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SCANNING ELECTRON MICROSCOPIC AUTORADIOGRAPHY OF LUNG

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Abstract

Scanning electron microscopic (SEM) autoradiography of the lung is being used to determine the distribution of inhaled, alpha particle-emitting, plutonium dioxide particles. SEM autoradiography provides high visual impact views of alpha activity. Particles irradiating the bronchiolar epithelium were detected both on the bronchiolar surface and in peribronchiolar alveoli. The technique is being used to obtain quantitative data on the clearance rates of plutonium particles from bronchi and bronchioles.

KEY WORDS: Scanning electron microscopy, Autoradiography, Lung, Plutonium.

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Introduction

The toxicity of α-emitting radionuclides, including transuranic compounds, is influenced by alpha particle interactions with tissues in space and time. Alpha particles from ^{239}Pu have a mean energy of about 5.1 MeV, penetrating about 45 microns in solid soft tissue or >250 microns in alveolar regions of the lung. The liquid photographic emulsion autoradiographic technique was developed about 45 years ago to visualize radionuclides in tissues. The spatial distribution of plutonium in the lung has been determined by light microscopic and transmission electron microscopic liquid photographic emulsion autoradiography (Adee et al., 1968; Sanders and Adee, 1970; Heppleston and Young, 1985), computer-simulated patterns (Diel, 1978; Simmons and Richards, 1984), and by nuclear track etching techniques (Henshaw et al., 1979). Scanning electron microscopy (SEM) has been used to detect tritium in the nuclei of cells (Hodges and Muir, 1973) and alpha tracks in nuclear film emulsion (Wittendorp-Rechenmann et al., 1983).

Non-radioactive particle deposition in the lung (Brody and Roe, 1983) and pulmonary pathology from inhaled $^{239}PuO_2$ (Nicholls et al., 1986) have been evaluated with SEM. We have developed a simple autoradiographic technique using SEM that provides comparatively large volume tissue sampling for alpha activity in the lung. The technique is particularly useful in localizing radionuclides in tissues, such as bronchiolar epithelium, that may contain relatively low amounts of radioactivity.

Materials and Methods

Fifty, 70-day-old, SPF, female, Wistar rats were exposed, nose-only for less than an hour, to a submicron-sized aerosol of $^{239}PuO_2$, tagged with ^{169}Yb to determine initial lung burden (ILB). Details of aerosol exposure have been previously published (Sanders et al., 1986). Mean ILB was 4.9 kBq ^{239}Pu and lung doses were 8.4 to 12.2 Gy at 60 to240 days after exposure. Rats were killed in groups of five at 1, 14, 28, 60, 90, 120, 150, 180, 210 and 240 days after exposure and their lungs prepared for SEM examination.

Rats were anesthetized with sodium barbital (50 mg/kg body weight), the trachea cannulated, the lung perfused with 10% glutaraldehyde and 10% formalin *in situ* at 4°C for 20 minutes at 25 cm of water pressure and then removed and placed in like

fixative for at least 1 week. Bisected tracheas and approximately 1-mm thick cross sections of the entire cardiac lobe were placed in distilled water overnight at 4°C, and gently blotted on filter paper. Tissue samples were adhered to a glass slide with double sticky tape, the slides dipped in 1:1 Ilford K-5 liquid photographic emulsion, and dried at room temperature. Slides were then placed in light-tight boxes, exposed at 4°C for 4 weeks, developed in Eastman Kodak D19, fixed in Kodak Rapid Fix, air-dried, mounted on aluminum studs, coated with 2 nm gold palladium and scanned with a JEOL-25S11 scanning microscope, operated at 15 keV. Micrographs were taken on Polaroid Type 55 film using a 50-second exposure time. The thickness of the emulsion layer when dried was about 1 micron. Approximately 1-mm^3 pieces of lung tissue were embedded in Lx-112 resin, covered with Ilford K-5 liquid photographic emulsion and exposed for 4 weeks for light microscopic examination.

