Helium and sulfur hexafluoride bolus washin in short-term microgravity

Dutrieue, Brigitte, Anne-Marie Lauzon, Sylvia Verbanck, Ann R. Elliott, John B. West, Manuel Paiva, and G. Kim Prisk. Helium and sulfur hexafluoride bolus washin in short-term microgravity. J. Appl. Physiol. 86(5): 1594–1602, 1999.—We performed single-breath washout (SBW) tests in which He and sulfur hexafluoride (SF6) were inspired throughout the vital capacity inspirations or were inhaled as discrete boluses at different points in the inspiration. Tests were performed in normal gravity (1 G) and in up to 27 s of microgravity (μG) during parabolic flight. The phase III slope of the SBW could be accurately reconstructed from individual bolus tests when allowance for airways closure was made. Bolus tests showed that most of the SBW phase III slope results from events during inspiration at lung volumes below closing capacity and near total lung capacity, as does the SF6:He phase III slope difference. Similarly, the difference between 1 G and μG in phase III slopes for both gases was entirely accounted for by gravity-dependent events at high and low lung volumes. Phase IV height was always larger for SF6 than for He, suggesting at least some airway closure in close proximity to airways that remain open at residual volume. These results help explain previous studies in μG, which show large changes in gas mixing in vital capacity maneuvers but only small effects in tidal volume breaths.

single-breath washout; phase III slope; phase IV height; closing volume; closing capacity; gas mixing

IN HUMAN LUNG, there are three major sources of ventilatory inhomogeneity: gravitational convection-dependent inhomogeneity (grav-CDI), such as that described by Milic-Emili et al. (9); nongravitational convection-dependent inhomogeneity (nongrav-CDI), and diffusion-convection-dependent inhomogeneity (DCDI). Both forms of CDI are believed to be related to ventilation differences between lung units sufficiently widely separated from each other for diffusion not to be an effective mechanism of abolishing concentration differences generated between them. In contrast, DCDI, which occurs where convective and diffusive transports are of the same order of magnitude, is believed to result in ventilatory inhomogeneity between intra-acinar lung units. This is seen as a substantially steeper phase III slope for sulfur hexafluoride (SF6) than for He in normal subjects (7, 11).

Weightlessness [microgravity (μG)] was expected to result in a significant reduction in ventilatory inhomogeneity by abolishing the grav-CDI, leaving nongrav-CDI and DCDI unaffected. Consistent with this, single-breath washout (SBW) experiments performed during parabolic flight (8) or in spaceflight (5) showed significant decreases in phase III slope and phase IV height, both of which are markers of ventilatory inhomogeneity. It was also expected that He and SF6 phase III slopes would be affected to a similar extent by the removal of gravity. However, when SBW tests were performed in sustained μG, the slopes for He and SF6 became identical, and after a 10-s breathhold SF6 slope was actually flatter than that for He (11). This unexpected result suggested that a change occurred in the geometry of the acinus in μG.

In an effort to understand what caused the results of these previous studies, we performed a number of SBW studies in the KC-135 microgravity research aircraft, which provided us with up to 27 s of μG. In one set of these experiments, trace amounts of He and SF6 were inhaled over the course of the entire vital capacity (VC) inspiration, as in the spaceflight study (11). It was found that the changes in He and SF6 phase III slope seen in sustained μG were not reproduced in the short-term μG and that it was the He phase III slope which behaved differently in short-term vs. sustained μG (7). These results suggested that 1) the cause of the acinar geometrical changes previously observed during sustained μG had a long (compared with 27 s) time course and thus possibly involved the pulmonary vasculature and 2) these long-term changes occurred in the airways around the acinar entrance, i.e., where the He diffusion front occurs.

