Deposition and dispersion of 1-µm aerosol boluses in the human lung: effect of micro- and hypergravity

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Deposition and dispersion of 1-µm aerosol boluses in the human lung: effect of micro- and hypergravity. J. Appl. Physiol. 85(4): 1252–1259, 1998.—We performed bolus inhalations of 1-µm particles in four subjects on the ground (1 G) and during parabolic flights both in microgravity (µG) and in ~1.6 G. Boluses of ~70 ml were inhaled at different points in an inspiration from residual volume to 1 liter above functional residual capacity. The volume of air inhaled after the bolus (the penetration volume ($V_p$)) ranged from 200 to 1,500 ml. Aerosol concentration and flow rate were continuously measured at the mouth. The deposition, dispersion, and position of the bolus in the expired gas were calculated from these data. For $V_p \geq 400$ ml, both deposition and dispersion increased with $V_p$ and were strongly gravity dependent, with the greatest deposition and dispersion occurring for the largest G level. At $V_p = 800$ ml, deposition and dispersion increased from 33.9% and 319 ml in µG to 56.9% and 573 ml at ~1.6 G, respectively ($P < 0.05$). At each G level, the bolus was expired at a smaller volume than $V_p$, and this volume became smaller with increasing $V_p$. Although dispersion was lower in µG than in 1 G and ~1.6 G, it still increased steadily with increasing $V_p$, showing that nongravitational ventilatory inhomogeneity is partly responsible for dispersion in the human lung.

Aerosol bolus inhalation; convective mixing; ventilation; gravity; aerosol bolus inhalation; convective mixing; ventilation;

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The bolus tube was filled with aerosol-containing monodisperse polystyrene latex particles (Duke Scientific). The particles were supplied in suspension (water), and the concentrate was diluted and dispensed via two Acorn II nebulizers (Marquest Medical Products). Before entering the bolus tube, the aerosol flows through a heated hose and a diffusion dryer to remove water droplets. The size of the spherical particles as specified by the manufacturer was 1.07 ± 0.014 (SD) μm. The aerosol concentration in the bolus tube was ~10^6 particles/ml air.

The aerosol generated by the nebulizers was checked with a particle sizer (PCS-2000 Special, PALAS). The size analysis confirmed the size of the particles given by the manufacturer and shows that the amount of doublets in the aerosol was <5%. Tests using conductive tubing showed no significant effect from possible electrostatic charges on the particles.

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**Data recording and analysis.** A personal computer (IBM ThinkPad 360 CSE) equipped with a 12-bit analog-to-digital card (National Instrument, DAQ700) was used for data acquisition. Signals from the photometer, a G sensor, a barometric pressure transducer, and the pneumotachograph were sampled at 100 Hz. Custom software was developed for the data acquisition using National Instruments Lab Win.

Data were collected on the ground and aboard the NASA Microgravity Research Aircraft. A typical flight consisted of a climb to an altitude of ~10,000 m, with the cabin pressurized to ~600 Torr. A “roller coaster” flight profile was then performed. The aircraft was pitched up to ~1.6 head-to-foot acceleration to a 45° nose-high attitude. 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 aircraft nose was 45° below the horizon. In this manner, μG was maintained for ~27 s. A pullout averaging ~1.6 head-to-foot acceleration was maintained for ~40 s, causing the nose to pitch up to a 45° nose-high altitude, and allowed the cycle to be repeated. For convenience, the ~1.6-G hypergravity phase will be referred to as 1.6 G in the text.

