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**NOTATION**

- $C(t)$: concentration at time $t$
- $\bar{c}$: radially averaged concentration
- $D_{App}$: apparent diffusion coefficient
- $D_L$: longitudinal diffusion coefficient
- $D_M$: molecular diffusion coefficient
- $HW_j$: volume width of bolus at half maximum response
- $j$: subscript to denote inspiration (i) or expiration (e)
- $l$: airway length
- $N_j$: number concentration
- $\eta$: dynamic viscosity of air
- $P$: pressure
- $Pe$: Peclet number = $ul/D$
- $r$: airway radius
- $R_a$: airway resistance to flow
- $R_T$: total lung resistance to flow
- $R_{Zj}$: resistance to flow for generation, $Z$
- $\sigma_{V_j}^2$: volume variance of bolus
- $u$: radially averaged axial velocity
- $V_{bol}$: bolus volume measured by volumetric width at half maximum
- $\bar{V}_j$: volumetric mean of bolus
- $\dot{V}(t)$: flow rate at time $t$
- $V(t)$: volume at time $t$
- $V_T$: tidal volume
- $Z$: lung generation from Weibel's model

- ERV: Expiratory Reserve Volume
- FVC: Forced Vital Capacity
- FEV$_j$: Forced Expiratory Volume in first second of forced expiration
- FRC: Functional residual capacity
- PEF: Peak expiratory flow
- Raw: Airway resistance to flow, $(P_{atmosphere} - P_{mouth})/\dot{V}$
- RV: Residual Volume
- TLC: Total Lung Capacity
- TLV: Threshold Limit Value
- TGV: Thoracic Gas Volume
- VC: Vital Capacity
ABSTRACT

An impulse-response method was utilized for studying the dispersion of an 0.5 μm aerosol bolus in the respiratory tract of 11 male and 12 female healthy non-smoking subjects. The increase in bolus volume variance ($\sigma_v^2$) between inspiration and expiration was used as an index of dispersion. The effects of bolus penetration ($\tilde{V}_i$), bolus volume ($V_{bol}$), lung inflation, and gender on $\sigma_v^2$ were investigated. The $\tilde{V}_i$ was varied in 250 ml increments over a subject’s tidal volume ($V_T$). Procedures were repeated for the $V_{bol}$ of 75, 150, and 300 ml in two $V_T$ (1.0 and 1.5 L in females, 1.0 and 2.0 L in males). For the experimental conditions, diffusion of the bolus was negligible due to the low intrinsic motion of 0.5 μm particles. $\sigma_v^2$ was found to increase in an exponential manner with $\tilde{V}_i$ over the range of experimental data ($250 < \tilde{V}_i < 1750$ ml). The only significant factors were $\tilde{V}_i$ and $V_{bol}$ (p<0.001 for both). The effects of gender, $V_T$, and all interactions were not significant.

A volume shift in the mean of the expired bolus toward the periphery was observed for all subjects. This shift decreased with increasing $\tilde{V}_i$ and was significant for all levels of $\tilde{V}_i$ (p<0.001). The effect of $V_{bol}$ and the volumetric shift in the center of gravity of expired aerosol toward the lung’s periphery show that expiration is not the reversal of inspiration and that asynchronous filling and emptying of the lung is present in healthy individuals. The continued increase in dispersion beyond a depth of 250 ml shows conclusively that convective mixing continues well beyond the anatomical dead space and into the lung’s periphery.
INTRODUCTION

The ability to measure changes in pulmonary function in humans is important for the detection of lung disease and acute responses to air pollutants. Early airways disease, as in the case of smokers, is thought to occur in the small airways, <2 mm in diameter (15,17,27,28). Short term exposures to the air pollutant ozone may also effect the small airways (11). Conventional pulmonary function measurements primarily reflect the condition of the large airways, >2 mm in diameter (15,27,28). By the time functional changes reveal chronic airway obstruction, the results of treatment are usually disappointing (28). Detection of airway disease, while in early stages may aid in slowing the progression of the disease. For example, a smoker may be informed of changes in lung function and encouraged to cease smoking.

Deposition in the lung is minimal for 0.5 \( \mu \text{m} \) particles (1,24). Half micron particles follow gas flow patterns very closely due to their low intrinsic motion, settling velocity of \( 1.0\text{E}-3 \text{ cm/s} \) and a molecular diffusion coefficient of \( 6.3\text{E}-7 \text{ cm}^2/\text{s} \) (6), which is over 6 orders of magnitude less than diffusion coefficients for most gases. Longitudinal mixing of 0.5 \( \mu \text{m} \) particles is likely to occur throughout the lung (24). Longitudinal mixing is analogous to axial mixing and refers to mixing along the length or axis of an airway as opposed to mixing in the radial direction.

In a tube, longitudinal mixing is proportional to the radial velocity gradient and the molecular diffusion coefficient. The longitudinal mixing of 0.5 \( \mu \text{m} \) particles, due to their slow rate of
molecular diffusion, reduces to a convective mixing situation resulting from the radial velocity gradient existing between the center and wall of an airway.

An impulse-response method with an aerosol of 0.5 μm particles may be used to characterize convective mixing in the lung. For a variety of mixing systems the increase in the variance of a tracer concentration profile with respect to time has been shown to be related to longitudinal dispersion or mixing (4). The impulse-response method consists of inserting a volumetrically small bolus of a tracer into a person's inspiratory volume. The term bolus refers to a non-ideal impulse which is a small volume of aerosol or gas confined between two larger volumes of clean air. An inhaled bolus is repeatedly divided as the flow of air passes through bifurcations and penetrates into the lung. On exhalation the air from the numerous branches recombines and moves aerosol toward the mouth. Aerosol concentration and subject flow rates are measured for inspiration and expiration as a function of time. Comparison of inhaled and exhaled bolus concentration shows the expired bolus to be distributed over a larger volume than the inspired bolus.

The impulse-response method has been utilized as a means of studying mixing in the lung (10,13,22). This method has detected early airway damage caused by smoking (13), acute ozone effects on the small airways (11), and has shown convective mixing to persist beyond the anatomical dead space and into the lung periphery (10). With the ability to detect convective mixing beyond the anatomical dead space, this method may lend itself to the determination of
changes in ventilation patterns (13) or regional time constants (11).

The purpose of this research was to investigate the role of physiological parameters in determining the dispersion of an aerosol bolus. Aerosol bolus dispersion reflects the irreversibility of convective mixing in the lung. If the temporal and spacial pattern of filling and emptying of the lung were perfectly reversible and particles followed the same flow streamlines on expiration as on inspiration, then an exhaled bolus would be identical to the inspired bolus.

BACKGROUND

Lung Structure and Function

The main purpose of the lung is for gas exchange between the air and blood. This exchange occurs across a blood gas barrier by molecular diffusion. From Fick’s law, diffusion is directly proportional to the surface area of the barrier and inversely proportional to the barrier thickness. The blood gas barrier is only approximately 0.5 to 1 μm thick and has a surface area approaching 50-100 square meters (28). The lungs' large surface area is provided by hundreds of millions of alveoli. Understanding alveolar ventilation requires some knowledge of lung morphology and physiology.

Weibel’s (26) lung model provides a simplified description of
airway structures. Weibel’s model ‘A’ assumes that the lung consists of 23 symmetric and dichotomously branching generations (\(Z\)) of airways. In Weibel’s model the nasopharyngeal region of the respiratory system is ignored. The trachea is designated as generation 0. Referring to figure 1, generation 1 starts at the end of the trachea where the airway divides and the left and right main bronchi begin. Generation 2 begins when the main bronchi bifurcates and two new airways begin. This branching process continues for 23 generations. For any given generation, \(Z\), there are \(2^Z\) airways. In actuality the lung is not symmetric and Weibel’s model overestimates the number of airways (31). It has, however, served as a useful model for understanding convection and diffusion in the lung.

