Bronchial airway deposition and retention of particles in inhaled boluses: effect of anatomic dead space

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Center for Environmental Medicine and Lung Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; GSF-National Research Center for Environment and Health, Institute for Inhalation Biology, D-85758 Neuherberg/ Munich, Germany; and Human Studies Division, National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina 27771

Bennett, William D., Gerhard Scheuch, Kirby L. Zeman, James S. Brown, Chong Kim, Joachim Heyder, and Willi Stahlhofen. Bronchial airway deposition and retention of particles in inhaled boluses: effect of anatomic dead space. J. Appl. Physiol. 85(2): 685–694, 1998.—The fractional deposition of particles in boluses delivered to shallow lung depths and their subsequent retention in the airways may depend on the relative volume and size of an individual’s airways. To evaluate the effect of variable anatomic dead space (ADS) on aerosol bolus delivery we had healthy subjects inhale radiolabeled, monodisperse aerosol (99mTc-iron oxide, 3.5 μm mean monodispersed aerosol diameter) boluses (40 ml) to a volumetric front depth of 70 ml into the lung at a lung volume of 70% total lung capacity end inhalation. By using filter techniques, aerosol photometry, and gamma camera analysis, we estimated the fraction of the inhaled boluses deposited in intrathoracic airways (IDF). ADS by single-breath N2 washout was also measured from 70% total lung capacity. Results showed that among all subjects IDF was variable (range 0.04–0.43, coefficient of variation = 0.54) and increased with decreasing ADS (r = –0.76, P = 0.001, n = 16). We found significantly greater deposition in the left (L) vs. right (R) lungs; mean L/R (ratio of deposition in L lung to R lung, normalized to ratio of L-to-R lung volume) was 1.58 ± 0.42 (SD; P < 0.001 for comparison with 1.0). Retention of deposited particles at 2 h was independent of ADS or IDF. There was significant retention of particles at 24 h postdeposition (0.27 ± 0.05) and slow clearance of these particles continued through 48 h postdeposition. Finally, analysis of central-to-peripheral ratios of initial deposition and 24-h-retention gamma-camera images suggest significant retention of insoluble particles in large bronchial airways at 24 h postdeposition (i.e., 24 h central-to-peripheral ratio = 1.40 ± 0.44 and 1.82 ± 0.54 in the R and L lung, respectively; P < 0.02 for comparison with 1.0). These data may prove useful for 1) designing aerosol delivery techniques to target bronchial airways and 2) understanding airway retention of inhaled particles.

aerosol deposition; aerosol drug delivery; retention of inhaled particles

FOR PURPOSES OF DRUG DELIVERY and airway clearance measurements, it may be desirable to restrict deposition of inhaled aerosols to the bronchial airways by inhalation of small aerosol boluses to shallow volumetric lung depths (18, 24). The general rationale for targeting therapeutic aerosol delivery to specific regions of the respiratory tract is to maximize the drug effect to the region desired while minimizing unwanted side effects to other regions (3). Targeting aerosolized drug delivery may also improve cost-effectiveness by minimizing the total delivered dose. The administration of gene therapy to the lungs of patients with cystic fibrosis is a recent example in which airway targeting of an inhaled aerosol is desirable (15).

An aerosol bolus is a discrete volume of air that contains particles sandwiched within an inhaled volume of particle-free air (19). The depth to which a bolus penetrates into the lung is determined by the volume of the bolus and the volume of air inhaled after its insertion into the air stream. An injection at the beginning of an inhalation would preferentially deliver the aerosol to the periphery, whereas inhalation of the bolus at the end of a breath tends to deliver the aerosol to the extrathoracic and conducting airways. The inhalation is generally followed by a period of breath-holding to maximize particle deposition on airway surfaces. It has been proposed that, in attempting to confine the aerosol to the anatomic dead space (ADS) of the lungs, the boluses be small (<50 ml) and delivered to shallow volumetric front depths (VFD), i.e., <150 ml (18–20, 24). The VFD is an estimate of the penetration of particles into the respiratory tract and represents the volume inspired from the point when the first particles enter the mouth to the end of inhalation (18, 19, 24).

Radiolabeled aerosol boluses have been used to measure clearance of particles from various depths in the lung (18, 20, 24). These studies have shown lung retentions as great as 60% at 24 h postdeposition for boluses delivered to shallow lung depths, i.e., less than the ADS. These investigators have found continued, significant clearance of particles between 24 and 72 h postdeposition; these results are contrast with experiments in which subjects inhaled large volumes of labeled aerosol. Presumably, this late phase of clearance represents a slow component from the airways. However, the degree of particle penetration to alveoli in those experiments (3) has been debated. Nonhomogeneous bolus distribution in the lung and axial streaming of the aerosol beyond the ADS might contribute to alveolar deposition (3, 24). In previous studies, boluses have been delivered to a constant volumetric lung depth regardless of the size of an individual’s ADS (7, 18–20, 24). In theory, increased ADS would decrease the relative penetration of the bolus into the lung, minimizing alveolar deposition from axial streaming but also shifting deposition toward the larynx and
METHODS

particle retentions (as a fraction of initial deposition) the fixed VFD (70-ml) measurements. In all subjects, boluses (40 ml) to a normalized VFD, a constant subset of the subjects also inhaled radiolabeled aerosol bolus technique at the same VFD (7, 19). Finally, a washout at 70% TLC end inhalation and measured with measurements of ADS by single-breath N₂ washout at 70% TLC end inhalation and measurements of effective air space dimensions (EAD) by the bolus technique at the same VFD (7, 19). Finally, a subset of the subjects also inhaled radiolabeled aerosol boluses (40 ml) to a normalized VFD, a constant fraction of their measured ADS, for comparison with the fixed VFD (70-ml) measurements. In all subjects, particle retentions (as a fraction of initial deposition) were measured through 48-h postdeposition.

