17	205.8	55.5	2.4	2.6
18	155.5	68.6	3.3	2.9
19	133.5	54.0	2.5	2.8

Table 7.5. ***Maximum and average inspiratory flow, volume inhaled and duration of inhalation for 19 asthmatic patients inhaling from an MDI. Each value is the mean obtained from the inhalation of two sequential doses.***

7.2.1 Correlation Between Salbutamol Deposition and Inspiratory Flow Parameters

The percentage radionuclide depositions in the whole lung, peripheral lung, oropharynx, and that remaining in the inhaler device were each compared with the inspiratory flow parameters obtained for the Diskhaler inhaler and the MDI. The Spearman rank correlation coefficients and probability values were calculated for each comparison and are given in Tables 7.6 - 7.13.

7.2.1.1 Studies with Normal Subjects

In the Diskhaler inhaler study there were no significant correlations between whole lung deposition and any of the flow parameters (Table 7.6). It was, however, correlated negatively with maximum flow rate in the MDI study (r_s = -0.56, $p < 0.05$). Whole lung deposition was also significantly correlated with duration of inhalation (r_s = 0.64, $p < 0.05$). Peripheral lung deposition (Table 7.7) was negatively correlated with maximum flow rate for both the Diskhaler (r_s = -0.67, $p < 0.05$) and the MDI (r_s = -0.85, $p < 0.001$). It was also correlated negatively with average flow rate, for the MDI (r_s = -0.66, $p < 0.05$), but it just failed to reach the 5% significance level for the Diskhaler.

Deposition in the oropharynx (Table 7.8) was significantly correlated with both maximum flow rate (r_s = 0.71, $p < 0.05$) and average flow rate (r_s = 0.60, $p < 0.05$), when the subjects inhaled from the Diskhaler inhaler. However, it was not significant when the subjects inhaled from the MDI.

The percentage of radionuclide remaining in the Diskhaler inhaler (Table 7.9) was correlated negatively with both maximum flow rate (r_s = -0.79, $p < 0.001$) and average flow rate (r_s = -0.70, $p < 0.05$). It was positively correlated with duration of inhalation (r_s = 0.74, $p < 0.05$). Percentage of radionuclide remaining in the MDI actuator was positively correlated with maximum flow rate (r_s = 0.59, $p < 0.05$) and negatively correlated with duration of inhalation (r_s = -0.76, $p < 0.01$).

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s= 0.23 p = 0.26	r_s= 0.11 p = 0.37	r_s= -0.28 p = 0.21	r_s= -0.13 p = 0.36
MDI	r_s = -0.56 p = 0.04	r_s = -0.15 p = 0.34	r_s = 0.64 p = 0.02	r_s = 0.53 p = 0.06

Table 7.6. *Spearman rank correlation coefficients showing strength of association between radionuclide deposition in the whole lung and inspiratory flow parameters for 10 normal volunteers inhaling from the Diskhaler inhaler and the MDI .*

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s= -0.67 p = 0.02	r_s= -0.53 p = -0.06	r_s= 0.31 p = 0.19	r_s= -0.33 p = 0.17
MDI	r_s= -0.85 p = 0.00	r_s = -0.66 p = 0.02	r_s = 0.46 p = 0.09	r_s = -0.26 p = 0.23

Table 7.7. *Spearman rank correlation coefficients showing strength of association between peripheral lung deposition and inspiratory flow parameters for 10 normal volunteers inhaling from the Diskhaler inhaler and the MDI .*

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s = 0.71 p = 0.01	r_s= 0.60 p = 0.03	r_s= -0.24 p = 0.25	r_s= -0.17 p = 0.31
MDI	r_s = -0.11 p = 0.4	r_s= -0.03 p = 0.47	r_s= -0.07 p = 0.50	r_s= -0.17 p = 0.32

Table 7.8. *Spearman rank correlation coefficients showing strength of association between radionuclide deposition in the oropharynx and inspiratory flow parameters for 10 normal volunteers inhaling from the Diskhaler inhaler and the MDI.*

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s= -0.79 p = 0.00	r_s= -0.70 p = 0.01	r_s= 0.74 p = 0.01	r_s= 0.07 p = 0.43
MDI	r_s= 0.59 p = 0.04	r_s= 0.44 p = 0.10	r_s= -0.76 p = 0.00	r_s= -0.26 p = 0.23

Table 7.9. *Spearman rank correlation coefficients showing strength of association between radionuclide remaining in the inhaler device and inspiratory flow parameters for 10 normal volunteers inhaling from the Diskhaler inhaler and the MDI.*

7.2.1.2 Studies with Asthmatic Subjects

In the asthmatic group, there were no significant correlations between whole lung deposition and any of the flow parameters for either the Diskhaler study or the MDI study (Table 7.10). These results differ from those of the normal group where, in the MDI study, there was a weak negative correlation between whole lung deposition and maximum flow rate and a stronger positive one with duration of inhalation.

The only flow parameter to give a significant correlation with peripheral lung deposition (Table 7.11) was volume inhaled in the MDI study (r_s = -0.46, $p < 0.05$). The only significant correlation with deposition in the oropharynx (Table 7.12) was duration of inhalation in the Diskhaler study (r_s = 0.52, $p = 0.01$). A weak positive correlation was indicted between percentage of radionuclide remaining in the Diskhaler inhaler (Table 7.13) and average flow rate (r_s = 0.40, $p < 0.05$). A weak negative correlation was also indicated between percentage in the Diskhaler inhaler and duration of inhalation (r_s = -0.41, $p < 0.05$).

7.3 Base Line Lung Function of Subjects

Tables 7.14 and 7.15 summarise the baseline lung function of the normal and asthmatic subjects participating in the 3-part study. The mean and standard deviations values from the three study days are given, for each subject. There were no major variations between the three study days.

7.3.1 Comparison of Flow Parameters with Baseline Lung Function

The flow data measured for the patients during the studies were compared with their baseline lung function. This was to investigate whether or not varying degrees of airways obstruction had any effect on inspiratory flow patterns. Although the lung function tests were based on forced expiration manoeuvres while the flow data was derived from unforced inspiration manoeuvres, it might be expected that the two would show some relationship. The patient results for the Diskhaler inhaler are shown in Table 7.16 and for the MDI in Table 7.17.

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s= -0.00 p = 0.50	r_s= -0.06 p = 0.40	r_s= -0.10 p = 0.34	r_s= 0.02 p = 0.47
MDI	r_s= 0.32 p = 0.1	r_s = 0.26 p = 0.14	r_s = 0.01 p = 0.48	r_s = 0.2 p = 0.21

Table 7.10. *Spearman rank correlation coefficients showing strength of association between radionuclide deposition in the whole lung and inspiratory flow parameters for 19 asthmatics inhaling from the Diskhaler inhaler and the MDI .*

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s= -0.06 p = 0.41	r_s= 0.02 p = 0.46	r_s= -0.07 p = 0.4	r_s= -0.14 p = 0.28
MDI	r_s= -0.29 p = 0.11	r_s = -0.19 p = 0.21	r_s = -0.37 p = 0.06	r_s = -0.46 p = 0.02

Table 7.11. *Spearman rank correlation coefficients showing strength of association between peripheral lung deposition and inspiratory flow parameters for 19 asthmatics inhaling from the Diskhaler inhaler and the MDI .*

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s = -0.17 p = 0.24	r_s= -0.08 p = 0.37	r_s= 0.52 p = 0.01	r_s= 0.34 p = 0.08
MDI	r_s = 0.0 7 p = 0.38	r_s = 0.00 p = 0.50	r_s = 0.12 p = 0.31	r_s = 0.15 p = 0.27

Table 7.12. *Spearman rank correlation coefficients showing strength of association between radionuclide deposition in the oropharynx and inspiratory flow parameters for 19 asthmatics inhaling from the Diskhaler inhaler and the MDI.*

Inhaler	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
Diskhaler	r_s= 0.24 p = 0.16	r_s= 0.40 p = 0.04	r_s= -0.41 p = 0.04	r_s= - 0.10 p= 0.34
MDI	r_s = 0.038 p = 0.44	r_s= -0.06 p = 0.407	r_s= -0.33 p = 0.08	r_s= -0.33 p = 0.08

Table 7.13. *Spearman rank correlation coefficients showing strength of association between radionuclide remaining in the inhaler device and inspiratory flow parameters for 19 asthmatics inhaling from the Diskhaler inhaler and the MDI .*

Normal	Base line PEF % Predicted (SD)	Base line FEV_1 % Predicted (SD)	Base line FVC % Predicted (SD)
1	* 127.5 (4.9)	112.3 (2.3)	102.7 (1.1)
2	* 105.0 (9.9)	89.0 (2.0)	90.3 (1.5)
3	* 138.0 (1.4)	87.3 (2.5)	91.0 (3.6)
4	* 108.0 (1.4)	91.7 (4.5)	104.7 (2.1)
5	* 124.0 (1.4)	104.0 (3.0)	117.0 (2.6)
6	* 100.0 (4.2)	110.0 (1.7)	110.3 (3.8)
7	131.7 (3.0)	120.0 (3.6)	112.3 (4.0)
8	121.7 (3.5)	96.0 (4.3)	112.0 (1.4)
9	* 94.0 (2.8)	* 90.0 (1.4)	* 93.0 (1.4)
10	120.3 (10.4)	111.3 (5.5)	113.3 (6.4)

Table 7.14. ***Mean (SD) base line lung function parameters, PEF, FEV_1 and FVC for the 10 normal volunteers, as a percentage of predicted values, prior to administration of salbutamol in the 3 studies. * indicates that the mean is derived from 2 measurements only.***

Patient	Base line PEF % Predicted (SD)	Base line FEV_1 % Predicted (SD)	Base line FVC % Predicted (SD)
1	55.0 (6.1)	52.3 (5.8)	89.3 (11.2)
2	46.3 (6.5)	37.3 (6.8)	74.3 (7.8)
3	64.3 (4.2)	62.0 (2.6)	100.3 (1.1)
4	74.0 (8.7)	48.3 (2.1)	106.7 (3.2)
5	45.0 (7.8)	32.3 (2.1)	63.7 (4.0)
6	52.0 (7.0)	38.6 (4.7)	75.3 (2.9)
7	45.7 (3.5)	32.3 (2.1)	64.7 (5.1)
8	52.3 (2.5)	57.3 (4.9)	83.0 (6.1)
9	66.7 (4.7)	53.3 (3.8)	94.0 (5.3)
10	42.3 (2.5)	28.7 (3.8)	64.7 (6.4)
11	39.7 (4.5)	57.7 (7.1)	79.3 (6.4)
12	66.0 (6.0)	49.7 (5.7)	74.0 (10.6)
13	38.7 (1.5)	37.3 (3.0)	96.3 (11.7)
14	65.0 (7.8)	45.7 (2.3)	69.3 (4.6)
15	49.2 (7.2)	22.0 (1.0)	50.7 (2.3)
16	89.3 (2.9)	75.7 (3.2)	88.0 (4.6)
17	65.3 (6.5)	78.0 (4.6)	112.7 (4.9)
18	69.7 (4.0)	56.0 (1.0)	99.3 (2.5)
19	41.7 (1.5)	30.3 (1.5)	77.0 (2.6)

Table 7.15. ***Mean (SD) base line lung function parameters, PEF, FEV_1 and FVC, as a percentage of predicted values, for the 19 asthmatic patients prior to administration of salbutamol in the 3 studies.***

Baseline Lung Function	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
PEF	r_s= 0.53 p = 0.01	r_s= 0.41 p = 0.04	r_s= 0.29 p = 0.11	r_s= 0.57 p= 0.00
FEV_1	r_s = 0.04 p = 0.43	r_s =0.15 p = 0.26	r_s = 0.45 p = 0.02	r_s = 0.43 p = 0.03
FVC	r_s = 0.09 p = 0.36	r_s= 0.15 p = 0.27	r_s= 0.49 p = 0.02	r_s= 0.58 p = 0.00

Table 7.16. *Spearman rank correlation coefficients showing strength of association between baseline lung function and inspiratory flow parameters for the 19 asthmatics inhaling from the Diskhaler inhaler.*

Baseline Lung Function	Maximum Flow Rate	Average Flow Rate	Duration of Inhalation	Volume Inhaled
PEF	r_s= 0.25 p = 0.15	r_s= 0.26 p = 0.14	r_s= 0.17 p = 0.24	r_s= 0.46 p = 0.02
FEV_1	r_s = 0.16 p = 0.25	r_s = 0.15 p = 0.26	r_s = 0.11 p = 0.33	r_s = 0.38 p = 0.05
FVC	r_s = - 0.03 p = 0.45	r_s= 0.04 p = 0.44	r_s= 0.43 p = 0.03	r_s= 0.53 p = 0.01

Table 7.17. *Spearman rank correlation coefficients showing strength of association between baseline lung function and inspiratory flow parameters for the 19 asthmatics inhaling from the MDI.*

Patient	% Change								
	PEF			FEV_1			FVC		
	D	M	S	D	M	S	D	M	S
1	61	27	33	33	30	45	17	39	22
2	22	17	32	41	28	41	23	24	28
3	48	37	52	44	39	52	27	22	23
4	-2	5	11	6	10	7	2	3	-2
5	27	25	-2	42	31	36	29	31	33
6	32	26	24	35	38	31	29	32	32
7	4	18	26	14	23	35	14	26	39
8	24	20	25	41	11	32	34	12	27
9	5	3	25	13	1	27	15	4	24
10	29	13	16	18	26	32	17	20	28
11	55	19	37	16	17	13	9	11	12
12	9	15	4	18	8	-1	8	-	1
13	16	16	38	19	28	50	5	12	30
14	19	13	30	36	39	35	30	30	36
15	2	4	19	29	14	16	18	18	21
16	9	6	9	2	6	2	2	3	2
17	20	74	21	15	34	24	6	20	17
18	28	24	21	33	35	27	2	2	0
19	50	20	30	59	33	38	34	32	20
Mean	24	20	24	27	24	28	17	19	21
S.D.	19	16	13	15	12	15	11	12	13

Table 7.18. ***Mean (SD) change in lung function parameters, PEF, FEV_1 and FVC, following administration of 200 μg of salbutamol to 19 asthmatic patients by Diskhaler inhaler (D), MDI (M) and MDI with spacer (S).***

Those correlations that were significant in the Diskhaler study were: PEF with maximum flow rate (r_s = 0.53, p = 0.01), PEF with average flow rate (r_s = 0.41, p < 0.05) and PEF with volume inhaled (r_s = 0.57, p < 0.001); FEV_1 with duration of inhalation (r_s = 0.45, p < 0.05) and volume inhaled (r_s = 0.43, p < 0.05); FVC with duration of inhalation (r_s = 0.49, p < 0.05) and volume inhaled (r_s= 0.58, P < 0.001). In the MDI study those correlations that were significant were: PEF with volume inhaled (r_s = 0.46, p < 0.05); FEV_1 with volume inhaled (r_s = 0.38, p= 0.05); FVC with duration of inhalation (r_s = 0.43, p < 0.05) and volume inhaled (r_s = 0.53, p = 0.01).

7.4 Clinical Effect of Deposited Salbutamol in Asthmatic Patients as Measured by Changes in Lung Function

Changes in PEF, FEV_1 and FVC, expressed as a percentage of baseline values for each asthmatic patient, and for each of the three inhalers are given in Table 7.18. There were no significant further improvements of lung function after 15 minutes following administration. Mean (SD) percentage changes in PEF for Diskhaler inhaler, MDI and MDI with spacer were 24 (19)%, 20 (16)% and 24 (13)% respectively. Mean (SD) percentage changes in FEV_1 for Diskhaler inhaler, MDI and MDI with spacer were 27 (15)%, 24 (12)% and 28 (15)% respectively. For FVC, the changes were 17 (11)%, 19 (12)% and 21 (13)% respectively. The three mean values of PEF, obtained for each inhaler, were not significantly different from each other. This was also true for FEV_1 and FVC.

7.4.1 Correlation of Improvement in Lung Function with Flow Parameters

Comparisons were made between improvements in lung function parameters and inspiratory flow parameters in order to investigate whether any correlations could be identified. Similar methods have been used in other studies to investigate optimum inspiratory procedures so as to gain maximum bronchodilation effect, without actually measuring aerosol deposition (Lawford and McKenzie, 1983). Here, improvements in PEF, FEV_1 and FVC were compared with maximum and average inspiratory flow rate, volume inhaled and duration of inhalation. The data indicated only one significant correlation. This was between maximum flow rate and improvement in PEF in the Diskhaler study (p < 0.05) and indicated that they were negatively associated.

7.4.2 Comparison of Clinical Effect With Lung Salbutamol Deposition for Each Patient

Figure 7.1 (a) - (s) shows, for each asthmatic patient in turn, the percentage of the drug dose deposited in the whole lungs for each inhaler study with the resulting percentage increases in PEF, FEV_1 and FVC. It has been shown that, for both the normal subjects and the asthmatic patients, the MDI and MDI with spacer, on average, deposit significantly higher proportions of the dose into the lungs. It is therefore important to investigate whether the larger amounts of drug delivered by these inhalers result in larger clinical effects.

The improvement in lung function parameters, when starting from similar baseline values, can be used to compare the clinical effect of a measured dose of drug deposited in the lung. It may then be possible to determine if a particular inhaler has a clear advantage over the others in providing benefit to most of the patients.

Patient 1 was unusual in that he achieved the highest lung deposition of 22.5% with the Diskhaler inhaler, compared to 16.6% for the MDI and 16.6% for the MDI with spacer. This was the largest lung deposition achieved for a Diskhaler inhaler in any of the subjects studied. The improvement in PEF of 60% in the Diskhaler study was also very much larger than for the other inhalers, although this was not so for FEV_1 and FVC. It is therefore difficult to interpret the relative advantage of one inhaler over another for this patient.

Patient 2 obtained the highest lung deposition of 28.4% with the MDI with spacer compared to 15.9% with the MDI and 11.1% with the Diskhaler inhaler. While the largest improvement in all lung function parameters was for the MDI with spacer the other two studies produced similar changes in PEF and FVC. FEV_1 improved as much for the Diskhaler inhaler as for the spacer despite the lower lung deposition.

Patient 3 obtained above average lung deposition for all three studies. The highest value was for the MDI study where 34% was achieved, while 15.9% was achieved for the Diskhaler inhaler and 21.9% was achieved for the MDI with spacer. Improvements in lung

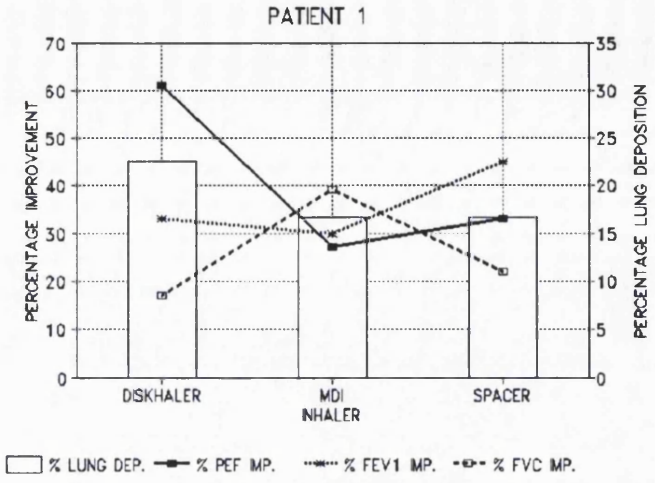


Figure 7.1 (a).

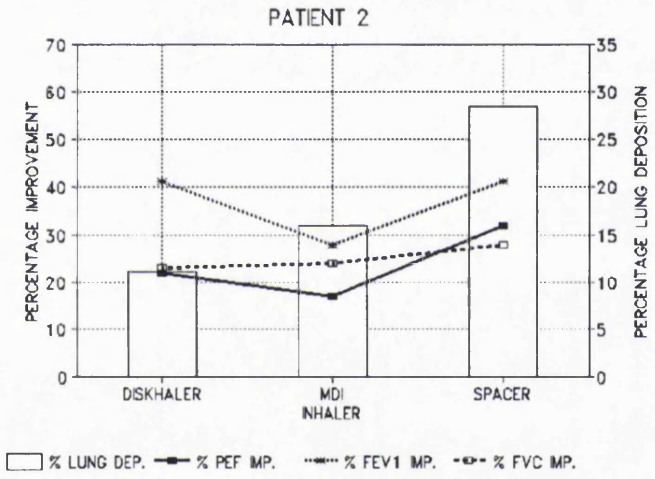


Figure 7.1 (b).

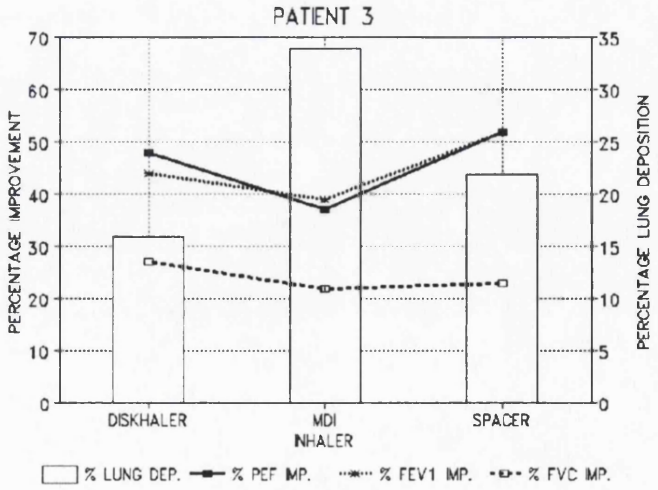


Figure 7.1 (c).

function were also above average particularly for PEF and FEV_1. The improvements were actually less for the MDI study than for the other two studies although this difference was not large.

Patient 4 achieved lung depositions of 7.9% for the Diskhaler inhaler, 24.4% for the MDI and 20.4% for the MDI with spacer. These were respectable quantities particularly for the MDI. However, little or no improvement in lung function was measured. The improvement values were all within the range considered normal for the purposes of these studies. Clearly this patient has demonstrated very poor bronchodilation in response to the salbutamol during these studies, despite the diagnosis of asthma and recent records showing a definite response to salbutamol in the past.

Patient 5 achieved lowest lung deposition of 6.2% with the MDI and highest lung deposition of 19.8% with the MDI with spacer. The Diskhaler inhaler lung deposition of 12.6% was a little above average. Similar improvements in lung function parameters were achieved in all three studies apart from the spacer study where PEF actually showed a negative change.

Patient 6 achieved lung depositions of 7.6%, 25.5% and 34% using the Diskhaler inhaler, the MDI and the MDI with spacer respectively. However, at around 30%, there were very little differences in the improvements in lung function parameters obtained with each method.

Patient 7 achieved lung depositions of 5.0%, 15.3% and 35.8% using the Diskhaler inhaler, the MDI and the MDI with spacer respectively. This resulted in improvements of PEF of 4%, 18% and 26% respectively; improvements of FEV_1 of 14%, 23% and 35% respectively; and improvements of FVC of 14%, 26% and 39% respectively. In this patient lung deposition and bronchodilator response were well matched.

Patient 8 obtained lung depositions of 7.8% for the Diskhaler inhaler, 23.0% for the MDI and 6.8% for the MDI with spacer. Even though highest lung deposition was achieved in the MDI study the lowest improvements in lung function parameters were also measured.

