Influence of surface chemistry and topography of particles on their immersion into the lung’s surface-lining layer

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Submitted 13 June 2002; accepted in final form 20 January 2003

Geiser, Marianne, Samuel Schürch, and Peter Gehr.
Influence of surface chemistry and topography of particles on their immersion into the lung’s surface-lining layer. J Appl Physiol 94: 1793–1801, 2003. First published January 24, 2003; 10.1152/japplphysiol.00514.2002.—Inhaled and deposited spherical particles, 1–6 µm in diameter and of differing surface chemistry and topography, were studied in hamster intrapulmonary conducting airways and alveoli by electron microscopy. Polystyrene and Teflon particles, as well as puffball spores, were found submersed in the aqueous lining layer and adjacent to epithelial cells. The extent of particle immersion promoted by a surfactant film was assessed in a “floating-drop-surface balance” by light microscopy. Teflon and polystyrene spheres were immersed into the subphase by 50–60% at film surface tensions of 25 and 30 mJ/m2, respectively, and totally submersed at 15 and 25 mJ/m2, respectively. Puffball spores were immersed by ~50% at 22 mJ/m2 and totally submersed at film surface tensions of ≤15 mJ/m2. These results suggest that the surface tension in the intrapulmonary conducting airways of hamsters may reach ≥15 mJ/m2 and that respirable particles (< 10 µm in diameter) are wetted and displaced into the surface lining layer, which may facilitate interactions with many lung cells.

aerosols; macrophages; respiratory tract; inhalation; surfactant

TO UNDERSTAND THE BIOLOGICAL RESPONSE to particles deposited on the inner surface of the lungs, it is essential to know the deposition site (conducting airways, alveoli) and the interaction of particles on their deposition with lung structures, including the surfactant film, the aqueous lining layer, and the cells.

It is generally accepted that most of the deposited insoluble particles leave the lungs via the bronchiocircular route by mucociliary action as free particles or within macrophages (19). Only very small particles (<1-µm diameter) or those persisting on the epithelial surfaces may be taken up by the eponymous cells (6) and even transferred through the epithelium (16, 24, 34). Lung fixation techniques that preserve the inner surface of the lungs and avoid displacement of particles and cells have helped to better localize the particles within the lungs (20, 27, 35). However, despite many studies on the deposition and clearance of particles, their interaction with the surface lining layer has barely been addressed.

The aqueous lining layer has been described as a two-phase system, consisting of a continuous aqueous layer of relatively low viscosity adjacent to the epithelial cells and above a gel phase; its existence, thickness, and continuity have been debated with respect to the different airway compartments and to the differing species. A thick mucus layer has never been established in small airways (11, 13). The aqueous phase is covered by a surfactant film at the air-liquid interface, and all evidence to date shows that the film is continuous between the alveoli and central airways (11, 13).

Studies in the surface balance have shown that surfactant promotes the displacement of polystyrene (PS) spheres from air into an aqueous subphase and that the extent of particle immersion depends on the film surface tension of the surfactant film. The lower the surface tension, the greater is the immersion of particles into the subphase (7, 30). In addition, there is evidence for particle displacement in lungs from animal studies (8–10, 12). So far, little is known how the shape, size, surface chemistry and topography of particles affect their interaction with the surface lining layer. A surface tension of 32 mJ/m2 was measured in the trachea of horses (18) and values of <2 mJ/m2 on expiration in alveoli (29). Surface tension in the intrapulmonary conducting airways is still unknown because this lung compartment is not accessible for measurements with present techniques. There is likely a surface-tension gradient from the alveoli to the central airways, but its shape is still a matter of speculation. It is not known how such a gradient would influence the displacement of particles into the lining layer or whether different types of particles would be affected in the same way.

The aim of this study was to localize particles with different surface chemistry and topography after their deposition in the intrapulmonary conducting airways and alveoli and to assess particle behavior at the air-liquid interface.