Results and Discussion

A thin, approximately 1-micron thick, opaque, homogeneous coating was seen on all tissue surfaces, covering subcellular structural details such as cilia on bronchiolar epithelial cells (Figure 2). However, overall tissue detail was remarkably good, for the purpose of microdosimetry applications. Plutonium particles were seen as individual particles in alveoli (Figure 1) during the first few weeks after exposure. By 28 days after exposure, there was a tendency towards aggregation of particles, particularly for those particles associated with the alveolar walls. Alpha tracks could be seen penetrating through the walls of alveoli from adjacent alveoli. Single plutonium particles and aggregates of particles were found on the bronchiolar epithelium and in locations that appeared to be beneath the bronchiolar epithelium, originating from adjacent alveoli (Figures 2, 3 and 4). The concentration of particles on tracheal epithelium (Figure 5) was considerably less than seen on bronchial and bronchiolar epithelium. An unusually homogeneous material uniformly emitting alpha particles was seen in a blood vessel of one rat (Figure 6). The material may be an iron-containing pigment, which is known to associate with plutonium in regions of inflammation and fibrosis. An example of the distribution of alpha activity at the pleural surface from plutonium particles located in subpleural alveoli is seen in Figures 7 and 8.

About 1% of particles deposited in the lung may be directly taken up by tracheal, bronchial or bronchiolar epithelium (Gore and Patrick, 1982; Briant and Sanders, 1987). Particle localization in these studies was accomplished by light microscopic autoradiographic techniques, which is often unable to distinguish between particles located within and beneath the mucosa. Many of these particles may not have been taken up by epithelial cells but were present in submucosal alveoli or lymphatics. SEM autoradiography of airways indicates that most plutonium particles irradiating bronchioles after the first few months after exposure, reside in adjacent alveoli (Sanders et al., in press). The terminal bronchioles are the most likely site of carcinoma formation from inhaled transuranics in the rat (Masse, 1980; Johnson et al., 1983). The prolonged retention of plutonium particles in peribronchiolar alveoli will expose bronchiolar epithelial cells to comparatively high radiation doses (Patrick, 1983), and become a significant factor in the induction of lung carcinomas. SEM autoradiography has recently been used to determine clearance rates of inhaled $^{239}PuO_2$ from regions of the rat respiratory tract (Sanders et al., in press). Using this clearance data, it will be possible to determine the radiation dose delivered to the bronchiolar epithelium from inhaled plutonium particles.

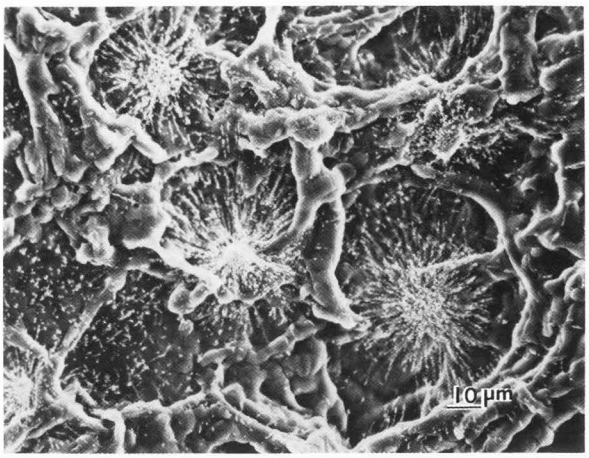

Figure 1. SEM autoradiography showing alpha-stars in the alveolar region at 15 days after inhalation of $^{239}PuO_2$.

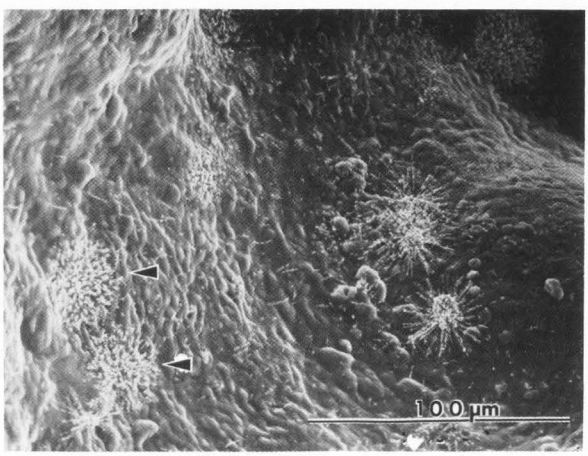

Figure 2. SEM autoradiography showing alpha stars on the surface of a bronchiole at 150 days after inhalation of $^{239}PuO_2$.