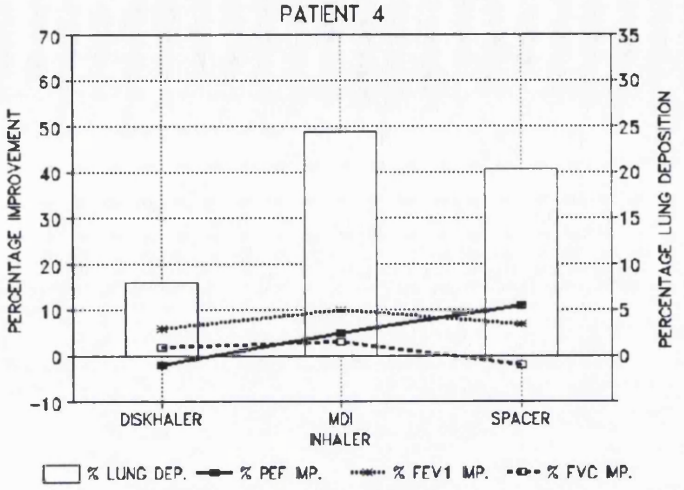


Figure 7.1 (d).

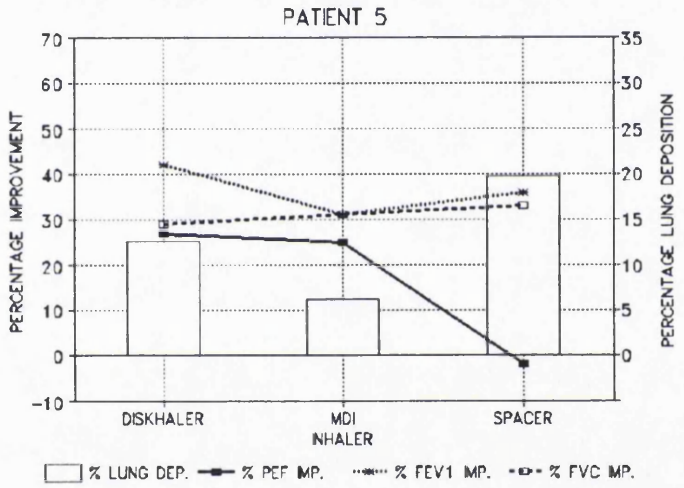


Figure 7.1 (e).

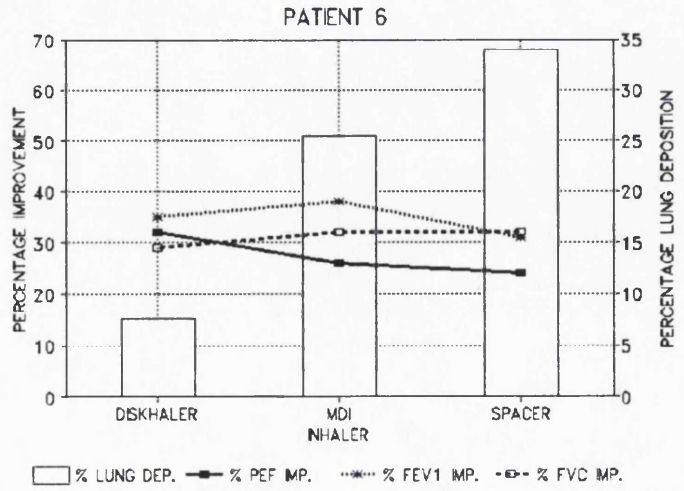


Figure 7.1 (f).

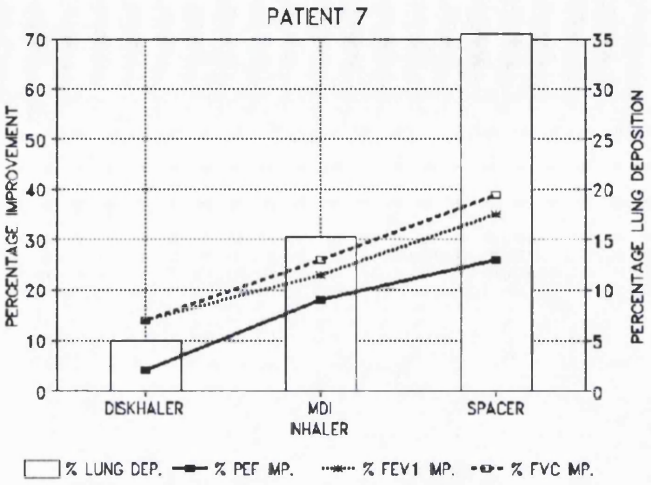


Figure 7.1 (g).

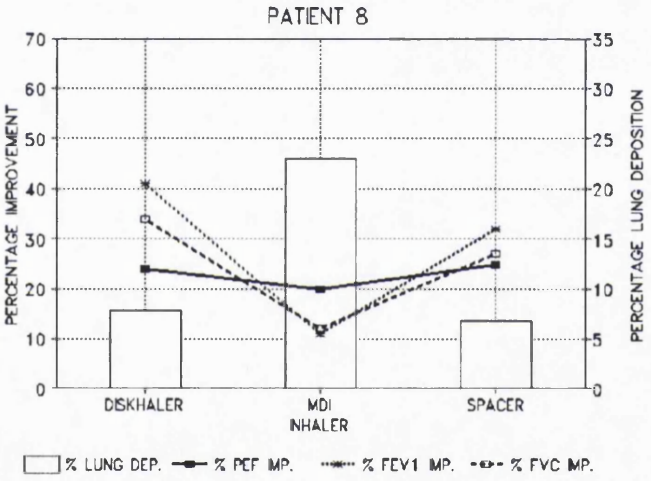


Figure 7.1 (h).

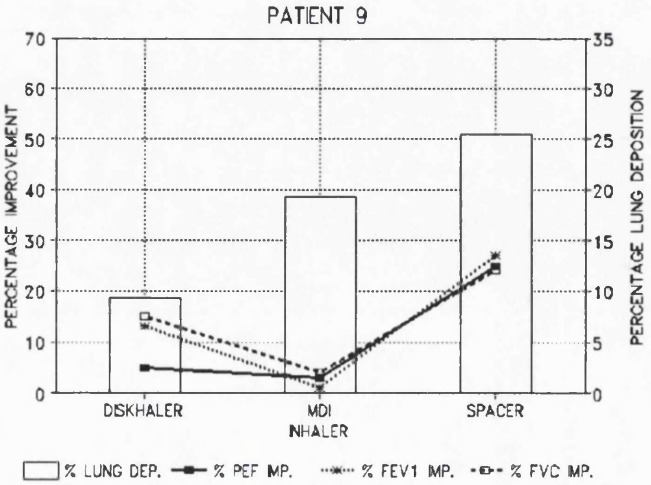


Figure 7.1 (i).

Largest improvements were recorded during the Diskhaler study. No direct relationship was evident between the quantities of drug deposited in the lungs and clinical effect. The patient's baseline lung function measurements did not vary greatly from one study day to the next.

Patient 9 achieved lung depositions of 9.4%, 19.3% and 25.5% for the Diskhaler inhaler, the MDI and the MDI with spacer respectively. Improvements in PEF, FEV_1 and FVC were very small for the Diskhaler inhaler and virtually zero for the MDI. However, using the Spacer resulted in improvements in PEF, FEV_1 and FVC of 25%, 27% and 24% respectively.

Patient 10 obtained a lung deposition of 20.8% when using the MDI. This was higher than the 15.5% resulting from the use of the MDI with spacer and the 6.1% from the Diskhaler inhaler. Both FEV_1 and FVC improved most for the MDI with spacer. However PEF improved greatest for the Diskhaler inhaler and shows the opposite trend to the other two.

Patient 11 obtained a lung deposition of only 0.5% of the dose when using the MDI. He achieved 8.9% in the lung using the Diskhaler inhaler and 11.8% using the MDI with spacer. This low MDI dose was due to his failure to inhale deeply enough. It is also likely that he failed to coordinate correctly. Improvements in FEV_1 and FVC were low for all three inhaler techniques. However the improvement in PEF was only 19.0% for the MDI compared to 55.0% for the Diskhaler inhaler and 37.0% for the MDI with spacer. The much lower improvement for the MDI is probably due to the lower salbutamol deposited in the lung in this study compared to the other studies. It may not have shown up in the other two parameters as their improvements were already small.

Patient 12 achieved lung depositions of 6.6%, 19.7% and 22.7% for the Diskhaler inhaler, MDI and MDI with spacer respectively. Improvements in lung function parameters were small for the Diskhaler and MDI studies and no measurable improvements were recorded for the MDI with spacer. The patient was feeling too unwell in the MDI study to complete the blowing tests following administration and the post FVC value was not measured.

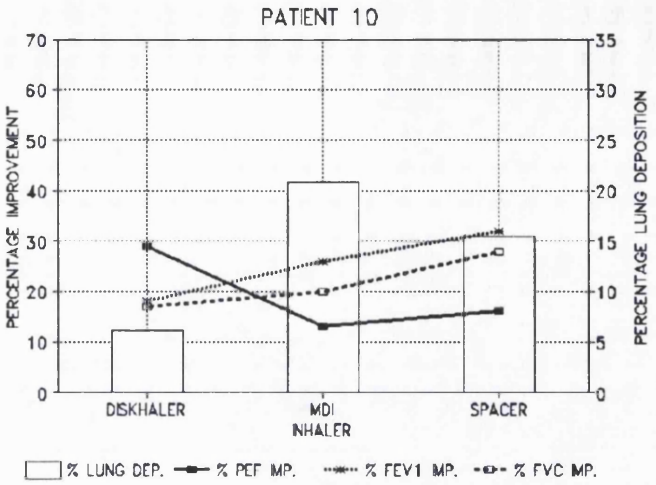


Figure 7.1 (j).

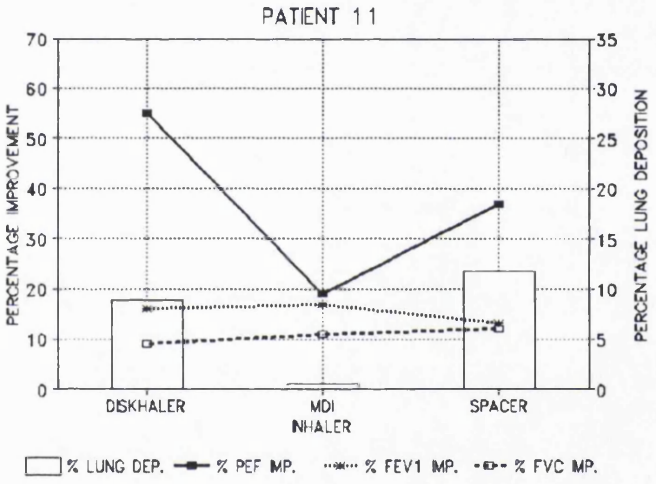


Figure 7.1 (k).

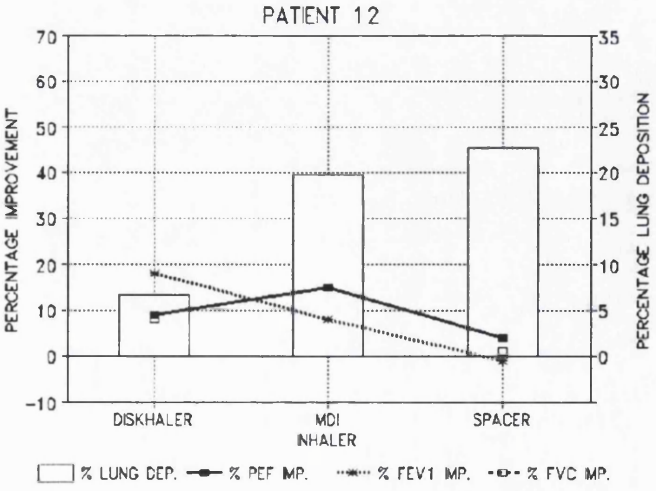


Figure 7.1 (l).

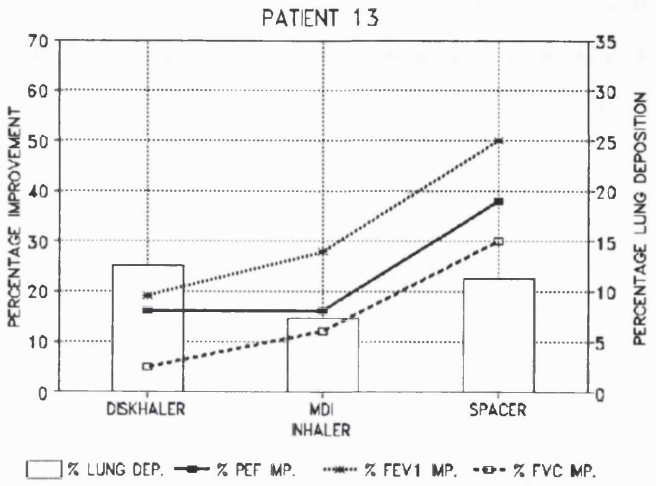


Figure 7.1 (m).

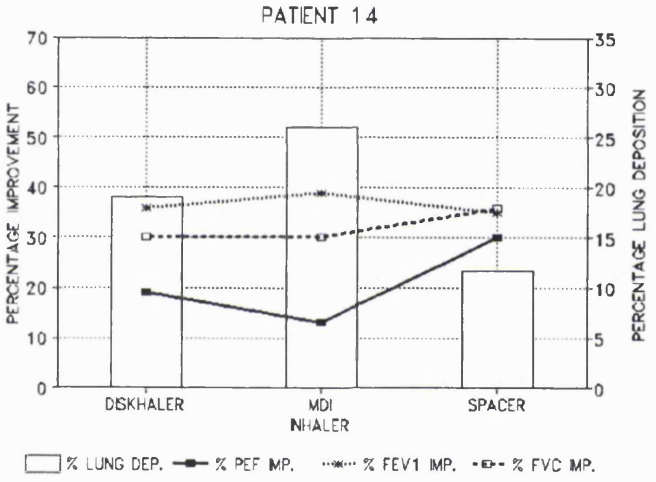


Figure 7.1 (n).

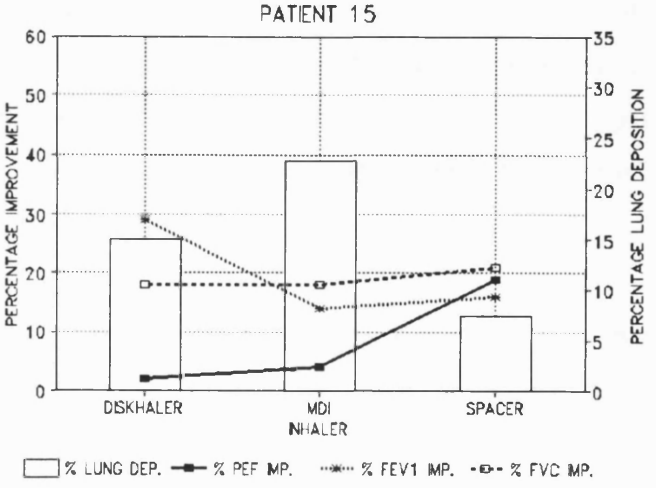


Figure 7.1 (o).

Patient 13 obtained 12.6% of the dose in the lungs for the Diskhaler inhaler, 7.3% for the MDI and 11.3% for the MDI with spacer. There were large variations in improvement in lung function with the greatest improvements obtained with the MDI with spacer while the least improvements were obtained with the Diskhaler inhaler. The clinical effect obtained in the study using the MDI with spacer was clearly larger than for the other two studies for this patient, despite the similar lung depositions achieved for all three.

Patient 14 achieved lung depositions of 19.0% for the Diskhaler inhaler, 26.0% for the MDI and 11.7% for the MDI with spacer. Improvements in FEV_1 and FVC were relatively high for all three methods of administration although changes in PEF were much lower. There were no obvious relationships between these parameters and lung deposition. All three methods were similarly effective, although the MDI with spacer gave the best all round improvements in the three parameters.

Patient 15 achieved lung depositions of 15.1% for the Diskhaler inhaler, 22.8% for the MDI and 7.4% for the MDI with spacer. Improvements in lung function were close to or below average, especially for PEF which showed no measurable improvements with the Diskhaler inhaler or the MDI. It was just below average for the MDI with spacer. There were no apparent relationships between these changes and lung drug deposition. Best overall improvements were obtained with the MDI with spacer.

Patient 16 achieved lung drug depositions of 6.0% for the Diskhaler inhaler, 13.5% for the MDI and 15.9% for the MDI with spacer. All improvements in lung function parameters were extremely low, and below 10% They were within the range considered normal. The baseline values were also high in comparison to other patients taking part in the study and were close to those of the normal subjects. They varied from 75% to 89% of predicted.

Patient 17 obtained lung depositions of 13.3% for the Diskhaler inhaler, 20.9% for the MDI and 31.1% for the MDI with spacer. Smallest improvements in lung function parameters were measured for the Diskhaler study. These ranged from 6% for FVC to 20% for PEF. Largest improvements occurred during the MDI study and ranged from 20% for FVC to 74% for PEF.

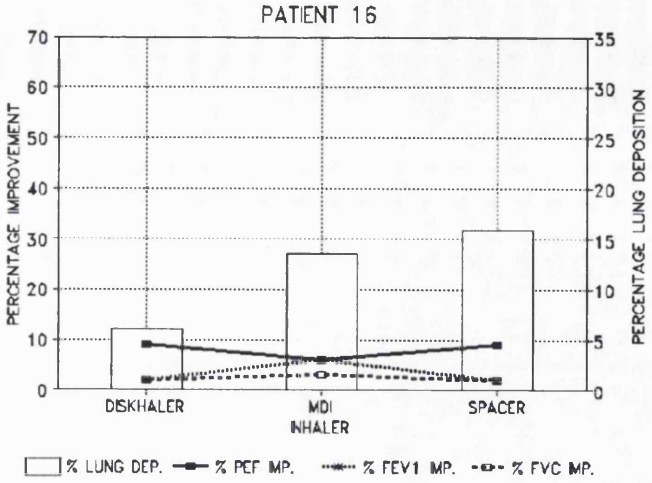


Figure 7.1 (p).

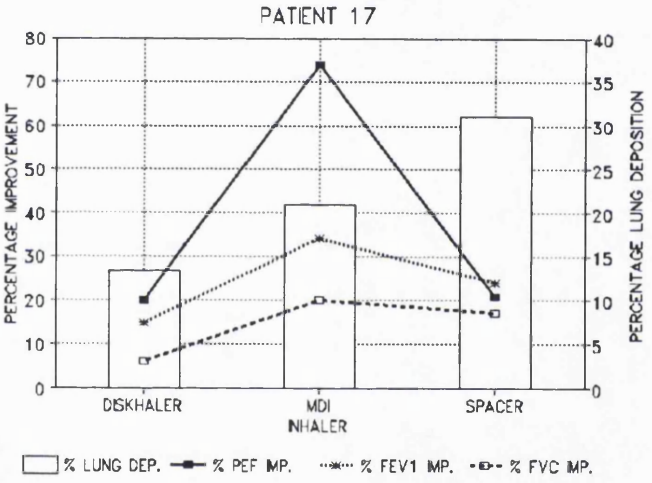


Figure 7.1 (q).

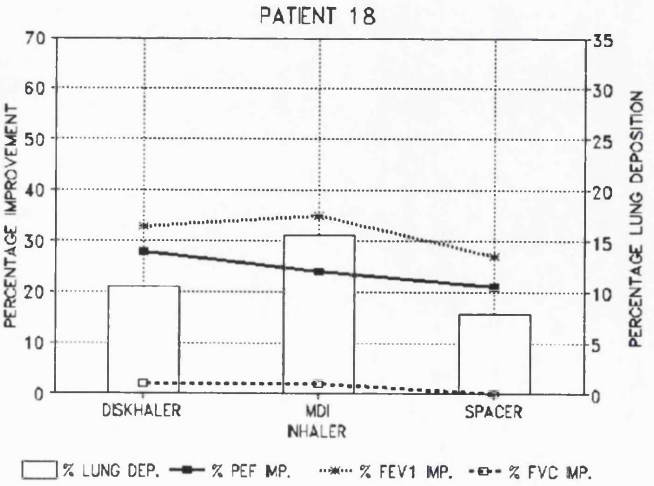


Figure 7.1 (r).

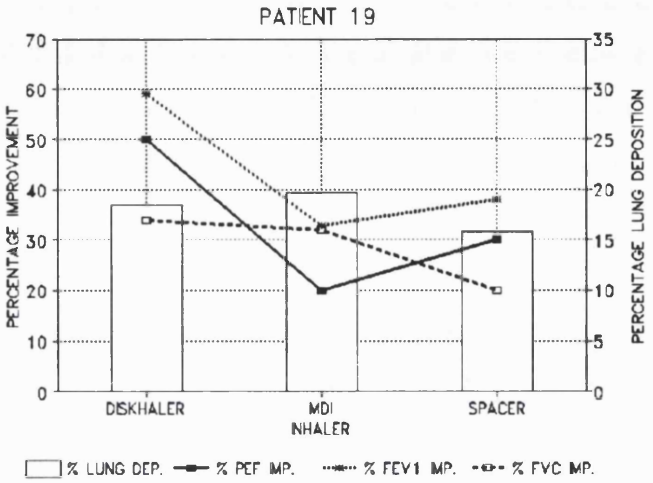

PATIENT 19
PERCENTAGE IMPROVEMENT
PERCENTAGE LUNG DEPOSITION
70
60
50
40
30
20
10
0
35
30
25
20
15
10
5
0
DISKHALER
MDI
INHALER
SPACER
% LUNG DEP.
% PEF IMP.
% FEV1 IMP.
% FVC IMP.

Figure 7.1 (s).

Patient 18 obtained lung depositions of 10.5% for the Diskhaler inhaler, 15.6% for the MDI and 7.8% for the MDI with spacer. All these values were below average particularly those obtained with the MDI with spacer. Improvements in PEF and FEV_1 were similar for all three inhalers and about average. However, improvement in FVC was extremely low in all cases.

Patient 19 obtained lung depositions of 18.5% for the Diskhaler inhaler, 19.6% for the MDI and 15.8% for the MDI with spacer. Improvements in PEF, FEV_1 and FVC were all high. The largest overall effect was measured during the Diskhaler study.

7.4.2.1 Summary

The data represented by these graphs show that there was no simple linear response to the amount of drug deposited in the lungs of the patients. Patient 7 is an obvious exception to this and the results from several other patients showed some relationship between response and lung deposition. There are several factors that can affect the improvement gained by a patient following use of an inhaler. In particular, variations in the patient's baseline bronchoconstriction between study days can influence the magnitude of the improvement measured (Permutt, 1978). In these studies this effect was kept minimal by proceeding with the study only if the patient's baseline lung function was close to that previously measured. Other effects such as the patient's responsiveness to bronchodilator drugs and the severity of their reversible airways obstruction during the studies are more difficult to control. It was therefore necessary to rely on the patient's recent history when deciding to include them in the study. It was also important to control adequately the bronchodilator medication that the patient had taken prior to the study. Occasionally the patient was not studied as they had forgotten not to take their morning dose.

Apart from the amount of drug deposited in the lungs, the different breathing patterns necessary to operate each inhaler may also have had some influence on the degree of bronchodilation measured. However it would be necessary to undertake parallel studies using placebo inhalers to measure the extent of this effect. The difficulty that some patients had in using the MDI is clearly indicated by the low amounts of lung deposition

measured for those studies. Somewhat surprisingly, this also appears to have been the case on several occasions with the MDI with spacer.

