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DEPOSITION PATTERNS AND THE TOXICITY
OF TRANSURANIUM ELEMENTS IN LUNG*

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Other than plutonium, very little information is available on the toxicology of transuranium elements in the respiratory tract. Thus, the title of this paper is somewhat misleading in that when referring to transuranic elements we shall be speaking primarily of plutonium.

There can be little doubt that the respiratory system is an important route of entry for plutonium compounds from nuclear weapons testing and accidental releases of plutonium into the atmosphere and that neoplasia is a potential consequence of inhaling plutonium.

Carcinogenesis. Deposition of radioactive compounds in the lung often creates special radiological conditions inasmuch as the distribution of radiation dose is non-uniform. Other than epidemiological studies of lung cancer in uranium and fluorspar miners, there are no data relating absorbed radiation dose from inhaled radioactive materials and tumor incidence in the human lung; in fact, there are no other data associating lung cancer with inhaled radionuclides in man. Evaluation of carcinogenic hazards from radioactive materials deposited in pulmonary regions must be extrapolated from data obtained in animal experiments.

The carcinogenesis of plutonium deposited in the lung has been the objective of several investigations. In the first demonstration of the carcinogenicity of plutonium in the lung, Temple et al (1960) produced two

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squamous cell carcinomas in 16 mice following an intratracheal injection of $^{239}\text{PuO}_2$ particles. About 50% of rats that survived 200-600 days post-exposure exhibited pulmonary neoplasms following intratracheal instillation of $^{239}\text{PuO}_2$ smoke (Lisco, 1959). The tumors were epidermoid carcinomas, adenocarcinomas and hemangioendotheliomas, and were usually associated with localized areas of plutonium induced fibrosis, mainly in the periphery of the lung. Pulmonary neoplasia has also been observed in dogs after inhalation of $^{239}\text{PuO}_2$. Of 35 dogs exposed to $^{239}\text{PuO}_2$, 32 have died 855-4068 days after exposure. In the dogs that died up to 1600 days after exposure, death was due to respiratory insufficiency from pulmonary fibrosis, edema and metaplasia. Twenty-four of the 29 remaining dogs (83%) have exhibited primary pulmonary neoplasia as the cause of death (Park, this symposium).

Several pulmonary studies with low levels of inhaled plutonium compounds have been reported in the Russian literature (Antonchenko et al, 1969; Erokhin et al, 1969). Control tumor incidences in their rat lungs was 0.39%. Pulmonary tumors were found in 4% of rats depositing 0.004 to 0.008 $\mu\text{Ci } ^{239}\text{Pu}$. The 4% incidence is probably significantly greater than controls since it followed a pattern of decreasing incidence which was as much as 20-30% at 0.040 to 0.080 $\mu\text{Ci/g lung}$. The induced tumors were mostly squamous cell carcinomas and adenocarcinomas. The lowest deposition that produced a biological effect in the lungs was 4 nanocuries/g of lung equivalent to a total radiation dose of 42 rads or 0.08 rads/day (Figure 1).

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The tumor incidence data from the Russian literature following inhalation of soluble plutonium compounds, from Yuile et al (1967) following inhalation of ^{210}Po by rats and from Park et al (this symposium) following inhalation of $^{239}\text{PuO}_2$ by dogs are plotted as a function of radiation dose to the lung (Figure 1). Significant tumor incidences are seen at accumulated average radiation doses of less than 100 rads to the lung. A 50% incidence may be expected at a dose of about 3000 rads.

In considering experimental dose-effect relationships it must be borne in mind that lung dose estimates are usually accumulative doses from the time of exposure to death or autopsy of the animal. The dose required to initiate uncontrolled malignant growth is less by an unknown amount than that which is conventionally estimated. The significance of these incidence figures is somewhat doubtful since the research effort required to define the relationship between dose and lung cancer incidence has not been made under conditions which allow comparison over the total dose range of interest.

