AEROSOL BOLUS DISPERSION (ABD) has been proposed as a sensitive measure of small airway function (3, 5, 13, 15, 20). This technique consists of inserting a volumetrically small bolus of aerosol into a subject’s tidal volume. As the aerosol travels through the airways of the lung, it becomes mixed with particle-free air, such that, on expiration, aerosol is dispersed over a larger volume of air than was the inhaled bolus. Relative to healthy nonsmokers, ABD has been found to be increased in asymptomatic smokers (3, 5, 15), in patients with cystic fibrosis (CF; Ref. 1), and in healthy young adults after acute ozone exposure (13). All studies have found ABD to increase with the volumetric penetration ($V_p$) of boluses, i.e., the volume of clean air inhaled following the center of a bolus (1, 3, 5, 7, 13, 15, 20). In the healthy lung, ABD is thought to be largely due to convective mixing and nonreversal of axial streaming (7, 20, 26). It has been speculated, however, that nonuniform distribution of ventilation due to regional differences in pulmonary resistance and compliance becomes an increasingly important factor affecting ABD in compromised lungs (1, 3, 4, 13, 19). Yet no studies have shown a direct relationship between dispersion and ventilation distribution indexes in normal subjects or diseased patients. Regional time constants, regional lung volumes, and $V_p$ may all play a role in determining the linear distance inhaled aerosol travels and the distribution of aerosol among airways at that distance. In a modeling study, Rosenthal (19) predicted that boluses penetrating a modest depth ($V_p$ of 400 ml) into the lungs should be more sensitive to nonuniform ventilation between parallel compartments than should deeper boluses, which may preferentially go to better ventilated compartments, or shallow boluses not penetrating beyond the dead space. At such an intermediate depth, an inhaled bolus might divide between fast- and slow-ventilated regions so that the sequential emptying of regions may cause particles, adjacent on inspiration, to be separated by volume on expiration, thereby increasing ABD. For two compartments of equal volume in parallel, Rosenthal (19) predicted ABD to increase 1) with increasing disparity in regional time constants and 2) with the magnitude of time constants when the ratio of time constants is maintained.

Dynamic convective inhomogeneities also influence multiple-breath gas washouts. The washout of either a single compartment or parallel compartments with equal time constants should be a monoexponential function of time when constant flow rates are maintained (10, 18). On the other hand, disparities in time constants between parallel compartments may cause a multieponential washout, the shape of which depends on the relative size of compartments, the concentration inhomogeneities between compartments before washout, and the magnitude of the difference in the time constants (18). Multiple-breath $^{133}$Xe washout ($MBW_{xe}$) has been utilized as a means of assessing regional ventilation distribution and whole lung washout (17, 21, 23–25). Patients with severe obstructive lung disease not only have slow total washouts but also have markedly nonuniform washout across the lungs (21).

We hypothesize that the increased ABD observed in CF patients, relative to normal subjects (1), is partially attributable to altered ventilation distribution in those patients. To investigate this hypothesis, we have compared aerosol bolus measurements with measures of $MBW_{xe}$ in healthy subjects and CF patients.

MATERIALS AND METHODS

Subjects. Fourteen patients with CF (4 men, 10 women; 20–45 yr of age) and nine healthy nonsmoking male volunteers (18–40 yr of age) were recruited to participate in this study. To be recruited for the study, healthy subjects were required to have a ratio of forced expired volume in 1 s (FEV$_1$) to forced vital capacity (FVC) ($FEV_1$/$FVC) \geq 70\%$ and $FEV_1 \geq 90\%$ of predicted, based on the equations of Knudson et al. (14). Healthy female subjects were not recruited to minimize the number of women of childbearing age exposed to radia-
tion in our study. Patients with CF had mild-to-moderate airway disease with FEV1 (%predicted) ranging from 49 to 77%. All subjects were free of acute respiratory infection for at least 2 wk before participation in the study. 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. Before participating in the study, all subjects were informed of study-associated risks and asked to sign a statement of informed consent.