MEASUREMENTS

Experiments were performed in a group of 16 healthy subjects (10 men, 6 women; ages 20–43 yr) with normal pulmonary function. In each subject, we measured forced expiratory volumes and flows, lung volumes, inspiratory capacity, and expiratory reserve volume by using spirometry. Airway resistance (Raw) and functional residual capacity (FRC) were measured by body plethysmography. The subjects had no smoking history, no history of lung disease, and no recent history of acute respiratory infection or viral illness within the previous 4 wk. Informed consent was obtained from each volunteer; the study had the approval of the University of North Carolina Committee on the Protection of the Rights of Human Subjects.

ADS

Single-breath N₂ washout was measured from 70% TLC, according to the technique of Fowler (10). Subjects inhaled a single breath of 100% O₂ from residual volume (RV) to 70% TLC and exhaled again to RV. A special mouthpiece designed to reduce the volume of the oral cavity was used for each individual. The mouthpiece was a metal tube that extended to the back of the mouth and was surrounded by individually fitted silicone dental compound. This same mouthpiece was used for the EAD and radiolabeled bolus inhalations described below. For each subject, the exhaled volumes associated with phase 1 and the ADS (exhaled volume through the midpoint of phase 2) of three N₂-washout curves were averaged. Phase 1 was determined as the exhaled volume (corrected for BTPS) associated with 0% exhaled N₂, and ADS was determined by the equal-area technique described by Fowler (10).

Xenon-Equilibrium Scan

While seated with his or her back to a gamma camera [Elscint Apex 415 large field-of-view (40 cm) gamma camera] interfaced with an Elscint model 109 computer, each subject rebreathed ¹³³Xe from a xenon-ventilation system to obtain an equilibrium scan of the lungs. This scan was used to define the outline of the lung and to normalize the particle-deposition scans to regional lung volume for regional-deposition analysis (described further in Data Analysis).

Radiolabeled Aerosol Bolus Inhalations

The aerosol, ⁹⁹ᵐTc-Fe₂O₃, was produced by spinning top generator (15), then passed through a tube furnace (800°C) and a concentrator before it was stored in a vertical 5-liter shielded cylinder attached above the bolus-delivery system. The detailed procedures for radiolabeling and producing these aerosols are described by Wales et al. (26). Some leaching of radiolabel from the particles has been found over time, but in vitro analysis in simulated body fluids suggests that the leaching rate is reproducible (26).

Before inhaling radiolabeled aerosol boluses, each subject began by sitting with his or her back against the gamma camera while a 15-min background image was recorded for later correction of lung images. The subject then inhaled the radiolabeled, monodisperse aerosol (3.5-µm mean monodisperse aerosol diameter (MMAD)) boluses (40 ml) delivered by a Pari bolus-delivery system [respiratory aerosol probe (RAP); Pari, Starnberg, Germany; Ref. 27] via the mouthpiece described above. Each bolus was inhaled to a 70-ml VFD during inhalation to 70% TLC, by using a constant inspiratory flow rate (250 ml/s) followed by an 8- to 10-s breath hold to maximize particle deposition in the airways. Each subject inhaled a total of 10–20 boluses. During the bolus inhalations, the relative aerosol concentration (Conc) and respired volumes were measured by photometry and a pneumotachograph at the mouth to determine the VFD of each inhaled bolus (18). Aerosol Conc values, as a function of the inhaled and exhaled volumes, were stored in a personal computer for further analysis. When ~10–15 µCi of activity were deposited in the subjects’ lungs, as determined by a single NaI detector placed against the subject’s back, inhalations of labeled aerosol ceased. This minimum level of activity was deemed necessary to obtain retention measurements through 48-h postdeposition. In most cases, the inhalations (10–20 boluses) took ~15 min; however, in some subjects, in whom lesser fractions of the bolus were deposited, inhalation of boluses took as long as 20 min.

The subject was then seated with his or her back to the gamma camera (in the same position as for the ¹³³Xe scan described above), and an initial 2-min posterior deposition scan was recorded. Sequential 2-min images were recorded for a period of 2 h and 30 min. Immediately before the third 2-min image, each subject ate and drank to wash deposited activity from the oropharynx and esophagus into the stomach. Before the fifth 2-min image, the subject turned, with the chest to the camera and while still seated, to obtain an anterior image of the deposition pattern (i.e., the fifth image). At 24 h postdeposition, the subjects returned for a 30-min posterior camera scan. At this time, subjects were also scanned for 10 min with a more sensitive detector consisting of six NaI crystals (Nuclear Data) (4). A 10-min background count with this detector was obtained before inhalation of the boluses the previous day. Finally, at 48 h postdeposition, the subject was again scanned with the Nuclear Data detector for a period of 30 min.

Radiolabeled bolus inhalations and subsequent scanning through 48 h, as described above, were repeated in a subset of seven subjects on a separate study visit at least 1 wk after the first study visit. For comparison with the delivery of boluses to a fixed VFD of 70 ml, these subjects inhaled the boluses (40
ml) to a VFD that was equal to a constant fraction (0.90) of the phase 1 volume (BTPS) (i.e., normalized VFD) from their single-breath N₂-washout curve measured at 70% TLC. All other inhalation conditions were similar to the fixed VFD experiments described above.

