BNWL-2000 PT1
UC-48, Biology
and Medicine

Pacific Northwest Laboratory Annual Report for 1975 to the ERDA Division of Biomedical and Environmental Research

Part 1 Biomedical Sciences

January 1976

Prepared for the U.S. Energy
Research and Development Administration
under Contract E(45-1):1830

Battelle
Pacific Northwest Laboratories

BNWL-2000 PT1

NOTICE

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BATTELLE
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U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
Under Contract E(45-1)-1830

Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151
Price: Printed Copy $8.50; Microfiche $2.25

BNWL-2000 PT1
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PACIFIC NORTHWEST LABORATORY ANNUAL REPORT FOR 1975 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,
Radiological Sciences Department,
and Systems Department

January 1976

Battelle
Pacific Northwest Laboratories
Richland, Washington 99352

PREFACE

The Annual Report for 1975 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 - W. R. Wiley	R. C. Thompson, Report Coordinator D. L. Felton, Editor
Part 2	Ecological Sciences Program Manager - B. E. Vaughan	B. E. Vaughan, Report Coordinator J. L. Helbling, Editor
Part 3	Atmospheric Sciences Program Manager - C. L. Simpson	C. E. Elderkin, Report Coordinator E. L. Owzarski, Editor
Part 4	Physical and Analytical Sciences Program Manager - J. M. Nielsen	J. M. Nielsen, Report Coordinator G. M. Garnant, Editor
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Reports previously issued are as follows:

Annual Report for

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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
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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
1974	BNWL-1950, Pt. 1-4

W. J. Bair, Manager
Biomedical and Environmental
Research Program

FOREWORD

The research described in this volume was conducted primarily in the Biology Department, PNL, with contributions from the Physics and Instrumentation, Radiological Sciences, and Systems Departments.

Reports on individual programs are grouped according to ERDA 189 projects. Each section is introduced by a "blue sheet" which summarizes the scope, objectives, and present status of the project. In addition to the normal DBER projects, five sections are devoted to projects covered by EPA "Pass Through" funds.

With the advent of ERDA, our research effort has expanded into many non-nuclear areas. Most of these projects were first funded in FY 1976 and few reportable results are available at this time. For such projects we include only the blue sheet, as an indication of the directions in which we are headed.

These new directions, while welcome, initially placed a severe strain on both facilities and personnel. Our professional and technical staff has expanded considerably this year, as evidenced by the listing, at the end of this volume, of staff who participated in the Biomedical Research Program. The new Life Sciences II Laboratory, constructed with Battelle funds and occupied this past summer, became available at a most opportune time and avoided a major facilities crunch. This building, specifically designed for research with experimental animals, effectively doubles the research and support facilities available for the biomedical and environmental research program. It is already fully occupied. Perhaps the most critical shortages are in the area of capital equipment, where funds have fallen far short of those necessary to fully implement the many new technologies involved in our expanded program.

Several changes in the organization and management of the Biology Department occurred during the past year. M. F. Gillis was named Manager, Project Development. J. E. Lund was named Manager and H. A. Ragan, Associate Manager, of the Experimental Pathology Section. B. O. Stuart was named Associate Manager, Inhalation Technology and Toxicology Section. There was a

general reshuffling of personnel among the research sections, with elimination of the Medical Sciences Section.

A highlight of the year was the Fifteenth Annual Hanford Life Sciences Symposium on the Biological Implications of Metals in the Environment, held September 29 to October 1, 1975 under the joint chairmanship of H. Drucker (Biology Department) and R. E. Wildung (Ecosystems Department). The proceedings will be published in the ERDA Symposium Series. The Sixteenth Annual Hanford Life Sciences Symposium on the Pulmonary Macrophage and Epithelial Cell is scheduled for September 26-28, 1976. We are also planning a radiation-associated immunology workshop for the spring of 1977.

A list of 1975 publications and presentations relevant to the ERDA programs reported in this volume appears on pages 207-212. Requests for reprints of publications will be honored as long as the supply lasts.

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DEVELOPMENT OF AEROSOL EXPOSURE AND ANALYTICAL TECHNIQUES

Person in Charge: W. C. Cannon

This project is concerned with the development of techniques for generation and characterization of aerosol exposure atmospheres; and with the development of radiochemical analytical techniques for biological and aerosol samples, particularly those associated with health effect studies related to nuclear power production. It is also concerned with relating physicochemical aerosol properties to respiratory deposition patterns. It also bears the cost of maintaining the inhalation exposure facilities.

Current research efforts in this project are primarily directed toward improving the capability for estimating the alveolar deposition during inhalation exposures. On-line instrumentation is being developed, which will provide data on experimental animal respiration parameters, aerosol concentration, and particle-size distribution. A computer will convert these data to running estimates of the integrated alveolar deposition, as the exposure proceeds, thus enabling the exposure to be terminated when the desired dose has been delivered.

Research in radiochemical analytical technology is primarily directed toward increasing the efficiency of routine sample processing. This is particularly important in the case of the very low-level radionuclide samples encountered in tissues from long-term, low-level effect studies, where improved column extraction processes appear feasible. This effort should result in increased sensitivity and accuracy of analyses, as well as a larger sample output rate.

PUBLICATIONS SINCE JANUARY, 1973

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Craig, D. K., R. L. Buschbom, and J. P. Herring. 1973. Relationships between nebulizer suspension concentration. Concentration and size distribution of $^{239}PuO_2$ aerosols generated for animal inhalation experiments. Health Phys. 24, 637-644.

Craig, D. K. 1973. Open end discussion on filtration of plutonium aerosols. Twelfth U.S.A.E.C. Air Cleaning Conference, vol. II (CONF-720823) NTIS, Springfield, VA, pp. 871-873.

Dionne, P. J., D. K. Craig, and J. R. Decker. 1973. Minicomputer control of aerosol inhalation exposure. Proc. IEEE Region Sixth Conference on Minicomputers and Their Applications, Institute of Electrical and Electronic Engineers, Inc., NY, pp. 159-162.

Craig, D. K., D. D. Mahlum, and E. L. Klepper. 1974. The relative quantity of airborne plutonium deposited in the respiratory tract and on the skin of rats. Health Phys. 27, 475-479.

Craig, D. K. and R. L. Buschbom. 1975. The alveolar deposition of inhaled plutonium aerosols in rodents. Amer. Ind. Hyg. Assoc. J. 36, 173-180.

Craig, D. K. and B. L. Klepper. 1975. The design and calibration of a low speed windtunnel for studying the foliar deposition and uptake of aerosols. Amer. Ind. Hyg. Assoc. J. 36, 692-699.

Craig, D. K., B. L. Klepper, and R. L. Buschbom. Deposition of aerosols of various plutonium compounds onto plant foliage at very low wind velocities. Proceedings of a Symposium on Atmospheric-Surface Exchange of Particulate and Gaseous Pollutants, Richland, WA, Sept. 4-6. AEC Symposium Series. (In Press)

Cataldo, D. A., E. L. Klepper, and D. K. Craig. Fate of plutonium Intercepted by leaf surfaces--leachability and translocation of seed and root tissues. Proceedings of Symposium on Transuranium Nuclides in the Environment, San Francisco, CA, November 17-21. International Atomic Energy Agency. (In Press)

IRON OXIDE INHALATION STUDIES IN BEAGLE DOGS

Investigators:
W. C. Cannon, R. F. Meginniss,[a]
D. K. Craig, and J. P. Herring
Technical Assistance:
E. F. Blanton

The retention of inhaled radioactively labeled iron oxide aerosols, as a function of particle size, was measured in beagle dogs.

Five beagle dogs were exposed to polydispersed aerosols of three different AMADs.[b] The aerosols were generated from suspensions of colloidal iron spiked with $^{198}AuCl_3$. Variation in AMAD was achieved by changing the concentration of the colloidal suspension. The aerosol AMADs varied from 1.1 μm to 3.8 μm but the GSDs[c] were all in the range from 1.65 to 1.80, indicating that the relative width of the distributions was about the same in all cases.

The retained activity was determined by whole-body counting and excreta analyses. The initially deposited activity was determined as the total inhaled volume times the aerosol concentration, minus the total exhaled activity; corrections were made for deposition in the sampling equipment. The retained activity is expressed as a percentage of the initially deposited activity.

Figure 1.1 shows the percent of retained activity after one day as a

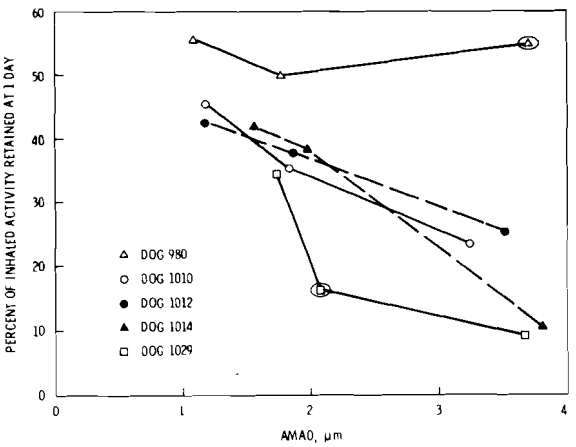

FIGURE 1.1. Percent of Retained Activity in Dogs Exposed to Iron Oxide Aerosols.

(a) Presently at College of Lake County, Grayslake, Illinois 60030. Was National Science Foundation Research Participation Fellow, June through August, 1975.

(b) activity median aerodynamic diameter.

(c) geometric standard deviation.

function of particle size in the range from 1 μm to 4 μm AMAD. The curves for three dogs (1010, 1012, and 1014) are quite similar. The curves for dogs 980 and 1029 would have similar slopes except for one point on each curve. Examination of respiration data for dogs 980 and 1029 during the exposures that produced the aberrant points showed breathing patterns that were markedly different from those usually observed.

Future exposures of these same dogs will employ aerosols generated by a spinning top or a vibrating orifice generator, to expand the range of aerosol particle sizes studied and to obtain better measurements of deposition as a function of specific particle size.

AN INTERCHANGEABLE FACILITY FOR EXPOSURE OF BEAGLE DOGS OR MINIATURE SWINE TO RADIOACTIVE AEROSOLS

Investigators:
J. R. Decker, W. C. Cannon, D. K. Craig, and J. L. Beamer
Technical Assistance:
E. F. Blanton and E. G. Kuffel

An animal-exposure facility has been built and tested which can be used to expose either beagle dogs or Hanford miniature swine to radioactive aerosols.

An aerosol inhalation glove box was designed which will accommodate either miniature swine or beagle dogs. It consists of three glove boxes: two interchangeable boxes for animal containment (one for dogs and one for swine), and another box to contain the aerosol generation and sampling equipment (Figure 1.2.). Either of the animal containment glove boxes may be attached to the aerosol generation box, depending upon which species is being exposed, thus increasing versatility and decreasing cost and storage problems.

Refinements of previously developed aerosol generation and sampling systems and respiratory monitoring systems in use at this laboratory for dogs were included in the new design. The aerosol generation glove box was enlarged for inclusion of additional sampling equipment such as the on-line particle size distribution monitor presently being constructed. To accommodate the greater tidal and minute volumes of swine (as contrasted with dogs), a larger aerosol chamber was required. The new chamber is constructed of stainless steel (previous chambers were polycarbonate) to prevent static-charge precipitation of the aerosol particles on the sides of the chamber. Loss of particles was also reduced by a new type of dispersion chamber at the top of the aerosol chamber, and a port was added at the bottom to facilitate thorough cleaning. Future

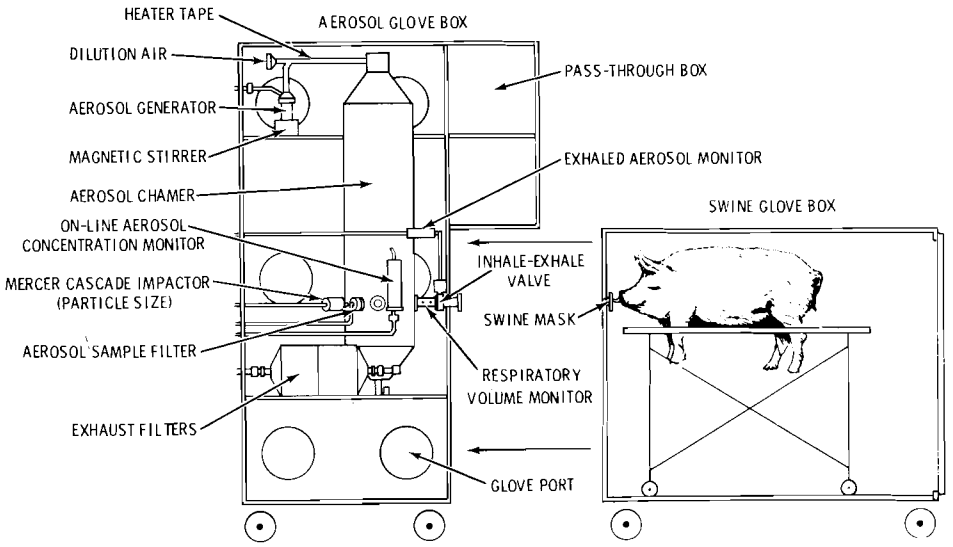

FIGURE 1.2. Inhalation Exposure Chamber for Dogs or Swine.

experiments will determine if cleaning between exposures will allow use of a single chamber for aerosols of various radionuclides, which would effect a considerable savings. Several improvements in the respiration monitoring system make disassembly and cleaning easier.

The on-line aerosol concentration monitoring system, part of the exposure facility, uses commercially available sample filter holders, thereby reducing costs. Error was reduced by considerable reduction of the dead air space in the sample line and by improving the geometry of the alpha detection system.

The swine containment glove box was designed to facilitate handling of a 250-pound, anesthetized and radioactive animal. The pig is placed on a special sling in a cart which can easily be rolled into and out of the containment box. The sling is hinged on one side to allow the pig to be rolled onto another cart for return to the metabolism cage following exposure.

BIOLOGICAL BEHAVIOR OF PLUTONIUM RELEASED WITH SODIUM

Person in Charge: D. K. Craig

Postulated LMFBR accidents involve the release of plutonium dioxide aerosols together with sodium. The objective of this project is to obtain data on the metabolic behavior of combinations of sodium compounds and plutonium following inhalation by rodents and dogs. This information is required in order to conduct more realistic accident analyses, and would confirm or contradict British studies which suggest that mixed aerosols of plutonium dioxide and sodium oxide are more transportable in the lung than aerosols of plutonium dioxide alone. It will also provide needed information on the toxicology of inhaled sodium aerosols alone.

In its first phase, this project will be principally concerned with the development of Pu-Na aerosol generation techniques. Previous investigations of Pu-Na aerosols have involved an exploding wire technique for aerosol generation, but this method is not satisfactory for the continuous generation required if exposures are to extend over several hours. Simulated LMFBR fuel will be vaporized under controlled conditions and brought in contact with sodium vapor. Aerosols will be characterized in terms of sodium concentration, plutonium concentration, ratio of sodium to plutonium, and aerodynamic particle size distribution.

Having established the aerosol generation techniques and incorporated them into nose-only animal inhalation apparatus, rats will be exposed to aerosols of various concentrations of Na only and various ratios of Na to Pu. Rats will be sacrificed at intervals extending to one year post-exposure, to follow the progression of lesions and the translocation of material from the lungs to other tissues. Should the early distribution and excretion data yield results significantly different from those obtained in previous experiments with inhaled $^{239}PuO_2$, we will extend these studies to include limited experiments using beagle dogs. With these data in hand a decision can be made as to the need for more extensive long-term toxicity studies.

This project was first funded in FY 1976 and reportable results are not yet available.

LOW LEVEL PLUTONIUM AND TRANSPLUTONIUM OXIDE INHALATION STUDIES IN BEAGLES

Person in Charge: J. F. Park

This project is concerned with long-term experiments to determine the dose-effect relationships of inhaled plutonium oxide in beagle dogs, and shorter-term experiments to study the kinetics and dosimetry of transplutonium elements of interest, such as $^{241}AmO_2$, $^{244}CmO_2$, and various oxide mixtures.

A major life-span study involving about 350 beagles is currently in progress. These animals received a single 5 to 30-minute exposure to a PuO_2 aerosol as shown in the following table:

Alveolar Deposition (μCi)	Number of Animals	
	$^{239}PuO_2$	$^{238}PuO_2$
3	10	10
1.25	20	20
0.25	20	20
0.05	20	20
0.01	20	20
0.002	20	20
Controls	40	

The ^{239}Pu dogs were exposed in FY 1971 and FY 1972; the $^{238}PuO_2$ dogs, in FY 1973 and FY 1974. The three highest deposition levels overlap those employed in previous studies that have been completed ($^{239}PuO_2$) or are currently in progress ($^{238}PuO_2$), and should predictably lead to a high incidence of lung and/or bone tumors. The lowest level corresponds to the presently established permissible lung burden, i.e., it will result in an estimated average dose of 15 rem/year to the lung. In addition to the dogs maintained for life-span observation, 50 dogs were exposed for periodic sacrifice to obtain information on deposition, retention, translocation, and excretion of inhaled plutonium, and on the pathogenesis of dose-related effects.

Deposition, retention, translocation and excretion data are also being obtained in beagles at intervals to 2 years following inhalation of $^{244}CmO_2$ and $^{241}AmO_2$ aerosols, for comparison with data previously obtained on $^{239}PuO_2$ and $^{238}PuO_2$. These data will be used for estimating the relative risk of these alpha-emitting radionuclides, that are prominent components of nuclear fuel cycle effluents.

PUBLICATIONS SINCE 1973

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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, 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. 1975. Late effects of inhaled plutonium in dogs, 1233-1247. In: Radiation Research. Biomedical, Chemical & Physical Perspectives, H. I. Adler and W. K. Sinclair (eds.). Academic Press, NY.

Olson, R. J. and C. R. Watson. 1975. A Computer-Aided Method for Analysis of SNOP-Coded Histopathology Observations, BNWL-B 379, Battelle-Northwest, Richland, WA.

Dagle, G. E. and J. F. Park. Plutonium-induced lymphadenitis in beagles. Proceedings of Hanford Biology Symposium on Radiation and the Lymphatic System, AEC Symposium Series. (In Press)

Ragan, H. A., J. F. Park, and R. J. Olson. Lymphocytopenia induced in beagles by inhalation of ^{239}Pu. Proceedings of Hanford Biology Symposium on Radiation and the Lymphatic System, AEC Symposium Series. (In Press)

Ragan, H. A. Density gradient separation of beagle lymphocytes. Bull. Amer. Soc. Vet. Clin. Path. (In Press)

Craig, D. K., J. R. Decker and R. L. Buschbom. Disposition of highly toxic radioactive aerosols inhaled by beagle dogs. Health Phys. (In Press)

DOSE-EFFECT STUDIES WITH INHALED PLUTONIUM OXIDE IN BEAGLES

Investigators:

J. F. Park, R. L. Buschbom, A. C. Case, D. L. Catt, D. K. Craig, G. E. Dagle, P. L. Hackett, S. M. Loscutoff, 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, G. L. Larson, S. J. Lepka, R. M. Madison, M. C. Perkins, L. R. Peters, and P. J. Raney

Beagle dogs given single exposure to $^{239}PuO_2$ or $^{238}PuO_2$ aerosols are being observed for life-span dose-effect relationships. The ^{239}Pu body burden of the nine dogs dying due to pulmonary fibrosis-induced respiratory insufficiency during the first 3 years after exposure was 1 to 12 μCi. One of these dogs had a pulmonary tumor. Three additional dogs with body burdens of 0.7 to 1.8 μCi died due to pulmonary neoplasia 4-1/2 years after exposure. None of the dogs exposed to ^{238}Pu have died during the first two postexposure years. After inhalation of $^{239}PuO_2$ or $^{238}PuO_2$ lymphocytopenia was the earliest observed effect, occurring 0.5 to 2 years after deposition of $\geq$ 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 $^{239}PuO_2$ (mean AMAD 2.3 μm, mean GSD 1.9), prepared by calcining the oxalate at 750°C for 2 hr; or to $^{238}Pu^{16}O_2$ (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 $^{239}PuO_2$ 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 3.1). One hundred thirteen dogs exposed to $^{238}Pu^{16}O_2$ in 1973 and 1974 were selected for life-span dose-effect studies (Table 3.2). Fourteen additional dogs for periodic sacrifice were exposed in 1975.

TABLE 3.1. Dose-Effects Studies with Inhaled $^{239}PuO_2$ in Beagles.

DOSE LEVEL GROUP	NUMBER OF DOGS MALE	NUMBER OF DOGS FEMALE	INITIAL ALVEOLAR DEPOSITION[a] nCi[b]	INITIAL ALVEOLAR DEPOSITION[a] 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 ± 95% CONFIDENCE INTERVALS AROUND THE MEANS

TABLE 3.2. Dose-Effect Studies with Inhaled $^{238}PuO_2$ in Beagles.

DOSE LEVEL GROUP	NUMBER OF DOGS MALE	NUMBER OF DOGS FEMALE	INITIAL ALVEOLAR DEPOSITION[a] nCi[b]	INITIAL ALVEOLAR DEPOSITION[a] 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) MEAN ± 95% CONFIDENCE INTERVALS AROUND THE MEANS

During the first 4-1/2 years following exposure to $^{239}PuO_2$, seven dogs in the highest level dose group and five 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 3.3 shows the causes of death and the distribution of ^{239}Pu in the tissues of these animals.

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. The fraction of plutonium in liver increased, accounting for 15 to 29% of final body burden 4-1/2 years after exposure. 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 fourth postexposure years. Dog 798 F had a larger fraction of plutonium in the thoracic lymph nodes.

The dogs that were euthanized due to respiratory insufficiency during the 3-year postexposure period 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. Dog 804 M also had a pulmonary tumor classified as a bronchiolar-alveolar carcinoma. The three dogs euthanized 4-1/2 years after exposure showed radiographic evidence of pulmonary neoplasia before respiratory insufficiency was observed. However, respiratory insufficiency was observed prior to euthanasia, probably primarily due to neoplasia in the lung. In two of the dogs the tumors were classified as bronchiolar-alveolar carcinoma and in the other dog as epidermoid carcinoma, following the World Health Organization classification of lung tumors in animals. The epidermoid carcinoma metastasized

TABLE 3.3. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}PuO_2$.

DOG NUMBER	TIME AFTER EXPOSURE, MONTHS	FINAL BODY BURDEN, μCi	PERCENT OF FINAL BODY BURDEN				CAUSE OF DEATH
			LUNGS	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	80	15	0.04	1.6	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	2.0	55	28	13.0	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	44	0.17	0.43	PERIODIC SACRIFICE
772 M	53	1.821	42	22	29.0	0.69	LUNG TUMOR
759 M	54	0.707	43	27	15.0	0.65	LUNG TUMOR
796 F	55	0.671	40	31	21.0	1.04	LUNG TUMOR

[a] TRACHEOBRONCHIAL, MEDIASTINAL AND STERNAL LYMPH NODES

to the skeleton and the bronchiolar-alveolar carcinomas metastasized to the heart and thoracic lymph nodes in one dog, and to the thoracic lymph nodes, mediastinum, kidney, skeleton and axillary lymph node in the other dog. Two of the dogs had lesions of secondary hypertrophic osteoarthropathy. Sclerosing lymphadenitis was associated with the high concentration of plutonium in the thoracic lymph nodes. There was also a generalized lymphoid atrophy which may be related to the debilitation in the dogs with respiratory insufficiency, or to lymphocytopenia.

