Mucociliary and long-term particle clearance in the airways of healthy nonsmoker subjects

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period of up to 1 yr and the prevention of artifacts in clearance measurements due to leaching of the radiolabel and metabolization of the dissolved material in organs. This method has been applied to investigate long-term clearance from the alveoli (36), which result in clearance half-times of ~120 days for healthy nonsmokers and impairment of clearance due to cigarette smoking and interstitial lung diseases, e.g., idiopathic pulmonary fibrosis and sarcoidosis.

Studies of airway clearance require a deposition of the test particles predominantly in the airways. Efforts were made to achieve this requirement by controlled inhalation of particle bolus at the end of tidal inhalation (8, 41). The phase 1 volume of the anatomic dead space was used as a threshold volume for the bolus penetration depth.

METHODS

Subjects and pulmonary function testing. Thirteen healthy subjects (age of 37 ± 11 yr), who had never smoked, participated in the study. Anamnestic data were collected using a questionnaire based on American Thoracic Society (ATS) recommendations (16), and all subjects were interviewed by a pulmonary specialist. None of the subjects reported a history of seasonal allergic rhinitis or the cardinal symptoms of airway hyperreactivity (wheezing, chest tightness, shortness of breath). None of the healthy subjects had a history of respiratory or cardiovascular disease or was receiving any long-term medication. The protocol was approved by the Ethical Committee of the Medical School of the Ludwig Maximilian University (Munich, Germany), and informed consent from each subject was obtained. Body plethysmography and spirometry were performed using a Jaeger Masterlab (Erich Jäger, Würzburg, Germany). Predicted values of conventional lung function parameters were calculated by normalizing to the reference values proposed by the European Community for Steel and Coal (39). A lung function test and a MPG measurement of the natural ferromagnetic contamination of the lungs of every subject were obtained before inhalation. MPG measurements were performed 30 min, 3 and 6 h, 1 and 2 days, 1 wk, and 1, 3, 6, and 9 mo after particle inhalation.

Volumetric dead space measurement. To measure the physiological dead space, a fast mass spectrometer (modified magnetic sector field mass spectrometer; DLT 1100 R, Dennis Leigh Technology, Sandbach, UK) was used, which allowed the measurement of the concentrations of various respiratory and nonrespiratory gases within the expired air as a function of the expired air volume. To measure the physiological dead space, a tracer gas mixture of 0.2% C18O2, 21% CO2, and 78.8% N2 was applied as a single-breath inhalation. The physiological dead space was obtained by the protocol of Fowler in part includes alveolar structures. In addition, a closer threshold volume for the conducting airways was estimated from the gas-expiration profile, where we analyzed the volume in which the C18O2 concentration dropped to below the 95% level (see Fig. 1A). This volume was called the phase 1 dead space volume (VDP1) and characterized the beginning of the diffusive process of the tracer gas into the gas-exchanging region of the lung. In all subjects, the dead space volumes were determined at an end-inspiratory level of 90% total lung capacity (TLC). The reason for the 90% TLC lung expansion was to have one protocol for healthy subjects and for patients (not included in this report). For example, chronic obstructive pulmonary disease patients with emphysema have a higher FRC due to trapped air.

Magnetic particle generation, inhalation, and MPG detection. Around 0.5–1 mg of spherical monodisperse ferrimagnetic iron oxide particles (Fe3O4; 4.2 μm aerodynamic, 1.9 μm geometric diameter, σg < 1.1) were deposited in the lungs by controlled voluntary inhalation. The particles were produced by a spinning top aerosol generator (37). A colloidal water solution of nonmagnetic iron oxide (Fe2O3) was nebulized into uniform droplets in an N2 atmosphere. After evaporation of the water, the colloidal subunits aggregated and formed compact spherical particles. With the use of a virtual impactor, the aerosol was concentrated 10–15-fold, and the flow rate was reduced from 40 to 1 l/min. After the concentration stage, H2 gas was added to a final concentration of 0.5%. The concentrated aerosol was fed through a furnace at 800°C, where the nonmagnetic iron oxide (hematite, Fe2O3) was reduced to the magnetic form of iron oxide, magnetite (Fe3O4). The particles were fed through a second virtual impactor, which reduced the aerosol flow from 2 to 0.2 l/min, resulting in a fivefold aerosol concentration.

