CHAPTER ONE

REVIEW

CHAPTER 1 - REVIEW

1.1 INTRODUCTION

The term 'aerosol' may be defined in two ways, namely a gaseous dispersion of particles or droplets or a 'pressurised pack'. The former definition is used throughout this thesis. 'Pressurised pack' is redefined as 'metered-dose inhaler' (MDI) which is a specific term used in inhalation therapy.

Inhalation aerosols have been used to treat respiratory diseases since conditions such as bronchial asthma were first recognised more than 2000 years ago. The clinical aerosols in common use today include mucolytics, bronchodilators, and prophylactic agents such as anti-inflammatory steroids and disodium cromoglycate. The present study is concerned with the bronchodilator drug, salbutamol. The historical development and mechanisms of action of modern bronchodilator therapy have been comprehensively reviewed (Paterson et al., 1979; Koch-Weser & Webb-Johnson, 1977a and 1977b; Austen & Lichtenstein, 1973). Efficacious β_2-stimulant bronchodilator drugs such as salbutamol, terbutaline and fenoterol when administered by inhalation, have the advantages of selectivity of action, rapid onset and low incidence of side effects (Ruffin et al., 1978d; Freedman, 1972; Williams & Kane, 1975; Pennock & Rogers, 1977). The importance of the route of administration in the kinetics and metabolism of anti-asthmatic drugs has been reviewed by Davies (1982). Only a small proportion of the aerosol dose discharged from most clinical aerosol delivery devices, when deposited in the lungs, is necessary to achieve the pharmacological effect (Davies, 1975; Newman et al., 1981a).

The site of action within the respiratory airways has not been well established, although Zimmerman & Ulmer (1980) and Godfrey et al. (1974) have suggested the 'lower airways' as the site of action for fenoterol and sodium cromoglycate, respectively.

The small proportion of aerosol delivered to the finer airways even under optimum inhalation conditions, demonstrates the efficiency of the human respiratory tract in preventing penetration of particles to this region. The respiratory system operates analogously to a series of filters which remove particles or droplets from the inhaled air according to their diameter, with the smaller particles penetrating further into the airways. Inhalation therapy is therefore involved in the optimum delivery of aerosol particles to a complex structure which is designed to minimise entry of such particles.

The particle size range of importance for therapeutic inhalation purposes is 10^{-2} to 10^{2}µm. (Morrow, 1981). Below or above these sizes, aerosols are normally much too unstable to remain airborne or their potential interaction with mammalian respiratory systems is negligible. Definitions of 'respirable' and 'inhalable' aerosols include only particles of < 15µm in size. (Raabe, 1982). These terms were established by the environmental health authorities in Britain and USA for the evaluation of the health risks of people exposed to dusts. Many of the basic theories and techniques used in inhalation research have been developed by toxicologists and health physicists concerned with the relation between the characteristics of airborne contaminants and their potential hazards after inhalation (Lippmann & Schlesinger, 1981; Andersen et al., 1979; Wolff & Dolovich, 1977; Black & Evans, 1974).

The mechanisms which govern aerosol deposition are common to all mammalian respiratory systems, and the understanding of these mechanisms is helped by a brief description of the morphology of the human airways.

The respiratory system forms the largest and most intimate contact between internal body tissues and the external environment.

An adult inhales daily about $10m^3$ of air which is distributed among several hundred million alveoli with a total surface area of about $30m^2$. (Hatch & Gross, 1964). Fig. 1.1 shows the basic structure of the human respiratory system which may be divided into the nasopharyngeal, tracheo-bronchial and pulmonary regions. The nasopharyngeal structure of ciliated nasal passages warms and moistens the inhaled air. Particles are deposited from the airstream by impaction on the mucus lining of the nasopharynx and subsequently removed by swallowing or sneezing. The nasal inhalation route will not be discussed in detail, since it was not used in these studies.

The tracheobronchial airways have the appearance of an inverted tree, with each generation of subdividing bronchi having a decreasing diameter. However the number of airways and total cross section increases in each generation so that the air velocity decreases distally. Inert, insoluble particles deposited on ciliated airways (ie. all generations from trachea to terminal bronchioles) are carried towards the larynx on the moving mucus blanket and then swallowed.

The pulmonary zone beyond the terminal bronchioles is the region where gas exchange takes place. The very thin epithelial wall of the respiratory bronchioles and alveoli allows rapid and efficient gaseous exchange with the pulmonary blood capillaries.

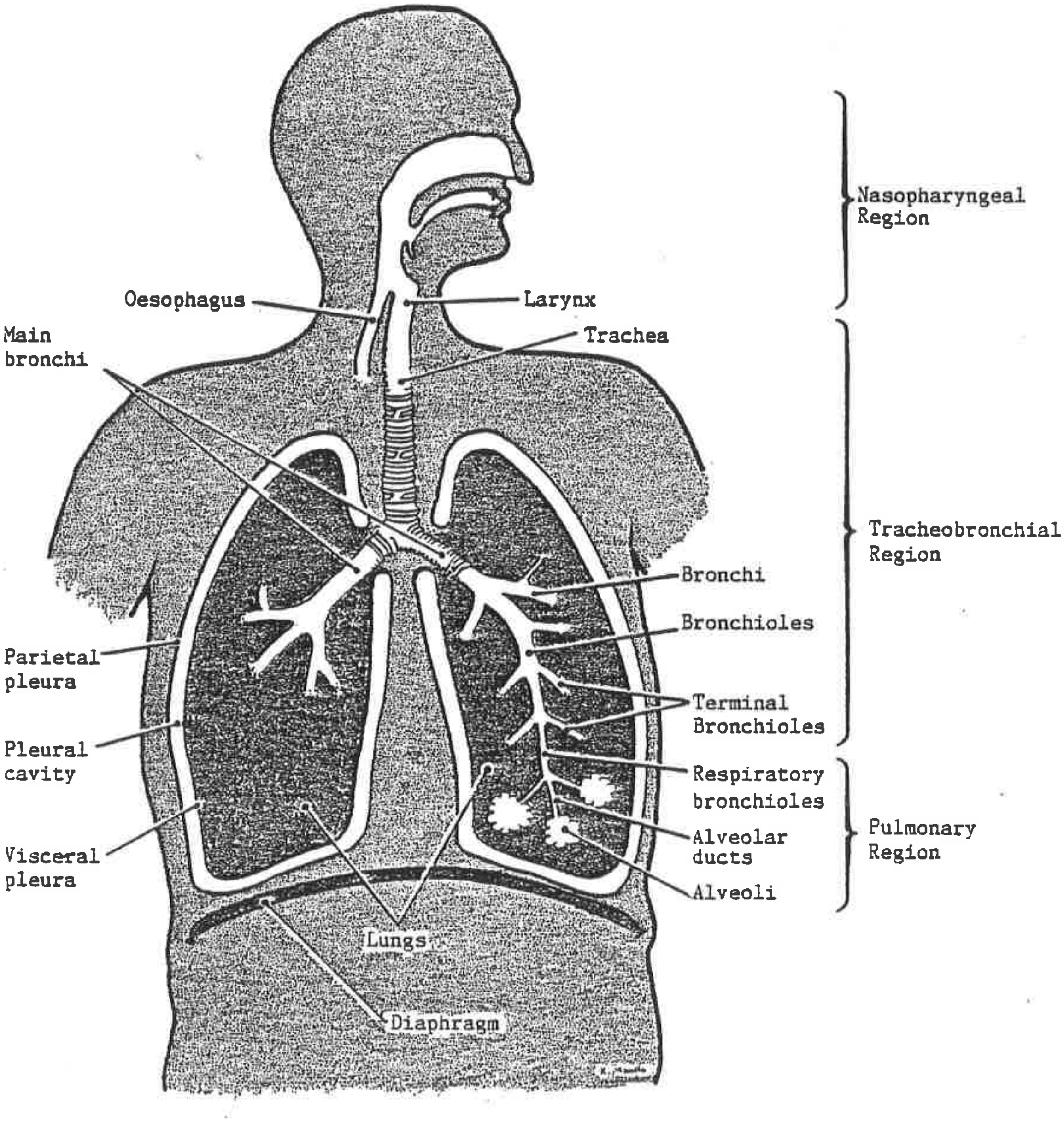
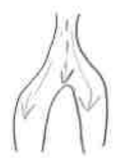