Histopathological examination of periodically sacrificed dogs indicated no plutonium-related lesions in dog 849 F (0.7 nCi). Dog 798 F (5.6 nCi) had slight lymphoid atrophy in the tracheobronchial lymph nodes, characterized by increased amounts of phagocytized pigment in the medulla, reduced numbers of mature lymphocytes and an apparent unmasking of the reticular cells in the outer area of the cortex. The tracheobronchial lymph nodes of dog 815 M (74 nCi) had moderate lymphoid atrophy characterized by decreased lymphoid follicles in the cortex, increased amounts of phagocytized pigment in the medulla, poorly defined medullary cords and increased fibrous connective tissue. The lungs of dog 798 F and 815 M were essentially normal with the exception of an occasional focal area of pleural or subpleural fibrosis associated with alpha stars on the autoradiographs.

The most consistent effect on the formed elements of blood in the $^{239}PuO_2$-exposed dogs has been a significant ($P < 0.05$), dose-related lymphocytopenia at mean initial alveolar depositions of 79 nCi and greater. This lymphocytopenia continued to be manifested through 47 months postexposure (Figure 3.1). At mean initial alveolar depositions of either 3.5 or 22 nCi, the mean lymphocyte concentrations declined after about 2 years and remained below that of control dogs, but the differences were not significant. With mean initial alveolar depositions of 5,800 nCi, pulmonary fibrosis and respiratory insufficiency developed relatively rapidly and only one dog survived beyond 31 months after exposure. The sustained lymphocytopenia in Groups 4 and 5 (mean initial alveolar depositions of 300 and 1,100 nCi), and the progressive lymphocytopenia in Group 3 (mean initial alveolar burden, 79 nCi) may be the result of continued irradiation of circulating lymphocytes. The progressive decrease in Group 3 lymphocytes is of particular interest, since by 3-1/2 years postexposure the values are not greatly different from those of Groups 4 and 5. These results might be obtained if a slowly replaced, radiosensitive subpopulation of lymphocytes were involved. In such a case, cell death could ultimately approach cell renewal rates, thus establishing a new equilibrium. At higher exposure levels (Groups 4 and 5) this subpopulation would be killed more rapidly, but they would plateau at a level similar to that of Group 3. Investigations are under way to define the subpopulations of lymphocytes that are affected by the ^{239}Pu inhalation.

The ^{239}Pu tissue distribution data (Table 3.3) show a decrease in the plutonium lung burden with time after exposure, therefore a probable source of the chronic irradiation causing the lymphocytopenia would be the tracheobronchial lymph nodes. Studies in rodents (Roser and Ford, Aust. J. Biol. Med. Sci. 50, 165, 1972) have shown that radionuclides injected into a single lymph node chain can result in a prolonged and sustained lymphocytopenia. In addition to direct radiation, indirect effects resulting in sustained lymphocytopenia must also be considered. 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 3.1). This change was due primarily to the reduction in lymphocytes with a lesser reduction in neutrophils, except in the highest dose-level group. 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 4-year postexposure period.

None of the dogs on the $^{238}Pu^{16}O_2$ life-span dose-effect study have died. The most obvious hematologic effect of ^{238}Pu was on circulating lymphocyte concentrations (Figure 3.2). There was a dose-related lymphocytopenia in Groups 3 through 6 similar to that found following inhalation of $^{239}PuO_2$. The lymphocytopenia after ^{238}Pu inhalation was more pronounced in Groups 3 and 4, both as to magnitude of depression and time of appearance, than in the comparable $^{239}PuO_2$ exposure groups. These results are probably related to the greater translocation of ^{238}Pu to extrapulmonary tissues including skeleton and bone. One dog in the periodic sacrifice group with a final body burden of 14 nCi one year after exposure had 52% of the final plutonium body burden in the lung with 9% in the thoracic lymph nodes, 6% in the liver, 16% in the skeleton and 11% in the kidney. Other experiments reported in this Annual Report show more translocation of ^{238}Pu to the skeleton than ^{239}Pu. Total leukocyte values (Figure 3.2) were most obviously depressed in Groups 5 and 6 but with a similar trend in Groups 3 and 4. This effect was due primarily to reduction in lymphocytes in Groups 3 and 4. A depressive effect of ^{238}Pu inhalation on neutrophils was manifested by 4 months postexposure in Group 6 dogs, and this became progressively more pronounced with time. A similar effect was noted in Group 5 dogs starting about 14 months postexposure. Eosinophil values in Group 6 dogs tend to be lower than in controls or other exposure groups from 1-26

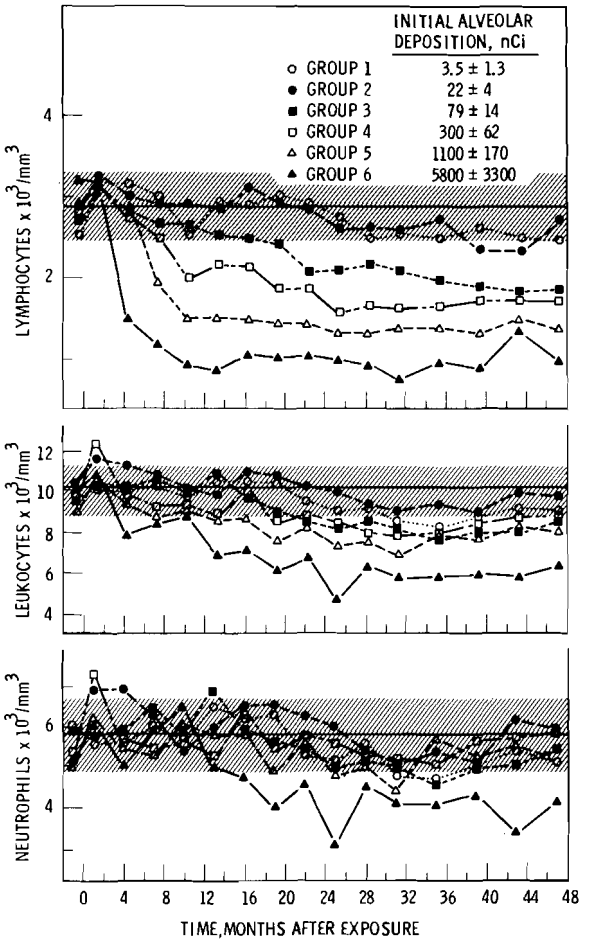

FIGURE 3.1. Mean Lymphocyte, Leukocyte and Neutrophil Values from Dogs after Inhalation of $^{239}PuO_2$. The shaded area represents mean values from age-related control dogs ± the mean 95% confidence interval.

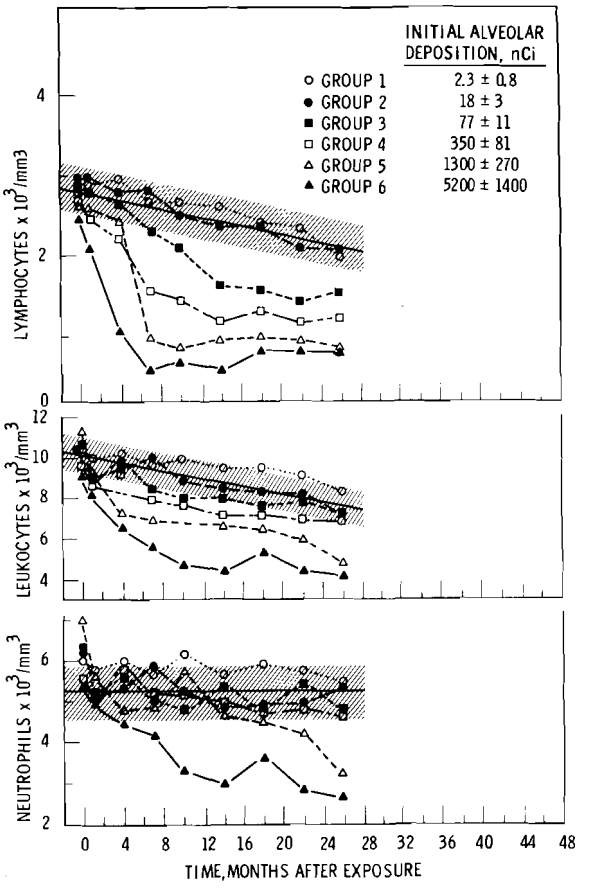

FIGURE 3.2. Mean Lymphocyte, Leukocyte, and Neutrophil Values from Dogs after Inhalation of $^{238}Pu^{16}O_2$. The shaded areas represent mean values from age-related control dogs ± the mean 95% confidence interval.

months following $^{238}PuO_2$ inhalation. The red blood cell concentrations also tended to be higher in Groups 5 and 6 for the first few months after exposure than were those of either the control or other exposed groups. There were no statistically significant differences in monocyte or eosinophil concentration or in values for red cell parameters between exposed and control animals.

In the control dogs there appears to be an age-related reduction in circulating leukocytes and lymphocyte concentrations which was not found in the control dogs for the ^{239}Pu inhalation study. Both groups of controls were similar ages at initiation of the study. They had similar breeding backgrounds, and were housed and handled under similar circumstances, so an explanation of this difference is not readily apparent.

Pulmonary function tests were performed during the fourth postexposure year, on representative dogs from each of the four highest dose-level groups exposed to $^{239}PuO_2$. The results of these tests are summarized in Table 3.1. Tests were divided into two groups: those which could be performed on awake dogs, and those requiring anesthesia to achieve specific ventilatory patterns. Because of the risks of anesthesia in animals with serious pulmonary impairment,

dogs with high respiratory rates and abnormal arterial blood gases were not anesthetized. Pulmonary function tests vary in their sensitivity to detect pulmonary abnormalities. Some of these tests, particularly those requiring anesthesia, have not been fully evaluated in dogs, although they are used routinely as indicators of pulmonary abnormalities in non-anesthetized humans. By using several tests to evaluate pulmonary function in these dogs, results of different tests can be compared in attempting to define a pattern of functional changes associated with plutonium exposure.

Tidal volume, respiratory rate and minute volume have been shown previously to be affected in serious pulmonary disease. Table 3.4 shows that dogs from the two highest dose groups had decreased tidal volumes and the single dog from Group 6 had an increased respiratory rate. Dogs from Groups 4 and 5 showed decreased minute volume. Pulmonary compliance measurements provide an assessment of lung elasticity, a decreased compliance indicating an increased lung stiffness. Table 3.4 shows that dynamic compliance was decreased in dogs from Groups 3, 4, and 5, while quasi-static compliance was decreased

TABLE 3.4. Summary of Pulmonary Function Tests (mean ± SD)

	CONTROL	GROUP 3	GROUP 4	GROUP 5	GROUP 6
AWAKE TESTS[b]					
NUMBER OF DOGS	7	3	11	10	1
TIDAL VOLUME (ml/kg)	20 ± 3.4	21 ± 4.5	18 ± 4	13 ± 3.1	12
RESPIRATION RATE (breaths/minute)	18 ± 6.6	14 ± 3.2	14 ± 1.8	25 ± 16.3	54
MINUTE VOLUME (ml/min/kg)	362 ± 168	291 ± 127	245[a] ± 44	284[a] ± 110	651
COMPLIANCE, DYNAMIC ($ml/cm\ H_2O$)	68.6 ± 8.4	35[a] ± 17	43.8[a] ± 24	24.7[a] ± 13	
HEART RATE (beats/minute)	127 ± 31	124 ± 24	112 ± 22	90[a] ± 33	168
PaO_2 ($mm\ Hg$)	93 ± 15.3	91.7 ± 3.1	97.4 ± 4.7	89.8 ± 12.7	82
$PaCO_2$ ($mm\ Hg$)	40 ± 6.1	42.6 ± 3.3	41.0 ± 2.4	44.5[a] ± 3.5	47.7
ANESTHESIA TESTS[b]					
NUMBER OF DOGS	7	3	10	9	
COMPLIANCE, QUASISTATIC ($ml/cm\ H_2O$)	105 ± 53	84 ± 19	99 ± 47	55[a] ± 23	
PULMONARY RESISTANCE ($cm\ H_2O$ / l/ sec)	5.8 ± 2	8.3[a] ± 1	7 ± 1.1	8.1 ± 1.5	
VITAL CAPACITY (ml/kg)	80 ± 14	82 ± 11	84 ± 12	64 ± 17	
M_3 ($\%\ N_2$/l)	1.3 ± 0.4	1.7 ± 0.6	1.6 ± 0.6	1.3 ± 0.6	
CLOSING VOLUME/ VITAL CAPACITY (%)	16 ± 4	17 ± 2.6	18 ± 6.5	24[a] ± 7	

[a] STATISTICALLY DIFFERENT ($P<0.05$) FROM CONTROLS BY DUNCAN'S MULTIPLE RANGE TEST

[b] TESTS EXPLAINED IN TEXT

only in dogs from Group 5. Pulmonary resistance, as measured in anesthetized dogs, was increased in dogs from Groups 3 and 5. Since most of the resistance to air moving in and out of the lung occurs in the large airways, this test suggests that the caliber of these airways may be affected by plutonium exposure.

Dogs from Groups 5 and 6 had $PaCO_2$ (arterial CO_2 partial pressure) indicative of minor pulmonary abnormalities. Extensive channels for collateral ventilation, present in dogs but not in humans, may allow blood gases to remain relatively normal despite severe pulmonary disease.

Vital capacity (the difference in lung volume between maximum exhalation and maximum inhalation) was not significantly affected in any of the exposure groups. M_3 is the slope of the alveolar nitrogen plateau measured during the closing volume maneuver, and reflects the distribution of gas in the lung. Closing volume maneuvers are performed by maximally inflating the lung with oxygen, then slowly deflating to maximum exhalation while plotting nitrogen concentration vs. volume. M_3 was not affected by plutonium exposure. Closing volume (a measurement related to the stability of the small airways) was increased in dogs from Group 5.

The results of pulmonary function tests are consistent with what would be expected from stiffer lungs due to pulmonary fibrosis, resulting in less efficient distribution of respired air. Whether dogs at lower dose-levels will develop changes similar to those seen in the dogs from Groups 3, 4 and 5, or whether a different pattern of changes will be seen at lower dose levels, will be determined in further studies.

LATE EFFECTS OF INHALED $^{238}PuO_2$ IN BEAGLES

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Osteosarcomas were the primary cause of death in beagle dogs 4 to 8 years after inhalation of $^{238}PuO_2$. The plutonium body burden at death ranged from 0.4 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 8 years ago to aerosols of $^{238}PuO_2$ for study of long-term disposition and biological effects. Nine of ten dogs exposed to 350°C-calcined $^{238}PuO_2$ (CMD, 0.1 μm), and ten of 12 dogs exposed to $^{238}PuO_2$ from crushed microspheres (CMD, 0.7 μm), died or were euthanized when death was imminent. Table 3.5 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.

During the 4- to 8-year postexposure period, eleven 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.

An osteosarcoma in the left humerus was surgically removed from dog 480 F 86 months after exposure (one month after radiographic diagnosis) by amputation of the leg. It was anticipated that removal of the osteosarcoma could prolong the survival of the dog and perhaps provide an opportunity to observe plutonium-induced tumor in other tissues, especially the lung. The dog was euthanized 13 months later (99 months after exposure) due to radiographic evidence of lung tumors and bone tumors in the right humerus and pelvis. Histopathology is in progress.

TABLE 3.5. Mortality and Tissue Distribution of Plutonium in Dogs after Inhalation of $^{238}PuO_2$.

DOG NUMBER	TIME AFTER EXPOSURE, months[a]	FINAL BODY BURDEN, μCi	% OF FINAL BODY BURDEN				CAUSE OF DEATH
			LUNGS	LYMPH NODES[b]	LIVER	SKELETON	
		(AFTER INHALATION OF $^{238}PuO_2$ 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	OSTEOSARCOMA
445 M	58	2.5	6	10	23	55	OSTEOSARCOMA
438 M	60	2.3	7	11	33	43	OSTEOSARCOMA
453 M	62	2.2	17	9	22	47	OSTEOSARCOMA
405 M	70	2.6	23	10	28	34	OSTEOSARCOMA & LUNG CARCINOMA
433 M	87	0.4	27	5	33	33	OSTEOSARCOMA
459 M	96	0.2[c, d]					
		(AFTER INHALATION OF $^{238}PuO_2$ 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 & OSTEOSARCOMA
489 F	62	2.5	7	26	27	32	OSTEOSARCOMA & LUNG ADENOMA
482 F	70	0.8	8	19	15	47	OSTEOSARCOMA
494 F	75	0.4	3	9	32	48	FIBROSARCOMA
488 F	76	1.4	9	15	29	41	OSTEOSARCOMA & LUNG ADENOMA
480 F	86 (99)[e]	0.6[c]					OSTEOSARCOMA
487 F	88	0.4	5	15	31	42	OSTEOSARCOMA
491 M	98	0.6[c, d]					
496 F	98	0.2[c, d]					

[a] TO DEATH OR TO OCTOBER 1975

[b] TRACHEOBRONCHIAL, MEDIASTINAL AND STERNAL LYMPH NODES

[c] ESTIMATED BODY BURDEN

[d] STILL ALIVE

[e] EUTHANIZED DUE TO RADIOGRAPHIC EVIDENCE OF BONE TUMORS AND LUNG TUMORS 13 MONTHS AFTER SURGICAL REMOVAL OF AN OSTEOSARCOMA AT 86 MONTHS AFTER EXPOSURE

The final plutonium body burden and its distribution among tissues for the dogs euthanized during the 4- to 7-year postexposure period is shown in Table 3.6. For all animals, the highest plutonium concentration occurred in the thoracic lymph nodes, followed in descending order by lungs or liver, and skeleton. The largest fraction of plutonium in the body was in the skeleton, 32-55%; followed by the liver, 15-33%, thoracic lymph nodes, 3-26%; and lung, 3-24%.

In addition to the biological effects causing death, 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 Reports for 1973 and 1974). 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 dogs with bone tumors, all but one had occasional aberrant alkaline phosphatase levels. Figure 3.3 shows the serum alkaline phosphatase profile of dog 480 F. Serum alkaline phosphatase activity was within control levels until 85 months after exposure. Following amputation of the osteosarcoma-bearing limb (86 months after exposure) enzyme levels returned to control levels within 6

TABLE 3.6. Plutonium Concentration in Tissues of Beagles After Inhalation of $^{238}PuO_2$.

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
433 M	87	0.4	1.4	61	0.4	0.1
(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
487 F	88	0.4	0.2	763	0.4	0.1

(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

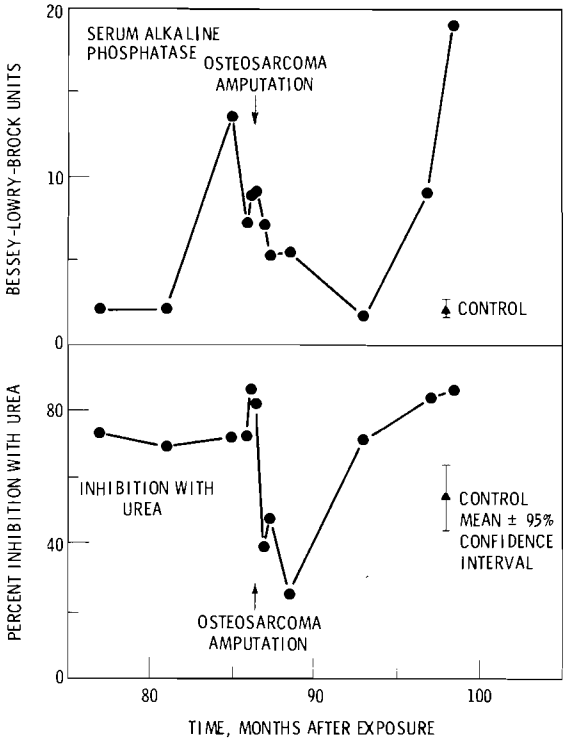

FIGURE 3.3. Serum Alkaline Phosphatase Profile of Beagle Dog Exposed to Inhaled $^{238}PuO_2$.

months, but then increased at 97 and 99 months after exposure. The dog was euthanized due to radiographic evidence of bone and lung tumors 99 months after exposure. Enzyme inhibition studies with urea, to which the bone isoenzyme is more sensitive than liver or intestinal isoenzymes, indicated a relative increase in bone isoenzyme prior to an increase in total serum alkaline phosphatase. For a period of at least 2 months following surgery, the bone isoenzyme was not a major fraction of the total activity.

Bone tumor locations are shown in Table 3.7. Seven of the eleven dogs had two or more bone tumor sites in the skeleton. Three dogs had metastases of the bone tumor to other organs. Two dogs with multiple bone tumor sites in the skeleton had metastases to the lungs. One dog with a single bone tumor site in the skeleton had metastases to the lungs,

TABLE 3.7. Location of Bone Tumors in Dogs after Inhalation of $^{238}PuO_2$.(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
453 M	X				X		2
405 M						X	1
433 M	X						1
481 F		X					1
489 F	X					X	2
482 F	X			X			2
488 F	X		X			X	3
487 F	X			X		X	3
TOTAL	7	3	1	3	4	8	26

(a) RADIOGRAPHIC, GROSS AND HISTOLOGIC DIAGNOSIS

spleen, liver, and kidney. Humerus and vertebrae were the most frequent bone tumor sites. All of the bone tumors in the humerus were in the proximal 1/3 of the bone. Bone tumors were located in the thoracic, lumbar, and sacral vertebrae.

The gross distribution of plutonium in the skeleton was evaluated by plutonium analyses of individual bones or groups of bones. If a tumor occurred in an individual bone, the contralateral or adjacent bone was analyzed. Table 3.8 compares the plutonium concentration in the individual bones of 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 8 years after inhalation of $^{238}PuO_2$. 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 all of these organs must be considered as possibly contributing to total late effects of inhaled $^{238}PuO_2$.

TABLE 3.8. Concentration of ^{238}Pu in the Skeleton After Inhalation of $^{238}PuO_2$ (nCi/g Wet Weight).

