JUL 21 1965

BNWL-SA-230
CONF-650624-2

PLUTONIUM PARTICLE-INDUCED NEOPLASIA OF THE CANINE LUNG
I. CLINICAL AND GROSS PATHOLOGY*

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This paper was prepared for presentation at the Second International Conference on Lung Tumors in Animals to be held June 24-29, 1965 at Perugia, Italy.

INTRODUCTION

Potential personnel contamination is associated with the production, fabrication, transportation, and use of ^{239}Pu . Experience has demonstrated that inhalation can be an important route of entry of ^{239}Pu into the body. Therefore, it is desirable to determine the toxicity of inhaled ^{239}Pu for the purpose of hazard evaluation and the establishment of permissible limits. Plutonium-239 decays by α -emission, emitting α -particles with an average energy of 5.15 Mev, and having a range of about 40 μ in water and soft tissue. It emits X-rays with energies of from 10-22 kev, and a few 380 kev γ -rays. Material produced at high power levels in addition emits fast neutrons (about 1 Mev) from spontaneous fission of ^{240}Pu , and a negligible amount of 60 kev γ -radiation from ^{240}Am . The radiological half-life of ^{239}Pu is 24,400 years. Sixteen micrograms of ^{239}Pu is equivalent to 1 μCi , or approximately 22.2×10^5 α -disintegrations per minute.

The toxicity of intratracheally administered ^{239}Pu for rats and mice has been demonstrated previously in several studies; death, pulmonary fibrosis, and neoplasia occurred 30 days to 2 years post-exposure (Abrams, et al., 1946; Lisco, 1959; Temple, et al., 1960).

*Work performed under Contract No. AT(45-1)-1830 between the Atomic Energy Commission and Pacific Northwest Laboratory, Battelle Memorial Institute, and under United States Air Force Contract Delivery Order No. (29-601)-62-1507 and the Atomic Energy Commission.

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Previous reports from this laboratory have described the toxicity of inhaled plutonium in beagle dogs. Respiratory insufficiency, lymphopenia, pulmonary fibrosis and metaplasia, and fibrosis of the bronchial lymph nodes were the primary pathologic effects observed in 32 beagle dogs that succumbed 2 to 14 months after exposure to $^{239}\text{PuO}_2$ aerosols. The body burdens at death ranged from 3 to 51 μCi , with 95% of the plutonium in the lungs and 4% in the bronchial and mediastinal lymph nodes (Bair and Willard, 1962; Park *et al.*, 1962; West and Bair, 1964; Clarke and Bair, 1964). One male dog, 150 days post-exposure, with a lung burden of 19 μCi , had bronchiolo-alveolar carcinomas detected by microscopic examination (Clarke, *et al.*, 1964).

This report describes the clinical and gross pathology in ten beagle dogs that died 29 to 56 months following exposure to $^{239}\text{PuO}_2$ aerosols. Five of these animals showed primary pulmonary neoplasia.

METHODS

Female purebred beagle dogs 12 to 43 months old, either raised in our colony or obtained from commercial breeders, were used for the experiments. The dogs were given routine physical examinations, immunizations, and medical treatment when necessary. The colony is remotely isolated and has been free of contagious and respiratory diseases for 5 years. After a suitable training period, the dogs were given a single 10 to 30 minute inhalation exposure to $^{239}\text{PuO}_2$ aerosols via a mask, without anesthesia or tranquilization. "Normal" breathing patterns were maintained during exposure; the exposure methods have been described previously (Bair and Willard, 1963). The aerosol was

produced by atomizing the plutonium dioxide suspension obtained by calcining ²³⁹-plutonium oxalate in air at 300° C, and suspending the oxide in an aqueous solution of 0.1% polypropylene-glycolethylene oxide polymer. The count median particle diameter of the aerosol ranged from 0.1 to 0.5 μ . After exposure, the dogs were held in metabolism cages for 30 days, then in inside runs with access to outside runs for the remainder of the study.

