PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1974
TO THE USAEC DIVISION OF BIOMEDICAL
AND ENVIRONMENTAL RESEARCH
PART 1 BIOMEDICAL SCIENCES

Battelle

Pacific Northwest Laboratories
Richland, Washington 99352

MARCH 1975

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PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1974
TO THE
ERDA DIVISION OF BIOMEDICAL AND ENVIRONMENTAL RESEARCH

PART 1 BIOMEDICAL SCIENCES

By

R. C. Thompson
and
Staff Members of Biology Department,
Physics and Instrumentation Department,
and Radiological Sciences Department

March 1975

On January 19, 1975, research and development programs of the U.S. Atomic Energy Commission (AEC) became part of the newly formed Energy Research and Development Administration (ERDA). In this report, since it refers to work done in 1974, most references are to AEC programs.

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RICHLAND, WASHINGTON 99352

PREFACE

The Annual Report for 1974 to the U.S. Energy Research and Development's Division of Biomedical and Environmental Research is organized by major program categories according to our schedule-189 submissions. Each part is directed toward a particular DBER Research and Development Program: Part 1 to Biomedical Programs, Parts 2 and 3 to Environmental Programs, and Part 4 to Physical and Technological Programs. Each part of the Annual Report comprises project reports authored by scientists from several research departments, reflecting the interdisciplinary nature of the research effort. The Annual Report consists of four parts:

Part 1 Biomedical Sciences	Program Manager: R. C. Thompson Editor: D. L. Felton
Part 2 Ecological Sciences	Program Manager: B. E. Vaughan Editor: J. L. Engstrom
Part 3 Atmospheric Sciences	Program Manager: C. E. Elderkin Editor: E. L. Nichols
Part 4 Physical and Analytical Sciences	Program Manager: J. M. Nielsen Editor: G. M. Garnant

Reports previously issued are as follows:

Annual Report for

1951	HW-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280, BNWL-235, Vol. 1-4
1966	BNWL-480, Vol. 1, BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1, BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2, BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2, BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2, BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2, BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2, BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4

W. J. Bair
Program Director, Life Sciences

FOREWORD

The research described in this volume is primarily that conducted in the Biology Department, with contributions under several projects from members of the Physics and Instrumentation Department, the Radiological Sciences Department, and the Systems Department. Biology Department research on blood irradiation and on problems related to the artificial heart program are included in another volume, Part 4 of this Annual Report.

Individual reports are grouped into sections, which correspond in title and content to AEC 189 Projects. These projects were realigned during the past year so that the work included under six projects in last year's report is this year divided among 15 projects. These 15 projects include none that are devoted to a totally new kind of effort, although the realignment will focus attention on certain areas that may have suffered neglect in larger conglomerate projects in the past. Thus, we now acknowledge the vital importance of aerosol exposure techniques to our inhalation program; we are emphasizing an attack on the problem of the mechanisms of inhaled plutonium toxicity, and upon the ancillary factors that may often modify radionuclide toxicity; and we have directed a specific project toward evaluating the acute toxicity of possible radionuclide releases following a catastrophic reactor accident.

The past year saw the accession of W. R. Wiley to the position of Manager of the Biology Department. He thus became the third manager in the 26-year history of the Department. W. J. Bair, who had served as Manager, Biology since 1968, assumed full-time responsibility as Program Director, Life Sciences, for Battelle-Northwest, a position he had filled in a part-time capacity for some time previously. A complete listing of Biology Department staff and organization charts for Battelle-Northwest are included at the end of this volume.

A highlight of the year was the Fourteenth Annual Hanford Biology Symposium on Radiation and the Lymphatic System held September 30 to October 2, 1974, under the chairmanship of J. E. Ballou. The proceedings will be published in the AEC Symposium Series. The Fifteenth Annual Hanford Biology Symposium on Biological Implications of Metals in the Environment is scheduled for September 29 to October 1, 1975.

At year end, the new Life Sciences II Laboratory was essentially completed. About two-thirds of this 100,000 square foot building, constructed with Battelle funds, is specifically designed for research with experimental animals. This effectively doubles the research and support facilities available to the Biology and Ecosystems Departments. About one-half of the new space will be available for AEC programs.

A list of 1974 publications relevant to AEC programs, by personnel of the Biology Department, appears at the end of this volume. Requests for reprints will be honored as long as the supply lasts.

R. C. Thompson
RX Program Director
Biology Department

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LOW-LEVEL PLUTONIUM AND TRANSPLUTONIUM OXIDE INHALATION STUDIES IN BEAGLES

This project consists of a large experiment to determine the life-time dose-effect relationships of inhaled plutonium oxide (^{238}Pu and ^{239}Pu), and several shorter-term experiments to study the kinetics and dosimetry of inhaled transplutonium oxides, such as those of americium and curium, and various oxide mixtures.

DOSE-EFFECT STUDIES WITH INHALED PLUTONIUM OXIDE IN BEAGLES

Investigators:

J. F. Park, R. L. Buschbom, D. L. Catt,
D. K. Craig, G. E. Dagle, P. L. Hackett,
G. J. Powers, H. A. Ragan, and C. R. Watson

Technical Assistance:

J. S. Barnett, E. F. Blanton, E. T. Edmerson,
V. T. Faubert, D. H. Hunter, S. J. Lepka,
R. M. Madison, M. C. Perkins, and L. R. Peters

Beagle dogs given single exposure to $^{239}\text{PuO}_2$ or $^{238}\text{Pu}^{16}\text{O}_2$ aerosols are being observed for life-span dose-effect relationships. The ^{239}Pu body burden of the nine dogs dying due to respiratory insufficiency during the first 3 years after exposure was 1 to 12 μCi . One of these dogs had a pulmonary tumor. None of the dogs exposed to ^{238}Pu have died during the first postexposure year. After inhalation of $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ lymphocytopenia was the earliest observed effect, occurring 0.5 to 2 years after deposition of ≥ 80 nCi plutonium in the lungs.

To determine the life-span dose-effect relationships of inhaled plutonium, 18-month-old beagle dogs were exposed to aerosols of $^{239}\text{PuO}_2$ (mean AMAD 2.3 μm , mean GSD 1.9), prepared by calcining the oxalate at 750°C for 2 hr; or to $^{238}\text{Pu}^{16}\text{O}_2$ (mean AMAD 1.8 μm , mean GSD 1.9), prepared by calcining the oxalate at 700°C and subjecting the product to H_2^{16}O steam in argon exchange at 800°C for 96 hr.

One hundred-twenty dogs exposed to $^{239}\text{PuO}_2$ in 1970 and 1971 were selected for long-term studies; 12 will be sacrificed to obtain plutonium distribution and pathology data, and 108 were assigned to life-span dose-effect studies (Table 1.1). One hundred-thirteen dogs exposed to

$^{238}\text{Pu}^{16}\text{O}_2$ in 1973 and 1974 were selected for life-span dose-effect studies (Table 1.2). Additional dogs for periodic sacrifice are scheduled for exposure in 1975.

During the first 3 years following exposure to $^{239}\text{PuO}_2$, seven dogs in the highest level dose group and two dogs in dose-level group 5 were euthanized when death was imminent due to respiratory insufficiency. Three dogs were euthanized from the low-level sacrifice group for comparison of plutonium tissue distribution. Table 1.3 shows the causes of death and the distribution of ^{239}Pu in the tissues of these animals.

TABLE 1.1. Dose-Effect Studies With Inhaled $^{239}\text{PuO}_2$
in Beagles

Dose Level Group	Number of Dogs		Initial Alveolar Deposition ^(a)			
	Male	Female	nCi ^(b)		nCi/g lung ^(b)	
0	10	10	0		0	
1	10	10	3.5	+ 1.3	0.04	+ 0.02
2	10	10	22	+ 4	0.3	+ 0.05
3	10	10	79	+ 14	1.1	+ 0.2
4	10	10	300	+ 62	3.9	+ 0.7
5	10	10	1100	+ 170	14	+ 2.0
6	3	5	5800	+ 3300	86	+ 44
	63	65				

(a) Estimated from external thorax counts at 14 and 30 days post-exposure and estimated lung weights.

(b) Mean $\pm$ 95% confidence intervals around the means.

TABLE 1.2. Dose-Effect Studies With Inhaled $^{238}\text{Pu}^{16}\text{O}_2$
in Beagles

Dose Level Group	Number of Dogs		Initial Alveolar Deposition ^(a)			
	Male	Female	nCi ^(b)		nCi/g lung ^(b)	
0	10	10	0		0	
1	10	10	2.3	+ 0.8	0.02	+ 0.01
2	10	10	18	+ 3	0.2	+ 0.04
3	10	10	77	+ 11	1.1	+ 0.4
4	10	10	350	+ 81	4.4	+ 0.9
5	10	10	1300	+ 270	17	+ 2.9
6	7	6	5200	+ 1400	69	+ 18
	67	66				

(a) Estimated from external thorax counts at 14 and 30 days post-exposure and estimated lung weights,

(b) Means $\pm$ 95% confidence intervals around the means.

TABLE 1.3. Tissue Distribution of Plutonium in Beagles
After Inhalation of $^{239}\text{PuO}_2$

Dog Number	Time After Exposure, Months	Final Body Burden, μCi	Percent of Final Body Burden				Cause of Death
			Lungs	Thoracic Lymph Nodes (a)	Liver	Skeleton	
910 M	11	12.3	84	15	0.06	0.04	Respiratory Insufficiency
747 F	12	5.4	71	29	0.07	0.07	Respiratory Insufficiency
906 F	13	6.2	88	12	0.03	0.05	Respiratory Insufficiency
849 F	13	0.0007	79	15	0.04	2.6	Periodic Sacrifice
896 F	15	4.1	81	15	0.23	0.12	Respiratory Insufficiency
817 M	21	3.8	64	34	1.4	0.19	Respiratory Insufficiency
815 M	25	0.074	64	32	0.08	0.10	Periodic Sacrifice
829 M	27	3.2	75	19	4.2	0.45	Respiratory Insufficiency
760 M	31	0.98	71	23	3.7	0.28	Respiratory Insufficiency
890 F	31	2	55	28	13	0.26	Respiratory Insufficiency
804 M	37	1.1	62	29	7.9	0.36	Respiratory Insufficiency and Lung Tumor
798 F	43	0.0056	55	43	0.15	0.05	Periodic Sacrifice

(a) Tracheobronchial, mediastinal and sternal lymph nodes

As survival time increased, the fraction of plutonium in the lung decreased. During the first postexposure year, plutonium was translocated primarily to the thoracic lymph nodes with little plutonium translocated to other tissues. During the second and third postexposure years, the fraction of plutonium in liver increased, accounting for 4 to 13% of final body burden. The organ distribution of plutonium in the three low-dose-level periodically sacrificed dogs was generally similar to that of the high-dose-level dogs sacrificed when death was imminent. The two exceptions were the larger fraction of plutonium in the skeleton of dog 849 F, and a smaller fraction in the liver of the low-level dogs sacrificed during the second and third postexposure years. Dog 798 F had a larger fraction of plutonium in the thoracic lymph nodes.

The dogs that were euthanized when death was imminent had increased respiration rates, hypercapnia and hypoxemia associated with lesions in

the lungs. Intermittent anorexia and body weight loss accompanied the respiratory insufficiency. Histopathologic examination of the lungs showed radiation pneumonitis characterized by interstitial and subpleural fibrosis, increased numbers of alveolar macrophages, alveolar epithelial hyperplasia, and foci of squamous metaplasia. Autoradiographs showed activity primarily composed of large stars that were more numerous in areas of interstitial and subpleural fibrosis (Figure 1.1). Dog 804 M also had a pulmonary tumor classified as a bronchiolar-alveolar carcinoma (Figures 1.2 and 1.3). Sclerosing lymphadenitis was associated with the high concentration of plutonium in the thoracic lymph nodes (Figure 1.4). There was also a generalized lymphoid atrophy which may be related to the debilitation in the dogs with respiratory insufficiency, or to lymphocytopenia.

X77

FIGURE 1.1. Autoradiograph of Lung Showing Alpha Stars in Areas of Interstitial Fibrosis. H&E.

FIGURE 1.2. Bronchiolar-alveolar Carcinoma in the Periphery of the Lung of Dog 804 M. H&E.

X6

FIGURE 1.3. Bronchiolar-alveolar Carcinoma in Dog 804 M Showing Necrosis with Extension of the Tumor into Adjacent Alveoli.

X92

FIGURE 1.4. Autoradiograph Showing Plutonium Associated With Sclerosing Tracheobronchial Lymphadenitis.

X15

A significant ($p < 0.05$) dose-related lymphocytopenia developed in the four highest dose-level group dogs exposed to $^{239}\text{PuO}_2$ (Figure 1.5). The lymphocytopenia appeared to be related to the initial alveolar burden, with a greater depression in lymphocytes in the higher dose-level groups; and to be related also to time after exposure, the lower dose-level groups developing lymphocytopenia later than the higher dose-level groups. The mean reduction was about 25%, 40%, 50% and 70%, respectively, with mean initial alveolar lung burdens of 0.1, 0.3, 1.1, and 5.8 μCi . Lymphocytopenia was first observed at 4, 8, 10, and 22 months, respectively, in the 0.1, 0.3, 1.1 and 5.8 μCi groups, respectively. The mean lymphocyte values in groups 1 through 5 were 14 to 20% greater 1 month after exposure than those observed pre-exposure. This trend was not seen in the group 6 or control dogs, and all values were within the 95% confidence intervals for age-related control dogs.

There was a progressive decrease in total leukocyte values and the two highest dose-level groups were significantly different ($p < 0.05$) from the controls (Figure 1.5). This change was due primarily to the reduction in lymphocytes with a lesser reduction in neutrophils, except in the highest dose-level group (Figure 1.5). In that group neutropenia developed about 16 months after exposure, but at that time only three animals remained in the group, including one which consistently had a very low leukocyte concentration even before exposure. At the three higher dose levels, a 20 to 30% increase in mean neutrophil values was observed 1 month postexposure but these values were not significantly different from those of the age-related control animals. There was no difference in either monocyte or eosinophil concentrations in peripheral blood between exposed and control animals. No significant changes in mean values for red cell parameters were observed during the 3-year postexposure period.

None of the dogs exposed to $^{238}\text{Pu}^{16}\text{O}_2$ have been sacrificed. The $^{238}\text{Pu}^{16}\text{O}_2$ -exposed dogs developed a dose-related lymphocytopenia (Figure 1.6). The mean reduction was about 36%, 49%, 68%, and 74%, respectively, with mean initial alveolar lung burdens of 0.1, 0.3, 1.3, and 5.2 μCi . Lymphocytopenia was first observed at 4 months after exposure in the 1.3 and 5.2 μCi dose-level groups, and at 7 and 10 months, respectively, in the 0.1 and 0.3 μCi dose-level groups. There was a decrease in total leukocyte values in the four highest dose-level groups, due primarily to the reduction in lymphocytes and to a reduction in neutrophils, especially in

the highest dose-level groups (Figure 1.6). There were no consistent differences in monocyte or eosinophil concentration or in values for red cell parameters, between exposed and control animals.

After inhalation of $^{239}\text{PuO}_2$ or $^{238}\text{Pu}^{16}\text{O}_2$, lymphocytopenia was the earliest observed effect, occurring after deposition of ≥ 80 nCi plutonium in the lungs. On a concentration basis 80 nCi dose level is about 40 times the 16 nCi maximum permissible human lung deposition, based on 0.3 rem/week to the lung.

FIGURE 1.5. Lymphocyte, Leukocyte and Neutrophil Values From Dogs After Inhalation of $^{239}\text{PuO}_2$. The shaded area represents mean values from age-related control dogs $\pm$ the mean 95% confidence interval.

FIGURE 1.6. Lymphocyte, Leukocyte and Neutrophil Values From Dogs After Inhalation of $^{238}\text{Pu}^{16}\text{O}_2$. The shaded areas represent mean values from age-related control dogs $\pm$ the mean 95% confidence interval.

LATE EFFECTS OF INHALED $^{238}\text{PuO}_2$ IN BEAGLES

Investigators:

J. F. Park, D. L. Catt, P. L. Hackett,
J. E. Lund, G. J. Powers, H. A. Ragan,
and C. R. Watson

Technical Assistance:

J. S. Barnett, E. T. Edmerson, V. T. Faubert,
D. H. Hunter, S. J. Lepka, R. M. Madison,
M. C. Perkins, and L. R. Peters

Bone tumors were the primary cause of death in beagle dogs 4 to 6 years after inhalation of $^{238}\text{PuO}_2$. The plutonium body burden at death ranged from 0.5 to 2.6 μCi with 32 to 55% of the plutonium in the skeleton. Pulmonary neoplasia was observed in three of the bone-tumor-bearing dogs.

Beagle dogs were exposed to aerosols of $^{238}\text{PuO}_2$ calcined at 350°C (CMD 0.1 μm) or $^{238}\text{PuO}_2$ from crushed microspheres (CMD 0.1 μm) to determine the long-term disposition and biological effects. Eight of the ten dogs exposed to 350°C -calcined $^{238}\text{PuO}_2$, and eight of the 12 dogs exposed to $^{238}\text{PuO}_2$ from crushed microspheres, died or were euthanized when death was imminent during the 7-year postexposure period. Table 1.4 shows the causes of death and the distribution of ^{238}Pu in the tissues of these animals.

Five dogs died during the first 3 years postexposure. Two of these dogs, with a 7-8 μCi final body burden, were euthanized because of respiratory insufficiency related to plutonium-induced pulmonary fibrosis. Two dogs died of causes apparently not related to plutonium exposure: wounds and encephalitis. It is not known whether the bone fracture was plutonium-related.

During the 4- to 6-year postexposure period, eight dogs were euthanized due to bone tumors. One dog, euthanized because of myelogenous leukemia, also had a bone tumor. Three of the dogs euthanized because of bone tumors also had lung tumors.

In one dog (405 M), the lung tumor was evident radiographically 15 months prior to euthanasia. The tumor was classified as a bronchiolar-alveolar cell carcinoma and had metastasized to the tracheobronchial lymph nodes. The other two dogs (488 F, 489 F) had small tumors which were composed of bronchiolar or alveolar cells, and were classified as adenomas for lack of histologic evidence of malignancy. One dog was euthanized due to a fibrosarcoma on the side of the face with no evidence of bone involvement. One dog died due to intestinal obstruction, probably not related to the plutonium exposure. It is not known whether the fibrosarcoma and myelogenous leukemia were related to the plutonium exposure. Considering their low incidence in controls, the lung and bone tumors were almost certainly caused by plutonium. During the 7th postexposure year, three more dogs have shown radiographic evidence of bone tumors. These dogs have not yet been euthanized.

The final plutonium body burden of the dogs euthanized during the 4- to 6-year postexposure period was 0.2-2.6 μCi . The largest fraction of plutonium in the body was in the skeleton, 32-55%; followed by the liver,

TABLE 1.4. Mortality and Tissue Distribution of Plutonium in Dogs After Inhalation of $^{238}\text{PuO}_2$.

Dog Number	Time After Exposure, months	Final Body Burden, μCi	% of Final Body Burden				Cause of Death
			Lungs	Thoracic Lymph Nodes ^(c)	Liver	Skeleton	
(After Inhalation of $^{238}\text{PuO}_2$ Calcined at 350°C)							
492 F	23	3	4	4	23	64	Bone Fracture
404 M	36	8.1	32	10	23	32	Respiratory Insufficiency
467 M	38	7	15	11	13	57	Respiratory Insufficiency
469 M	54	2.6	34	5	17	41	Bone Tumor
445 M	58	2.5	6	10	23	55	Bone Tumor
438 M	60	2.3	7	11	33	43	Bone Tumor
453 M	62	2.2	17	9	22	47	Bone Tumor
405 M	70	2.6	23	10	28	34	Bone & Lung Tumor
433 M	74	0.7 ^(a,b)					
459 M	74	0.2 ^(a,b)					
(After Inhalation of $^{238}\text{PuO}_2$ Crushed Microspheres)							
485 M	22	3.1	72	7	7	12	Encephalitis
500 M	34	1.1	39	21	12	24	Dog Fight Wound
497 F	52	0.2	16	3	31	46	Intestinal Obstruction
481 F	60	0.5	13	23	23	37	Myelogenous Leukemia & Bone Tumor
489 F	62	2.5	7	26	27	32	Bone & Lung Tumor
482 F	70	0.8	8	19	15	47	Bone Tumor
494 F	75	0.4	3	9	32	48	Fibrosarcoma
488 F	76	1.4	9	15	29	41	Bone & Lung Tumor
480 F	76	0.6 ^(a,b)					
491 M	76	0.6 ^(a,b)					
487 F	76	0.3 ^(a,b)					
496 F	76	0.2 ^(a,b)					

(a) Estimated body burden

(b) Still alive

(c) Tracheobronchial, mediastinal and sternal lymph nodes

15-33%; thoracic lymph nodes, 3-26%; and lung, 3-24%. In the crushed-microsphere-exposed dogs, the fraction of plutonium retained in the lung decreased, and the fraction in the skeleton increased during the 2- to 6-year postexposure period. Fractions of plutonium in the lymph nodes and liver varied from one animal to another, with no clear time-related trend. In the dogs exposed to 350°C -calcined $^{238}\text{PuO}_2$, there is no apparent time-related trend toward translocation of plutonium from the lung,

or toward accumulation in the thoracic lymph nodes, liver, or skeleton. For all animals the highest plutonium concentration occurred in the thoracic lymph nodes, followed in descending order by lungs or liver, and skeleton (Table 1.5).

In addition to the biological effects causing death, all of the dogs had fibrotic thoracic lymph nodes, focal pulmonary fibrosis and hepatic nodular hyperplasia. The plutonium-exposed dogs had a chronic absolute

lymphocytopenia and a higher frequency of elevated serum glutamic pyruvic transaminase (SGPT) values (See Annual Report, 1973). Some dogs had consistently elevated SGPT for several months prior to death. Increases in serum alkaline phosphatase activity were moderate, infrequent, and usually paralleled the elevated

SGPT. Of the nine dogs with bone tumors, all but one had occasional aberrant alkaline phosphatase levels. Figure 1.7 shows a serum enzyme profile during the 50-month period prior to euthanasia for a dog with bone tumors in the scapula, vertebrae, and humerus, as well as hepatic nodular hyperplasia.

TABLE 1.5. Plutonium Concentration in Tissues of Beagles After Inhalation of $^{238}\text{PuO}_2$.

Dog Number	Time After Exposure, months	Final Body Burden, μCi	Plutonium Concentration, nCi/g ^(a)			
			Lungs ^(b)	Lymph Nodes ^(c)	Liver	Skeleton
(After Inhalation of ²³⁸ PuO ₂ Calcined at 350°C)						
492 F	23	3	2	200	3.2	2.3
404 M	36	8.1	7.7	560	5.8	1.9
467 M	38	7	14	6900	4	1.2
469 M	54	2.6	14	220	1.6	0.8
445 M	58	2.5	2.1	510	1.8	1
438 M	60	2.3	1.1	890	3.4	0.6
453 M	62	2.2	4.2	230	1.9	1
405 M	70	2.6	7	180	1.9	0.6
(After Inhalation of ²³⁸ PuO ₂ Crushed Microspheres)						
485 M	22	3.1	25	460	0.7	0.3
500 M	34	1.1	5.6	1000	0.4	0.3
497 F	52	0.2	0.4	14	0.3	1
481 F	60	0.5	0.9	260	0.2	0.2
489 F	62	2.5	3.2	2300	4	0.8
482 F	70	0.8	0.8	900	0.6	0.2
494 F	75	0.4	0.2	92	0.6	0.2
488 F	76	1.4	2.2	1600	1.8	0.6

(a) nCi/g wet tissue

(b) Estimated normal weight of the lung was used to calculate concentration due to lesions in the lung

(c) Highest concentration in tracheobronchial, mediastinal or sternal lymph nodes

SERUM ENZYMES IN DOGS AFTER INHALATION OF $^{238}\text{PuO}_2$

FIGURE 1.7. Serum Enzymes in Dog 488 F After Inhalation of $^{238}\text{PuO}_2$. SGPT in Wroblewski-LaDue Units, Alkaline Phosphatase in Bessey-Lowry-Brock Units.

X224

The bone tumors were classified as osteosarcomas. The tumor cells were morphologically variable. In areas of the tumors where there was ample tumor bone formation, the cells were polyhedral, with a single nucleus. The cells tended to line the irregular surface of the bone spicules (Figure 1.8). In other areas of the tumors, the cells were spindle-shaped, resembling fibroblasts. They were arranged in large bundles and whorls. Tumor bone and/or osteoid was scarce in these areas, which constituted a small part of most tumors. Nearly all tumors were characterized by large amounts of tumor bone formation. The tumors appeared to grow by expansion and infiltration into adjacent tissues. Blood vessel invasion was common. In two dogs (469 M and 453 M) there were tumor emboli in the smaller branches of the pulmonary arteries, along with established pulmonary metastases (Figure 1.9). There were numerous single tracks in the photographic emulsion of the autoradiographs over the bone and bone marrow (Figure 1.10), indicating deposition of plutonium. The strongest focal activity was observed over

FIGURE 1.8. Area of Osteosarcoma Where Tumor Bone Formation Predominates. Note the irregular surface of the bone spicule, lined by small, polyhedral-shaped cells. H&E

hemosiderin granules in the cytoplasm of the marrow macrophages (Figure 1.11). Deposition in the bone-proper generally appeared uniform. However, occasional focal areas of high activity were observed over bone. The bone tumors contained less activity than the adjacent normal bone.

The location of the bone tumors in the dogs are listed in Table 1.6. Four of the nine dogs had two or more bone tumor sites in the skeleton; five dogs had tumors of the humerus, all in the proximal 1/3 of the bone. Five dogs had tumors in the vertebrae, located in the lumbar vertebrae in four of the five dogs.

The gross distribution of plutonium in the skeleton was evaluated by plutonium analyses of individual

X38

FIGURE 1.9. Metastatic Osteosarcoma in the Periphery of Lung from a ^{238}Pu -Exposed Dog. H&E

X215

FIGURE 1.10. Autoradiograph of the Cortex of the Femur of a Dog 5 Years After Inhalation of $^{238}\text{PuO}_2$. Note the relatively uniform distribution of silver grain tracks in the emulsion over the bone compared to the distribution in the marrow in Figure 1.11. Methylene blue.

X332

FIGURE 1.11. Bone Marrow Adjacent to Site Shown in Figure 1.10, Illustrating Localization of Activity Over Hemosiderin-Containing Macrophages. Methylene blue.

Table 1.6. Location of Bone Tumors in Dogs After Inhalation of $^{238}\text{PuO}_2$. (a)

Dog Number	Humerus	Femur	Scapula	Pelvis	Rib	Vertebrae	Total
469 M	X			X	XX	XXX	7
445 M		X			X	X	3
438 M		X					1
435 M	X				X		2
405 M						X	1
481 F		X					1
489 F	X					X	2
482 F	X			X			2
488 F	X		X			X	3
Total	5	3	1	2	4	7	22

(a) Radiographic, gross and histologic diagnosis.

bones or groups of bones. If a tumor occurred in an individual bone, the contralateral or adjacent bone was analyzed. Table 1.7 compares the plutonium concentration in the individual bones of four dogs with bone tumors. Although plutonium concentration in the individual bones varied, there did not appear to be a consistent trend within the individual animals toward tumors in bones with higher plutonium concentration.

Osteosarcomas, related to skeletal deposition of plutonium, were the primary cause of death in beagles 4 to 7 years after inhalation of $^{238}\text{PuO}_2$. However, concomitant lesions were observed in the lung, liver, and lymph nodes. Since there appears to be considerable animal-to-animal variability in the fraction of plutonium retained in these tissues, the effects in several organs must be considered in estimating the late effects of inhaled $^{238}\text{PuO}_2$.

Table 1.7. Concentration of ^{238}Pu in the Skeleton
nCi/g wet weight.

	Dog Number			
	<u>405M</u>	<u>453 M</u>	<u>445 M</u>	<u>469 M</u>
Pelvis	1.3	1.3	1.3	1.4 ^(a)
Rib	0.6	1.4 ^(a)	1.5 ^(a)	1.1 ^(a)
Scapula	0.5	1.2	1.8	1.1
Vertebrae	0.6 ^(a)	0.9	1.4 ^(a)	0.8 ^(a)
Femur	0.6	0.8	1.3 ^(a)	1.1
Humerus	0.7	0.6 ^(a)	0.7	1.5 ^(a)
Cranium	0.3	0.6	0.7	0.5
Mandible	0.3	0.6	0.6	0.3
Maxilla	0.3	0.6	0.6	0.3
Extremities ^(b)	0.2	0.5	0.8	0.4
Total Skeleton	0.6	1	1	0.8

(a) Bone Tumor

(b) Distal to the humerus and femur

DISTRIBUTION OF ^{241}Am AND ^{244}Cm IN DOGS AFTER
INHALATION OF THE OXIDES

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Dogs were exposed to $^{241}\text{AmO}_2$ or $^{244}\text{CmO}_x$ aerosols and sacrificed at various times postexposure. Both isotopes are readily translocated from the lungs to liver, skeleton and muscle, ^{244}Cm twice as rapidly as ^{241}Am .

To further our knowledge of the biological disposition and metabolism of transplutonium oxides, beagle dogs were exposed to three aerosol concentrations of $^{241}\text{AmO}_2$ or $^{244}\text{CmO}_x$. Five dogs were exposed at each dose level and scheduled for sacrifice at 10, 30, 90, 270, and 810 days.

Both materials were prepared by calcining the oxalate at 700 to 750°C for 2 to 4 hours. One hundred mg of the resulting powder was suspended in triple-distilled water, shaken vigorously and allowed to settle for 10 minutes before decanting the top 3.5 cm of the suspension. This decanted fraction was assayed before further dilution to obtain the isotope concentration desired for each dose level. These suspensions were nebulized according to established procedures to produce the $^{241}\text{AmO}_2$ or $^{244}\text{CmO}_x$ aerosols which the animals inhaled. The aerosol concentration and particle size distribution data, together with thorax counting data obtained 7 days postexposure, are given in Table 1.8.

Since the experiments were short-term (a maximum of 27 months post-exposure), retired breeding colony dogs averaging 5 years of age were used. Daily excreta samples were collected until the first thorax count, followed by weekly samples for the first month and a week-long sample just before sacrifice. Additional thorax counts were scheduled for all dogs at each of the sacrifice times. Routine hematology and clinical chemistry measurements were made pre-exposure and at 4-month intervals thereafter.

Nine americium- and 12 curium-exposed dogs have been sacrificed to date and tissue and excreta samples submitted for radiochemical analyses. The available tissue distribution data are summarized in Table 1.9. Both isotopes were readily transported from the dogs' lungs, curium-244 more rapidly than americium-241. While most of the material is translocated to liver and skeleton, there is also a considerable quantity in muscle and other soft tissues.

TABLE 1.8. Aerosol and Thorax Count Data (average values for 5 dogs)

Isotope	Dose Level	Aerosol Data			Thorax Count at 7 Days	
		Conc., nCi/l	AMAD, μ m	GSD	Alveolar Burden, nCi	Percent of Inhaled Dose Deposited
^{241}Am	Low	0.66	0.72	2.41	1.2	13
^{241}Am	Medium	42.1	1.35	1.71	124	30
^{241}Am	High	336	1.45	1.68	1150	35
^{244}Cm	Low	1.27	0.45	2.01	13	41
^{244}Cm	Medium	13.2	0.52	2.14	71	27
^{244}Cm	High	109 ^(a)	0.47	2.23	415 ^(a)	22

AMAD = Activity median aerodynamic diameter (μ m)

GSD = Geometric standard deviation of distribution

(a) Mean of 4 values. The concentration for Dog #598 F was 358 nCi/l; thorax count at 7 days was 2360 nCi.

TABLE 1.9. Tissue Distribution (percent of body content at sacrifice)

Isotope:	Americium-241				Curium-244			
	10 Days		30 Days		10 Days		30 Days	
Time Post-exposure:								
Dose Level: ^(a)	M	H	M	H	M	H	M	H
Dog #:	473 F	651 F	529 F	614 F	633 F	655 F	686 F	598 F
Lung	79	82	55	53	33	33	20	23
Liver	4	9	19	23	29	29	37	25
Skeleton	5	3	11	17	18	23	20	37
Muscle	8	3	11	5	13	9	13	9
Other	4	3	4	3	7	6	10	6
Total Body Content (nCi)	130.3	1062	112.8	1296	184.9	502	69.5	405

(a) M = Medium
H = High

TOXICOLOGY OF INHALED PLUTONIUM AND TRANSPLUTONIUM ELEMENTS IN RODENTS

This project is principally concerned with the effect of temporal and spatial distribution of dose from inhaled plutonium and transplutonium elements on the induction and pathogenesis of cancer in the lungs and other organs of rats and hamsters.

EFFECTS OF PuO₂ PARTICLES DEPOSITED IN RAT LUNG
FOLLOWING INTRAPERITONEAL INJECTION

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier

From 0.2 to 2.0% of intraperitoneally injected ²³⁹PuO₂ was found in the alveolar septa of rats at 1 to 2 years after injection, resulting in a cumulative radiation dose to the lung of up to 600 rads. Only one lung tumor was seen in 108 injected rats. It was concluded that sequestration and immobilization of large PuO₂ particles in the alveolar septa results in nil or low lung tumor incidence as compared to the high lung tumor incidence seen when PuO₂ is delivered to the lung as an aerosol.

Previous studies have shown that particulate plutonium injected into the blood or peritoneum will be partially transported to the lung, with the degree of uptake by the lung capillary network increasing with increasing particle size. Female Sprague Dawley rats were given a single intraperitoneal injection of either 72 nCi (36 rats), 360 nCi (38 rats), or 2900 nCi (35 rats) ²³⁹PuO₂ particles, suspended in 1 ml saline. The count median diameter of the PuO₂ was ~ 0.2 μm. Controls consisted of 92 rats. All rats were held for life span observation.

Discrete PuO₂ particles were seen on autoradiograms of the lung, nearly all within the alveolar septa. No differences in PuO₂ distribution were seen from 1 month to 2 years after injection; subpleural or peribronchiolar concentration of PuO₂ was not observed. Most of the sequestered

particles exhibited sizes estimated to be greater than 0.3 μm. The amount of ²³⁹Pu found in the lung at about 1 year after injection ranged from 0.2 to 0.3% of injected ²³⁹Pu for groups injected with 72 or 360 nCi, and 1.5% for groups injected with 2900 nCi. The amount of ²³⁹Pu in the lungs increased with age. Cumulative average radiation doses delivered to lungs at 1 year after injection were 600 rads (2900 nCi), 40 rads (360 nCi), and < 10 rads (72 nCi). Median survival times were 350 days, 470 days, and 580 days, respectively, and 760 days for controls.