Fig 1.1 Structure of the human respiratory tract.

In disease states such as bronchitis and emphysema the epithelium thickens which decreases gas exchange. The same effect results from increased intra-alveolar fluid in pulmonary oedema and pneumonia. Other diseases, such as pneumoconiosis, which are caused by high levels of occupational dusts depositing in the lower regions of the lung, prompted research work on aerosol deposition mechanisms (Brain & Valberg, 1979).

The most important mechanisms of particle deposition are impaction, sedimentation and diffusion. Electrostatic precipitation and interception are also important mechanisms for highly charged, or fibrous particles, respectively (Lippmann, 1977). Impaction occurs when the direction of airflow in the branching vessels changes and particles of sufficient inertia in the airstream have a trajectory in a different direction from the airflow. The impaction mechanism is particularly important in the larger airways since the velocity of air is greater and impaction may be enhanced by turbulent patterns of airflow. Deposition by impaction is more likely for particles travelling along the centre of the airway when a bifurcation is reached. Schlesinger & Lippmann, (1972) have shown preferential deposition at bifurcations for particle sizes ranging from 1.1 to 7.7µm. Sedimentation under gravity becomes an important mechanism for deposition in the smaller bronchi, the bronchioles and the alveolar spaces where the airways are small and the air velocity is low. Particles below 0.5µm in size have terminal settling velocities which are too low for effective sedimentation. In this case diffusion becomes more important and the Brownian motion experienced by submicron particles becomes an effective mechanism for deposition in small airways and alveoli.

is there any evidence of this?



Is terminal velocity dependent on particle size?

Fig. 1.2 shows the major factors which influence deposition efficiency in the respiratory tract; the most important

Fig 1.2. MAJOR FACTORS AFFECTING DEPOSITION

PARTICLE PROPERTIES :-	DIAMETER.
	DENSITY.
	SHAPE.
	CHARGE.
	CHEMICAL COMPOSITION - SOLUBILITY, HYGROSCOPICITY.
AEROSOL PROPERTIES :-	CONCENTRATION.
	PARTICLE SIZE RANGE.
	BOLUS OR CONTINUOUS CLOUD.
RESPIRATORY TRACT PROPERTIES:-	GEOMETRY (VARIABILITY).
	PRESENCE OF DISEASE.
	HUMIDITY.
BREATHING PATTERNS :-	RESIDENCE TIME (BREATH-HOLDING).
	VOLUMETRIC FLOWRATE (BREATHING RATE, TIDAL VOLUME).
	MOUTH OR NASAL BREATHING.

factor is particle diameter. A useful definition of particle size which is commonly employed in aerosol research is 'aerodynamic diameter'. This is defined as the diameter of a unit density sphere that has the same settling velocity as the particle in question.

x74
2c
explanation required, How is AD determined?

Deposition of aerosol particles of different materials and shapes can be compared when they are characterised by aerodynamic diameter which incorporates both particle density and aerodynamic drag which is dependent on particle shape.

Several reviews have estimated the influence of other deposition factors, but the effect of each variable and the interaction between them is poorly understood (Altshuler, 1969; Stuart, 1973; Morrow, 1974b; Lippmann *et al.*, 1980).

1.2 MODEL SYSTEMS

The complex structure of the respiratory tract makes predictions of aerosol deposition fractions difficult. Nevertheless, various idealised respiratory models have been developed in an attempt to estimate deposition *in vivo* from *in vitro* parameters which are more easily measured such as particle size.

Many deposition studies use 'model' particles which are stable, non-hygroscopic and monodisperse. In practice, it is conventional to define 'monodisperse' for this purpose as a size distribution with a geometric standard deviation (σ_g) less than 1.1. Monodisperse particles allow greater differentiation in deposition pattern with particle size, since by accepted deposition theory, an aerosol with a very narrow distribution of sizes would be more locally deposited in the respiratory tract than an aerosol with a wide distribution of particle diameters. Amongst the many materials used for model particles are ferric oxide, aluminosilicates, albumin, polystyrene and Teflon (Hurford, 1981; Kotrappa & Moss, 1971; Bailey & Strong, 1980). Several reviews of the materials and methods have been published (Philipson, 1981; Spurny & Lodge, 1968; Newton et al., 1980). γ-emitting radiolabels may be incorporated into inert aerosol particles for in vivo studies. Addition of the radiolabel to the initial preparation ensures homogenous particles with minimum leaching of the radioactive tag (Few et al., 1970).

of Vortan?

The most common devices for producing monodisperse aerosols are the spinning disc generator (May, 1949) and the vibrating orifice generator (Berglund & Liu, 1973). Both instruments produce monosized droplets which are dried to form aerosol particles. The required particle size is obtained by controlling the operational parameters.

The complex variations in anatomy, physiology and breathing patterns can be avoided by using in vitro model airway systems, which vary from simplified branching tubes (Yang et al., 1974) to silicone casts made from excised lungs (Horsfield et al., 1971).