EAD Measurements

In each subject, we also measured the EAD at 70 ml VFD (EAD₇₀ ml) and at a VFD of 0.9 phase 1 N₂ washout by determining the recovery of particles from inhaled boluses (also 40 ml) as a function of breath-holding time. The technique used in these experiments is described in detail elsewhere (7, 19). In brief, the EADs were determined by analysis of exhaled aerosol recovery after inhalation of 40-ml boluses to the prescribed VFD and breath holds at 70% TLC for 0–10 s. A 2-μm MMAD monodisperse aerosol (geometric standard deviation = 1.1), composed of diethylhexyl sebacate and salt nuclei, was generated by a Monodisperse Aerosol Generator (MAGE; TSI, Minneapolis, MN) for use in the EAD measurements. If we assume that the lung is composed of a system of randomly oriented tubes, the rate of decline (slope) of the recovery vs. breath-hold time relationship is inversely proportional to the effective inner diameter of those tubes (7). Although the assumption of random orientation may not be valid for boluses delivered to very shallow depths, as is the case here, the measured EADs should still be reflective of the average linear, vertical dimension of the air spaces to which the radiolabeled aerosol boluses were delivered in each subject (19).

Data Analysis

Fractional deposition of the bolus. Using filter techniques, aerosol photometry, and gamma-camera analysis, we estimated the fraction of the inhaled boluses that actually deposited in intrathoracic airways (IDF). During the inhalation of the boluses, a filter (Pall BBSOT) was placed on the expiratory port of the Pari bolus-delivery system to collect total activity exhaled by the subject (Aₑₙ). After the subject completed inhalation of the aerosol boluses, a test (inspiratory) filter was attached to the mouthpiece of the RAP system, and a single 40-ml bolus was drawn onto the filter with a 1-liter syringe (Aₐₙ) to measure the aerosol delivered (inhaled) by a single bolus. In addition, the peak aerosol number Conc for each bolus was measured by laser photometry at the mouth during both the subject’s bolus inhalations (Concn) and this postinhalation filter sample (Concnf). The total inhaled activity (A₁₈₅) could then be determined as

\[ A₁₈₅ = n(Aₐₙ)(\text{mean } \text{Concn}/\text{Concnf}) \]  

where \( n \) was the total number of boluses inhaled by the subject. The total deposition fraction (DF) for each subject could then be determined as

\[ \text{DF} = 1 - \left( \frac{Aₑₙ}{A₁₈₅} \right) \]  

The aerosol number Conc measured by photometry on expiration (Concn) was generally either too noisy or was affected by exhaled water condensate (11) to be useful in determining DF.

Using region-of-interest (ROI) gamma-camera analysis (6), we estimated the relative amount of deposited particles in the lungs (intrathoracic lung activity) vs. mouth and larynx (extrathoracic = head + stomach activity) in each subject. For each subject, we analyzed the third 2-min posterior deposition gamma-camera image (i.e., the image after eating and drinking). Separate, subsequent tests showed that 85% of mouth activity is washed into the stomach by the eating and drinking procedure used here. Thus much of the “head” activity in this image likely reflected deposition in the larynx. There may have been a small portion of activity in the mouth that was missed by this analysis, especially in the tallest subjects. Activity in each region (lung, head, and stomach) was multiplied by an attenuation factor to correct for different gamma attenuations for the lungs (2.5), head (2.0), and stomach (4.0; see Ref. 25). IDF was then calculated as

\[ \text{IDF} = \frac{\text{Lung activity}}{\text{Total activity}} \times \text{DF} \]

where total activity is the sum of lung, head, and stomach activity, and each is corrected for attenuation.

Correcting for attenuation differences between head, lung, and stomach with these factors provided more realistic estimates of activity in these regions. Although the mean attenuation factors used were determined for 111In (25), it is the relative differences in attenuation between the three regions that is important for estimates of IDF, not the absolute values. 99mTc, with a 140-KeV gamma emission, has a linear-attenuation coefficient in water of 0.16, whereas 111In, with gamma energies of 171 and 245 KeV, has coefficients of 0.15 and 0.13, respectively.

Regional lung deposition of the inhaled bolus. Just as ROI analysis was used to determine intra- vs. extrathoracic deposition of the inhaled boluses, we also used these techniques to determine regional deposition within the lung itself. To assess the degree of central (C) vs. peripheral (P) airway deposition within the lung, we calculated a C/P ratio of 99mTc activity (14, 23), normalized to the 133Xe-equilibrium scan for each subject. This normalization was done to account for the difference in relative lung areas and thickness between the C and P regions. While both the C and P regions overlap alveoli and small airways, the C region also incorporates large, bronchial airways not present in the P region. Thus increases in C/P to values >1.0 reflect increased deposition in large airways. The C/P ratio was determined for both the right (R) and left (L) lungs on the initial, 2-min deposition scan to minimize interference from stomach activity (i.e., before eating and drinking).

