Dosimetry of Inhaled Plutonium-239 Dioxide in Rodent Lung - A Morphometric Study

Kathleen Rhoads

June 1979

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Abstract

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- A MORPHOMETRIC STUDY -

By Kathleen Rhoads

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Wistar rats and Syrian hamsters were exposed to aerosols of $^{239}\text{PuO}_2$ via inhalation at doses near those for maximum tumor response in rat life-span studies. Clearance of inhaled Pu from the lung was similar in both species, and less than 6% of the administered dose was translocated to extrapulmonary tissues up to 1 year postexposure. Morphometric analysis was performed on light and electron micrographs in order to characterize the structure and composition of lung in control and exposed animals of both species. Inhalation exposure to $^{239}\text{PuO}_2$ had little effect on the overall structure or composition of lung as estimated by morphometric methods. Focal lesions typical of radiation damage were found during microscopic observation, however these lesions were of such limited extent that they did not significantly affect the outcome of the study. Most of the differences noted were between the two species, although there did not appear to be a direct relationship between the type of differences observed and the markedly different lung tumor response of rats and hamsters in life-span studies of inhaled $^{239}\text{PuO}_2$.

Application of morphometric techniques to autoradiograms of lung tissue did reveal some species differences in particle distribution.

Rats showed a greater long-term association of particles with the subpleural region and major airways than hamsters. It was also found that the spatial arrangement of particles was Poisson distributed in hamsters, whereas it was not in rats. In a direct species comparison, the particles showed a greater tendency to occur in groups for rats, and this species difference was more pronounced in the subpleural region than in the remainder of the lung. The major airways and the subpleural region contain the principal target cells for tumor production and serve as foci for the origin of tumors. Different dose distributions within these regions may therefore affect the tumor response to inhaled $^{239}\text{PuO}_2$.

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INTRODUCTION

Life-span studies of inhaled $^{239}\text{PuO}_2$ aerosols in rodents have shown that rats develop a high incidence of lung tumors in response to this treatment (1-3), whereas hamsters develop very few (4-5). This study focused on two factors which may have influenced susceptibility to lung tumor induction in these species; lung composition and structure, and the distribution of inhaled $^{239}\text{PuO}_2$ particles within the lung. These parameters were investigated during the first year postexposure, which corresponded to the latent period for the earliest tumors in rats.

The effects of radiation on the lung, both from external sources and internally deposited radionuclides, have been studied and documented in a wide variety of animal models. The principal early effects include radiation pneumonitis and fibrosis which lead to respiratory impairment and death at high doses. The early effects of pulmonary irradiation result from damage to the air-blood barrier in the alveolar septa, whereas the larger airways and blood vessels show minimal change. Of greater practical concern are potential late effects which occur at low doses, the most significant being the ability of radiation to induce proliferative changes in lung tissue which may progress to malignant tumors. Lung tumors attributable to the effects of radiation alone or in combination with other factors have been found in man and produced in many species of experimental animals (1-3, 6-16).

Interest in inhaled $^{239}\text{PuO}_2$ stems from the fact that this compound is both a by-product of light-water reactors now in use and the

principal fuel for breeder reactors currently under development.

Plutonium-239 in various physical and chemical forms has been shown to produce lung tumors in a number of animal models, mainly rats and dogs (1-3, 10-12). Plutonium-239 has a half-life of 24,400 years and emits a 5.1 Mev decay alpha particle. Due to the short range of alpha particles in tissue (45 μ m in tissue of density 1.0), the potential biological hazard for ^{239}Pu occurs only when the material is deposited internally. The fate of internally deposited Pu depends on the route of administration and the physical and chemical form of the compound. The critical organs are lung and, in the case of soluble Pu compounds, bone. Inhalation represents the most likely route of exposure for any significant number of people in the event of an accidental release.

In two life-span studies of inhaled $^{239}\text{PuO}_2$, 460 rats developed 143 lung tumors, the maximum incidence being 65-70% at doses ranging from 1000-2000 rads over a period of 350-620 days (1,2). In contrast, hamsters exposed to $^{239}\text{PuO}_2$ developed a total of 3 malignant tumors in 764 animals, all at doses greater than 3500 rads over 1 year (4,5). The normal incidence of lung tumors in both species is reported to be very low (1,2,4,5), although about 7% of untreated hamster lungs develop adenomatous metaplasia which does not progress to neoplasia within the life span of the animals (4).

The most frequently observed tumor in rats at relatively low doses, and in those hamsters which developed tumors, was adenocarcinoma of peripheral origin, arising from bronchiolar or perhaps alveolar epithelium (1,2,17,18). At higher doses in rats squamous cell carcinoma, also of bronchiolar origin, became the predominant tumor

type (1,2). A small number of hemangiosarcomas arising from vascular endothelium were found at the highest doses (1). Epithelial tumors arose preferentially in the regions where accumulation of plutonium particles had caused the lung tissue to become scarred and fibrotic (1,3,9,17). Low-level exposures produced adenocarcinomas without eliciting an inflammatory or fibrotic response, indicating that the presence of lung damage was less important in the development of these tumors than for the other two types (1). Adenocarcinoma is also the most common naturally occurring tumor type in rodents, and in human populations not exposed to cigarette smoking or other carcinogenic agents (16,19). The composition of lung, especially the population of susceptible target cells and the nature of early morphologic changes following radiation exposure, may therefore determine the ultimate potential for tumor induction.

The susceptibility of hamsters to radiation-induced lung tumors does appear to be a function of spatial and temporal dose distribution. Although inhalation exposures to the alpha-emitters $^{238}\text{PuO}_2$, $^{239}\text{PuO}_2$ and $^{241}\text{AmO}_2$ rarely produced tumors (4,5,20,21), such a response was readily obtained following multiple intratracheal instillations of ^{210}Po , also an alpha-emitter (13,14), and by external irradiation of the thorax (15). The observation that lung tumors can be induced in hamsters by radiation, under some circumstances, and by chemical carcinogens argues against any non-specific anti-tumor mechanism in explaining the resistance of this species to inhaled $^{239}\text{PuO}_2$.

The distribution of $^{239}\text{PuO}_2$ in dog and rodent lung depends on a number of factors, including the initial deposition pattern and the

forces which then respond to the presence of deposited material. Inhaled $^{239}\text{PuO}_2$ particles of respirable size are deposited in a relatively uniform fashion throughout the lung (4,9,22), following which several mechanisms function to redistribute the material within the lung and the body. Particles deposited in the upper respiratory tract are cleared by ciliary action within 1-7 days following exposure (22,23), whereas particles deposited in the alveoli may be retained for months or years (1,2,12).

Many particles in the alveoli are phagocytized by macrophages which are cleared via the upper respiratory tree. Other particles may be trapped within alveolar epithelial cells or the pulmonary lymphatic system and retained for relatively long periods (12,23). This pattern of progressive clearance from large to small airways and long-term retention in peripheral lung has also been observed in man following insufflation of a non-radioactive particulate (24). A small amount of $^{239}\text{PuO}_2$ is translocated from lung via the bloodstream to other parts of the body. This type of translocation is more extensive with soluble plutonium compounds such as nitrates and citrates than with relatively insoluble PuO_2 (1,11).

The PuO_2 particles which are retained in the lung become redistributed with time, tending to accumulate in the subpleural region (see Plate I) and, to a lesser extent, in the peribronchial and perivascular spaces (1,3,4,9,17). This has also been observed with soluble plutonium compounds (10). The non-uniform distribution of inhaled particulate radionuclides apparently influenced the subsequent development of biological effects, and should be taken into account when considering lung dosimetry.

Plate I. Concentration of $^{239}\text{PuO}_2$ particles in the subpleural region of rat lung:

- A. 150 days postexposure
Terminal lung burden, 35 nCi
- B. 240 days postexposure
Terminal lung burden, 20 nCi
- C. 350 days postexposure
Terminal lung burden, 7 nCi.

(Autoradiogram, 2 week exposure, 65 X).

A

B

C

Morphometric methods developed by Weibel for the study of lung and other biological material have been adapted for this study of the distribution of inhaled $^{239}\text{PuO}_2$ particles and their effect on the structure and composition of lung (25-28). These topics were chosen for emphasis because of their relevance to the tumor development problem and because they lend themselves well to analysis by morphometric methods. This investigation was designed to yield information about the physical make-up of lung tissue in both control and exposed animals, and data for use in radiation dosimetry. Comparison of a susceptible and a resistant species also provides insight into the factors most significant for development of radiation-induced tumors in the lung, with the aim of predicting human responses to inhaled alpha-emitting radionuclides.

METHODS AND MATERIALS

Animal Data

Thirty-five each of young-adult female rats and hamsters were obtained for this study. The rats were randomly-bred, specific pathogen free animals of the Wistar strain born on March 10, 1975¹. They arrived in this laboratory on May 22, 1975 at 73 days of age. Randomly-bred Syrian golden hamsters arrived from the supplier on May 27, 1975 at age 65 days². All animals were isolated for a period of 20 days to allow for adaptation to the new environment and to ensure that no latent illness was present at the time of exposure. The animals were exposed to $^{239}\text{PuO}_2$ in early adulthood, a stage at which they attain relative physiological stability, yet have a remaining life span sufficient to allow study of neoplastic responses. The most prominent pathological conditions found in rats allowed to live out their normal life span are chronic murine nephropathy, fatty degeneration of the liver, adrenal sinusoidal ectasia, and mammary fibroadenoma (1). The most common natural cause of death in hamsters appears to be liver and kidney disease associated with amyloidosis (4).

Animals were housed together in a small animal facility from 1 week postexposure until necropsies were performed. Rats were housed 5 per cage, and hamsters were housed individually to prevent cannibalism. Clean cages and corncob bedding were provided twice

¹ Hilltop Laboratory Animals, Inc., Chatsworth, CA
² Engall's Laboratory, Farmersburg, IN

weekly, and food and water were given ad libitum. The animal facility was maintained on a 12 hour photoperiod at a temperature of 72°F with 30-50% humidity.

Exposure Techniques

Ten animals of each species were kept as controls, and the remaining 25 rats and 25 hamsters were given single, nose-only exposures to aerosols of $^{239}\text{PuO}_2$ on June 11, 1975. Rats were exposed for 45 minutes and hamsters for 30 minutes in order to achieve the desired lung burdens of 160 and 90 nCi respectively. Techniques for nose-only inhalation exposure of the animals in this study were described by Craig and Buschbom (29). The inhalation suite utilizes four levels of containment for aerosols of hazardous materials such as $^{239}\text{PuO}_2$. Within the suite are individual exposure rooms with apparatus appropriate for the type of animal to be exposed. For rodents, this consisted of a glove box containing a cylindrical lucite exposure chamber (volume 14 liters).

Unanesthetized animals were inserted into 'coke' bottles from which the bottoms and part of the tops had been removed. Paper towel wadding and rubber stoppers taped into the wide end secured the animals so their noses were close to the narrow opening of the bottles. The bottles were then inserted into ports in the exposure chamber along with aerosol sampling devices; an 8-stage cascade impactor, an electrostatic precipitator, and filters for determining the overall aerosol concentration.

The $^{239}\text{PuO}_2$ was prepared by calcination of plutonium oxalate for 2 hours at 750°C and administered within 2 days after preparation. The size of $^{239}\text{PuO}_2$ particles in aqueous suspension remains stable; neither the X-ray diffraction pattern nor the ultrafilterability ($< 0.2\%$) changes over a period of several months (1). The aerosol was generated with a Lovelace Nebulizer from a suspension having a specific activity of $239 \mu\text{Ci/ml}$. The particle morphology is shown in Plate II. The particle size distribution as measured in a cascade impactor (30) was considered log-normal for both exposures. The activity median aerodynamic diameter (AMAD) of the particles was 3.8μ and 3.4μ for the rat and hamster exposures, respectively. Detailed aerosol data appear in Appendix I.

Necropsy Schedule

Animals were necropsied at intervals up to about 1 year post-exposure in order to observe time-dependent changes in lung tissue composition. Two exposed rats and two exposed hamsters were necropsied at 1 day postexposure in order to estimate alveolar deposition and to observe the very early response to the inhalation process. Necropsies were performed on two exposed animals from each species at 14 and 30 days in order to observe early morphological changes. One control of each species was also necropsied at 14 days to establish a normal baseline for morphometric parameters. After 1 month postexposure, animals were necropsied at intervals of 30-60 days. One control animal and two exposed animals of each species were included in most necropsy

Plate II. Morphology of $^{239}\text{PuO}_2$ particles as collected on an electrostatic precipitator. (Electron Micrograph, 10,800 X)

groups. Rat necropsies were performed at 1, 14, 30, 62, 90, 124, 152, 180, 240, 303, and 349 days postexposure. The schedule was identical for hamsters up to 240 days, with 1 additional group of hamsters at 260 days as the final group.