Using Weibel’s model, the lung is divided into two broad functional regions; tracheobronchial and pulmonary. Generations 0 through 16 compose the tracheobronchial region, which is sometimes referred to as the conducting zone, through which air is transported to and from the distal airways. Rings of cartilage maintain the shape and size of the bronchi in the conducting zone to about the twelfth generation. Cartilage tapers off toward the end of the tracheobronchial region and is completely gone by respiratory bronchioles (\(Z=17\)), which is where the pulmonary region begins. At the beginning of the pulmonary zone the airways are partially alveolated. The airways are fully alveolated with the onset of the 20th generation.

The movement of air in the lung occurs by the combined actions
of convection and diffusion. In the proximal airways of the conducting zone, convective motion of the air predominates. After generation 9 the total cross sectional area of the airways rapidly increases and linear velocity decreases making diffusion increasingly more important. The movement of air in the pulmonary zone in the lung is predominately by diffusion.

Inflation of the lung is caused mainly by the contraction of the diaphragm and the external intercostal muscles. As these muscles contract the chest cavity expands. The expansion reduces the pressure in the lung and air flows down the resulting pressure gradient into the lung. Deflation of the lung requires no muscle energy during normal breathing. The elastic properties of the tissue in the alveoli provide the pressure to deflate the lung. When the diaphragm and the intercostals are relaxed the lung naturally recoils and air is exhaled. At the end of a normal expiration the outward retraction of the chest wall balances the elastic recoil of the lung. When all muscles are relaxed, the lung volume at end of a expiration is defined as the functional residual capacity (FRC).

Figure 2 shows FRC in relation to other static lung volumes. Whites and males typically have larger lung volumes than blacks and females (14). Referring to figure 2, normal resting breathing consists of inhaling and exhaling 500-750 ml above FRC at a frequency of 12-15 breaths per minute. The volume inhaled and exhaled above FRC is referred to as a tidal volume ($V_T$). Total lung capacity (TLC) is the volume of the lung at maximum
inspiration. A complete expiration lowers the lung volume to residual volume (RV).

An understanding of convective flow in the lung may be approached by considering flow through individual airways. Assuming Poiseuille flow in an airway, the flow (\( \dot{V} \)) of gas is proportional to the driving pressure (\( P \)) and inversely proportional to the resistance (\( R \)).

\[
\dot{V} = \frac{P}{R}
\]  

(1)

The resistance (\( R_a \)) to flow in an airway is

\[
R_a = \frac{8\eta l}{\pi r^4}
\]  

(2)

where \( \eta \) is dynamic viscosity of air, \( l \) is the airway length, and \( r \) is the radius of the airway. The dimensions of unsupported airways in the lung vary with lung inflation. Changes in airway dimensions are invariant to direction and these changes in \( l \) and \( r \) are proportional to the cube root of changes in TLC (24). \( R_a \) decreases with increasing lung volume as it is inversely proportional to \( r^4 \).

Using Weibel's symmetric and dichotomous branching lung model, the combined airway resistance (\( R_z \)) for a generation (\( Z \)) is given by

\[
R_z = \frac{R_a}{2^Z}.
\]  

(3)

From equation 3, the generation having the maximal resistance is
expected in the region of $Z=6$. Total airway resistance ($R_T$) to flow in the lung is

$$R_T = \sum_{Z=0}^{Z=23} R_z.$$ (4)

Airway resistance and total lung volume are usually measured in humans by constant volume body plethysmography. This method requires a person to be placed in a box of constant volume. While a subject inhales and exhales air from outside the box the air pressure in the box and at the subject's mouth are measured along with flow at the mouth. To determine FRC a shutter at the person's mouth is closed at the end of a normal expiration. When the person tries to inhale against the closed shutter the rib cage expands, decreasing the pressure at the mouth and increasing the pressure in the box. Boyle's law ($P_1V_1=P_2V_2$) may then be applied to determine FRC. The total resistance to flow between the mouth and the alveoli may be obtained similarly by having the person breathe, with the shutter open. Recall that flow of gas is proportional to pressure and inversely proportional to resistance. Total airway resistance ($Raw$) may be obtained by measuring the change in volume and pressure required to produce a given flow. Obstructive lung diseases caused by airway narrowing increase $Raw$.

Dynamic lung volumes are used as another measure of airway function. Two commonly measured volumes for expiratory maneuvers are forced vital capacity (FVC) and forced expiratory volume in the first second (FEV$_1$). During normal expiration, muscles are relaxed
and the lung naturally recoils. Forced maneuvers require muscular compression to increase intra-alveolar pressure, which drives air out of the lung. During forced expiratory maneuvers there is dynamic compression the airways. This compression causes flow limitation and flow rates become effort independent, i.e. an increase in effort also increases the compression on the airway and no increase in flow is obtained. The driving pressure on expiration is equal to the sum of pleural and alveolar elastic pressures. As air flows through an airway the resistance to flow causes a pressure drop. Once the airway pressure drops below the pleural pressure the airway becomes compressed and flow limited. Early in expiration, airway and pleural pressures reach equilibrium in the region of generation 2, the lobar bronchi (27). As expiration progresses, airways narrow causing increased resistance to flow and the equal pressure point moves distally, deeper into the lung. The increased pressure on the lung may lead to premature closing of airways and thus a reduction in the volume exhaled. In healthy individuals FVC is nearly the same as the person’s vital capacity (VC). Normally FEV₁ is about 80% of the FVC (28).

Bronchi less than 2 mm in diameter only account for about 20% of the lungs airway resistance (27). Due to the low resistance to flow offered by small airways, measurements of FEV₁, FEV₁/FVC, and Raw primarily reflect the condition of the large airways, >2 mm (15,28). It is important to note that much early airway damage, as in the case of smoking, occurs in the small peripheral airways of the lung (15,17,27,28). Since conventional pulmonary function
measurements may not measure the function of the small airways, they have been referred to as a "silent" or "quiet" zone.

McDonnell and Seal (14) have developed prediction equations based on subject's gender, race, and height for normal static and dynamic lung volumes and airway resistance. These equations were determined from analysis of data on 314 volunteers recruited from the area in and around Chapel Hill, NC.

Longitudinal Mixing

A viscous fluid moving through a tube under laminar flow conditions develops a radial velocity gradient due to the frictional forces at the walls of the tube, resulting in a parabolic flow profile. If there were no velocity gradient, plug flow would exist and relative motion between molecules would be due entirely to diffusion. Since a velocity profile does exist, molecules near the center of the tube are displaced down the length of the tube relative to molecules near the wall (see figure 3). This relative motion of molecules near the center of the tube is a form of longitudinal mixing. Since longitudinal mixing is proportional to the radial velocity gradient, mixing along the axis of a tube is generally expected to be less for turbulent than for laminar flow.

Longitudinal mixing in a straight tube is traditionally described for the one-dimensional case (22).

\[ \frac{\partial c}{\partial t} + u \frac{\partial c}{\partial x} = D_L \frac{\partial^2 c}{\partial x^2} \]
Where $D_L$ is an effective mixing coefficient which is equal to the sum of a molecular diffusion coefficient ($D_M$) and an apparent diffusion coefficient ($D_{Ap}$) due to the flow profile, and $u$ and $c$ are the radially averaged velocity and concentration, respectively, at an axial tube position ($X$).

