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PLUTONIUM INHALATION STUDIES

(A series of lectures given in Japan in 1969
at the invitation of the Japanese
Atomic Energy Commission)

W. J. Bair

February 1970

AEC RESEARCH & DEVELOPMENT REPORT

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UC-48, Biology
and Medicine

PLUTONIUM INHALATION STUDIES

(A series of lectures given in Japan in 1969 at
the invitation of the Japanese Atomic Energy Commission)

by

W. J. Bair

Biology Department
Environmental and Life Sciences Division

February 1970

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PLUTONIUM INHALATION STUDIES

FOREWORD

These notes were prepared for the presentation of a series of lectures at the National Institute of Radiological Sciences, Chiba, Japan, February 15, 1969 to March 3, 1969, at the invitation of the Japan Atomic Energy Commission. The lectures reviewed the most significant aspects of the research on inhaled plutonium performed at the Pacific Northwest Laboratory during the period 1956 to 1969, and are derived primarily from published reports in documents and the open literature. The research was accomplished by the extensive efforts of numerous scientists and technologists working cooperatively. Major contributors include:

Bruce O. Stuart, Ph.D.
James F. Park, D.V.M.
Charles L. Sanders, Ph.D.
William J. Clarke, D.V.M., Ph.D.
Edwin B. Howard, D.V.M., Ph.D.
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James V. Dilley, Ph.D.
Elias G. Tombropoulos, Ph.D.
M. Gilbert Brown

Those contributing specific technologies include:

Drs. J. M. Thomas and R. J. Olson - statistics
P. J. Dionne - simulation model
R. W. Perkins and P. O. Jackson - Pu-Am analyses
Dr. L. J. Kirby, J. P. Herring, R. F. Keough,
G. J. Powers, Dr. Beatrice J. McClanahan, and
A. C. Case - radiochemistry
Dr. W. C. Roesch, K. L. Swinth, C. R. Watson -
in vivo counting
Dr. Patricia L. Hackett and Glenda S. Vogt -
hematology
Dr. L. A. George and R. R. Adey - electron microscopy

Visiting scientists who contributed to these studies include:

Dr. J. E. West, Capt., USAF (VC)

Dr. J. L. Murray, Capt., USAF (VC)

Dr. H. E. Casey, Capt., USAF (VC)

Dr. N. Matsusaka, National Institute of Radiological
Sciences, Japan

Of the numerous technicians who made important contributions to the study, the following deserve special recognition for their long association and major involvement with the program:

M. D. Snyder

W. Skinner

K. E. McDonald

T. C. Kinnas

J. C. Gaven

P. E. Bergam

L. R. Richardson

For those interested in further details of these studies, a complete bibliography is included.

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PLUTONIUM INHALATION STUDIES

LECTURE 1TECHNOLOGY USED IN PLUTONIUM INHALATION STUDIES*HISTORY OF PROGRAM

Our plutonium inhalation program began in about 1955. In beginning the program, we had not yet developed techniques for exposing animals to radioactive aerosols, so we used intra-tracheal injections as a method for introducing the plutonium into the lungs of mice, rats, and dogs. However, in these first experiments, it quickly became apparent that intra-tracheal injection was a poor substitute for inhalation of aerosols, because the particulate material was highly concentrated in two or three lobes of the lung instead of being uniformly dispersed. Therefore, we began development of techniques to expose animals to radioactive aerosols under controlled conditions.

In our inhalation program, we have generally followed the practice of doing preliminary type experiments on mice, rats, and more recently, hamsters. To facilitate extrapolation of the results of animal studies to man, we usually follow the rodent experiments with dog experiments. For this purpose we have established our own beagle dog colony so that we would have a reliable source of healthy, nearly parasite-free dogs for experiments.

In establishing our inhalation program, we made the decision that our point of interest would begin when the aerosol was deposited in the body. While knowledge of the quantitative aspects of deposition of inhaled aerosols is of considerable interest and importance, rather elaborate technology is required to make the necessary measurements. Further, because the deposition of particulate materials is highly variable depending upon factors such as the anatomy and size of the

* *Principal Investigators: J. F. Park, B. O. Stuart,
D. H. Willard, W. J. Bair*

respiratory tract, respiratory rate and volume, etc., it is difficult to extrapolate the results from animal studies to the human, except in a qualitative way. Because of this decision we were able to initiate aerosol exposures much earlier than if we had first tried to develop methods to measure deposition. As a result, our long-term dog experiments have been in progress more than 10 years.

The objectives of our inhalation program are:

- Provide guidance for establishment of permissible limits by identifying and defining hazards of inhaled radionuclides.
- Develop therapeutic methods for removal of inhaled radionuclides.

These objectives are being met by experimentally determining the disposition of various forms of inhaled material in the body and the acute and chronic biological effects which may occur.

A number of fission products have been studied. These are identified in Table 1.1.

TABLE 1.1. *Completed Inhalation Studies in Mice, Rats, and Dogs*

<u>Radionuclide</u>	<u>Retention and Distribution</u>	<u>Biological Effects (Acute and Long Term)</u>
^{131}I	I_2 , AgI, Stable I_2	
^{90}Sr	SrSO_4 , SrO , SrF_2 , SrHPO_4 SrTiO_3 , SrCO_3	SrSO_4
^{106}Ru	RuO_2	RuO_2
^{144}Ce	CeO_2	CeO_2
^{147}Pm	Pm_2O_3 , $\text{Pm}(\text{ClO}_4)_3$	Pm_2O_3
^{237}Np	Np dust	

Other: Removal of $^{144}\text{CeO}_2$ with DTPA

Effects of Cigarette Smoking on Lung Clearance

Using mice, rats, and dogs, we have studied retention, distribution, and biological effects of inhaled ^{131}I , ^{90}Sr , ^{106}Ru , ^{144}Ce , ^{147}Pm , and ^{237}Np . Most of these studies have been phased out and current emphasis is in the four following areas:

- Plutonium and curium.
- Uranium.
- Lung biochemistry and cytology.
- Removal therapy.

The most extensive program involves plutonium. Studies of curium are just getting started. Another part of the program is concerned with problems associated with the uranium mining and milling industry. The long-term effects of combined exposure to uranium ore dust, radon daughters, diesel engine exhaust fumes, and tobacco smoke are being studied in hamsters and dogs. To understand some of the processes fundamental to the disposition and biological effects of inhaled particles, the biochemistry of the lung, particularly in regard to the biosynthesis of surfactant, is being investigated. Also, cellular reactions to inhaled particles are being examined quantitatively and morphologically using light and electron microscopy. Because we have firsthand knowledge of the toxicity of inhaled plutonium and other radionuclides, we are extremely interested in developing therapeutic procedures to remove them from the body.

The extent of our plutonium studies is shown in Table 1.2. These will be dealt with in more detail in subsequent lectures. The disposition of inhaled plutonium has been compared for several compounds and for several particle size aerosols. Acute and chronic effects of inhaled plutonium have been defined in clinical studies. A significant response has been the development of lung tumors. Lymphopenia and immunosuppression are other effects which have been of interest. These will be discussed in a later lecture.

TABLE 1.2. *Plutonium Inhalation Studies*

1. Retention, Distribution and Excretion	2. Biological Effects (Acute and Long Term)
PuO_2 , $\text{Pu}(\text{NO}_3)_4$, PuF_4	$^{239}\text{PuO}_2$ vs. $^{238}\text{PuO}_2$
Particle Size Effects	$^{239}\text{Pu}(\text{NO}_3)_4$
Biliary Excretion	Lymphopenia
^{239}Pu vs. ^{241}Am	Immunology
Pu Microspheres	Reticuloendothelial System
3. <u>Removal Therapy</u>	

INHALATION EXPOSURE LABORATORY

To introduce you to our plutonium studies, something should be said about the laboratory in which the animal exposures are accomplished. We have not required an elaborate facility, although it would have been helpful and we are looking forward to our new laboratory. Our requirements have been reasonably satisfied by a laboratory area which is only partially isolated from the other work area. The ventilation of the exposure laboratory is designed so that the air pressure in the exposure laboratory is negative with respect to the other space in the building. The exhaust air from the room goes through an absolute filter. The exhaust from aerosol chambers in the glove boxes in which the aerosols are generated is filtered at the chamber, at the glove box, and again when it leaves the laboratory. However, the lowest air pressure is maintained in the aerosol chamber to minimize direct contamination of the room air. The laboratory is equipped with two air sampling systems. One provides a direct measurement of alpha radiation levels. The second is a high volume filter sampler. The filters are removed for counting in proportional counters.

A fresh air breathing system supplies air to masks worn by personnel when aerosols are being generated or when room air contamination is known or suspected. Radiation monitoring personnel are always available when radioactive aerosols are being handled. The air monitoring system and the fresh air breathing system are equipped with alarms in the event of an emergency condition. Floor contamination occurs occasionally, but heavy tar paper is usually placed on the floor before experiments are started so that clean up is relatively easy.

AEROSOL EXPOSURE CHAMBERS AND GLOVE BOXES

To study inhaled plutonium in experimental animals, it is desirable to cause the deposition of plutonium in a manner closely simulating that which would occur in a human exposure to contaminated air. Also to minimize radiological hazards to the researcher and avoid numerous other problems, it is necessary to prevent external contamination of the experimental animal. This requires that the aerosol be introduced to just the nasal and oral regions of the animal. This is done by using suitable containers to hold rodents and masks for dogs.

Chamber and Glove Boxes for Rodents

Figure 1.1 shows a series of aerosol chambers used for mice exposed daily to ^{90}Sr . The external contamination of these animals was acceptable because the aerosol was very low level and could be cleaned up. However, because plutonium presents more difficult decontamination problems than ^{90}Sr , such a chamber could not be used for plutonium inhalation experiments.

Instead, it was necessary to confine the aerosol to an inner chamber, through ports in the wall of which the rodents were allowed to inhale the aerosol. An example is shown in Figure 1.2. Mice were individually placed head first into

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FIGURE 1.1. Aerosol Exposure Chambers for Mice

Neg 15789-1
FIGURE 1.2. Aerosol Exposure Chamber and Glove Box for Nose Only Exposure of Mice

50 ml conical centrifuge tubes with the bottom 1/2 in. cut off to give a 3/8-in. opening. The tubes containing the mice were inserted into ports drilled into the wall of a vertical aerosol chamber constructed of a 16-in. length of 8-in. (OD) lucite tubing. The exposure chamber could hold as many as 84 mouse tubes, in seven rows of 12 mice each row. The aerosol was admitted at the top and exhausted at the bottom through an electrostatic precipitator and two sets of membrane filters to collect plutonium and prevent contamination of the room air. In such a chamber, the aerosol concentration varied 25% from top to bottom of the chamber.

Figure 1.3 shows a chamber and glove box used for rats. The principle is the same as for the mice except that it is slightly scaled up. Containers to hold rats were fabricated from Coca Cola bottles, the bottoms of which were removed with a glass saw. The rats were inserted through the bottoms of the bottles and held in place with large rubber stoppers. The bottles were then inserted into ports drilled into the wall of the aerosol chamber. Exposing mice and rats to radioactive aerosols in this manner restricted the radioactive contamination mainly to the head region. Following exposure to the aerosol, the heads of the rats were washed with water and detergent.

Another glove box and chamber used for exposure of rats to plutonium and other radionuclides are shown in Figure 1.4. This is a relatively inexpensive glove box and is used for routine rat exposures. When they become heavily contaminated and there is difficulty in confining the contamination to the inside of the glove box, the boxes are sealed in a large wooden box and buried.

Figure 1.5 shows a more elaborate version of a glove box for exposing rats to radionuclides. The aerosol chamber is

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FIGURE 1.3. *Aerosol Exposure Chamber and Glove Box for Nose
Only Exposure of Rats*

Neg 29715-10

FIGURE 1.4. Aerosol Exposure Chamber and Glove Box for Rats

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FIGURE 1.5. *Glove Box Containing Aerosol Exposure Chamber and Cage Storage Space for Rats*

shown in the center. Ports into which the bottled rats are placed are closed with rubber stoppers when exposures are not in progress. This glove box provided space for storage of waste. A dust generator is shown at the top of the chamber. Everything taken into or out of the box passes through an air lock. Electrostatic precipitators used to decontaminate the exhaust from the chamber are also shown.

Our work with ^{238}Pu posed serious potential contamination problems. We were interested in the acute lethal dose range. This meant that we would be giving each rat 50 to 100 μCi . In addition we had learned that ^{238}Pu seemed to be more mobile than ^{239}Pu in that we occasionally found ^{238}Pu on the floor of the laboratory. This was seldom our experience with ^{239}Pu . The explanation for this is probably the fact that for a given mass, ^{238}Pu is about 280 times more radioactive than ^{239}Pu . Because of this, ^{238}Pu is more readily detected than an equal mass of ^{239}Pu . This glove box was designed to house the rats as well as contain the aerosol exposure chamber (Figure 1.6). The glove box is divided into areas. The compartment containing the aerosol chamber is at one end. Another compartment above the chamber houses the aerosol generator. After the rats are exposed to the plutonium aerosol, the bottles containing the rats are dropped through the chute into the housing compartment. The bottles are dropped into the waste bag and the rats are put into cages.

We have been interested in using hamsters in our pulmonary studies. Rats and, to lesser extent, mice are prone to respiratory tract infection, whereas hamsters seem to be free of such problems. Hamsters have been successfully used at other sites for whole body type of aerosol exposures. However, we have had difficulty in confining them to soft drink bottles or other type containers. The hamsters are capable of reversing themselves in most types of such confinement, and can suffocate

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FIGURE 1.6. Multi-Compartment Glove Box Containing Aerosol Exposure Chamber, Cage Storage Area, Radioactive Waste Storage, and Aerosol Generators for High Level Radionuclide Exposures of Rats

in the bottles. Therefore, we have been trying to develop a hamster chamber for nose only type of exposure. Hamsters are being used in a study of inhaled radon, radon daughters, and uranium dust. The chamber being used in this project may interest you (Figure 1.7). It is comprised of two lucite hemispheres. A turntable floor is located at the equator. Cages containing hamsters are loaded into the chamber through the port and placed on the turntable. Excreta is collected on absorbent paper placed on a shelf below the cages. Aerosol is introduced at the top and exhausted at the bottom. The chamber can be washed out through a port in the bottom. Figure 1.8 shows the cage used. It is constructed of stainless steel. Each hamster is in a separate compartment.

Aerosol Chambers and Glove Boxes for Dogs

A number of different aerosol chambers and glove boxes for exposing dogs have evolved. In Figure 1.9, a large dust chamber is shown suspended in one compartment of the glove box. The dog, placed in the compartment to the right, inhaled the aerosol through a mask attached to the bottom of the tank. A plastic bag inside the aerosol chamber collapsed as the dog inhaled the aerosol. Dry dust can be fired into this chamber with an air pistol. There are also other ways of introducing dust into such a chamber. The technician is shown wearing a fresh-air breathing mask. The recorder is used to monitor temperature and relative humidity within the chamber. The relative humidity of the chamber seldom exceeds 40% even when aerosols are generated from aqueous solutions or suspensions.

A similar glove box is shown in Figure 1.10. However, a different aerosol chamber was used. It consisted of a large octagon with conical ends. The aerosol generator is shown at the top of the chamber. The instrumentation shown measured pressures and air flows within the system.

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FIGURE 1.7. Spherical Aerosol Chamber for Daily Exposure of Hamsters to Constituents of Uranium Mine Environment

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FIGURE 1.8. *Hamster Cages for Spherical Aerosol Chamber*

Neg 17649-3

FIGURE 1.9. Glove Box and Aerosol Chamber for Exposure of Dogs (Plutonium dioxide dust was fixed into the aerosol chamber with an air pistol.)

Neg 17649-4

FIGURE 1.10. *Glove Box and Aerosol Chamber for Exposure of Dogs (Plutonium dioxide aerosols were produced from an aqueous suspension and delivered to the conical top of the octagon chamber.)*

In those experiments where daily exposures of dogs were required, it was necessary to devise a system by which more than one dog could be exposed at one time. The glove box and chamber shown in Figure 1.11 accommodates five dogs simultaneously. The dogs are trained to sit in the boxes. The masks through which they inhale the aerosol are rigidly attached to the aerosol chamber, in this case a 3-ft diameter hexagon.

The most recent design for an aerosol exposure system for dogs is shown in Figure 1.12. The aerosol chamber is contained in the large compartment of the glove box. The dog sits in another compartment with his nose in a mask. The mask is fabricated from liquid latex and plaster. It is attached to a three-way respiratory valve which is controlled by electronic circuits housed in the boxes above the dog. The floor upon which the dog sits can be altered by an electrically operated jack to keep the dog comfortable.

The aerosol generator is located in the compartment above the aerosol chamber. Air flows and pressure in the equipment are controlled at the console. Three 1-in. membrane filter samplers operate simultaneously, at about $100 \text{ cm}^3/\text{min}$. Thermal precipitators are used to sample the air exhaled by the dog as well as the air within the aerosol chamber for particle size measurements by electron microscopy. Air is exhausted from the aerosol chamber through an electrostatic precipitator and at least two membrane filters, at a rate of about 6 l/min. This is a slight excess of the rate of air introduced through the aerosol-generating system to ensure that the pressure in the chamber is negative with respect to the glove box. Figure 1.13 shows a close-up of the respiratory valve which is attached to the aerosol chamber and the mask of the dog. The respiratory valve was a linear valve operated by a linear motor, Figure 1.14.

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FIGURE 1.11. *Glove Box and Aerosol Chamber for Simultaneous Exposure of Five Dogs*

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FIGURE 1.12. Glove Box and Aerosol Chamber for Exposure of Dogs
(Exposure of the dog occurs through a three-way respiratory valve. Respiratory rate and volume and exhaled air are measured.)

Neg 38997-6

FIGURE 1.13. *Three-Way Respiratory Valve for Exposure of Dogs to Radio-active Aerosols*

Neg 38997-5

FIGURE 1.14. Three-Way Respiratory Valve Showing Linear Motor

In this system, the exhaled air was measured and also filtered to remove the radionuclide for analyses. This provided data to estimate deposition, although as mentioned earlier this has not been a major objective of our studies.

An example of a 3-way respiratory valve is shown in Figure 1.15. This is a rotary type designed in our laboratory. At the top of the valve is a motor which actuates the valve. The motor is controlled electronically by a system which monitors the respiration of the dog. Figure 1.16 shows the valve disassembled. Although this is one of the best we have used, we have not been satisfied with the technique to measure respiratory parameters during exposure. A new system is under development to monitor respiratory excursions during the exposure of dogs to radioactive aerosols.

PLUTONIUM AEROSOLS

Preparation of Plutonium

Plutonium used in our studies was prepared to our specifications in some cases and in others it was from a production run without further manipulation. Oxides were prepared by calcining the oxalate at temperatures from 300 to about 1000 °C. Two oxides were prepared by oxidation of the delta form of plutonium metal. Sedimentation techniques were used on aqueous suspensions of oxide particles to narrow the particle size distribution of the aerosols generated from the suspensions. X-ray diffraction analyses of PuO_2 preparations confirmed the crystalline state.

Ultrafilterability Tests

PuO_2 suspensions were tested for the presence of ionized plutonium to assure that the results obtained in the animal studies were correctly attributed to PuO_2 particles. In early studies, PuO_2 suspensions were filtered through a fine-grade

Neg 38997-2

FIGURE 1.15. *Rotary Type Three-Way Respiratory Valve for Exposure of Dogs to Radioactive Aerosols*

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FIGURE 1.16. Rotary Type Three-Way Respiratory Valve for Exposure of Dogs
to Radioactive Aerosols - Disassembled

membrane filter having an average pore size of $0.3\text{ }\mu\text{m}$. The filter was expected to retain all particles larger than $0.005\text{ }\mu\text{m}$ with at least 95% efficiency.

More recently, the method of Lindenbaum and Westfall has been used to test the ultrafilterability of plutonium particle suspensions. By this method the plutonium suspension in a dialysis bag is centrifuged at $1000 \times$ gravity for 40 minutes. The plutonium in the ultrafiltrate passing through the dialysis membrane having an average pore size of $24\text{ }\text{\AA}$ is measured.

We routinely test the integrity of the membrane with a hemoglobin solution. The hemoglobin molecule, about $70\text{ }\text{\AA}$ by $40\text{ }\text{\AA}$, should not pass the dialysis membrane with a pore size of $24\text{ }\text{\AA}$. Hemoglobin in the ultrafiltrate is taken as evidence for a disrupted membrane. In our tests hemoglobin was added to the plutonium suspensions in the dialysis tubing before centrifugation. Hemoglobin was found in the ultrafiltrate from ^{238}Pu suspensions with a higher frequency than from ^{239}Pu suspensions. Also, in some cases we found that the fraction of plutonium in the ultrafiltrate increased with the amount of plutonium placed in the dialysis tubing. Some of these results are shown in Table 1.3. The amount of plutonium in the ultrafiltrate varied by a factor of 10 depending upon the amount of plutonium placed in the dialysis tubing. In a number of these there was evidence of hemoglobin. Therefore, we suspect that plutonium may in some cases alter the pore size of the dialysis membrane.

Generation of Aerosols

Plutonium aerosols have been produced from dry powder or from suspensions and solutions. Because the mass of material is small, conventional aerosol-generating systems such as dust mills cannot be used. Therefore, new or modified methods were required.