The second set of experiments was He and SF6 bolus studies (Bolus), also performed in the short-term μG available in the KC-135. A Bolus test uses the same lung volume (LV) maneuver as a single-breath test, but the air is breathed for the entire inspiration, except for a small bolus containing He and SF6, which is inserted into the test inspiration at a particular point in the breath. The rationale for using the Bolus tests was that the SBW test could be decomposed into several Bolus tests in which the bolus is positioned at different points in the inspiration. We had originally hoped to use these Bolus tests to shed light on the unexpected behavior of He and SF6 in sustained μG (11), but the different behavior seen in short-term μG (7) precluded that. Nevertheless, the results shed light on the relative importance of different LV ranges in the generation of
the commonly used markers of ventilatory inhomogeneity, phase III slope and phase IV height.

METHODS

Experimental system. Flow was measured with a heated Fleish no. 2 pneumotachograph connected to the subject's mouthpiece. The two ports of the pneumotachograph were connected via short tubes to a differential pressure transducer (MP-45, ±2 cm H2O; Validyne, Northridge, CA) coupled to a CD-101-871 carrier demodulator. The diaphragm of the pressure transducer was mounted vertically along the axis of the aircraft to minimize gravitational sensitivity. The other side of the pneumotachograph was connected to a two-way non-rebreathing valve (Hans Rudolph 2600, Kansas City, MO). This valve had an outlet to room air and an inlet connected to a pneumatics sliding valve (Hans Rudolph 8500B) activated by a valve controller (Hans Rudolph 4285B). When the sliding valve was in one position, the subject inspired air from an open port. With the valve in the other position, the subject inspired through a 150-ml tube containing the Bolus test gas, followed by air, or from a bag containing the SBW test gas. A sampling capillary was connected from the mouthpiece to a rapidly responding magnetic mass spectrometer (MGA 1100, Perkin-Elmer, Pamona, CA) for gas-concentration measurements. The mass spectrometer was equipped with a summing circuit to eliminate changes in output due to changes in barometric pressure, kinematic viscosity, or water vapor. Barometric pressure and vertical acceleration (Gz) were also measured.

A portable computer (IBM ThinkPad 360CSE) was used for data acquisition and valve control. Signals from the mass spectrometer, gravity sensor, and flow and barometric pressure transducers were sampled at 100 Hz by a 12-bit analog-digital board (DAQ-700, National Instruments). The pneumatic valve controller was connected to the digital output of the DAQ-700 for computerized control of the valve. Custom software was developed for data acquisition and valve control by using National Instruments Lab Windows CVI.

The data were collected in the National Aeronautics and Space Administration KC-135 microgravity research aircraft. A typical flight consisted of a climb to an altitude of ~10,000 m, with the cabin pressurized to ~600 mm Hg. A "roller-coaster" flight profile was then performed. The aircraft was pitched up at 1.8 Gz to a 45° nose-high altitude. Then the nose was lowered to abolish wing lift, and thrust was reduced to balance drag (thus maintaining µG). A ballistic flight profile resulted and was maintained until the nose of the aircraft was 45° below the horizon. In this manner, µG was maintained for ~27 s. A 1.8-Gz pullout maintained for ~40 s caused the nose to pitch up to a 45° nose-high attitude and allowed the cycle to be repeated.

Subjects and data collection. We studied eight healthy nonsmoking subjects. Only in six subjects did we obtain boluses within the same 10% LV range in both normal gravity (1 G) and µG to enable comparison (see Test maneuver). The subjects' relevant anthropometric data are shown in Table 1. These six subjects are the same as those studied previously (7), and they retain their subject number. None of the subjects reported pulmonary problems on questioning. Except for subjects 1 and 5, all subjects took anti-motion-sickness drugs (0.4 mg scopolamine, 5 mg dexedrine) on the days of flight. The data were collected with the subjects sitting in a standard aircraft seat in 1 G on the ground and during the µG phases of the parabolic flights. All ground data were collected under normobaric (~760 mm Hg), normoxic (21% O2) conditions. The µG data were collected under normoxic conditions at a barometric pressure of ~600 mm Hg. In addition, the barometric pressure decreased by ~20 mm Hg during the µG period as a result of the changes in aircraft engine power required to fly the maneuver.