Levenspiel and Smith (12) showed that longitudinal mixing may be characterized in a manner similar to molecular diffusion. That is, a longitudinal dispersion coefficient, $D_L$, may be determined which is analogous to the molecular diffusion coefficient. Using an impulse-response type experiment, see figure 4, Levenspiel and Smith introduced the concept of using the variance of a concentration profile with respect to time as a method for evaluating the longitudinal dispersion coefficient. Levenspiel and Smith's method was limited to the case of a tracer injected as a delta function into an infinite length pipe. Producing a delta function of the tracer would require that some finite amount of the tracer be inserted in zero time. Van Der Laan (25) extended this concept to the case of a finite pipe. Aris (3) showed that a non-ideal tracer injection could be used as long as the tracer concentration was measured at two positions down stream of the injection. Bischoff (4) later showed that the change in the variance between the two positions was identical to the variance in the case of the ideal tracer injection.

Taylor laminar diffusion is the balance developed in a sufficiently long tube between radial diffusion and axial convection due to a velocity profile (21). Figure 5 illustrates
axial stream and Taylor diffusion. Taylor laminar diffusion may increase longitudinal mixing in generations 8 to 12 (24,29,30). When passing through a bifurcation a velocity profile is altered. It has been shown that the inspiratory profile is sharper than the more flattened expiratory profile (8,20). Without a well developed velocity profile Taylor diffusion is reduced or eliminated.

Ultman and Blatman (21) recommended a developing dispersion theory for inspiration and a redeveloping dispersion theory for expiration. Developing dispersion theory refers to the case where the effect of Taylor diffusion on longitudinal mixing is continually increasing as depth into the lung increases. On exhalation, four vortices flatten the velocity profile at bifurcations and so redeveloping dispersion theory was adopted. For redeveloping theory, velocity profiles are destroyed at each bifurcation and so must be redeveloped. As the velocity profile reforms the effect due to Taylor diffusion increases. Figure 6, adapted from Schroter and Sudlow (20), illustrates the velocity profiles expected for inspiration and expiration. Empirical relations for inspiratory-like and expiratory-like flow mixing coefficients were developed by Scherer et al. (19). These experimental mixing coefficients are similar to theoretical coefficients developed by Ultman and Blatman (21).

Half micron particles follow gas flow patterns very closely due to their low intrinsic motion, settling velocity of 1.0E-3 cm/s and a molecular diffusion coefficient of 6.3E-7 cm²/s (6). The Peclet number (Pe), which relates the magnitude of convection to
diffusion, is expected to be in excess of 2000 throughout the lung for 0.5 μm particles during normal breathing (24). Longitudinal mixing of these particles therefore reduces to the case of convective mixing. Longitudinal mixing of particles in this size range is likely to occur throughout the lung (24).

The use of a bolus composed of a suspension of 0.5 μm particles should provide more information on the convective mixing in the periphery of the lung than a gaseous bolus. Dispersion of gases distal to the terminal bronchi is dominated by molecular diffusion in both the radial and axial directions. Due to the high rate of diffusion, gaseous molecules are exchanged with residual air or lost to the walls and insufficient concentrations of the gas bolus are recovered for dispersion analysis. Helium and sulfur hexafluoride have molecular diffusivities of 0.698 and 0.0923 cm²/s, respectively. For comparison, recall the molecular diffusivity of a 0.5 μm particle is only 6.3 x 10⁻⁷ cm²/s (6). Compared to other particles the use of particles in the 0.5 μm size maximizes recovery by reducing deposition by diffusion, sedimentation, and impaction (24,31).

When using the impulse-response method it has been customary to determine the moments of the tracer concentration with respect to time. The application of this method to the studies of the human respiratory tract requires that the moments of the tracer concentration be determined with respect to volume to account for varying flow rates.

The calculation of the zeroth moment quantifies the amount of
tracer passing the mouth during inspiration or expiration. Determination of the zeroth moment of an aerosol bolus gives the number of particles \( N_j \) passing through the mouth:

\[
N_j = \int_{a_j}^{b_j} C(t) \dot{V}(t) \, dt \quad (6)
\]

Where \( C \) is number concentration, \( \dot{V} \) is flow, and the subscript \( j \) refers to inhalation (i) or exhalation (e). The integration limits, \( a \) and \( b \), refer to the ends of the concentration profile where the concentration signal has reached 0. By calculating the number of inhaled \( (N_i) \) and exhaled \( (N_e) \) particles it is possible to determine the fraction of aerosol recovery \( (N_e/N_i) \).

The mean volume \( \left( \bar{V}_j \right) \) of the bolus concentration profile with respect to the end of inspiration is the first normalized moment or center of mass:

\[
\bar{V}_j = \frac{\int_{a_j}^{b_j} C(t) \dot{V}(t) V(t) \, dt}{\int_{a_j}^{b_j} C(t) \dot{V}(t) \, dt} \quad (7)
\]

where \( V \) is cumulative volume.

The depth of penetration of a bolus is defined as the mean volume, \( \bar{V}_i \), of an inhaled bolus. This terminology is used because the first moment is the volume of air following the center of mass for inspired bolus. Assuming no deposition, if the pattern or sequence of filling and emptying of the lung is reversible and the first air in is the last air out of the lung, then the center of
mass of the exhaled bolus, $\bar{V}_e$, will be equal to $\bar{V}_i$. Deposition of aerosol in the lung from a bolus would probably be greater for the beginning of the bolus as it would penetrate most deeply into the lung. This non-uniform deposition from the bolus would tend to cause $\bar{V}_e$ to be slightly less than $\bar{V}_i$.

The second moment of the bolus is the volume variance ($\sigma_v^2$):

$$
\sigma_{v_j}^2 = \frac{\int_{t_0}^{b_j} C(t) V(t)^2 \dot{V}(t) \, dt}{\int_{a_j}^{b_j} C(t) \dot{V}(t) \, dt} - \bar{V}_j^2
$$

(8)

The volume variance of an inhaled aerosol bolus ($\sigma_{v_i}^2$) describes the distribution of aerosol as it is inhaled. As the aerosol moves through the lungs it becomes more disperse. On exhalation $\sigma_{v_e}^2$ reflects the sum of the initial aerosol dispersion and any subsequent dispersion occurring in the lungs. If flow streamlines were exactly the same for inspiration and expiration, then particles would be expected to retrace their paths and an exhaled bolus would appear the same as the inspired bolus and $\sigma_{v_i}^2$ would equal $\sigma_{v_e}^2$. This is only valid if we assume nondiffusing particles, which is not a bad assumption for particles in the 0.5 $\mu$m size range. In the lung, velocity profiles differ between inspiration and expiration which could lead to not completely reversing the axial streaming process.

$$
\sigma_v^2 = \sigma_{v_e}^2 - \sigma_{v_i}^2
$$

(9)
where $\sigma_y^2$ describes the net pulmonary mixing (24). Bischoff (4) showed that the increase in $\sigma_y^2$ between two measurement points was mathematically equal to the increase in $\sigma_y^2$ between a perfect impulse and a downstream measurement point.

Dispersion Studies

Recent studies (2,11,13) have shown correlations between the dispersion of an aerosol bolus and lung function. Anderson et al. (2) showed an increase in bolus half width (the volumetric width of a bolus measured at one half the maximum concentration) and a shift in the mode of expired aerosol concentration with the degree of lung damage caused by cystic fibrosis in patients compared to healthy subjects. The mode shift of the expired bolus was found to move proximally toward the mouth in patients and distally toward the periphery in healthy subjects. Investigators concluded that the shift in the bolus mode was an indicator of asynchronous filling and emptying of large units of the lungs, such as lobes. $FEV_1/FVC$ was correlated to dispersion in cystic fibrosis patients. This correlation, however, was not seen in normal subjects.

A study involving healthy subjects exposed to ozone, conducted by Keefe et al. (11), found an increase in bolus half width after ozone exposure. Spirometric data showed FVC and FEV1 to be significantly reduced by ozone exposure. Although both spirometry and the aerosol bolus were changed significantly by ozone exposure, there was not a significant correlation between bolus dispersion and spirometric data, suggesting that the alteration in bolus
dispersion may have reflected different changes in lung function than those detected by standard pulmonary function tests.