There was no pathological reaction evident in areas of alveolar septum surrounding sequestered PuO₂ particles. Only one lung tumor was seen in all groups, an adenocarcinoma, at 823 days after injection of 72 nCi ²³⁹PuO₂. No pulmonary metaplasias were seen in any group.

Other studies in rats with intraperitoneally injected $^{238}\text{PuO}_2$, $^{239}\text{PuO}_2$ + asbestos or $^{239}\text{PuO}_2$ + benzpyrene also failed to demonstrate induction of lung tumors. In comparison, $^{239}\text{PuO}_2$ given by inhalation produces many lung tumors in rats at comparable radiation doses to lung.

Plutonium dioxide particles immobilized in the alveolar septa will expose only those epithelial cells within the limited range of alpha emissions. Particles deposited in the alveolar air spaces will be phagocytized by macrophages or type I

alveolar epithelium, with some particles being cleared from the lung, some particles being translocated to other organs, and some particles being transported and concentrated in subpleural peribronchiolar or perivascular regions of the lung. The results of such continuous movement of particles in the lung is the exposure of a much greater number of epithelial cells to alpha irradiation than would be expected from $^{239}\text{PuO}_2$ particles immobilized in the alveolar endothelium. It is concluded that stationary $^{239}\text{PuO}_2$ particles in the alveolar septa are much less carcinogenic than $^{239}\text{PuO}_2$ particles delivered by inhalation.

CARCINOGENICITY OF INTRATRACHEALLY INSTILLED $^{239}\text{PuO}_2$ AND ASBESTOS IN RATS

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier

Immobilization and concentration of $^{239}\text{PuO}_2$ in asbestos-induced scar tissue in peribronchiolar regions of the rat lung reduces the incidence of lung tumors. No mesotheliomas were observed.

Earlier studies with asbestos + $^{239}\text{PuO}_2$ in the peritoneal cavity of rats demonstrated an additive effect of the two carcinogens in the induction of peritoneal mesotheliomas (Annual Report, 1971). Similar studies were performed in the lung for comparison.

Female rats were given a single intratracheal instillation of saline, 0.9 mg chrysotile asbestos, 29 nCi

$^{239}\text{PuO}_2$, or 0.9 mg chrysotile asbestos + 51 nCi $^{239}\text{PuO}_2$, each mixed thoroughly in saline prior to instillation. Animals were held for life-span observation (Annual Report, 1973).

The alveolar retention half-time for ^{239}Pu was about 200 days in rats given only PuO_2 but 450 days in rats given PuO_2 + asbestos (Figure 2.1). The cumulative radiation dose to lung

Figure 2.1 Effect of Asbestos on Retention of $^{239}\text{PuO}_2$ in the Lung of Rats.

at 2 years after instillation was 425 rads for rats given only PuO_2 and 1200 rads for rats given PuO_2 + asbestos. PuO_2 particles given with asbestos were rapidly sequestered and retained more tenaciously within the peribronchiolar areas of the lung because of asbestos-induced inflammatory and progressive formation of scar tissue. No differences in animal mortality patterns were seen among groups. At two years after instillation, the incidence of pulmo-

nary tumors was as follows: 0 in rats given saline, 9% in rats given asbestos, 32% in rats given PuO_2 and 25% in rats given asbestos + PuO_2 . One pulmonary sarcoma was found in each of the groups given asbestos. No mesotheliomas were observed in any group. Squamous carcinomas were seen after PuO_2 administration but not after asbestos or asbestos + PuO_2 administration. The majority of lung tumors were adenocarcinomas (Table 2.1). A greater incidence of adenomatosis was seen in rats given PuO_2 than in those given PuO_2 + asbestos (21% and 8%, respectively). The adenocarcinomas found in rats given PuO_2 + asbestos appear to be less differentiated and more invasive in the lung than adenocarcinomas seen after only PuO_2 administration.

In contrast to observations following intraperitoneal administration, asbestos did not act in an additive manner with PuO_2 in the induction of pulmonary tumors. Indeed, the incidence of tumors was decreased by the addition of asbestos with PuO_2 . The incidence of lung carcinomas in rats given $^{239}\text{PuO}_2$ was about four times greater per rad absorbed dose to lung than for rats given $^{239}\text{PuO}_2$ + asbestos. Despite the small number of animals and the differences in initial deposition of $^{239}\text{PuO}_2$ in the lung, this investigation suggests that reducing the number of pulmonary epithelial cells hit by alpha irradiation will decrease the induction of pulmonary carcinoma.

TABLE 2.1. Neoplastic Response of Lung to Intratracheally Instilled Asbestos, PuO_2 or Asbestos + PuO_2 .

Group	Number of Animals	Incidence of Pulmonary Tumors, %			
		Adenocarcinoma	Squamous Carcinoma	Sarcoma	Total
Saline	26	0	0	0	0
Asbestos	22	4	0	4	9
$^{239}\text{PuO}_2$	22	21	11	0	32
$^{239}\text{PuO}_2$ + Asbestos	27	21	0	4	25

TOXICOLOGY OF INHALED $^{238}\text{PuO}_2$ AND $^{239}\text{PuO}_2$ IN HAMSTERS

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier

Male and female Syrian golden hamsters were exposed to $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ aerosols for study of plutonium metabolism and pathologic effects. Alveolar clearance decreased with increasing amounts of deposited plutonium. The metabolism of $^{238}\text{PuO}_2$ and that of $^{239}\text{PuO}_2$ were similar but gave evidence of a greater $^{238}\text{PuO}_2$ solubility. Adenomatous-squamous metaplasia was a common finding in the lung, particularly at the highest depositions. Only one primary lung tumor was seen in 172 necropsied hamsters up to a year after exposure.

Studies with inhaled plutonium in beagle dogs, albino rats and baboons indicate a high primary lung cancer incidence at optimal carcinogenic radiation doses to the lung of a few thousand rads. This study provides data on an additional species, the Syrian golden hamster, and its toxicologic response to inhaled $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$.

Both male and female Syrian golden hamsters were exposed at about 70 days of age to freshly prepared $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ aerosols by a single, nose-only, 30-minute exposure. Hamsters were exposed in groups of 35 animals at initial alveolar deposition levels of about 3 nCi to 200 nCi for each sex and each plutonium isotope. Initial alveolar deposition was determined for females at all exposure levels and for males at one exposure level, as the amount of plutonium in the lung at 30 days plus the amount of plutonium in excreta

from 4-30 days after exposure. The initial alveolar depositions of plutonium in males at other exposure levels were estimated by construction of clearance curves from the data on individual lung plutonium contents as hamsters died. Approximately 180 male and 180 female exposed, and 85 male and 85 female unexposed controls, are being held for life-span observation.

The initial (30-day) alveolar clearance and long-term alveolar clearance of PuO_2 was clearly influenced by the amount of deposited plutonium, with an increase in deposition causing a decrease in both phases of alveolar clearance (Tables 2.2., 2.4, 2.5). Initial alveolar depositions for female exposure groups were 4.0, 9.5, and 200 nCi of $^{238}\text{PuO}_2$ and 3.1, 35 and 160 nCi of $^{239}\text{PuO}_2$. Initial alveolar depositions for male exposure groups were estimated at 5.0, 15, and 200 nCi for $^{238}\text{PuO}_2$ and 3.0, 19, and 160 nCi for $^{239}\text{PuO}_2$.

TABLE 2.2. Deposition and Clearance of Inhaled Plutonium Dioxide by Female Hamsters (Average Values from 3 to 5 Animals).

Isotope	Excreta, 4-30 Days After Exposure	Amount of Pu, nCi		Initial Alveolar Deposition	Alveolar Clearance at 30 Days After Exposure, %
		Excreta, 4-30 Days After Exposure	Lung at 30 Days After Exposure		
$^{238}\text{PuO}_2$	2.5	1.4	+ 0.7	4	66
	5.4	3.9	+ 2	9.5	59
	34	160	+ 56	200	18
$^{239}\text{PuO}_2$	2.1	0.91	+ 0.32	3.1	71
	21	14	+ 12	35	62
	(9.7) (a)	(9	+ 6.3) (a)	(19) (a)	(53) (a)
	63	95	+ 31	160	41

(a) Male hamsters

TABLE 2.3. Distribution of ^{238}Pu or ^{239}Pu in Tissues of Female Hamsters as Percent of Terminal Body Burden at 30 Days After Inhalation. (Average Values from 3 to 5 Animals).

Tissue	Initial Alveolar Lung Burden, nCi					
	$^{238}\text{PuO}_2$			$^{239}\text{PuO}_2$		
	4.0	9.5	200	3.1	35	160
Lung	88	96	98	92	94	98
Skeleton	2.2	0.12	0.24	0.53	0.58	0.12
Liver	0.26	0.59	0.08	0.53	2.4	0.22
Pulmonary Lymph Nodes	1.6	1.2	1.3	0.58	0.66	0.84

TABLE 2.4. Distribution of ^{238}Pu in Tissues of Male and Female Hamsters as Percent of Initial Alveolar Lung Burden at 3-12 Months After Inhalation of $^{238}\text{PuO}_2$.

Sex	Female			Male		
Initial Alveolar Lung Burden, nCi	<u>4</u>	<u>9.5</u>	<u>200</u>	<u>5</u>	<u>15</u>	<u>200</u>
Number of Animals	<u>3</u>	<u>7</u>	<u>20</u>	<u>8</u>	<u>13</u>	<u>8</u>
Lung	*	11	34	12	20	37
Skeleton	*	*	1	0.36	0.90	0.98
Liver	*	0.25	0.89	0.33	0.51	0.68
Pulmonary Lymph Nodes	*	0.43	4	*	1.2	2.8

* Insufficient data.

At 30 days after exposure, the lung contained from 88% to 98% of terminal body burden of either ^{238}Pu or ^{239}Pu . No differences were seen in liver or skeleton plutonium contents; pulmonary lymph nodes contained about twice the amount of ^{238}Pu as of ^{239}Pu (Table 2.3).

nephritis. Six carcinomas in various extrapulmonary organs were seen scattered among the various groups. Only one primary lung tumor was seen in the 172 necropsied hamsters, an adenocarcinoma of a male hamster in the highest $^{239}\text{PuO}_2$ exposure group (Table 2.6).

TABLE 2.5. Distribution of ^{239}Pu in Tissues of Male and Female Hamsters as Percent Initial Alveolar Lung Burden at 3-12 Months After Inhalation of $^{239}\text{PuO}_2$.

Sex	Female			Male		
Initial Alveolar Lung Burden, nCi	3.1	35	160	3	19	160
Number of Animals	13	13	18	5	12	14
Lung	4.2	15	41	*	13	36
Skeleton	0.08	0.15	0.22	*	0.21	0.24
Liver	0.04	0.07	0.08	*	0.52	0.14
Pulmonary Lymph Nodes	0.09	1.1	1.1	*	0.77	2.6

* Insufficient data.

At later times of from 3 to 12 months after exposure, the liver and skeleton accumulated more ^{238}Pu than ^{239}Pu , although the difference was small. Pulmonary lymph nodes continued to accumulate more ^{238}Pu than ^{239}Pu , although, again, the difference between the two isotopes was small (Tables 2.4 and 2.5). No difference in the metabolism of PuO_2 was seen between male and female hamsters (Tables 2.2, 2.3, 2.4, 2.5).

Of 360 hamsters exposed to PuO_2 , 134 had died by 12 months after exposure; of 170 unexposed hamsters, 38 died during the same period. The survival of male hamsters was somewhat better than that of females.

The primary cause of death at the higher exposure levels was radiation pneumonitis; at lower exposure levels and in controls, the primary cause of death was amyloidosis and chronic

Hamsters did, however, exhibit a high incidence of pulmonary adenomatous-squamous metaplasia (so-called focal adenomatous lesion, sometimes seen associated with squamous metaplasia) in the highest exposure groups; the incidence of these lesions decreased with decreasing deposition level, although they were also seen in unexposed controls (Table 2.6).

It is concluded that the behavior of either $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ in the hamster is similar to that seen in the albino rat, and that no differences are seen in the behavior of plutonium between male and female hamsters. As in the rat, $^{238}\text{PuO}_2$ shows evidence of being more soluble than $^{239}\text{PuO}_2$.

The pathologic reaction of the lung of the hamster was quite different from that seen in the rat, particularly in the lack of lung tumor formation, which in the rat at similar times and radiation doses, was much more prevalent.

TABLE 2.6. Pathogenicity of Inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ in Hamsters

Initial Alveolar Deposition, nCi	Isotope	Sex	Number of Animals Dead Alive		Median Life Span, days ^(a)	Incidence of Pulmonary Pathology, %		Extrapulmonary Tumors
						Adenomatous-Squamous Metaplasia	Tumors	
3.1	$^{239}\text{PuO}_2$	Female	13	17	---	15.4	0	---
35	"	"	13	17	---	15.4	0	Carcinoma of stomach
160	"	"	18	12	324	38.9	0	---
3.0	$^{239}\text{PuO}_2$	Male	5	25	---	0	0	---
19	"	"	12	18	---	7.7	0	Esophageal Carcinoma, Renal Carcinoma
160	"	"	14	16	---	42.9	7.1 ^(b)	---
4.0	$^{238}\text{PuO}_2$	Female	3	27	---	0	0	---
9.5	"	"	7	23	---	0	0	---
200	"	"	20	10	288	15	0	Intestinal Carcinoma
5.0	$^{238}\text{PuO}_2$	Male	8	22	---	0	0	---
15	"	"	13	17	---	7.7	0	---
200	"	"	8	22	---	25	0	---
Control I	---	Female	13	32	---	0	0	Biliary Carcinoma
Control I	---	Male	7	38	---	0	0	---
Control II	---	Female	10	30	---	0	0	Adrenal Carcinoma
Control II	---	Male	8	32	---	0	0	---

(a) After exposure at about 70 days of age.

(b) Adenocarcinoma

TOXICOLOGY OF INHALED $^{239}\text{PuO}_2$ IN RATS.
PROGRESS REPORT.

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier

Preliminary results are reported from an experiment still in progress. About 50% of the initial alveolar deposition of inhaled $^{239}\text{PuO}_2$ was cleared from the lung in the first 30 days; the remainder was retained with a biological half-life of about 200 days. The incidence of lung tumors may be increased following initial alveolar deposition of as little as 0.15 nCi. Squamous carcinomas in lung predominate at high deposition levels and adenocarcinomas at lower levels.

Additional information is needed on the carcinogenicity of inhaled $^{239}\text{PuO}_2$ at low exposure levels, and for comparison with similar data on $^{238}\text{PuO}_2$. Experiments with $^{239}\text{PuO}_2$ are described in this report and similar studies with $^{238}\text{PuO}_2$ in the succeeding report.

Several groups of Wistar, SPF female rats were exposed to aerosols of $^{239}\text{PuO}_2$ in 1972. Exposure groups consisted of rats with the following initial alveolar lung burdens (number of animals shown in parentheses): 180 nCi (30), 47 nCi (32), 42 nCi (60),

8.1 nCi (60), 0.20 nCi (60), and 0.15 nCi (60). These rats were held for life-span observation. An additional five rats at each exposure level were killed at 1 day, and another five at 30 days after inhalation for determination of ^{239}Pu content of tissues. Initial alveolar lung burdens were determined as either (1) 1-day burdens, or (2) the total amount of ^{239}Pu in excreta collected from 4-30 days after exposure plus body burden at 30 days after exposure. Results from these two methods showed reasonable agreement (Table 2.7).

TABLE 2.7 Preliminary Data on Deposition and Alveolar Clearance of Inhaled $^{239}\text{PuO}_2$ by Rats

<u>Initial Alveolar Deposition, nCi</u>		<u>Alveolar Clearance at</u>		<u>Biological Half Life</u>
<u>1-Day Lung</u>	<u>Excreta + 30-Day</u>	<u>30 Days After</u>		<u>of Long-Term</u>
<u>Burden</u>	<u>Body Burden</u>	<u>Exposure, %</u>		<u>Component in Lung</u>
0.15 $\pm$ 0.07	---	---		---
0.20 $\pm$ 0.05	---	---		---
4.4 $\pm$ 1.6	8.1 $\pm$ 3.1	52		---
56 $\pm$ 13	42 $\pm$ 16	51		---
---	47 $\pm$ 23	55		130
180 $\pm$ 54	---	---		240

From 51-55% of the initial alveolar deposit of ^{239}Pu was cleared from the lung within 30 days after exposure. The biological half-life for ^{239}Pu following early clearance was estimated at about 200 days (Table 2.7).

There was a greater translocation of ^{239}Pu to extrapulmonary tissues at the lower deposition levels. About 97% of initial alveolar deposits of 40 nCi or less had cleared from the lung by 1-2 years after exposure; but only 86% of alveolar ^{239}Pu had cleared at the highest dose level. The amount of plutonium retained in pulmonary lymph nodes at 1-2 years was usually less than 1% of alveolar deposition (Table 2.8).

About 75% of the animals in this experiment are now dead. Only at the

two highest exposure levels was there evidence of a decreased life span (Table 2.9). Squamous metaplasia has been seen only at the highest exposure level. Alveolar adenomatous metaplasia or adenomatosis, on the other hand, was seen with decreasing frequency at lower doses, including controls. No primary lung tumors were found in controls. No carcinomas were found in the two lowest exposure groups, although two of these rats developed lymphosarcomas. Hemangiosarcoma and squamous carcinoma were more prevalent at the higher deposition levels, while the incidence of adenocarcinoma was greater at lower deposition levels. There is a clearly increased incidence of total tumors with increasing plutonium exposure (Table 2.9).

TABLE 2.8. Distribution of ^{239}Pu in Tissues at 1-2 Years After Inhalation of $^{239}\text{PuO}_2$. Values listed are mean percent of initial alveolar lung burden.

Tissue	Initial Alveolar Lung Burden			
	8.1 nCi	42 nCi	47 nCi	180 nCi
Lung	2.1	3.3	2.7	14
Skeleton	0.08	0.08	0.05	0.06
Liver	0.18	0.07	0.04	0.07
Pulmonary Lymph Nodes	0.61	0.47	0.59	1.5

TABLE 2.9. Preliminary Data on the Pathogenicity of Inhaled $^{239}\text{PuO}_2$ in Rats

Initial Alveolar Deposition, nCi	Number of Animals Dead Alive		Median Life Span, days ^(a)	Incidence of Pulmonary Pathology, % of Dead Animals						
				Adeno-matosis	Squamous Metaplasia	Adeno-carcinoma	Squamous carcinoma	Hemangio-sarcoma	Lympho-sarcoma	Total Tumors
Controls	33	17	716	3	0	0	0	0	0	0
0.15	41	19	731	0	0	0	0	0	4.9	4.9
0.20	43	17	652	2.3	0	0	0	0	0	0
8.1	49	11	679	0	0	4.1	2	2	2	12.2 ^(b)
42	43	17	641	11.6	0	18.6	9.3	2.3	0	30.2
47	32	0	586	25	0	25	9.4	3.1	0	37.5
180	30	0	510	30	10	20	23.3	10	0	53.3

(a) After exposure at about 70 days of age.

(b) Also one primary fibrosarcoma.

The incidence of extrapulmonary, nonmammary tumors was not influenced by deposition of $^{239}\text{PuO}_2$. From 17% to 57% of these tumors metastasized to the lung, the incidence of metastasis being somewhat greater at the moderate deposition levels (Table 2.10).

From these preliminary results, it is already evident that the lung is the principal target organ for neoplastic response, and that relatively little plutonium reaches other organs.

TABLE 2.10. Preliminary Data on the Percentage Incidence of Extrapulmonary Tumors and Frequency of Metastases to Lung Following Inhalation of $^{239}\text{PuO}_2$.

Site of Tumor	Initial Alveolar Deposition						
	Controls	0.15 nCi	0.20 nCi	8.1 nCi	42 nCi	47 nCi	180 nCi
Skin	3	4.9	2.3	4.1	2.3	3.1	0
Uterus	3	2.4	0	2	2.3	0	0
Adrenal	0	0	2.3	2	0	0	0
Kidney	0	2.4	0	0	2.3	0	0
Ovary	0	0	2.3	0	0	0	0
Spleen	0	0	2.3	0	0	0	0
Abdomen	0	0	0	2	0	0	0
Thorax	3	2.4	0	0	0	0	0
Leukemia	6.1	2.4	4.7	4.1	0	0	0
Total	15.1	14.5	13.9	14.2	6.9	3.1	0
% Extrapulmonary Tumor Metastatic to Lung (Excluding Leukemia)	20	17	50	57	33	0	0

TOXICOLOGY OF INHALED $^{238}\text{PuO}_2$ IN RATS. PROGRESS REPORT

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier

Preliminary results are reported from an experiment still in progress. Alveolar clearance of $^{238}\text{PuO}_2$ was somewhat less than that for $^{239}\text{PuO}_2$, despite the greater in vivo solubility of $^{238}\text{PuO}_2$. The incidence of lung tumors may be increased following initial alveolar deposition of as little as 0.07 nCi. Squamous carcinoma in lungs predominated at high deposition levels and adenocarcinoma at lower levels. No hemangiosarcomas were seen with $^{238}\text{PuO}_2$.

A study of the comparative toxicology of ^{238}Pu and ^{239}Pu is important because of the higher specific activity of ^{238}Pu and the greater in vivo solubility of $^{238}\text{PuO}_2$ as compared with $^{239}\text{PuO}_2$.

Several groups of Wistar, SPF female rats were exposed to aerosols of freshly prepared $^{238}\text{PuO}_2$ in 1972. Exposure groups consisted of rats with the following initial alveolar lung burdens (number of animals shown in parentheses): 890 nCi (27), 220 nCi (30), 13 nCi (60), 6.9 nCi (60), 0.2 nCi (60), 0.07 nCi (60), and unexposed controls (50). All rats were

held for life-span observation. An additional five rats at each exposure level were killed at 1 day, and another five at 30 days after inhalation for determination of ^{238}Pu content of tissues. Initial alveolar lung burdens were determined as either (1) 1-day lung burdens, or (2) the total amount of ^{239}Pu in excreta collected from 4-30 days after exposure plus body burden at 30 days after exposure. Results from these two methods showed reasonable agreement (Table 2.11).

From 36% to 53% of initial ^{238}Pu deposited in the alveoli was cleared

TABLE 2.11. Preliminary Data on Deposition and Clearance of Inhaled $^{238}\text{PuO}_2$ by Rats (Average $\pm$ SD).

Initial Alveolar Deposition, nCi		Excreta + 30-Day Body Burden		Alveolar Clearance at 30 Days After Exposure, %	Biological Half-Life of Long-Term Component in Lung, days
1-Day Lung Burden					
0.07 $\pm$	0.02	---		---	---
0.20 $\pm$	0.04	---		---	---
10 $\pm$	2.8	6.9 $\pm$	4.1	53	---
12 $\pm$	4	13 $\pm$	2.9	42	---
---		220 $\pm$	65	36	---
890 $\pm$	250	---		---	150

from the lung by 30 days after exposure. Increasing the amount of deposited $^{238}\text{PuO}_2$ decreased the fraction of ^{238}Pu cleared during this early clearance phase. The biological half-life for ^{238}Pu in the lung during the long-term, second clearance phase was estimated at 150 days (Table 2.11).

A greater fraction of ^{238}Pu was translocated from the lung with smaller amounts of initial alveolar deposition. All exposure groups showed consistently high deposition of ^{238}Pu in bone, and somewhat less in liver. The amount of ^{238}Pu found in bone or in pulmonary lymph nodes was usually about half of that found in the lung at 1-2 years after exposure. No differences in lung retention or tissue burdens of ^{238}Pu were seen at these times over the deposition range of 6.9 nCi to 220 nCi, despite rather marked differences seen at 30 days after exposure (Table 2.12).

About 60% of the animals in this experiment have died to date. Only at the highest exposure level, 890 nCi,

was there evidence of a reduced life span compared to other groups (Table 2.13).

Squamous metaplasia in the lung has been seen only in the highest exposure level, while squamous carcinomas were found as the predominant lung tumor type in the two highest deposition levels. No evidence of primary neoplasia or metaplasia was seen in the lungs of control animals. No hemangiosarcomas were seen in any group; one lymphosarcoma was seen in the lowest exposure group. Pulmonary adenocarcinomas and adenomatosis were the predominant indicators of plutonium deposition at the moderate-to-low deposition levels (Table 2.13).

The incidence of extrapulmonary, nonmammary tumors was not influenced by deposition of $^{238}\text{PuO}_2$. Primary sarcomas, carcinomas, and melanomas were found in skin, uterus, adrenal, spleen, thoracic cavity (esophagus and pleural cavity), and urinary bladder. Several leukemias were also seen. Up to 60% of these tumors, other than leukemia, had metastasized to the lung. No apparent differences were seen among the dose level groups (Table 2.14).

TABLE 2.12. Distribution of ^{238}Pu in Tissues at 1-2 Years After Inhalation of $^{238}\text{PuO}_2$. Values listed are mean percent of initial alveolar lung burden.

Tissue	Initial Alveolar Lung Burden			
	6.9 nCi	13 nCi	220 nCi	890 nCi
Lung	6.2	7.3	8.6	43 ^(a)
Skeleton	13	3.4	4	2.9 ^(a)
Liver	1.1	0.65	0.44	0.41 ^(a)
Pulmonary Lymph Nodes	3.6	4	2.1	4.2 ^(a)

(a) 100-300 Days After Inhalation

TABLE 2.13. Preliminary Data on the Pathogenicity
of Inhaled $^{238}\text{PuO}_2$ in Rats.

Initial Alveolar Deposition, nCi	Number of Animals		Median Life Span, days ^(a)	Incidence of Pulmonary Pathology, % of Dead Animals						
				Adeno- matosis	Squamous Metaplasia	Adeno- carcinoma	Squamous Carcinoma	Hemangio- sarcoma	Lympho- sarcoma	Total Tumors
Controls	34	16	637	0	0	0	0	0	0	0
0.07	32	28	696	0	0	3.1	0	0	3.1	6.3
0.2	29	31	---	0	0	0	0	0	0	0
6.9	28	32	---	3.6	0	7.1	0	0	0	7.1
13	33	27	682	6.1	0	6.1	0	0	0	6.1
218	30	0	627	36.7	0	23.3	33.3	0	0	56.7
890	27	0	133	7.4	18.5	3.7	14.8	0	0	18.5

(a) After exposure at about 70 days of age.

TABLE 2.14. Preliminary Data on the Percentage Incidence of Extrapulmonary Tumors and Frequency of Metastases to Lung Following Inhalation of $^{238}\text{PuO}_2$.

Site of Tumor	Initial Alveolar Deposition						
	Control	At 0.07 nCi	0.20 nCi	6.9 nCi	13 nCi	220 nCi	890 nCi
Skin	---	3.1	3.4	---	3	3	---
Uterus	5.9	3.1	3.4	---	6.1	---	---
Adrenal	---	---	3.4	---	3	---	---
Kidney	---	---	---	---	---	---	---
Ovary	---	---	---	---	---	---	---
Spleen	---	---	---	---	---	3.3	---
Thorax	---	6.3	---	---	---	---	---
Bladder	---	---	---	---	3	---	---
Leukemia	2.9	---	---	7.1	---	---	---
Unknown Origin	<u>2.9</u>	<u>---</u>	<u>---</u>	<u>3.6</u>	<u>---</u>	<u>---</u>	<u>---</u>
Total	11.7	12.5	10.2	10.7	15.1	6.3	0
% Extrapulmonary Tumors Metastatic to Lung (Excluding leukemia)	50	0	33	33	60	50	0

TOXICOLOGY OF INHALED $^{244}\text{CmO}_2$ in Rats.

PROGRESS REPORT

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier

Preliminary results are reported from an experiment still in progress. About 50% of initial alveolar deposition of $^{244}\text{CmO}_2$ was cleared from the lung during the first day and about 90% by 30 days after exposure. $^{244}\text{CmO}_2$ is more readily absorbed from the lung than plutonium oxides, as evidenced by higher concentrations in bone and liver. Adenocarcinoma is the predominant tumor type thus far observed in the lung. Bone tumors were also observed.

Curium-244, with a physical half-life of 17.6 years, is a product of nuclear fuel cycles. Due to its high specific activity, $^{244}\text{CmO}_2$ is physically unstable and behaves in vivo like a more soluble material.

Several groups of Wistar, SPF female rats were exposed to aerosols of freshly prepared $^{244}\text{CmO}_2$ in early 1973. Exposure groups consisted of rats with the following initial alveolar lung burdens (number of animals shown in parentheses): 1600 nCi (24), 710 nCi (44), 32 nCi (60), 6.0 nCi (60), 0.44 nCi (60), and unexposed controls (40). All of these rats were held for life-span observation. In addition five rats at each exposure level were killed at 1 day and

another five at 30 days after inhalation for determination of ^{244}Cm content of tissues.

From 86% to 97% of initially deposited ^{244}Cm was cleared from the lung within 30 days after exposure. The amount of ^{244}Cm deposited in the lung had no effect on clearance of the element from the lung. The amount of ^{244}Cm found in the body at 30 days, plus that found in excreta collected from 4-30 days, was about twice that found in the lung at 1 day after exposure. Where excreta data were unavailable, initial alveolar deposition was assumed to be twice the amount present in the lung at 1 day (Table 2.15).

TABLE 2.15. Preliminary Data on Deposition and Clearance of Inhaled $^{244}\text{CmO}_2$ by Rats.

Initial Alveolar Deposition, nCi		Alveolar Clearance at		Biological Half Life of Long-Term Component in Lung, days
1-Day Lung Burden	Excreta + 30-Day Body Burden	30 Days After Exposure, %		
0.22 ± 0.08	---	---	---	---
2.9 ± 0.42	6 ± 0.9	97	---	---
15 ± 4.1	32 ± 29	86	---	---
---	710 ± 100	95	---	---
790 ± 240	---	---	---	70

About as much ^{244}Cm was found in the skeleton or liver as in lung at 30 days after exposure, with skeletal ^{244}Cm burdens normally the greatest (Table 2.16). Less than 4% of initially deposited ^{244}Cm remains in the lung at periods of greater than 1 year after exposure.

Only 38% of the animals in this experiment have died (Table 2.17). Rats with an initial alveolar burden

of 1600 nCi experienced an early high mortality; the last of these died at 83 days after exposure. Rats with an initial alveolar burden of 710 nCi had a much longer survival time; two died early with squamous carcinomas. Other pulmonary preneoplastic and neoplastic responses took the form of either adenomatosis or adenocarcinoma formation and have thus far been observed only at the 710 nCi and 32 nCi deposition levels.

TABLE 2.16. Distribution of ^{244}Cm in Tissues at 30 Days After Inhalation. Values listed are mean percent of terminal body burden.

Tissue	Initial Alveolar Lung Burden				
	0.44 nCi	6.0 nCi	32 nCi	710 nCi	1600 nCi
Lung	4.8	16	25	15	17
Skeleton	10	15	42	52	52
Liver	15	10	19	16	14
Pulmonary Lymph Nodes	--	3.9	1.1	0.80	0.69

TABLE 2.17. Pathogenicity of Inhaled $^{244}\text{CmO}_2$ in Rats.

Initial Alveolar Deposition, nCi	Number of Animals		Median Life Span, days ^(a)	Incidence of Pulmonary Pathology, % of Dead Animals						
				Adeno-matosis	Squamous Metaplasia	Adeno-carcinoma	Squamous carcinoma	Hemangi-osarcoma	Lympho-sarcoma	Total Tumors
Unexposed Controls	7	33	--	0	0	0	0	0	0	0
0.44	15	45	--	0	0	0	0	0	0	0
6.0	15	45	--	0	0	0	0	0	0	0
32	17	43	--	0	0	11.8	5.9	0	0	17.6
710	31	13	505	25.8	0	25.8	3.2	0	0	29.0
1600	24	0 ^(a)	44	0	0	0	0	0	0	0

(a) After exposure at about 70 days of age.

The incidence of extrapulmonary, nonmammary tumors does not seem to be related to ^{244}Cm deposition, except for the seven bone tumors (Table 2.18). Four of these tumors were diagnosed as osteosarcomas, two as chondrosarcomas, and one as an undifferentiated fibrosarcoma. Preliminary estimates of cumulative radiation doses to bone

for the groups with bone tumors range from about 10 to 1000 rads. All of the bone tumors had metastasized to the lung.

From these early results it would appear that both lung and bone are important target organs for neoplastic response to inhaled $^{244}\text{CmO}_2$.

TABLE 2.18. Preliminary Data on the Percentage Incidence of Extrapulmonary Tumors and Frequency of Metastasis to Lung Following Inhalation of $^{244}\text{CmO}_2$.

Site of Tumor	Initial Alveolar Deposition				
	0.44 nCi	6 nCi	32 nCi	710 nCi	1600 nCi
Skin	---	6.7	5.9	3.2	---
Uterus	---	---	---	---	---
Adrenal	---	---	---	---	---
Kidney	6.7	---	---	---	---
Ovary	---	---	---	---	---
Spleen	---	---	---	---	---
Thorax	---	---	---	3.2	---
Bladder	---	---	---	---	---
Leukemia	6.7	---	---	3.2	---
Unknown Origin	---	---	---	---	---
Bone	---	6.7	11.8	12.9	---
Total	13.4	13.4	17.7	22.5	0
% Extrapulmonary Tumors metastatic to Lung (excluding leukemia)	0	100	67	57	0

LONG-TERM EFFECTS OF INTRATRACHEALLY INSTILLED
 $^{253}\text{EsCl}_3$ IN RATS

Investigators:

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Technical Assistance:

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Rats administered $^{253}\text{EsCl}_3$ by intratracheal instillation developed more bone tumors and fewer lung tumors than similar rats administered $^{239}\text{Pu}(\text{NO}_3)_4$. In explanation, it is suggested that ^{253}Es may irradiate bone surface cells more effectively while ^{239}Pu may irradiate a greater total number of cells in the lung.