Dose Distribution and Carcinogenesis. The number of cells in the lung irradiated by a given mass of plutonium dioxide particles will increase with decreasing size of the deposited plutonium particles. Because of the dissatisfaction with the ICRP technique for estimating lung exposure from inhaled alpha-emitting particles, the Los Alamos Biomedical Group has developed a theoretical approach intended to identify the variables which influence dose to a cell. The cell doses are compared with data on the radiation dose to an epithelial cell of the rat skin which is associated

Figure 1. Relationship of the average lung dose (rads) and the incidence of pulmonary neoplasms in animals following inhalation of alpha emitting radionuclides. The study of ^{210}Po in rats is from Yuile, Berke and Hull (1967), ^{239}Pu citrate and ammonium pentacarbonate in rats is from Antonchenko et al (1969) and Erokhin et al (1969) and of $^{239}\text{PuO}_2$ in dogs is from Park et al (this symposium). The incidence of pulmonary neoplasms in non-exposed control animals was less than 1% in all groups.

with a given skin cancer incidence during the life-span of the rat (Dean and Langham, 1969). They then estimate the tumor probability for pulmonary cells exposed to various sized PuO_2 particles totaling 16 nanocuries in activity. According to their model, concentration of plutonium in larger but fewer particles, would increase the probability for tumor formation. In our dog studies fibrotic zones in the lung periphery were associated with plutonium concentrations. These fibrotic areas seemed to serve as the nidus for future neoplastic development with epithelial metaplasia in these areas being a possible, preneoplastic, intermediate stage (Clarke et al, 1966). The study in rats by Sanders and Park (1971) in which rats were given focal deposits of $\text{Pu}^{239}\text{O}_2$ by transthoracic injection appears to bear out the validity of these observations in the dog. Epithelial metaplasia was seen early in this study, prior to the development of neoplasms, and was seen in all animals with neoplasms. The type of metaplasia and neoplasia in dogs and rodents argues strongly for the epithelial, either alveolar or bronchiolar, origin of the observed tumors. These aforementioned studies indicate that the most effective method for producing lung cancers with plutonium may be to limit the irradiated volume of the lung. However, we do not know if there is a minimum radiation dose or a critical mass of tissues; also unknown is the species sensitivity and the time required in the various mammals for induction of neoplasms.

Structural Alterations. Many of the ultra-structural alterations in the lung of rats which have inhaled $^{239}\text{PuO}_2$ particles (Sanders et al, 1971) are similar to those described following external irradiation of the lung (Goldenberg et al, 1968; Garbagni, 1964; Phillips, 1966). The alveolar

alterations following inhalation of $^{239}\text{PuO}_2$ were progressively more severe with increasing lung doses ranging from 300-13,000 rads and were similar in many aspects to those previously described with the light microscope (Clarke et al, 1966). There was an increase in the number of Type II alveolar epithelial cells and in the size and number of their osmiophilic inclusions, occurring during the middle phase of the developing pneumonitis. Other general features were characterized by the accumulation of a membranous-proteinaceous exudate in the air spaces, accumulation of lipid in the septal walls, severe disruption of the endothelium and accumulation of collagen, elastin, fibroblasts, plasma cells and mast cells in greatly thickened septal walls (Sanders et al, 1971). Compared to Type II epithelium, the Type I alveolar epithelial cells remained remarkably intact, exhibiting only a few areas of focal cytoplasmic disruption. Generalized edema and swelling of Type I cells were observed about one week prior to the death of the animals. The response of the Type II epithelium is particularly interesting since these cells may serve as precursors for epithelial neoplasms induced by plutonium (Clarke et al, 1966; Howard, 1970).

Depressed Macrophage Function. Plutonium interferes with the normal cellular processes in the lung; among the most important are those associated with alveolar macrophages. Among the alterations in macrophage function attributable to plutonium are a decreased incorporation of ^3H -thymidine into macrophages (Masse, 1971), reduced phagocytosis of latex test particles by $^{239}\text{PuO}_2$ laden macrophages (Sanders, 1970) and a depressed clearance of iron oxide dust following a prior exposure to ^{239}Pu aerosol (Nenot, 1971).

Inhaled plutonium also exhibits a cytocidal action on macrophages. In rats, following a pulmonary deposition of about 10 nanocuries/g lung, there was observed a decrease in the number of saline extractable alveolar macrophages to about 65% of control numbers at 300 days after inhalation of $^{239}\text{PuO}_2$ particles (Table 1). This indicated a long-term cytocidal effect of PuO_2 on macrophages.