The flowmeter was calibrated by integration of the flow from strokes of a 3-liter calibration syringe (model 5530, Hans Rudolph). A flow display was mounted in front of the subject for flow control. The calibration was performed on the ground and in flight in both 1 G and µG. The in-flight 1-G and µG calibrations differed from the ground calibration by ~10% because of the lower in-flight barometric pressure, and the appropriate calibrations were applied to the in-flight data. Furthermore, the Fleisch pneumotachograph flow-resistance characteristics are sensitive to changes in gas viscosity (14). The viscosity of our SBW test gas was greater than that of air, resulting in a measured increase of 1.107 in the resistance of the pneumotachograph compared with air. This factor was applied to correct for volume overestimation induced by the high viscosity of the test gas for the SBW tests. No correction was applied for the Bolus tests, because the inspired gas was air during the major portion of time.

The mass spectrometer was calibrated before and after use by sampling of the SBW test-gas mixture and air. The transit time of the mass spectrometer was determined 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.

Test maneuver. For the subjects, the single-breath Bolus tests involved exactly the same breathing maneuver as the SBW test described by Lauzon et al. (7). A period of quiet breathing through the mouthpiece was followed by a moderately rapid expiration to residual volume (RV). At RV, the sliding-valve control program was actuated, and the subject inspired up to total lung capacity (TLC) and expired back to RV at a controlled flow rate. The synchronization of the breathing maneuver with the aircraft profile was as follows. The first expiration to RV was performed during the transition between 1.8 Gz and µG, and both the inspiration from RV to TLC and the expiration from TLC to RV were performed during the µG phase. Because the µG phase lasted only ~27 s, the flow rate of the subjects was adjusted according to their VC to ensure that the whole maneuver would be performed in µG. The flow rates varied from 0.5 to 0.8 l/s but were the same for a given subject under all gravity conditions; individual flow rates are given in Table 1.

The Bolus tests only differed from the SBW tests by the gas-concentration profile during inspiration and subsequent expiration. For the SBW test, the entire inspiration consisted of 5% He-1.25% SF6-balance O2 (7). For the Bolus test, the inspiration consisted of air until a predetermined LV had been reached. The valve was then switched to the 150-ml bolus of 66% He-1.3% SF6-balance O2, followed by air to TLC. The bolus gas mixture provided end-inspiratory gas concentrations of ~1.6% He and ~0.3% SF6. The normalization process (see Data analysis below) allowed for the resulting gas-

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<th>Table 1. Anthropometric data and test flow rates</th>
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All subjects retain the same no. as in previous publications (7, 11). M, male; F, female.
concentration differences. Target LV was set at 0, 30, 60, and 90% of inspired VC (IVC). Each test was performed at least three times. Figure 1 superimposes a SBW test and four different LV Bolus tests obtained in 1G in the same subject.

Data analysis. The Bolus data were analyzed by using the same analysis techniques applied to the SBW tests by Lauzon et al. (7). Briefly, He and SF₆ concentrations were plotted vs. volume, and corresponding phase III slopes were computed by an iterative procedure using linear regression (5). In most cases, phase IV volume was taken to be the volume from the end of phase III to the end of expiration. However, when the phase III-phase IV transition was not clear-cut, we used the onset of phase IV determined from the corresponding SBW. This was done in the case of all boluses in µG for LV $30\%$ IVC. In both cases, the maximum phase IV height was referenced to the extrapolated phase III slope line (5). Each test was analyzed individually.

Changes in barometric pressure in the aircraft during the µG maneuvers lead to an error in VC. For our subject with smallest VC, this resulted in a $3\%$ error in phase III slope for all gases (see Ref. 7 for details of how this error was estimated). The magnitude of this error is exceeded by the variability of the measurements, and no attempt was made to correct for the changing barometric pressure in the aircraft.

Finally, He and SF₆ phase III slopes and phase IV heights were normalized by considering the pretest concentration as 100% and the inspired concentration as 0%, treating He and SF₆ as if they were resident gases. For the SBW tests, this results in positive phase III slopes and phase IV heights, and for the Bolus tests, the He and SF₆ phase III slopes and phase IV heights range from negative to positive values as the LV ranges from 0 to 100% IVC. SF₆-He slope difference was also calculated for each test.