Necropsy and Tissue Preparation Procedures

Lung Fixation by Instillation

It was important that the degree of inflation of the lungs be carefully controlled in order to achieve comparable results in morphometric work. This was accomplished by instillation of fixative into the trachea while the lungs were still within the chest cavity. A fixative pressure of 20-25 cm above the trachea has been found to be the minimum necessary for full inflation of all parts of the lung, however increasing the pressure above 30-40 cm may result in collapse and distortion of alveolar septa and capillary walls (31). For this reason, the fixative pressure was maintained at 20 cm above the trachea for all instillations. The fixative used in this experiment was a glutaraldehyde-formaldehyde combination reported by McDowell and Trump (32) to yield excellent results for electron microscopy (Appendix II-A).

Prior to instillation the animals were weighed and anesthetized with Halothane. An incision was made from the upper abdominal cavity to the trachea. The lungs were collapsed to allow better penetration of the fixative by making two small incisions in the diaphragm near the rib cage. The trachea was then exposed and severed well above the

bifurcation. Tissue attached to the trachea was secured with a clamp to prevent it from retracting into the chest cavity. During instillation the animals were suspended in vertical position on a lucite stand constructed for this purpose. The clamp attached to the trachea was taped to the stand to keep the trachea extended and open. The apparatus containing the fixative consisted of a 25 ml buret with an 18 gauge needle attached to the tip. Fixative was continuously supplied to the buret through a siphon tube from a 120 ml bottle reservoir. This ensured that the fixative level in the buret was constant during the course of the instillation. The fixative was fed from the buret into the trachea using a #160 polyethylene catheter. The tracheas of the youngest hamsters were too small to allow insertion of the catheter, so a piece of smaller #60 catheter was fitted into the end of the larger one before insertion. The catheter was inserted about 1-1.5 cm into the trachea depending on the size of the animals. The trachea was not secured around the catheter during instillation so that air could escape and the overflow of fixative could be observed when the lungs were completely filled. A total of 5 or 10 ml was allowed to run into the lungs of hamsters or rats respectively, due to the difference in lung volume. At completion of the instillation, the trachea was ligated. The intact lungs and trachea were then removed from the thorax and immersed overnight in fixative prior to preparation of the tissues for microscopic examination and plutonium analysis.

Tissue Sampling

The lung, liver, tracheobronchial lymph nodes, bone and carcass from exposed animals were taken for plutonium analysis. The lung and liver were removed at the time of necropsy, and the remaining body was placed in a large beaker and boiled for 2-3 hours to facilitate separation of the bone from the remainder of the carcass. After the 24 hour fixation period, the five lung lobes and the heart were dissected away from the trachea and other thoracic contents. The remaining thoracic tissue was taken to be the tracheobronchial lymph nodes. All five lung lobes were weighed, following which the left lobe was returned to fixative and reserved for microscopic examination. The remaining 4 right lobes were weighed and analyzed for plutonium content. The total lung plutonium burden was determined from the concentration in the right lobes, assuming that the concentration in all 5 lobes was similar.

For control animals, the body, liver, and lung weights were taken at necropsy, and the entire body except for the lung was analyzed to determine the background level of plutonium in controls. Control lungs were not analyzed for plutonium content.

Plutonium Analysis

The concentration of plutonium in biological materials was determined by the method of Keough and Powers (33). Tissues to be analyzed were placed in pyrex beakers, reduced to ash in a muffle furnace, and dissolved in a boric-nitric acid mixture for counting in a liquid scintillation system. Lung, liver, and lymph nodes were diluted to a

total volume of 60 ml; bone and carcass were diluted to 120 ml. Five ml aliquots of each sample and toluene base scintillation fluid were placed in a 20 ml scintillation vial. The mixture was shaken vigorously to extract plutonium from the aqueous phase into the scintillator phase. Each sample was pipetted in triplicate. One vial was spiked with a known amount of plutonium to serve as an internal standard; the others provided duplicate counts of the sample alone. Samples were counted to 10,000 counts or for 10 minutes. Total sample count rates and tissue plutonium burdens were then calculated from the aliquot count rates and the tissue weights recorded at necropsy.

Preparation of Tissues for Light and Electron Microscopy

The left lobe of the lung was reserved for microscopic analysis, and was assumed to be representative of the total lung. The top third of the lobe was prepared for histopathologic analysis and autoradiography. This tissue was cut into two cross-section slices approximately 5 mm thick, processed in an Autotechnicon, and embedded in paraffin (see Appendix II-B). Sections 5 μ thick were cut from the paraffin block and stained with hematoxylin and eosin for histopathology (Appendix II-C). Autoradiograms were prepared from 5 μ paraffin sections of exposed animals only. Sections were cut and floated on water in individual dishes. These were transferred in a darkroom onto microscope slides coated with emulsion³ to detect alpha particles

³ Kodak NTA Plates, Emulsion Thickness 25 μ .

emitted by ^{239}Pu . The plates were exposed for 2 weeks, developed (Appendix II-D), and stained with hematoxylin and eosin.

In addition to the left lung sections indicated previously, any other lung tissue with an unusual appearance was embedded in paraffin for histopathology. The tissues were examined for lesions indicative of radiation damage. Abnormal conditions were noted, including pneumonitis, metaplasia, hyperplasia, and any indication of neoplastic changes. Body weights and organ weights for lung and liver were also compared.

The remainder of the left lobe was prepared for electron microscopy. Only a very limited quantity of the total lung tissue could be examined with the electron microscope. Therefore, the tissue for observation was chosen by careful systematic and random sampling methods in order to ensure that a representative sample was obtained. The lobe was first sliced in 2-3 mm thick cross-sections from which a systematic sample was chosen for further preparation. From hamsters every other slice was taken, and from rats every third slice was taken so that 3-4 slices per animal were selected. These slices were cut with a sharp razor blade into cubes 1-2 mm on a side ($2-3 \text{ mm}^3$ volume), producing a total of 300-400 cubes per animal. From this pool of cubes 10 were selected at random for embedding. For this purpose, all of the cubes in fixative were placed into a 6" diameter petri dish with a numbered square grid attached to the bottom. Random numbers were chosen from a statistical table (34) and the tissue cubes above corresponding numbers on the grid were chosen until a sample of 10 was obtained. These ten cubes were washed overnight in cacodylate buffer (Appendix II-A), post-fixed in osmium tetroxide (1% solution in cacodylate

buffer), and embedded in Spurr's Resin (Appendix II-B). Five of these 10 cubes were chosen at random for light microscopic analysis, the remaining 5 being reserves in case one of the primary sample should be technically unsuitable. The resin-embedded cubes were sectioned on an ultramicrotome using glass knives. Sections 1μ thick were stained according to the method of Richardson et al (35) for light micrographs (Appendix II-C). Ultrathin sections (90-100 nm) were cut from 3 blocks, placed on 200 mesh copper grids, and stained in uranyl acetate and lead citrate for electron micrographs (Appendix II-E).

Morphometric Analysis

Lung Composition and Structure

Analysis of lung composition and structure was carried out at both the light and electron microscopic levels of magnification. A multi-purpose test system similar to that described by Weibel (36) was used for the majority of the analyses carried out in this part of the study. A description of this test system and the theory behind its application are given in Appendix III (See Plate III).

Light micrographs were used to determine the overall air-tissue distribution in the lungs. The 1μ thick sections of resin-embedded tissue were used for this purpose since they allowed greater resolution than paraffin sections, thus minimizing errors due to section thickness. Light micrographs were prepared from sections of the five resin-embedded blocks chosen for study from each animal. They were recorded

Plate III. Application of test system for morphometric analysis.

- M Alveolar macrophage
 - E Capillary endothelium
 - I Type I alveolar epithelium
 - II Type II alveolar epithelium
 - RBC Capillary erythrocyte
 - I_a Intersection of test line with air-tissue interface
 - I_c Intersection of test line with capillary lining
- (electron micrograph, 5500 X)

on Polaroid film⁴ at a magnification of 220 X using red and neutral-density filters. The field to be photographed was chosen systematically to avoid bias. The 8.5 cm x 10.5 cm negatives were printed on 8" x 10" paper at an enlargement of 2.55 X for a final print magnification of 560 X. Morphometric analysis was performed to determine the relative volumes of major lung components, including major blood vessels, conducting airways (bronchi and bronchioles), respiratory air space (alveoli and ducts), alveolar septa, free cells in alveolar spaces, and pleural surface. Structural parameters analyzed on these micrographs were the surface area/volume ratio and arithmetic mean thickness of septal wall, and the number of alveoli/mm³.

Analysis of the fine structure of alveolar septa required the use of electron microscopy. Electron micrographs were prepared from thin sections of resin-embedded lung on 8.5 cm x 10 cm plate film⁵. Only parenchymal lung was considered in this portion of the study. The fields chosen for recording were taken from a specific corner of the copper grid mesh to avoid bias. Six micrographs were taken on each of the 3 tissue sections, for a total of 18 fields per animal. When a section had greater than 6 potential fields, they were numbered and 6 were chosen at random for recording. The micrographs were taken at an accelerating voltage of 60 kv and at a magnification of 2050 X on the negative. The negatives were printed on 8" x 10" high-contrast paper at enlargements of 2.55 X and 5 X for different phases of the morphometric analysis. The final print magnifications were 5250 X and 10,400 X.

⁴ Polaroid Type 105 Positive/Negative Film.

⁵ Kodak Electron Microscope Film 4489.

A tissue differential was performed on micrographs at the lower magnification using point-counting volumetry. Nuclear profiles of all cellular constituents were also recorded as an index of relative cell numbers. The alveolar tissue components studied at this level were:

- I. Epithelium
 - A. Type I
 - B. Type II
- II. Capillaries
 - A. Endothelium
 - B. Lumen
 - 1. Plasma + erythrocytes
 - 2. Leukocytes
- III. Interstitium
 - A. Cellular Components
 - 1. Fibroblasts
 - 2. Inflammatory Cells
 - 3. Unidentified Cellular Processes
 - B. Noncellular Components
 - 1. Basement Membrane
 - 2. Ground Substance
 - 3. Collagen
 - 4. Elastin
- IV. Free Cells (Macrophages and desquamated epithelium).

Structural parameters included in the electron microscopic analysis were arithmetic mean thickness of alveolar septa and surface area/volume ratio of septa and capillaries. The test system for this portion of the analysis was the same as that used on the light level micrographs.

The higher magnification electron micrographs were used to study the air-blood barrier thickness. Areas containing capillaries in alveolar septa were chosen for enlargement, maximizing the amount of tissue available for analysis. Both arithmetic and harmonic mean thickness were measured, as well as the tissue distribution in the air-blood barrier. Arithmetic mean thickness (AMT) of the air-blood barrier represented the mass of tissue present between alveolar air space and capillary blood. AMT and the tissue distribution were determined simultaneously using the multipurpose test system referred to previously. Harmonic mean thickness (HMT) corresponded to the diffusion resistance of the air-blood barrier. Estimation of HMT required direct measurement of random distances across the air-blood barrier using a different test system (See Plate IV and Appendix III) (25).

Distribution of $^{239}\text{PuO}_2$ in Lung

The autoradiograms of paraffin-embedded sections were used to investigate time-dependent changes in the distribution of plutonium dioxide particles within the lung. Sections were photographed at 4.5 X magnification on 35 mm film⁶ using a green interference filter

⁶ Kodak Tri-X Pan, ASA 400.

Plate IV. Test system for measurement of harmonic mean thickness of the alveolar air-blood barrier.

Top: Application of test system to micrograph.

Bottom: Scale for measurement of air-blood barrier transsections.

(Electron micrograph, 10,300 X)

to improve contrast. These were printed onto 8" x 10" high contrast paper at an enlargement of 7 X, for an overall print magnification of 30 X. Montages of the entire lung cross-sections were prepared and subjected to morphometric analysis.

For this portion of the study, lung tissue was divided into 6 major components as illustrated in Plate V. Subpleural was defined as the area within 1 cm of the pleural surface on montages, corresponding to a distance of 0.33 mm from the pleural surface of fixed lung. Parenchymal consisted of all alveolar septa and respiratory air spaces not designated as subpleural. Bronchial-bronchiolar and vascular included only the lumen of major airways and blood vessels. Peribronchial-bronchiolar and perivascular were defined as the entire wall of an airway or blood vessel including lining cells, connective tissue, and spaces. The fraction of lung volume occupied by each of these components was estimated by point-counting methods as described in Appendix III.

Tracks from plutonium dioxide particles form stars on the autoradiograms. These were identified under the microscope at 63 X or 100 X, and the location of each was marked on the photomontage of the section. The smallest recorded stars contained about 20 tracks, corresponding to a minimum particle diameter of 0.1 - 0.2 μ (23). The PuO₂ particle distribution was obtained by counting the stars and recording the location of each in one of the six tissue components.

These 2 sets of data were used to define an absolute and relative particle concentration for each component. The absolute particle concentration (APC) represented the number of particles per unit area

Plate V. Method for determining $^{239}\text{PuO}_2$ particle distribution.