McCawley and Lippmann (13) found increased bolus dispersion in smokers compared to a matched pair of non-smokers. Spirometric tests did not detect any differences between the two groups. This study also addressed the effect of changing the $V_T$ and preinspiratory volume on dispersion as measured by a peak dispersion index, which is the percentage of the maximum bolus concentration exhaled to the maximum bolus concentration inhaled. A linear regression of the data showed dispersion to depend only on the penetration volume of the bolus and showed no effect from changing either the $V_T$ or the preinspiratory volume. Regardless of the method of measuring dispersion other studies have also found linear relationships between bolus dispersion and penetration volume (2,10).

Heyder and Davies (9) using a 300 cubic centimeters (cc) bolus, released at the beginning of a subject's inhalation, found dispersion to increase with $V_T$ at constant expiratory reserve volume (ERV). ERV is the lung volume between FRC and RV (see figure 2). Since the bolus was released at the beginning of the subject's inhalation, an increase in $V_T$ was also an increase in the depth of penetration. Changes in the ERV were not found to influence dispersion at a constant $V_T$. However, there was an increase in the recovery of particles with increasing ERV's.

Ultman (21-24) has extensively studied the theory of longitudinal mixing and gas transport and has conducted studies in
humans with gaseous boluses composed of helium and sulfur hexafluoride. Based on these studies, mixing in the conducting airways ($0 \leq Z \leq 17$ in Weibel model) is principally caused by convective mixing. Distal to the terminal bronchi ($Z > 17$), diffusion predominates due to decreased axial velocity as a result of increased cross sectional area of the lung.

Heyder et al. (10) studied convective mixing in the lung using a bolus composed of 1 μm particles of di(2-ethyl-hexyl)sebacate. As previously stated, longitudinal mixing reduces to the convective mixing case for an aerosol with low intrinsic motion. By using an aerosol as a tracer of air flow patterns, Heyder et al. (10) were able to show that convective mixing continues to be an important transport process in the lung periphery.

STATEMENT OF PURPOSE

The purpose of this study was to investigate the role of physiological parameters on longitudinal dispersion of an aerosol bolus. Of primary interest were:

1. Depth of bolus penetration, $\bar{V}_i$, into the lung,
2. Degree of lung inflation,
3. Bolus volume, $V_{bol}$, and
4. Subject’s gender.
From previous studies, convective mixing was expected to increase bolus dispersion as a function of $V_i$ to at least one liter $(2,10,13)$. This trend was explored and extended to greater depths in the lung.

The effect of lung inflation was explored by comparing the aerosol dispersion as a function of the depth of bolus penetration for different $V_T$. All $V_T$ were started from functional residual capacity (FRC). Airway caliper is directly related to lung inflation. As the lung inflates airways beyond the anatomic dead space increase in diameter. Thus the volume of individual lung generations increases with increasing lung volume. Consider a bolus penetrating 500 ml into the lung and recall that $V_i$ is defined as the volume of air from the bolus center of mass and the end of inspiration. This bolus would be expected to pass more bifurcations in a one liter $V_T$ than in a two liter $V_T$. Dispersion measurements were be made in two $V_T$ for each subject.

The effect of $V_{vol}$ on dispersion is, of yet, unexplored. If mixing in the lung is purely longitudinal, then regardless of the input the overall mixing should be the same. Differences in the sequence of filling and emptying of lung units constitutes differences in the regional time constants of the units and would not be a non-longitudinal processes. If differences in time constants do exist, then dispersion measurements may vary with $V_{vol}$.

Male and female subjects were also compared in a common $V_T$ of one liter. Females typically have smaller lungs than males. If dispersion is a function of the number of generations through which
a bolus passes, then females may have greater bolus dispersion for a given depth of penetration into the lung than males. Information on aerosol dispersion in females is sparse or nonexistent. This study provides dispersion data on normal, healthy females.

METHODS AND MATERIALS

Aerosol

The experimental setup is shown in figure 7. Nonhygroscopic, 0.5 μm, triphenyl phosphate (TPP) particles were produced by nebulizing a solution comprised of 2.0 gram TTP in 100 ml of 94.5% ethanol (U.S.P. alcohol). Droplets of solution arising from a three jet Collison nebulizer passed through a heating coil (1 inch inner diameter, 2 ft length) for ethanol evaporation. Clean air then diluted the flow before passing through a charcoal filter for ethanol removal. The flow was then diverted through a T-connection. One branch of the connection allowed flow to be continuously exhausted. The other branch lead to a solenoid valve. Opening the solenoid valve allowed aerosol to mix with a subject’s inhaled air.

The aerosol generation system is the same as used by Keefe et al. (11) and similar to that used by McCawley and Lippman (13). The particles had a geometric standard deviation between 1.2 to 1.5, as reported by McCawley and Lippman (13) and Keefe et al. (11), respectively.
Triphenyl phosphate has been used in recent studies (11,13,18) with no reports of adverse health effects. The American Conference of Governmental Industrial Hygienist (ACGIH) has recommended a Threshold Limit Value (TLV) of 3 mg/m³, which was recommended on the basis of a study on 32 men employed in the manufacture of TPP (32). Fourteen of these workers were exposed to an estimated daily time weighted average airborne concentration of 3.5 mg/m³ for as long as ten years. No ill effects were observed in these workers although there was a slight, but statistically significant, decrease in red blood cell cholinesterase activity.

The risks to subjects from the inhalation of TPP in this study were considered minimal. This conclusion of minimal risks was based on the use of TTP in previous studies and the low level of subject exposure. Possible exposure of subjects to TTP was limited to approximately a four hour time period and to less than 100 breaths containing aerosol. Total possible subject exposure to the TTP was less than 1/75 of the TLV.

Subjects

A group of 11 male and 12 female, nonsmoking, healthy volunteers were recruited from the area in and around Chapel Hill, NC. Prior to the recruitment of subjects, this study was approved by the Committee on the Protection of the Rights of Human Subjects, School of Medicine, University of North Carolina at Chapel Hill. Subjects recruited for the study were informed of the purpose and procedures of the study and of any potential risks from
participation. After an opportunity to have questions related to this study answered, subjects were asked to sign a statement of Informed Consent.

To be eligible for recruitment, all potential subjects were required to be between the ages of 18 and 40 years, non-smokers for at least the past one year and no history of more than 0.2 pack years (a pack year is 20 cigarettes per day for a year), no recreational drug use within six months and no history of regular drug use exceeding once per week, no history of cardiorespiratory disease, no history of hay fever or asthma, and no viral illness within the past month. Testing for pregnancy in female subjects and sickle cell disease in black subjects was also conducted. Pregnancy or sickle cell excludes potential subjects from the study. Recruited subjects were trained in the performance of spirometry and plethysmography. Measures of peak expiratory flow (PEF), FVC, FEV1, Raw, and Thoracic Gas Volume (TGV), which is approximately equal to FRC, were completed in triplicate the day of the study. Spirometry was conducted in a standing position on a dry seal spirometer (CPI model 220). A subject’s PEF, FEV1, and FVC were taken from the maneuver with the greatest sum of FEV1 and FVC. Plethysmography was performed in the sitting position in a constant volume body Plethysmograph (CPI model 2000TB). Three plethysmography measurements were averaged to determine a subject’s Raw and TGV.