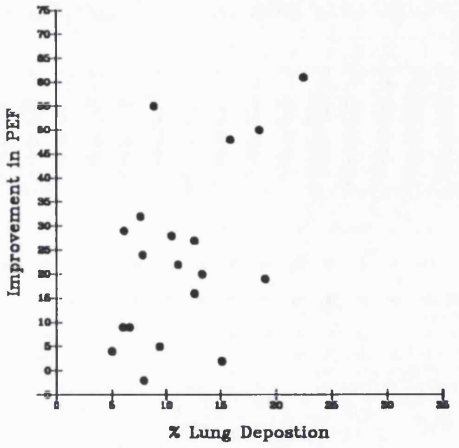
7.4.3 Correlation of Improvement in Lung Function with Salbutamol Lung Deposition

The lung deposition and lung function improvement data for the nineteen patients were also analysed using correlation methods. This was to see if associations could be identified that were not apparent when inspecting the data of individuals. The data for the Diskhaler inhaler, MDI and MDI with spacer were first plotted as scatter graphs (Figure 7.2 - 7.4).

The improvements in lung function parameters measured for patients 4 and 16 were all within the range considered normal for the purposes of these studies. Their base line values were also close to the normal range. These results cannot be explained by low salbutamol deposition in the lungs as each achieved a dose comparable to other patients who showed far larger improvements in their lung function.

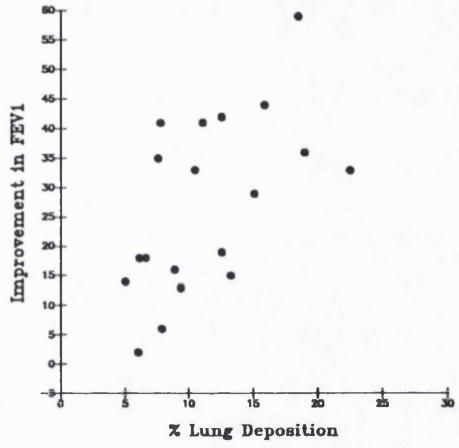
The correlation analysis was repeated after removing the data from these two patients so as to show how they influenced the results. Patient 12 also showed small improvements in lung function in all three studies despite achieving average lung drug deposition in the MDI and spacer studies. However this was not removed as some improvements were larger than the normal range.

When all nineteen subjects were included there were significant correlations between the percentage of salbutamol deposited in the whole lung and two lung function parameters, in the Diskhaler study. These were percentage improvement in FEV_1 ($r_s = 0.57$, $p < 0.01$) and percentage improvement in FVC ($r_s = 0.40$, $p < 0.05$). However, the correlation with improvement in PEF was not significant. Correlation between percentage of drug deposited in the lungs and improvement in FEV_1, FVC and PEF for both the MDI and the MDI with Spacer were poor and none were significant (Figure 7.2 - 7.4; Table 7.19) .



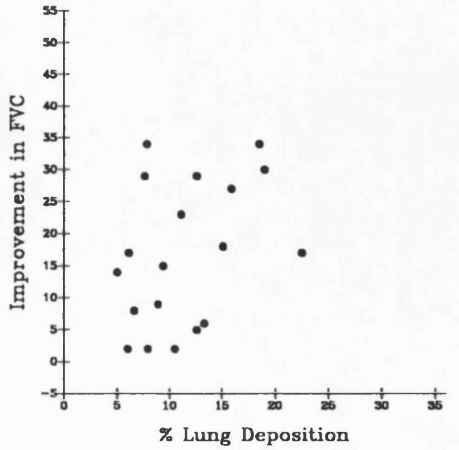
(a)

r_s = 0.33, p = 0.08



(b)

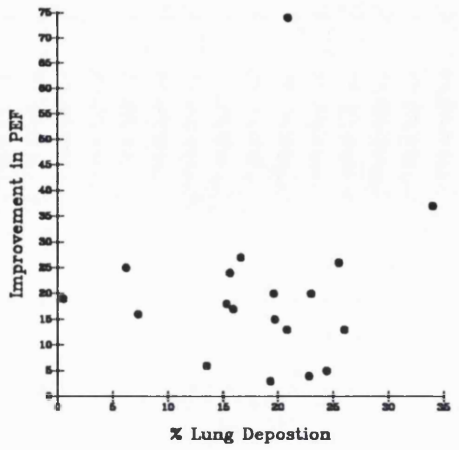
r_s = 0.57, p = 0.006



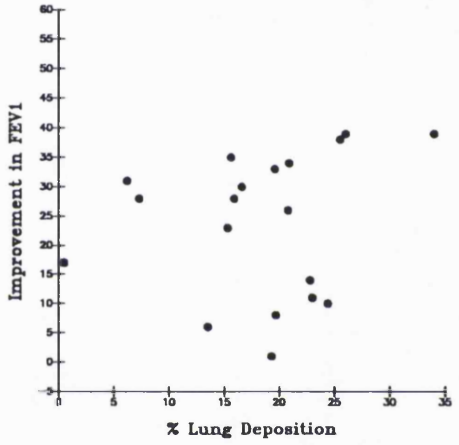
(c)

r_s = 0.40, p = 0.04

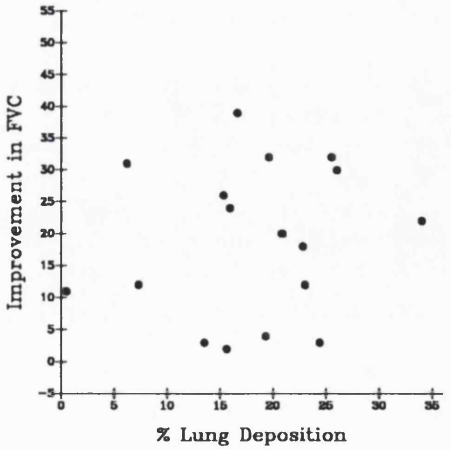
Figure 7.2 . *Association between lung deposition of ^{99m}Tc labelled salbutamol and improvement in: a) PEF; (b) FEV_1 ;(c) FVC; for 19 asthmatic patients, following inhalation from a Diskhaler inhaler.*



(a)
r_s = 0.02, p = 0.46

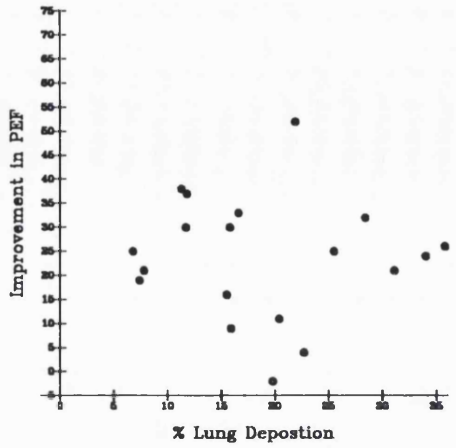


(b)
r_s = 0.28, p = 0.12

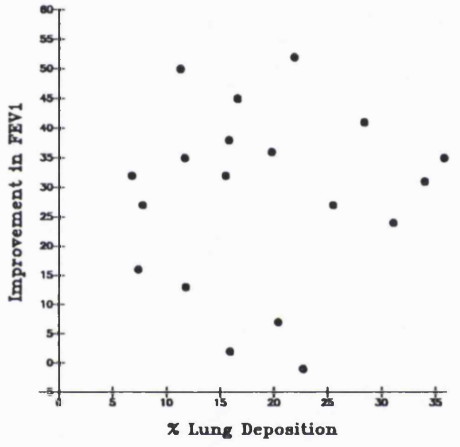


(c)
r_s = 0.14, p = 0.28

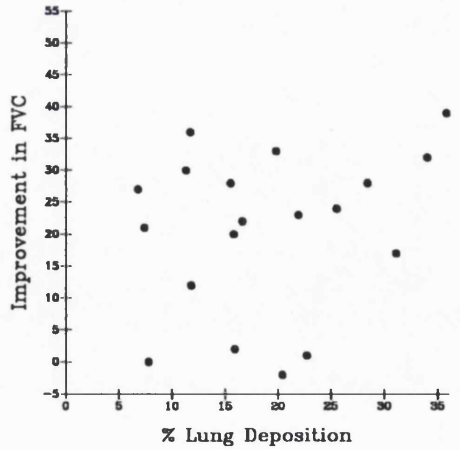
Figure 7.3. *Association between lung deposition of ^{99m}Tc labelled salbutamol and improvement in: a) PEF; (b) FEV_1; (c) FVC; for 19 asthmatic patients, following inhalation from an MDI.*



(a)
r_s = -0.06, p = 0.40



(b)
r_s = 0.01, p = 0.49



(c)
r = 0.16, p = 0.25

Figure 7.4. *Association between lung deposition of ^{99m}Tc labelled salbutamol and improvement in: a) PEF; (b) FEV_1; (c) FVC; for 19 asthmatics, following inhalation from an MDI with spacer.*

Inhaler	% Improvement in PEF	% Improvement in FEV_1	% Improvement in FVC
Diskhaler	r_s= 0.33 p = 0.08	r_s= 0.57 p = 0.006	r_s= 0.40 p = 0.04
MDI	r_s = 0.02 p = 0.46	r_s = 0.28 p = 0.12	r_s = 0.14 p = 0.28
Spacer	r_s= -0.06 p = 0.40	r_s = 0.01 p = 0.49	r_s = 0.16 p = 0.25

Table 7.19. ***Spearman rank correlation coefficients showing strength of association between improvement in lung function and lung deposition for 19 asthmatics inhaling from the Diskhaler inhaler, the MDI and the MDI with spacer.***

When patients 4 and 16 were removed the only difference to these conclusions was that the correlation of improvement in FVC with lung deposition in the Diskhaler study was no longer significant.

7.4.3.1 Subgroups with Low Lung Deposition

The data from all the asthmatic patients studies were pooled together and those from studies in which the patient achieved less than 7% drug deposition in the lung placed in group 1. The data from the other studies were placed in group 2. A comparison was made between the two groups, for percentage improvements in PEF, FEV_1 and FVC, using independent t-tests.

The mean (SD) percentage lung deposition of groups 1 and 2 were 5.2 (2.4) and 17.9 (7.5) respectively, and were significantly different from each other ($p < 0.01$). In groups 1 and 2 respectively, the mean (SD) percentage improvements in PEF were 18.3 (10.0) and 25.3 (16.0); the mean (SD) percentage improvements in FEV_1 were 21.5 (7.9) and 29.8 (13.1); and the mean (SD) percentage improvements in FVC were 17.9 (9.0) and 20.8 (11.2). None of these comparisons reached statistical significance.

7.5 Discussion of Results

The importance of inspiratory flow rate on the effectiveness of aerosol drug particles depositing in the lower airways has been demonstrated previously (Pavia et al, 1977; Newhouse and Ruffin, 1978). When using an MDI, several studies have recommended that, for optimum lung drug deposition (Dolovich *et al*, 1981; Newman *et al*, 1982a) and clinical efficacy (Newman *et al*, 1981b; Lawford and McKenzie, 1983), the user should actuate the device in the early stages of a slow, deep and steady inhalation. A crucial feature is an inspired airstream that carries some of the aerosol into the lungs. However, if the inhalation is too rapid most of the particles will be impacted on the back of the throat. The timing of actuation of the canister is also very important. There may be a reduction in lung deposition if actuation occurs before inhalation, and a complete absence of lung dose if actuation occurs after inhalation (Newman *et al*, 1981a).

Since dry powder inhalers are breath actuated the efficient drug delivery from these devices requires a sufficiently rapid inspiratory flow rate to de-aggregate drug/carrier complexes or large agglomerates of drug particles, and provide an airstream to transport some of the drug into the lungs. Pedersen (1986) found that both medium inhalation (60 - 80 l/min) and fast inhalation (71 - 130 l/min) through a Rotahaler inhaler gave better bronchodilator response than slow inhalation (36 - 50 l/min).

It has not been one of the objectives of these studies to find the optimum technique for inhaling bronchodilator drug as, for the MDI, this has already been thoroughly covered in other studies. The inspiratory flow rate and lung volume at inspiration were, therefore, not strictly controlled as in some of the above studies. Instead, the emphasis has been on following the recommended method for each device and then monitoring the resulting flow profile, deposition pattern and clinical effect. These techniques were identical with those employed in some earlier studies (Short *et al*, 1981; Zainudin *et al*, 1990) and as such the results are directly comparable. The simple instructions that were given to the subjects are the same as those that are given at the clinic and in the manufacturer's literature. For example, subjects were not asked to inhale at an exact flow rate but were first asked to empty their lungs. For the MDI they were next asked to inhale slowly and deeply, while for the Diskhaler inhaler they were asked to inhale rapidly. The inspiratory flow rates measured were therefore a fairly wide spread of values, but reflected well the range expected in typical usage of the devices.

The range of maximum and average inspiratory flow rates obtained when the patients inhaled from the MDI are very similar to those obtained by Newman *et al* (1981a) with a group of asthmatic and chronic bronchitic patients. In the present studies the normal subjects inhaled larger volumes of air and at higher flow rates than the asthmatics. The average flow rates obtained here, are in general, higher than the 30 l/min recommended by some authors (Clarke, 1988; Newman, 1984a) for obtaining optimum lung deposition. Subjects found it easy to inhale fast from the MDI, as its resistance was low, and their interpretation of "slow" tended to be relatively high.

Conversely, the need for an adequate inspiratory flow rate with the MDI was demonstrated in patient 11 who failed to inhale deeply and fast enough. He obtained less than 1% of the dose in his lungs. Patient 10 also failed to inhale deeply but managed to obtain a higher inspiratory flow rate resulting in over 20% of the dose depositing in his lungs.

Analysis of the MDI data from the normal group showed a strong negative correlation between the deposition in the peripheral lung and both maximum and average inspiratory flow rates. There were somewhat weaker negative associations between flow rate and whole lung deposition, and these correlations reached significance only for maximum flow rate. These results suggest a trend towards lower and less peripheral lung deposition as inspiratory flow rates increase and is consistent with the recommendations for slow and deep inhalations discussed earlier. None of the above trends were as strong in the asthmatic group and none reached statistical significance.

This is the first published study to investigate the delivery of salbutamol from the Diskhaler inhaler and to measure inspiratory flow rates simultaneously. It is therefore important to compare the results obtained here with those obtained from other dry powder inhalers. The results of a previous study, obtained using indirect labelling methods, have shown that the Diskhaler inhaler and the Rotahaler inhaler deliver a similar proportion of particles to the lungs of asthmatic subjects (Roberts, Unpublished data). Clinical trials have also shown that the Diskhaler inhaler is as effective as the Rotahaler inhaler in asthmatic adults but that more patients prefer the newer device (Faurschou *et al*, 1988).

None of the patients had previously used a Diskhaler inhaler, although several had used a Rotahaler inhaler. They therefore had to rely solely on the instructions given at the time of the study. None had any difficulty in using this device, although some said they preferred the MDI. All were able to obtain a sufficiently high air flow to remove most of the dose from the chamber. As for the MDI, patient 11 achieved a much lower flow rate than the others but still managed to obtain 8.9% of the dose in his lungs.

The absence of correlation between lung deposition and maximum or average flow rates in both groups of subjects might at first seem surprising. It has already been stated that, in studies of other DPIs, high flow rates produced better bronchodilation than low flow rates. However, the chief reason for the high flow is to aerosolise the drug. Once the minimum flow rate to achieve this has been reached the airstream is already higher than that necessary to carry the drug particles into the lungs without loses in the back of the throat. In theory even higher flow rates would lead to larger impaction losses in the throat. The range of maximum inspiratory flow rates measured during the studies were 85.7 - 241.5 l/min. for the normals and 59.4 - 170.0 l/min. for the patients. These were all above the minimum flow rates required to remove most of the drug from the inhaler chamber.

In the normal group, despite the lack of correlation with whole lung deposition, the significant negative correlation between maximum flow rate and peripheral lung deposition suggests that higher flow rates are associated with more central lung deposition. However this effect was not evident in the asthmatic group.

The significant correlations, in the normal group, between deposition in the oropharynx and both maximum and average flow rates is an indication of increased inertial losses as air velocity increases above the minimum necessary to get the drug particles airborne. As stated above, this is in line with theory predicting the behaviour of aerosol particles in the respiratory tract.

The absence of many of these correlations in the asthmatic group is difficult to explain. It is possible that there were much wider variations in the inhalation techniques employed by the patients, compared to the normals, such that the statistical analysis was not able to identify any relationships. However, if this was the case it appears not to have affected the overall drug deposition patterns in the two groups, which were very similar.

The deposition patterns and bronchodilator responses of salbutamol delivered from the three delivery systems to the asthmatic patients were compared. After starting with an identical drug dose the Diskhaler inhaler deposited significantly less of the dose within

the lungs than either the MDI or the MDI with spacer. However, all three inhalers produced almost identical mean improvements in PEF, FEV_1 and FVC, with no significant differences between them. Analysis of the data from individual subjects showed no clear relationship between the amount of drug deposited in the lungs, achieved in each inhaler study, and the improvements in lung function measured. In addition, the only significant correlations in the data for all of the subjects were in the Diskhaler study between percentage improvements in FEV_1 and FVC and lung deposition. The other inhaler studies gave no significant correlations.

These observations are consistent with the results of Hartley *et al*, (1977) and Hetzel and Clark (1977). Neither of these studies measured the actual lung deposition patterns of drug. However they both compared the bronchodilator effects of equal administered doses of dry salbutamol powder from a Rotahaler inhaler with those from a pressurised MDI. Hartley *et al* (1977) found that 200 μg of drug delivered as a powder produced a similar bronchodilator response to 200 μg delivered from the MDI. Although for the latter inhaler the response tended to be maintained better at three and four hours after inhalation. They also reported that there was no evidence of a dose-response effect between 50 μg, 100 μg and 200 μg of salbutamol delivered as a powder and showed that each of these doses produced a similar response to 200 μg of salbutamol delivered by MDI. However, they found that 400 μg of salbutamol powder gave a larger response.

Hetzel and Clark (1977) found that there were slightly reduced improvements in FEV_1 and FVC with the Rotahaler inhaler compared with the MDI in asthmatic patients with good MDI technique. However, these differences were not significant and other patients with poor MDI technique derived much greater benefit from using the Rotahaler inhaler. In another study Duncan *et al*, (1977) found a small improvement in FEV_1 following inhalation of 200 μg of salbutamol delivered by MDI compared with 200 μg delivered by DPI.

The evidence, both from this study and from other studies, indicate that there is very little difference, if any, in the bronchodilator response achieved by 200 μg of salbutamol delivered by a correctly used MDI or by a dry powder inhaler. This is despite the lower

lung dose shown here for the Diskhaler inhaler. However, the data of Hartley *et al*, (1977) suggest that additional bronchodilator response may be achieved by increasing the dose of salbutamol administered to 400 μg.

Peripheral lung deposition might be a more important determinant of clinical response than whole lung deposition since there is a greater abundance of receptor sites in this region. Of the three delivery methods, the MDI with spacer data would be expected to indicate this effect most clearly since a greater proportion of the dose was deposited in the peripheral region with this method than with the other two (Table 6.8). There is some evidence for this in Table 7.18 which shows that the MDI with spacer gave marginally the best mean improvement in FEV_1. However, analysing the data in more detail failed to demonstrate any correlation between improvement in FEV_1 and salbutamol deposition in the lung peripheral region as was the case for whole lung deposition (Figure 7.4(b)).

Chapter 8. Assessment of Errors, Summary and Further Work.

8.1 Introduction

The investigation would not be complete without an assessment of the radiolabelling technique and the accuracy of the data recorded. The main limitation of the labelling technique, also noted by Vidgren *et al*, (1987) and Köhler *et al* (1988), is the rapid dissolution of the radionuclide from the drug when in the lung environment. This prevents its wider application to studies requiring much more than 5 minutes of imaging time. Its effect on the drug deposition results recorded in this investigation and the reasons for it are considered in more detail. The variation in total recovery, noted in chapter 6, is also considered. Finally a summary is made of the present investigation and the possibilities for future work and applications of the methods considered.

8.2 Dissolution of Radiolabel

While imaging the thorax it was observed that the lung counts in each successive 1-minute time frame progressively decreased. There was a substantial decrease during the first five minutes of counting (Figure 8.1). Some subjects were imaged over a longer period so as to follow this effect further. Small quantities of radioactivity were then noticed in the thyroid gland. The half-time of clearance of lung radioactivity was about 10 minutes. This was compatible with the value observed when $^{99m}TcO^-_4$ is absorbed from the lungs (Yeates *et al*, 1973; Rinderknecht *et al*, 1980) and suggests that the pertechnetate became separated from the salbutamol and the two were absorbed and distributed separately by the pulmonary and bronchial blood system. Shenfield *et al*, (1976) showed that salbutamol itself is rapidly absorbed from the lungs into the blood plasma with a peak plasma level within 10 minutes of drug administration. However, the detection of activity in the thyroid gland indicates that free pertechnetate was present in the blood during the studies since this is one of the organs in which the pertechnetate ion (TcO^-_4) accumulates following distribution by the blood circulatory system (Miller, 1975).

It is known that soluble particles are readily absorbed into the bloodstream when they are deposited in the lungs (Newhouse and Ruffin, 1978; Yeates *et al*, 1973), especially from the alveolar regions. The clearance rate of insoluble particles, unable to diffuse through the pulmonary membrane, is much slower (Thomson and Pavia, 1974; Short *et al*, 1979) and largely due to mucociliary transport and cough. The absorption of pertechnetate from

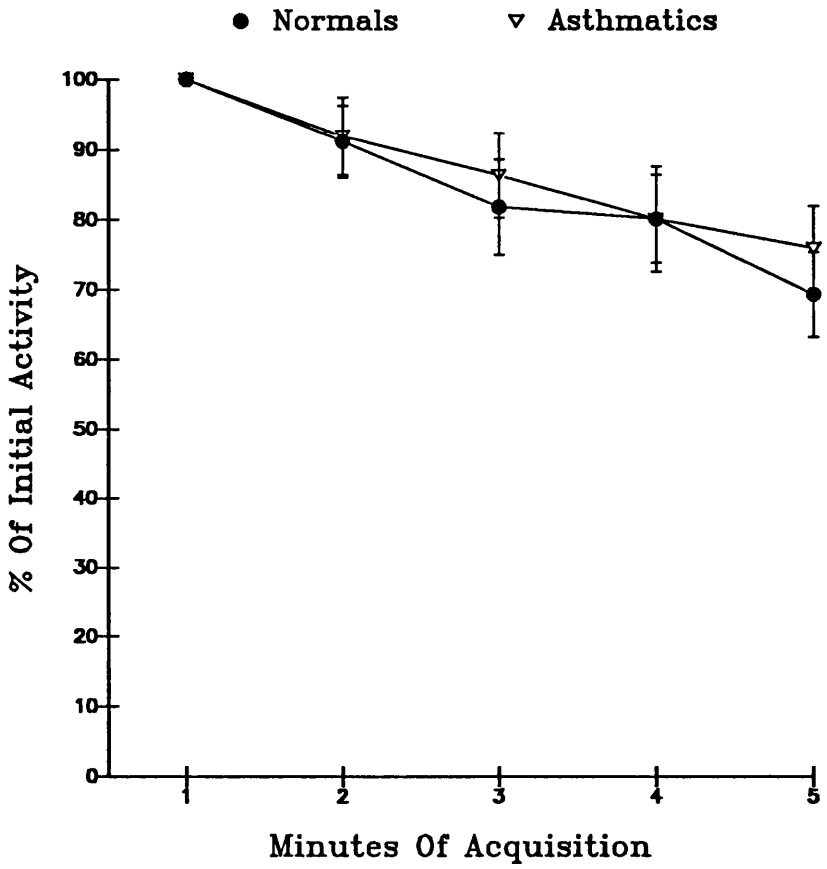


Figure 8.1. ***Clearance of radionuclide from the lungs of the normal and asthmatic subjects. Error bars indicate standard deviation.***

the gastrointestinal tract is also slower (Hays, 1973) and is unlikely to have contributed greatly to the losses sustained during the study period.

