Multiple-breath washout of helium and sulfur hexafluoride in sustained microgravity

G. KIM PRISK, ANN R. ELLIOTT, HAROLD J. B. GUY, SYLVIA VERBANCK, MANUEL PAIVA, AND JOHN B. WEST
Department of Medicine, University of California, San Diego, La Jolla, California 92037; Akademisch Ziekenhuis, Vrije Universiteit Brussel, 1090 Brussels; and Biomedical Physics Laboratory, Université Libre de Bruxelles, 1070 Brussels, Belgium

Prisk, G. Kim, Ann R. Elliott, Harold J. B. Guy, Sylvia Verbanck, Manuel Paiva, and John B. West. Multiple-breath washout of helium and sulfur hexafluoride in sustained microgravity. J. Appl. Physiol. 84(1): 244–252, 1998.—We performed multiple-breath washouts of N2 and simultaneous washouts of He and SF6 with fixed tidal volume (~1,250 ml) and preinspiratory lung volume (approximately the subject’s functional residual capacity in the standing position) in four normal subjects (mean age 40 yr) standing and supine in normal gravity (1 G) and during exposure to sustained microgravity (µG). The primary objective was to examine the influence of diffusive processes on the residual, nongravitational ventilatory inhomogeneity in the lung in µG. We calculated several indexes of convective ventilatory inhomogeneity from each gas species. A normal degree of ventilatory inhomogeneity was seen in the standing position at 1 G that was largely unaltered in the supine position. When we compared the standing position in 1 G with µG, there were reductions in phase III slope in all gases, consistent with a reduction in convection-dependent inhomogeneity in the lung in µG, although considerable convective inhomogeneity persisted in µG. The reductions in the indexes of convection-dependent inhomogeneity were greater for He than for SF6, suggesting that the distances between remaining nonuniformly ventilated compartments in µG were short enough for diffusion of He to be an effective mechanism to reduce gas concentration differences between them.

Humans; spaceflight; zero gravity; distribution of pulmonary ventilation; phase III of expiration; specific ventilation; intra-acinar inhomogeneity; convective inhomogeneity

Inhomogeneity of ventilation is known to be caused by convective (bulk flow) processes and by interactions between diffusion and convection. The absence of gravity [microgravity (µG), weightlessness] has long been postulated to cause a reduction in the convective inhomogeneity of ventilation (1, 14). However, until recently, it was not possible to measure the behavior of the lung in µG.

During vital capacity maneuvers, a reduction in ventilatory inhomogeneity was observed in short periods of µG during parabolic flight (13) and in sustained µG (9). This reduction was in the convective component of the overall inhomogeneity of ventilation, as evidenced by marked reductions in the size of cardiogenic oscillations and the height of the terminal rise in gas concentration after the onset of airway closure. The observations were consistent with the removal of gravitationally induced distortion of the lung parenchyma. However, only minor reductions in phase III slope were observed in transient and sustained µG (9, 13).

This observation is consistent with model predictions of diffusion-convection-dependent inhomogeneity (DCDI) (16) and showed that, at the acinar level, considerable inhomogeneity of ventilation persisted irrespective of the presence or absence of gravity. More recently, by use of gases of widely differing diffusivity (He and SF6), it was shown that, although this inhomogeneity at the acinar level persists in µG, its nature was altered considerably (21). In normal subjects in normal gravity (1 G) the phase III slope was steeper for SF6 than for He because of the effects of DCDI at the acinar level and the more rapid diffusional spreading of He. However, in µG this slope difference was abolished, and the two slopes became the same. Furthermore, after a 10-s breath hold in µG, the slope difference was reversed, with the SF6 slope actually being flatter than the He slope. The genesis of this unexpected change is unknown, but it is likely due to a widespread change in acinar geometry, perhaps as a result of alterations in pulmonary blood volume, or to alterations in cardiogenic mixing as a result of altered propagation of the cardiac pressure wave through the lung.

In contrast to the results obtained during vital capacity maneuvers, µG resulted in only small changes in convection-dependent inhomogeneity (CDI) and DCDI when measurements were made with near-normal tidal volumes from functional residual capacity (FRC) (19). This surprising result indicates that, during normal breathing, inhomogeneities in lung mechanical properties, rather than gravitational distortion, are dominant in determining the inhomogeneity of ventilation. Use of an independent technique for measuring inhomogeneity of specific ventilation (SV) using rebreathing data collected in µG resulted in a similar conclusion (22).