TABLE 1.3. *Ultrafilterability of Plutonium Solutions and Suspensions*

Compound	Concentration, $\mu\text{Ci/ml}$	Particle Size CMD, μm	Percent Filtered
$^{239}\text{Pu}(\text{NO}_3)_4$	400	---	10
$^{239}\text{PuO}_2$	40	0.05	1.5
	3	0.05	0.15
$^{239}\text{PuF}_4$	40	~ 0.2	0.2 - 1
	20	~ 0.2	0.2 - 0.7
$^{238}\text{PuO}_2$ (Plasma Torch)	500	0.04	2
	100	0.04	0.5
$^{238}\text{PuO}_2$ (300°)	1500	0.06	1 - 2.5
	400	0.06	1 - 2
$^{238}\text{PuO}_2$ (750°)	500	0.04	1

Dispersion of Solutions and Suspensions

A modified Laterbach-type generator was used for dispersion of solutions and suspensions. The aerosol generator was constructed from 1-in. OD plastic pipe or from an 8-in. long, 1-in. plastic rod with a 3/4-in. hole drilled from one end to within 1/2 in. of the other end (Figure 1.17). A side arm was attached near the open end to lead the aerosol into the aerosol chamber. A length of 1/4-in. stainless steel tubing sealed at the bottom end with the top end extending through a rubber stopper was inserted into the generator. One to seven very fine holes, drilled in the wall of the tubing about 1/4-in. from the sealed end, were located at the surface of the plutonium suspension or solution. Filtered dry air delivered at 30 psi to the tubing produced a fog consisting of plutonium particles. This was directed into the aerosol chamber.

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FIGURE 1.17. Aerosol Generator

Dry Dusts

Aerosols were produced from dry plutonium dust by two methods. In one, the dry dust was placed in No. 4 gelatin capsules and fired into an aerosol chamber with an air pistol. In a second method, the aerosols were produced by directing dry air at a rate of $100 \text{ cm}^3/\text{min}$ through a small diameter tube attached as a side arm to a 25 ml flask containing the dry plutonium dioxide dust. The flask was vibrated with an electric test tube shaker to keep the dust in motion. The top of the flask was connected to the top opening of the aerosol chamber by flexible tubing.

Aerosol Sampling

Aerosol samples are collected for two purposes: measuring average concentration of the aerosol and determining particle size characteristics of the aerosol. Membrane filter samples, collected at about 100 to $150 \text{ cm}^3/\text{min}$, provide estimates of the aerosol concentration. For particle sizing, a thermal precipitator collects samples directly on electron microscope grids. The Walkenhorst-type thermal precipitator is shown in Figure 1.18. The power supply provides current at about 2 A. Air is drawn through the sampler at a rate of $40 \text{ cm}^3/\text{min}$. Four electron microscope grids are mounted on platinum posts in each of the two plugs. The grids are parallel to the hot wire in the precipitator and perpendicular to the air flow. A membrane filter backs up the thermal precipitator. We have also used a point-to-plane electrostatic precipitator. The one shown in Figure 1.19 was designed at the University of Rochester. The aerosol sample is collected directly on an electron microscope grid.

Particle Sizing

The electron microscope grids are shadowed with chromium at an angle of about 20 degrees. The electron micrographs are

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FIGURE 1.18. Walkenhorst-Type Thermal Precipitator

Neg 38997-14

FIGURE 1.19. Point-to-Plane Electrostatic Precipitator
(from University of Rochester Design)

made at a magnification of about 30,000. A Zeiss particle size analyzer is used to size the particles and the results are processed by a computer program to give several parameters of a log-normal distribution. These include:

- Geometric mean.
- Geometric standard deviation.
- Average diameters:
Count, specific surface, surface area, volume,
volume/unit surface area.
- Median diameters:
Count, surface, mass.

Figure 1.20 is an electron micrograph of PuO_2 particles with a CMD of $0.6 \mu\text{m}$ and MMD of $4.3 \mu\text{m}$. The third dimension, height, of the particle is indicated by the length of the shadows.

Figure 1.21 is an electron micrograph of PuO_2 particles with a CMD of $0.05 \mu\text{m}$ and MMD of $0.12 \mu\text{m}$.

The PuO_2 particles have a density of about 11 and are essentially opaque to the electrons. However, there is always a degree of uncertainty about whether all the particles sized are PuO_2 or some contaminating dust. Therefore, a technique was developed to examine autoradiograms of aerosol samples with the electron microscope. Figure 1.22 is an example. Particles from which alpha tracks originated can be identified as being plutonium. There were particles in this sample which were not plutonium, although they were dense particles.

IN VIVO MONITORING

We have used in vivo monitoring techniques in our plutonium studies since 1956. Because in vivo counting of plutonium is subject to errors, we have not always been willing to accept the results in a quantitative sense. However, in vivo counting has been extremely useful to the investigator

1
μ

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FIGURE 1.20. *Electron Micrograph of PuO_2 Particles*
(Count median diameter = $0.6 \mu\text{m}$)

FIGURE 1.21. *Electron Micrograph of PuO_2 Particles*
(Count median diameter = $0.05\ \mu\text{m}$)

1 μ

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FIGURE 1.22. *Electron Micrograph of an Autoradiogram of $^{239}\text{PuO}_2$ Particles*

in a qualitative sense in following the progress of a given experiment. With constant careful recalibration the results can be very reliable, especially in rodents.

Figure 1.23 shows a whole body counting assembly for rodents. The cast lead shield housed three 1 mm × 2 in. NaI (Th) crystals and the photomultiplier tube assemblies. The horizontal animal tunnel was 2-1/2 in. in diameter to admit rats placed in 7-in. long perforated cardboard cylinders. Signals from three detectors were integrated into a single-channel pulse height analyzer with a window of 14 to 43 keV. This instrument is used for both ^{238}Pu and ^{239}Pu . The 17 keV X rays from ^{239}Pu and the attenuated 43 keV gammas from ^{238}Pu are detected. Frozen rats containing known amounts of ^{238}Pu or ^{239}Pu are used for calibration. Figure 1.24 shows the energy spectra obtained for ^{238}Pu and ^{239}Pu .

With this instrument, a counting rate of 1000 cpm is equivalent to 0.16 μCi of ^{239}Pu or 0.095 μCi of ^{238}Pu . An example of a plutonium retention curve obtained for a rat is shown in Figure 1.25. The data are adequate for determining the retention half time and other parameters.

The rodent counter can be used to obtain longitudinal scans of the animals by adjusting the shield so that the crystals monitor only a fraction of the animal at one time. The animal is then moved through the counter at a predetermined rate.

A dog counter was developed along the same general design as the rodent counter, except that the whole dog could not be monitored at once, but had to be scanned. The instrument is shown in Figure 1.26. The crystal detectors are mounted in the shadow shield. Three of the five crystals surrounding the dog in a vertical plane are 2 in. × 2 in. NaI (Tl) and two are 1 mm × 2 in. crystals. The two thin crystals, mounted opposite

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FIGURE 1.23. Whole Body Counting Assembly for Rodents

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FIGURE 1.24. X-ray and Gamma Energy Spectra of ^{238}Pu and ^{239}Pu Sources in Rat Standards

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FIGURE 1.25. Typical Whole Body Retention Curve for Inhaled Plutonium Oxide in Rats (Initial alveolar deposition is taken as value of intercept of retention curve on ordinate.)

Neg 29715-12
FIGURE 1.26. Whole Body Longitude Scanning System for Detecting Radionuclides
in Dogs

each other, are used for plutonium while the three thick crystals are used for gamma emitters. The crystals are enclosed in lead collimators which assure that the crystals monitor only a small volume of the dog. The dog is propelled past the detectors on a motor-driven cart at a constant rate (Figure 1.27). For counting, the dog is restrained in a counting box as shown in Figure 1.28. This holds the dog in a constant position.

An example of a dog scan is shown in Figure 1.29. The scan indicates the longitudinal distribution of the radioactivity in the animal. In this case the dog had inhaled $^{59}\text{Fe}_2\text{O}_3$. In our studies of insoluble compounds, the radioactivity is primarily in the lung, and this scan shows a typical lung peak. The area under the curve is compared with that obtained with a standard source in a dog phantom to obtain a quantitative estimate of the body burden.

The dog counter is also used for in vivo monitoring of rodents. This is shown in Figure 1.30. The rodent in the cardboard container is placed in a fixed position between the crystals.

Another counter is also used for monitoring plutonium in dogs. This was designed as a chest counter for humans and has been quite useful for monitoring dogs. A description of this instrument will be given in a later lecture.

PLUTONIUM ANALYSES

An important aspect of our program is the plutonium analyses of tissues and excreta. The first step is drying of the samples at 150 degrees. Then the samples are muffled at 450 to 500 °C until the residues are carbon free. This may require alternate nitric acid wet ashing and muffling to produce carbon free residues. The residues are dissolved with 2M HNO_3 and 5% HF evaporated to dryness at 150 °C, redissolved

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FIGURE 1.27. Whole Body Longitude Scanning System for Detecting Radionuclides in Dogs (Transport system for propelling dog scintillation crystals mounted in iron shield.)

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FIGURE 1.28. Plastic Box Used to Contain Dogs for Counting

Neg 0651463-1

FIGURE 1.29. *Examples of Longitudinal Scans of Dogs*

Neg 29715-1

FIGURE 1.30.

*Adaptation of the Dog Counter for Counting Rodents
(The rodents are contained in cylindrical cardboard cartons.)*

with nitric acid and evaporated at 300 °C. It is finally dissolved in 2M HNO_3 + 1% H_3BO_3 , treated with urea, and extracted with the D_2EHPA [bis(2-ethylhexyl) hydrogen phosphate] scintillating solution. Measurements are made by liquid scintillation counting. A preliminary concentration by coprecipitation with lanthanum fluoride or bismuth phosphate is useful for samples in which the plutonium content approaches background levels.

The results of the plutonium analyses, the weights of tissues and excreta samples, and other pertinent data provide the input for a computer program which performs all calculations required to convert the data into a form for interpretation.

SUMMARY

We have gained enough experience through the years to standardize most of the procedures used in our plutonium studies. However, we are continually searching for better methods to improve our experiments, both quantitatively and qualitatively. Studies with plutonium are expensive and time consuming. Therefore, it is imperative to gain as much information as possible from each experiment. Subsequent lectures will summarize the results of our studies and indicate where additional information is needed to complete our knowledge of inhaled plutonium.

LECTURE 2

THE DISPOSITION OF INHALED PLUTONIUM

PART I. COMPARISON OF CHEMICAL FORMS*

Three plutonium compounds have been studied in more than 200 beagle dogs to determine the dynamics of plutonium retention, distribution, and excretion following inhalation. While some studies have been done with $\text{Pu}(\text{NO}_3)_4$ and PuF_4 , the major interest has been PuO_2 , which seems to be the plutonium compound most likely to be encountered in an accident situation.

Experimental Procedures

Experimental Design

The experimental procedures used in these studies have been similar, although improvements in the technology have been achieved through the years. Each experiment was comprised of four phases. During the first or pre-exposure phase, the dogs were trained to accept the aerosol exposure equipment, given extensive physical examinations which included the establishment of several physiological base lines, measured for background radioactivity, and acclimated to the metabolic cage. The second phase was the actual exposure to the aerosol for periods ranging from 10 minutes to 2 hours. Measurements made during this period have varied among the many experiments performed. In some cases the total exhaled air was collected for measurement of the exhaled plutonium. Continuous measurements of respiratory rates and volumes have also been accomplished. The purpose in making these measurements was to relate respiratory parameters to the deposition and retention of the inhaled aerosol. The third or postexposure phase began immediately following the exposure when the animal was taken from the glove box, washed to remove external contamination, counted in the whole body or chest counter and placed in the

* *Principal Investigators: J. V. Dilley, J. F. Park, B. O. Stuart, D. H. Willard, W. J. Bair*

metabolism cage. Urine and feces were collected at the same time each day for plutonium analysis. Venous blood samples were collected periodically for plutonium analyses and in vivo counting was performed at regular intervals. Physiological parameters were measured and clinical tests including chest radiography were performed on a routine basis. The fourth phase, sacrifice of the animal, occurred at a prescribed time after exposure or when death due to plutonium toxicity appeared imminent. The dogs were sacrificed under anesthesia by exsanguination from the carotid artery. Detailed autopsies were performed. All organs were carefully examined, dissected, weighed, sections removed for histology, and the remainder reweighed and digested with HNO_3 for plutonium analysis.

In all experiments, daily excreta samples were collected for at least the first month after exposure. In several experiments daily excreta samples were collected for as long as 5 months. In longer term experiments after the first month, excreta samples were collected at intervals of 1 month to 6 months. These collections consisted of daily samples for 1 week, or in some cases 2 weeks.

Development and Analysis of Whole-Body Clearance Curves

All plutonium analytical data were introduced into a computer program. For those dogs on the shorter term experiments where it was feasible to collect and analyze all excreta as well as analyze all tissues collected at necropsy, the total quantity of plutonium deposited at the time of the inhalation exposure was computed. Whole body clearance curves were constructed for each dog by subtracting cumulative excretion values from the total quantity of plutonium deposited. An example is given in Figure 2.1. Extrapolation of the clearance curve to the ordinate at zero time gives an estimate of the fraction of the deposited plutonium that was deposited below

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 FIGURE 2.1.1. Whole Body and Lung Retention of ^{239}Pu

the ciliated epithelium of the terminal bronchioles and was, therefore, unavailable for early clearance by ciliary processes. This fraction was assumed to have been deposited in the alveoli and accounted for the long-term retention of plutonium in the dogs. Since subsequent clearance of the fraction deposited in the alveoli was exponential with time, the expression, $p = Ae^{-bt}$, was evaluated empirically for each dog. Here, p is the quantity of plutonium present in the dog at a given time; t is the quantity given after exposure; A is a constant related to the quantity initially deposited in the alveoli; and b is a constant describing the rate of clearance and excretion in urine and feces of the plutonium deposited in the alveoli.

The amount of plutonium found in the lung at time of sacrifice was expressed as a fraction of the total amount initially deposited. Assuming the clearance of plutonium was exponential with time, an expression for lung retention, $L = Ae^{-bL^t}$, similar to the whole body retention equation, was obtained for each dog.

In most experiments, whole body or chest counting techniques were also used to obtain estimates of whole body and lung retention of plutonium. Comparisons of these results with the information obtained from the radiochemical analysis of tissues and excreta have been useful in interpreting the results of in vivo counting during the course of long-term studies. In vivo counting techniques, even under the most optimum conditions, are not totally reliable for estimating lung clearance of plutonium. Because of the accumulation of plutonium in the lymph nodes of the thorax, chest counts reflect the results of at least two processes, the clearance of plutonium from the lung and the accumulation in the lymph nodes. Therefore, direct measurement of the clearance of plutonium from the lung presents problems other than those

associated with the detection of the low energy X rays or gamma rays emitted by plutonium (or americium). Because of these, we have preferred to estimate lung clearance rates as shown in Figure 2.1.

It was generally assumed that if a "soluble" form of plutonium were inhaled, it would rapidly clear from the lungs and be deposited in bone and liver. However, this had not been demonstrated in the laboratory. Therefore, we initiated experiments to determine the fate of an inhaled "soluble" form of plutonium. For our studies, we chose $\text{Pu}(\text{NO}_3)_4$ because it was more commonly encountered as a contaminant of air than the citrate complex studied in most laboratories.

In a preliminary experiment, three dogs were exposed to an aerosol produced from a solution of plutonium in 0.14N HNO_3 and three were given intravenous injections of the same solution. In both cases, the dogs received about 20 μCi of plutonium. Total excreta were collected for 1 month and the animals were sacrificed. The distribution of plutonium in the tissues of these dogs is shown in Figure 2.2. The lungs of the dogs which inhaled plutonium contained 60 to 70% of the body burden. The skeleton contained about 20% and the liver about 12%. Only small percentages occurred in the other tissues. In the dogs given $\text{Pu}(\text{NO}_3)_4$ intravenously, the liver contained more than 80% and the skeleton only about 7%.

These results showed that plutonium nitrate is not rapidly translocated from the lung to skeleton and liver. The intravenous data are also interesting in that it differed substantially from that reported by others for intravenously administered plutonium citrate complex. For example, it is generally found that the liver contains only about 20% while the skeleton contains 60 to 70%. Therefore, it is probable

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 FIGURE 2.2 Distribution of Plutonium in Body Tissues of Dogs

that the injection of uncomplexed plutonium nitrate resulted in the formation of colloidal particles sufficiently large to be removed by the liver and spleen. After inhalation of the same material, autoradiographs showed much particulate plutonium in the lung, also probably due to colloid formation in the aerosol droplets.

Another study has been completed which was designed to provide information about the toxicity of inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ as well as further data on the disposition of the plutonium in the body. Twelve dogs were exposed 10 to 60 minutes to an aerosol from a 0.27N HNO_3 solution of plutonium. The aerosol particles in this as well as the previous experiment had a count median diameter of about 0.12 μm . Figure 2.3 illustrates the whole body retention of the inhaled $\text{Pu}(\text{NO}_3)_4$.

The whole body retention half time was about 720 days for this dog. Alveolar deposition in these dogs ranged from 50 to 93% of the total Pu deposited. Figure 2.4 shows retention half times for all of the dogs. The whole body half times ranged from 400 to nearly 1000 days. The larger values were generally seen over the longer time periods because the rate of clearance tends to decrease with time after inhalation. The pulmonary retention half times were about 100 days, but the two dogs studied for 200 to 300 days showed values of about 200 days.

The disposition of alveolar-deposited plutonium is shown in Figure 2.5. The lung retained between 40 to 50% of the alveolar-deposited plutonium in those dogs which lived from 75 to 119 days after exposure. At longer times the percentage dropped to between 30 and 40%. Of that which was cleared from the lungs, between 10 to 20% was excreted in the feces and only 1 to 2% in the urine. About 20 to 25% was translocated to the skeleton and 5 to 10% to the liver. The fraction in

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FIGURE 2.3. Whole Body Retention of an Acute Lethal Quantity of Inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ in Beagle Dogs

FIGURE 2.4. Retention Half-Times for Inhaled $^{239}\text{Pu}(\text{NO}_3)_4$

FIGURE 2.5. Disposition of Alveolar-Deposited $^{239}\text{Pu}(\text{NO}_3)_4$ in Dogs

the liver appeared to increase with time, possibly as a result of continued translocation from the lung. The tracheobronchial lymph nodes contained about 2% of the alveolar-deposited plutonium.

Further comparison of the tissue distribution of plutonium is shown in Figure 2.6. The tissue burdens are expressed as percent of the total body burden at death. Included are the three dogs sacrificed after 30 days in the earlier experiment. Forty to 70% of the body burden was in the lungs, 13 to 37% in the skeleton, 9 to 27% in the liver, 1 to 5% in the tracheobronchial and mediastinal lymph nodes, and 3 to 5% in the other tissues. The concentrations of plutonium in the tissues are shown in Table 2.1. The highest concentration occurred in the tracheobronchial lymph nodes followed in descending order by lungs, liver, and skeleton. As we will see later, these results are qualitatively similar to those obtained with a very insoluble plutonium compound, the dioxide. However, the distribution of plutonium within the tissue differed from PuO_2 in that both aggregates and ionic plutonium were found. This is seen in the autoradiographs.

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FIGURE 2.6. Distribution of ^{239}Pu in Tissues of Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$ (Percent of Whole Body Burden)

TABLE 2.1. Concentration of ^{239}Pu in Tissues of Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$
(nCi/g wet tissue)

Tissue	Dog Number											
	424	420	396	458	340	376	419	421	423	461	447	388
Tracheobronchial-Lymph Nodes	2000	7200	3900	420	1900	1400	780	340	140	280	160	160
Lungs*	420	450	390	140	240	240	150	63	41	50	36	51
Liver	20	44	31	6	19	22	15	8	8	4	5	4
Skeleton	13	14	16	5	5	12	8	2	2	2	2	1

* Lungs were 2 to 3 times normal weight due to pathology, therefore calculated normal weights were used to determine concentration.

Figures 2.7, 2.8, and 2.9 are autoradiographs of lung sections. The presence of alpha "stars" indicate the plutonium either formed large aggregates or was accumulated by cellular action. There are single tracks, also, indicative of small particles or isolated molecules of plutonium. Figures 2.10 and 2.11 are autoradiographs of tracheobronchial lymph node sections. The presence of large aggregates is evident. Figures 2.12, 2.13, and 2.14 are autoradiographs of bone. Only single tracks were seen in bone tissues. However, particulates or aggregates were seen in liver, Figures 2.15 and 2.16.

A typical excretion curve for inhaled $\text{Pu}(\text{NO}_3)_4$ is shown in Figure 2.17. The daily excretion of plutonium in the urine is about one and one-half orders of magnitude below the excretion in feces. The data were fitted by power function equations. These are represented by the straight-line curves.

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FIGURE 2.7. *Autoradiograph of Lung Sections from Dogs after Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$*

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FIGURE 2.8. Autoradiograph of Lung Sections from Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Neg 0693716-48

FIGURE 2.9. Autoradiograph of Lung Sections from Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

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FIGURE 2.10. Autoradiograph of Tracheobronchial Lymph Node
Section from Dog After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

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FIGURE 2.11. Autoradiograph of Tracheobronchial Lymph Node
Section from Dog After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Neg 0693716-30

FIGURE 2.12. Autoradiograph of Bone Section from Dog After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

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FIGURE 2.13. Autoradiograph of Bone Section from Dog After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Neg 0693716-43

FIGURE 2.14. Autoradiograph of Bone Section from Dog After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

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FIGURE 2.15. Autoradiograph of Liver Section from Dog After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

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FIGURE 2.16. Autoradiograph of Liver Section from Dog After
Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Neg 0682337

FIGURE 2.17. Excretion of Plutonium Nitrate in Dogs

The results of our studies with inhaled $\text{Pu}(\text{NO}_3)_4$ suggest that, at least over a 1-year time period, the skeleton would not be the critical tissue for this generally considered soluble form of plutonium. This will be discussed further in regard to biological effects.