SP Subpleural

P Parenchymal

PB Peribronchial-Bronchiolar

B Bronchial-Bronchiolar

PV Perivascular

V Vascular

Pu Plutonium dioxide particle

(Autoradiogram, 2 week exposure, 30 X).

on the montages, not normalized for section size or $^{239}\text{PuO}_2$ lung burden, and was used to study the rate of clearance from various components of lung. The relative particle concentration (RPC) in each region was calculated in such a way that a completely uniform distribution would yield a value of 1 for all regions at all times, and be independent of the number of particles counted or size of the tissue section analyzed.

The subpleural and internal regions were divided into sections approximately 2 cm^2 in area, and the number of particles in each was tabulated to study the local distribution of particles for grouping effects. The range of ^{239}Pu alpha particles in lung is such that where 2 or more particles were present in a section, the fields irradiated by the particles had a high probability of overlapping. Subpleural in this case was defined in the same way as previously, and internal was a combination of all other lung regions.

Statistical Analysis

Several different types of statistical analyses were used in this study, depending on the type of data to be compared. The majority of volumetric and structural data involved comparison of 4 group means, consisting of control and treatment groups in each species. For this type of data, either analysis of variance (ANOVA) (34) or Tukey's T method for multiple comparisons (37) were used. Results from the ANOVA were reported as 'control vs. exposed' for each species separately and 'rat vs. hamster' which was a species

comparison with control and exposed groups combined. This latter test was probably only meaningful where the exposed vs. control comparisons within each species showed no difference. The Tukey method was a more versatile but less powerful method of comparing multiple groups where specific comparisons not available from the ANOVA were desired. In this method, the significance level was set at $p \leq 0.05$ for the group of comparisons as a whole, the individual comparisons then having p values smaller than the overall value. In cases where two group means were compared or where one mean was compared to a constant value, a 2-sided t -test was used to determine the level of significance. For those analyses involving discrete grouped data, a chi-square test was used.

Linear regression analysis was performed on some data in order to observe time-dependent trends. The regression coefficients were compared to a constant or to average lung values to establish changes over time or differences between lung regions. Correlation coefficients were used to detect similarity or difference in 2 sets of data showing time-dependent trends.

An observed difference is considered statistically significant at $p \leq 0.05$, and levels of significance down to $p \leq 0.001$ are reported in tables. P values between 0.20 and 0.05 are also indicated and referred to as "potentially" significant, since the same numerical results would be significant at $p \leq 0.05$ in a larger group of animals.

RESULTS

Metabolism of Plutonium Dioxide

The $^{239}\text{PuO}_2$ lung burdens at sacrifice for all animals were fit to an exponential clearance model to estimate initial deposition and radiation dose in lung. Clearance from rat lung was best fit by a 2 compartment model, $Y = 56.9 e^{-0.0805t} + 43.1 e^{-0.00549t}$ ($Y = \%$ of initial alveolar deposition remaining at time t postexposure). According to this model, 57% of the PuO_2 was cleared rapidly with a half-time of 8.6 ± 5.6 days (mean $\pm$ standard deviation) and the remaining 43% more slowly with a half-time of 126 ± 56 days. Clearance data from hamsters were more variable than for rats, and the best fit was a single compartment model, $Y = 100 e^{-0.0115t}$ in which the PuO_2 was cleared with a half-time of 60.5 ± 13.5 days, intermediate between those in the rat model. The initial alveolar deposition (IAD) and dose accumulated to time of sacrifice were estimated by applying these clearance models to the final $^{239}\text{PuO}_2$ lung burdens (1). The mean IAD was 160 ± 71 nCi for rats and 91 ± 94 nCi for hamsters. The accumulated dose in rats averaged 483 ± 400 rads with a maximum of 1628 rads; for hamsters the corresponding doses were 534 ± 702 and 2451 rads. In order to compare the effect of a one vs. two compartment model, the rate at which dose accumulated in the lung over time was calculated for a hypothetical 'average' animal of each species using the group means for initial alveolar deposition and lung weight. The results in Figure 1 showed a very similar dose rate

Figure 1. Accumulation of radiation dose in rodent lung following a single inhalation exposure to $^{239}\text{PuO}_2$.
(calculated on the basis of the lung clearance equation in the text and the equation for conversion to dose in reference 1)

for both species over the period of study, despite the difference in form for the clearance models. Projecting these doses to 620 days for comparison with Figure 2 yields a value of 822 rads for rats and 736 rads for hamsters. According to the figure, the rat group would have been expected to develop about 50% lung tumors (1).

The $^{239}\text{PuO}_2$ burdens in other body tissues were an indication of the extent to which the particles initially in the lung were translocated to other parts of the body. These data are reported as percentage of initial alveolar deposition at time of sacrifice in extrapulmonary tissues of interest; with < 0.6% translocated to liver, < 3.7% to thoracic lymph nodes, < 0.5% to the skeleton, < 2.8% to the remaining carcass, or < 6% to all extrapulmonary tissues combined (Tables I and II).

General Pathology

The results of all pathological analyses appear in Table III. A comparison of lung, liver, and body weights showed no demonstrable effect on treated animals of either species relative to controls. The lesions observed in H&E-stained paraffin sections of lung were principally focal and of 2 types -- inflammatory and proliferative. Inflammatory lesions of varying extent, with or without fibrosis, were found in 2 of 11 control rats, 9 of 24 exposed rats, 0 of 7 control hamsters, and 3 of 20 exposed hamsters. Proliferative lesions were absent in control rats and present in 3 exposed rats, 1 control hamster, and 5 exposed hamsters. In hamsters, these were all

Figure 2. Incidence of lung tumors in rats following a single inhalation exposure to $^{239}\text{PuO}_2$. Radiation doses are calculated at 620 days postexposure.
(Adapted from information in reference 1)

Table I. Clearance and Metabolism of Inhaled $^{239}\text{PuO}_2$ in Rats

Days Post-exposure	Number Animals	Estimated IAD (nCi)	Percent of Initial Alveolar Deposition in Organs ^a					Total Extra-pulmonary
			Lung	Thoracic Lymph Node	Liver	Bone	Remaining Carcass	
1	2	161 $\pm$ 21	95	1.01 $\pm$ 0.47	0.23 $\pm$ 0	1.73 $\pm$ 0.72 ^b	130 $\pm$ 30 ^b	1.24 $\pm$ 0.47
14	2	199 $\pm$ 24	58	2.06 $\pm$ 0.55	0.13 $\pm$ 0	0.15 $\pm$ 0.07	1.57 $\pm$ 0.11	3.91 $\pm$ 0.51
30	2	76 $\pm$ 29	42	2.04 $\pm$ 0.42	0.082 $\pm$ 0.006	0.28 $\pm$ 0.014	0.80 $\pm$ 0.48	3.21 $\pm$ 0.96
62	2	266 $\pm$ 107	31	1.59 $\pm$ 0.26	0.076 $\pm$ 0.006	0.13 $\pm$ 0.054	0.18 $\pm$ 0.049	1.97 $\pm$ 0.35
90	2	126 $\pm$ 11	26	0.97 $\pm$ 0.76	0.089 $\pm$ 0.008	0.20 $\pm$ 0.035	0.24 $\pm$ 0.064	1.48 $\pm$ 0.79
124	2	170 $\pm$ 104	22	0.70 $\pm$ 0.11	0.073 $\pm$ 0.001	0.11 $\pm$ 0.014	0.10 $\pm$ 0.040	0.99 $\pm$ 0.064
152	2 (1) ^c	174 $\pm$ 19	19	1.18 $\pm$ 0.014	0.080 $\pm$ 0.006	0.012 ^c	0.086 $\pm$ 0.035	1.31 $\pm$ 0.11
180	2	194 $\pm$ 97	16	0.92 $\pm$ 0.50	0.066 $\pm$ 0.001	0.12 $\pm$ 0.078	0.094 $\pm$ 0.052	1.20 $\pm$ 0.63
240	2	126 $\pm$ 67	12	1.05 $\pm$ 0.11	0.075 $\pm$ 0.031	0.21 $\pm$ 0.085	0.15 $\pm$ 0.12	1.49 $\pm$ 0.13
303	3	110 $\pm$ 85	8	1.00 $\pm$ 0.49	0.064 $\pm$ 0.015	0.12 $\pm$ 0.069	0.18 $\pm$ 0.15	1.37 $\pm$ 0.69
349	3	178 $\pm$ 64	6	1.08 $\pm$ 0.27	0.041 $\pm$ 0.037	0.31 $\pm$ 0.38	0.10 $\pm$ 0.035	1.54 $\pm$ 0.59

^aAll values are mean $\pm$ standard deviation.

^bValues may be affected by external contamination and are eliminated from total extrapulmonary value.

^cOnly one sample available.

Table II. Clearance and Metabolism of Inhaled $^{239}\text{PuO}_2$ in Hamsters

Days Post-exposure	Number Animals	Estimated IAD (nCi)	Percent of Initial Alveolar Deposition in Organs ^a					Total Extra-pulmonary
			Lung	Thoracic Lymph Node	Liver	Bone	Remaining Carcass	
1	2 (1) ^c	21 $\pm$ 26	99	2.35 $\pm$ 1.15	0.57 $\pm$ 0.057	16.00 ^{b,c}	843 ^{b,c}	2.92 $\pm$ 1.20
14	2	77 $\pm$ 27	85	1.13 $\pm$ 0.93	0.21 $\pm$ 0.064	0.39 $\pm$ 0.078	2.77 $\pm$ 1.07	4.48 $\pm$ 2.14
30	2	45 $\pm$ 25	71	3.69 $\pm$ 2.20	0.22 $\pm$ 0.049	0.17 $\pm$ 0.14	1.05 $\pm$ 1.05	5.12 $\pm$ 0.96
62	2	49 $\pm$ 11	49	0.62 $\pm$ 0.16	0.13 $\pm$ 0.021	0.099 $\pm$ 0.002	0.42 $\pm$ 0.20	1.26 $\pm$ 0.38
90	2	46 $\pm$ 10	36	0.72 $\pm$ 0.19	0.16 $\pm$ 0.035	0.081 $\pm$ 0.028	0.29 $\pm$ 0.014	1.24 $\pm$ 0.24
124	2	96 $\pm$ 124	24	0.41 $\pm$ 0.26	0.030 $\pm$ 0.010	0.14 $\pm$ 0.10	0.56 $\pm$ 0.73	1.13 $\pm$ 0.56
152	2 (1) ^c	78 $\pm$ 94	17	0.78 $\pm$ 0.30	0.13 $\pm$ 0.081	0.11 ^c	0.39 $\pm$ 0.47	1.40 $\pm$ 0.85
180	2	45 $\pm$ 59	13	0.55 $\pm$ 0.21	0.42 $\pm$ 0.49	0.16 $\pm$ 0.18	0.78 $\pm$ 1.00	1.92 $\pm$ 1.89
240	2	245 $\pm$ 33	6	0.57 $\pm$ 0.43	0.049 $\pm$ 0.002	0.045 $\pm$ 0.009	0.054 $\pm$ 0.003	0.71 $\pm$ 0.44
260	2	209 $\pm$ 182	5	0.24 $\pm$ 0.15	0.019 $\pm$ 0.008	0.043 $\pm$ 0.030	0.038 $\pm$ 0.029	0.34 $\pm$ 0.22

^aAll values are mean $\pm$ standard deviation.

^bValues may be affected by external contamination and are eliminated from the total extrapulmonary value.

^cOnly one sample available.

Table III. Pathological Observations in Rats and Hamsters Following Inhalation of $^{239}\text{PuO}_2$

Organ	Weight in Grams ^a			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Lung	3.80 $\pm$ 1.30	4.16 $\pm$ 1.21	2.73 $\pm$ 0.28	2.81 $\pm$ 0.69
Liver	16.06 $\pm$ 2.74	15.57 $\pm$ 2.43	5.84 $\pm$ 1.43	6.18 $\pm$ 0.92
Whole Body	440 $\pm$ 75	423 $\pm$ 75	112 $\pm$ 19	113 $\pm$ 9

Lung Lesions	Number of Animals Affected			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Inflammatory	2	9	0	3
Proliferative	0	3	1	5
Abnormal Type II Cells	0	10	1	7
Vacuolated Erythrocytes	0	0	4	15
Total Number of Animals ^b	11	24	7	20

^a Values are mean $\pm$ standard deviation.

^b These values also apply to corresponding groups in Tables IV-XIV.

adenomatous metaplasias. In rats there was an advanced adenomatous metaplasia, a squamous metaplasia, and a fibroblastic hyperplasia. Statistical comparison of treatment groups revealed no significant differences in the incidence of these lesions for either species.

Two abnormalities were noted in the course of analyzing electron micrographs, one of which appeared to be a treatment effect, and the other, a species characteristic. Type II alveolar epithelial cells which were abnormally large and sometimes bizarre in form were found in 10 exposed rats and 7 exposed hamsters, as compared to 0 in control rats and 1 in control hamsters. This represented a significant treatment effect for rats ($p = 0.011$) but not for hamsters, and probably was related to hyperplasia of type II epithelium which has been demonstrated following lung damage of various types (15, 38-46). These abnormal cells first appeared at about 90 days postexposure. The other abnormality noted in electron micrographs was a distortion and vacuolation of erythrocytes, which was seen in 4 control and 15 exposed hamsters, but was completely absent in rats. This did not represent a treatment effect for hamsters, but the difference was highly significant ($p < 0.001$) when hamsters were compared to rats with treatment groups combined. The source of this abnormality was not determined.