SBW slope reconstruction from Bolus slopes. The basis of the proposed SBW reconstruction is to consider a set of gas boluses that, by their volumetric width and number, cover exactly and without overlap a full inspiration. In that case, the sum of the inspired and expired gas concentrations from all these Bolus tests should coincide with the concentration curve from the SBW test. As a consequence, the SBW phase III slope should equal the sum of the individual Bolus phase III slopes. Because the Bolus tests were performed at four LV values and bolus volume was only 150 ml, the IVC was not fully covered. Therefore, an interpolation had to be applied to the experimental Bolus slopes to reconstruct adequately the SBW slope. Two interpolation methods were used and are illustrated in Fig. 2.

In both methods, data points corresponding to LV values lying in a 3% IVC window were pooled (circled data points in Fig. 2) and represented by their average LV and slope. To obtain an interpolated function for slope $S(LV)$ over the 0–100% IVC range, we proceeded as follows. In LV range of 30–95% IVC, each two subsequent data points, or pooled data points, were interpolated linearly. The data point, or pooled data points, corresponding to the largest LV level in a given subject, was extrapolated to zero slope for 100% IVC, since no bolus is inspired at this LV level. The difference between method 1 and 2 concerns the interpolation between 0 and 30% IVC. Method 1 simply involves linear interpolation in the range of 0–30% IVC with extension to 0% IVC (solid line in Fig. 2). Method 2 takes into account the closing volume (CV), which was between 0 and 30% IVC for all subjects under study. In this method, the interpolation line above 30% IVC was extended to $LV = CV$, and a linear interpolation in the range 0–CV was computed with extension to 0% IVC (dashed line in Fig. 2). CV was determined from the corresponding gas SBW by subtracting the phase IV volume from the expired VC.

Finally, the reconstructed slope ($S_2$) is the mean over LV of the interpolated function $S(LV)$ multiplied by the number of

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Fig. 1. Representative traces from a subject of He concentration vs. volume obtained in normal gravity (1 G). Single-breath washout (SBW) and 4 different lung volume Bolus tests were represented simultaneously. Apparent width of inspired bolus is exaggerated because mass spectrometer is driven off-scale by the high bolus-gas concentration. IVC, inspired vital capacity.

Fig. 2. Illustrative example of application of the 2 reconstruction methods for 1 subject with sulfur hexafluoride (SF₆) in 1 G. Closing volume (CV) = 13.26% IVC for this subject. Experimental slopes are represented by the points. These were averaged to provide points for interpolation, as indicated by circles. Method 1, continuous line; method 2, broken line. LV, lung volume.
bolus ($n_{bol}$) needed to cover IVC

$$S_E = \frac{n_{bol}}{100\%} \int_{0\%}^{100\%} S(LV) \, dLV$$  \hspace{1cm} (1)

Concentration and volume normalization. To compare phase III slopes and phase IV height from different subjects and gravity conditions, two subsequent normalizations were carried out. 1) Slope and height normalization: the Bolus phase III slopes and phase IV height were normalized by the dilution factor, $V_{bolus}/TLC \times 100\%$, to account for different dilution of a fixed bolus volume ($V_{bolus}$) of 100% concentration in different subject TLC. Following this normalization, phase III slopes were expressed in liters$^{-1}$, and phase IV heights were dimensionless. TLC was obtained from mass balance computed on the SBW tests for each subject in each gravity condition by using the gas concentration from extrapolated phase III as that remaining in the lungs at RV. 2) Volume normalization: LV was rescaled as %TLC$_{1G}$ to compare slopes at the same absolute LV.

RESULTS

Reconstruction of SBW phase III slope from Bolus phase III slopes. Using data points for both gases and both gravity conditions, we compared the SBW slopes ($S_{SBW}$) with the corresponding $S_E$ obtained by using interpolation method 1 (Fig. 3A) or method 2 (Fig. 3B). The regression slopes (given with confidence interval at $P = 0.001$) were $0.91 \pm 0.36$ and $1.12 \pm 0.29$, respectively, and the corresponding intercepts were $-0.13 \pm 0.33$ and $-0.24 \pm 0.26$. When either method 1 or 2 was used, the regression lines were similar and did not differ significantly from the line of identity. The correlation coefficients between $S_{SBW}$ and $S_E$ were 0.81 (method 1) and 0.91 (method 2). The same regression analyses were performed with data subsets including only the 1-G or the µG data, yielding the same result, namely, that the regression lines did not differ from the line of identity.

