• Cigarette Smoke and Plutonium

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The major objective of this project is to obtain experimental data that are directly applicable to resolving the question of whether cigarette smokers are at greater risk than nonsmokers to potential health effects of inhaled plutonium. Because cigarette smokers constitute a large fraction of the population, a synergistic effect of plutonium and cigarette smoke might influence estimates of the health risk for plutonium and other transuranics released to the environment.

An autoradiographic technique developed for detection of plutonium on the interior surface of pulmonary airways (Annual Report, 1978) was used on the lungs of rats from an earlier experiment. The data demonstrated the effect of cigarette-smoke exposure on the mucociliary clearance mechanism. A similar technique, using cellulose nitrate track-etch film, is being applied to the pulmonary airways of dogs.

Distribution and Retention of $^{239}PuO_2$ in Pulmonary Airways of Rats

Results of a previously reported experiment with rats showed a marked cigarette-smoke-induced reduction in pulmonary clearance of inhaled $^{239}PuO_2$. A group of 35 rats was exposed to cigarette smoke for 7 mo; another group of 35 rats was sham-exposed for the same period of time. Both groups were exposed, by inhalation, to $^{239}PuO_2$ (approximately 80 nCi initial lung burden [ILB]), and smoke and sham exposures were resumed 7 days afterward. In vivo (whole-body) counts indicated that smoke- and sham-exposed rats retained means of 64 and 39% of their ILBs, respectively, 6 wk after inhalation of plutonium (Table 1); the difference was highly significant ($P < 0.0002$).

The pulmonary airways of these rats were examined using an autoradiographic technique developed for quantifying the distribution of plutonium particles. The technique involved isolation of large airways from lung parenchyma, separation of the mucosa from cartilage and adventitia, and mounting the sheet of epithelium on a glass microscope slide so that the inside surface of the airway faced outward. This surface was coated with liquid photographic emulsion, exposed, developed, and coverslipped. The final preparation consists of epithelium from approximately 2.5 cm of trachea and 2.0 cm of both the right and left bronchi. A transparent grid was superimposed over the slide, and the alpha "stars" within each grid square (area, $\sim$1 mm^2) were counted. Figure 1 is a photomicrograph of the preparation as it appears during evaluation. Data recorded include the location of each grid square with respect to the total airway surface and the number of alpha stars each grid square contains.

A summary of the data collected from rats killed at 1, 7, and 42 days after inhalation is given in Table 1. In sham-exposed rats, the concentration of plutonium particles in pulmonary airways on day 42 was less than that on day 1 by a factor of almost five. Corresponding data from smoke-exposed rats show a concentration reduced by a factor of 14. Smoke-exposed rats had significantly ($P < 0.01$) lower concentrations of plutonium in their airways than did sham-exposed rats regardless of when they were killed.

Microscopically, it is impossible to distinguish whether the plutonium particles causing the autoradiographic star are located within or beneath the mucosal blanket. The concentrations of plutonium particles in the airways of smoke-exposed rats were consistently low, compared to those of sham-exposed rats, even though in vivo counts verify approximately equal amounts of plutonium inhaled by both groups. This indicates that particle concentrations reflect clearance rate at the time of death. This observation is also indicated by the inverse relationship between airway particle concentration and in vivo counts; apparently, plutonium particles in the airways have little effect on total body count.

Two observations made during the evaluation of the airway preparation are noteworthy. First, aggregations of plutonium particles were not necessarily associated with bifurcation in the airways. Many of the bifurcations are clearly visible in the bronchial preparations; there was no readily apparent selective deposition or retention

TABLE 1. The Effect of Cigarette Smoke Exposure on Clearance of $^{239}PuO_2$ From the Lungs of Rats (Mean ± SD)

Exposure Group	Sacrifice Time Postexposure, days	In Vivo Counts: Day 4 Postexposure[a], cpm	In Vivo Counts: 1 Day Before Sacrifice, cpm	In Vivo Counts: 1 Day Before Sacrifice, % of day 4	Pu Particles per mm²: Trachea	Pu Particles per mm²: Right Bronchus	Pu Particles per mm²: Left Bronchus	Pu Particles per mm²: Airway Average
Pu Only	1				9.2 ± 6.6 (6)	15.2 ± 8.4 (6)	9.5 ± 6.0 (10)	11.2 ± 4.9 (22)
Smoke + Pu	1				4.4 ± 1.4 (9)	4.6 ± 3.1 (10)	4.4 ± 3.3 (9)	4.2 ± 1.8 (28)
Pu Only	7	169 ± 32 (10)[b]	147 ± 27 (10)	88	6.8 ± 3.6 (6)	9.4 ± 4.4 (8)	11.8 ± 7.6 (8)	10.4 ± 6.4 (22)
Smoke + Pu	7	159 ± 30 (10)	147 ± 25 (10)	94	0.8 ± 0.6 (8)	1.6 ± 0.7 (9)	2.2 ± 1.2 (7)	1.4 ± 0.6 (24)
Pu Only	42	163 ± 22 (10)	64 ± 11 (9)	39	2.1 ± 1.6 (7)	2.6 ± 1.7 (8)	2.2 ± 1.4 (6)	2.3 ± 1.5 (21)
Smoke + Pu	42	161 ± 47 (9)	103 ± 24 (9)	67	0.3 ± 0.1 (9)	0.3 ± 0.2 (9)	0.3 ± 0.1 (8)	0.3 ± 0.1 (26)