Figure 8.1 shows the clearance, in the first five minutes of imaging, of the radionuclide from the lungs of the normal and asthmatic subjects. There was no difference in the mean clearance rates of both groups of subjects.

The uptake of radioactivity in the thyroid gland was small and the count rate was only slightly above background level. This problem was noticed after the first study and it was thought that it might be necessary to use a thyroid blocking agent in order to reduce the dose to this organ. However the ARSAC "Notes For Guidance On The Administration Of Radioactive Substances To Persons For Purposes of Diagnosis, Treatment Or Research", January 1993, states that it is unnecessary to administer blocking agents to reduce radiation dose when using ^{99m}Tc. It also states that 40 MBq was the usual maximum allowed uptake per test with an allowed EDE of 0.5 mSv. The amount taken up by the thyroid in these studies was well below this.

The major concern was the reducing count-rate from the lung ROIs. In order to minimise this effect, the data from the first three 1-minute time frames only were used to form the final image. The data indicate an approximate count loss of 7% for each minute of imaging time over the first three minutes. Over this time, approximately 21% of the lung counts are lost. When averaged over all the studies, this would lead to an underestimation of lung deposition of approximately 2.5% for the Diskhaler studies and 4% for each of the MDI and MDI with spacer studies.

8.3 Variability of Recovered Dose

For the MDI, it is possible that the low recovery values are due to overestimation of the total dose administered. Conversely, for recovery greater than 100%, the total dose administered may have been underestimated. Since it was impossible to measure the amount of radioactivity in the actual dose administered to the subject, the radioactivity was estimated by comparison with the average of five other doses from the same canister actuated into a special collection bag after the study. It is possible that a significant

variation between the dose delivered to the subject and the average of those delivered to bag was the cause of discrepancies in dose percentage recovery values.

In order to measure the degree of variation in "shot weight" a typical 240 dose canister was tested. After shaking the canister five shots were actuated. The mean shot weight was measured by weighing the canister before and after the five actuations. This was repeated a number of times. In this way the mean shot weight for groups of five actuations were sampled throughout the life of the canister. A plot of mean shot weight against canister shot is shown in Figure 8.2.

The Mean (SD) shot weight was 83.8 (3.1) mg and the coefficient of variation of shot weights was 3.7%. This does not fully explain why, in the MDI studies, the activity recovery in five of the patients was between 117 and 130%. In most of the patient studies, the weight of the can was measured before and after administration as well as after the five shots had been discharged to the bag. This permitted measurement of the actual weight of dose delivered to the patient as well as the mean dose weight discharged to the bag. Table 8.1 shows percentage recovery, shot weight during the study and mean shot weight to the bag afterwards. The three studies with the highest recovery corresponded to a much higher dose delivered to the patient than to the bag. They also corresponded to exceptionally high actuator deposition suggesting that much of this extra dose was deposited there.

The data from Table 8.1 also show that the mean dose delivered to the patients was higher and more variable than the dose delivered to the bag despite the same canister being used for the two measurements (91.2 SD 9.3 mg compared to 84.2 SD 1.9 mg). This may correspond to an observation, made during the studies, about the way some patients used their MDI. They tended to continue to squeeze the canister well after the initial depression and inhalation. It is possible that this allowed extra dose to be released from the stem, due to partial refilling of the metering chamber. Drug recovery, in most of the studies, was either very close to or less than 100%. Some of the dose may be lost to the surroundings due to the failure of the subject to inhale the whole dose with some being blown back into the air instead of being inhaled. The low recovery cannot be

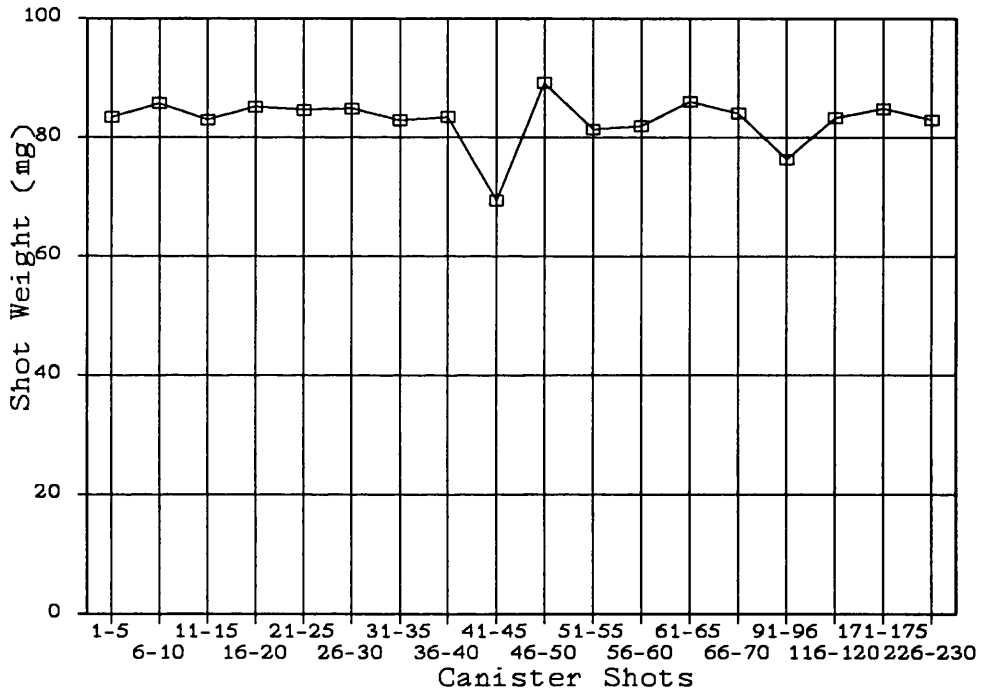


Figure 8.2. ***Variation of shot weight delivered from an MDI.***

PATIENT	% OF DOSE RECOVERED	MEAN SHOT WEIGHT (mg)	
		DURING STUDY	TO BAG
1	96.0	N/A	N/A
2	87.6	N/A	N/A
3	100.1	88.5	82.5
4	78.6	N/A	N/A
5	107.4	85.8	85.9
6	126.7	103.0	85.1
7	96.6	89.5	85.6
8	77.2	87.0	81.3
9	75.3	90.1	81.0
10	81.5	86.9	83.1
11	71.0	86.4	82.1
12	117.4	88.6	83.1
13	68.1	86.5	84.4
14	70.1	89.6	84.4
15	91.3	88.0	86.0
16	129.3	122.4	86.8
17	123.9	92.7	83.2
18	85.7	89.6	85.7
19	119.0	85.4	87.0

Table 8.1 ***Percentage of dose recovered, shot weight during study and mean shot weight to the bag for the 19 patients during the MDI study. N/A indicates data is not available.***

explained by the proportion of dose exhaled as this was always less than 1%. If, as in other studies, total recovery had been derived by summing the individual contributions from each deposition site, the estimate of the mean proportion of dose deposited in the lungs would have been, on average, slightly higher than the values given here. With recovery taken as 100%, the calculated values for lung dose, in the Diskhaler studies, would be 15% for the normals and 12.1% for the patients; in the MDI studies they would be 24.9% for the normals and 19.8% for the patients; In the MDI with spacer studies they would be 26% for the normals and 21.9% for the patients. This gives average increases, for the three studies, of 3.6% for the normals and 1.7% for the patients.

Despite the limitations in calculating the total dose delivered by the MDI, the methods used in these studies should give more accurate lung deposition results. This is because percentage values are derived from an estimate of the dose delivered instead of summing the proportion of the dose recovered from all the deposition sites. They therefore take into account the possibility that some of the dose is either lost to the air, due to incorrect use of the device, or lost to the systemic circulation due to the rapid redistribution of the radionuclide from the original deposition sites.

8.4 Summary and Future Work

A new method for the radiolabelling of salbutamol in MDIs and Diskhaler inhalers has been validated and applied to the study of drug deposition in normal and asthmatic subjects. It was initially based on the method of Köhler *et al*, (1988) but was developed in order to meet the requirements of the present studies and has a number of advantages over previously reported techniques. One of the most important is that it is relatively easy to perform and requires no specialised equipment; inhalers are prepared from basic constituents according to typical formulations and, apart from the addition of technetium-99m, do not require substantial modification of the formulation. The method is more accurate than indirect labelling methods since the drug and salbutamol are attached to each other while depositing in the respiratory tract. Hence the measurements reflect actual drug deposition rather than the deposition of an inert analogue. The method is versatile and may be applied to both MDIs and DPIs allowing a novel technique for the labelling of dry salbutamol sulphate powder to be developed. Using this technique, this is the first

study to measure the deposition pattern of salbutamol delivered from the Diskhaler inhaler.

Since the active drug is radiolabelled, pulmonary function can be studied simultaneously enabling it to provide a simple and effective means of investigating the clinical effects of a known distribution of bronchodilator drug on patients suffering from respiratory disease. Quality control of inhalers was achieved using the liquid twin impinger which ensured that they were of an acceptable performance. Good quality inhalers were produced which enabled deductions to be made about the way standard inhalers perform under clinical conditions.

The validation tests, using the Andersen cascade impactor, confirmed a close association between the properties of the radionuclide and the drug and showed that the radiolabel acted as a marker for the presence of the salbutamol over a wide range of particle sizes. The addition of the radiolabel had no measurable effect on the distribution of the drug within the impactor system. This was demonstrated by comparing the distributions of labelled drug particles with those of unlabelled drug particles. Repeating the tests in a humid environment showed that while the proportion of larger particles/droplets increased the ratio of drug to radionuclide remained the same.

The nature of the labelling is not fully understood. However, it is important to note that, although the salbutamol is not radiolabelled by chemical bond in the same way that ipratropium bromide is with bromine-77 (Short *et al*, 1981), the radioactive marker travels with the drug in such a way that an image of its distribution closely mirrors the distribution of the drug. It is probable that the radionuclide simply coats the surface of the drug particles. The reason that the technetium ion is localised on the drug in the propellant/drug suspension is probably due to its greater solubility in the β_2-agonist molecule than in the propellent as suggested by Köhler *et al*, (1988). This would also explain why it dissociates so easily in the humid lung environment following evaporation of the propellant droplets since the technetium ion is likely to be more soluble in water than in drug. However, further investigation is required to confirm this.

The rapid absorption of the radionuclide from the lungs into the systemic circulation means that the count period is limited to around 3 minutes. This is a major restriction to the application of this technique preventing it from being used for longer investigations such as the study of the fate of drug as it is metabolised, circulated in the bloodstream and excreted via the kidneys into the urine. It also prevents the assessment of any long term lung retention or the rate of absorption and clearance of the drug from the lungs. However, its main application is to provide information about the initial deposition sites of salbutamol or other drugs following delivery by aerosol. The cascade impactor investigation has shown that it is sufficiently stable for this type of study.

These studies are the first to attempt to account for the total dosage of the radionuclide delivered in the various deposition sites. Although the mean percentage of the estimated total dosage accounted for was in the region of 82 - 95% for the various studies the range within each study was wide and in some cases well over 100%.

The drug was administered under conditions as similar as possible to those in which subjects would normally use their inhalers. Subjects were therefore not asked to follow highly specific breathing patterns but instead were instructed to use their devices in the "recommended" fashion. Some subjects were more experienced in the use of a particular inhaler while a few of the normals had not used an inhaler before. Any obvious mistakes or bad practice were corrected before the actual study by allowing the subjects some practice with a placebo inhaler. This was important since the presence of the inhaler adaptor was the main deviation from normal conditions and it was necessary for the subjects to get used to the feel of using it. The aim was to enable each subject to use the inhalers competently.

For the MDI, the percentage deposition of the radiolabelled drug in the lungs in both normal subjects and patients with reversible airflow obstruction is nearly double that previously reported using indirect labelling techniques. These results are particularly reliable since both potential sources of error, the dissolution effect and the low mean recovery values, when taken into account suggest even higher lung deposition. The results are in line with those obtained from studies of other directly labelled $beta_2$-agonists.

Deposition in the oropharynx was reduced, on average, by 86% when the MDI was used with the spacer compared to when it was used on its own. Significantly more dose was deposited in the lungs for both the MDI and MDI with spacer than for the Diskhaler inhaler. This, however, was not reflected in the amount of improvement of lung function, with no significant differences in the improvement in PEF, FEV_1 and FVC between the three methods. There were large variations between each patient and it was difficult to correlate improvement in lung function with proportion of drug dose depositing in the lung.

Inspiratory flow rates were also measured and a number of significant correlations were identified between flow parameters and drug deposition. Several of these confirmed previously reported results. However, due to the wide range of inhalation manoeuvres it was difficult to draw definite conclusions about some of the data. An alternative way of performing the studies would be for each subject to perform a discrete and predetermined set of inspiratory manoeuvres for each inhaler. For example, the lung depositions resulting from three different inspiratory flow rates or three different lung volumes could be compared using appropriate statistical tests. However, a large number of studies would need to be performed on each subject and this would be deviating from the objective of performing each study under conditions as close to normal clinical practice as possible. This type of study could be undertaken in the future using the same flow measuring equipment used here.

It is anticipated that this method of aerosol labelling could be applied to other drug formulations delivered from pressurised metered dose inhalers or dry powder inhalers. It may also be possible to adapt the technique for drug delivery by nebuliser. The main requirement would be for the radionuclide to localise and remain with the drug at least until *in-vivo* deposition. For any drug delivered by these inhalers for which particle size and lung deposition characteristics are required, it is necessary to measure the particle size distribution before and after treatment to ensure that it has not been altered. As so many drugs are available there is a wide scope for potential further studies. These include the new longer acting β_2-agonists such as salmeterol. Similar methods are known to work for

sodium cromoglycate (Newman *et al*, 1989) and it may also be possible to use the technique with topically acting corticosteroids to measure lung deposition.

In the future it will be necessary for therapeutic inhalers to contain only propellants that do not degrade the ozone layer of the atmosphere. This would rule out the present CFC based propellants. Dry powder inhalers are likely to become more important than they are at present. Sweden has already banned CFC based propellants. In response, the country's pharmaceutical industry has concentrated on the development of alternative inhalers particularly advanced dry powder inhalers. At present there is pressure on the pharmaceutical industry world wide to develop "ozone friendly" inhalers. When the CFC ban becomes more widespread and the new inhalers become available there will be the requirement to develop methods of assessment of inhaler performance. It is likely that radiolabelling techniques such as this one will be employed or adapted in the future to quantify drug delivery from these inhalers.

Appendix 1

Estimated Radionuclide Dosimetry for Radioaerosol Studies.

A maximum of 18 MBq of radioactivity (technetium-99m) was administered to each subject during one study. The radioactivity delivered to the lungs is approximately 20 % of that released by the MDI and MDI with spacer and 12 % of that released from the Diskhaler inhaler. For the MDI study the remaining 80 % of radioactivity comprised 50 % in the stomach, 20 % in the actuator with 10 % unaccounted for by the methods of quantification adopted. For the MDI with spacer study the remaining 80 % comprised 7 % in the stomach 17 % in the actuator, 41 % in the spacer and 15 % unaccounted for. For the Diskhaler inhaler study the remaining 88 % comprised 60 % in the stomach, 15 % remaining in the device and 13 % unaccounted for. The radiation doses were calculated using the MIRD (Medical Internal Radiation Dose) notation. It was estimated that the biological half-life of clearance of radioactivity from the lungs was 10 minutes, while the half-life of clearance from the stomach was of the order of 1 hour.

Calculations

Total lung burden for the three studies = 9 .4 MBq = 254 μCi.

Total stomach burden for three studies = 21.1 MBq = 570 μCi.

Cumulated Activity for Lung = initial activity x mean life,

where mean life = 1.44 x effective half-life (T_E)

and $1/T_E = 1/T_{Physical} + 1/T_{Biological}$, and $T_{physical}$ = 6 hours for ^{99m}Tc.

Therefore cumulated activity = 254 x 1.44 x 0.162 μCi - h.

= 59.3 μCi - h.

Similarly

Cumulated activity for the stomach

= 570 x 1.44 x 0.86 μCi - h

= 705.9 μCi - h

Average Absorbed Dose to Lungs (D_L)

Using MIRD S-factors, dose to lungs (D_L) is given by

$D_L = (59.3 \times 5.2 \times 10^{-5}) + (705.9 \times 1.7 \times 10^{-6})$

$= 0.0043$ rads

$=$ 4.3 mrads or 0.04 mGy

Average Absorbed Dose to Stomach (D_S)

$D_S = (705.9 \times 1.3 \times 10^{-4}) + (59.3 \times 1.8 \times 10^{-6})$

$= 0.092$ rads

$=$ 92 mrads or 0.92 mGy

The average absorbed dose to the gonads, breast, red bone marrow, thyroid, bone, pancreas, spleen, kidneys and adrenals were also calculated using the appropriate MIRD S-factors.

The Effective Dose Equivalent (EDE)

This is calculated by adding the weighted average absorbed dose to each organ. Using the weighting factors given in the ICRP publication 53 the contribution of all relevant organs to the EDE due to cumulated activity in the lungs and stomach were as follows:

Gonads	0.5 μSv	Stomach	55.2 μSv
Breast	0.6 μSv	Pancreas	7.7 μSv
Red Bone Marrow	1.4 μSv	Spleen	4.3 μSv
Lungs	4.8 μSv	Kidneys	1.5 μSv
Thyroid	0.03 μSv	Adrenals	1.2 μSv
Bone	0.2 μSv		

EDE = 77.4 μSv

This is 1.5 % of the current statutory permitted annual dose limit of 5 mSv for a member of the public. However, this EDE is 7.7 % of the recommended annual dose limit of 1 mSv for a member of the public (ICRP publication 60) which is due to form the basis of future legislation.

Dose to Thyroid Gland

A small amount of radiation was measured in the thyroid gland some 10 to 20 minutes following inhalation. This was due to the release of technetium pertechnetate from the drug in the lungs. However, the recorded counts were close to background levels and were not included in the calculation of EDE.

Dose Due to Krypton-81m Scan

The EDE due to the krypton scan was calculated to be 2 μSv, based on ICRP publication 53.

Dose Due to Transmission Scan

The effective dose rate at the surface of the flood source containing 100 MBq of 99m-Technetium was measured to be 80 μSv per hour. Since the subject was only close to this source for 2 minutes the dose was extremely low.

Appendix 2

Patient/Volunteer Information and Consent Form

We have developed a method of labelling Salbutamol with a radioactive tracer (Technetium - 99m). This is attached to the drug in the salbutamol inhaler and also to the drug as a dry powder capsule.

We would like to study how much drug, when inhaled in the usual way, actually enters the lungs. In order to do this, we would like to ask you to perform three studies in random order.

1) To inhale two puffs of salbutamol from a pressurised inhaler.
2) To inhale two puffs of salbutamol squirted into a volumatic spacer.
3) To inhale a 200 μg salbutamol dry powder capsule.

Before, and for one hour after each inhalation, we will measure simple breathing tests.

The radiation dose from the three tests, represents 1 % of the annual whole body dose limit for a member of the public.

If you are willing to help with this study, we will require your witnessed signature below. Please note that you may withdraw from this study at anytime without stating a reason or without any prejudice whatsoever.

Name .....

Date .....

WITNESSED BY:-

Name .....

Date .....

References

Agnew JE, Pavia D, Clarke SW. 1981. Airways penetration of inhaled radioaerosol: an index to small airways function? Eur. J. Respir. Dis. 62; 239 - 255.

Agnew JE, Bateman JRM, Pavia D, Clarke SW. 1984. Radionuclide demonstration of ventilatory abnormalities in mild asthma. Clin. Sci. 66; 525 - 531.

Agnew JE. 1984. Physical properties and mechanisms of deposition of aerosols. In: Clarke SW, Pavia D eds. Aerosols and the lung. Butterworth & Co. (Publishers) Ltd. pp 49 - 70.

Ahlquist RP. 1948. A study of the adrenotropic receptors. Am. J. Physiol. 153; 586 - 600.

Alexander HL. 1929. Bronchial asthma, its diagnosis and treatment. Ballière Tindall and Cox, London.

American Thoracic Society. 1962. Definitions and classification of chronic bronchitis, asthma and pulmonary emphysema. Am. Rev. Respir. Dis. 85; 762 - 768.

American Thoracic Society. 1986. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am. Rev. Respir. Dis. 136; 225 - 244.

Anderson SD, Seale JP, Rozea P, Bandler L, Theobald G, Lindsay DA. 1976. Inhaled and oral salbutamol in exercise-induced asthma. Am. Rev. Respir. Dis. 114; 493 - 500.

Armitage P, Berry G. 1987. Statistical methods in medical research, 2nd edition. Blackwell scientific publications, Oxford.

Ashworth HL, Wilson CG, Sims EE, Wotton PK, Hardy JG. 1991. Delivery of propellant soluble drug from a metered dose inhaler. Thorax 46; 245 - 247.

Barnes PJ. 1992. Neural mechanisms in asthma. In: Clark TJH, Godfrey S and Lee TH eds. Asthma 3rd edn. Chapman & Hall Medical, London.

Borgström L, Nilsson M. 1990. A method for determination of the absolute pulmonary bioavailability of inhaled drugs: terbutaline. Pharm. Res. 7; 1068 - 1070.

Brain JD, Valberg PA. 1979. Deposition of aerosol in the respiratory tract. Am. Rev. Respir. Dis. 120; 1325 - 1366.

British Thoracic Society, Research Unit of the Royal College of Physicians of London, King's Fund Centre, National Asthma Campaign. Guidelines for management of asthma in adults: I - chronic persistant asthma. 1990. Br. Med. J 301; 651 - 653.

Brittain RT, Farmer JB, Jack D, Martin LE, Simpson WT. 1968. α-[(t-butylamino)methyl]-4-hydroxy-*m*-xylene-χ^1,α^3-diol (AH.3365): a selective β-adrenergic stimulant. Nature 219; 862 - 863.

Burney PGJ. 1992. Epidemiology. Br. Med. Bull. 48; 10 - 22.

Buxton-Thomas M. 1989. Chapter 10. The lungs. In: Sharp PF, Gemmell HG, Smith FW eds. Practical Nuclear Medicine. IRL Press, Oxford. pp 161 - 177.

Campbell MJ, Machin D. 1990. Correlation and linear regression. In: Medical statistics a commonsense approach. John wiley & sons, Chichester. pp 80 - 96.

Carstairs JR, Nimmo AJ, Barnes PJ. 1985. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. Am. Rev. Respir. Dis. 132; 541 - 547.

Carswell H, Nahorski SR. 1983. Beta-adrenoceptor heterogeneity in guinea-pig airways: comparison of functional and receptor labelling studies. Br. J. Pharmac. 79; 965 - 971.