**Subjects and protocol.** Four healthy subjects participated in the study. These were the same four subjects that participated in the previous study (8), and we retained their subject number for comparison purposes. Their relevant anthropometric data are listed in Table 1. After a few normal breaths, the subject exhaled to residual volume (RV) to ensure a known lung volume starting point. As functional residual capacity (FRC) varies significantly with G level (9,17), we chose to use the more stable RV as the starting point for the test breath. Although there is some small change in RV in μG (9), the effect on our measurements is small. The test breath consisted of an inspiration from RV to FRC + 1 liter at a flow rate of ~0.45 l/s, immediately followed by an expiration to RV, also at a flow rate of ~0.45 l/s. A flowmeter provided visual feedback to the subject. FRC refers to the seated 1-G FRC of the subject, and it was fixed for all experiments on that subject. During the inspiration, an aerosol bolus of ~70 ml was introduced at different penetration volumes (Vp) ranging from 200 to 1,500 ml. The Vp was defined as the volume of air inhaled from the mode of the aerosol bolus to the end of the inhalation.

The protocol was repeated four times for each Vp, both in μG and in 1.6 G. Before the flight, a set of data was also collected on the ground (1 G) with the same protocol. The protocol was approved both by the Committee on Investigations Involving Human Subjects at the University of California.
nia, San Diego, and by the Institutional Review Board at the
 Johnson Space Center, Houston, TX.
 Data analysis. For each bolus test, three different param-
eters were calculated. They were the aerosol deposition, the
aerosol dispersion, and the mode shift (MS). Deposition (DE)
was calculated by using the following equation

\[ \text{DE} = 1 - \frac{N_{\text{ex}}}{N_{\text{in}}} \]  

(1)

where \(N_{\text{in}}\) and \(N_{\text{ex}}\) are the number of particles in the inspired
and expired bolus, respectively. \(N_{\text{in}}\) and \(N_{\text{ex}}\) were calcu-
lated from the integration of the aerosol concentration as a function
of the inspired air. The integration was done when the
concentration exceeded 5% of the maximal inspired concentra-
tion to reduce error due to the noise of the signal. An original
tracing is shown in Fig. 2 for a bolus inhaled at a Vp of 500 ml.

The shaded areas represent the portion of the inhaled and
expired bolus, respectively. Only the volumes at one-half
the maximum concentration of the bolus are required to
compute \(H_{\text{in}}\) and \(H_{\text{ex}}\). Therefore, their computation is not
influenced by the 5% cutoff of the signal used in the deposition
calculation.

The MS was defined as the difference between the position
of the peak of the expired bolus (\(M_{\text{ex}}\)) and the volume
penetration of the inspired bolus (Fig. 2)

\[ \text{MS} = M_{\text{ex}} - V_p \]  

(3)

A negative value of MS indicates that the mode of the
expired bolus has shifted to a smaller lung volume than the
location of the inspired bolus, i.e., that the bolus has
moved toward the mouth.

Statistical analysis was performed by using Systat V5.0
(Systat, Evanston, IL). Measurements performed for the
same experimental conditions with the same subject were not
averaged before the statistical analysis was performed. Data
were grouped in different categorial variables such as G level
(µG, 1 G, and 1.6 G), Vp (200, 400, 600, 800, 1,000, 1,200,
and 1,500 ml), and subject number. A two-way analysis of vari-
ance was performed to test for differences between the chosen
categorial variables. Post hoc testing using Bonferroni adjust-
ment was performed for tests showing significant F-ratios.
Significant differences were accepted at the \(P < 0.05\) level.

RESULTS

Bolus inhalations were performed on four healthy
subjects for all Vp values ranging from 200 to 1,500 ml.
However, at 1.6 G, because of high deposition, the
expired bolus tracings we recorded for Vp > 800 ml had
too high a noise-to-signal ratio and the data were
discarded.