In addition to analysis of C vs. P airway deposition, we analyzed the uniformity of deposition throughout the lungs (13) by using the first 2-min-deposition scan. Outline ROIs of the L and R lungs created from the 133Xe-equilibrium scan were equally divided into an upper (U) and lower (L) region (i.e., UL, LL, UR, and LR). The 99mTc activity in each of the four regions was divided by total 99mTc activity for all regions combined. A similar ratio for 133Xe-equilibrium activity was also calculated for each region. Each 99mTc ratio was then divided by the corresponding 133Xe ratio for each region to account for differences in lung volume in the four regions, e.g., UL/total = (A₁₈₅UL/A₁₈₅total)/(A₁₈₅UL/A₁₈₅total). If the deposited particles from the bolus were evenly distributed throughout the lungs, this ratio for each region should be close to 1.0. An increase or decrease in deposition out of proportion to the volume in that region will increase or decrease the ratio to >1.0 or <1.0, respectively. We then defined the SD (as a measure of variance) for the average ratio for all four regions as the evenness index (EI) for bolus deposition (13). If all regions have ratios of 1.0 (i.e., a uniform distribution), EI = 0. EI then increases with increasing unevenness as the four ratios diverge from 1.0.
Pulmonary retention of deposited particles. A rectangular region bordering the R and L lung (defined by the $^{133}$Xe-equilibrium scan) was used to determine, by computer analysis, the lung particle retention as a fraction of the initial counts (background and decay corrected) in each lung over the gamma-camera scanning period at 2 and 24 h ($R_2$ and $R_{24}$, respectively). For comparison with the initial deposition C/P ratio, we also calculated C/P ratios (see Regional lung deposition of inhaled bolus) at 2 and 24 h postdeposition for the R lung (23). Because high levels of stomach activity tended to interfere with the 2-h image of the lung we did not determine L lung C/P at this time point. The Nuclear Data six-crystal detector was used to monitor clearance during the 24- to 48-h period after particle deposition. There were three detectors for each lung, but we only utilized the upper two detectors on each side to minimize detection of activity in the gastrointestinal tract. A 20% window, centered around the 99mTc peak for each crystal, was used to determine each crystal’s total detected counts. The activities measured by each of these detectors were summed, and decay/background was corrected to 24-h postdeposition to provide a mean whole lung retention at 48 h (expressed as a fraction of the initial 24-h counts).

Because the boluses were very small and deposition fractions might be low in some individuals, we established a criterion for net counts above background at 24 and 48 h to accept the respective retention values at those times. As the radioactivity in the lung approaches background activity (either by clearance or by decay), its measure becomes highly variable (i.e., %SD of measured counts) over a given measurement period (8). For 24-h retention data to be acceptable (for either R, L, or both lungs combined) in a given subject, the %SD (8) of the net counts could not exceed 20%. Similarly, if the 24-h retention data were acceptable in a given subject, the 48-h retention had to meet the same criterion to be acceptable. The same criterion was applied to measured activity in each lung for acceptance of 24-h C/P data. This error, %SD, associated with the net counts (i.e., background corrected) at either 24 or 48 h was calculated as the square root of the sum of total and background counts divided by the difference in these measured counts (expressed as a percentage).

Statistical analysis. Comparison of parameters between the two bolus depths (i.e., fixed VFD and the normalized VFD) and comparison of C/Ps at different time points postdeposition were made by repeated-measures analysis (Systat for Macintosh). Differences in parameters between men and women were determined by analysis of variance. Linear regression and stepwise multiple-regression analysis (Systat for Macintosh) were used to determine the relationship between bolus deposition-retention parameters and other lung parameters (e.g., ADS, lung volumes, Raw).

### Fractional Deposition of the Bolus

Table 1 summarizes the mean fractional deposition data (DF and IDF) of the inhaled boluses for all subjects studied, for men vs. women, and for the fixed VFD vs. normalized VFD. Also given are the mean phase 1 and ADS of N$_2$ washout and the EAD$_{70\text{ ml}}$ in these subjects. Although 48% of the particles in the bolus were deposited in the respiratory tract (DF), only 26% actually deposited in the intrathoracic airways (IDF). Almost one-half of the particles deposited in the respiratory tract (i.e., 1 - IDF/DF) were found in the mouth or larynx (swallowed into the stomach). Women had twice the IDF of men; this result is consistent with their having smaller ADS and EAD$_{70\text{ ml}}$ than the men. When we compared bolus deposition at a fixed depth (VFD = 70 ml) to a normalized depth (VFD = 0.9 phase 1) we found that both DF and IDF were significantly increased at the normalized depth. Because 0.9 phase 1 in this group represented a mean VFD of 95 ± 11 ml (BTPS), a deeper penetration than the fixed VFD (71 ± 2 ml), it was not surprising that DF and IDF were increased. More importantly, the results showed that, among all subjects studied at 70% TLC and VFD = 70 ml, IDF was quite variable [range = 0.04–0.43, coefficient of variation (CV) = 0.54]. Figure 1 shows that the IDF for all subjects correlated significantly with the ADS of the subjects ($r = -0.76$, $P = 0.001$, $n = 16$). When other variables (i.e., EAD$_{70\text{ ml}}$, Raw, TLC) were considered in a multiple-regression analysis (Systat for Macintosh)
Macintosh) for IDF, only ADS significantly predicted IDF (P = 0.02). There was also a tendency for TLC to predict IDF (IDF increased with decreasing TLC; P = 0.08). In measurements of IDF where VFD was normalized to the subject’s phase 1 N2 washout (n = 7), the variability in IDF was reduced (CV = 0.33 vs. 0.61 for the fixed VFD) and less associated with ADS (r = −0.39 vs. −0.60, normalized VFD vs. fixed VFD, respectively, in these subjects). EAD was also much less variable between subjects when measured at a constant fraction of phase 1 N2 washout (Table 1) and was not different between men and women.