$^{239}\text{PuF}_4$

Another plutonium compound of potential interest is $^{239}\text{PuF}_4$. Since there was no knowledge of the behavior of this compound in the body after inhalation, a preliminary experiment was completed. The results are of interest in that they demonstrate the varied behavior of inhaled plutonium.

Six beagle dogs were exposed to dry aerosols of $^{239}\text{PuF}_4$. The PuF_4 was difficult to size because it appeared to consist of thin flakes. The best estimate of the size was that it was about 0.2 μm . Daily urine and fecal samples were collected for analyses and the dogs were sacrificed after 85 days. Three of the dogs were treated with DTPA (dicalcium trisodium diethylenetriaminepentaacetate). This seemed to be completely ineffective in altering the disposition of PuF_4 in the dogs.

The whole body clearance curves are shown in Figure 2.18. The percentage deposited in the alveoli of these dogs was low in all cases, from 2 to 18.5% of the total deposited in the dogs. This compared to values as high as 93% in the dogs which inhaled $\text{Pu}(\text{NO}_3)_4$.

The retention half times are given in Figure 2.19. The whole body half times ranged from 127 to 405 days. The pulmonary half times ranged from 78 to 266 days. The clearance of PuF_4 from one dog was much more rapid than in the others, and this dog was not one of those treated with DTPA.

The disposition of the alveolar-deposited plutonium fluoride is shown in Figure 2.20. Excluding the dog with the

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FIGURE 2.18. Retention of ^{239}Pu in Dogs After Inhalation of $^{239}\text{PuF}_4$

FIGURE 2.19. Retention Half Times for Inhaled $^{239}\text{PuF}_4$

FIGURE 2.20. Disposition of Alveolar-Deposited $^{239}\text{PuF}_4$ in Dogs

high rate of clearance, the lung retained 70 to 80% of the alveolar-deposited plutonium for three months. Excretion in the feces accounted for about 20%, while the tracheobronchial lymph nodes accumulated about 5%. Between 2 and 9% was excreted in the urine while less than 2% was translocated to the other tissues in the body. More plutonium was translocated to the skeleton than to the liver.

The tissue distribution expressed as percent of the body burden at sacrifice is given in Table 2.2. With the exception of the one dog, 90% or more of the plutonium was in the lung and 2 to 14% in the tracheobronchial lymph nodes. The other tissues in the body contained only fractions of 1%. The tissues showing the highest concentrations were the tracheobronchial lymph nodes. This is shown in Table 2.3. The average concentration in these lymph nodes was from 5 to 10 times that in the lungs. The concentrations in the other tissues were quite low. Autoradiographs show that the plutonium was in particulate form in the lung, Figures 2.21 and 2.22. Particulate plutonium also occurred in the tracheobronchial lymph nodes, Figures 2.23 and 2.24.

Figure 2.25 shows the excretion of plutonium by one of the dogs which inhaled PuF_4 . This indicates the general relationship between the body burden and the amount excreted each day. These will be compared among all types of plutonium in a later part of this discussion.

Comparison of Four Different Plutonium Dioxides

Plutonium dioxide is produced in a number of different ways under a wide range of temperatures, pressures, etc. The resulting oxide may also undergo a wide variety of other stresses. For example, plutonium oxide may occur as a result of oxidation of the metal in air, high explosive detonation and incineration of nuclear weapons, reactor burn-ups,

TABLE 2.2. Distribution of ^{239}Pu in Dogs 90 Days After Inhalation of $^{239}\text{PuF}_4$

Tissue	Dog Number					
	429	441	448	434*	436*	451*
Lung	94	93	82	93	89	97
Tracheobronchial	4.1	5.5	14	5.9	8.7	2.4
Lymph Nodes						
Liver	0.3	0.3	0.9	0.0	0.1	0.02
Skeleton	0.8	0.8	1.2	0.5	1	0.2
Muscle	0.2	0.1	0.5	0.1	0.5	0.1
Skin	0.2	0.1	0.7	0.03	0.1	0.4
Brain	0.1	0.01	0.6	0.2	0.01	0.01
All Other	0.2	0.3	0.5	0.5	0.7	0.3
Body Burden - μCi	0.33	1.1	0.17	2.3	0.29	0.39

* Treated with DTPA

TABLE 2.3. Concentration of ^{239}Pu in Dogs 90 Days After Inhalation of $^{239}\text{PuF}_4$

Tissue	Dog Number					
	429	441	448	434*	436*	451*
Lymph Nodes						
Tracheobronchial	20	122	33	188	38	25
Mediastinal		0.2	0.06		1.5	0.1
Lungs	4	15	2.1	32	3.3	5.3
Thyroid	0.03	0.04	<0.001	<0.001	<0.001	<0.001
Ovaries	0.02	0.03	<0.001	0.02	<0.001	0.002
Liver	0.005	0.02	0.008	<0.001	0.001	<0.001
Skeleton	0.004	0.02	0.004	0.02	0.005	0.001
Body Burden- μCi	0.33	1.1	0.17	2.3	0.29	0.39

* Treated with DTPA

Neg 0693716-44

FIGURE 2.21. Autoradiograph of Lung Section from Dog After Inhalation of $^{239}\text{PuF}_4$

Neg 0693716-42

FIGURE 2.22. Autoradiograph of Lung Section from Dog After Inhalation of $^{239}\text{PuF}_4$

Neg 0693716-46

FIGURE 2.23. *Autoradiograph of Tracheobronchial Lymph Node
Section from Dog After Inhalation of $^{239}\text{PuF}_4$*

Neg 0693716-45

FIGURE 2.24. *Autoradiograph of Tracheobronchial Lymph Node
Section from Dog After Inhalation of $^{239}\text{PuF}_4$*

Neg 0690411-1

FIGURE 2.25. Daily Excretion of ^{239}Pu After Inhalation of $^{239}\text{PuF}_4$ (Dog 429)

incineration of plutonium thermoelectric power sources, calcination of oxalates, and by preparation of ceramics in high-temperature plasma jets. Thus, it is likely that plutonium dioxide particles may have a wide range of physical properties depending upon how it was formed and the stresses to which it has been exposed. It is equally likely that plutonium particles having different physical properties will behave differently in the body. A number of our studies were designed to determine the possible influence of different physical properties of inhaled plutonium dioxide on its retention, translocation, and excretion.

Experimental Design

The disposition of four types of inhaled plutonium dioxides which were prepared by different methods and oxidized at different temperatures were compared. The experimental design is shown in Table 2.4.

TABLE 2.4. *Comparative Study of Four Different Plutonium Dioxides - Experimental Protocol*

Group Number	Dog Numbers	$^{239}\text{PuO}_2$ Aerosol Inhaled	Particle Size, μm		Percent Ultra-Filterability
			CMD	MMD	
I	1*, 2 ⁺ , 3*	Oxalate Calcined at 1000°	0.51	2.8	0.04
II	4 ^Δ , 5, 6 ⁺	Oxalate Calcined at 350°	0.45	2.8	0.02
III	7 ⁺ , 8 ^Δ , 9	Metal Oxidized at 458°	0.50	4.8	0.006
IV	10 ^Δ , 11, 12	Metal Oxidized at 123°	0.46	1.3	0.006

*, +, ^Δ Litter Mates

Four groups of beagle dogs were given a single exposure to one of four different plutonium dioxide aerosols. Two were prepared by calcining the oxalate at 1000 °C or at 350 °C. Another was produced by the ignition at 450 °C of the stabilized delta metal and the other by the slow oxidation of the pure metal at 123 °C.

The particle size characteristics of the four aerosols were similar, with count median diameters of about 0.5 μm . Mass median diameters were 2.8 μm for the calcined oxalates, 4.8 μm for the metal oxidized at 450 °C, and 1.3 μm for the metal oxidized at 123 °C. The oxides prepared from the oxalates were about 0.03% ultrafilterable and the oxidized metals about 0.006%. X-ray diffraction analyses indicated that all four were pure crystalline plutonium dioxides.

Figure 2.26 shows electron micrographs of the four oxides: The oxalate calcined at (I) 1000 °C is characterized by rounded corners and the apparent fusion of several smaller particles. The oxalate calcined at (II) 350 °C and the metal oxidized at (III) 450 °C contain many particles of similar shape and are essentially indistinguishable. The particles of metal oxidized at (IV) 123 °C are all irregular in shape, many of which appear to be aggregates. This material is said to be very friable, breaking down into smaller particles.

Whole Body and Lung Retention

In all dogs except two, the alveolar deposition ranged between 20 and 45% of the total quantity of plutonium deposited. The whole body retention half times ranged from about 1000 days to more than 3400 days, Figure 2.27. The largest values were observed for dogs which inhaled metal oxidized at 450 °C. The lung retention half times ranged between 600 to 1000 days for all dogs except those which inhaled the oxalate calcined at 350 °C. For these dogs, the retention half time was 300 to 400 days.

Neg 0663031

FIGURE 2.26. *Electron Micrographs of Four Different Plutonium Dioxides*

Neg 0662004-2

FIGURE 2.27. Comparison of Whole Body and Lung Retention of Four Plutonium Dioxides in Dogs

Disposition of Alveolar-Deposited Plutonium

Figure 2.28 shows the complete disposition of the alveolar-deposited plutonium. The lungs contained more than 90% of the alveolar-deposited plutonium 3 months after exposure in all dogs except those which inhaled the oxalate calcined at 350 °C. In these dogs the lungs contained only 80 to 87%. The difference in lung burden is largely accounted for by the amounts found in the bronchial and mediastinal lymph nodes. The accumulation of plutonium in the bronchial lymph nodes was greatest after inhalation of the oxalate calcined at 350 °C. Fecal elimination of alveolar-deposited plutonium was least in the dogs which inhaled the metal oxidized at 450 °C. These dogs showed the longest whole body retention half times.

Translocation of plutonium to tissues outside of the lung and lymph nodes was least for the dogs which inhaled oxalate calcined at 1000 °C. Urinary excretion of plutonium as well as translocation appeared to be greatest for the oxalate calcined at 350 °C. This is clearly shown in Table 2.5 which gives the tissue distribution expressed as percent of the body

TABLE 2.5. *Tissue Distribution of ^{239}Pu in Dogs 90 Days After Inhalation (Comparison of four plutonium dioxides)**

<u>Tissue</u>	<u>1000° Oxalate</u>	<u>350° Oxalate</u>	<u>450° Metal</u>	<u>123° Metal</u>
Lung	97	88	95	96
Tracheobronchial Lymph Nodes	2.7	11	5	3.4
All Other Lymph Nodes	0.009	0.02	0.02	0.02
Liver	0.025	0.16	0.05	0.04
Skeleton	0.09	0.16	0.1	0.09
Muscle	0.034	0.036	0.06	0.05
Spleen	0.0001	0.006	0.001	0.001

* Means of Three Dogs Each Group (Percent of body burden)

Neg 0662004-1

FIGURE 2.28. Disposition of Alveolar-Deposited $^{239}\text{PuO}_2$ in Dogs (Comparison of four plutonium dioxides)

burden. Mean values for the three dogs in each group are shown. However, the only really significant difference is the larger fraction accumulated in the tracheobronchial lymph nodes of the dogs which inhaled oxalate calcined at 350°.

Excretion Rates

The amount of ^{239}Pu excreted each day in urine and feces was expressed as the percent of the body burden of plutonium at the beginning of the 24-hour period during which the excretion occurred. These data were programmed for curve fitting by least squares analyses using a digital computer. Curves were fitted to each set of data for individual dogs and to daily mean values for the three dogs in each group to obtain equations representing the mean excretion rates for each dog and each group of dogs, respectively. Logarithmic equations of the form, $Y = At^b$, where Y is the percentage of plutonium excreted each day in urine or feces, were fitted to all data. Power functions did not always provide the best visual fit, but are probably adequate to compare the four plutonium dioxide aerosols.

Excretion data are shown for a representative dog in Figure 2.29. The plots of individual excretion values for the dog show an example of the daily fluctuations in levels of ^{239}Pu in urine and feces. The values for urine are about two and one-half orders of magnitude below the fecal values. Figure 2.30 gives the curves with 95% confidence levels fitted to the mean daily values of all three dogs in each group. Comparison of these excretion data for the four groups indicates that there were some differences. After the first 20 days, the rate of fecal excretion tended to be less for dogs in Group III than for the other dogs. This correlates with the generally longer whole body retention half times calculated for these dogs. The rate of excretion of plutonium in urine

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FIGURE 2.29. Daily Excretion of ^{239}Pu After Inhalation of Plutonium Metal Oxidized at 450°

Neg 0661922-2

FIGURE 2.30. Daily Excretion of ^{239}Pu (Comparison of four plutonium dioxides)

tended to be greatest for dogs which inhaled oxalate calcined at 350° and those which inhaled metal oxidized at 450°.

Interpretation of Excretion Data

The extensive plutonium excretion data available from this study has been further analyzed to obtain more information relative to the estimation of body burdens from excretion data. Figure 2.31 shows the mean daily excretion values for three dogs which were exposed to PuO_2 formed by calcination of the oxalate at 1000 °C (Group I). The curve for urinary excretion lies two and one-half orders of magnitude below the fecal excretion curves, with highest urinary excretion rates of <0.02%, compared to 37% for the feces. The low urinary levels show a wider scattering about the fitted line than do fecal excretion values. Inspection of the fecal excretion points show that while a single power function could be fitted to the first 10 to 15 days after exposure, the same equation does not describe the values for 15 to 90 days. The majority of the fecal radioactivity excreted during the first few days after exposure represents material that was cleared from the nasopharynx and tracheobronchial regions of the respiratory tract, plus a contribution from the rapid phase of pulmonary clearance. Since we are concerned with estimating the level of plutonium retained in the pulmonary lung, where clearance is very slow and the total alpha irradiation dose builds up almost linearly with time, the first 3, 5, 7, and 14 days of excretion values were successively dropped from the computer program and new curves were refitted to the remaining points in order to obtain the best fit to a single "long-term" power function. While this process resulted in little change of the curve for urinary excretion, both slope and intercept (i.e., day - one) values of fecal excretion curves decreased markedly up to removal of the first 7 days, with little change between "-7" and "-14" day curves.

Neg 0671626-3

FIGURE 2.31. Daily Excretion of ^{239}Pu After Inhalation of Oxalate Calcined at 1000°

Figure 2.32 shows the same "biphasic" logarithmic pattern of fecal excretion values after beagle dogs inhaled PuO_2 prepared by oxidation of the metal at 450 °C (Group III). Again there was a marked decrease of slopes and intercepts when the first 3, 5, 7, and 14 days of fecal excretion values were omitted, with comparatively little difference between -7 and -14 day curves. Urinary excretion rates were two orders of magnitude below fecal excretion rates, and showed little change in the fitted curve when initial points were omitted.

Figure 2.33 illustrates the fecal and urinary excretion curves fitted to excretion values from dogs exposed to PuO_2 formed by oxidation of plutonium metal at 123 °C (Group IV). This is the temperature of spontaneous oxidation in air. The curve fitted to the values of fecal excretion of plutonium showed very pronounced changes in slope and intercept when initial values were omitted, while again there was comparatively little effect on the urinary excretion curve. Urinary excretion rates were nearly three orders of magnitude below those of fecal excretion.

Figure 2.34 shows similar curves for fecal and urinary excretion levels from three dogs which were exposed to PuO_2 formed by calcination of the oxalate at 350 °C. The slopes and intercepts of curves fitted to the fecal excretion values when the first 3, 5, 7, and 14 days were omitted showed the same decreasing pattern that was found for the other three oxides. Once more the urinary excretion levels were less than 1/100th of the fecal excretion levels, and showed a greater degree of point scattering about the fitted curves. The dotted lines show the best fitting of a single exponential function to the 15- through the 90-day values for both urinary and fecal excretion; linear functions were also tested to determine which mathematical relationship would best fit the excretion data beginning 2 weeks after exposure. These data were

Neg 0671626-4

FIGURE 2.32. Daily Excretion of ^{239}Pu After Inhalation of Metal Oxidized at 450°

Neg 0671626-8

FIGURE 2.33. Daily Excretion of ^{239}Pu After Inhalation of Metal Oxidized at 123°

Neg 0671626-5

FIGURE 2.34. Daily Excretion of ^{239}Pu After Inhalation of Oxalate Calcined at 350°

subjected to a digital computer program which performed an iterative minimizing of residual sums of squares by repeatedly altering slope and intercept values until convergence was obtained. These tests showed that power functions provided minimal residual variation for both urinary and fecal excretion, and thus gave the best fit to the experimental data.

The results of this study show the relatively long-term pulmonary retention of inhaled PuO_2 , its gradual accumulation in bronchial and mediastinal lymph nodes, and the accompanying low rate of translocation and excretion. None of the four types of PuO_2 deviated from this general pattern, although certain differences in their behavior were apparent, demonstrating the ability of biological processes to discriminate among compounds which were crystallographically indistinguishable.

Certain radiation protection considerations are also implied by these results. Because of its greater accumulation in lymph nodes, the oxalate calcined at 350 °C may be less hazardous than the other oxides.

LECTURE 3THE DISPOSITION OF INHALED PLUTONIUMPART II. EFFECT OF PARTICLE SIZE*²³⁹PuO₂ - Short Term Studies

The common parameter in the previous study was the nearly same particle size characteristics of the four different plutonium oxide aerosols used. Other experiments have been completed in which the particle size of the aerosols was the variable, the method of preparing the plutonium dioxide being constant. The plutonium dioxide was prepared by calcining the oxalate at a temperature of about 350 °C.

Pulmonary Retention

An effect of particle size on pulmonary retention of PuO₂ is shown in Figure 3.1. The data are mean values for three to six dogs at each point. When dogs inhaled PuO₂ with a MMD of 0.23 μm, only 50% of the alveolar-deposited plutonium was retained in the lungs after 30 days compared with about 88% following inhalation of an aerosol with a MMD of 3.3 μm and more than 95% following inhalation of an aerosol with a MMD of 4.3 μm. In the case of all three aerosols, the major clearance occurred within 1 or 2 weeks after exposure.

Translocation and Excretion

The routes by which plutonium was cleared from the lung are illustrated in Figure 3.2. Cumulative 30-day values for lung retention, translocation to the bronchial lymph nodes or to other tissues, and excretion are given for five aerosols. For all aerosols, the majority of the plutonium cleared from the lungs was excreted in the feces. Assuming that there was no secretion of systemic plutonium into the gastrointestinal tract, the amount excreted in the feces provides an estimate of the plutonium cleared from the deep respiratory tract by

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Neg 0631707-4

FIGURE 3.1. Effect of Particle Size on Lung Retention of $^{239}\text{PuO}_2$ in Dogs

Neg 0631707-2

FIGURE 3.2. Effect of Particle Size on Retention, Translocation and Excretion of Inhaled $^{239}\text{PuO}_2$ 30 Days After Exposure (Each value is mean of three dogs)

mucus-ciliary processes which may or may not have involved phagocytosis. This route was most effective for the inhaled aerosol with a MMD of $0.23\ \mu\text{m}$ and least effective for aerosols with MMD's of 2 to $4\ \mu\text{m}$. The data for the aerosol with a MMD of $7.6\ \mu\text{m}$ suggest that the importance of this route also may increase with particle size. These data indicate that minimal translocation of PuO_2 to the bronchial lymph nodes occurs for aerosols with MMD's in the range of 2 to $3\ \mu\text{m}$.

The percentage of alveolar-deposited plutonium that was translocated to other tissues in the body or excreted in urine was greatest following inhalation of PuO_2 with the smallest MMD and decreased with increasing particle size of the aerosol. Since the clearance of plutonium from the lungs by this route probably involved transport by the blood, it is reasonable to conclude that solubilization of the PuO_2 occurred in the lungs. This is consistent with the fact that for a given total mass, small particles present a greater surface area and are more soluble than large particles. It was also found that plutonium excreted in urine readily passed membrane filters. This suggests that it was either excreted in an ionic form, as a soluble complex, or as very fine particles. There is doubt that all of the plutonium was in an ionic form when it left the lung because it did not selectively accumulate in those tissues, liver and bone, which generally accumulate plutonium following inhalation or injection of soluble forms such as the nitrate or the citrate complex.

The distribution of ^{239}Pu among the tissues of these dogs is compared in greater detail in Table 3.1. In animals of Groups II, III, V, and VI, the small amount of plutonium found outside of the lung was primarily in the tracheobronchial lymph nodes, liver, muscle and skeleton. The fractions found in tissues varied somewhat with the particle size of the aerosol to which the dogs were exposed. When aerosols with MMD's

TABLE 3.1. *Distribution of Plutonium in Tissues of Dogs*

Group	Particle Size, μm CMD MMD	Percent Filterable (a)	Percent of Body Burden One Month After Exposure						
			Bronchial Lymph Nodes	Lung	Spleen	Liver	Kidney	Muscle	Skeleton
I	0.12 0.23	0.5	2.7	71	0.6	0.87	0.56	4.4	1.3
II	0.32 2.3	0.2	0.75	98	0.01	0.17	0.018	0.008	0.51
III	0.43 3.3	0.2	0.25	98	0.008	0.28	0.022	0.85	0.64
IV	0.24 6.1	2	0.54	96	0.014	1.6	0.02	0.25	1.0
V	0.3 7.6	0.03	1.4	98	0.009	0.097	0.0019	0.071	0.057
VI	0.60 4.3	--	1.5	98	0.0008	0.049	0.0018	0.10	0.029
VII	0.13 0.88	85	0.47	67	0.17	12	0.21	1.5	16
VIII (Intravenous Injection)	85	=	0.014	0.28	6.6	82	0.11	3.0	6.4

Groups I to VI exposed to $^{239}\text{PuO}_2$.