Lung Composition

Major Components

The results of the lung composition analysis at the light microscopic level and corresponding statistical analyses are listed in Tables IV and V. The distribution of major lung components was similar for rats and hamsters and in control and exposed groups. Parenchymal tissue accounted for about 90% of lung volume, of which 60 - 70% was respiratory air space (alveolar sacs and ducts) and the remaining 20 - 30%, alveolar septa in both species. Alveolar macrophages occupied less than 0.5%, and pleural surface less than 0.1%, of the total lung volume. Figures for conducting airways (bronchi and bronchioles) and major vascular regions were much more variable, ranging from about 0.5-3% and 3.5-9% respectively. The volume of conducting airways decreased in exposed rats as compared to controls, due to decreases in epithelial and luminal volumes. The volume of major blood vessels decreased in exposed hamsters while exposed rats showed a smaller fraction of major vascular endothelium relative to control groups. In a comparison of the two species, rats were found to have a greater relative volume of conducting airway and vascular endothelium, and the volume of parenchymal lung was lower in exposed rats than exposed hamsters.

Alveolar Septa

The composition of alveolar septa, analyzed at the electron microscopic level, appears in Table VI with statistical analyses in

Table IV. Volume Distribution of Major Lung Components
in Rats and Hamsters Following Inhalation of
 $^{239}\text{PuO}_2$

Tissue Component	Percent of Lung Volume ^a			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Parenchymal Lung	88 $\pm$ 6	92 $\pm$ 7	90 $\pm$ 10	96 $\pm$ 3
Septal Tissue	28 $\pm$ 8	25 $\pm$ 10	19 $\pm$ 5	25 $\pm$ 11
Respiratory Airway	60 $\pm$ 11	67 $\pm$ 14	71 $\pm$ 7	71 $\pm$ 11
Cells in Air Spaces	0.4 $\pm$ 0.2	0.4 $\pm$ 0.3	0.4 $\pm$ 0.3	0.5 $\pm$ 0.3
Conducting Airway	2.9 $\pm$ 3.4	1.0 $\pm$ 2.0	1.2 $\pm$ 2.0	0.6 $\pm$ 1.6
Wall	1.5 $\pm$ 1.9	0.6 $\pm$ 1.5	0.5 $\pm$ 0.8	0.2 $\pm$ 0.6
Epithelium	0.6 $\pm$ 0.8	0.2 $\pm$ 0.3	0.2 $\pm$ 0.4	0.06 $\pm$ 0.14
Lumen	1.4 $\pm$ 1.8	0.4 $\pm$ 1.0	0.7 $\pm$ 1.3	0.4 $\pm$ 1.0
Major Vascular	8.3 $\pm$ 5.2	6.8 $\pm$ 5.9	9.2 $\pm$ 8.1	3.4 $\pm$ 2.5
Wall	4.4 $\pm$ 2.2	4.1 $\pm$ 4.1	5.3 $\pm$ 7.2	2.0 $\pm$ 1.5
Endothelium	0.7 $\pm$ 0.5	0.4 $\pm$ 0.3	0.6 $\pm$ 0.7	0.3 $\pm$ 0.3
Lumen	3.9 $\pm$ 4.3	2.9 $\pm$ 4.6	3.7 $\pm$ 1.8	1.6 $\pm$ 1.4
Pleural Surface	0	0.004 $\pm$ 0.02	0.01 $\pm$ 0.04	0.1 $\pm$ 0.3
Test Points Counted per Animal	704 $\pm$ 24	670 $\pm$ 51	693 $\pm$ 12	674 $\pm$ 49

^a Values are mean (%) $\pm$ standard deviation.

Table V. Statistical Analysis for Volume Distribution of Major Lung Components in Rats and Hamsters Following Inhalation of $^{239}\text{PuO}_2$

Tissue Component	Probability of Difference in Four Groups	ANOVA			Extended Tukey T Method					
		Control Rat vs. Exposed Rat	Control Hamster vs. Exposed Hamster	Rat vs. Hamster	Control Rat vs. Control Hamster	Control Rat vs. Exposed Rat	Control Hamster vs. Exposed Hamster	Exposed Rat vs. Exposed Hamster	Rat vs. Hamster	Control vs. Exposed
Parenchymal Lung	0.0088	--	--	--	NS	NS	NS	*	NS	NS
Septal Tissue	NS	NS	+	NS	--	--	--	--	--	--
Respiratory Airway	0.11	+	NS	+	--	--	--	--	--	--
Cells in Air Spaces	0.067	NS	NS	NS	--	--	--	--	--	--
Conducting Airway	0.0507	*	NS	**	--	--	--	--	--	--
Wall	0.068	--	--	--	NS	NS	NS	NS	NS	NS
Epithelium	0.023	*	NS	+	--	--	--	--	--	--
Lumen	0.011	*	NS	NS	--	--	--	--	--	--
Major Vascular	0.0265	NS	*	+	--	--	--	--	--	--
Wall	0.11	--	--	--	NS	NS	NS	NS	NS	NS
Endothelium	0.0039	**	NS	**	--	--	--	--	--	--
Lumen	NS	NS	+	NS	--	--	--	--	--	--
Pleural Surface	0.13	NS	+	+	--	--	--	--	--	--
Tests Points Counted	0.16	*	NS	NS	--	--	--	--	--	--

NS Not Significant
 + Significant with $p \leq 0.20$
 * $p \leq 0.05$
 ** $p \leq 0.01$
 -- Not Analyzed

Table VI. Volume Distribution of Alveolar Tissue Components in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

Tissue Component	Percent of Alveolar Tissue ^a			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Epithelium	13.9 $\pm$ 2.1	14.6 $\pm$ 3.0	17.3 $\pm$ 5.8	16.7 $\pm$ 3.2
Type I	10.6 $\pm$ 1.7	10.2 $\pm$ 2.6	13.1 $\pm$ 3.9	11.7 $\pm$ 2.7
Type II	3.3 $\pm$ 2.0	4.4 $\pm$ 1.5	4.1 $\pm$ 2.3	5.0 $\pm$ 1.7
Capillary				
Endothelium	13.0 $\pm$ 2.7	11.8 $\pm$ 2.1	14.9 $\pm$ 2.7	14.9 $\pm$ 2.1
Lumen	42.1 $\pm$ 8.4	37.2 $\pm$ 9.1	38.7 $\pm$ 12.6	35.8 $\pm$ 7.1
Blood Leukocyte	1.6 $\pm$ 0.8	1.6 $\pm$ 0.8	2.3 $\pm$ 1.4	1.8 $\pm$ 1.0
Interstitial	29.3 $\pm$ 6.3	32.9 $\pm$ 8.5	26.2 $\pm$ 8.1	27.9 $\pm$ 8.1
Cellular	10.1 $\pm$ 2.5	12.5 $\pm$ 7.8	12.5 $\pm$ 4.8	14.7 $\pm$ 7.3
Fibroblast	4.4 $\pm$ 1.6	5.8 $\pm$ 4.3	4.7 $\pm$ 2.6	6.1 $\pm$ 5.6
Inflammatory Cells	0.2 $\pm$ 0.3	0.6 $\pm$ 1.0	0.04 $\pm$ 0.08	0.5 $\pm$ 1.1
Not Identified	5.5 $\pm$ 2.1	6.2 $\pm$ 3.0	7.7 $\pm$ 3.3	8.2 $\pm$ 2.0
Noncellular	19.2 $\pm$ 4.2	20.3 $\pm$ 3.6	13.7 $\pm$ 5.1	13.2 $\pm$ 3.4
Basement Membrane	7.2 $\pm$ 1.5	7.7 $\pm$ 1.7	7.4 $\pm$ 2.0	6.8 $\pm$ 1.9
Ground Substance	7.5 $\pm$ 2.7	8.7 $\pm$ 2.5	4.4 $\pm$ 2.9	4.7 $\pm$ 2.1
Collagen	4.2 $\pm$ 1.8	3.6 $\pm$ 1.7	1.7 $\pm$ 0.8	1.6 $\pm$ 1.0
Elastin	0.4 $\pm$ 0.4	0.3 $\pm$ 0.4	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2
Alveolar Macrophage	1.2 $\pm$ 1.3	2.1 $\pm$ 1.8	1.8 $\pm$ 1.6	2.2 $\pm$ 1.6
Test Points Counted per Animal	826 $\pm$ 308	860 $\pm$ 347	641 $\pm$ 75	774 $\pm$ 335

^a All values are mean $\pm$ standard deviation.

Table VII. The distribution by volume of alveolar tissue components showed capillary lumen occupying 36-42% of the alveolar septa in both species; interstitial tissue, 26-33%; epithelium, 14-17%; capillary endothelium, 12-15%; alveolar macrophages, 1-2%; and blood leukocytes, about 2%. All of the differences observed in this part of the study were between species, neither of the treated groups showing a significant change from the controls in either rats or hamsters. Rats contained a lower fraction of epithelium, principally type I, and of capillary endothelium than hamsters. Interstitium occupied a larger fraction of alveolar tissue in rats than in hamsters, due to the larger proportions of 2 noncellular components, ground substance and collagen.

In the distribution of alveolar cell types (Table VIII), capillary endothelium was the most frequently observed, accounting for 35-40% of cells in all groups. Interstitial cellular elements accounted for 25-31%; epithelial cells, 17-21% (approximately evenly divided between type I and II); blood leukocytes, 10-12%; and alveolar macrophages, 2-5%. The fraction of type I epithelial cells was higher in rats than in hamsters (Table IX), whereas in the volume distribution, type I cells occupied a smaller fraction of the total alveolar volume in rats than in hamsters. These findings may indicate that rat lung contained a higher proportion of noncellular elements in general (capillary lumen as well as interstitial elements) thus decreasing the relative volume of all cellular components compared to hamster. An alternative explanation may be that type I epithelial cells in hamsters cover a greater area per cell, and have a higher

Table VII. Statistical Analysis for Volume Distribution of Alveolar Tissue Components in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

Tissue Component	Probability of Difference in Four Groups	ANOVA			Extended Tukey T Method					
		Control vs. Exposed Rat	Control vs. Exposed Hamster	Rat vs. Hamster	Control vs. Exposed Rat	Rat vs. Exposed Hamster	Control vs. Exposed Hamster	Exposed Rat vs. Exposed Hamster	Rat vs. Hamster	Control vs. Exposed
Epithelium	0.040	NS	NS	**	NS	NS	NS	NS	*	NS
Type I	0.055	NS	NS	**	NS	NS	NS	NS	*	NS
Type II	0.089	+	NS	+	NS	NS	NS	NS	NS	NS
Capillary										
Endothelium	0.00016	+	NS	***	NS	NS	NS	**	**	NS
Lumen	NS	+	NS	NS	--	--	--	--	--	--
Blood Leukocyte	NS	NS	NS	+	--	--	--	--	--	--
Interstitial	NS	NS	NS	*	--	--	--	--	--	--
Cellular	NS	NS	NS	+	--	--	--	--	--	--
Fibroblast	NS	NS	NS	NS	--	--	--	--	--	--
Inflammatory Cells	NS	NS	NS	NS	--	--	--	--	--	--
Not Identified	0.020	NS	NS	**	NS	NS	NS	*	NS	NS
Noncellular	0.17×10^{-6}	NS	NS	***	*	NS	NS	**	**	NS
Basement Membrane	NS	NS	NS	+	--	--	--	--	--	--
Ground Substance	0.29×10^{-5}	+	NS	***	NS	NS	NS	**	**	NS
Collagen	0.40×10^{-5}	NS	NS	***	**	NS	NS	**	**	*
Elastin	NS	NS	NS	+	--	--	--	--	--	--
Alveolar Macrophage	NS	+	NS	NS	--	--	--	--	--	--
Test Points per Animal	NS	NS	NS	+	--	--	--	--	--	--

NS Not Significant
 + Significant with $p < 0.20$
 * $p < 0.05$
 ** $p < 0.01$
 *** $p < 0.001$
 -- Not Analyzed

Table VIII. Distribution of Alveolar Cell Types in Rat
and Hamster Lung Following Inhalation of
 $^{239}\text{PuO}_2$

Cell Type	Percent of Alveolar Cells ^a			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Epithelium	20.8 ± 4.8	19.3 ± 5.5	17.3 ± 4.0	17.3 ± 6.8
Type I	11.5 ± 3.7	10.2 ± 4.8	8.9 ± 5.1	6.7 ± 5.1
Type II	9.4 ± 5.9	9.0 ± 4.6	8.4 ± 6.0	10.6 ± 4.7
Capillary				
Endothelium	39.5 ± 6.2	35.3 ± 9.2	39.9 ± 11.5	38.3 ± 10.4
Blood Leukocyte	10.1 ± 6.5	9.7 ± 6.2	12.3 ± 10.7	9.9 ± 5.8
Interstitial	26.9 ± 6.3	30.9 ± 11.2	25.6 ± 9.5	30.3 ± 11.3
Fibroblast	22.7 ± 4.6	25.2 ± 9.3	20.9 ± 9.9	25.8 ± 8.8
Inflammatory Cells	1.9 ± 2.2	3.1 ± 4.9	0.6 ± 1.0	1.6 ± 3.7
Not Identified	2.3 ± 2.5	2.7 ± 2.4	4.1 ± 2.8	2.9 ± 2.2
Alveolar Macrophage	2.1 ± 3.1	3.6 ± 3.5	5.0 ± 3.3	4.1 ± 3.8
Cells Counted per Animal	71 ± 17	62 ± 29	47 ± 8	52 ± 27

^a All values are mean ± standard deviation.