Table 2 summarizes the regional deposition data of the inhaled boluses for all subjects. There was a tendency for average C/P to increase with decreasing ADS (r = −0.46, P = 0.08). There were no differences in C/P between the R and L lungs nor in C/P or EI between men and women. Although the normalized VFD was, on average, 24 ml deeper into the lung, the C/P for the normalized VFD was not different from that of the fixed 70-ml depth. It was clear, however, from the deposition scans that there was a difference in L vs. R lung deposition. Figure 2 illustrates the deposition scans of one subject (posterior on the right and anterior on the left). Because this was a fairly consistent observation, we calculated L/R ratio (L/R) for each subject’s deposition scan, again normalized to a similar ratio for the xenon-equilibrium scan to account for lung volume differences between the two lungs. Consistent with lung morphology (12), mean L/R for 133Xe-equilibrium scans was 0.79, or 44% L lung volume and 56% R lung volume. Mean L/Rs for the posterior (L/Rp; first image) and the anterior (L/Ra; fifth image) are shown in Table 2. L/Rp for all subjects and each subgroup were significantly >1.0, indicating greater deposition in the L compared with the R lung. For all subjects, L/Ra was significantly less than L/Rp. Nevertheless, the mean L/Ra for all subjects was still significantly >1.0, indicating greater deposition in the L lung, regardless of which image was considered. In an attempt to understand the mechanism of this L-R asymmetry, we performed stepwise multiple-regression analysis for L/Rp and L/Ra considering ADS, EAD, TLC, and Raw as dependent variables. Again, only ADS was significant as a predictor of L/Ra or L/Rp (P = 0.02 and 0.06, respectively). L/R increased with increased ADS; this suggests greater L/R with decreasing volumetric penetration into the airways. On the other hand, there was no difference in L/R between the fixed and normalized VFD bolus delivery, despite the fact that the normalized VFD was, on average, 24 ml deeper into the lung. In fact, for this subset of subjects, there was a tendency for the L/R.
Table 3. Retention of deposited boluses at 2 h

<table>
<thead>
<tr>
<th></th>
<th>All Subjects</th>
<th>Men</th>
<th>Women</th>
<th>VFD_{fixed}</th>
<th>VFD_{fixed1}</th>
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<tr>
<td></td>
<td>n</td>
<td>16</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Left lung</td>
<td>0.70 ± 0.11</td>
<td>0.72 ± 0.13</td>
<td>0.68 ± 0.06</td>
<td>0.65 ± 0.12</td>
<td>0.64 ± 0.07</td>
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<tr>
<td>Right lung</td>
<td>0.70 ± 0.09</td>
<td>0.71 ± 0.10</td>
<td>0.69 ± 0.08</td>
<td>0.64 ± 0.11</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>Whole lung</td>
<td>0.70 ± 0.10</td>
<td>0.71 ± 0.11</td>
<td>0.69 ± 0.07</td>
<td>0.65 ± 0.10</td>
<td>0.64 ± 0.05</td>
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Values are means ± SD; n, no. of subjects.

to be greater at the deeper depth (i.e., at VFD = 0.9 phase).

The retention data for the deposited boluses are shown in Table 3. Despite the differences in L vs. R lung distribution (L/R in Table 2), there was no difference in 2-h retention (R2) for the L vs. R lung. On the other hand, this L-R similarity in R2 is consistent with the C/Ps being similar between L and R lungs (Table 2). The relationship between C/P and R2 for all 16 subjects studied at the fixed 70-ml VFD is shown in Fig. 3. Data for both the L and R lungs are shown. There is a significant negative correlation between C/P and R2 for all subjects and both lungs combined (r = −0.50, P < 0.05). Of the two lungs, only the R lung has a significant negative correlation (r = −0.67, P < 0.01) for R2 vs. C/P. Despite our attempts to eliminate the stomach from the C/P analysis on the left side, the L lung C/P may have been confounded by high levels of stomach activity in many of the subjects. There was no significant relationship between whole lung R2 and ADS (r = 0.24). R2 was less variable between individuals when the boluses were delivered to a normalized depth (0.9 phase 1) into the lung. But again, despite the fact that the normalized VFD was an average 24 ml deeper into the lung, R2 was not different from the fixed depth.

At 24 and 48 h, four men were excluded from the retention analysis because of insufficient activity in their whole lung for the fixed 70-ml VFD inhalation maneuver (see METHODS). These individuals had low initial deposition: three subjects had the lowest IDFs, and one subject with low initial specific activity associated with the inhaled particles. Three of these subjects were also among the seven subjects studied at a normalized VFD. The three individuals with low IDFs also had lower than average C/P ratios. Consequently, comparisons of these retentions (e.g., between men and women, fixed vs. normalized VFD) may not be valid, especially to relate to regional deposition parameters for which a greater range of data was available. The mean R2_{48} for the 12 subjects included in this analysis was 0.27 ± 0.05. At 48 h postdeposition, the average whole lung retention (R_{48}/R_{24}) was 0.85 ± 0.16 (n = 12); i.e., 15% of particles retained at 24 h were cleared by 48 h postdeposition.

Figure 4 compares the C/P ratios at deposition (initial) vs. at 2- and 24-h postdeposition. Only 10 of the 16 subjects had sufficient activity in each lung at 24 h to allow C/P analysis at that time (i.e., n = 10 for comparisons in Fig. 4). As discussed in METHODS, the L lung C/P at 2 h was too confounded by high levels of stomach activity to be considered reliable. By repeated-measures analysis, the R lung C/P was significantly decreased with time (P = 0.03 for comparison of 3 time points). The 2- and 24-h C/P were significantly less than the initial C/P (P = 0.03 and 0.01, respectively) but were not different from each other. All three C/Ps on the right side, however, were significantly >1.0 (P < 0.02). The L lung C/Ps were not different, by paired analysis, between initial deposition and 24 h postdeposition. Again, the C/P at 24 h for the L lung was also significantly >1.0 (1.82 ± 0.54, P < 0.001 for comparison to 1.00).

