Ventilation heterogeneity in excised lobes: effect of tidal volume

MICHAEL J. EMERY,1 JACOB HILDEBRANDT,1,2 AND MICHAEL P. HLASTALA1,2
Departments of 1Physiology and Biophysics and 2Medicine, University of Washington, Seattle, Washington 98195

Emery, Michael J., Jacob Hildebrandt, and Michael P. Hlastala. Ventilation heterogeneity in excised lobes: effect of tidal volume. J Appl Physiol 88: 1659–1671, 2000.—Although several factors are known to influence nonuniformity of ventilation, including lung mechanical properties (regional structure and compliance), external factors (chest wall, pleural pressure, heart), and ventilatory parameters (tidal and preinspiratory volume, flow rate), their relative contributions are poorly understood. We studied five excised, unperfused, canine right-middle lobes under varied levels of tidal volume (VT), thus eliminating many factors affecting heterogeneity. Multiple-breath washouts of N2 were analyzed for anatomic dead space volume (VDanat), nonuniformity of N2 washout, and nonuniformity between joined acinar regions vs. that occurring between larger joined regions. Approximately 80% of ventilation heterogeneity was found among joined acinar regions at resting levels of VT, but increasing VT reduced intra-acinar heterogeneity to about 25% of that found at resting levels. Increasing VT had essentially no effect on VDanat and heterogeneity among larger joined regions. The results indicate that the magnitude of VT is a major influence on the dominant intra-acinar component of ventilation heterogeneity and that VT effects on VDanat are likely due to perfusion and/or influences normally external to the lobar structure.

intraregional ventilation distribution; phase III slope; multiple breath inert-gas washout; normalized phase III slope

INCREASING TIDAL VOLUME (VT) has long been known to increase exhaled anatomic dead space volume (VDanat) and alter the uniformity of ventilation distribution (1, 6, 15, 24, 27). Human studies suggest that increasing VT tends to increase the uniformity of gas distribution among very small joined airway regions on the scale of acini (6, 24), whereas ventilation between larger regions becomes more uneven (6). Quantitative and mechanistic understanding of these VT-dependent changes remains incomplete, in part because most evidence is from studies of intact animals and humans in which many influences on distribution of ventilation act simultaneously. In addition, methodological constraints have limited measurement of intra-acinar ventilation heterogeneity, and this component may dominate the nonuniformity of gas distribution in the whole lung (8). Also, although it is generally accepted that VT-dependent changes in VDanat are due to enlargement of proximal airways (1), computer models designed to test mechanisms of gas mixing within peripheral or proximal airways also predict VT effects on VDanat (9, 17, 25), and the relative contributions of these mechanisms have not been clearly demonstrated.

We undertook a reductionist approach to this problem by measuring ventilation heterogeneity in excised, suspended canine right-middle lobes under tightly controlled conditions of negative-pressure breathing and varied VT. Other ventilatory parameters, including preinspiratory volume, inspiratory time (T1), expiratory time (T2), and volume history, were held constant. This preparation removed most extralobar influences on ventilation distribution, including chest wall and diaphragm, the beating heart, and the normal gravity-determined pleural pressure gradient. Presently, the most sensitive and advanced method of assessing the uniformity of ventilation distribution is normalized phase III slope (SIII) analysis of exhaled inert gas from multiple breath washout (MBW) (12, 13). A biexponential curve-fit approach to SIII analysis was developed for these studies (see APPENDIX A) and used to separate diffusive-convective-dependent inhomogeneity (dci) of gas mixing that occurs among joined acinar airways (intraregional) from that which occurs between airways separating larger regions (interregional) due to only convective-dependent inhomogeneity (cdi). VDanat was assessed from the same data. The results clearly demonstrate that, in this preparation, increasing VT over the normal physiological range largely eliminates the dominant intraregional component of ventilation heterogeneity found at resting levels of VT but has little or no influence on interregional heterogeneity and VDanat.

METHODS

Apparatus and General Procedures

All animal procedures were reviewed and approved by the University of Washington’s Animal Care Committee.

Anesthesia was induced in five mongrel dogs (20–30 kg) of either sex by bolus injection of thiopental sodium (20 mg/kg iv) followed, as needed, by bolus injections of pentobarbital sodium (30–120 mg iv) to maintain a deep surgical plane. After induction, the animals were intubated and ventilated by piston pump ventilator (model 608, Harvard Apparatus, South Natick, MA) at a fixed VT of 15 ml/kg with zero end-expiratory pressure (EEP). Ventilatory rate was varied to maintain normal end-expired CO2, arterial O2, and arterial
pH status, and an occasional "sigh" was given by double inflation. Concentrations of inspired and expired gases were continuously monitored with a respiratory mass spectrometer (model 1100, Perkin-Elmer, Pomona, CA), sampling gas midstream in a connector between the endotracheal tube and ventilator at a rate of 1 ml/s. The animals were anticoagulated with -6,000 units of sodium heparin and exsanguinated, and the thorax was then accessed via sternotomy. EEP was increased to +5 cmH2O to maintain the lung near normal end-expiratory volume, except when partial deflation was needed during the excision procedure to avoid pleural damage. The right-middle lobar bronchus was exposed by dissecting away surrounding blood vessels and connective tissue, carefully sparing the lobar pleura. The lobar bronchus was cross-clamped near the hilum at the end of a tidal inspiration, transected central to the cross-clamp, and mounted on a semirigid plastic tube (Tygon). The lung surface was occasionally misted with normal saline throughout the excision procedure.

The lobe was then suspended in a sealed plethysmograph (constructed for these experiments) that allowed air from outside of the box to flow into and out of the lobe through the mounting tube (Fig. 1). Rigid, closed-cell foam was used to fill much of the chamber volume not occupied by the lobe. The lobe was inflated by a negative box pressure (Pbox) of -5 cmH2O [i.e., to a transpulmonary pressure (Ptp) of +5 cmH2O] by a small vacuum pump adjusted to maintain a stable EEP. A computer-controlled linear-motor ventilator (custom designed and locally built) was used to remove and replace a volume of the box gas to produce the desired swing of Ptp and ventilate the lobe. This pressure-controlled system allowed VT, Ti, Te, and breath-hold time (after inspiration and/or expiration) to be varied independently. To achieve constant instantaneous gas flow rates during inspiration and expiration given the nonlinear compliance characteristics of the system (box, gas, lobe), a custom software program was developed and used to modify instantaneous computer voltage output controlling the ventilator. Negative Pbox was measured by a differential pressure transducer (±2.5 psi, Statham Instruments, Hato Rey, PR) and delivered to a chart recorder (DMS 1000, Western Graphtech, Irvine, CA). The signal was also fed to a separate computer for digital recording in a data file and for visual monitoring of pressure levels on a screen display during the experiments. The pressure transducer voltage signal was calibrated over the range of -55 cmH2O to +5 cmH2O.