Einsteinium-253 is a transuranic element having a half-life of 20.5 days and alpha decay energy of 6.6 MeV. Following intratracheal administration of $^{253}\text{EsCl}_3$ in rats, the radionuclide is retained mainly in lung, skeleton, liver, and kidney, a disposition pattern quite similar to that seen with soluble plutonium compounds. The great difference in physical half-life between ^{253}Es and ^{239}Pu ($T_{1/2} \sim 24,000$ years) offers the unique opportunity to investigate the effect of dose rate, and to some extent the effect of dose distribution, on the development of delayed biological effects. Whereas the differences in dose rate can be clearly identified as functions of the physical half-lives of the radionuclides, the differences in dose distribution are less well-defined. The latter are subject only to speculation in light

of our current thinking about the dynamic state of translocation and redistribution of the nuclides within the major tissues at increasing periods of time after exposure.

The experimental outline is shown in Table 2.19. The cumulative radiation doses are approximately one-half those estimated in earlier progress reports, principally because of refinements in the determination of retention parameters and in the choice of tissue or organ weights.

The number of malignant tumors and tumor types found are listed in Table 2.20. It is noteworthy that osteosarcomas were the most frequent, with lung tumors (8) and leukemias (9) the next most common malignancies. Einsteinium increased tumor incidence over that observed in controls except

TABLE 2.19. Experimental Protocol

Administered Dose, $\mu\text{Ci/kg}$	Number of Rats	Cumulative Radiation Dose, rads		
		Lung	Kidney	Skeleton
47.2	29	9800	560	1200
10.7	48	1900	110	230
0.214	48	38	2	5
0.01 N HCl	43	--	--	--

TABLE 2.20. Malignant Tumors Induced in Rats by Intratracheally Instilled $^{253}\text{EsCl}_3$

	0.01 N HCl	0.21 $\mu\text{Ci/kg}$	10.7 $\mu\text{Ci/kg}$	47.2 $\mu\text{Ci/kg}$
Osteosarcoma			20	1
Pulmonary Adenocarcinoma		1	4	
Pulmonary Squamous Cell Carcinoma			1	
Pulmonary Hemangiosarcoma			1	
Pulmonary Fibrosarcoma		1		
Renal Carcinoma			4	
Lymphocytic Leukemia	1	4	2	
Myelogenous Leukemia			2	
Subcutaneous Fibrosarcoma		1		
Abdominal Fibrosarcoma		1		
Pelvic Fibrosarcoma		1		
Heart Fibrosarcoma		1		
Urinary Bladder Fibrosarcoma	1			
Extremity Fibrosarcoma	1	1		
Abdominal Hemangiosarcoma		1	1	
Abdominal Liposarcoma			1	
Intestinal Leiomyosarcoma		1		
Pancreatic Islet Carcinoma			2	
Mammary Adenocarcinoma		1	1	
Gastric Adenocarcinoma		1		
Mammary Carcinoma	1			
Skin Squamous Cell Carcinoma	1	1		
Thymic Carcinoma	1			
Total Malignant Tumors	6	16	39	1

at the highest dose where early deaths due to radiation pneumonitis precluded tumor development.

The distribution of tumors induced by ^{253}Es and ^{239}Pu is compared in Table 2.21. The plutonium data is taken from the Russian literature (Koshurnikova and Lyubchanskii, 1969; Buldakov and Lyubchanskii, 1970). Although these data are from different laboratories and should be compared with caution, it seems clear that there are differences in tumor site. Where groups with comparable tissue doses and similar survival times can be compared, ^{239}Pu is the more effective inducer of lung tumors, while ^{253}Es produced more bone tumors. More detailed statistical comparisons will be possible when studies with ^{239}Pu , underway in our laboratory, are completed.

The 20.5-day physical half-life of ^{253}Es and the consequent short time for redistribution within bone may result in a large fraction of the radiation dose being delivered to sensitive cells on the bone surfaces, thus explaining its greater effectiveness in the induction of bone tumors. By comparison, the dose to skeleton from ^{239}Pu is accumulated throughout the life-span, with some fraction "wasted" within the bone matrix due to bone remodeling and redistribution of the plutonium. The difference in lung tumorigenicity between ^{253}Es and ^{239}Pu may also be related to the changing pattern of dose distribution. In the lung, unlike the bone, we know of no uniquely radiosensitive grouping of cells that may be selectively irradiated, and the continuing redistribution of ^{239}Pu throughout the animal's life should result in more cells exposed and a consequent increase in tumorigenicity.

TABLE 2.21. Carcinogenicity of Intratracheally Instilled ^{239}Pu (a) and ^{253}Es Compounds in Rats.

Agent	Dose	Cumulative Dose, rads		Average Survival, days	Tumor Incidence, %	
		Lung	Skeleton		Lung	Skeleton
$^{253}\text{EsCl}_3$	47.2 $\mu\text{Ci/kg}$	9800	1200	181 ^(c)	0	3
$^{253}\text{EsCl}_3$	10.7 $\mu\text{Ci/kg}$	1900	230	475 ^(c)	12.5	42
$^{253}\text{EsCl}_3$	0.21 $\mu\text{Ci/kg}$	38	5	707 ^(c)	4	0
^{239}Pu Citrate	0.305 μCi ^(b)	2230	374	184	5.88	0
^{239}Pu Sodium triacetate	1.0 μCi	1570	705	387	34.7	17.3
$^{239}\text{Pu}(\text{NO}_3)_4$	1.0 μCi	5855	880	285	20.3	4.3
$^{239}\text{Pu}(\text{NO}_3)_4$	0.42 μCi	2755	418.5	415	29.1	7.3
$^{239}\text{Pu}(\text{NO}_3)_4$	0.048 μCi	317	51.3	618	6.45	1.07
$^{239}\text{Pu}(\text{NO}_3)_4$	0.0042 μCi	28	---	626	1.08	0

(a) Data from Russian studies cited in text.

(b) Retained in lung 5 days after single exposure by inhalation.

(c) Median survival time.

DISPOSITION OF INHALED $\text{Pu}(\text{NO}_3)_4$ AND $\text{Es}(\text{NO}_3)_3$ IN RATS

Investigator:

J. E. Ballou

Technical Assistance:

R. A. Gies

Preliminary results indicate ^{253}Es is cleared from the lung more rapidly and translocated to liver and skeleton more extensively than either ^{238}Pu or ^{239}Pu .

The long-term biological effects of "soluble" forms of the alpha-emitting transuranic elements are being studied for comparison with ongoing studies employing the more insoluble oxides. Elements having a wide range in specific activities are employed to investigate the influence of dose rate and dose distribution on the biological effect.

Seventeen hundred, male Wistar rats, 60 days of age, were administered a single, 30-minute, nose-only exposure to aerosols of $^{239}\text{Pu}(\text{NO}_3)_4$, $^{238}\text{Pu}(\text{NO}_3)_4$, or $^{253}\text{Es}(\text{NO}_3)_3$ generated from 0.27 N nitric acid solution. The initial lung burdens ranged from 0.06 to 900 nCi ^{239}Pu , 0.02 to 1500 nCi ^{238}Pu and 10 to 1500 nCi ^{253}Es . Approximately 65% of the rats were

retained for life-span study of long-term biological effects, while the remainder were sacrificed periodically to determine retention kinetics in order to estimate radiation dose. Animals were killed immediately after exposure, at 7, 14, 30, 60, and 100 days following $^{253}\text{Es}(\text{NO}_3)_3$ inhalation, and at 30, 60, 100, 150 and 200 days following $^{239}\text{Pu}(\text{NO}_3)_4$ or $^{238}\text{Pu}(\text{NO}_3)_4$ inhalation.

Preliminary results summarizing retention kinetics in lung, liver and skeleton are shown in Figure 2.2. The plotted values are averages from 2 to 5 different exposure groups (10 to 25 rats per group) which varied widely in initial lung burden. There is some indication that clearance kinetics may be influenced by the magnitude of the initial lung burden; however, since the data are incomplete, they have simply been pooled for this presentation.

The rate of early lung clearance appears to be in the order $^{253}\text{Es} > ^{238}\text{Pu} > ^{239}\text{Pu}$, apparently related to the mass of radionuclide initially

deposited in lung (see Annual Report for 1972). The fraction of the initial lung burden translocated to liver and skeleton follows the same order of progression as that seen for lung clearance. The clearance curves for all three radionuclides appear to have roughly similar shapes after the first 30 days, although they may differ quantitatively because of the variations in translocation rate from lung.

Retention parameters derived from Figure 2.2 are shown in Table 2.22. The lung curves could be reasonably well described by two exponential functions with an additional fraction cleared with a half-life of < 1 day. The rapidly cleared fraction has been neglected in subsequent calculations of cumulative radiation dose. Retention in liver and skeleton was approximated by single exponentials. In the case of skeleton the data had not been collected for a long enough period to confirm the anticipated long retention half-life. Published values for effective half-life were therefore employed.

FIGURE 2.2. Disposition of Inhaled Plutonium and Einsteinium in Rats.

Estimated radiation dose, accumulated for 500 days, is shown in Table 2.23 for lung, liver, and skeleton. The overlap in the range of computed doses for the various tissues is encouraging and suggests that comparisons of long-term biological effects will be possible among the various dose groups for the different radionuclides. It should be noted that

the dose from ^{253}Es is delivered in considerably less than 500 days (about 95% of the skeleton dose is delivered in 85 days), while the Pu dose will be accumulated for the animal's life-span.

It is therefore anticipated that the dose ranges for the Pu isotopes will ultimately be somewhat greater than those indicated in Table 2.23.

TABLE 2.22. Retention Parameters for Inhaled Pu and Es Nitrates in Rats.

Agent	Lung		Liver		Skeleton	
	Fraction of ILB	T _{eff} , days	Fraction of ILB	T _{eff} , days	Fraction of ILB	T _{eff} , days(a)
$^{239}\text{Pu}(\text{NO}_3)_4$	0.19	< 1	0.02	55	0.11	500
	0.25	7				
	0.46	55				
$^{238}\text{Pu}(\text{NO}_3)_4$	0.15	< 1	0.03	70	0.17	500
	0.70	10				
	0.15	80				
$^{253}\text{Es}(\text{NO}_3)_3$	0.35	< 1	0.035	10	0.35	20
	0.30	3				
	0.35	10				

(a) Values for the effective half-life of Pu in bone are estimated from the literature. Einsteinium is assumed to be lost solely by radioactive decay.

TABLE 2.23. Estimated Range of Doses in Groups of Rats Exposed to ^{238}Pu , ^{239}Pu , and ^{253}Es . (a)

Agent	Estimated 500-Day Cumulative Dose, rads		
	Lung	Liver	Skeleton
$^{238}\text{Pu}(\text{NO}_3)_4$	0.08 - 5000	0.006 - 13	1 - 500
$^{239}\text{Pu}(\text{NO}_3)_4$	0.2 - 4000	0.001 - 20	0.02 - 350
$^{253}\text{Es}(\text{NO}_3)_3$	10 - 1200	0.1 - 13	1 - 180

(a) Range is inclusive for 5 dose levels of ^{238}Pu , 6 of ^{239}Pu and 3 of ^{253}Es .

LONG-TERM EFFECTS OF INHALED $\text{Pu}(\text{NO}_3)_4$ AND $\text{Es}(\text{NO}_3)_3$ IN RATS

Investigators:

J. E. Ballou and G. E. Dagle

Technical Assistance:

R. A. Gies

Ongoing studies with rats exposed to a wide range of lung burdens of inhaled $^{239}\text{Pu}(\text{NO}_3)_4$, $^{238}\text{Pu}(\text{NO}_3)_4$, and $^{253}\text{Es}(\text{NO}_3)_3$ show little effect on weight gain; the highest dose levels significantly affect survival. Information on the long-term carcinogenic effects is not yet available.

Preliminary observations on weight gain and gross mortality are available from a continuing study of the long-term effects of inhaled actinides. Thus far, 1700 male Wistar rats, 60 days of age, in exposure level groups of 100, have received single, nose-only exposures to $^{239}\text{Pu}(\text{NO}_3)_4$, $^{238}\text{Pu}(\text{NO}_3)_4$, or $^{253}\text{Es}(\text{NO}_3)_3$ aerosols. Animals from each exposure group were sacrificed periodically to determine parameters of clearance kinetics required for dose calculations. Other animals are being retained for life-span study to determine the dose-response relationships for tumor induction. The investigation has been in progress for more than 800 days and will eventually include additional animals exposed to curium and americium nitrates.

Monthly weight averages for rats exposed to Pu or Es aerosols (Figures 2.3, 2.4, 2.5) were little influenced by the radiation insult, although the initial lung burdens (ILB) varied

over a wide range. The median survival times (MST) appeared to decrease as the lung burdens were increased; however, this effect is probably not statistically significant except at the highest level of lung deposition. Mortality curves are shown in Figures 2.6 and 2.7.

Mortality curves are not available for $^{253}\text{Es}(\text{NO}_3)_3$ since this part of the study has been in progress for only 10 months. The highest dose level of ^{253}Es (group 13 in Figure 2.5) was the most acutely toxic (MST = 46 days) of any of the materials administered.

Preliminary observations suggest that both $^{238}\text{Pu}(\text{NO}_3)_4$ and $^{239}\text{Pu}(\text{NO}_3)_4$ will principally induce lung and other soft tissue tumors rather than the bone tumors one might expect from the more mobile, soluble Pu compounds. Tissues from rats exposed to $^{253}\text{Es}(\text{NO}_3)_3$ have not yet been examined for histopathologic effects.

FIGURE 2.3. Effect of Inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ on Weight Gain of Rats.

FIGURE 2.4. Effect of Inhaled $^{238}\text{Pu}(\text{NO}_3)_4$ on Weight Gain of Rats.

FIGURE 2.5. Effect of Inhaled $^{253}\text{Es}(\text{NO}_3)_3$ on Weight Gain of Rats.

FIGURE 2.6. Effect of Inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ on Survival of Rats.

FIGURE 2.7. Effect of Inhaled $^{238}\text{Pu}(\text{NO}_3)_4$ on Survival of Rats.

TOXICOLOGY OF INHALED BERYLLIUM COMPOUNDS

This project is concerned with the retention and translocation of inhaled beryllium compounds, and with their possible granulomatous or carcinogenic effects in the lungs of rats and hamsters.

PULMONARY CLEARANCE AND PATHOGENICITY OF INHALED HIGH-FIRED
BERYLLIUM OXIDE. COCARCINOGENIC STUDY WITH $^{239}\text{PuO}_2$

Investigators:

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Technical Assistance:

D. M. Meier and E. F. Blanton

The alveolar retention half-life of beryllium oxide inhaled by rats was about 6 months. The alveolar clearance of $^{239}\text{PuO}_2$ was decreased to 60% of normal when PuO_2 was given at 1, 30, or 60 days after beryllium oxide. These results demonstrate the important role played by the alveolar macrophage in beryllium-induced granulomatous disease, and the rapid impairment of alveolar macrophage function by phagocytized beryllium oxide.

It has recently been found that high-fired beryllium oxide, previously thought innocuous, is indeed toxic. Studies are being conducted in this laboratory on the pathogenicity of the material. Beryllium oxide is the air contaminant common to most situations in which beryllium disease has been seen in man. Recent studies have demonstrated the aerosolization of beryllium from newly ignited mantle-type camp lanterns, indicating the possibility of widespread population exposure to beryllium oxide.

Female Wistar SPF rats were given a single, nose-only exposure to high-fired beryllium oxide, calcined at about 1000°C. In the first study, 210 male and 210 female rats were exposed to beryllium oxide with the animals equally divided among three dose levels of initial alveolar deposition of beryllium: 1 to 2 µg, 12 to 18 µg and 62 to 104 µg. Several animals were killed at 8 and 12 months post-exposure for histopathological examination and study of beryllium clearance. In the second study, 25 rats

were exposed to beryllium oxide with an initial alveolar deposition of 30 µg beryllium. Three groups of five rats (I, II, III) were then given an additional inhalation exposure to $^{239}\text{PuO}_2$ at 1 day, 30 days, or 60 days after beryllium exposure, and the alveolar clearance of ^{239}Pu measured.

The amount of beryllium retained in the lung at 8 months after exposure ranged from 24% to 36% of initial alveolar deposition. Analysis of this and earlier data indicated an estimated biological half-life for beryllium oxide of about 6 months. The amount of beryllium in pulmonary lung nodes increased to about 4% of initial alveolar deposition by 8 months after exposure.

Moderately developed granulomatous lesions were seen in the lungs at 8-12 months after exposure. These lesions consisted of loosely to moderately packed, dust-laden macrophages, usually of a histiocytic appearance, surrounding bronchiolar or vascular areas or in subpleural

zones of the lungs. Lymphocyte involvement was moderate; mast cells were rarely seen in these lesions. Only one primary lung tumor was seen in 60 male rats killed at 1 year after exposure: this was a sarcoma which was found in several lung lobes. No alveolar adenomatous or squamous metaplasias were seen in any lungs.

In the second study, mean retention of 20 μg and 12 μg of beryllium, respectively, were found in the lungs of groups sacrificed 60 and 90 days after exposure. The initial alveolar deposition was estimated at about 30 μg beryllium. $^{239}\text{PuO}_2$ given by itself exhibits a two-phase alveolar clearance, with about 50% of initial alveolar deposition cleared during the first 30 days after exposure and an approximate 200-day biological half-life in the lung for ^{239}Pu thereafter.

For all groups given $^{239}\text{PuO}_2$ and/or beryllium oxide, 90% of the ^{239}Pu found in the body at 30 days was in the lung. The amount of ^{239}Pu cleared from the alveoli during the first 30 days after exposure to $^{239}\text{PuO}_2$ ranged from only 46 to 50% of initial alveolar deposition (Table 3.1). Alveolar clearance of ^{239}Pu was depressed in rats given beryllium oxide prior to PuO_2 , with 30-day alveolar clearance in these groups amounting to only 26 to 34%. This amounts to a 40% reduction in early alveolar clearance of ^{239}Pu , irrespective of the time $^{239}\text{PuO}_2$ was given after beryllium oxide deposition (Table 3.1).

Although alveolar clearance of ^{239}Pu was depressed as greatly at 1 day as at 60 days after beryllium oxide deposition, there was no evidence of any fine structural damage

TABLE 3.1. Alveolar Clearance of Inhaled $^{239}\text{PuO}_2$ Deposited in the Lung at 1 Day, 30 Days, or 60 Days After Inhalation of Beryllium Oxide. About 35 μg of beryllium was initially deposited in the alveolar region. All values are means $\pm$ standard deviation ($n = 5$). Each paired group has the same common PuO_2 exposure, with half of that group having previously been exposed to beryllium oxide.

Treatment	Amount of ^{239}Pu , nCi		% Initial Alveolar Burden of ^{239}Pu Cleared in 30 Days(c)
	Excreta(a)	Lung(b)	
I PuO ₂ only	8.5 $\pm$ 4.6	11 $\pm$ 4.9	48 $\pm$ 9.6
I PuO ₂ , at 1 day after BeO	5.9 $\pm$ 1.2	18 $\pm$ 10	26 $\pm$ 8.9
II PuO ₂ only	13 $\pm$ 4.9	16 $\pm$ 6.4	50 $\pm$ 8.8
II PuO ₂ , at 30 days after BeO	9.9 $\pm$ 5.1	18 $\pm$ 9.2	34 $\pm$ 5.7
III PuO ₂ only	15 $\pm$ 8.5	16 $\pm$ 6.6	46 $\pm$ 17
III PuO ₂ , at 60 days after BeO	12 $\pm$ 4.5	25 $\pm$ 4.6	30 $\pm$ 8.4

(a) From 4-30 days after exposure to $^{239}\text{PuO}_2$ following rapid upper tracheobronchial clearance and excretion in the feces.

(b) At 30 days after exposure to $^{239}\text{PuO}_2$.

(c)
$$100 - \left[\frac{\text{Amount } ^{239}\text{Pu in Lung}}{\text{Amount } ^{239}\text{Pu in total body + excreta at 4-30 days}} \times 100, \% \right]$$

to alveolar macrophages, which had phagocytized both particulates, incorporating them into phagosomes.

It is concluded that high-fired beryllium oxide produces rapid functional damage to the alveolar macrophage, possibly inhibiting its movement to the ciliated portions of the terminal bronchioles, resulting in the more prolonged retention of a test aerosol of $^{239}\text{PuO}_2$. The significant impact of high-fired beryllium

oxide in the lung may not be so much the origin of beryllium disease as the significant impairment of pulmonary clearance to other inhaled toxic particulates to which the individual may be exposed.

Several groups of rats have been exposed to two dose levels of $^{239}\text{PuO}_2$ and beryllium oxide in combination for life-span toxicology studies, particularly carcinogenic effects on the lung. Results are not yet available.

DEVELOPMENT OF AEROSOL EXPOSURE AND ANALYTICAL TECHNIQUES

This project is concerned with the improvement of animal inhalation technology, and of techniques for radiochemical analysis, and with basic research on the deposition and retention of aerosols in the respiratory systems of various experimental animal species.

DEPOSITION OF INHALED POLYDISPERSED AEROSOLS IN BEAGLES
AS A FUNCTION OF PARTICLE SIZE

Investigators:

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 W. C. Cannon and J. P. Herring

Technical Assistance:

E. F. Blanton

A new technique is described for determining the percentage of aerosols deposited as a function of particle size in the lungs of beagle dogs during inhalation exposure. An example of the results is presented.

For comparison with man and for control of dose delivered during inhalation exposure of dogs, it is of interest to know the percentage of inhaled aerosols in each size range that will be deposited in the lungs. A new technique for determining this percentage, based upon direct measurement of the particle size distributions of the inhaled and exhaled aerosols, has been developed.

The aerodynamic size distribution of radioactive aerosols inhaled (IN) and exhaled (OUT) by adult beagle dogs was measured using 8-stage Mercer cascade impactors. This size distribution is characterized by the activity median aerodynamic diameter (AMAD) and geometric standard deviation (GSD). Aerosols of known concentration were inhaled through a venturi to measure inspired volume and respiration parameters. The exhaled aerosol was drawn through an impactor and/or a filter.

Using measured size distribution parameters $AMAD_{IN}$ and GSD_{IN} for inhaled, and $AMAD_{OUT}$ and GSD_{OUT} for exhaled aerosol, the total initial deposition curve as a function of aerodynamic equivalent size can be

computed. The fractional deposition of inhaled aerosols in i th size range (t_i) is given by the expression,

$$t_i = 1 - \frac{g_i Q_{OUT}}{f_i Q_{IN}} \quad (1)$$

where: f_i = fraction of inhaled aerosol in the i th size range,
 g_i = fraction of exhaled aerosol in the i th size range
 Q_{IN} = total quantity of inhaled aerosol,
 Q_{OUT} = total quantity of exhaled aerosol.

Values for Q_{IN} and Q_{OUT} can be estimated from the total activity (A) collected per unit time in the two impactors and their respective flow rates (F), i.e.,

$$Q = A/F \quad (2)$$

substituting in equation (1),

$$t_i = 1 - \frac{g_i A_{OUT} F_{IN}}{f_i A_{IN} F_{OUT}} \quad (3)$$

Since the f_i and g_i values are available from the inhaled and exhaled aerosol size distributions, t_i values can be calculated from equation (3). The results of such a calculation are shown in Figure 4.1. Histograms are shown of the fractional aerosol distribution obtained from the 8-stage cascade impactor samples of inhaled and exhaled aerosols of $^{241}\text{AmO}_2$. Values for t_i are plotted against the mean aerodynamic equivalent diameter for each size range.

This new technique for determining total initial deposition in dogs as a function of particle size is now

being applied to data obtained from the exposure of 15 dogs to $^{241}\text{AmO}_2$ and 15 dogs to $^{244}\text{CmO}_x$, and from three dogs each exposed six times to ^{198}Au -labeled gold aerosols. AMAD_{IN} values ranged from 0.4 to 1.9 μm , while GSD_{IN} values were from 1.3 to 2.4 for the different materials and concentrations. There was little difference between AMAD_{IN} and AMAD_{OUT} , but GSD_{OUT} was significantly smaller than GSD_{IN} for all but the gold aerosols. These calculations will enable the influence of other factors, such as the respiration parameters of each dog, to be examined.

FIGURE 4.1. Initial Deposition of $^{241}\text{AmO}_2$ Aerosol in Dog as a Function of Particle Size.

Tidal Volume, 177 ml

Respiratory rate, 13.4 min^{-1}

$\text{AMAD}_{\text{IN}} = 0.748 \mu\text{m}$

$\text{AMAD}_{\text{OUT}} = 0.752 \mu\text{m}$

$\text{GSD}_{\text{IN}} = 2.456$

$\text{GSD}_{\text{OUT}} = 2.112$

$i = 1, 2, 3$, etc. identifies cascade impactor stage.

MINICOMPUTER PROCESSING FOR ON-LINE TIDAL
VOLUME AEROSOL MEASUREMENTS

Investigator:

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Technical Assistance:

P. C. Stover^(a)

A small computer installed in the Inhalation Toxicology Laboratory is used to monitor aerosol inhalation exposure of dogs. It is designed to provide data acquisition, processing, and reporting during an exposure to improve experimental exposure techniques.

The current configuration of the aerosol inhalation control computer system is shown in Figure 4.2. The basic computer system consists of a Data General Corporation NOVA 1220 with an ASR-33 Teletype and a high-speed paper-tape reader/punch. The

control processor has 24,576 words of 16-bit core memory with automatic program load and a programmable real-time clock. The system is designed as a general-purpose laboratory tool for the Inhalation Toxicology program.

FIGURE 4.2. Aerosol Inhalation Control Computer System.

(a) Systems Department

Communication links to the computer have been developed for three laboratory instruments: the Plutonium Aerosol Monitor (PAM), which measures aerosol activity to determine concentrations; the Tidal Volume Aerosol Monitor (TVAM), which measures breath-by-breath inhaled volume; and the Particle Size Aerosol Monitor (PSAM), which measures particle size distribution in four partitions. A system has been developed which contains programs for each of the laboratory instruments. The programs can be called up (invoked) by typing appropriate names through the teletype keyboard. Programs are written in modular form so that they are relatively independent, yet permit data to be transferred between them.

Eight major programs have been written for aerosol-exposure-related research:

- the executive program, the program supervisor which includes the keyboard monitor used to call other programs
- the PAM program, for reading filter activity data into the computer and calculating aerosol concentrations
- a TVAM exposure program which uses concentration data from the PAM program, operator-supplied data, and TVAM instrument data to calculate total inhaled volumes and doses during aerosol exposures
- a tidal volume distribution program which generates a histogram (tidal volume versus number of breaths) from either the TVAM exposure program or the TVAM training program.
- a PSAM program to control the automatic operation of the PSAM and record particle size distribution data
- a series of support programs which include an editor for inserting comments on experiment reports printed by the teletype, a clock handler, arithmetic routines, and all input/output handlers; and finally,
- an off-line program which calculates the desired volume to be inhaled by each dog to reach the desired deposited dose.

The TVAM program is the basic program used to monitor all aerosol exposures with dogs, and is invoked once for each dog being exposed. Inputs to the program are the data on the concentration of the aerosol, derived from the PAM program, operator-entered data (including the dog identification code, the date, and the deposition coefficient), and either the desired dose or the desired exposure volume. Once the experiment starts, following the initialization procedure, the computer system monitors the TVAM instrument taking data on an interrupt basis throughout the experiment. Computer outputs occur both during and after the experiment.

During the experiment, the system monitors the inhaled volume and dose, and compares them against the limits entered prior to the exposure. The system keeps track of the percentage of the desired dose or volume attained and prints percent completion messages every 10% from 50 to 100% so that the exposure progress can be monitored by the user. The system does not terminate the experiment, but only reports progress. In addition, the system punches a complete record of the experimental data on the paper tape in order to provide a record of the experiment and record the data on a medium suitable for post-processing. One paper-tape record is automatically punched for each dog. Each record consists of a header followed by data. The header contains the date, dog identification, deposition coefficient, aerosol concentration, and the starting time of the experiment. Recorded data include a breath-by-breath recording of the inhaled tidal volume and the time of occurrence of each breath.

At the termination of each exposure, a summary of the experimental data is printed on the teletype. The report is formatted on 8 1/2 by 11-inch pages and contains (1) the DATE, (2) DOG IDENTIFICATION, (3) DESIRED DOSE (nCi), (4) INHALED DOSE (nCi), (5) TOTAL INHALED VOLUME (Liters), (6) TIME OF EXPOSURE (Minutes), (7)

NUMBER OF BREATHS, (8) AVERAGE RESPIRATION RATE (Breaths/Minute), (9) MINUTE VOLUME (Liters/Minute), and (10) AVERAGE TIDAL VOLUME (Liters/Breath). In addition, the program contains a simple edit routine which allows the investigator to insert typed comments on the output report. Comments are free format and can be entered at any time.

The final program designed for the TVAM exposure component of the system is the Tidal Volume Distribution Program. This program generates a tidal volume frequency distribution histogram from the data recorded on paper tape during an exposure. The user selects the minimum and maximum volume limits and the number of partitions. The program reads an exposure

tape, prints the dog identification code, the date of the experiment, and the distribution of number of breaths in each volume partition. In addition, an optional histogram plot of the data is available and the program indicates the number of breaths, if any, that lie outside the specified range of volumes.

The TVAM training program is used during the period when the dogs are trained to breathe in the experimental environment. It is basically the same program as the exposure program, but all dose-related elements have been deleted from it. Outputs include punched paper tape and printed reports. The paper tape can be used with the tidal volume distribution program to obtain breath distribution histograms.

ON-LINE MEASUREMENT OF PARTICLE SIZE
DISTRIBUTION OF RADIOACTIVE AEROSOLS

Investigators:

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Technical Assistance:

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A device based upon the varying impaction efficiencies of particles as a function of their aerodynamic equivalent diameters has been designed and built. It is being installed and calibrated in a dog exposure chamber where it will be used to determine in real time the particle size distribution of the aerosols being inhaled by the animals.

Particle deposition data from previous exposures of beagle dogs to plutonium oxide aerosols have shown a strong correlation between particle size and deposition. Thus, in order to accurately control deposition, the particle size distribution of the aerosol must be known. Heretofore this information was available only after the experiment was complete. In order to improve deposition control we have designed and fabricated an on-line particle size aerosol monitor (PSAM) which will provide up to a 16-point particle size histogram just prior to exposure of the experimental animal. At the present time the PSAM is arranged to calculate a %-point histogram.

The PSAM is based on the collection of particles by their impaction on a fixed plate. When an aerosol stream of given velocity impinges upon such a plate, adherence of individual particles to the plate is a function of aerodynamic equivalent diameter (AED), which depends upon the density and shape as well as the actual size of the particle. The relationship between stream velocity,

AED and impaction efficiency has been well documented by other investigators.

A simplified block diagram of the PSAM is shown in Figure 4.3. It consists basically of a four-position turnstile (Figure 4.4) into which filter pucks are loaded from an external cartridge, rotated into position to sample the aerosol, rotated into position to count the sample, then finally ejected from the turnstile. The instrument contains four selectable orifice sizes and four selectable flow velocity control valves which allow up to 16 AED cut-off values to be measured. The AED is selected by computer control, allowing a defined volume of aerosol to flow through the selected orifice at a defined velocity. Most particles with an AED less than the cut-off value will not impact on and adhere to the plate but will flow around it and be collected on the membrane filter which is contained in a puck. Once the defined volume of aerosol is drawn through the system, the turnstile is rotated by a motor so that the filter is facing a nuclear detector. The activity on the filter is

(a) Systems Department

(b) Engineering Technology Department

FIGURE 4.3. Block Diagram of Particle Size Aerosol Monitor (PSAM).

FIGURE 4.4. Four-Position Turnstile for PSAM.

counted and the computer calculates the concentration in the aerosol of particles below the chosen cut-off. The procedure is then repeated, using a new orifice and/or new flow velocity to define a new AED cut-off.

The time required to calculate the concentration at each cut-off may be from 10 to 200 sec, depending on the total activity of the aerosol. A new filter puck is inserted in the turnstile for each cut-off value chosen. The used filter pucks are collected

and can be recounted at a later time as a check. Once the desired number of cut-offs has been obtained, the computer will automatically compute and display the total aerosol concentration and PSD.

A dual-mode PSAM control system has been developed so the unit can be operated by a hardwire, integrated circuit control system or under the control of a small laboratory computer system. The two systems are interlocked as shown in the block

FIGURE 4.5. Block Diagram of Computer and Manual Control System for PSAM.

diagram, Figure 4.5, so the manual system can override the automatic system at the discretion of the operator.

The manual control system is fitted with a set of control switches to operate the PSAM and a set of indicators that show the status of the unit by monitoring sensors mounted on the PSAM. Each switch controls a single function. Available controls are INSERT PUCK, MOVE TABLE, SELECT AEROSOL VALVE. The control system allows single function selection, but is designed so that only a limited number of function sequences can be actuated. For example, if no filter puck is present under the exposure position, the aerosol valve actuation control is inhibited. Similarly, it is not possible to insert a puck while the turnstile is in motion. The manual control system is designed to provide the maximum degree of flexibility in operational procedure, while limiting action when the requested command does not meet sequencing rules.

In a computer mode, the PSAM program is entered from the executive program by issuing a command via the teletype; the user can then enter operating commands. There are five basic commands used to input data to the PSAM program: VALVE TIME, COUNT

TIME, FLOW RATE, DETECTOR EFFICIENCY, AND BACKGROUND. Following input parameters, the user can actually expose and count pucks in either semi-automatic or fully automatic mode.

There is one basic command, SINGLE COUNT, to cause the PSAM to position, expose and count one puck for a specified orifice number (table position). At the completion of the exposure, the PSAM will automatically advance to the count position, counting occurs, and the computer calculates the aerosol concentration and prints the results via the teletype.

There is also one basic command, AUTO COUNT 4 PUCKS, which causes the PSAM to automatically position, expose, and count four pucks. At the completion of each filter puck reading, concentration is calculated and printed by teletype. After all four filter pucks are processed, the particle size distribution is calculated and printed. The distribution consists of four values, each corresponding to a particle size range. The particle size distribution calculated in this way for each aerosol prior to exposure of the animal can be used by the computer to estimate deposition and control the quantity of material inhaled by the animal.

ADAPTATION OF A MAY SPINNING TOP MONODISPERSED AEROSOL
GENERATOR TO ANIMAL INHALATION STUDIES

Investigators:

W. C. Cannon and D. K. Craig

Technical Assistance:

E. F. Blanton and E. J. Rossignol

Studies of respiratory deposition of aerosols in the beagle dog will be undertaken using monodispersed aerosols of ^{198}Au tagged iron oxide or metallic gold. In preparation for this study a dog exposure glove box was modified to accommodate a May spinning top aerosol generator. This generator is capable of generating monodispersed aerosols in the size range of $0.5\ \mu\text{m}$ to $5\ \mu\text{m}$ aerodynamic equivalent diameter, a range useful for such a study.