DISCUSSION

In the present study, we have determined the efficiency of aerosol bolus delivery to the bronchial airways and its relationship to airway volumes and sizes. Under our experimental conditions, ∼26% of the inhaled particles at 2 h postdeposition (R2) as function of central-to-peripheral (C/P) ratio of initial deposition. Each symbol represents value for 1 subject; n = 16 subjects. □, Left lung; ♦, right lung; r = −0.50, P < 0.05 for both lungs combined.
3.5-µm MMAD particles in shallow boluses actually deposited in the intrathoracic airways (IDF in Table 1). Among all subjects, the IDF for fixed VFD of 70 ml increased with decreasing ADS (Fig. 1). In subjects with very large ADS (>250 ml) <10% of the boluses were deposited in the intrathoracic airways when delivered to the fixed VFD. This is likely to be caused by the bolus' penetrating less deeply into the subjects' lungs and the settling distances, or sizes of the airways, being larger in these subjects. The difference between men and women provides a good illustration of these differences in airflow volume and size. The women had smaller ADS and about one-half the EAD at the VFD of 70 ml (Table 1). Consequently, the IDF in women was twice that of men. On the other hand, EADs measured at the normalized, constant fraction of phase 1 N2, were not different between men and women. Thus it is likely that the greater IDF in women at the fixed depth of 70 ml was primarily influenced by deeper bolus penetration into more distal airway generations. Finally, in support of airflow volume being an important determinant of bolus-deposition efficiency, IDF was less variable between subjects when the VFD was adjusted to correspond with the size of each subject's ADS (i.e., CV = 0.33 for normalized depth VFD compared with 0.61 for the fixed 70-ml VFD; see Table 1).

To maximize the dose of aerosol to the airways (i.e., IDF), it is important to consider that IDF is a function of particle size, VFD, and breath-hold time. For clinical applications, it may not be practical to increase the breath-hold time much beyond the 8–10 s used in these experiments. Certainly, increasing the VFD would also increase the IDF, as illustrated in Table 1 by the increased IDF (33%) when the bolus was delivered at a normalized VFD, that on average was 24 ml deeper into the lung than the fixed 70-ml VFD (IDF = 23%). At some point, however, increasing VFD will result in a greater fraction of the deposited particles from boluses residing in alveolated air spaces. In the case of the normalized VFD, this did not seem to occur as the R24 and R48 values (which should reflect an increase in alveolar deposition) were not different between the fixed and normalized VFD conditions (Table 3). Finally, increasing particle size (e.g., to 5–6 µm) may increase IDF via increased settling velocities (by the square of the aerodynamic particle size) during the breath-hold period. However, increasing particle size may also result in increased extrathoracic deposition by impaction during inhalation, in effect preventing the particles from penetrating to the intrathoracic region. It may be that a combination of both increasing particle size and decreasing inspiratory flow may prove to be the most efficient for delivering the greatest fraction of the inhaled bolus to the intrathoracic airways (1).

The C/P distribution of deposited boluses within the intrathoracic airways may have been somewhat influenced by variations in ADS. At the fixed VFD, there was some tendency for C/P to increase with decreasing ADS, but this trend was not quite significant (P = 0.08). This tendency may be a reflection of slightly smaller large airways (i.e., main stem and lobar bronchi) in individuals with the smaller ADS (e.g., women), which should contribute to greater central airway deposition. But this effect may be offset by the fact that the bolus is penetrating deeper (at a fixed VFD) with decreasing ADS, enhancing peripheral airway deposition relative to the central airways. These two offsetting influences may explain why there was no clear effect of ADS on the C/P ratio. In fact, when VFD was normalized to a constant fraction of phase 1 N2, C/P tended to increase with decreased EAD (r = −0.67, P = 0.10, n = 7), supporting the concept that, when penetration depth is normalized, greater deposition in the central airways will favor those with the smallest airway dimensions. The average C/P (~1.6–1.7) was less than that we found recently in a similar group of normal subjects inhaling larger (5-µm) monodisperse particles with a rapid, shallow breathing pattern (C/P = 2.1) (4). It was also much less than we achieved with normal subjects inhaling a shallow breath of a 2-µm polydisperse aqueous aerosol followed by a forced expiration (C/P = 2.9) (5). These data suggest that, although the boluses may have primarily deposited in the airways, most of the deposition likely occurred distal to the very large airways, i.e., lobar and main stem bronchi, which were included in our central region of analysis. In fact, the greatest deposition likely occurred in the smallest airways reached by the leading edge of the inhaled boluses. The settling distances associated with the very large airways make deposition less efficient. For example, particles used in these radiobolus experiments had settling velocities of 300 µm/s, which means that in 8 s each particle settles 2.4 mm. In other words, all particles are deposited that reached airways with a vertical linear intercept of <2.4 mm, but particles in larger airways have less complete deposition. It may be that the most efficient way of depositing particles in the very large airways is to incorporate a forced expiration after the breath-holding period (5) to enhance deposition by impaction at sites of expiratory flow limitation (22).