Initially, air was removed from the box with a large syringe to produce several Ptp swings of 30 cmH2O (Pbox reduced from -25 to -235 cmH2O) to apply a standard volume history. A resting-level tidal breath was defined as a Ptp swing of 12 cmH2O (Pbox from -25 to -217 cmH2O). Ti and Te were set at 2 s and 4 s, respectively. The mass spectrometer sample inlet was positioned to access the center of the bronchial tube.
where it exited the box. A Krogh pediatric spirometer (locally built) was attached to this mounting tube when measurement of VT was needed. The spirometer was then removed and replaced on the mounting tube by a pediatric T-piece two-way valve (2230 series, Hans Rudolph, Kansas City, MO) arranged to deliver the marker and washout gases. Lobs were ventilated at 10 breaths/min with a gas mixture of 1% He-1% SF6 (balance air). Signals for concentrations of He, N2, and SF6, ventilated at 10 breaths/min with a gas mixture of 1% He-1% SF6 (balance air). Signals for concentrations of He, N2, and SF6, were stored in a computer file as digital values sampled at 36 Hz for each data-acquisition channel.

Initial gas concentrations were considered to be uniform throughout the lobe when concentrations of gases at the sampling site were invariant over the entire ventilatory cycle. After several minutes of ventilation with the chosen pattern, the inspired gas mixture was changed to 100% O2, and MBW of indicator gases was continued and recorded for at least 30 breaths. The lobar surface was maintained in moist condition by injecting ~50 ml of room-temperature normal saline through a misting system surrounding the lobe between each MBW.

Six end-inspiratory pressures (EIP) were chosen in a random manner to produce tidal Ptp swings from 5 to 35 cmH2O in 5-cmH2O increments. Apparatus dead space volume (VDapp) was ~6 ml, Ti and Te remained at 2 and 4 s, respectively, and EEP remained at ~5 cmH2O. After each washout, VT was returned to the resting level, a double inflation "sigh" was given, the next EIP was selected, VT was measured, and another MBW was performed. The final (seventh) MBW was carried out under the same conditions as the first to assess reproducibility and stability of measurements over the course of the experimental procedures.

Volume at end-expiration (lobe volume (VL)) for each lobe was estimated only from N2 MBW. With each breath of O2, the alveolar N2 is diluted by the fraction Vl/(Vl + VA) = 1/(1 + x), where alveolar volume (VA) equals VT - total dead space volume (VD), and x equals VA/VL. Thus after n breaths the ratio of the final phase III N2 concentration (F0N2) to the initial value (F0N2) is

\[ \frac{F_nN2}{F0N2} = \left( \frac{1}{1 + x} \right)^n \]  

or, in the log form, \( \log(F_n/F_0) = -n \log(1 + x) \). The slope of a graph of \( \log(F_n/F_0) \) vs. n has slope \( -\log(1 + x) \). Hence, x and then VL can be derived from washout data. In the presence of substantial heterogeneity of ventilation, this method is weighted toward the compartments with early washouts (i.e., less than the total VL).

Measurement of Exhaled Gas Dead Space Volume and Heterogeneity

VDanat - V0 was quantified from the N2 concentration profile of the second washout breath (Fig. 2A) by a method similar to that described by Young (36) but replacing CO2 by N2, in which volume is assessed to the point at which exhaled indicator gas concentration reaches 50% of the alveolar level (defined at 0.75 VT). VDanat was then obtained by subtracting VDapp from V0 (VDanat = VD - VDapp).

Early and late phase III slopes. MBW results in a series of diminishing exhaled marker gas concentration profiles that each display the three phases classically labeled as I, II, and III (Fig. 2B). Slopes of two portions of the phase III plateau were determined from each washout breath as indicators of alveolar gas concentration uniformity (Fig. 2A). Early emptying units were represented by marker gas measured from 0.5 to 0.75 of the exhaled VT (phase III(E)), and late emptying units were represented by 0.75 to 0.95 of VT (phase III(L)).

Fig. 2. Data analysis. A: a single exhaled N2 profile showing volumes defined as total dead space (Vo) and early (E) and late (L) portions of phase III slope. VT, tidal volume. B: an example of exhaled N2 concentration vs. time from first 2 breaths of a multiple-breath washout using 100% O2, showing characteristic phases I, II, and III. C: an example of concentration-normalized N2 phase III slopes (SnIII) vs. breath number, showing measured values (observed) and those determined by curve fitting, including total double exponential fit and its diffusive-convective-dependent inhomogeneity (dcdi) and convective-dependent inhomogeneity (cdi) components, as determined by the methods described in the text. Slope for final breaths (Sn) is determined from average of breath 21–25 (*); cdi max (liter−1) is value of SnIII cdicdili (liter−1) determined for breath 25.
Slopes were determined as a function of volume by best fitting a linear equation to data points obtained from phase IIIE and phase IIL.

Normalization and further analysis of phase III slopes. To discern contributions of only cdi and dcdi to nonuniformity of exhaled gas, a normalization procedure was employed in a manner similar to that described by Crawford et. al. (8), with several modifications (see Ref. 11 and APPENDIX A). To remove the effect of progressive gas dilution on slopes, the normalized slopes of phase IIIE ($S_{IIIE}$) and phase IIL ($S_{IIL}$) were found by dividing best fit slopes by the average concentration of marker gas in the respective intervals (i.e., E or L).

Measured values of $S_{IIIE}$ ($S_{IIIE_{observed}}$ and $S_{IIIE_{observed}}$) from each washout breath were plotted as a function of breath number (Fig. 2C). Contributions of convective-dependent inhomogeneity ($S_{IIL_{cdi}}$) and diffusive-convective-dependent inhomogeneity ($S_{IIL_{dcdi}}$) to the total calculated inhomogeneity of each washout breath ($S_{IIIE_{total}}$) were estimated by a curve-fitting procedure (Fig. 2C). For each breath of a MBW, cdi is assumed to add a slightly diminishing, but nearly continuous, additional interregional inhomogeneity of marker gas dilution to that present in each previous breath (23). Therefore, $S_{IIIE_{cdi}}$ as a function of $n$ is approximated by an equation similar in form to Eq. 2:

$$S_{IIIE_{cdi}} = m_3(1 - e^{-k_1 \cdot n})$$

where $m_3$ is the extrapolated asymptote of $S_{IIIE_{cdi}}$ vs. $n$ and $k_1$ is the rate constant of the cdi component.

The contribution of dcdi to the inhomogeneity of marker gas dilution during MBW has been found by model simulation (22) and experimental evidence (8, 32) to increase by sequentially reduced increments over only the first few breaths and to become constant after approximately breath 5 or 6. Therefore, diffusion-convection-dependent gas mixing is described by an equation similar in form to Eq. 2:

$$S_{IIIE_{dcdi}} = m_3(1 - e^{-k_2 \cdot n})$$

where $m_3$ is the asymptote of $S_{IIIE_{dcdi}}$ vs. $n$, and $k_2$ is the rate constant of the dcdi component.