MATERIALS AND METHODS

Study design. The animal experiments were performed in accordance with the Swiss Federal Act on Animal Protection...
and the Swiss Animal Protection Ordinance and approved by the Cantonal Veterinary Department, Bern (Bern, Switzerland). Hamsters inhaled aerosols of PS particles, Teflon spheres, or puffball (Calvatia excipuliformis) spores. Lungs were fixed immediately after inhalation by intravascular triple perfusion. Deposited particles were examined in intrapulmonary conducting airways and alveoli by electron microscopy. The extent of particle immersion promoted by a surfactant film formed on an aqueous substrate was assessed in a specially designed “floating-drop-surface balance” by light microscopy.

Particles. Spherical particles of differing surface chemistry and surface topography were used in this study (Table 1 and Fig. 1). PS microspheres (Polybead, Polysciences, Eppelheim, Germany), sizes of 1, 3, and 6 μm, have a smooth surface and a surface tension or surface free energy of ~33 mJ/m². Teflon particles, sizes of 3.8 and 12.0 μm, were kindly produced for us by Dr. Klas Philipson (Karolinska Institute, Solna, Sweden) (26). The 12.0-μm Teflon microspheres were used for the in vitro studies only. The surface of these particles appears smooth at light microscopic magnification. However, on higher magnification with the scanning electron microscope, the surface has a cobblestone-like appearance, consisting of partially fused Teflon nanospheres of 0.1–0.2 μm in diameter. Teflon has a surface free energy of ~18 mJ/m². Teflon microspheres are the most hydrophobic particles available.

The surface free energy of both the PS and Teflon microspheres were taken from publications by Neumann’s group (e.g., Ref. 21).

Puffball spores, size of 3.5 μm, collected by shaking them out from dried mushrooms (Calvatia excipuliformis), have a spiny surface topography with wart-like protrusions. The fungal cell wall is 80–90% polysaccharides with the remainder consisting of protein and lipid (5). Thus, from this chemical composition, we would expect the surface free energy to be between 40 and 50 mJ/m² for the surface tension of biopolymers, see Neumann et al. (23)). However, this estimate is not consistent with the water-repellent property of the spores.

Wettability of puffball spores. We conducted the following experiments to study the wettability of puffball spores. 1) Water droplets (1–2 mm in diameter) formed from double distilled water with a surface tension of at least 72.0 mJ/m² at 22°C (as determined by the Wilhelmy plate method) were formed at the tip of a glass micropipette connected to a 50-μl Hamilton microsyringe. While still hanging on the micropipette, the water droplet was pulled down and lifted off the mushroom surface repeatedly (the dry mushroom was previously cut open with a razor blade). The droplet did not adhere to the surface at all and, in addition, did not collect any spores, albeit the mushroom surface was covered with spores that tended to form an aerosol on the generation of an air current. 2) Droplets were deposited onto a horizontal part of the mushroom surface, and the advancing contact angle of the droplets was determined with a protractor eyepiece of a Wild microscope. The contact angles were always between 160 and 170°, which proves the extreme water repellency of the inner surface of the mushroom. In contrast, Teflon has a water contact angle of 105°, which translates into a surface free energy of ~18 mJ/m² (33). 3) Puffball spores were dusted onto a microscope glass slide by squeezing the mushroom repeatedly. Water droplets were then placed with a micropipette onto the glass slide. Again, the contact angles were between 160 and 170°, although only ~50% of the glass surface was covered with spores. 4) The experiments described above were repeated by placing saline droplets that contained 10 mg/ml bovine lipid extract surfactant (BLES; Biochemicals, London, ON, Canada) on the surface. The surface tensions of the droplets were at equilibrium (23–25 mJ/m²) as determined by the pendant drop method. The surfactant contact angle on the layers of spores were between 110 and 120° for at least 15 min and between 100 and 110° for the next 20 min. Only when we replaced the aqueous droplets by a perfluorocarbon fluid [fluorocarbon (FC) 43, 3M, St. Paul, MN, surface tension of 16 mJ/m² at 22°C] was there immediate spreading of the fluid and wetting of the spores.

The above experiments demonstrated that the spores are extremely water repellent likely because of their microstructure. This characteristic is not unique to puffball spores. Neinhuis and Barthlott (22) have presented an investigation and review on the surface structural design of water repellent, antiadhesive leaves. For example, the water contact angle on the leaves of the Lotus plant (Nelumbo nucifera) and on the leaves of St. John’s wort (Hypericum agypticum) is 162°. The surface of the Lotus leaf under the microscope looks similar to that of the puffball spores.