Blood samples were collected from the jugular vein for routine hematologic determinations. Starting in 1962, arterial blood-gas analyses were performed yearly, or more frequently when respiratory effects were seen. Oxygen content, hemoglobin saturation, and CO₂ content were determined on femoral arterial blood sampled while the dogs were at rest. Thoracic radiographs were taken yearly, or more frequently when clinical changes occurred. Radiographs of the cleaned disarticulated skeleton were obtained at necropsy. Respiration rates, tidal volume and body weights were recorded monthly. At necropsy, all tissues (except those sections collected for histopathology) were weighed and digested with HNO₃. After ashing in a muffle furnace, the residue was dissolved in 0.3 N HCl, the plutonium extracted with thenoyltrifluoroacetone, and aliquots counted with an α -proportional counter.

Representative tissue samples from each organ were fixed in 10% neutral buffered formalin for histopathologic study. Each lobe of the lung, all bronchial lymph nodes, and all gross lesions were sampled individually.

RESULTS

Mortality and Plutonium Content of the Tissues

Ten dogs died or were sacrificed when death was imminent during the 3 to 5 year post-inhalation period. Four other exposed dogs and one control were also sacrificed during this period to determine the tissue distribution and pathologic effects of ^{239}Pu in asymptomatic animals. Twenty-six exposed and 22 control dogs alive 3 to 5 years post-exposure are being maintained for long-term effects studies.

The body burdens at death ranged from 0.9 to 2.7 μCi (table I). The lungs and bronchial and mediastinal lymph nodes contained 76 to 95% of the body burden. Forty-two to 75% of the plutonium was in the lungs, while 17 to 49% was in the bronchial and mediastinal lymph nodes. The plutonium in the liver ranged from 2 to 15%, and 1 to 5% of the body burden was in the bone. Assuming uniform distribution of plutonium in the lung and a biological half-life of about 750 days, the average total radiation dose to the lung tissue was 9,000 to 23,000 rads (table I).

Clinical Signs

The first indication of a pathologic change in the exposed animals was a lymphopenia. Lymphocyte counts, approximately one-half of control values 33 weeks post-exposure, decreased to one-third before death (fig. 1). There was no associated decrease in eosinophils, and the monocytes and basophils were within normal range. Total leukocytes decreased proportionally to the decrease in lymphocytes, while neutrophils were not significantly different from control values.

Table I

Mortality and tissue distribution of ^{239}Pu in dogs after inhalation of $^{239}\text{PuO}_2$

Dog number	Time of death (months after exposure)	Age at death (months)	Plutonium (μCi)		Distribution of plutonium at death (percent of body burden)				Average radiation dose to lungs ^{††} (estimated rads)
			Deposited in lungs [†]	Body burden at death	Lungs	Bronchial lymph nodes	Liver	Bone	
182	29	42	5.8	2.7	74	21	2	1	12,000
184	31	49	5.9	2.5	75	17	5	1	12,000
215	38	81	3.9	1.4	50	42	2	4	9,000
* 83	40	52	5.3	1.8	59	27	6	2	12,000
*173	44	58	6.9	2.1	46	48	2	2	19,000
*106	45	66	9.0	2.7	50	37	6	2	23,000
*183	45	77	3.1	0.9	52	38	6	3	10,000
180	48	65	5.1	1.4	42	49	5	3	15,000
76	51	73	5.7	1.4	47	45	3	4	15,000
*213	56	76	5.7	1.2	49	27	15	5	15,000

*Primary pulmonary tumors.

[†]Estimated amount deposited below ciliated epithelium, where clearance processes were not effective in causing rapid removal.

^{††}Average radiation dose assumes uniform distribution of absorbed α - energy from plutonium particles in the lung.

Packed cell volume, hemoglobin, and erythrocyte counts were normal, except for elevated terminal measurements associated with dehydration and hemoconcentration.