Volume change between 1-G and µ-G. Average values of the volumes involved in the VC maneuver performed under 1-G and µ-G conditions are presented in Table 2. RV did not change significantly from 1-G to µ-G, whereas TLC and IVC decreased by 13% ($P < 0.004$) and 17% ($P < 0.002$), respectively, in µ-G compared with 1-G. Closing capacity (CC) did not differ between He and SF$_6$ SBW tests, and the values shown in Table 2 correspond to the average of all He and SF$_6$ measurements in all subjects. CC decreased by 4% TLC$_{1G}$ from 1-G to µ-G ($P < 0.03$). In subsequent Figs. 4–7, the 1-G and µ-G data are represented by using TLC$_{1G}$ value as a reference.

He and SF$_6$ Bolus phase III slopes. Figure 4 shows the 1-G and µ-G Bolus phase III slopes plotted against the corresponding LV at which the boluses were inhaled. Figure 4, A and B, represents He and SF$_6$ slopes, respectively, with each panel showing 1-G (solid symbols) and µ-G (open symbols) data.

To pool the data of Fig. 4 into distinct lung levels where He and SF$_6$ data in 1-G and µ-G could be compared, the entire LV range was divided into four categories: LV < CC$_{1G}$, CC$_{1G}$ < LV < 60% TLC$_{1G}$, 60% TLC$_{1G}$ < LV < 80% TLC$_{1G}$, and LV > 80% TLC$_{1G}$ delimited in Fig. 4.

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<td>RV, liters</td>
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<td>CC, %TLC$_{1G}$</td>
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Values are means ± SE of lung volumes over all subjects in normal gravity (1 G) and in microgravity (µG). TLC, total lung capacity; IVC, inspired vital capacity; RV, residual volume; CC, closing capacity = closing volume + RV. Note: RV was calculated from the nitrogen single-breath washout tests and reflects average RV between the beginning and the end of maneuver. *Significantly different between 1 G and µ G, $P < 0.05$. 

Fig. 3. Reconstructed slopes ($S_E$) vs. SBW slopes ($S_{SBW}$) obtained with method 1 (A) and method 2 (B). Regression lines are shown, and lines of identity are indicated by dashed lines. µ-G, microgravity.
by vertical dashed lines. The upper limit of the lowest LV category was fixed at \( CC_{1G} = 33\% \text{TLC}_{1G} \), which corresponded to the average CC of all subjects in 1 G. Unpaired t-tests were performed to compare the mean ± SE values between 1 G and \( \mu G \) in each LV categories. The resulting pooled representation of He and SF\(_6\) data under 1-G and \( \mu G \) conditions is shown in Fig. 5.

For LVs below \( CC_{1G} \), the mean value of He and SF\(_6\) absolute phase III slopes flattened significantly from 1 G to \( \mu G \). In the two middle-LV ranges, i.e., between \( CC_{1G} \) and 80\% TLC\(_{1G}\), the 1 G slopes for He and SF\(_6\) were smallest in absolute value and changed sign with increasing LV. \( \mu G \) did not significantly affect the He or SF\(_6\) phase III slope behavior in LV range between 60 and 80\% TLC\(_{1G}\). For LVs over 80\% TLC\(_{1G}\), He and SF\(_6\) slopes (which are positive in this range) decreased significantly from 1 G to \( \mu G \). However, in \( \mu G \), we were unable to perform Bolus tests at as high an absolute LV as we could in 1 G (Fig. 4), and thus the difference in slope between 1 G and \( \mu G \) above 80\% TLC\(_{1G}\) is probably exaggerated.