The first respiratory models (Landahl, 1963; Beeckmans, 1965; Task Group, 1966) used simplified anatomical systems with symmetrical dichotomous branching defined by the length and diameter of each airway (Weibel, 1963). The Task Group model further limited the calculations to nasal breathing at a fixed breathing rate and tidal volume. (Tidal volume is the volume of air inhaled or exhaled during one breath). Fig. 1.3 shows the Task Group (1966) estimates for regional deposition fractions based on a single aerosol parameter - the mass median aerodynamic diameter. Comparisons with more recent data (Mercer, 1975) indicate that this model adequately describes total deposition during nasal breathing, but provides incorrect estimates of regional deposition for mouth breathing.

does this differ from AD on p.13?

Nasal breathing patterns are more important for environmental aerosol exposure experiments; whereas therapeutic

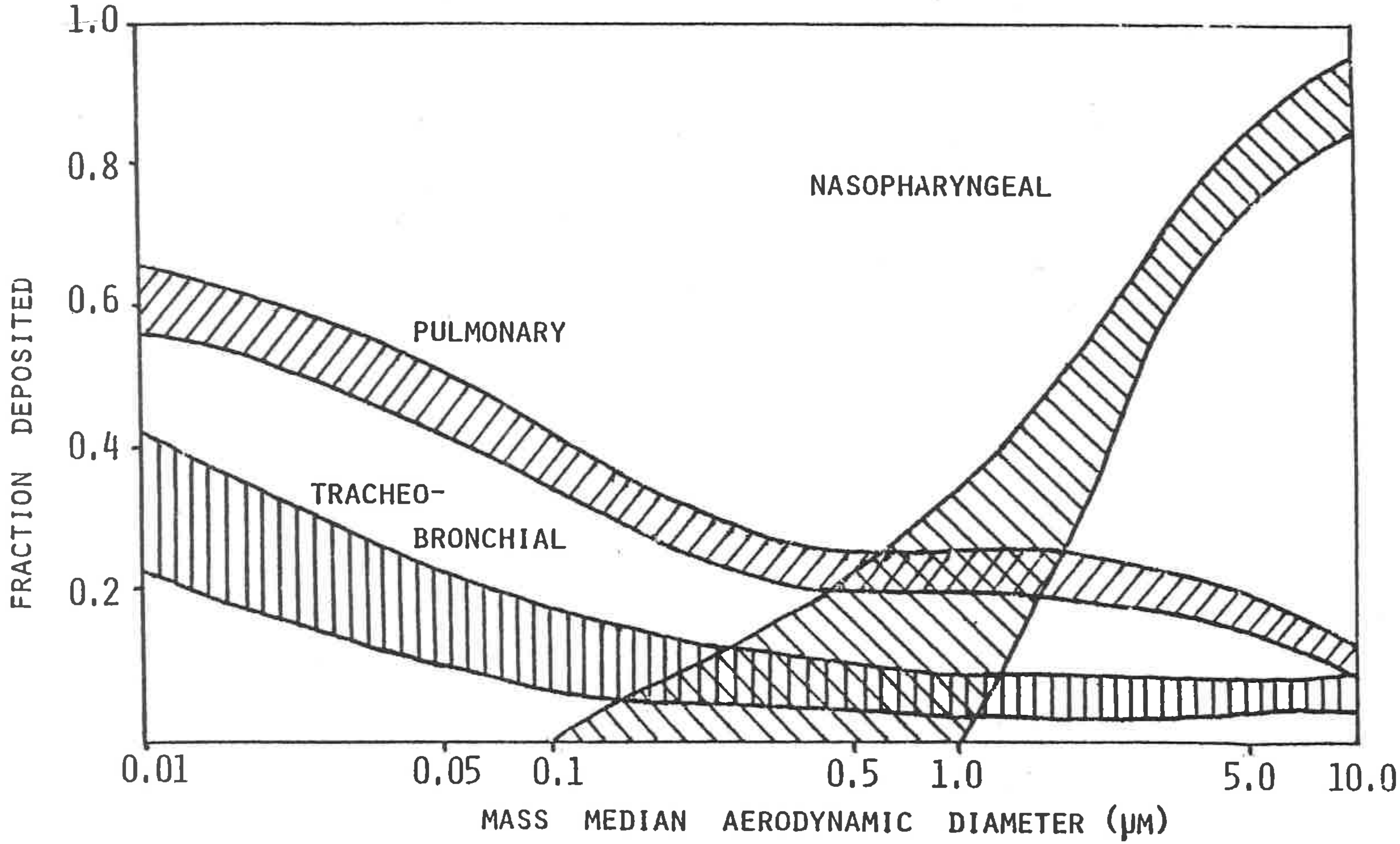


Fig 1.3. Deposition in the respiratory tract for nasal breathing as predicted by the International Committee on Radiation Protection model for 3 compartments. ~~The amount~~ by which different geometric standard deviations affect the curves at a given MMAD is depicted by the width of the curves.

aerosol administration is usually by mouth inhalation. Camner (1981) used the Task Group Model to show that an assumption of 100% nasal breathing underestimates the lung deposition of any particles larger than 2-3µm. Additional studies showed that normal subjects average about 80% nasal breathing at rest and about 40% during conversation (Camner & Bakke, 1980). During exercise the proportion of nasal breathing would be even less, since the high nasal resistance to airflow favours mouth breathing at higher total volumetric flow rates (Hounam & Black, 1971).

Several anatomical models have attempted to improve on the shortcomings of Weibel's data (Weibel, 1963). Horsfield & Cumming (1968) and Shiah (1981) proposed anatomical models with asymmetrical branching systems which included defining the major and minor daughter branches and the branching angles in two and three dimensions, respectively. Hansen & Ampaya (1975) also measured the detailed geometry of a human acinus (ie. a terminal bronchiole and distal airways and air spaces).