(AFTER INHALATION OF $^{238}PuO_2$ CALCINED AT 350°C)

DOG NUMBER	469 M	445 M	438 M	453 M	405 M	433 M
PELVIS	1.4[a]	1.3	1.3	1.3	1.3	0.14
RIB	1.1[a]	1.5[a]	1.1	1.4[a]	0.6	0.13
SCAPULA	1.1	1.8	0.8	1.2	0.5	0.09
VERTEBRA	0.8[a]	1.4[a]	0.6	0.9	0.6[a]	0.11
FEMUR	1.1	1.3[a]	0.9[a]	0.8	0.6	0.11
HUMERUS	1.5[a]	0.7	1.1	0.6[a]	0.7	0.13
CRANIUM	0.5	0.7	0.5	0.6	0.3	0.07
MANDIBLE	0.3	0.6	0.4	0.6	0.3	0.06
MAXILLA	0.3	0.6	0.3	0.6	0.3	0.06
EXTREMITIES[b]	0.4	0.8	0.3	0.5	0.2	0.03
TOTAL SKELETON	0.8	1	0.6	1	0.6	0.09

(AFTER INHALATION OF $^{238}PuO_2$ CRUSHED MICROSPHERES)

DOG NUMBER	481 F	489 F	482 F	488 F	487 F
PELVIS	0.4	---	0.3[a]	0.7	0.1[a]
RIB	0.2	1.3	0.3	4.7	0.2
SCAPULA	0.3	0.9	0.2	0.8[a]	0.1
VERTEBRA	0.3	0.9[a]	0.2	0.8[a]	0.1[a]
FEMUR	0.1[a]	0.9	0.3	0.3	0.1
HUMERUS	0.3	1.6[a]	0.3[a]	1.1[a]	0.1[a]
CRANIUM	0.2	0.8	0.2	0.3	0.1
MANDIBLE	0.2	0.6	0.2	0.2	---
MAXILLA	0.1	0.4	0.2	0.3	---
EXTREMITIES	0.1	0.4	0.1	0.2	0.1
TOTAL SKELETON	0.2	0.8	0.2	0.6	0.1

[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 $^{241}AmO_2$ or $^{244}CmO_x$ aerosols and sacrificed at various times postexposure. Both isotopes were more readily translocated from the lungs to liver, skeleton and muscle than plutonium. Initially, ^{244}Cm translocated more rapidly than ^{241}Am; but by 270 days postexposure, the tissue distribution of both isotopes was similar.

Beagle dogs were exposed to three aerosol concentrations of $^{241}AmO_2$ or $^{244}CmO_x$. Five dogs were exposed at each dose level and scheduled for sacrifice at 10, 30, 90, 270 and 810 days postexposure. Both materials were prepared by calcining the oxalate at 700 to 750°C for 2 to 4 hours. The aerosol concentration, particle size distribution data, and external thorax counting data obtained 7 days postexposure are given in Table 3.9. For $^{241}AmO_2$, the AMAD was smaller for the low dose level dogs. The AMAD of the $^{244}CmO_x$ aerosol was consistently smaller than

TABLE 3.9. Aerosol Concentration, Particle Size Distribution Data and Initial Alveolar Distribution in Beagle Dogs 7 Days Postexposure to ^{241}Am and ^{244}Cm Aerosols.

		AEROSOL DATA			INITIAL ALVEOLAR DEPOSITION, nCi[a]
ISOTOPE	DOSE LEVEL	CONC., nCi/ℓ	AMAD, μm	GSD	
^{241}Am	LOW	0.66	0.72	2.41	1.2
^{241}Am	MEDIUM	42.1	1.35	1.71	124.0
^{241}Am	HIGH	336.0	1.45	1.68	1150.0
^{244}Cm	LOW	1.27	0.45	2.01	13.0
^{244}Cm	MEDIUM	13.2	0.52	2.14	71.0
^{244}Cm	HIGH	109.0[b]	0.47	2.23	415.0[b]

AMAD = ACTIVITY MEDIAN AERODYNAMIC DIAMETER (μm)
GSD = GEOMETRIC STANDARD DEVIATION OF DISTRIBUTION
(a) ESTIMATED FROM EXTERNAL THORAX COUNTS AT 7 DAYS POSTEXPOSURE
(b) MEAN OF 4 VALUES

that of the $^{241}AmO_2$ aerosol. Ultrafilterability tests (suspended fraction of activity < 24Å) were conducted within 24 hours of placing the calcined material in water suspension. The $^{241}AmO_2$ yielded ultrafilterability values of < 0.1%. The $^{244}CmO_x$ gave values of 1 to 3%.

Twelve americium- and twelve curium-exposed dogs have been sacrificed to date. The americium-241 tissue distribution data for the medium and high dose level dogs are given in Table 3.10. The curium-244 data are in Table 3.11. Radiochemical analyses for the low dose level dogs are still in progress. There were frequently large differences between the tissue distribution of the high dose level dogs and the medium dose level dogs sacrificed at the same time postexposure. There was no clear dose-related trend except that the fraction of the final body burden in the muscle and other tissues was higher in the medium dose level dogs. The fraction of the final body burden retained in the lungs was usually less for the medium dose level dogs, especially at later times postexposure.

Means of the tissue distribution data for the high and medium dose level dogs are shown in Figure 3.4. Translocation of both ^{241}Am and ^{244}Cm following inhalation of the oxides was rapid, the ^{244}Cm moving out of the lung twice as fast as the ^{241}Am,

TABLE 3.10. Tissue Distribution Data in Dogs After Inhalation of $^{241}AmO_2$ (% of final body burden).

	10 DAY PE		30 DAY PE		90 DAY PE		270 DAY PE	
	M	H	M	H	M	H	M	H
DOG NO.	473 F	651 F	529 F	614 F	532 F	563 F	579 F	638 F
LUNG	78.5	81.3	55.4	53.0	29.4	44.4	13.8	16.7
LIVER	4.1	8.9	18.9	22.7	38.8	21.4	32.4	48.8
SKELETON	5.1	4.0	10.9	16.6	18.2	23.6	45.1	31.2
MUSCLE	8.0	3.2	11.3	5.0	9.9	8.0	4.4	1.7
OTHER	4.3	2.6	3.5	2.7	3.7	2.6	4.3	1.7
FINAL BODY BURDEN (nCi)	131.1	1073.0	112.8	1297.0	119.6	1198.0	153.7	590.0

M - MEDIUM DOSE LEVEL
H - HIGH DOSE LEVEL
PE - POST EXPOSURE

TABLE 3.11. Tissue Distribution Data in Dogs After Inhalation of $^{244}CmO_2$ (% of final body burden).

	10 DAY PE		30 DAY PE		90 DAY PE		270 DAY PE	
	M	H	M	H	M	H	M	H
DOG NO.	633 F	655 F	686 F	643 F	548 F	598 F	658 F	635 F
LUNG	32.9	32.9	19.9	23.2	18.0	17.5	8.1	15.2
LIVER	29.1	29.0	37.1	25.1	35.5	48.4	33.6	42.1
SKELETON	18.4	23.2	19.8	36.8	29.7	24.6	26.6	31.5
MUSCLE	12.5	9.4	13.0	8.5	10.0	4.3	26.7	6.1
OTHER	7.1	5.5	10.2	6.4	6.8	5.2	5.0	5.1
FINAL BODY BURDEN (nCi)	185.0	502.0	69.5	405.0	190.0	3300.0	77.5	955.0

M - MEDIUM DOSE LEVEL
H - HIGH DOSE LEVEL
PE - POSTEXPOSURE

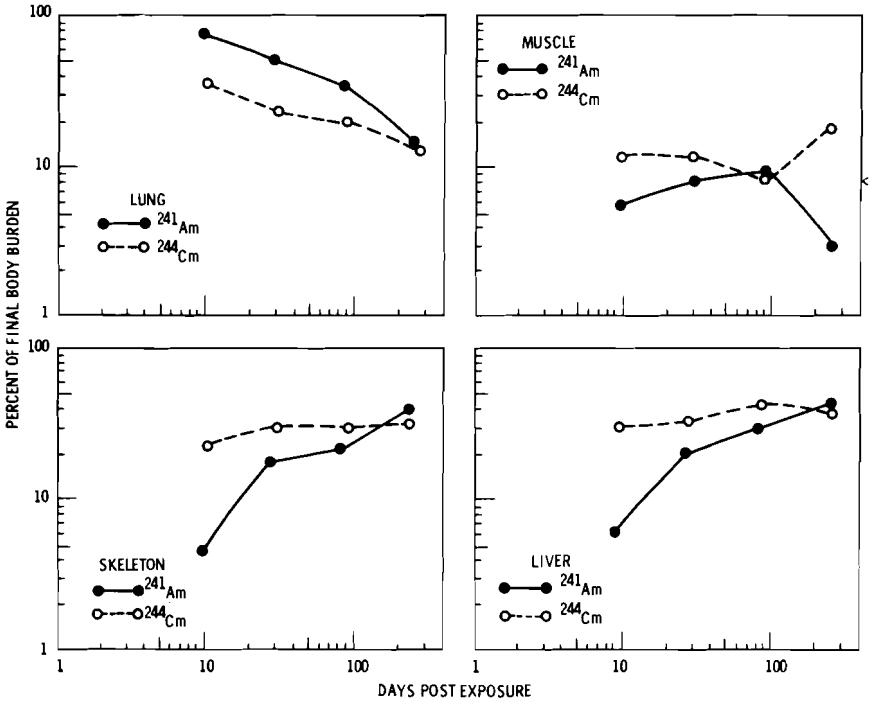

FIGURE 3.4. Distribution of ^{241}Am and ^{244}Cm in Tissues After Inhalation of the Oxides.

initially. Twenty percent of the ^{241}Am and 62% of the ^{244}Cm was found in tissues other than the lung 10 days after exposure; therefore, 7-day postexposure external thorax counts cannot be assumed to give good estimates of initial alveolar deposition. There was little change in the ^{244}Cm tissue distribution after 30 days postexposure. However, americium-241 continued to accumulate in the liver and skeleton and the fractions of ^{244}Cm and ^{241}Am in the lung, skeleton and liver were similar at 270 days postexposure.

After the first week, and until 30 days postexposure, about 80% of the excreted ^{241}Am was found in the feces compared with about two-thirds of the ^{244}Cm. However, by 90 days postexposure, roughly equal quantities of both isotopes were excreted in feces and urine.

It is not clear whether differences in the translocation rate of ^{241}Am and ^{244}Cm are due to chemical differences, specific activity differences, or particle size differences. However, the oxides of americium and curium clearly do not behave like the oxides of plutonium. Figure 3.5 compares the isotope tissue distribution in dogs from one week to about a year after inhalation of the oxides of four transuranic isotopes (^{239}Pu, ^{238}Pu, ^{241}Am and ^{244}Cm). The oxides were prepared by similar methods of calcining the oxalate at 700 to 750°C. For all four oxides the density range from 9.8 to 11.4 g/cm^3 and initial ultrafilterability varied from 0.002% for $^{239}PuO_2$ to 2.24% for $^{238}PuO_2$. The rate of translocation of material from lung to other tissues increased from ^{239}Pu to ^{238}Pu to ^{241}Am to ^{244}Cm, possibly reflecting the decrease in mean particle size from an MMD of 0.7 μm for $^{239}PuO_2$ to 0.6 μm for $^{238}PuO_2$ to 0.4 μm for $^{241}AmO_2$ to 0.1 μm for $^{244}CmO_x$. Accumulation of the isotopes in the liver and skeleton, as a percentage of final body burden, was 1% for ^{239}Pu, and 7 to 23% for ^{238}Pu at about a year postexposure; while at 270 days postexposure, values were 40% for ^{241}Am and 30 to 40 for ^{244}Cm. Less than 1% of the ^{244}Cm and ^{241}Am, compared to 5 to 10% of the plutonium, was in the thoracic lymph nodes.

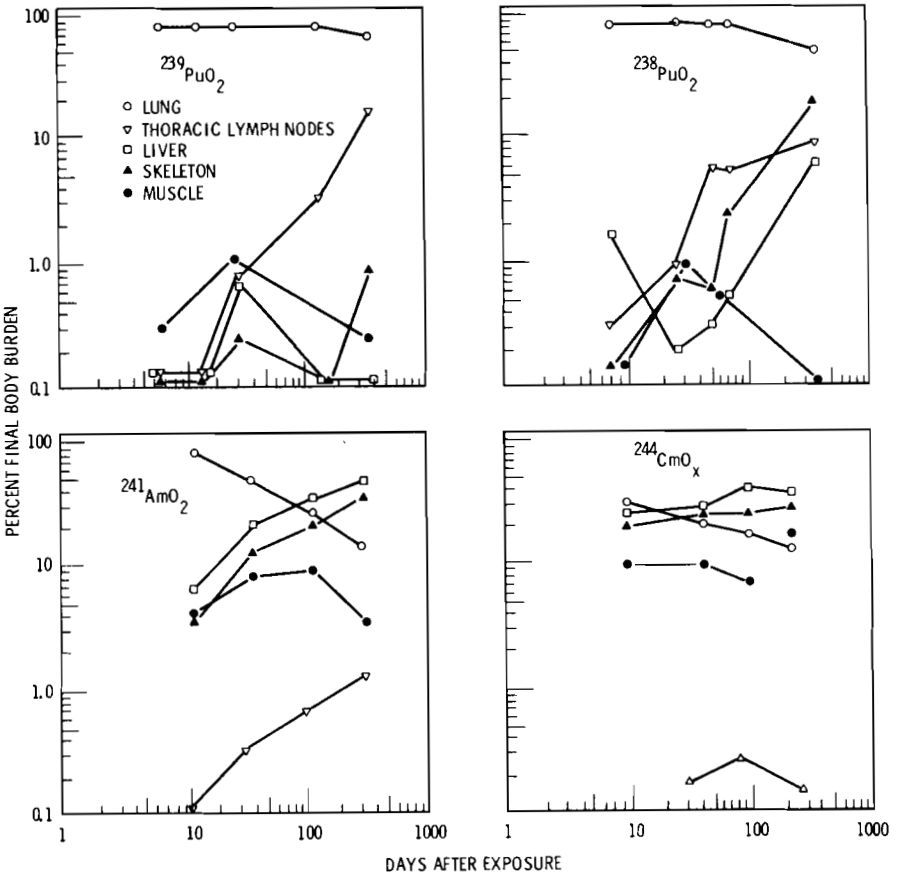

FIGURE 3.5. Distribution of ^{239}Pu, ^{238}Pu, ^{241}Am and ^{244}Cm in Tissues After Inhalation of the Oxides.

LOW LEVEL PLUTONIUM NITRATE INHALATION STUDIES IN BEAGLES

Person in Charge: G. E. Dagle

The objective of this study is to determine the dose-effect relationships of inhaled plutonium nitrate in life-span studies in beagle dogs. The critical tissue after inhalation of "soluble" plutonium (such as plutonium nitrate) has been generally considered to be the skeleton or liver, on the assumption that such plutonium will be rapidly translocated from the lung to skeleton and liver. In several rodent studies, however, inhalation of "soluble" plutonium has resulted in lung tumors as well as skeletal tumors.

The experimental design, patterned after the PuO_2 studies in progress in this laboratory, is shown below:

Initial Alveolar Deposition, μCi	Number of Animals	
	$^{239}Pu(NO_3)_4$	$^{238}Pu(NO_3)_4$
3	10	10
1.25	20	20
0.25	20	20
0.05	20	20
0.01	20	20
0.002	20	20
Vehicle Control	20	20
Unexposed Control	20	20

Beagles will be exposed to aerosols of $^{239}Pu(NO_3)_4$ during FY 1976 and 1977, and to $^{238}Pu(NO_3)_4$ during FY 1978 and 1979. Additional dogs will be exposed for periodic sacrifice to provide information on deposition and translocation of plutonium nitrate and to study the pathogenesis of dose-related effects.

This project was first funded in FY 1976. Preliminary work, such as the breeding of animals for use in the experiment, was performed under another project and animals have already been exposed for obtaining kinetic data on deposition and early translocation and for the standardization of exposure techniques.

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TOXICOLOGY OF INHALED PLUTONIUM AND TRANSPLUTONIUM ELEMENTS IN RODENTS

Person in Charge: C. L. Sanders

The broad objective of this project is to obtain from experimental animals that have inhaled compounds of plutonium and transplutonium elements, the biological data that will assist in hazard evaluation and in the establishment of permissible exposure limits for man. The project is concerned primarily with carcinogenic effects in rats and hamsters, particularly in relation to alveolar deposition, radiation dose, and its spatial-temporal distribution in lung. Emphasized in this project are the induction and and pathogenesis of pulmonary metaplasia and neoplasia, and neoplasia in extrapulmonary sites. Studies of pulmonary clearance, translocation, microdosimetry, biochemistry, and cocarcinogenesis as related to the transuranic-induced pulmonary neoplasia are also included.

The major concern of this project in the recent past has been with life-span studies. These have included the following inhalation treatments, with the numbers of animals involved shown in parentheses: hamsters - $^{238}PuO_2$ (180), $^{239}PuO_2$ (180), untreated controls (170); Wistar rats - $^{238}Pu(NO_3)_4$ (500), $^{239}Pu(NO_3)_4$ (500), $^{238}PuO_2$ (350), $^{239}PuO_2$ (350), "aged" $^{238}PuO_2$ (70), $^{244}CmO_2$ (280), air oxidized $^{239}PuO_2$ (70), $^{253}Es(NO_3)_3$ (180), nitric acid controls (100), untreated controls (250). Life-span studies with $^{241}AmO_2$ in Wistar rats will be initiated in the near future.

As these life-span studies are concluded, it is anticipated that increased emphasis will be placed on experiments that are designed to provide insight into the mechanisms responsible for observed effects. Such experiments will involve autoradiographic evaluation of microdosimetry, electron microscopic study of the pathogenesis of lung tumors, biochemical study of species differences in tumor susceptibility, and in-vitro studies with cultured lung cells. It is anticipated that as new chemical and physical forms of transuranic elements are identified to be significant potential hazards of fuel cycle operations, the toxicity of these compounds will be studied in rodents under this project.

PUBLICATIONS SINCE 1973

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INHALATION TOXICOLOGY OF $^{238}PuO_2$, $^{239}PuO_2$, AND $^{244}CmO_2$ IN RATS

Investigator:
C. L. Sanders
Technical Assistance:
D. M. Meier

Wistar rats were given a single, nose-only exposure to freshly prepared, high-fired transuranic oxides and observed for their life span. Of 294 rats exposed to $^{238}PuO_2$, 33 developed lung tumors; of 295 exposed to $^{239}PuO_2$, 56 developed lung tumors; and of the 239 exposed to $^{244}CmO_2$, 23 developed lung tumors, 12 developed bone tumors and two developed liver tumors. Bone or liver tumors were not seen with inhaled $^{238}PuO_2$ or $^{239}PuO_2$. No lung, bone, or liver tumors were observed in 118 unexposed control rats. Squamous carcinoma of the lung was seen mostly at high doses of PuO_2; adenocarcinoma was the predominant lung tumor type at lower doses for PuO_2 and at all doses for $^{244}CmO_2$. Pulmonary hemangiosarcoma was seen only with $^{239}PuO_2$.

Preliminary results of these experiments were reported in last year's Annual Report. Complete results are now available except for retention and radiation dosimetry data for ^{244}Cm. Comparisons of these data are of particular interest because the specific activity of ^{238}Pu is about 280 times that of ^{239}Pu, while the specific activity of ^{244}Cm is about 1400 times that of ^{239}Pu; $^{238}PuO_2$ exhibits a greater mobility in tissues than does $^{239}PuO_2$, and $^{244}CmO_2$ is considerably more mobile than either.

Wistar rats were exposed to transuranic dioxides within 48 hours after their calcination at 750°C. Exposure data and particle size characteristics of the generated aerosols are shown in Table 5.1. Particle sizes decreased with increasing specific activity of the transuranic dioxides.

The average activity for alpha emissions per particle of transuranic dioxide was about 10 times less for $^{239}PuO_2$ than for $^{238}PuO_2$. Such calculations were not made for $^{244}CmO_2$

TABLE 5.1. Exposure Data and Particle Size Characteristics of $^{238}PuO_2$, $^{239}PuO_2$, and $^{244}CmO_2$ Inhaled by Rats.

INITIAL ALVEOLAR DEPOSITION, nCi [a]		AVERAGE AMOUNT OF TRANSURANIC IN AEROSOL CHAMBER,	PARTICLE SIZE CHARACTERISTICS [b]				ALPHA DISINTEGRATIONS PER WEEK PER CMD PARTICLE
METHOD A	METHOD B	nCi/ℓ	AMAD, μm	GSD	CMD, μm	$\Sigma\chi^2$	
			$^{239}PuO_2$				
0.15 ± 0.07	---	3.0	1.6	1.9	0.13	21.0	1.5×10^1
0.20 ± 0.05	---	1.0	2.2	1.8	0.21	13.0	6.5×10^1
4.4 ± 1.6	5.5 ± 2.3	28.0	2.2	1.7	0.28	5.4	1.6×10^2
56 ± 29	43 ± 12	210.0	2.4	1.8	0.28	6.5	1.6×10^2
---	47 ± 25	720.0	3.3	1.7	0.47	14.0	8.0×10^2
184 ± 54	---	1300.0	3.4	1.7	0.43	7.5	6.0×10^2
			$^{238}PuO_2$				
0.07 ± 0.04	---	0.21	2.3	1.8	0.25	19.0	3.1×10
---	0.20 ± 0.04	0.16	2.6	2.7	0.04	11.0	1.0×10
9.9 ± 4.9	9.0 ± 3.2	34.0	1.6	2.8	0.02	5.1	1.3×10
12 ± 9.0	13 ± 2.9	56.0	1.8	2.3	0.07	23.0	6.2×10
---	218 ± 97	450.0	1.2	1.7	0.14	1.0	5.3×10
887 ± 245	---	1800.0	1.3	1.7	0.16	4.0	7.8×10
			$^{244}CmO_2$				
0.22 ± 0.08	---	0.4	0.7	3.1	<0.01	50	
2.9 ± 0.4	6.0 ± 0.9	3.6	1.3	2.1	0.07	25	
15 ± 4.1	32 ± 29	58.0	0.9	2.4	0.04	34	
---	710 ± 100	640.0	0.7	2.6	0.01	28	
790 ± 240	---	660.0	0.5	2.6	0.01	54	

[a] $\bar{X}$ ± S.D.; METHOD A, AMOUNT IN LUNG AT 24 HOURS AFTER EXPOSURE; METHOD B, AMOUNT IN BODY AT 30 DAYS AMOUNT EXCRETED FROM 4-30 DAYS AFTER EXPOSURE

[b] CASCADE IMPACTOR ANALYSES; IF $\Sigma\chi^2$ IS <15, PARTICLES ARE NORMALLY DISTRIBUTED WITH RESPECT TO THE LOGARITHM OF THEIR AERODYNAMIC EQUIVALENT DIAMETERS. AMAD = ACTIVITY MEDIAN AERODYNAMIC DIAMETER, GSD = GEOMETRIC STANDARD DEVIATION, CMD = COUNT MEDIAN DIAMETER.

because of particle-size uncertainties associated with its non-log-normal distribution.

Of the 1061 rats employed in these studies, 118 were unexposed controls and 115 were sacrificed at intervals following exposure to determine early retention and tissue distribution of the inhaled transuranics. The remainder of the animals were observed for their life span: for ^{238}Pu, 294 animals; for ^{239}Pu, 295; and for ^{244}Cm, 239.

Plutonium was cleared from the lung in a biphasic pattern; the more rapidly clearing component exhibited a half-time of about 30 days; the slower component exhibited a half-time of about 180 days for ^{238}Pu and 250 days for ^{239}Pu. For ^{238}Pu, the rate of alveolar clearance was influenced by the quantity deposited, the clearance being more rapid at the lower exposure levels (Figure 5.1). The clearance of ^{239}Pu was little affected by the quantity deposited except at the highest deposition level (Figure 5.2). Curium was cleared much faster than Pu, with about 90% eliminated within 30 days, and the remainder lost, with a half-time of about 1000 days.