Check WA, Kaliner MA. 1990. Pharmacology and pharamacokinetics of topical corticosteroid derivatives used for asthma therapy. Am. Rev. Respir. Dis. 141; S44 - S51.

Choo-Kang YFJ, Simpson WT, Grant IWB. 1969. Controlled comparison of the bronchodilator effects of three β-adrenergic stimulant drugs administered by inhalation to patients with asthma. Br. Med. J. 2; 287 - 289.

Chung KF, Clark TJH. 1992. Corticosteroids In: Clark TJH, Godfrey and Lee TH eds. Asthma 3rd edition. Chapman & Hall Medical, London. pp 416 - 448.

Clark TJH. 1992. Definition and clinical categories of asthma. In: Clark TJH, Godfrey S and Lee TH eds. Asthma 3rd edn. Chapman & Hall Medical, London. pp 1 - 13.

Clarke SW, Newman SP. 1984. Therapeutic aerosols 2 - Drugs available by the inhaled route. Thorax 39; 1 - 7.

Clarke SW. 1988. Inhaler therapy. Q. J. Med. 253 (New series 67); 355 - 368.

Cochrane GM. 1992. Management of adult asthma. In: Clark TJH, Godfrey and Lee TH eds. Asthma 3rd edition. Chapman & Hall Medical, London. pp 506 - 550.

Colthup PV, Dallas FAA, Saynor DA, Carey PF, Skidmore LF, Martin LE. 1985. Determination of salbutamol in human plasma and urine by high-performance thin-layer chromatography. J. Chromatogr. 345; 111 - 118.

Crofton and Douglas' Respiratory Diseases, 1989. Seaton A, Seaton D, Leitch AG. Chapter 1: The development and structure of the respiratory tract. Blackwell Scientific Publications, Oxford.

Cullum VA, Farmer JB, Jack D, Levy GP. 1969. Salbutamol: a new selective β-adrenoceptive receptor stimulant. Br. J. Pharmac. 35; 141 - 151.

Davies DS. 1975. Pharmacokinetics of inhaled substances. Postgrad. Med. J. 51 (suppl. 7); 69 - 75.

Davies PJ, Hanlon GW, Molyneux AJ. 1976. An investigation into the deposition of inhalation aerosol particles as a function of air flow rate in a modified 'Kirk Lung'. J. Pharm. Pharmac. 28; 908 - 911.

Davies PJ, Pepys J. 1977. Occupational asthma. In: Clark TJH & Godfrey S eds. Asthma 1st edn. Chapman and Hall, London. pp 190 - 213.

Davis SS, Bubb MD. 1978. Physico-chemical studies on aerosol solutions for drug delivery III. The effect of relative humidity on the particle size of inhalation aerosols. Int. J. Pharm. 1; 303 - 314.

De Blaquiere P, Cristensen DB, Carter WB, Martin TR. 1989. Use and misuse of metered-dose inhalers by patients with chronic lung disease. Am. Rev. Respir. Dis. 140; 910 - 916.

Dolovich M, Sanchis J, Rossman C, Newhouse MT. 1976. Aerosol penetrance: a sensitive index of peripheral airways obstruction. J. Appl. Physiol. 40; 468 - 471.

Dolovich M, Ruffin RE, Roberts R, Newhouse MT. 1981. Optimal delivery of aerosols from metered dose inhalers. Chest 80 (suppl.); 911 - 915.

Duncan D, Paterson IC, Harris D, Crompton GK. 1977. Comparison of the bronchodilator effects of salbutamol inhaled as a dry powder and by conventional pressurised aerosol. Br J. Clin. Pharmac. 4; 669 - 671.

Ellul-Micallef R. 1976. Asthma: A look at the past. Br. J. Dis. Chest 70; 112 - 116.

Eriksson S. 1991. Emphysema: Historic perspectives. In: Weinbaum G, Giles RE, Krell RD eds. Pulmonary Emphysema. Annals of the New York Academy of Science 624; 1 - 6.

Evans ME, Walker SR, Brittain RT, Paterson JW. 1973. The metabolism of salbutamol in man. Xenobiotica 3; 113 - 120.

Farmer JB and Levy GP. 1969. Comparative β-adrenoceptive stimulant properties of salbutamol (AH 3365), orciprenaline and soterenol (MJ 1992). Br. J. Pharmac. 35; 358 - 359.

Faurschou P, Dahl R, Hyldebrandt N, Rasmussen FV, Svendsen UG. 1988. The effects of beclomethasone diopropionate delivered from the Diskhaler inhaler compared to the Rotahaler inhaler. Eur. Respir. J. 1 (suppl. 2); 376.

Fleming JS. 1979. A technique for the absolute measurement of activity using a gamma camera and computer. Phys. Med. Biol. 24; 176 - 180.

Fletcher C, Peto R, Tinker C, Speizer FE. 1976. Chapter 1. In: The Natural History Of Chronic Bronchitis and Emphysema. Oxford University Press. pp 1 - 9.

Freedman T. 1956. Medihaler therapy for bronchial asthma: a new type of aerosol therapy. Postgrad. Med. 20; 667 - 673.

Gray BJ, Frame MH, Costello JF. 1982. A comparative double-blind study of the bronchodilator effects and side effects of inhaled fenoterol and terbutaline administered in equipotent doses. Br. J. Dis Chest 76; 341 - 350.

Gross NJ. 1980. What is this thing called love? - or, defining asthma. Am. Rev. Respir. Dis. 121; 203 - 204.

Gross NJ, Skorodin MS. 1984. Anticholinergic, antimuscarinic bronchodilators. Am. Rev. Respir. Dis. 129; 856 - 870.

Guildry GG, Brown WD, Stogner SW, George RB. 1992. Incorrect use of metered dose inhalers by medical personnel. Chest 101; 31 - 33.

Hall IP, Tattersfield AE. 1992. β-Agonists. In: Clark TJH, Godfrey and Lee TH eds. Asthma 3rd edition. Chapman & Hall Medical, London. pp 341 - 365.

Hallworth GW, Andrews UG. 1976. Size analysis of suspension inhalation aerosols by inertial separation methods. J. Pharm. Pharmac. 28; 898 - 907.

Hallworth GW, Westmoreland DG. 1987. The twin impinger: a simple device for assessing the delivery of drugs from metered dose pressurized aerosol inhalers. J. Pharm. Pharmacol. 39; 966 - 972.

Hannan WJ, Emmett PC, Aitken RJ, Love RG, Millar AM, Muir AL. 1982. Effective penetration of the lung periphery using radioactive aerosols: concise communication. J. Nucl. Med. 23; 872 - 877.

Hartley D, Jack D, Lunts LHC, Ritchie AC. 1968. New class of selective stimulants of β-adrenergic receptors. Nature 219; 861 - 862.

Hartley JPR, Nogrady SG, Gibby OM, Seaton A. 1977. Bronchodilator effects of dry salbutamol powder administered by Rotahaler. Br. J. Clin. Pharmac. 4; 673 - 675.

Hays MT. ^{99m}Tc-pertechnetate transport in man: absorption after subcutaneous and oral administration; secretion into saliva and gastric juices. J. Nucl. Med. 1973; 14: 331 - 335.

Hetzel MR, Clark TJH. 1976. Comparison of intravenous and aerosol salbutamol. Br. Med. J. 2; 919.

Hetzel MR, Clark TJH. 1977. Comparison of salbutamol Rotahaler with conventional pressurized aerosol. Clin. Allergy 7; 563 - 568.

Heyder J. 1981. Mechanisms of aerosol particle deposition. Chest 80 (suppl.); 820 - 823.

Heyder J, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W. 1986. Deposition of particles in the human respiratory tract in the size range 0.005 - 15 μm. J. Aerosol. Sci. 17; 811 - 825.

Hidy GM. 1984. Health effects of inhaled aerosols. In: Aerosols. An industrial and environmental science. Academic press, Inc. pp 578 - 644.

Hiller FC, Mazumder M, Wilson D. 1978. Aerodynamic size distribution of metered-dose bronchodilator aerosols. Am. Rev. Resp. Dis. 118; 311 - 317.

Hiller FC, Mazumder MK, Smith GM, Bone RC. 1980. Physical properties, hygroscopicity and estimated pulmonary retention of various therapeutic aerosols. Chest 77; 318 - 321.

Hindle M, Chrystyn H. 1991. Preliminary assessment of a method to measure relative lung bioavailability after inhalation. J. Pharm. Pharmac. 43 (suppl.); 46p.

Hindle M, Chrystyn H. 1992. Determination of the relative bioavailability of salbutamol to the lung following inhalation. Br. J. Clin. Pharmac. 34; 311 - 315.

Hindle M, Newton DAG, Chrystyn H. 1993. Investigations of an optimal inhaler technique with the use of urinary salbutamol excretion as a measure of relative bioavailability to the lung. Thorax 48; 607 - 610.

Hinds WC. 1982a. Properties of gases. In:. Aerosol Technology. John Wiley & Sons. pp 13 - 37.

Hinds WC. 1982b. Acceleration and curvlinear particle motion. In: Aerosol Technology. John Wiley & Sons. Chap. 5.

Ho KKL. 1989. Particle size characterisation by laser light scattering. The Pharmaceutical Journal (July); 51 - 56.

Holgate ST, Church MK. 1992. The mast cell. Br. Med. Bull. 48; 40 - 50.

Kamburoff PL, Prime FJ. 1970. Oral and inhaled salbutamol as a bronchodilator. Br. J. Dis. Chest 64; 46 - 54.

Kennedy MCS, Simpson WT. 1969. Human pharmacological and clinical studies on salbutamol: a specific β-adrenergic bronchodilator. Br. J. Dis. Chest 63; 165 - 174.

Kirk WF. 1972. *In vitro* method of comparing clouds produced from inhalation aerosols for efficiency in penetration of airways. J. Pharm. Sci. 61; 262 - 265.

Kirkwood BR. 1992a. Comparison of two means. In: Essentials of medical statistics. Blackwell scientific publications, Oxford. pp 41 - 45.

Kirkwood BR. 1992b. Comparison of several means - analysis of variance. In: Essentials of medical statistics. Blackwell scientific publications, Oxford. pp 46 - 56.

Kirkwood BR. 1992c. Transformations. In: Essentials of medical statistics. Blackwell scientific publications, Oxford. pp 138 - 146.

Köhler D, Fleischer W, Matthys H. 1988. New method for easy labelling of beta-2-agonists in the metered dose inhaler with technetium-99m. Respiration 53; 65 - 73.

Kuitert LM. 1992. β Agonists in asthma - state of the art: report on a Royal Society of Medicine seminar. Thorax 47; 568 - 569.

Lands AM, Arnold A McAuliff JP, Luduena FP, Brown TG. 1967. Differentiation of receptor systems activated by sympathomimetic amines. Nature 214; 597 - 598.

Larsson S, Svedmyr N. 1977. Bronchodilating effect and side effects of $beta_2$-adrenoceptor stimulants by different modes of administration. (Tablets, metered aerosol, and combinations thereof). A study with salbutamol in asthmatics. Am. Rev. Respir. Dis. 116; 861 - 869.

Lawford P, McKenzie D. 1983. Pressurized aerosol inhaler technique: how important are inhalation from residual volume, inspiratory flow rate and the time interval between puffs? Br. J. Dis. Chest 77; 276 - 281.

Leifer, KN, Wittig HJ. 1975. The beta-2 sympathomimetic aerosols in the treatment of asthma. Ann. Allergy 35; 69 - 80.

Lung Defence and Immunology. 1989. In: Seaton A, Seaton D, Leitch AG eds. Crofton and Douglas's Respiratory Disease. Blackwell Scientific Publications. pp 95 - 103.

Martin LE, Hobson JC, Page JA, Harrison C. 1971. Metabolic studies of salbutamol-^{3}H: a new bronchodilator, in rat, rabbit, dog and man. Eur. J. Pharmac. 14; 183 - 199.

Martin GP, Bell AE, Marriott C. 1988. An in vitro method for assessing particle deposition from metered pressurised aerosols and dry powder inhalers. Int. J. Pharm. 44; 57 - 63.

Martonen, 1986. Chapter 38. Surrogate experimental modes for studying particle deposition in the human respiratory tract: an overview. In: Aerosols. Research, risk assessment and conrol strategies. Lee SD, Schbeider T, Grant LD, Verkerk PJ eds. Lewis publishers inc. Michigan. pp 547 - 568.

Matthews C. 1910. The use of adrenaline in acute asthma. Br. med. J. 1; 441.

Matthys H, Eltschka R, App EM. 1988. Deposition of a labelled β_2-agonist Aerosol. Atemw.-lungenkrkh 14; 485 - 488.

May KR. 1949. An improved spinning top homogeneous spray apparatus. J. Appl. Physics 20; 932 - 938.

May KR. Multistage liquid impinger. Bacteriological Reviews. 1966. 30; 558 - 570.

May CS, Spiro SG, Johnson AJ, Paterson JW. 1975. Intravenous infusion of salbutamol in the management of asthma. Thorax 30; 236.

Miller WF. 1973. Aerosol therapy in acute and chronic respiratory disease. Arch. Intern. Med. 131; 148 - 155.

Miller W. 1975. Technetium 99m biorouting. In: Nuclear Medicine Technology. C.V. Mosby Co, St. Louis. pp 255 - 277.

Morén F. 1978. Drug deposition of pressurized inhalation aerosols II. Influence of vapour pressure and metered volume. Int. J. Pharm. 1; 213 - 218.

Morén F. 1982. Drug deposition of pressurized inhalation aerosols. Eur. J. Respir. Dis. 63 (suppl. 119); 51 - 55.

Morgan DJ, Paull JD, Richmond BH, Wilson-Evered E, Ziccone SP. 1986. Pharmacokinetics of intravenous and oral salbutamol and its sulphate conjugate. Br. J. Clin. Pharmac. 22; 587 - 593.

Morrow PE. 1974. Aerosol characterization and deposition. Am. Rev. Respir. Dis. 110 (suppl.); 88 - 99.

Morrow PE. 1981. An evaluation of the physical properties of monodisperse and heterodisperse aerosols used in the assessment of bronchial function. Chest 80 (suppl.); 809 - 813.

Newhouse MT, Ruffin RE. 1978. Deposition and Fate of aerosolized drugs. Chest 73 (suppl.); 936 - 943.

Newman SP, Pavia D, Morén F, Sheahan NF, Clarke SW. 1981a. Deposition of pressurised aerosols in the human respiratory tract. Thorax 36; 52 - 55.

Newman SP, Pavia D, Clarke SW. 1981b. How should a pressurised β-adrenergic bronchodilator be inhaled? Eur. J. Respir. Dis. 62; 3 - 21.

Newman SP, Pavia D, Garland N, Clarke SW. 1982a. Effects of various inhalation modes on the deposition of radioactive pressurized aerosols. Eur. J. Respir. Dis. 63 (suppl. 119); 57 - 65.

Newman SP, Morén F, Pavia D, Corrado O, Clarke SW. 1982b. The effects of changes in metered volume and propellant vapour pressure on the deposition of pressurized inhalation aerosols. Int. J. Pharm. 11; 337 - 344.

Newman SP, Clarke SW. 1983. Therapeutic aerosols 1 - physical and practical considerations. Thorax 38; 881 - 886.

Newman SP, Killip M, Pavia D, Morén F, Clarke SW. 1983. Do particle size and airway obstruction affect the deposition of pressurized inhaltion aerosols? Thorax 38; 233 (abstract).

Newman SP. 1984a. Therapeutic aerosols. In: Clarke SW, Pavia D eds. Aerosols and the lung. Butterworth & Co. (Publishers) Ltd. pp 197 - 224.

Newman SP. 1984b. Production of radioaerosols. In: Clarke SW, Pavia D eds. Aerosols and the lung. Butterworth & Co. (Publishers) Ltd. pp 71 - 91.

Newman SP, Millar AB, Lennard-Jones TR, Morén F, Clarke SW. 1984a. Improvement of pressurised aerosol deposition with Nebuhaler spacer device. Thorax 39; 935 - 941.

Newman SP, Killip M, Pavia D, Morén F, Clarke SW. 1984b. The effect of changes in particle size on the deposition of pressurized inhalation aerosols. Int. J. of Pharm. 19; 333 - 337.

Newman SP, Clark AR, Talaee N, Clarke SW. 1989. Pressurised aerosol deposition in the human lung with and without an "open" spacer device. Thorax 44; 706 - 710.

Newman SP, Weisz AWB, Talaee N, Clarke SW. 1991. Improvement of drug delivery with a breath actuated pressurised aerosol for patients with poor inhaler technique. Thorax 46; 712 - 716.

Newman SP, Clarke SW. 1992. Inhalation devices and techniques. In: Clark TJH, Godfrey S, Lee TH, eds. Asthma 3rd edn Chapman & Hall, London. pp 470 - 505.

Orehek J, Gayrard P, Grimaud CH, Charpin J. 1976. Patient error in use of bronchodilator metered aerosols. Br. Med. J. 1; 76.

Paterson JW, Courtenay Evans RJ, Prime FJ. 1971. Selectivity of bronchodilator action of salbutamol in asthmatic patients. Br. J. Dis. Chest 65; 21 - 38.

Paterson IC and Crompton GK. 1976. Use of pressurised aerosols by asthmatic patients. Br. Med. J.1; 76 - 77.

Paterson JW, Woolcock AJ, Shenfield GM. 1979. Bronchodilator drugs. Am. Rev. Respir. Dis. 120; 1149 - 1188.

Pauwels R. 1992. Non-steroidal prophylactic agents. In: Clark TJH, Godfrey and Lee TH eds. Asthma 3rd edition. Chapman & Hall Medical, London. pp 449 - 468.

Pavia D, Thomson ML, Clarke SW, Shannon HS. 1977. Effect of lung function and mode of inhalation on penetration of aerosol into the human lung. Thorax 32; 194 - 197.

Pearson MG. 1993. Asthma guidelines: who is guiding whom and where to? Thorax 48; 197 - 198.

Pedersen S. 1986. How to use a rotahaler. Arch. Dis. Child. 61; 11 - 14.

Pepys J, Davies RJ. 1977. Allergy. In: Clark TJH & Godfrey S eds. Asthma 1st edn. Chapman and Hall, London. pp 126 - 161.

Permutt S. 1978. What should we measure to evaluate bronchodilator drug response? Chest 73 (suppl.); 944 - 948.

Polli, GP, Grim WM, Bacher FA, Yunker MH. 1969. Influence of formulation on aerosol particle size. J. Pharm. Sci. 58; 484 - 486.

Porstendörfer J. 1971. Untersuchungen zur frage des wachstums von inhalierten aerosolteilchen im atemtrakt. Aerosol Sci. 2; 73 - 79.

Pride NB. 1992. Definition and clinical spectrum. Br. Med. Bull. 48; 1 - 9.

Rinderknecht J, Shapiro L, Krauthammer M, Taplin G, Wasserman K, Uszler JM, Effros R M. 1980. Accelerated clearance of small solutes from the lungs in interstitial lung disease. Am. Rev. Resp. Dis. 121; 105 - 117.

Ruffin RE, Montgomery JM, Newhouse MT. 1978. Site of beta-adrenergic receptors in the respiratory tract. Chest 74; 256 - 260.

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

Scadding FH. 1970. Diseases of the Respiratory System. In: Conybeare's Textbook of Medicine. Mann WN, Lessof MH eds. E & S Livingstone, Edinburgh & London. pp 537-598.

Scadding JG. 1977. Definition and clinical categories of asthma. In: Clark TJH & Godfrey S, eds. Asthma 1st edn. Chapman and Hall, London. pp 1 - 10.

Shanks RG, Brick I, Hutchison K, Roddie IC. 1967. Stimulation of adrenergic β-receptors by orciprenaline. Br. Med. J. 1; 610 - 612.

Sharp PF. 1989. Chapter 1. General principles. In: Sharp PF, Gemmell HG, Smith FW eds. Practical Nuclear Medicine. IRL Press, Oxford. pp 1 - 5.

Shenfield GM, Evans ME, Paterson JW. 1974. The effect of different nebulizers with and without intermittent positive pressure breathing on the absorption and metabolism of salbutamol. Br. J. Clin. Pharmac. 1; 295 - 300.

Shenfield GM, Evans ME, Paterson JW. 1976. Absorption of drugs by the lung. Br. J. Clin. Pharmac. 3; 583 - 589.

Short MD, Dowsett DJ, Heaf PJD, Pavia D, Thomson ML. 1979. A comparison between monodisperse Tc-99m-labeled aerosol particles and Kr-81m for the assessment of lung function. J. Nucl. Med. 20; 194 - 200.

Short MD, Singh CA, Few JD, Studdy PR, Heaf PJD, Spiro SG. 1981. The labelling and monitoring of lung deposition of an inhaled synthetic anticholinergic bronchodilating agent. Chest 80 (suppl.); 918 - 921.

Smith FW. 1989. Chapter 19. ^{99m}Technetium pertechnetate. In: Sharp PF, Gemmell HG, Smith FW eds. Practical Nuclear Medicine. IRL Press, Oxford. pp 329 - 338.

Spiro SG, May CS, Johnson AJ, Paterson JW. 1975. Intravenous injection of salbutamol in the management of asthma. Thorax 30; 236.

Spiro SG, Singh CA, Tolfree SEJ, Partridge MR, Short MD. 1984. Direct labelling of ipratropium bromide aerosol and its deposition pattern in normal subjects and patients with chronic bronchitis. Thorax 39; 432 - 435.

SPSS/PC + Statistics 4.0 manual. 1990. pp B-49 - B-66.

Sterling GM. 1983. Respiratory disease. Chapter 4 Lung mechanics II: the airways. William Heinemann Medical Books Ltd, London. pp 51 - 71.

Tattersfield A.E. 1992. Bronchodilators: New developments. Br. Med. Bull. 48; 190 - 204.

Thomson ML, Pavia D. 1974. Particle penetration and clearance in the human lung. Arch. Environ. Health 29; 214 - 219.

Thomson NC. 1992a. Anti-inflammatory therapies. Br. Med. Bull. 48; 205 - 220.

Thomson NC. 1992a. Anticholinergic drugs. In: Clark TJH, Godfrey S and Lee TH eds. Asthma 3rd edn. Chapman & Hall Medical, London. pp 366 - 389.

Toogood JH, Baskerville J, Jennings B, Lefcoe NM, Johansson SA. 1984. Use of spacers to facilitate inhaled corticosteroid treatment of asthma. Am. Rev. Respir. Dis. 129; 723 - 729.

Tothill P & Galt JM. 1971. Quantitative profile scanning for the measurement of organ radioactivity. Phys. Med. Biol. 16; 625 - 634.

Ullman A, Svedmyr N. 1988. Salmeterol, a new long acting inhaled β_2 adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. Thorax 43; 674 - 678.

Ulrik CS, Backer V, Dirksen A. 1992. A 10 year follow up of 180 adults with bronchial asthma: factors important for the decline in lung function. Thorax 47; 14 - 18.

Van Oort M, Hagan R, Gollmar R, Childers AG. The effect of temperature on results obtained from the twin impinger and cascade impactor. Glaxo inc, Research Triangle Park, NC.

Vidgren MT, Kärkkäinen A, Karjalainen P, Paronen TP. 1987. A novel labelling method for measuring the deposition of drug particles in the respiratory tract. Int. J. Pharm. 37; 239 - 244.