In addition to individual He and SF\(_6\) behavior in Fig. 5, LV dependence of the difference between both gas slopes is shown in Fig. 6. The SF\(_6\)-He phase III slope difference in 1 G reveals a pattern that mimics that of the individual gases (Fig. 5): SF\(_6\)-He phase III slope difference is negative for LV below \( CC_{1G} \), increases progressively, and changes sign over the middle-LV ranges to produce a positive SF\(_6\)-He slope difference for LV over \( CC_{1G} \). Also, in the LV range where SF\(_6\) and He slopes changed significantly from 1 G to \( \mu G \), SF\(_6\)-He phase III slope differences followed the same pattern of significant changes.

He and SF\(_6\) Bolus phase IV. He and SF\(_6\) Bolus phase IV heights obtained in 1 G and \( \mu G \) were pooled into the same four LV categories as was done for the phase III slopes, and the pooled phase IV data are shown in Fig. 7. In 1 G, average He and SF\(_6\) phase IV heights were negative for LV < \( CC_{1G} \) and positive for all other LV ranges. For LV < \( CC_{1G} \), He and SF\(_6\) absolute phase IV heights decreased significantly from 1 G to \( \mu G \). For all LVs above 60\% TLC\(_{1G}\), He and SF\(_6\) phase IV heights
were reduced in µG to the extent that they were no longer significantly different from zero. In 1 G, He phase IV height was significantly less than SF₆ phase IV height in absolute value in each of the four LV ranges (\( P < 0.005 \), paired t-test), whereas in µG He phase IV height was slightly smaller in absolute value than was SF₆ phase IV height but only in the LV range below CC₁G (\( P < 0.001 \)). In the LV range where phase IV heights were significantly different from zero, the average of He and SF₆ phase IV volumes did not change and were 11.4 ± 0.4% TLC₁G in 1 G to 9.7 ± 0.9% TLC₁G in µG (not significant).

**DISCUSSION**

The present study was conducted in two parts. First, we validated the reconstruction of SBW phase III slope from Bolus phase III slopes and confirmed that the reconstruction technique remains valid under different gravity conditions. Then we used the He and SF₆ Bolus tests, as opposed to the traditional SBW test, where He and SF₆ gases are inhaled over the entire course of inspiration, to investigate LV dependence of ventilation distribution under different gravity conditions.

Reconstruction of single-breath tests from Bolus tests. We have demonstrated the possibility of reconstructing the phase III slope obtained from a traditional SBW test using those measured in the corresponding Bolus tests. From a theoretical point of view, this is a consequence of the independence of transport between molecules inhaled at different LVs. From an experimental point of view, the quality of reconstruction depends on the ability to deliver sharp boluses at different points in breathing maneuvers that are reproducible. Figure 3 shows that experimental SBW phase III can be considered equivalent to an adequately weighted summation of multiple-bolus phase III slopes and that this is true for He and SF₆ slopes under different gravity conditions. Despite removal of the gravity-dependent effects in those tests performed in µG, the addition algorithm remains valid.

We considered the possibility that alterations in the interpolation algorithm might improve the fit. The only seemingly reasonable alteration beyond simple linear interpolation we could devise a priori was to allow for a difference in behavior above and below CV. We did this by assuming that the phase III slope behavior was...
linear above CV until 60% of VC, and we backextrapolated the slope as a function of LV back to CV (see Fig. 2). The result of this (Fig. 3B) was an improvement of the fit of the reconstructed slope ($S_{o}$) to the SBW slope ($S_{SBW}$).

Phase III slope LV dependence and effect of gravity. Before discussing the effect of LV and gravity on phase III slope, we should note the effect of $\mu$G on LV, since $\mu$G reduced VC in our subjects by 17%. This reduction is much greater than the 5% VC reduction observed by Elliott et al. (3) in sustained $\mu$G and it also exceeds the 8% VC reduction previously reported in transient $\mu$G (10). This is likely caused by a combination of failure to reach TLC in the limited time available for these complex maneuvers, requiring careful flow control on the part of the subject, and a translocation of blood into the thorax, which may be larger during the roller-coaster flight profile used here than in previous studies in which periods of normal gravity occurred between parabolic maneuvers (10). However, the exact cause of the observed change in VC remains unclear. With our choice to represent our 1-G and $\mu$G data sets as a function of absolute LV (Fig. 4), the 17% VC reduction in $\mu$G led to fewer $\mu$G than 1-G points in the LV range above 80% TLC$^{1G}$. Therefore, any statistical result obtained in this LV range (Figs. 5–7) should be interpreted with caution, as the differences may be somewhat exaggerated by this problem.