Aerosol bolus measurements. Detailed methodology for the bolus technique has been presented previously (7). Aerosol boluses were inserted into a 1.25-liter tidal volume, inhaled from functional residual capacity, at the Vp values of 250 mL (Vp50) and 500 mL (Vp500). Vp is the volume of air following the center, i.e., the volumetric mean, of a bolus. After a bolus inspiration, subjects exhaled to residual volume. Inspiratory and expiratory flow rates were kept constant at 0.4 l/s. Inhaled boluses were targeted to have a volumetric width of 150 mL. Experimentally, 95% of aerosol was confined in 180 mL of the inhaled breath. Boluses were composed of a 0.5-µm polydisperse (geometric SD = 1.5) aerosol of triphenyl phosphate (7, 13). All measurements were conducted with the subject in a seated position. Three to four maneuvers were conducted at each Vp. Flow and aerosol concentration from each maneuver were acquired at a 200-Hz sampling rate and were saved for subsequent data analysis.

Data analysis was based on the first three moments of the bolus distribution with respect to respired volume (i.e., the integrated respired flow). The zeroth moment is the area under the curve (AUC). Aerosol recovery (R) is the ratio of the AUC exhaled to the AUC inhaled. The first moment is the center of mass of a bolus with respect to volume and is also referred to as the mean volume of a bolus. Volumetric mean shift (ΔV) is the mean volume of an expired bolus minus that of the inspired bolus. The second moment is variance. Volume variance was used as an index of ABD and is the variance of an exhaled bolus minus that of the inhaled bolus. If the pattern of lung emptying exactly mirrored that of filling and all particles retracted streamlines, then an exhaled bolus would be exactly the same as an inhaled bolus, i.e., R would equal 1.0 and both ΔV and ABD would be zero. The indexes of ABD, R, and ΔV from the individual maneuvers were averaged for comparisons to gas washout tests.

133Xe washout test. MBW133Xe was conducted with subjects seated in front of a gamma camera (Elscint Apex 415; large field of view). During the washout, subjects breathed a 1.2-liter tidal volume with tidal flows of 0.4 l/s to mimic the breathing pattern used for the bolus maneuver. Subjects rebreathed 133Xe in air until equilibrium was reached, i.e., until a constant count rate was detected by the camera. At equilibrium, acquisition was initiated with a total of 32 images collected: 4 at equilibrium and 28 during a washout period where 133Xe-free air was inhaled and exhaled 133Xe was captured in a charcoal filter. Each image was acquired for 6 s, which was the subject’s breathing period.

Whole lung washout was characterized by fitting single- and double-exponential decay curves to each subject’s washout, i.e., $Xe(t) = e^{-t}$ and $Xe(t) = F_1 e^{-t} + F_2 e^{-t}$, respectively, where $Xe(t)$ is 133Xe counts at time t, normalized to 133Xe counts at t = 0; $t_1$ and $t_2$ are washout rate (min$^{-1}$); $F_1$ and $F_2$ are coefficients, which sum to one, and represent the relative amount (fraction) of 133Xe in the early (fast) and late (slow) phase of washout, respectively; and $F_1$ and $F_2$ are coefficients, which sum to one, and represent the relative amount (fraction) of 133Xe in the early (fast) and late (slow) phase of the washout, respectively. 133Xe washout curves were generated by summing the counts in both lungs for each 6-s image, dividing by the sum of lung counts from the fourth equilibrium image, and plotting as a function of time after equilibrium. Double-exponential decay curves were fit to each subject’s washout by using commercially available curve-fitting software (CurveExpert 1.3, Starkville, MS). For each subject, the $t_1$ and the squared sample correlation coefficient or goodness of fit (GoF) for the single-exponential function, and the $F_2$ for the double-exponential function were determined for comparison to bolus parameters. GoF describes how closely a subject’s washout follows a single-compartment washout. GoF has an upper limit of one for a perfect single-exponential fit and decreases as a washout deviates toward a multiexponential decay.

A regional analysis of each subject’s 133Xe washout was also performed on the 50% washout image. Before analysis, the 50% washout image was normalized for lung volume by dividing by the fourth equilibrium image. Each lung was then divided into thirds by height, establishing a total of six regions of interest. Two indexes of the regional distribution were determined. One index was an apex-to-base ratio (A/B), which is the total counts in the two apical regions divided by the total counts in the two basal regions. Differences in regional ventilation distribution are reflected by deviations in A/B from one. The second index was the SD of the counts for all six lung regions at 50% 133Xe washout (SD50%). A perfectly even washout of all six regions would result in a SD50% equal to zero. SD50% increases with increasing heterogeneity of the 133Xe washout.

Pulmonary function tests. Forced-expiratory maneuvers were conducted with the subject in a standing position. Three acceptable trials were obtained from each subject. The largest single FEV1 and FVC values, regardless of trial, were used in the calculation of FEV1/FVC and for percent predicted FEV1. Percent predicted FEV1 (14) was determined for comparisons with bolus data.