Groups VII and VIII given plutonium nitrate.

a. Percent of plutonium suspension.

of 4.3 and 7.6 μm were inhaled, the percent in the tracheo-bronchial lymph nodes was greater and percents in the liver, spleen, kidney, muscle, and skeleton were less than when aerosols with MMD's of 2.3 and 3.3 were inhaled. Comparison of Group I dogs (those exposed to the aerosol with the smallest MMD) with those in Group IV (exposed to an aerosol containing 2% ionic Pu) showed that in the case of Group I dogs the Pu was fairly uniformly distributed throughout all tissues. However, in Group IV dogs, Pu was translocated mainly to liver and skeleton. This was similar to that seen after inhalation of $\text{Pu}(\text{NO}_3)_4$.

The rate of excretion of plutonium in urine and feces, Figures 3.3 and 3.4, further demonstrates the effect of particle size. Data for inhaled and intravenously injected plutonium nitrate are included for comparison. The rate of excretion in both urine and feces decreased rapidly with time after exposure to the PuO_2 aerosols with large MMD's. However, with decreasing particle size of the aerosol, the rates of excretion in urine showed less decline with time, and in the case of the aerosol with the smallest MMD, were comparable to those for relatively soluble plutonium nitrate. The rates of excretion in feces showed less effect of the particle size. The smallest particle size Pu was excreted at the highest rate.

Since these results indicate that the pulmonary retention of inhaled large PuO_2 particles is greater than for small particles, the autoradiograms prepared from tissue sections of the dog lungs were examined to determine the size of the PuO_2 particles retained. Figure 3.5 is an autoradiograph of lung tissue. Single tracks were accepted as originating from a particle rather than from ionic plutonium. This was done on the basis that the PuO_2 suspensions used for production of the aerosols contained only small traces of ^{239}Pu in the ionic state. From ultrafiltration studies, it was estimated that

Neg 0621077-2

FIGURE 3.3. Daily Excretion of ^{239}Pu in Urine of Dogs

Neg 0621077-1

FIGURE 3.4. Daily Excretion of ^{239}Pu in Feces of Dogs

Neg 0693716-8

FIGURE 3.5. *Autoradiograph of Lung Section from Dog After Inhalation of $^{239}\text{PuO}_2$*

the amount of ionic ^{239}Pu varied from 0.03 to 0.5% for the suspensions discussed here. All autoradiographic exposures were for 14 days. Dense tracks were classified as emanating from particles larger than a given size.

The particle size distribution of $^{239}\text{PuO}_2$ in lungs from dogs sacrificed immediately after exposure, 1, 7, 14, or 30 days are compared in Figure 3.6 for exposures to these different aerosols. The particle-size distributions of the aerosols are also included.

It is immediately obvious that the particle-size distribution of PuO_2 in the lungs of dogs sacrificed immediately after exposure differed significantly from the size distribution of the aerosol to which the dogs were exposed, particularly for those aerosols having large MMD's. Apparently the large particles appearing in the aerosol were either not inhaled due to prior deposition on the walls of the apparatus

Neg 0631707-1

FIGURE 3.6. Relationship Between Particle Size Distribution of $^{239}\text{PuO}_2$ in Lungs of Dogs and in the Inhaled Aerosol

or if they were inhaled, they made up the bulk of the plutonium cleared and excreted immediately after exposure. It is also true that the size distribution of PuO_2 in the lungs was different for each inhaled aerosol and the differences were consistent with size distribution in the aerosol. For example, in the case of the aerosol with an MMD of $0.086 \mu\text{m}$, there was a small percentage of particles greater than $0.36 \mu\text{m}$ and, consequently, these made up a small percentage of the particles in the lung. In general, for all aerosols inhaled, the fraction of small particles retained in the lung decreased with time. This resulted in the larger particles, between 0.06 and $0.36 \mu\text{m}$, making up increasingly larger percentages of those retained in the lung. With minor exceptions there was no increase with time of the percentage contributed by particles larger than $0.36 \mu\text{m}$.

It is difficult to determine the fate of those particles which were not retained in the lung since the particles excreted in the feces were not sized and it was impossible to size the particles removed via the lymphatic and circulatory routes. Some information was obtained by examining the particles accumulated in the bronchial lymph nodes, Figure 3.7. However, in this case it was only possible to size the particles in the lymph nodes of dogs exposed to the aerosol with the largest MMD, $4.3 \mu\text{m}$, since there were insufficient particles in the lymph nodes of dogs exposed to the other aerosols to count. The particle size distributions are shown in Figure 3.8. With minor exceptions, the particle size distributions in lungs and lymph nodes were similar at all three time periods. This is in apparent contradiction to the data in Figure 3.2, which shows that percentage accumulation of plutonium mass in lymph nodes may be particle size dependent. This has not been resolved.

Neg 0693716-7

FIGURE 3.7. *Autoradiograph of Tracheobronchial Lymph Node
Section from Dog After Inhalation of $^{239}\text{PuO}_2$*

Further Studies of Small Size PuO_2 Particles

The results of the previous series of experiments indicated that the smaller the particle size of the inhaled aerosol, the greater the translocation from the lung and excretion in urine. These experiments were performed with the PuO_2 which showed the highest rate of clearance from the lung of the four different plutonium dioxides compared in the first study discussed. Therefore, it was of interest to determine whether PuO_2 calcined at higher temperatures was subject to relative high rates of pulmonary clearance, translocation, and excretion. Two experiments have been completed, one with $^{238}\text{PuO}_2$ calcined at about 750 °C and another with $^{239}\text{PuO}_2$ calcined at about 900 °C.

$^{238}\text{PuO}_2$

Twelve dogs were exposed to $^{238}\text{PuO}_2$ prepared by calcining the oxalate at 750 °C. The CMD of the aerosol was 0.05 μm and

Neg 0631707-3
 FIGURE 3.8. Particle Size Distribution of ²³⁹PuO₂ in Lungs and Bronchial Lymph Nodes of Dogs

the MMD was about $0.1 \mu\text{m}$. In these dogs, the total deposition ranged from 10 to 27% of the inhaled aerosol and the alveolar deposition ranged from 4 to 20%. The amount of ^{238}Pu deposited in the alveoli of these dogs ranged from 40 to more than 200 μCi . Because of the toxicity of this quantity of plutonium, one dog died 27 days after exposure and the others at times up to about 100 days.

The retention half times for the whole body and lung are shown in Figure 3.9. The whole body retention half times ranged from 400 to 1500 days. The shorter half times were observed for those dogs which died within 2 months after exposure. Because of the severe toxicity of the Pu in these animals and the short observation time, the half times are probably not very meaningful. The longer half times, observed in those animals that survived more than 2 months, are probably more applicable to low level situations.

The pulmonary half times ranged from 200 to 400 days. Again the longer times of about 400 days may be more characteristic of the material inhaled than the short half times seen in dogs which survived less than 2 months due to severe pulmonary pathology.

The disposition of the alveolar-deposited ^{238}Pu is shown in Figure 3.10. The lungs retained between 80 and 90% of the plutonium deposited, 1 to 4% was translocated to the tracheo-bronchial lymph nodes, and 4 to 7% was translocated to other tissues in the body, primarily the skeleton. Less than 0.5% was excreted in the urine and 2.5 to about 7% in the feces.

Table 3.2 shows the distribution of ^{238}Pu among the tissues. The data are expressed as percent per tissue of the total body burden at the time of death. More than 90% of the body burden was in the lungs. The skeleton and tracheo-bronchial lymph nodes contained about equal amounts of ^{238}Pu ,

FIGURE 3.9. Inhaled $^{238}\text{PuO}_2$ Retention Half Times

FIGURE 3.10. Disposition of Alveolar-Deposited $^{238}\text{PuO}_2$ in Dogs
(CMD = 0.05 μm , MMD = 0.1 μm)

TABLE 3.2. *Inhaled $^{238}\text{PuO}_2$ Distribution of ^{238}Pu in Beagle Dogs
(Percent of body burden)*

Tissue	Time of Death, days											
	27	30	35	56	56	61	70	76	77	94	94	
Lungs	92	94	93	92	91	94	90	90	91	91	91	
Skeleton	2.4	1.9	3.3	3.7	3.9	2.9	4.5	3.1	2.1	4.5	4.5	
Tracheobronchial Lymph Nodes	4.1	2.0	1.4	2.5	4.1	1.7	2.5	3.0	4.7	3.3	3.3	
Liver	1.3	1.5	1.8	0.9	0.5	1.0	1.9	1.3	1.0	0.3	0.3	
Muscle	0.2	0.2	0.2	0.6	0.2	0.1	0.3	1.3	0.4	0.1	0.1	
Pelt	0.1	0.2	0.3	0.1	0.1	0.3	0.1	0.1	0.1	0.01	0.01	
GI Tract	0.1	0.06	0.05	0.08	0.04	0.06	0.07	0.2	0.03	0.05	0.05	
Spleen	0.02	0.01	0.07	0.08	0.02	0.03	0.03	0.03	0.01	0.3	0.3	
Blood	0.05	0.07	0.03	0.02	0.02	0.03	0.05	0.02	0.02	0.004	0.004	
Body Burden, μCi	261	167	168	112	74	140	84	88	58	44	44	

between 2 and 4%. About 1% was in the liver. The other tissues contained less than about 1% of the total body burden.

The highest mean concentration of ^{238}Pu occurred in the tracheobronchial lymph nodes, exceeding that in the lungs by factors of 2 to 10. This is given in Table 3.3. The mean concentration of Pu was about equal in liver, skeleton, and spleen. The tissue distribution of ^{238}Pu in these dogs was similar to that seen in most studies of PuO_2 .

Considering the small particle size of the inhaled aerosol and the relative duration of this experiment, the total fractions translocated from the lung to other tissues and excreted in the urine were less than would be predicted from the results of the 30-day $^{239}\text{PuO}_2$ studies. Only the fractions of ^{238}Pu translocated to the skeleton and liver were consistent with the results of the small particle size ^{239}Pu aerosol experiment. However, comparison of the results of this ^{238}Pu experiment with the ^{239}Pu experiments is difficult because of the high doses employed. The severe pathology which developed in the ^{238}Pu dogs may have altered the disposition of the plutonium.

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

Results from another experiment are available for comparison with both the $^{238}\text{PuO}_2$ experiment and the short-term ^{239}Pu experiment. Six dogs were exposed to an aerosol produced from a suspension of $^{239}\text{PuO}_2$ which had been calcined at about 900 °C. The CMD was about 0.05 μm and the MMD was about 0.12 μm - very similar to the $^{238}\text{PuO}_2$ aerosol just discussed. Three of these dogs were sacrificed after 150 days, the other three are still under observation. Results are given for the three dogs sacrificed.

TABLE 3.3. Inhaled $^{238}\text{PuO}_2$ Concentration of ^{238}Pu in Dog Tissues (nCt/g)

Tissues	Time of Death, days									
	27	30	35	56	56	61	70	76	77	94
Tracheobronchial Lymph Nodes	14,000	5,000	4,000	4,100	3,400	2,000	1,200	4,100	6,200	940
Lungs	2,900	2,300	2,300	1,300	880	1,500	840	1,000	620	520
Mediastinal Lymph Nodes	20,000	501	240	100	570	180	110	230	360	100
Liver	12	15	21	5	2	7	4	7	4	1
Skeleton	6	4	5	5	3	4	3	2	1	3
Spleen	4	2	1	2	1	2	1	2	0.3	9
All Other Lymph Nodes	2	3	3	2	2	1	2	3	5	1
Adrenal	15	1	1	1	1	1	1	1	1	0.3
Ovary		1	1		1			1		0.2
Testes	0.2			0.1		0.2	0.2		0.1	
Body Burden, μCi	261	167	168	112	74	140	84	88	38	44

The whole body retention curves are shown in Figure 3.11. About 2 μCi were deposited in these dogs. The alveolar deposition was 75 to 90%. This was relatively high compared with the other experiments. This indicates that little of the inhaled aerosol was deposited in the tracheobronchial and nasal pharyngeal region of the respiratory tract.

The calculated whole body and pulmonary retention half times are given in Figure 3.12. The whole body retention half times were about 400, 700, and 1000 days. These compare with those of the ^{238}Pu experiments. The pulmonary retention half times were about 350, 500, and 600 days. These were somewhat greater than observed for ^{238}Pu .

The disposition of alveolar-deposited plutonium is shown in Figure 3.13. The lungs retained 74 to 82%. The amounts translocated to the tracheobronchial lymph nodes were 2 to about 7%. Only about 1% was translocated to all other tissues including liver and skeleton. Excretion in urine accounted for less than half a percent. Therefore, nearly all of the plutonium which was cleared from the lungs was excreted in the feces.

This tissue distribution of ^{239}Pu is shown in Table 3.4. Although well over 90% of the body burden was in the lungs, the highest concentration occurred in the tracheobronchial and mediastinal lymph nodes. The skeleton and liver burdens comprised a smaller percent of the body burden than was seen in the ^{238}Pu experiment described.

Total daily excreta from these dogs was collected for the duration of the experiment and analyzed for plutonium. These data are plotted in Figure 3.14 for one of the dogs. The daily variation in amounts excreted is typical of that seen in our plutonium studies. Urinary excretion was about two orders of magnitude less than fecal excretion of plutonium.

Neg 0690411-3
 FIGURE 3.11. Whole Body Retention of ^{239}Pu in Dogs After Inhalation of $^{239}\text{PuO}_2$

FIGURE 3.12. Inhaled $^{239}\text{PuO}_2$ Retention Half Times
(CMD = 0.05 μm , MMD = 0.12 μm)

FIGURE 3.13. Disposition of Alveolar-Deposited $^{239}\text{PuO}_2$
(After 150 days) (CMD = $0.05\ \mu\text{m}$, MMD = $0.12\ \mu\text{m}$)

TABLE 3.4. Distribution of ^{239}Pu in Dog Tissues 150 Days After Inhalation of $^{239}\text{PuO}_2$
(CMD = 0.05 μm , MMD = 0.12 μm)

Tissues	Percent of Body Burden			Concentration, nCi/g		
	Dog Number			Dog Number		
	330	338	350	330	338	350
Lymph Nodes						
Tracheobronchial	2	4	8	34	41	98
Mediastinal	0.4	0.14	0.002	31	18	0.15
All Other	0.003	0.02	0.01	0.006	0.01	0.01
Lungs	96	94	91	16	8.4	11
Liver	0.7	0.6	0.3	0.05	0.02	0.01
Skeleton	0.4	0.5	0.3	0.007	0.005	0.003
Spleen	0.007	0.005	0.02	0.006	0.002	0.01
Adrenals	0.002	0	0.0007	0.04	0	0.004
Testes	0.0006	0.002	0.0007	<0.001	0.001	<0.001
Body Burden, μCi				2	0.9	1.3

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Neg 0690411-4

FIGURE 3.14. Daily Excretion of ^{239}Pu After Inhalation of $^{239}\text{PuO}_2$ (CMD = $0.05 \mu\text{m}$, MMD = $0.12 \mu\text{m}$)

These results, even more than those obtained with ^{238}Pu , seem inconsistent with the results of the short-term 30-day experiments. The short-term experiments indicated that the smaller the particle size, the greater the translocation of plutonium from lung to other tissues and the greater the excretion in urine. The amount of plutonium inhaled by these dogs was relatively small so that the possible influence of pathology on the disposition of plutonium in the body was minimal. However, properties of PuO_2 other than particle size were shown to be important to the relative disposition of inhaled PuO_2 . The $^{239}\text{PuO}_2$ used in this experiment was calcined at 900°C . In comparing the relative disposition of PuO_2 calcined at different temperatures, it was found that those calcined at about 1000°C showed greater retention and less translocation than those calcined at lower temperatures. The results of this experiment suggest that particle size may be less an influencing factor in translocation when the PuO_2 has been exposed to high temperatures. The comparative studies of particle size described earlier were done with a PuO_2 particle calcined at the relatively low temperature of 300°C .

Summary Comparison of Various Pu Compounds

Studies of a number of different plutonium compounds have been described. To evaluate the significance of these results and the differences seen, it will be helpful to review some of the results. These are summarized in several figures.

Whole body retention half times are compared in Figure 3.15. Maximum retention occurred in those animals which inhaled an oxide prepared by heating plutonium metal to 450°C and the least retention occurred in those which inhaled PuF_4 . Of the oxides, the 900°C calcined oxalate with a particle size of $0.05\text{ }\mu\text{m}$, CMD, was retained the least, similar to the nitrate. It can be seen that particle size was an important factor by

FIGURE 3.15. Summary - Whole Body Retention Half Times for Inhaled Plutonium in Dogs

comparing the retention of the small particle size 900 °C oxide with the 1000 °C oxide which had a particle size a factor of ten larger. The larger particle size oxide showed greater whole body retention than the small sized PuO_2 .

In Figure 3.16, the pulmonary retention half times are compared. The oxides separate into two groups. Those showing the greatest pulmonary retention were the 1000 °C oxalate and the two oxides prepared from the metal. Those showing the most rapid clearance, or least retention, were the two oxides with the smallest particle size of about 0.05 μm CMD, both calcined at relatively high temperatures. The third oxide in this group showing the lowest retention was the one prepared by calcining oxalate at 350 °C; the particle size was a factor of 10 above those of the other two in this group. The conclusion, then, seems to be that in terms of retention, both particle size and the process by which the oxide is formed are important.

The pulmonary retention of PuF_4 was less than the oxide, but still somewhat greater than for the $\text{Pu}(\text{NO}_3)_4$.

Figure 3.17 shows the comparative disposition of the plutonium oxides. Several points are obvious.

- The oxalate calcined at 350° showed the greatest accumulation in the tracheobronchial lymph nodes.
- The 750 °C $^{238}\text{PuO}_2$, with a particle size of 0.05 μm CMD, showed the greatest translocation to other tissues.
- The 900 °C $^{239}\text{PuO}_2$, also with a particle size of 0.05 μm CMD, showed the greatest excretion in the feces.

The fact that this was 150 days total excretion compared to 90 days for the other experiments shown, accounts for some of the difference, but not for all of it. Thus, the relative roles of three major routes for clearance of plutonium from the lung depend upon the physical properties of the inhaled

FIGURE 3.16. Summary - Pulmonary Retention Half Times for Inhaled Plutonium in Dogs

A series of five experiments was performed. This is shown in Table 4.1. First, 22 dogs were exposed to air in which 50- μm $^{238}\text{PuO}_2$ particles were dispersed. Second, single or several 50- μm particles were placed in the lungs of six dogs. Third, 120- μm particles were given 22 dogs by intubation under sodium pentothal anesthesia. Fourth, two dogs were given 120- μm particles by intravenous injection. In the final experiment, six dogs were exposed to aerosols of ^{238}Pu dust prepared by crushing the $^{238}\text{PuO}_2$ microspheres. This discussion will be concerned only with deposition and retention of large particles.

TABLE 4.1. *Experimental Plan for Studies of $^{238}\text{PuO}_2$ Microspheres in the Lungs of Dogs*

<u>Material</u>	<u>Method of Administration</u>	<u>Particle Size, μm</u>	<u>Number of Dogs</u>
Microspheres	Inhalation	50	22
Microspheres	Intubation	50	6
Microspheres	Intubation	120	22
Microspheres	Intravenous	120	2
Crushed Microspheres	Inhalation		6

In all of these experiments, whole body longitudinal scanning techniques were used to follow the course of the particles in the dogs.

Dogs were exposed for 10 minutes to 50- μm particles dispersed in air. Because of the density of the particles and the heterogenous dispersion of particles in the chamber it was not possible to obtain a value for aerosol concentration with which to relate deposition in the dogs. Approximately 12 to 85 mCi or 4,000 to 29,000 particles were passed through the chamber. Amounts deposited in the dogs were estimated from whole body counts as 10 to 900 μCi .

Figure 4.1 is an example of scans from a dog which inhaled 50- μ m particles. A significant fraction of the plutonium deposited was deposited in the nasal pharynx region. Subsequent scans show the passage of the plutonium through the animal. Comparison of the animal scans with a scan of a dog phantom with ^{238}Pu particles in the lung region indicate that either no $^{238}\text{PuO}_2$ particles were deposited in the lung or if they were, they were cleared very rapidly. Complete elimination of the ^{238}Pu particles occurred in all dogs by 16 days after exposure. One dog was killed 20 minutes postexposure and analysis showed 83% in stomach and 17% in the tongue. Another dog sacrificed 2-1/2 minutes postexposure revealed 9.7% in the oral cavity, 80.4% in the stomach, 4.4% in the esophagus, 3.2% in the nasal cavity, 0.8% in the trachea, and approximately 1.3% in the head and pelt of the head.

In the next experiments, one or several 50- μ m particles were placed in lungs of six anesthetized dogs by means of a bronchoscope. Figure 4.2 shows typical scans. Most particles were cleared after 2 to 8 days, although several were retained for 2 months or longer. One remained for 3 months and another has been retained for more than 6 months.