Of the initial alveolar deposit, about 5% of ^{239}Pu, 10% of ^{238}Pu and 50% of ^{244}Cm was retained within the body at 2 years after exposure, mostly in pulmonary lymph nodes, liver, and bone. The more insoluble the transuranic dioxide, the smaller the uptake by bone and liver, and the smaller the total body burden at 2 years after exposure.

Autoradiographic studies indicated that plutonium particles were concentrated in subpleural and peribronchiolar regions of the lung within several months after exposure, at alveolar depositions greater than

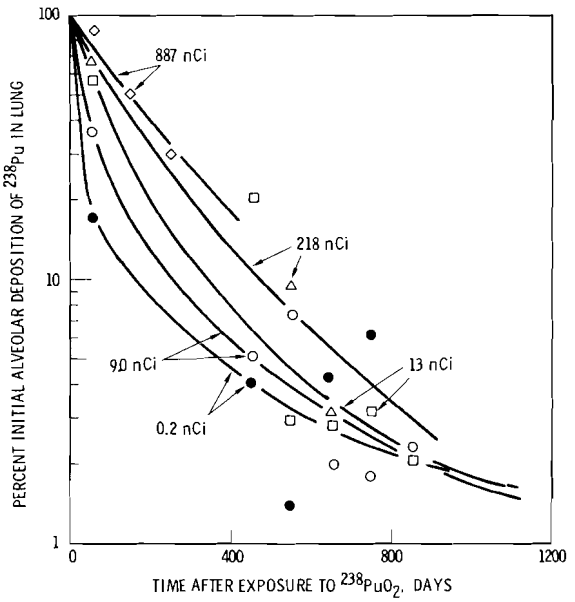

FIGURE 5.1. Alveolar Clearance of Inhaled $^{238}PuO_2$. Each point represents a mean for all rats in a group necropsied at 100-day intervals with data being plotted on the 50-day points for each interval.

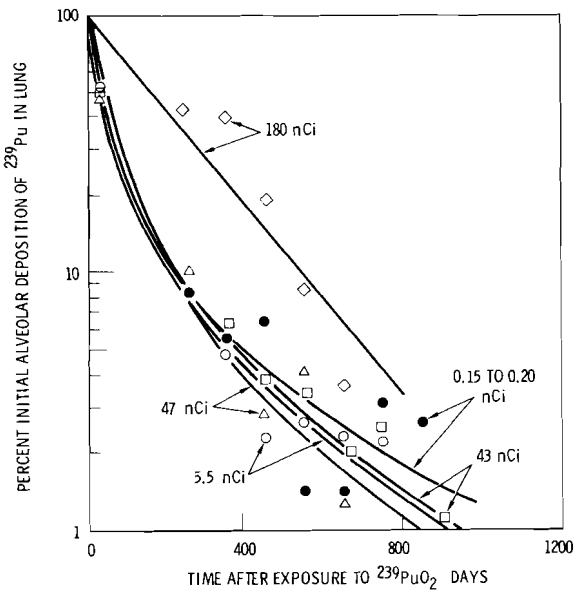

FIGURE 5.2. Alveolar Clearance of Inhaled $^{239}PuO_2$. Each point represents a mean for all rats in a group necropsied at 100-day intervals with data being plotted on the 50-day points for each interval.

10 nCi. There was little such concentration of Cm at any deposition level, most of the alpha tracks being single or in aggregates associated with macrophages or hemosiderin-like pigments.

Estimates of the radiation dose to the lung (average organ dose) were made for each animal based on the radionuclide content of the lung at autopsy and the average retention kinetics for the radionuclide in lung. Animals were divided into radiation-dose-to-lung cohorts for consideration of dose-effect relationships (Table 5.2).

Rats accumulating less than about 1000 rads to the lung at 620 days after exposure showed no significant life-shortening (Table 5.2). Radiation pneumonitis was of greater severity at the higher Pu deposition levels, being generalized at doses that resulted in significant life shortening. Foci of radiation fibrosis were present at lower doses, associated with PuO_2 particle concentrations. Radiation pneumonitis following Cm exposure was present only in the two highest dose groups, being more diffuse than that seen with Pu. Foci of alveolar fibrosis were seldom seen with Cm.

Metaplastic lesions were frequently seen associated with foci of fibrosis following Pu exposure; such an association was not seen with Cm, although the incidences of metaplasia following Cm exposure were similar to that seen following comparable Pu exposures. Alveolar adenomatous metaplasia appeared to develop into adenocarcinoma, and squamous metaplasia into squamous carcinoma.

A total of 33 lung tumors were found in rats exposed to ^{238}Pu, 56 lung tumors in rats exposed to ^{239}Pu and 33 lung tumors in rats exposed to ^{244}Cm. No lung tumors were seen in unexposed controls. The median time of appearance of lung tumors was similar to the median life span of the group in which the tumors occurred. The predominant tumor type at all but the highest doses of Pu was adenocarcinoma, with high incidences of squamous carcinoma seen at high Pu doses (Table 5.2, Figure 5.3).

TABLE 5.2. Tumor Types Found in Rats Exposed to $^{238}PuO_2$, $^{239}PuO_2$, and $^{244}CmO_2$ Inhaled by Rats.

RADIATION DOSE TO LUNG, rads[a]	MEDIAN SURVIVAL, days [b]	NUMBER OF LUNG TUMORS/NUMBER OF RATS	INCIDENCE OF PULMONARY TUMORS, %			
			TOTAL	ADENO-CARCINOMA	SQUAMOUS CARCINOMA	SARCOMA[c]
			$^{239}PuO_2$			
<0	665	0 / 48	0	0	0	0
<1	696	0 / 63	0	0	0	0
4.2 ± 2.3	704	2 / 68	2.9	1.5	1.5	0
27 ± 12	622	4 / 51	7.8	3.9	0	3.9
78 ± 17	628	9 / 26	34.6[e]	30.8	3.8	0
255 ± 132	591	17 / 38	44.7[e]	36.8	5.3	2.6
680 ± 120	598	5 / 16	31.3	18.8	6.3	6.3
2100 ± 1210	525	12 / 18	66.7[e]	33.3	22.2	11.1
>10,000	379	7 / 15	46.7[e]	6.7	33.3	6.7
			$^{238}PuO_2$			
0	641	0 / 50	0	0	0	0
<1	655	1 / 71	1.3	0	0	1.3
3.0 ± 1.5	740	2 / 39	5.1	5.1	0	0
26 ± 11	693	1 / 50	2.0	2.0	0	0
56 ± 11	682	3 / 33	9.1	9.1	0	0
153 ± 81	625	2 / 34	5.9	5.9	0	0
1720 ± 994	570	13 / 27	48.1[e]	25.9	18.5	3.7
8340 ± 3230	561	6 / 6	100.0[e]	33.3	66.7	0
>10,000	129	5 / 26	19.2	3.8	15.4	0
			$^{244}CmO_2$			
0	698	0 / 20	0	0	0	0
0.4[d]	651	1 / 57	1.8	1.8	0	0
6.0[d]	676	2 / 61	3.3	3.3	0	0
32.0[d]	637	6 / 54	11.1	11.1	0	0
710[d]	505	14 / 43	32.6	30.2	2.3	0
1600[d]	44	0 / 24	0	0	0	0

[a] AT 620 DAYS AFTER EXPOSURE; MEAN ± STANDARD DEVIATION

[b] AFTER EXPOSURE AT 70 DAYS OF AGE

[c] MOSTLY HEMANGIOSARCOMAS

[d] INITIAL ALVEOLAR DEPOSITION, nCi

[e] SIGNIFICANT AT $P < 0.05$ LEVEL USING KOLMOGOROV-SMIRNOV TWO-SAMPLE TEST

Inhalation of either ^{238}Pu or ^{239}Pu had no effect on the induction of extrapulmonary tumors. However, following exposure to ^{244}Cm, 12 bone tumors (classified as chondrosarcoma or osteosarcoma) and two hepatomas were observed. No bone or liver tumors were seen in unexposed rats.

Four rats exposed to Pu developed lung tumors at estimated average radiation doses to lung of less than 10 rads. These tumors are of considerable importance since the present limit for occupational radiation exposure to the lung from Pu is 1.5 rads/year. While the number of observations is too small for statistical significance and the dose estimates are somewhat uncertain, the adequacy of present exposure limits must be questioned in light of these results.

Plutonium-239 appeared to be a more effective carcinogen in the lung than was either ^{238}Pu or ^{244}Cm, particularly in terms of adenocarcinoma incidence at intermediate dose levels (Figure 5.3). This may be due to the larger number of deposited $^{239}PuO_2$ particles for a given radiation dose, resulting in the irradiation of a larger number of epithelial target cells.

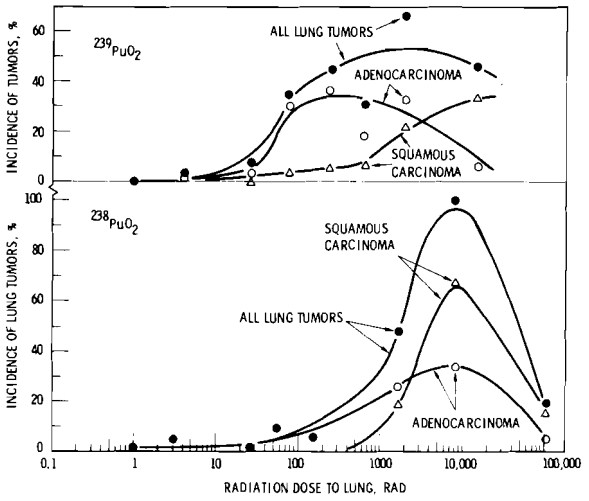

FIGURE 5.3. Relationship of Cumulative Radiation Dose to the Lung at 620 Days After Exposure to Incidence of Primary Lung Tumors, Following Inhalation of $^{238}PuO_2$ or $^{239}PuO_2$.

LATE EFFECTS OF INHALED $Pu(NO_3)_4$ AND $^{253}Es(NO_3)_3$ IN RATS

Investigators:

J. E. Ballou, G. E. Dagle and K. E. McDonald

Technical Assistance:

R. A. Gies

Preliminary results indicate that lung tumors will be more numerous than bone tumors in rats administered inhaled $^{239}Pu(NO_3)_4$, $^{238}Pu(NO_3)_4$ or $^{253}Es(NO_3)_3$ aerosols.

Male Wistar rats, 60 days of age, were administered a single, nose-only inhalation exposure to $^{239}Pu(NO_3)_4$, $^{238}Pu(NO_3)_4$ or $^{253}Es(NO_3)_3$ aerosols two to three years ago. The experimental protocol and current progress on these experiments are presented in Table 5.3.

The radiation dose estimates for Pu animals were based on the Pu content of lung and skeleton at death and retention kinetics determined from serially sacrificed animals and animals that died during the long-term phase of the study. The accumulated radiation dose from ^{253}Es was

TABLE 5.3. Occurrence of Malignant Lung and Bone Tumors in Rats Exposed to Plutonium or Einsteinium Nitrate Aerosols.

TREATMENT [a]	INITIAL LUNG BURDEN (nCi ± SD)	NUMBER OF RATS	ESTIMATED 500-DAY ACCUMULATED DOSE, rads		INCIDENCE OF MALIGNANT TUMORS [b]	
			SKELETON	LUNG	SKELETON	LUNG
$^{239}Pu(NO_3)_4$	0.06 ± 0.02	64	0.02	0.2	0/23	0/23
	1.25 ± 0.30	64	0.5	5	0/14	0/14
	2.43 ± 1.8	66	0.9	10	0/15	0/15
	142 ± 47	60	50	600	0/33	19/33
	919 ± 359	64	350	4000	1/8	4/8
$^{238}Pu(NO_3)_4$	0.02 ± 0.01	60	--	0.08	0/6	0/6
	0.52 ± 0.16	63	--	2	1/10	0/10
	3.38 ± 0.76	59	2	11	0/11	0/11
	70 ± 27	53	66	225	3/17	6/17
	1514 ± 782	72	500	5000	1/11	6/11
$^{253}Es(NO_3)_3$	3	60	0.3	3	--	--
	60	60	8	50	--	--
	850	60	110	750	0/60	0/60
NONTREATED CONTROLS		100			0/98	0/98

[a] ANIMALS WERE EXPOSED FOR 30 MINUTES TO AEROSOLS

[b] OSTEOSARCOMAS OF SKELETON; ADENOCARCINOMAS, HEMANGIOSARCOMAS AND SQUAMOUS CELL CARCINOMAS OF THE LUNG

estimated by other means because its short physical half-life (20.5 days) precluded measurement except at early times after administration. External counts (NaI crystal) were made 7 days after $^{253}Es(NO_3)_3$ inhalation, by which time external contamination was substantially reduced. Several rats were killed immediately after external counting to determine the ratio of total body count to ^{253}Es content of the lungs. The 7-day lung value estimated from this ratio was adjusted for biological losses and physical decay during the initial week following exposure. The skeletal content of ^{253}Es was estimated from serially sacrificed animals to be 35% of the initial lung burden. The retention half time of ^{253}Es in skeleton was the same as its 20.5-day physical half-life.

Approximately one-third of the animals have been examined by the pathologist, leaving about 600 rats still in process. The animals thus far examined were selected because of interesting or obvious lesions and do not represent a random sample. The preliminary results shown in Table 5.3 cannot, therefore, be extrapolated for predictive purposes. It seems noteworthy, however, that the incidence of lung tumors far exceeds the incidence of bone tumors in animals examined thus far. This seems reasonable, since the estimated radiation dose to lung is 5 to 10 times higher than the skeletal dose. Since relatively "soluble" forms of the radionuclides were employed in these studies, it might have been expected that a greater fraction of the radiation dose would have accumulated in bone. This might, indeed, be the case for a long-lived animal such as the dog, or in man, where the dose to skeleton could accumulate over a long period of time; but it is apparently not true for rats exposed to inhaled $^{238}Pu(NO_3)_4$ or $^{239}Pu(NO_3)_4$ aerosols. With $^{253}Es(NO_3)_3$ there is no possibility of long-term accumulation of skeletal radiation dose because of its short physical half-life.

INHALATION TOXICOLOGY OF AIR-OXIDIZED $^{239}PuO_2$ IN RATS

Investigator:
C. L. Sanders
Technical Assistance:
D. M. Meier

Wistar rats inhaled air-oxidized $^{239}PuO_2$ obtained from the surface of ^{239}Pu metal. The transportability of air-oxidized $^{239}PuO_2$ from the lung is considerably greater than that of high-fired $^{239}PuO_2$. Autoradiographs indicated rapid clearance from the lung of the "single track" fraction of plutonium deposited in the lung.

The temperature at which PuO_2 is formed has been shown to influence its biologic behavior following inhalation. Air-oxidized plutonium from metal surfaces could be a significant hazard for exposure of man in the nuclear industry, but this form of PuO_2 has received little study.

The $^{239}PuO_2$ employed was obtained by lightly brushing the surface of weapons grade plutonium metal, 32 months after its fabrication. Significant contaminating elements in the PuO_2 preparation were: 525 ppm ^{241}Am, 450 ppm C and 166 ppm Mg. The plutonium isotopic composition was 93.37% ^{239}Pu, 0.017% ^{238}Pu, 6.13% ^{240}Pu, .45% ^{241}Pu. Larger-sized PuO_2 particles were removed by sedimentation in a water column. The activity median aerodynamic diameters (AMAD) of the remaining smaller particles ranged from 2.1 to 2.3 μm. The ultrafilterability of this PuO_2, after 10 days in water suspension, was 0.48% which increased to 2.32% when suspended for 4 days in molar DTPA. These data indicate a substantially greater in vitro solubility for the air-oxidized $^{239}PuO_2$ as compared to high-fired $^{239}PuO_2$ of comparable AMAD.

Thirty-two rats were exposed to air-oxidized $^{239}PuO_2$, with an average initial alveolar deposition of 36 nCi. These rats were killed for tissue distribution data at intervals up to 93 days after exposure. Five rats were placed in individual metabolism cages and excreta collected for 93 days. Two other groups of 33 rats each were exposed to air-oxidized $^{239}PuO_2$ with initial alveolar deposition of 9.5 nCi and 540 nCi, respectively; these rats will be held for life-span observation (Table 5.4).

Air-oxidized $^{239}PuO_2$ moved from the lung more rapidly than high-fired $^{239}PuO_2$ (Figure 5.4). About 70% of air-oxidized $^{239}PuO_2$ was cleared from the lung by 30 days after exposure and 80% by 90 days after exposure. The skeleton and liver accumulated from 10 to 50 times more ^{239}Pu following exposure to air-oxidized $^{239}PuO_2$. There was a net loss of ^{239}Pu from liver after about 15 days postexposure; the ^{239}Pu content of skeleton increased throughout the 93-day observation period. The body burden of ^{239}Pu decreased by about 70% during the 93-day observation period, with about six times more ^{239}Pu being excreted in the feces than in the urine.

Autoradiographs showed the presence of particulate $^{239}PuO_2$, as well as single tracks, in the lung at one day after exposure. The single tracks were largely cleared from the lung by 30 days after exposure, leaving particulate $^{239}PuO_2$, distributed in a manner similar to that seen with high-fired $^{239}PuO_2$. No lung tumors were seen during the first 8 months after exposure.

TABLE 5.4. Exposure Data for Rats Inhaling Air-oxidized $^{239}PuO_2$.

INITIAL ALVEOLAR[a] DEPOSITION, nCi	NUMBER OF RATS	PARTICLE SIZE CHARACTERISTICS[b] AMAD, μm	GSD	CMD, μm	ΣX^2	TYPE OF STUDY
36 ± 27[(A)]	32	2.2	2.5	0.05	5.9	TISSUE DISTRIBUTION FOR 93 DAYS
9.5 ± 8.0[(B)]	33	2.1	1.7	0.26	7.8	LIFE SPAN
540 ± 40[(B)]	33	2.3	1.9	0.21	32	LIFE SPAN

(a) MEAN ± STANDARD DEVIATION: ESTIMATED AS Pu IN BODY AT 93 DAYS + Pu EXCRETED FROM 4-93 DAYS POST EXPOSURE (A); OR AS AMOUNT DETERMINED BY EXTERNAL COUNT AT ONE DAY AFTER EXPOSURE (B)

(b) CASCADE IMPACTOR ANALYSES: IF $\Sigma X^2 < 15$, PARTICLES ARE NORMALLY DISTRIBUTED WITH RESPECT TO THE LOGARITHM OF THEIR AERODYNAMIC EQUIVALENT DIAMETERS; AMAD = ACTIVITY MEDIAN AERODYNAMIC DIAMETER; GSD = GEOMETRIC STANDARD DEVIATION; CMD = COUNT MEDIAN DIAMETER

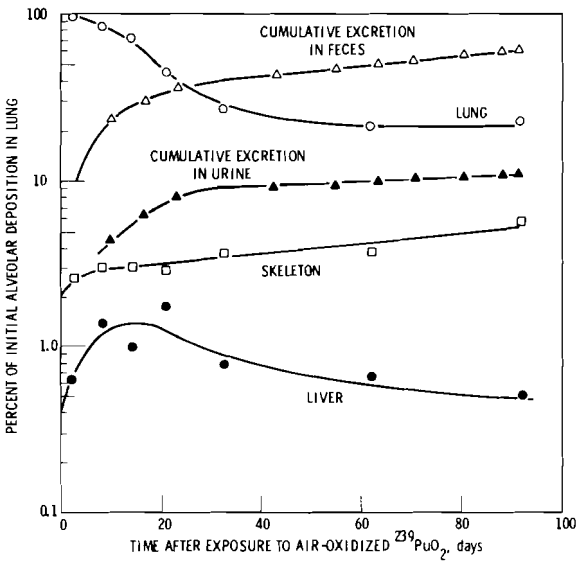

FIGURE 5.4. Kinetics of Plutonium Distribution and Excretion Following Inhalation of Air-Oxidized $^{239}PuO_2$.

INHALATION TOXICOLOGY OF $^{238}PuO_2$ AND $^{239}PuO_2$ IN SYRIAN HAMSTERS

Investigator:
C. L. Sanders
Technical Assistance:
D. M. Meier

Of 292 hamsters exposed to $^{238}PuO_2$ or $^{239}PuO_2$, only three developed malignant lung tumors, all adenocarcinomas, at initial alveolar depositions of > 160 nCi. Adenomatous metaplasia was seen in both unexposed and exposed hamsters, its severity and incidence increasing with increasing plutonium deposition. The translocation of plutonium from the lung was similar to that observed in the rat, with $^{238}PuO_2$ being more transportable than $^{239}PuO_2$.

Parallel studies have been completed, comparing the inhalation toxicology of $^{238}PuO_2$ in the rat and hamster. Preliminary results were reported in last year's Annual Report. An account of the rat data will be found elsewhere in this Annual Report.

Syrian golden hamsters were exposed to PuO_2 within 48 hours after calcination at 750°C. The particle-size characteristics were similar to those of the PuO_2 employed in the rat exposures, with a smaller particle size for $^{238}PuO_2$ than for $^{239}PuO_2$. The average particle disintegration rates for $^{238}PuO_2$ were about 100 times greater than for $^{239}PuO_2$. Both male and female hamsters were exposed, nose-only, for 30 minutes in groups of 35 animals to one of three aerosol concentrations (Table 5.5). The procedures employed are more fully described in the report on the rat studies

Of a total of 448 hamsters, about equally divided between male and female, 156 were unexposed controls, 134 were exposed to $^{238}PuO_2$ and 158 were exposed to $^{239}PuO_2$ (Table 5.8).

Plutonium deposited in the alveoli was cleared from the lung in a biphasic manner. With increased deposition of Pu, alveolar clearance decreased due to a decrease in the magnitude of the rapid clearance component (Figures 5.5 and 5.6). Distribution of Pu between lung and extrapulmonary tissues at 30 days after exposure was similar for rat and hamster, for male and female, and for $^{238}PuO_2$ and $^{239}PuO_2$ (Tables 5.6 and 5.7). Significant solubilization of $^{238}PuO_2$ occurred at later times, resulting in about 10 times more ^{238}Pu than ^{239}Pu being eventually translocated to liver and skeleton.

Plutonium dioxide particles were randomly distributed in alveolar regions of the lung at early times after exposure. Essentially all the alpha tracks on autoradiograms were in star configurations indicative of their particulate nature. Autoradiograms of lung at several months to two years after aerosol exposure demonstrated an often marked concentration of PuO_2 in subpleural or peribronchiolar regions of the lung.

TABLE 5.5. Deposition and Particle Size for Hamsters Inhaling PuO_2.