The output of the second concentrator fed into a vertical tube, which acted as an aerosol storage tank. This tank was coupled to the

from functional residual capacity (FRC), subjects inhaled a volume of the tracer gas and then exhaled to a lung volume below the FRC. This breathing maneuver was performed three times at a constant air flow, controlled by the subject using a visual flow signal. Figure 1A shows a typical expiration diagram of C18O2 together with the estimated volumes. The physiological dead space was derived from the C18O2 expirogram using the method of Fowler (19) and Meyer et al. (35). C18O2 labeled with the stable oxygen isotope 18O (C18O2) was completely taken up in the gas-exchanging region of the lung but not from the airways. Therefore, C18O2 was only expired from the dead space of the lung and not from the alveolar region. Hence, C18O2 allows the measurement of the respiratory dead space not only in healthy subjects but also in patients with chronic obstructive pulmonary disease and lung emphysema. As has been previously shown, the physiological dead space measured by this technique showed a high correlation with the dead space measured conventionally with nitrogen. The dead space volume obtained by the protocol of Fowler in part includes alveolar structures. In addition, a closer threshold volume for the conducting airways was estimated from the gas-expiration profile, where we analyzed the volume in which the C18O2 concentration dropped to below the 95% level (see Fig. 1A). This volume was called the phase 1 dead space volume (VDP1) and characterized the beginning of the diffusive process of the tracer gas into the gas-exchanging region of the lung. In all subjects, the dead space volumes were determined at an end-inspiratory volume of 90% total lung capacity (TLC). The reason for the 90% TLC lung expansion was to have one protocol for healthy subjects and for patients (not included in this report). For example, chronic obstructive pulmonary disease patients with emphysema have a higher FRC due to trapped air.

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aerosol input channel of the respiratory aerosol probe (8). The respiratory aerosol probe consisted of a computer-controlled valve system, which can switch between clean air and aerosol during inhalation. The flow rate and aerosol concentration were continuously measured during in- and exhalation. The mean inhalation and exhalation flow rates were kept at 250 ml/s. The aerosol was administered as a 100-ml bolus at the end of inhalation to a defined volumetric front depth (VF). At the end of inhalation, an 8-s breath hold was performed to enhance the particle deposition. The end-inspiratory volume was 1 liter above the FRC. The lung expansion of the dead space measurements was 90% TLC and, therefore, larger compared with that of the aerosol administration. Therefore, the volumetric dead space during aerosol administration requires a 10% reduction, as can be estimated from the data in Bennett et al. (6), where the dead space volume was measured at different levels of lung expansion. About 20–30 breath were necessary to deposit 0.5–1 mg of magnetite particles in the lung.

Directly after inhalation, the particles were detected by the MPG system (47). The subject lay on a bed with the lungs directly under the superconducting loop array, where the weak magnetic field of the lungs was detected by a superconducting quantum interference device. The entire system was enclosed in a magnetically shielded room. After natural ferromagnetic contamination was corrected for, the remanent magnetic field detected was shown to be a reliable measure of the amount of particles retained within the lungs (38). Subjects were studied over a 8- to 9-mo postinhalation period.

Data analysis. Particles deposited in the lung by the shallow bolus technique showed at least two different mechanisms of clearance. The first fast phase happened within the first day, and later proceeded into the slow phase of clearance. The course of the clearance curve was fitted by the sum of two exponential functions according to:

\[
B(t) = B_0 \left[ (1 - A_S) \exp\left(-\frac{t}{T_F}\right) + A_S \exp\left(-\frac{t}{T_S}\right) \right]
\]

where \(B_0\) describes the amount of retained magnetic material directly after inhalation, \(1 - A_S\) describes the amount of fast-cleared material with the time constant \(T_F\), \(A_S\) describes the amount of slowly cleared material, and \(T_S\) is the time constant of the slowly cleared material. Other authors suggest more than two clearance phases and therefore use a multiterm exponential function, but our data do not suggest more than the two proposed mechanisms. Incorporating an additional intermediate clearance phase results only in a small fraction with less statistical significance. Additionally, the amount of retained material after 24 h was analyzed.