The sum of Eqs. 2 and 3 yields Eq. 4, which is then fit to the plot of $S_{IIIE_{observed}}$ vs. $n$.

$$S_{IIIE_{total}} = m_3(1 - e^{-k_1 \cdot n}) + m_2(1 - e^{-k_2 \cdot n})$$

Parameters in Eq. 4 were simultaneously determined by a standard least squares method. To begin the iterative best fit procedure, initial values of $m_3$ and $k_2$ were set ~2 orders of magnitude larger than values of $m_2$ and $k_1$, respectively.

Values of $S_{IIIE_{observed}}$ were weighted in the curve-fitting procedure by $1/n$ so that later breaths have less influence on best fit parameters (see APPENDIX A). On a few occasions, extreme outliers of $S_{IIIE}$ values were found for late washout breaths and excluded from analysis.

We have designated measured $S_{IIIE_{observed}}$ values from breath 1 as $S_{IIIE}$. $S_{IIIE}$ values obtained for the first breath by curve fitting are identified as $S_{IIIE_{total}}$, $S_{IIIE_{dcdi}}$, and $S_{IIIE_{cdi}}$, unless otherwise indicated. In similarity to previous investigations, cdi information contained in breaths near the end of a washout (see APPENDIX A) was considered by averaging $S_{IIIE_{observed}}$ values from the final washout breaths (in this case breaths 21 through 25), to obtain $S_n$ (5, 8). In this study we also considered the curve-fit value of $S_{III_{cdi}}$ determined for washout breath 25 ($cdi_{max}$) as an alternate method of obtaining cdi information contained in later washout breaths (Fig. 2C).

A separate investigation of $V_{Dapp}$ effects on $S_{IIIE}$ was undertaken as a component of these studies (see APPENDIX B).

Empirically determined correction factors were quantified to allow for comparison of results from lobes with varied $V_{Dapp}/V_T$ and applied to the results of these experiments where indicated (corrected to $V_{Dapp}/V_T = 0$).

Only results from analysis of $N_2$ washout are given in this report (except in APPENDIX B, where He and SF$_6$ results are also mentioned).

Statistical Analysis

Paired $t$-tests and the Wilcoxon signed-rank test were used for comparison of the following: 1) $V_T$, $V_L$, and $S_{IIIE_{total}}$, $S_{IIIE_{cdi}}$, and $S_{IIIE_{dcdi}}$, was described by the slope of the best fit linear and single exponential regression equations, but the results are reported for only the best fit solutions (linear or single-exponential; see RESULTS). For this analysis, the six individual values of $S_{IIIE_{total}}$, $S_{IIIE_{cdi}}$, and $S_{IIIE_{dcdi}}$ per lobe were normalized by the average of those measurements for that lobe to control for average differences between lobes. The statistical significance of any EIP influence on $V_{Dnat}$ and $S_{IIIE}$ results (normalized) was also tested by paired $t$-test comparison of best fit slope values to a zero slope predicted by the null hypothesis.

RESULTS

General Lobar Measurements

None of the lobes exhibited visible increases in gas trapping at the end of the experimental procedures. The average amount of time from the first to the last MBW was 1.7 ± 0.5 h (mean ± SD).

Table 1 lists the values of $V_T$, $V_L$, and $N_2$ $S_{IIIE_{total}}$ obtained for each lobe from the first and last MBW runs performed under equal conditions of EIP. Compared with the initial washout, the same EIP used for the final washout produced an ~4% increase in $V_T$, which was not found to be statistically significant. An ~18% greater $V_L$ measured in the final vs. initial washout was found to be significant by the Wilcoxon signed-rank test ($P < 0.05$) but not the paired $t$-test. $N_2$ $S_{IIIE_{total}}$ measured from the final washout averaged 0.76 ± 0.21 (mean ± SD) of that obtained from the initial washout, but this potential trend of decreasing $N_2$ $S_{IIIE_{total}}$ over the course of the procedures was not found to be statistically significant.

Exhaled Dead Space Volume

$V_{Dnat}$ was not markedly altered by increasing EIP as $V_T$ increased by approximately twofold (Fig. 3). Regressions of $V_{Dnat}$ were calculated against both EIP and In(EIP) (best correlations found against EIP, $R^2$ range;
Table 1. The effect of time on VT, VL, and SnIIILtotal, as determined for each lobe from the initial and final N2 washouts under the given conditions of EIP

<table>
<thead>
<tr>
<th>Lobe</th>
<th>EIP, cmH2O</th>
<th>VT, ml</th>
<th>VL, ml</th>
<th>SnIIILtotal, liter</th>
<th>VT, ml</th>
<th>VL, ml</th>
<th>SnIIILtotal, liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-20.0</td>
<td>63.0</td>
<td>148.0</td>
<td>1.72</td>
<td>67.5</td>
<td>155.4</td>
<td>1.01</td>
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<td>2</td>
<td>-30.0</td>
<td>74.0</td>
<td>160.2</td>
<td>0.86</td>
<td>74.0</td>
<td>175.0</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>-10.0</td>
<td>36.0</td>
<td>177.0</td>
<td>7.55</td>
<td>36.0</td>
<td>192.4</td>
<td>4.89</td>
</tr>
<tr>
<td>4</td>
<td>-15.0</td>
<td>65.0</td>
<td>162.3</td>
<td>0.77</td>
<td>70.0</td>
<td>206.4</td>
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</tr>
<tr>
<td>5</td>
<td>-25.0</td>
<td>94.5</td>
<td>226.2</td>
<td>1.36</td>
<td>99.0</td>
<td>305.7</td>
<td>0.81</td>
</tr>
</tbody>
</table>

EIP, end-inspiratory pressure; EEP, end-expiratory pressure; VL, preinspiratory lobar volume; VT, tidal volume; VDapp, apparatus common dead space volume between the Rudolph valve and the site of gas sampling. SnIIILtotal, total calculated inhomogeneity of each washout breath of the late portion of the concentration-normalized N2 phase III slope. EEP was always -5 cmH2O. VDapp = 6 ml. *P < 0.05, initial vs. final, Wilcoxon signed-rank test. All other paired comparisons, initial vs. final, P was not significant by paired t-test and Wilcoxon signed-rank test.