Deposition. The effect of G level on the deposition of
the aerosol bolus is displayed in Fig. 3. Figure 3A shows
the deposition averaged over the four subjects (means ± SD).
The data were not significantly different from one G level
to the other for Vp = 200 ml and were signifi-
cantly different for Vp > 200 ml (\(P < 0.05\)), with lower
deposition being present at lower G levels. At Vp = 400
and 600 ml, significant differences were found between
data in µG and 1.6 G and between data in 1 G and 1.6 G
but not between data in µG and 1 G. At each G level,
deposition increases with increasing Vp. In µG, depo-
sition varies from 18% at Vp = 200 ml to 50% at Vp = 1,500 ml.
In 1 G, it varies from 14% at Vp = 200 ml to 69% at Vp = 1,500 ml;
and at 1.6 G, it varies from 14% at Vp = 200 ml to 57% at Vp = 800 ml.
The slope of the regression lines of deposition as a function of Vp is
displayed in Fig. 3B (means ± SD). They are 0.023,
0.046, and 0.075%/ml in µG, 1 G, and 1.6 G, respec-
tively.

Dispersion. Figure 4A shows the dispersion as a
function of Vp for each G level. Data were averaged over
the four subjects (means ± SD). For Vp = 400 ml,
dispersion was strongly G dependent, with the greatest
dispersion occurring for the largest G level. For Vp =

\[ H = (H_{\text{in}}^2 - H_{\text{ex}}^2)^{0.5} \]  

(2)

where \(H_{\text{in}}\) and \(H_{\text{ex}}\) are the half-widths of the inspired
and expired boluses, respectively. Only the volumes at one-half
the maximum concentration of the bolus are required to
compute \(H_{\text{in}}\) and \(H_{\text{ex}}\). Therefore, their computation is not
influenced by the 5% cutoff of the signal used in the deposition
calculation.
function of the \( V_p \). Deposition increases with increasing \( V_p \) for each G level. Deposition is usually attributed to three main mechanisms: inertial impaction, gravitational sedimentation, and Brownian diffusion. For 1-\( \mu \)m-diameter particles, gravitational sedimentation is the mechanism that affects the most deposition in 1 G. The settling velocity of the spherical particles is \( \sim 33 \mu \text{m/s} \) in 1 G, whereas the Brownian displacement in 1 s is only \( \sim 13 \mu \text{m} \) (3). Inertial impaction is negligible for this particle size. In a previous study by our group (8), we computed the contribution of these three deposition mechanisms for a full inspiration of 1-\( \mu \)m-diameter particles with a one-dimensional model of the aerosol transport and deposition in the human lung. In 1 G, we found that deposition by impaction, sedimentation, and Brownian diffusion was 2.2, 78.6, and 19.2\% of total deposition, respectively.

Gravitational sedimentation takes place primarily in the distal part of the lung where residence time in airways is high and airway dimensions are small. At each G level, deposition increases with \( V_p \) as the aerosol bolus probes more distal airways (Fig. 3A). This can be referred to as the “penetration effect.” The increase in deposition may also be attributed to a “time effect.” By increasing the \( V_p \), the bolus enters the respiratory tract earlier in the inspiration and, therefore, more time is available to the particles to deposit. Both effects con-

**DISCUSSION**

Deposition. This study reports the effect of G level on the deposition and dispersion of 1-\( \mu \)m aerosol boluses inhaled to various volumetric depths within the lung. Figure 3 illustrates the deposition of aerosol as a 200 ml, there was a significant difference in dispersion values between \( \mu \)G and 1.6 G but not between \( \mu \)G and 1 G and between 1 G and 1.6 G. For each G level, dispersion was an approximately linear function of \( V_p \). The slope of the linear regressions between dispersion and \( V_p \) are displayed in Fig. 4B (means \( \pm \) SD). They are 0.19, 0.53, and 0.65 ml/ml in \( \mu \)G, 1 G, and 1.6 G, respectively.