RESULTS

Data of pulmonary function testing, anatomic dead space, and particle inhalation. Lung function data of the study groups are shown in Table 1. The lung function data of all subjects are within the band of healthy subjects. None of the subjects had a history of cigarette smoking or gave rise to any chronic inflammatory processes within the lungs. Figure 1A shows a typical washout profile of the test gas C\(^{18}\)O\(_2\) together with the estimation of the Fowler dead space volume (V\(_{DF}\)) and V\(_{DP1}\) (threshold volume of 95% C\(^{18}\)O\(_2\) concentration). The mean values for V\(_{DF}\) and V\(_{DP1}\) at a lung inflation of 90% TLC are shown in Table 2 and are \(282 \pm 49\) and \(164 \pm 34\) ml, respectively. In the healthy subjects, V\(_{DF}\) and V\(_{DP1}\) show a high correlation (coefficient of correlation, \(r = 0.91, P < 0.001\)). The aerosol bolus was administered at the end of a 1-liter breath from FRC, in which the mean lung expansion was \(70 \pm 7\%\) in the healthy nonsmokers. To adapt the lung inflation of the dead space measurements to the aerosol inhalation, a reduction of the dead space volumes of \(10–15\%\) is necessary, according to data in Ref. 6 and our few measurements. Figure 1B shows a typical concentration profile of an inhaled and an exhaled aerosol bolus in one subject. The breath holding time of 8 s between inhalation and exhalation is not shown. The bolus penetration (front depth), as shown in Fig. 1B in relation to the Fowler dead space and the phase 1 dead space, is \(59\%\) of V\(_{DF}\) and \(100\%\) of V\(_{DP1}\) as corrected to 70% TLC lung expansion. The mean volume of aerosol penetration front depth during bolus inhalation was V\(_F\) = 150 ± 27 ml, where the mean deposition was \(51 \pm 8\%\) after 8 s of breath-holding time. The deposition without breath hold was below \(20\%\). The bolus penetration depth V\(_F\) and V\(_{DP1}\) are correlated \((r = 0.82, P < 0.01)\).

The aerodynamic particle size was measured using a sedimentation cell, and particle concentration was measured using a laser aerosol spectrometer. The particle size distribution obtained by sedimentation cell measurements revealed a geometric standard deviation of \(\sigma_g < 1.1\); therefore, the particles can be characterized as monodisperse. The particles were very compact (density \(\rho = 4.9\ g/cm^3\)), were chemically stable, and resisted dissolution in physiological saline, body fluids, and the lungs for several months.

Fast clearance of particles from the airways. The retention of the ferromagnetic iron oxide particles was measured in the MPG system directly after, and 3 h, 6 h, 1 day, 2 days, 1 mo, 3 mo, 6 mo, and 9 mo after inhalation. The mean course of the retention of all healthy nonsmokers within the first 24 h is shown in Fig. 2. The data follow a two-phase decay with a fast phase within the first day and a slow phase over the following months. The mean data of the half-times of the two-phase decay and the fraction of clearance after the slow decay (As) are given in Table 2. After 24 h, \(49 \pm 8\%\) of the particles were retained in the lung. Extrapolating the long-term decay back to time 0 reveals that \(50 \pm 8\%\) of the particles follow the slow phase of retention. Only \(50\%\) of the particles depend on the mucociliary fast clearance mechanism, which happens with a half-time of \(3.0 \pm 1.6\ h\).

Table 1. Age and lung function data of healthy subjects

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>TLC, liters</th>
<th>FEV(_1), liters</th>
<th>RV, liters</th>
<th>FEV(_1)/% TCL</th>
<th>RV% TLC</th>
<th>R(_{max}), kPa</th>
<th>SVF, liters</th>
<th>FEV(<em>1)/% VC(</em>{max})</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.1±11.0</td>
<td>6.6±1.1</td>
<td>3.6±0.7</td>
<td>1.7±0.5</td>
<td>4.9±0.8</td>
<td>4.7±0.8</td>
<td>0.2±0.1</td>
<td>3.8±0.7</td>
<td>0.2±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; VC\(_{max}\), maximal vital capacity; FVC, functional vital capacity; R\(_{max}\), total resistance; FEV\(_1\), forced expiratory volume in 1 s.

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Slow clearance of particles from the airways. The slow phase of airway clearance of 1.9-μm geometric diameter iron oxide particles is shown in Fig. 3. Within the first day, 50% of the particles were cleared via the mucociliary apparatus, and the remaining particles followed a mean clearance half time of 109 days (270 days of measurement time). After 9 mo, 9.8% of the initially deposited particles were retained in the lungs. Attempts to include an intermediate clearance phase into the model failed. Only 10% of the long-term-retained particles might follow a clearance mechanism with a half time of 15 days, but the level of statistical significance debased.