We report the results of multiple-breath washout (MBW) studies performed in µG, in which tidal volume and preinspiratory lung volume (PILV) were fixed and He and SF6 were used as tracers in the test gas mixture. By fixing PILV, we eliminated the confounding influence of changes in FRC in different positions or in µG that limited the interpretation of previous data (19). The addition of small amounts of He and SF6 to the test gas mixture allowed us to examine the effects of diffusive gas mixing on the residual nongravitational inhomogeneity in the lung in µG. Because these gases differ widely in their diffusivity, their behavior in the periphery of the lung is markedly different, and these differences can provide information on the nature of the inhomogeneity. Data were collected during the 14-day Spacelab Life Sciences-2 (SLS-2) mission of the space
He AND SF₆ WASHOUT IN µG

E. Samuel Redmond, Donald S. G. Chappell, and Michael V. Decker

He and SF₆ washout in flight microgravity: data collection and analysis

METHODS

Experimental system: We used the same experimental system used for previous studies of ventilatory inhomogeneity in µG (9, 19, 21). Briefly, the system centers around a bag-in-box with separate bags for inspired and expired gas. The subject breathes through a nonrebreathing valve, inspiring air or test gas. Flow was measured with a linearized (25) Fleisch no. 2 pneumotachograph in the wall of a bag-in-box system, and gas concentrations were measured with a rapidly responding magnetic sector mass spectrometer sampling at the lips of the subject, with all signals sampled at 160 Hz. Inspired gas was contained in a bag within the bag-in-box and was composed of 5% He-1.25% SF₆-balance O₂.

The system was arranged to allow the subject to act as the operator for all measurements, except those pre- and postflight measurements performed in the supine position, when assistance was provided. Subjects were trained to maintain a constant body position while standing and in µG, with their hands on the valves at the level of the mouthpiece.

The mass spectrometer was calibrated immediately before and after use by sampling gases carried on board. These data were also used to determine and correct for fragment ion cross talk between channels. The flowmeter was calibrated by integration of the flow from strokes of a 3-liter calibration syringe. Mass spectrometer transit time was determined daily by measuring the time required for a sharp puff of gas containing CO₂ to be detected by the mass spectrometer, and the data were then aligned accordingly. The time delay was taken as the time to the midpoint of the rise in CO₂ and thus included the lag time and dynamic response time components. The total time delay was ~30 ms greater for SF₆ than for He because of a 10–90% response that was ~30 ms greater for CO₂ than for He. On the basis of previous experience and simulation studies on test data, these differences are sufficiently small to be ignored in the context of these measurements.

Subjects and data-collection schedule: The anthropometric data for the subjects are shown in Table 1. The subjects were numbered as in previous studies so that comparisons can be made. Subject 2 flew on SLS-1 and SLS-2 and retains her subject number. All subjects were healthy, nonsmokers for at least 2 yr before the start of data collection, had normal lung function, and reported no pulmonary problems.

The data were collected during the 14-day flight of SLS-2 in October 1993. Subjects were studied before the mission at 123 days before flight (L-123) and at L-110, L-87, and L-19. In-flight data were collected on subjects 2 and 10 on flight day 3 (FD-3) and on all subjects on FD-7, FD-9 or FD-10, and FD-13. In flight, only the SF₆ data from FD-13 could be analyzed for phase III slope as a function of breath number because of noise in this channel in one of the mass spectrometers, although breath 1 of the washouts was analyzed for all tests performed. Data were collected on subjects 2, 8, and 11 on the day of return to 1G (R + 0, ~10 h after landing) and then on R + 1, R + 2, R + 6 or R + 7, and R + 9. Not all subjects participated in every postflight session because of logistical constraints, but all subjects participated in at least three postflight test sessions.

Pre- and postflight data were collected in the upright and supine positions, except on R + 0, when subjects were studied only in the upright position. All pre- and postflight data collection was performed under normobaric (~760 Torr) normoxic (21% O₂) conditions. In-flight data were collected under normobaric, normoxic conditions, except for environmental CO₂ levels, which were slightly elevated, ranging from 0.2 to 0.4%. All experiments were reviewed and approved by the University of California, San Diego and the National Aeronautics and Space Administration-Johnson Space Center Institutional Review Boards, and informed consent was obtained from all subjects.

Test maneuver: The subject breathed air through the mouthpiece and, when comfortable, turned a valve beginning the test. The subject then expired to residual volume (RV), and this minimum volume was detected by the system. From this volume, a predetermined volume was added, defining the PILV to be used for the rest of the test. This volume was determined preflight in the standing position for each subject and set so that it was approximately equal to the subject’s upright FRC. The same PILV was used for all tests performed on a subject, regardless of the posture or gravity level. Subjects were then prompted by the alphanumeric display to “breathe in,” and the inspiratory line was closed with large-bore solenoid valves, preventing exhalation. Once the inspired volume reached the PILV plus a predetermined tidal volume (typically ~1,250 ml; Table 1), the inspiratory line was closed, and simultaneously the expiratory valves opened and the subject was prompted to “breathe out.” After exhalation of the preset tidal volume, the cycle repeated itself.

Three controlled breaths of air were taken in this manner, and then the subject was prompted to turn a rotary valve during exhalation so that the next inspiration was taken from the bag prefilled with the test gas mixture. The subject then continued to inspire the test gas, with tidal volume being controlled for 12 breaths. At the completion of breath 12 of the test gas, the subject was prompted to exhale to RV, completing the test.