Heterogeneity of Exhaled N2

Measured values of SnIII. SnIII1 was greater than SnIIIL1 when measured with EIP = -35 cmH2O and -10 cmH2O, in every instance (average values, Fig. 4). SnIII1 was found to be significantly greater than SnIIIL1 for EIP = -35 cmH2O by the two-tailed paired t-test (P < 0.04) and Wilcoxon signed-rank test (P < 0.05), but a significant difference was only found with EIP = -10 cmH2O by the Wilcoxon signed-rank test (P < 0.05). When normalized by the difference between VDapp, simultaneous measurement of SnIII, SnIIIL and VT plotted as functions of end-inspiratory pressure (EIP). End-expiratory pressure (EEP) was constant at -5 cmH2O. EIPs were chosen in random order. Solid symbols, VT; open symbols, VDapp.

SnIII1 and SnIIIL1 found with EIP = -10 cmH2O, the difference value was found to be significantly reduced with EIP increased to -35 cmH2O, when analyzed by the paired t-test (P < 0.04) but not the Wilcoxon signed-rank test.

As suggested by Fig. 4, statistical significance of the apparent reduction in SnIII1 and SnIIIL1 when measured with EIP = -35 cmH2O vs. -10 cmH2O, as tested by the paired t-test, was precluded by the wide range of SnIIIL1 values found for the group with EIP = -10 cmH2O. However, the consistent reductions in SnIII1 and SnIIIL1 with increased VT were found to be significant by the Wilcoxon signed-rank test (P < 0.05).

When compared with L SnI, simultaneously measured E SnI values were found to be significantly larger for EIP = -10 cmH2O by the Wilcoxon signed-rank test (P < 0.05) but not for EIP = -35 cmH2O (Fig. 5A). SnI was not found to be significantly larger for the E vs. L portions of the phase III slope with EIP = -10 cmH2O or -35 cmH2O, when tested by the paired t-test. SnI for the E and L portions of the phase III slope were not found to be significantly different when measured with EIP = -10 cmH2O vs. -35 cmH2O, as tested by the paired t-test and Wilcoxon signed-rank test.

Curve-fit values of SnIII and comparison with measured values. In regard to goodness of fit, curve-fit results for N2 SnIII observed vs. breath number were very similar to results found for N2 SnIII observed vs. breath number. In the interest of brevity, parameters that produced the best fit solutions of Eq. 4 to measured values of N2 SnIII observed are only presented for the more commonly studied L portion of the phase III slope (Table 2). Observed SnIII1 and SnIIIL1 values were highly correlated with the corresponding best fit SnIII1 total and SnIIIL1 total values, respectively (R² = 1.0), and weighted correlation of the best fit solutions to each
values of cdi max were not found to be different when these comparisons of cdi max were made by the paired t-test.

As suggested by the grouped mean best fit values from the first washout breath (Figs. 6), S_nIIIE_total was greater than simultaneously measured S_nIIIL_total in every instance (individual data not shown, V_0app/VT = 0). Increasing EIP resulted in a large decline in N_2 S_nIIIE_total and S_nIIIL_total primarily because of the dcdi component. The dominant S_nIIIdcdi component of S_nIIIL_total varied widely among the five lobes at the lowest levels of EIP.

When within-lobe normalized S_nIIIL_total values were calculated as functions of both EIP and ln(EIP), the best correlations of both S_nIIIE_total and S_nIIIL_total values vs. breath number were found when ln(EIP) was the independent variable. Slopes of normalized S_nIIIE_total vs. ln(EIP) for the five lobes averaged −0.064 ± 0.023 l/[ln(cmH_2O)] (R^2 = 0.72 ± 0.08). For normalized N_2 S_nIIIL_total vs. ln(EIP), slopes averaged −0.047 ± 0.013 l/[ln(cmH_2O)] (R^2 = 0.78 ± 0.04). For the group of five lobes, slopes were significantly greater than zero as tested by the paired t-test (P < 0.048 and P < 0.024 for E and L values, respectively).

N_2 S_nIIIE_dcdi and S_nIIIL_dcdi results mirrored those of S_nIIIE_total and S_nIIIL_total. Fig. 7 contains the within-lobe normalized values of S_nIIIE_dcdi and S_nIIIL_dcdi vs. EIP for the five lobes tested (V_0app/VT = 0), with lines representing the best fit solutions. For normalized N_2 S_nIIIE_dcdi vs. ln(EIP), slopes averaged −0.068 ± 0.022 l/[ln(cmH_2O)] (R^2 = 0.67 ± 0.13, however, for one lobe R^2 = 0.16 and for the other four lobes R^2 = 0.80 ± 0.06). For normal EIP, slopes averaged −0.054 ± 0.013 l/[ln(cmH_2O)] (R^2 = 0.79 ± 0.04). For the group of five lobes, slopes were significantly greater than zero as tested by the paired t-test (P < 0.039 and P < 0.014 for E and L values, respectively).

For normalized N_2 S_nIIIE_dcdi vs. ln(EIP), slopes averaged −0.038 ± 0.017 l/[ln(cmH_2O)] (R^2 = 0.44 ± 0.18). Slopes of normalized S_nIIIL_dcdi vs. ln(EIP) averaged −0.021 ± 0.011 l/[ln(cmH_2O)] (R^2 = 0.27 ± 0.14). Slopes of S_nIIIE_dcdi and S_nIIIL_dcdi vs. ln(EIP) were not found to be significantly different from zero by the paired t-test (P = 0.084 and P = 0.442, respectively).

**DISCUSSION**

Overview

The present study was designed to improve understanding of how intralobar V_Danat and heterogeneity of gas distribution are influenced by altering VT. Increasing VT by varying EIP was found to have little influence on V_Danat (Fig. 3), but significantly decreased E and L exchanged S_nII (Figs. 4, 6, and 7), primarily because of decreasing dcdi (Figs. 6 and 7). Maintaining EEP fixed and controlling EIP, as opposed to predefining levels of VT, facilitates interpretation of results from lobes of varied size. Assuming that compliance characteristics of lobes are similar, equal conditions of Ptp will favor 1) the same degree of stress applied to all lobes regardless of size, 2) equal specific ventilation, and 3) diffusion-convection fronts in similarly sized airways. Ventilatory patterns were delivered to the lobes with a very
A high degree of repeatability and accuracy. The effect of time on the preparation was to increase parenchymal compliance, as evidenced by generally increased VT and VL when measured under equal conditions of EIP for the initial and final washouts. Although there was an apparent temporal trend in four out of five lobes toward decreased SnIIILtotal when the conditions of the first MBW were repeated for the last, this was not found to be statistically significant. The randomized nature in which EIP test conditions were applied also largely removes concerns that trends in the results were influenced by time. However, it remains a possibility

Table 2. Curve-fit results for SnIIIL

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<td>R²</td>
<td>0.99</td>
<td>0.98</td>
<td>0.91</td>
<td>0.96</td>
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EEP = -5 cmH2O; m1, the SnIIILcdi asymptote, where cdi is convective-dependent inhomogeneity; m2, the SnIIILdcdi asymptote, where dcdi is diffusive-convective-dependent inhomogeneity; k1, curvature of the cdi component; k2, curvature of the dcdi component; R², correlation coefficient. Two-exponential solutions of N2 SnIIILtotal vs. breath number (n), best fit to SnIIILobserved measured from multiple breath washout with given EIP. R² shown for curve-fit values vs. unweighted data from all 25 breaths. In all instances, R² ≥ 0.98 for curve fit vs. the measured values weighted by 1/n.