The experimental arrangement is shown in Figure 4.6. Air enters the chamber through filters at the chamber bottom, flows up past the generator and is exhausted at the top to a

filtered vacuum system. Aerosol will be inhaled by the dog through a venturi tube which is part of a respiration monitoring system. A system of valves will separate the inspired from the expired air. Independent particle size and aerosol concentration measurements will be made on inspired and expired air.

Preliminary operation checks of the aerosol system have begun using uranine dye aerosols in place of the ^{198}Au tagged aerosols. A mass concentration $2.9\ \text{mg}/\text{m}^3$ of $2\ \mu\text{m}$ AED particles has been obtained. Such a concentration will be sufficient for tagged aerosols to be used in the study.

FIGURE 4.6. May Spinning Top Monodispersed Aerosol Generator Adapted for Animal Inhalation Studies.

DEVELOPMENT OF A BENZO(A)PYRENE AEROSOL DELIVERY
SYSTEM FOR ANIMAL INHALATION STUDIES

Investigators:

W. C. Cannon and R. H. Starkey

Technical Assistance:

E. F. Blanton and J. P. Herring

Apparatus and techniques have been developed to expose experimental animals to aerosols of benzo(a)pyrene, a chemical carcinogen found in urban atmospheres and tobacco smoke.

Benzo(a)pyrene (BaP) is a carcinogenic hydrocarbon, detected in the atmospheres of many American cities and known to be a component of tobacco smoke. While fairly large amounts of BaP delivered by intratracheal instillation have been required to produce tumors in experimental animals, it is possible that smaller amounts acting with cocarcinogens might result in additive or synergistic effects. It is of particular interest at this laboratory to know if such an additive or synergistic effect exists with PuO_2 and BaP. We have therefore developed methods for generating and characterizing BaP aerosols. To produce aqueous suspensions of small BaP particles suitable for nebulization, a concentrated solution of BaP in acetone was introduced slowly into water with constant stirring. The BaP precipitates as small particles. A small amount of gelatin in the water considerably increases the concentration of BaP suspensions which can be produced in this way.

Concentration of BaP in the nebulized aerosol was determined by collecting the particles on a membrane filter, which was then analyzed for BaP. Particle size was determined using a cascade impactor. Analyses were carried out by extracting the

BaP from filter papers or impaction stages with cyclohexane, followed by spectrofluorimetric measurement.

Since BaP is a carcinogen, the aerosol was generated in a glove-box-enclosed aerosol chamber of the type commonly used with plutonium. Other manipulations of BaP were carried out in a double-filtered fume hood; waste solvents and contaminated materials were disposed of by incineration.

Test aerosols of BaP were generated using the Lovelace nebulizer and the Retec Model PN7002 aerosol generator. The aerosols were passed through a drying tube and diluted with air before introduction to the aerosol chamber. Characterization data for these test aerosols are given in Table 4.1.

Figure 4.7 is an electron micrograph of the aerosol particles, shadowed with chromium. The spheroidal particles are probably BaP. The larger, irregularly shaped particles may be agglomerates or possibly flakes of solidified gelatin. Since such particles did not show up in microscopic examination of the suspensions prior to nebulization, we assume they are artifacts of the generation and/or collection of the aerosol.

TABLE 4.1. Characterization Data for Test Aerosols

Suspension Concentration ($\mu\text{g/ml}$)	Generator Type	Mean Aerosol Conc. ($\mu\text{g/liter}$)	AMAD ^(a) (μm)	GSD ^(b)
10	Lovelace	29.3	3.56	2.05
10	Lovelace	34	3.19	1.96
5	Lovelace	24.4	1.84	2.45
5	Retec	84.7	3.03	1.76

(a)AMAD = activity median aerodynamic diameter

(b)GSD = geometric standard deviation

X5550

FIGURE 4.7. Photomicrograph of Benzo(a)pyrene Particles Collected on Electron Microscope Grids. (A) Spherical BaP Particles; (B) Large Agglomerates, Probably Gelatin and BaP; (C) Wet BaP Particles.

RADIOANALYTICAL TECHNIQUES FOR ^{241}Am and ^{244}Cm
DETERMINATIONS IN BIOLOGICAL SAMPLES

Investigator:

G. J. Powers

Technical Assistance:

A. C. Case, J. S. Barnett,

K. R. Hanson, and W. A. Zimmerman

Increasing interest in the toxicity of americium and curium as products of nuclear reactors has prompted the development of new analytical procedures for assaying the wide range of activity usually encountered in an organ distribution study.

Two different approaches may be used to assay the amount of these radionuclides in biological samples: liquid scintillation counting and alpha energy analysis (AEA).^(a) For liquid scintillation counting a Nuclear Chicago Mark II Model 6847 Scintillation System was used. Typical backgrounds ranged from 18 to 25 c/m. The instrument counted both nuclides, ^{241}Am and ^{244}Cm , with 100% efficiency. The scintillation reagent (TT21) was made by dissolving in one liter of toluene 8.0 g 2, 5-diphenyloxazole (PPO), 0.2 g 1, 4-bis-2, (5 phenyloxazoly1)-benzene (POPOP) and 500 ml of Triton X100.^(R) Dowex 50W-X8,^(RR) 50-100 mesh of the hydrogen form, was the exchange resin used.

In the other method, alpha energy analysis was performed on a Nuclear Data Analyzer 2400. This analyzer contains four Ortec detectors with background counts of 0.01 c/m and operates at 25% efficiency.

For samples to be analyzed by either method, biological tissues and excreta were alternately wet-ashed with nitric acid and muffled in a furnace at 400-500°C until free of carbonaceous material. The resulting inorganic ash was then treated with a

combination of nitric and hydrofluoric acid to assure complete breakdown of silicates and refractor oxides. The sample was then evaporated to dryness and baked on a hot plate until excess hydrofluoric acid was evaporated. The inorganic ash was then dissolved and diluted to a specific volume in 2 M nitric acid from which various aliquots were taken for radiological assay of ^{241}Am or ^{244}Cm .

For the liquid scintillation procedure, samples containing a minimum of 1 nCi activity were counted directly by liquid scintillation. A sample aliquot, not to exceed 2 ml, was pipetted into 17 ml of TT21 contained in a scintillation vial. The vial was capped, shaken, and counted. If the background count of the 2-ml aliquot was less than 25 c/m, the sample had to be re-aliquoted and processed for an alpha energy analysis.

Samples having a level of activity < 1 nCi required alpha energy analysis. For this analysis an isotopic tracer of known amount was added to a larger sample aliquot. The aliquot was evaporated to dryness and converted to the chloride form and subsequently passed through a Dowex 50W-X8 resin in 1 M HCl. The sample was then eluted from the resin, using 100 ml 8 N HNO_3 . The eluent was evaporated to dryness, redissolved with 1:1 H_2SO_4 and pH adjusted to 2.3-2.6 with water and NH_3 to a final volume of 10 ml. The resulting solution was placed in a disposable, polyethylene, electrolytic cell containing a

(a) Performed by Radiological Sciences Dept.

(R) Rohm & Haas, Philadelphia, PA

(RR) Biorad Labs., Richmond, CA

3/4" stainless steel plate. The cell was then subjected to 1 amp of current at 8 volts for 45 min. The electrodeposited plate was then rinsed with distilled water, dried and counted on the alpha energy analyzer. The amount of nuclide recovered was calculated from the amount of isotopic tracer remaining.

The chemistry of americium and that of curium are so closely related

that by judicious handling, the isotope of one nuclide may be used for determination of the chemical yield of the other. The differences between the alpha energies of ^{244}Cm (5.80 MeV) and ^{241}Am (5.7 MeV) are easily resolved on an alpha energy spectrum. The limit of detection for AEA is estimated to be approximately 0.05 d/m for 1000 min count. The range in chemical yield was between 45 and 70%.

MECHANISMS OF INHALED PLUTONIUM TOXICITY

This project is concerned with effects of plutonium on the primary biochemical functions leading to cell death or carcinogenesis, including in particular, damage within the immune system, damage at the level of the cell genome, and the immediate expression of such effects in terms of altered biochemical function.

MOLECULAR HYBRIDIZATION: DEVELOPMENT OF METHODOLOGY
FOR INVESTIGATING MECHANISMS OF PLUTONIUM
INDUCED NEOPLASIAS

Investigator:
M. E. Frazier

The method for making tritium-labeling DNA copies from oncornaviruses is described as are the means for removing normal host sequences that may have been copied. Several probes have been made, from known oncornaviruses, and these probes have been checked for specificity of the reaction.

Renaturation of single-stranded nucleic acids, from different sources, to form a double-stranded nucleic acid structure (either DNA:RNA or DNA:DNA) is commonly referred to as molecular hybridization. This technique depends on the ability of any segment of single-stranded nucleic acid to find either its complementary RNA or DNA (depending on the experimental protocol) and form a stable, double-stranded hybrid structure. This reaction is highly specific, proceeding in the presence of vast excesses of non-complementary nucleic acids. Molecular hybridization has been shown to be extremely useful for investigating the molecular aspects of many problems including such diverse areas as gene regulation and viral oncology.

We have developed this capability in our laboratory in order to investigate the role of viruses (either RNA or DNA) in the development of the lung and bone tumors occurring in experimental animals following exposure to inhaled plutonium. Initially the research will center on the search for the presence of oncornaviruses (RNA tumor viruses) in these tumors, since the methodology is well established.

Cells infected with RNA tumor viruses contain a cytoplasmic particulate fraction of a density ~ 1.16 g/ml and 550S sedimentation coefficient. These particles contain a unique RNA-instructed DNA polymerase (RIDP) closely associated with a high molecular weight (70S) RNA. The RIDP catalyzes the synthesis of a complementary DNA copy (cDNA) from the 70S RNA template. The steps in this reaction are RNA $\rightarrow$ RNA:DNA hybrid $\rightarrow$ DNA:DNA duplex. A portion of the cDNA is then integrated into the cell genome.

The radioactive single-stranded DNA, needed for a probe in the molecular hybridization studies, is prepared by disrupting the tumor cells and isolating the particulate fraction with the physical and biochemical properties of RNA tumor viruses. The DNA is then synthesized by supplying the detergent-disrupted (NP-40) viruses with magnesium and the four deoxynucleoside triphosphates, one of which is radioactively labeled with tritium (Figure 5.1), and allowing the endogenous RIDP to synthesize the ^3H -DNA copy. When synthesis is terminated, the protein and RNA present are eliminated by phenol-cresol extraction and

FIGURE 5.1. Endogenous Synthesis of Radioactive DNA Probe.

sodium hydroxide. The remaining radioactive single-stranded ^3H -DNA is purified on a Sephadex G50 column. This probe DNA is then analyzed to determine its ability to hybridize selectively by challenging it with various DNA and RNA preparations.

Since RNA tumor viruses contain some normal cell sequences as well as virus-specific sequences, it is necessary to remove the normal cell sequences from the probe DNA. These "recycling" experiments are carried out by absorbing out the normal sequences during a hybridization reaction. Separation of the specific probe is performed using hydroxylapatite column chromatography. Hydroxylapatite in low concentrations of sodium phosphate buffer binds double-stranded nucleic acids while allowing the single-stranded nucleic acids to be eluted from the column. Using this procedure we can remove single-stranded probe ^3H -DNA, based on the degree of mismatch (of base pairs) with the DNA being tested, by simply raising the column to the desired temperature and then washing the column with sodium phosphate buffer. Material eluted at 60°C did not hybridize; material denaturing to single-stranded nucleic acid and subsequently eluting from the column below 80°C is considered poorly matched; and nucleic acid denatured only by temperatures higher than 86°C is considered to be composed of well-matched Watson-Crick pairs. In

the recycling experiments we will collect the labeled single-stranded nucleic acid that elutes at 60°C . This single-stranded material represents virus-specific sequences (Figure 5.2) which can then be used to test various nucleic acid samples for the presence of virus-specific sequences with standard hybridization techniques.

In addition to the attempts to prepare probes by endogenous RIDP activity present in plutonium-induced lung and bone tumors, we have already prepared, and tested for specificity, probes from a number of known RNA tumor viruses (i.e., Rauscher leukemia virus, Moloney sarcoma virus, Kirsten leukemia virus, feline leukemia virus, feline sarcoma virus, etc.) These probes will be used in hybridization studies to check for related sequences in normal tissues and tumors from plutonium-exposed animals.

The use of molecular hybridization techniques in the search for viruses in tumors affords distinct advantages over other conventional (i.e., immunological) methods since they can be used to detect the presence of specific nucleic acid sequences regardless of whether the genetic information is being transcribed into proteins. The exquisite sensitivity of the hybridizations (given a probe of sufficiently high specific activity) will easily allow the detection of less than one complete RNA virus

FIGURE 5.2. Preparation of Leukemia Virus - Specific Probe.

copy per 200 cells, or an average of 0.5% of a virus RNA copy per cell. In addition, the melting temperature of the hybrids can be accurately used to determine the degree of mismatching between the base pair sequences

of any two nucleic acids. This technique thus provides us with the necessary methodology for investigating the role of exogenous or genetically-inherited viruses in the molecular pathology of radiation-induced carcinogenesis.

EFFECTS OF PLUTONIUM ON THE IMMUNE SYSTEM: IgG IN
BEAGLE DOGS EXPOSED TO PLUTONIUM AND IN NONEXPOSED CONTROLS

Investigators:

J. G. Hadley and J. E. Morris

Serum IgG levels in plutonium-oxide-exposed and unexposed beagle dogs was assayed with the Mancini system. With this system, there was no significant difference between IgG levels of exposed dogs and controls, nor among exposed dogs with different degrees of lymphopenia.

A study was initiated to extend our understanding of the immunological effects of inhaled plutonium oxide ($^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$) and to acquire baseline information on the normal humoral immunity of unexposed beagle dogs.

The normal humoral immunity was evaluated in terms of total immunoglobulin-G (IgG) serum levels in exposed and unexposed beagle dogs, by means of a Mancini assay. This assay is a single diffusion system with antiserum (monospecific for canine IgG) incorporated in an agar layer. Wells are cut in the agar layer and are loaded with canine serum of unknown concentration and standard concentrations of purified canine IgG. The migration distance of precipitin rings around the wells is related to temperature, time of incubation, and the concentration of IgG in the material placed in the wells.

During the past year over 1600 serum samples from exposed and unexposed male and female beagle dogs were assayed. The age of the animals ranged from approximately 1 to 5 years. In unexposed controls, IgG levels increased during this time period with the average IgG levels in females higher than those of comparably aged males (Figure 5.3).

FIGURE 5.3. IgG Serum Levels in Control Dogs as Related to Age.

The IgG serum levels in beagle dogs, 1 to 12 months after exposure to $^{238}\text{PuO}_2$, were not statistically different from those of unexposed controls. The range of $^{238}\text{PuO}_2$ deposition in the lung was from approximately 0.002 to 6 μCi . Neither was there a difference in serum IgG levels 12 to 36 months after lung depositions of $^{239}\text{PuO}_2$ ranging from approximately 0.001 to 2 μCi . No correlation was discerned between the levels of lymphopenia produced in these animals and serum IgG levels.

A SIMPLE METHOD FOR THE ISOLATION OF
CANINE IgM FROM NORMAL SERUM

Investigator:

J. E. Morris

Technical Assistance:

R. S. Moore

A simple system is described for the purification and partial characterization of canine IgM from serum.

Immunoglobulin-M (IgM), associated with humoral immunity, is the second highest in concentration of the immunoglobulins found in normal serum. Techniques previously used for isolating this protein were either laborious and time-consuming or the degree of purity and yield were low. The system to be described offers a simple and efficient means of obtaining purified canine IgM in reasonable amounts.

The procedure employed ammonium sulfate fractionation of the serum followed by column chromatography on G-200 and Sepharose-4B. Canine serum was precipitated with ammonium sulfate twice at a final concentration of 1.6 M at 4°C. The precipitate was dissolved in phosphate-buffer-saline (PBS) (0.075 M sodium phosphate, 0.075 M sodium chloride, pH 7.2). After exhaustive dialysis against PBS,

the protein was eluted through a column of G-200 equilibrated with PBS. The first peak from this column was pooled, concentrated and filtered through a Sepharose-4B column equilibrated with PBS. By pooling the first half of the second eluting component from the Sepharose-4B column, purified canine IgM was obtained. Human IgM was used as a column marker.

The purified canine IgM, when subjected to immunoelectrophoresis, migrated in the beta region and had an observed sedimentation coefficient of 15.6 S at 6.2 mg/ml in PBS.

This material will serve as a source of immunogen for the preparation of monospecific antiserum used in the quantitation of serum IgM levels and IgM antigenic sites on lymphocytes of exposed and unexposed beagle dogs.

**TOXICOLOGY OF RADIONUCLIDES IN THE
FETAL AND JUVENILE MAMMAL**

This project seeks to develop information on distribution, retention, and biological effects of radionuclides in the prenatal and juvenile animal, which is needed for the establishment of appropriate human exposure limits for these age groups.

INFLUENCE OF AGE ON THE DEVELOPMENT OF BONE
TUMORS IN RATS INJECTED WITH ^{239}Pu CITRATE

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

J. O. Hess and J. D. Stearns

Rats of various ages, ranging from prenatal to young adult, were exposed to monomeric ^{239}Pu and bone tumor incidence established. The younger rats were less sensitive on an administered dose basis, but perhaps more sensitive on the basis of total cumulative radiation dose. The extremities were the preferential site of bone tumor development in the adults; tumors of the head predominated in the newborn and prenatal groups.

We previously described the protocol employed to study the long-term response of rats injected at various ages with ^{239}Pu citrate (Annual Report, 1972). Last year (Annual Report, 1973) the alterations in median survival times produced by plutonium in the different age groups was compared.

Table 6.1 shows the age at time of administration of the Pu and the doses ($\mu\text{Ci/kg}$) employed. The doses were selected to produce similar radiation doses to the bone of all ages at early times after administration. As can be seen from this table, the radiation doses during the first 10 days were similar, particularly for the three older age groups. These estimates of radiation dose are based on analyses of Pu in the femurs of serially sacrificed animals. Although the dosimetric data at extended postexposure times are not

quite complete, tentative averages are shown in the table for each age-dose group, irrespective of sex. Although some quantitative change may be anticipated, it is clear that the cumulative radiation doses received by the four age groups to 2 years postexposure, which approximates the median survival time for most of the groups, was highly dependent on the age at time of exposure.

These animals showed an increased incidence of bone tumors; substantially higher than that seen in rats exposed to the same dose prenatally and kept with their mothers. The reason for this increased incidence, and for the incidence of bone tumors in controls, is unknown and will be further examined.

As indicated above, when the bone tumor response is expressed as a function of administered dose in $\mu\text{Ci/kg}$,

TABLE 6.1. Influence of Age at Time of Administration
on ^{239}Pu -Induced Bone Tumors in the Rat.

(Average values for approximately 25 rats per group)

Administered Dose, $\mu\text{Ci/kg}$	Radiation Dose to Femur, rads				Median Survival Time, days		Incidence of Grossly Iden- tifiable Bone Tumors, %	
	10 days	45 days	9 months	2 years	Male	Female	Male	Female
Adult								
0	0	0	0	0	780	810	0	0
0.5	5	22	92	320	620	700	30	4
1	16	73	308	1080	485	555	52	44
3	50	220	920	3200	460	5100	65	40
Weanling								
0	0	0	0	0	730	800	2	0
0.3	8	20	36	75	710	765	5	0
1	25	63	120	210	650	620	30	10
3	75	190	360	610	500	620	32	40
Newborn								
0	0	0	0	0	625	810	0	5
3	6	20	45	80	640	780	20	5
10	18	64	145	260	570	615	17	23
30	55	190	440	840	130	475	10	10
Prenatal								
0	0	0	0	0	795	640	0	6
6	3	4	5	8	670	>800	8	0
20	9	13	16	27	730	730	2	0
60	27	37	47	80	660	630	3	5
Cross-Fostered (in utero Exposure)	<27	<37	<47	<80	650	760	20	20
Cross-Fostered (milk only)	<<27	<<37	<<47	<<80	696(a)	714(a)	0	0

(a) Mean survival time

the rats exposed as adults appear to be the most sensitive. Plots of bone tumor incidence as a function of radiation dose to the femur result in curves which are unique to each age group. These differences in the shapes of the curves, as well as the indicated differences in survival times, obviate straightforward inter-age comparisons on a radiation dose basis. Nevertheless, when the incidence is considered relative to radiation doses up to 300 rads, the adults do not appear to be more sensitive than the younger animals. A more precise interpretation of the results must await completion of histologic and dosimetric evaluations.

The anatomic distribution of the tumors was markedly influenced by age at the time of injection (Figure 6.1). In the adults the extremities were

the predominant site of bone tumor development, with a somewhat lesser fraction in the vertebrae; a number of tumors were also observed at other sites. The fraction of the tumors developing in the extremities was unchanged in the weanlings; the incidence of tumors of the vertebral column was reduced, with a concomitant increase in osseous tumors appearing in the head. In the two younger age groups, over 50% of all tumors found were in the head, with very few in the extremities or vertebrae; the fraction found at other sites was similar to that in the adult. It appears that the difference in anatomic location may relate to the somewhat greater concentration of radioactivity in the head of the younger animals relative to that found in the extremities.

FIGURE 6.1. Anatomic Distribution of Bone Tumors in Rats Exposed as Adults (A), Weanlings (W), Newborns (NB), or Prenatally (PN) to ^{239}Pu Citrate.

METABOLISM OF ^{241}Am CITRATE IN RATS RELATIVE TO AGE

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

J. O. Hess, M. J. Kujawa, and P. L. Savignac

The biological disposition of ^{241}Am was studied in newborn, weanling, and adult rats. The initial deposition in liver was greater in the adults than in the newborns, and least in the weanlings. Skeletal deposition showed the inverse pattern.

Previous studies in this laboratory with ^{239}Pu demonstrated marked differences in metabolism relative to the age of the rat at the time of injection. Metabolic differences were also seen with other radionuclides,

although the patterns depended on the characteristics of the particular nuclide. The data available in the literature on the distribution and retention of ^{241}Am citrate have been somewhat contradictory and only a few

data are available as to the role of age in its metabolism. To study this, newborn (0 days), weanling (21 days), and adult rats (approximately 4 months) were injected with a solution of ^{241}Am citrate at a pH of 4.0-4.5, approximately 85% ultrafilterable. Because of the effect of dose reported by some investigators, two dose levels, 1 and 10 $\mu\text{Ci/kg}$ body weight, were employed. Approximately 50 rats in each age group were injected with each dose, administered intravenously in the weanling and adult groups and intracardially in the newborn. Six animals of each age-dose group were killed at 4 hours, 1, 3, 7 and 14 days postinjection and additional animals will be killed at 28, 56, and 90 days postinjection. A number of tissues, some of which are shown in Table 6.2, were taken for radioanalysis and autoradiography. The amount of radioactivity in the residual carcass was measured by whole-body counting in an Armac scintillation counter.

Results for the period 1 to 14 days postadministration are complete and are tabulated in Table 6.2. The difference between the animals receiving the high and low dose usually was small and the direction of

the difference was inconsistent. Accordingly, a combined average has been prepared for this report. About half of the administered nuclide appeared in the liver of the adult animals at 1 day postadministration and fell to about one-third this value in the succeeding 2 weeks. Only about one-third of this level, 17.6% of the dose, appeared in the liver of newborn animals at 1 day postadministration, and this was retained quite tenaciously. The greater decrease in concentration (%/g) in the newborn animals reflected the growth of the liver during this period. An even smaller fraction, less than 10%, of the administered activity appeared in the liver of the weanling animals at 1 day and this also was retained quite tenaciously. This pattern was also seen in previous experiments with ^{239}Pu , and presumably relates to the high affinity of the skeleton for nuclides at this time of life, probably associated with rapid mineralization. The fraction of the dose, and the concentration, seen in other soft tissues such as the spleen and kidney was much less than that in the liver and was little affected by age.

TABLE 6.2. Distribution and Retention of ^{241}Am as a Function of Age at Exposure.

(fraction and concentration as percent of administered dose)

Days Postadministration		Femur		Skull	Liver	
		%	%/g	%/g	%	%/g
Adult	1	1	1	1	51	4
	7	1	1	1	40	4
	14	1	1	1	16	1
Weanling	1	3	11	5	10	5
	7	3	8	7	9	2
	14	3	5	5	7	1
Newborn	1	1	25	26	18	61
	7	1	13	27	15	34
	14	1	8	24	12	12

In the adult animals, the fraction of the dose and the concentration in the femur remained constant throughout the period for which data are presently available. The concentration of ^{241}Am in the skull also remained constant with time, but was only about 70% of that in the femur. The fraction of the dose which appeared in the femur of the weanling animals was substantially greater than that in the adults, probably relating to the rapid mineralization taking place at this time of life. The concentration fell in the weanlings, however, reflecting the growth of the femur taking place during this 2-week period. Subsequent to 1 day postadministration, the concentration in the skull approximated that in the femur and did not show the rapid decline in concentration, which is reasonable since the skull grows less

than the femur at this time of life. In the newborn animals the percent of the dose in the femur at 1 day was substantially less than in the other groups, but showed a progressive increase with time; although, as with the weanling, the concentration fell during the 2-week period studied. The concentration in the skull was identical to that in the femur at 1 day, and remained constant, again reflecting the minimal skull growth.

The limited data thus far available show marked age differences in the distribution and retention of ^{241}Am relative to age and the time of administration. Results at longer times following administration should better define the nature and direction of these differences and provide a better basis for comparison with other transuranic elements.

THE TOXICITY AND DISTRIBUTION OF ^{241}Am AND ^{244}Cm IN THE RAT AFTER ADMINISTRATION AT NINE DAYS OF GESTATION

Investigators:

M. R. Sikov and D. D. Mahlum

Technical Assistance:

J. O. Hess, M. J. Kujawa, and P. L. Savignac

Intravenous doses as high as 60 $\mu\text{Ci/kg}$ of ^{241}Am or ^{244}Cm to the pregnant rat at 9 days of gestation were neither embryocidal nor teratogenic. This lack of effect appears to be associated with a lower deposition of nuclide in the fetoplacental unit than has been found with other transuranic elements.

We have found general similarities in the distribution of heavy metals within the fetoplacental unit (FPU) of the rat. Differences relating to the specific material, physicochemical state, and stage of gestation are also apparent. In earlier studies, the importance of specific activity in determining the distribution and toxicity of plutonium at

nine days of gestation was apparent in a comparison of ^{238}Pu and ^{239}Pu . Doses of 10-15 $\mu\text{Ci/kg}$ of these isotopes produced a substantial incidence of embryonic death when given at 9 days of gestation, while doses of approximately 30-40 $\mu\text{Ci/kg}$ were 100% lethal.

The present studies with ^{241}Am and ^{244}Cm were undertaken to extend the range of heavy metals examined with respect to the FPU in order to provide a basis for prediction of the factors controlling their distribution and toxicity. Protocols and procedures similar to those used with other nuclides were employed in these studies. Citrated solutions of these nuclides were prepared and administered intravenously to the dam after 9 days of gestation.

Neither ^{241}Am nor ^{244}Cm was found to be embryocidal, even at doses as high as $60 \mu\text{Ci/kg}$, in contrast to plutonium which was embryocidal at much lower doses. Gross examination of the fetuses showed no malformations at any dose level. More detailed analyses have been performed on fetuses from the highest dose groups; no visceral or skeletal abnormalities have been found.

Additional animals were injected under the same conditions at 9 days of gestation for determination of the distribution and retention of ^{241}Am and ^{244}Cm . The results with ^{241}Am are presented in Table 6.3. Although not complete, analyses of comparable tissues from animals injected with ^{244}Cm appear to give almost identical results. As with plutonium, only a small fraction of the activity reaching the FPU passes into the embryo. However, the total incorporation in the FPU is an order of magnitude less

than that seen with plutonium or with most of the other heavy metals studied to date. The ratio of activity in the fetal membranes to that in the placenta does not seem to show the marked gradient seen with plutonium and other heavy metals studied. In previous studies it appeared that the embryocidal effect of the plutonium was due to activity in the fetal membranes and placenta. The low activity present in the membranes and placenta in the present experiments probably accounts for the lack of mortality observed.

It is premature to speculate on the reasons for the reduced activity of these radionuclides in the FPU. One major difference in the metabolism does appear to bear upon this, however. As discussed elsewhere in this Annual Report, the liver shows a marked avidity for ^{241}Am in the dam, and it may be removed from circulation too rapidly for significant transport to the FPU. There are other possible explanations which must be explored.

Autoradiographs of fetoplacental units from each litter are being prepared. Studies on the incorporation and distribution of these radionuclides in the fetoplacental unit after administration at 15 and 19 days of gestation are also in progress. Data from these studies are not yet available.

TABLE 6.3. Deposition of ^{241}Am Citrate in the Fetoplacental Unit (FPU) of the Rat After Injection at Day 9 of Gestation.

Time Postinjection, Days	Percent Injected Dose/gram Wet Weight (Mean + S.E.)			
	FPU	Membranes	Placenta	Fetus
1	0.048 ± 0.001	---	---	---
3	0.017 ± 0.002	0.039 ± 0.004	0.013 ± 0.001	---
5	0.0032 ± 0.002	0.008 ± 0.009	0.006 ± 0.0004	0.0003 ± 0.001

EFFECTS OF ^{253}Es INJECTED IN WEANLING AND ADULT RATS

Investigators:

D. D. Mahlum, M. R. Sikov, and F. P. Hungate

Technical Assistance:

J. O. Hess and J. D. Stearns

Weanling and adult Wistar and adult Fischer rats were intravenously injected with ^{253}Es and observed until death or to 30 months of age. In the adult Wistars there was a peak incidence of bone tumors at about 5.0 $\mu\text{Ci/kg}$ with a lower rate at higher doses, but in the weanlings the incidence continued to increase up to 100 $\mu\text{Ci/kg}$. Bone tumor incidence in the Fischer rat tended to be lower than in the Wistar strain.

In the previous reports we recounted differences in the response to ^{253}Es of adult and weanling Wistar and of adult Fischer rats. Differences in both early and late mortality, as well as other changes such as impaired dentition, were affected by dose, age, and strain. In this report we will consider the age-related differences in tumor development as a function of administered dose in these two strains of rats.

In the first experiment male adult and male and female weanling rats of the Wistar line were injected intravenously with ^{253}Es as the chloride (pH 2) at doses ranging from 0.4 to 50 $\mu\text{Ci/kg}$. In the second experiment, adult male Wistar rats were injected with doses of 0.1 to 5 $\mu\text{Ci/kg}$, while the Fischer rats of both sexes received 0.4 to 5 $\mu\text{Ci/kg}$. An additional group of Wistar animals received 2.0 $\mu\text{Ci/kg}$ as the citrate for comparison of chemical form, and another group was subjected to removal of approximately one-half of their blood on two successive days, 28 days after injection of 2.0 $\mu\text{Ci/kg}$.

Gross and radiographic examinations for detection of bone tumors were performed on all animals at

death or sacrifice at 30 months of age. Microscopic examination has been performed on only a limited number of specimens; the results confirm the gross diagnoses.

In Experiment I both adult and weanling groups showed a dose-dependent response in bone tumor incidence after Es administration (Table 6.4). The patterns of response, however, were dissimilar for the two ages. The incidence in the adults was maximal at 10 $\mu\text{Ci/kg}$ and declined at higher doses. In the weanling, the incidence continued to increase to 100 $\mu\text{Ci/kg}$ and then fell.

The bone tumor responses of the Wistar animals in Experiment II were similar to those in Experiment I at doses of 0.4 and 2.0 $\mu\text{Ci/kg}$. The incidence at 5.0 $\mu\text{Ci/kg}$ was higher than at any dose in the first experiment, suggesting that we may have missed the peak in Experiment I. The bone tumor incidence in the Fischer rats did not appear to be increased as markedly as in the Wistars.

In animals injected either as adults or weanlings, no increase in mammary tumor incidence was found among the females, and there was a

tendency for a decrease at the higher doses relating, perhaps, to decreased longevity. Although the mammary tumor incidence was unaffected among males injected as weanlings, it was increased by doses of 0.1-5.0 $\mu\text{Ci/kg}$ in those injected as adults. Adult males injected with higher doses as adults had an incidence equal to or lower than that of the controls.

A variety of other tumors were noted on gross examination although they have not yet been characterized histologically. It is apparent, however, that the overall incidence of tumors was elevated in animals injected with Es, although no clear relationship to dose level has been discerned.

TABLE 6.4. Bone Tumor Incidence in Weanling and Adult Wistar and Fischer Rats Administered ^{253}Es .

Experiment I	Dose of ^{253}Es Administered, $\mu\text{Ci/kg}$								
	0	0.1	0.4	2	5	10	50	100	150
Wistar Weanlings	0/58 ^(a)	---	---	3/27	---	2/29	5/20	8/20	1/20
Wistar Adults	1/53	---	1/31	3/40	---	11/50	6/40	---	---
Experiment II									
Wistar Adults	1/20	2/20	2/20	7/51	8/20	---	---	---	---
Fischer Adults	1/19	---	0/19	4/19	---	---	---	---	---

(a) (Number of tumors)/(number of animals exposed)

EFFECT OF IRRADIATION ON THE DEVELOPMENT OF FETAL
RAT LUNG IN ORGAN CULTURE AND IN UTERO

Investigators:

J. C. Vogt, M. R. Sikov, and D. D. Mahlum

The development of fetal lungs and their response to radiation was studied in vivo and in organ culture. Culturing at 13 or 15 days of gestation permitted good growth and differentiation. The damage produced by radiation was partially repaired in vivo, but was irreversible in vitro. Morphologic and biochemical studies indicated that connective tissue biosynthesis is favored over general protein synthesis during in vitro development of irradiated lung.

Malformations of the embryo are considered to be the result of a series of chemical alterations which lead to net morphologic change. Ionizing radiation is recognized as a potent teratogenic agent which also produces alterations in organogenesis. The effects of radiation on a fetal organ are often obscured by changes occurring in the uterine environment as well as in the mother. Studies using tissue culture have contributed to our understanding of the cellular response to radiation but they are inappropriate for measuring the effects on cell differentiation. Organ culture has been used to elucidate the mechanisms of organ development and was thought also to be amenable to measure postirradiation cellular interactions independently of abscopal effects. The relating roles of direct and indirect effects were studied by comparing the effects of X-irradiation on in vivo and in vitro histogenesis of the lung.