DISCUSSION

Many open questions arise from the bronchial clearance measurements after shallow bolus inhalation compared with the recent understanding of MC. The fact that not all particles are removed from the bronchial tree within 24 h can have several reasons. Despite the possibility that a fraction of particles may reach alveolar structures, even with the use of the bolus technique, the remaining part may get lost from the mucociliary escalator. Our studies do not provide data on how this may happen. A complex interaction between ventilation, deposition, and mucus properties and transport may be involved. The mechanism underlying the long-term clearance phase cannot be identified. However, compared with other histological studies, we can address airway macrophages as being possible target cells in the long-term clearance mechanisms.

Long-term airway clearance. Further studies have shown that, after shallow bolus inhalation, a fraction of particles is long-term retained within the conducting airways of the human lung and that the fraction of long-term-retained particles depends primarily on the geometric particle size (46, 48). The fraction of long-term-retained particles decreases with increasing particle size and vanishes for particles larger than 6 μm. Part of this particle size-dependent clearance may be caused by decreasing deposition fraction of larger particles in the periphery. All of these studies were performed using radiolabeled particles of different chemical composition and different density, which were detected in the lung by a sensitive lung counter. The results of this study using magnetic particles in combination with the MPG detection technique coincide with the above data, where 2-μm particles result in 50% long-term particle retention in the airways. Therefore, effects due to the labeling technique can be excluded.

It has been suggested that the main reason for the long-term retention was the penetration of particles into peripheral (alveolar) structures (17, 44), where the particles are subject to the macrophage-mediated clearance, which has a half-time of several months for iron oxide particles (36). The low deposition of only 50% after 8-s breath hold in this study (below 20% without breath hold) suggests that the inhaled bolus primarily stays in bronchial structures with diameters in the millimeter range. The settling distance of 4.2-μm aerodynamic diameter particles is 4.2 mm in 8 s. Inhaling the aerosol into tubes with a smaller width will collect most particles and cause a high

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**Table 2.** Results of measurement of airway dead space volumes using C\(^{18}\)O\(_2\) tracer gas method of particle properties and inhalation parameters, and of the clearance curve parameters

<table>
<thead>
<tr>
<th>Aerosol inhalation</th>
<th>Fowler DS (C(^{18})O(_2)), ml</th>
<th>281.8±48.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 DS (C(^{18})O(_2)), ml</td>
<td>164.2±34.0</td>
<td></td>
</tr>
<tr>
<td><strong>Aerosol inhalation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus front depth, ml</td>
<td>150.0±26.9</td>
<td></td>
</tr>
<tr>
<td>Lung expansion, %TLC</td>
<td>70.4±7.1</td>
<td></td>
</tr>
<tr>
<td>Aerodynamic diameter, μm</td>
<td>4.15±0.18</td>
<td></td>
</tr>
<tr>
<td>Geometric diameter, mm</td>
<td>1.88±0.08</td>
<td></td>
</tr>
<tr>
<td>Deposition (8 s BH)</td>
<td>0.51±0.08</td>
<td></td>
</tr>
<tr>
<td><strong>Clearance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A_S)</td>
<td>0.50±0.08</td>
<td></td>
</tr>
<tr>
<td>(T_{1/2F}, h)</td>
<td>3.0±1.6</td>
<td></td>
</tr>
<tr>
<td>(T_{1/2S}, days)</td>
<td>109±78</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. DS, dead space; \(A_S\), amount of slowly cleared material; \(T_{1/2F}\), half-time of fast-cleared material; \(T_{1/2S}\), half-time of slowly cleared material.

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**Fig. 2.** Retention of 2-μm-diameter ferromagnetic iron oxide particles in the airways of 13 healthy nonsmokers within the first 24 h. Data show mean values (thick curve) ± SD (thin curve).

**Fig. 3.** Long-term retention of 2-μm-diameter ferromagnetic iron oxide particles in the airways of 13 healthy nonsmokers within 250 days postinhalation. Data show mean values (thick curve) ± SD (thin curve).
deposition, even in a system of randomly oriented tubes. In addition, the mean bolus penetration front depth \( (V_F = 150.0 \pm 26.9 \text{ ml}) \) coincides with the mean \( V_{DPI} \), which suggests that the bolus primarily remains in the airways, followed by a subsequent particle deposition in this region. The possibility of transporting the particles deeper into the lung, for example due to asynchronous filling or cardiogenic mixing, must also happen during normal breathing and, therefore, should be incorporated into the dead space measurements using the \( \text{C}^{18} \text{O}_2 \) method, because gas reaching alveolar structures is absorbed and not exhaled. Therefore, \( V_{DPI} \) can be assumed as a safe threshold volume for the gas and particle penetration into the airways.