TABLE 1

Effect of a single inhalation of $^{239}\text{PuO}_2$ particles on the number of mononuclear cells removed from the lung by 19-10 ml saline washings. About 10 nanocuries were deposited in the alveoli of rats.

<u>Age of Animal (Days)</u>	<u>Time After Inhalation (Days)</u>	<u>Number of Animals</u>	<u>Number of Mononuclear Cells in Lung Washings X 106</u>
90	---	5	10.15 ± 2.07
400	---	5	9.93 ± 1.88
110	20	10	10.32 ± 2.37
240	150	5	8.09 ± 2.50
290	200	5	7.36 ± 2.11
340	250	5	6.80 ± 1.20
390	300	5	6.71 ± 1.35

Long-Term Clearance. Long-term clearance is the summation of several physiologic processes such as the transport of plutonium compounds to the ciliated epithelium, the solubilization of particles and the transport of particles within lymphatic and blood capillaries. Because all these processes are occurring simultaneously, total clearance cannot be described by first order kinetics.

Plutonium is peculiar compared to most inhaled materials in that it exhibits such a tenacious retention in the lung. Only for the case of PuO_2 in the dog is there a sufficient body of long-term data to warrant detailed consideration (Table 2). Stuart et al (1970) have employed hybrid computer to model the data from more than 60 beagle dogs surviving as long as 11 years after inhalation of $^{239}\text{PuO}_2$ particles. They find that a biological half-life of 4 years best describes the retention of 85% of the pulmonary deposited plutonium. The alveolar retention of PuO_2 in the mouse is somewhat similar to that in the dog (Bair et al, 1961). The more soluble plutonium compounds exhibits retention times 30-50% of that observed for PuO_2 . The much less extensive animal data for soluble compounds suggest that they are lost more rapidly from the lung, deposited more quickly in bone and liver and to a lesser degree in lymph nodes than is PuO_2 . Differences in alveolar retention may also be attributed to variations among the animal species studied and to the particle sizes administered to these animals (Table 2).

The immobilization of Pu-laden macrophages in the alveoli, the rapid engulfment of Pu by the alveolar epithelium, the transport of Pu into the pulmonary lymphatic system and the sequestration of Pu in fibrotic zones

TABLE 2.
Long-term clearance of alveolarly deposited inhaled plutonium compounds

<u>Animal Species</u>	<u>Chemical Form</u>	<u>MMD</u>	<u>Particle Size(μm)</u>	<u>Retention Half-Time(Days)</u>	<u>Reference</u>
Mouse	$^{239}\text{PuO}_2$	---	0.2 CMD	460	Bair et al, 1961
Dog	$^{239}\text{PuO}_2$	---	0.6 CMD	1600	Bair and McClanahan, 1961
Dog	$^{239}\text{PuO}_2$	2.8	0.51 CMD	780	Bair and Park, 1968
Dog	$^{239}\text{PuO}_2$	2.8	0.45 CMD	370	Bair and Park, 1968
Dog	$^{239}\text{PuO}_2$	4.8	0.50 CMD	840	Bair and Park, 1968
Dog	$^{239}\text{PuO}_2$	1.3	0.46 CMD	690	Bair and Park, 1968
Dog	$^{238}\text{PuO}_2$	1.12	0.05 CMD	~300	Park et al, 1970
Dog	$^{238}\text{PuO}_2$	1.1-2.9	---	270-320	Morrow et al, 1967
Dog	$^{239}\text{PuO}_2$	0.5-1.5	---	~400	Morrow et al, 1967
Rat	^{239}Pu Citrate	---	---	173	Lyubchanskii, 1967
Rat	Sodium ^{239}Pu -Triacetate	---	---	209	Lyubchanskii, 1967
Rat	Ammonium ^{239}Pu -Pentacarbonate	---	---	122-167	Lyubchanskii, 1967
Rat	$^{239}\text{Pu}(\text{NO}_3)_4$	---	---	212	Lyubchanskii, 1967
Rat	$^{239}\text{PuCl}_3$	---	---	169	Lyubchanskii, 1967

of pulmonary tissue may account for its relatively long residence time.