Data analysis: Data from each test were identified within the data stream, and calibrations were applied. To allow ready comparison between the resident gas washout (N₂) and the nonresident gas washin (He or SF₆) and to account for differences in the inspired concentrations of He and SF₆, gas

Table 1. Anthropometric data

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>FVC, %pred</th>
<th>FEV₁/FVC, %pred</th>
<th>Tidal Volume, ml (1G)</th>
<th>No. of Washouts</th>
<th>Standing</th>
<th>Supine</th>
<th>µG</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>F</td>
<td>45</td>
<td>159</td>
<td>56</td>
<td>100</td>
<td>107</td>
<td>925</td>
<td>11</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>37</td>
<td>178</td>
<td>82</td>
<td>104</td>
<td>96</td>
<td>1,237</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>36</td>
<td>173</td>
<td>62</td>
<td>101</td>
<td>94</td>
<td>1,240</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>42</td>
<td>185</td>
<td>79</td>
<td>101</td>
<td>91</td>
<td>1,225</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

All subjects retain the same number from prior publications (Refs. 7–9, 18–21). Subject 2 flew on Spacelab Life Sciences (SLS)-1 and SLS-2. Age, age at date of launch of SLS-2; FVC, forced vital capacity; FEV₁, forced expired volume in 1 s; %pred, percent predicted; Tidal volume, average tidal volume used in washouts performed in normal gravity (1 G); µG, microgravity. Values in parentheses indicate SF₆ data of adequate quality to analyze for slope and dead space; in other cases, only valid He and N₂ data were available for analysis.
concentrations were normalized by considering the pretest concentration of gas in the lung as 100.0 and the inspired concentration of gas as 0.0. Thus N<sub>2</sub> was simply rescaled to cover the range 0–100%, whereas He and SF<sub>6</sub> were inverted and then rescaled to cover the range 0–100%. This results in all data appearing as a washout from an initial concentration of 100% to a final concentration of 0%, with positive phase III slopes for all gases.

Data were first corrected for mass spectrometer transit time, and the flow was converted to BTPS conditions. For each of the three tracer gases (N<sub>2</sub>, He, SF<sub>6</sub>), end-tidal gas concentrations were measured, and the mixed expired gas concentration was determined from the integration of the product of flow and instantaneous gas concentration. The cumulative expired gas volume over the course of the washout was also determined in milliliters (STPD). For all gases (N<sub>2</sub>, He, SF<sub>6</sub>) we calculated the distribution of SV (from end-tidal and mixed expired data), the slope ratio of the washout, the normalized phase III slope, and the contribution of CDI to the washout as detailed below.

Distribution of SV. Distribution of SV was calculated using the method described by Lewis et al. (12). This calculation was performed twice for each washout: once for the mixed expired gas concentrations and once for the end-tidal gas concentrations. In both cases 50 compartments of SV were chosen to span the range of SV from 0.01 to 10 on a uniformly distributed logarithmic scale. The distribution of fractional ventilation values providing the best fit to the observed 12 breaths of gas concentration was then determined using the technique of enforced smoothing (12). The first two moments of the distribution (mean, log-SD) were then calculated.

Slope ratio of MBW. The logarithm of the mixed expired gas concentration was plotted as a function of lung turnover (cumulative expired volume divided by PILV) for each breath over each measurement of the maneuver, and the slope of the washout was calculated using the method described by Crawford et al. (4). This calculation was performed twice for each washout: once for the mixed expired gas concentrations and once for the end-tidal gas concentrations. In both cases 50 compartments of SV were chosen to span the range of SV from 0.01 to 10 on a uniformly distributed logarithmic scale. The distribution of fractional ventilation values providing the best fit to the observed 12 breaths of gas concentration was then determined using the technique of enforced smoothing (12). The first two moments of the distribution (mean, log-SD) were then calculated.

Results

We found no significant differences in the variables between pre- and postflight. This is similar to our other studies on the distribution of ventilation in µG (9, 19, 21), and for that reason we report only the combined pre- and postflight (standing and supine) in-flight (µG) results.

S<sub>n</sub>. The S<sub>n</sub> of N<sub>2</sub> is shown in Fig. 1 for all four subjects. There was no consistent difference in S<sub>n</sub> between the standing and supine positions in 1 G. Similarly, the S<sub>n</sub> for each subject was determined from the least-squares best-fit line of gas concentration fitted against volume over the range 700–1,200 ml of expired tidal volume for each breath (500–900 ml for subject 2). Phase III slope was expressed as the normalized expired slope (S<sub>n</sub>) by dividing the slope by the end-tidal gas concentration for that breath (19). For each subject in each state, the progression of S<sub>n</sub> with breath number was determined by averaging the values for each breath for each performance of the maneuver, and the results are expressed as means ± SE. For each data set, the final S<sub>n</sub> (S<sub>n</sub>final) for the washout was determined as the average over the final two breaths. S<sub>n</sub>final was used by Crawford et al. (4, 5) to compare end points of washouts. We use it here because it allows a reduction of the noise associated with end points of the washouts when gas concentrations are very low.