Table IX. Statistical Analysis for Distribution of Alveolar Cell Types in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

Cell Type	Probability of Difference in Four Groups	ANOVA			Extended Tukey T Method					
		Control Rat vs. Exposed Rat	Control Hamster vs. Exposed Hamster	Rat vs. Hamster	Control Rat vs. Control Hamster	Control Rat vs. Exposed Rat	Control Hamster vs. Exposed Hamster	Exposed Rat vs. Exposed Hamster	Rat vs. Hamster	Control vs. Exposed
Epithelium	NS	NS	NS	+	--	--	--	--	--	--
Type I	0.036	NS	NS	**	NS	NS	NS	NS	NS	NS
Type II	NS	NS	NS	NS	--	--	--	--	--	--
Capillary										
Endothelium	NS	NS	NS	NS	--	--	--	--	--	--
Blood Leukocyte	NS	NS	NS	NS	--	--	--	--	--	--
Interstitial	NS	NS	NS	NS	--	--	--	--	--	--
Fibroblast	NS	+	*	NS	--	--	--	--	--	--
Inflammatory	NS	NS	NS	+	--	--	--	--	--	--
Not Identified	NS	NS	NS	NS	--	--	--	--	--	--
Alveolar Macrophage	NS	NS	NS	+	--	--	--	--	--	--
Cells per Animal	NS	NS	NS	*	--	--	--	--	--	--

NS Not Significant
 + Significant with $p \leq 0.20$
 * $p \leq 0.05$
 ** $p \leq 0.01$
 -- Not Analyzed

cytoplasm/nuclear volume ratio. A similar scarcity of epithelial and endothelial nuclear profiles has been reported in other small mammals where the thickness of the air-blood barrier is minimized for greater respiratory efficiency (47). The only treatment effect observed in this portion of the study was an increase in the relative number of fibroblasts for treated hamsters over controls. The corresponding difference in rats was numerically similar but not significant.

The composition of the air-blood barrier differed from the alveolar volume distribution in that it included only tissue in close proximity to alveolar capillaries, and did not include the capillary lumen itself as a component. This represented the relative amounts of various tissue types directly involved in the gas exchange apparatus of the lung. The results in Table X indicated that epithelium and endothelium occupied roughly equal fractions of the barrier volume, ranging from 20-29%. Interstitium occupied the largest share at 44-54%, being roughly equal to the combined volumes of epithelium and endothelium. There were no differences between treatment groups in the air-blood barrier composition (Table XI), however species differences were found in endothelium and interstitial components. Hamsters had a higher percentage of capillary endothelium than rats when treatment groups were combined. Rats had a larger proportion of interstitium than hamsters in both control and exposed groups, due to the greater relative volume of noncellular components.

Table X. Volume Distribution of Air-Blood Barrier in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

Tissue Component	Percent of Air-Blood Barrier ^a			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Epithelium	24 $\pm$ 4	26 $\pm$ 5	27 $\pm$ 5	27 $\pm$ 7
Type I	19 $\pm$ 2	20 $\pm$ 5	21 $\pm$ 3	20 $\pm$ 5
Type II	6 $\pm$ 5	6 $\pm$ 5	6 $\pm$ 4	7 $\pm$ 4
Capillary Endothelium	22 $\pm$ 4	20 $\pm$ 3	29 $\pm$ 6	23 $\pm$ 9
Interstitial	54 $\pm$ 3	54 $\pm$ 5	44 $\pm$ 3	47 $\pm$ 9
Cellular	19 $\pm$ 4	21 $\pm$ 6	20 $\pm$ 4	24 $\pm$ 7
Fibroblast	11 $\pm$ 5	9 $\pm$ 4	6 $\pm$ 5	10 $\pm$ 7
Not Identified	8 $\pm$ 2	11 $\pm$ 4	14 $\pm$ 4	15 $\pm$ 3
Noncellular	35 $\pm$ 5	34 $\pm$ 6	23 $\pm$ 6	23 $\pm$ 4
Basement Membrane	13 $\pm$ 2	12 $\pm$ 3	12 $\pm$ 3	10 $\pm$ 2
Ground Substance	17 $\pm$ 4	17 $\pm$ 4	9 $\pm$ 3	11 $\pm$ 4
Collagen	5 $\pm$ 1	4 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1
Elastin	0.9 $\pm$ 0.8	0.6 $\pm$ 0.7	0.3 $\pm$ 0.3	0.2 $\pm$ 0.1

^a All values are mean $\pm$ standard deviation.

Table XI. Statistical Analysis for Volume Distribution of Air-Blood Barrier in Rats and Hamsters Following Inhalation of $^{239}\text{PuO}_2$

Tissue Component	ANOVA	Extended Tukey T Method					
	Probability of Difference in Four Groups	Control Rat vs. Control Hamster	Control Rat vs. Exposed Rat	Control Hamster vs. Exposed Hamster	Exposed Rat vs. Exposed Hamster	Rat vs. Hamster	Control vs. Exposed
Epithelium	NS	--	--	--	--	--	--
Type I	NS	--	--	--	--	--	--
Type II	NS	--	--	--	--	--	--
Endothelium	0.010	NS	NS	NS	NS	*	NS
Interstitial	0.00011	*	NS	NS	**	**	NS
Cellular	NS	--	--	--	--	--	--
Fibroblast	NS	--	--	--	--	--	--
Not Identified	NS	--	--	--	--	--	--
Noncellular	0.30×10^{-9}	**	NS	NS	**	**	NS
Ground	0.34×10^{-6}	**	NS	NS	**	**	NS
Collagen	0.21×10^{-5}	**	NS	NS	**	**	NS
Elastin	0.032	NS	NS	NS	NS	**	NS
Basement Membrane	0.012	NS	NS	NS	NS	NS	NS

NS Not Significant

* $p < 0.05$

** $p < 0.01$

-- Not Analyzed

Lung Structure

The information about lung structure in Table XII was obtained in all parts of the analysis. At the light level, the number of alveoli/mm³ ranged from 75-80 in all groups except for control rats, in which it was 130. This was greater than the value for exposed rats or control hamsters (Table XIII). The S/V ratio of alveolar spaces ranged from $0.11\mu^{-1}$ to $0.13\mu^{-1}$ in all groups, with no difference due to treatment or species.

The arithmetic mean thickness (AMT) of alveolar septa was analyzed at both the light and electron microscopic levels. Values for AMT were generally about 2 times larger at the light level because of the error introduced by the greater thickness of tissue sections used in light microscopy. The patterns were very similar, however, with AMT ranging from 6.7-7.3 μ for light and 3.5-3.9 μ for EM in all groups except control hamster, which was consistently smaller at 4.9 μ and 2.8 μ respectively. This difference was not significant at either level, however.

The surface/volume ratio (S/V) of alveolar septa decreased in value as the thickness of septa increased, since it is a reciprocal function of AMT for a flat tissue sheet. The EM results were again about a factor of 2 different from the light level values but had a similar pattern. S/V ratios were smaller in the exposed groups than for the corresponding control groups in all cases except for rats in the light level analysis. This was consistent with a thickening of alveolar septa in exposed animals, however none of the differences

Table XII. Lung Structure in Rats and Hamsters Following Inhalation of $^{239}\text{PuO}_2$

	Structural Parameters ^a			
	Rat		Hamster	
	Control	Exposed	Control	Exposed
Alveoli per mm ³	130 $\pm$ 57	80 $\pm$ 27	75 $\pm$ 17	78 $\pm$ 22
Alveoli Surface/Volume Ratio (μ^{-1})	0.13 $\pm$ 0.03	0.11 $\pm$ 0.04	0.11 $\pm$ 0.02	0.11 $\pm$ 0.03
Alveolar Septa Arithmetic Mean Thickness (μ)				
Light	7.3 $\pm$ 1.5	6.9 $\pm$ 2.9	4.9 $\pm$ 0.6	6.7 $\pm$ 5.1
E.M.	3.5 $\pm$ 1.1	3.9 $\pm$ 1.6	2.8 $\pm$ 0.2	3.7 $\pm$ 2.8
Surface/Volume Ratio (μ^{-1})				
Light	0.29 $\pm$ 0.06	0.33 $\pm$ 0.09	0.42 $\pm$ 0.06	0.35 $\pm$ 0.08
E.M.	0.67 $\pm$ 0.48	0.55 $\pm$ 0.15	0.73 $\pm$ 0.05	0.63 $\pm$ 0.15
Capillaries Surface/Volume Ratio (μ^{-1})	1.1 $\pm$ 0.3	1.2 $\pm$ 0.3	1.4 $\pm$ 0.5	1.3 $\pm$ 0.2
Air-Blood Barrier Arithmetic Mean Thickness (μ)	1.11 $\pm$ 0.23	1.14 $\pm$ 0.40	0.89 $\pm$ 0.20	1.07 $\pm$ 0.43
Epithelium	0.26 $\pm$ 0.08	0.27 $\pm$ 0.08	0.23 $\pm$ 0.06	0.26 $\pm$ 0.05
Interstitium	0.59 $\pm$ 0.13	0.63 $\pm$ 0.25	0.39 $\pm$ 0.10	0.52 $\pm$ 0.30
Endothelium	0.25 $\pm$ 0.05	0.24 $\pm$ 0.07	0.27 $\pm$ 0.06	0.28 $\pm$ 0.06
Harmonic Mean Thickness (μ)	0.36 $\pm$ 0.04	0.38 $\pm$ 0.05	0.30 $\pm$ 0.05	0.35 $\pm$ 0.05
AMT/HMT	3.08 $\pm$ 0.50	2.93 $\pm$ 0.71	2.96 $\pm$ 0.51	3.01 $\pm$ 0.90

^a All values are mean $\pm$ standard deviation.

Table XIII. Statistical Analysis for Lung Structure in Rats and Hamsters Following Inhalation of $^{239}\text{PuO}_2$

Structural Parameters	Probability of Difference in Four Groups	ANOVA			Extended Tukey T Method					
		Control vs. Exposed Rat	Rat vs. Hamster	Control Hamster vs. Exposed Hamster	Control Rat vs. Exposed Rat	Control Rat vs. Exposed Hamster	Control Hamster vs. Exposed Rat	Control Hamster vs. Exposed Hamster	Rat vs. Hamster	Control vs. Exposed
Alveoli per mm ³	0.00020	***	NS	*	*	**	NS	NS	*	NS
Alveoli - S/V Ratio	NS	--	--	--	--	--	--	--	--	--
Alveolar Septa										
AMT										
Light	NS	NS	NS	NS	--	--	--	--	--	--
E.M.	NS	--	--	--	--	--	--	--	--	--
S/V Ratio										
Light	0.011	+	+	**	*	NS	NS	NS	**	NS
E.M.	NS	--	--	--	--	--	--	--	--	--
Capillaries - S/V Ratio	NS	--	--	--	--	--	--	--	--	--
Air-Blood Barrier										
AMT	NS	--	--	--	--	--	--	--	--	--
Epithelium	NS	--	--	--	--	--	--	--	--	--
Interstitialium	NS	--	--	--	--	--	--	--	--	--
Endothelium	NS	--	--	--	--	--	--	--	--	--
HMT	0.0031	--	--	--	NS	NS	NS	NS	**	NS
AMT/HMT	NS	--	--	--	--	--	--	--	--	--

NS Not Significant
 + Significant with $p < 0.20$
 * $p < 0.05$
 ** $p < 0.01$
 *** $p < 0.001$
 -- Not Analyzed

were significant. The control hamster S/V ratios, $0.42\mu^{-1}$ for light and $0.73\mu^{-1}$ for EM, were greater than the corresponding values in control rats at $0.29\mu^{-1}$ and $0.67\mu^{-1}$, which was a significant difference at the light level. The S/V ratios for exposed hamsters, $0.35\mu^{-1}$ at the light level and $0.63\mu^{-1}$ for EM, were also larger than for exposed rats, $0.33\mu^{-1}$ and $0.55\mu^{-1}$ respectively, but not significantly so at either level. The S/V ratio of alveolar septa in the light microscopic analysis was smaller for rats than for hamsters when treatment groups were combined.