Translocation to Pulmonary Lymphatics. The ratio of the amount of inhaled plutonium in the lung to that in the pulmonary lymph nodes does not appear to be dependent on the animal species, the amount of activity deposited in the lung or the solubility or particle size of the inhaled plutonium compound, but is only a function of the time after exposure to the aerosols. Less than 10% of the terminal body burden of inhaled $^{239}\text{PuO}_2$ was found in pulmonary lymph nodes during the first year after exposure whereas more than 50% may be found in these nodes after 5 years (Figure 2).

Initially, plutonium particles in lymph nodes are located mostly in macrophages found in the medullary sinuses, and later move into cortical regions (Bair, Willard and Temple, 1961; Clarke and Bair, 1964). There is a suggestion from the data of Morrow and Casarett (1961) that the smallest particles are the most tenaciously retained in the lung parenchyma while the larger particles are selectively translocated to the lymph nodes.

Transuranics Other Than Plutonium. Plutonium-239 contains significant quantities of ^{241}Am as a contaminant. Following inhalation of $^{239}\text{PuO}_2$ by beagle dogs, the ^{241}Am was found to be excreted faster in the urine with a relatively greater translocation to liver and less to pulmonary lymph nodes than for ^{239}Pu (Bair and Perkins, 1968). The excretion of ^{241}Am in the urine of humans accidentally exposed to an $^{239}\text{PuO}_2$ aerosol, during a 6 month period of observation, was greater than for ^{239}Pu , even though the amount of inhaled ^{241}Am was considerably less than ^{239}Pu (Hammond, Lagerquist and Mann, 1968). Following inhalation exposure of rats to ^{241}Am or ^{242}Cm

Figure 2. The relationship of the amount of plutonium present in the lung to the amount of plutonium present in pulmonary lymph nodes as a function of time after inhalation of various plutonium compounds in the rat and the dog.

salts, 15-45% of the activity deposited in the alveoli was cleared within 15 minutes. Plutonium-239 was retained in the lung considerably longer than either ^{241}Am or ^{242}Cm (Nenot et al, 1971). In another experiment, dogs were exposed to aerosols of $^{244}\text{CmCl}_3$, or $^{244}\text{CmO}_2$. Curium-244 was rapidly translocated to liver and skeleton such that by 65 days, skeleton, liver, and lung ranked in decreasing order as fractions of terminal body burdens for ^{244}Cm , respectively. No significant differences were seen in the tissue distribution of ^{244}Cm when administered either as the dioxide particle or the chloride forms (Gallegos et al, 1970). These studies indicate that the skeleton and not the lung is the tissue which receives the greatest radiation dose from inhaled americium and curium.

Cellular Distribution of Dose: Alveolar Epithelium. The fundamental mechanisms of alveolar clearance and redistribution and therefore the distribution of radiation dose from inhaled Pu, have to do with the degree and rapidity of the phagocytosis of inhaled particles in the alveoli. Casarret and Milley (1964) postulated that alveolar epithelial cells were phagocytic and serve as at least one source of alveolar macrophages. This assumption has been proven partly correct by the studies of Sanders et al (1970, 1971) who demonstrated the endocytic uptake of inhaled $^{239}\text{PuO}_2$, Cr_2O_3 and NiO particles by the Type I alveolar epithelium.

The hypothesis that alveolar epithelial cells, once having phagocytized particles, will then desquamate and be cleared out the lung like a macrophage (Casarret and Milley, 1964) has not been proven. Fine structural studies of the lung at radiation doses up to 13,000 rads from inhaled $^{239}\text{PuO}_2$,

have demonstrated the relative radioresistance of the Type I epithelium. No evidence of Type I cell detachment or removal was found. On the other hand, Type II alveolar epithelial cells were very reactive, often exhibiting proliferation, desquamation and probable removal out to the ciliated bronchioles. However Type II epithelial cells do not phagocytize inhaled or intratracheally injected ink, latex, $^{239}\text{PuO}_2$ or other metal oxide particles (Sanders et al, 1970, 1971; Esterly and Faulkner, 1970) and thus are not directly involved in the clearance of particles from the lung. It is thus probable that once PuO_2 particles are taken up by Type I alveolar epithelium, they are difficult to physiologically remove and may thus account for a portion of the long retention time of PuO_2 in the lung.