Contribution of CDI. The contribution of CDI to the overall washout was calculated using the method outlined by Crawford et al. (4). Briefly, because the contribution to the S<sub>n</sub> from DCI effects is essentially constant from breath 5 of the washout, any subsequent rise in S<sub>n</sub> is due to CDI effects. We calculated this effect as the slope S<sub>CDI</sub> per unit lung turnover obtained from a linear least-squares fit to the S<sub>n</sub> data from breath 5 to breath 10 of the washout (beyond which noise in the gas concentration signals is too large for accurate slope determination) and use this index (ΔS<sub>CDI</sub>) directly.

PILV. PILV was calculated from the volume of N<sub>2</sub> washed out over the course of the maneuver. At the beginning of the washout, the lung was assumed to be uniformly filled with gas at ambient concentration (which was set to 100% by the calibration procedure described above). At the end of the washout the lung was assumed to be uniformly filled with gas at the end-tidal gas concentration of the last breath of the washout (typically <10%). By use of mass balance, the average PILV of the washout was then determined.

Statistical methods. We followed the procedure used in our earlier studies of pulmonary function in µG (7–9, 18–21). Subjects acted as their own controls. Statistical analysis was performed using Systat version 5.0 (Systat, Evanston, IL) or Microsoft Excel (Microsoft, Redmond, WA). Data were grouped according to subject and position (standing, supine, µG), and two-way analysis of variance was performed. In cases where there were significant F ratios, post hoc testing was performed using the Bonferroni adjustment to determine significance levels. Simple comparisons were performed using t-tests. Significance was accepted at P < 0.05, and values are means ± SE.
calculated over the final two breaths of the washout ($S_n$) was unaltered by position in 1 G. Although they are not shown, the equivalent plots for He and SF$_6$ are qualitatively similar; however, the reduction in $S_n$ over the course of the washout from 1 G to µG was considerably smaller for He than for SF$_6$. There was no difference in the behavior of He or SF$_6$ between the standing and the supine position.

Figure 2 shows the individual $S_n$ values calculated from the He and SF$_6$ washouts in the standing position in 1 G and µG. For clarity, we do not show the $S_n$ data from the supine washouts, since these were similar to those obtained in the standing position in 1 G. In all four subjects the difference between the $S_n$ values for SF$_6$ and He are much smaller in µG than in 1 G, and, whereas $S_{n_0}$ is significantly different in three of four subjects in 1 G, there are no significant differences in µG. The $S_n$ values for SF$_6$ over the later breaths of the washout are subject to considerable noise. The inspired concentration of SF$_6$ was only 1.25%, a value we could...
not exceed because of possible contamination of the cabin of the spacecraft. Thus, by the end of the washout, the inspired-expired gas concentration difference becomes very small.

In µG, \( S_n \) for all gases was significantly lower than that measured in 1 G. The magnitude of this decrease was dependent on the diffusivity of the gas, with \( \text{He} \) showing only slight reductions in \( S_n \) and \( \text{SF}_6 \) showing a much larger reduction. The reduction was greatest in the three largest subjects (8, 10, and 11), whereas in subject 2, in whom the tidal volume was a larger proportion of vital capacity, the change between 1 G and µG was small. The greater reduction in the \( S_n \) for \( \text{SF}_6 \) (0.064 ± 0.012 liter\(^{-1}\)) than in the \( S_n \) for \( \text{He} \) (0.028 ± 0.012 liter\(^{-1}\)) in µG means that the \( \text{SF}_6 \)-\text{He} difference in \( S_n \) is greatly reduced in µG compared with the standing and supine positions in 1 G.

CDI contribution. Figure 3 shows the results from the washouts performed in the standing position and in µG. In µG, \( \Delta S_{\text{CDI}} \) was reduced (but not significantly) for \( \text{He} \) and largely unaltered for \( \text{N}_2 \) and \( \text{SF}_6 \) (Fig. 3). The CDI contribution to \( S_n \) (\( \Delta S_{\text{CDI}} \)) could not be determined from the supine data because of the large effects on \( S_n \) caused by cardiogenic oscillations in this position.

Distribution of SV. The distributions we obtained from the end-tidal gas concentrations in these very well controlled experiments were always unimodal. In subject 11 the distributions derived from mixed expired gas concentrations showed evidence of a second mode at high values of SV, but this was small. Because the distributions themselves are unremarkable, they are not included in this report. However, the mean and log-SD values for end-tidal and mixed expired data for each position and for each of the three gas species are shown in Table 2. Because we controlled tidal volume and PILV in each of the positions, the mean SV of the distributions is not indicative of the values that would normally be obtained in these positions but is increased due to the larger than normal tidal volume used. The mean SV values for each position are the same in all conditions when a specific gas is considered.