Many workers have investigated the pattern of airflow in ? model respiratory systems. The simplest flow pattern in airways consists of a parabolic axisymmetrical velocity profile but this has been shown to be inaccurate. Complex velocity profiles and flow instabilities have been demonstrated in a model system of repeatedly branching tubes (Pedley *et al.*, 1971) and in a replica cast of the human airway system (Olson *et al.*, 1973) during inspirational flow. Schroter & Sudlow (1969) established the patterns of turbulence in a more realistic system using inspiratory and expiratory flows in a human cast. Dekker (1961) and Chan *et al.* (1980) have shown further discrepancies in model systems due to the effect on airflow of the larynx. Chan *et al.* (1980) demonstrated enhanced tracheal deposition for particles greater than 2µm due to turbulence created by the 'laryngeal jet', in a human cast and an idealised airway model of branching tubes. Dekker (1961) calculated critical flow velocities of $100cm^3$ s^{-1} and $350cm^3$ s^{-1} in a human tracheal cast with and without a larynx, respectively.

up to here

Theoretical estimates of total and regional deposition have been evaluated in increasingly complex models by several workers (Taulbee & Yu, 1975; Shiah & Wang, 1980; Goldberg & Smith, 1981; Gerrity et al., 1981). Taulbee & Yu (1975) and Gerrity et al., (1981) applied the deposition probability equations of Landahl (1963) and Beeckmans (1965) to the symmetrical and asymmetrical anatomical models of Weibel (1963) and Horsfield et al. (1971), respectively. The particle sizes studied were 0.05 - 5μm and 4μm, with fixed values for tidal volume and inspiratory flow rate. The mathematical model developed by Shiah & Wang (1980) may be more relevant to therapeutic inhalation systems as it predicts the deposited fractions of inhaled hygroscopic particles in each generation of an airway model. Several reviews of theoretical lung deposition models have been published (Mitchell, 1960; Brain & Valberg, 1974; Morrow, 1974a; Chen, 1981).

The use of complete or partial casts of the human tracheobronchial tree in deposition studies (Schlesinger et al., 1977; Martonen, 1983) has the advantage of utilising a more realistic anatomical structure than either theoretical models or simplified models of branching tubes. The airflow can be controlled precisely so that deposition effects of changes in breathing pattern are estimated. In addition, one-way airflow may be employed so that inspirational and expirational deposition fractions are separated. Repeat studies are possible without the large intersubject scatter in results experienced in *in vivo* studies (Tarroni et al., 1980; Stahlhofen et al., 1981). Estimates of deposition efficiencies may therefore be made using a single parameter (usually particle size) and controlling other variables such as breathing patterns and complex anatomical differences.

The comparison of deposition patterns in respiratory models, lung casts and _in vivo_ studies provides useful data for the understanding of _in vitro/in vivo_ correlations in aerosol behaviour. Several workers have shown good agreement in deposition data from hollow casts and _in vivo_ studies (Schlesinger & Lippman, 1976; Chan & Lippman, 1980). The regional deposition fractions are related to a single particle size parameter such as Stokes number (Chan & Lippmann, 1980) or mass median aerodynamic diameter (MMAD) (Schlesinger & Lippmann, 1976). Chan & Lippmann (1980) introduced a new anatomical parameter, the bronchial deposition size (BDS) which allows classification of individuals and populations according to their tracheobronchial deposition efficiency.

Total and regional deposition in the human respiratory tract has been studied using 'model' aerosol particles such as di-2-ethylhexylsebacate (DES) and ferric oxide. Their monodispersity and nonhygroscopicity means that they may be produced in predetermined sizes which are likely to remain unchanged at the high relative humidities experienced after inhalation. Heyder and coworkers (Heyder _et al._, 1975; Heyder _et al._, 1980a) have used these materials. DES was used to assess total lung deposition for different breathing patterns by measuring the concentrations of inhaled and exhaled particles at the mouthpiece. Ferric oxide particles radiolabelled with gold (^{198}Au) were used to assess regional lung deposition by measuring the thoracic activity remaining after 24 hours. This fraction was assumed to be deposited on non-ciliated airways in the pulmonary region. The results from these studies were also used to estimate pulmonary airspace dimensions (Gebhart _et al._, 1981) and to establish particle transport mechanisms onto airway surfaces in different lung regions (Heyder, 1982).

The number of factors which affect deposition in the respiratory tract provide very complex conditions for realistic estimates. The effects on total deposition of several factors (particle diameter, particle density, period of breathing cycle and respiratory volumetric flow rate) were described by a single deposition parameter (Heyder et al., 1980b). However, this parameter may only be applied to uncharged, spherical, non-hygroscopic aerosol particles. The use of 'model' particles and model respiratory systems provided estimates of effects of electrostatic charge on deposition of particles (Fraser & Hill, 1966; Wasan et al., 1973; Chan et al., 1978). Chan et al. (1978) used ferric oxide particles (MMAD 2-7µm) with 360-1000 negative charges per particle in a hollow cast of the human tracheobronchial tree. Studies of particle shape, particle dissolution and respiratory parameters were simplified similarly by using inert dusts and gases (Van de Vate & Leeuwen, 1980; Mercer, 1967; Scherer et al., 1972; Bake et al., 1974).

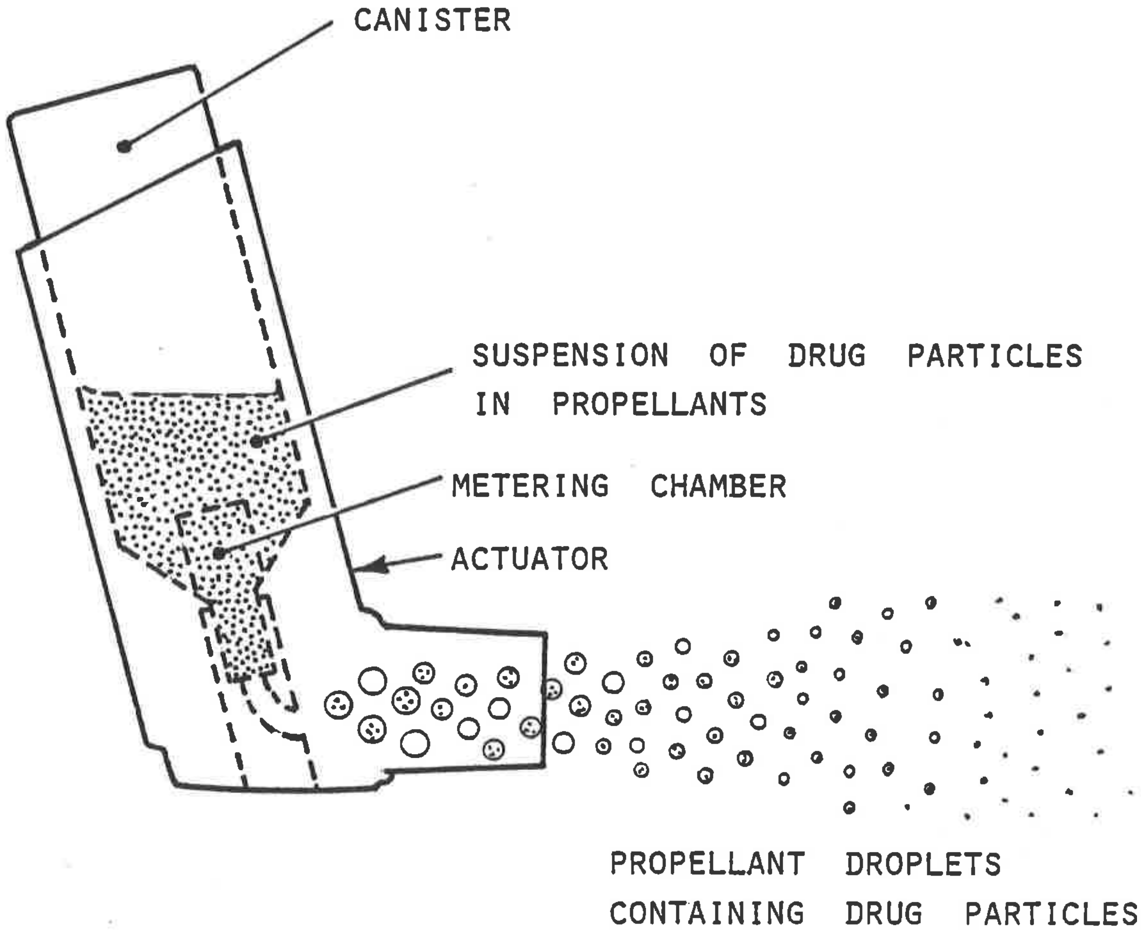
Fitzgerald (1975) and Held & Cooper (1979) have measured the hygroscopic growth of aqueous droplets of simple salts such as NaCl and H_2SO_4 in respirable size ranges. Ferron (1977) has also calculated the mean increase of initial aerodynamic diameter at high humidities for 0.4-6µm particles of twelve different salts. The results were applied to the Task Group deposition model and considerable differences in the deposition estimates demonstrated when compared with nonhygroscopic particles.