Animals, aerosol generation and inhalation, lung fixation, and tissue sampling. The purpose of the inhalation experiments with hamsters was twofold. First, the purpose was to assess the regional distribution and the clearance of particles in lungs by unbiased stereology on light microscopic sections; these results were reported elsewhere (8–10, 12). The second purpose was to investigate the interaction of particles with the lung lining layer at the deposition sites by electron microscopy. This was the aim of the present study. The special design for tissue sampling (systematic sampling) allowed us to take tissue samples for both microscopic analyses from each animal (8).

Deposited particles were studied in 29 male Syrian Golden hamsters, weighing 112–183 g, which had inhaled aerosols of either PS spheres (n = 16), Teflon particles (n = 6), or puffball spores (n = 7) (Table 1). Animals, aerosol generation

Table 1. Particle characteristics and film surface tension for particle immersion

<table>
<thead>
<tr>
<th>Particle Characteristics</th>
<th>Particle Characteristics</th>
<th>Film Surface Tension, mJ/m²</th>
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<tr>
<td>Size, dₜ (dₜw), μm</td>
<td>Surface free energy, mJ/m²</td>
<td>Surface topography</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>1(1)</td>
<td>~33</td>
</tr>
<tr>
<td></td>
<td>3(3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6(6)</td>
<td></td>
</tr>
<tr>
<td>Teflon</td>
<td>3.8(5.9)</td>
<td>~18</td>
</tr>
<tr>
<td></td>
<td>12.0°</td>
<td></td>
</tr>
<tr>
<td>Puffball spores</td>
<td>3.5(3.1)</td>
<td>~45</td>
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Microspheres of 1 to 6-μm geometric (dₜ) and aerodynamic (dₜw) diameter were used for the inhalation and in vitro studies. Teflon consists of partially fused nanospheres of 0.1–0.2 μm in diameter. The puffball spores are the least wettable particles because of their surface protrusions. *Particles were used in vitro only.
The deeply anesthetized hamsters inhaled the aerosol pneumotachography and laser-light scattering photometry. The number of inhaled and exhaled particles were measured by exhalation channel. The breathing parameters, as well as the stream. The particles were then dried, charge equilibrated, dropped of suspensions of spheres into a particle-free air. A styrene spheres have a smooth surface. and inhalation, lung fixation, as well as tissue sampling have been described previously (8–10, 12, 20, 36).

Briefly, aerosols were generated by jet nebulization of droplets of suspensions of spheres into a particle-free air stream. The particles were then dried, charge equilibrated, and concentrated before being transferred to the inhalation/exhalation channel. The breathing parameters, as well as the number of inhaled and exhaled particles, were measured by pneumotachography and laser-light scattering photometry. The deeply anesthetized hamsters inhaled the aerosol through an intratracheal cannula, either during spontaneous (slow, shallow) breathing or during continuous negative-pressure ventilation (slow, deep breathing), the latter at a level of respiration (mean lung inflation) of ~85% of the total lung capacity (TLC) (36). Aerosols were inhaled during 4–30 min, until a sufficient number of particles for microscopic analysis was deposited.

Immediately thereafter, the lungs were prepared for fixation by sequential intravascular perfusion of buffered 2.5% glutaraldehyde, 1% osmium tetroxide, and 0.5% uranyl acetate. With this triple-perfusion system, the surface layers of small airways and alveoli, including the phospholipids of the surface lining layer and the surfactant film at the air-liquid interface, are well preserved (1, 11, 13). In addition, the membrane lipids of the phagocytic cells are also preserved. All lungs were fixed within <50 min of the initial inhalation. The short time lapse between the start of the inhalation and lung fixation allows the investigation of particles as close to their deposition sites as possible.

After fixation, the respiratory organs were removed from the thorax. The trachea and main stem bronchi were cut off at the lung hilum. The left and right lung as well as the accessory lobe were then subjected to systematic sampling of lung tissue (8).