INITIAL ALVEOLAR DEPOSITION, nCi [a]	SEX	AVERAGE AMOUNT OF ^{238}Pu IN AEROSOL CHAMBER, nCi/ℓ	PARTICLE SIZE CHARACTERISTICS [b] AMAD, μm	GSD	CMD, μm	ΣX^2	ALPHA DISINTEGRATIONS PER WEEK PER CMD PARTICLE
			$^{239}PuO_2$				
3.0 ± 1.3	FEMALE	9	1.6	1.8	0.18	7.2	4.0×10^1
31 ± 24	FEMALE	160	2.3	1.8	0.25	7.2	1.1×10^2
160 ± 52	FEMALE	990	2.4	1.9	0.21	15	6.5×10^1
3.0 *	MALE	13	1.9	1.8	0.18	18	4.0×10^1
19 ± 13	MALE	110	2.3	1.9	0.22	19	7.4×10^1
200 *	MALE	1200	2.8	2.0	0.18	36	4.0×10^1
			$^{238}PuO_2$				
3.7 ± 2.0	FEMALE	11	1.1	1.7	0.14	5.5	5.3×10^3
9.8 ± 4.3	FEMALE	23	1.6	1.7	0.18	1.3	1.1×10^4
205 ± 50	FEMALE	400	1.5	1.9	0.11	4.4	2.6×10^3
5 *	MALE	10	1.1	1.6	0.15	2.9	6.4×10^3
15 *	MALE	110	1.4	1.7	0.18	1.8	1.1×10^4
200 *	MALE	400	1.6	1.9	0.12	8.2	3.4×10^3

(a) MEAN ± STANDARD DEVIATION; AMOUNT OF ^{239}Pu IN BODY AT 30 DAYS + AMOUNT IN EXCRETA AT 4-30 DAYS AFTER EXPOSURE (*ESTIMATES BASED ON AEROSOL CONCENTRATION AND PARTICLE SIZE)

(b) CASCADE IMPACTOR ANALYSES; AMAD = ACTIVITY MEDIAN AERODYNAMIC DIAMETER, GSD = GEOMETRIC STANDARD DEVIATION, CMD = COUNT MEDIAN DIAMETER. IF ΣX^2 IS <15, PARTICLES ARE NORMALLY DISTRIBUTED WITH RESPECT TO THE LOGARITHM OF THEIR AERODYNAMIC EQUIVALENT DIAMETERS

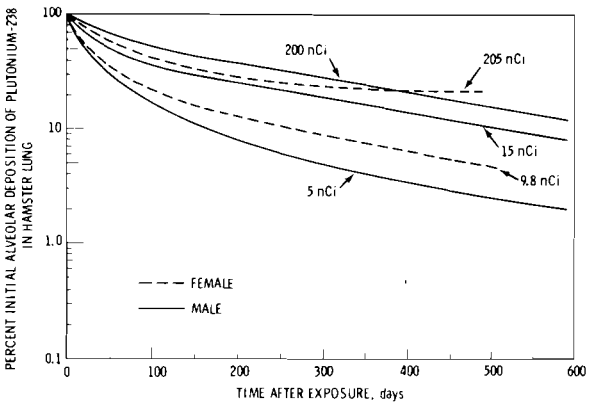

FIGURE 5.5. Alveolar Clearance of Inhaled ^{238}Pu in Male and Female Hamsters Following Inhalation of $^{238}PuO_2$.

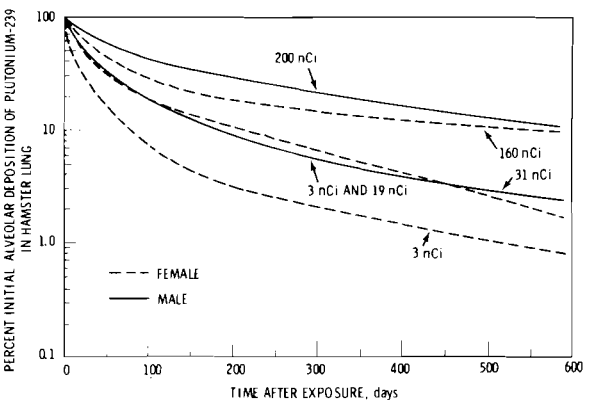

FIGURE 5.6. Alveolar Clearance of Inhaled ^{239}Pu in Male and Female Hamsters Following Inhalation of $^{239}PuO_2$.

TABLE 5.6. Distribution of ^{238}Pu in Hamster Tissues and Excreta at 30 days After Inhalation of $^{238}PuO_2$. Values are means ± S. D. (females only).

TISSUE	INITIAL ALVEOLAR DEPOSITION, nCi 3.7	9.8	205
	(PERCENT OF INITIAL ALVEOLAR DEPOSITION)		
LUNG	36 ± 19	44 ± 20	81 ± 28
PULMONARY LYMPH NODES	0.68 ± 0.62	0.51 ± 0.66	1.1 ± 0.20
LIVER	0.11 ± 0.17	0.24 ± 0.14	0.07 ± 0.03
SKELETON	0.91 ± 0.79	0.06 ± 0.10	0.24 ± 0.17
STOMACH AND INTESTINES	1.2 ± 0.46	0.65 ± 0.18	0.21 ± 0.08
KIDNEY	0.15 ± 0.12	0.09 ± 0.07	0.10 ± 0.12
SPLEEN	0.11 ± 0.17	0.03 ± 0.04	<0.01
REMAINING TISSUES	1.2 ± 0.45	0.06 ± 0.12	0.12 ± 0.05
EXCRETA[a]			
URINE	3.3	3.0	2.0
FECES	57	53	15

[a] FROM 4-30 DAYS AFTER EXPOSURE

Exposure to PuO_2 appreciably decreased life span only in males that received the highest amount of $^{239}PuO_2$ (Table 5.8). All hamsters, irrespective of treatment, exhibited amyloidosis in the liver and kidney, of sufficient severity to be the probable cause of death in most of the animals.

At the highest deposition levels, there was observed an early acute inflammatory reaction in the lung, consisting of numerous mononuclear cells filling the air spaces, prominent and numerous type II alveolar epithelial cells on the alveolar septa, edema of the alveolar septa and, later, thickening of the septa due to interstitial fibrosis. The radiation pneumonitis syndrome in hamsters exhibited a more reactive inflammatory phase than in the rat. Radiation fibrosis was most severe in distal regions of lung lobes and in subpleural regions. Overall, however, radiation pneumonitis was less severe in hamsters than in rats at comparable deposition levels.

TABLE 5.7. Distribution of Inhaled ^{239}Pu in Tissues and Excreta at 30 Days After Inhalation of $^{239}PuO_2$. Values are means ± S. D.

TISSUE	INITIAL ALVEOLAR DEPOSITION, nCi 3.0	19[a]	31	160
	(PERCENT OF INITIAL ALVEOLAR DEPOSITION)			
LUNG	31 ± 13	46 ± 32	46 ± 21	59 ± 19
PULMONARY LYMPH NODES	0.14 ± 0.12	0.45 ± 0.68	0.32 ± 0.06	0.51 ± 0.25
LIVER	0.13 ± 0.11	0.12 ± 0.05	0.20 ± 0.15	0.10 ± 0.12
SKELETON	0.19 ± 0.30	0.41 ± 0.23	0.29 ± 0.22	0.07 ± 0.02
STOMACH AND INTESTINES	1.2 ± 0.40	0.65 ± 0.31	0.51 ± 0.04	0.48 ± 0.06
KIDNEY	<0.01	0.06 ± 0.04	0.03 ± 0.04	0.01 ± 0.01
SPLEEN	0.15 ± 0.30	0.04 ± 0.06	0.03 ± 0.02	0.01 ± 0.02
REMAINING TISSUES	1.0 ± 0.43	2.0 ± 1.0	0.27 ± 0.15	0.12 ± 0.10
EXCRETA[b]				
URINE	6.2	4.9	7.9	4.0
FECES	60	45	44	36

[a] MALES, ALL OTHER GROUPS ARE FEMALES

[b] FROM 4-30 DAYS AFTER EXPOSURE

TABLE 5.8. Pulmonary Toxicology in Hamsters Following Inhalation of Plutonium.

INITIAL ALVEOLAR DEPOSITION, nCi	NUMBER OF ANIMALS	SEX	RADIATION[a] DOSE, rad	MEDIAN[b] SURVIVAL, days	INCIDENCE OF PULMONARY PATHOLOGY, % TUMORS	ADENOMATOUS METAPLASIA	SQUAMOUS METAPLASIA
			CONTROLS				
0	74	FEMALE	---	353	0	7	1
0	82	MALE	---	471	0	16	0
			PLUTONIUM-238 DIOXIDE				
9.8	29	FEMALE	210	357	3[c]	14	0
205	29	FEMALE	4500	288	0	34	3
5	16	MALE	110	307	0	38	0
15	29	MALE	330	382	0	24	0
200	31	MALE	4400	413	3[d]	58	3
			PLUTONIUM-239 DIOXIDE				
3	23	FEMALE	66	329	0	9	0
31	23	FEMALE	680	362	0	26	4
160	30	FEMALE	3500	324	3[d]	47	7
3	24	MALE	66	440	0	38	0
19	34	MALE	420	498	29[c]	29	0
200	24	MALE	4400	246	8[c], [d]	58	8

[a] AT ONE YEAR AFTER EXPOSURE, AS ESTIMATED FROM INITIAL ALVEOLAR DEPOSITION AND LUNG RETENTION DATA

[b] AFTER EXPOSURE AT 70 DAYS OF AGE

[c] ADENOMA

[d] ADENOCARCINOMA

The only statistically significant pathological finding in the hamster lung was the development of adenomatous metaplastic lesions. These consisted of focal to widespread proliferations of bronchioloalveolar epithelium which appeared to arise from the superficial, distal bronchiolar epithelium.

Alveolar adenomatous metaplasia was found in 6.8% of unexposed females and in 15.9% of unexposed male hamster lungs, the increased incidence in males presumably being due to their longer life span. The incidences of adenomatous metaplasia tended to increase with increasing radiation doses to lung in both male

and female and with both $^{238}PuO_2$ and $^{239}PuO_2$, to a maximum incidence of 58% at 200 nCi Pu alveolar deposition. The severity of the adenomatous lesions also increased with increasing radiation dose. Several hamsters also exhibited squamous metaplasia in the lung, although mostly at the higher deposition levels, and in association with adenomatous metaplasia.

A total of six lung tumors were found in 292 exposed hamsters; of these six tumors, three were benign pulmonary adenomas and three were malignant pulmonary adenocarcinomas. The adenocarcinomas were found only in the highest exposure groups for both sexes and both plutonium isotopes (Table 5.8). No lung tumors were seen in unexposed hamsters.

The Syrian golden hamster appears to be a poor animal model for the study of plutonium-induced pulmonary carcinogenesis because of the high spontaneous incidence of amyloidosis leading to early death, because of the high incidence of adenomatous metaplasia in unexposed animals, and because of the rarity with which lung tumors are induced by plutonium.

DISPOSITION OF INHALED $^{241}Am(NO_3)_3$ IN THE RAT

Investigators:
J. E. Ballou and R. A. Gies
Technical Assistance:
E. F. Blanton and J. P. Herring

Approximately twice as much inhaled $^{241}Am(NO_3)_3$ was excreted in urine during the first week following exposure as was the case for $^{238}Pu(NO_3)_4$, $^{239}Pu(NO_3)_4$ or $^{253}Es(NO_3)_3$. Immediately after exposure little ^{241}Am was translocated from the lung to tissues; however, after 30-60 days, the skeleton was the major site of deposition.

Preliminary investigations with inhaled nitric acid aerosols of the transuranic elements are being conducted in rodents before studying the long-term biological effects in beagle dogs. Results in rats administered $^{238}Pu(NO_3)_4$, $^{239}Pu(NO_3)_4$ and $^{253}Es(NO_3)_3$ are presented elsewhere in this Annual Report. Preliminary results of an ongoing study with $^{241}Am(NO_3)_3$ are reported here.

Thirty-five male Wistar rats, 60 days of age, were administered $^{241}Am(NO_3)_3$ aerosols generated from a 0.27N nitric acid solution of the radionuclide. A Lovelace nebulizer was employed to generate an aerosol with activity median aerodynamic diameter (AMAD) of 2.1 μm and geometric standard deviation (σ_g) of 2.2. Americium in the solution from which the

aerosol was generated was 92% ultrafilterable through Visking cellulose tubing. The rats were exposed for 30 minutes, nose-only, to an aerosol containing 170 nCi ^{241}Am per liter.

The disposition of ^{241}Am immediately after the 30-minute aerosol exposure is compared in Table 5.9 with similar results for other transurnics. Relatively less ^{241}Am was found in the lung, possibly because of the larger particle size of the $^{241}Am(NO_3)_3$ aerosol. As would be expected of larger particles, deposition was heavier in the upper respiratory tract, thus limiting pulmonary deposition. A large fraction of the total burden was cleared to the "stomach and jejunum" compartment during the 30-minute exposure. This is apparently material that has cleared from the mouth or ciliated airways and is subsequently ingested. As with the other transuranics, there was little immediate translocation of ^{241}Am from the lung to other tissues such as liver, bone or muscle.

An average of 17% of the initial lung burden of ^{241}Am was excreted in the urine during the first day after inhalation exposure; 30% was excreted in the urine during the first week. Comparable values for Es and Pu were, respectively, 5 and 8% for the first day and 8 and 18% for the first seven days. An explanation for the greater excretion of americium is not apparent.

Since this is a continuing study, only partial results are available for ^{241}Am retention and distribution. The lung, skeleton and associated soft tissues are the major reservoirs of ^{241}Am retention at both 30 and 60 days postexposure. The lung contained 17% of the body burden 30 days after inhalation exposure; this was reduced to 8% at 60 days. The skeleton contained 50% of the body burden at 30 days, and 60% at 60 days postexposure.

TABLE 5.9 Disposition of Transuranic Nitrate Aerosols in Rats Immediately After 30-Minute Inhalation Exposure. (Average values from 10 rats expressed as percent of total burden.)

AEROSOL CHARACTERISTICS[a]	$^{241}Am(NO_3)_3$	$^{253}Es(NO_3)_3$	$^{239}Pu(NO_3)_4$	$^{238}Pu(NO_3)_4$
AMAD	2.1	0.5	1.0	0.4
σ_g	2.2	1.7	1.7	1.6
UPPER RESPIRATORY TRACT				
TRACHEA	2.3	2.0	2.0	2.0
NOSE	6.0	3.0	3.0	3.0
TONGUE AND ESOPHAGUS	10.0	4.0	3.4	3.6
HEAD	6.0	6.0	3.0	2.0
STOMACH AND JEJUNUM	34.0	9.5	20.0	7.0
TOTAL	58.3	24.5	31.4	17.6
TRANSLOCATED TO TISSUE				
LIVER	0.1	0.1	0.01	0.05
RESIDUAL CARCASS	1.6	2.0	7.9	1.55
TOTAL	1.7	2.1	7.91	1.60
RETAINED IN LUNG	6.0	28.0	24.0	28.0
RETAINED IN PELT	34.0	45.0	36.0	53.0
INITIAL LUNG BURDEN (nCi)[b]	52±23	423±62	78±18	70±27

(a) AVERAGE OF THREE DETERMINATIONS

(b) AVERAGE OF 10 RATS, WITH STANDARD DEVIATION

DISPOSITION AND BIOLOGICAL EFFECTS OF INHALED PLUTONIUM IN MINIATURE SWINE

Person in Charge: M. T. Karagianes

This proposed project has not been funded, but preliminary experiments have been performed using discretionary funds. Estimates of the possible effects of inhaled plutonium in man are based largely on data from studies with rodents and dogs. Data from another larger species would be useful in extrapolation of this animal data to man. The miniature swine has served as a useful experimental model for man in many biomedical investigations because of physiologic and anatomic similarities.

This project would expose swine to $^{239}PuO_2$ aerosols, by inhalation, to determine short- and long-term effects. Short-term studies (up to 1 year) would investigate plutonium deposition, distribution and excretion, providing information necessary to the design and interpretation of long-term studies. Long-term experiments would be primarily concerned with evaluating carcinogenic effects, but would also provide material for the study of other possible effects and the mechanisms responsible for these effects. The results obtained from all studies would be compared with other animal and human data and would be used to assess the swine as a model for predicting effects in humans.

$^{239}PuO_2$ AEROSOL EXPOSURE OF MINIATURE SWINE

Investigators:
M. T. Karagianes, J. L. Beamer, D. K. Craig,
W. T. Kaune, J. R. Decker, W. C. Cannon
Technical Assistance:
A. J. Clary, J. P. Herring, E. L. Blanton,
M. C. Miller, E. G. Kuffel

Techniques were developed for the exposure of miniature swine to inhaled aerosols. Preliminary distribution data are reported for two swine exposed to inhaled $^{239}PuO_2$.

Initial work on this project was directed at modifying existing aerosol equipment and establishing proper techniques for the inhalation exposure of miniature swine. Instrumented swine were exposed to dye-labeled aerosols in order to characterize the anatomy of respiratory tract lymph nodes and to accumulate other physiologic data such as tidal and minute volumes. These measurements were useful in the modification of existing dog aerosol chambers (Annual Reports for 1974 and 1975) to accommodate the larger swine. The pig exposure chamber is described elsewhere in this Annual Report.

A face mask was designed and fabricated to fit the elongated snout of the pig. This mask (Figure 6.1) provides for exposure by the intranasal pathway; inflatable cuffs on the intranasal catheters prevent aerosol leakage around the external nares. All face-mask attachments and connectors are sealed with medical grade silastic to further ensure against aerosol leakage. Swine exposed by this method had an average tidal volume of 530 ml and an average minute volume of 4.7 ℓ.

Two swine were exposed to $^{198}AuCl_3$-colloid aerosols to test and evaluate chamber modifications and face-mask

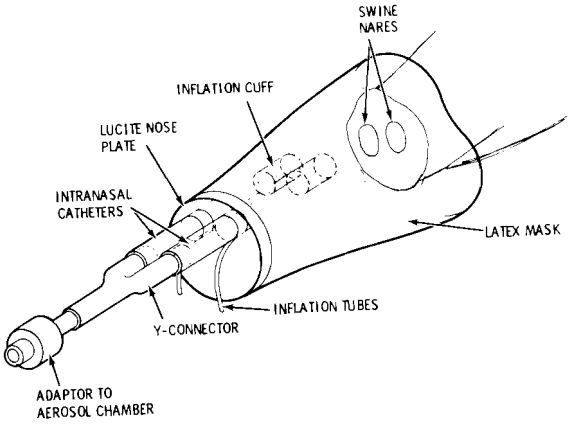

FIGURE 6.1. Face Mask for $^{239}PuO_2$ Aerosol Exposure of Miniature Swine.

exposure techniques. Minor improvements were made and two additional animals were then exposed to $^{239}PuO_2$ aerosols having an activity median aerodynamic diameter (AMAD) of 3.0 to 3.5 μm. Both animals were killed 10 days following exposure. Preliminary data are available from one of these pigs.

Total plutonium recovered at necropsy, as determined by liquid scintillation counting, was 1.72 μCi, with 94.2% of this retained in the lungs, 0.6% in the respiratory tract lymph nodes, and the remainder in the other body organs and tissues. Plutonium concentration in the lungs was highest in the larger diaphragmatic lobes with decreasing concentrations in the smaller apical, cardiac and intermediate lobe, respectively. These are data derived from only one animal but are encouragingly similar to those reported for rodents and dogs at 10 days postexposure.

The exposure of swine via the inhalation route has been demonstrated to be a feasible procedure.

INHALATION HAZARDS TO URANIUM MINERS

Person in Charge: B. O. Stuart

The goal of this project is to identify the specific uranium mine air contaminants, and the critical levels of these contaminants, that initiate or promote the development of respiratory tract pathology, including carcinoma, fibrosis, and emphysema. The high incidence of lung cancer among uranium miners of the Colorado plateau, in a period of increasing demand for uranium ore, is a matter of national concern. Epidemiological studies among uranium miners have been based on very uncertain exposure histories. Studies with experimental animals offer the only hope of unraveling the complex etiology of observed effects. Of particular concern are possible effects of the drastically increased mine ventilation rates demanded by new regulations, which will markedly alter radon:radon daughter ratios, and create new patterns of radiation dose, possibly altering the tissue at risk.

Under this project, large and small laboratory animals have received daily, life-span exposures, by realistic inhalation techniques, at levels and ratios of mine air contaminants that have been correlated with actual human exposure conditions. Massive fibrosis, bullous emphysema, and respiratory tract neoplasia have been produced in beagle dogs after 4 to 5-1/2 years of daily exposures to radon daughters with uranium ore dust, with and without concurrent cigarette smoking, as well as in hamsters following life-span exposures to radon daughters, with and without uranium ore dust.

Current research efforts include studies of pulmonary pathogenesis induced by chronically inhaled radon daughters, uranium ore dust, and cigarette smoke in beagle dogs, together with interspecies comparisons in simultaneously exposed mice, hamsters, and rats. Data from these comparative studies will permit extrapolation of the dose-effect relationships to man. Also in progress are studies of the biological consequences of varying the attached versus unattached ratios of radon daughter radionuclides. These studies will provide data to evaluate the relative hazards of dusty versus minimal-dust conditions in uranium mine operations. Studies to assess the relative hazards of Rn, RaA, and RaC' will provide data essential to the evaluation of inhalation hazards in future, underground, uranium mining as compared to conditions prevalent in the early 1950s. All of these investigations have a direct bearing upon permissible mine-air concentrations, and indirectly on the risk-benefit assessment of nuclear energy utilization.

PUBLICATIONS SINCE 1973

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PULMONARY NEOPLASTIC RESPONSE IN BEAGLE DOGS EXPOSED DAILY TO RADON DAUGHTERS, URANIUM ORE DUST, AND CIGARETTE SMOKING

Investigators:
R. F. Palmer, R. E. Filipy,
G. E. Dagle, and B. O. Stuart
Technical Assistance:
W. Skinner, K. C. Upton, and C. R. Petty

Beagle dogs that received daily exposures for up to 5 years to radon daughters (600 WL) with ore dust and/or cigarette smoke developed a variety of severe pulmonary diseases including squamous carcinoma, bronchiolar-alveolar carcinoma, and fibrosarcoma.

To determine the combined and separate roles of radon daughters, uranium ore dust, and cigarette smoking in the development of lung cancer and other respiratory tract pathology, two groups of beagle dogs received inhalation exposures to 600 WL of radon daughters and 15 mg/m^3 uranium ore dust, with and without concomitant exposure to daily cigarette smoking. A third group was exposed only to daily cigarette smoking, and a fourth served as control animals. Table 7.1 shows the original experimental design, and the number of animals remaining alive in each exposure group on November 1, 1975. Due to the rapidly deteriorating health of the animals in Groups 1 and 2, exposures to radon daughters and uranium ore dust were discontinued after August 15, 1974--5 years after exposures started.