Pieces of lung, less than 2 mm in the greatest dimension, were explanted from fetuses of known gestational age and cultured in L-15 (Liebowitz) medium with 20% fetal calf serum. The lungs were exposed

to radiation either after explantation or prior to sacrifice of the pregnant rat and studied over the period of 4 to 72 hr after irradiation.

Culturing at 13 or 15 days of gestation allowed good growth and differentiation of the fetal lung, as indicated by progressively increasing numbers of terminal bronchioles and budding of the bronchial tree. The 17- and 19-day fetal lungs did not tolerate in vitro conditions as readily. Notwithstanding the excellent growth and differentiation in the 13- and 15-day fetal lungs, development in vitro showed a number of differences from development in vivo, including an attenuation of the epithelium surrounding the lumina, which were filled with debris. Degeneration and engorgement of the vascular system occurred, and pyknosis in association with the mesenchyme increased with incubation time. There was usually a relative increase in the amount of connective tissue, which appeared fibrous and basophilic. The 13- and 15-day lungs showed an initial inhibition of growth followed, after the first day, by a resurgence which was best exemplified by the increased mitotic frequency noted in the epithelium.

Exposure to 435 R of X-ray at either 13 or 15 days of gestation resulted in fewer terminal bronchioles, tended to disrupt the capillaries, and altered the histology of the epithelium surrounding the lumina. Radiation produced a further increase in the relative amount of connective tissue above that noted with culture alone. The lung explants which had been irradiated in vivo showed a particular increase in fibroblastic growth between 1 and 3 days in culture, followed by disintegration of the cultures after 3 days. This is in contrast to the recovery seen during this postirradiation time interval when the fetal lungs were allowed to develop in utero. In conclusion, radiation accelerated and magnified the changes associated with culturing. In the organ cultures the effects of irradiation were progressive and irreversible during the 3-day postirradiation period studied. In contrast, recovery was observed between 1 and 3 days postirradiation in the lungs which were allowed to develop in vivo.

Explants at 15 days of gestation were incubated for a 4-hr period with ^{14}C -leucine and ^3H -proline. After incubation, the tissue was rinsed with medium, homogenized and the proteins precipitated with trichloroacetic acid (TCA). The precipitates and supernatants were analyzed for radioactivity and other constituents.

The protein-to-DNA ratios remained nearly constant for unirradiated lung cultured for 4, 24 or 72 hr but increased in lungs developing in vivo. The protein:DNA ratios for irradiated lung also increased when development occurred in vivo, but decreased when irradiated tissue was cultured.

The incorporation of both ^3H -proline and ^{14}C -leucine into TCA-precipitable material was depressed by irradiation whether development occurred in vitro or in vivo. The depression was markedly greater under in vitro conditions. The ratio of ^3H to ^{14}C in unirradiated lungs was similar for in vivo and in vitro development, showing about a 40% gradual increase between 15 and 18 days (Figure 6.2). Irradiated lung developing in vitro showed a large increase in the

^3H : ^{14}C ratio at 1 day after explantation, followed by a smaller increase between 1 and 3 days. In contrast, irradiated lung developing in utero tended to have a decreased ratio of ^3H to ^{14}C at 1 day, followed by an increase between 1 and 3 days.

These data suggest that the synthesis of connective tissue relative to total protein is similar whether development occurs in vivo or in vitro. In contrast, in vitro development of irradiated lung appears to favor connective tissue biosynthesis over general protein synthesis.

FIGURE 6.2. Effects of X-irradiation at 15 Days of Gestation on the Ratio of ^3H -proline/ ^{14}C -leucine Incorporation into the TCA-precipitable Fraction by Explants of Fetal Lung After Development in Vivo or in Vitro. Labeled medium was introduced 4 hr prior to termination.

FACTORS MODIFYING RADIONUCLIDE METABOLISM AND EFFECT

This project is concerned with the effects on radionuclide toxicity of factors normally excluded in controlled laboratory studies, such as concurrent nutritional deficiencies, drug abuse, infections, variation in hormonal state, and exposure to other toxic substances.

TISSUE DISTRIBUTION OF INJECTED PLUTONIUM IN IRON-DEFICIENT MICE

Investigators:

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Technical Assistance:

D. H. Hunter and M. C. Perkins

When killed 6 days after injection, iron-deficient mice to whom ^{239}Pu citrate was administered intraperitoneally had body burdens approximately two-fold greater than did iron-replete mice. With the exception of the liver, all soft tissues, including testis, of the iron-deficient mice had a greater plutonium concentration than those of the control mice.

Plutonium has an affinity for the physiologic iron-binding proteins transferrin, ferritin, and hemosiderin. Because of this relationship, the body iron status might influence absorption, retention and/or distribution of plutonium. In a previous study (Annual Report, 1973), it was shown that iron-deficient mice, administered ^{239}Pu citrate by gastric intubation, had body burdens of plutonium about fourfold greater than age-related controls. In addition, the absorbed plutonium translocated from soft tissue to bone in the iron-deficient mice, rather than to liver, as in the control animals. To study these interrelationships further, plutonium was administered by intraperitoneal injection rather than by gastric gavage.

Two groups of SJL/J white male mice were fed either an iron-deficient (3.9 μg iron/gram) or iron-replete (39.8 μg iron/gram) diet when they were between 30 and 93 days of age. At 88 days of age animals in each group were administered 0.05 μCi ^{239}Pu citrate by intraperitoneal injection and killed 6 days later in order to determine tissue distribution and retention of the injected plutonium. At the time of death, eight mice from each group were selected at random for hematologic evaluation and to determine serum iron concentration. At that time mice on the iron-deficient diet were moderately anemic with microcytosis of the erythrocytes evident, and their serum iron values were ~50% that of the control group (Table 7.1).

TABLE 7.1. Hematologic and Serum Iron Values in Control and Iron-Deficient Mice 93 Days of Age. (Mean $\pm$ SD)

	No.	Weight (grams)	WBC ^(a) ($\times 10^3/\text{mm}^3$)	RBC ^(b) ($\times 10^5/\text{mm}^3$)	HGB ^(c) (g/100 ml)	VPRC ^(d) (ml/100 ml)	MCV ^(e) (μ^3)	Pooled Serum Iron ($\mu\text{g}/100\text{ ml}$)
Iron Deficient	8	25.8 ± 1.3	5.8 ± 1.3	7.73 ± 0.8	10 ± 0.7	29.6 ± 2.8	38.3 ± 1.2	68
Control	8	24.2 ± 2.5	6.4 ± 3.8	9.47 ± 0.6	14.6 ± 0.8	45.1 ± 3.2	47.8 ± 1.2	142

- (a) WBC - White Blood Cells
 (b) RBC - Red Blood Cells
 (c) HGB - Hemoglobin
 (d) VPRC - Volume of Packed Red Cells
 (e) MCV - Mean Corpuscular Volume

The percent of injected plutonium retained in various tissues, urine and serum 6 days after administration is shown in Figure 7.1. The mean plutonium content of all tissues examined, with the exception of the liver,

was 1.5-2 times higher in the iron-deficient mice. The liver plutonium content of the iron-deficient mice was ~5% less than in iron-replete mice.

FIGURE 7.1. Retention of Plutonium in Iron-Deficient and Control Mice 144 Hours After the Administration of ^{239}Pu Citrate by Intraperitoneal Injection (Mean $\pm$ SEM).

This study, like the gastric gavage study, demonstrates increased body burdens of plutonium when body iron stores are reduced from normal. The animals in the present study were about 20 days older when started on the iron-deficient diet than were the mice on the plutonium absorption study. About three times as long a period was therefore required to produce a marginal iron deficiency. This was due to the fact that the mice had been provided adequate dietary iron during the neonatal growth phase when the hematopoietic system was expanding rapidly. Since body iron stores were less depleted in this study than in the previous one, this could account for the failure to find a greater difference in plutonium retention between the iron-deficient and iron-replete groups.

Of particular interest is the fact that the plutonium content of the liver was no different between the iron-deficient and the control groups. This confirms the results from the absorption study, in which there was a 50% decrease in liver plutonium content in the iron-deficient animals between 24 and 96 hours after gavage, and the concentration of plutonium in the femur increased about 95%. In the control group during the same time period, the liver plutonium increased about 33% with only a 20% increase in femur concentrations. These observations could have implications for therapy, since in cases of accidental plutonium exposure it

might be possible to saturate serum transferrin with iron, thus preventing some translocation of plutonium to the skeleton with alternative deposition in the liver, where it would be more available for chelation therapy.

Autoradiographs were prepared from testis sections and examined for distribution of ^{239}Pu within the organ. The total number of tracks per 25 oil immersion fields for interstitial, basal tubular and spermatid cell types are given in Table 7.2. Both the total counts for testis tissue and the ^{239}Pu tracks per 25 fields increased by 64% in iron-deficient compared with control mice. Plutonium-239 was distributed evenly between the endocrine component and the germinal or sustentacular component of testis tissue, in both groups, 6 days after intraperitoneal injection.

Because of the prevalence of iron deficiency in certain segments of the human population, further evaluations of the interrelationships between plutonium metabolism and iron deficiency are needed. Of particular concern will be investigations with experimental animals to determine if transplacental movement of plutonium is enhanced in iron-deficient dams, and also if neoplasia is increased, since iron deficiency (at least in some species) can result in impaired immunocompetence.

TABLE 7.2. ^{239}Pu Tracks per 25 Oil Immersion Fields

		Interstitial	Basal Layers of Tubules	Spermatids
Control (N=10)	$\bar{x}$	10.8	7.1	3.6
	SD	5.1	3	1.9
	Relative Frequency of Tracks	1	0.7	0.3
Iron Deficient (N=12)	$\bar{x}$	17.3	11.0	7.1
	SD	9.3	4.1	3.5
	Relative Frequency of Tracks	1	0.6	0.4

TOXICOLOGY OF CHRONICALLY FED STRONTIUM-90 IN MINIATURE SWINE

This project has sought to establish the dose-effect relationships following daily exposure to ingested ^{90}Sr through three generations of miniature swine. As this experiment approaches completion, primary attention is directed toward the interpretation of observed effects in relation to cellular mechanisms and in comparison with the effects of similarly deposited alpha emitters.

EFFECTS OF CHRONIC ^{90}Sr INGESTION IN MINIATURE SWINE

Investigators:

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and B. J. McClanahan

Technical Assistance:

E. T. Edmerson, D. H. Hunter, and M. C. Perkins

Daily ^{90}Sr feeding of $> 125 \mu\text{Ci/day}$ resulted in pancytopenia with death usually due to hemorrhage. At $125 \mu\text{Ci}$, pancytopenia and myelolymphoproliferative disorders appeared. At $< 125 \mu\text{Ci/day}$ a dose-related neutropenia was evident. There is an apparent increased incidence of both benign and malignant tumors of soft tissue in animals fed $25 \mu\text{Ci/day}$ for their lifetime. Benign uterine tumors, dental defects and marked arthritis continued to be the major diseases necessitating euthanasia in aged animals from all groups, including control swine.

In continuing studies over the past 16 years, more than 700 miniature swine have been exposed to chronic ^{90}Sr feeding as outlined in Figure 8.1. Currently 36 animals remain from this portion of the study; 12 controls, 12 at $1 \mu\text{Ci/day}$, 1 at $5 \mu\text{Ci/day}$, 1 at $25 \mu\text{Ci/day}$, 4 at $125 \mu\text{Ci/day}$, and a group of 6 at $250 \mu\text{Ci/day}$ which was removed from ^{90}Sr feeding at 210 days of age in November 1970. The causes of death for animals from the various groups during 1974 are shown in Table 8.1.

Analysis of cumulative mortality data by a life-table method showed no difference between life spans of controls and those of parent and offspring generations fed 1 or $5 \mu\text{Ci/day}$.

The parent generation started on $25 \mu\text{Ci/day}$ at 9 months of age had life spans comparable to those of controls. However, the offspring of this group ($25 \mu\text{Ci/day}$) had an increased mortality rate after about 8 years of age. The primary causes of death in the various treatment groups are summarized in Table 8.2.

In general, miniature swine exposed to ^{90}Sr at feeding levels $> 125 \mu\text{Ci/day}$ died of the effects of ^{90}Sr on the marrow cells with resulting pancytopenia. At $125 \mu\text{Ci/day}$, pancytopenia occurred, but there was a high incidence of myeloproliferative and lymphoproliferative neoplasms. The only hematologic manifestation of daily ^{90}Sr feeding at the 1, 5 and

FIGURE 8.1. Experimental Design, Chronic ^{90}Sr Ingestion in Miniature Swine.

25 $\mu\text{Ci}/\text{day}$ levels has been a significant ($P < 0.05$) and chronic neutropenia. The primary indications for euthanasia of aged swine in these three groups, and in the control animals, have been uterine tumors, severe endometritis, dental defects, marked erosive arthritis, and ankylosing spondylitis. There does not appear to be any dose-effect relationship between these conditions and the ^{90}Sr feeding level, but as these data are completed, they will be statistically evaluated.

Some of the more common non-neoplastic lesions observed at the different ^{90}Sr treatment levels are shown in Table 8.3. These lesions are not graded as to severity in this table. Nephritis has been commonly observed both grossly and histologically and generally consists of varying degrees of interstitial fibrosis. Vascular lesions have most frequently been classified as atheromatous intimal plaques or frank arteriosclerosis. These lesions have been observed in animals on the 250 $\mu\text{Ci}/\text{day}$ level as young as 3 months. Of particular interest is the increased incidence

of pneumonia at certain dose levels, especially at 25 $\mu\text{Ci}/\text{day}$, where overt radiation effects are minimal. The majority of these have been classified as chronic in nature and many as granulomatous.

The incidence of neoplasms observed at different ^{90}Sr feeding levels in animals over one year of age is shown in Table 8.4. Although not statistically evaluated, it appears that animals ingesting 25 $\mu\text{Ci}/\text{day}$ have an increased incidence of malignant tumors of various types and also an increase in benign tumors of the liver. The only bone tumors observed have been at the 125 μCi and 625 $\mu\text{Ci}/\text{day}$ levels.

Strontium-90 assays of femur samples obtained from animals up to 2500 days of age indicated that the concentration of ^{90}Sr in bone ash had attained a constant level relative to daily intake (Annual Report, 1968). However, there appeared to be a slight downward trend which has been verified by additional samples from animals up to 5100 days of age. This trend, observed in all the

groups, could be attributed to a naturally occurring and/or radiation-induced osteoporosis, with ^{90}Sr leaving the skeleton more rapidly than calcium. An alternative or additional factor might be a reduction in intestinal absorption of strontium without a concomitant reduction in calcium absorption. This would result in lower ^{90}Sr levels in all tissues of the body, not merely in the skeleton. Non-skeletal tissues have not been routinely assayed so this information is not available.

Since there is a paucity of information concerning the effects of deposited radionuclides on immunocompetence, and because of the apparent increase in neoplasia and/or chronic pneumonia at certain feeding levels, investigation into the immune status of ^{90}Sr is indicated. Future studies will be directed toward defining these interrelationships, as well as viral infections, and kinetics of the hematopoietic system.

TABLE 8.1. Causes of Death During 1974 in Chronic ^{90}Sr Study in Miniature Swine.

^{90}Sr Level, $\mu\text{Ci/day}$	Animal Number	Age (years)	Type of ^(a) Death	Dental Lesions	Arthritis	Uterine Pathology	Other Causes
0	362	14.9	D	++++	++++	++++	Fibrinous peritonitis
	783	13.5	E	++	+++	++++	
	960	12.7	D	++++	++++	+++	Enteritis
	961	12.4	E	++	+++	++++	Enteritis
	1190	12	D	++	++++	--	
1	305	15.2	E	+++	++++	--	Rectal prolapse
	624	13.4	D	+++	++	++++	Fibrinous peritonitis
	658	13.5	D	++	++	++++	Fibrinous peritonitis
	668	14	E	++++	++	+	Hepatic cirrhosis
	669	13.5	E	++++	++++	++	Emaciation; posterior paralysis
	862	13.1	E	++	++++	++	Posterior paralysis
	1017	12.5	D	++	++	++++	Fibrinous peritonitis
5	883	13.3	E	++++	+++	+	Lymphocytic leukemia
	1177	11.8	E	+	++	--	Severe nephritis
125	3580	5.2		+	--	+	Severe thrombocytopenia
	3583	5.7	E	++++	--	+	Osteomyelitis
	3998	4.2	E	+	--	--	Marked pancytopenia
	4012	5	E	++	+	+	Anemia; pancytopenia
	4054	4	E	++	++	--	Anemia; thrombocytopenia
250 (b)	4124	4.5	E	++	+	+	Pancytopenia; hemorrhage

(a) E = euthanasia; D = died

(b) Discontinued ^{90}Sr feeding at 210 days of age

TABLE 8.2. Summary of ^{90}Sr Effects in Miniature Swine.

^{90}Sr level, $\mu\text{Ci/day}$	Observed Effects	Approximate Survival Time
3100	Pancytopenia-Hemorrhagic Crisis	3 months
625 (F_1)	Pancytopenia-Hemorrhagic Crisis- Myeloid Metaplasia	3 months
313 (F_1)	Pancytopenia-Hemorrhagic Crisis- Myeloid Metaplasia	5 months
250 (F_1)	Pancytopenia-Hemorrhagic Crisis	7 months
125 (F_1 & F_2)	Pancytopenia-Hematopoietic Neoplasia	3 years
25 (F_1 & F_2)	Neutropenia-Neoplasia	> 9 years
5 (F_1 & F_2)	Neutropenia	> 11 years
1 (F_1 & F_2)	Neutropenia	> 11 years

TABLE 8.3. Non-Neoplastic Lesions in Miniature Swine
Ingesting ^{90}Sr Daily for Their Lifetime.

^{90}Sr Dose Levels, $\mu\text{Ci/d}$ Number of Animals ^(a)	Incidence of Lesions, %					
	0	1	5	25	125	250 ^(b)
	74	52	29	47	40	7
Cystic endometritis	27	39	41	45	8	14
Nephritis	38	37	48	47	22	43
Pneumonia	4	2	10	36	19	14
Vascular Lesions	18	23	10	19	11	29
Myeloid Metaplasia	7	12	10	15	18	43

(a) Animals > 1 year of age on which necropsy and histologic examinations are completed.

(b) Removed from ^{90}Sr feeding at 210 days of age.

TABLE 8.4. Neoplasms in Miniature Swine Ingesting ^{90}Sr Daily for Their Lifetime.

^{90}Sr Dose Levels, $\mu\text{Ci/d}$	Tumor Incidence, %					
	0	1	5	25	125	250
Number of Animals ^(a)	74	52	29	47	40	6 ^(b)
<u>Uterine</u>						
Benign	38	50	41	28	5	
Malignant		4	7	4		
<u>Ovarian</u>						
Malignant	1			9		
<u>Intestinal</u>						
Malignant	1	4		6		
<u>Liver</u>						
Benign	8	8	17	21		
Malignant				2		
<u>Myelolymphoid</u>						
Myeloid	4			9	38	33
Lymphoreticular		6	7	9	15	
<u>Miscellaneous</u>						
Benign	5	10	3	9	3	
Malignant				6		17
<u>Bone</u>					10	

(a) Animals > 1 year of age on which necropsy and histologic evaluations are completed.

(b) Removed from ^{90}Sr feeding at 210 days of age (Nov. 1970).

GUT RELATED STUDIES OF RADIONUCLIDE TOXICITY

This project is concerned with the behavior and effect of radionuclides that enter the gastrointestinal tract by ingestion or subsequent to inhalation. Current emphasis centers on the absorption of actinide elements as influenced by age and compound form.

ABSORPTION OF URANIUM-233, NEPTUNIUM-237, PLUTONIUM-238,
AMERICIUM-241, CURIUM-244, AND EINSTEINIUM-253 FROM
THE GASTROINTESTINAL TRACT OF NEWBORN AND ADULT RATS

Investigators:

M. F. Sullivan and A. L. Crosby

Absorption of the nitrates of ^{233}U , ^{237}Np , ^{238}Pu , ^{241}Am , ^{244}Cm , and ^{253}Es by the adult rat was estimated at 0.03%, 1.0%, 0.006%, 0.012%, 0.04%, and 0.014%, respectively; absorption of the oxides of ^{241}Am and ^{244}Cm was estimated at 0.01% and 0.003%, respectively. These estimates are based on retention in skeleton and liver. Absorption of the nitrates of ^{237}Np , ^{238}Pu , ^{241}Am , ^{244}Cm , and ^{253}Es by the newborn rat was estimated at 6.6%, 2.9%, 5.0%, 2.0%, and 5.1%, respectively; absorption of the oxides of ^{241}Am and ^{244}Cm was estimated at 1.1% and 1.7%, respectively. The amount retained by the lower small intestine of newborn rats was frequently several times more than the amount absorbed, which could be a significant factor in evaluating the hazard potential of these radionuclides.

Preliminary observations on the enhanced absorption of curium and californium from the gastrointestinal tract of newborn rats were reported in the 1973 Annual Report. Plutonium absorption is also known to be enhanced in the newborn. We have now completed observations on a number of actinide elements and compounds.

"Soluble" forms of the radionuclides were prepared by diluting nitric acid solutions and adjusting the acidity to pH 2. Americium oxide was suspended in water a few days before administration. Curium oxide was administered either as a fresh

suspension or after standing in water for several months. Adults were administered, by gavage, 5 to 8 μCi contained in 0.5 to 1.0 ml of solution. Two-day-old rats received about 2 μCi in a volume of 0.05 to 0.1 ml, administered carefully through a 0.024 in. polyethylene stomach tube. Higher radionuclide concentrations were administered to some rats whose tissues were used for preparation of autoradiographs. Excreta from adult rats was collected daily for 4 days and pooled for the next 3 days. All animals were killed at 7 days and tissues sampled for analysis.

Distribution and excretion data for adult rats are shown in Table 9.1. The sum of skeletal and liver retention is probably the best estimate of total absorption. Urinary excretion during the 7-day period between gavage and sacrifice should also be included; however, it seems likely that a major portion of the radioactivity

measured in the urine results from fecal contamination. The urine values in Table 9.1 are usually several times larger than skeleton plus liver, which is not in accord with many observations of minimal urinary excretion following parenteral administration of these radionuclides.

TABLE 9.1. Distribution of Radionuclides in Adult Rats Seven Days After Administration by Gavage of Nitrates and Oxides of ^{233}U , ^{237}Np , ^{238}Pu , ^{241}Am , ^{244}Cm , and ^{253}Es .

Radio-Nuclide	Number of Rats	Percent of Gavage Dose				Skeleton + Liver + Urine
		Skeleton ^(a)	Liver	Skeleton + Liver	Urine	
Nitrates						
²³³ U	11	0.03	0.003	0.03	0.13	0.16
²³⁷ Np	11	1	0.06	1.1	0.4	1.5
²³⁸ Pu	12	0.006	0.001	0.007	0.02	0.03
²⁴¹ Am	11	0.012	0.003	0.015	0.05	0.06
²⁴⁴ Cm	14	0.04	0.004	0.04	0.08	0.12
²⁵³ Es	6	0.014	0.001	0.015	0.02	0.04
Oxides						
²⁴¹ Am	6	0.003	0.01	0.013	0.11	0.12
²⁴⁴ Cm	3	0	0.003	0.003	0.03	0.03
	11 ^(b)	(0.02)	(0.014)	(0.04)	(0.06)	(0.1)

(a) Skeleton estimated as 23 X single femur content.

(b) Values in parentheses obtained using curium oxide suspended in water for 4 months prior to administration. Values not in parentheses were obtained using freshly suspended material.

The approximately 1% gastrointestinal absorption of neptunium in adult rats is markedly higher than that of the other actinides studied, which fall within a rather narrow range of about 0.005% to 0.05%. Absorption of americium and curium oxides was not greatly different from absorption of the nitrates, except

that freshly prepared curium oxide was absorbed to a 10-fold lower degree than an aged suspension. The enhanced absorption of the aged suspension is consistent with other observations of increased biological mobility of high specific activity oxides, due presumably to radiolytic decomposition of the oxide.

Of particular interest is the high retention of actinides by the newborn within the intestine--both in the contents and within the intestinal wall (Figure 9.1). While this material cannot be considered to be absorbed, it may be of hazard significance, as indicated by substantial damage observed, particularly in the lower small intestine (Figure 9.2). These observations are being extended.

Absorbed actinide nitrates show a preference for deposition in bone

rather than liver, in the case of both adult and newborn rats. This preference is reversed when oxides are fed to adult rats, but no such reversal is observed in the newborn. This argues, again, for a different mechanism of absorption in the newborn. Closer study of such differences might lead to a basic understanding of the mechanisms involved, and the factors that may influence these mechanisms.

FIGURE 9.1. Autoradiograph of an Ileum Section From a Newborn Rat Given 10 μ Ci of ^{238}Pu Nitrate 2 Days Before Sacrifice.

FIGURE 9.2. Histologic Section of the Ileum From a Newborn Rat Given 5 μ Ci of ^{253}Es Nitrate 7 Days Before Sacrifice. H&E.

Distribution data for the newborn rats are shown in Table 9.2. Excreta were not collected from these animals. The column headed "total absorbed" is therefore a minimum estimate, not including re-excreted material. Absorption in the newborn is enhanced over that in the adult by factors of 50 to 500, except for a factor of only 6 for neptunium. This

low enhancement ratio in the case of neptunium is quite possibly due to the older rats (8 days) employed in the neptunium experiments. The newborns did not seem to discriminate between the aged and fresh suspensions of curium oxide, suggesting that the mechanism of absorption in the newborn is different from that in the adult.

TABLE 9.2. Distribution of Radionuclides Seven Days After Administration by Gavage to Two-Day-Old Rats of Nitrates and Oxides of ^{237}Np , ^{238}Pu , ^{241}Am , ^{244}Cm , and ^{253}Es .

Radio-nuclide	Number of Rats	Percent of Gavage Dose				Total Absorbed ^(b)
		Skeleton ^(a)	Liver	Wall	Intestine Contents	
Nitrates						
²³⁷ Np	10 ^(c)	3.6	0.10	5.5	7.5	6.6
²³⁸ Pu	11	2.6	0.3		13	2.9
²⁴¹ Am	8	4.5	0.3	11	11	5
²⁴⁴ Cm	7	1.8	0.2			2
²⁵³ Es	8	4.4	0.4	0.8	6.8	5.1
Oxides						
²⁴¹ Am	10	0.14	0.02	0.8	0.8	1.1
²⁴⁴ Cm	5	1.9	0.16	2.2	2.8	1.7
	(4) ^(d)	(2.3)	(0.16)	(7.0)	(11.)	(1.8)

(a) Skeleton estimated as $0.22 \times \text{body wt (g)} \times \text{concentration in femur (\%/g)}$

(b) Total absorbed estimated as sum of separate analyses on femur, liver, and residual carcass (not including intestinal wall or contents).

(c) Gavigated at 8 days of age.

(d) Values in parentheses obtained using curium oxide suspended in water for 4 months prior to administration. Values not in parentheses were obtained using freshly suspended material.

ACUTE TOXICITY OF REACTOR CATASTROPHE EFFLUENT

This project has the specific objective of determining the acutely lethal effects of ingested or inhaled radionuclides of the type that would be released in a catastrophic reactor accident.

ACUTE TOXICITY OF INGESTED RUTHENIUM-106

Investigators:

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and J. L. Beamer

The median lethal ingested dose of ^{106}Ru for adult, weanling, and newborn rats was about 9, 20, and 2 mCi/kg, respectively. Death from gastrointestinal damage in dogs occurred at doses between 3.5 and 4.0 mCi/kg. Death was usually caused by severe damage to the lower bowel where the radiation dose was highest due to the prolonged residence time in that segment. Incorporation of ^{106}Ru in the epithelium of the small intestine of the newborn contributed to the sensitivity of that age group.

The Rasmussen Study Report (WASH-1400) indicated a need for information on the acute toxicity of ingested radionuclides that might be released to the environment as a result of a reactor accident. Of particular concern are beta emitters of widely varying energies. At the high end of the energy spectrum are radionuclides like ^{106}Ru , whose ^{106}Rh daughter emits a 3.5 MeV maximum energy beta. Such energetic betas should be effective in irradiating the sensitive cells at the base of intestinal crypts. On the low end of the energy spectrum are such radionuclides as ^{103}Ru with a 0.2 MeV maximum energy beta particle, which would be effective only in irradiating the surface of the mucosa. The results of studies with ^{106}Ru are described in this report.

The ^{106}Ru was obtained as a chloride in acid solution. The pH was adjusted to 1 by addition of NaOH and the solutions diluted so that adult rats received the appropriate gavage dose in a 1 to 2 ml volume. Two-day-old rats were given 0.1 ml through a

polyethylene tube (0.024" O.D.). Adult beagle dogs were fed the appropriate ^{106}Ru doses by mixing concentrated solutions with about 100 g of their diet. Controls were given similar preparations made from stable ruthenium chloride. Blood was withdrawn before treatment and at intervals thereafter when effects were anticipated. Mortality data are shown in Table 10.1.

The median lethal dose for adult rats was about 9 mCi/kg and for weanling rats about 20 mCi/kg. The median lethal dose for the newborn rats was less than 2 mCi/kg. Death of the adult rats was caused by severe damage to the large bowel, in which ingesta remains for about 18 hours. The calculated dose to that gut segment from 9 mCi/kg of ^{106}Ru is 6000 rad. The greater toxicity in the newborn may be due to the incorporation of ^{106}Ru into the epithelium of the lower small intestine (Figure 10.1A), where it caused complete denudation by 8 days (Figure 10.1B).

TABLE 10.1. Survival of Rats and Dogs After
Oral Administration of Ruthenium-106

Species	^{106}Ru Dose, mCi/kg	Mortality, No. Deaths/ No. Exposed	Average Survival Time, Days
Adult Rats	2-5	0/12	---
	7	1/4	9
	8	4/10	9
	9	5/12	7.6
	10	5/7	7.8
Weanling Rats	5-16	0/22	---
	20	2/2	5
	24	3/3	4.3
	28	3/3	4.3
Suckling Rats	1	0/2	---
	2-	5/5	12
	3	6/6	8.7
	4	9/10	8.2
	5	14/15	7.9
	7	18/18	7.8
	9	3/3	7.3
Adult Dogs	2.2-3.2	0/7	---
	3.5-3.7	4/4	15
	4	2/2	15

FIGURE 10.1A. Autoradiogram of the ileum of a Two-Day-Old Rat Given 4 mCi/kg ^{106}Ru by Gavage and Killed 4 Days Later.

FIGURE 10.1B. Photomicrograph of the ileum of a Two-Day-Old Rat Given 4 mCi/kg ^{106}Ru by Gavage and Killed 8 Days Later. H&E.

The blood cell counts of adult rats made during the acute injury stage (Figure 10.2A) showed that only the lymphocyte values were depressed, probably due to local irradiation of the lymphatic tissue in the intestine during passage. The neutrophil values increased in a dose-dependent manner (Figure 10.2B) but hemoglobin values did not change appreciably, suggesting that hemoconcentration was not substantial. These hematologic data indicate that death from ruthenium ingestion was not due to a total irradiation effect but to localized exposure of the intestine.

The large bowel also received the highest radiation dose in the dogs. The calculated dose to the surface of that segment from 40 mCi (3.7 mCi/kg) is approximately 10,000 rad. Anorexia and rectal hemorrhage were more noticeable symptoms in dogs than in rodents. These responses contributed to death, which occurred 3 weeks after treatment although much of the early gastrointestinal damage had by then been repaired.

FIGURE 10.2A. Lymphocyte and Neutrophil Counts of Adult Rats Injected with ^{106}Ru by Gavage.

FIGURE 10.2B. Lymphocyte and Neutrophil Counts of Adult Rats Injected with ^{106}Ru by Gavage.

INHALATION HAZARDS TO URANIUM MINERS

This project has sought to establish the synergistic effects of uranium mine air pollutants following prolonged inhalation by rodents and dogs, and to compare the data obtained with the results of similar observations on uranium miners.

BIOLOGICAL EFFECTS IN BEAGLE DOGS OF INHALED RADON DAUGHTERS,
URANIUM ORE DUST AND CIGARETTE SMOKE

Investigators:

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Technical Assistance: J. C. Gaven, W. Skinner,
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After 5 years of daily inhalation exposures to 600 WL radon daughters plus uranium ore dust and/or cigarette smoking, observed pulmonary lesions include macrophage proliferation, septal fibrosis, epithelial hyperplasia, emphysema, endothelial proliferation, and bronchiolar-alveolar epithelial changes involving multiple foci of squamous metaplasia with atypia. Epithelial neoplasms were found in the respiratory tracts of three dogs.

The high incidence of lung cancer among uranium miners of the Colorado plateau is a matter of national concern in a period of increasing demand for uranium ore. The miners are exposed to a variety of inhalation hazards, including radon daughters, uranium ore dust, and cigarette smoking, that may cause or contribute to respiratory tract pathology. To determine the combined or separate roles of radon daughters and cigarette smoking in the development of lung cancer and other respiratory tract pathology, groups of 20 dogs each received daily life-span exposures to 4 hr of 600 Working Levels of radon daughters with ore dust, and/or cigarette smoking, over 16 hr/day, 7 days per week, or both; control dogs received sham-smoking.

Table 11.1 shows the experimental design and also the number of animals remaining alive in each exposure group approximately 5 years after the start of exposures. In Figure 11.1 are the survival curves of groups 1

and 2. The survival curves of these two groups are not felt to be significantly different. All deaths in the smoking-only and control groups have been due to planned sacrifice or to causes unrelated to exposure.

Mean minute volumes of exposed dogs have not differed significantly from those of control dogs, but respiratory rates of the surviving dogs of groups 1 and 2 are considerably elevated. There has been a wide variation among values for dogs within each of the two groups, with some animals showing rates of 100 to 120 respirations per minute with severe respiratory insufficiency characterized by anoxemia and hypoxemia immediately prior to sacrifice.

Since February 1974, 11 dogs from group 1 and 13 dogs from group 2 were sacrificed in poor condition caused by progressive pulmonary insufficiency. These dogs had been exposed daily for 4 to 4.5 years.

TABLE 11.1. Experimental Design and Animals Alive on 12/1/74

Group	Number of Animals		Exposure Regimen
	Original	Alive 12/1/74	
1	20	3	600 WL ^(a) radon daughters with uranium ore dust (carnotite, 15 mg/m ³), plus sham smoking
2	20	2	Cigarette smoke ^(b) plus 600 WL ^(a) radon daughters with uranium ore dust (carnotite, 15 mg/m ³)
3	20	18	Cigarette smoke ^(b)
4	9	8	Controls, with sham smoking

(a) Working Level - Any combination of short-lived radon daughters in one liter of air that will result in the ultimate emission of 1.3×10^5 MeV of alpha energy from radioactive decay.