1.3 THERAPEUTIC AEROSOLS

Several reviews have outlined the problems associated with prediction of therapeutic aerosol deposition patterns from experimental data which used controlled inhalation of stable dust particles and theoretical models (Lourenco & Cotromanes, 1982; Gonda & Byron, 1978; Clarke & Newman, 1981; Moren, 1981). Obvious shortcomings of such models include the intersubject biological variability in deposition and the changes brought about by the presence of disease. Most research studies use stable, nonhygroscopic, monodisperse particles, whereas aerosols used in therapy are usually heterodisperse and sometimes unstable liquid or solid particles. In addition, therapeutic aerosols are generated from metered-dose inhalers (MDI), powder inhalers or nebulisers.

The fundamental differences between 'model' aerosols and those generated from metered-dose inhalers have been defined by Clarke & Newman (1981). MDI's contain either solutions or suspensions of drug in a mixture of propellants, and are packed in the inhaler under the high vapour pressures (typically 50 psi) of the propellants (Fig. 1.4). The initial velocity of the propellant droplets on actuation is about 30 ms^{-1} (Rance, 1974). There is some evidence to show that the propellants do not completely evaporate in the first few seconds after actuation (Wiener, 1958) and droplet sizes at the actuator orifice may exceed 35µm (Moren & Andersson, 1980). The droplets are delivered as a concentrated bolus containing several million particles and inhaled during a single breath (Clarke & Newman, 1981). In contrast, inert model particles are usually delivered as a more dilute cloud during steady tidal breathing. The mean particle size from several commercial MDI's after propellant evaporation was measured as 2.8-4.3µm MMAD with a polydispersity (σg) of 1.5-2.1 (Hiller <u>et al.</u>, 1978a). These are in a suitable size range for effective therapy. The particle size distribution may be influenced by changes in formulation such as the proportion of propellants used, the input particle size of the drug and the presence of surfactant (Polli <u>et al.</u>, 1969; Newman <u>et al.</u>, 1982a).

Fig 1.4. A METERED-DOSE INHALER



Therapeutic aerosols generated from nebulisers consist of droplets of aqueous drug solution, which begin to evaporate before inhalation. (Mercer, 1981). Commonly used medicinal nebulisers may be driven by compressed air or a piezoelectric crystal and generate polydisperse aerosols with mass median diameters in the range 1-7µm, σg 1.7-2.5 (Tillery et al., 1976). Modifications have been made to the standard designs to increase the output of both droplet concentration and aerosol volume (Esmen et al., 1982; Litt & Swift, 1972). Mercer has studied the output characteristics of nebulisers extensively. He measured the output in ml/min, the particle size distribution of the generated aerosol and the effect of auxiliary airflow in both compressed air and ultrasonic nebulisers (Mercer et al., 1968a; Mercer et al., 1968b; Mercer et al., 1969).

Powder inhalers often contain an excipient such as lactose for dilution of small drug quantities. The capsules used in powder inhalers may contain as little as 100µg of drug, so that excipients are required to facilitate manufacture and powder dispersion in use. The powder cloud of drug and excipient discharged from these inhalers is extensively agglomerated (Bell et al., 1971; Hallworth, 1977).

Bronchodilator drugs are often hygroscopic at high humidities. Many workers have studied the influence of high humidities on the particle size distribution and the effect on deposition probabilities of therapeutic aerosols. (Byron et al., 1977; Porstendorfer et al., 1977; Bell & Ho, 1981; Gonda et al., 1982; Lewis et al., 1982; Groom, 1981). Hiller et al. (1980b) showed significant increases in count and mass median aerodynamic diameters for aerosols from MDI's and powder inhalers at ambient and high humidities (95%RH). Bell & Ho (1981) and Martonen et al. (1982) measured the retarding influence of glycerin on growth rates of aerosols containing isoprenaline. The hygroscopic effects of in vivo inhalation clearly also apply to aqueous droplets of bronchodilator drugs from nebulisers (Ferron et al., 1976; Davis & Bubb, 1978) and to finely divided particles of drug from powder inhalers (Hallworth, 1977; Bell et al., 1971; Hiller et al., 1978b).

The particular problems of aerosol delivery from metered-dose inhalers have been extensively investigated. Extension devices or 'spacers' have been introduced which allow propellant evaporation between actuation of the inhaler and inhalation by the patient (Moren, 1978; Corr et al., 1982; Sackner et al., 1981). Nebulised aqueous droplets have also been reduced in size by passing through a heating system before inhalation. (Lunt et al., 1981; Alison, 1982; Arborelius, 1982).

The advantages of spacer devices have been shown in many clinical trials (Lindgren et al., 1980; Hidinger & Perk, 1981; Hodges et al., 1981). Aerosol delivery to the lungs is improved due to the decrease in both the particle size and velocity of the spray droplets, giving reduced impaction of high velocity droplets in the oropharynx (Newman et al., 1981b).