Fig. 6. Grouped average of best fit, first-breath values (corrected to VD(app)/VT = 0 ml) of N2 SnIIIL (A) and N2 SnIIIL (B), including total and cdi and ddi components, plotted as functions of EIP.
that variability in the measurements was increased by aging of the preparation and thus potentially reduced or obscured minor trends in the data.

Tidal Volume Influences on Exhaled Dead Space Volume

The absence of a measured change in exhaled $V_{Danat}$ with increasing VT is an important finding of this study and is at odds with the results and conclusions of most other investigations of $V_{Danat}$ vs. VT (9, 17–19, 31). Increasing VT is usually found to affect $V_{Danat}$ regardless of the method of measurement and has generally been explained by expansion of proximal airways (1) and various mechanisms that influence gas mixing within peripheral or proximal airways (9, 18, 25). $V_{Danat}$ derived from clearance curves increased with increasing VT in two human studies (9, 25), in agreement with predictions of mathematical models representing intra-airway and cardiogenic gas mixing mechanisms that were sensitive to peripheral airway geometry (25). $V_{Danat}$ was also found to increase with VT when estimated from human exhaled gas profiles by use of methods similar to this study (18), in agreement with model predictions that included gas dispersion in relatively proximal airway regions (19). However, the same modeling study did not find the VT influence on $V_{Danat}$ to depend on distal airway geometry (19). At least one study found no change in Fowler $V_{Danat}$ when VT was varied in humans, but no explanation was offered for the discrepancy with other studies (26). The present results from excised canine lobes are sufficient to allow hypotheses of intra-airway gas-mixing mechanisms to be discounted when addressing the influence of variable VT on $V_{Danat}$. Canine and human lungs appear to be similar in regard to heterogeneity of distal airway branching geometry (E. R. Weibel, personal communication), and properties of more proximal airways that are predicted to influence gas dispersion are probably also similar.

It is unlikely that this result is due to errors in the procedures of the experiment, in which the average SD of $V_{Danat}$ was $0.6 \text{ ml}$ (allowing for a resolution of about 1 ml). The second washout breath was used for measurement of $V_{Danat}$ to avoid variability in first-breath values due to manual valving. $V_{Danat}$ is known to vary with progressive washout breaths to a small degree (8), but this effect is not expected to alter our overall conclusions. The method used to estimate $V_{Danat}$ in these studies (36) is similar to the method proposed by Fowler that has often been used to determine this value (14), and differences are not expected to affect the overall conclusions of this study.

Along with every rise in VT, inspiratory flow rate also increased proportionally in this study because of fixed TI. Increasing inspiratory flow rate is predicted to move the diffusion-convection front position toward the lung periphery (12, 23). $V_{Danat}$ represents the summed average appearance on expiration of gas from the vicinity of the diffusion-convection front of all parallel airway regions (13), and therefore increasing inspiratory flow rate is predicted to increase $V_{Danat}$ (12, 22, 23). However, increasing airway cross-sectional area during inspiration tends to decrease convective velocity and to move the front position proximally (22). In this study, the average volume from the gas-sampling site in the lobar bronchus to the diffusion-convection front position appears to have remained unaltered. This finding implies that the average front position must have moved mouthward because increasing VT increases the volume of the airways. Therefore, under the conditions of these experiments, the increase in airway cross-sectional area during inspiration tends to decrease convective velocity and to move the front position proximally (22). In this study, the average volume from the gas-sampling site in the lobar bronchus to the diffusion-convection front position appears to have remained unaltered. This finding implies that the average front position must have moved mouthward because increasing VT increases the volume of the airways. Therefore, under the conditions of these experiments, the increase in airway cross-sectional area was a greater influence on the average position of the diffusion-convection front than was the increase in convective flow rate. In partial support of this result, $V_{Danat}$ was found to remain unchanged when inspiratory flow rate was varied over a wide range in intact dogs mechanically ventilated with a constant VT (2). In that study, molecular diffusivity was found to be a greater determinant of the diffusion-convection front position than was convection.

The lack of VT influence on $V_{Danat}$ in this study of excised, unperfused lobes leads to the conclusion that,
when such an influence is found in more intact preparations and humans, it is due to extralobar mechanisms and/or perfusion. Cardiogenic mixing and the nonuniform intrapulmonary pressure gradient are two extralobar mechanisms that are known to influence ventilation distribution (13), but it is not clear how those influences would produce the VT effect on VD\textsubscript{anat}. A need for the presence of these two extralobar mechanisms to produce the reported correlation between VT and VD\textsubscript{anat} is contradicted by the results of one experiment in which an anesthetized, perfused lung preparation was used to demonstrate the VT influence on dead space ventilation (31).

One explanation for the discrepancy between our present results and those of previous studies where VD\textsubscript{anat} correlated with VT may be found in the known effects of gas exchange on alveolar gas concentrations. As pointed out by Cormier and Bélanger (4), the exhaled N\textsubscript{2} profile during a washout with 100% O\textsubscript{2} in perfused lungs is determined by several factors involving gas exchange. Alveolar N\textsubscript{2} is initially diluted slightly in the beginning of O\textsubscript{2} inspiration because of increased alveolar CO\textsubscript{2} transfer, a fairly constant O\textsubscript{2} consumption, and the low rate of N\textsubscript{2} transfer between the liquid and gas phases. For a given CO\textsubscript{2} production and a given Ti and Te, the time required for the respiratory quotient to become <1.0 during a breath is longer for a large compared with a small-volume inspiration, presumably resulting in a large dilution of early-exhaled N\textsubscript{2}. Further investigation is required to address whether this mechanism contributes to the increase in VD\textsubscript{anat} found when VT is increased in perfused lungs.

Uniformity of Exhaled Alveolar Gas and Influences of Tidal Volume

Contributions of dcdi and cdi to overall heterogeneity. S\textsubscript{nIII} was a far greater influence on the uniformity of exhaled alveolar gas than was S\textsubscript{nIIId}. In this preparation. The dcdi component was responsible for ~85% of N\textsubscript{2} S\textsubscript{nIII} when values for the lowest two conditions of EIP and VT were averaged and for ~70% of the total at the two highest levels of EIP and VT (corrected to VD\textsubscript{anat}/VT = 0, Fig. 6B). Human MBW studies have found the dcdi component of S\textsubscript{nIII} to be the major contributor to the phase III slope (6,8). In a previous study of upright and awake humans breathing 1-liter volumes at a moderate rate, the dcdi contribution to the total N\textsubscript{2} S\textsubscript{nIII} (measured for late-exhaled gas) was found to be ~62% after gas exchange was taken into account (8). The relatively greater contribution of dcdi in this excised lobar preparation was expected because of the absence of most external influences on ventilation distribution.