Twenty dogs from Groups 1 and 2 died of pulmonary insufficiency during the past year. Pathologic changes in the lungs of the Group 1 and Group 2 dogs included severe discoloration of the pleural surfaces and pleural thickening with small areas of scar tissue. 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

TABLE 7.1. Experimental Design and Animals Alive on 11/1/75

GROUP	NUMBER OF ANIMALS ORIGINAL	NUMBER OF ANIMALS ALIVE 11/1/75	EXPOSURE REGIMEN
1	20	0	600 WL RADON DAUGHTERS WITH URANIUM ORE DUST (CARNOTITE) 15 mg/m^3 (4 HR/DAY, 5 DAYS/WEEK)
2	20	1	CIGARETTE SMOKE PLUS 600 WL RADON DAUGHTERS WITH URANIUM ORE DUST (CARNOTITE) 15 mg/m^3
3	20	14	CIGARETTE SMOKE (10 CIGARETTES/DAY, 7 DAYS/WEEK)
4	9	5	CONTROLS (SHAM SMOKED)

to marked emphysema occurred beneath the parietal pleura in the thoracic cavity of many dogs. Extensive pleural and alveolar septal fibrosis, as well as chronic inflammatory changes caused by uranium ore-induced pneumoconiosis, were observed, and radiation-induced epithelial changes had occurred. Adenomatous proliferation of alveolar epithelium and squamous metaplastic changes in the alveolar epithelium became more extensive as the duration of the exposure increased. Infiltration of mononuclear inflammatory cells in perivascular and peribronchiolar areas and dilation of bronchial mucus glands were frequent observations. These changes are associated with chronic bronchitis, and they 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 the lungs of all dogs from these two groups.

Epithelial neoplasms have been found in the respiratory tracts of six dogs. Two pulmonary bronchiolar-alveolar carcinomas and a pulmonary squamous cell carcinoma occurred in Group 1 dogs; one dog in Group 1 and one dog in Group 2 developed squamous cell carcinomas of the nasal turbinates; and one dog in Group 2 developed a pulmonary fibrosarcoma.

One of the pulmonary neoplasms was a well circumscribed, 2-mm-diameter mass in the deep lung parenchyma of the apical lobe of a dog which had been exposed to radon daughters and uranium ore dust for 51 months. This tumor was considered a low-grade bronchiolar-alveolar carcinoma. Another pulmonary neoplasm was a more anaplastic bronchiolar-alveolar carcinoma. It was observed subpleurally in the right apical lobe of the lung from a dog exposed to radon daughters and uranium ore dust for 54 months.

A squamous carcinoma was present in the lung of a dog after 54 months of exposure to radon daughters and uranium ore dust. It originated in the metaplastic alveolar epithelium of the right diaphragmatic lobe, adjacent to and walling off an emphysemic bulla. Squamous metaplasia of alveolar epithelium adjacent to emphysemic bullae was seen in the lungs of the same dog.

A Group 1 dog developed a neoplasm on the left side of the face after 48 months of exposure. The tumor was grossly apparent and upon dissection found to be raised and approximately 4 to 5 cm in diameter. It had nearly occluded the nasal passage and invaded the bony structure of the maxilla and turbinates. No metastases to other organs were found. The tumor was a squamous cell carcinoma and probably originated in the metaplastic mucosa of the nasal turbinates. Another squamous cell carcinoma involved the nasal turbinates in a Group 2 dog after 51 months of exposure to radon daughters and ore dust, and 57 months to cigarette smoke. This tumor had invaded the adjacent bone of the turbinates, but invasion was not as extensive as that seen in the first dog.

A dog exposed to radon daughters and uranium ore dust for 54 months and killed 8 months after cessation of exposure because of increasing respiratory distress, showed a well circumscribed thoracic mass on radiographs, which gross necropsy revealed as a tumor, roughly spherical and approximately 3 cm in diameter, in the anteroventral portion of the right apical lobe of the lung. Microscopically, the tumor consisted of irregular, spindle-shaped cells separated by strands of collagen (Figure 7.1). Numerous mitotic figures

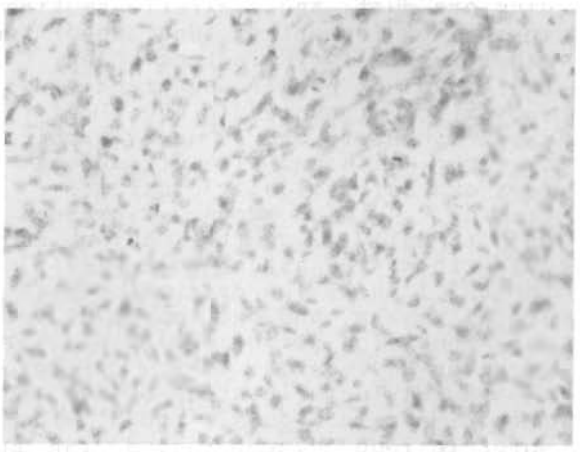


FIGURE 7.1. Pulmonary Fibrosarcoma Occurring in the Right Apical Lobe of a Dog Exposed Daily to Inhaled Radon Daughters With Uranium Ore Dust. H&E. Magnification 130X.

were present in the tumor, as were necrotic areas. The lung appeared to be the primary site, and the tumor was diagnosed as a fibrosarcoma, a rare tumor in the lungs of dogs.

These findings show for the first time that it is possible to induce primary neoplasms in the lungs of large experimental animals by chronic daily exposure to uranium mine air contaminants, i.e., radon, radon daughters, and uranium ore dust, with and without cigarette smoking. These exposures to known levels of air contaminants establish a direct cause-and-effect relationship between lung cancer and inhaled uranium mine aerosols, and will provide a much firmer basis for determining the absorbed radiological dose required to produce lung cancer. The contribution of the ore dust to the observed developing neoplastic changes is under study.

DOSIMETRIC STUDIES OF INHALED RADON DAUGHTERS IN DOGS

Investigators:

R. F. Palmer, P. O. Jackson,

J. C. Gaven, and B. O. Stuart

Technical Assistance:

A. C. Case, J. Forsythe, and W. Skinner

Radon daughter exposure-chamber aerosol parameters and dose rates to respiratory tracts of dogs breathing these aerosols are reported.

Short-lived radon daughters were measured by gamma spectrometry in excised respiratory tract tissues of beagle dogs after 4-hour exposures to the same radon daughters plus uranium ore dust aerosol used for our long-term studies. During the exposure periods the exposure chamber atmosphere was characterized by measuring condensation nuclei concentrations, particle size distribution of attached radon daughters, fraction of unattached radon daughters, and the concentrations of uranium ore dust and radon daughters. The chamber parameters measured are shown in Table 7.2 and the dose rates to various areas of the respiratory tract in Table 7.3. For dose calculations, the measured concentrations of RaA, RaB, and RaC were corrected for decay back to the time of sacrifice--immediately after the cessation of the 4-hour exposure period.

The data in Table 7.2 show that greater than 90% of the radon daughters were attached to uranium ore dust of a respirable size. This is further evidenced by the high concentrations of radon daughter products measured in the deep lung. Following Run 1, each lung lobe was fractionated into proximal, medial and distal thirds prior to analysis. In all cases but one, the distal fraction showed a higher concentration of radon daughters than did the others.

TABLE 7.2. Aerosol Parameters Measured in Exposure Chamber Dosimetry Runs.

	RUN 1	RUN 2
WORKING LEVEL [a]	494 ± 34	428 ± 14
UNATTACHED FRACTION OF RaA	0.07 ± 0.02	0.07 ± 0.02
RaA (nCi/ℓ)	102 ± 3	87 ± 3
RaB (nCi/ℓ)	52 ± 4	45 ± 3
RaC (nCi/ℓ)	34 ± 3	29 ± 2
RADON (nCi/ℓ)	146	131
URANIUM ORE DUST (μg/cc)	15.8	13.3
CONDENSATION NUCLEI (10^3/cc)	89 ± 8	98 ± 10
AMAD/GSD [b]		
RaA	0.55 / 1.74	0.55 / 1.65
(μm) RaB	0.61 / 1.68	0.61 / 1.65
RaC	0.62 / 1.67	0.63 / 1.78

[a] AVERAGE ± S.D.

[b] ACTIVITY MEDIAN AERODYNAMIC DIAMETER / GEOMETRIC S.D.

TABLE 7.3. Calculated Dose Rates to Various Areas of the Respiratory Tracts of Dogs Inhaling Radon Daughters Plus Uranium Ore Dust.

	RUN 1 (mrads/WLM ± S.D.)	RUN 2 (mrads/WLM ± S.D.)
LUNG	391 ± 153	237 ± 22
BIFURCATION	690	90
TRACHEA	100	20
LARYNX	540	15
TURBINATES	170	90

Alpha dose rates were determined by calculations based on measurements of gamma emissions from individual radon daughters, corrected for radiological decay. The alpha dose rates of 0.39 and 0.24 rads/WLM delivered to the parenchymal lung are somewhat higher than the average of 0.17 rads/WLM observed by Morken at the University of Rochester in dogs that received radon daughters in the absence of ore dust particles, where unattached fractions were much higher. The correspondingly much lower dose rates observed in the bifurcations and trachea (0.69 and 0.09 rads/WLM in the present studies, versus a range of 0.28 to 12.5 rads/WLM for radon daughters alone at the University of Rochester) indicate that absorbed alpha dose is not as highly concentrated in the upper respiratory tract when uranium mine exposure conditions are simulated, i.e., low unattached radon daughter fractions in the presence of airborne uranium ore dust. The wide variation in measured dose rates between individual animals exposed to the same aerosols, observed in both laboratories, shows that it is not possible to adopt a single Work-Level-Exposure to Absorbed-Dose conversion factor without incorporation of both physical and physiological parameters.

INTERSPECIES COMPARISON OF THE BIOLOGICAL EFFECTS OF INHALED RADON DAUGHTERS AND URANIUM ORE DUST

Investigators:
R. F. Palmer, K. E. McDonald
J. E. Lund, and B. O. Stuart
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 ± 0.7 μCi/ℓ. 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 ± 5 μg of uranium ore dust and 2.6 ± 0.3 μCi 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.

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. Also, rats were less affected by the presence of the uranium ore dust than were the other two species, but were somewhat more sensitive to the radiation from radon and its daughters in the absence of the ore dust.

Histopathologic examinations of the respiratory tracts and other selected organs showed pathological changes present in the experimental groups of rats to be more severe than in either hamsters or mice, in spite of the simultaneous exposure of the three species in the same chamber. Two rats in the group exposed to radon daughters without ore dust had extreme squamous metaplasia of pulmonary alveolar epithelium with keratin pearls, but without evidence of vascular invasion. These lesions are preneoplastic. One rat in the radon daughter-plus-ore group had a pulmonary squamous carcinoma with evidence of vascular invasion. Squamous metaplasia of nasal and turbinate epithelium occurred in slight and moderate degrees of severity in rats of the radon daughter-plus-ore group and the radon-daughters-only group, respectively. Rhinitis ranged in severity from slight in radon daughters-plus-ore to moderate and suppurative in the radon-daughters-only rats. Squamous metaplasia was also a feature of laryngeal, tracheal, and bronchiolar epithelium but was slightly more severe in the radon daughter-plus-ore group of rats. Other pulmonary changes included radiation pneumonitis and emphysema. The pneumonitis

was present to a greater degree of severity and with higher incidence in the radon daughters-plus-ore group. Other than the slight degree of pulmonary emphysema in 7 of 16 rats, the control rats were relatively free of pulmonary lesions.

The most significant changes observed in the control hamsters were related to amyloid deposition in the kidney, liver, and spleen with occasional deposition in other tissues, including the adrenal glands and thyroid. Occasional calcification of lung, muscle, kidney, trachea and other tissues was also observed, possibly related to decreased renal function resulting from amyloid deposition in the kidney.

Changes in hamsters, related to exposure to radon daughters or radon daughters-plus-uranium-ore, were restricted to the respiratory tract. The radon daughters group showed rhinitis with metaplastic changes of the simple columnar epithelium lining the nasal passages and the turbinates. The metaplastic change ranged from a substitution of simple squamous cells for the columnar cells, to the presence of stratified squamous epithelium with keratin formation. Metaplastic changes were also observed in the trachea, bronchioli, and alveoli. The tracheal and bronchiolar changes were less severe than those observed in the nasal passages and ranged from slight cellular atypia and piling of cells to formation of stratified squamous epithelium with keratin formation. Keratin formation was observed in only one hamster. The alveolar changes consisted mostly of cuboidal cells with large nuclei lining the alveolar spaces. In most hamsters the cellular changes were focal and scattered through the lung sections. In some, the metaplastic lesions were large and discrete, with and without the presence of stratified squamous epithelium and keratin. There was no evidence of vascular invasion or cellular changes which would indicate neoplasia in these large lesions. They were therefore classified as metaplastic rather than neoplastic. The precancerous nature of these lesions in hamsters has been established in preceding studies by observation of such changes in conjunction with neoplasia.

The radon daughter-plus-uranium-ore group of hamsters showed results similar to those seen in the radon daughter group except that metaplastic changes were nearly absent in the nasal and bronchiolar epithelium and were less prevalent in the trachea. This strongly suggests an altered deposition pattern of radon daughters in the presence of ore dust; i.e., deeper penetration into the parenchyma of the lungs.

Mice exposed in the same chamber exhibited less severe changes than either rats or hamsters. The most severe pathologic changes occurred in the epithelium of the respiratory system, especially that of the nasal passages and turbinates. Those changes ranged from very slight squamous metaplasia in the radon daughter-plus-ore group to slight squamous metaplasia in the radon-daughter mice.

Thirteen of the latter mice had small areas with sheets of squamous cells several layers in thickness accompanied by very slight keratinization in one of these animals. Slight rhinitis was present in 22 of the 32 mice in the two exposed groups. Other lesions in the respiratory tracts of mice included laryngeal, tracheal, and bronchiolar epithelial hyperplasia and squamous metaplasia, as well as radiation pneumonitis and emphysema. The incidence and degree of severity of those lesions were low, however, and no differences existed among the treated groups. Pathological changes in the control animals were restricted to very slight respiratory epithelial squamous metaplasia in two animals and peribronchiolar and perivascular infiltration of mononuclear inflammatory cells. There was one nonpulmonary neoplastic mass in a control animal which has been tentatively diagnosed as a reticulum lymphosarcoma of the intestinal tract.

These studies indicate that rats and hamsters are appropriate rodent models for degenerative and proliferative diseases (including squamous carcinoma) occurring in uranium miners, and that both species show appropriate pathologic changes related to variations in deposition and response patterns caused by the presence of uranium ore as it is encountered in the mines.

DOSE-RATE STUDIES OF RESPONSE TO INHALED URANIUM MINE AIR CONTAMINANTS IN RODENTS

Investigators:
J. C. Gaven, R. F. Palmer, and B. O. Stuart
Technical Assistance:
H. D. Steele

Hamsters and rats were exposed to radon daughters, with and without concomitant uranium ore dust, 84 hours per week for five months. Results will be compared with those from earlier studies to evaluate dose-rate effects.

An experiment was conducted to compare the nature and severity of effects from short-term, high-level radon daughter product exposures with those from protracted lower-level exposures, in order to test the validity of the current assumption that total integrated exposure is an adequate description of lung cancer risk in uranium miners. Rats and hamsters were exposed simultaneously in groups of 32 and 34, respectively, for 84 hours per week to aerosols consisting of radon plus approximately 1200 Working Levels of radon daughters, with and without 15 mg/m^3 carnotite uranium ore dust. Controls were housed in identical cages and chambers, but exposed to room air only. Exposures were stopped after 5 months, at which time the animals had received approximately 12,000 Working Level Months of cumulative exposure to radon daughters.

The animals will be held for their life spans and results will be compared with those from previous experiments in which hamsters were exposed for their full lifetimes to identical radon daughter and uranium ore dust atmospheres, but at exposure rates of only 30 hours per week. Previous exposures to 1200 WL with and without ore dust at 6 hrs/day, 5 days/week had no effect on hamster lifespans.

Figures 7.2 and 7.3 show the effects of the exposures on animal

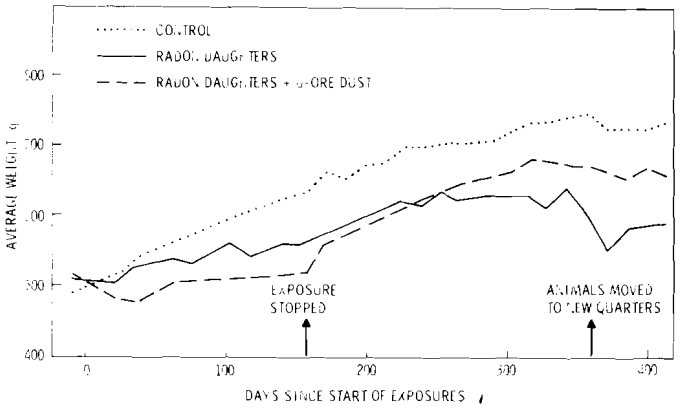


FIGURE 7.2. Weights of Rats Exposed to Radon Daughters, With and Without Concomitant Uranium Ore Dust.

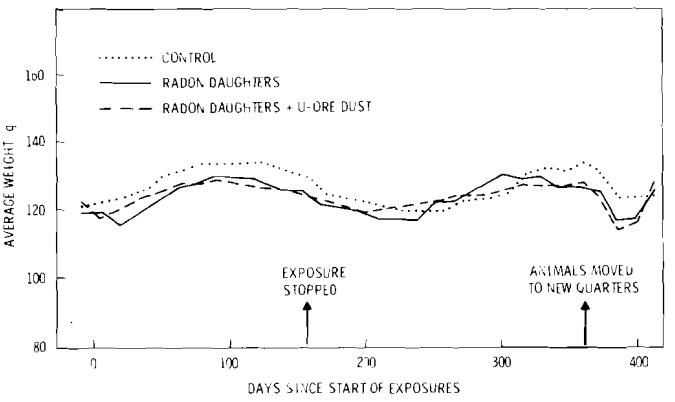


FIGURE 7.3. Weights of Hamsters Exposed to Radon Daughters, With and Without Concomitant Uranium Ore Dust.

weights; Figures 7.4 and 7.5, the effects on their survival to date. For hamsters, there is little or no difference among the three groups. The rats, however, show significant differences among the groups for both parameters. The weight data indicate that the additional insult of uranium ore dust enhances the effect of exposure to radon daughters alone during the period of exposure, but is associated with somewhat lesser mortality over a longer time span. Results of histopathologic examination of the respiratory tracts and other organs from these animals may explain these responses.

Although the experiment is still in progress, a variety of pathological changes have been observed, notably the occurrence of malignant pulmonary neoplasia in rats. The lung of a rat that died 426 days following the start of exposure to 1200 WL of radon daughters and 15 mg/m^3 of U-ore dust presented the classic appearance of squamous cell carcinoma at necropsy. It has been verified microscopically to be an invasive squamous cell carcinoma with metastases to the heart and tracheobronchial lymph nodes (Figures 7.6 and 7.7).

An invasive squamous cell carcinoma was diagnosed in the lung of a second rat from the group exposed to approximately 12,000 WLM of radon daughters with concomitant uranium ore dust. The animal had survived for 445 days since the start of exposures.

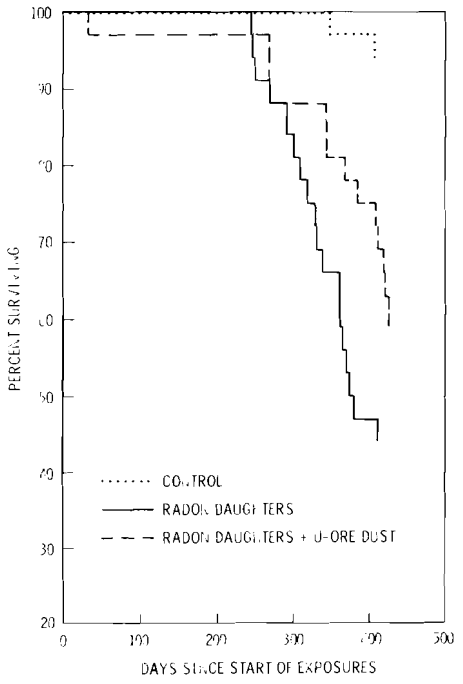


FIGURE 7.4. Survival of Rats Exposed to Radon Daughters, With and Without Concomitant Uranium Ore Dust.

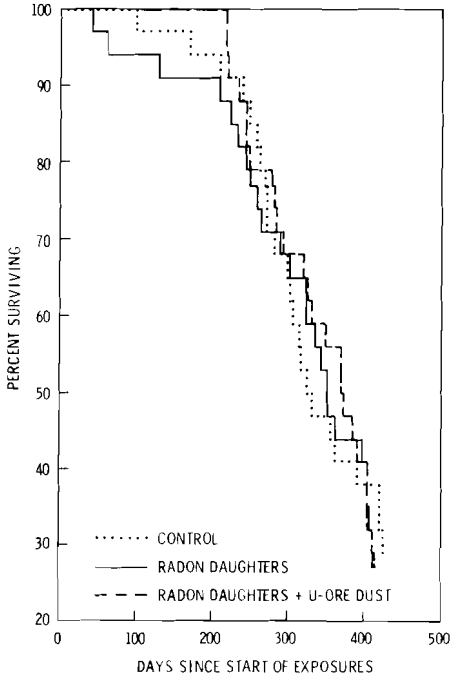
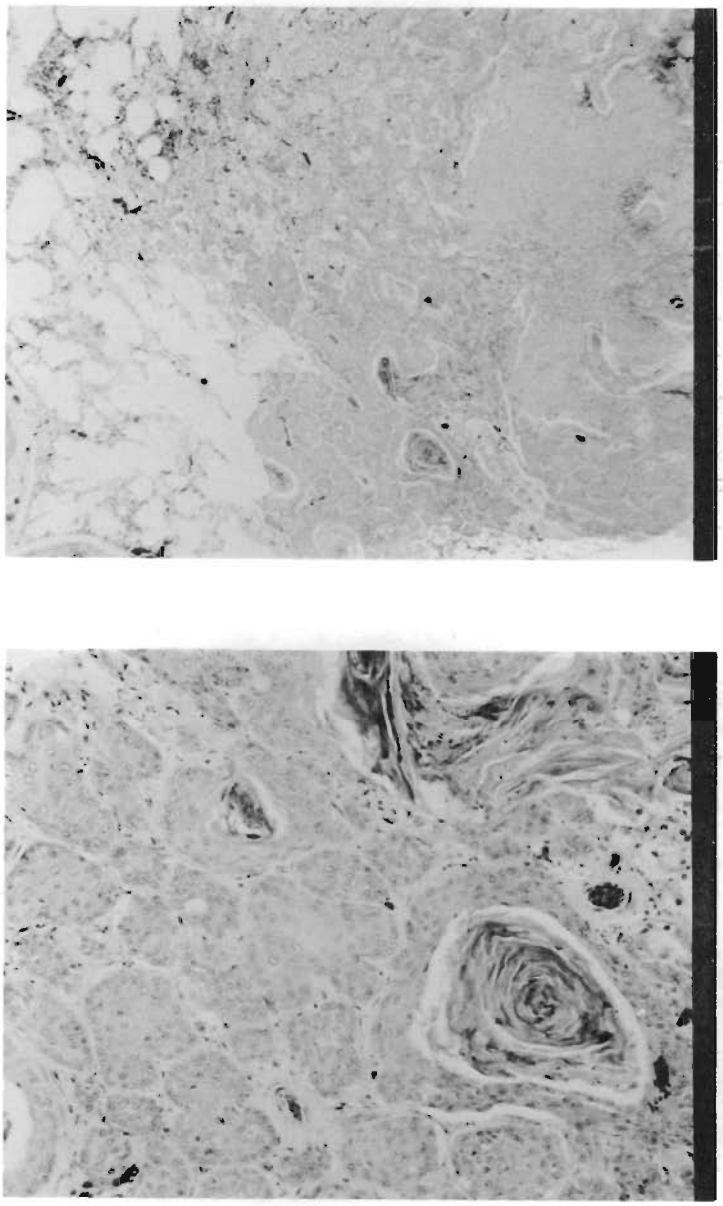


FIGURE 7.5. Survival of Hamsters Exposed to Radon Daughters, With and Without Concomitant Uranium Ore Dust.