Log-SD, which describes the width of the distribution, was highest in the supine position and lowest in µG, except for \( \text{SF}_6 \), although in general these differences were not statistically significant. The log-SD for \( \text{He} \) was similar to that for \( \text{SF}_6 \) in standing and supine positions; however, in µG the log-SD for the end-tidal \( \text{He} \) data was less (the distribution was more homogeneous) than that for \( \text{SF}_6 \) (P = 0.045).

Slope ratio of MBW. Slope ratios calculated between 10–50% and 50–100% of cumulative expired volume are shown in Table 2. With the exception of \( \text{SF}_6 \), the smallest slope ratio (which indicates the greatest degree of inhomogeneity) was found in the supine position, although the differences were small. In µG the slope ratio for \( \text{SF}_6 \) was reduced compared with the standing position (P < 0.00), and the reduction for \( \text{He} \) bordered on being statistically significant (P = 0.054). However, the slope ratio for \( \text{N}_2 \) was unchanged.

### Table 2. Specific ventilation slope ratio and PILV

<table>
<thead>
<tr>
<th></th>
<th>Standing</th>
<th>µG</th>
<th>Supine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{N}_2 )</td>
<td>( \text{He} )</td>
<td>( \Delta )</td>
</tr>
<tr>
<td>ME SV</td>
<td>0.400</td>
<td>0.355</td>
<td>0.035</td>
</tr>
<tr>
<td>( \Delta )</td>
<td>± 0.048</td>
<td>± 0.044</td>
<td>± 0.004</td>
</tr>
<tr>
<td>Log-SD</td>
<td>0.734</td>
<td>0.639</td>
<td>0.054</td>
</tr>
<tr>
<td>( \Delta )</td>
<td>± 0.054</td>
<td>± 0.020</td>
<td>± 0.050</td>
</tr>
<tr>
<td>ET SV</td>
<td>0.316</td>
<td>0.299</td>
<td>0.035</td>
</tr>
<tr>
<td>( \Delta )</td>
<td>± 0.035</td>
<td>± 0.028</td>
<td>± 0.051</td>
</tr>
<tr>
<td>Log-SD</td>
<td>0.585</td>
<td>0.499</td>
<td>0.054</td>
</tr>
<tr>
<td>( \Delta )</td>
<td>± 0.024</td>
<td>± 0.019</td>
<td>± 0.053</td>
</tr>
</tbody>
</table>

Values are means ± SE. Mean and log standard deviation of distribution (log-SD) were calculated from average distribution of specific ventilation (SV) for each subject. PILV, preinspiratory lung volume; ME, mixed expired; ET, end tidal; slope ratio, curvilinearity of washout; \( \Delta \), results of statistical comparisons. *µG or supine significantly different from standing (P < 0.05). ‡Significant difference between \( \text{He} \) and \( \text{SF}_6 \) (P < 0.05). (*) and (†), 0.1 < \( P < 0.05 \). §, \( P \) = not significant.
Lung volumes. The mean tidal volumes of breath 1 of the washouts were 1,150 ± 23 (SE), 1,130 ± 28, and 1,220 ± 40 ml in standing and supine positions and in µG, respectively. The tidal volume in µG was significantly greater than that measured in the supine position. This small difference results from timing differences in the volume control systems used on the ground and in µG (19). As in the previous study (19), we consider these differences to be of minor physiological significance. Tidal volume measured in the standing position was not different from that measured in the supine position or in µG. There were no differences between the tidal volumes measured on successive breaths of the washouts.

PILV values as calculated from the N₂ washout data were 2,985 ± 115, 2,917 ± 135, and 3,199 ± 216 ml in the standing and supine positions and in µG, respectively. There was no difference in PILV among the three positions. The volume between the PILV selected and maintained by the system and the subject's RV (effectively the expiratory reserve volume) was not different among positions, nor was it different between the beginning and end of the washouts.

DISCUSSION

Sₜ. In the previous study of MBW data in µG (19), we found a small increase in Sₜ in the supine compared with the standing position. In this study we fixed PILV to be the same in all three positions and, in doing so, eliminated any change that might be caused by changes in lung volume per set that, at least in 1 G, would tend to decrease Sₜ at lower lung volumes (3). Under these conditions of fixed lung volume, Sₜ was largely unaffected by gravitational orientation in 1 G (Fig. 1). This suggests that the degree of inhomogeneity caused by gravity during breathing at near-normal tidal volumes from FRC is largely independent of position, in contrast to the situation during vital capacity single-breath tests. It further suggests that the influence of the changes in thoracic blood volume that occur in transition from the standing to the supine position (18) is negligible with respect to its effect on the distribution of ventilation.

In contrast to the previous study (19), Sₜ was moderately reduced in µG over most of the washout in this study. We expected to see such a reduction in Sₜ if the CDI contribution to overall inhomogeneity were decreased, since there is clear evidence for a gravitational influence on alveolar ventilation (1, 10, 14), and we were surprised not to see such a reduction in µG in an earlier study (19). Removing the confounding influence of a change in PILV likely allowed us to see a reduction in Sₜ in this case.