Prior to making bolus dispersion measurements, subjects were required to meet pulmonary function criteria. Requirements for
continuation in the study were FVC ≥ 80% predicted, FEV₁/FVC ≥ 75%, and Raw ≤ 2.0 (cm H₂O)/liter/sec. Possible obstructive and restrictive diseases would have been indicated by FEV₁/FVC < 75% and by FVC < 80% predicted, respectively. Predicted lung volumes were determined using regression equations developed by M'Donnell and Seal (14) for the study population. An elevation in Raw would also have been an indicator of obstructive disease.

Experimental Design

The strategy developed for this study was designed to investigate the effect of four parameters on dispersion of an aerosol bolus with a minimum number of maneuvers. The parameters under investigation were depth of bolus in the lung, degree of lung inflation, Vₙα, and gender. Table 1 shows all the possible combinations of parameters.

To determine effect of penetration of a bolus into the lung, dispersion measurements were made at 250 ml increments in each Vₜ. Subject maneuvers are illustrated in figure 8. Identical procedures were followed for three Vₙα of 75, 150, and 300 ml.

After a bolus release during inhalation, subjects continued to inhale to their target Vₜ and then were instructed to exhale to residual volume. Subjects were given approximately a one minute rest between maneuvers. The average of three to four maneuvers were taken for a given dispersion measurement.

Measurements were made at two Vₜ for each subject, to investigate the effect of lung inflation. The 1 liter Vₜ was used
for all subjects. The 1.5 and 2 liter $V_r$ were reserved for female and male subjects, respectively. All $V_r$ were started from functional residual capacity. A flow rate of 40 lpm was used for all maneuvers. This flow was chosen because of its use in previous studies (11,13).

Data Acquisition

A Dell 386 computer equipped with analog to digital board (Data Translation, model 2801A) was used to control bolus release and to acquire data. Flow, volume, and concentration signals were acquired at the rate of 200 Hz and saved for subsequent dispersion analysis. Figure 9 illustrates the three signals acquired for each bolus. The flow signal was determined using a Fleisch pneumotachograph and pressure transducer. The concentration signal was obtained by means of an in-line Sinclair-Phoenix aerosol photometer with a linearizing circuit. Analog integration of the flow signal was used to trigger the opening of a solenoid valve releasing aerosol into a subject's inhaled airstream at a predetermined volume. The solenoid valve was closed when the volume signal reached the sum of the trigger volume and the $V_{tot}$.

As an aid for the bolus dispersion measurements, a VGA monitor was used for a real time display of a subject's inspired and expired volume. By following a grid on the screen, subjects were taught to control both their tidal volume and flow rate.
Data Analysis

Bolus data analysis was based on the first three moments of the concentration profile with respect to volume. Figure 10 illustrates a bolus plotted as a function of volume. This form of analysis was chosen due to the physical meanings of the moments and relation of the second moment to the coefficient of longitudinal dispersion. All integrations used in moment calculations were performed with respect to the end of inspiration.

Prior to signal analysis the concentration signal was smoothed at 50 Hz with a digital low pass filter. The cumulative volume used in calculations was obtained by time integration of the flow signal.

The second moment, $\sigma_v^2$, was approximated by assuming normality of the concentration profile and relating the full width at half maximum response, termed half width (HW) to variance.

$$
\sigma_v^2 = \frac{\text{HW}^2}{8 \ln(2)}
$$

(10)

Assuming that the bolus is normally distributed in volume, 95% of the particles inhaled are located within $2\sigma$ of the mean volume of the inhaled bolus. This relation provides a more reproducible method of obtaining $\sigma_v^2$ since it is less sensitive to the low signal to noise ratio at the edges of the profile than the more formal computation presented in equation 8.
Statistical Analysis

The variable of main interest in the study was $\sigma_y^2$, which is the difference between $\sigma_{v_t}^2$ and $\sigma_{v_l}^2$. This measure has been used in other studies as an index of longitudinal dispersion in the human lung (22,23). The average of three to four maneuvers were taken for a given dispersion measurement. The first step in the analysis was to determine if a transformation of $\sigma_y^2$ was needed in order for the analysis of variance assumptions to be satisfied. A procedure due to Box and Cox (33) was used to determine what if any transformation was appropriate. This method indicated that the logarithm of $\sigma_y^2$ should be used in the statistical analysis. Figure 11 illustrates the increase in the statistical variance of $\sigma_y^2$ with increasing $\sigma_y^2$. A log transformation of $\sigma_y^2$ achieves the homogeneity of the statistical variance necessary for analysis.

The experimental design indicated in Table 1 is an unbalanced for factor design with subjects nested under the sex factor. The other three factors are $V_t$, $\bar{V}_i$, and $V_{bol}$. A four factor analyses of variance with the dependent variable $\log_{10}(\sigma_y^2)$ was used to test for significance effects.

The common 1 liter $V_t$ was considered for detection of an effect due to gender. Similarly, only $\bar{V}_i \leq 750$ ml were considered for the comparison of dispersion as a function of lung inflation. These $\bar{V}_i$ were common to the small (1.0L) and large (1.5L females, 2.0L males) $V_t$. The restrictions in the data used to compute the means were necessary so that comparisons would not be confounded by the unbalanced experimental design. Since as the results will show
gender and \( V_T \) are not significant they were ignored and an further analysis was for this reduced model.

The Ryan-Einot-Gabriel-Welsch (REGW) multiple comparison procedure (34-37) was used on the \( \log_{10}(\sigma_v^2) \) means to determine if levels of \( \bar{V}_i \) were different from each other. The same procedure was also used on the \( \log_{10}(\sigma_v^2) \) means to determine if levels of \( V_{bo} \) were different from each other.

A volume shift between \( \bar{V}_e \) and \( \bar{V}_i \) was investigated to determine what if any factors were affecting this difference. An analysis of variance for an unbalanced four factor design was done on the difference between \( \bar{V}_e \) and \( \bar{V}_i \) with subjects nested under the sex factor. The other factors were \( V_T, \bar{V}_i, \) and \( V_{bo} \). No interactions were included in this analysis. The Box and Cox (33) procedure was used to determine if a transformation of the difference was necessary. The results will show significant \( \bar{V}_i \) and \( V_{bo} \) effects. At each level of these two effects, the hypothesis that the mean is zero was tested by a one-sample t-test using the error mean square from the above analysis of variance as the variance estimate.
RESULTS

Height, weight, and pulmonary function data for study subjects are provided Table 2. Height, weight, peak expiratory flow (PEF) and dynamic lung volumes differ between males and females. These differences were expected as they have been observed by others (14). Although differences exist between the male and female subject's pulmonary function data, these differences are normal and both groups contain health subjects.

The results of the four factor analyses of variance of \( \log_{10}(\sigma_y^2) \) are given in Table 3. The only significant effects are the main effects for \( \bar{V}_t \) and \( V_{bd} \) (\( p<0.001 \) for both). The main effects of sex and \( V_t \) and all interactions were not significant.

Table 4 compares the male and female means of \( \log_{10}(\sigma_y^2) \) at \( V_t=1000 \) ml. Dispersion measurements of males and females in a 1 liter \( V_t \) are compared in figures 16, 17, and 18 for the \( V_{bd} \) of 75, 150, and 300 ml, respectively. Though not statistically significant, these figures show the average dispersion in males to be greater than that in females in all cases except the 300 ml bolus at a shallow penetration of 250 ml.

Results appearing in tables 5 and 6 show that dispersion is unaffected by the degree of lung inflation. This observation held for both males and females. Figures 12 and 13 show the dispersion of a 150 ml bolus in females and males, respectively. These figures are in agreement with the statistical findings as the data for the small and large \( V_t \) appear to follow the same trends.

Table 7 compares the means of \( \log_{10}(\sigma_y^2) \) for each level of \( \bar{V}_t \).
Each level of $V_i$ was found to be significantly different from all others ($p<0.05$). The trend is for dispersion to increase with increasing $V_i$.