Evidence for diffusive-convective-dependent gas mixing during exhalation. A decrease in expired gas heterogeneity as exhalation proceeds (curvilinearity of the phase III slope) has been predicted by computer models of intrapulmonary ventilation distribution (24,25) and is considered to be evidence for continued homogenization of pulmonary gas due to diffusive-convective interaction (6,25). In agreement with those predictions and a previous demonstration in humans (6), we found that values representing early-exhaled gas (S\textsubscript{nIIIE} \textsubscript{total} and S\textsubscript{nIIIE} \textsubscript{dcdi}) were greater than corresponding values representing late-exhaled gas (S\textsubscript{nIII} \textsubscript{total} and S\textsubscript{nIII} \textsubscript{dcdi}) in every instance. Results from multiple 1.5-liter breath washouts in humans analyzed for the slope of phase III from 0.75 to 1.0 liters and from 1.0 to 1.5 liters suggested this result, when slopes from the later expire were found to be consistently less than from the earlier expire (6). A single-breath, inspiratory-capacity washout study in humans failed to detect a difference in heterogeneity of early- vs. late-exhaled alveolar gas (24); however, the disparity may be due to added interregional heterogeneities imposed by vital capacity maneuvers and/or the decrease in intraregional heterogeneity expected from large volume breathing. The ability to resolve this decreasing heterogeneity of exhaled gas for both small and large tidal breaths in these experiments was probably enhanced by the lack of change in VD\textsubscript{anat} (an effect that would influence early-exhaled alveolar gas), and because heterogeneity of exhaled gas was not influenced by gas exchange (an effect that increases heterogeneity of later exhaled alveolar gas; Ref. 4).

VT-dependent alteration of total heterogeneity and the dcdi and cdi components. The measured values of S\textsubscript{nIII} were found to be decreased when tested with the largest vs. the smallest level of VT in every instance, for both early- and late-exhaled phase III gas. However, the wide variability in this index among the lobes with EIP = ~10 cmH\textsubscript{2}O limited the statistical significance of this result (Fig. 4). Variation in S\textsubscript{nIII} among the various lobes was greatly reduced with EIP = ~35 cmH\textsubscript{2}O. Best fit values of S\textsubscript{nIIId} were essentially identical to the measured S\textsubscript{nIIId} values and also displayed wide variability among the lobes (Fig. 6). Most human studies have found heterogeneity of exhaled alveolar gas concentrations to decrease with increased VT, beginning with early investigations of exhaled gas profiles (15,27). More recently, single-breath washouts in humans revealed phase III slopes that were larger for 1-liter inspirations than for an inspiratory capacity maneuver (24), in general agreement with human N\textsubscript{2} clearance studies (3,28). Hyperventilation (increased VT and frequency) decreased N\textsubscript{2} clearance in one human study (38), but comparison with the present results is limited because voluntary hyperventilation is known to alter chest wall deformation patterns and interregional sequencing in addition to gas flow rate (13). On the whole, increasing VT has been found to result in more uniform mixing of tidal and resident gas as measured by N\textsubscript{2} clearance or exhaled gas uniformity. The decline in N\textsubscript{2} S\textsubscript{nIIId} with increased VT was significant for both early- and late-exhaled alveolar gas, because of the significant decline in S\textsubscript{nIIId} dcdi. In support of this result was the greatly reduced difference between directly measured early- vs. late-exhaled S\textsubscript{nIIId} observed. The stepwise increase in EIP decreases S\textsubscript{nIIId} \textsubscript{total} and S\textsubscript{nIIId} \textsubscript{dcdi} to a lesser degree, reflecting smaller increments in VT due to the
normal pulmonary pressure-volume relationship (Fig. 6). Dcdi is produced between fine airways sharing branch points in the lung periphery (22), and therefore our results indicate a large influence of VT on ventilation distribution in those airway regions. The cdi component of SnIIItotal was not found to decrease significantly with increased EIP for either early- or late-exhaled gas, thus reducing the significance of EIP-dependent changes in SnIIItotal compared with the SnIIIdcdi component alone.

These study results suggest that altered intra-regional gas distribution may be responsible for much of the VT effect on heterogeneity of exhaled gas, and they partially support the presence of two opposing influences on the phase III slope when VT is altered in more intact preparations (5, 6). Human studies have suggested that increased uniformity of gas mixing with increased VT reduces cdi in the peripheral lung (6, 24), whereas cdi between larger airway regions increases (6). The cdi was a very small component of exhaled gas nonuniformity in these excised and unperfused lobes, possibly because of the removal of extralobar influences, and we failed to find a significant influence of increasing VT on first-breath values of SnIcdi. The SnI and cdimax results indicate that within these lobes the cdi influence on ventilation heterogeneity is actually decreased in early-exhaled phase III gas when VT is increased (Fig. 5), an effect that may serve to reduce the measured increase in cdi found in more intact preparations (6). The statistical significance of this result was minimal, but the conclusion is reinforced by the significantly greater differences between E SnI and E cdimax vs. corresponding L SnI and L cdimax at low VT, which were essentially eliminated by increasing VT. However, SnIIIdcdi, L SnI, and L cdimax were not significantly altered by VT, and this portion of the expire corresponds more closely to the exhaled volume examined in most previous investigations. Overall, the results suggest that a VT-dependent alteration in cdi may not be an important inherent property of the canine pulmonary lobe but rather an extralobar effect on interregional heterogeneity.

Potential mechanisms for VT-dependent effects on dcdi and cdi. Decreased intraregional heterogeneity with increased VT may be the result of mouthward movement of the diffusion-convection front, decreased resistance to diffusive mixing, and/or increased uniformity of acinar inflation. Heterogeneity of airway branching geometry and nonuniform airway and parenchymal mechanical characteristics are inherent properties of the normal human lung structure that increase among progressively smaller regions and are generally thought to influence heterogeneity of intraregional ventilation (20, 23, 29, 33, 35). As previously discussed, the VTanat results argue for a mouthward movement of the average diffusion-convection front position as VT was increased. This shift in the front position is predicted to allow increased diffusive mixing of parallel airway regions and increase the uniformity of exhaled alveolar gas concentrations (22, 23). Resistance to diffusive gas transfer is expected to be nonuniform among parallel airways with nonuniform geometry due to differences in path length for molecular travel (24), and this nonuniformity is expected to decrease with increased tidal volume due to increased airway dimensions (6).