Full vital capacity, single-breath nitrogen washouts were conducted with the subject in a seated position (8, 9). Trials for which the mean expiratory flow exceeded 0.5 l/s in the first 0.5 liter of expiration were discarded (9). Phase III slope ($N2$) was estimated for each maneuver (11). The $N2$ values from acceptable maneuvers were averaged for comparisons with bolus parameters.

Data analysis (comparisons of tests). The primary focus of this study was to compare ABD with MBW133Xe indexes of ventilation distribution. Pearson correlation coefficients were utilized for comparisons between ABD for each $V_p$ and A/B, SD50%, $\lambda$, GoF, and $F_2$. As a secondary analysis, we also compared R and $\Delta V$ with these parameters. Because each bolus parameter (ABD, R, $\Delta V$) was measured at two depths into the lung, there were 10 comparisons between each bolus parameter and other indexes. A Bonferroni correction was applied, and a significance level of 0.005 was adopted. For comparison with the findings of Anderson et al. (1, 3), correlations were also determined between bolus parameters of R and ABD and both spirometry (%predicted FEV1) and $\Delta N_2$. Statistical significance for within- and between-group differences were tested by dependent (paired) and independent two-tailed Student’s t-test, respectively.

RESULTS

Table 1 provides a summary of mean data for the normal and CF groups. Bolus measurements and spirometry were available on all subjects. Nitrogen washout was not conducted in one CF patient because of instrument malfunction. The MBW133Xe data of one CF patient were inadvertently deleted during analysis so that indexes of A/B and SD50% were not available.
Table 1. Mean experimental data grouped by disease status

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects</th>
<th>Cystic Fibrosis Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>26.4 ± 6.2</td>
<td>29.9 ± 8.3</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>105.8 ± 9.0</td>
<td>62.6 ± 8.7*</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>78.8 ± 5.0</td>
<td>62.9 ± 5.8*</td>
</tr>
<tr>
<td>FEF25–75, l/s</td>
<td>4.25 ± 1.00</td>
<td>1.12 ± 0.33*</td>
</tr>
<tr>
<td>∆N2, %/s</td>
<td>0.70 ± 0.25</td>
<td>4.97 ± 1.57*</td>
</tr>
<tr>
<td>A/B</td>
<td>23 ± 9</td>
<td>33 ± 10†</td>
</tr>
<tr>
<td>ABD250, ml/l</td>
<td>35 ± 8</td>
<td>49 ± 18†</td>
</tr>
<tr>
<td>R250, %</td>
<td>97 ± 6</td>
<td>82 ± 9†</td>
</tr>
<tr>
<td>R500, %</td>
<td>93 ± 8</td>
<td>69 ± 10*</td>
</tr>
<tr>
<td>∆V250, ml</td>
<td>22 ± 25</td>
<td>50 ± 30†</td>
</tr>
<tr>
<td>∆V500, ml</td>
<td>6 ± 11</td>
<td>-18 ± 45</td>
</tr>
<tr>
<td>A/B</td>
<td>1.27 ± 0.13</td>
<td>1.66 ± 0.30*</td>
</tr>
<tr>
<td>SD50%</td>
<td>0.07 ± 0.02</td>
<td>0.12 ± 0.04†</td>
</tr>
<tr>
<td>GoF</td>
<td>0.95 ± 0.06</td>
<td>0.89 ± 0.06†</td>
</tr>
<tr>
<td>λ, min⁻¹</td>
<td>0.89 ± 0.11</td>
<td>0.62 ± 0.12*</td>
</tr>
<tr>
<td>F5, %</td>
<td>18 ± 25</td>
<td>35 ± 15†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. FEV1, forced expired volume in 1 s; FVC, forced vital capacity; FEV1/FVC, ratio of FEV1 to FVC; FEF25–75, average forced expiratory flow over middle half of FVC; ∆N2, phase III slope; ABD250 and ABD500, aerosol bolus dispersion at volumetric depth of 250 and 500 ml, respectively; R250 and R500, aerosol recovery at 250 and 500 ml, respectively; ∆V250 and ∆V500, mean shift at 250 and 500 ml, respectively; A/B, 133Xe counts in apex divided by that in base; SD50%, SD of counts between 6 lung regions at 50% 133Xe washout; GoF, goodness of fit; λ, 133Xe washout rate; F5, coefficient of fraction of 133Xe in a slow compartment. Significantly different from normal subjects at *P < 0.001, †P < 0.005, ‡P < 0.01.