Characteristic of the 1-G phase III data as a function of LV in Fig. 5 is that in the extreme LV ranges, i.e., below CC$^{1G}$ or above 80% TLC$^{1G}$, Bolus phase III slopes are largest in absolute value and opposite in sign. The comparison of 1-G and $\mu$G Bolus phase III slopes also shows that the reduction in SBW phase III slope between 1 G and $\mu$G largely results from alterations in the behavior of the lung at high and low LVs and not from the middle-LV ranges where little difference appears (Fig. 5).

Michels and West (8) and Guy et al. (5) used argon boluses inhaled at the start of inspiration from RV and found absolute argon phase III values reduced by 39% (short-term $\mu$G) and 29% (long-term $\mu$G) of their 1-G value, respectively. This is of the same order of magnitude as the range of absolute He and SF$_6$ slope decrease we found (29–36% of their 1-G value) in the LV range below CC$^{1G}$ (Fig. 5). Figure 5 also shows that, if only the very low LVs contributed to the $\mu$G effect on phase III slope, the overall SBW slope would actually be increased in $\mu$G (because of the less negative slope contribution seen below CC$^{1G}$ in $\mu$G). However, bolus measurements made over the full LV range show that there is a counteracting effect of slope reductions with opposite sign at high volumes, which results in an overall SBW phase III slope reduction (5, 7, 8). Thus, despite the fact that we have data covering a higher LV range in 1 G, there must, nevertheless, be a marked reduction in Bolus slope at high LV in $\mu$G to explain the decrease in SBW slope in $\mu$G.

The SF$_6$-He slope difference, which is due to ventilatory inhomogeneity that is predominantly DCDI in origin, is also reduced in absolute value by $\mu$G in the low-LV range below CC$^{1G}$ (Fig. 6), pointing to an intra-acinar effect of $\mu$G at this LV. However, in the case of SF$_6$-He slope difference, this low-LV effect is countered by a similar effect, but with opposite sign, in the high-volume range. The overall effect is that the SBW SF$_6$-He slope difference is unaffected by short-term $\mu$G (7).

Boluses inhaled in the middle-LV range produce relatively small Bolus phase III slopes, with a virtual zero contribution to the overall phase III slope around LV = 60% TLC$^{1G}$, and are unaffected by the removal of gravity. When the near-zero phase III slopes in 1 G are considered, this could imply either that 1) at the end of inspiration He or SF$_6$ gas, which was inhaled in the middle-LV range, reaches homogeneous concentration between all lung units where mechanisms of ventilatory inhomogeneity (grav-CDI, nongrav-CDI, and DCDI) are operational or 2) the different mechanisms generate slopes of opposing sign so as to compensate each other. Both possibilities are not mutually exclusive in that the condition of homogeneous end-inspiratory concentration may occur at the acinar level where DCDI is operational, and the compensation of slopes...
may occur between more widely separated units subject to CDI. However, the fact that in µG, where grav-CDI has been eliminated, phase III slopes in the middle-LV range remain unchanged argues against the hypothesis of compensating slopes.

Phase IV: LV dependence and effect of gravity. The difference between events below and above CC1G appears very clearly in both 1-G and µG phase IV data (Fig. 7). Based on the onion-skin model of Milic-Emili et al. (9), the following predictions can be made. 1) For a bolus inhaled below CC1G, closed airways at the onset of inhalation introduce preferential ventilation of non-dependent lung regions, generating concentration differences resulting in a negative phase IV. 2) For boluses inhaled at LVs above CC1G, the preferential ventilation of the dependent lung units would lead to a positive phase IV height because of airway closure in the dependent lung region at the end of expiration. The resulting phase IV height would be expected to decrease slightly at higher values of LV as the difference in expansion ratios between upper and lower lung units becomes less near TLC. 3) These interregional mechanisms would produce the same phase IV height for He and SF6, since they deal with widely separated lung units.