Spacers are particularly useful in children and patients with poor co-ordination, since a major problem with MDI's is actuating the inhaler at the beginning of an inspiration (Coady et al., 1976; Shim & Williams, 1980; Crompton, 1982). Some of the extension devices are fitted with one-way valves which allow normal inspiration and expiration without removing the spacer from the mouth. Various training aids and breath-actuated devices have also been developed to overcome this problem (McIlreath et al., 1972; Woodcock, 1980).

Several workers have also studied the inhalation technique of patients using metered-dose inhalers. Some agreement exists on the optimum delivery conditions. The preferred pattern is a slow, deep inhalation (< 1 ls^{-1}), with firing of the inhaler early in the inspiration, followed by a prolonged 10s breath-holding pause before expiration. The actuator is preferably placed 4cm from the open mouth (Woolf, 1979; Newman et al., 1980; Dolovich et al., 1981a). However, Lawford & McKenzie (1982) have found no significant difference between open or closed mouth around the actuator mouthpiece and 4 or 10 seconds breath-holding. Roberts et al. (1982) studied the performance of a six-step inhalation technique recommended by MDI manufacturers as guidelines for correct administration. They found the mean number of steps correctly performed was only 2.9 out of the possible six, using 42 patients.

Additional factors involved in deposition of therapeutic aerosols in lung regions has made useful theoretical lung models extremely difficult to develop. Gonda et al. (1982) estimated the effects on deposition of hygroscopic growth of aerosol particles and Groom (1981) measured the growth factors for several bronchodilator drugs. Gonda (1981) also estimated the effects of polydispersity of aerosols on regional deposition.

The difficulties in predicting in vivo behaviour from in in vitro methods or deposition models, have encouraged the use of in vivo methods in the majority of deposition research work concerning therapeutic aerosols.

1.4 IN VIVO METHODS

The in vivo methods for assessing deposition of therapeutic aerosols in humans include the following:

(a) clinical assessment of respiratory functions. e.g. measurement of FEV_1 (forced expiratory volume in 1 second) before and after treatment (Choo-Kang & Grant, 1975).

(b) measurement of the particle size distribution or concentration of inhaled and exhaled aerosol. This technique often employs 'model' particles. (Przyborowski, 1969, Heyder *et al.*, 1973).

(c) Radiolabelling the drug molecule with tritium (^3H) or carbon-14 with subsequent radioassay of body fluids (Martin *et al.*, 1971).

(d) Radiolabelling the aerosol with a γ-emitting radionuclide and subsequent external measurement of γ-activity (West, 1967). The γ-label may be introduced by:-

(i) radiolabelling model particles

(ii) adding a radiotracer to a therapeutic inhalation system e.g. sodium pertechnetate ($Na^{99m}TcO_4$) added to an aqueous solution for nebulisation (Dashe, 1974).

(iii) radiolabelling the drug molecule. (Short & Few, 1981).

Horton (1974) and Taplin (1979) have reviewed the advantages of using γ-labelled aerosols and the most commonly used radionuclides.

These *in vivo* methods have been used to study the following:-

(1) The additional problems of optimum delivery of aerosols to the respiratory tract when administration is from therapeutic inhalation devices such as the metered-dose inhaler (Moren, 1982a).

(2) The effect of factors such as particle size and breathing manoeuvres on therapeutic aerosol deposition patterns (Simonsson, 1982).

(3) Estimates of the site of action of inhaled drugs in the respiratory tract (Newhouse & Ruffin, 1978).

(4) The deposition and clearance of inhaled particles, the measurement of clearance rates and the efficacy of mucolytic drugs (Pavia et al., 1980; Yeates et al., 1981). This subject will not be discussed in detail, since this thesis is primarily concerned with deposition of bronchodilator drugs.

Many clinical trials have compared the efficacy of different methods of administration (Smelzer & Barnett, 1973). For example, delivery of aerosols from MDI's and powder inhalation devices were assessed in terms of clinical response (Chambers et al., 1980; Lal et al., 1980). Most studies showed no difference between the two methods, although the powder inhaler may be more effective in patients with poor co-ordination (Hartley et al., 1979; Chatterjee & Butler, 1980).

It has been recognised that the most useful technique for obtaining the complete deposition pattern of MDI's is the addition of a radiotracer to the aerosol (Moren, 1982a). The particular problems of administration from a metered-dose inhaler have been studied by the research group at the Royal Free Hospital, London. Many of their studies have used 'model' particles for measuring deposition fractions to establish optimum inhalation techniques for MDI's (Newman et al., 1981b; Newman et al., 1980; Newman et al., 1982b). The radiolabelled particles used for the above studies were Teflon spheres labelled with technetium-99m (Newman et al., 1981a). They were manufactured by atomising droplets with a spinning disc generator from a Teflon microfine suspension containing Tc-99m. The droplets were collected and heated to fuse the Teflon thus binding the radiolabel. The resultant particles were added to a mixture of propellants in a typical MDI formulation. Newman's experiments are among the few published studies which examine the deposition of particles from metered-dose inhalers. The γ-activity was measured by a whole body counter. Typical results for the body distribution are shown in Fig. 1.5 (Newman et al., 1981a). 80.4% of activity was deposited in the mouth and orophyarynx.

A reduction of about 20% in this figure was achieved by use of a spacer device, with a slight increase in the tracheo-bronchial deposition from 5.8% to about 9.5% (Newman et al., 1981b). The figures are in agreement with pharmacokinetic studies (Davies, 1975). However, the disadvantages of using Teflon spheres in these studies are common to most model particles namely that unlike therapeutic aerosols they are monodisperse and nonhygroscopic. The authors argued that according to the Task Group model (Task Group, 1966) deposition is virtually independent of the degree of polydispersity, but Gonda (1981) has suggested otherwise. They also argued that the hygroscopic growth of bronchodilator particles is less than 25% diametrically at high humidity (Hiller, 1980a). However, the relative humidity used was 95% which is rather low for the simulation of the conditions of the respiratory tract (99%rH) (Ferron, 1977). Direct extrapolation of Newman's results to bronchodilator aerosol deposition may therefore be unwarranted.