Further studies have shown that the fraction of slowly cleared particles was constant for as long as the bolus remained in the conducting airways (48). A small bolus with a width of 30 ml was used, and the front depth of this bolus was varied between 40 and 180 ml. The fraction of slowly cleared particles increased for boluses penetrating deeper than 200 ml, which accounts for increasing alveolar deposition of the particles. This also suggests that, as long as the particles do not penetrate deeper than the phase I dead space, we get particle deposition primarily in the conducting airways. These results in humans are, to some degree, in contradiction to clearance measurements in beagle dogs after shallow bolus inhalation (32), which show that the fraction of long-term-retained particles increase with increasing penetration depth. This may be due to different anatomic structures and different mechanisms of lung ventilation in the beagle dog compared with the human lung. Nevertheless, the clearance studies in beagle dogs after shallow bolus inhalation demonstrate the long-term clearance in the airways but predict a fraction of ~50% of the long-term-retained particles originating from alveolar deposition. Additionally, it was shown in humans that the fraction of slowly cleared particles did not depend on either inhalation flow rate or on breath-holding time (48).

Bolus inhalation studies using radioactive labeled particles and gamma camera imaging showed a left to right lung asymmetry in lung deposition using the shallow bolus technique (5, 6), which depended on the lung volume (%TLC of end-inspiratory lung inflation) but not on the anatomic dead space. The reason for this inhomogeneity may be an inhomogeneous particle deposition (i.e., due to heartbeat) or inhomogeneous ventilation. The effect may cause deviations from bulk particle transport with the possibility of a deeper penetration into smaller airways. On the other hand, both studies demonstrated a 24-h retention, which was independent of the left to right deposition asymmetry, of the lung inflation and of the anatomic dead space, suggesting that the effect may not have a strong impact on the regional particle distribution and the slow clearance mechanisms.

The detection of long-term-retained particles in the airways may imply a loss of particles from the mucociliary transport machinery and a transport of deposited particles to the submucus space. Morphometric studies revealed that the particle surface properties and the interaction with surfactant seem to play a key role (20, 43). After deposition, the particles are coated with surfactant and then get displaced into the aqueous subphase, where they may be submerged and penetrate between the cilia. Additionally, it was shown that the mucus fluid does not form a continuous layer (25, 26). Particle deposition in such holes allows direct contact with beating cilia. With decreasing particle size, the probability increases that particles can penetrate between cilia or between cilia supporting cells, where they can easily be engulfed by macrophages.

**Mechanisms of long-term airway clearance.** In each subject, the long-term clearance was recorded over a 270-day period. Figure 4 summarizes the mean long-term retention curve of this study compared with the clearance of 1.3-μm geometric diameter (2.9-μm aerodynamic diameter) iron oxide particles from the alveolar region of healthy nonsmokers and smokers (36). The mean age of the subjects, who participated in the alveolar clearance study, was 30 ± 4 yr, and the cigarette consumption of the smokers was 11.7 ± 10 pack-years (1 pack-year corresponds to the smoking of one pack of cigarettes per day over a period of 1 yr). The alveolar clearance study showed a small fraction of fast-cleared particles (5% in nonsmokers and 11% in smokers), which accounts for a small fraction of bronchial particle deposition. The alveolar long-term clearance kinetics revealed a mean half-time of 124 ± 66 days in nonsmokers and 208 ± 82 days in smokers. In nonsmokers, the half-times of the bronchial and the alveolar clearance of iron oxide particles are comparable, and the logarithmic plot in Fig. 4 shows a parallel slope. Despite the possibility that a small fraction of the long-term clearance after bolus inhalation may reflect alveolar clearance, we can suggest that the long-term clearance underlies comparable mechanisms in the bronchial and in the alveolar region. Because alveolar clearance is primarily a function of alveolar macrophages, we can suggest that the long-term particle retention in the airways may be a function of bronchial macrophages.