The first two predictions are confirmed by our 1-G data (Fig. 7), whereas the third prediction is contradicted, i.e., He phase IV height is significantly less than SF6 phase IV height at all LVs in 1 G. This suggests that at least some of the lung units that are flow limited—or effectively closed—are sufficiently near to lung units that remain open, so that He diffusion is an effective mechanism at reducing concentration gradients between them. Such an argument is at least plausible given the size of the human acinus, ~1 cm in length (6). The typical inspiration time of our subjects was ~7.5 s, and the binary diffusive coefficient is $D = 0.6 \text{ cm}^2/\text{s}$ for He ($D = 0.1 \text{ cm}^2/\text{s}$ for SF6). The “mean square path” is given by $\Delta x = \sqrt{2D\tau}$, leading to 3 cm for He (1.2 cm for SF6), results that are compatible with diffusive mixing for He between adjacent or nearby acini.

![Fig. 8. Data from this study (closed symbols) compared with data from study by Anthonisen et al. (1) (x). A: He phase III slopes; B: He phase IV heights.](http://jap.physiology.org)
Equilibration of He between adjacent lung units subject to CDI was previously suggested by the differential behavior in μG of He and SF₆ multiple-breath washouts (in the tidal LV range) (12). The difference between He and SF₆ phase IV height observed here suggests a similar diffusion mechanism, occurring even in μG. In fact, if phase IV had only a gravity-dependent interregional basis, phase IV height would become zero at all LVs in μG. In the lowest LV range (Fig. 7), a significant phase IV height persists, and it was reduced by 51% compared with 1 G. This is similar to the 78% reduction found by Michels and West (8) under short-term μG conditions and the 64% reduction reported by Guy et al. (5) under long-term μG. However, in all LV ranges above CC₁G, there is no phase IV height of physiological significance in μG. Verbanck et al. (13) showed that at middle LVs in μG there are units of the lung with high specific ventilation, probably with low-end expiratory LVs. The absence of a distinct phase IV height for tests in which the bolus was inspired above CC₁G shows that the lung units that close are not necessarily those with low-end expiratory LVs.

The remaining phase IV in μG for the low-LV range implies that the previously hypothesized patchy airways closure (2) still occurs in μG, consistent with results of SBW tests in μG (5, 8). A similar He and SF₆ difference is present here as in 1 G.

Comparison with previous studies. Bolus tests have previously been used to measure CV under different gravity conditions (5, 8) or to study the effect of inhaled bolus at different LVs on phase III slope and phase IV height in 1 G (1, 4). To facilitate comparison with phase III and phase IV data obtained by Anthonisen et al. (1), who used He boluses inhaled in the LV range between 0 and 80% IVC, we transformed our LV scale to %IVC, as shown in Fig. 8A (phase III slope) and Fig. 8B (phase IV height). Whereas this comparison clearly shows a good agreement between our 1-G data set and the previous results of SBW tests in μG (5, 8), the phase IV height for tests in which the bolus was inspired above CC₁G shows that the lung units that close are not necessarily those with low-end expiratory LVs.

In summary, bolus inspirations at different LVs of He and SF₆ were performed in both 1 G and in short periods of μG. The resulting phase III slopes could be combined (with appropriate interpolation) to produce the phase III slopes obtained during conventional SBW tests in which the test gas is inspired over the entire VC range. The Bolus tests showed that almost all of the phase III slope seen in conventional SBW tests is a result of events below CC or near to TLC, with little slope resulting from gas inspired in the middle-LV range. The phase IV height for SF₆ was always greater than that for He, suggesting that diffusion must play a role in eliminating concentration differences established by convective inhomogeneities during inspiration. For this to be the case, some flow-limited lung units must be close to units that remain open at RV, a result consistent with previous studies.

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