The dispersion for the 300 ml bolus was found to be significantly less than that of the 75 and 150 ml bolus, which were not different from each other ($p<0.05$). This comparison appears in table 8. Although there is an $V_{bol}$ effect, the magnitude of the difference in dispersion between the $V_{bol}$ is small. Figures 14 and 15 compare the dispersion of the $V_{bol}$ in a large $V_T$ for females and males, respectively. In these figures it is difficult to discern the difference in dispersion as a function of $V_{bol}$, but in general the dispersion for the 300 ml $V_{bol}$ is lower than that of the smaller $V_{bol}$.

A shift in the mean volumes, $\bar{V}_e > \bar{V}_i$, was found in the data for all subjects. In all cases the exhaled bolus had a greater volumetric mean than the inhaled bolus. The illustration of a bolus in figure 9 shows a shift of approximately 100 ml. This shift gradually decreases with increasing penetration. The difference in volume means for levels of $V_i$ and $V_{bol}$ are given in tables 9 and 10, respectively. At each level of these two factors, the hypothesis that the mean difference, $\bar{V}_e - \bar{V}_i$, was equal to zero was rejected. The volume shift was significantly different from zero for all levels of bolus $V_i$ and $V_{bol}$ ($p<0.001$). This shift in the means was also consistently larger in males than in females as is illustrated in figure 19.
DISCUSSION

We studied the effects of bolus penetration ($\bar{V}_i$), lung inflation, $V_{\text{inl}}$, and gender on mixing in the lung. The significance effect of $\bar{V}_i$ on dispersion is consistent with the findings of others (2,10,13).

The observation that changing $V_{\text{inl}}$ had a significant effect on dispersion is inconsistent with longitudinal mixing theory (4). In a longitudinal mixed system, the error associated with an imperfect impulse is eliminated by defining dispersion as $\sigma_v^2$ (21,22). It should be noted, however, that although the difference in dispersion measurements between the $V_{\text{inl}}$ was highly significant it was small in magnitude, as is illustrated in figures 14 and 15.

There was no significant difference existing in the dispersion between males and females. Figures 16-18, however, show that males tended to have increased dispersion over females. Referring to table 2, females have smaller dynamic lung volumes than males. From figure 8, predicted TLC is also smaller for females (5.2 liters) than males (6.9 liters). Using Weibel’s (26) dimensions and modifying them to the predicted TLC for study subjects, females would be expected to have airways of only approximately 91% the diameter of the airways in males at the same flows. Due to the difference in airway diameters, more turbulence is expected in the airways of females than males. Laminar longitudinal dispersion, i.e. axial streaming, is generally greater than turbulent longitudinal dispersion (24).

If increased turbulence is a cause of decreased dispersion,
then a difference would also be expected to exist between large and small $V_t$. Airway diameter increases with lung inflation. For the differences in lung inflation occurring in this experiment, the airways during a maneuver at a small $V_t$ would be approximately 95% the diameter of the airways when using a larger $V_t$. Reducing airway diameter would again be expected to increase turbulence and decrease longitudinal dispersion.

Consistent with the lack of an effect of airway diameter of dispersion, Siekmeier et al. (38), made measurements before and after bronchoconstriction and showed dispersion as measured by $HW$ to be largely unaffected by increasing airway resistance. Airway resistance increases as airway diameter decreases (see equation 2).

An argument for increasing dispersion with decreasing airway diameter may also be made. Smaller airways would cause a bolus to travel further into the periphery passing through more bifurcations (16). Using flow visualization of neutrally buoyant and nondiffusing beads, Haselton and Scherer (8) found for cyclic flow, that axial streaming was not fully reversed on expiration. These authors concluded that the more pointed inspiratory velocity profiles lead to net transfer of the beads to deeper volumes. Scherer et al. (19) using a five generation glass model of the bronchial tree, found axial dispersion to be approximately three times greater for inspiration than for expiration. This inconsistency in dispersion was considered to be due the difference between inspiration and expiratory velocity profiles at bifurcations. An incomplete reversal of axial streaming would
result in an exhaled bolus being distributed over a larger volume than the inhaled bolus. This incomplete reversal is measured by the dispersion index $\sigma_v^2$. Since there is an increasing number of bifurcations with increasing $\tilde{V}_i$, flow irreversibility would be expected to increase $\sigma_v^2$ as a function of $\tilde{V}_i$.

In all measurements there was a volumetric shift in $\tilde{V}_e > \tilde{V}_i$. The bolus mean for expiration was larger than for inspiration. This shift toward the periphery was also observed by Keefe et al. (11) and Anderson et al. (2) in healthy individuals. In patients with cystic fibrosis, Anderson et al. (2) found a shift toward the mouth $\tilde{V}_i > \tilde{V}_e$. A shift toward the mouth was also observed to increase with bronchoconstriction (38). Anderson et al. (2) felt the shift in patients may reflect asynchronous filling and emptying of large lung units such as lobes. Siekmeier et al. (38) recognized the volume shift to be a measure of the symmetry of lung ventilation. As already discussed the leading front of an inhaled bolus penetrates deeper into the lung and this volume shift toward the mouth may also reflect the non-uniform deposition of a bolus.
CONCLUSION

For the range of volumetric penetrations explored in this study we have found the log of axial dispersion to increase as a linear function of $\bar{V}_i$. By using an aerosol bolus, dispersion was reduced to mixing by convection. Our data confirms that convective mixing continues beyond the anatomical dead space and into the lungs periphery, which is in agreement with the findings of Heyder and coworkers (10).

The significant effect of $V_{bol}$ on dispersion shows the lung not to be a purely longitudinally mixed system. The larger $V_{bol}$ (300 ml) was expected to have a more balanced distribution in each generation of airways through which it passed (11) than small $V_{bol}$. Changes in regional time constants between inspiration and expiration may have caused the dissimilar dispersion between the $V_{bol}$. Despite the significant effect of $V_{bol}$ on dispersion the magnitude of the effect is small and may be physiologically unimportant.

The volume shift, $\bar{V}_e > \bar{V}_i$, reflects variations in filling and emptying (2). This shift indicates that the pattern of filling and emptying is not reversible. This indicates mixing mechanisms other than longitudinal mixing are occurring. A likely mechanism would be the nonsequential filling and emptying in the lung. In the nonsmoking healthy subjects used in this study, $\bar{V}_e$ was always larger than $\bar{V}_i$. The decrease in the volume shift with increasing $\bar{V}_i$ may indicate that there are several mechanisms effecting the volume shift.
Perhaps the dispersion of boluses penetrating deeply into the lung, i.e., a large $V_t$, are influenced most by variations in parallel ventilation. A bolus introduced to the tidal air early in inspiration may go to a different region of the lung than those released late in inspiration. Variations in lung compliance may lead to the parallel ventilation of a deeply penetrating bolus. Parallel ventilation may be important at or near the end of inspiration when unobstructed regions could begin to ventilate obstructed areas. Interestingly, a volume shift where $\bar{V}_t$ is larger than $\bar{V}_r$ has been observed for cystic fibrosis patients (2) and normals after bronchoconstriction (38).

The lack of significance effect of either $V_t$ or gender on dispersion indicates that small changes in airway diameters do not affect aerosol dispersion. For the same flow rate, greater the linear velocities would have been expected in the airways of females and the 1 L $V_t$. The lack of $V_t$ and gender effects may also imply the same changes in flow rates will not effect dispersion.