Cellular Distribution of Dose: Alveolar Macrophage. Alveoli contain reticuloendothelial cells (RE) derived from circulating monocytes which originate from the bone marrow (Pinkett et al, 1966). These consist of mononuclear cells and histiocytes in the septal walls and alveolar macrophages in the air spaces. The passage of alveolar macrophages with engulfed particles to the ciliated epithelium constitutes a prime mechanism by which particles are cleared from the alveoli.

Inhaled $^{239}\text{PuO}_2$ particles deposited in the alveoli are phagocytized by alveolar macrophages within a few hours after exposure (Sanders, 1969). Subcellular studies of plutonium deposits in Kupffer cells (Rahman and Lindenbaum, 1964), in peritoneal macrophages (Sanders and Adey, 1969) and in alveolar macrophages (Sanders and Adey, 1970) have demonstrated the localization of plutonium particles in phagolysosomes of these RE cells.

Figure 3. The cumulative removal of inhaled $^{239}\text{PuO}_2$ and alveolar macrophages in lung washings as a function of the number of successive lung washings. Female albino rats received an initial alveolar lung deposition of less than 30 nanocuries. The amount of plutonium removed in all the washes amounts to about 45-50% of the lung burden. Animals washed at 1-34 days after exposure.

The fraction of alveolar macrophages engaged in phagocytosis of inhaled plutonium particles is proportional to the amount of plutonium deposited in the deep lung, with up to 80% of alveolar macrophages in pulmonary washings containing one or more PuO_2 particles at lung burdens of greater than $1.0 \mu\text{Ci}/\text{gram lung}$. Over 95% of PuO_2 particles in the alveoli after the second day are intracellular, mostly within macrophages (Sanders, 1969).. Based on the ability to remove plutonium in lung washings and the presence of nearly all PuO_2 in extractable macrophages, it was estimated that over 50% of the lung burden was within alveolar macrophages during the first few weeks after exposure. Autoradiographic examination of washed lung showed numerous remaining macrophages containing PuO_2 particles still present in the alveoli. Extrapolation of a curve plotting cumulative removal of plutonium in washings versus number of lung washings, performed during the first week after deposition of less than $0.1 \mu\text{Ci PuO}_2/\text{gram lung}$, indicated that approximately 70% of the plutonium lung burden could be removed within alveolar macrophages by about 50 consecutive lung washings (Figure 3). The remaining lung plutonium activity was found within Type I alveolar epithelium, in peribronchiolar vascular areas and in pulmonary lymphatics.

Cellular Distribution of Dose: Influence of PuO_2 Lung Burden. Much of the experimentation with inhaled plutonium in animals has been performed by depositing relatively large amounts of plutonium in the lungs. The fate of plutonium deposits in the lung may be quite different at comparatively high lung burdens than at lower burdens. We have carried out experiments in rats exposed to $^{239}\text{PuO}_2$ aerosols, depositing various amounts

of ^{239}Pu in their lungs. The count median diameters of the PuO_2 aerosols ranged from 0.11-0.13 μm . Following an average lung deposition of 0.03 $\mu\text{Ci } ^{239}\text{PuO}_2$, there was an equivalent count median diameter for PuO_2 in macrophages of about 0.12 μm for macrophages washed from the lungs at 1-3 hours or 11-15 days after exposure to the plutonium aerosol. The equivalent PuO_2 particle diameter in an extracted alveolar macrophage was determined using the technique of particle sizing by counting alpha tracks on autoradiograms of cytosmeears. It was assumed that only one PuO_2 particles of diameter, d , was present in a macrophage, even though many particles may be concentrated in phagolysosomes of RE cells (Sanders and Adee, 1969; Sanders, 1969). Increasing the plutonium lung burden greatly increased the concentration of plutonium in individual macrophages (Table 3).