Thorax 1993;48:506–511

Lung deposition patterns of directly labelled salbutamol in normal subjects and in patients with reversible airflow obstruction

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Abstract

***Background*—Earlier studies of aerosol deposition in the lungs have relied on indirect labelling of Teflon spheres of a similar size distribution to the drug in question and have assumed similar aerodynamic properties. Using a modification of a new technique for directly labelling salbutamol, the deposition of salbutamol within the lungs of normal subjects and patients with asthma has been studied with the use of a metered dose inhaler (MDI) alone, an MDI with a spacer device, and a dry powder inhaler (DPI).**

***Method*—Salbutamol was directly labelled with technetium-99m and placed in an MDI or DPI. Ten normal subjects and 19 patients with asthma inhaled 200 μg of salbutamol by means of the MDI alone, the MDI with a spacer device attached, and by DPI on separate days. Deposition was assessed by a dual headed gamma camera after inhalation of the drug.**

***Results*—The total mean (SD) percentage deposition of the drug in the normal subjects was 21·6% (8·9%) with the MDI alone, 20·9% (7·8%) with the MDI with spacer, and 12·4% (3·5%) with the DPI. For the patients, the mean percentage deposition was 18·2% (7·8%) with the MDI alone, 19·0% (8·9%) with the MDI and spacer, and 11·4% (5·0%) with the DPI. Bronchodilatation achieved by the patients was similar with all three techniques. Mean peripheral lung deposition was significantly greater with a spacer device than when the MDI was used alone in both normal subjects (49·4% (6·1%) *v* 44·1% (9·9%)) and patients (38·6% (11·1%) *v* 30·4% (9·4%)).**

***Conclusions*—The deposition of directly labelled salbutamol from an MDI is greater than previously estimated by indirect labelling techniques. The deposition of labelled salbutamol from a DPI, however, is little different from that measured by indirect techniques.**

(*Thorax* 1993;48:506–511)

Radiolabelling techniques have provided an important source of information on the deposition patterns of therapeutic aerosols in the lungs. Until recently most information has been obtained from indirect labelling techniques because of the inability to attach a gamma emitting radionuclide to the drug itself. An inert substance, usually Teflon or polystyrene, with size characteristics similar to the drug in question, is labelled and used either as a substitute for the drug itself[1] or mixed in with the drug in a reconstituted metered dose inhaler (MDI).[2] The latter method has the advantage of allowing bronchodilator responses to be measured at the same time as the distribution pattern of the radiolabelled particles. These techniques make the basic assumption, however, that the radiolabelled carrier and the unlabelled drug particles have similar physical properties and distribution when inhaled into the thorax.

There has been scanty information on deposition patterns of directly labelled drug within the lung. Short *et al*[3] radiolabelled ipratropium bromide with bromine-77, and a new method for directly labelling β_2 agonists has recently been described by Köhler and colleagues.[4] A similar technique to that of Köhler has been applied by Newman *et al*[5] to sodium cromoglycate with technetium-99m (^{99m}Tc) in normal subjects and also to salbutamol in asthmatic subjects using a breath actuated MDI.[6] We have recently reported a modification of Köhler's technique for the preparation of an MDI and also have developed a dry powder inhaler (DPI) containing salbutamol directly labelled with ^{99m}Tc.[7] The present study uses these techniques to assess the deposition patterns and bronchodilator responses of directly labelled salbutamol both in normal subjects and in patients with asthma using an MDI, an MDI with a spacer device, and a DPI, each with similar doses of salbutamol.

The study provided an opportunity to assess whether the commonly quoted percentage deposition of salbutamol in the lungs of normal subjects and patients with airflow obstruction (9–14%)[1-3 8 9] was the same if directly labelled salbutamol was used instead of radiolabelled substitution particles.

Methods

The detailed methods for making the MDI and DPI, together with their validation, is reported elsewhere.[7] The technique is briefly described below.

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Received 21 July 1992
Accepted 4 January 1993

METERED DOSE INHALER

A small amount of ^{99m}Tc as sodium pertechnetate was eluted from a molybdenum-99/technetium-99m generator into butanone. Approximately 60% of the $^{99m}TcO^-_4$ was transferred to the butanone phase. After allowing the two phases to separate, the lower aqueous phase was discarded and the organic phase collected and evaporated to dryness, leaving $^{99m}TcO^-_4$ on the surface of a glass vial. A mixture of oleic acid and trichlorofluoromethane (propellant 11) was added to the micronised salbutamol and mixed in an ultrasonic bath during which the $^{99m}TcO^-_4$ was adsorbed onto the salbutamol. Further propellant 11 was then added and the mixture was carefully weighed into empty MDI canisters together with liquid dichlorodifluoromethane (propellant 12). A metering valve was crimped on to each canister. The canisters were made according to a formula, containing propellant and drug, of a typical MDI.

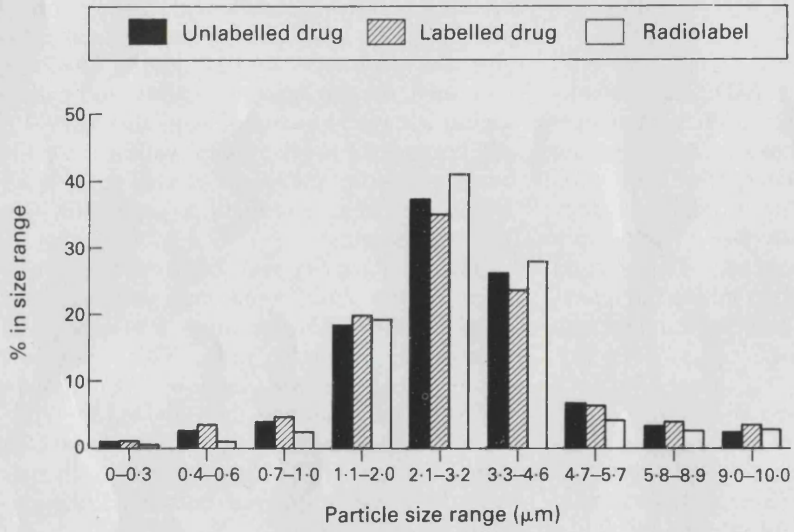

Figure 1 Mean distribution of radiolabel and salbutamol for four MDIs actuated into an Anderson Mark II cascade impactor (operated at 28·3 l/min), shown as a percentage of the total recovery for eight stages plus filter. Also shown is the mean percentage drug distribution for four MDIs containing unlabelled salbutamol.

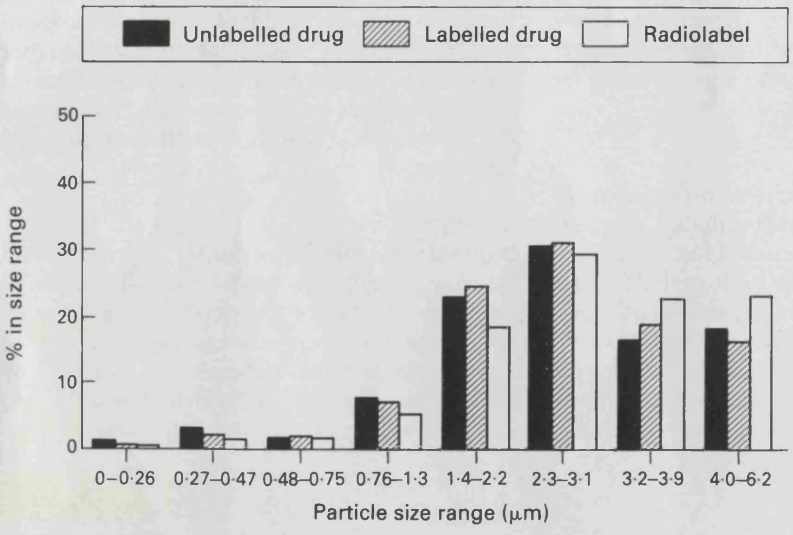

Figure 2 Mean distribution of radiolabel and salbutamol from the contents of three DPIs sampled by an Anderson Mark II cascade impactor (operated at 60 l/min), shown as a percentage of the total recovery for seven stages plus filter. Also shown is the mean percentage drug distribution for three DPIs containing unlabelled salbutamol.

DRY POWDER INHALER

For the DPI, the drug was in the form of micronised salbutamol sulphate. The salbutamol sulphate was mixed with $^{99m}TcO^-_4$ in propellant 11 in an ultrasonic bath. The propellant 11 was then evaporated off and the dried labelled salbutamol sulphate recovered and blended with lactose carrier before being dispensed into unit dose blisters, each containing 200 μg salbutamol.

VALIDATION

The distribution of the radionuclide and salbutamol particles were compared with an Anderson 8 stage cascade impactor. This device is modelled to the particle carrying characteristics of the human respiratory tract so that the potential lung penetration by both solid and liquid airborne particles can be predicted by the instrument. The percentages of the radionuclide and salbutamol measured in each stage were similar for both the MDI and DPI (figs 1 and 2). The proportion of particles of labelled and unlabelled drug and the radionuclide showed concordance throughout the range of particle sizes when each device was tested at its appropriate flow rate. With the MDI, the mass median aerodynamic diameters (MMAD) were similar for labelled (2·8 μm) and unlabelled (2·9 μm) salbutamol, and for the radionuclide (2·7 μm). The MMAD with the DPI was 2·7, 2·7, and 3·0 μm for labelled and unlabelled salbutamol, and for the radionuclide, respectively (figs 1 and 2).

Quality control of each MDI canister and DPI blister was verified on the day of each experimental study with a Twin Impinger. This device measures the proportion of small particles or droplets with an aerodynamic diameter of 6·4 μm which have a 50% probability of progressing and depositing in the second stage.

SUBJECTS

Ten normal subjects and 19 patients with asthma were studied. The normal subjects comprised seven men and three women with a mean age of 34·3 (range 23–49) years; nine were non-smokers and none had a history of any respiratory illness. The 19 patients (13 men, mean age 49·7 (range 22–71) years) all had a documented history of reversible airflow obstruction in response to bronchodilators. The patients were asked to withhold all bronchodilator therapy for at least six hours before the investigation. All subjects gave written informed consent and the study was approved by the local ethics committee.

LUNG FUNCTION TESTS

Lung function measurements were performed with a portable pneumotachograph (Flowmate, Jaeger). Each subject was tested 15 minutes before and 30 minutes after the administration of salbutamol. On each

occasion the best of three satisfactory efforts was used. Measurement of the peak expiratory flow rate (PEF, l/min), forced expired volume in one second (FEV_1), and the forced vital capacity (FVC) were made.

ADMINISTRATION OF RADIOLABELLED SALBUTAMOL

All the subjects performed the three separate parts of the study in random order on different days with at least a week between each study. The subjects were given instructions and demonstrations of how to use each type of inhaler and were allowed to practise with placebo inhalers.

With the DPI, 200 μg salbutamol was inhaled from a single unit dose containing 6–18 MBq radioactivity. With the MDI the subjects inhaled 200 μg salbutamol containing 6–18 MBq as two separate actuations of 100 μg.

The three studies were conducted as described below.

Metered dose inhaler

Subjects inhaled from the MDI after breathing out to residual volume. They then performed a steady deep inhalation actuating the MDI themselves immediately after they started to inhale. At the end of an inhalation they held their breath for 10 seconds before gently exhaling into a collecting bag. The second dose of salbutamol was then inhaled in exactly the same fashion and subjects exhaled into the collecting bag again.

Metered dose inhaler with spacer device

Before inhaling from an MDI through a spacer device (Volumatic) the subjects were first asked to breathe out. No emphasis was made to ask them to reach residual volume. They then actuated the MDI into the spacer and performed a slow gentle inhalation, effectively a large tidal breath. They held their breath for 10 seconds and then expired into a collecting bag. A second inspiration was carried out in exactly the same fashion without actuating the MDI into the spacer. After exhaling into a collecting bag the MDI was shaken vigorously and a second actuation of the MDI was performed into the spacer; this was followed by two similar inhalations.

Dry powder inhaler

With the DPI all subjects breathed out to residual volume and rapidly inhaled the contents of one unit dose prepared for use by one of the investigators. They then held their breath for 10 seconds and exhaled gently into a collecting bag. In the normal subjects two full breaths were taken from the same unit dose, but in the patients the procedure was carried out only once in accordance with the instructions of the manufacturer.

IMAGING PROCEDURES

All radiological information was acquired with a Siemens dual headed rota camera on line to a DPS-3300 nuclear medicine computer system (ADAC Laboratories). Anterior and posterior views were acquired simultaneously. Before adminstration of the radiolabelled drug all subjects underwent a krypton-81m lung scan to provide an outline image of the lung fields. The lung fields were divided into two regions of interest, a central third and peripheral two thirds.[3]

Subjects were seated between the heads of the rota camera with the mid point of their chest at the centre of the field of view. Data collection was split into five 60 second time frames so that movement of the label could be followed as well as measurement of the initial deposition. After imaging the lungs, the subject was repositioned and the throat and stomach imaged separately for 120 seconds each.

All data analysis was carried out with the ADAC computer. Regions of interest were drawn on the lung image with a light pen, the krypton image acting as a template to provide the lung outline. The anterior and posterior counts obtained for each region were multiplied together and their square root taken to give the geometric mean counts. This was corrected for background counts and radiological decay. The counts were also individually corrected for attenuation within the body because of the thickness of the chest wall. Details of this attenuation correction are given elsewhere.[7]

The total activity available from two actuations of the MDI used was calculated by actuating the MDI five times into a bag and measuring the count rates. This was done before the MDI was used by a subject or patient. The result was then divided by a factor of 2·5 to give the activity from two actuations. For the DPI, the activity of the single unit dose was measured before administration.

Dissolution of radiolabel

It was observed that the lung counts in each successive one minute time frame progressively decreased. There was a substantial decrease during the five minutes of counting—that is, 2–7 minutes after inhalation of the radio aerosol. The half life of clearance of lung radioactivity was about 10 minutes,[7] but adequate counts for analysis were obtained on each subject by five minutes after inhaling the salbutamol—that is, after three minutes of counting.

STATISTICS

Normal statistical methods of mean and standard deviation were used. Differences between the three methods of inhalation within groups were tested with the paired t test, and between patients and normals by the Student's t test. A level of $p < 0{\cdot}05$ was considered significant.

Results

Lung function for the normal subjects and the patients did not vary between the three separate days of each study (table 1). In the patients the mean bronchodilatation 30

Table 1 Mean (SD) baseline results of lung function tests in normal subjects and patients before performing the studies on each of the three separate occasions

	Normal subjects (n = 10)		*Patients (n = 19)*	
	FEV_1(l)	*FVC (l)*	*FEV_1 (l)*	*FVC(l)*
MDI alone	4·02 (0·95)	4·83 (1·04)	1·45 (0·51)	3·22 (0·95)
MDI + spacer	3·94 (0·85)	4·89 (1·05)	1·42 (0·48)	3·07 (0·89)
DPI	3·89 (0·88)	4·85 (1·03)	1·44 (0·55)	3·12 (0·98)

FEV_1—forced expiratory volume in one second; FVC, forced vital capacity; MDI—metered dose inhaler; DPI—dry powder inhaler.

Table 4 Mean (SD) percentage of total lung deposition in the peripheral portion of the lung in normal subjects and patients for the three inhalation methods.

	Normal subjects (n = 10)	*Patients (n = 19)*
MDI alone	44·1 (9·9)*	30·4 (9·4)
MDI + spacer	49·4 (6·1)*†	38·6 (11·1)†
DPI	39·4 (8·6)*	28·1 (9·6)

MDI—metered dose inhaler; DPI—dry powder inhaler. *Significantly different from patients, $p < 0·05$; †significantly different from MDI alone and DPI, $p < 0·05$.

minutes after administration of salbutamol by MDI, with or without a spacer, or by DPI were similar (table 2).

Table 3 summarises the deposition, expressed as a percentage of the total dose available of the radionuclide, in normal subjects and patients for each study in the lungs, throat, mediastinum, and stomach, and for each inhaler device. The mean (SD) percentage deposition in the lungs was least with the DPI, 12·4% (3·5%) being deposited in the normal subjects and 11·4% (5·0%) in the patients. The mean total lung deposition was significantly greater in both normal subjects and patients with the MDI than with the DPI ($p < 0·05$). The mean total lung deposition was not significantly improved by the addition of the spacer device in either group. The use of a spacer did, however, significantly improve the peripheral deposition (expressed as a percentage of total lung deposition) in both groups ($p < 0·05$, table 4). Peripheral deposition was also significantly greater in the normal subjects than in patients with all three methods (table 4). There was a wide range between individuals for each method.

With both the DPI or the MDI without the spacer, large amounts of activity were counted over the throat, mediastinum, and stomach, with small quantities remaining in the actuator of the MDI or the DPI. When the Volumatic spacer was used, significantly less of the inhaled dose was deposited in the throat, mediastinum, and stomach in both normal subjects and patients (table 3), with 44·8% and 37·9%, respectively, of the mean activity remaining within the spacer.

Because the normal subjects took two inhalations from a single unit dose with the DPI and the patients took only a single breath, there was significantly less activity remaining in the device in the normal group (11·5% (7·1%)) than in the patients (18·9% (8·9%)), $p < 0·05$. There was, however, no significant difference in mean total lung deposition between the two groups with the DPI.

By adding up the percentage activity at the different sites, the total dose accounted for was calculated. The mean values for these was similar in all the experiments in both the normal subjects and patients, but the range was wide (table 3).

Table 2 Mean (SD) percentage change from baseline lung function in patients (n = 19) after inhaling salbutamol with each of the inhalation methods. No significant difference in percentage change in FEV_1 nor FVC was seen between the three methods.

	PEF % change (SD)	*FEV_1 % change (SD)*	*FVC % change (SD)*
MDI alone	20 (16)	24 (12)	19 (12)
MDI + spacer	24 (13)	29 (15)	21 (13)
DPI	24 (19)	27 (15)	17 (11)

PEF—peak expiratory flow rate; FEV_1—forced expiratory volume in one second; FVC—forced vital capacity; MDI—metered dose inhaler; DPI—dry powder inhaler.

Discussion

This study has examined the deposition of directly radiolabelled salbutamol in patients and normal subjects with different inhaler devices. Our validation of the technique with the Anderson cascade impactor confirmed that the radiolabelled drug was deposited in a similar fashion to the unlabelled drug. For the MDI the Anderson cascade impactor was

Table 3 Mean (SD) percentage of total available activity in each of the sites studied for the three inhalation methods in normal subjects and patients.

	Lungs	*Throat/ mediastinum/ stomach*	*Actuator/ device*	*Spacer*	*Exhaled*	*Recovered*
Normal subjects (n = 10)						
MDI alone	21·6 (8·9)*	47·0 (12·6)	18·0 (4·8)	—	0·5 (0·5)	87·1 (14·8)
MDI + spacer	20·9 (7·8)*	7·7 (6·1)†	14·8 (2·9)	37·9 (14·9)	0·5 (0·5)	81·8 (18·3)
DPI	12·4 (3·5)	59·2 (7·4)	11·5 (7·1)	—	0	83·1 (5·4)
Patients (n = 19)						
MDI alone	18·2 (7·8)*	50·1 (13·9)	26·2 (15·8)	—	0·4 (0·5)	94·9 (20·4)
MDI + spacer	19·0 (8·9)*	6·3 (3·8)†	18·9 (10·4)	44·8 (16·2)	0·3 (0·3)	89·3 (14·4)
DPI	11·4 (5·0)	63·9 (9·6)	18·9 (8·9)	—	0·2 (0·5)	94·4 (6·9)

MDI—metered dose inhaler; DPI—dry powder inhaler.
*Significantly different from DPI, $p < 0·05$; †significantly different from MDI alone and DPI, $p < 0·001$.

operated at a standard configuration and a gas flow rate of 28·3 l/min. For the DPI it was operated at a flow rate of 60 l/min and the configuration included a preseparator stage for collecting the larger particles. The particle size cutoffs for each stage were therefore recalculated for the higher rate and hence the size ranges for each stage are slightly different (figs 1 and 2). Both figures illustrate the similar distribution of labelled and unlabelled salbutamol and radiolabel over a wide range of particle sizes.[7]

The percentage total deposition in the lungs of the normal subjects is higher than those previously reported from our laboratory with inhalers containing radiolabelled Teflon spheres. We have previously reported a mean deposition of 11·2% in the lung with a reconstituted MDI and a mean of 9·1% deposited with a DPI device in patients with airflow obstruction.[2] The values reported here are also higher than those obtained with either a labelled substitute,[1 8 9] or with directly labelled sodium cromoglycate.[5]

Values similar to those obtained in the present study have been reported by others who have also used directly labelled β_2 agonists. Matthys *et al*[10] reported a mean deposition of 26% directly labelled salbutamol in the lung of four normal subjects with an MDI, and 34% with the addition of a spacer device. They also obtained a lung deposition of 18·7% when inhaling from residual volume and 33% when inhaling from 50% of vital capacity.[4] Newman *et al*,[6] using a technique similar to that of Köhler to directly label salbutamol, have also found a mean total lung deposition of 22·8% in asthmatic patients when the inhaler was used properly, but only 7·2% when inhaler technique was faulty. Another study with MDIs containing propellant soluble drug has reported lung deposition of over 39%.[11] These results suggest that radiolabelled Teflon spheres probably do not have the same aerodynamic properties within the lung as the drug being tested, and that drug deposition is higher than the 10% deposition usually quoted for MDIs.[1 2 8 9 12]

The wide range of percentage deposition within the lungs in both normal subjects and patients obtained in the present study was to be expected as no attempt was made to control flow rates or the volume inhaled. It is reported that deposition may vary according to the inhalation flow rate and the lung volume at the beginning of inhalation, being optimum at an inspiratory flow rate of 30 l/min from 50% of vital capacity.[8] Our subjects were allowed to use the inhalers in their own fashion after some tuition. They inhaled according to typical instructions of the manufacturer, and these techniques were identical to those employed by us in earlier studies on patients with airflow obstruction.[2 3] It was our intention to obtain a range of deposition that would occur in clinical practice, and not to impose a rigid volume or flow governed system of inhalation. The results of this current study are therefore directly comparable to our earlier study in asthmatic patients where lung deposition from an MDI of labelled Teflon particles of similar MMAD to salbutamol was 11·2%.[2]

Our results with the addition of a spacer device showed an improvement only in peripheral lung deposition rather than in the total lung deposition as reported by others.[8] The present study does, however, confirm the dramatic reduction in the percentage deposition in the oropharynx and stomach which may be of greater relevance in reducing the ingested dosage of inhaled corticosteroids to reduce the potential for systemic side effects.

With the DPI, although total mean lung deposition was similar in normal subjects and patients, the significant improvement in peripheral lung deposition in the normal subjects, with less activity remaining in the DPI, suggests that taking two inhalations from a single blister may be more effective than one. The measurements may, however, also reflect better function of normal lungs.

The rapid dissolution of the radiolabel in the lungs was also noted by Köhler *et al*.[4] This is probably the result of the radiolabel becoming dissociated from the drug within the lungs because it is water soluble. Although we counted for five minutes after inhalation of the drug, only the first three minutes of data were used in assessing deposition because of the rapid clearance of activity from the lungs. It was not possible to count for less than three minutes as insufficient counts were accumulated. Because of this clearance the present results may well underestimate the percentage deposition in the lungs immediately after inhalation.