Three to 24 months before death, respiratory rates steadily increased from the normal of 20 to 40/minute to over 150/minute the week before death. Respirations were shallow with characteristic abdominal breathing movements. When respiratory rates exceeded 100/minute, vesicular sounds could no longer be detected by auscultation, and harsh bronchial sounds were heard throughout the thorax, indicating progressive consolidation of the lung. Auscultation of the heart, although difficult due to the lung sounds, indicated that the second heart sound was more pronounced than the first. As respiration rates increased, respiratory movements were exaggerated and the thorax was held in a position of nearly permanent inspiration. The dogs were less active, had a slightly depressed attitude, and seemed to prefer a peculiar upright sitting position with elbows rotated outward. The dogs showed intermittent anorexia, increased time needed for drinking and eating, occasional vomiting and coughing, dehydration, and a 20 to 30% body weight loss during the month prior to death. At 1 week prior to death, the dogs became severely depressed and a general weakness was noted. In addition, a clear nasal discharge was present, the nares were dilated, the mucus membranes were cyanotic, and their rectal temperatures were subnormal.

Associated with the increased respiratory rate and decreased tidal volume 3 to 12 months prior to death, arterial blood CO_2 increased and O_2 and hemoglobin saturation decreased (table II).

Table II.

Arterial blood gas values in dogs after $^{239}\text{PuO}_2$ inhalation

Dog number	Months post-exposure	O ₂ content (ml/100 ml blood)	Hemoglobin O ₂ saturation (percent)	CO ₂ content (ml/100 ml blood)	pH
Controls	-	21.5 ± 0.7*	94.6 ± 2.0*	37.7 ± 1.0*	7.416 ± 0.01*
215	35	19.1	95.1	52.0	7.26
	38†	14.7	74.6	53.9	7.20
83	35	20.2	94.3	39.7	-
	40	21.7	91.5	41.3	7.39
173	38	19.2	95.2	55.0	7.27
	40	17.6	89.0	50.6	7.31
	43	17.4	82.0	48.9	7.34
106	44†	15.6	68.9	50.0	7.35
183	29	21.2	95.5	48.2	-
	37	16.5	91.1	44.8	7.39
	45†	15.6	65.0	48.0	7.36
180	42	16.0	82.3	45.9	7.41
	47	18.0	83.2	44.6	7.24
	48	16.5	82.5	42.5	7.27
76	41	19.6	96.1	44.2	7.39
	47	18.8	91.5	42.3	7.26
	51†	12.2	59.3	49.4	7.21
213	43	22.76	74.17	44.87	7.41
	48	17.09	88.09	47.08	7.38
	54	17.57	93.26	47.71	7.48

*Mean and 95 percent confidence intervals around the mean.

†Analysis completed 12-48 hours prior to death.

Thoracic radiographs at 6 to 12 months prior to death showed uniform mottling of the lung field, due to pulmonary fibrosis, and cardiac enlargement. At one month before death the lung densities became more diffuse, the heart greatly enlarged, and the trachea showed a dorsal deviation.

A well demarcated peripherally located nodule was detected in the left diaphragmatic lobe of dog 213 on routine thoracic examination 48 months post-exposure, 8 months before death. Figures 2 and 3 show the thoracic radiographic changes in this dog, and indicate the location and growth rate of the pulmonary tumor.

The lateral thoracic radiographs of dog 83 showed a small area of increased density in the diaphragmatic lobes 31 months post-exposure (fig. 4). The lesion increased in size and density through 36 months post-exposure, indicating an actively changing area. Forty months post-exposure, the month before death, the neoplasm had increased markedly in size and density, had indistinct margins and cavitations, and appeared confined primarily to the left diaphragmatic lobe (fig. 5).

The thoracic radiographs of dog 106 indicated a suspicious area of increased density in the diaphragmatic lobe 8 months before death. The lesion was not sufficiently delineated from the surrounding fibrotic tissue for definitive diagnosis; however, retrospective examination of the radiographs after necropsy confirmed the presence of the tumor (fig. 6). The neoplasms in dogs 215 and 173 could not be demonstrated radiographically. Radiographs of the disarticulated skeleton were normal.

Gross Pathology

Gross pathologic changes were seen in the lung, bronchial and mediastinal lymph nodes, heart, and liver at necropsy.

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. In some dogs the spots were so dense that the lung had a confluent, whitish appearance. 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 2 to 3 times normal weight.