The most striking effect on regional bolus deposition was the significantly greater deposition in the L vs. the R lung (Table 2 and Fig. 2). This was true for either posterior or anterior camera images, although the L-R asymmetry was greatest in the posterior scans. This suggested that deposition in the L lung tended to be more posterior, whereas in the R lung deposition tended toward the anterior portion of the lung. The anterior image may also have been more affected by increased 99mTc gamma attenuation by the heart on the left side. Because we didn't have an anterior image for the 133Xe-equilibrium scan (for normalization purposes) and had to use the posterior 133Xe L/R, the L/R may be underestimated by this effect. It is not clear why this L-R asymmetry in bolus deposition occurs. It may be that the anatomy and/or geometry of the bronchial airways is such that particles deposit more efficiently in the L lung vs. the R lung. This would require that the mean linear intercept, through which particles settle to deposit, be less in the L than in the R lung. In the asymmetric Horsfield et al. model of the lung (12), the
main stem and lobar bronchi tend to be oriented more horizontally to the direction of gravity in the L vs. the R lung, i.e., to have shorter settling distances for particles to deposit. L-R asymmetries have not generally been noted when $^{133}$Xe boluses have been used in regional ventilation studies (2, 9). The early study of Dollfuss et al. (9), however, used a column of scintillation detectors that did not distinguish the L and R lungs. Although Bake et al. (2) did employ scintillation counters over each lung, they, like others who have utilized gas boluses to determine regional lung ventilation, introduced the boluses at the beginning of inhalation from FRC, rather than at the very end of inhalation as we did here. An earlier pilot study by Ilowite et al. (13) also showed L-R asymmetries in deposition when the aerosol boluses were delivered at 90% TLC. However, they also found that small $^{133}$Xe gas boluses, delivered in the same manner as the aerosol boluses, were unevenly distributed between L and R lungs (3). These results would suggest that a greater fraction of the bolus volume is delivered to the L lung than to the R lung. It may be that, as the lung approaches end inhalation at this lung volume (70% TLC), the L and R lungs are not expanding uniformly. The base of the R lung is opposed by the relatively rigid liver, whereas the base of the L lung is opposed by the more distensible stomach, possibly allowing the L lung to expand more easily at higher lung volumes. The dependence of L/R on ADS (multiple-regression analysis), i.e., increasing L/R with increased ADS, is not clear, because L/R tended to be greater at the deeper penetration in the subjects studied at normal VFD. The mechanism responsible for the L-R asymmetry in bolus deposition requires further study.

Despite the differences between men and women in IDF and the L-R asymmetry in deposition for all subjects, we found no differences in $R_2$ for either of these comparisons (Table 3). There was also no relationship between ADS and $R_2$ at any time postdeposition. The clearance through 2 h ($R_2$) correlated with the regional C/P deposition (Fig. 3) (14); this suggests that early phase clearance was consistent with deposition patterns within the Airways. The variability in $R_2$ was reduced when subjects inhaled to a normalized depth (Table 3; CV = 0.08 vs. 0.16, for VFD$_{70\text{ml}}$ vs. fixed VFD$_{70\text{ml}}$), respectively, but the mean $R_2$ was not different, despite the fact that the normalized depth was an average 24 ml deeper into the lung for the normalized VFD. Similarly, there was no difference in $R_2$ between men and women, despite the likelihood that the boluses penetrated deeper into the women, who had the smaller ADS. There was also no correlation between $R_2$ and ADS, again an unexpected result if the boluses were more likely to penetrate to alveolated air spaces in individuals with the smaller dead space. These data, the significant $R_2$ (0.27), and the continued clearance of particles between 24 and 48 h are consistent with the conclusions of Stahlhofen et al. (24) that particle clearance is not only a function of airway deposition site but that, at a given airway site, particles may be cleared either in a rapid clearance phase (e.g., during the first few hours) or in a slower delayed phase (extending beyond 24 h).

As further support of particle retention in the airways at 24 h postdeposition, the C/P ratio did not decrease to 1.0 at 24 h in either lung. Because the central region of analysis contains large bronchial airways that are not present in the peripheral lung region, this result suggests that particles are still retained in these airways at 24 h postdeposition, consistent with conclusions of Stahlhofen et al. (24). The R lung C/P at 24 h was decreased relative to initial deposition, but the C/P for the L lung was not. It may be that we underestimated the initial L lung C/P because of stomach activity that would be reflected primarily in the peripheral region. A previous analysis (23) of C/Ps over time showed that the C/P decreases to average values closer to 1.0 (1.13 ± 0.13) at 24 h for normal subjects breathing large volumes of inhaled aerosol with rapid inhalations. We also have similar data (unpublished observations) in normal subjects from our laboratory. These data show 24-h C/P ratios different from 1.0 when subjects inhaled the same particles used here ($^{99m}$Tc-iron oxide) with tidal-breathing maneuvers (mean C/P = 1.10 ± 0.39 at 24 h compared with 1.77 ± 0.47 initially; n = 17). However, in both these studies (Ref. 23; unpublished observations), alveolar deposition and retention was probably a much greater component both initially and at 24 h. Thus the contribution of large airway retention at 24 h was less likely to be detected in such an analysis. On the other hand, deposition in the bolus experiments described here occurred predominantly in either large or small bronchial airways. Thus the peripheral region primarily reflected small airways from which particles could also be cleared, albeit at a slower rate than from the large airways. In fact, our data, especially the R lung C/Ps, suggest that, as predicted, the large, central airways clear more rapidly than do the peripheral airways through 2 and 24 h.

Alternatively, the elevated C/P ratios at 24 h may be interpreted as preferential ventilation of lung segments associated with the central regions, i.e., along the shortest pathways from the main stem bronchi. This may be especially true for boluses that are delivered at the very end of an inhaled breath, such as those in this study. In other words, there may not have been uniform delivery of particles to small airways (and possibly alveoli) between the C and P lung regions. If so, the elevated activity in the C region at 24 h may still be associated with small airway (or alveoli) but not large airway retention in this region. This interpretation is more consistent with recent findings (16) that show that insoluble particles, sprayed via a microspray nozzle onto a very localized surface of large airways in dogs, are entirely cleared from the lung 24 h later. However, it may be difficult to compare that study and the present study because of the different techniques used for delivering particles to the airways.