The surface/volume ratio in capillaries was determined only from electron micrographs, as capillaries were not easily visible with light microscopy. The S/V ratios for rats, at $1.1\mu^{-1}$ and $1.2\mu^{-1}$ for control and exposed groups, were smaller than the corresponding results in hamsters at $1.4\mu^{-1}$ and $1.3\mu^{-1}$, but no differences could be demonstrated between treatment groups or species.

The air-blood barrier thickness measured from electron micrographs followed the same pattern when measured either as arithmetic mean thickness (AMT) or harmonic mean thickness (HMT). The total air-blood barrier thickness, due to the thickness of interstitial components, tended to be greater in rats than in hamsters, and in exposed animals as compared to the corresponding controls. The epithelial and endothelial layers were relatively constant for all groups. Total AMT ranged from 0.89μ to 1.14μ in all groups, with interstitium accounting for roughly half of the thickness at 0.39 - 0.63μ and the remainder about evenly divided between epithelium at 0.23 - 0.27μ and endothelium at 0.24 - 0.28μ . This corresponded well to the air-blood

barrier composition data, in which the relative volumes of each component were nearly identical in proportion. However, volumetric analysis appeared to be the more sensitive measure of composition, revealing a number of species differences, whereas none were found in the AMT data. The only demonstrable difference in air-blood barrier thickness was for HMT, rat being greater than hamster when the treatment groups were combined. HMT for rats was 0.36μ in control and 0.38μ in exposed animals, and the corresponding values for hamsters were 0.30μ and 0.35μ .

Distribution of $^{239}\text{PuO}_2$

The distribution of $^{239}\text{PuO}_2$ in 6 major components of lung tissue was analyzed in order to detect changes in distribution over time and differences between rats and hamsters. The raw data in this part of the study consisted of simple counts of the number of PuO_2 particles associated with each of the tissue components and the number of points from the morphometric test system falling on each component. These data were analyzed in several different ways to describe the partitioning of particles among different lung regions.

Absolute Particle Concentration

A measure of number of particles per unit volume, or absolute particle concentration (APC), was defined as: number of particles associated with each of the 6 tissue components/number of morphometric test points counted on that component. An estimate of the

fraction of particles retained in each component at long vs. short times was obtained by the ratio: APC at > 120 days postexposure/APC at < 90 days postexposure. This value, expressed as percent and labeled retention in Table XIV, was compared statistically to 100% (i.e., no change) in order to detect differences in particle concentration over time. With 2 exceptions, all components in both species showed retention values lower than 100%. Total retention (T) was 32% and 33% and in rats and hamsters respectively; subpleural (SP), 47% and 48%; parenchymal (P), 23% and 26%; and bronchial-bronchiolar (B), 14% and 0%. No particles were found in vascular lumen at any time. Two components in rats showed a retention greater than 100%, indicating a potential increase in particles/unit area. The value for perivascular (PV) was 406% and peribronchial-bronchiolar (PB) was 228%, whereas in hamsters, PV was 0 and PB was 16%. Neither of the rat values was statistically different from 100%, but the hamster PB value was less than 100%. Species differences were found in 2 components, where the retention in both PB and B for rats was greater than in hamsters.

Linear regression analysis of APC values provided information about initial distribution of particles and changes in concentration over time. The best-fit curves are illustrated graphically in Figure 3, and the results in numerical form with statistical analyses appear in Table XV, part A. In fitting the APC data vs. time to an equation of the form $Y = \beta_0 + \beta_1 X$, the initial APC was represented by β_0 in the table and by the Y intercept in the figures. The tendency

Table XIV. Retention of Inhaled $^{239}\text{PuO}_2$ Particles in Rat and Hamster Lung

Lung Region	Retention (%) ^a		Statistical Analysis ^b		
	Rat	Hamster	Rat vs. 100%	Hamster vs. 100%	Rat vs. Hamster
Subpleural	47 $\pm$ 55	48 $\pm$ 66	**	**	NS
Parenchymal	23 $\pm$ 34	26 $\pm$ 40	***	***	NS
Bronchial-Bronchiolar	14 $\pm$ 26	0	***	--	*
Peribronchial-Bronchiolar	228 $\pm$ 434	16 $\pm$ 45	+	***	*
Perivascular	406 $\pm$ 1122	0	+	--	+
Total Lung	32 $\pm$ 43	33 $\pm$ 48	***	***	NS

^a Retention = $\frac{\text{Particle Concentration at } > 120 \text{ days}}{\text{Particle Concentration at } < 90 \text{ days}} \times 100$

Values are mean $\pm$ standard deviation.

^b Statistical Analysis

NS Not Significant

+ Retention in rat or hamster is different from 100%, or rat is different from hamster with $p \leq 0.20$.

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

-- Not Analyzed

Figure 3. Absolute particle concentration of $^{239}\text{PuO}_2$ in rodent lung.
Linear regression analysis.

Table XV. Linear Regression Analysis for Absolute Particle Concentration in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

A. Linear Regression Coefficients for APC as a Function of Time^a

Lung Region	Rats ^b		Hamsters ^b	
	$\hat{\beta}_0 \pm S_{\beta_0}$	$\hat{\beta}_1 \pm S_{\beta_1}$	$\hat{\beta}_0 \pm S_{\beta_0}$	$\hat{\beta}_1 \pm S_{\beta_1}$
Subpleural (SP)	0.56 $\pm$ 0.089	-0.0014 $\pm$ 0.00045**	0.20 $\pm$ 0.047	-0.00065 $\pm$ 0.00031*
Parenchymal (P)	0.53 $\pm$ 0.086	-0.0016 $\pm$ 0.00044**	0.27 $\pm$ 0.075	-0.00099 $\pm$ 0.00049+
Bronchial-Bronchiolar (B)	0.069 $\pm$ 0.031***	0.000061 $\pm$ 0.00016	0.10 $\pm$ 0.042*	-0.00046 $\pm$ 0.00027
Peribronchial (PB)	0.17 $\pm$ 0.19	-0.00019 $\pm$ 0.00098	0.031 $\pm$ 0.013***	-0.00014 $\pm$ 0.000082+
Perivascular (PV)	0.14 $\pm$ 0.058**	-0.00037 $\pm$ 0.00030	0.066 $\pm$ 0.028**	-0.00030 $\pm$ 0.00018
Total	0.47 $\pm$ 0.079	-0.0012 $\pm$ 0.00040**	0.26 $\pm$ 0.057	-0.00096 $\pm$ 0.00040*

B. Correlations Between Components (for APC)

Correlation Between	Correlation Coefficient ^b	
	Rats	Hamsters
SP, P	0.91***	0.92***
SP, B	0.31	0.22
SP, PB	0.51**	0.44*
SP, PV	0.59**	0.14
P, B	0.17	0.15
P, PB	0.51**	0.30
P, PV	0.54**	0.097
B, PB	0.57**	0.099
B, PV	-0.046	0.88***
PB, PV	-0.053	0.032

^a Values are mean $\pm$ standard error of the mean.

^b Statistical Analysis

+ β_0 in each region is different from the total lung coefficient; β_1 is different from 0; or the correlation coefficient is different from 0 with $p \leq 0.10$.

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

of APC to change over time was illustrated by the slopes of the regression lines in the figures, which correspond to β_1 in the table.

The pattern for initial APC (β_0) was similar in both species, with SP and P having higher concentrations than B, PB, and PV. The β_0 values for individual regions were compared to the initial APC for total lung. In rats, the β_0 values for SP, P, and PB were not different from the total lung value, whereas β_0 was smaller for B and PV. In hamsters, β_0 was less than the total lung value for all components except SP and P, which showed no difference. When β_0 was compared between species, initial APC was greater in rats than in hamsters for total lung ($p < 0.05$), SP ($p < 0.01$), and P ($p < 0.05$); PV, PB and B showed no species difference.

With the exception of B in rats, values for β_1 were negative, indicating a possible decrease in APC over time. The rate of decrease was apparently greater for the SP and P components than for B, PB and PV in both species. The β_1 values were compared statistically to 0 in order to determine whether the observed values represented a significant rate of change. APC decreased in SP and P for rats and in SP for hamsters, whereas none of the other components were different from 0. No difference was found when the values of β_1 were compared between rats and hamsters.

Correlation coefficients (Table XV, part B) were used to detect similarities or differences in trends for particle concentration between components. All of the correlations in APC were positive except those between B/PV and PB/PV in rats, neither of which were highly correlated. Those components showing high positive correlations

for APC in both species were SP/P and SP/PB. There were also high positive correlations between SP/PV, P/PB, P/PV and B/PB in rats and between B/PV in hamsters.

Analysis of absolute particle concentration showed that the initial distribution of $^{239}\text{PuO}_2$ particles was not uniform in all lung components, the concentration in SP and P being greater than in B, PB, and PV. With a few exceptions, the trends in APC over time indicated a decrease for all components, consistent with overall clearance of PuO_2 from the lung. The rate of decrease was greater in SP and P, which began with higher APC values, than in the other three components where APC was initially low. The patterns of particle deposition and clearance were similar in rats and hamsters, the exception being a higher initial APC in the SP and P components, and a greater tendency to retain particles associated with major airways in rats than in hamsters.

Relative Particle Concentration

A further refinement of the particle distribution data led to definition of a quantity called relative particle concentration (RPC): fraction of particles associated with each component/fraction of lung volume occupied by that component. When the particle concentration was normalized in this way, a completely uniform particle distribution would yield a value of 1 for all components, and would be independent of the ^{239}Pu lung burden or the size of tissue section analyzed. As such, values greater or less than 1 indicated the relative partitioning of particles between components, and provided a measure of dose in

each according to the fraction of particles contained therein. RPC was subjected to linear regression analysis in the same way as APC, and the results appear in Table XVI, part A and Figure 4. The initial RPC values (β_0) for SP and P were generally higher than for B, PB and PV, except in hamsters where the B value was similar to that for P. The β_0 values were compared to unity, the hypothetical value for uniform distribution. In rats, SP and P had identical initial values at 1.17, but only that for P was different from 1 due to the smaller variability in the data. The values for B at 0.25, PB at -0.15, and PV at 0.09 were all less than 1 in rats. For hamsters, the SP and P values were not as uniform, at 0.85 and 1.06 respectively, but neither differed from 1. The values for B at 1.08 and PB at 0.50 were likewise not different from 1, but PV at 0.19 was less than 1. Comparison of β_0 values between rats and hamsters revealed no species differences.

When trends in RPC over time were examined, the β_1 values were compared to 0, or a state of no change. In rats, β_1 was positive for PB and potentially so for SP, indicating an increase in RPC over time. This confirmed the results of the retention analysis, which also showed an apparent increase in particle concentration for the PB region. β_1 was negative for P, and the values for B and PV were not different from 0. In hamsters, β_1 indicated a potential decrease in RPC for P, with none of the other components showing a definite temporal trend. A species comparison revealed no difference in β_1 between rats and hamsters for any component. The only correlation between components (Table XVI, part B) was negative, between SP/P in rats.

Table XVI. Linear Regression Analysis for Relative Particle Concentration in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

A. Linear Regression Coefficients for RPC as a Function of Time^a

Lung Region	Rats ^b		Hamsters ^b	
	$\hat{\beta}_0 \pm S_{\beta_0}$	$\hat{\beta}_1 \pm S_{\beta_1}$	$\hat{\beta}_0 \pm S_{\beta_0}$	$\hat{\beta}_1 \pm S_{\beta_1}$
Subpleural (SP)	1.17 $\pm$ 0.14	0.0014 $\pm$ 0.00071 [†]	0.85 $\pm$ 0.21	0.0014 $\pm$ 0.0014
Parenchymal (P)	1.17 $\pm$ 0.066*	-0.00099 $\pm$ 0.00034**	1.06 $\pm$ 0.15	-0.0020 $\pm$ 0.0010 [†]
Bronchial-Bronchiolar (B)	0.25 $\pm$ 0.14***	-0.000079 $\pm$ 0.00069	1.08 $\pm$ 0.73	-0.0043 $\pm$ 0.0047
Peribronchial (PB)	-0.15 $\pm$ 0.47*	0.00050 $\pm$ 0.0024*	0.50 $\pm$ 0.30	-0.0012 $\pm$ 0.0020
Perivascular (PV)	0.090 $\pm$ 0.23**	0.0015 $\pm$ 0.0012	0.19 $\pm$ 0.13***	-0.00078 $\pm$ 0.00085

B. Correlations Between Components (for RPC)

Correlation Between	Correlation Coefficient ^b	
	Rats	Hamsters
SP, P	-0.85***	0.37 [†]
SP, B	0.055	0.15
SP, PB	-0.15	0.051
SP, PV	-0.021	0.059
P, B	-0.24	-0.079
P, PB	-0.21	0.14
P, PV	-0.12	0.10
B, PB	-0.0023	0.096
B, PV	-0.17	-0.059
PB, PV	0.063	-0.081

^a Values are mean $\pm$ standard error of the mean.

^b Statistical Analysis

[†] β_0 is different from unity; β_1 is different from zero; or the correlation coefficient from 0 with $p \leq 0.10$.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Figure 4. Relative particle concentration of $^{239}\text{PuO}_2$ in rodent lung.
Linear regression analysis.