Tissue processing. All tissue samples were dehydrated in graded series of ethanol. Samples for transmission electron microscopy were embedded in Epon, and ultrathin sections were cut and stained with uranyl acetate and lead citrate. The sections were examined in a Philips 300 transmission electron microscope (Philips, Zurich, Switzerland) operating at 60 kV. Tissue blocks destined for scanning electron microscopy were critical-point dried, sputtered with platinum, and viewed in a Philips XL 30-FEG scanning electron microscope operating at 10 kV. Particles were studied in intrapulmonary conducting airways and alveoli from different anatomical regions.

Particle immersion by surfactant on a floating-drop-surface balance. The extent of particle immersion promoted by a surfactant film formed on an aqueous substrate of 0.9% NaCl and 55% sucrose (density 1.23 g/ml) was assessed on a drop, whose surface area could be changed like the film area in a Langmuir-Wilhelmy balance. The aqueous drop was floating on a substrate of FC-5312 [density 1.93 g/ml, surface tension (at 25°C) 18 mJ/m²; 3M] (Fig. 2). A surfactant film of dipalmitylphosphatidylcholine (DPPC) (Sigma-Aldrich, Oakville, ON, Canada) or of BLES was formed at the air-water and FC-water interfaces by either spreading the DPPC from a solution of 2 mg/ml in chloroform:n-hexane, 1:4 by volume, or by spreading 2 μl of BLES of a concentration of 27 mg/ml of phospholipid as provided. The experiments were conducted at 22 ± 1°C.

Apparatus. The FC fluid was filled into a cylindrical well that was 2 cm in diameter and 1 cm deep (Fig. 2). An aqueous droplet (0.9% NaCl) was placed onto the center of the FC surface. Because the density of the FC fluid is greater than that of water and the mutual solubility of the two fluids is negligible, the water drop formed a lens floating on top of the FC. A stainless steel needle reached the center of the water droplet, which allowed us to adjust its volume by either moving water into the droplet or withdrawing water from it to increase or reduce the droplet’s surface area. This allowed us to change the surface tension of the film at the surface of the droplet. The initial film surface tensions after film formation were 25.0 ± 0.5 mJ/m² for the DPPC and 24 ± 1 mJ/m² for the BLES films. The particles were dusted onto the surfactant film surfaces from Q-tip cotton balls by knocking the Q-tip stems gently with a metal rod. Particle immersion

Fig. 1. Scanning electron micrographs of native particles. A: polystyrene spheres have a smooth surface. B: Teflon particles have a knobby surface. C: puffball spores have a spiny surface with wart-like protrusions. Bars = 2 μm.
was investigated with a Nikon Optiphot metallurgical microscope (Nikon, Mississauga, ON, Canada) that was equipped for differential interference contrast and dark field for epillumination. In a second approach, to improve the imaging of partially immersed particles, we used a confocal microscope (Tracor Northern Tandem Scanning Reflected Light Microscope). With the use of a $\times 40$ objective (Zeiss antiflex), the resolution in the vertical direction was better than 1.0 $\mu$m, which allowed us to visualize the deformations of the fluid surface due to capillary rise around the protrusions of the puffball spores.

The film surface tension of the floating drop was estimated as described previously (31). Briefly, a Teflon tubing was connected to a screw mount 0.5-ml Hamilton gastight syringe (Hamilton) that contained the test fluid, a mixture of dimethylphthalate:n-octanol (DMP/O; Fluka, Buchs, Switzerland), 4:1 by volume. The fluid was doped with 4 mg/ml of crystal violet (Sigma Chemical, St. Louis, MO) to facilitate imaging of the test fluid. The Teflon tubing was connected to a micropipette (tip diameter of $\sim 20$ $\mu$m). Droplets of DMP/O, 0.2–0.5 mm in diameter ($D_0$), were then placed onto the surfactant film of the floating drop. From the diameter of the test fluid droplet, after it had spread to a diameter ($D$) characteristic for a particular surface tension, the film surface tension could be determined from a calibration curve, from $D/D_0$ (18). For surface tensions of $<25$ mJ/m$^2$, the test fluid DMP/O was replaced by FC-43 (3M) (29). In previous control experiments, we had determined that the test fluids in combination with the staining agents did not change the film surface tension. Only if the film surface tension exceeded 32 mJ/m$^2$ did the test fluid droplets start to disintegrate and interfere with the film analysis. In the present work, the film surface tension never exceeded 30 mJ/m$^2$, so there was no measurable influence of the test fluid on the film surface tension.