MS. Figure 5 shows the effect of the G level on the MS. The MS is plotted on Fig. 5A as a function of \( V_p \). At each G level, the MS becomes increasingly negative with increasing \( V_p \). For a given \( V_p > 800 \) ml, significant differences were found between G levels, with MS becoming more and more negative (the bolus moving mouthward) with increasing G level. For \( V_p < 800 \) ml, there were no significant differences. At \( V_p = 800 \) ml, there was a significant difference only between \( \mu \)G and 1.6 G. Regression between MS and \( V_p \) at each G level was calculated. The slopes of these lines are displayed in Fig. 5B (means \( \pm \) SD). Whereas the slope in \( \mu \)G was lower than in 1 G, no significant differences were found between the slope in 1 G and in 1.6 G.

**Fig. 3.** Deposition of aerosol bolus. A: data are means \( \pm \) SD, averaged over the 4 subjects as a function of \( V_p \). \( \bigcirc \), Microgravity (\( \mu \)G); \( \bullet \), 1 G; \( \triangle \), 1.6 G. *Significantly different compared with 1-G data, \( P < 0.05 \). B: slope of the regression lines between deposition and \( V_p \) for different gravity levels.

**Fig. 4.** Aerosol bolus dispersion. A: data are means \( \pm \) SD, averaged over the 4 subjects as a function of \( V_p \). \( \bigcirc \), \( \mu \)G; \( \bullet \), 1 G; \( \triangle \), 1.6 G. *Significantly different compared with 1-G data, \( P < 0.05 \). B: slope of regression lines between dispersion and \( V_p \) for different gravity levels.
For a given $V_p$, deposition increases with G level. Of the three mechanisms of deposition, sedimentation is the only G-dependent process. Sedimentation is, therefore, the mechanism to account for the increased deposition in 1.6 G compared with 1 G. For $V_p < 400$ ml, deposition was, however, not significantly different from one G level to the other, confirming that deposition by sedimentation is a process that takes place primarily in the periphery of the lung.

In $\mu$G, as inertia is negligible, deposition can only be attributed to diffusion. However, Brownian diffusion by itself does not explain the deposition values we measured in $\mu$G. Indeed, for $V_p > 400$ ml where significant differences were found between G levels, deposition in $\mu$G is more than one-half the deposition in 1 G, which would imply that Brownian diffusion is the predominant mechanism of transfer of particles to the lung. This is in contradic- tion with the difference between the settling and diffusive velocity of these particles.

In a previous study (8), we observed a higher intrapulmonary deposition for small particles in $\mu$G than predicted by numerical models. The higher deposition was then explained by the nonreversibility of the flow and by a larger deposition by diffusion due to a higher alveolar concentration of aerosol in the absence of G. The nonreversibility of the flow was mainly explained by the asymmetry in the velocity profiles between inspiration and expiration. Very recently, Otani et al. (16) studied low-Reynolds-number cyclic flows in an alveolus model. They showed that these flows are irreversible, despite the low Reynolds number, causing substantial convective mixing in the periphery of the lung. The nonreversibility of the flow results in an additional mixing effect that moves the particles in the direction of the alveoli, increasing the apparent contribution of diffusional loss of particles in the lung. This increase in diffusional loss might explain the high deposition values we measured in $\mu$G in the present study.

Aerosol dispersion. Figure 4 illustrates the dispersion of aerosol as a function of the $V_p$. Dispersion is attributed to Brownian diffusion and convective mixing. As defined by Heyder et al. (11), convective mixing refers to all the mechanisms, except Brownian diffusion, which transport particles from tidal to residual gas. Convective mixing causes dispersion of aerosol boluses, depending on factors such as velocity patterns, airway and alveolar geometries, asymmetries between inspiratory and expiratory flows, inhomogeneous ventilation of the lung, and cardiogenic mixing.

Velocity profiles in the airways are not uniform and allow particles at different radial positions in the airways to travel at different speed (21). The branching structure of the bronchial tree also imparts a directional asymmetry to the flow field, manifested by a difference in the shape of the velocity profile during inspiration and expiration. During inspiration, airflow is most rapid at the center of the airways, so that the aerosol bolus entering the lung is surrounded by a sheath of residual gas that is largely particle-free. Thus particles in the center of the airways reach more distal generations of the respiratory tract than do particles located near the walls of the airways, where velocities are smaller. During expiration, the velocity profile is more blunted, and particles in the center of the airways travel at a slower rate than during inspiration, whereas particles near the walls travel faster, preventing the bolus from recovering its original shape.