The retention time of PuO_2 within macrophages was influenced both by the time after exposure and by the amount of plutonium deposited in the lung. Since greater than 95% of PuO_2 in alveoli is within macrophages after the first day, we have been able to estimate retention time for PuO_2 in macrophages from lung washing data. At a mean lung burden of 1.7 $\mu\text{Ci/gram lung}$, the retention half-life for PuO_2 in macrophages was only about 3 days, while at a mean lung burden of 0.06 $\mu\text{Ci/gram lung}$, it was estimated at greater than 30 days (Figure 4).

The clearance of inhaled $^{239}\text{PuO}_2$ from the alveoli exhibited a readily discernable biphasic pattern in the group with less than 0.1 $\text{PuO}_2/\text{gram lung}$. The early alveolar clearance phase was completed within 30 days, followed by a much slower alveolar clearance phase. Less early alveolar

TABLE 3

Influence of the amount of alveolar $^{239}\text{PuO}_2$ on the concentration of PuO_2 in alveolar macrophages. Alveolar macrophages were removed from rat lung at 1-15 days after inhalation of PuO_2 by one 12 ml saline washing. Autoradiograms of macrophages on cytospins were exposed for 14 days. Only alpha tracks passing outside the cell were counted.

Mean Distribution in Each Class (%)

<u>α Tracks per Macrophage</u>	<u>Mean Amount of ^{239}Pu in Lung (μCi)</u>		
	<u>0.03</u>	<u>0.4</u>	<u>1.7</u>
2-5	51.3	18.5	8.2
6-30	39.8	36.1	25.4
>30	8.9	45.4	66.2

Figure 4. Influence of the amount of inhaled plutonium deposited in the lungs of rats on the ability to remove plutonium in 19 successive lung washes as a function of time after exposure. Less than 0.1 uCi/gram lung of plutonium was deposited in the low exposure group, while more than 1.0 uCi/gram lung of plutonium was deposited in the high exposure group. Each point represents the cumulative amount of plutonium found in one animal.

Figure 5. Influence of the amount of plutonium deposited in the lung on the rate of alveolar clearance from the lung as a function of time after inhalation of $^{239}\text{PuO}_2$. The first data points are at one day after exposure. Each point represents one rat.

AMOUNT OF PLUTONIUM IN THE LUNG
(μCi)

clearance was seen following a mean alveolar deposition of about 0.4 μCi PuO_2 /gram lung, while essentially no early alveolar clearance was seen following deposition of greater than 1.0 μCi /gram lung (Figure 5). The fraction of lung plutonium cleared from the alveoli during the first 30 days after exposure was 6-8 times greater following deposition of less than 0.1 μCi /gram lung than following depositions of greater than 1.0 μCi /gram lung. Thus there is a very significant difference, when comparing 0.1 μCi /gram and 1.0 μCi /gram lung, in the early distribution and fate of inhaled $^{239}\text{PuO}_2$ particles. At less than 0.1 μCi /gram lung there were fewer macrophages with engulfed PuO_2 particles, smaller mean equivalent PuO_2 size per macrophage, a greater retention time for PuO_2 in macrophages and a larger fraction of alveolar PuO_2 cleared in the first 30 days than were observed at lung burdens of greater than 1.0 μCi /gram lung. One may also conclude from this data that the retention of PuO_2 in macrophages appears to be required to assure significant early alveolar clearance of PuO_2 particles. It is also evident that proportionately greater radiation doses are delivered to the lung and to macrophages following deposition of large amounts of plutonium, due to the greater retention and cellular concentration of PuO_2 in the lung than is seen at relatively smaller lung burdens.

Cellular Distribution of Dose: PuO_2 Particle Size Distribution. The nonuniformity in dose distribution is further exemplified when examining the PuO_2 distribution within macrophages at lung burdens where concentration of more than one PuO_2 particle in a macrophage does not occur to any significant degree. The amount of plutonium washed from the lungs of rats

during the first 30 days after inhalation exposure, or about a mean of 45% of the lung burden, was divided into particle size classes using the aforementioned autoradiographic technique. Over 85% of the plutonium activity or radiation dose, originated from only 2% of the sampled pulmonary macrophages which contained PuO_2 particles (Table 4). Assuming a similar particle size distribution in the entire lung, as was seen in macrophages it can be shown that a larger fraction of the total lung dose was delivered by a very small number of deposited PuO_2 particles. Thus a recommended maximum dose to the lungs of plutonium workers of 15 rems actually means nearly 700 rads delivered to 2% of the lung volume assuming a PuO_2 particle size distribution as was observed in our studies with PuO_2 in alveolar macrophages of rats.