In addition to the above lesions, the anterior, dorsal part of the left diaphragmatic lobe of dogs 83 and 213, and the right diaphragmatic lobe of dog 106, contained dense, white, irregularly defined areas. These areas ranged from 2 to 5 cm in diameter, protruded slightly above the surface of the lung and had cystic, necrotic centers (figs. 6 and 7). The anterior, ventral, lateral part of the left diaphragmatic lobe of dog 106 and the right apical lobe of dog 173 contained single peripherally located, firm, creamy-white, well demarcated nodules, 1 cm in diameter, that were raised slightly above the surface of the lung (figs. 8 and 9). Dog 213 had multiple smaller, white, firm, poorly defined nodules, less than 0.5 cm in diameter, scattered throughout the lung parenchyma, primarily associated with the subpleural areas. Dog 215 had no nodules in the lung that could be differentiated from the surrounding fibrotic tissue.

The tumors were peripherally located in the lung parenchyma and subpleural areas. Grossly, the tumors did not appear to originate from or penetrate the primary bronchi. Pleural or pericardial effusion and osteoarthropathy was not present, and metastases were not evident.

The bronchial lymph nodes were small, indurated, and had dark centers, while the mediastinal nodes appeared hypertrophied and congested.

The right ventricular wall of the heart appeared flabby, and the chambers were dilated. Right ventricular wall thickening was not apparent.

The livers of all the animals were markedly congested.

DISCUSSION

Clinical signs were not diagnostically helpful in differentiating the dogs with pulmonary neoplasia from the dogs with chronic pulmonary fibrosis. It was apparent, however, that the clinical signs and death of the animals were probably caused by the chronic obliterative lung disease. Also, a surviving dog five years post-exposure with a large, rapidly growing, peripherally located lesion in the right diaphragmatic lobe, suspected to be a pulmonary tumor, showed no respiratory insufficiency, further indicating that pulmonary neoplasia was not the primary cause of death (fig. 11).

The diffuse irregular radiographic lesions were at first nonspecific in dogs 83 and 106 (figs. 5 and 6); however, subsequent radiographs showing a marked increase in size and density of the areas indicated potential neoplastic lesions. The well demarcated, rapidly growing nodule in dog 213 was more characteristic of a tumor (figs. 2 and 3).

The radiographic lesions of bronchiolo-alveolar tumors in man have been described as single nodules, fairly well defined, or as diffuse lesions simulating a pneumonic process (Kress and Allan, 1963). Few descriptions of the radiographic lesions of specific pulmonary neoplasias have been reported in veterinary literature. Most of the reported cases were incidental findings at necropsy. Although radiographic evidence of tumor formation was seen in the lungs of 3 of the 4 animals as early as 9 months before death, this is probably a poor estimate of when the neoplastic process actually started, since the surrounding fibrotic processes limited the detection of increased densities in the lung. The fibrosis also limited the detection of small neoplastic lesions at necropsy. Tumors were detected grossly in only 4 of the 6 animals in which they were found microscopically. Also, multicentric origin was apparent in only 2 and metastases were not evident in any of the animals. However, microscopic examination indicated multicentric origin in 5 of 6 animals and lymphogenous metastases to the bronchial lymph node in 1 animal. These findings indicate the importance of representative tissue sampling for the evaluation of experimental neoplastic processes.

Necropsy material from aging control dogs from this and other beagle colonies, although apparently negative for pulmonary tumors, is not sufficient to establish a pulmonary tumor incidence figure for colony raised dogs. Therefore, the 0.2% incidence figure for canine necropsy material appears to be the best data available for comparison (Nielsen and Horava, 1960; and Sedlmeier and Dahme, 1958). It is evident that the 14% pulmonary tumor incidence, at 4 to 6 years of age, in the dogs that inhaled plutonium in this study was probably caused

by the plutonium retained in the lung, since there was no indication that the pathology in the bronchial lymph nodes was a concomitant cause of death or neoplasia. However, it is possible that obstruction of lymphatic drainage from the lung contributed to the pulmonary pathology. Since the animal evidenced a marked lymphopenia, immunological effects should also be considered in the pathogenesis of these tumors.