Two interesting methods have recently been published which study the deposition *in vivo* with respect to particle size. Hiller and coworkers (Hiller et al., 1982) have measured the deposition fraction of a multimodal aerosol consisting of a mixture of three sizes of polystyrene latex spheres. By calculation of the difference in concentration between inhaled and exhaled aerosol, they have demonstrated a possible method for accurately assessing deposition of

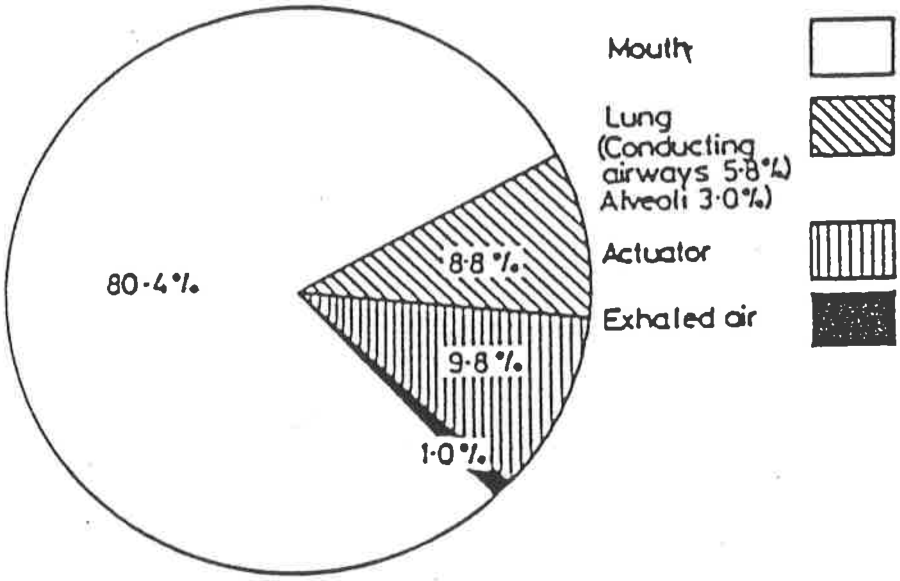


Fig 1.5 *Fractional deposition of ^{99m}Tc-labelled Teflon particles, administered from pressurised canisters.*

(Newman et al. (1981) Thorax 36 52-55)

polydisperse aerosols.

Rees et al. (1982) have measured the bronchodilator response to terbutaline aerosols of three different size ranges in metered-dose inhalers. Unfortunately, the size fractions were rather broad with some degree of overlap. Nevertheless, they showed a significantly greater response to the smallest size fraction compared with the largest (mass median diameters 5.6 and 13.6μm).

Many studies since that of Wilson & LaMer (1948) have demonstrated the effect of particle size on regional deposition by using radiolabelled model particles (Pavia & Thomson, 1976; Foord et al., 1978). The results show increasing head and tracheobronchial deposition with increasing particle size, and the greater significance of pulmonary deposition as particle size decreases.

Dolovich et al. (1981b) have reviewed their work which studied the influence of changes in aerosol particle size and method of inhalation on the site of deposition and physiological response. The addition of Tc-99m to a fenoterol solution administered from an ultrasonic nebuliser allowed direct measurements of the dose and aerosol distribution in humans using a gamma camera (Ruffin et al., 1978a). In another study, the same group of workers demonstrated that with a nebulised aerosol, a decrease in inspiratory time from 8 sec to 2 sec resulted in a lower total lung dose and relatively more aerosol deposited in the central airways (Ryan et al., 1981). Similarly, measurements of airway response were used to assess the value of specialised breathing techniques such as intermittent positive pressure breathing (IPPB) and positive end expiratory pressure (PEEP) (Shenfield et al., 1973; Dolovich et al., 1977; Andersen & Klausen, 1982). Pavia et al. (1977) also studied the effect of mode of inhalation of aerosol on the depth of penetration of particles in the lungs using model particles containing a radiotracer. In 50 patients with airways obstruction, they demonstrated that penetration of particles is directly related to the volume inspired per breath and forced expiratory volume in one second, and inversely related to flow rate during inhalation.

Ruffin and coworkers have used a radiotracer method to confirm patterns of aerosol deposition and measured airway responses to establish receptor sites for histamine (Ruffin et al., 1978a). Measurement of responses after central or peripheral aerosol deposition has also been used to determine receptor sites for isoproterenol (Ruffin et al., 1981), fenoterol (Ruffin et al., 1978b) and sites of airway obstruction and dilatation (Strohl et al., 1981). Radioaerosols such as ^{99m}Tc-macro-aggregated albumin and radioactive xenon gas were used to assess deposition patterns (Chopra et al., 1979). Riley et al. (1979) showed that an enhanced response to isoproterenol was achieved by inhaling near maximal inspiration (80% VC) i.e. more central deposition, and in divided doses sequentially rather than a single large dose.

Respiratory disease may cause alterations in the pattern of breathing and changes in airway diameter and geometry which may influence the mechanisms of deposition (Goldberg & Lourenco, 1973). The use of γ-radionuclides for studying different disease states and drug formulations is well established (Lopez-Majano, 1976; Hardy & Wilson, 1981). Radioaerosols are frequently used to establish the presence of respiratory diseases (Siegel & Potchen, 1973; Wagner, 1976; Hayes & Taplin, 1980). Lung ventilation is measured using radioactive gases such as Kr-81m and Xe-113 (Short et al., 1979; Fazio et al., 1982; Taplin et al., 1974). Radioaerosols such as ^{99m}Tc- or ^{113m}In-labelled human serum albumin microspheres are used for lung perfusion measurement

(Rhodes et al., 1969; Santolicandro et al., 1975). Results from gamma camera images indicate the presence of diseases such as chronic obstructive airway disease (COAD), chronic bronchitis and bronchial carcinoma.

The only direct method of measuring bronchodilator aerosol deposition with a γ-radiolabel in a suspension type MDI has recently been published by Short & Few (1981). They used ipratropium bromide labelled with bromine-77, administered from metered-dose inhalers to healthy volunteers. Their preliminary results were in broad agreement with previous studies (Newman et al., 1981a) showing about 80% oropharyngeal deposition and about 15% lung deposition. This method cannot normally be used for direct measurements of bronchodilators because the elements present in the drug molecules do not have suitable γ-emitting radionuclides.

Several studies of bronchodilators have used ^{3}H- or ^{14}C-labelled drugs which require biological assay methods for indirect assessment of in vivo aerosol behaviour. Most experiments of this kind determine the pharmacokinetics, metabolism and excretion of inhaled bronchodilators such as terbutaline (Nilsson et al., 1976), fenoterol (Laros et al., 1977) and disodium cromoglycate (Walker & Evans, 1972a). Tritiated salbutamol has been used to demonstrate bronchodilation from the inhaled, rather than swallowed, portion of a dose (Walker et al., 1972b); and to assess the value of IPPB with nebulised aerosols (Shenfield et al., 1974).