In another group of dogs, 300-to 120- μ m particles were placed in the lungs by intubation. Figure 4.3 shows the passage of a 120- μ m particle through the dog. The particle cleared the lung within 45 minutes. Retention data are summarized in Table 4.2. In all but five dogs, the 120- μ m particles were cleared within 10 days. One dog retained the particle 2 months, another 3 months, and two dogs have retained the particles more than 6 months. One dog has retained a 120- μ m particle and another dog a 300- μ m particle for nearly 3 years.

tract and excreted in the feces over a period of a few days. Apparently no particles were deposited in the lower respiratory tract, or if they were, clearance was rapid and complete.

- In 30 dogs, 50-, 120-, and 300- μm $^{238}\text{PuO}_2$ deposited in the lungs by intubation were cleared within 10 days. However, nine dogs retained particles for several months, up to a year in two cases. No biological effects have been seen except for a possible lymphopenia in one which has retained a 300- μm particle for over a year.

PART IV. LONG-TERM $^{239}\text{PuO}_2$ STUDIES

Introduction

The most extensive inhalation studies have been done using $^{239}\text{PuO}_2$ prepared by calcining the oxalate at a temperature of about 350 °C. The particle size of this material was about 0.3 to 0.5 μm , CMD, and about 3 μm , MMD. Figure 4.9 shows the comparative disposition of several plutonium oxides. The PuO_2 studied most extensively is the one showing the greatest accumulation in the tracheobronchial lymph nodes. As a result, this PuO_2 showed relatively rapid clearance from the lung during the early weeks after exposure.

Nearly 100 dogs have been exposed to this PuO_2 . The deposited dose varied from less than a microcurie to about 50 microcuries. Therefore, in some cases severe damage occurred and the dogs died early after exposure. Others died as a result of radiation injury at longer times after exposure and others were sacrificed before pathology developed. From these dogs, over a period of nearly 10 years, we have accumulated extensive data on the disposition of this one particular plutonium dioxide.

Long-Term Tissue Distribution

Long-term excretion data and in vivo counting of dogs after inhalation of $^{239}\text{PuO}_2$ suggest that long-term whole body retention of plutonium follows a logarithmic relationship with time, Figure 4.10. For example, for data from five dogs, single regression curves were obtained with slopes varying from -0.023 to -0.037. Therefore, using the equation, $Y = At^{-0.03}$, we calculated the initial alveolar deposition, A, of plutonium in all of the long-term dogs. Tissue distributions for all dogs were expressed in terms of alveolar deposition. This has provided an estimate of the long-term retention and translocation of this one particular PuO_2 ,

Neg 0673019-9

FIGURE 4.9. Comparative Disposition of Four Different Plutonium Dioxides After Inhalation by Dogs

Neg 0681612-1
 FIGURE 4.10. Whole Body Retention of ^{239}Pu in Dogs After Inhalation of $^{239}\text{PuO}_2$

as shown in Figure 4.11. This is still tentative because a number of long-term dogs are still alive and results from these dogs may alter the shapes of the curves.

In Figure 4.11, time is expressed as hundreds of days. Therefore, the time period covers about 9 years postexposure. The data for retention of plutonium in the lung appears to be exponential. A straight line can be reasonably fitted to the data. The half time for lung retention of plutonium is about 1000 days.

The accumulation of plutonium in the tracheobronchial lymph nodes largely occurred early, within the first year after exposure, but continued for several years. Recent data suggests that plutonium levels in the lymph nodes may tend to decrease 5 years after exposure. However, this may not prove to be true since one dog showed a very high value 9 years after exposure. The tracheobronchial lymph nodes appear to accumulate at least 40% of the amount of plutonium initially deposited in the alveoli.

Accumulation of plutonium in liver did not become very significant until about 2 years after exposure. The liver burden increased markedly to about 15 to 20% of alveolar deposition and remained at that level.

Translocation to the bone occurred more gradually, reaching 3 to 4% of the alveolar deposition after 4 to 5 years. Maximum values were nearly 5% in several dogs, but the skeleton of one dog had about 9.5% of the alveolar-deposited plutonium after about 6-1/2 years.

Figure 4.12 shows the tissue distribution expressed as percent of the body burden at death. The tracheobronchial lymph nodes contained nearly as much of the body burden as the lungs beginning 5 to 6 years after exposure. The percentage found in the liver and skeleton increased with time but has

Neg 0690411-2

FIGURE 4.11. Retention and Translocation of Alveolar-Deposited $^{239}\text{PuO}_2$ in Dogs
(Data from over 50 dogs)

Neg 0673019-10

FIGURE 4.12. Distribution of ^{239}Pu in Dogs After Inhalation of $^{239}\text{PuO}_2$

not equaled the amount in the lung and tracheobronchial lymph nodes. However, if the study continues long enough, it is possible that this could occur.

The relative concentration of plutonium in these tissues is of special interest because it indicates the relative radiation doses to the tissues. Examples of these values are shown in Table 4.3. The average concentration of plutonium was about an order of magnitude higher in the bronchial lymph nodes than in the lung. In most dogs, the average concentration was higher in the mediastinal lymph nodes than in the lung. The concentration of plutonium in the liver was about an order of magnitude less than in lung, and the concentration in bone about two orders of magnitude less. Based on the results to date, it can be concluded that the average concentration of plutonium in lung and tracheobronchial lymph nodes following inhalation of PuO_2 , having properties similar to that used in this study, will be much greater than in other tissue, and of course these two tissues will receive the greatest radiation exposures.

Development of Simulation Model for Inhaled Plutonium

A model is being developed to simulate the disposition of inhaled PuO_2 in the dog. The purpose of this is twofold. For one, the development of such a model facilitates maximum use of the experimental data already collected. Second, in developing such a model, the need for additional experimental data can be readily identified. We could also hope to avoid collecting needless data.

A dynamic simulation model for inhaled plutonium oxide was constructed using hybrid computer facilities. Blood, tissue, and excretion data collected from more than 50 beagle dogs up to 7 years after inhaling $^{239}\text{PuO}_2$ were incorporated into a program for predicting long-term retention and translocation of inhaled insoluble plutonium.

into the long-term inhalation model which predicts the long-term burdens for the deep lung, lymph nodes, and systemic organs after a single inhalation exposure. Figure 4.15 shows typical results from the long-term simulation, as well as the experimental points to which the model was fitted. Figure 4.16 shows the long-term simulation for three different types of lung clearance - varying from a half time of 2 years to an increasing half time of 1 to 4 years.

Neg 0673019-1

FIGURE 4.15. *Retention and Translocation of Plutonium in Beagle Dogs After $^{239}\text{PuO}_2$ Inhalation (Typical results from the long-term simulation model with experimental data superimposed on the curves.)*

The model has served to point out areas where additional data are needed. For example, experimental data are unavailable on the dynamics of plutonium entering the blood from the lung. Additional long-term data are needed to test compartment retention times and to verify the model.

This current long-term model for retention and translocation of inhaled plutonium oxide predicts that 15% of the plutonium deposited in the deep lung will remain in the deep lung

Neg 0672122-6

FIGURE 4.16. Long-Term Retention and Translocation of Plutonium in Beagle Dogs After $^{239}\text{PuO}_2$ Inhalation. Dynamic Model Based on Slow Pulmonary Clearance. (Three different pulmonary clearance models are simulated.)

and 60% will remain in the lymph nodes 15 years after exposure. This model also predicts that the whole body burden decreases less than 10% after the initial rapid clearance which occurs during the first 2 weeks after exposure. However, it is expected that these predicted values will be altered as more long-term data are incorporated in the model.

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LECTURE 5CELLULAR INTERACTIONS WITH PLUTONIUM PARTICLES*INTRODUCTION AND EXPERIMENTAL METHODS

To understand the disposition of inhaled plutonium in the body and the subsequent biological effects, it is helpful to know something about the cellular reactions to plutonium. Except for the work done by Dr. L. Casarett at the University of Rochester, little has been done to determine the response of a cell to the presence of a plutonium dioxide particle. Therefore, we initiated a study to qualitatively and quantitatively characterize the cellular processes involved in the initial distribution and subsequent fate of $^{239}\text{PuO}_2$ particles deposited in the lung. To develop our experimental techniques, we began by studying the peritoneal macrophage of the rat. Subsequently, studies of lung macrophages were developed. In both the lung and the peritoneal cavity macrophages are thought to be important vectors of clearance of foreign particles due to their ability to phagocytize particulate material and transport these particles to the lymphatics or to clear them from the site of deposition.

The experimental approach has been to deposit the plutonium dioxide in the peritoneal cavity or lung and then wash the cavity or lung at the appropriate time with saline to obtain suspensions of cells.

For the intraperitoneal experiments, female, Sprague-Dawley rats were injected intraperitoneally with 1 to about 20 μCi ^{239}Pu dioxide particles, suspended in 2 ml sterile, pyrogen-free saline.

The plutonium particles were prepared by calcining plutonium oxalate at 300 °C. The particles had a count mean diameter of 0.12 microns as measured from electron micrographs.

* *Principal Investigator: C. L. Sanders*

Less than 2% of the ^{239}Pu was lost by 2-hour dialysis or was found in the saline supernatant after 3 hours centrifugation at 5000 rpm.

Anesthetized rats were exsanguinated by cardiac puncture. The peritoneal cavity was washed with 10 ml saline. The saline wash was given by intraperitoneal injection and the abdomen massaged for one minute. The abdomen was then opened along the mid-ventral line and the intestines were allowed to fall from the abdomen into a beaker where the wash was collected. Peritoneal cells and plutonium particles in washings were removed by centrifugation.

The method of cell counting by DNA assay was adapted from Tedesco and Mellman. The total, nucleated, cell number in the wash was estimated on the assumption that nucleated cells contain an average diploid amount of DNA-deoxyribose.

For electron microscopy, cell pellets from peritoneal cavity washings at 15 and 60 minutes after injection of $19\ \mu\text{Ci}\ ^{239}\text{PuO}_2$ were fixed in cold 1% glutaraldehyde in 0.1M cacodylate buffer, pH 7.3, for 1 to 2 hours and post fixed in osmium tetroxide for 1 hour. The cells were dehydrated through a series of graded alcohols and embedded in Epon 812. Silver sections were stained with aqueous uranyl acetate and then with lead citrate and examined with a RCA EMU-4 electron microscope.

For the lung macrophage experiments, Sprague-Dawley female rats were exposed to aerosols of $^{239}\text{PuO}_2$ particles with count mean diameters ranging from 0.1 to 0.2 μm . Less than 0.5% of ^{239}Pu was in the ionic state, as determined by ultra-centrifugation and dialysis. At 15 minutes to 25 days, after a 5 to 15 minute aerosol exposure, rats were anesthetized and exsanguinated. The lung was removed and washed with 12 ml of saline. The wash was introduced with a syringe into the trachea and then collected by inverting the lung. Cells in the wash were isolated by centrifugation.

Both lung and peritoneal cells were resuspended in normal rat sera and smears prepared. For autoradiography, slides were coated with Ilford K-5 emulsion and exposed for 14 days. The percentage of particles which had been phagocytized by macrophages and the phagocytic index, i.e., the percent of macrophages with engulfed particles, were determined from autoradiograms. Two or more alpha tracks on autoradiograms, originating from the same point were considered as identifying a particle. The plutonium contents of washed tissues and selected washes were determined by dissolving samples in acid and counting in a liquid scintillation system.

To determine the amount of phagocytosis occurring in centrifuge tubes during isolation of cells and particles from washes, 0.1 μCi $^{239}\text{PuO}_2$ particles were added to lung washings from three unexposed rats and the cells were isolated for autoradiographic examination. Less than 4% of the particles were phagocytized and less than 5% of the macrophages had engulfed particles, indicating minimal, in vitro, phagocytosis of plutonium particles during the time required to prepare the smears.

PHAGOCYTOSIS OF PuO_2 PARTICLES

The macrophage is characterized by a large cytoplasmic volume as compared to the nuclear volume, irregularly shaped nucleus, abundant endoplasmic reticulum and ribonucleoprotein particles, numerous lysosomal bodies and cytoplasmic vesicles and an irregular cell surface, Figure 5.1. This macrophage is from the peritoneal cavity, but is similar in many aspects to the structure of macrophages found in the lung. Biochemically, there are some differences.

Plutonium particles were rapidly phagocytized by macrophages in both the peritoneal cavity and the lung. The percentage of plutonium particles phagocytized by peritoneal

Neg 0673470-3

15,000X

FIGURE 5.1. Normal Rat Peritoneal Mononuclear Phagocyte (N) Nucleus, (nu) Nucleolus, (ER) Endoplasmic Reticulum, (L) Lysosomal-like Bodies, (M) Mitochondria, (V) Vacuoles, (F) Finger-like Cytoplasmic Projections, (G) Golgi Apparatus

macrophages at intervals following intraperitoneal injection of plutonium particles is shown in Figure 5.2 (closed circles, after 1.4 μ Ci; open circles, after 9 μ Ci, 14 days and 3 days autoradiogram exposure, respectively). The amount of activity administered did not change rate of particle engulfment within macrophages.

Neg 0682562-1

FIGURE 5.2. Phagocytosis of $^{239}\text{PuO}_2$ Particles by Rat Peritoneal Mononuclear Cells After Intraperitoneal Injection (● 1.4 μCi ^{239}Pu , Δ 9 μCi ^{239}Pu)

The rate of phagocytosis of plutonium particles by alveolar macrophages is shown in Figure 5.3. The macrophages were obtained by saline washing of lung. These data were determined by autoradiographic techniques. However, the intracellular accumulation of particles determined by the technique of autoradiography from cytosmeears is an underestimate. Under the resolution of the light microscope, many particles accumulated within a phagosome, must be counted only as one particle. As will be shown, macrophages are capable of accumulating a large number of particles. From 20 to 50% of all plutonium particles found in the first wash were phagocytized by pulmonary

Neg 0683210-6

FIGURE 5.3. Phagocytosis of Inhaled $^{239}\text{PuO}_2$ Particles in Rat Lung. (Results derived from lung washes. Each symbol represents a different ^{239}Pu lung burden ranging from 0.01 to 0.3 μCi with the exception of those represented by the open triangles which were 1 to 4 μCi).

macrophages during the first 3 hours; over 80% and usually over 95% of these particles were found within macrophages after the first day. The rate of phagocytosis of plutonium particles in the lung during the first 3 hours was similar to that observed in peritoneal mononuclear phagocytes. Those particles falling on the mucus blanket lining the trachea, bronchi and bronchioles are mostly cleared from the lung and excreted in the feces during the first few days. Over half of the particles on

smears of lung washings taken during the first few hours had not been phagocytized. These particles which were not phagocytized have been initially deposited on the mucus lining of the trachea, bronchi and bronchioles.

INTRACELLULAR LOCALIZATION OF PLUTONIUM

The intracellular localization of plutonium particles within pulmonary macrophages was demonstrated by autoradiography of smears and lung sections. Figure 5.4 is an autoradiographic (light level) demonstration of the phagocytosis of plutonium particles by lung macrophages.

Plutonium particles were subcellularly identified and localized within macrophages by the technique of electron microscopic autoradiography, Figure 5.5. A large mass of plutonium is found in this cell, providing an obvious "star" formation of the electron microgram, comprised of tracks of reduced silver halide grains, representing the path of emitted alpha particles.

Figure 5.6 gives a more detailed view of several plutonium particles grouped within phagosomes near the cell surface. This is shown at the top. Below is an autoradiogram of particles in same configuration. The tracks confirm the identification of the mass of material in the cell as plutonium.

Plutonium particles are concentrated within phagosomes of macrophages. The amount of plutonium which can be accumulated within a macrophage is striking. The accumulation of plutonium within macrophages was seen in Figure 5.6. Figure 5.7 further illustrates this concentrating of plutonium particles in a macrophage. Insert is an electromicrogram of particles deposited directly on an electron microscopic grid from a saline suspension (1.4 μCi). Marked particle accumulation in macrophage, at 1 hour after 19 μCi injected, is shown in Figure 5.8. Even larger quantities of plutonium seem to be

Neg 0681872

- A. Section of lung removed at 7 days after inhalation, fixed in glutaraldehyde and embedded in epon. Autoradiogram, 14-day exposure, Richardson's Stain (12). Note alveolar macrophage in upper left-hand corner within an alveolus. 1300X

Neg 0681872

- B. Cytosmeared pulmonary macrophage isolated from lung lavage at 1 hour after inhalation of particles. Autoradiogram, 14-day exposure, Giemsa Stain. 1300X

FIGURE 5.4. Autoradiographic Demonstration of $^{239}\text{PuO}_2$ Particle Phagocytosis by Rat Lung Macrophage. A. Lung Section; B. Cytosmeared from Lung Wash

Neg 2689A

FIGURE 5.5. *Electronmicrogram of an Autoradiogram of a Rat Macrophage Containing ^{239}Pu Particles*

Neg 0673470-4

A. Plutonium particles are grouped within vacuoles (46,000X).

Neg 0673470-4

B. Autoradiograph of phagocyte showing reduced silver grains along path of alpha particles emanating from plutonium particles (18,000X).

FIGURE 5.6. Engulfed $^{239}\text{PuO}_2$ Particles in a Rat Peritoneal Phagocyte 2 Hours After Injection

Neg 0673470-5

34,000X

FIGURE 5.7. Engulfed $^{239}\text{PuO}_2$ Particles in a Rat Mononuclear Phagocyte 24 Hours After Injection. (The insert shows plutonium particles in the injected suspension.)

Neg 0680716-1

23,000X

FIGURE 5.8. Rat Peritoneal Mononuclear Phagocyte 1 Hour After Intraperitoneal Injection of $19\ \mu\text{Ci}\ ^{239}\text{PuO}_2$

in the cell in Figure 5.9. There is so much plutonium in the cell that shattering and breaks in the section occurred due to action of the sectioning knife striking hard plutonium particles compared to the softer embedding medium.

The structure of these cells appeared comparatively normal despite considerable radiation dose accumulating from such a large particle mass.

<u>Equivalent Particle Diameter (μm)</u>	<u>Dose to Phagocyte (rad/hr)</u>
0.5	40.0
1.0	225. *
2.0	1000. *
4.0	4000. *

KINETICS OF PLUTONIUM PHAGOCYTOSIS

Kinetic studies in the peritoneal cavity following injection of 1.4 μCi plutonium indicated that each phagocyte which had engulfed particles had concentrated them about 500-fold over the initial distribution found in the peritoneal cavity. These results are shown in Figure 5.10.

The amount of plutonium phagocytized per cell was calculated from measurements of the plutonium in the wash, the % of plutonium particles phagocytized, the total number of mononuclear cells and the fraction of cells containing plutonium. The accumulation of particles was highest at 30 minutes and then fell during the ensuing 3 hours to about half of the 30-minute value. During the period from 3 to 7 hours, the average active phagocyte contained the equivalent of about 500, 0.12 μm Pu particles.

* Significant self-absorption, value corrected for this error-dose is approximation.

Neg 0680716-2

23,000X

FIGURE 5.9. Rat Peritoneal Mononuclear Phagocyte 1 Hour After Intraperitoneal Injection of $19 \mu\text{Ci } ^{239}\text{PuO}_2$

Neg 0682562-5

FIGURE 5.10. *Calculated Average Equivalent Numbers of 0.12 μ Diameter Particles Phagocytized by Individual Rat Peritoneal Mononuclear Phagocytes After Intraperitoneal Injection of 1.4 μCi $^{239}\text{PuO}_2$*

Cell counts in washings of peritoneal cavity were determined by assaying for DNA using modification of Schneider's procedure (diphenylamine reaction following TCA precipitation).

Concentration of plutonium particles also occurred in lung macrophages, following inhalation of PuO_2 . Alpha tracks counted from individual macrophages could be related to particle diameter, which in this case would actually be the diameter of the total mass of plutonium accumulated in the cell. The phagocytic index (percentage of macrophages on cytosmear which

have engulfed particle(s) increased rapidly following inhalation of PuO_2 . The phagocytic index remained at a constant level for a few weeks and then tended to decrease (Figure 5.11). These changes were probably related in time with the amount of plutonium initially deposited in the alveoli. In dogs, one animal whose lung was washed 2 years after exposure had a P.I. of about 20%, indicating long-term retention of particles in macrophages; not in one macrophage but probably in a series of macrophages. Particles are probably transferred to other cells following death of individual cells.

Neg 0683210-5

FIGURE 5.11. Phagocytic Indexes of Pulmonary Macrophages Obtained from Rat Lung Washes After Inhalation of $^{239}\text{PuO}_2$

The number of macrophages phagocytizing plutonium particles was directly related to the amount of plutonium deposited in the lung (1 to 28 days after exposure). This is illustrated in Figure 5.12 which shows the correlation between the phagocytic index (%) and the log of the amount of plutonium present in the lung. The lung had been washed once with saline. Less than 10% of the plutonium present in the lung was removed by one saline wash. Each symbol represents data from one animal, sampled at from 1 hour to 25 days after inhalation of plutonium particles. The center solid line is the regression line (correlation coefficient, 0.91, for the line). The outer solid lines represent the 95% confidence intervals around the regression line. The outermost dashed lines represent the 95% confidence intervals for individual samples. The phagocytic index rose with increasing amounts of Pu in the lung. Thus it is possible to predict, within certain limits of confidence, the amount of plutonium present in the lung during the first few weeks following exposure by washing macrophages from the lung and determining their P.I.