Analysis of RPC data showed that the $^{239}\text{PuO}_2$ particle concentration was shifting from parenchymal lung to the subpleural and, to a lesser extent, the PB and PV regions. This effect was less pronounced in hamsters than in rats, where it was statistically demonstrable. As with APC, RPC values tended to fall into 2 groups, with the SP and P regions containing a larger share of particles than B, PB and PV at most times. This effect was more pronounced in rats and tended to diminish over time for both species as particles were redistributed within the lung components. Although most of the observed changes appeared to be more dramatic in rats than in hamsters, a direct comparison of the two species did not reveal any differences for initial particle distribution (β_0) or change in concentration over time (β_1).

Spatial Association of Particles

The local distribution of particles in the subpleural and internal regions, as determined by counting the number of particles in 2 cm^2 sections, was analyzed in ways which emphasized different biological aspects of the problem. In Figure 5, the distribution was expressed as percent of particles in groups of various sizes. This represented the fraction of dose to subpleural or internal lung delivered by isolated PuO_2 particles or by groups where the radiation fields from 2 or more particles overlap.

In the subpleural region for rats (Figure 5, part A), 31% of the particles appeared as singles, 63% in groups of 2-10, and 6% in groups of >10; for hamsters, the values were 52% singles and 48% in

Figure 5. Group size distribution of $^{239}\text{PuO}_2$ particles in rodent lung.

- A. Subpleural
- B. Internal

groups of 2-10, with no groups of >10 observed. In a species comparison, rats had a lower fraction of single particles, and greater fraction of groups >10 than hamsters. Species differences for individual group sizes are indicated on the figure, and there was no difference for the 2-10 particle categories combined.

The particle distribution for the internal region (Figure 5, part B) was highly correlated with that in the subpleural region, with correlation coefficients of 0.991 for rats and 0.983 for hamsters. The species difference was not as pronounced for the internal region as in the subpleural, however. In rats, the internal particle distribution was 39% singles, 59% in groups of 2-10 and 3% >10; for hamsters, the corresponding distribution was 48%, 49% and 4%. There were no species differences for any group category in the internal region.

In Figure 6 the results were expressed as percentage of 2 cm^2 sections containing a given number of particles, which corresponded to the fraction of the subpleural or internal region exposed to radiation at various dose levels. In rats, about 52% of the subpleural sections contained no particles; 17% contained 1 particle; 31% between 2 and 10 particles; and 0.5%, greater than 10 particles (Figure 6, part A). In hamsters, the corresponding values were 70%, 18%, 13% and 0%. A comparison between rats and hamsters showed that hamsters had a higher percentage of empty sections than rats. There was no difference in the fraction of one-particle sections, and rats had a higher proportion of sections in the 2-10 ($p < 0.01$) and >10 particle categories than hamsters. Differences for individual group sizes are noted in the figure.

Figure 6. $^{239}\text{PuO}_2$ particle distribution in 2 cm^2 sections of rodent lung.

- A. Subpleural
- B. Internal

The subpleural and internal particle distributions were again highly correlated, with correlation coefficients of 0.992 for rats and 0.995 for hamsters. The internal distribution for rats (Figure 6, part B) was 63% of sections containing no particles, 17% with one particle, 20% with 2-10 particles, and 0.4% with >10 particles. In hamsters, the corresponding distribution was 76%, 12%, 12% and 0.05%. Rats had a higher percentage of sections than hamsters in the 2 particle category. The mean number of particles in subpleural sections which were not empty was also greater in rats, at 2.33 ± 1.06 particles per section, than in hamsters, at 1.73 ± 0.68 ($p < 0.05$). The values for the internal region, 1.90 ± 0.91 in rats and 1.72 ± 0.63 in hamsters, were not different.

Both the subpleural and internal distributions of $^{239}\text{PuO}_2$ were compared to the Poisson model of spatial distribution in order to test for a particle grouping effect. This model represents the distribution of events that occur independently and at a constant rate. In the case of inhalation exposure, the model was applicable if particles were deposited individually, rather than as aggregates, and at a constant rate over the exposure period. These assumptions were considered to be valid for the exposure conditions in this experiment.

Particle distributions for individual animals were compared to the model using a chi-square test, and the subpleural particle distribution in rats as a group was found to be different from the model ($p < 0.001$), whereas that in hamsters was not different. In comparing the observed and expected values for each particle group

size in rats, it was found that there were consistently too few sections with 0 or 1 particle and too many sections containing more than 2 particles. Thus, the subpleural particle distribution in rats differed from the Poisson model due to the presence of many large groups of particles. This comparison to a theoretical model confirmed the difference found by direct rat vs. hamster comparison. The particles in rat lung showed a greater tendency to aggregate into groups of 2 or more, and the very large groups of >10 particles found in subpleural rat lung were absent in hamsters.

Comparison of the internal particle distribution to the Poisson model yielded results very similar to those for the subpleural region. Rats again differed from a Poisson distribution due to an excess of group sizes greater than 2 particles ($p < 0.001$), and hamsters showed no difference.

Linear regression analysis of the Poisson parameter estimates over time yielded additional information about the particle distribution. The Poisson parameter λ is estimated by $\hat{\lambda}$, the fraction of sections with 1 or more particles. The regression equations for rats were

$$\hat{\lambda} = 0.751 - 0.00173 t$$

in the subpleural region, and

$$\hat{\lambda} = 0.638 - 0.00173 t$$

for the internal region. The corresponding equations for hamsters were

$$\hat{\lambda} = 0.429 - 0.00108 t$$

and

$$\hat{\lambda} = 0.340 - 0.00121 t.$$

For both the subpleural and internal regions, the intercepts showed species differences and were different from 0. The intercept represented the initial fraction of sections with 1 or more particles. The slope parameters were all less than 0, which indicated that the number of sections containing particles decreased over time. This was expected because of overall clearance of $^{239}\text{PuO}_2$ from the lung. A species comparison of slopes showed no differences for either the subpleural or internal region. The differences in particle distribution between rats and hamsters were therefore constant over time. The median values of $\hat{\lambda}$ also showed species differences in both the subpleural ($p = 0.018$) and internal ($p = 0.018$) regions.

DISCUSSION

A study of lung structure and $^{239}\text{PuO}_2$ particle distribution in rats and hamsters was undertaken to determine whether these factors may account for the apparent species difference in susceptibility to lung tumor induction by inhaled radionuclides. Analysis of lung composition and structure using morphometric methods revealed some

subtle differences between species, and a small number of changes following exposure, but none of these could be directly related to the observed species difference in tumor response.

In the distribution of major components, lung was found to be roughly 90% parenchyma and 10% major airways and blood vessels. The relative volume of major airways and blood vessels did show some differences due to treatment or species, whereas the volume of parenchymal lung was similar in all groups. These results corresponded well to other published data for rodents (48,49) as well as for human beings (50,51), although the relative fractions of parenchymal airspace and septal tissue were different in man and rodents. The relative volume of septal tissue was only 6% in human lung as compared to 20-30% in rodents, indicating that the alveoli in human lung are larger in size than those in rodents. This was confirmed by calculation of the number of alveoli/mm³ in this study and from the total number of alveoli in human lung (50). The number of alveoli/mm³ in parenchyma of adult human lung was lower by a factor of 2-4 than the rodent values in this study, whereas the values for immature human and rodent lungs were comparable. Alveolar size could affect clearance and mobility of foreign material in the lung, and should be taken into account in extrapolation of data between species. The difference in alveolar size between rats and hamsters in this experiment may have affected ²³⁹PuO₂ particle distribution.

A more detailed analysis of alveolar septa using electron micrographs again revealed some species differences, but there were few demonstrable effects due to treatment. Capillaries occupied

between $1/3$ and $1/2$ of the septal volume in all groups, interstitial components accounted for another $1/3$, and the remainder was taken up by epithelium and capillary endothelium in roughly equal amounts. Rat alveolar septa contained a larger fraction of noncellular interstitial components and a smaller fraction of epithelium and endothelium than hamsters. Comparable data on the distribution of alveolar tissue components in the literature was neither extensive nor consistent, although some data from both rats and human beings was in good agreement with the results of this study (48, 51-53).

Structural analysis of lung revealed that the dimensions of such features as alveolar septa, capillaries, and the air-blood barrier were slightly greater in rats than in hamsters. This is in agreement with the results of another species comparison, in which it was demonstrated that some structural parameters bore a direct relationship to body weight, including lung volume, alveolar surface area, and harmonic and arithmetic mean thickness of the air-blood barrier (47).

The actual reported values for arithmetic and harmonic mean thickness of the air-blood barrier (27,31,47-49,51,54) were usually somewhat greater than those obtained in this study. However, they were in the same general range, and the ratio AMT/HMT corresponded well to the value of 3 reported for most mammals (47). The reason for this discrepancy in values was probably due to the sampling of fields for analysis. The values in other studies appear to have been calculated from data on total alveolar tissue, rather than on fields weighted toward regions containing capillaries as was done in this study.

No changes due to treatment were demonstrated for any structural parameters, although changes in all components of alveolar septa have been shown to occur following external and internal irradiation. Among the observed changes are vacuolation and eventual disintegration of both capillary endothelium and type I alveolar epithelium accompanied by hyperplasia of type II alveolar epithelium, inflammation, edema, and fibrosis in the interstitial region (15,45,46,52,53,55-58). These types of damage have previously been detected using morphometric methods in the lungs of rodents damaged by oxidant gases (48,49,54,59) and radiation (52,53), therefore morphometric techniques should have been sufficiently sensitive to detect such changes in this study, had they been present. Inflammation, fibrosis, and proliferative changes did occur following inhalation of $^{239}\text{PuO}_2$, but these changes were focal in nature and of such limited extent that they did not affect the overall composition or structure of lung at the doses administered. A similar lack of change in lung tissue composition was found following inhaled $^{238}\text{PuO}_2$ in hamsters receiving lung burdens comparable to those of the hamsters in the present investigation (60).

An attempt was made to quantitate the distribution of inhaled $^{239}\text{PuO}_2$ in different regions of the lung, and to determine whether there were differences in the spatial association of particles within these regions. Quantitative analysis of $^{239}\text{PuO}_2$ distribution within the lung confirmed earlier qualitative observations that particles were not uniformly distributed throughout the various lung regions.

Linear regression analysis of absolute and relative concentration of $^{239}\text{PuO}_2$ particles on autoradiograms demonstrated that the initial partitioning of particles into the various lung regions was very similar for rats and hamsters, although the number of particles per unit volume in the parenchymal and subpleural regions was smaller for hamsters. In general, the concentration of particles associated with major airways and blood vessels was lower than that in parenchymal lung. A similar pattern was found for ^{210}Po in saline administered intratracheally to hamsters (61), where the alveoli and small bronchioles contained the largest share of particles in comparison to larger airways at both long and short times postexposure. Both intratracheal ^{210}Po and inhaled $^{238}\text{PuO}_2$ (62) in hamsters showed a transient increase in association of material with large airways during the early clearance phases, however. This pattern may also have been reflected in the RPC values for hamsters, where the concentrations in and around major airways initially were comparable to those in parenchymal and subpleural lung and then show a rapid decrease to very low values at long times postexposure. In contrast, the relative particle concentration in and around major airways for rats was initially lower than in hamsters and was maintained at a relatively constant level over a much longer time. Although this effect was not demonstrable by direct species comparison in linear regression analysis of APC or RPC, there was a species difference in retention of $^{239}\text{PuO}_2$ for major airway wall and lumen at long vs. short times. The apparent relative concentration of $^{239}\text{PuO}_2$ in the subpleural region was not demonstrated directly either by linear

regression analysis or retention values, although indirect evidence was afforded by the negative correlation for RPC in the subpleural and parenchymal regions of rats. Concentration of particles near the edges of the lung and in foci of damaged tissue was also found in a study of hamster lung following inhalation of $^{238}\text{PuO}_2$ (60).

The local distribution of particles in the subpleural and internal regions was also different for rats and hamsters, although the overall partitioning of particles into these components was very similar for both species. The particles showed a greater tendency to accumulate in large groups for rats than for hamsters, as demonstrated in both a direct species comparison and in the comparison of particle distribution to a theoretical model. The tendency for particles which were initially distributed in a random fashion to become grouped appears to depend on the dose level and on time elapsed postexposure for hamsters following inhalation of $^{238}\text{PuO}_2$. Particles were randomly grouped at low doses and at relatively early times after exposure, whereas those animals with large lung burdens showed a tendency for particles to become grouped at times greater than 135 days postexposure (60,63). Analysis of distances between particles revealed that the grouping resulted from differential clearance rather than from active accumulation of particles in regions of relative concentration (60). Although a grouping effect was not found for hamsters in this study, the retention values showed net clearance from all regions of the lung, which was consistent with the mechanism of particle concentration by differential clearance as opposed to active accumulation. The picture for rats was not as

clear, with both the peribronchial and perivascular regions showing an apparent increase in particle concentration. Although this increase was not statistically significant, it indicated that there were regions in the rat lung where little or no clearance had taken place.