Clearly, deposition of therapeutic aerosols in vivo is largely established by indirect methods because of the difficulties in labelling drug particles. Additional problems with human studies include the ethical considerations of repeated doses and the use of non-medicinal materials, particularly for radiolabelled 'model' particles. Due to these difficulties, many *in vivo* aerosol studies have used mammalian subjects such as rats, rabbits, dogs and donkeys.

Several authors have compared the morphometry of different mammalian species with humans (Schlesinger & McFadden, 1981; Schreider & Raabe, 1981; Stauffer, 1975). Schlesinger and McFadden (1981) compared the length, diameter and angle of branching of respiratory airways in six species: the donkey, rabbit, rat, hamster, dog and human. They concluded that the dog exhibits geometric relationships of the upper bronchial tree which were closest to man, and the rabbit and rat show the greatest differences from man. Crosfill and Widdicombe (1961) have studied various respiratory measurements in eight mammalian species. Some of their published results are shown in Table 1.1, demonstrating the large differences in breathing patterns.

The difficulties in administration of aerosols to experimental animals is another disadvantage. Many of the techniques have been developed for toxicological studies which require repeated nasal inhalation exposures under controlled conditions in many subjects (Sachsse *et al.*, 1973; Carpenter *et al.*, 1979). Specialised exposure systems have been developed for toxicity testing of medicinal aerosols in metered-dose inhalers which utilise nasal inhalation from a chamber (Swann, 1972; Smith & Spurling, 1974). However, correct inhalation of MDI's is particularly difficult to achieve because of the necessity of firing the aerosol at the required point in an oral inspiration (Poynter & Spurling, 1971).

Theoretical models have been developed to predict deposition in mammalian airways for inert particles (Schum & Yeh, 1980; Kliment, 1974; Schreider & Hutchens, 1979). Many *in vivo* studies in animals have used radiolabelled model particles to evaluate aerosol deposition (Holma, 1967;

Table 1.1 Mean Values for various respiratory measurements in eight species.

	Body Weight (kg)	Lung Weight (g)	Functional Residual capacity (ml)	Tidal Volume (ml)	Minute Volume (1 min^{-1})	Frequency (Breaths min^{-1})	Mean alveolar diameter (µm)
Mouse	0.032	0.20	0.29	0.18	0.021	109	38.69
Rat	0.25	1.6	1.55	1.55	0.16	97	59.13
Guinea-pig	0.69	3.2	4.75	3.7	0.13	42	83.43
Rabbit	2.4	9.1	11.3	15.8	0.62	39	93.97
Monkey	2.45	22.3	87.5	20	0.70	33	89.06
Cat	3.7	20	66	34	0.96	30	133.19
Dog	12.6	82	252	144	3.1	21	74.05
Man	70	1065	2000	400	6.4	16	166.11

(Crossfill & Widdicombe, 1961)

Cuddihy et al., 1973; Bianco et al., 1980). These may provide initial results before human experiments (Patrick, 1982), or use materials which cannot be tested in humans (Taplin et al., 1977). Valuable data from mammalian studies is also obtained by using autoradiography (Barnes, 1971) and excised lungs (Tomenius, 1977). Valberg et al. (1982) examined how breathing patterns influenced aerosol deposition sites in excised dog lungs. They found that total deposition decreased as breathing frequency increased and the most uniform deposition was produced by slow-deep breathing. The aerosol used was nebulised sulphur colloid labelled with technetium ($^{99m}Tc_2S_7$).

In vivo animal models are frequently used to assess the pharmacokinetics of fluorocarbon propellants used in MDI's. (Shargel & Koss, 1972; Paulet et al., 1975; Niazi & Chiou, 1977).

Clarke (1973) and Muacevic (1975) have established the value of dog and rat experimental models for the toxicity testing of MDI's. Chowhan & Linn (1979) have used an in vitro rat lung model to assess drug deposition sites from a powder inhalation aerosol. They claim a good simulation of the human respiratory tract by comparison with size distributions measured in the Andersen cascade impactor. In vivo animal models have been used for drug deposition studies by measuring radiotracer in a nebulised formulation (Theodorakis et al., 1983) and using a fluorescent tracer in a MDI formulation (Tilov et al., 1978).

Animal models are therefore valuable tools in therapeutic aerosol research work, provided the difficulties in aerosol administration are resolved. The lung morphology of the animal species should be ideally close to that of humans, and should be well established so that the results obtained may be confidently extrapolated to human inhalation. As the size of the animal decreases, the relative surface area and ventilation rate per kg body weight increase, thereby ensuring more upper airway particle deposition in small animals (Palm et al., 1956).

Animals are particularly useful in the initial in vivo evaluation of a new formulation or when aerosol deposition studies use radiolabelled model particles of materials which are not toxicologically tested for human use.

Aims and objectives

The main objective of the present study was to examine in vivo deposition of aerosols from metered-dose inhalers, and to correlate the findings with in vitro aerosol parameters such as particle size. Estimates of regional deposition of therapeutic aerosols based on in vitro experiments with inert particles from other aerosol delivery devices are inaccurate. Additional factors must be taken into account with metered dose inhalers, such as polydispersity, hygroscopicity of the drug particles, and the high velocity and high particle concentration of the discharge. The use of model airways is limited because it ignores the complicated structure of respiratory airways. In addition, there is uncertainty about reproducing appropriate conditions of humidity and airflow.

It was decided to obtain direct measurements with a gamma camera of deposition sites in two animal models, the rabbit and the dog, using the same subjects for repeated experiments. The use of animals rather than humans enabled different formulations, some containing substances not fully tested toxicologically for clinical use, to be investigated. Conscious animals were used to avoid the effects of anaesthetics on respiratory parameters (Rich et al., 1979; Dain et al., 1975; Marsh et al., 1981). It was necessary to develop the animal models and to establish their validity as models for deposition studies of inhalation aerosols.

The use of a gamma camera for in vivo experiments, necessitated the introduction of a γ-emitting label into the aerosol formulation. In particular, it was intended originally to radiolabel the salbutamol particles in aerosols so that the resultant in vivo γ-camera images of radioactivity would indicate the body distribution of drug.

The relative in vivo distribution of therapeutic aerosols of different mean sizes was also required to provide correlations of in vivo deposition patterns with measurements of particle size.

Accurate measurements of particle size distribution of aerosols were required. Two methods of size analysis were chosen for study, cascade impaction to measure aerodynamic size, and a laser light-scattering method to provide projected area distributions rapidly. The two methods were correlated. A sampling chamber was developed with the aim of delivering representative samples of therapeutic aerosols to both particle size measuring instruments.