A large fraction of the inhaled plutonium can be removed from the lung by serial washing of the lung with saline. In this experiment, Figure 5.13, rat lungs were washed 19 times. After the first few days, essentially all the plutonium removed by washing was within macrophages. Within a few hours after exposure, over 50% of the lung burden was removed from animals given 1 to 2 μCi plutonium by washing the lungs with saline. This value decreased rapidly with time such that only about 10% was available in washes after 2 weeks. In animals given only 0.1 μCi , from 40 to 65% of the lung burden was removed by serial washing during the first 40 days after exposure. This indicates that at the higher doses, significant damage to macrophages and lungs occurs such that particles more readily penetrate into the alveolar walls and thus cannot be removed

FIGURE 5.12. *Correlation Between Phagocytic Index (%) and Log of Amount of Plutonium Present in Rat Lung (Samples obtained from 1 hour to 25 days after exposure. Correlation coefficient is 0.91. Outer solid lines represent 95% confidence intervals around regression line and outer dashed lines represent 95% confidence intervals for individual samples).*

Neg 0683210-1

FIGURE 5.13. Amount of Plutonium Removed from Rat Lungs by 19 Lung Washings at Intervals After Inhalation of PuO_2

by washing. Such penetration is not so significant in the lower exposure groups.

Pulmonary lavage has proved useful in the treatment of certain lung disorders, particularly pulmonary alveolar proteinosis. The techniques of pulmonary lavage in living or killed animals offers an experimental method for the quantitative study of the deposition, phagocytosis, distribution and retention of inhaled radioactive particles. Also of significant interest is the possibility that pulmonary lavage may offer a practical method for the therapeutic removal of

insoluble radioactive particles accidentally deposited in the lungs of man.

The phagocytic index for peritoneal macrophages is given in Figure 5.14. A rapid rise was followed by an equally rapid fall in the fraction of macrophages containing plutonium particles in washes from the peritoneal cavity. Particles were essentially all intracellular after the second hour; thus macrophages must be going somewhere from the peritoneal fluid.

Neg 0682562-3

FIGURE 5.14. Percentage of Rat Peritoneal Mononuclear Cells That Have Phagocytized Plutonium Particles at Intervals Following Intraperitoneal Administration of $1.4 \mu\text{Ci } ^{239}\text{PuO}_2$

The plutonium contents of washings from the peritoneal cavity are shown in Figure 5.15. About 80% of the injected activity was removed by 2-10 ml saline washes during the first few minutes after injection. This value dropped during the next 7 hours to less than 10%, at the same time that there was observed a fall in the macrophages containing ingested plutonium particles.

After the first week, there was a rise in plutonium found in washes, which was associated with damage and fibrosis in the peritoneum at the site of particle transfer and accumulation.

Neg 0682562-2

FIGURE 5.15. Total ^{239}Pu in Washings of the Peritoneal Cavity at Intervals Following Intraperitoneal Injection of $1.4 \mu\text{Ci } ^{239}\text{PuO}_2$

(Figures 5.14, 5.15, 5.16, and 5.17, are from rats given 1.4 μCi PuO_2 .) Associated with the loss of plutonium activity in the washings was a concomitant gain in the plutonium content of the mesenteries and omentum (Figure 5.16). Over 50% of injected plutonium was found in these tissues by 7 hours. A loss of activity was observed at 1 week corresponding to a gain in activity in the peritoneal washings at this time.

Neg 0682562-4

FIGURE 5.16. ^{239}Pu Content of the Rat Omentum and Mesentery at Intervals Following Intraperitoneal Injection of 1.4 μCi $^{239}\text{PuO}_2$

Figure 5.17 is an autoradiogram of omentum tissue from a rat given 1.4 μCi PuO_2 by intraperitoneal injection. The plutonium particles in the omentum and mesenteries were specifically concentrated within lymphatic nodules studding the surface of these tissues. Loss of lymphocytes was observed at 1 week after injection of PuO_2 .

Neg 0680716-4

FIGURE 5.17. Autoradiogram of Omentum Tissue from Rat
Given 1.4 μCi $^{239}\text{PuO}_2$

ELECTRON MICROSCOPIC STUDIES

The structural alterations occurring in phagocytes which have ingested PuO_2 particles have been studied by light and electron microscopy. Figure 5.18 shows the changes observed at the light level. This is a section of an epon embedded pellet of peritoneal cells:

Neg 0673470-1

1100X

FIGURE 5.18. *Light Microscopic Views of Peritoneal Cells After Plutonium Deposition. Epon Section, Richardson's Stain.*

- A. *Control, showing mononuclear phagocytes (MP), and mast cell granules (MS).*
- B. *Two hours after plutonium deposition. Little change is seen at this time.*
- C. *Twenty-four hours after plutonium deposition. Note evidence of cytonecrosis (arrow).*
- D. *Seven days after plutonium deposition. Note marked swelling of cells, cytoplasmic vacuolization and large inclusions (arrows) within cells. This is almost a pure population of macrophages.*

FIGURE 5.18. (contd)

Figure 5.19 is an electron microgram of macrophages 7 days after PuO_2 deposition.

Neg 0673470-9

FIGURE 5.19. Electronmicrogram of Rat Peritoneal Macrophages 7 days After $^{239}\text{PuO}_2$ Deposition

- A. Acid phosphatase precipitate was used as an indicator of lysosomal activity in microbodies.
- B. Lysosomal body with numerous small plutonium particles found lining its periphery. Lysosome is quite large compared to controls.
- C. Myelin-like material accumulated in many macrophages; probably the result of peroxidation and destruction of membranous material; digestion of lipid fraction of cell membranes is slower than protein fraction, thus these figures will accumulate in damaged areas.
- D. Mitochondrial degeneration.

FIGURE 5.19. (contd)

Figure 5.20 is also an electron microgram of macrophages 7 days after PuO_2 deposition. Note large size, ingested cytoplasmic debris, large lysosomal bodies and cytoplasmic vacuoles.

Neg 0673470-8

FIGURE 5.20. Electronmicrogram of Rat Peritoneal Macrophages
7 Days After $^{239}\text{PuO}_2$ Deposition

Figure 5.21 shows, effectively, a dead macrophage containing plutonium particles. Loss of ribonucleoprotein particles and mitochondrial damage is also shown. Other changes include dilatation of endoplasmic reticulum; highly vesiculated with overall loss of electron opacity and separation of nuclear membrane. This cell was collected 1 hour after 19 μCi $^{239}\text{PuO}_2$ were injected.

Neg 0680716-3

FIGURE 5.21. Rat Peritoneal Mononuclear Phagocyte 1 Hour After Injection of 19 μCi of $^{239}\text{PuO}_2$

TESTS OF PHAGOCYTOTIC CAPABILITY

The macrophages are some of the most radioresistant of all the hematopoietic cells, demonstrated mostly in studies with external irradiation. We were interested in testing the phagocytic capabilities of the reticuloendothelial system following deposition of large quantities of PuO_2 . The phagocytic function of macrophages in the peritoneal cavity was determined by administering latex beads at intervals after injection of plutonium particles. The results are shown in Figure 5.22. Samples of cells were taken 7 to 14 days after deposition of $19 \mu\text{Ci } \text{PuO}_2$ and 1 mg latex beads.

Neg 0683669

- a. Engulfed latex beads and a large plutonium particle (arrow) within a phagocyte 1 hour after the simultaneous injection of 1 mg latex beads and $19 \mu\text{Ci } ^{239}\text{PuO}_2$ particles.
- b. Autoradiographic demonstration of phagocytized small plutonium particles in a cell also containing latex beads; 7 days after injection of $1.4 \mu\text{Ci } ^{239}\text{PuO}_2$ particles and 60 minutes after injection of latex beads.

FIGURE 5.22. Photomicrograms Demonstrating the Phagocytosis of Plutonium Particles, Latex Beads, and Cell Debris by Peritoneal Mononuclear Cells (Giemsa Stain)

Neg 0683669

- c. Large plutonium particle (arrow) found within a large cytoplasmic vacuole of a phagocyte; 7 days after injection of 19 μCi $^{239}\text{PuO}_2$ particles and 60 minutes after injection of latex beads. Note the whole cell and cell debris and the absence of latex beads.
- d. Engulfment of latex beads by phagocytes, 14 days after the injection of 19 μCi $^{239}\text{PuO}_2$ particles and 60 minutes after injection of latex beads. Note the whole cell and cell debris also present within these phagocytes.

Neg 0683669

- e. Same as d; note the engulfed large plutonium particle (arrow) and cell debris and the absence of latex beads.
- f. Same as d; note the large engulfed plutonium particle (arrow) and the destruction of the cell's nucleus.

FIGURE 5.22. (contd)

It was found that phagocytic function was not reduced at 14 days after administration of 19 μ Ci. Significant reduction in phagocytosis of latex beads occurred only in those phagocytes which had previously engulfed large plutonium particles. Thus, damage was seen only in individual macrophages; the cell system responded to such large doses probably by proliferation of macrophages in the peritoneum.

CONCLUSIONS

- Plutonium particles phagocytized rapidly; about 60 minutes within administration.
- The amount of particles phagocytized was nearly 90% or more in the peritoneal cavity and lung.
- The amount of activity administered, up to 19 μ Ci, did not prevent the subsequent phagocytosis of particles; blockage of particle uptake by macrophages was not observed.
- Particles were concentrated in macrophages; the net result is the creation of "hot spots" of intense alpha activity, more so than would be seen with only the limited range of 5 MeV alphas in tissue. This contributes to the already difficult problem of dosimetry.
- Particles are transported within macrophages to lymphatics. Mechanism in the lung has only been inferred by these studies in the peritoneal cavity.
- Particles not phagocytized by pulmonary macrophages are rapidly cleared. Lung clearance after the first few days is nearly all by pulmonary macrophages.
- The retention time of particles in macrophages (not necessarily in the same macrophage) is considerable; certainly of the order of several months.
- The distribution and retention of particles in macrophages appears to be related in time with the amount of plutonium deposited in the alveoli.

- Structurally the macrophage is more radioresistant than other cells found in the peritoneal fluid. Particles are found in large lysosomes at 1 week after injection. Functional (latex bead test) and structurally the macrophage exhibits a remarkable ability to ingest large amounts of plutonium and these cells also exhibit radioresistance.
- The behavior of plutonium particles in the body seems to be largely determined in the early phases by the kinetic behavior of macrophages.

LECTURE 6BIOLOGICAL EFFECTS OF INHALED PLUTONIUM*INTRODUCTION

The most needed information about the toxicity of a potential environmental contaminant such as plutonium is its low-level long-term effect on human life. Such information has to be derived from animal experimentation. Further, the long-term experiments required to provide this information are costly, difficult to perform, and even more difficult to interpret. The latter occurs because in such long-term studies there is opportunity for other factors to enter the picture, for example, common disease processes and extraneous environmental forces. Therefore, it is important to know the biological responses which can be attributed to a substance such as plutonium deposited in the respiratory tract. One of the objectives of our research program is to identify the biological effects which can be caused by inhaled plutonium. To do this we have investigated the biological response to very high doses of inhaled plutonium as well as those occurring at moderately low doses. Hopefully, these results can be extrapolated satisfactorily to the very low dose situations, those which man is most likely to encounter, until the results of the long-term low dose studies are available. In this discussion, I will review our knowledge of the biological effects of inhaled plutonium gained from numerous experiments with rodents and dogs.

Modes of Death

As in the case of most toxic materials, the criterion of greatest interest is the shortening of life span. Depending upon the dose, inhaled plutonium will shorten the life span. The mode of death varies from the acute to the long-term or chronic.

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Acute Toxicity

When we began these studies, it was generally believed unlikely that enough plutonium could be inhaled to cause acute toxicity and death. However, we have demonstrated two modes of acute toxicity and death. In the first, deposition of large amounts of plutonium in the lungs can cause death within a week. This acute respiratory death is characterized by severe inflammatory reaction, edema, hemorrhage, and generalized destruction of the functional tissue of the lung. The animal essentially drowns in its own fluids.

The second mode of acute death occurs at somewhat lower doses and at times ranging from about 1 month to several months after exposure. This mode of death differs from the first in that the development of extensive fibrosis is a contributing factor to the loss of functional tissue in the lung. Death is a result of respiratory insufficiency and is preceded by rapidly increasing respiratory rates and by high arterial blood CO_2 and low O_2 .

Subacute Toxicity

The third mode of death, subacute, is also a respiratory death but occurs at lower plutonium doses. The syndrome is similar to that of the second mode of acute toxicity except that fibrosis develops more gradually and death may occur from 1 year to 3 or 5 years after exposure. In this case the total volume of lung tissue irradiated is much less than in the two acute modes of death, but with time fibrosis initiated at the sites of irradiation progressively infiltrates the rest of the lung. Death is preceded for about 2 months by a rapidly increasing respiratory rate. In some cases there was evidence of repair taking place, because transient increased respiratory rates were observed at intervals over a 1- or 2-year period before the terminal stage.

Carcinogenic Death

Pulmonary carcinogenesis occurred in many dogs which died a respiratory death 3 to 5 years after exposure. However, carcinogenesis was the cause of death in a number of animals which survived beyond 5 or 6 years postexposure. Most of these animals showed fibrotic lesions as well, but enough lung tissue remained functional to maintain respiratory requirements.

Chronic or Long-Term Death

This mode of death has not yet been observed, but is speculative. It is defined as shortening of life by the more subtle effects of inhaled plutonium, acting on the lung, tracheobronchial lymph nodes and/or other tissues to which the plutonium is translocated.

DOSE-MORTALITY RELATIONSHIP

Sufficient data have been obtained to begin establishment of the quantitative relationship between the amount of plutonium deposited in the lung and mortality. These data provide an opportunity to attempt to extrapolate the results of animal studies to man.

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

In these studies, about 100 dogs were given a single 10 to 30 min exposure to $^{239}\text{PuO}_2$. In the earliest experiments, we found that dogs depositing more than 0.1 $\mu\text{Ci/g}$ of lung died within about a year due to respiratory insufficiency caused by severe irradiation injury of the respiratory tissues. The longer term effects have been studied in 40 dogs which deposited less than 0.1 $\mu\text{Ci/g}$ of lung. To date, 6 to 9 years after exposure, 25 have died or were sacrificed when death was imminent, and an additional five were sacrificed to obtain tissue distribution data. Seventeen of the 25 dogs held for duration of life have had primary pulmonary tumors, including

all dogs which survived as long as 4-1/2 years postexposure. Of the 10 dogs still alive, five show confirmed radiographic densities indicative of pulmonary tumors. In the animals with pulmonary tumors, plutonium body burdens at autopsy ranged from 2.7 to 0.8 μCi . Values for 14 dogs are shown in Table 6.1. Analysis of the tissues from the other three dogs were not completed. Some of the animals still alive, but showing radiographic evidence of lung tumors, have estimated body burdens as low as 0.2 μCi . The tissue distribution of plutonium in these dogs was discussed previously. The lungs contained 40 to 70% of the body burden, 20 to 40% was in the tracheobronchial lymph nodes, 7 to 15% in liver and 2 to 5% in the bone.

The tumors were classified as bronchiolo-alveolar carcinomas. One dog also showed a lymphangiosarcoma which appeared to originate in the vicinity of the capsule of a mediastinal lymph node. It is believed that the tissue distribution was altered by the pathology. This is illustrated in Figure 6.1 which shows the amounts of plutonium in the lungs and tracheobronchial lymph nodes of all dogs which were sacrificed or died during the course of this study. The data are expressed as percent of the body burden of plutonium at death. The fraction in the lungs dropped off almost linearly with time as the fraction in the tracheobronchial lymph nodes increased. There are several values for tracheobronchial lymph nodes, however, which do not fit the pattern established by the values from all the other dogs. The amounts in the tracheobronchial lymph nodes were less than what would be predicted from the other data. These dogs were the only ones showing extensive metastatic tumors in the tracheobronchial lymph nodes. Therefore, we suspect that the metastatic process may have either decreased the translocation of plutonium to the lymph node or caused the release of plutonium from the lymphatic tissue. Thus, developing pathology may have altered the disposition of the inhaled

TABLE 6.1. Bronchiolo-Alveolar Carcinomas in Dogs After Inhalation of $^{239}\text{PuO}_2$

Time of Death (Years)	Lung Burden (μCi)	Lung Dose (Rad)	Time of Death (Years)	Lung Burden (μCi)	Lung Dose (Rad)
3	0.7	5,000	4.7	0.6	7,000
3.3	1.1	7,000	5.0	0.3	3,000
3.5	1.0	11,000	5.5	0.5	10,000
3.7	1.3	14,000	5.5	0.4	9,000
4.4*	0.6	6,000	6.1***	0.2	7,000
4.4	0.4	5,000	6.5	0.4	8,000
4.4**	1.0	8,000	8.5	0.05	2,000

* Also bronchial carcinoma and lymphangiosarcoma in the lung

** Also capillary hemangioma in the lung

*** Also lymphangiosarcoma in a mediastinal lymph node

Neg 0693716-5

FIGURE 6.4. Autoradiograph of Section from Rat Lung Containing
 $2.5 \mu\text{Ci } ^{239}\text{PuO}_2$

Neg 0693716-6

FIGURE 6.5. Autoradiograph of Section from Rat Lung Containing
 $> .5 \mu\text{Ci } ^{238}\text{PuO}_2$

Neg 0693716-11

FIGURE 6.6. *Autoradiograph of Section from Rat Lung Containing
1.1 μCi $^{238}\text{PuO}_2$*

particle. This poses the question--which plutonium isotope is more toxic? Or, is it more hazardous for the total alpha energy to be dissipated in a small volume of lung tissue or distributed through a large volume of the lung? Acute toxicity tests have been completed in rats and dogs. The results are similar. Figure 6.7 shows the relationship between the amount of plutonium in the lung at death and the time of death for the two isotopes. There was no significant difference between the two isotopes in causing acute mortality. Similar results were obtained in dogs. Longer term studies are not complete. It is possible that over a longer period of time and at low dose levels the difference in volume of lung tissue irradiated and the difference in dose per particle could cause one or the other of the two isotopes to be more hazardous.

Neg 0682197-4

FIGURE 6.12. Arterial Blood Gas Analyses of Beagles After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

TABLE 6.3. *Arterial Blood Characteristics in Dogs After $^{239}\text{PuO}_2$ Inhalation*

Dog Number	Months Post-Exposure	O ₂ Content (ml/100 ml blood)	O ₂ Saturation (%)	CO ₂ Content (ml/100 ml blood)
Controls (6 dogs)	-	21.07 $\pm$ 1.85	94.19 $\pm$ 1.86	37.36 $\pm$ 1.02
182	30	12.73	49.61	46.01
215	35	19.12	95.12	51.98
	38	14.65	74.63	53.90
83	35	20.21	94.27	39.67
	40	21.65	91.47	41.30
173	38	19.20	95.24	55.00
	40	17.60	89.02	50.60
	42	18.00	89.55	51.60
	43	17.35	82.03	48.94
106	44	15.62	68.90	49.97

Effects of Inhaled $^{239}\text{PuO}_2$ on Body Weight.

Effects of Inhaled $^{238}\text{PuO}_2$ on Body Weight.

Neg 0682196-2

FIGURE 6.13. Effects of Inhaled $^{238}\text{PuO}_2$ on Body Weight

This has been determined by injecting the animals with 10^6 red cells from sheep and making subsequent measurements of antibody titer. An example of the results obtained in this test is shown in Figure 6.14. The rats had lung burdens of about $0.05 \mu\text{Ci } ^{239}\text{PuO}_2$. One week postexposure, there appeared to be an increased titer. This apparent stimulation was followed by a sharp drop which continued for 6 weeks more. This response has also been seen in dogs at both early and long times after exposure to PuO_2 .

Taurine Excretion

Another response to inhaled PuO_2 is an increased excretion of taurine in the urine. An example is shown in Figure 6.15. The increase of taurine in the urine showed a possible relationship to the decrease in circulating lymphocytes. It is thought that radiation-destroyed lymphocytes could give rise to the increased taurine in the urine. However, this theory has not been completely substantiated.

GROSS PATHOLOGY

Gross pathologic changes were severe in those animals which died following inhalation of plutonium. The lungs were firm, uncollapsed, did not pit with pressure and were not buoyant in formalin. Their surfaces were dark red and showed many small, creamy-white spots extending from 1 to 5 mm into the parenchyma. The cut surfaces were firm, dark red, and exuded serosanguineous fluid. The bronchi contained clear or blood-tinged fluid and froth. All the lungs were two to three times normal weight. The next two Figures illustrate the general gross appearance of lungs from dogs which have inhaled plutonium (Figures 6.16 A and B and 6.17 A and B).

The bronchial lymph nodes were generally small, indurated, and had dark centers. No other tissue showed gross changes, except for the enlarged hearts of some dogs which died of

Neg 0693716-52

A. Left Diaphragmatic Lobe

Neg 0693716-50

B. Right Diaphragmatic Lobe

FIGURE 6.16. Left and Right Diaphragmatic Lobes of Lung from a Dog 50 Days After Inhalation of $^{238}\text{PuO}_2$ (Lung burden $\sim 70 \mu\text{Ci}$)

Neg 0693716-51

FIGURE 6.17A. Gross Appearance of Lungs from a Dog 66 Months After Inhalation of $^{239}\text{PuO}_2$ (Lung burden $\sim 0.4 \mu\text{Ci}$)

Neg 0693716-49

FIGURE 6.17B. Left Diaphragmatic Lobe - Cross Section

cardiopulmonary insufficiency. The right ventricular wall of the heart appeared distended and the chambers were dilated.