FIGURES 7.6 & 7.7. Squamous Carcinoma in the Lungs of a Rat Exposed to Radon Daughters With Uranium Ore Dust; 10X (Fig. 7.6), 40X (Fig. 7.7).

INHALATION HAZARDS TO COAL MINERS (COAL AND DIESEL ENGINE EXHAUST)

Person in Charge: B. O. Stuart

This project is concerned with the biological disposition and effects of present and projected coal mine air contaminants, with particular emphasis on the etiology of the chronic respiratory disease, "coal workers' pneumoconiosis" (CWP). More than 120,000 coal workers are estimated to suffer from CWP and one-fourth of these will probably develop crippling massive fibrosis. Pathogenesis is controversial and there is little in the way of effective treatment.

There is strong interest in promoting the use of diesel engines for coal handling in mines in order to avoid the disasters of fire, explosion and asphyxiation caused by arcing from the presently used electric systems. Our experiments on uranium mine air contaminants have shown, however, that chronic exposure of rodents to exhaust fumes from an inefficient diesel engine can produce interstitial fibrosis, vesicular emphysema, bronchiolar epithelial hyperplasia and cuboidal metaplasia of the bronchial epithelium. We will therefore study the possibly synergistic effects of diesel exhaust together with other coal mine air contaminants.

The pathogenesis of coal-induced respiratory diseases will be investigated in hamsters and rats, exposed 6 hr/day, 5 days/week, for their lifetimes, to aerosols prepared from selected coals. Parallel groups will be similarly exposed to aerosols of coal dust mixed with diesel-engine exhaust. Detailed organic and physicochemical analyses for specific gas-aerosol constituents (composition, particle spectrum, aerosol behavior) will be carried out to correlate with the developing pathology, as observed in animals sacrificed at intervals during the course of the exposure. Limited confirmatory studies in a larger, long-lived experimental animal will be later initiated to aid in the extrapolation of dose-effect relationships to man.

This project was first funded in FY 1976 and reportable results are not yet available.

TOXICOLOGY OF TRITIUM AND THE NOBLE GASES

Person in Charge: J. E. Ballou

This project is concerned with evaluating the biological hazard of exposure to elemental tritium and krypton-85 by studying effects in laboratory animals. Present assessment of the hazard from these radioactive gases is based on radiation dose calculations which derive largely from physical, rather than biological, data. With continuing expansion of the nuclear industry and the planned development of fusion technology, it is critically important that biological effects data be obtained to support these hazard estimates. It has been predicted, for example, that nuclear power expansion may ultimately be limited by the capacity of the environment to safely absorb and dilute the radioactive gaseous effluents. Tritium and krypton-85 are the major long-lived (half-life 12.3 and 10.4 years, respectively) radioactive gases associated with the nuclear fuel cycle.

The proposed work is divided into three chronological categories. In Category I are efforts to develop techniques for gas atmosphere exposures, analytical capabilities, radiation dosimetry, and to design and construct exposure chambers. Category II includes short-term exposure studies with ^{85}Kr, and, later, with ^{3}H, when a tritium effluent cleanup system is in place. A major early effort will investigate transfer kinetics of ^{85}Kr across the lung and into the fetus, since the major route of entry is via inhalation, and the most radiosensitive part of the general population will probably be the fetus. Acute exposure effects on lung biochemistry, pulmonary physiologic function and hematology will also be investigated. Category III will include studies of the long-term biological effects in rats and dogs following chronic exposure to ^{85}Kr or ^{3}H gas atmospheres. The tumorigenicity of ^{85}Kr and ^{3}H atmospheres after whole-body and nose-only exposures will be a major endpoint for comparison with similar results that have been obtained for other radionuclides.

This project was first funded in FY 1976. Category I studies have been initiated but reportable results are not yet available.

DELAYED EFFECTS OF EXPOSURE TO ACID AEROSOLS

Person in Charge: J. E. Ballou

This project is an outgrowth of earlier investigations of the toxicity of inhaled soluble nitrate forms of plutonium and the transplutonium elements. In these studies, control rats, exposed only to the aerosolized 0.27 N nitric acid, developed a significant number of osteosarcomas: at least a 4% incidence compared to a spontaneous incidence in the Wistar rat of <0.1%. To our knowledge, this was the first observation of osteosarcoma induction by inhaled nitric acid.

Since acid aerosols are well known constitutents of industrial atmospheres and effluents, including those of the nuclear industry, it is important to acquire additional information on their carcinogenicity. A consistent finding of bone tumors rather than lung tumors, after acid inhalation, might also lead to a better understanding of the mechanism of bone tumor induction.

Projected experiments will determine the long-term biological effects following inhalation of commonly employed mineral acids including nitric, sulfuric and hydrochloric acid. The studies will involve both single and multiple exposures to aerosol concentrations ranging from the threshold limit value to concentrations approximately 200 times this level. Wistar rats will be utilized initially; however, dogs may be employed in later studies if the rat data suggest that experiments with a longer-lived species are desirable.

This project was first funded in FY 1976 and no results from newly initiated experiments are as yet available.

DELAYED EFFECTS OF INHALED NITRIC ACID AEROSOLS IN THE RAT: PRELIMINARY STUDIES

Investigators:
J. E. Ballou, G. E. Dagle, and K. E. McDonald
Technical Assistance:
R. A. Gies

Rats that inhaled transuranic nitrate aerosols in a study of the toxicology of "soluble" forms of these elements were simultaneously exposed to aerosols of the suspending solution, 0.27N nitric acid. Because of the well known acute toxicity of mineral acids, "treated control" groups exposed to nitric acid alone were added to this study, along with other perturbations shown in Table 10.1.

Although the experiment outlined in Table 10.1 is not complete, it seems clear that exposure to nitric acid is associated with the finding of bone tumors in these animals. Other rats, exposed to low levels of inhaled $Pu(NO_3)_4$ and accumulating radiation doses to skeleton of only a few rads, showed one osteosarcoma in the 79 rats thus far examined. The spontaneous incidence of osteosarcomas in the Wistar rat is extremely low, probably <0.1%.

The finding of bone tumors after inhalation of nitric acid came as a surprise, although others have observed primary lung tumors when this agent was inhaled. The relatively mild treatment that produced the bone tumors [30-minute exposures at approximately 7 X Threshold Limit Value (TLV)] cautions against any unnecessary exposures to aerosolized nitric acid.

TABLE 10.1. Effect of Inhaled Nitric Acid on Bone Tumor Incidence in Rats.

AEROSOL TREATMENT	NUMBER OF RATS	MEDIAN SURVIVAL TIME, days	NUMBER OF OSTEOSARCOMAS
NITRIC ACID [a]	100	795	4/58
NITRIC ACID [a] + Ca-DTPA [b]	68	732	1/57
NITRIC ACID [a] + SHAM Ca-DTPA [c]	30	712	1/27
NONTREATED + SHAM Ca-DTPA [c]	30	665	0/28
NONTREATED CONTROL	100	706	0/98

(a) 30-MINUTE EXPOSURE TO A NITRIC ACID ATMOSPHERE APPROXIMATELY 7 TIMES THE TLV OF 5 mg/m^3.

(b) 6 WEEKLY, 1-HOUR EXPOSURES TO AN AEROSOLIZED CHELATING AGENT CALCIUM TRISODIUM DIETHYLENETRIAMINEPENTAACETATE (Ca-DTPA) STARTING 20 DAYS AFTER NITRIC ACID INHALATION. RATS DEPOSITED 3 mg Ca-DTPA PER TREATMENT

(c) THE SHAM Ca-DTPA EXPOSURES SIMULATED THE SIX WEEKLY CHELATE EXPOSURES, USING ROOM AIR IN PLACE OF Ca-DTPA AEROSOL.

TOXICOLOGY OF INHALED BERYLLIUM COMPOUNDS

Person in Charge: C. L. Sanders

Beryllium compounds, particularly beryllium oxide, constitute a potential toxicological problem in many of their applications. This project is concerned with the retention and translocation of inhaled beryllium oxide and its biological effects, particularly granulomatous and carcinogenic, in lung and extrapulmonary tissue.

This project was undertaken at the behest of the then Division of Biology and Medicine of the USAEC and funded for the period FY 1973 to FY 1975. The project is now terminated.

PUBLICATIONS SINCE 1973

Sanders, C. L., W. C. Cannon, G. J. Powers, and R. R. Adee. 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., W. C. Cannon, G. J. Powers, R. R. Adee, and D. M. Meier. 1975. Toxicology of inhaled high-fired BeO in rodents. I. Metabolism and early effects. Arch. Environ. Health 30, 546-551.

INHALATION TOXICOLOGY OF BERYLLIUM OXIDE IN RATS

Investigator:
C. L. Sanders
Technical Assistance:
D. M. Meier

Male or female Wistar rats given single, nose-only exposures to beryllium oxide developed granulomatous lesions in their lungs which increased in incidence and severity with increasing amount of deposited beryllium. Two carcinomas in the lung and two hepatomas were seen in 325 rats exposed to beryllium; none of these tumor types were seen in 164 unexposed rats.

This report described the pathological effects of beryllium oxide in rats up to 23 months after exposure, at which time the study was terminated.

A total of 184 female Wistar rats and 141 male Wistar rats were exposed, nose-only, at 70 days of age, to one of three levels of beryllium oxide calcined at 1000°C. Initial aveolar depositions of deposited beryllium, as estimated from animals sacrificed at one day postexposure, ranged from 1-2 µg, 12-18 µg and 57-109 µg, in the three exposure groups. Tissue levels of beryllium were determined by atomic absorption spectroscopy. Unexposed control rats numbered 80 females and 84 males. The study was terminated at 645 to 662 days postexposure (Table 11.1).

Moderately developed granulomatous lesions were seen in the lungs at 8-12 months after exposure to beryllium. These lesions consisted of focal accumulations of histiocytes or macrophages, often containing dust particles. These cells were usually found aggregated in subpleural or peribronchiolar regions of the lung. Inflammatory lymphoid cell accumulation, alveolar interstitial fibrosis and epithelial hyperplasia of the septa occurred in more advanced lesions. These lesions, as observed at the termination of the experiments, were graded according to number of lung lobes involved (Table 11.1).

Mild forms of granulomatous lesions were seen in unexposed Wistar rat lungs. There appeared to be a trend toward increasing severity of granulomatous lesions with increasing amount of deposited beryllium, with no difference being seen between male and female. The most severe lesions were confined to rats receiving the highest amounts of deposited beryllium (Table 11.1).

Of the total of 489 rats, only 16 developed malignant tumors. No statistically significant differences were seen between any of the groups and the controls with respect to the induction of malignant tumors, as a function of sex. The incidence of all malignant tumors in male rats (13/141), irrespective of treatment, was higher than for female rats (3/184). Two rarely observed tumor

TABLE 11.1. Experimental Design and Results of BeO Inhalation Study in Rats.

INITIAL ALVEOLAR[a] DEPOSITION, µg BERYLLIUM	NUMBER OF RATS[b]	SEX	TERMINATION TIME, DAYS POSTEXPOSURE	DEGREE OF SEVERITY OF PULMONARY GRANULOMATOUS LESIONS, AS % OF RATS KILLED AT TERMINATION OF EXPERIMENT[c]			
				o	+	++	+++
0	80 (50)	FEMALE	625	34	58	8	0
0.9 ± 0.3	61 (30)	FEMALE	660	33	57	10	0
16.2 ± 15.0	65 (43)	FEMALE	653	12	58	30	0
57.3 ± 17.2	58 (29)	FEMALE	661	10	34	41	14
0	84 (20)	MALE	626	33	56	10	0
2.0 ± 0.7	46 (12)	MALE	662	58	42	0	0
12.3 ± 11.3	43 (18)	MALE	641	28	50	22	0
109 ± 58.0	52 (16)	MALE	645	19	19	38	25

[a] MEAN ± STANDARD DEVIATION

[b] NUMBER IN PARENTHESIS INDICATES RATS ALIVE AT TERMINATION OF EXPERIMENT

[c] DEGREE OF SEVERITY:

o NONE OR MINIMAL RESPONSE

\+ FOCAL LESIONS IN ONE OR TWO LOBES

++ FOCAL LESIONS IN MORE THAN TWO LOBES; WIDESPREAD LESIONS IN LESS THAN TWO LOBES

+++ WIDESPREAD LESIONS THROUGHOUT MOST LOBES

types were seen in exposed rats which were not seen in unexposed rats: lung carcinoma and hepatoma. Two of each of these tumor types were seen; three were in males and one in a female; three were seen in the highest dose groups. Although not seen in statistically significant numbers, the rarity with which these tumors are seen in unexposed rats suggests that beryllium may have been involved in their induction.

In contrast to soluble beryllium compounds, insoluble high-fired beryllium oxide is only marginally carcinogenic in the lung of rats, even though there is a substantial, macrophage-related granulomatous effect. The deposition of high-fired beryllium oxide may significantly influence the fate and effects of other, stronger pulmonary carcinogens, particularly those whose pathogenesis is linked to the alveolar macrophage clearance mechanism.

INTERNAL EMITTER HAZARDS OF THE THORIUM FUEL CYCLE

Person in Charge: J. E. Ballou

This project will investigate the special hazards associated with the uranium-thorium breeder reactor; in particular the mixed U-Th oxide fuels, the nitric acid process solutions of these fuels, the high specific-activity uranium isotopes and the radioactive decay products unique to the thorium fuel cycle. Although biological information is available for many of the individual radionuclides of interest, there is little information on the mixtures of radioactive elements that will result from this fuel cycle.

Preliminary to biological studies, it will be necessary to identify the most probable fuel element matrices and the potential radioactive pollutants that may result from their irradiation and processing. A major effort will be required to develop analytical techniques for quantitating the significant radionuclides in these complex mixtures. Of particular hazard concern will be the determination of ^{232}U and its daughter products, the ^{228}Th decay series, which constitute major contaminants of the breeder-produced fissile ^{233}U.

As soon as analytical techniques are developed, biological studies will be initiated with ^{232}U and the mixed oxide fuel materials. These initial studies will be relevant to problems that may be anticipated to arise in the protection of occupationally exposed workers in the nuclear industry. Gastrointestinal absorption, skin absorption and decontamination, wound absorption, decorporation therapy, and retention and distribution kinetics for the several modes of exposure will be among the factors investigated. Studies of long-term biological effects in rats, and possibly in beagle dogs, will be undertaken for comparison with similar studies on the transuranic elements. Since breeder reactor materials are potential environmental contaminants, studies will also be made of their toxicity as a function of animal age, of their transfer across the placenta, and their teratogenic potency. The possibility of synergistic biologic effects, or of other unusual expressions of toxicity, will be investigated as part of the overall health-safety evaluation of the thorium fuel cycle.

The total evaluation of internal emitter hazards of the thorium fuel cycle is obviously beyond the scope of any single project in any single laboratory,

and will require efforts extending over many years. It is the limited objective of this project to perform short-term studies that will define the specific areas requiring more intensive study, and to initiate some obviously desirable long-term studies so that results may be available at the earliest possible moment.

This project was first funded in FY 1976. Efforts are underway, particularly in the area of analytical development, but reportable results are not yet available.

TOXICOLOGY OF RADIONUCLIDES IN FETAL AND JUVENILE MAMMALS

Person in Charge: M. R. Sikov

Many of the biological parameters used to calculate permissible levels of exposure of adults to radioactive materials are inappropriate for the rapidly growing infant or child or for the pregnant female. These differences, when considered in conjunction with the greater intrinsic radiosensitivity of the immature organism, emphasize the need for more detailed information on the metabolism and toxicity of radionuclides in the prenatal and juvenile mammal. The aim of this project has therefore been to obtain such quantitative data.

Past efforts have included studies of the metabolism of ^{131}I, ^{137}Cs, ^{144}Ce, and ^{239}Pu, relative to age, and to a variety of nutritional and biochemical factors. Prenatal and neonatal rats were shown to be more sensitive than weanlings or adults to ^{131}I impairment of thyroid function and to carcinogenic effects. The metabolism and subacute toxicity of intravenously injected ^{239}Pu in newborn, weanling, and adult rats showed patterns unique to each age; studies of long-term effects at lower dose levels are nearing completion--a major effort is currently directed toward completing histopathologic evaluation and data analysis. The metabolism of intravenously injected ^{241}Am and ^{253}Es has also been studied as a function of age, and acute toxicity evaluated for the latter radionuclide.

The cross-placental transfer of ^{239}Pu, its distribution in the fetoplacental unit, and its effects on development have been studied in detail. To provide a basis for extrapolation to man, we are currently exploring the factors controlling transplacental passage and fetoplacental distribution in several species, including subhuman primates. Because prenatal exposure to plutonium results in a relatively high radiation dose to the yolk sac, where primitive cells of the gonads and the hematologic system originate, we are investigating the postnatal sequelae of prenatal exposure. Organ culture techniques are being used to study embryotoxic effects, including the effects of single particles of plutonium oxide.

Studies were recently begun on the inhalation of $^{239}PuO_2$ particles by newborn rats. Animals are being sacrificed at intervals following exposure to establish the deposition, retention and translocation of plutonium relative to age and particle size.

Future emphasis will be directed toward elucidating the biochemical and physiological processes in the immature mammal that may be responsible for the differences in metabolism and effects of radionuclides, as compared to their metabolism and effects in adults. A continuing effort will be made to interpret the findings of this project in terms of appropriate population exposure standards.

PUBLICATIONS SINCE JANUARY, 1973

Sikov, M. R., D. D. Mahlum, and W. J. Clarke. 1973. Effect of age on the carcinogenicity of ^{131}I in the rat--Interim Report. In: Radionuclide Carcinogenesis, C. L. Sanders, R. H. Busch, J. E. Ballou, and D. D. Mahlum (eds.) (CONF-720505), NTIS, Springfield, VA.

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.

Sikov, M. R. and D. D. Mahlum. 1975. Influence of age and physicochemical form on the effects of ^{239}Pu on the skeleton of the rat. In: Biological Effects and Toxicity of ^{239}Pu and ^{226}Ra, Jee, W. S. S. (ed.). (In Press)

Mahlum, D. D., M. R. Sikov, and F. P. Hungate. 1975. Influence of temporal distribution of alpha dose in bone tumor induction. In: Biological Effects and Toxicity of ^{239}Pu and ^{226}Ra, Jee, W. S. S. (ed.). (In Press)

INFLUENCE OF AGE AND PHYSICOCHEMICAL FORM ON THE EFFECTS OF ^{239}Pu ON THE SKELETON OF THE RAT

Investigators:
M. R. Sikov and D. D. Mahlum
Technical Assistance:
J. O. Hess and D. L. Catt

Studies were made of the skeletal distribution and effects of monomeric and polymeric ^{239}Pu injected intravenously in adult, weanling, and newborn rats. Autoradiographs suggested that periosteal deposition of activity was greater in the weanlings than in adults and newborns. Abnormal healing of pathologic fractures was observed only in rats exposed as weanlings. Other histologic changes of bone, including exostosis, necrosis, and epiphyseal abnormalities, were most marked in the weanlings injected with monomer and least evident in the newborns.

This report continues the description of experiments (Annual Reports 1967, 1968) involving the intravenous injection of monomeric and polymeric ^{239}Pu preparations into newborn, weanling, and adult rats. Dose levels ranged from 6 to 90 μCi/kg body weight. Animals were killed and studied at intervals ranging from 1 day through 9 months postexposure. Since it was anticipated that maturational changes in the skeleton would influence its response, a detailed examination was made of the radiation doses, microscopic distributions and histologic changes in the femur.

The divergence in age-related average radiation doses to the femur, seen earlier in examination of data from the initial 70-day postexposure period, progressively increased with time (Figure 13.1). In a subsequent experiment, it was found that the decreases in bone strength and dry and ash weight, observed previously in survivors at 9 months, were present by three months after exposure of weanlings to 60 μCi/kg, and were accompanied by a twofold increase in serum alkaline phosphatase levels.

Autoradiographs of decalcified femurs showed that, in the animals exposed as adults, there were heavy concentrations of single tracks along the endosteal surfaces and along the margins of the bony trabeculae near the epiphyseal cartilage. The track density on the periosteal surface was much lower than on the endosteal surface (Figure 13.2), and there were still fewer tracks within the deeper compact bone or trabeculae. The few

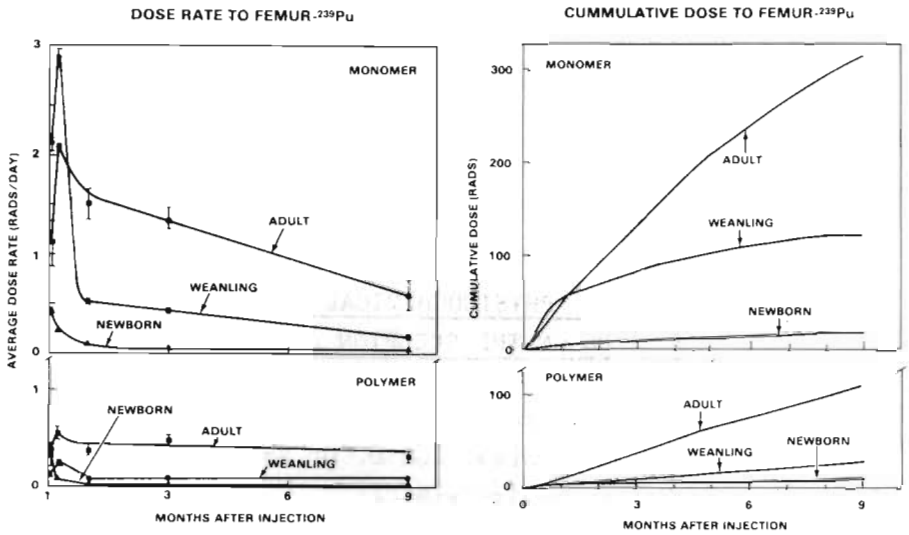


FIGURE 13.1. Dose Rate and Cumulative Radiation Doses to the Femur, Normalized to an Injected Dose of 1 μCi/kg to Adult, Weanling, and Newborn Rats.