(b) Ten cigarettes per day over a 16-hour period, 7 days per week.

FIGURE 11.1. Survival Time of Beagle Dogs Exposed to Radon Daughters and Uranium Ore Dust, With and Without Concomitant Exposure to Cigarette Smoke.

All lungs showed gray, green, and brown discoloration of large irregularly shaped areas of the pleural surfaces. In many lungs, pleural thickening was evident, and small areas of scar tissue were visible on the pleural surface. Multifocal bullous emphysema was observed beneath the pleural surface in the lungs of 4 of 11 dogs from group 1 and 8 of 13 dogs from group 2. Mild to marked emphysema occurred beneath the parietal pleural surface in the thoracic cavity of many dogs.

One control dog (sham exposure) was sacrificed for histopathologic comparison with the dogs of groups 1 and 2. The respiratory tract of this dog was generally normal in gross appearance. A few small foci of subpleural interstitial fibrosis with associated alveolar epithelial hyperplasia and cuboidal squamous metaplasia were present in the lungs. These lesions are considered spontaneous in nature and are commonly found in beagle dogs as they grow older.

With the exception of three neoplastic lesions described later, the tissues examined from dogs of groups 1 and 2 showed histological findings similar to those described in the previous annual report. Extensive pleural and alveolar septal fibrosis, as well as chronic inflammatory changes caused by uranium ore-induced pneumoconiosis, were observed. The radiation-induced epithelial changes previously described were also seen. Both types of changes are observed with a slightly greater degree of severity as length of exposure increases. Adenomatous proliferation of alveolar epithelium and squamous metaplastic changes in the alveolar epithelium are also more extensive as the duration of exposure increases. Infiltration of mononuclear inflammatory cells in perivascular and peribronchiolar areas and dilation of bronchial mucus glands are frequent observations. These changes are associated with chronic bronchitis, and are probably induced by the continued irritation of the airways by uranium ore dust, cigarette smoke, or both. Vesicular emphysema is a micropathologic feature of all lungs of dogs from these two groups.

Epithelial neoplasms were found in the respiratory tracts of three dogs. A microscopic bronchiolo-alveolar carcinoma, approximately 2 mm in diameter, was present in the deep parenchyma of the apical lobe of a group 1 dog that had been exposed to radon daughters and uranium ore dust for 51 months (Figure 11.2). This was a low-grade, well circumscribed neoplasm with no evidence of invasion or metastases.

X39

FIGURE 11.2. Bronchiolo-alveolar Carcinoma From the Lung of a Dog Which was Exposed to Radon Daughters and Uranium Ore Dust for 55 Months. H & E.

Another dog from group 1 developed a maxillary tumor which was grossly visible on the left side of its face after 48 months of exposure. The tumor was approximately 4 cm in diameter and upon dissection was found to nearly occlude the nasal passages. Micropathologically, it was a squamous cell carcinoma and probably originated in the mucosa of the turbinates on the left side (Figure 11.4).

A squamous cell carcinoma involving the nasal turbinates was also found in a group 2 dog after 51 months of exposure to radon daughters and uranium ore dust, and 57 months of cigarette smoke. This neoplasm had invaded the bony structure of the turbinates but invasion of adjacent tissue was not as extensive as that seen in the earlier case.

Microscopic examination of turbinates from dogs of groups 1 and 2 has revealed an apparent progression of pathologic lesions from normal mucosa to neoplastic changes. The histogenesis of these squamous carcinomas presumably includes the following steps:

- (1) Loss of cilia and flattening of normally ciliated columnar epithelium.
- (2) Hyperplasia and squamous metaplasia of the epithelium.
- (3) Pseudo-epitheliomatous hyperplasia (Figure 11.3).
- (4) Squamous cell carcinoma (Figure 11.4).

In many cases, two of the non-neoplastic changes may be observed in

the turbinates of a single dog. This progression is currently being documented in detail.

Because of the rapidly deteriorating health of the animals, exposures to radon daughters and ore dust were stopped on August 15, 1974. All dogs are continuing to be smoked or sham-smoked during day shift only, 7 days per week. Cessation of exposure to radon daughters and uranium ore dust may allow the dogs to live longer and increase the possibility for development of pulmonary neoplasms before the dogs succumb to the progressive pulmonary effects of the uranium ore dust. Also, it could provide information regarding the possible regression of lesions including epithelial hyperplasia and squamous metaplasia.

X99

FIGURE 11.3. Pseudoepitheliomatous Hyperplasia of the Epithelium in the Nasal Turbinates of a Dog which was Exposed to Radon Daughters, Uranium Ore Dust, and Cigarette Smoke for 53 Months.
H & E.

X99

FIGURE 11.4. Squamous Cell Carcinoma of Nasal Turbinates from a Dog which had been Exposed to Radon Daughters, Uranium Ore Dust, and Cigarette Smoke for 51 Months. The tumor had invaded the maxilla as well as the nasal passages.
H & E.

DISPOSITION OF LONG-LIVED URANIUM CHAIN ALPHA EMITTERS
FOLLOWING REPEATED INHALATION EXPOSURES OF
BEAGLE DOGS TO URANIUM ORE AND RADON DAUGHTERS

Investigators:

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Technical Assistance:

J. C. Gaven, H. G. Steele and K. C. Upton

Thorium-230 was found to separate readily in vivo from parent uranium, following long-term inhalation of carnotite uranium ore by beagle dogs. Thorium-230 should be considered as an individual hazard in uranium mining and milling operations.

For more than 10 years we have been concerned with whether retention of ^{230}Th inhaled as a constituent of airborne uranium ore dust is governed by the characteristics of the parent particles. In 1963, preliminary studies of rats inhaling aerosols of crushed pitchblende ore in secular equilibrium showed a rapid in vivo separation of ^{230}Th from parent uranium in all tissues, including lungs and tracheobronchial lymph nodes. Subsequent studies with beagle dogs inhaling pitchblende ore dust also indicated an independent fate of the uranium and thorium components.

After 4.5 years of exposure, four beagle dogs inhaling carnotite uranium ore and radon daughters, two with and two without concomitant exposure to cigarette smoke, showed marked nonequilibrium ratios of $^{230}\text{Th}/^{234}\text{U}$, ranging from 5.6 to 7.1 in lungs and 6.3 to 9.1 in tracheobronchial lymph nodes. Although these ratios were quite similar in the two tissues, the average concentrations of the radionuclides were

13 to 16 times higher in lymph nodes than in lung.

The ratios of $^{210}\text{Pb}/^{230}\text{Th}$ measured in the nasal turbinates of two of these dogs were 7 to 12 times higher than those in the lungs. As ^{210}Pb concentrations were about the same in these two tissues, there was a much lower concentration of ^{230}Th in turbinates than in lung. It is not known whether the higher ratio in the turbinates results from solubilization of ^{210}Pb from ore in the lung and its translocation to the spongy bone of the turbinates, or from the higher deposition of radon daughters in turbinates than in lung and their subsequent decay to ^{210}Pb . This latter mechanism would result in a higher radiation dose to turbinates than to lung, and would tie in with the finding of squamous cell carcinoma in the turbinates of two of our exposed dogs. Planned analyses of bones other than turbinates and measurement of radon daughter deposition should help to resolve these uncertainties.

COMPARATIVE EFFECTS IN HAMSTERS, RATS, AND MICE OF
EXPOSURE TO SIMULATED URANIUM MINE ATMOSPHERES

Investigators:

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Technical Assistance:

H. G. Steele and J. C. Gaven

Hamsters, rats, and mice were chronically exposed to radon daughters, with and without uranium ore dust, to provide an interspecies comparison of the effects of these uranium mine inhalation hazards.

Syrian golden hamsters, C57BL mice, and specific pathogen-free rats were exposed simultaneously in groups of 16 animals each for 30 hr/week to aerosols consisting of radon plus radon daughters, with and without carnotite uranium ore dust. In the chamber without uranium ore dust the radon concentration was reasonably constant, averaging $4.7 \pm 0.7 \mu\text{Ci}/\ell$. The concentrations of radon daughters and condensation nuclei, however, were quite erratic, varying with humidity and the number of animals in the chamber. Working levels of radon daughters averaged 1,520 during the first 265 days of the exposure period but increased to over 16,000 by day 378 when only one hamster remained alive in the chamber. Condensation nuclei concentrations varied from 2,100/cc to a high of 87,000/cc over the same time period. In the chamber with $17 \pm 5 \mu\text{g}$ of uranium ore dust and $2.6 \pm 0.3 \mu\text{Ci}$ of radon per liter, radon daughter concentrations ranged between 5,400 and 7,400 working levels, and condensation nuclei concentrations between 66,000/cc and 111,000/cc.

Figure 11.5 shows that all three species showed more drastic life shortening in the chamber containing uranium ore dust in addition to radon and its daughters than they did in

the one without the ore dust. This is not surprising when one considers the greater radiation insult and the additional stress of breathing ore dust that they experienced.

In Table 11.2 are seen the days of survival since the start of exposures, and the cumulative Working Level Months of radon daughter exposure received by each species of animal in the two chambers, at the time 50% of the animals had died. It can be seen that rats were less affected by the presence of the uranium ore dust than were the other two species, but were somewhat more sensitive than the others to the radiation from radon and its daughters in the absence of the ore dust. Planned studies involving measurement of radioactivity and ore concentration in tissues throughout the respiratory tracts of each species should aid in explaining these effects.

The results of the histopathologic examinations of the respiratory tracts and other selected organs are not available at this time. At necropsy, several animals showed macroscopic lesions suggestive of metaplastic changes, but their final diagnosis must await microscopic verification.

FIGURE 11.5. Comparative Effects of Exposure of Hamsters, Rats and Mice to Simulated Mine Atmospheres.

TABLE 11.2. Survival Time and Working Level Months of Exposure to Radon Daughters when 50% of the Animals had Died.

	Radon Daughters Chamber		Radon Daughters + U-Ore Chamber	
	Median Survival Time, Days	Cumulative WLM	Median Survival Time, Days	Cumulative WLM
Mice	269	9,400	104	14,700
Hamsters	263	8,900	126	18,000
Rats	215	7,600	156	23,000

EVALUATION OF RADIONUCLIDES IN MAN

This project is concerned with the development and application of methods for evaluating the radiologic impact of the nuclear industry on its workers and on residents of the local environs.

EVALUATION OF POSTMORTEM TISSUE SAMPLES

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Technical Assistance:

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Collection and radiochemical analysis of postmortem tissue samples (lung, liver, bone, and tracheobronchial lymph nodes) from individuals residing or formerly residing in the vicinity of the Hanford Project continued during the past year. Emphasis on this work has been increasing and the region of interest of sample collection has been enlarged, as have our processing capabilities. Postmortem tissue samples and blood samples were also analyzed for the U.S. Transuranium Registry (USTR). Since January 1, 1974, 72 plutonium analyses have been performed on 36 samples from the Hanford locality, and 56 analyses on 26 samples obtained from the USTR. In process at this writing are 43 samples from the Hanford locality and 16 USTR tissue samples. Eighty-six Hanford locality samples and 59 USTR tissue samples are awaiting processing.

Analysis of interlaboratory comparison samples continues. These latter samples consist of bovine bone, liver and lung samples which will be analyzed independently by several AEC laboratories currently engaged in plutonium analysis. Comparison of the results reported by the various laboratories will form a basis for mutual information exchange among the laboratories leading to improved analytical techniques and improved assurance of consistency of analytical results.

During the year substantial improvements were made in the radiochemical procedure used to separate and measure the plutonium content of the tissue samples. Use of the procedure as diagrammed in Figure 12.1 has resulted in radiochemical yields frequently exceeding 90%, and even large bone and liver samples, which heretofore had been exceedingly troublesome, will have yields exceeding 70 or 80%, respectively. Error estimates on these yields at 1 σ are nominally $\pm 10\%$ at 0.4 d/m-aliquot including propagation of calibration and yield uncertainties and counting statistics for 1000 minute counts. That point at which the total propagated error (1 σ) about the estimates approaches $\pm 100\%$ is nominally 0.03 d/m-aliquot for 1000 minute counts indicating that so-called "less than" values will probably appear as 0.05 to 0.06 d/m-aliquot is approached. Although the apparent sensitivity may not be quite as good using alpha energy analysis in comparison to alpha track counting, which uses 10,000 minute exposures, the incorporation of a radiochemical yield tracer in the form of ^{242}Pu or ^{236}Pu , as circumstances warrant, results in analyses in which considerable confidence may be placed in the overall analytical accuracy. The ability to distinguish between ^{238}Pu and $^{239,240}\text{Pu}$ is also advantageous.

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PROCEDURE FOR DETERMINING Pu ALPHA IN AUTOPSY SAMPLES

(SEPTEMBER 1974)

FIGURE 12.1. Evaluation of Postmortem Tissue Samples.

Internal audit procedures were undertaken to assure the accuracy of analysis in this program. Twenty-four 100 ml urine samples to which the Westinghouse-HEDL Radiological Chemistry Section had added known amounts of ^{239}Pu were prepared in triplicate and in batches of six samples each containing from 0.1 d/m to 2 d/m ^{239}Pu per sample. Except for one sample lost in process, the ^{239}Pu activity determined in the remaining 23 samples agreed within counting statistics with the stated values. In addition, audit samples have been obtained from the International Atomic Energy Agency to further assure the accuracy of our procedures. These samples will enter the system blind and will be measured by several laboratories.

In addition to four gold-surfaced diodes used in the AEA system, four additional ruggedized detectors were obtained for use primarily in the analysis of USTR samples which generally contain more activity and consequently pose a greater potential for contamination. The ruggedized detector may be cleaned whereas the gold-surfaced detectors are usually destroyed by almost any physical impact.

The results of plutonium analyses on present and former residents of the Hanford area, as reported in 1974,¹ were re-examined to see if more information could be extracted from the data. Based on this re-examination, the following conclusions seem warranted:

- Of the individuals residing locally, none showed a measured plutonium concentration in lung or liver exceeding 5 fCi Pu/g. About one-half of the results for each of these tissues were less than 0.5 fCi Pu/g. The largest positive measurements were 2.6 fCi Pu/g of lung and 2.3 fCi/g of liver.

- Of those individuals not residing locally, the measured concentration of plutonium in lung and liver exceeded 5 fCi Pu/g only in one liver specimen with a measured concentration of 6.5 fCi/g. About one-half of these results were less than 0.5 fCi Pu/g. The largest positive measurements obtained since 1950 were 1.8 fCi Pu/g for lung and 1.1 fCi Pu/g for liver.
- Among local residents, the highest concentration in bone was 49 fCi/g. More than three-fourths of the measured concentrations in bone of residents and nonresidents were less than 5 fCi/g.

The above results are in substantial agreement with analytical results obtained elsewhere for the general population in New Mexico, Colorado, Georgia, South Carolina, Tennessee, and New York. There is no indication that past Hanford operations have been an important contributor to the plutonium burden of residents in the Hanford area.

Procedures have been developed for the analysis of uranium in tissues from former workers exposed to uranium. Analysis is by fluorophotometry of 100 λ aliquots of dissolved samples, with a detection limit of 0.5 ng per 100 λ aliquot. No significant problems were encountered and the laboratory is prepared to process human tissue samples for uranium as requested.

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1. Nelson, I. C., K. R. Heid, P. A. Fuqua, and T. D. Mahony. 1972. Plutonium in Autopsy Tissue Samples. Health Phys. 22, 925-930.

QUANTIFICATION OF INHALED PROMETHIUM

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Human data on the retention of inhaled promethium would be useful for diagnosing the retention of $^{147}\text{Pm}_2\text{O}_3$ in accidentally exposed workers. Exposure of human volunteers to the readily detectable ^{143}Pm seems feasible, providing a suitable carrier can be found that will provide the necessary mass and that will behave like promethium in the human body. Samarium has been proposed as such a carrier and experiments comparing the behavior of intravenously injected and inhaled samarium and promethium in rats were described in last year's Annual Report. The results of these rat studies indicated that, for a reasonable time after exposure, samarium could serve as a suitable carrier for promethium.

An experiment was performed this year in which a mixed promethium-samarium oxide aerosol was inhaled by

beagle dogs. Although evaluation of tissue distribution and excretion data from this experiment has not been completed, preliminary results indicate that for a few weeks following exposure, promethium and samarium are sufficiently similar in their behavior to qualify samarium as a suitable carrier for promethium in beagle dogs.

Plans will therefore proceed for the generation of suitable samarium-promethium aerosols for use in the exposure of human volunteers, with review of proposed experimental protocol by the Battelle-Northwest Human Subjects Committee, for the development of a diagnostic model for the excretion of inhaled promethium oxide.

(a) Physics and Instrumentation Dept.

RADIONUCLIDES IN RICHLAND SCHOOL CHILDREN

Investigators:

I. C. Nelson^(a) and G. W. R. Endres^(a)

Technical Assistance:

F. N. Eichner^(a) and M. E. Castillo^(a)

An investigation was initiated on the occurrence of certain gamma-emitting radionuclides, notably ^{137}Cs , in local residents. The objective of this investigation is to verify the presence or absence of Hanford radioactive wastes in Hanford residents as a result of possible ground water and Columbia River contamination. Cesium-137 is the principal radionuclide of interest since it has a long half-life (30 years) and could persist in ground water for some time. Because ^{137}Cs has also occurred in fallout and from nuclear testing, small amounts are expected to occur in people from that source. Measurement of ^{137}Cs in control groups whose drinking water is not derived from ground water which could be contaminated by Hanford wastes or from the Columbia River is therefore necessary.

The subjects chosen for these studies are Richland school children, primarily because knowledge of the occurrence of radioactive nuclides in these individuals is highly important and because they will be available for repeated examinations over the years. These data will also form a background based directly on human measurements for impact evaluation if future plant sitings should result in the dispersal of cesium-bearing or similar contaminants.

Measurements were made on 181 Richland school children using a mobile whole body counter, shown in Figure 12.2. Although measurements have not been made on control groups, preliminary analysis of the data suggests that no unusual results will be found. Only very small amounts of ^{137}Cs were found, in addition to the naturally occurring radionuclide, ^{40}K .

FIGURE 12.2. Mobile Whole Body Counter at a Richland School.

(a) Physics and Instrumentation Dept.

TREATMENT FOR RADIONUCLIDE INCORPORATION

This project is concerned with developing methods for decreasing the damage from internally deposited radionuclides by hastening their removal or otherwise decreasing the radiation dose to sensitive tissues. It seeks to be responsive to the practical needs of those responsible for the treatment of internal or external radionuclide contamination.

LONG-TERM EFFECTS OF INHALED Ca-DTPA IN RATS PREVIOUSLY
EXPOSED TO $^{239}\text{Pu}(\text{NO}_3)_4$ AEROSOLS

Investigators:

J. E. Ballou, G. E. Dagle, and R. L. Buschbom

Technical Assistance:

R. A. Gies

Inhaled Ca-DTPA given 20 days after $^{239}\text{Pu}(\text{NO}_3)_4$ inhalation was marginally effective ($p = 0.10$) in reducing the amount of Pu in rat lung. This reduction in Pu lung burden did not, however, affect the later induction of lung tumors. An increased osteosarcoma incidence was noted in control rats exposed only to nitric acid aerosols. Complete tumor incidence data are not yet available.

One-hundred male Wistar rats were exposed to inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ (78 nCi initial lung burden) and after 20 days were administered 6 weekly, hr-long, nose-only exposures to Ca-DTPA aerosols. Animals were sacrificed periodically after plutonium exposure to determine clearance kinetics and efficacy of delayed therapy. The remaining animals have been retained in an ongoing study for life-span observation of long-term biological effects. Also included in this study are 200 treatment control animals which received 0.27 N nitric acid aerosols, nitric acid plus Ca-DTPA, or nitric acid with sham Ca-DTPA exposure.

The efficacy of inhaled Ca-DTPA in the removal of inhaled ^{239}Pu from lung is indicated in the retention

curve shown in Figure 13.1. Because of the wide range in initial lung burdens within the treated and non-treated groups, it is not possible to demonstrate a clear-cut effect of Ca-DTPA treatment in these animals. There appears, however, to be a trend for the Ca-DTPA treated lungs to fall below the fitted curve, indicative of some increased removal of ^{239}Pu . A nonparametric sign test, when applied to this data, showed that the differences are significant at the 0.10 level.

Rats that died during the experiment showed a variety of tumors, both benign and malignant. Approximately one-third of the animals exposed to ^{239}Pu have been examined for pathologic lesions; approximately one-third of those examined, including

FIGURE 13.1. Retention of ^{239}Pu in Lungs of Rats Treated with Inhaled Ca-DTPA Aerosols.

those treated with Ca-DTPA and untreated animals, exhibited pulmonary carcinomas. Of 40 rats thus far examined, exposed only to Ca-DTPA aerosols, one had a pulmonary adenocarcinoma. This single lung tumor cannot be attributed to Ca-DTPA treatment at this time.

It is interesting that three control rats exposed only to nitric acid aerosols have developed osteosarcomas. Several hundred animals previously exposed to nitric acid along with low levels of $\text{Pu}(\text{NO}_3)_4$ (bone dose <10 rads) have not developed these lesions.

OBSERVATIONS IN RATS 2 YEARS AFTER EXPOSURE
TO Ca-DTPA AEROSOLS

Investigators:

J. E. Ballou and G. E. Dagle

Technical Assistance:

R. A. Gies

Inhaled Ca-DTPA in 6 weekly 1-hr exposures, ~5 mg per exposure, did not affect weight gain or long-term survival of rats. One pulmonary adenocarcinoma was found in the group exposed to Ca-DTPA aerosols; however, this cannot be attributed to chelate treatment at this time.

One-hundred male Wistar rats, 60 days of age, were given nose-only exposure to Ca-DTPA aerosols or simulated aerosol exposure (sham) as part of a study of the latent toxicity of this chelating agent when administered by the inhalation route. The study is still in progress.

The rats were given 6 weekly 1-hr, nose-only exposures to the drug (or sham exposed) and were subsequently retained for life-span observation. The inhaled dose of Ca-DTPA was estimated to be approximately 5 mg per exposure, based on breathing parameters and the concentration of Ca-DTPA in the aerosol chamber. The absorbed dose of Ca-DTPA will be calculated from the amount of the drug excreted in urine when these analyses are completed.

The animals were weighed at monthly intervals after exposure; dead animals were necropsied, and major tissues and lesions were processed for histopathologic analysis.

Treatment with Ca-DTPA or sham treatment had no significant effect on weight gain compared to that of nontreated controls. On the other hand, the mortality curves shown in Figure 13.2 suggest that there may have been some differences in survival. These differences cannot, however, be attributed to treatment. The early deaths in the group exposed

to Ca-DTPA occurred during the period of handling associated with exposure to the aerosols. The later peak at 19 months postexposure was due to the loss of temperature control in the animal room which housed the treated and sham-treated rats. The nontreated control animals were unaffected since they were housed in an adjacent room that was not influenced by the temperature excursion. These data do not suggest that there is any unusual hazard associated with inhaled Ca-DTPA. Of 40 rats thus far examined, only one had a pulmonary adenocarcinoma. This single lung tumor cannot be attributed to Ca-DTPA treatment at this time.

FIGURE 13.2. Mortality Curves for Treated (Ca-DTPA Aerosols) and Untreated Rats Up to 2 Yr Postexposure.

TERATOLOGIC EFFECTIVENESS AND FETAL TOXICITY
OF DTPA IN THE RAT

Investigators:

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Technical Assistance:

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and K. A. Strikwerda

Ca-DTPA, injected intraperitoneally in rats from day 8 through 12 of gestation, was embryocidal in doses larger than 8-HD (eight times the human dose of 28.7 $\mu\text{mol/kg}$ body weight). The effect was 100% fatal at doses > 16-HD. No fetal deaths occurred from Zn-DTPA, even at 64-HD. Some fetuses from dams receiving > 8-HD of either salt showed an increase in hydronephrosis and some alteration in kidney structure. A few minor skeletal abnormalities were also observed at these doses. In general, the teratologic effects of DTPA, if any, were minor and sporadic.

As part of the toxicity evaluation of Ca- and Zn-DTPA, the teratologic potential and fetal toxicity were studied in the Wistar rat. Ca-DTPA is known to increase Zn excretion, at least temporarily, and Zn deficiency is teratologic, Zn being required for certain steps in DNA synthesis. The next lower homolog of DTPA, EDTA, has been shown by Swenerton and Hurley (*Science* 173, 62-64, 1971) to have a teratogenic effect, which could be reversed by supplementing the Zn content of the diet. Accordingly, these studies were undertaken using both the Ca and Zn salts of DTPA. All doses are given as multiples of the commonly employed intravenous human dose (HD) which is 0.0287 mmol/kg. This is equivalent to 14.3 mg Ca-DTPA/kg or 15.0 mg Zn-DTPA/kg.

Pregnant rats, six per group, were injected intraperitoneally on days 8 through 12 of gestation in various multiples of the human dose (HD) as indicated in Table 13.1; pregnant

control rats received equivalent volumes of 0.9 NaCl over the same gestation interval. The rats were killed after 20 days of gestation, the fetuses removed, examined for gross malformation, weighed and either fixed in 95% ethanol or Bouin's fixative for study of teratologic change. Those fixed in alcohol were stained with alizarin red and cleared with glycerol for investigation of skeletal abnormalities; those in Bouin's fixative were transferred to 70% ethanol and sectioned with a razor blade for detection of soft tissue malformations.

Zinc-DTPA at 64-HD produced a slight decrease in the normal weight gain experienced by the dam in the period between 8 and 12 days of gestation, but it did not affect weight at 1- or 8-HD. The average weight of the fetuses at 20 days of gestation was unaffected by this treatment and no increase in prenatal death was noticed at any dose level, including the 64-HD dose.

TABLE 13.1. Effects of Intraperitoneal Administration of Ca- or Zn-DTPA to Pregnant Rats on Days 8 through 12 of Gestation.

Treatment	Dose (xHD) (a)	Maternal Weight Changes, grams (Mean $\pm$ s $\bar{x}$)		Fetal Weight ^(b) grams (Mean $\pm$ s $\bar{x}$)	Fetal Mortality (number dead) Total	Average Number of Embryos per Dam
		8-13th Day	8-20th Day			
0.9% NaCl	0	18 $\pm$ 3.5	96 $\pm$ 12	3.45 $\pm$ 0.06	4/92	11.5
Zn-DTPA	1	29 $\pm$ 1.3	113 $\pm$ 12	4.59 $\pm$ 0.10	0/49	12.2
	8	26 $\pm$ 3.7	100 $\pm$ 11	4.36 $\pm$ 0.10	0/65	10.5
	64	12 $\pm$ 3.9	92 $\pm$ 5.7	3.98 $\pm$ 0.06	0/66	11
Ca-DTPA	1	19 $\pm$ 3.5	97 $\pm$ 7.6	3.91 $\pm$ 0.03	0/52	13
	8	26 $\pm$ 5.0	111 $\pm$ 6.5	3.75 $\pm$ 0.04	4/67	13.4
	16	8 $\pm$ 1.9	26 $\pm$ 9.6	2.92 $\pm$ 0.16	44/51	12.8
	32	- 4 $\pm$ 3.4	8 $\pm$ 6.5		34/34	8.5
	48	-12 $\pm$ 5.2	- 2 $\pm$ 7.8		38/38	12.6
	64	- 4 $\pm$ 4.5	8 $\pm$ 5.9		105/105	13.1

(a) HD = human dose = 0.0287 mmol/kg.

(b) Based on total number of live fetuses.

Neither 1- nor 8-HD doses of calcium-DTPA affected the weight increase of pregnant animals, the average weight of the fetuses, or the incidence of prenatal death. After 64-HD, however, there was a decrease in maternal weight rather than the normal increase, and all embryos died. To better define this phenomenon in the intermediate dose range between 8- and 64-HD, additional animals were exposed to doses of 16-, 32-, 48-, and 64-HD, as noted in Table 13.1. The 32- and 48-HD levels resulted in a decrease in maternal weight and in death of 100% of the embryos. At 16-HD there was a slight diminution in the weight gain of the pregnant female and an 80% mortality among the embryos; the mean weight of the fetuses which survived until 20 days of gestation was greatly reduced.

It is apparent that the threshold for the embryocidal effect of calcium-DTPA lies between 8 and 16 times the human dose, since these doses result in 0 and 80% mortality, respectively. Most of the deaths which occurred took place at 9 to 10 days of gestation so that essentially all those to whom the dose is fatal died before the second or third injection of the DTPA was delivered. It is unlikely that the fetal deaths were due to local hypertonicity from the intraperitoneal injection, since the zinc-DTPA was as concentrated and likely exerted similar osmotic effects, but

was not embryocidal even at the 64-HD dose level. Since Zn-DTPA does not remove zinc as does the calcium salt, it is likely that these results are similar to those reported by Swenerton and Hurley, and may be attributed to a depletion of zinc from the mother and/or the embryo, although specific experiments would be required to prove this conclusively.

No consistent increases in the incidence of major visceral malformations were noted in any group, whether control or experimental, although one fetus from the 8-HD Ca-DTPA group was anophthalmic, one from the 8-HD Zn-DTPA group displayed hydrocephalus, and one from the 64-HD Zn-DTPA group had a marked thinness of the walls of the cardiac ventricles. The most striking change was an increase in hydronephrosis in surviving animals which received the higher doses of either zinc- or calcium-DTPA. In addition, the kidneys of a number of animals in these dose groups showed a peculiar honeycombed appearance on gross examination. The viscera of a number of the surviving animals exposed to either salt showed a darkened appearance compared to controls. Although histologic evaluation of these observations is not yet complete, the darkening appears to result from a finely granular golden pigment.

A few of the fetuses exposed to 8-HD Ca-DTPA showed minor skeletal abnormalities. These consisted primarily of slightly decreased ossification of the bones of the skull and of short bone spurs appearing below the 13th rib in a few litters. With Zn-DTPA at the 8- and 64-HD levels, two fetuses of one litter at each

level showed spurs lying below the 13th rib. Although not observed in the present control series, this has been a common finding among the controls of this strain of rat and is probably not significant. Thus, the teratologic effects of DTPA appear to be quite minor and sporadic, if, in fact, they do exist.

TOXICITY OF INHALED Ca-DTPA IN THE DOG

Investigators:

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Technical Assistance:

M. J. Hummel, G. D. Irwin, K. A. Allen,
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R. M. Madison

Anesthetized beagle dogs inhaled Ca-DTPA daily for 5 days through a tracheal catheter. No gross pathology specifically ascribable to the treatment was observed at necropsy 18 weeks later. Clinical chemistry, urinalysis, and bone marrow findings were essentially negative. While histopathologic and hematologic examination is still underway, gross observation and tests would indicate no toxic manifestations from aerosolized Ca-DTPA under the conditions of the experiment.

As a treatment for internally deposited radionuclides, it is important that calcium tri-sodium diethylenetriaminepentacetate (Ca-DTPA) be administered promptly following exposure. It is more conveniently administered by aerosol inhalation than by intravenous drip, the older method of administration. The inhalation route is being studied for possible side effects. Results of an acute experiment in rats and hamsters (Annual Report, 1973) suggested the occurrence of a transitory emphysema in the first 2 weeks following a series of

five daily exposures to aerosolized Ca-DTPA. The same experiment has been run in beagle dogs with monitoring of the blood, marrow, and urine for any clinical evidence of pathology.

Male beagle dogs, about 13 months of age, were anesthetized with intravenously administered sodium pentothal. A Foley catheter was then inserted by mouth into the trachea, and a respirator (either a Bird Mark 7 or Monaghan 225) was used to force aerosol at 20 psi maximum inspiration

pressure into the catheter. Minute volumes averaged 10 liters at 24-26 inhalations per minute. The Retec nebulizer operated on the inspiration cycle only and produced an aerosol of $2.4 \mu\text{m}$ AMAD, $\sigma_g \approx 2.2$. The dose of Ca-DTPA administered averaged 60 $\mu\text{mole/kg}$ body weight per exposure. Average dog weight was 11.7 kg. Aerosol retention in the lung, as measured by the amount of Ca-DTPA excreted in the urine, was about 27% of that nebulized.

Dogs were acclimated to metabolism cages for a minimum of 1 week before exposure to insure that representative pretreatment clinical chemistry and hematologic values were obtained. Blood, bone marrow, feces and urine samples were routinely taken for clinical examination prior to the first exposure; at 3 days, 1, 4, 8, and 18 weeks following the last exposure; and prior to sacrifice. Total urine and feces were collected during, and for 3 days to 1 week following exposure for DTPA analysis. Dogs were sacrificed by exsanguination following deep pentothal anesthesia in groups of six (four treated and two control dogs), at 1, 4, 8, and 18 weeks following their last aerosol treatment.

No gross lesions were observed at necropsy that could be attributed to the inhalation treatments. Histopathologic and detailed hematologic results are not yet available. Urinalyses showed the presence of phosphate crystals in both treated and control animals and generally alkaline urine following treatments. Glucose, casts, and blood were not present in the urine; the presence of WBCs, RBCs and epithelial cells did not correlate with DTPA exposure. There was occasional evidence of high urinary protein (300 mg/100ml) in both DTPA and control animals.

The bone marrow was essentially normal with no effects observed at 1 and 4 weeks following treatment, although a mild erythroid hyperplasia was seen in some controls and in some DTPA-treated dogs sacrificed after 8 and 18 weeks. Changes in serum parameters of experimental dogs following exposures were comparable to those observed in control animals. Final conclusions must await the completion of histopathologic and hematologic evaluations.

EFFECTS OF INGESTED, LOW LEVELS OF CHELATING AGENTS ON
THE EXCRETION OF WELL-FIXED ^{238}Pu FROM THE RAT

Investigator:

V. H. Smith

Technical Assistance:

M. H. Hummel and K. A. Allen

Ethylenediaminetetracetate (EDTA), diethylenetriaminepentacetate (DTPA), and oxytetracycline (OTC) were administered in the drinking water to rats intramuscularly injected 6 months previously with $^{238}\text{Pu}(\text{NO}_3)_4$. At dose levels roughly equivalent to those encountered by man in food, drink and medicament, EDTA, DTPA and OTC elicited no elevation in Pu excretion. The presence of these chelons would therefore not explain the occasional high urinary Pu output in untreated humans.