[a] The count at 4 days postexposure was considered the initial lung burden.
[b] Number of observations upon which each mean is based

FIGURE 1. Alpha "Stars" in an Autoradiograph of the Inside Surface of a Rat Trachea

at these sites. However, there were distinct regional differences in particle concentration in the tracheal preparations. Higher particle concentrations were frequently noted at the dorsal wall of the trachea. Since, at necropsy, the lungs were inflated with air and fixed by vascular perfusion with 2% glutaraldehyde, plutonium in the airways at the time could not have been translocated by the fixative. Subsequent scanning electron micrographs of the tracheal wall of a rat revealed that the dorsal wall was much more ciliated than the ventral wall. The micrographs (Figures 2 and 3) show the trachea of an unex-

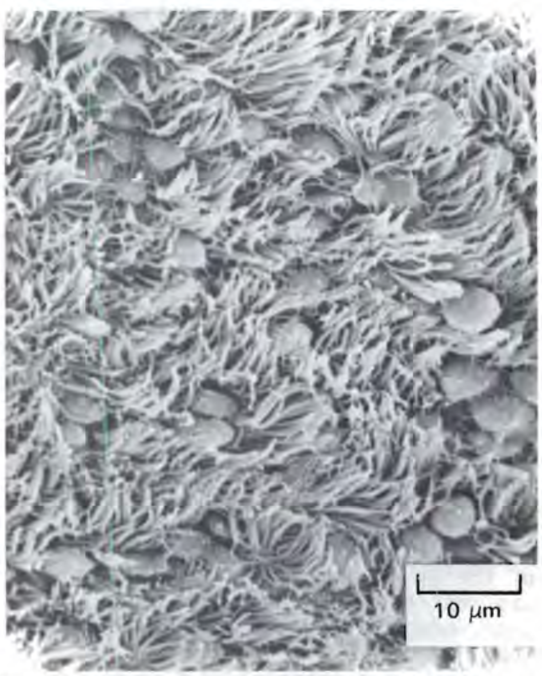

FIGURE 2. Scanning Electron Micrograph of the Dorsal, Inside Surface of a Rat Trachea.

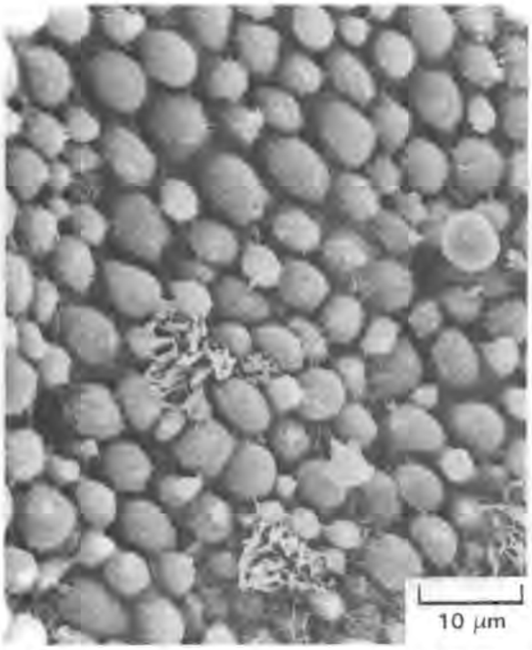

FIGURE 3. Scanning Electron Micrograph of the Ventral, Inside Surface of a Rat Trachea.

posed, control rat. Although this difference has been alluded to in light microscopic studies of the trachea, the magnitude of the difference shown by scanning electron microscopy was quite surprising. Further investigation of airway anatomy will include microscopy of rat bronchi and of the airway of at least one other species.

When this program began, one hypothesis considered was that areas of pulmonary airway mucosa would be denuded of cilia by cigarette smoke, resulting in selective retention of plutonium in those areas. The data collected thus far do not indicate such selective retention. We conclude, rather, that the greater pulmonary retention of plutonium by smoke-exposed rats (compared to sham-exposed rats) demonstrated by whole-body counting is a direct result of the diminished mucociliary clearance induced by cigarette smoke. More data will be available in the future from autoradiographs of lung sections from these rats and from radiochemical analysis of remaining lung tissue.

Distribution and Retention of $^{239}PuO_2$ in Pulmonary Airways of Dogs

Cigarette-smoke-exposed and sham-exposed dogs were killed approximately 60 wk after inhalation exposure to a $^{239}PuO_2$ aerosol. Lungs were inflated with air and fixed by vascular perfusion. Retention and distribution of plutonium in pulmonary airways will be characterized by a form of autoradiography, using cellulose nitrate track-etch film. The refined technique for this purpose is as follows: lung parenchyma is stripped from the tracheobronchial tree down to approximately the level of the secondary bronchi of each lung lobe. These are split, flattened, and presented so that track-etch film is in contact with the internal surface. After an empirically determined exposure time, the film is removed and etched with NaOH solution, which results in the appearance of alpha "stars" where the film was in contact with a plutonium particle. A disadvantage of this technique is that the film is no longer in contact with the tissue at evaluation; however, photographs taken of the tissue preparation during exposure are expected to compensate. On the other hand, a marked advantage to this technique over conventional autoradiography is that several films may be made of the same tissue, if necessary. Because this work is in its initial stages, no results are available at this time.