SUMMARY

Cellular sites of plutonium localization and later redistribution of plutonium in the lung influences the toxicity of inhaled plutonium compounds. The loss of plutonium from macrophages, the death of macrophages, the engulfment of plutonium by the alveolar epithelium and the sequestration of plutonium in foci of fibrosis accounts for the relatively long retention times for PuO_2 in the lung. The concentration of plutonium in particles, disproportionately in a few larger particles, in specific cellular elements of the lung and in subpleural, fibrotic areas of the lung, provides for high radiation dose rates in limited tissue volumes. Such "hot spots" of alpha irradiation may result in the picture of epithelial metaplasia,

TABLE 4

Distribution of ^{239}Pu activity within alveolar macrophages obtained by saline washings at 1-34 days after inhalation of $^{239}\text{PuO}_2$ in rats. The amount of plutonium in 800 macrophages was autoradiographically determined in 8 exposed rats.

Fraction of $^{239}\text{PuO}_2$ Laden Macrophages (%)	Equivalent ^a Particle Diameter (μm)	Amount of ^{239}Pu in Particle (μCi)	Number of Macrophages ^b with plutonium particles of selected size	Amount of macrophage ^c Laden plutonium in each size class (μCi)	Fraction of ^d Macrophage Laden Plutonium in each size class (%)	Radiation Dose ^e to Macrophage (rads/hour)
50	0.12	5.8×10^{-10}	5.6×10^5	.00033	2.5	0.5
24	0.16	1.4×10^{-9}	2.7×10^5	.00038	2.9	1.2
10	0.18	2.0×10^{-9}	1.1×10^5	.00023	1.7	1.8
4	0.20	2.9×10^{-9}	0.4×10^5	.00013	1.0	2.4
4	0.22	3.6×10^{-9}	0.4×10^5	.00016	1.2	3.2
4	0.26	6.0×10^{-9}	0.4×10^5	.00027	2.0	5.3
2	0.33	1.3×10^{-8}	0.2×10^5	.00030	2.3	11.
2	>0.36		0.2×10^5	.01150	86.4	>15.

a - Equivalent to $0.0388 \sqrt[3]{\frac{\text{alpha track counts}}{\text{sensitivity (0.13)}}$

b - Product of total number of macrophages x fraction of macrophages with engulfed PuO_2 x fraction of PuO_2 -laden macrophages in each class.

c - Product of the number of macrophages with plutonium particles of selected size x amount of ^{239}Pu in particle of that size.

d - Fraction of amount of macrophage laden plutonium in each size class/amount of plutonium in washings.

e - Assuming macrophage with diameter of $12.7 \mu\text{m}$.

preceding alveolo-bronchiolar carcinoma formation arising from Pu-laden areas of the lung. Such factors may be of great importance when determining the acceptable limits of exposure to man to air-borne plutonium and other transuranic elements.