There are sometimes great variations in the daily excretion of Pu, which complicates the estimation of body burden using the Langham curves. The possibility that such high values in urinary plutonium output might be due to chelating agents in the diet or in medicaments was investigated in this experiment.

The daily Pu excretion was measured over a 3-week period in adult, female, Wistar-strain rats injected intramuscularly 6 months previously with $3.01 \mu\text{Ci } ^{238}\text{Pu}(\text{NO}_3)_4$. These rats were then exposed for 2 days to drinking water containing 1 or 100 ppm Na_2EDTA , 100 ppm $\text{Na}_3\text{H}_2\text{DTPA}$, or 633 ppm oxytetracycline HCl . This treatment was repeated for 2 days after a 5-day interval. Each treatment was given to five rats in single-rat metabolism cages and to five rats housed collectively.

The agents in the drinking water were well tolerated by the rats and consumption of water was similar to that measured without additives. The daily intake at the higher dose levels was about $0.16 \mu\text{mol/kg}$, for EDTA and DTPA, and $10.8 \mu\text{mol/kg}$ for OTC. Since oral absorption of EDTA and DTPA is less than 5%, the amounts reaching the blood stream would be more than 100 times less than the usual intravenously administered human treatment dose of $28.7 \mu\text{mol/kg}$. The antibiotic chelating agent was in oral form and the dose administered was comparable to that for man.

Plutonium excretion during and following the treatment periods was within the range of observations made during the preceding non-treatment periods. The chelon doses, while extremely low compared to the human therapeutic dose, were theoretically capable of chelating more than 300 X the quantity of Pu initially injected and were reasonably representative of what humans consume from certain foods and beverages.

INAPPLICABILITY OF HIGH PRESSURE SPRAY INJECTION
FOR CHELATE ADMINISTRATION

Investigator:

V. H. Smith

Technical Assistance:

M. J. Hummel

A high-pressure spray injector was tested for use in injecting chelating agents around radionuclides in wounds. It was difficult to employ because of the force required for proper injection, and it did not improve the effectiveness of the injected chelate in removing intramuscularly injected ^{238}Pu .

High-pressure spray injectors are capable of subcutaneous or intramuscular penetration, depending on the orifice used to direct the spray through the skin. Several investigators have shown that injection of the chelating agent, DTPA, around a wound can enhance the movement of Pu from the site. While excision is probably the first choice in treating an actinide-contaminated puncture wound, there may be occasions when this is impractical and spray injection of a chelating agent might be efficacious.

A foot-operated, Porton Jet Injector (purchased from SCHUCO, 110 Fifth Avenue, New York, NY) was tested by injecting various volumes of fluorescein solution through the skin of deeply anesthetized dogs just prior to their necropsy. The various injected regions were examined under ultraviolet light for measurement of the inundated tissue volumes. With the subcutaneous orifice in place, 0.5 ml of injectant penetrated the skin and spread asymetrically to cover areas of 8 to 20 cm². Delivery from the intramuscular orifice gave a narrow column of dye about 2 to 3 cm long which abruptly terminated and spread horizontally for 10 or more cm² onto the fascia surrounding the next bundle of muscle; most of the injectant was located at the lower fascia depot, with about one-fourth deposited along the injection column. Injectant volumes less than 0.5 ml gave lesser penetration; higher doses increased

spreading, but not penetration. Good penetration was markedly dependent upon good contact between the delivery orifice and the skin, which must be very tightly pressed against the underlying tissues. Efforts to inject close to bone or tendons, as in the paw of the dog, resulted in little penetration to bone or into tendons; the injection column was occasionally reflected back through the skin and onto the operator.

Rats were injected in the right rear ham with 1.2 $\mu\text{Ci } ^{238}\text{Pu}(\text{NO}_3)_4$ in 10 μl of 1 M HNO_3 . One hour later six rats were treated in the left ham with 0.2 ml of a 25% solution of Ca-DTPA (pH 7.3); six rats were injected at six sites surrounding the radionuclide injection site with a total of 0.2 ml of the same agent, using a 30-gauge needle; and six rats were treated with two 0.1-ml spray injections, attempting to spray through the skin at two locations above a line where the radionuclide was thought to be deposited. In untreated rats, killed 30 hours after radionuclide injection, 68% of the Pu was retained at the site of injection. Animals treated in the non-radionuclide-injected leg retained about 20% less than controls; those treated in the Pu-injected leg retained about 50% less than controls. There was no statistically valid difference between the spray-injected and needle-injected rats.

CHEMICAL RADIATION PROTECTION

The immediate objectives of this project are to determine the tissue distribution and intracellular localization of the protective agent WR2721, and to evaluate its efficacy in modulating damage to tissues acutely exposed to both internal and external radiation sources.

LOCALIZATION OF ^{35}S -WR2721 IN CELLS

Investigator:

J. C. Hampton

Technical Assistance:

R. R. Adee

Compound WR2721 protects mice exposed to 1500 R X-rays. Using the intestinal mucosa as a model of a rapidly proliferating cell population, the results of this work show that the compound has little effect on proliferating cells, but rather protects postmitotic cells.

Acute intestinal radiation death occurs in mice at 3-7 days after whole-body exposure to 1500-3000 R X-rays. Hemorrhage, edema and villus epithelial cell loss are major pathologic findings in the gut, inhibition of mitosis in the crypts being the cause of villus epithelial denudation. Cells remaining on the villus at the time of death are characterized by stunted microvilli, loss of ribosomes and of intracellular membranes and the cells are squamous rather than columnar in shape. Compound WR2721 (S-2(3-aminopropylamino) ethyl phosphorothioic acid hydrate) administered prior to exposure protects mice to 1500 R; the animals survive and show no signs of acute intestinal syndrome.

Mice received WR2721, 600 mg/g body wt, 10 min prior to exposure to 1500 R X-rays; irradiated, unprotected mice served as controls. Figure 14.1 shows a portion of ileum from a protected animal fixed 2 hrs after exposure. Crypt damage in the form of cell debris in crypt lumens is evident but villus structure is normal. Figure 14.2 shows ileum at 3 days postexposure in an unprotected animal. Villus structure has been

obliterated, patchy epithelial loss is present and dilated crypts reflect excessive cell death, despite occasional mitotic figures. Figure 14.3 shows the ileum of a protected mouse killed at 3 days postexposure. Crypt damage appears to be about the same as in the unprotected animals but villus structure is still intact, including the epithelial covering on the shortened villi. As shown in this figure, cells on the villus tip are rounded, pale and swollen; sometimes cells in this position contained many small, clear vacuoles. Cells showing similar features frequently occur on normal villi, evidence of impending extrusion into the gut lumen. Figure 14.4 shows the fine structure of an epithelial cell from a protected animal fixed 3 days postexposure. Microvilli and cell organelles appear normal at mid-villus levels. Cells at the villus tip show degenerative changes resembling those seen in Figure 14.5 but without lipid inclusions. Figure 14.5 is an epithelial cell from a 3-day postexposure animal showing the characteristic stunted microvilli, paucity of rough endoplasmic reticulum (ER), and accumulation of large lipid droplets. Comparison of Figure 14.4 and 14.5

X280

FIGURE 14.1. Protected, Irradiated Ileum 2 hrs Postexposure. Villi are normal; crypt (upper left) contains cell debris.

X280

FIGURE 14.3. Protected, Irradiated Ileum 3 Days Postexposure. Dilated crypts, shortened but covered villi.

X280

FIGURE 14.2. Unprotected, Irradiated Ileum 3 Days Postexposure. Villi obliterated, patchy denudation.

X14,000

FIGURE 14.4. Protected, Irradiated Ileum 3 Days Postexposure. Normal microvilli, ER and Mitochondria.

X17,500

FIGURE 14.5. Unprotected, Irradiated Ileum 3 Days Postexposure. Large lipid droplets, few ribosomes and intracellular membranes.

shows that cells protected with WR2721 retain intact organelles necessary for cell viability, and this probably accounts for the failure of protected animals to develop the acute radiation syndrome.

Within 1/2 hr after injection into mice, tritiated thymidine is incorporated into newly synthesized DNA by cells preparing to divide. This provides a marker upon which an estimate of cell killing in proliferating cell populations can be based for protected and unprotected animals and a judgment can be made as to whether WR2721 has any protective effect on dividing cells.

Labeling by ^3H -TdR in the crypts of both groups was about the same with regard to grain counts/nucleus and labeling index, Figures 14.6 and 14.7, so WR2721 probably provides little protection for the proliferative

cells. The protective effect seems to be on postmitotic cells so that they remain on the villus longer and retain sufficient functional capacity to allow time for the generative compartments to recover and to repopulate the villi.

FIGURE 14.6. Labeling Index for Jejunum of Treated (WR2721) and Untreated Mice Exposed to 1500 R X-Rays.

FIGURE 14.7. Labeling Index for Ileum of Treated (WR2721) and Untreated Mice Exposed to 1500 R X-Rays.

MECHANISMS OF RADIATION EFFECTS

This project is concerned with some of the basic biological and chemical indices that may aid in understanding the primary effect of the radiation insult and also serve in the diagnosis of early effects. Of particular interest are membrane structure and function, the role of virus in radionuclide-induced malignancy, and the role of radiation-induced free radicals in biological damage.

COORDINATE REGULATION OF THE EXOCELLULAR
PROTEASES IN *NEUROSPORA CRASSA*

Investigators:

B. Cohen, J. E. Morris and H. Drucker

Technical Assistance:

J. Hadley and L. E. Neil

When cells of *N. crassa* are starved for nitrogen, sulfur, or carbon and energy, and protein is sole source of these nutrilites, specific inhibition studies, electrophoresis and immunological assays suggest that at least two of the exocellular proteases synthesized (an alkaline and a neutral protease) in response to these conditions are the same, regardless of limiting nutrilitite. This implies a coordinate regulation of these enzymes.

In previous studies, we found that the fungus *N. crassa*, when grown on a medium where protein was sole carbon and energy source, would produce, as judged by pH optima and specific enzyme inhibitors, three exocellular proteolytic enzymes (acidic, neutral, and alkaline protease).

Other studies from this laboratory and the laboratory of Dr. George Marzluf had shown that *N. crassa* could grow on protein substrate as sole source of the nutrilites nitrogen or sulfur. These investigations, along with the studies on induction of protease with protein as sole carbon and energy source, posed a rather interesting question: were the same exocellular proteases produced under all three sets of nutrilitite conditions (protein as sole source of nitrogen and/or sulfur and/or carbon), or were different enzymes produced in response to these different forms of starvation? This problem can be restated in a more general sense: Can

synthesis of the same protein from a given structural gene be called forth under different conditions of cell growth; i.e., stage of differentiation, presence or absence in the environment of specific metabolites or toxins, etc.?

To answer this question, exponential phase cells of *Neurospora crassa* were placed in medium void of carbon, nitrogen, or sulfur, where the protein bovine serum albumin served as sole source of these nutrilites and as the inducer for protease biosynthesis. The culture filtrates were assayed as a function of time by the following techniques:

- i. Protease assays in the presence and absence of the alkaline protease inhibitor, phenylmethylsulfonylfluoride (PMSF).
- ii. Zone electrophoresis on cellulose strips, with analyses of the strips for protease activity.

- iii. Immunologic analyses using antisera against crystallized alkaline protease produced by carbon-starved cells.

It was determined that the kinetics of protease biosynthesis were approximately the same under the three sets of inducing conditions. Further, the amount of enzyme inhibitable at any time point in the induction of protease by PMSF appeared to be constant regardless of limiting nutrilitate.

Zone electrophoresis of samples from all three conditions of culture demonstrated two bands of protease activity (Figure 15.1). These bands at all time intervals were electrophoretically distinct from the neutral and alkaline proteases produced by cells grown on protein as sole carbon source.

The kinetics of accumulation of protein immunoreactive to antisera (prepared to the crystallized alkaline protease isolated from the culture filtrate of carbon-starved cells) were the same under all three conditions of protease induction. Further, Ochterlony analyses of filtrates suggested that only one protein reacting with identity to the crystallized alkaline protease was present in culture filtrates from the three different culture sets.

These data show clearly, by reasonable criteria, that the alkaline protease produced under conditions of carbon-, nitrogen-, or sulfur-starvation, with protein as sole

source of these nutrilitates, is the same. They show with somewhat less clarity that the neutral protease produced under the three conditions of culture is the same.

It could be assumed that for each of these enzymes there are three structural genes controlled by three operators responding to a lack of carbon, nitrogen, or sulfur, and the presence of protein, by the biosynthesis of protease. If this were the case, then lack of any two of the repressing compounds (i.e., C or N) or all three of the nutrilitates (C, N and S) should lead to a rate and extent of protease synthesis greater than the lack of any one of the nutrilitates and, perhaps, approaching additivity. In point of fact, this is not observed: The same amount of enzyme is made at approximately the same rate whether one, two, or three of these nutrilitates are missing from the culture medium.

These results suggest that there is one structural gene for the alkaline and neutral protease of *N. crassa*. The regulatory gene for these structural genes is responsive, in some fashion, to the lack of any one of three metabolites and thus appears to be multivalent. Induction of protease in *Neurospora crassa*, therefore, appears to be controlled both at the level of the cell membrane, perhaps by way of the model described in previous reports, and at the level of the nutrilitate concentration of the cell, regardless of the nature of the limiting nutrilitate.

FIGURE 15.1. Electrophoretic Separation of Proteases from Culture Filtrates of *Neurospora Crassa*. Contact prints of substrate-gel plates; zones of protease activity appear dark. The electrophoretic origin is indicated by an arrow; the polarity by + and -; the position of neutral protease by P1; and of alkaline protease by P2. These bands (P1 and P2) were identified as neutral and alkaline proteases by reaction with the inhibitors o-phenanthroline (for neutral protease) and PMSF (for alkaline protease).

- A. Time course of protease release by albumin-induced, nitrogen-starved mycelium.
- B. (i) Five-hour culture filtrate of albumin-induced, sulfur-starved mycelium.
(ii) Five-hour culture filtrate of albumin-induced, carbon-starved mycelium.
- C. (i) Culture filtrate of albumin-induced, carbon-starved mycelium stored for three days at 5°C. Neutral protease activity disappears.
(ii) Crystallized alkaline protease of *Neurospora crassa*.

INDUCTION OF EXOCELLULAR PROTEASES FROM *N. CRASSA*
UNDER CONDITIONS OF NITROGEN STARVATION

Investigators:

B. Cohen and H. Drucker

Technical Assistance:

L. E. Neil

Protease induction from *N. crassa* starved for nitrogen was found to be similar in requirements to protease induction for carbon-starved cells. The primary difference between the two processes is that the nitrogen-starved cells do not require added protease for induction; however, concentration effects for protein substrate, nature of amino acid repression of the process, and kinetics of protease synthesis were almost identical in the two systems.

Previous studies had delineated the requirements for induction of exocellular protease from the fungus *N. crassa* when grown on protein as sole carbon and energy source. In this report, we will discuss the requirements for protease biosynthesis when *N. crassa* is grown on protein as sole nitrogen source, and compare these conditions to those previously observed.

Rates of protease biosynthesis under conditions where protein is sole N-source are proportional to the concentration of protein inducer, with proportionality similar to that observed when protein serves as sole carbon source. Induction of enzyme under conditions of nitrogen starvation requires the presence of protein substrate throughout the time-course of induction. If protein substrate is removed by filtration at any time during induction and cells are resuspended in a medium void of protein and lacking nitrogen, no protease appears to be synthesized. Again, this is similar to the situation with protein inducer as carbon and energy source.

There is one important difference in requirements for the induction of exocellular protease under conditions

of nitrogen deprivation versus conditions of carbon deprivation: It appears that small quantities of added specific protease are not essential for protease induction when protein is sole N-source, whereas added enzyme has been shown to be a requirement for induction of protease from carbon-starved cells.

Protease biosynthesis, when protein is the carbon and energy source, is catabolite repressed by substrate amounts of a readily metabolized sugar such as glucose or sucrose. The induction of protease under conditions of nitrogen deprivation is repressed to this level when ammonium ion is added as N-source at concentrations as low as 1 mM. Ammonium ion thus appears to behave as a specific repressor. When the effects of common amino acids on protease induction were examined under conditions of N-deprivation, effects on protease biosynthesis were similar to those observed when the same amino acids were examined for effect on carbon-starved cells. The amino acids tryptophan, arginine, and methionine all effected concentration-dependent specific repressions of the induction, with kinetics of repression similar to those observed for carbon-starved cells.

In the previous report, it was demonstrated that the same proteases may be produced under conditions where protein serves as sole carbon and energy, nitrogen, and sulfur source for cell growth. The similarities in requirements for the biosynthesis of protease under conditions of carbon

and nitrogen deprivation are striking, as are the similarities in response to added amino acids (Table 15.1). It would appear that the fine genic "tuning" under both sets of conditions is the same. This, in turn, may imply the existence of common regulatory genes within the two systems.

TABLE 15.1. A Comparison of Protease Biosynthesis Under Conditions of Nitrogen-Deprivation versus Protease Biosynthesis Under Conditions of Carbon-Deprivation

	<u>Protein as Sole C-Source</u>	<u>Protein as Sole N-Source</u>
Requires specific protease to initiate induction	Yes	No
Effective protein concentration range	0.1-2%	0.1-2%
Requires protein throughout induction	Yes	Yes
Potential "Physiological" Repressors	Sugars (Glucose, Sucrose)	NH ₄
Nature of repression	Catabolic	Absolute
Amino acids affecting absolute repression	Tryptophan Arginine Methionine	Tryptophan Arginine Methionine
Enzymes produced	(Acid)? Neutral Alkaline	(Acid)? Neutral Alkaline

INDUCTION OF EXOCELLULAR PROTEASES FROM
N. CRASSA BY "CRUDE CELL" PREPARATIONS

Investigators:

B. Cohen and H. Drucker

Technical Assistance:

L. E. Neil

A fraction was isolated from cells of *N. crassa* by mild treatment which could effect induction under conditions of carbon or nitrogen starvation. Both the protein present in this fraction and a protease present at low levels in this material appear to play a role in the induction process.

In the past year, studies on induction of protease under conditions of nitrogen deprivation resulted, quite accidentally, in a series of observations which may have real bearing on the entire problem of protease regulation in *Neurospora*.

Briefly, we found that nitrogen-starved *Neurospora* mycelium, upon blending for five seconds in a Waring blender, and removal from the extracting medium (called "blendate" here) appeared to make exocellular protease in the absence of protein inducer (Figure 15.2A). The blendate itself, when added to nitrogen-starved cells of *N. crassa*, effected induction, with induction rate proportional to blendate concentration (Figure 15.2B). We wished to examine the mechanism of this induction.

We found that the inducing activity of blendate was thermolabile (Figure 15.2C) and that blendate contained a concentration of protein varying between 0.64 mg/ml and 1.55 mg/ml. This is a low concentration of protein inducer relative to bovine serum albumin as inducer, implying that the specific activity of inducing factor in blendate must be high relative to that of albumin. The material also contained protease in low level (0.03 PU/ml); again, a level of protease activity which, in carbon-starved cells, will no more than initiate induction of protease.

We were thus faced with two primary possibilities on what component of blendate might be involved in the induction of protease from nitrogen-starved cells: specific protein elements within the blendate, or specific proteolytic enzymes released from the cell surface during the mild extraction. It would appear that induction is effected primarily by the protease in blendate. The primary proteolytic activity in the material is an alkaline protease which appears to be totally inhibited by phenylmethylsulfonylfluoride or diisopropylfluorophosphate. Preincubation of blendate with these inhibitors results in almost total loss of the inducing ability of blendate.

We found that blendate would also effect induction from carbon-starved cells; again, using specific inhibitors, the inducing constituent appeared to be the alkaline protease in the blendate.

The dramatic decreases in inducing activity upon incubation of blendate with known inhibitors of proteolytic activity show in relatively clear fashion that specific protease is involved in the induction of protease from either nitrogen-starved or carbon-starved cells. It suggests that the apparent lack of requirement for specific protease in the induction of protease from cells grown on protein as sole nitrogen source may be more a matter of degree (level of activation protease needed) than a matter of kind.

FIGURE 15.2. Time Course of Accumulation of Extracellular Protease in Culture Filtrates of *Neurospora crassa*. Cells were grown for 12 hrs on medium consisting of 1% sucrose, and Vogel's salts, and then transferred to a medium of nitrogen-free Vogel's salts + 1% sucrose.

A. Induction of cells by blending and demonstration of activity of blendate.

(O—O) 1% albumin-induced, nitrogen-starved control

(Δ—Δ) Nitrogen-starved, blended 60 sec

(□—□) Nitrogen-starved, blended 60 sec, filtered, washed, and resuspended in fresh nitrogen-free Vogel's medium

B. Induction of nitrogen-starved cells by blendate.

(O—O) Undiluted blendate

(Δ—Δ) Blendate diluted 1 + 3 with nitrogen-free Vogel's medium

(□—□) Blendate diluted 1 + 1 with nitrogen-free Vogel's medium

(▼—▼) Blendate diluted 3 + 1 with nitrogen-free Vogel's medium.

C. Heat-inactivation of blendate.

(O—O) Induction of nitrogen-starved cells by untreated blendate

(Δ—Δ) Induction of blendate treated at 70° for 2.5 min.

ROLE OF THE CELL MEMBRANE IN VOLUME REGULATION

Investigator:

R. P. Schneider

Technical Assistance:

L. M. Butcher

The mechanism for the stimulation of Na^+ efflux in swollen cells was studied in red cells with increased and decreased cell volume. Results suggest that this response is the result of an increased affinity of the system for Na^+ caused by reduced inhibition by intracellular K^+ resulting from simple dilution during swelling, and a direct effect of membrane stretching on conformation of the transport system.

In the absence of active membrane transport, the concentration of non-diffusible ions and proteins in mammalian cells is higher than that in the external solution. This gradient, which results from the presence of internal protein, generates inward osmotic flow of water which ultimately ruptures the cells. The outward transport of sodium, catalyzed by the sodium-potassium transport system, corrects this tendency by maintaining the level of internal sodium below the equilibrium concentration.

Previous work in this laboratory has produced a model of regulation of Na^+ transport which proposes that intracellular K^+ levels act as a feedback (allosteric) inhibitor of Na^+ transport. Internal K^+ levels inhibit Na^+ efflux and cause the response of efflux to internal Na^+ concentration to be sigmoidal (i.e., dependent on the square of Na^+ concentration) rather than linear. Increased cell volume could elevate Na^+ efflux by either increasing affinity of the transport system for Na^+ or by generally increasing activity. It also seemed possible that this regulatory response could represent another facet of allosteric control.

In order to examine the nature of the response of Na^+ efflux to cell volume, the response of efflux to cell sodium was measured in both swollen and shrunken cells. Cells

containing a series of Na^+ concentrations and normal $\text{Na}^+ + \text{K}^+$ levels were loaded with $^{22}\text{Na}^+$ and suspended in media of either 240 mOsm, which swelled the cells, or 500 mOsm, which reduced cell volume. The response of active efflux to intracellular Na^+ concentration was then measured in the two sets of cells. In both MgCl_2 and NaCl -containing media, the concentration of internal Na^+ which resulted in half-maximal efflux ($S_{0.5}$) was lower in swollen than in shrunken cells (Figure 15.3). This indicates that the affinity of the Na^+ pump for Na^+ was increased in swollen cells. In a typical experiment, cells suspended in 240 mOsm MgCl_2 medium had a cell volume relative to normal (V_r) of 1.16 and a $S_{0.5}$ for Na^+ of 8.4 $\mu\text{moles/ml}$ cells. On the other hand, cells in 500 mOsm media with a V_r of 0.80 had a $S_{0.5}$ of 18 $\mu\text{moles/ml}$ cells. In NaCl -medium, swollen cells ($V_r = 1.11$) had a $S_{0.5}$ of 15 $\mu\text{moles/ml}$ cells and shrunken cells ($V_r = 0.78$) of 45 $\mu\text{moles/ml}$ cells.

If cell swelling had an allosteric effect on the transport system, i.e., relieving the inhibition by internal K^+ , one would expect the dependence on Na^+ to exhibit a lower $S_{0.5}$ and decreased sigmoidicity (Hill coefficient). In spite of the fact that significantly lower $S_{0.5}$ values were observed in swollen cells, no consistent correlation between the sigmoidicity of the Na^+ dependence and that

FIGURE 15.3. The Response of Sodium Efflux to Intracellular Sodium Concentration in Swollen and Shrunken Red Blood Cells. The osmotic strength of the dilute medium (open circles) was 68% of body fluids, and that of the concentrated medium (closed circles) was 140% of body fluids.

of cell volume was observed. The data from these flux experiments were not of sufficient precision to distinguish changes in Hill coefficient of less than 25%. Despite the uncertainty regarding the order of Na^+ dependence, the increased affinity observed in swollen cells suggests that the conformation of the system was altered. This could occur via dilution of the intracellular media and resulting relief from K^+ inhibition due to the decreased K^+ concentration. Alternatively, mechanical stretching of the membranes during swelling could alter the conformation.

In order to ascertain which of the above alternatives operate to regulate efflux, a series of cells with V_r ranging from 0.82 to 1.25 were prepared and efflux was compared at fixed concentrations of internal Na^+ . In this series, the internal K^+ concentration increased as the cell volume increased, rather than the normal behavior in which the K^+ concentrations are diluted by swelling. In spite of the increasing K^+ concentration, the efflux at internal Na^+ levels of 10 mM increased with cell volume. The increase in efflux in the swollen cells must have been caused by a more direct sensing of cell volume than of the internal ion

concentrations. The most likely explanation is a direct effect of membrane stretching on the conformation of the Na^+ pump. When the data from the experiment just described were plotted as Na^+ efflux in $\mu\text{moles ml}^{-1} \text{ hr}^{-1}$ versus V_r , the points formed a straight line with a slope of 1.3. A similar experiment was performed on normal cells, i.e., where the K^+ concentrations decreased with increased cell volume. A similar plot of these data described a straight line with a slope of 4.2. This suggests that the stimulation of Na^+ transport observed in swollen cells is the result of both a dilution of intracellular K^+ and stretching of the membrane.

During the course of measuring cell volume in various media we observed that cell volume increased as medium osmolarity decreased until V_r was about 1.2. Further reductions in medium strength then caused a decrease in cell volume, even when cell rupture had not occurred. Subsequent experiments in MgCl_2 media showed that when medium concentration was less than that required to cause a V_r of 1.2, the cell membranes developed a several-fold increase in the passive leak of Na^+ and K^+ out of the cell. When suspended in dilute NaCl media the net movements were K^+ out and Na^+ inward.

The response of erythrocytes to extreme swelling, then, is to immediately allow passive movements of Na^+ and K^+ to reduce the ionic (osmotic) gradient and reduce hemolysis; this movement causes a rapid net movement of Na^+ into the cell. The Na^+ transport system then pumps out the additional Na^+ , restoring the cell to normal volume over several hours in spite of the reduced medium osmolarity. When the swelling is less than that required to increase the leak flux, the volume is reduced by increased efflux of Na^+ . The increase occurs because intracellular K^+ has been slightly reduced and because stretching of the membrane increases the affinity of the pump for internal Na^+ .

This study of regulation of sodium transport by cell volume represents the final phase of the work on regulation of sodium transport. Effort formerly applied to this program has been directed to study of the surface of a leukemia virus.

THE ORIGIN OF ENVELOPE PROTEINS OF A LEUKEMIA VIRUS

Investigator:

R. P. Schneider

Technical Assistance:

L. M. Butcher

The roles of avian myeloblastosis virus (AMV) and host myeloblast cells in controlling the protein composition of virus envelope and host cell membrane are being studied by examining an ATPase enzyme in the virus and cells. New culture techniques for virus-producing myeloblasts have been developed.

Viruses which cause leukemia in animals (RNA tumor viruses) are enclosed in an envelope of lipid, protein and glycoprotein which resembles cell membranes. These viruses are formed by budding through the host cell membrane, which is a known source of the envelope lipid, but the contribution of the membrane to the envelope protein is presently unknown. Avian myeloblastosis virus (AMV) is found in the plasma of leukemic chickens in high concentration (2×10^{12} /ml), and its envelope and the host cell membrane contain an ATPase of high specific activity. These properties provide an opportunity for the biochemical study of the source of an envelope protein and its structural relationships in the outer structure of the virus. The ultimate goals of the study are to ascertain the role of the virus in controlling the structure of the host cell membrane and how this control relates to alterations of membranes observed in malignant cells. It seems likely that at least some of these changes may occur at loci of virus maturation or budding. Understanding of the control processes may aid in detection of infected (potentially malignant) cells. The expertise developed in this program should ultimately be relevant to virology and immunology studies of radionuclide-induced carcinogenesis.

Since much of the research planned for this study requires the in vitro culture of virus-producing myeloblasts, considerable effort was expended in the development of a convenient system for culturing these in gram quantities. Three culture media, two salts solutions and several concentrations of chicken serum were tested in spinner and shaking flasks. Initial attempts failed because chicken serum from commercial sources killed the cells. Serum collected in this laboratory, however, was found to be satisfactory. The system developed is: Eagle's Basal Medium and Earle's salts with added glucose, vitamins, glutamine, antibiotics and 20% chicken serum in a 5% CO₂ atmosphere. The cells grew with a doubling time of about 2 days at a density of 5×10^6 /ml and required fresh medium only every third day. Virus production was constant for 2 months until growth was stopped in order to perform other work.

Most of the work on the ATPase itself has been devoted to attempts to solubilize it in active form. This is a necessary step for planned isolation of the enzyme. Several attempts have been made to extract enzymes, including: sonication, high salt concentration, low ionic strength extraction, variations in pH, freeze-thaw, extended dialysis

against EDTA, butanol, glycerol, treatment with several proteases, bile salts, and several nonionic detergents. All of the above treatments either failed to separate the ATPase from the virus or destroyed most of the activity. A 5-min exposure of AMV to 1% concentrations of sodium cholate, or one of several non-ionic detergents, followed by a tenfold dilution, releases about 10 to 15% of the activity while inactivating from 60 to 80% of the total activity. The released ATPase activity floats on 20% sucrose after centrifugation for 1 hr at 100,000 x g. This suggests that the enzyme is bound to a lipid-containing particle, possibly the viral envelope.

Although treatment with hydrolytic enzymes did not successfully solubilize the enzyme, it did provide some clues concerning its structural relationships in the envelope. As shown in Figure 15.4, the proteases pronase and trypsin inactivated the

FIGURE 15.4. The Effect of 1 mg/ml Phospholipase C, 5 mg/ml Trypsin and 0.25 mg/ml Pronase on ATPase Activity of Avian Myeloblastosis Virus. Incubation with phospholipase C was conducted at 22°; the proteases were incubated at 37°C.

enzyme, demonstrating that at least a portion of the ATPase is exposed to the external surface of the envelope. Phospholipase C, which hydrolyzes lecithin to phosphocholine and diglyceride in the presence of Mg²⁺ or Ca²⁺, also inhibited the enzyme. This suggests that the enzyme requires phospholipid and that it is an integral part of the lipid bilayer portion of the envelope. Studies of the specific phospholipid requirement of the enzyme are presently under way. Previous studies have suggested that only surface glycoproteins of viruses are derived from the host cell and that all other proteins are virus-specific. Our findings contradict this view, since the ATPase occurs in AMV from myeloblasts but not fibroblasts; yet it seems to be an integral component of the envelope.

Mitochondrial and membrane ATPases are inhibited by sulfhydryl-reactive compounds such as organic mercurials and the alkylating agents, e.g. iodoacetamide, and protected by sulfhydryl-reducing reagents such as dithiothreitol and mercaptoethanol. Recent studies in this laboratory have shown that the ATPase of AMV, however, is inhibited by dithiothreitol, while iodoacetamide and p-chloromercuribenzoate partially protect against inhibition. This suggests that oxidized sulfhydryl groups are required for activity. The ATPase activity in cell membranes of virus-producing myeloblasts will be characterized and treated with enzymes, and properties and structural relationship of the ATPase in the membrane compared to those of the virus enzyme. The unique reactivity toward sulfhydryl reagents should enable us to identify this ATPase among the many found in the cells.

These studies should provide information concerning structural transitions of the budding loci which occur during formation of the envelope. Attempts to isolate the ATPase will resume as more is learned about its characteristics.

VIRAL AND MOLECULAR ASPECTS OF RADIATION (^{90}Sr)-INDUCED
LEUKEMIA IN MINIATURE SWINE

Investigators:

M. E. Frazier and J. E. Morris

Technical Assistance:

T. K. Andrews

Biochemical evidence of oncornavirus infection has been found in the spleens of irradiated miniature swine (both leukemic and nonleukemic) but not in spleens from normal unirradiated miniature swine. In the spleens from irradiated swine, we have demonstrated a true RNA-instructed DNA polymerase (RIDP). Using this RIDP we have generated tritium-labeled DNA probes of high specificity which can now be used in hybridization studies to examine the molecular pathology of radiation-induced leukemia of swine.

Last year we reported finding RNA-instructed DNA polymerase (RIDP) and high molecular weight (70S) RNA in a particulate fraction isolated from the plasma of leukemic swine. Similar activity could not be detected in plasma from either radiation-exposed or normal nonleukemic swine. We have now extended these findings to include detection of RIDP and 70S RNA from the spleens of leukemic miniature swine. Attempts to characterize and purify the preparations resulted in the sequential use of sucrose and glycerol gradients. A peak of RNase-sensitive DNA polymerase activity was found in the 1.14-1.18 g/ml region of the isopycnic gradient. However, while that region contained about 80% of the RIDP it also contained nearly 75% of the protein added to the gradient. These fractions were pooled and added to a velocity (glycerol) gradient. There was a clearing of the OD₂₈₀ absorbing material (contaminating proteins) from the portion of the gradient containing RIDP. Sixty-eight percent of the enzyme activity was found in the region containing 500-700S particles while only about 25% of the protein was still present. The resultant fraction was used for synthesis of the endogenous ^3H -DNA product.

The kinetic curve of DNA polymerase activity showed a peak of DNA synthesis at 15-30 min which was ~75% RNase-sensitive. There was a marked decrease in DNA synthesis with the omission of one of the deoxynucleoside triphosphates, indicating a heteropolymeric polydeoxynucleotide was being synthesized.