One of the most striking aspects of the Sₜ data is the difference in the magnitude of the reduction in Sₜ from 1 G to µG between He and SF₆. Compared with the standing position, Sₜ was reduced in µG on average for all breaths by 0.028 ± 0.012 and 0.0640 ± 0.012 liter⁻¹ for He and SF₆, respectively. The reduction occurred in all four subjects (Fig. 2) in whom a clear difference exists between SF₆ and He in all the standing data (and in the supine data, although these are not shown). However, in the µG data, this difference is much smaller. In particular, for subjects 2 and 8, there is little difference in Sₜ between SF₆ and He. These are the same two subjects who exhibited negative SF₆-He phase III slope differences in vital capacity single-breath tests performed during the same Spacelab flight (21). A direct comparison is provided in Fig. 4, which plots the phase III slopes recorded from vital capacity washouts of He and SF₆ performed without an end-inspiratory breath hold (21) and the phase III slopes from breath 1 of the multiple-breath study reported here. The breath 1 data are equivalent to a single-breath washout test performed starting at PILV and with an inspiration of ~1,200 ml. Figure 4 shows that, as was the case in the vital capacity breaths, there is a reduction in the SF₆-He phase III slope difference in µG. However, the magnitude of this reduction is much smaller in these breaths near tidal volume. A large part of the sloping alveolar plateau is thought to be due to the interaction between convective and diffusive transport processes that occur in a human at a zone about the level of the entry to the acinus. For the more diffusive gas (He), this zone is more proximal by two to three airway generations than it is for the less diffusible gas (SF₆) (16). Modeling studies have shown that the critical determinant of phase III slope due to these effects is the degree of volumetric asymmetry and/or cross-sectional area asymmetry of daughter branches in this zone. Thus a change in the difference between He and SF₆ slopes suggests a change in acinar conformation due to the removal of gravity or a change in the mechanics of gas mixing in this zone, perhaps as a result of changes in cardiogenic mixing. The reduction in SF₆-He slope difference in µG observed in this study also indicates that the changes in acinar gas mixing previously reported for breaths that encompass lung volumes near RV and total lung capacity (21) are also operating,
although to a lesser extent, in breaths that are more representative of physiologically normal breathing.

CDI contribution. The slope of 

$S_n$ as a function of breath number after breath 5 of the washout ($\Delta S_{CDI}$) is due solely to CDI effects (4, 5). The $\Delta S_{CDI}$ we calculated indicates only a small (or no) reduction in the CDI contribution for SF$_6$ and N$_2$ in $\mu G$ (Fig. 3). The result for N$_2$ is consistent with our previous study, in which we found only a modest reduction in the inhomogeneity of ventilation measured with multiple-breath N$_2$ washouts (19), and with the lack of change we see in other indexes of CDI in this study (e.g., log-SD) for N$_2$ and SF$_6$. However, for He, there is a substantial (although not statistically significant) reduction in $\Delta S_{CDI}$ in $\mu G$. This reduction is consistent with the notion that the (necessarily) nongravitational CDI existing in $\mu G$ is located in units that are relatively close to one another, such that diffusion of a highly diffusible gas is an effective mechanism to reduce concentration gradients set up by CDI. That a reduction in CDI is seen in the He data suggests that this nongravitational CDI exists between acini or between groups of a few acini.

The original technique of determining CDI contribution (percent CDI) or, originally proposed by Crawford et al. (5), used much longer washouts, and a single exponential was fitted to the data beginning with breath 5 and then backextrapolated to obtain the CDI contribution on breath 1. In these shorter washouts with larger tidal volume (leading to more rapid reduction in gas concentration), we were not confident of the process of fitting an exponential, and so we followed the later procedure of Crawford et al. (4) and fitted a straight line. Even so, the fits were of poor quality at times, and backextrapolation is of doubtful validity, and so we confine ourselves to reporting the rate of increase in $S_n$ beyond breath 5, which is due to the continuing effects of CDI ($\Delta S_{CDI}$).

The gravitational contribution to phase III slope may be estimated from the phase III slopes of breath 1 ($S_{n(1)}$) in 1 G and $\mu G$. This contribution

$$\left[100 \times \frac{1 - S_{n(1)}(\mu G)}{S_{n(1)}(1 \text{ G})}\right]$$

is relatively small in all gases: ~17% for N$_2$, 22% for He, and 15% for SF$_6$. The low value for the gravitational contribution to phase III slope is consistent with previous single-breath studies (9) and shows that the overall contribution of gravity to phase III slope is small in normal subjects. In a previous study (5) an upper limit of 28% was estimated for the total CDI contribution, consistent with our estimates.

Distribution of SV. Although we calculated the distribution of SV from all three gases (N$_2$, He, SF$_6$), there was little to distinguish between the different gas species, except that in the standing position and in $\mu G$ the log-SD of the distributions was consistently higher for SF$_6$ than for He (Table 2).