CONCLUSIONS

Morphometric analysis of rat and hamster lung did not demonstrate any extensive changes in lung composition or structure following inhalation exposure to $^{239}\text{PuO}_2$ at levels near that for maximum tumor yield in rats. The problem of dosimetry for this compound thus appears to be relatively uncomplicated by any major radiation-induced pathological alterations in the lung. Rat and hamster lung were found to be similar in structure and composition, with few significant differences which could be directly related to the different tumor responses. The potential target cells for tumor formation occupied a small fraction of total lung volume in all groups, regardless of treatment or species. Bronchiolar epithelium accounted for less than 1%; alveolar epithelium, less than 5%; and capillary endothelium, less than 4% of the lung volume.

The distribution of $^{239}\text{PuO}_2$ particles was not uniform in all regions of the lung; thus estimation of the dose to specific tissues or regions within the lung requires a correction for this effect. Species differences were found for particle distribution in the sub-pleural region and major airways, and in the spatial association of

particles, both of which may affect the tumor development process.

These regions contain the principal target cells for tumor production and serve as foci for the origin of tumors. Different dose distributions within these regions may therefore explain, at least in part, the difference in tumor response to inhaled $^{239}\text{PuO}_2$ for rats and hamsters.

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Appendix I
Aerosol Data

	RATS	HAMSTERS
Aerosol Flow Rate	0.687 l/min	0.706 l/min
Amount of Suspension Used	6.1 ml	6.1 ml
Duration of Exposure	45 min	30 min

Particle Size Data

AMAD ^a	3.75 μm	3.40 μm
GSD ^b	2.5	2.4
MMD ^c	1.1 μm	1.0 μm
CMD ^d	0.1 μm	0.1 μm
* ΣX^2	3.8	4.2

a. AMAD - Activity Median Aerodynamic Diameter

b. GSD - Geometric Standard Deviation

c. MMD - Mass Median Diameter

d. CMD - Count Median Diameter

* The critical value for ΣX^2 at $p=0.10$ is 9.236. The particle size distribution may be considered log-normal at this level of significance if $\Sigma X^2 < 9.236$.

Appendix II-A

Fixative and Buffer Solutions

McDowell-Trump Fixative (modified) (32)

Per Liter

11.6 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
2.7g NaOH
40 g Paraformaldehyde
20 ml Glutaraldehyde (50%)

Add sodium phosphate, NaOH, and paraformaldehyde to 980 ml distilled water and heat until the paraformaldehyde dissolves. Add glutaraldehyde, filter, and adjust the pH to 7.2-7.4.

Cacodylate Buffer

Per Liter

42.8g Na Cacodylate $(\text{CH}_3)_2\text{AsO}_2\text{Na}$

Bring to volume with distilled water, titrate with 1N HCl to pH 7.2-7.4, and add 1 g/liter CaCl_2 to neutralize absorbed CO_2 from air.

Appendix II-B

Tissue Embedding Procedures

Embedding in Paraffin for Light Microscopy

Autotechnicon Schedule:

formalin	1/2 hour
70% ethanol	1/2 hour
70% ethanol	1/2 hour
80% ethanol	1 hour
95% ethanol	1 hour
95% ethanol	1 hour
100% ethanol	1 hour
100% ethanol	1 hour
Xylene	1 hour
Xylene	1 hour
melted paraffin	2 hours
paraffin	2 hours

Tissues remain in hot paraffin until they are embedded in molds.

Preparation and Embedding in Resin for Light and Electron Microscopy

1. Small tissue blocks were placed in cacodylate buffer overnight, changing the solution one time in this period. This removes traces of phosphate present in the fixative which react with osmium tetroxide during the post-fixation step to produce an undesirable granularity in the tissues.

2. Tissue cubes were post-fixed in a 1% solution of osmium tetroxide in cacodylate buffer for 1-1/2 to 2 hours.
3. Dehydration, infiltration, and embedding procedure:
 - 50% ethanol a quick rinse to remove OsO_4
 - 70% ethanol 5-10 min
 - 95% ethanol 5-10 min
 - 100% ethanol 5-10 min
 - 100% ethanol 5-10 min
 - 100% ethanol 5-10 min
 - propylene oxide 15 min
 - propylene oxide 15 min
 - 1:1 propylene oxide/Spurr's Resin* 1 hour
 - 1:3 propylene oxide/Spurr's Resin 1 hour

Tissues are placed in the above solutions on a rotating wheel to facilitate mixing. Place tissue in pure resin overnight, under partial vacuum in a refrigerator. The next day, tissues are placed in molds with fresh resin and cured at 70°C for a minimum of 24 hours.

* Spurr's Resin is available commercially from:

Polysciences, Inc.
Paul Valley Industrial Park
Warrington, PA 18976

Appendix II-C

Stains for Light Microscopy

Hematoxylin and Eosin

Hematoxylin (DeLafield) was purchased in ready-to-use form from the supplier*

Eosin-Phloxine B - Working Solution

1% Eosin (stock)	100 ml
1% Phloxine B (stock)	10 ml
95% ethanol	780 ml
Glacial acetic acid	4-5 ml

1% Eosin (stock)

Eosin Y, water soluble	1 g
distilled water	100 ml

1% Phloxine B (stock)

Phloxine B	1 g
distilled water	100 ml

H & E staining procedure for paraffin sections on regular microscope slides or NTA plates: For regular slides, deparaffinize in 3 changes of xylene, 5 min. in each change. NTA plates receive this treatment at the beginning of the developing procedure.

Hematoxylin	6-8 min.
running water	until the water runs clear

*Carolina Biological Supply Co., Gladstone, OR

very dilute ammonia water (a few drops of NH_4OH solution in a stain dish)	dip several times
running water	3-5 min
Eosin (working solution)	dip 10 times
running water	rinse to clear
100% ethanol	5 min

Dry thoroughly in air or a 35°C oven before coverslipping.

Richardson's Stain (35)

Solution A

Azure II	1 g
distilled water	100 ml

Solution B

Methylene Blue	1 g
Sodium Borate	1 g
distilled water	100 ml

Mix solutions A and B 1:1 just before use. Dry sections to the slide on a hot plate, cover with stain and allow to sit on the hot plate until the pool of stain begins to dry around the edges. Rinse in tap water, dry and mount.

Appendix II-D

Autoradiogram Developing (NTA Plates)

Working in a darkroom, place exposed plates in a staining tray for developing.

Developing Schedule:

Xylene	5 min
Xylene	5 min
Xylene	5 min
100% ethanol	3 min
95% ethanol	3 min
70% ethanol	3 min
running water	2 min
D-19 developer	5 min
water rinse	1 min
Rapid Fix	10 min total in 3 separate dishes
running water	30 min
Photo-Flo	dip for 30 sec, then allow to dry.

Appendix II-E

Stains for Ultrathin Sections in Electron Microscopy

Uranyl Acetate

2% solution in distilled water at pH 5.0

or 2% solution in maleate buffer at pH 5.4

Maleate Buffer (per liter)

5.8 g Na maleate

2.4 g NaOH

dilute to volume with distilled water

Lead Citrate

Pb nitrate	1.33 g
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Na citrate	1.76 g
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boiled distilled water	30 ml
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Shake the above together for 1 min, then allow to stand 30 min with intermittent shaking.

Add 8 ml of 1N NaOH (carbonate free)

Dilute this suspension to 50 ml with distilled water and mix by inverting. The solution should clear and have a final pH around 12. Any turbidity which appears may be removed by filtering or centrifugation.

Appendix III

Application of Morphometric Techniques

Morphometry or stereology is a method by which quantitative information about an object can be obtained from a small representative sample of that object by application of a suitable test system to the chosen sample. It has been employed in the fields of geology and metallurgy for a number of years and has been adapted for use in biological materials as well (26). In the present study, the composition and structure of lung were investigated by the application of appropriate test systems in the form of transparent overlays to photographs of lung tissue sections. These tissue sections were selected by careful systematic and random sampling techniques and were assumed to be two-dimensional representations of the lung as a whole. By careful analysis of these sections, it is possible to obtain information about the structure of lung in its normal three-dimensional state. This discussion will be confined to the methods by which data was obtained and the formulas which were used to calculate each parameter from the raw data. An extensive explanation of the theory by which the formulas were derived will not be attempted here as this has been done elsewhere (26-28).

Relative Volume. Perhaps the most straightforward application of morphometric technique is its use in determining the relative volumes of different components within a larger composite structure. The relative volume of each component in the whole object is directly proportional to the area occupied by the component on a 2-dimensional

section of the object (26). This can be carried a step further by making use of the fact that the area of the component is proportional to the number of uniformly spaced points falling on the component in this flat representation. This results in a relatively simple and efficient method for obtaining relative volumes. The 2-dimensional sample is covered by a test system consisting of uniformly spaced points. A hexagonal arrangement of points on the test system gives a more uniform distribution than a quadratic grid, but either system may be used. The number of points falling on the component to be studied is counted and compared to the total number of points on the whole sample. The fractional volume occupied by that component is then: number of points falling on the component/total number of points falling on the object sample. This method was used to calculate the various fractional volumes occupied by the different lung components listed in the text at the light and electron microscopic levels as well as in the $^{239}\text{PuO}_2$ particle distribution.

Surface Area and Volume Relationships. Information about the surface area of a structure may be obtained by applying a test system of straight lines to a random representation of the structure. In a 2-dimensional representation, a flat surface appears as a line or contour, the length of which is related to the surface area of the sheet. When a test system of straight lines is applied to this section, the number of times the straight lines intersect the surface contour will be proportional to the length of the contour. This principle can be combined with relative volume determination to estimate the surface area/volume ratio of an object. The test

system consists of a series of short lines of length z , the 2 ends of which are used for point counting. By counting the number of points falling on an object (P) and the number of test line intersections (I) with the surface of the object, one can calculate the surface area/volume ratio:

$$S/V = \frac{4}{z} \frac{I}{P} \quad (25-28).$$

The surface area/volume ratios of alveoli, alveolar septa, and capillaries were calculated using this principle.

Thickness of a Tissue Sheet. The principle of S/V ratio can also be used to estimate the arithmetic mean thickness $\bar{\tau}$ of a broad sheet, for which $S/V = 2/\bar{\tau}$. Therefore: $\bar{\tau} = \frac{z}{2} \frac{P}{I}$. $\bar{\tau}$ represents the average mass of tissue present in the sheet, however if the sheet thickness varies, it may not provide any useful information about processes which involve diffusion of material across the sheet. For this purpose, the harmonic mean thickness τ_h may be calculated, as it is related to the diffusion resistance of a barrier (26). The test system for determining τ_h is different from those described previously. Estimation of τ_h involves direct measurement of random dimensions of a barrier using a logarithmic scale. The random lengths to be measured are selected by overlaying the sections with a square grid of lines and measuring the barrier at those points where the lines cross it (25). In this case the barrier thickness was divided into ten classes represented by a midpoint L . If f_j represents the frequency with which a thickness in each class j was

observed, then:

$$\frac{1}{\tau_h} = \frac{\frac{3}{2} \left(\frac{10}{\sum_{j=1}^{10} f_j} \cdot \frac{1}{L_j} \right)}{\left(\frac{10}{\sum_{j=1}^{10} f_j} \right)}$$

τ_h was calculated for the air-blood barrier and $\bar{\tau}$ for the air-blood barrier and for whole alveolar septa.

Number of Structures. It is possible to estimate the number of discrete objects in a unit volume by counting the number of trans-sections of such objects (N) in a known area (A) on a two-dimensional section. This formula requires some knowledge of the shape and size distribution of the objects to be counted. β is the correction for shape and relates the average cross-section area of an object to its volume. The size distribution parameter K was assumed to be negligible and taken as 1 (28). The number of structures per unit area is:

$$\# \text{ structures} = \frac{K}{\beta} \cdot \frac{(N/A)^{3/2}}{V}$$

where V is the fractional volume of the structure as determined by point-counting methods described previously. In calculating the number of alveoli / mm³ using this relationship, β was estimated as 1.55. The test area for this analysis was the total area of the multipurpose test system used for volumetric and structural analysis.

Test Systems. The multipurpose test system used in these analyses was a hexagonal array of points 18 mm apart and joined at alternating intervals by short line probes 18 mm long as illustrated in Plate III. The test system included 140 points and 70 lines with a total line length of 1260 mm. The total area of the test system was approximately 180 mm x 220 mm. Volumetric analysis in the PuO_2 particle distribution was performed using a hexagonal array of points 8 mm apart. The test system for determining τ_h was a 1" square grid of lines. The midpoints of the classes for measurement of the barrier were:

<u>Class #</u>	<u>Midpoint</u>
1	0.5 mm
2	1.5
3	2.5
4	3.5
5	5.0
6	8.0
7	12.5
8	20.0
9	32.5
10	50.0 (see Plate IV)

All dimensions must be divided by the appropriate magnification for use in the formulas.

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