In Cs_2SO_4 gradients RNA bands at a density of 1.65 g/ml, DNA at 1.45 g/ml, and hybrids (RNA:DNA) at an intermediate density. When early DNA products (5-min reactions) of the endogenous RIDP reaction were subjected to analysis in Cs_2SO_4 there were peaks of acid-precipitable radioactivity in the RNA and hybrid region of the gradient. These shifted to the DNA region of the glycerol gradient under conditions that hydrolyze RNA but not DNA (alkali or ribonuclease) or that disrupt hydrogen bonds but not covalent bonds (heat or alkali). These results proved that an RNA:DNA hybrid was formed during the reaction and that the product of the reaction was DNA.

In order to prove that the RNA portion of the hybrid was a 70S molecule these same early reaction products were analyzed in velocity gradients

against RNA molecules of known size. Considerable acid-precipitable radioactivity was consistently found in the 60-70S region of these gradients. This activity was easily shifted to small sedimenting particles by treatment with ribonuclease, which destroyed the RNA component of the hybrid.

Definitive proof of the presence of RIDP rests on the demonstration that the DNA product of the reaction is complementary to heteropolymeric regions of the RNA template. In our system we checked the ^3H -DNA product by hybridization against RNA isolated from: C-type particles produced in porcine tissue cultures originating from leukemic swine, avian myeloblastosis virus (AMV), synthetic polyadenylic acid, and Rauscher leukemia virus (RLV). The DNA probe appeared to be specific for the porcine DNA. Eighty percent of the ^3H -DNA hybridized to the porcine RNA, 25% to the RLV-RNA, and less than 3% to the Poly A and AMV RNA. The specificity of this reaction, including both the extent of hybridization and high average temperature of melting ($\sim 84^\circ\text{C}$ with porcine RNA), indicate the probe

annealed specifically with the heteropolymeric regions of the porcine C-type virus RNA.

Synthesis of RNA-DNA hybrids by an endogenous reaction has also been found in metaplastic spleens from irradiated swine. However, there is considerably less synthesis as a result of this RIDP-like activity and we have not yet made sufficient probe from irradiated (nonleukemic) swine for hybridization with the porcine type C virus RNA to determine absolutely that heteropolymeric regions of the RNA are being copied.

Immunological analysis of the porcine C-type virus is continuing. Antisera prepared against the porcine isolates have demonstrable cross-reactivity with Rauscher leukemia virus; RLV antisera likewise show some cross-reaction with the porcine isolates.

Transmission studies, with purified virus isolated from cell cultures and spleens from leukemic swine, have been initiated in neonatal swine.

FREE-RADICAL DAMAGE TO MAMMALIAN ERYTHROCYTES

Investigators:

D. R. Kalkwarf and W. D. Felix

Free radicals derived from irradiated crystals of L-serine hemolyzed canine erythrocytes, while radicals derived from irradiated glucose did not. These results further confirm the hypothesis that radicals capable of reducing flavin mononucleotide are also capable of hemolyzing erythrocytes.

In previous studies we demonstrated the hemolytic effect of free radicals on porcine erythrocytes in aqueous suspension. The radicals

were derived from gamma-irradiated crystals of pure biochemicals and were released into the suspensions upon dissolution. Results indicated that

only radicals capable of chemically reducing flavin mononucleotide caused hemolysis. We have now extended the tests of this correlation to other radiation-induced radicals and to canine erythrocytes. In addition, initial erythrocyte concentrations were increased to more nearly approach the value in whole blood.

Erythrocyte suspensions were prepared from freshly drawn canine blood. The red cells were washed free of plasma and resuspended in isotonic phosphate solution at pH 7.3 (1.06 g NaH_2PO_4 and 4.37 g Na_2HPO_4 per liter).

Irradiated crystals of glucose and L-serine were used as the source of the free radicals. The concentrations of radicals in the crystals were determined by electron spin resonance spectrometry. Weighed samples of these preparations were rapidly dissolved in samples of the cell suspensions. Since the added crystals made these suspensions slightly hypertonic, exposure was limited to 1 min. This was considered to be ample time for the free radicals to reach the erythrocyte surfaces. Each cell suspension was then centrifuged, the supernatant solution discarded, and the cells were resuspended and gently agitated in the same phosphate buffer at 37°C. Hemolysis was followed as a function of time by periodically removing small samples of the suspensions, centrifuging and measuring the optical absorbance of the supernatant solution at 412 nm. The extent of hemolysis caused by nonirradiated crystals was measured in the same way, and hemolysis, due to nonradical irradiation products, was evaluated by adding the cell suspensions to solutions of predissolved irradiated crystals.

Free radicals derived from irradiated crystals of L-serine increased the rate of erythrocyte hemolysis beyond that caused by either nonirradiated crystals or nonradical radiolysis products. As in previous studies of this type, samples attained

relatively constant levels of hemolysis 10 hours after exposure. At that time, samples initially at 0.1 of the packed-cell concentration and exposed to 1.0×10^{17} radicals per milliliter were 36% hemolyzed. Control samples exposed to equal quantities of nonirradiated crystals or nonradical radiolysis products were only 2% or 14% hemolyzed, respectively. When the erythrocyte concentration was decreased below 0.05 of the packed-cell concentration, no hemolysis in excess of control values was observed.

No hemolysis could be attributed to free radicals derived from irradiated glucose. Significant hemolysis occurred; but in each case, it was either equaled or exceeded by hemolysis in the control samples.

These results support the correlation between the abilities of radicals to both reduce flavin mononucleotide and to hemolyze mammalian erythrocytes. Serine is the only amino acid whose radiation-induced radicals have been found to reduce flavin mononucleotide while glucose and fructose are the only sugars whose derived radicals have not been found to do so.

The increased effectiveness of serine radicals at higher erythrocyte concentrations is clearly consistent with a competitive mechanism in which radicals either attack the red cells or recombine with each other. A more-detailed demonstration of a similar effect was obtained by allowing radicals derived from irradiated serine crystals to bleach p-nitrosodimethylaniline (PNDA) in the same type of competitive reaction. The number of molecules bleached per added radical increased linearly from 0.025 at 5×10^{-5} M PNDA to 0.35 at 1×10^{-3} M PNDA. Since, in the experiments described above, erythrocyte suspensions were used at values up to only 0.1 of the packed-cell concentrations, increased hemolytic effectiveness of serine radicals on erythrocytes is predicted at whole-blood concentrations.

FREE-RADICAL INACTIVATION OF MUSCLE ALDOLASE

Investigators:

W. D. Felix^(a) and D. R. Kalkwarf^(a)

Rabbit muscle aldolase has been shown to be deactivated by addition of irradiated crystals of various sugars and amino acids. Inactivation observed immediately upon dissolution is ascribed to reaction with free radicals, whereas post-dissolution inactivation is ascribed to acid-catalyzed reaction with nonradical radiolysis products.

Previous studies have shown the effectiveness of materials released during dissolution of irradiated biochemical crystals in disrupting enzymatic functions. Free radicals which are formed by inactivation of crystalline biochemicals are proposed as probable deactivation agents. Further details of the inactivation process are examined here.

Radicals were formed within crystalline biochemicals by gamma irradiation. Radical concentrations in the solid were determined by electron spin resonance techniques. Rabbit muscle aldolase is a single-substrate enzyme which catalyzes the conversion of fructose-1, 6-diphosphate to an acetone and an aldehyde. Enzyme activity was measured as a function of the production rate of the latter compounds.

Predissolution of the irradiated crystals followed by addition of aldolase gave a measurement of the reactivity of the radiolysis products with the enzyme. Following dissolution of the irradiated crystals the concentration of free radicals in solution was below the detection level of ESR (within minutes after dissolution).

Aldolase activity following addition of irradiated crystals directly to an aldolase suspension gave a measure of deactivation caused by the extremely short-lived transient compounds plus that caused by the stable radiolysis products. The short-lived transients were assumed to be free radicals. Both natural and neutral pH solutions were used. The natural pH which resulted after addition of irradiated crystals was usually between 4 and 5 at the concentration levels used. Neutral pH was maintained in a phosphate buffer solution with an osmolarity equivalent to isotonic saline.

At the pH following addition of compounds such as galactose, fructose, glucose, lactose, or glycine to unbuffered solutions (natural pH), deactivation was evident from both radical reaction and from nonradical radiolysis product reaction. Enzymatic activity decreased as a function of time following initial exposure indicative of extensive, continuing reaction with the radiolysis products at natural (or acidic) pH.

At neutral pH in buffered solution, however, reaction with stable radiolysis products was barely detectable,

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whereas reaction with short-lived transient products resulting from direct addition of irradiated crystals to the aldolase solution was of primary importance.

Methylglyoxal, gluconic acid, glucuronic acid, other aldehydes, and unidentified peroxides which are expected products of sugar radiolysis, may be responsible for enzyme inactivation at low pH. However, the nature of the primary inactivation agent among the stable radiolysis products has yet to be identified. Aldolase is known to be irreversibly denatured at pH values less than 4.5. The deactivation observed at natural pH was possibly a function of acid catalysis reactions with nonradical products.

Inactivation seen on initial addition of the irradiated crystals, observed at both natural and neutral pH

levels, is apparently justifiably ascribed to reaction of free radicals formed within the irradiated biochemical crystal. As with the stable radiolysis products, the nature of the active free radical in solution has not been completely established. With ESR studies, the nature of the solid radicals in most solid materials can be readily established. Limitations of present ESR techniques, and experimental problems in delivery of solution to an ESR cavity within milliseconds of crystal dissolution, have precluded the detection and identification of these radicals in solution. The time frame within which we must work in attempting to detect these active agents is a direct indication of extreme reactivity and is commensurate with the expected short lifetime of radicals in solution.

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BIOLOGY DEPARTMENT PUBLICATIONS

Reprints may be requested from the author at the above address, except for those articles marked with an asterisk, which are no longer available.

- *Bair, W. J. 1974. Consideration of Reactor Accident Exposure Guides for Plutonium, Pl. In: Conference on Fast Reactor Safety (CONF-740401). NTIS, Springfield, VA.

Bair, W. J., D. H. Willard, I. C. Nelson, and A. C. Case. 1974. Comparative Distribution and Excretion of ^{237}Pu and ^{239}Pu Nitrates in Beagle Dogs. Health Phys. 27, 392-395.

Bair, W. J., C. R. Richmond, and B. W. Wachholz. 1974. A Radiobiological Assessment of the Spatial Distribution of Radiation Dose from Inhaled Plutonium, WASH-1320, United States Atomic Energy Commission, Washington, D.C., 47 pp.

- *Bair, W. J. 1974. Toxicology of Plutonium, pp. 255-315. In: Vol. 4 of Advances in Radiation Biology, J. T. Lett, H. Adler, and M. Zelle (eds.). Academic Press, Inc. New York, NY.

- *Bair, W. J. 1974. The Effects on Populations of Exposure to Low Levels of Ionizing Radiation. Health Phys. 26, 588-591. (book review).

Craig, D. K., W. C. Cannon, R. E. Filipy, D. D. Mahlum, and V. H. Smith. 1974. SNS Source Term Evaluation Program - Annual Report FY 1974, BNWL-1856, Battelle-Northwest, Richland, WA.

- *Craig, D. K. 1974. Methods of Introducing the Fibres into Experimental Animals: Inhalation Experiments, pp. 65-73. In: Fibres for Biological Experiments, P. V. Pelnar (ed.). Institute for Occupational and Environmental Health, Montreal, Quebec, Canada.

Decker, J. R. and M. F. Gillis. 1973. A Completely Implantable Three Channel Temperature Biotelemetry System. ISA Trans. 12 (2), 97-102.

- *Drucker, H. 1974. The Effects of Sugars on the Induction of Exocellular Proteases from Neurospora crassa, p. 187. In: Abstracts of the 74th Annual Meeting, American Society for Microbiology, Washington, D.C. (abstract)

- *Drucker, H., R. E. Wildung, T. R. Garland, and M. P. Fujihara. 1973. Influence of Seventeen Metals on Microbial Population and Metabolism in Soil, p. 90. In: Agronomy Abstracts, 1973 Annual Meeting, American Society of Agronomy, Madison, WI. (abstract)

- *Gomez, L. S., R. W. Bistline, G. E. Dagle, J. L. Lebel, and R. L. Watters. 1974. Effect of Delayed Lymphadenectomy on $^{239}\text{PuO}_2$ Translocation in Dogs. Health Phys. 27, 213-216.

- *Gruber, H. E. and B. Rosario. 1974. Variation in Eyespot Ultrastructure in Chlamydomonas Rienhardi (ac-31). J. Cell Sci. 15, 481-494.

- *Hampton, J. C. and P. H. Grundy. 1974. The Fine Structure of Intestinal Epithelial Cells of Mice Irradiated after Treatment with WR2721, pp. 556-557. In: Proceedings of the 8th International Congress of Electron Microscopy, Canberra, Australia. (abstract)

- *Hungate, F. P., W. R. Riemath, L. R. Bunnell, and M. F. Gillis. 1974. Chronic Irradiation of Blood with a Portable Irradiator. Radiat. Res. 59, 169. (abstract)

- *Jaton, J., D. G. Braun, A. D. Strosberg, E. Haber, and J. E. Morris. 1973. Restricted Rabbit Antibodies: Amino Acid Sequences of Rabbit H Chains of Allotype a1, a2, and a3 in the Region 80 to 94. J. of Immunol. 111, 1838-1843.

- *Klepper, B. L. and D. K. Craig. 1974. Deposition of Airborne Particulates onto Plant Leaves. Plant Physiol. 53 (Suppl. 30).
- *McClanahan, B. J., H. A. Ragan, and D. D. Mahlum. 1974. Plutonium Metabolism in Newborn and Weanling Pigs. Radiat. Res. 59, 171. (abstract)
- Mahlum, D. D. and M. R. Sikov. 1974. Distribution and Toxicity of Monomeric and Polymeric ^{239}Pu in Immature and Adult Rats. Radiat. Res. 60, 75-88.
- *Mahlum, D. D. and M. R. Sikov. 1974. Influence of Age on the Late Effects of Plutonium-239 in Rats. Radiat. Res. 59, 171. (abstract)
- *Park, J. F., D. L. Catt, D. K. Craig, R. J. Olson, and V. H. Smith. 1974. Solubility Changes of ^{238}Pu Oxide in Water Suspension and Effect on Biological Behavior after Inhalation by Beagle Dogs, pp. 719-724. In: Third International Congress of the International Radiation Protection Association, Vol. 1, W. S. Snyder (ed.) (CONF-730907). NTIS, Springfield, VA.
- *Park, J. F. 1974. Late Effects of Inhaled Plutonium in Dogs. Radiat. Res. 59, 161. (abstract)
- *Ragan, H. A., B. J. McClanahan, and P. L. Hackett. Blood and Bone Marrow Effects of $^{253}\text{Einsteinium}$ in Miniature Swine. Radiat. Res. 59, 151. (abstract)
- Sanders, C. L. and E. H. Groff. 1974. Bibliography: Effects of Radiations on the Lung with References Through 1972, BNWL-1870, Battelle-Northwest, Richland, WA.
- *Sanders, C. L., D. K. Craig, and V. H. Smith. 1974. Early Fate of Inhaled $^{238}\text{PuO}_2$, $^{239}\text{PuO}_2$ and ^{244}CmO in Rodents. Radiat. Res. 59, 257. (abstract)
- Sanders, C. L., W. C. Cannon, G. J. Powers, and R. R. Ade. 1974. Toxicology of Inhaled Low-Fired and High-Fired Beryllium Oxides in the Rat and Hamster, BNWL-1818, Battelle-Northwest, Richland, WA.
- Sanders, C. L. 1973. Carcinogenicity of Inhaled Plutonium-238 in the Rat. Radiat. Res. 56, 540-553.
- *Schneider, R. P. 1973. Evidence for Allosteric Regulation of Na^+ Transport by K^+ . Fed. Proc. 32, No. 2372. (abstract)
- Schneider, R. P. 1974. Regulation of Sodium Transport in Erythrocytes. Arch. Biochem. Biophys. 160, 552-560.
- *Sikov, M. R. and D. D. Mahlum. 1974. Influence of Age on ^{239}Pu Distribution, Dosimetry, and Effects. Trans. Amer. Nuclear Soc. 18, 389. (abstract)
- *Sikov, M. R. and D. D. Mahlum. 1974. Carcinogenicity of ^{131}I Relative to Age at Exposure. Radiat. Res. 59, 102. (abstract)
- *Sikov, M. R. 1974. Biological Evaluations of IUD Materials. Med Instrumen. 8, 169. (abstract)
- *Smith, V. H. 1974. The Biological Disposition of $\text{Es}(\text{NO}_3)_3$ in Rats after Intravenous, Intramuscular and subcutaneous administration, pp. 725-730. In: Third International Congress of the International Radiation Protection Association, Vol. 1, W. S. Snyder (ed.). (CONF-730907). NTIS, Springfield, VA.
- Sullivan, M. F., J. L. Beamer, and T. D. Mahony. 1974. Effects of Intracavitary Irradiation by Californium-252, pp. 299-310. In: Biological Effects of Neutron Irradiation. IAEA, Vienna.
- *Thompson, R. C. 1974. Effects of Plutonium in Animals. Trans. Amer. Nuclear Soc. 18, 389. (abstract).
- *Thompson, R. C. 1974. Summary and Speculative Interpretation Relative to Exposure Limits. Radiat. Res. 59, 162. (abstract).
- *Vogt, J. C. Histogenesis of Fetal Rat Lung and Liver after X-Irradiation in Organ Culture and In Utero, BNWL-5109, Battelle-Northwest, Richland, WA.

*Wildung, R. E., T. R. Garland, and H. Drucker. 1973. The Potential Role of the Soil Microbiota in Increasing Plutonium Solubility in Soil and Plutonium Uptake by Plants. Agronomy Abstracts, 1973 Annual Meeting, American Society of Agronomy, Madison, WI.

Wolf, E. G. and R. J. Olson. 1974. A Computer Program Used to Estimate Primary Productivity from pH and Carbon Dioxide Data Employing the Upstream-Downstream Method, BNWL-1828, Battelle-Northwest, Richland, WA.

PRESENTATIONS

Adee, R. R. Electron Microscopic X-Ray Energy Spectrometry - The State-of-the-Art. Seminar, Biology Dept., Battelle-Northwest, Richland, WA, February 19.

Bair, W. J. Considerations of Reactor Accident Exposure Guides for Plutonium. American Nuclear Society Meeting, Los Angeles, CA. April 2-4.

Bair, W. J. Considerations in Assessing the Potential Harm to Populations Exposed to Low Levels of Plutonium in Air. IAEA Seminar on Radiological Safety Evaluations to Population Doses and Application of Radiological Safety Standards to Man and the Environment, Portoroz, Yugoslavia, May 20-24.

Bair, W. J. Potential Impact of the Nuclear Power Controversy on Medical-Dental Uses of Ionizing Radiation. Nuclear Medicine Seminar, Richland, WA, June 16-19.

Bair, W. J. Carcinogenicity of Inhaled Radionuclides. Health Physics Society Meeting, Houston, TX, July 7-11.

Bair, W. J. Biomedical Aspects of Plutonium. Nuclear Power Special Series of Discussions, Institute of Technology, Continuing Education and Extension, University of Minnesota, Minneapolis, MN, December 4.

Bair, W. J. The Biological Effects of Transuranium Elements in Experimental Animals. Environmental Protection Agency Hearings on Plutonium and the Transplutonium Elements, Washington, D.C., December 10, 1974.

Ballou, J. E., G. E. Dagle, and W. G. Morrow. Long-Term Effects of Intratracheally Instilled $^{253}\text{EsCl}_3$ in Rats. Health Physics Society Meeting, Houston, TX, July 7-11.

Beamer, J. L. Practical Laboratory Applications of the Zeiss Tessovar Macrozoom Camera. Washington State University, Biological Photographic Association Meeting, Pullman, WA, May 15.

Clary, A. J. Miniature Swine in Biomedical Research. Annual District 8 Convention of the American Association for Laboratory Animal Science, Portland, OR, June 5-7.

Cohen, B., J. Morris, K. Miyata, and H. Drucker. Exocellular Protease Biosynthesis in Neurospora crassa Under Conditions of Carbon, Nitrogen, or Sulfur Starvation. Neurospora Conference, Monterey, CA, March 27.

Cohen, B. Regulation of Extracellular Proteases in Aspergillus nidulans and Neurospora crassa. Seminar, Biology Department, Battelle-Northwest, Richland, WA, May 28.

Craig, D. K. and R. L. Buschbom. The Alveolar Deposition of Inhaled Plutonium Aerosols in Rodents. American Industrial Hygiene Conference, Miami, FL, May 12-17.

Craig, D. K. and E. L. Klepper. The Design and Calibration of a Low Speed Windtunnel for Studying the Foliar Deposition and Uptake of Aerosols. American Industrial Hygiene Association Conference, Miami, FL, May 12-17.

Craig, D. K., D. D. Mahlum, and E. L. Klepper. The Relative Quantity of Airborne Plutonium Deposited in the Respiratory Tract and on the Skin of Rats. Health Physics Society Meeting, Houston, TX, July 7-11.

Craig, D. K., E. L. Klepper, and R. L. Buschbom. Deposition of Aerosols of Various Plutonium Compounds onto Plant Foliage at Very Low Wind Velocities. Symposium on Atmospheric-Surface Exchange of Particulate and Gaseous Pollutants, Richland, WA, September 4-6 and 7th Aerosol Technology Meeting, IIT Research Institute, Chicago, IL, October 10-11.

Dagle, G. E. and J. F. Park. Plutonium-induced Lymphadenitis in Beagles. 14th Hanford Biology Symposium "Radiation and the Lymphatic System", Richland, WA, September 30-October 2.

Dagle, G. E. and J. F. Park. Pulmonary Pathology Induced by Plutonium in Beagles. Plmonary Pathology Session of the Annual Meeting of the American College of Veterinary Pathologists, Chicago, IL, December 6.

Drucker, H., K. Miyata, B. Cohen, and J. E. Morris. Carbon Starvation and Exocellular Protease Biosynthesis in *Neurospora crassa*. *Neurospora Conference*, Monterey, CA, March 27.

Drucker, H. The Effects of Sugars on the Induction of Exocellular Proteases from *Neurospora crassa*. American Society of Microbiology, Chicago, IL, May 12-17.

Drucker, H. Regulation of the Synthesis of Exocellular Enzymes. Botany Department, Washington State University, Pullman, WA, October 25.

Filipy, R. E., B. O. Stuart, R. F. Palmer, H. A. Ragan, and P. L. Hackett. The Effects of Inhaled Uranium Mine Air Contaminants in Beagle Dogs. Symposium on Experimental Lung Cancer. Carcinogenesis and Bioassays. Battelle Seattle Research Center, Seattle, WA, June 23-26.

Frazier, M. E., J. E. Lund, and R. H. Busch. In Vitro Interactions of Lymphocytes and Cultured Cells from Beagles with Pu-Induced Bone Tumors. 14th Hanford Biology Symposium "Radiation and the Lymphatic System", Richland, WA, September 30-October 2, 1974.

Frazier, M. E. Radiation (^{90}Sr) Induced Leukemia in Miniature Swine. Seminar, University of Utah Medical School, Salt Lake City, UT, November 6, 1974.

Hampton, J. C. and P. H. Grundy. The Fine Structure of Intestinal Epithelial Cells of Mice Irradiated After Treatment with WR2721. 8th International Congress of Electron Microscopy, Canberra, Australia, August 25-31.

Horstman, V. G. Radiation Biology at Hanford. Engineering Wives Club, Richland, WA, September 9 and Noon Lions Club, Richland, WA, November 14.

Howard, R. F. Biology at Hanford. Kiona-Benton City School, Benton City, WA, December 5, 1974.

Hungate, F. P., W. R. Riernath, L. R. Bunnell, and M. F. Gillis. Chronic Irradiation of Blood with a Portable Irradiator. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Hungate, F. P., W. F. Riernath, L. R. Bunnell, and M. F. Gillis. A New Irradiator for Chronic Irradiation of Blood. 14th Hanford Biology Symposium "Radiation and the Lymphatic System", Richland, WA, September 30-October 2.

Klepper, E. L. and D. K. Craig. Deposition of Airborne Particulates onto Plant Leaves. Annual Meeting of the American Society of Plant Physiologists, Ithaca, NY, June.

Loscutoff, S. M. Carbon Monoxide and Low Oxygen Effects on Peripheral Circulation of Dogs. Seminar, Biology Department, Battelle-Northwest, Richland, WA, September 24.

Lund, J. E. Mechanism of Cyclic Hematopoiesis. Meeting of the Federated American Societies for Experimental Biology, Atlantic City, NJ, April 8.

Mahlum, D. D. Influence of Age on ^{239}Pu Distribution, Dosimetry, and Effects. American Nuclear Society Meeting, Philadelphia, PA, June 23-27.

Mahlum, D. D. and M. R. Sikov. Influence of Age on the Late Effects of Plutonium-239 in Rats. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

McClanahan, B. J., H. A. Ragan, and D. D. Mahlum. Plutonium Metabolism in Newborn and Weanling Pigs. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Olson, R. J. Processing and Analysis of Data from a Long-Term Study of Free Ranging Pocket Mice. Seminar, Oak Ridge National Laboratory, Oak Ridge, TN, July 11.

Park, J. F. Late Effects of Inhaled Plutonium in Dogs. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Park, J. F., R. J. Olson, W. J. Bair, and G. E. Dagle. Accumulation and Retention of Inhaled $^{239}\text{PuO}_2$ in the Lymphatic System of Beagles. 14th Hanford Biology Symposium "Radiation and the Lymphatic System", Richland, WA, September 30-October 2.

Park, J. F., J. E. Lund, H. A. Ragan, P. L. Hackett, and M. E. Frazier. Inhaled $^{238}\text{PuO}_2$ -Induced Bone Tumors in Dogs. Symposium on Malignant Bone Tumors, Dusseldorf, Germany, October 15-18.

Ragan, H. A., B. J. McClanahan, and P. L. Hackett. Blood and Bone Marrow Effects of ^{253}Es in Miniature Swine. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Ragan, H. A. Strontium-90 Induced Hematodegenerative and Hematoproliferative Disorders in Miniature Swine. Annual Meeting of the American Society of Veterinary Clinical Pathologists, Denver, CO, July 21.

Ragan, H. A., J. F. Park, and R. J. Olson. Lymphocytopenia Induced in Beagles in Inhalation of ^{239}Pu . 14th Hanford Biology Symposium "Radiation and the Lymphatic System", Richland, WA, September 30-October 2.

Sanders, C. L. and G. E. Dagle. Studies of Pulmonary Carcinogenesis in Rodents Following Inhalation of Transuranic Compounds. Symposium on "Experimental Lung Cancer. Carcinogenesis and Bioassays", Battelle Seattle Research Center, Seattle, WA, June 23-26.

Sanders, C. L., D. K. Craig, and V. H. Smith. Early Fate of Inhaled $^{238}\text{PuO}_2$, $^{239}\text{PuO}_2$, and $^{244}\text{CmO}_x$ in Rodents. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Sanders, C. L. Effects of Transuranics in Pulmonary Lymph Nodes of Rodents. 14th Hanford Biology Symposium "Radiation and the Lymphatic System", Richland, WA, September 30-October 2.

Schaffner, T. The Bursa of Fabricius and Uptake of Cloacal Particulates. Seminar, Biology Department, Battelle-Northwest, Richland, WA, February 5.

Schneider, M. J., C. D. Becker, D. H. Fickeisen, T. O. Thatcher, E. G. Wolf, and E. L. Hunt. The Aquatic Physiology of Thermal and Chemical Discharges. Symposium on Physical and Biological Effects on the Environment of Cooling Systems and Thermal Discharges at Nuclear Power Stations, Oslo, Norway, August 26-30.

Sikov, M. R. and D. D. Mahlum. Influence of Age on ^{239}Pu Distribution, Dosimetry, and Effects. American Nuclear Society Special Session on Dosimetry for Internal Emitters, Philadelphia, PA, June 23-26.

Sikov, M. R. and D. D. Mahlum. Carcinogenicity of Iodine-131 Relative to Age at Exposure. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Sikov, M. R. The Use and Biological Effects of Ultrasound. Food and Drug Administration Panel on Ob-Gyn Devices, Washington, D.C., December 9.

Stuart, B. O., R. E. Filipy, R. F. Palmer, H. A. Ragan, and P. L. Hackett. The Biological Effects of Inhaled Radon Daughters With Uranium Ore and Cigarette Smoking in Beagles. Health Physics Society Meeting, Houston, TX, July 7-11.

Sullivan, M. F. Neutrons in Radiotherapy. Seminar, University of Washington, Seattle, WA, March 6.

Sullivan, M. F. Sustained Release of Narcotic Antagonists. Conference on Drug Therapy and Novel Delivery Systems, Battelle Seattle Research Center, Seattle, WA, April 29-May 1.

Thompson, R. C. Effects of Plutonium in Animals. Meeting of the Advisory Committee on Reactor Safeguards of the United States Atomic Energy Commission, Los Alamos, NM, January 4-5 and American Nuclear Society Meeting, Philadelphia, PA, June 23-28.

Thompson, R. C. Animal Data on Plutonium Toxicity. Life Sciences Symposium, Los Alamos Scientific Laboratory, Los Alamos, NM, May 22-24.

Thompson, R. C. Symposium on Trans-uranium Element Toxicity - Dose Response Relationships at Low Exposure Levels. Summary and Speculative Interpretation Relative to Exposure Limits. 5th International Congress of Radiation Research, Seattle, WA, July 14-20.

Thompson, R. C. Implications With Respect to Protection Criteria. Environmental Protection Agency Hearings on Plutonium and the Trans-plutonium Elements, Washington, D.C., December 10, 1974.

Vogt, J. C. Histogenesis of 15-Day Fetal Rat Lung After X-Irradiation in Organ Cultures and In Utero. Seminar, Biology Department, Battelle-Northwest, Richland, WA, August 8, 1974 and Presentation of Thesis at Oregon State University, Corvallis, OR, August 13.

Wierman, E. L., G. L. Webb, and D. L. Catt. A Technique for Training Unanesthetized Beagle Dogs to Aerosol Inhalation Exposures. Annual District 8 Convention of the American Association for Laboratory Animal Science, Portland, OR, June 5-7.

ORGANIZATION CHARTS

RESEARCH AND DEVELOPMENT

1974

BIOLOGY DEPARTMENT STAFF

William J. Bair, Ph.D. - Manager (1)
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 Judith A. Harrison - Clerk

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 Maurice F. Sullivan, Ph.D. - Staff Scientist

Roy C. Thompson, Ph.D. - RX Program Director
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 Patricia M. Bresina - Clerk
 David W. Goiri - Technician (3)
 Jocelyn Leemhuys - Typist (4,5)
 Marilyn J. Love - Stenographer (6)

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 Gertrude G. Haggard - Clerk
 Helen W. Buescher - Receptionist (6,3)
 Nell P. Couch - Receptionist (6)
 Catherine M. Praino - Stenographer (6)

-
- (1) Present position - Director, Life Sciences Program
 - (2) Formerly Associate Manager and Coordinator of Battelle Institute Life Sciences Program
 - (3) Transferred
 - (4) Youth Opportunity Program (YOP)
 - (5) Terminated
 - (6) Part Time

BIOMETRICS CENTER

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 (Systems Department)
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 John R. Tadlock, A.B.
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 Jacqueline C. Wallace
 (Systems Department)

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 Barbara G. Moore
 Martha C. Perkins
 Janet R. Smith (3)
 Mary S. Tobin (2)
 Gary L. Webb

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 Oregon State University
 Corvallis, Oregon

Theresa L. Felten, M.S.
 Oregon State University
 Corvallis, Oregon

Kim D. Nordberg
 Pacific Lutheran
 Tacoma, Washington

-
- (1) Transferred
 (2) Terminated
 (3) Inquiry into Science Program

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 Sharon A. Clemetson - Secretary (1)
 Mona G. Edwards - Technical Typist

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 Dennis L. Catt
 John C. Gaven
 John P. Herring
 Susan M. Loscutoff, Ph.D.
 Richard J. Olson, M.S. (1)
 Ray F. Palmer, M.S.
 Gerald J. Powers
 Charles L. Sanders, Ph.D.
 Thomas Schaffner, M.D. (3)
 Bruce O. Stuart, Ph.D. (4)
 Alfred P. Wehner, Dr. Med. Dent.
 Deborah J. Wiggins, B.S. (5)

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 Wilbur Skinner
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 Dale C. Wardell
 Marvin H. Young (6,1)
 William A. Zimmerman

Consultant

G. Saccomanno, M.D.
 St. Mary's Hospital
 Grand Junction, Colorado

Northwest College and University
 Association for Science Fellows (NORCUS)

Ronald H. Starkey, Ph.D.
 University of Wisconsin
 Madison, Wisconsin

-
- (1) Terminated
 - (2) Transferred
 - (3) Visiting Scientist from the University of Bern, Switzerland
 - (4) Temporary assignment to USAEC, Washington, D.C.
 - (5) Science and Engineering Program
 - (6) Inquiry into Science Program
 - (7) Summer Student

MEDICAL SCIENCES SECTION

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 Janet R. Warren (2)

Consultants

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D. K. Clawson, M.D. University of Washington Seattle, Washington	D. K. Merkeley, M.D. Laboratory of Clinical Medicine 67 Cobb Bldg. Seattle, Washington
D. S. Coffey, Ph.D. The Johns Hopkins University School of Medicine Baltimore, Maryland	C. A. Paulsen, M.D. U.S. Public Health Service Seattle, Washington
J. E. Davis, M.D. New York Medical College New York, New York	S. S. Schmidt, M.D. Private Practice Eureka, California
R. Jaffee, M.S. University of California Medical Center San Francisco, California	E. Steinberger, M.D. University of Texas Medical School Houston, Texas
S. Katsh, Ph.D. University of Colorado Medical Center Denver, Colorado	G. D. Stibbs, D.M.D. School of Dentistry University of Washington Seattle, Washington

Northwest College and University Association for Science Fellows (NORCUS)

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Boston University
Boston, Massachusetts

Robert M. Riner
University of Puget Sound
Tacoma, Washington

Charlene Curtis
Eastern Washington State College
Cheney, Washington

Judith C. Vogt, Ph.D.
Oregon State University
Corvallis, Oregon

Carol M. Radosevich
Ft. Wright College
Spokane, Washington

-
- (1) Transferred
 - (2) Terminated
 - (3) Inquiry into Science Program

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 - (2) Battelle Institute Fellow
 - (3) Leave of Absence
 - (4) Hourly Call In
 - (5) Transferred

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 - (3) Deceased
 - (4) Youth Opportunity Program (YOP)
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 - (3) Temporary
 - (4) Sick Leave

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