Radiographs of dogs showed the developing fibrosis and tumors in the dogs which inhaled plutonium. Figure 6.18 is a radiograph which compares a control dog with one in which fibrotic lesions are extensive.

The development of a tumor is illustrated in the next three Figures. The thoracic radiograph showed a well demarcated, peripherally located tumor in the dorsal part of the right diaphragmatic lobe 50 months postexposure, Figure 6.19. The tumor more than doubled in size by 54 months postexposure (Figure 6.20). At 60 months postexposure, the tumor occupied nearly all of the right diaphragmatic lobe forcing the heart to be in contact with the left thoracic wall. On retrospective examination of the radiographs a small density was seen at the site of tumor formation 37 months postexposure, 23 months before death (Figure 6.21).

We are currently measuring the tumors on the radiographs to obtain an estimate of the rate of growth of the tumors. By extrapolation of the growth curves, we hope to obtain an estimate of the time the tumor growth began. Calculation of the radiation dose to the lung for this period rather than waiting until death should give a better dose value to relate to tumor incidence.

HISTOPATHOLOGY

A major objective of our plutonium studies has been to define the histopathology resulting from the inhalation of varying amounts of several plutonium compounds. The most extensive information has been obtained for $^{239}\text{PuO}_2$. Histopathologic changes occurring after the inhalation of other ^{239}Pu compounds and ^{238}Pu are being compared to the changes seen after inhalation of $^{239}\text{PuO}_2$. For this reason, $^{239}\text{PuO}_2$ will be emphasized in this discussion.

Neg 0682154-1

FIGURE 6.18. Lateral Radiograph of Control Dog (top) and Dog 75 Days After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$ (Lung burden $\sim 40 \mu\text{Ci}$)

Neg 38391-5

FIGURE 6.20. Lateral Radiograph of Dog
60 Months After Inhalation
of $^{239}\text{PuO}_2$. Tumor in
Right Diaphragmatic Lobe
(Lung burden $\sim 0.25 \mu\text{Ci}$)

Neg 38391-11

FIGURE 6.19. Lateral Radiograph of Dog
50 Months After Inhalation
of $^{239}\text{PuO}_2$. Tumor in
Right Diaphragmatic Lobe
(Lung burden $\sim 0.25 \mu\text{Ci}$)

Neg 38391-7

FIGURE 6.21. Lateral Radiograph of Dog 37 Months After Inhalation of $^{239}\text{PuO}_2$. Tumor in Right Diaphragmatic Lobe (Lung burden $\sim 0.25 \mu\text{Ci}$)

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

Histopathologic changes following inhalation of $^{239}\text{PuO}_2$ have been studied in dogs from immediately after exposure to about 9 years after exposure.

Immediately following particle exposure, there was a heavy, diffuse distribution of activity throughout the entire lung (Figure 6.22). By the seventh postexposure day, the conducting and respiratory lumina were devoid of activity; the remaining medium to large sized particles were located mostly in the walls of the terminal bronchioles and the smaller particles in the alveolar septa (Figure 6.23). The residual particles, tending to adhere to the less mobile parts of the moist intra-bronchiolar and alveolar surfaces, had been engulfed by macrophages or had penetrated the cytoplasm of the alveolar

Neg 0693716-12

82X

FIGURE 6.22. Diffuse Distribution of Plutonium Particles in Lung Immediately After Exposure (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-9

224X

FIGURE 6.23. $^{239}\text{PuO}_2$ Particles in Alveolar Septa 7 Days After Exposure (Autoradiograph, Hematoxylin and Eosin)

epithelial cells to reach the connective tissue beneath the alveolar epithelium. During this period, other than a slight septal cell proliferation and a moderate loss of mature lymphocytes in the bronchial lymph nodes, the lungs and lymph nodes of these animals appeared normal. This would be expected since the dose to the lung tissue at this time did not exceed 150 rad and was much less for the lymph nodes.

At 14 to 30 days postexposure, moderate alveolar septal cell proliferation ensued, accompanied by increased phagocytic pickup of the smaller particles (Figure 6.24). This activity was confined to the apparently fixed septal cells and was not seen in the alveolar macrophages. The larger particles continued to remain in the vicinity of the smooth muscle in the terminal bronchioles (Figure 6.25). With this interstitial concentration of the remaining particles, some of the small bronchioles showed "ballooning" and desquamation of the epithelial cells (Figure 6.26), and a few small foci of peribronchiolar fibrosis and lymphocytic and macrophagic infiltration. At 30 days postexposure, multiple small foci of septal, peribronchiolar and perivascular fibrosis, and acute inflammation were present. Macrophages were especially apparent in the alveolar lumina of the affected areas (Figure 6.27). These changes did not differ to any extent from those described by Warren for the mild and marked reactions of X-ray induced pneumonitis and were produced by comparable approximate doses of 700 to 1900 rad to the lung.

The bronchial lymph nodes of these dogs showed a continuing moderate to severe depletion of lymphocytes in the central areas, leaving a layer of normal appearing nodules in the periphery (Figure 6.28). This loss of lymphocytes resulted in the "unmasking" of background reticulum cells. The mediastinal nodes continued to appear normal. Particulate deposition in the bronchial lymph nodes did not occur to any great

Neg 0693716-10

224X

FIGURE 6.24. Phagocytosis of PuO_2 Particles by the Septal Cells, 14 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)

224X

FIGURE 6.25. Concentration of PuO_2 Particles in the Vicinity of the Muscular Wall of a Terminal Bronchiole, 30 Days Postexposure (Autoradiograph)

224X

FIGURE 6.26. "Ballooning" of Bronchiolar Epithelial Cells,
30 Days Postexposure (Hematoxylin and Eosin)

Neg 0693716-15

224X

FIGURE 6.27. Septal Fibrosis and Alveolar Macrophage Increase,
30 Days Postexposure (Hematoxylin and Eosin)

88X

FIGURE 6.28. *Moderate Depletion of Lymphocytes in the Medullary Cords of a Bronchial Lymph Node, 30 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)*

extent until after 7 days postexposure (Figure 6.29). This would seem to indicate that with the low concentrations of inhaled particulate matter used in this experiment, lymphatic translocation was not of importance during the first 7 days but became significant at 10 to 14 days postexposure in conjunction with increased septal cell activity.

By 65 days postexposure, septal cell radioactivity appeared to decrease while alveolar macrophage activity increased (Figure 6.30). Particles located within the terminal bronchiole walls, however, did not seem to vary in number or location with time. Septal and focal fibrosis became increasingly severe, resulting in a partial obliteration of the pulmonary architecture in some areas. The appearance of alveolar cell metaplasia was focal and moderate in amount, but was overshadowed by a

Neg 0693717-16

224X

FIGURE 6.29. *Medium Sized PuO₂ Particle in Medullary Sinus of a Bronchial Lymph Node, 7 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)*

224X

FIGURE 6.30. *PuO₂ Particles in Alveolar Macrophages, 65 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)*

mild to severe peribronchiolar squamous and bronchiolar-type metaplasia, the alveolar lining cells taking on the appearance of squamous or simple columnar epithelium (Figures 6.31 and 6.32). These metaplastic changes were especially prominent in the areas of heaviest deposition of radioactivity (Figure 6.33). Concurrent with these changes was the appearance of increasing amounts of activity in the region of the peribronchial and bronchiolar, and sub-pleural lymphatics, and in the bronchial lymph nodes (Figure 6.34).

The bronchial lymph nodes showed an extensive loss of lymphocytes, small scattered foci of necrosis, moderate to severe fatty change, small islands of residual lymphatic tissue, and many macrophages containing hemosiderin pigment (Figure 6.35). The mediastinal nodes were unaffected. During this period, the estimated radiation dose to the lung was from 4,000 to 13,000 rad.

224X

FIGURE 6.31. *Severe Peribronchiolar Squamous Metaplasia, 79 Days Postexposure (Hematoxylin and Eosin)*

224X

FIGURE 6.32. *Bronchiolar-Type Metaplasia 79 Days Postexposure (A dumbbell-shaped giant cell is seen in the center of the field. The lumina are filled with fibrin, macrophages and debris). (Hematoxylin and Eosin)*

Neg 0693716-13

224X

FIGURE 6.33. *Hyalinization of Alveolar Septum, 855 Days Postexposure. $^{239}\text{PuO}_2$ Particles Surround Affected Areas (Autoradiograph, Hematoxylin and Eosin)*

88X

FIGURE 6.34. *PuO₂ Particle Deposition in the Lymphatic Sinuses and Nodule Peripheries of a Bronchial Lymph Node, 14 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)*

88X

FIGURE 6.35. *Extensive Loss of Lymphocytes, Necrosis and Residual Lymphatic Tissue in Bronchial Lymph Nodes, 79 Days Postexposure (Hematoxylin and Eosin)*

At 124 through 384 days postexposure, there was extensive squamous and alveolar cell metaplasia present in groups of alveoli around some of the bronchioles. Fibrous thickening of alveolar septa and foci of advanced dense fibrosis were prominent. Hyperplasia with tufting of the epithelium could be seen in some bronchi (Figure 6.36). The epithelial cells revealed a marked variation in size and shape. In some bronchi the lining surface was either covered by a thin layer of flattened cells or, in the more advanced regions, was focally denuded of epithelium. Focal areas of alveolar fibrin "balls," neutrophil clusters, giant cells, and hemorrhage were present (Figure 6.37). A large pleural scar was seen in both the right apical and cardiac lobes (Figure 6.38) and numerous parenchymal scars were observed in the right intermediate and diaphragmatic lobes of the lung of the 384-day animal.

Neg 0693716-14

208X

FIGURE 6.36. *Bronchiolar Epithelial Hypertrophy, Edema and Inflammatory Cell Infiltration (Autoradiograph, Hematoxylin and Eosin)*

Neg 0693716-20

224X

FIGURE 6.37. Alveolar Hemorrhage, 120 Days Postexposure
(Hematoxylin and Eosin)

Neg 0693716-19

88X

FIGURE 6.38. Large Pleural Scar in Lung, 384 Days Post-
exposure (Hematoxylin and Eosin)

The bronchial lymph nodes showed large areas of lymphocytic depletion and replacement by reticulum cells, leaving only small residual lymphatic nodules. The medial bronchial lymph nodes were the most severely damaged, showing focal edema, hemorrhage, hemosiderin-filled macrophages, and necrosis that surrounded small peripheral islands of residual lymphatic tissue (Figure 6.39). By 855 days postexposure, severe septal and peribronchiolar fibrosis and alveolar cell metaplasia were prominent. Hemosiderin-filled macrophages were present in some of the more heavily fibrosed areas. There was a diffuse increase in alveolar macrophages, the appearance of a few giant cells in the alveoli, hyalinization of some of the alveolar septa (Figure 6.40), and a scattered infiltration of neutrophils around some of the terminal bronchioles. Early bronchopneumonia was apparent in the left cardiac lobe. Focal areas of pleural fibrosis, necrosis, and a slight fibrosis of the blood vessels were seen.

The bronchial lymph nodes of this animal showed a complete loss of lymphocytes with fibrous connective tissue replacement of the lymphatic tissue and fibrosis of the vessels (Figure 6.41). The mediastinal nodes, on the other hand, showed only a moderate loss of lymphocytes with concomitant fibrosis and an increase of reticulum cells.

The particles remaining in the lung tissue from 124 days postexposure on were primarily of medium to large size and were "trapped" by surrounding fibrous connective tissue. The earlier clearance of the smaller particle was in accord with previously published data. Subsequent necrosis of some of these fibrotic and scarred areas, however, released some of these "immobilized" particles and stimulated increased macrophage activity (Figure 6.42). The pleural and subpleural fibrosis noted in the lungs of some of these longer lived animals was suggestive of the fibrinous pleural adhesions seen

FIGURE 6.39.

88X

FIGURE 6.41. Complete Replacement of Lymphatic Elements by Fibrous Connective Tissue in Bronchial Lymph Gland, 855 Days Postexposure (Hematoxylin and Eosin)

Neg 0693716-17

224X

FIGURE 6.42. Necrosis and Scarring in Areas of $^{239}\text{PuO}_2$ Particles (Autoradiograph, Hematoxylin and Eosin)

in X-irradiation pneumonitis. However, the pleural scars noted in this experiment were associated with heavy depositions of radioactivity in the regions of the pleural lymphatics resulting in fibrous condensation of the lung parenchyma in that area (Figure 6.43). The total radiation dose to the lungs from 124 days postinjection on ranged from 5,000 to 12,000 rad.

There appeared to be a good correlation between the deposited activity and the pathologic damage observed in the bronchial and mediastinal lymph nodes. In general, the germinal centers were gradually replaced by fibrous connective tissue and reticulum cells (Figure 6.44).

88X

FIGURE 6.43. *Pleural Fibrosis Associated with a Heavy Deposition of PuO₂ in the Region of the Subpleural Lymphatics, 384 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)*

88X

FIGURE 6.44. *PuO₂ Deposition and Fibrosis in a Bronchial Lymph Node, 855 Days Postexposure (Autoradiograph, Hematoxylin and Eosin)*

With special staining techniques it was seen that at 55 days postexposure, there was a generalized thickening of the reticulum and elastic fibers in the alveolar walls (Figure 6.45) that markedly increased in intensity through 168 days (Figure 6.46). From 346 days on, there was a striking change in these fibers. They became thickened, clumped, fragmented, and presented a very disorganized appearance (Figure 6.47). Concurrent with these changes there was an elastotic degeneration of the collagen fibers, patchy deposits of fibrin, and an increase in PAS positive material in the alveolar walls, alveolar and bronchiolar lumina, and in the cytoplasm of the alveolar macrophages.

Seventeen dogs have evidenced bronchiolo-alveolar carcinomas. The tumors were peripheral or subpleural in location,

560X

FIGURE 6.45. *Thickening of the Reticulum and Elastic Fibers in the Alveolar Walls, 55 Days Postexposure (Gridley's Reticulum Stain)*

Neg 0693716-18

560X

FIGURE 6.46. *Marked Increase in Thickening of the Alveolar Reticulum and Elastic Fibers, 168 Days After Exposure (Gridley's Reticulum Stain)*

560X

FIGURE 6.47. *Clumping and Fragmentation of the Alveolar Reticulum and Elastic Fibers, 384 Days Post-exposure (Gridley's Reticulum Stain)*

and all were multicentric in origin, except in one dog where only one neoplasm in the right apical lobe was observed.

The lungs of these animals showed marked pleural, sub-pleural, parenchymal, and alveolar septal fibrosis and sclerosis. In addition, other large areas appeared to be undergoing consolidative processes, consisting of septal cell proliferation, alveolar macrophage increase, giant cell formation, hemorrhage, and varying degrees of fibroplastic response. Scattered throughout, in association with some of these fibrosed and sclerotic areas, were numerous foci of alveolar and bronchiolar type metaplasia. Transition from metaplasia to neoplasia could be seen in many of these regions as pleomorphic cells with epithelioid or fusiform characteristics infiltrated the surrounding parenchyma. In some instances, the neoplastic cells formed the basis of frond-like or papillary projections (Figure 6.48), while in others a bronchiolar

Neg 0693716-23

82X

FIGURE 6.48. *Bronchiolar-Alveolar Carcinoma with Papillary Character. (Hematoxylin and Eosin)*

or glandular structure was apparent (Figure 6.49). Ciliation was not seen in any of the metaplastic or neoplastic cells.

In the areas of bronchiolar or gland-like pattern, the cells often took on the appearance of squamous or glandular epithelium. Those areas with a papillary semblance, in contrast, have very anaplastic looking cells showing loss of polarity and nuclear displacement toward their luminal tips. Throughout the parenchyma of many of the lungs, small focal clumps of hyperchromatic, anaplastic cells could be seen, suggestive of nests of early neoplasia. In addition, some of the metaplastic foci showed gradational transition to a loose, streaming pattern of fusiform or spindle cells similar to those found in undifferentiated bronchiolar carcinoma (Figure 6.50). Mitotic figures, though not numerous, were present in all neoplasms.

Due to compression atelectasis of the surrounding lung parenchyma, there was a tendency toward encapsulation in some

Neg 0693716-24

82X

FIGURE 6.49. *Bronchiolar-Alveolar Carcinoma with Glandular Character (Hematoxylin and Eosin)*

Neg 0693716-22

208X

FIGURE 6.50. *Fusiform or Spindle-Like Cells (Hematoxylin and Eosin)*

of these tumors; however, encapsulation in most instances was not complete and there was extension of the tumor cells into the surrounding tissue. In the larger, more anaplastic neoplasms, focal areas of necrosis and calcification of the necrotic debris could be seen. Although local invasiveness was apparent in all of these tumors, metastases were found only in one animal. In this dog, metastatic foci from the primary lung tumors were seen in the middle and left bronchial lymph nodes (Figure 6.51). Extension to other tissues or organ systems, however, was not apparent.

The origin of these bronchiolo-alveolar tumors appeared to be from the bronchiolar and/or alveolar epithelium in all cases. In no instance could derivation be associated with bronchial epithelium or bronchial glands.

Neg 0693716-21

82X

FIGURE 6.51. Metastases of Primary Lung Tumor to Left Bronchial Lymph Node (Hematoxylin and Eosin)

Perivascular and peribronchiolar fibrosis was generalized and severe. Many of the pulmonary vessels showed a marked thickening of their media, intimal proliferation, and in the case of the arteries, fracture and disorganization of the elastica interna, and an increase in and vacuolation of the sub-endothelial connective tissue in the tunica intima.

Terminal bronchioles in these damaged areas either manifested a denudation or bizarre squamous forms in their epithelial linings.

Serial autoradiographs of these lung sections showed moderate numbers of radioactive particles associated with the desmoplastic and metaplastic processes (Figure 6.52). All of the neoplasms originated in peripheral lung areas of particle retention, fibrosis, and bronchiolo-alveolar metaplasia. The irradiated proliferating cells, comprising the metaplastic response to lung scarring, appeared to be the nidus of tumor formation.

Neg 0693716-25

224X

FIGURE 6.52. $^{239}\text{PuO}_2$ Particles in Region of Neoplasia (Autoradiograph, Hematoxylin and Eosin)

The bronchial lymph nodes of these animals were composed of dense, sclerotic connective tissue and were practically devoid of lymphoid elements. Masses of hemosiderin and anthracotic particle-bearing macrophages were prominent in the central portions of the nodes. Serial autoradiographs of these sections showed a heavy, diffuse distribution of radioactivity throughout the scarred parenchyma. The mediastinal nodes, on the other hand, evidenced varying degrees of fibrosis and sclerosis, dependent on their radioactive particle content.

$^{238}\text{PuO}_2$

The histologic response to inhaled $^{238}\text{PuO}_2$ has been compared to $^{239}\text{PuO}_2$ in both rats and dogs. The findings are similar in both species.

Gross pathologic examinations of sacrificed animals from high levels of either $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ showed essentially the same patterns and degree of damage for the two radioisotopes. There was frequently foam in the trachea, and the tracheobronchial lymph nodes were dark red and swollen. The lungs appeared mottled and were often hemorrhagic. This severe pulmonary congestion resulted in massive fluid accumulation in the lung tissue, probably due to radiation-induced leakage of blood serum through the alveolar capillaries.

Microscopically, however, certain differences were noted between the effects of these two isotopes. At 7 days post-exposure, the principal lung lesions in the $^{238}\text{PuO}_2$ animals consisted of alveolar flooding by an eosinophilic, proteinaceous fluid, and fibrinous plugs in the alveolar sacs and terminal bronchioles. Mononuclear inflammatory cells were prominent, with small foci of suppuration. Alveolar cell proliferation, though present, was only moderate in degree. Early fibrosis and septal thickening appeared to be minimal. In contrast, the $^{239}\text{PuO}_2$ animals at 7 days postexposure showed much

more extensive proliferative changes and alveolar macrophage formation than the $^{238}\text{PuO}_2$ animals. Evidence of fibrosis was also more. However, vascular fluid leakage appeared to be the same in the two groups.

At 21 days postexposure, there was extensive damage to the vascular system of the lung, accompanied by fibrinous organization of the material in the terminal airways. The extent of these deposits was greater in the ^{239}Pu rats than in the ^{238}Pu rats. Alveolar cell proliferation and septal thickening and fibrosis continued to be greater in the ^{239}Pu animals. In addition, one of the ^{239}Pu animals showed a focal area of bronchiolar hyperplasia and metaplasia in the lung periphery, typical of a pre-neoplastic lesion.

Autoradiographic studies of lungs from animals which inhaled either $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ showed heavy concentrations of particles toward the pleural surfaces and apexes of the lobes (Figures 6.53 and 6.54). Lung sections from ^{238}Pu -exposed animals showed some diffusely scattered single tracks which could be ionic material, or small particles (Figures 6.55 and 6.56). In general, the pathologic changes mirror the tissue distributions with time for ^{238}Pu and ^{239}Pu . Rats which inhaled $^{239}\text{PuO}_2$ aerosols showed responses of early pulmonary edema and infiltration of serum proteins which persisted over several months after exposure. The lungs of these animals also showed progressive hyperplastic proliferation of alveolar lining cells and increasing fibrosis up to a year after exposure.