Typical aerosol boluses for a normal subject and CF patient are illustrated in Fig. 1. The MBWXe indexes in the normal subjects.

ABD and R were significantly different between the CF and normal groups at both Vp levels (Table 1). Within groups, measurements made at Vp250 were significantly different from those at Vp500 for both ABD and R (P < 0.02). As seen in our previous study (7), ABD and ∆V were correlated in both groups at Vp250 (P < 0.005) and Vp500 (P < 0.05). No associations were observed between ABD and R at either Vp or in either CF patients or normal subjects.

In the CF patients, but not in the normal subjects, we found significant correlations between bolus parameters and ventilation indexes obtained by MBWXe (Table 2). The highly significant associations observed in the CF patients between ABD and λ, as well as F5 at Vp500, are illustrated in Figs. 2 and 3, respectively. As shown in Table 2, weak associations (not significant after Bonferroni correction) were also observed between ventilation parameters (λ and F5) and both ABD at Vp250 and ∆V at Vp500. The associations observed between R and GoF at Vp500 are illustrated in Fig. 4. Neither of the regional ventilation indexes, A/B and SD50%, was correlated with bolus parameters. No significant correlations were observed between bolus parameters and MBWXe indexes in the normal subjects.

Consistent with the results of Anderson et al. (1), significant correlations between spirometry and ABD were also observed in the CF patients: FEV1 (%predicted) correlated with ABD at Vp500 (r = -0.63, P = 0.02) and to a lesser extent at Vp250 (r = -0.51, P = 0.06). As in smokers (3), there were no significant correlations between the single-breath nitrogen ∆N2 and bolus measurements observed in either normal subjects or CF patients.

DISCUSSION

We investigated the influence of ventilation distribution on aerosol bolus measurements in health and disease. ABD, R, and ∆V were compared with MBWXe in
normal subjects and CF patients. We made comparisons with five indexes of ventilation distribution from the MBW\textsubscript{Xe} test: 1) $\lambda$, which is the total washout rate of the lungs and is decreased by poor ventilation of obstructed regions; 2) $F_s$, which is the fraction of the lung that was poorly ventilated; 3) GoF, which reflects the degree to which the lungs work as a single, presumably well-ventilated compartment and the uniformity of ventilation between compartments; 4) A/B, which indicates uniformity of ventilation between apical and basal regions of the lungs; and 5) SD\textsubscript{50\%}, which reflects the heterogeneity of washout rates across six gross regions of the lung. Comparisons between these indexes and ABD and R suggest that these bolus parameters are both affected by nonuniform ventilation distribution induced by airway obstruction.

Associations between bolus measurements and ventilation distribution were only observed in the CF patients. Spirometric data show that these patients had significant airway obstruction, relative to normal subjects, based on decreased $FEV_1/FVC$ and the forced expiratory flow over the middle half of FVC (Table 1). Patients also exhibited marked nonuniform ventilation distribution, relative to normal subjects, as evidenced by slow washouts (increased $\lambda$) and multiexponential $^{133}\text{Xe}$ washouts (decreased GoF and increased $F_s$). We have made no attempt to quantify the degree to which convection vs. diffusion-dependent inhomogeneities are affecting ventilation distribution. However, for particles in the size range of the experimental aerosol and larger, it is well accepted that aerosol mixing in the lung is predominantly convective (20, 26). Thus the associations between bolus parameters and the MBW\textsubscript{Xe} indexes observed in the CF patients are likely attributable to the influence of convective inhomogeneities on these measurements.

ABD in the CF patients was found to be associated with measures of ventilation distribution in a depth-dependent fashion, i.e., ventilation distribution had a greater influence on ABD at the deeper $V_p$ ($V_{p500}$) into the lung. In the CF patients at $V_{p500}$, ABD was correlated with $F_s$ ($r = 0.89, P < 0.001$) and $\lambda$ ($r = -0.76, P < 0.005$). The $\lambda$ is decreased by the poor ventilation of obstructed regions in the CF patients, and the fraction of the lung that is poorly ventilated is reflected by $F_s$. The strong correlations between ABD and both $\lambda$ and $F_s$ suggest that, with increasing severity of obstruction, there is an increasing probability of an inspired bolus being divided between fast and slow (i.e., obstructed) regions and that differing time constants between these regions will cause sequential emptying, thereby enhancing ABD. The late expiration of aerosol from the slow regions may also exhibit itself as a long tail on the exhaled bolus and account for the positive association observed between $F_s$ and $\Delta V$ ($r = 0.56, P < 0.05$) in the patients at $V_{p500}$. At $V_{p500}$ on the basis of the correlations with $F_s$ and $\lambda$, ABD is clearly influenced by inhomogeneities in the distribution of ventilation between compartments as well as the pattern or sequence of lung filling and emptying.