**RESULTS**

*Particle retention in airways and alveoli.* Regardless of the sampling site and of particle nature, all particles were submersed in the aqueous phase or coated by the lining film material (Figs. 3 and 4). The particles were in close association with the epithelial cells, which were often indented (Figs. 3A and 4, A and B). The protrusions of the spores indented the alveolar type I cells to such an extent that the apical and basal membranes came into close contact. In these situations, the spores were separated from the blood by $<$100 nm (Fig. 4B). Quantitative light microscopic analyses of particle deposition reported elsewhere (9, 10, 12) showed that the majority of particles had been immersed into the lining layer after their deposition in the respiratory tract.

In addition, particle displacement facilitated further interaction with cells, including phagocytosis of particles by resident macrophages (Fig. 5). A considerable number of particles taken up by macrophages were found by stereological investigation (8–10, 12). Internalization of particles by epithelial cells was not observed.

*Particle immersion by a surfactant film in vitro.* The Teflon microparticles of 12.0 and 3.8 $\mu$m in diameter were studied on DPPC surfactant films at differing surface tensions by using various light microscopic techniques (Figs. 6 and 7). When the particles were sprinkled on a DPPC film at a surface tension of 25.0 $\pm$ 1.0 mJ/m$^2$, the average diameter of the approximately circular three-phase line was within 10% of the average particle diameter, indicating particle immersion of
of the single particles are totally submersed because they do not show any air exposure (Fig. 7).

The wetting and displacement of the puffball spores by a surfactant film at differing surface tensions was studied by confocal microscopy (Fig. 8). At a film surface tension of \( \sim 25 \text{ mJ/m}^2 \), the amount of immersion cannot be determined from this micrograph as it could be at anything up to \( \sim 50\% \) (Fig. 8A). When spores were placed on a DPPC film at a surface tension of \( \sim 22 \text{ mJ/m}^2 \), the particle immersion is at least 50\%, otherwise deformations of the fluid surface due to the protrusions would not be visible (Fig. 8B). Because of the limited resolution of the light microscope, the protrusions cannot be seen directly. However, capillary forces likely have deformed the fluid surface at the periphery of the spores during the wetting process. At a film surface tension of \( \sim 15 \text{ mJ/m}^2 \), most of the spores are totally submersed (Fig. 8C).

**DISCUSSION**

**Particle displacement in airways and alveoli.** The microscopic analyses of inhaled and deposited particles in intrapulmonary conducting airways and in alveoli of hamsters revealed that, regardless of the anatomical site and particle nature, all of the particles were submersed in the aqueous lining layer or coated by the lining film material.

Particles were studied in lung compartments that contain rather thin surface lining layers, so the immersed particles were found in close contact with the epithelial cells, which were even indented by the particles. It is still unclear whether particles will reach the epithelial cells in airways that contain thick and viscous mucus layers like in cystic fibrosis, chronic obstructive pulmonary disease, or asthma. Nevertheless, the presence of mucus does not prevent particles from being wetted and displaced.

A study with hamsters suggests that the immersion of particles into the airway lining layer does not interfere with fast airway clearance because, on average, 87\% of the originally submersed particles were cleared within 24 h (8).

The displacement of particles into the surface lining layer might be beneficial for particle clearance from small airways and alveoli, as the particles are brought into the compartment of resident macrophages, which comprise the major clearance pathway in the peripheral lung. However, the displacement of particles toward the epithelial cells facilitates the interaction of the particles or any parts of them with many other lung cells, which may have consequences for lung disease.