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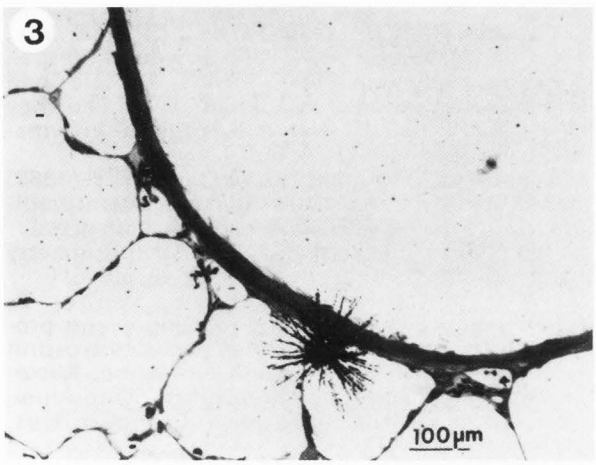

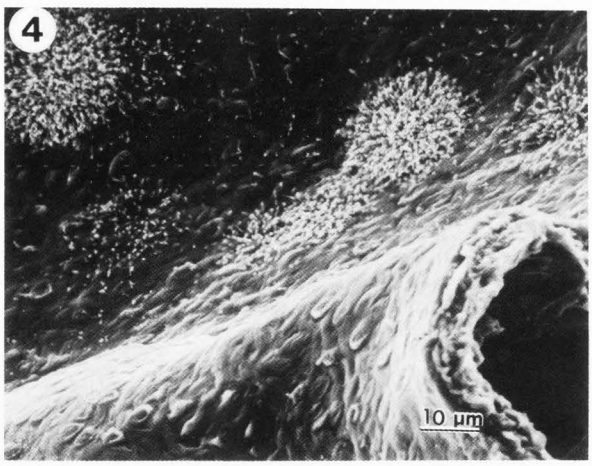

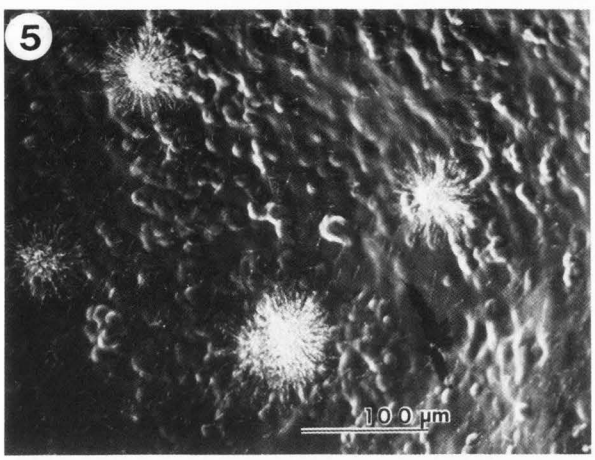

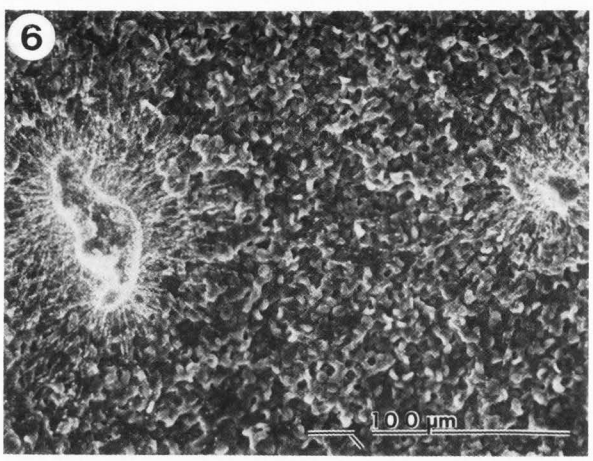

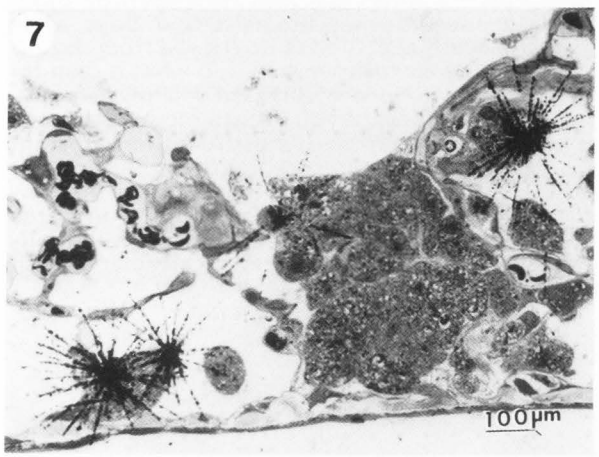

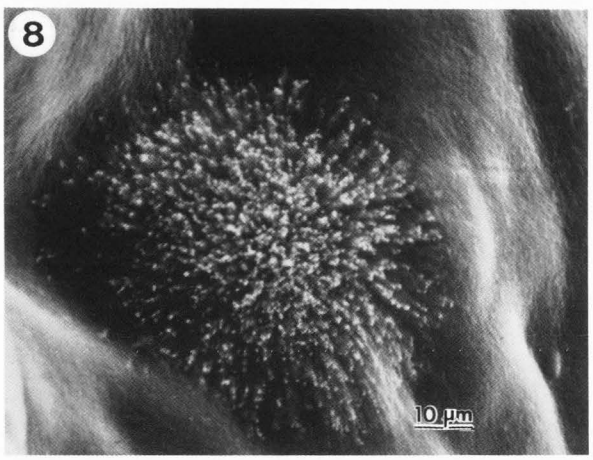

Figure 3. Light microscopic autoradiography of lung showing location of a plutonium particle in an adjacent alveolus at 30 days after inhalation of $^{239}PuO_2$.

Figure 5. SEM autoradiography showing alpha stars on the surface of the trachea at 1 day after inhalation of $^{239}PuO_2$.

Figure 7. Light microscopic autoradiography of subpleural alveoli showing location of plutonium particles irradiating through the pleural at 60 days after inhalation of $^{239}PuO_2$.

Figure 4. SEM autoradiography showing alpha tracts originating from plutonium particles in adjacent alveoli penetrating through the bronchiolar epithelium at 30 days after inhalation of $^{239}PuO_2$.

Figure 6. SEM autoradiography showing an unknown material in a pulmonary blood vessel with uniformly emitted alpha particles at 180 days after inhalation of $^{239}PuO_2$.

Figure 8. SEM autoradiography of pleural surface showing alpha tracks emitted from a subpleural plutonium particle at 90 days after inhalation of $^{239}PuO_2$.

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Discussion with Reviewers

Godfried M. Roomans: What is your evidence that the fixation procedure used does not displace inhaled particles from their original location in the lungs?

Authors: This study was not originally designed to examine particle distribution in the lung. Ideally lung fixation by formalin vapor and/or vascular perfusion with formaldehyde would maximize the integrity of particle distribution in the lung, particularly during the first few weeks following inhalation when many particles are being cleared from the lung. However, many particles were seen on bronchiole surfaces during the first few days after exposure, which appeared to be cleared from the airways in a biphasic manner similar to what is seen for whole lung clearance of inhaled $^{239}PuO_2$ in rats. There were few particles on bronchiole surfaces seen 30 to 700 days after exposure. A rather constant plutonium particle concentration in peribronchiolar alveoli during this time (>30 days) indicates that few plutonium particles were transported into this area by the fixative, but that they were preferentially retained in this area following initial alveolar deposition.