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1. L. A. Temple, S. Marks and W. J. Bair, Int. J. Radiat. Biol. 2, 143 (1960).
2. H. Lisco, Lab. Invest. 8, 162 (1959).
3. G. P. Antonchenko, N. A. Koshurnikova and E. R. Lyubchanskii, Radiobiology, USSR (Engl.) 9 (1), 97 (1969).
4. R. A. Erokhin, N. A. Koshurnikova, V. K. Lemberg, A. P. Nifatov and A. A. Puzyrev, Gig. truda 5, 61; 10, 33 (1969).
5. C. L. Yuike, H. L. Berke and T. Hull, Radiat. Res. 31, 760 (1967).
6. P. N. Dean and W. H. Langham, Hlth. Phys. 16, 79 (1969).
7. W. J. Clarke, J. F. Park, J. L. Palotay and W. J. Bair, Hlth. Phys. 12, 609 (1966).
8. C. L. Sanders and J. F. Park, Inhaled Particles, Vol. III (C. N. Davies, Editor) Pergamon Press (London). in press
9. C. L. Sanders, R. R. Adey and T. A. Jackson, Archs. envir. Hlth. 22, 525 (1971).
10. V. E. Goldenverg, S. Warren and R. Chute, Lab. Invest. 18, 215 (1968).
11. R. Garbagni, S. Chiarle and B. Bellion, Minerva nucl. 8, 245 (1964).
12. T. L. Phillips, Radiology 87, 49 (1966).
13. E. B. Howard, Morphology of Experimental Respiratory Carcinogenesis. P. Nettesheim, M. G. Hanna, Jr., and J. W. Deatherage, Jr. (Editors) p. 147. ORNL Conf. 700501, NTIS, Springfield, VA. (1970).
14. R. Masse, Inhaled Particles, Vol. III. C. N. Davies (Editor) Pergamon Press (London). in press

15. C. L. Sanders, Hlth. Phys. 18, 82 (1970).
16. J. C. Nenot, Inhaled Particles, Vol. III. C. N. Davies (Editor) Pergamon Press (London). in press
17. B. O. Stuart, C. L. Sanders, W. J. Bair and P. J. Dionne, Pacific Northwest Laboratories, BNWL-1306, Vol. I, Pt. 1, p. 47. Battelle-Northwest, Richland, WA (1970).
18. W. J. Bair, D. H. Willard and L. A. Temple, Hlth. Phys. 7, 54 (1961).
19. W. J. Bair and B. J. McClanahan, Archs. envir. Hlth. 2, 648 (1961).
20. W. J. Bair and J. F. Park, Radiation Protection, p. 181 Pergamon Press (London) (1968).
21. J. F. Park, E. B. Howard and W. J. Bair, Pacific Northwest Laboratories, Tech. Rept. AFWL-TR-69-75. Richland, WA (1969).
22. P. E. Morrow, F. R. Gibb, H. Davies, J. Mitola, D. Wood, N. Wraight and H. S. Campbell, Hlth. Phys. 13, 113 (1967).
23. E. R. Lyubchanskii, Radiobiology, USSR (Engl.) 7, 84 (1967).
24. W. J. Clarke and W. J. Bair, Hlth. Phys. 10, 391 (1964).
25. P. E. Morrow and L. J. Casarett. Inhaled Particles and Vapours, p. 167. C. N. Davies (Editor) Pergamon Press (Oxford) (1961).
26. W. J. Bair and R. W. Perkins, Pacific Northwest Laboratories, BNWL-714, Vol. 1, p. 3.12. Battelle-Northwest, Richland, WA (1968).
27. S. E. Hammond, C. R. Lagerquist and J. R. Mann, Amer. indust. Hyg. Ass. J. 29, 169 (1968).
28. J. C. Nenot, M. Morin and J. LaFuma, Hlth. Phys. 20, 167 (1971).
29. A. F. Gallegos, H. A. Boyd, R. G. Thomas and R. O. McClellan, Lovelace Foundation for Medical Education and Research, LF-43, p. 100. Albuquerque, N.M. (1970).

30. L. J. Casarett, and P. S. Milley, Hlth Phys. 10, 1003 (1964).
31. C. L. Sanders and R. R. Adee, Hlth. Phys 18, 293 (1970).
32. C. L. Sanders, T. A. Jackson, R. R. Adee, G. J. Powers and A. P. Wehner, Archs. intern. Med. 127, 1085 (1971).
33. J. R. Esterly and C. S. Faulkner, II. Am. Rev. resp. Dis. 101, 869 (1970).
34. M. O. Pinkett, C. R. Cowdrey and P. C. Nowell, Am. J. Path. 48, 859 (1966).
35. C. L. Sanders, Archs. Envir. Hlth. 18, 904 (1969).
36. Y. E. Rahman and A. Lindenbaum, Radiat. Res. 21, 575 (1964).
37. C. L. Sanders and R. R. Adee, J. Reticulo-Endothelial Soc. 6, 1(1969).