**Displacement of smooth spherical particles.** We had previously shown by a force analysis for PS or polymethylmethacrylate particles, whose radii were \(< 100 \mu\text{m} \), that the surface forces are the dominating factor in particle displacement. For small particles with a radius of \(10 \mu\text{m} \) and a surface tension (surface free energy) of \(30 \text{ mJ/m}^2 \), the vertical component of the surface force (sinking force) is several orders of magnitude greater than the forces related to gravity (7, 30).
In the present study, the extent of immersion, as assessed in the floating-drop-surface balance (for summary, see Table 1), demonstrated that Teflon spheres were immersed into the subphase by 50–60% at film surface tensions of 25 mJ/m² and totally submersed at 15 mJ/m², except where the particles formed aggregates. When we placed the 3.8-μm Teflon microspheres onto films of either DPPC or lipid extract surfactant at an equilibrium surface tension of ~25 mJ/m², the extent of particle immersion for such small particles was difficult to quantify, even by using confocal microscopy. Thus we conducted additional in vitro experiments using 12.0-μm Teflon microspheres. The immersion of those particles was consistent with that of the smaller ones. We were not able to discover an influence of particle size on the displacement of these two kinds of microspheres.

Puffball spores: surface topography, wetting, and immersion. Puffball spores were immersed by <50% at the film surface tension of 25 mJ/m². This is consistent with the contact angle of ~110° formed in vitro by a surfactant droplet of a surface tension of 23–25 mJ/m² on a layer of puffball spores. Zero immersion (no wetting at all) would be consistent with a contact angle of 180° [see Schürch et al. (30) for the force analysis of particle displacement].

At a film surface tension of ~22 mJ/m², the spores appeared immersed by at least 50%. This follows from the deformation of the fluid surface (menisci) around the particles due to capillary forces acting on the protrusions of the spores. If the immersion had been less than ~50%, we would not have been able to see the deformations because the micrographs were taken by epi-illumination from above. When the surface tension

![Fig. 4. Transmission (A and B) and scanning (C and D) electron micrographs of puffball spores deposited on alveolar surfaces. A: the spore (its wall and protrusions are electron dense and appear black) is totally submersed in the surface lining layer. The epithelium was indented on displacement by surfactant, even by the spore's spiny protrusions. B: detail of indentations of epithelial cells by the spore's protrusions (not the same spore as in A). At these locations, the spore is separated from the circulating blood by <100 nm. C and D: the spore is totally covered by the surface lining layer; its surface topography is visible through the thin lining layer. A, alveolar lumen; C, blood capillary; EN, endothelial cell; EP, epithelial (type 1) cell; LC, leukocyte; LL, osmiophilic lining layer material. Bars = 2 (A and D), 0.5 (B), or 5 μm (C).](image_url)

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![Fig. 5. Transmission electron microscopy micrographs of phagocytosed polystyrene microspheres (P). A: 6-μm particle taken up by an airway macrophage. B: 1-μm particles within an alveolar macrophage. AW = airway lumen. Bars = 5 (A) or 1 μm (B).](image_url)
of the film, onto which the particles had previously been placed at 25 mJ/m², was decreased, the wetting process did not progress gradually or smoothly. Parts of the spores appeared to be wetted suddenly because the menisci seemed to move up jerkily. Finally, on reaching a film surface tension of \(~15\) mJ/m², most of the spores, except for some aggregates, became submersed within a few seconds.

The surface free energy of the puffball spores was estimated to be \(~45\) mJ/m² from their chemical composition (5, 15, 17). Smooth, spherical particles of such relatively high surface free energy should have been submersed at film surface tensions of \(~25\) mJ/m² (30). The wetting behavior of puffball spores can only be explained by their complicated, spiny surface topography, since corrugated or spiny surfaces as well as plates or particles with sharp edges resist wetting to a greater degree than smooth, spherical particles (25).

**Surface tension in the airways.** The in vitro studies on the wetting and immersion of Teflon particles and puffball spores in combination with the measurements of the contact angle on layers of spores demonstrated that the surfactant film surface tension had to be lowered to at least \(~15\) mJ/m² to submerse the particles. These observations suggest that the surface tension in the intrapulmonary conducting airways of the hamster may reach a minimum surface tension of \(\leq 15\) mJ/m². This is considerably below the surface tension of \(32\) mJ/m² measured in the trachea of horses (18) and substantially below the equilibrium surface tension of \(~25\) mJ/m² for pulmonary surfactant films. Because surface tensions below \(~25\) mJ/m² can only be achieved by mechanically compressed films, the conclusion is that the surfactant film in the conducting airways of hamsters at least temporarily may reach a compressed state. Changes in airway surface area are very modest during regular breathing and are likely not sufficient to compress the surfactant film from \(25–30\) to \(15\) mJ/m².