Increasing EIP in this preparation increases stretching force uniformly throughout the lung, but heterogeneous airway and parenchymal compliance characteristics must produce nonuniform expansion, perhaps resulting in more uniform ventilation with greater VT. Modeling studies that utilize only documented nonuniformity of peripheral airway geometry generally underestimate the magnitude of exhaled gas nonuniformity and its VT dependence in humans (24, 33). Sufficient modification of model geometry can bring results of simulations closer to those actually observed experimentally (33), but the lung structure clearly possesses heterogeneous mechanical properties that should also be taken into account when interpreting the results of these experiments (29, 35). Heterogeneity of intra-regional compliance may be able to account for discrepancies between model predictions and experimental results, but the complexity of modeling mechanical heterogeneities in the fine airways still precludes their use in detailed computer simulations of intrapulmonary gas mixing.

The configuration of the excised lobe preparation may have influenced the cdi results to a small degree, in particular the gravity-determined nonuniform tissue stretch that is expected due to hanging lobes by their bronchi. The weight of the blood-free lobe is supported by tissue adjacent to the bronchus, and this local stress progressively diminishes peripherally. Distortion-dependent changes in ventilation distribution are expected to be manifest primarily as altered cdi, reflecting regional differences in lung expansion between top and bottom. The small decrease in cdi found for early-exhaled alveolar gas when VT was increased is consistent with the presence of this potential distortion effect.

Increasing ventilatory flow rate has been shown to have mixed influences on the uniformity of exhaled indicator gas (15, 16, 25, 37, 38). Alteration of interregional sequencing is probably responsible for much of the flow rate effect on ventilation heterogeneity in spontaneously breathing humans (37), but peripheral airway effects of flow rate have also been supported by human investigations (25). In contrast to increasing heterogeneity found when inspiratory and expiratory flows were voluntarily increased during vital capacity maneuvers (25), these experiments demonstrate the opposite effect in response to increased VT and flow rate. Furthermore, in related experiments using this preparation, we found that altering inspiratory time from ½ to 8 s (fixed VT) failed to significantly alter SnIIIdcdi or SnIIItotal (11). Results from the present study argue against a role for the unperfused lobar structure in producing increased ventilation heterogeneity often associated with increasing convective flow rate (over the range tested).

Unlike healthy young humans, dogs possess extensive collateral ventilation that has been implicated in homogenizing gas distribution between pulmonary re-
regions (10) and may account for some of the improved uniformity of exhaled gas when VT is increased. However, several factors decrease the possible importance of this mechanism in producing the VT effect. Communications in the walls of acinar and alveolar regions, as represented by pores of Kohn, are expected to enlarge with increased lung stretch, but the normal liquid lining probably reduces the free communication of gases. Also, the N₂ diffusion-convection front position is predicted to be near the entrance of acinar regions, resulting in uniform mixing within more distal regions, regardless of the path (22). Interregional communications exist at higher levels of the canine bronchial tree (10), and these pathways may increase diffusive- and/or convective-dependent mixing of gases with increased VT. Although interregional communications may have contributed to these results to some degree, the generally similar improvement of gas mixing in human and canine lungs with increased VT argues against the necessity of such communications for the VT effect.

Conclusions

These studies of the effects of VT on ventilation heterogeneity and VT on in excised canine lobes have demonstrated that larger VT significantly decreases intraregional heterogeneity of ventilation within and among acinar regions. In contrast, VDT was found to be unaltered by changes in VT, and also interregional heterogeneity of ventilation that occurs among more proximally branching regions was essentially unchanged or slightly decreased. These results indicate that the magnitude of VT is an important consideration when interpreting results of other investigations in which uniformity of exhaled gas is a measured parameter. Furthermore, the ability to detect changes in S III is reduced with large VT maneuvers because of substantial VT effects on the dominant dcDI component of ventilation heterogeneity. Thus large-volume maneuvers cannot accurately represent the heterogeneity of ventilation present under conditions of resting VT. These results also suggest that VT-dependent influences on VDT and interregional heterogeneity require the presence of normal extravascular influences on the lung parenchyma and/or perfusion.

APPENDIX A

Modified SIII Analysis

Procedures used in this study for determination of S III and its cdi and dcdi components contain several modifications of the method previously introduced by Crawford et al. (8), which had evolved from work by Paiva (21) and Paiva and Engel (22). Normalizing the phase III slope by the mean gas concentration of the specific volume of interest (e.g., E or L in this study, Fig. 2A) is a variation of the original method by which the mean gas concentration of the entire exhaled volume was utilized (8, 11) and essentially removes the assumption that early- and late-exhaled marker gases are washed out at similar rates. The present procedure for determining cdi and dcdi components of S III is the first to utilize a biexponential equation best fit to measured slope values from MBW. In the original method, only the cdi process is represented by a best fit function, and here we add a second single-exponential equation representing the dcdi process in the complete equation. The form of this second equation is appropriate when considering previous descriptions and simulations of the dcdi contribution to S III during MBW (7, 8, 22).

The addition of a second equation in this version of S III analysis, as well as the weighting of data in the curve-fit procedure, imposes certain restrictions on the possible solutions. In addition to requiring a solution with more components, the 1/n weighting procedure gives earlier washout breaths more influence over the best fit solution of Eq. 4, thus reducing the influence of increased error in values measured from later washout breaths due to falling marker gas concentrations (Fig. 2C). This S III analysis procedure probably gives slightly smaller values of S III and greater values of S III than would have been found previously. Model analysis predicts that the dcdi process does not actually reach dynamic equilibrium before the fifth breath (7, 8), and the exact number of breaths required to reach dynamic equilibrium of dcdi in lungs of humans and dogs is not known. Left unaccounted for, a residual influence of dcdi on the rate of increase in S III vs. n beyond breath 4 likely leads to an overestimated rate of increasing S III . By Eq. 4, the best fit solution is required to pass through the origin. This requirement essentially formalizes previously stated assumptions that all influences on the normal- ized slope values are generated by cdi and/or dcdi (excluding gas exchange; Ref. 4) and that marker gas concentrations are uniformly distributed in the lung before washout (8, 21, 34). Although some objections may be raised concerning the effect of this restriction on the possible best fit solutions, this method conveys some theoretical and practical advantages over earlier methods (8, 21, 34). By the original method, a single-exponential fit to slope values obtained after breath 4 represents the cdi process, and the solution was back- extrapolated to the y-axis (in which the model function is zero) (8). S III for breath 1 was the difference between the back-extrapolated values of S III from breath 1 and the y-intercept, and S III was the difference between the first-breath measured S III and the back-extrapolated value of S III (8). By the original method and recent variations, indexes representing ventilation heterogeneity in peripheral airways are subject to all error present in single measurement of alveolar slope from the first washout breath (8, 34). We have found that, in many experimental situations, slope values from the first washout breath are often prone to errors for a variety of reasons. An inherent advantage of this fitting procedure lies in the use of all data points from the washout to determine both peripheral and proximal airway contributions to ventilation heterogeneity, reducing the influence of single-measurement errors. In addition, required passage of solutions through the origin prevents generation of solutions with negative y-intercepts. Such solutions lead to either subjective judgment that they are not appropriate or difficult theoretical explanation for potentially negative values of S III and/or S III . Earlier detailed comparison of this, earlier, and other S III methodologies found that solutions for most sample MBW data sets were very similar (11). However, methods that employed the 1/n weighting procedure and/or required passage through the origin were superior for allowing computer-generated solutions to be found for difficult data sets (i.e., data sets containing outlying values or wide variations in measured breath-to-breath values of S III ). In our experience, the present methodology allows nonsubjective S III analysis to be applied more successfully in situations in which such variations normally occur (e.g., spontaneously
breathing humans, manual valving, low marker gas concentrations, etc.).