Ventilation inhomogeneities may be described by two mechanisms: convection-dependent ventilatory inhomogeneities (15) and diffusive processes. Whereas diffusive processes are responsible for \(\sim 30\%\) of inhomogeneities of gas concentrations in 1 G (6), this mechanism is negligible for particles of 1 µm in size, as their diffusional coefficient is more than an order of magnitude lower than that of gases. Prisk et al. (18) showed in a study on ventilatory inhomogeneities determined from multiple-breath washouts during sustained $\mu$G that there was a reduction in the contribution of convection-dependent ventilatory inhomogeneities to overall ventilatory inhomogeneity. They concluded, however, that a considerable proportion of the inhomogeneity of ventilation present in the upright lung at 1 G during tidal breathing is nongravitational in origin. These ventilatory inhomogeneities also increase dispersion.
Rosenthal (19) showed in a theoretical study that ventilatory inhomogeneities should increase dispersion. Indirect experimental evidence of this statement can be found in a study by Anderson et al. (2) in which bolus aerosol dispersion was measured in normal subjects and in patients with cystic fibrosis (CF). CF is an obstructive disease that is known to increase inhomogeneities of ventilation. According to this paper, studies of pulmonary mechanics in patients with CF have shown an increase in the inhomogeneities of ventilation distribution. Anderson et al. showed that patients with CF exhaled boluses that were broader than those exhaled by normal subjects at all V_p values they examined (V_p = 100–700 ml). These authors believed that the increased bolus spreading in patients with CF was partly due to the increased inhomogeneities in ventilation distribution, compared with normal subjects. In our study, we modified the level of ventilatory inhomogeneities in healthy subjects by increasing the test in µG where ventilatory inhomogeneities are still present (18, 23) but reduced, compared with normal G. We obtained lower dispersion values in µG than in 1 G.

Particles sediment during their transport in the airways and, therefore, do not follow the airstream. This effect is negligible in the first generations of the lung where bulk velocity is an order of magnitude larger than the settling velocity of the 1-µm-diameter particles. In the distal part of the lung, the radial position of the particles may significantly change while flowing through airways. However, according to Schulz et al. (22), who studied the convective and diffusive gas transport in canine intrapulmonary airways, the sedimentation rate does not affect significantly aerosol dispersion. They performed aerosol bolus inhalations in 1 G with 0.86 and 2.38-µm-diameter particles (a 7.7 times difference in settling velocity) and yet found no systematic differences between the dispersion of the smaller and the larger particles.

The convective mixing occurring over a single breathing cycle is the phenomenon assessed by aerosol bolus dispersion. The data we collected in this study highlight the effect of G on this phenomenon. As shown in Fig. 4A, for each G level, dispersion increases continuously with increasing V_p, indicating that each subsequent airway generation of the lung contributes to convective mixing. In the studied range of V_p values, this increase is approximately linear and is shown in the slope of the regression lines of dispersion as a function of V_p (Fig. 4B). For a given V_p, dispersion increases with G level: likely factors include the uneven distribution of ventilation and the asynchrony in regional filling and emptying of the lung, both of which increase with G.

However, while dispersion is lower in µG than in 1 G, it still increased steadily with increasing V_p, showing that nongravitational ventilatory inhomogeneity is partly responsible for dispersion. Although total ventilation inhomogeneity is reduced in µG, G-independent ventilatory inhomogeneities are still present and contribute to aerosol dispersion, as do cardiogenic oscillations, differences in velocity patterns between inspiratory and expiratory, the effect of secondary flow mixing at bifurcations, and laryngeal jetting. Although all these mechanisms may contribute to aerosol dispersion, the continuing presence of ventilatory inhomogeneities in µG (18, 23) suggests that aerosol dispersion in µG is largely due to nongravitational ventilatory inhomogeneities.