The fact that the mean SV values were similar within a gas species independent of position is a good indication of the technical validity of these measurements. We fixed tidal volume and PILV to eliminate the influence of changes in lung volume per se on the inhomogeneity of ventilation. Previous studies have shown that measures of the inhomogeneity of ventilation vary markedly in the same subject when the studies are performed at different lung volumes (3, 17).

In our previous studies of the multiple-breath N$_2$ washout in $\mu G$, we found that resting FRC changed (19), as was the case in other studies of lung volume in $\mu G$ (8). By controlling tidal volume and PILV, we sought to study the lung at a similar absolute lung volume to observe the effects of the removal of gravity on the inhomogeneity of ventilation.

The difference between the mean SV measured by N$_2$ and that measured by He or SF$_6$ (the results of which were similar to each other, except in the case of the supine data, which are affected by cardiogenic oscillations) is likely caused by the effects of continuing gas exchange in the lung (2). Although we normalized the gas data so that the washout data appeared as washout data, washin gases will tend to reach their equilibrium concentration more rapidly as the shrinkage due to removal of respiratory gases concentrates them toward the inspired level. In contrast, the washout of the resident gas is delayed as the concentrating effect of gas exchange raises its concentration from the inspired (zero) level. Thus the effective PILV of a resident gas is lowered (less gas is washed out), raising the mean SV.

The log-SD results from these washouts provide a direct measure of how evenly the inspired gas is distributed on the basis of convective flow. For N$_2$, log-SD was greatest in the supine position and smallest in $\mu G$, although these differences were not significant. This is as might be expected from the effect of gravity on the convective distribution of gas in the lungs. In $\mu G$, where we would expect differences in alveolar size to be minimized, the distributions of SV are narrower than in 1 G, although again the differences were not significant. A similar result was seen in the previous study when PILV was not fixed (19). However, this study produces a more consistent result in the supine position than the earlier study, with log-SD generally being greater in the supine (less homogeneous) than in the standing position. This is similar to the effect seen in single-breath washouts (9, 21), which showed significantly greater inhomogeneity of ventilation in the supine than in the standing position.

Direct comparison of log-SD for He and SF$_6$ with that for N$_2$ may not be valid, but because He and SF$_6$ are washin gases, they may be compared with each other. To verify that the wider distributions for SF$_6$ (larger log-SD) were not the result of more noise in the SF$_6$ data, we performed a simple simulation. A typical He washout was analyzed using the techniques described and taken as a reference. Normally distributed random noise was added to these data at a level approximately twice that seen in the SF$_6$ data, thus testing a worst-case scenario, and the analysis was repeated. This was performed eight times on the same data set, and the log-SD change was averaged. An increase of the noise in the signals resulted in no change in log-SD for the end-tidal data (0.5554 reference, 0.5548 ± 0.0037 SD, noisy simulations) or in the mixed expired data (0.6529...
In conclusion, studies of MBWs in \( \mu G \), performed at a fixed PILV and with tracers of differing diffusivity in the inspirate, largely confirm the somewhat surprising result of previous studies. Specifically, it appears that, despite reductions in CDI in the lung during large breaths, during tidal breathing, CDI remains largely unchanged in \( \mu G \). However, on the basis of the calcula-

---

reference, 0.6536 \( \pm \) 0.0011 SD, noisy simulations), indicating only a small sensitivity of log-SD to the noise levels in the underlying signals.

In general, the log-SD was greater for SF\(_6\) than for He, although these differences were small. However, in the end-tidal data collected in \( \mu G \), the log-SD for He was considerably less than that for SF\(_6\). This likely reflects the enhancement in alveolar mixing provided by the higher diffusivity of He. If significant CDI exists between closely placed units of lung, then diffusion will be an effective mechanism in reducing this inhomogeneity. Evidence for considerable nongravitational CDI has been obtained from experiments performed in \( \mu G \) (9, 19, 21, 22) and from studies of excised lungs (15, 23, 24). The markedly lower log-SD for He than for SF\(_6\) in \( \mu G \) is strong evidence for nongravitational CDI between lung units that are sufficiently close to each other that diffusion of He can reduce the concentration differences generated by CDI and is consistent with the calculations of CDI contribution performed above.

Slope ratio of MBW. The slope ratio is affected by the degree of CDI in the lung. We had expected to see an increase in the slope ratio in \( \mu G \) and failed to observe this in the previous MBW study (19). We attributed this in part to the reduction in lung volume in \( \mu G \). In this study we obtained absolute values for the slope ratio similar to those in the previous study and also failed to see an improvement in this index in \( \mu G \), despite the fact that lung volume was held constant. In fact, SF\(_6\) showed a significant reduction in slope ratio (became more inhomogeneous) in \( \mu G \), and the change for He bordered on statistically significant (Table 2).