Quantitative comparison of the dcdi vs. cdi contribution to total ventilation heterogeneity is maximal for values determined for the first washout breath, because starting conditions for both processes are equal only before MBW. The dcdi dominates first-breath SnIII values (8, 12, 32), and later washout breaths contain increased information regarding cdi (23). The components of SnIII have been quantified in other ways that allow for more detailed examination of the cdi process (5, 7). The change in SnIII beyond five or six normal tidal breaths (or approximately >1.5 lung volume turnovers) is predicted to be almost entirely determined by the cdi process (8, 34), and SnIII of some or all of those breaths can be averaged as other indications of cdi influence on SnIII (5–7). SnIII will usually be a minor contributor to SnIII for the first washout breath, but small changes in cdi measured for washout breath 1 can produce large changes in SnIII of later washout breaths because of compounded influence of cdi. We have found that SnIII and SnIII are only adequate for detecting relatively large changes in cdi. The values of SnIII were usually a small fraction of the already small values of SnIII, resulting in a small signal-to-noise ratio. Similarly, SnIII observed from later washout breaths normally exhibits wide variability due to increasing measurement error of decreasing marker gas concentrations and phase III slope values, thus limiting resolution of SnIII. Therefore, we quantified cdmax as the value of SnIII determined by curve fit for breath 25 of the MBW. In theory, cdmax contains the same cdi information found in SnIII but excludes the influence of dcdi and is less prone to single measurement errors.

**APPENDIX B**

**Influences of Apparatus Dead Space on SnIII**

Even small changes in the ratio of VDapp to VT can have large influences on SnIII. Aside from any true alteration in the distribution of inspired gas, an alteration in VDapp and/or VT can introduce variability in VDapp/VT and SnIII. To control for the influence of VDapp, a subset of He, N2, and SF6 MBWs was performed under varied conditions of VDapp. SnIII results were then evaluated to create correction factors that were applied to other SnIII results from these studies.

**Methods**

Right middle lobes were removed from four mongrel dogs and prepared for negative-pressure ventilation as described in the text. The data acquisition system sampled each channel at 24 Hz. The minimum VDapp (~10 ml) consisted of the mounting tube and pediatric one-way valve. VDapp was then varied in a random manner from 10 to 45 ml in 5-ml increments by altering the length of tubing between the gas sampling site and the gas source/exit. All other parameters of ventilation were kept constant. Ti and TE remained at 2 and 4 s, respectively, and EEP remained at ~5 cmH2O throughout the experiment. VT was kept constant by continuing 12-cmH2O swings of Ptp, except for a double inflation approximately once every 5 min between MBW runs. A final MBW was performed on each lobe at the end of the protocol under conditions identical to that of the first MBW to test for the effects of time and manipulation on stability of SnIII total. On completion of the entire MBW series, lobes were removed from the apparatus and visually inspected for evidence of gas trapping while deflated.

Each SnIII result determined for the first washout breath was used to find the best fit linear regression equation to describe changes in ln(SnIII) with varied VDapp/VT (range; 0.2 to 0.8). The relationship is representative by a decaying exponential y = Ae b, so that ln(y) = ln(A) – bx. Slopes (b) of the best fit equations obtained for SnIII total and the cdi and dcdi components for each gas species (He, N2, SF6) were then compared by paired t-tests to quantify the significance of differences in b between variables.

**Results**

None of the lobes developed observable gas trapping over the course of the experiment. The average amount of time from the initial MBW to the end of the last MBW in this series was 2.2 ± 0.4 h (mean ± SD). The ratio of the final to initial measurements of N2 SnIII total averaged 0.96 ± 0.05, indicating that the preparation was stable with respect to the SnIII total.

On the average, a fourfold increase in VDapp decreased SnIII total by at least fourfold. Best fit solutions were well correlated with the data for all three gases and the cdi and dcdi components of SnIII total (overall, R2 = 0.77 ± 0.05). The degree to which SnIII total decreased when VDapp was increased was not found to be influenced by gas species as tested with SF6, N2, and He, as was also the case for SnIII dcdi and SnIII cdi. For all gases, bcdi determined from ln(SnIII total) vs. VDapp/VT (~1.66 ± 0.19) was significantly less negative than btotal determined from ln(SnIII total), (~1.82 ± 0.19). The bcdi determined from ln(SnIII dcdi) vs. VDapp/VT (~3.4 ± 0.29) was found to be significantly more negative than btotal and bcdi.

**Discussion**

An increase in the volume of reinflated dead space gas serves to reduce the range of possible gas concentration differences in the more distal airways (30). VDapp is present with all MBW maneuvers and varies among differing apparatus configurations and applications. In addition, normal variations in VT between individuals and test maneuvers guarantees that VDapp/VT is usually an independent variable.

Increasing VDapp was found to decrease the measured values of SnIII in a manner best described as a decreasing exponential function of VDapp/VT. Therefore, the following correction equation can be utilized

\[ Sn_{III \text{L corrected}} = Sn_{III \text{L uncorrected}} \cdot e^{-b \cdot \text{VDapp/VT}} \]  

(A1)

The b is unique for SnIII total and each of its components but not appreciably different for SnIII values obtained with inert gases of varied molecular weight as represented by SF6, N2, and He. Therefore

\[ b_{\text{total}} = -1.82 \quad b_{\text{cdi}} = -1.66 \quad b_{\text{dci}} = -3.41 \]

These results should allow for better comparison of SnIII values when VDapp/VT is an uncontrolled variable, for example by back-extrapolation of measured SnIII values to VDapp/VT = 0, using these empirically derived values of b with known values of VDapp and VT. Only SnIII was evaluated to create these correction factors, in part because SnIII corresponds most closely to that portion of the exhaled volume examined in most studies of heterogeneous ventilation. Although an error may have been introduced by applying these correction factors to SnIII values in this report, it is not expected to alter our overall conclusions.

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Address for reprint requests and other correspondence: M. P. Hlastala, Pulmonary Medicine, Box 356522, Univ. of Washington, Seattle, WA 98195 (E-mail: Hlastala@u.washington.edu).

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