At the shallower $V_p$, ABD did not show the strong associations with ventilation distribution apparent at $V_{p500}$. At $V_{p250}$ ABD was only weakly associated with $F_s$ ($r = 0.60, P < 0.05$) and $\lambda$ ($r = -0.58, P < 0.05$). These weaker associations at $V_{p250}$ suggest that deeper aerosol bolus inhalations may be important to detect nonuniform ventilation distribution brought about by airway obstruction. It may be that $V_{p250}$ was close enough to the end of inspiration so as to have the inhaled aerosol preferentially enter slow filling regions. Aerosol inhaled into slow regions may be exhaled out later than expected because convective inhomogeneities may alter the sequence of lung emptying from that of “last in, first out” to “first in, last out” (10, 16). The CF patients showed peripheral $\Delta V$ (50 ± 30 ml) that was significantly greater than the $\Delta V$ (22 ± 25 ml) observed in normal subjects at $V_{p250}$ ($P < 0.05$). Despite weak associations with our measures of ventilation distribution, ABD was still significantly greater in patients than in normal subjects at $V_{p250}$ ($P < 0.05$).

The R correlated well with GoF at both $V_{p250}$ ($r = 0.73, P = 0.003$) and $V_{p500}$ ($r = 0.72, P = 0.003$). As the lungs shift from a single, presumably well-ventilated system toward a multicompartment system with healthy and obstructed lung regions, R decreases (Fig. 4). Particles that entered poorly ventilated regions will
be exhaled slower and later than particles that entered healthy regions, even though the two sets of particles were similarly localized within the inspired bolus. Increased residence time in slow regions and sites of airway obstruction, which may have induced the ventilatory inhomogeneities, could enhance deposition (12). Indeed, an increased regional deposition fraction for poorly ventilated areas has been reported for aerosols in the 0.5- to 1.0-µm size range and has been attributed to increased residence time (23, 25). It has been demonstrated in healthy children with total lung capacity (TLC) ranging from 2.1 to 5.3 liters that, for a given Vp, into the lung, deposition of inhaled particles increases with decreasing TLC, whereas ABD is unaffected (22). The enhanced deposition by sedimentation and diffusion is attributed to smaller dimensions associated with smaller TLC. Similar to the effect of TLC on deposition, the preferential ventilation suspected at Vp 250 may produce an increase in deposition by taking aerosol to smaller airway dimensions than would occur with uniform ventilation patterns.

There were no correlations found between ABD or R and regional measures of ventilation distribution, SD50% and A/B, nor were these latter parameters correlated with other indexes of ventilation distribution. Both SD50% and A/B were significantly increased in patients compared with normal subjects, indicating larger inter-regional disparities in ventilation within the patients. Specifically, A/B and SD50%, both increase with decrements in ventilation to the apical regions of the lungs relative to the basal regions. Gross interregional differences in ventilation, i.e., partial obstruction of an entire lung or multiple lobes, should, theoretically, increase ABD, and they have been shown clinically in one patient to do so (4, 19). For CF patients in our study, however, the damage to the lungs was not so distinct between lung lobes (e.g., a unilateral bronchial stenosis) (4) so as to lead to correlations between ABD and the indexes SD50% and A/B.

In conclusion, the results of this study show that aerosol bolus measurements of ABD and R are related to convective inhomogeneity of ventilation in patients with CF. The influence of uneven ventilation on ABD in these patients was greater when boluses were delivered deeper (500 vs. 250 ml) into the lungs. On the other hand, given the lack of correlations between indexes of ventilation distribution and ABD in normal subjects, it appears that convective inhomogeneity of ventilation is not a major mechanism of dispersion in the normal lung.

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Address for reprint requests: J. S. Brown, Center for Environmental Medicine and Lung Biology, Univ. of North Carolina at Chapel Hill, CB #7310, 104 Mason Farm Rd., Chapel Hill, NC 27599-7310.


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