Heavy depositions of particles of all sizes were found in the tracheobronchial lymph nodes of animals after inhalation of $^{239}\text{PuO}_2$. These sections showed marked hyperplastic proliferation of large mononuclear cells and large numbers of pigmented macrophages. The tracheobronchial lymph nodes of $^{238}\text{PuO}_2$ animals contained fewer particles in some cases but

Neg 0693716-26

25X

FIGURE 6.53. $^{238}\text{PuO}_2$ Particles in Rat Lung (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-28

25X

FIGURE 6.54. $^{238}\text{PuO}_2$ Particles in Rat Lung (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-27

63X

FIGURE 6.55. $^{238}\text{PuO}_2$ Particles in Rat Lung (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-29

63X

FIGURE 6.56. $^{238}\text{PuO}_2$ Particles in Rat Lung (Autoradiograph, Hematoxylin and Eosin)

these were much more radioactive. Examples of autoradiograms of dog tracheobronchial lymph nodes are shown in Figures 6.57, 6.58, and 6.59. The $^{238}\text{PuO}_2$ particles caused the same types of response, with mononuclear cell proliferation, including cords of plasma cells adjacent to sinusoids which were filled with macrophages. Erythrophagia was also present.

Other organs generally show little or no damage. However, exceptions are the spleens of $^{238}\text{PuO}_2$ rats. All of these animals showed a moderate lymphoid hyperplasia and an increase in plasma cells and macrophages in their spleens.

Autoradiographs of the femurs from animals which have inhaled ^{238}Pu show extensive translocation of ionic Pu from the lungs to the bone with time. This radioactivity appeared to be associated more with the endosteum and trabecular bone rather than cortical bone. This translocation of ^{238}Pu resulted in some marrow destruction with fibrous connective tissue deposition in the marrow spaces. There was also active remodeling of the bone architecture in these regions. In contrast, radioactivity was practically nonexistent in autoradiographs of $^{239}\text{PuO}_2$ animals.

The livers of animals which inhaled $^{238}\text{PuO}_2$ generally show single alpha tracks--from either ionic plutonium or very small particles. Occasionally, a larger particle is seen. Figure 6.60 is an excellent example. This would certainly lead one to believe that the transport of intact particles from the respiratory or perhaps gastrointestinal tract to the liver can occur.

Neg 0693716-32

125X

FIGURE 6.57. $^{238}\text{PuO}_2$ Particles in Dog Tracheobronchial Lymph Nodes (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-36

125X

FIGURE 6.58. $^{238}\text{PuO}_2$ Particles in Dog Tracheobronchial Lymph Nodes (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-33

63X

FIGURE 6.59. $^{238}\text{PuO}_2$ Particles in Dog Tracheobronchial Lymph Nodes (Autoradiograph, Hematoxylin and Eosin)

Neg 0693716-34

208X

FIGURE 6.60. Example of a $^{238}\text{PuO}_2$ Particle in Dog Liver (Autoradiograph, Hematoxylin and Eosin)

CONCLUSIONS

The biological effects of inhaled plutonium are manifest principally in three tissues: the lung, tracheobronchial lymph nodes and the circulatory lymphocyte. In our studies, death caused by inhaled Pu, whether PuO_2 or $\text{Pu}(\text{NO}_3)_4$, has always been due to irradiation of the pulmonary tissue and the resulting cardiopulmonary insufficiency and/or bronchiolo-alveolar carcinoma.

LECTURE 7

IN VIVO COUNTING OF PLUTONIUM IN DOGS*INTRODUCTION

Instrumentation for measuring plutonium in dogs and rodents was developed in our laboratory in about 1956. The dog scanning instrument was discussed in an earlier lecture. These instruments used a detector system of 2 in. by 1 mm thick, thallium-activated sodium iodide scintillation crystals. Using single channel analyzers, we looked primarily at the 17 keV X rays from plutonium. These instruments were very useful but we encountered difficult calibration problems associated with the high attenuation of the low energy X rays. We have not solved all of these problems, but considerable progress has been made in adapting a counter designed for human chest counting to in vivo counting of dogs. To understand the problems, factors important to successful in vivo counting of plutonium will be reviewed.

X- and Gamma-Ray Emissions from Plutonium

Gamma-ray emissions of 100 keV and at the 384 keV complex lack intensity for the detection of plutonium at maximum permissible body burden levels. For such low levels of plutonium, the significant radiations are the L X rays from uranium daughters. The 13.6, 17, and 20.2 keV X rays result from the internal conversion of gamma rays from excited states in the uranium daughters. For pure ^{239}Pu , about 4% of the alpha particle events produce X rays. Other X rays may arise when other plutonium isotopes are present.

 ^{241}Am as a Tracer for Pu

A small amount of ^{241}Am may also occur from the beta decay of ^{241}Pu , which has a 10-year half life. The ^{241}Am emits a 60 keV gamma which can be used as a tracer for plutonium in

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most cases; however, ^{241}Am also gives rise to L X rays which complicates the interpretation of X-ray data. The 60 keV photon is emitted at a rate of about 35.9% of the ^{241}Am alpha particle events. The 60 keV gamma of ^{241}Am is not as severely affected by variations in tissue distribution, tissue absorption, or surface contamination as are the L X rays. Other minor low energy radiations present include 39 keV (^{241}Am) and 52 keV (^{239}Pu).

Important to the detection and measurement of plutonium by in vivo counting of these photon emissions is knowledge of the isotopic composition of the contaminating material. It will change with irradiation time of the plutonium (Figure 7.1). The percentage of ^{239}Pu decreases with irradiation time as the higher isotopes increase. The composition will also change with time after removal from the neutron field (Figure 7.2). The buildup of ^{241}Am increases the total X-ray activity. If

FIGURE 7.1. Change in Isotopic Composition of Plutonium with Irradiation Time⁽¹⁾

FIGURE 7.2. *Change in Activity of a Typical Plutonium Sample as a Function of Time⁽¹⁾*

there is no separation of americium from plutonium, the buildup of ^{241}Am in the lung can be appreciable (Figure 7.3). The importance of these factors to the interpretation of in vivo counting data has stimulated further work in this area.

For our animal studies we have used scintillation counters rather than proportional counters. The relatively high efficiency of scintillation counters is an attractive feature. In addition they are more easily used for unanesthetized animals than proportional counters.

FIGURE 7.3. Ratio of $^{239}\text{Pu} + ^{240}\text{Pu}/^{241}\text{Am}$ in the Lung (Pulmonary + Lymph) as a Function of Time for an Initial Pulmonary Deposition of 13 nCi of Plutonium ($^{239}\text{Pu} + ^{240}\text{Pu}$) and 1 nCi of ^{241}Am for the Assumed ^{241}Pu Weight Percents in the Initial Deposition - for Oxide Inhalation Only⁽²⁾

BIOLOGICAL RELATIONSHIP BETWEEN Pu AND Am

An important aspect of our use of in vivo counters for our experimental animals is a study to determine whether ^{241}Am can be used as a tracer for plutonium. This study was also motivated by the need for this information to evaluate the results of in vivo counting of plutonium in humans. The objective was to determine whether plutonium and americium separated in the body after inhalation of various plutonium compounds containing ^{241}Am in varying amounts. This study was in two parts, radiochemical and biological. To obtain the biological information it was necessary to develop analytical techniques for plutonium isotopes and ^{241}Am in biological tissues.

Determination of Plutonium-to-Americium Ratios in Biological Samples

In the evaluation of the relative behavior of plutonium and americium in exposed animals, a very precise method of measurement is required. Two techniques were developed and are illustrated with spectra in Figure 7.4. In each, the plutonium and americium were not chemically separated from each other since this could result in preferential loss of one radioisotope, but direct counting techniques were employed which allowed their simultaneous measurement. In one case, following wet ashing, all salts were converted to chlorides and the plutonium and americium were electroplated from an ammonium chloride electrolyte. This method had typical plating recoveries exceeding 99% when there were no interfering trivalent elements present in the dissolved sample. Following electroplating, alpha pulse height analysis was used to measure the ratio of 5.15 MeV alphas to the 5.48 MeV alphas. The 5.15 MeV alphas are emitted by ^{239}Pu and ^{240}Pu while the 5.48 MeV alphas are emitted by both ^{241}Am and ^{238}Pu . The additional peak at 4.18 is due to ^{238}U which is added as a

carrier for electrodeposition. To use this method for plutonium-ameridium analysis, it is necessary that the ratios of the plutonium isotopes be known.

Neg 0673920

FIGURE 7.4. *Plutonium-Ameridium Measurements in Biological Materials⁽³⁾*

Photon spectrometry, the second method, illustrated in Figure 7.4, is preferable where it is applicable. Here, the plutonium X rays and the low energy ameridium gamma rays are measured with a recently developed lithium-drifted silicon

diode having approximately 1 keV resolution. The excellent resolution permits separation of the 13 keV, 17 keV, and 20 keV X-ray lines from plutonium decay, from the 26 keV and 60 keV gamma rays from ^{241}Am decay. The greater penetration of X rays allows direct counting of wet-ashed samples if corrections for self-absorption effects are made. These effects depend on the sample mounting technique and a very uniform mount must be used. Calibration curves for the corrections are made by preparing standards of known plutonium-ameridium content but with different weights of tissue ash.

The primary limitation of the X-ray spectrometry method is that present silicon diode detectors are small and the X-ray fluorescence yields for ^{239}Pu low. Therefore, the overall counter efficiency is rather low. Where sufficiently high levels of exposure have occurred, however, the technique offers the advantage that no chemical separation is required for measuring both absolute amounts of plutonium and ameridium and their ratios. Samples which could be analyzed by both methods gave results which were in good agreement.

Pu-Am Ratios in Dogs After Inhalation of Plutonium

The relative retention, translocation, and excretion of plutonium and ameridium are being studied under several conditions by comparing the ratio of ^{238}Pu and ^{239}Pu to ^{241}Am in dog tissues with the ratio in the aerosol sample collected at the time of an inhalation exposure. The following conditions are being studied:

- Dogs exposed up to 10 years ago to relatively pure $^{239}\text{PuO}_2$.
- Dogs exposed 3 months previously to one of four different types of PuO_2 , relative pure ^{239}Pu .
- Dogs exposed to $^{239}\text{Pu}(\text{NO}_3)_4$ and died within a year.
- Dogs exposed to $^{239}\text{PuF}_4$ containing higher isotopes of Pu and ^{241}Am . Three of six dogs were treated with DTPA.
- Dogs exposed to PuO_2 prepared by calcining oxalate enriched with higher isotopes of plutonium.

Preliminary results have been obtained. ^{239}Pu - ^{241}Am ratios in tissues of dogs sacrificed 3 months after inhalation of one of four different $^{239}\text{PuO}_2$'s are given in Table 7.1. These ratios are compared with the ratios in the sample of aerosol collected at the time of exposure. In dogs which inhaled these oxides, there was no evidence for separation of plutonium from ^{241}Am .

TABLE 7.1. 239 - ^{240}Pu -to- ^{241}Am Ratios in Dog Tissues 90 Days After Inhalation of Plutonium

Plutonium Compound	Aerosol	Lung	Tracheobronchial Lymph Nodes	Liver
$\text{Pu}(\text{NO}_3)_4$	38	70	50	12
PuF_4	6.5	5.5	5.5	--
	6.5	8.3	6.6	--
PuO_2				
1000° Oxalate	14	13	12	--
350° Oxalate	13	11	13	--
450° Metal	31	32	34	--
123° Metal	31	32	30	--
PuO_2 (Enriched)	1.9	1.9	1.8	--

The averaged results of a $^{239}\text{Pu}(\text{NO}_3)_4$ experiment are given in Table 7.1. In all dogs analyzed to date there was an increased ^{239}Pu - ^{241}Am ratio in lungs and lymph nodes and a decreased ratio in liver relative to the aerosol inhaled. This is tentatively interpreted as being due to relatively greater translocation of ^{241}Am to the liver from the lung than ^{239}Pu . The high ratios in the lymph nodes could be a result of ^{241}Am translocation from the lymph nodes, or the translocation may have occurred while the ^{241}Am was still in the lung before it had a chance to accumulate in the lymph nodes.

Data for inhaled $^{239}\text{PuF}_4$ are also shown in Table 7.1. The plutonium-ameridium ratios in the tissues were about the same as for aerosol. Therefore, separation of plutonium from ameridium appeared to be very slight.

Similar results showing no separation of plutonium from ameridium were obtained for PuO_2 which was highly enriched with ^{240}Pu , ^{241}Pu and ^{241}Am when the aerosol was inhaled.

Dogs which had been exposed to $^{239}\text{PuO}_2$ prepared by calcining the oxalate at 350° yield data for long times after exposure, up to 78 months. These data are shown in Table 7.2. The ratios of ^{239}Pu - ^{241}Am in the tissues were not greatly different from the aerosol samples. Possibly the liver shows a reduced ratio--greater accumulation and/or retention of ^{241}Am than ^{239}Pu . Additional data will be obtained from dogs exposed 5 to >8 years ago.

TABLE 7.2. $^{238}, ^{239}, ^{240}\text{Pu}$ -to- ^{241}Am Ratios in Dog Tissues After Inhalation of PuO_2

Dog Number	Time After Exposure (Months)	$^{238}, ^{239}, ^{240}\text{Pu}$ -to- ^{241}Am Ratios*			
		Aerosol	Lung	Lymph Nodes	Liver
268	40	12.6	12.8	10.5	
257	44	13.0	15.8	17.2	
259	54	13.6	13.4	11.4	
246	54	13.1	11.8	9.8	
216	60	13.1	15.4	15.6	7.5
85	66	13.1	15.4		10.6
86	67	13.1	6.3	13.3	
81	73	13.1	11.8	14.3	
212	78	13.2	14.3	9.0	

* $^{238}\text{Pu} = 1.1\%$

In these studies, there was little indication that ^{239}Pu and ^{241}Am were significantly separated in the body after inhalation of $^{239}\text{PuO}_2$ containing amounts of ^{241}Am . However, since ^{241}Am was clearly translocated to the liver more rapidly than ^{239}Pu after inhalation of $\text{Pu}(\text{NO}_3)_4$, it cannot be concluded that ^{241}Am is a valid tracer for plutonium in all cases.

IN VIVO COUNTING OF PLUTONIUM

Counter Design

The counter consists of 52 detectors, each composed of a photomultiplier optically coupled to a 1 mm thick NaI (Tl) crystal, Figure 7.5. The cleaved crystals are 1-3/4 in. in diameter, mounted on a quartz base and with a 1 mil aluminum window. These detectors are arranged into four arrays of 13 each. Each array is housed in a 6 in. x 12 in. by 7 in. deep stainless steel, light-tight box with a 10 mil mylar window. The anodes of the phototubes are connected in parallel and the signal is fed to a pulse-height analyzer. Counting is done in a lead cave or in a steel room. Dogs were counted in the position shown in Figure 7.5 or placed in plastic box on which two banks of crystals were mounted. This is shown in Figure 7.6.

Dog Phantom

A dog phantom was used to calibrate the counter. The phantom was fabricated by a commercial firm using the skeleton of one of our mature control dogs. Figure 7.7 compares the phantom with a live dog. The lung area was filled with a lung equivalent material and the phantom was divided into 33 2-cm thick sections containing a grid of holes on 2-cm centers. The phantom is shown with a section removed in Figure 7.8. The section is shown in Figure 7.9. The holes are shown filled with tissue-equivalent plugs. The plugs can be replaced by tissue-equivalent capsules containing a radionuclide standard.

*Neg 39326-9**(a)* *Neg 39326-10**(b)*

FIGURE 7.5. Scintillation Counter Used for Measurement of ^{239}Pu in Thorax of Dogs (52 Detectors in four arrays of 13 each.)

Neg 0690341-8

FIGURE 7.6. Scintillation Counter for Measurement of ^{239}Pu in Dog Thorax (Two arrays of 13 detectors mounted on opposing sides of dog-holding box.)

Neg 39326-1

FIGURE 7.7. Comparison of Dog Phantom with Anesthetized Dog

Neg 39326-5

FIGURE 7.8. Dog Phantom Showing Section Removed

Neg 39326-4

FIGURE 7.9. *Section of Thorax Removed from Dog Phantom [Tissue equivalent plugs (white) can be replaced with tissue equivalent capsules containing radionuclide standard sources.]*

Such a capsule is shown in the photograph. Figure 7.10 is a radiograph of two sections removed from the phantom thorax and shows the grid of holes and the extent of the lung-equivalent material. The tissue-equivalent plugs and capsules can be seen in the lung area.

Figure 7.11 is a ventral-dorsal radiograph of the phantom. The ribs and vertebrae can be identified. Also the stomach can be seen. Figure 7.12 is a lateral radiograph showing the rib cage.

Neg 39571-1

FIGURE 7.10. Radiograph of Two Sections Removed from Thorax of Dog Phantom Showing Dog Skeleton and Spaces Where a Number of Tissue Equivalent Plugs Have Been Removed (Fourteen radionuclide-containing capsules can be seen in the section on the right.)

Neg 39571-3

FIGURE 7.11. Radiograph of Dog Phantom - Dorsal-Ventral View

Neg 39571-2

FIGURE 7.12. Radiograph of Dog Phantom - Lateral View

Tissue and fat equivalent pads representing the addition of 2 kg of tissue can be used to adjust the phantom weight.

Figure 7.13 shows the phantom in the counting box with the two banks of detectors attached. Figure 7.14 shows the phantom in the box used for the scanning counter. The phantom is shown in a chest counter in Figure 7.15. This counter has four 2 mm x 5 in. crystals surrounding the chest. The background for this counter was about 50 cpm in the low energy region, 13.6 to 25.6 keV, and 200 cpm in the second, 13.6 to 66.3 keV. The lower detection limit for the 17 keV X-ray region is about 8 nCi and for the 60 keV photon region, the limit is about 7 nCi.

Factors Influencing In Vivo Counting

Bone shielding is a significant problem in detecting the 17 keV X rays. The half value layer for 17 keV X rays is

Neg 0690341-8

FIGURE 7.13. Dog Phantom in Dog Counting Box on Which 2-13
Detector Arrays are Mounted

Neg 0690341-9

FIGURE 7.14. Dog Phantom Being Placed in Counting Box for Longitudinal Scanning in Dog Counter

Neg 0690341-10

FIGURE 7.15. Dog Phantom in Dog Thorax Counter Consisting of Four 2 mm x 5 inch Crystals Arranged Around the Thorax Region

0.6 cm in tissue but only 0.03 cm in bone. Therefore, the skeleton is essentially opaque to 17 keV X rays. In the dog, the half value layer for a plutonium source in the chest was 1.3 cm. This value is higher than for tissue or skeleton because of the relatively low density of tissue in the chest.

Changing distribution of plutonium within the dog is an important factor. This occurs after inhalation of plutonium as it slowly accumulates in the tracheobronchial lymph nodes and more slowly is translocated to liver, etc. An example of how this changes the sensitivity of in vivo counting is shown in Figure 7.16. As the percent of the body burden accumulates in the lymph nodes, the sensitivity decreases. This Figure also shows that the sensitivity increases as more of the plutonium is translocated to the liver.

Neg 0651580-35

FIGURE 7.16. Effect of Distribution of ^{239}Pu in Dog Phantom on Counting Sensitivity

Table 7.3 shows a comparison between the X-ray sensitivity obtained for four dogs and the value obtained using the same plutonium distribution in the dog phantom. The close agreement illustrates the value of the phantom.

TABLE 7.3. Effect of Organ Distribution of ^{237}Pu on Calibration of Counter

Dog Number	Percent in Tissue				Calibration Factor	
	Lung	Bronchial Lymph Nodes	Liver	Other	Measured	Predicted From Phantom
76	47	45	3	5	2.1	2.14
183	52	38	6	4	2.1	2.19
213	49	27	15	8	2.3	2.35
272	63	17	14	6	2.45	2.42

Neg 0651580-3

Another factor of importance to in vivo counting was mentioned earlier. This is the isotopic composition of the plutonium. The effect of isotopic composition on sensitivity of counting is shown in Figure 7.17. Isotope A identifies a relatively old PuO_2 source in which ^{241}Am has grown. Isotope B was a relatively pure ^{239}Pu source. The sensitivity for detection of Isotope A was about twice that of Isotope B.

Whole body burden values obtained with the whole body counter have also been compared with values estimated from the analysis of excretion data. An example is shown in Figure 7.18. The whole body burden is expressed in microcuries. When the animal was sacrificed after 90 days and analyzed for plutonium, the body burden was found to be 7.5 μCi . The in vivo counter

Neg 0682155-1

FIGURE 7.17. Effect of Isotopic Composition
on Counting Sensitivity

Neg 0682155-3

FIGURE 7.18. Comparison of Whole Body Burden of ^{239}Pu Determined from Analyses of Excreta with that Obtained with Whole Body Counter

estimate was 7.8 μCi , an error of about 5%. In about 12 dogs which had body burdens ranging from 1 to 45 μCi , estimates obtained by counting the 60 keV region were within a few percent. Estimates obtained by counting the 17 keV region were generally higher than the actual analyzed burdens.

One of the causes for the largest errors encountered in in vivo counting of plutonium in experimental animals is external contamination. A very small amount of plutonium on the outer surface of skin is counted with a very high efficiency, relative to that within the animal because of the lack of shielding by body tissues. This is particularly troublesome in inhalation studies where it is very difficult to avoid small amounts of external contamination. Therefore, washing of the animal with detergent is generally required each time the dog is counted until levels in the excreta are so low that they constitute a negligible contaminating source.

SUMMARY

The use of in vivo counting for experimental animals has proved feasible in our laboratory. A number of factors have been identified which constitute potential problems to correct interpretation of the data. Among these are isotopic composition of the plutonium, distribution of plutonium within the animal, separation of plutonium from americium in the case of inhaled $\text{Pu}(\text{NO}_3)_4$, and external contamination of the animal.

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