Further experimental work is necessary to better understand the occurrence of longitudinal dispersion in lung. The use a lung model composed of a network of tubes, which would eliminate the variability between subjects, may be usefully in studies to better evaluate the effects of airway caliper and lung volume. It would also be beneficial to examine unidirectional and bidirectional axial streaming in a straight tube to avoid the complexities of flow patterns in bifurcations.
REFERENCES


APPENDIX

Figure Captions

1. **Idealization of Human Airways**: Weibel's symmetric lung model assumes a dichotomous branching system consisting of 23 generations \((Z)\). Generations 0 through 16 make up the conducting zone and generations 17 through 23 make up the pulmonary zone. \(a\): \(0 \leq Z \leq 6\), subsequent to the trachea \((Z=0)\), branching rapidly increases the number of airways by \(2^Z\). \(b\): 19\(\leq Z \leq 23\), a respiratory bronchiole leads into three generations of alveolar ducts, which in turn lead to alveolar sacs.

2. **Static Lung Volumes**: Functional residual capacity (FRC) is the lung volume at which the elastic recoil of the lung balances the outward force of the rib cage. During normal breathing, a person inhales and exhales at a tidal volume \((V_t)\) of 500-750 ml above FRC at a frequency of 12-15 breaths per minute. Total lung capacity (TLV) and residual volume (RV) are the limits of inspiration and expiration, respectively.

3. **Velocity profiles and tracer distributions**: Inertial and viscous forces act on a fluid as it flows through a tube. The distribution of a tracer of neutrally buoyant non-diffusing particles suspended in the fluid depends on the radial velocity gradient in the tube. \(a\): No frictional forces at the wall or a fluid with zero viscosity would result in uniform velocity or plug flow. \(b\): Laminar flow, \(Re \leq 2000\), viscous forces predominate.
Assuming a no slip condition, the velocity will be 0 at the tube wall and reach a maximum at the center of the tube. The tracer distribution is altered by the velocity gradient through the process of axial streaming. Particles near the center of the tube have greater axial transport than those near the wall. \( \text{a)} \): Turbulent flow, Re>3000, inertial forces predominate. Vortices or eddy currents diminish the radial velocity gradient, so that axial distortion of the tracer distribution is reduced.

4. Impulse-Response Experiment: As a tracer moves down a length (L) of a tube it mixes with the surrounding fluid. The extent of axial displacement and mixing may be determined by measuring the tracer’s concentration at two positions downstream of an injection point. The normalized first moment of the concentration profile with respect to volume is the center of gravity or volumetric mean (\( \bar{V} \)) of the distribution. The increase in the mean volume (\( \bar{V}_2 - \bar{V}_1 \)) from the first to the second sampling point is equal to the system volume between the sampling points. The centralized second moment of the profile is the volume variance (\( \sigma^2 \)). The increase in volume variance (\( \sigma^2_2 - \sigma^2_1 \)) between the sampling points represents the degree of mixing (\( \sigma^2_v \)).

5. Mixing Mechanisms: Longitudinal mixing occurs by the combined actions of convection and molecular diffusion. The contribution of convection to longitudinal mixing is proportional to the velocity gradient established in a tube. \( \text{a)} \): If the time
necessary for diffusion is much greater than the time necessary for convection, then velocity gradients axially distort the tracer distribution establishing an axial and radial concentration gradient. During this process, radial mixing does not occur and the particles follow the flow stream lines increasing axial dispersion by the mechanism of axial streaming. If the time required for convection is roughly equal to or less than the time required for diffusion, then diffusion contributes to the degree of longitudinal mixing. Taylor diffusion occurs when diffusion and convection are at equilibrium. Diffusion reduces the concentration gradient.

6. Velocity Profiles at Airway Bifurcations: Velocity profiles are deformed as flow passes through a bifurcation. Inspiratory velocity profiles tend to be more parabolic than expiratory profiles. During inspiration, the region of maximum velocity at the center of a parent airway reaches a stagnation point at a bifurcation. At the beginnings of daughter airways, the velocity profile is skewed and the maximum velocity occurs near the inside edge of the airway. Secondary flows curve around the wall from the inside to the outside edges. On expiration two daughter airways empty into the parent airway. Four vortices are formed as the two flows combine. The occurrence of these vortices reduces the radial velocity gradient.
7. Aerosol Bolus Delivery and Acquisition System: Aerosol is produced by nebulizing a solution of 1 gram TTP dissolved in 50 ml ethanol. The evaporation of ethanol produces particles of TTP in the 0.5 μm size range. Aerosol passes through a charcoal filter for ethanol vapor removal and flow is continuously exhausted. A bolus is produced by the activation of a solenoid valve, which allows aerosol to dilute with a volumetric small portion of a subject’s inspiratory volume. Subject flow rates and aerosol concentration are monitored by a pneumotachograph and photometer, respectively. Data acquisition is started near the end of an expiratory cycle, a bolus is delivered into the subsequent inspiration and data is recorded at a rate of 200 Hz for 10 seconds.

8. Subject Maneuvers: All maneuvers were started at functional residual capacity. A 1 liter tidal volume ($V_T$) was common to both males and females. Due to male-females differences in total lung capacity, a larger $V_T$ of 2 liters was used for males and 1.5 liters was used for females. Bolus were positioned at 250 ml increments in each $V_T$. Following an inspiration containing a bolus, subjects were instructed to exhale completely. For each $V_{T_{max}}$ there were a total of 24 different maneuvers performed by females and 30 performed by males. Flow rates were maintained at 40 lpm for all maneuvers.
9. **Signals used in Data Analysis:** Signals were acquired at a 200 Hz sampling rate. Prior to signal analysis the concentration signal was smoothed at 50 Hz with a digital low pass filter. The cumulative volume used in calculations was obtained by time integration of the flow signal.

10. **Schematic of a Bolus:** The above bolus was targeted for a volumetric penetration ($\bar{V}_t$) of 750 ml in a 2 liter $V_T$. In this example, the actual $V_T$ was 50 ml greater than the target $V_T$. The extra inspiratory volume slightly increased the actual $\bar{V}_t$. Insufficient inhalation would have caused an decrease in $\bar{V}_i$. The points $a_j$ and $b_j$, are the integration limits for a bolus, where the subscript $(j)$ denotes either inspiration $(i)$ or expiration $(e)$. On the volume axis, $0$ is the point of maximum inspiration.

11. **Statistical Variance in Dispersion measurements:** Volume variance, $\sigma^2$, is used as an experimental measure of dispersion. The statistical variance in measurements escalated with increasing dispersion. A log transformation of the dispersion measurements improves the homogeneity of statistical variance across the experimental data range.

12&13. **Effect of Tidal Volume:** Comparison of the dispersion of a 150 ml bolus shows no effect of the inspiratory volume in females or males. This trend was also observed with the 75 and 300 ml bolus.
14&15. **Effect of Bolus Volume:** Dispersion of the 300 ml bolus was found to be significantly lower than the dispersion of the 75 and 150 ml bolus in females and males.

16-18. **Gender Effects:** Although no statistical effect of gender was found, dispersion appears to be greater in males than females.