Due to the continued slow excretion of plutonium, translocation to the bronchial lymph nodes, liver and bone, and the unknown time of tumor induction, it is extremely difficult to determine exact amounts of plutonium responsible for tumor production. The radiation dose to the lung and initial deposition in these animals is based on whole-body retention data of dogs that died less than 1 year after exposure. This dose calculation neglects the translocation of plutonium from the lung and any change in excretion rate which may have occurred in the long-term dogs. Also, it is an estimate of the average uniform α -radiation dose to the lung. Since the plutonium was deposited in particulate form, and the effective range of α -particles in tissue is 40 μ , the radiation dose is not uniform throughout the lung.

To the authors' knowledge, there have been no previous reports of experimentally produced primary pulmonary neoplasia in dogs.

SUMMARY

Previous reports from this laboratory described the pathologic effects in 32 beagle dogs that succumbed 2 to 14 months after exposure to $^{239}\text{PuO}_2$ aerosols. One male dog, 150 days post-exposure, with a lung burden of 19 μCi , had bronchiolo-alveolar carcinomas. Inhalation of $^{239}\text{PuO}_2$ caused spontaneous death in 10 female beagle dogs 29 to 56

months post-exposure. The body burden at death ranged from 1 to 3 μCi . Cardiopulmonary insufficiency and lymphopenia were the primary clinical signs associated with severe fibrosis of the lung and bronchial lymph nodes. These organs contained 76 to 95 percent of the terminal body burden. Five of these animals showed primary pulmonary tumors. Three of the animals showed radiographic lesions suggestive of pulmonary neoplasia 4 to 9 months before death. The tumors were grossly multicentric in 2 of the 4 animals and metastases were not evident; however, microscopic examination indicated multicentric origin in 5 animals and metastases to the bronchial lymph nodes in 1 dog. Six of the 42 dogs exposed to $^{239}\text{PuO}_2$ showed bronchiolo-alveolar carcinomas, an incidence of 14 percent compared to a primary lung tumor incidence of 0.2 percent reported for canine necropsy material.

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LEGENDS

Fig. 1. Leukocyte values in dogs after $^{239}\text{PuO}_2$ inhalation.

Fig. 2. Thoracic radiographs of dog 213 (48 months post-exposure) showing cardiac enlargement, increased density in the lung and indication of a well-demarcated, peripherally located nodule in the left diaphragmatic lobe.

Fig. 3. Thoracic radiograph of dog 213 (56 months post-exposure) showing cardiac enlargement, dorsal deviation of the trachea, diffuse increased density in the lung, and a peripherally located, well-demarcated nodule in the left diaphragmatic lobe indicating the location of the tumor.

Fig. 4. A small increased density in the diaphragmatic lobe of dog 83 (31 months post-exposure).

Fig. 5. Thoracic radiograph of dog 83 (40 months post-exposure) showing cardiac enlargement, dorsal deviation of the trachea, and an irregularly increased density in the diaphragmatic lobe of the lung. Indistinct margins and cavitations indicate the tumor location.

Fig. 6. Thoracic radiograph of dog 106 (45 months post-exposure) indicating the appearance of the tumors in the diaphragmatic lobes the month before death.

Fig. 7. Irregularly defined pulmonary tumor with a cystic center in the anterior dorsal part of the left diaphragmatic lobe of dog 213.

Fig. 8. Pulmonary tumor, with necrotic center and indistinct margins in the anterior, dorsal area of the left diaphragmatic lobe of dog 83.

Fig. 9. Well-demarcated, peripherally located tumor in the anterior, ventral, lateral part of the left diaphragmatic lobe of dog 106.

Fig. 10. Well-defined, peripherally located, pulmonary tumor in the anterior, ventral, lateral part of the right apical lobe of dog 173.

Fig. 11. Thoracic radiograph of dog 216 (54 months post-exposure) showing a large space-occupying lesion in the diaphragmatic lobe.

LEUKOCYTE VALUES OF DOGS AFTER PLUTONIUM INHALATION

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11