The current study is also the first to attempt to account for the total dosage of the radiolabel delivered in the various areas where it may have been distributed. Although the mean percentage of the estimated total dosage delivered accounted for was in the region of 82–95% for the various studies, the range within each study was wide and in some cases over 100%. A possible cause for the recovered amount falling below 100% could be the rapid clearance of the radiolabel from the lung; very little of the radioactivity was found in the expired breath.

In conclusion, we have described a relatively simple new technique for the direct labelling of salbutamol and have shown that the percentage deposition of the directly labelled drug in the lungs in both normal subjects and patients with reversible airflow obstruction is nearly double that previously reported with indirect labelling techniques when using an MDI. This technique may enable the investigation of deposition of bronchodilator drugs within the lung to be related to their clinical effect.

Volumatic is a trademark of the Glaxo Group of companies.
We thank RJ Marriott and AJ Taylor of Glaxo Group Research Ltd for their assistance during the study, Dr R Melchor was sponsored by Fondo de Investigaciones Sanitarias de la Seguridad Social, Spain.

1 Newman SP, Pavia D, Moren F, Sheahan NF, Clarke SW. Deposition of pressurised aerosols in the human respiratory tract. *Thorax* 1981;**36**:52–5.
2 Zainudin BMZ, Biddiscombe M, Tolfree SEJ, Short MD, Spiro SG. Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurised metered dose inhaler, as a dry powder, and as a nebulised solution. *Thorax* 1990;**45**:469–73.
3 Short MD, Singh CA, Few JD, Studdy PR, Heaf PJD, Spiro SG. The labelling and monitoring of lung deposition of an inhaled synthetic anticholinergic bronchodilating agent. *Chest* 1981;**80**(Suppl):918–21S.
4 Köhler D, Fleischer W, Matthys H. New method for easy labelling of β_2 agonists in the metered dose inhaler with technetium-99m. *Respiration* 1988;**53**:65–73.
5 Newman SP, Clark AR, Talaee N, Clarke SW. Pressurised aerosol deposition in the human lung with and without an "open" spacer device. *Thorax* 1989;**44**:706–10.
6 Newman SP, Weisz AWB, Talaee N, Clarke SW. Improvement of drug delivery with a breath actuated pressurised aerosol for patients with poor inhaler technique. *Thorax* 1991;**46**:712–6.
7 Biddiscombe MF, Melchor R, Mak VHF, Marriott RJ, Taylor AJ, Short MD, *et al.* The lung deposition of salbutamol, directly labelled with technetium-99m, delivered by pressurised metered dose and dry powder inhalers. *Int J Pharm* 1993 (in press)
8 Newman SP, Pavia D, Garland N, Clarke SW. Effects of various inhalation modes on the deposition of radioactive pressurised aerosols. *Eur J Respir Dis* 1982; **63**(Suppl):119.
9 Dolovitch MB, Ruffin RE, Roberts R, Newhouse MT. Optimal delivery of aerosols from metered dose inhalers. *Chest* 1981;**80**(Suppl):911–5.
10 Matthys H, Eltschka R, App EM. Deposition of a labelled β_2 agonist aerosol. *Atemwlungenkrkn* 1988;**14**:485–8.
11 Ashworth HL, Wilson CG, Sims EE, Wotton PK, Hardy JG. Delivery of propellant soluble drug from a metered dose inhaler. *Thorax* 1991;**46**:245–7.
12 Clarke SW. Inhaler therapy. *Q J Med* 1988;**67**:355–68.

Adventitia

Asthma and the psyche

John was a scruffy, doe eyed lad of 13 admitted with acute asthma. It was his 12th admission, all the previous ones being under a paediatrician. My registrar noted that he improved very quickly and he was prompted to look for precipitating factors. He found none and I also had a go at John with similar results. He was well when he went home. Two days later he was back with another attack which was again shortlived. John assured me that there were no problems at home or at school, but at a second talk he burst into tears and said: "Well, if you want to know, I hate my mum." Mum was a poor widow, big and fat, and with a terrible squint. At night she went to the pub to wash up. At school John was ashamed of his poor clothes and shoes (it was long before torn jeans became fashionable). Other boys had nice presents at Christmas and on birthdays but John had none. His sister had young children and when her lorry driver husband was away on long journeys, John babysat so that she could "carry on" elsewhere. He felt ashamed of her activities, as he was of his mother's job.

After a couple of days he said he was ready to go home and that he wouldn't get asthma again. On the way home he ran away from his mother and later he telephoned me. I picked him up and took him home with me for crumpets and tea, after which John was in hospital for the 14th time.

After a few more crises it was decided that he should be found foster parents. His new mother was also fat but had no squint. She was well dressed and exuded warmth. John lost his asthma and eczema.

Five years later he was admitted again. He was working but had quarrelled with his foster mother and gone back to his old home. A few weeks later his asthma and eczema were bad again. No, I don't think it had anything to do with the house dust mite.

Mr West, a sock manufacturer, developed asthma when he was 47 and was admitted with a bad attack which was resistant to treatment. The sister mentioned that his wife and secretary visited at different times and that he seemed to be worse after such visits. After probing he also burst into tears. His secretary was his mistress and during their liaison she had acquired half his business so he could not get rid of her, as he now wanted to. He accepted that anger, fear, and frustration were precipitants of his asthma and, after a troublesome year, peace and contentment were somehow restored and he became much better.

Some six years later I was standing at a bus stop. He drove by and stopped to pick me up. Our journey was long enough to establish that he was free of asthma but not to find out how peace had been achieved. He made very good socks and I still have a pair of them in a drawer.

I met these cases long ago, but since then I have never recognised such a clear connection between psyche and asthma. How many have I missed? As a young consultant I spent all my working hours on the wards and in outpatients. As the years went by other matters took up time and more layers of junior staff intruded between me and my patients. These days continuity of care seems even worse, with more of a consultant's time being occupied away from patients. Most asthmatics attending hospitals are likely to be seen by different doctors on most occasions. The accent seems to be increasingly on medication with scant regard for causation.

D DAVIES

International Journal of Pharmaceutics, 91 (1993) 111–121

IJP 02990

The lung deposition of salbutamol, directly labelled with technetium-99m, delivered by pressurised metered dose and dry powder inhalers

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(Received 22 June 1992)
(Accepted 24 July 1992)

Key words: ^{99m}Tc; Salbutamol; Label; Metered dose inhaler; Dry powder inhaler; Cascade impactor; Deposition pattern

Summary

A method is described for the radiolabelling of the β_2-agonist, salbutamol, with the radionuclide ^{99m}Tc. The technique was used to prepare metered-dose inhalers and dry powder inhalers for inhalation by six normal subjects and the deposition of drug within the lungs was measured. In vitro data are presented from studies using an Andersen cascade impactor which show that salbutamol and ^{99m}Tc in the aerosol discharged by the metered dose inhaler, or drawn through the instrument from a dry powder inhaler, have a closely matched particle size distribution. Data from inhalers containing unlabelled salbutamol showed that the addition of the radiolabel had not significantly altered its distribution. Using a dual headed gamma camera (Siemens Rota Camera), we studied six normal volunteers and measured a mean (±SD) lung deposition of 11.3 (2.2)% of the dose discharged from a dry powder inhaler and 24.1 (8.5)% from the metered dose inhaler. The deposition values from the metered dose inhaler are considerably greater than those observed using indirect labelling techniques.

Introduction

Radiolabelling has been an important technique for obtaining information concerning the deposition of bronchodilator aerosols in the lungs. Until recently most data have been obtained from indirect labelling techniques because of the inability to attach a gamma-emitting radionuclide to the drug itself. An inert substance, usually teflon or polystyrene, with size characteristics similar to the drug in question is labelled and used either as a substitute for the drug itself (Newman et al., 1981), or mixed in with the drug in reconstituted metered dose inhalers (MDI) (Zainudin et al., 1989). The latter technique had the advantage of allowing bronchodilator responses to be measured at the same time as the distribution pattern of the radiolabelled particles. However, these techniques made the basic as-

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sumption that the radiolabelled carrier and the unlabelled drug particles have similar physical properties and distribution when inhaled into the thorax.

Until recently, there has been scanty information on deposition patterns of directly labelled drug deposition. Short et al. (1981) radiolabelled ipratropium bromide with Bromine-77, and a new method for directly labelling β_2-agonists has been recently described (Köhler et al., 1988). A similar technique to Köhler has been applied by Newman et al (1989) to sodium cromoglycate and to salbutamol (Newman et al., 1991) using ^{99m}Tc. We describe a modification of Köhler's technique for the preparation of an MDI and a dry powder inhaler (DPI) containing salbutamol that is directly labelled with ^{99m}Tc.

The technique, and results of validation, are presented here including data on the deposition within the thorax in six normal subjects.

Materials and Methods

Labelling of salbutamol in a metered dose inhaler

On each study day, three canisters containing labelled salbutamol and propellants were prepared. The radionuclide ^{99m}Tc was present as the sodium salt of the pertechnetate ion (TcO_4^-).

First, a small volume of sodium pertechnetate, eluted from a molybdenum-^{99m}Tc generator and containing 11 000 Mbq of radioactivity, was placed into a 20 ml separating funnel together with 4 ml of sterile water and 5 ml of butanone. Because of the high activity involved, large amounts of shielding were required.

The funnel was shaken for 3 min so that the contents were well mixed. During this process approx. 60% of the $^{99m}TcO_4^-$ was transferred to the butanone phase. After allowing the two phases to separate, the lower aqueous phase was discarded and the organic phase collected into a 25 ml screw top glass vial.

The open vial was then placed on a hot plate at 100°C and evaporated to dryness, assisted by a stream of warm air, leaving the $^{99m}TcO_4^-$ dried onto the base of the vial. After cooling, a mixture of surfactant (oleic acid) and trichlorofluoromethane (propellant 11) was added to the vial together with micronised salbutamol. The lid was firmly screwed onto the glass vial which was then placed in an ultrasonic bath for 20 min. During this process the TcO_4^- was adsorbed on to the salbutamol. Further propellant 11 was added to ensure that the correct weight of suspension was achieved.

The suspension was then transferred to a 250 ml separating funnel before being carefully weighed into empty MDI cans. Liquid dichlorodifluoromethane (propellant 12) was dispensed from a vacuum flask into the canisters. Finally a metering valve was crimped on to each canister, which was then placed in the ultrasonic bath for 5 min before use.

Labelling of salbutamol as a dry powder

For use as a dry powder, salbutamol was in the sulphate form, and for this delivery method surfactant was not required. The salbutamol sulphate was mixed with propellant 11 in a glass vial containing dried $^{99m}TcO_4^-$ prepared as above. This was placed in the ultrasonic bath for 20 min as before. The propellant 11 was evaporated to dryness by passing warm air over the vial. The dried labelled salbutamol sulphate was then recovered from the vial and a carefully weighed amount was blended with lactose carrier and then dispensed into unit doses, each containing 200 μg of salbutamol.

In vitro validation

Initially, the association of radionuclide and salbutamol was evaluated by taking a sample of the radiolabelled micronised salbutamol in suspension, and placing it into two test-tubes. After centrifuging at 2000 rpm for 20 min, the salbutamol visibly creamed to the surface leaving the clear propellant in the body of the test tube. Each test tube was then imaged close to the collimator of a gamma camera (Siemens Rota camera). Three regions of interest were drawn over the acquired image of the test-tube dividing it into top, middle and bottom.

In a separate experiment no salbutamol was added to the preparation, but every other step in the process was the same as before. The propel-

lant 11/oleic acid solution was transferred to two test tubes and centrifuged again at 2000 rpm. for 20 min. They were then imaged and the activity measured.

Aerodynamic particle size distribution of radiolabelle salbutamol

Metered dose inhalers

To evaluate how closely the radionuclide and the salbutamol particles follow each other within a range of aerodynamic particle sizes, the aerosols generated by 30 actuations from each of four MDIs, containing labelled salbutamol, were sampled into an Andersen MkII cascade impactor. The Andersen cascade impactor is an eight stage instrument modelled to the particle collecting characteristics of the human respiratory tract. Both solid and liquid airborne particles are classified according to their aerodynamic particle size by the amount of radioactivity and salbutamol measured at each of the calibrated stages. Air was drawn through the device at a standardised flow rate of 28.3 l/min measured at the inlet to the impactor. In addition to the radiolabelled inhalers, the aerosol generated by 30 actuations from each of four MDIs containing unlabelled salbutamol were sampled into the Andersen impactor.

After dismantling the instrument, the throat, actuator, filter and the eight stages were individually washed with methanol to dissolve and remove the salbutamol, which was collected into separate samples. The number of radioactive counts in each sample was measured with the gamma camera. This process was performed for each of the four canisters containing labelled salbutamol.

The amount of salbutamol in each set of samples from each of the eight MDIs was determined using ultraviolet spectrophotometry at a wavelength of 246 nm. For the radioactive samples, measurement was performed several days later to allow the radioactivity to decay, and to eliminate any potential hazard during handling of the samples.

Dry powder inhalers

The radiolabelled contents of unit doses from three DPIs were drawn into the Andersen cascade impactor at a flow rate of 60 l/min measured at the inlet of the impactor. The configuration was different to that used for the MDIs and included a pre-separator stage for collecting the large particles. The particle size cut-off for each stage of the impactor was recalculated using Eqn 1 (Hinds 1982), rearranged to give Eqn 2. It is assumed that the Stokes number for each stage of the impactor is constant for the two different flow rates.

$$\mathrm{Stk} = \frac{\rho_p d_\mathrm{p}^2 U C_\mathrm{c}}{9\mu D_\mathrm{j}} \tag{1}$$

where Stk represents the Stokes number, ρ_p the particle density, d_p the particle diameter, U the jet velocity, C_c the slip correction factor, μ the viscosity of air an D_j the jet diameter.

$$d_2 = \sqrt{\frac{d_1^2 U_1}{U_2}} \tag{2}$$

where d_1 is the cut off diameter at the original velocity U_1 an d_2 denotes the corresponding value for the stage at velocity U_2.

The radionuclide content of each stage was measured with the gamma camera in a similar fashion to the MDI samples, and the drug content was measured using high-pressure liquid chromatography (HPLC). The contents of three inhalers containing unlabelled salbutamol were also sampled by the impactor and the drug deposited at each stage similarly measured.

Quality control of MDI and DPI

A Twin Impinger apparatus (Apparatus A, Appendix XVII C, p. 204; British Pharmacopoeia, 1988) in which the proportion of small droplets/particles with an aerodynamic diameter of 6.4 μm has a 50% probability of progressing and depositing in the second stage, has been used throughout the studies to ensure that the technetium labelled inhalers prepared on each study day were consistent in their performance.

Administration of salbutamol to normal volunteers

To evaluate the effectiveness of the labelling method, six normal volunteers were studied on 2

separate days at least 1 week apart. Each subject gave informed written consent and the study was approved by the local ethics committee. The subjects were considered normal if their FEV_1 and FVC were $>80\%$ predicted for their age, sex and height, and the values did not increase by more than 10% after receiving 200 μg of salbutamol from a commercial MDI.

Each subject inhaled from the two different types of inhaler and in addition underwent a ^{81m}Kr scan to provide a clear outline of their lungs. When using the MDI, subjects inhaled 200 μg of salbutamol containing 6–18 Mbq as two actuations, each containing 100 μg. For the DPI study, 200 μg of salbutamol was inhaled from a single unit dose containing 6–18 Mbq of radioactivity.

Each subject was given simple instructions on how to use the inhaler, and allowed some practice runs with placebo inhalers. For the MDI, subjects inhaled steadily and deeply from residual volume actuating the device soon after starting the manoeuvre, followed by a 10 s breath hold at the end of inspiration before gently exhaling into a collecting bag. For the DPI, subjects inhaled from residual volume as quickly and as deeply as

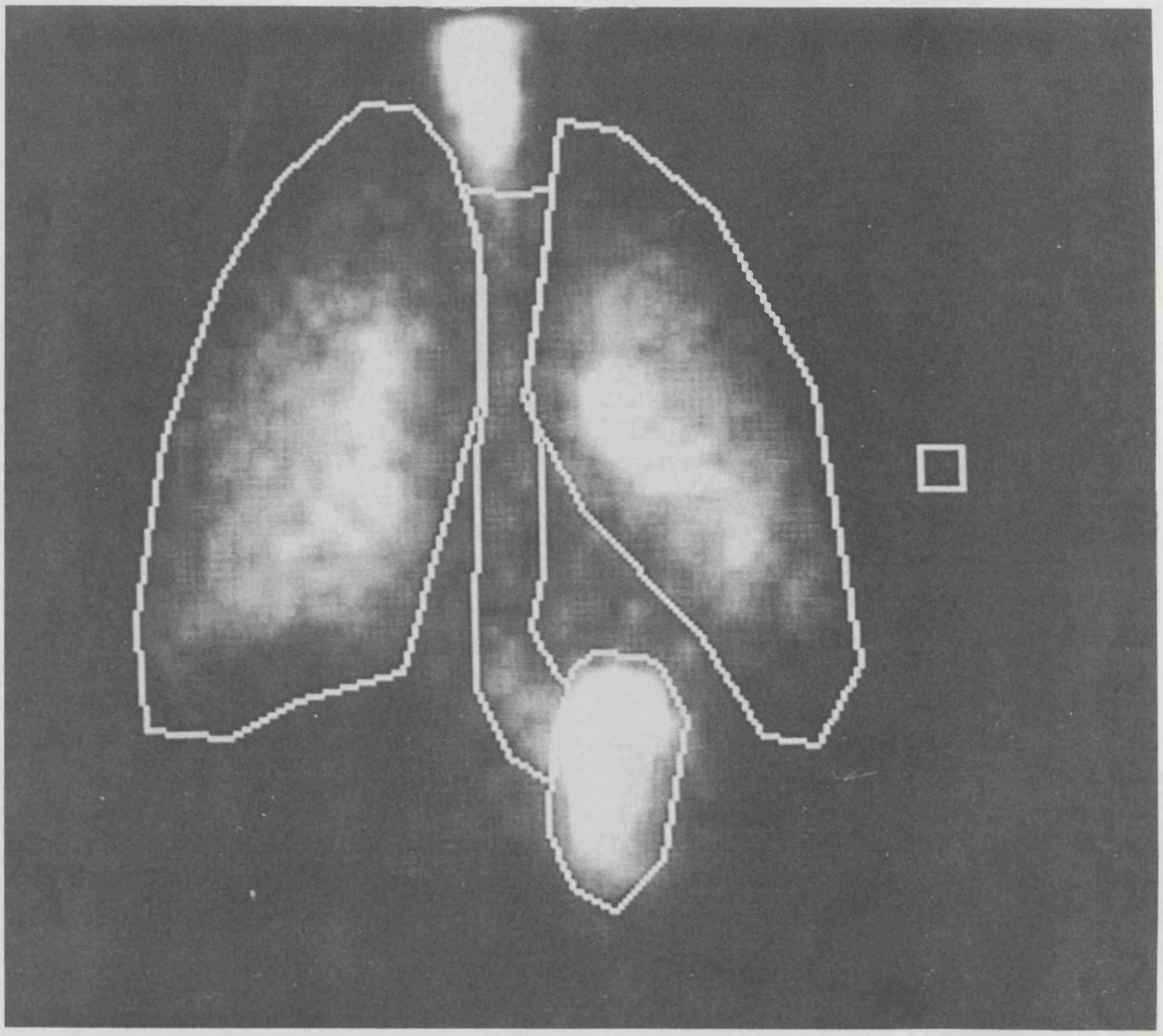

Fig. 1. Gamma camera view showing a typical deposition pattern of the anterior chest after inhalation by a normal subject from a dry powder inhaler. Regions of interest have been drawn around the lungs, mediastinum and stomach.

possible followed by a 10 s breath hold before gently exhaling into a collecting bag, and then repeated the manoeuvre.

Image collection by the gamma camera commenced within 2 min of administration and continued for 5 min.

Imaging procedures

All radionuclide images were acquired using a Siemens Dual headed Rota Camera on line to a DPS-3300 Nuclear Medicine Computer System (ADAC Laboratories). This system is able to acquire simultaneous anterior and posterior views. Each subject was seated between the heads of the camera with the mid point of their chest at the centre of the field of view.

Data collection was split into five 60-s time frames so that movement of the label could be followed as well as measuring the initial deposition. Then, the subject was repositioned and the throat and stomach imaged separately for 120 s each.

All data analysis was carried out with the DPS-3300 computer system. Regions of interest (ROI) were drawn on the lung image using a light pen (Fig. 1), the krypton image acting as a template to provide the lung outline. The anterior and posterior nett counts obtained for each region were multiplied together and their square root taken to give the geometric mean (GM) counts. This was then corrected for radioactive decay and attenuation within the body. The potential activity delivered from two actuations of each MDI used was calculated by actuating the MDI five times into a bag and measuring the count rate. This result was then divided by a factor of 2.5 to give the activity for two actuations. For the DPI, the activity was measured by counting the single dry powder unit dose before administration.

Attenuation correction

Gamma-ray photons emitted by the ^{99m}Tc located within the body are attenuated by body tissues before reaching the gamma camera detectors. This means that the counts detected are less than would be observed if the source were in air alone. A method to correct for this attenuation using gamma-ray transmission, and based on the principles outlined by Tothill and Galt (1971) for quantitative profile scanning, has been adopted.

A source of known radioactivity was sandwiched between different thicknesses of tissue equivalent perspex and between the heads of the camera. The geometric mean (GM) of the counts obtained is not dependent on the depth of the

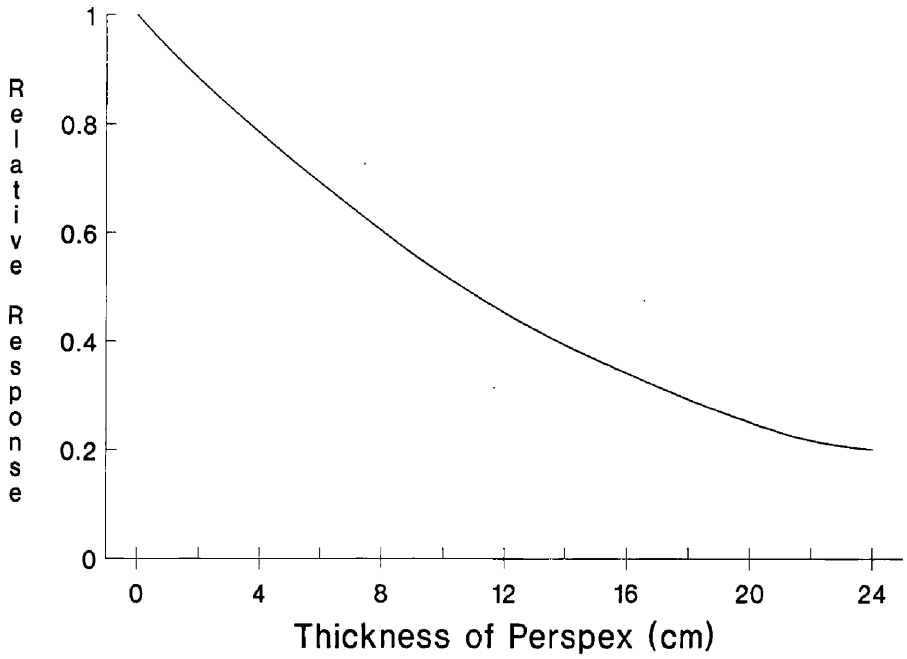

Fig. 2. The geometric mean response from the two heads of the gamma camera to a ^{99m}Tc point source between layers of perspex, expressed relative to the same source in air.