19. **Volume Shift in Males and Females:** $\tilde{V}_c$ was greater than $\tilde{V}_i$ for all subjects. The mean shift was slightly larger for males than females.
Figure 1: Idealization of Human Airways
(Adapted from ER Weibel, 1963)
Figure 2: Static Lung Volumes
Figure 3: Illustration of Velocity Profiles and Tracer Distribution
Figure 4: Impulse Response Experiment
Figure 5: Mixing Mechanisms

(a) AXIAL STREAMING
Convection without Diffusion

(b) TAYLOR DIFFUSION
Diffusion and Convection Equilibrium
Figure 6: Velocity Profiles at Airway Bifurcations
(Adapted Schroter and Sudlow, 1969)
Figure 7: Aerosol Bolus Delivery and Acquisition System
Figure 8: Subjects Maneuvers

**Females**

- TLC
- FRC
- RV

Lung Volume (liters)

- 79% TLC
- 92% TLC
- 62% TLC
- 3 sec
- 4.5 sec

**Males**

- TLC
- FRC
- RV

Lung Volume (liters)

- 62% TLC
- 75% TLC
- 3 sec
- 6 sec
Figure 9: Signals Used in Data Analysis
Figure 10: Schematic of a Bolus
Dispersion in Males at TV = 2, VBOL = 150

Figure 11
Dispersion in Females, $VBOI = 150$

![Graph showing dispersion in females with VBOI = 150. The x-axis represents volumetric penetration (I) and the y-axis represents log volume variance by HW (ml²). The graph includes data points for different tidal volumes (1.0 and 1.5) with error bars indicating variability.](image)
Dispersion in Males at \( VBOL = 150 \)

Figure 13
Dispersion in Females at TV = 1.5

**Figure 14**

Log [Volume Variance (m²/ℓ)]

- **Volumetric Penetration (ℓ)**
  - Bolus Volume
    - 75
    - 150
    - 300

Axial plane: Axial plane 0.6 0.8 1 Volumetric Penetration (I)

Z-axis: Bolus Volume

Figure 14
Dispersion in Males at TV = 2

![Figure 15](image)

**Figure 15**
Dispersion in Males and Females at $TV = 1$, $VBOL = 75$

Figure 16
Dispersion in Males and Females at TV = 1, VBOL = 150

Figure 17
Dispersion in Males and Females at TV = 1, VBOL = 300

Figure 18
Figure 19

Volume Shift in Males and Females

Subject Gender

+ Male

Diamond Female
Table 1. Experimental Design of Bolus Experiment

<table>
<thead>
<tr>
<th>( V_T ) (ml)</th>
<th>( \bar{V}_i ) (ml)</th>
<th>FEMALE (N=12)</th>
<th>MALE (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vbol (ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>150</td>
</tr>
<tr>
<td>1000</td>
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<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>X</td>
<td>X</td>
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<tr>
<td></td>
<td>750</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1500</td>
<td>250</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>1250</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2000</td>
<td>250</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>1250</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>1750</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
### Table 2: Subject and Primary Function Data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Function</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The table includes columns for subject data, age, gender, function, and various data points. The specific data values are not legible due to the image quality.
## Table 3. Analysis of Variance of $\log_{10}(\sigma_y^2)$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEX</td>
<td>1</td>
<td>1.1335</td>
<td>1.55</td>
<td>0.227</td>
</tr>
<tr>
<td>ERROR A</td>
<td>21</td>
<td>0.7326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_T$</td>
<td>2</td>
<td>0.0107</td>
<td>1.95</td>
<td>0.143</td>
</tr>
<tr>
<td>$\bar{V}_i$</td>
<td>6</td>
<td>10.1459</td>
<td>1853.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vbol</td>
<td>2</td>
<td>0.0739</td>
<td>13.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$V_T \times \bar{V}_i$</td>
<td>4</td>
<td>0.0067</td>
<td>1.22</td>
<td>0.300</td>
</tr>
<tr>
<td>$V_T \times$ Vbol</td>
<td>4</td>
<td>0.0035</td>
<td>0.63</td>
<td>0.640</td>
</tr>
<tr>
<td>VP x Vbol</td>
<td>12</td>
<td>0.0052</td>
<td>0.94</td>
<td>0.503</td>
</tr>
<tr>
<td>$V_T \times \bar{V}_i \times$ Vbol</td>
<td>8</td>
<td>0.0025</td>
<td>0.46</td>
<td>0.887</td>
</tr>
<tr>
<td>SEX x $\bar{V}_i$</td>
<td>2</td>
<td>0.0073</td>
<td>1.34</td>
<td>0.263</td>
</tr>
<tr>
<td>SEX x Vbol</td>
<td>2</td>
<td>0.0025</td>
<td>0.46</td>
<td>0.630</td>
</tr>
<tr>
<td>SEX x $\bar{V}_i \times$ Vbol</td>
<td>4</td>
<td>0.0101</td>
<td>1.85</td>
<td>0.119</td>
</tr>
<tr>
<td>ERROR B</td>
<td>543</td>
<td>0.0055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>617</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Effect of Gender on Dispersion

<table>
<thead>
<tr>
<th>Variable</th>
<th>SEX</th>
<th>N</th>
<th>MEAN</th>
<th>S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log_{10}(\sigma_v^2) )</td>
<td>FEMALE</td>
<td>108</td>
<td>4.19</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>MALE</td>
<td>99</td>
<td>4.34</td>
<td>0.086</td>
</tr>
</tbody>
</table>

* Computed using the error mean square from the analysis of variance in Table 3.

Table 5. Effect of Lung Inflation in Females

<table>
<thead>
<tr>
<th>Variable</th>
<th>( V_T )</th>
<th>N</th>
<th>MEAN</th>
<th>S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log_{10}(\sigma_v^2) )</td>
<td>1000</td>
<td>108</td>
<td>4.19</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>108</td>
<td>4.18</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* Computed using the error mean square from the analysis of variance in Table 3.

Table 6. Effect of Lung Inflation in Males

<table>
<thead>
<tr>
<th>Variable</th>
<th>( V_T )</th>
<th>N</th>
<th>MEAN</th>
<th>S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log_{10}(\sigma_v^2) )</td>
<td>1000</td>
<td>99</td>
<td>4.34</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>99</td>
<td>4.32</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* Computed using the error mean square from the analysis of variance in Table 3.
### Table 7. $\tilde{V}_1$ Main Effect Means for $\log_{10}(\sigma_y^2)$

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\tilde{V}_1$</th>
<th>N</th>
<th>Mean</th>
<th>S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\log_{10}(\sigma_y^2)$</td>
<td>250</td>
<td>138</td>
<td>4.00</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>138</td>
<td>4.27</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>138</td>
<td>4.49</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>69</td>
<td>4.68</td>
<td>0.009</td>
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<tr>
<td></td>
<td>1250</td>
<td>69</td>
<td>4.88</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>33</td>
<td>5.08</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>1750</td>
<td>33</td>
<td>5.24</td>
<td>0.013</td>
</tr>
</tbody>
</table>

* From Analysis of Variance

### Table 8. $V_{bol}$ Main Effect Means for $\log_{10}(\sigma_y^2)$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vbol</th>
<th>N</th>
<th>Mean</th>
<th>S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\log_{10}(\sigma_y^2)$</td>
<td>75</td>
<td>206</td>
<td>4.48</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>206</td>
<td>4.48</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>206</td>
<td>4.44</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* From Analysis of Variance
Table 9. Volume Shift by Levels of Penetration

<table>
<thead>
<tr>
<th>( \bar{V}_i )</th>
<th>N</th>
<th>( \bar{V}_c-\bar{V}_i )</th>
<th>S.E.*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>138</td>
<td>115</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>500</td>
<td>138</td>
<td>110</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>750</td>
<td>138</td>
<td>102</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1000</td>
<td>69</td>
<td>106</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1250</td>
<td>69</td>
<td>80</td>
<td>2.2</td>
<td>&lt;0.001</td>
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<tr>
<td>1500</td>
<td>33</td>
<td>72</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1750</td>
<td>33</td>
<td>25</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* From Analysis of Variance
** Ho: \( \bar{V}_c-\bar{V}_i=0 \)

Table 10. Volume Shift by Levels of Bolus Volumes

<table>
<thead>
<tr>
<th>VBOL</th>
<th>N</th>
<th>( \bar{V}_c-\bar{V}_i )</th>
<th>S.E.*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>206</td>
<td>95</td>
<td>1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>150</td>
<td>206</td>
<td>99</td>
<td>1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>300</td>
<td>206</td>
<td>104</td>
<td>1.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* From Analysis of Variance
** Ho: \( \bar{V}_c-\bar{V}_i=0 \)