Equilibrium surfactant films need to be compressed by \(15–20\)% to reach values below \(~2\) mJ/m² or by \(~5\)% to reach \(15\) mJ/m² under dynamic compression (28).
However, the alveolar surfactant film reaches minimum surface tensions below ~2 mJ/m² under in vivo conditions (4). It is clear that there must be a surface tension gradient between the compressed alveolar surfactant film and that in the trachea or large bronchi, because all evidence to date shows that the film is continuous between the alveoli and central airways (11, 13). Very little is known with regard to the compression state of the surfactant film in the conducting airways. We also do not know the rate of change in airway surface tension at a particular location and at a particular time during the breathing cycle. However, we know from the in vitro studies that particles placed on a surfactant film can be submersed within seconds on a momentary reduction of the surface tension from equilibrium of 23–25 to ~15 mJ/m².

Additional, more direct evidence for surface tensions far below the equilibrium value of ~25 mJ/m² in the central airways of rabbit has been obtained by Bachofen and Schürch (3). The shape of bronchiolar macrophages beneath the surfactant film in the surface lining layer depends on the local surface tension and can serve to estimate the airway surface tension. At a low surface tension at 40% TLC, the macrophages were shown to freely bulge into the bronchiolar lumen. At high surface tension (~80% TLC), the macrophages were flattened and pressed into the bronchiolar epithelium.

It has recently been shown that the ability of a surfactant film to reach near zero value on film compression is not damaged during fixation with glutaraldehyde and osmium tetroxide (2). Thus it is unlikely that the different shape of the macrophages at differing lung volumes is an artifact due to fixation. It is also unlikely that particle immersion is due to a fixation artifact.

How can a compressed surfactant film be generated in the conducting airways when the airway system does not seem to have a confining barrier that would prevent or slow down film leakage? There are two characteristics that may facilitate the generation of a temporary compressed nonequilibrium surfactant film in the airways, which may reduce surface leakage up the airways. One is the anatomic feature of the enormous decline in the surface film area from the alveoli to the conducting airways; the other is the complex structure of the pulmonary surfactant film. Highly compressed alveolar surfactant films exhibit viscoelastic properties. These films frequently appear multilaminated and relax over periods of several seconds from surface tensions near zero to higher values on extension of the film area (14, 32). Thus the surfactant film may resist fast changes in surface tension, especially on film area extension. A compressed film of relatively low surface tension may reach beyond the alveolar region into the airways.

In summary, this study has shown that inhaled particles, regardless of the nature of their surfaces, will be submersed into the lining layer after their deposition in small airways and alveoli. The displacement is promoted by the surfactant film at the air-liquid interface, whose surface tension falls to relatively low values temporarily. Complete wetting and immersion of some particle types require surface tensions far below the equilibrium value of ~25 mJ/m². The finding that such particles are also immersed in the lining layer gives additional evidence that the surfactant film in

Fig. 8. Confocal images of puffball spores dusted onto a DPPC surfactant film at differing surface tensions. A: spores at a film surface tension of ~25 mJ/m². The amount of immersion cannot be determined from this micrograph, because it could be anything up to ~50% immersion. B: spores at ~22 mJ/m². The particle immersion is at least 50% because the surface deformations of the fluid due to the protrusions can be visualized. These deformations appear irregular and star-like around the particle. C: spores at a film surface tension of ~15 mJ/m². The light gray areas (arrowhead) represent submerised particles or particle aggregates, whereas at the left bottom some spores still appear partially exposed to air (arrow). The diagonal lines on the figure originate from the aperture in the rotating disc confocal microscope. Bars = 10 μm.
small airways may reach a compressed state with a surface tension substantially below that of an equilibrium film. Particle displacement likely enables further interaction with many lung cells.

We thank V. Im Hof, I. Maye, and U. Waber for help with the inhalation and the lung fixation and K. Babi, S. Frank, B. Kuper-schmid, and B. Krieger for excellent technical assistance.

The study was supported by the Swiss National Science Foundation Grant 32-65352.01, the Silva Casa Foundation, the Canadian Institutes of Health Research, and the Alberta Heritage Foundation for Medical Research.

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