Further studies show that a separate population of macrophages can be found in the airways (7), which have specific characteristics that distinguish them from alveolar macrophages (1, 24). Histological and stereological studies have revealed that, already 20 min after inhalation of Latex or Teflon particles, a certain fraction can be found in airway macrophages (22, 30), and 24 h after particle inhalation >80% of the remaining particles are phagocytized by airway macrophages. The present study together with the histological results

![Fig. 4. Comparison of the mean long-term retention of iron oxide microparticles from the airways of healthy nonsmokers (AW, NS) with retention from the alveolar region in healthy nonsmokers (AL, NS) and smokers (AL, S). 20–39, Age group of the subjects involved in the alveolar clearance study.](http://jap.physiology.org/ by 10.2309/June 10, 2017)
suggests that long-term particle retention in the airways may be a function of airway macrophages.

Studies by Alexis et al. (2) have shown that a fraction of the radiolabeled particles being deposited by the aerosol bolus technique can be removed from the lung by induced sputum. During the first hours, sputum induction significantly enhanced the clearance of particles, whereas after 24 h there was no effect of induced sputum on clearance, and ~40% of the particles were long-term retained within the lungs.

**MC studies compared with the bolus technique.** MC in humans was intensively studied in the past, and pathophysiological conditions and pharmacological interventions were investigated (4, 9, 18, 27, 28, 40). All of these studies use radiolabeled aerosols (mostly tagged with \(^{99m}\)Tc). The observation time is limited to the decay time of the radiotracer. Most of these studies detect the clearance of the radiotracer from the lung by gamma camera imaging in a hospital; therefore, most studies have observation times of not more than 2 h. This is not sufficient to characterize the kinetics of MC (half-time of ~3 h), and it is impossible to characterize its effectiveness. At least 24 h of detection time is required for a characterization of airway clearance. Studies that fulfill this criterion show a significant particle retention after 24 h, which might indicate either peripheral deposition or long-term airway clearance (2, 13). There are few studies that detected airway clearance over longer time periods (up to 6 mo) using different radiotracers and very sensitive lung counters (14, 15). These studies use a specific slow inhalation technique in combination with large particles (6-μm aerodynamic diameter) to deposit particles primarily in small airways. The data recording in these studies started at 24 h post inhalation, and therefore no data about MC were presented. In the bronchial clearance study by Svartengren et al. (49), the slow inhalation technique and the bolus technique were directly compared and showed only small differences in the 24-h retention, although both techniques are presumed to have different main sites of particle deposition. By modeling considerations, it has been suggested that the slow inhalation technique has the highest deposition probability in the small airways, whereas the shallow bolus technique primarily deposits particles in the larger airways.

Many of the studies using radiotracers need corrections because of a leaching of the radiotracer. Because the kinetics of \(^{99m}\)Tc in the human body are very complex (<50% of soluble \(^{99m}\)Tc is excreted via urine within 24 h (3)), these corrections bear some uncertainty. Additionally, in those studies, the site of particle deposition is not very well controlled. One attempt is to inhale large particles with high flow rates, which should primarily induce deposition on bifurcations due to impaction. This approach may not deposit particles homogeneously in the airways but on sites that may have less density of cilia and reduced mucus transport. Another attempt is to inhale larger particles at slow inhalation flow, which allows the particles to pass the larynx and the airway bifurcations and to fall out in the small airways. This is a strong attempt to better control the site of deposition and to reduce extrathoracic deposition.

In conclusion, with the use of the shallow bolus technique, it has been shown that clearance of particles from the conducting airways shows two distinct phases. MC does not eliminate all particles within the first 24 h after particle deposition. Depending on the particle size, a fraction of particles (50% for 2-μm-diameter particles) are retained in the long term and follow clearance mechanisms that are comparable to those in the alveolar compartment. Although a certain fraction of the long-term-retained particles may originate from particle deposition in the lung periphery, the data suggest that part of the long-term clearance mechanism is a function of airway macrophages. The data require a new interpretation of the mechanisms of particle clearance in the airways and a new assessment of particle dosimetry. Pathophysiological and pharmacological influences on the long-term particle retention in the airways (24-h retention and long-term clearance kinetics) were not yet investigated. Because macrophage-mediated clearance mechanisms play an important role in the lung periphery, cigarette smoking, lung diseases, and drugs that modulate alveolar clearance may also be of relevance in the airways and have to be investigated in the future.

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**REFERENCES**