The relative contribution of G to dispersion also increases with V_p (Fig. 4). At V_p = 200 ml, no significant differences were found between dispersion at different G levels, whereas at V_p = 1,500 ml, dispersion in µG is only 51% of dispersion in 1 G. Based on the slope of the regression lines (Fig. 4B), dispersion due to G-independent factors is 36% of overall dispersion in 1 G and 30% of overall dispersion in 1.6 G. According to Verbanck et al. (23), the G-independent ventilatory inhomogeneities are at least as large as the G-dependent ventilatory inhomogeneities for gases. Our data show a lower contribution of the G-independent inhomogeneities to the overall dispersion than would be expected from the study by Verbanck et al. This might be explained by the absence of diffusion inhomogeneities in the aerosol experiments, which reduces the G-dependent ventilation inhomogeneities.

Comparison with previous 1-G studies. The deposition, the dispersion, and the MS at 1 G were compared with data previously obtained by Brand et al. (4) and Darquenne et al. (7). Brand et al. (4) studied 79 healthy subjects and used V_p values ranging from 20 to 800 ml. Darquenne et al. (7) performed the bolus tests in 10 healthy subjects with V_p values ranging from 200 to 1,500 ml. The two previous studies used 0.87-µm-diameter particles and a flow rate of 0.25 l/s, compared with 1-µm-diameter particles and a flow rate of 0.45 l/s in our experiments. The three sets of data (means ± SD) for deposition, dispersion, and MS are displayed in Fig. 6, A-C, respectively. Given the differences in the protocols, our small subject population, and the inter- and intrasubject variabilities, we found the values for all three parameters similar to those of other investigators. The deposition data shown in Fig. 6A are, however, higher than deposition obtained by Ander-
we were able to collect data at 1 G and compare the measurements with those obtained on the ground in the same subject. The data are displayed in Fig. 7 for subject 2 used as an example. The comparison shows that the results obtained in both environments are similar, with no statistical differences between aircraft and ground data. This means that there were no

Fig. 6. Comparison of our 1-G data (●) with bolus studies of Brand et al. (4) (○) and Darquenne et al. (7) (△). Data are means ± SD. In our study, particle size (dₚ) is 1 µm and flow rate (Q) is 0.45 l/s. In Brand et al. (4) and Darquenne et al. (7) studies, dₚ is 0.87 µm and Q is 0.25 l/s. A: deposition, B: dispersion, C: mode shift.

Fig. 7. Comparison between data obtained for subject 2 on the ground (○, mean ± SD) and aboard the aircraft at 1 G (●). A: deposition, B: dispersion, C: mode shift.

son et al. (2) and Kim et al. (13) for 1-µm-diameter particles.

Effect of changes in gas density caused by altitude. Several flights were performed to collect all the data. During each flight, before the start of the first parabola,
systematic differences in our data introduced by the lower barometric pressure in the aircraft cabin and that we may, therefore, reliably compare aircraft and ground data. Similar comparisons were made for the three other subjects, and the results were the same.

In summary, aerosol bolus inhalations were performed with 1-µm-diameter particles in four subjects on the ground and aboard the KC-135 aircraft during both weightlessness and the hypergravity phases. The boluses were inhaled at different $V_p$ values ranging from 200 to 1,500 ml. The data showed that both bolus dispersion and deposition increased with $V_p$ and that they were strongly $G$ dependent, with the largest dispersion and deposition occurring for the largest $G$ levels. Even if dispersion was reduced in µG, it still increased steadily with $V_p$. The dispersion observed in µG can be explained by $G$-independent ventilatory inhomogeneities, the nonreversibility of the flows between inspiration, and expiration and cardiogenic mixing.

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