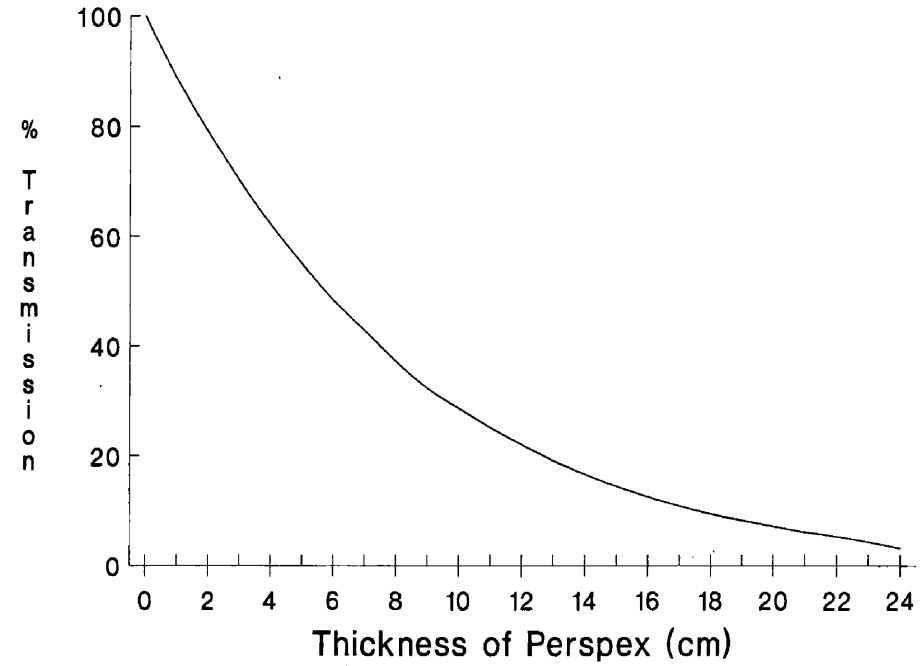

Fig. 3. Transmission of ^{99m}Tc gamma-rays through layers of perspex.

source within the perspex but only on the thickness of perspex that lies between the two heads (Tothill and Galt, 1971). The GM counts for several thicknesses of perspex were obtained and then divided by the GM counts gathered when the source was in air alone to give relative response factors for each thickness of perspex. The relative response factors were then plotted as a function of perspex thickness (Fig. 2).

For ^{99m}Tc deposited in the abdomen or pelvis these factors are very similar to the actual attenuation of the tissue overlying the source and can be directly applied to correct for the attenuation by measuring the patient with callipers. However,

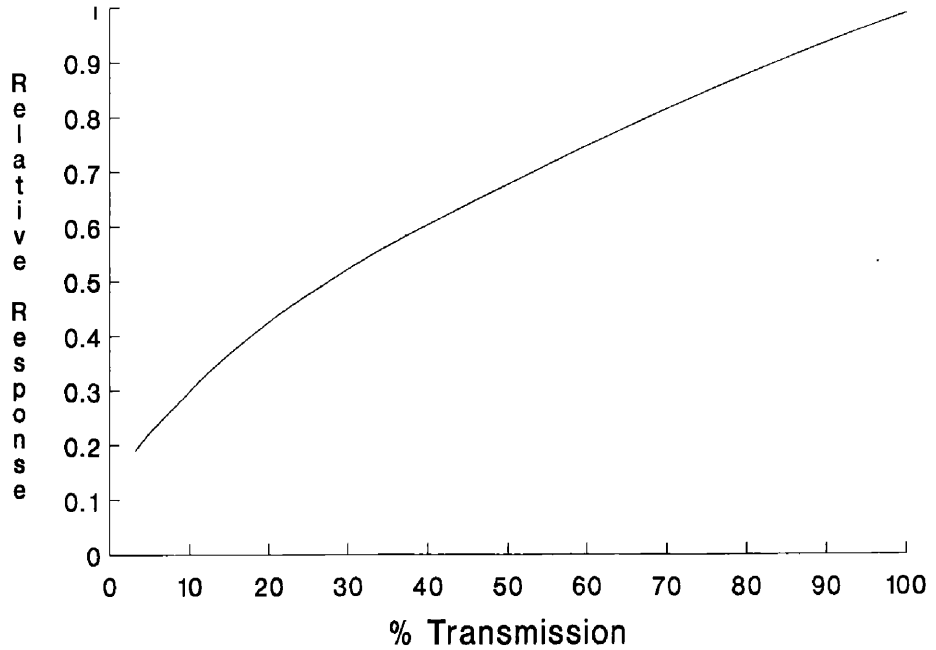

Fig. 4. Relative response to a ^{99m}Tc point source between layers of perspex and in air, plotted as a function of percentage gamma-ray transmission through perspex.

the thorax has a much lower and less uniform density and these factors over-estimate the amount of ^{99m}Tc within the lungs. Therefore, an effective tissue thickness for the throat and thorax must be obtained.

To do this the percentage of gamma-ray photons transmitted through known thicknesses of perspex was determined. Different thicknesses of perspex were placed between a flood source of ^{99m}Tc and one of the heads of the Rota camera. The percentage transmission for each thickness of perspex was obtained by comparing the counts acquired for a given thickness with those obtained when no perspex was present. The percentage transmission was plotted as a function of perspex thickness (Fig. 3). The two plots were then combined to obtain a third plot of relative response as a function of percentage transmission (Fig. 4).

A transmission image of the chest, throat and abdomen of each subject was obtained using the 99m-technetium flood source, with the patient seated between the source and one of the imaging heads. Regions of interest were drawn over the lungs, throat and stomach, and counted. The image was compared to the 'in air image' of the flood source alone to derive the percentage transmission for each region. This was then used to obtain the modified relative response factors to correct for attenuation in each separate region. The attenuation correction factors (inverse of the relative response factors) for the lung of each individual tested is summarised in Table 1.

TABLE 1

Attenuation factors (inverse of relative response factors) used to correct for the attenuation in body tissues in the thorax of the six subjects studied

Subject	Attenuation factor
1	1.90
2	2.03
3	1.66
4	1.88
5	1.92
6	2.10

In order to assess the accuracy of this method, a phantom (Alderson Research Labs Inc.) of the upper torso was used. Water was placed in the chest/head compartment and a transmission scan was obtained. Small amounts of activity comparable with that expected in the lungs during a study were counted first in air and then in the lung compartment of the phantom. Relative response factors were derived as explained above. A mean

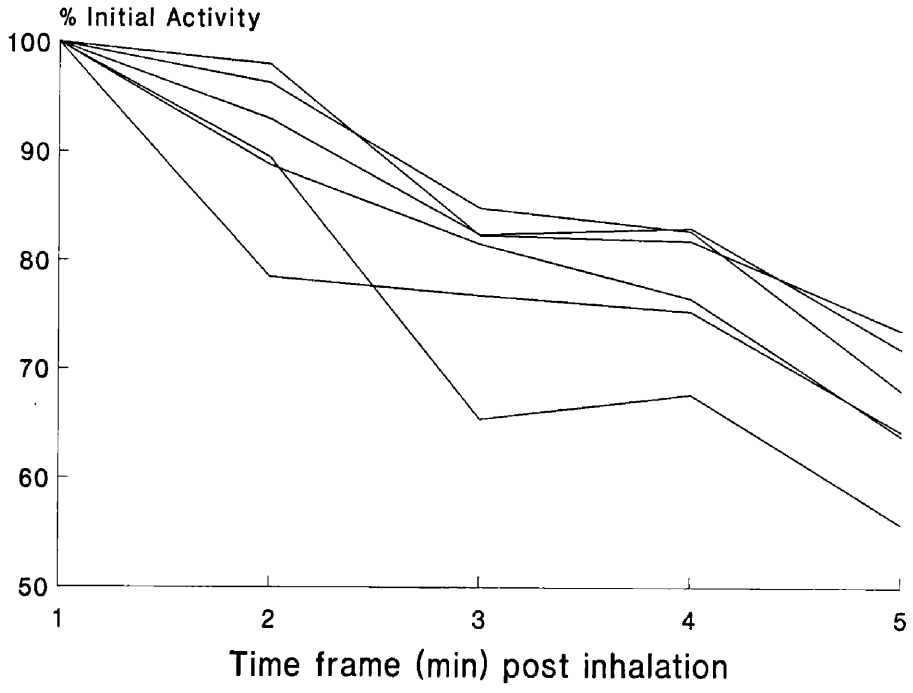

Fig. 5. The dissolution of ^{99m}Tc from the lungs of six normal subjects after inhalation from an MDI.

discrepancy between the in air counts and the corrected phantom counts of 3% (range −1.3% to 10%) was obtained.

Dissolution of radiolabel

The lung counts in each successive one minute time frame after inhalation progressively decreased. There was a substantial decrease during the 5 min of counting (i.e., 2–7 min after inhalation) of the radio-aerosol. Some subjects were imaged over a longer period in order to follow this further. The half-life of clearance of lung radioactivity was about 10 min. This was compatible with that observed when $Na^{99m}TcO_4^-$ alone is absorbed from the lungs (Rinderknecht et al., 1980), and suggests that the pertechnetate dissolves in the lung mucosa after deposition, with subsequent systemic absorption and distribution in the blood. Fig. 5 shows the dissolution effect of the ^{99m}Tc from the lungs in the six normal subjects.

Results

Distribution of activity in labelled drug suspension

Fig. 6a shows the gamma camera image of the radiolabelled salbutamol suspension after centrifugation. Over 90% of counts were in the top region of interest. Visual inspection of the test tube showed that most of the salbutamol was in a layer on the surface of the propellant. Repeating the experiment for six further preparations of suspension gave a mean (±SE) of 85 (3.2)% of the radioactivity found in the top layer of the test tubes.

When the same process was performed without salbutamol, the distribution of the radioactivity was quite different (Fig. 6b), with most of the activity in the bottom of the test tube. In addition, only 12% of the $^{99m}TcO_4^-$ that was available in the glass vial during the preparation was transferred to the propellant/surfactant alone, which was in contrast to more than 80% that was transferred when salbutamol was present. This suggests that the radionuclide binds mainly to salbutamol, and not to propellant nor surfactant.

Results from Andersen impactor

Figs. 7a and b shows the relative percentage of radioactive counts and salbutamol content of each of the stages of the Andersen impactor expressed as a percentage of the total for all the stages, for the MDI and DPI respectively. The actuator and throat counts were not included. These results show a close similarity between the radioactivity and the drug dose for each stage, and indicate that the two are closely associated over a wide range of particle sizes. The close association between the unlabelled and labelled drug indicated that the particle size distribution had not been significantly altered by the addition of the ra-

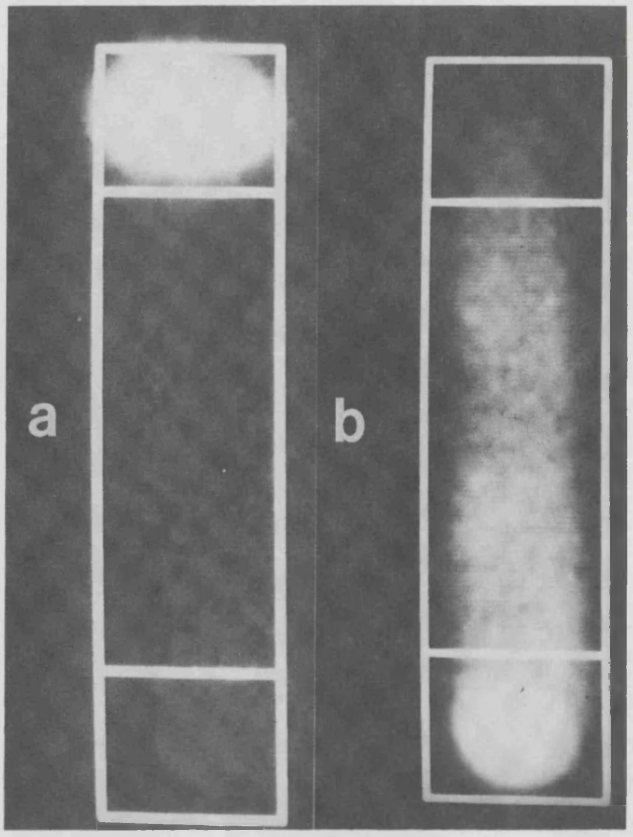

Fig. 6. (a) Gamma camera image showing a sample of salbutamol labelled with ^{99m}Tc suspended in propellant 11 and oleic acid in a test tube. Regions of interest have been drawn to obtain counts in the top, middle and bottom portions. The test tube has been centrifuged at 2000 rpm. for 20 min. The majority of salbutamol has visibly creamed to the surface of the suspension. Over 90% of counts are seen in the top region of the test tube. (b) Gamma camera image showing a test tube containing propellant 11 and oleic acid and ^{99m}Tc. Unlike panel (a), no salbutamol has been added. Every other condition is the same. Few counts are recorded in the top region although there is higher activity at the bottom.

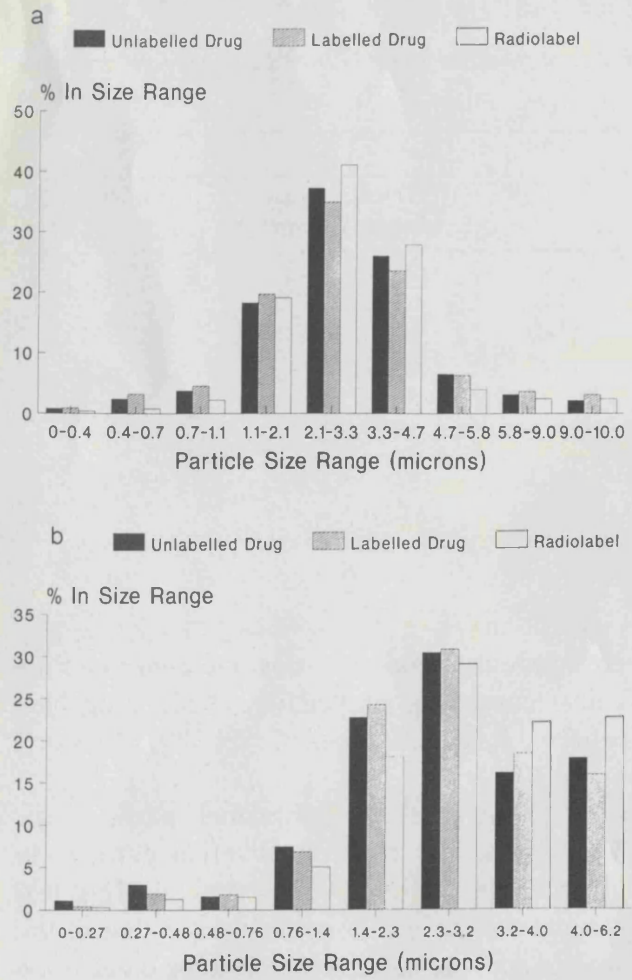

Fig. 7. (a) Mean distribution of radiolabel and salbutamol for four MDIs actuated into an Anderson MkII cascade impactor (operated at 28.3 l/min), shown as a percentage of the total recovery for eight stages plus filter. Also shown is the mean percentage drug distribution for four MDIs containing unlabelled salbutamol. (b) Mean distribution of radiolabel and salbutamol from the contents of three DPIs sampled by an Anderson MkII cascade impactor (operated at 60 l/min), shown as a percentage of the total recovery for seven stages plus filter. Also shown is the mean percentage drug distribution for three DPIs containing unlabelled salbutamol.

dionuclide. For the MDIs the mass median aerodynamic diameter (MMAD) for the unlabelled and labelled drug was 2.9 (GSD 1.5) and 2.8 (1.6) μm, respectively, while the MMAD for the radionuclide was 2.8 (1.5) μm. For the DPIs, the MMAD for the unlabelled drug was 2.7 (1.6) μm, whilst the MMADs for the labelled drug and radionuclide were 2.7 (1.5) and 3.0 (1.5) μm, respectively.

Lung deposition in normal volunteers

The results obtained from the six volunteers studied are listed in Table 2 and an example of a lung image is shown in Fig. 1. Mean ($\pm$SD) deposition of radionuclide in the lungs using the MDI was 24.1 (8.5) and 11.3 (2.2)% for the DPI, and the difference between the two delivery devices was statistically significant (paired t-test, $P < 0.05$). The small difference between the deposition in the throat, stomach and mediastinum for the two techniques was not significant. After using the MDI, the mean dose of radioactivity not accounted for was 14.1% of the total administered, and 17.5% for the DPI.

Discussion

This study has modified an original method of directly labelling β_2-agonists (Köhler et al., 1988), in which commercial MDIs were used. After cooling to -60°C, they pierced the canisters and added further propellant and surface active agent, previously labelled with radionuclide. The radionuclide was reported to transfer to the bronchodilator drug within the canister.

In our present study, the MDI is prepared from its basic constituents so as to produce a canister as close as possible in configuration and performance to the commercially available product. Also, the method of labelling employed for the MDI has been developed into a novel technique of labelling salbutamol sulphate for use in dry powder inhalers.

The validation tests show a close association between the properties of the radionuclide and the drug. The percentages of radionuclide and drug on each stage of the Andersen impactor were similar, although for the DPI the particle size distribution of the radionuclide was slightly coarser than that observed from the drug. Particles of the size reported are suitable for inhalation to all parts of the lungs (Morrow, 1974; Newman and Clarke, 1983).

The absorption of the radiolabel from the lungs into the systemic circulation limited the counting period for the lungs to 5 min; 7 min after inhala-

TABLE 2

Percentage deposition of radiolabelled aerosol following inhalation by six normal volunteers from a metered dose inhaler and dry powder inhaler; less than 1% was exhaled in each study

Subject	MDI			DPI		
	Lungs	Throat/media-stinum/stomach	Actuator	Lungs	Throat/media-stinum/stomach	Device
1	21.4	37.1	27.7	8.5	67.0	7.3
2	17.0	45.2	14.3	10.8	62.0	9.0
3	15.0	31.6	17.7	14.0	52.6	11.7
4	31.6	48.2	13.5	9.7	63.7	13.4
5	37.0	46.6	15.0	10.8	52.5	28.0
6	22.6	58.1	15.4	13.8	48.0	12.1
Mean	24.1	44.5	17.3	11.3	57.6	13.6
S.D.	8.5	9.2	5.3	2.2	7.6	7.4

tion. This phenomenon was also noted by Köhler et al. (1988). The rates of clearance from the lungs of the six subjects were similar (Fig. 5). Whilst counting over the lungs was for 5 min, only the first 3 min of data were used in assessing deposition. It was not possible to reduce count time further because the low count rate within the lungs gave inadequate count statistics.

The percentage total deposition in the lungs in the normal subjects in this study is higher than those previously reported both in this laboratory using inhalers containing radiolabelled teflon spheres and by other workers. A mean of 11.2% was deposited in the lung from a reconstituted MDI and a mean of 9.1% deposited from a dry powder inhaler device in patients with airflow obstruction (Zainudin et al., 1990). The values presented here are also higher than those obtained by groups using either a labelled substitute (Newman et al., 1981) or with directly labelled sodium cromoglycate (Newman et al., 1989). However, Matthys has recorded a mean of 26% lung deposition with directly labelled salbutamol in an MDI in normal subjects (Matthys et al., 1988). They also measured a lung deposition of 18.7% when inhaling from residual volume and 33% when inhaling from 50% of vital capacity (Köhler et al., 1988). Also, Newman et al. (1991), using directly labelled salbutamol, measured a mean total deposition of 22.8% in the lungs of asthmatic patients using their inhalers in the correct fashion. Workers using metered dose inhalers containing propellant soluble drug have reported lung depositions of over 39% (Ashworth et al., 1991).

The finely milled salbutamol particles are smaller and less dense than teflon particles or other drugs that have been studied. This may account for the increased lung deposition of this study compared to those reported by other workers.

The portion of the activity released from the DPI and MDI that is unaccounted for may be partially explained by the dissolution of the activity within the lungs, with a substantial portion being redistributed systemically and therefore not counted. This may imply an even higher lung deposition than has been reported. Very little is expired by the subjects after breath holding indicating that most of the particles inhaled are deposited during this manoeuvre.

The advantages of the current method are that the production of the labelled drug is relatively easy to perform and requires no special equipment. It is able to give an accurate indication of the distribution of the drug and, apart from the technetium, does not require substantial modification of inhaler formulation. Pulmonary function can be studied simultaneously enabling it to provide a simple and effective means of observing the effects of a known distribution of drug on patients suffering from respiratory disease.

References

Ashworth, H.L., Wilson, C.G., Sims, E.E., Wotton, P.K. and Hardy, J.G., Delivery of propellant soluble drug from a metered dose inhaler. *Thorax*, 46 (1991) 245–247.

Hinds, W.C., Acceleration and curvilinear particle motion. In *Aerosol Technology*, Chap. 5, Wiley, New York, 1982.

Köhler, D., Fleischer, W. and Matthys, H., New method for easy labelling of β_2-agonists in the metered dose inhaler with technetium-99m. *Respiration*, 53 (1988) 65–73.

Matthys, H., Eltschka, R. and App, E.M., Deposition of a labelle β_2-agonist aerosol. *Atemw, lungenkrkh.*, 14 (1988) 485–488.

Morrow, P.E., Aerosol characterization and deposition. *Am. Rev. Respir. Dis.*, 110 (Suppl.) (1974) 88–99.

Newman, S.P., Clark, A.R., Talaee, N. and Clarke, S.W., Pressurise aerosol deposition in the human lung with and without an 'open' spacer device. *Thorax*, 44 (1989) 706–710.

Newman, S.P. and Clarke, S.W., Therapeutic aerosols. 1: Physical and practical considerations. *Thorax*, 38 (1983) 881–886.

Newman, S.P., Pavia, D., Morén, F., Sheahan, N.F. and Clarke, S.W., Deposition of pressurised aerosols in the human respiratory tract. *Thorax*, 36 (1981) 52–55.

Newman, S.P., Weisz, A.W.B., Talaee, N. and Clarke, S.W., Improvement of drug delivery with a breath actuated pressurised aerosol for patients with poor inhaler technique. *Thorax*, 46 (1991) 712–716.

Rinderknecht, J., Shapiro, L., Krauthammer, M., Taplin, G., Wasserman, K., Uszler, J.M. and Efros, R.M., Accelerated clearance of small solutes from the lungs in interstitial lung disease. *Am. Rev. Respir. Dis.*, 121 (1980) 105–117.

Short, M.D., Singh, C.A., Few, J.D., Studdy, P.R., Heaf, P.J.D. and Spiro, S.G., The labelling and monitoring of lung deposition of an inhaled synthetic anticholinergic bronchodilating agent. *Chest*, 80 (Suppl.) (1981) 918S–921S.

Tothill, P. and Galt, J.M., Quantitative profile scanning for the measurement of organ radioactivity. *Phys. Med. Biol.*, 16 (1971) 625–634.

Zainudin, B.M.Z., Biddiscombe, M., Tolfree, S.E.J., Short, M. and Spiro, S.G., Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurised metered dose inhaler, as a dry powder and as a nebulised solution. *Thorax*, 45 (1990) 469–473.

Zainudin, B.M.Z., Tolfree, S.E.J., Biddiscombe, M., Whitaker, M., Short, M.D. and Spiro, S.G., An alternative to direct labelling of pressurised bronchodilator aerosol. *Int. J. Pharm.*, 51 (1989) 67–71.