The average $R_{24}$ in our study (0.27) was less than that observed in previous bolus studies ($R_{24}$ = 0.4–0.6) (18, 24). We subsequently found that the leaching rate of
radioisotope $^{99m}$Tc from the particles was greater in the present experiments than these previous studies. To estimate the in vivo rate of leaching, we performed a series of experiments in three subjects who inhaled boluses of 1-µm MMAD similarly labeled particles delivered early in a deep inhalation. In other words, we designed an experiment to maximize delivery of particles to alveoli, where little or no clearance should occur in 24 h. We found a consistent measure of $R_{24} = 0.62 \pm 0.03$ and $R_{48}/R_{24} = 0.95 \pm 0.04$. This corresponded to mean exponential leaching rates of 2%/h for the first 24 h and 0.2%/h for the period 24–48 h. If we use this rate to correct our $R_{24}$ measures (i.e., divide $R_{24}$ by 0.62), we obtain values of 0.4–0.5 that more closely approximate those in previous studies. We have subsequently found that the leaching rate is minimized by the heating of the particles to high temperatures after generation by placing the tube furnace downstream from the concentrator (see Methods). Although the absolute values of $R_{24}$ and $R_{48}/R_{24}$ in our experiments may be lower than actual particle retention for these time periods, our data still suggest significant retention of particles at 24 h and continued airway clearance of particles between 24 and 48 h. Similarly, the C/P analysis at 24 h should be unaffected, unless much different leaching rates occur in large vs. small airways. Because we have found 24-h C/P ratios decrease to 1.0 (discussed above) in subjects tidally breathing these particles (prepared in the same manner as in this study), it seems unlikely that leaching rates are different in the C and P regions of the lung.

How can we be sure that an insignificant portion of the bolus was deposited in alveolar air spaces? The relatively large mean EAD$_{70\,mm}$ of 6.6 mm suggests that the mode of the boluses was centered within fairly large airways (lobar to segmental bronchi), although certainly the leading edge of the bolus may have penetrated much deeper. If we calculate the fraction of the inhaled bolus that is retained in the lungs at 24 h ($F_{24}$)

$$F_{24} = \text{IDF} \times R_{24} \text{ (corrected for leaching)}$$

we find mean $F_{24} = 0.14 \pm 0.05$ for the fixed VFD of 70 ml ($n = 12$), i.e., only 14% of the inhaled bolus was retained at 24 h. In other words, only 14% of the bolus, on average, would need to penetrate beyond the ADS for $R_{24}$ to reflect alveolar retention of particles. On one hand, with axial streaming and asymmetric bolus delivery, it seems possible that such a small portion of the bolus might penetrate to alveolar air spaces. Although there is an excellent correlation between $F_{24}$ and IDF ($r = 0.87$), there is no such correlation between $F_{24}$ and $R_{24}$ ($r = 0.08$). Until recently, it has been thought that $R_{24}$ represents alveolar retention of particles. If that were the case in our experiments, we would have expected there to be an excellent correlation between it and $F_{24}$; that is, as $F_{24}$ (the fraction of inhaled bolus that may have penetrated beyond the ADS) increases, $R_{24}$ should have also increased. The fact that no such relation exists supports the conclusion that $R_{24}$ is not dominated by alveolar retention of particles in these experiments. As a further illustration, we compare the $F_{24}$ for women ($n = 6$) vs. men ($n = 6$); $F_{24} = 0.16 \pm 0.05$ and 0.11 $\pm$ 0.04, respectively; $P = 0.07$. Despite this tendency for greater $F_{24}$ in women and the possibility that the normalized bolus penetrated deeper into their lungs compared with men's lungs, the $R_{24}$ was not different between the genders and, paradoxically, tended to be less in women ($R_{24} = 0.25 \pm 0.05$ vs. 0.29 $\pm$ 0.05, women vs. men, respectively). To further characterize the degree of bolus penetration to alveolated air spaces or the gas exchange area of the lung, future experiments might utilize inert gas boluses delivered in the same manner as was the aerosol boluses (21). The recovery and dispersion of such inert gas boluses should provide information on their penetration into pulmonary air spaces that may parallel aerosol bolus behavior. Finally, in our experiments in which 1-µm particles were deposited in the alveolar region, only 5% of the particles were cleared between 24 and 48 h (probably reflecting leaching of the radionuclide and/or early alveolar clearance). But the retention data at the shallow volumetric depth show that 15% of the particles deposited were cleared between 24 and 48 h later. This indicates a faster clearance than can be observed when particles are primarily deposited in the alveolar region and is also consistent with recent findings of Anderson et al. (1), who deposited particles preferentially in airways by using a slow-inhalation technique.

In conclusion, using gamma-camera analysis of aerosol boluses delivered to shallow volumetric depths (less than the subjects' ADS), we found that 1) fractional deposition of the boluses was strongly dependent on the size of the subject's ADS; 2) there was significantly greater deposition in the L vs. the R lungs; and 3) a significant number of particles were retained in the airways at 24 h postdeposition, and these particles continued to clear at a slow rate through 48 h postdeposition. To maximize bolus deposition to the airways, the volumetric depth to which the bolus is to be delivered should be normalized to the individual's ADS. This normalization will provide for less intersubject variability in the distribution of particles within the airways. The bolus delivery technique described here should be compared with other techniques designed to restrict deposition of particles to the airways (1, 5). It may be that different features of the various techniques may be combined to provide the optimal airway-delivery technique. The asymmetry in L vs. R lung deposition requires further study to determine how to provide for the most homogeneous delivery of the aerosol to the airways. Finally, the retention data substantiate findings in previous studies (18, 24) that insoluble particles may be retained in airways much longer than previously assumed (i.e., beyond 24 h postdeposition).

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