This result confirms our previous observation that at normal or near-normal tidal volumes the bulk of the CDI-induced inhomogeneity of ventilation is nongravitational in origin and must, therefore, reflect differences in alveolar size or alveolar expansion between regions of the lung that are likely close together.

Lung volumes. The differences in tidal volume (which was controlled by the system), although significant in a statistical sense, are sufficiently small to be ignored in a physiological sense, inasmuch as they are only 50–100 ml. In the previous MBW study (19), we also saw a higher tidal volume in \( \mu G \) than on the ground, which we attributed to differences in system timing with respect to closing of the valves. We believe the same to be the case here.

The constancy of the PILV above RV is a good indication of the degree of control we were able to impose on our subjects’ breathing pattern and allows us to be very confident in the quality of our results. Similarly, the absolute PILV was unchanged between positions, suggesting that any changes in RV, such as have been seen previously (8), were sufficiently small so as to not perturb our measurements and allow us to compare the inhomogeneity of ventilation at similar absolute lung volumes.

Ventilatory inhomogeneity during tidal breathing. Previous studies of ventilatory inhomogeneity in \( \mu G \) led to equivocal results. Single-breath washout studies have provided strong evidence for a marked reduction in CDI effects, as evidenced by reductions in markers of inhomogeneity such as the height of the terminal rise after airway closure and the size of the cardiogenic oscillations (9, 13). On the basis of previous studies of the effect of gravity on the lung (1, 10, 14), these reductions were expected. However, considerable inhomogeneity that was clearly convective in origin remained, indicating that regional differences in mechanical behavior of the lung play a significant role in the overall degree of convective inhomogeneity. In a recent study of the degree of SV derived from rebreathing data (22), it was concluded that, in 1 G, gravitationally independent convective inhomogeneities were at least as large as gravitationally dependent inhomogeneities.

These same single-breath studies cited previously (9, 13) showed that, in \( \mu G \), most of the phase III slope persisted, indicating that considerable acinar inhomogeneity remains in \( \mu G \). This was not unexpected, since inhomogeneity at the acinar level was expected to be largely independent of gravity. However, when He and SF\(_6\) single-breath washouts were studied in \( \mu G \) (21), marked changes in diffusion-dependent inhomogeneity occurred, with unexpected slope reversals between SF\(_6\) and He. This suggested widespread changes in acinar conformation or alterations in cardiogenic mixing in the lung. Similar studies in short periods of \( \mu G \) (25 s), however, failed to reproduce these results, suggesting that the time course leading to these alterations in acinar gas mixing was >25 s but less than several hours (11). This in turn suggested that changes in the vascular compartment must play a role, since the known time course of viscoelastic settling of the lung is on the order of only a few seconds (6). These studies also suggested that the principal change in acinar gas mixing in \( \mu G \) was in the more proximal He diffusion front, as opposed to the more distal SF\(_6\) diffusion front, consistent with the changes in He observed in this study.

When multiple-breath N\(_2\) washouts were performed in the same subjects used for single-breath studies (9, 19), there were no significant reductions in the CDI components of ventilatory inhomogeneity, with the exception of a slight reduction in normalized phase III slope. These results were, however, somewhat confounded by the changes in PILV between the states studied. However, the overall conclusion from that study was that, during breathing close to that seen tidally, gravity played a largely unimportant role in the total amount of inhomogeneity seen in the normal human lung. The results of this study support that conclusion, with little or no change in CDI-dependent inhomogeneity in N\(_2\) or SF\(_6\), gases in which diffusion is less effective at abolishing the residual nongravitational concentration differences due to CDI.
tions of the CDI contribution to total inhomogeneity, it appears that this nongravitational CDI may exist between lung units that are relatively close together (at the level of a single acinus or a few acini) where diffusion of He can reduce the concentration gradients generated by CDI. The changes in interacinar gas mixing likely stem from enhanced spreading of the diffusion front for He, possibly caused by changes in cardiogenic mixing. However, the exact mechanism of these changes is unclear.

We acknowledge the support and cooperation of the crew of SLS-2 and the National Aeronautics and Space Administration and Martin Marietta Services personnel who supported the mission. We also thank Jim Billups, Mel Buderer, Charlie Davis, Marsha Dodds, Brian Dubow, Gerald Kendrick, Pat Kincade, Janelle Fine, Mary Murrell, and Gloria Salinas.

This work was supported by National Aeronautics and Space Administration Contract NAS9-16037 and contract Prodex with the Belgian National Policy Office.

This work was supported by National Aeronautics and Space Administration and Martin Marietta Services personnel who supported the mission. We also thank Jim Billups, Mel Buderer, Charlie Davis, Marsha Dodds, Brian Dubow, Gerald Kendrick, Pat Kincade, Janelle Fine, Mary Murrell, and Gloria Salinas.

Address for reprint requests: G. K. Prisk, Dept. of Medicine, 0931, University of California, San Diego, San Diego, 9500 Gilman Dr., La Jolla, CA 92039-0931.

Received 31 March 1997; accepted in final form 4 September 1997.

REFERENCES