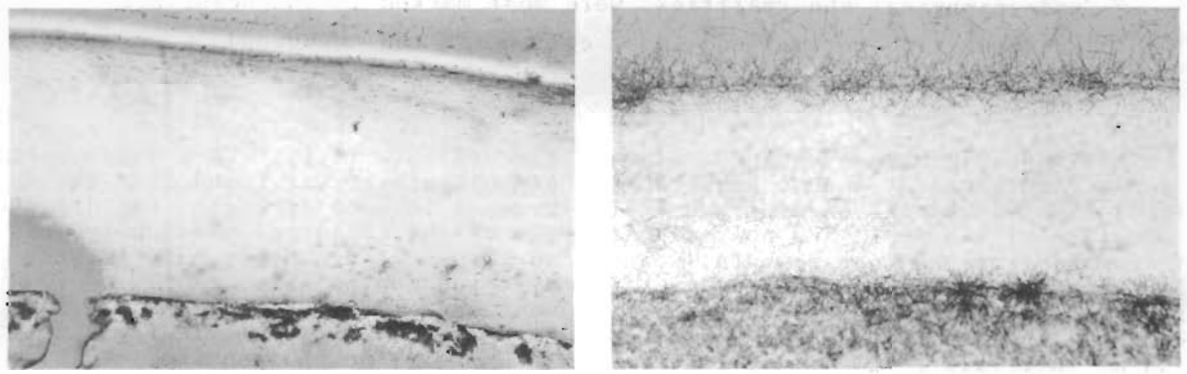


FIGURE 13.2. Photomicrographs of Autoradiographs of Shaft of Femur from Adult (left) and Weanling (right) Rats Killed 7 Days after Intravenous Injection of Monomeric ^{239}Pu. (300X)

alpha stars seen were primarily in the epiphyseal marrow. Activity in cortical bone increased with time, although even at 9 months, the activity was predominantly on endosteal surfaces.

In the animals exposed as weanlings, dense single-track activity was noted along the endosteal borders and in the margins of the trabeculae, as well as in areas of provisional calcification. There was a greater tendency toward localization along the periosteal surfaces (Figure 13.2). A significant fraction of the activity was seen in compact bone at earlier times than in the animals exposed as adults. The pattern of distribution after administration of either monomer or polymer to newborn animals tended to be more uniform than that observed in the animals exposed as weanlings, and there was little difference between the behavior of monomer and polymer.

The histopathologic changes seen in the adults were those that have been described by others. Typically, this included progressive distortion and malalignment of the epiphyseal cartilage with occasional small nuclei of disorganized cartilage. At the higher doses, necrosis of the bone as well as marked destruction of the marrow were seen. At 9 months the major effect, especially at low doses, was an increased remodeling in some regions, particularly near the epiphyses. Some of the marrow spaces were still devoid of cellular elements, but in the central portions of the shaft the marrow was generally hypercellular.

Most aspects of the pattern in the weanlings were the same as in the adults, although there were some major differences in the type of response, including early-appearing pathologic fractures (Annual Report, 1967). These fractures did not repair and, microscopically, heavy callus formation was found to overlie the areas of fracture. This callus was composed of dense connective tissue which, in some regions, was undergoing transition to mature cartilage and, in most instances, contained small fragments of bone. There was pronounced osteoclastic activity associated with the fracture and remodeling process (Figure 13.3).

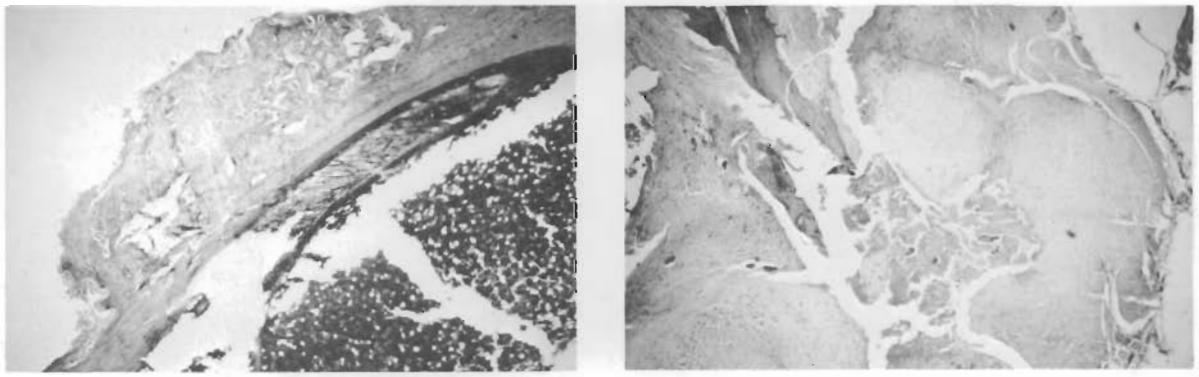


FIGURE 13.3. Photomicrographs of Femurs from Weanling Rats at 3 Months after Injection of Monomeric ^{239}Pu Showing Exostosis (left) and Pathologic Fracture (right) with Callus Formation. (120X)

As in the adults, necrosis involving the cortical and the trabecular bone was present in many instances. However, in the weanlings, osteoclastic activity--indicating a remodeling process in the cortical bone--was frequently observed along the periosteal border. This was accompanied by a proliferation of fibers of connective tissue with maturation of the cartilage, which gave an appearance similar to exostosis (Figure 13.3). At the higher doses of monomer there was some necrosis of the marrow and partial obliteration by proliferating mature fibers of connective tissue as early as 1 month after injection.

There was less histopathologic damage to the femurs of the rats exposed at birth than in the two older age groups. Only sporadic and mild early damage was seen, even at the highest dose levels. At 90 days, and thereafter when changes similar to those in the adults and weanlings were present, they tended to be independent of physicochemical form. The major difference was perhaps the fact that there was a considerable amount of cartilage present throughout the length of the cortical bone, suggesting that it had not ossified properly in many of the animals.

The lower incorporation and uniform distribution of ^{239}Pu in the newborn are in keeping with reports of a lower rate of mineralization and interstitial deposition, while the high initial concentrations in the weanling are to be expected from the normally high rate of mineralization and increased vascularity at that age. In both cases the fall in average concentration and dose rate is attributable to the high growth rate and consequent dilution of the activity. However, the calculation of average dose does not give a true picture since, at the histologic level, there is enhanced deposition along the periosteal surfaces in the weanlings. This results in differences in microdosimetry and may give rise to differences in mechanisms of damage. Although this is purely speculative, it may be that the dose to the periosteum leads to a relative ischemia or malnutrition of the femur, resulting in devitalization. The effects on mechanical strength were much greater than on chemical composition, implicating alterations in structural organization.

INFLUENCE OF TEMPORAL DISTRIBUTION OF ALPHA DOSE IN BONE TUMOR INDUCTION

Investigators:
D. D. Mahlum, M. R. Sikov and
F. P. Hungate
Technical Assistance:
J. O. Hess and J. D. Stearns

Bone tumor induction data from adult and weanling rats intravenously injected with ^{239}Pu and ^{253}Es were compared. Most of the radiation dose to the skeleton from ^{253}Es was received during the first month postexposure, while that from ^{239}Pu was distributed over the 30 months of the study. The results suggest that the shorter duration radiation dose is less conducive to the induction of bone tumors than the dose delivered at a lower rate over a longer period of time.

Low LET radiation is often less effective when given over a protracted time at low dose rates than when given acutely at high dose rates. In contrast, there is evidence which indicates that alpha radiation may be more carcinogenic when the dose is extended over a longer time period. The results in adult and weanling rats from concurrent experiments with ^{239}Pu and ^{253}Es (Annual Report, 1974) were analyzed to determine whether, in these experiments, the tumorigenicity of alpha radiation was enhanced by protraction. The earlier incidence values, revised with new input, are presented in Figure 13.4 as a function of cumulative radiation dose.

In the adult, approximately 60% of the total radiation dose from ^{253}Es was delivered in the first month postexposure, while only 5% of the total dose from ^{239}Pu was delivered during this same period. In weanlings, the fraction of the total dose delivered during the first month was somewhat higher for both nuclides--85% for ^{253}Es and 38% for ^{239}Pu. The incidence of bone tumors is based on radiographic diagnosis or gross examination at necropsy. Histopathologic examination may alter the incidence slightly. Data for only male adults are shown in Figure 13.4 because no females were used in the Es experiment; weanlings of both sexes were used in both experiments and the data have been combined because no sex-dependent differences in tumor incidence were detectable.

The responses of adults and weanlings to Es at equivalent doses are not greatly different. Weanlings exposed to Pu, however, showed two-to-threefold greater incidence of osteosarcoma than did the adults exposed to comparable doses. Our data suggest that Es may be more effective than Pu at the lower radiation doses

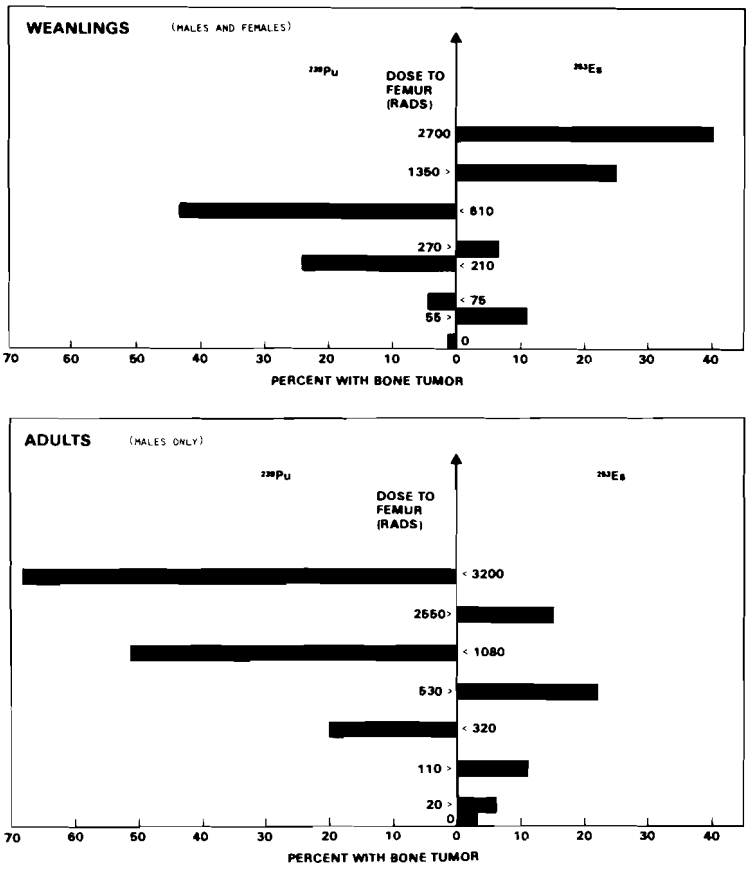


FIGURE 13.4. Cumulative Average Radiation Doses to the Femur, and Bone Tumor Incidence After Intravenous Injection of ^{239}Pu or ^{253}Es in Adult and Weanling Rats.

in the weanling. At higher doses our data support the conclusion that protracted alpha radiation is more effective in inducing osteosarcomas than is a more acute exposure.

The noted differences in bone tumor incidence cannot be unequivocally attributed to differences in the temporal distribution of dose. The alpha energy of ^{253}Es (6.3 Mev) is greater than that of ^{239}Pu (5.1 Mev) and the micro-distribution of Es in bone may also differ somewhat from that of Pu. It seems unlikely, however, that these differences would play a critical role.

COMPARATIVE CROSS-PLACENTAL TRANSFER AND FETOPLACENTAL DISTRIBUTION OF PLUTONIUM-237,-238,-239

Investigators:

M. R. Sikov and D. D. Mahlum

Technical Assistance:

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

Pregnant rats were injected with 1 μCi of ^{237}Pu, ^{238}Pu, or ^{239}Pu citrate after 15 or 19 days of gestation and killed 24 hr later. No consistent differences in the percentage depositions of the three isotopes in the component structures of the fetoplacental unit were found. This is interpreted to indicate that specific activity (the number of atoms injected) is not an important factor in the fetoplacental dynamics and resulting cross-placental transfer of Pu.

In an earlier study (Annual Report 1973) we measured the cross-placental transfer of ^{238}Pu and its distribution in the fetoplacental unit (FPU) of the rat. Comparisons of these values with data from concurrent and previous experiments with ^{239}Pu indicated that there were some differences in the partition of the two isotopes within the FPU and suggested the possibility of a difference in transfer dynamics. The recent availability of ^{237}Pu, and the fact that the earlier comparisons were based on data which were obtained in experiments which were not completely concurrent, led us to perform a study in which the cross-placental transfer and distribution of the three Pu isotopes were measured under identical conditions.

All three isotopes were prepared to allow injection of a constant dose of 1 μCi, in 1.0 ml solution, containing 1 mg sodium citrate at pH 4.0-4.5. Mated female rats of Wistar derivation (day of positive vaginal smear = 0 days of gestation) were injected via tail vein after 15 or 19 days of gestation. The three or four pregnant rats in each isotope-time group were killed 24 hr postinjection and the uterus was removed. Some FPUs were selected for radioanalysis or autoradiography in toto and others were dissected for analysis. Several tissues from the dam were also weighed and taken for radioanalysis.

There were some differences in the partition among the tissues of the dams at the two times of gestation; most notably, a 40% decrease in liver activity at 20 days of gestation relative to that at 15. This probably reflects the much greater fraction of the dose present in the FPUs at the later time of gestation. Since there were a limited number of rats in each time group, however, the overall means were calculated. The pooled data on partition among the tissues of the dams at the two times of gestation are presented in Table 13.1. The spleen was the only major organ for which significantly different activities of the three isotopes were found.

There was a clear general pattern of concentration differences within the FPU for all three isotopes of Pu (Figure 13.5). This progression of

TABLE 13.1. Percent of Plutonium Isotope Dose (±S.E.) in Liver, Spleen, Femur, and Residual Carcass at 24 hr Postinjection of Rats at 15 or 19 Days of Gestation.

TISSUE	^{237}Pu (8)[a]	^{238}Pu (7)	^{239}Pu (7)
LIVER	29 ± 5	33 ± 4	26 ± 6
SPLEEN	0.22 ± 0.02	1.0 ± 0.04	0.68 ± 0.17
FEMUR	1.7 ± 0.10	1.4 ± 0.2	1.4 ± 0.1
CARCASS	42 ± 3	48 ± 6	56 ± 9

[a] NUMBER OF RATS

concentrations: membranes > placenta > fetus, resembled that found in earlier experiments. Likewise, the concentrations in the placenta and membranes were much greater after injection at 19 days than at 15 days of gestation, although there was less stage-dependent difference in concentration in the fetuses. The total content of all three components was much greater at the later time, however, reflecting the greater weights.

The activities and concentrations of ^{238}Pu and ^{239}Pu in the FPU and its components are in general accord with those found in our earlier experiments. No consistent pattern of difference between the partition of the three isotopes within the FPU was observed in the present studies. This suggests that specific activity is not an important factor in the cross-placental transfer of Pu or in its distribution within the FPU. The 1-μCi dose injected represents 2.1×10^{11}, 1.5×10^{14}, and 4.1×10^{16} atoms of Pu for the 237, 238 and 239 isotopes, respectively. This further suggests that the dynamics within the FPU are independent of the number of atoms, within this range and under the conditions of a high excess of citrate used in this study, and that this is not limited by overloading of the FPU by an excess of Pu atoms.

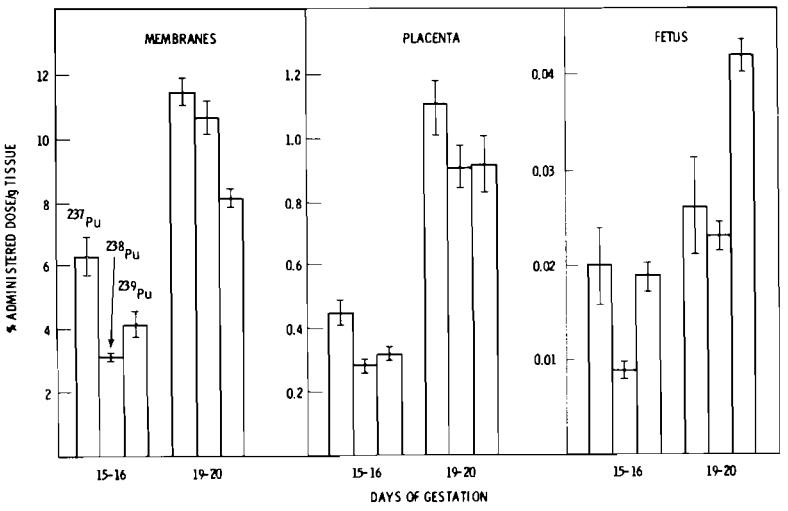


FIGURE 13.5. Concentrations of Pu Isotope in the Fetoplacental Unit at 24 hr Postinjection at 15 or 19 Days of Gestation (Each Value Based on about 20 [range 13-28] Determinations). Vertical bars indicate standard error.

FACTORS MODIFYING RADIONUCLIDE METABOLISM AND EFFECTS

Persons in Charge: D. D. Mahlum/M. R. Sikov

Studies of radionuclide metabolism and effects have usually employed a single radionuclide administered under carefully controlled conditions. Animals are supplied adequate amounts of all essential nutrients and are maintained in an environment of controlled temperature and humidity; often a pathogen-free situation is created. These are common and reasonable experimental precautions taken to reduce effects from uncontrolled variables. People who may be exposed to radionuclides do not, however, live under such ideal conditions. They are subjected to many agents (ubiquitous, local, or self-imposed), and to a variety of environmental and health situations, which may influence the biological disposition and effect of these radionuclides. Examples include suppressed or accentuated immunologic status, dietary deficiencies and excesses, use of drugs (those medically prescribed as well as those abused), normal variations in hormonal state, inadvertent exposure to harmful agents in the environment such as pesticides and known carcinogens, and various common physiopathologic disorders. The consequences of radionuclide exposure may either be exacerbated or ameliorated depending on the conditions extant, and it is particularly important that we know of those that may increase radiotoxicity.

This project was therefore initiated in FY 1973, to provide a quantitative assessment of the influence of various agents and health situations on the biological disposition and resulting effects of important radionuclides. The following factors are presently being investigated.

- The influence of iron reserves on plutonium metabolism. Iron deficiency is one of the most common nutritional diseases of young children and women, and exposure of this population to plutonium or other radionuclides may result in effects not found in iron-replete individuals.
- The effects of alcohol on the metabolism of plutonium and other radionuclides. Alcohol ingestion results in a great number of physiologic perturbations, including morphologic and functional disturbances in the liver, as well as disturbances in iron metabolism. The use of alcohol by large numbers of people and the role of the liver in the metabolism of Pu and other radionuclides suggest the possible importance of this factor in radionuclide toxicity.

- Induction of mammary tumors in rats injected with plutonium, to determine whether the induction process involves a direct effect of Pu on mammary tissue or an indirect effect through other systems. Currently, the incorporation of ^{239}Pu in the mammary gland (and other tissues) during pregnancy and lactation is being measured and the subsequent fate of the incorporated material evaluated.

PUBLICATIONS SINCE JANUARY, 1973

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BODY IRON STATUS AND PLUTONIUM METABOLISM IN RATS

Investigator:
H. A. Ragan
Technical Assistance:
G. Edmerson, S. English, D. Hunter,
M. Perkins, M. Pipes, and J. Smith

Rats were fed an iron-deficient diet for 75 days, after which ^{239}Pu citrate or ^{239}Pu citrate and iron dextran was administered. Results suggest that plutonium retention may be related more to serum iron concentrations than to the status of body iron stores.

Previous Annual Reports have described the enhanced absorption and retention of plutonium in iron-deficient mice, and the altered tissue distribution of plutonium. To further study these changes, 80 female Wistar rats were fed either a control or an iron-deficient diet, starting at 21 days of age. At 88 days of age, ten rats from each dietary group were sacrificed for evaluation of their hematologic status (Table 14.1). Eight days later, the remaining 30 rats in the iron-deficient and control groups were given 0.4 μCi ^{239}Pu citrate by intraperitoneal injection (IP) or 0.4 μCi ^{239}Pu citrate IP plus 10 mg of iron as iron dextran. Five animals from each of the four groups were killed 1, 5, and 12 days later to determine the tissue distribution of plutonium.

TABLE 14.1. Volume of Packed Red Cells (VPRC), Mean Corpuscular Volume (MCV) and Serum Iron (SI) Values in Iron-deficient and Control Rats (Mean ± SD).

	N	VPRC, ml/100 ml	MCV, μ^3	SI, μg/100 ml
AT 88 DAYS OF AGE				
IRON-DEFICIENT	10	26.1±9.5	38±4	83±49
CONTROL	10	50.7±1.9	53±2	275±55
AT 96-108 DAYS OF AGE				
IRON-DEFICIENT	12	37.6±4.6	45±3	230±108
CONTROL	13	43.4±2.4	51±2	226±66

There were no differences in plutonium retention between iron-deficient and control rats given plutonium only (Figure 14.1). When iron was given concurrently, the concentration of ^{239}Pu in the femur was reduced and that in the spleen markedly increased in both the iron-deficient and control rats. The higher plutonium concentrations in the spleen remained constant through 12 days post-injection in both the iron-deficient and control groups given iron.

Hematologic evaluation of the iron-deficient rats strongly suggests that they had somehow managed to ingest iron prior to administration of plutonium (Table 14.1). The VPRC and MCV of iron-deficient rats had increased markedly from values obtained approximately 10 days earlier, and there was no difference in serum-iron values between the iron-deficient and control groups, whereas earlier, the controls had serum iron values approximately threefold higher. Iron stores were still depleted in the

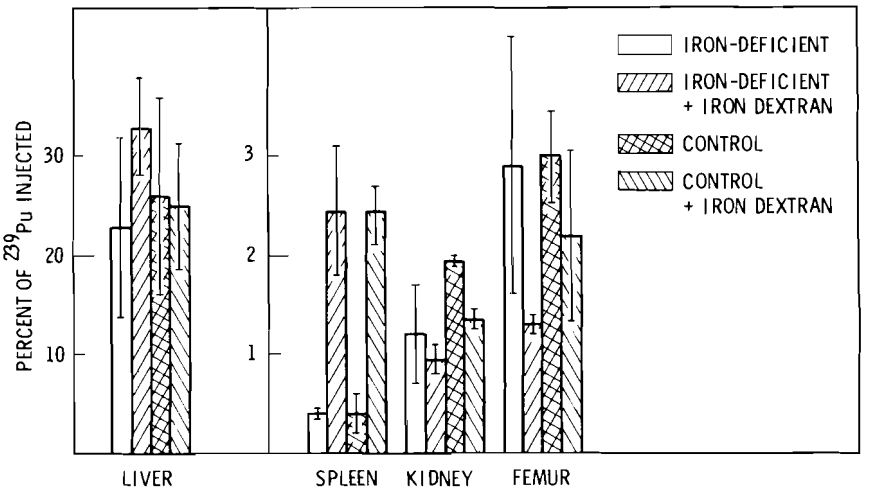

FIGURE 14.1. ^{239}Pu in Rat Tissues 24 Hours Following Injection of ^{239}Pu Citrate or ^{239}Pu Citrate Plus Iron Dextran (Mean ± SD).

iron-deficient groups, as indicated by bone marrow examination. These observations could be logically explained if the iron-deficient rats had inadvertently been allowed to ingest iron between the initial and the later measurements. The results suggest that plutonium retention and distribution, like that of iron, is more dependent on erythroid demands as reflected in plasma iron turnover than by levels of storage iron.

Iron dextran is known to be mobilized from the injection site as a colloid which is processed through the reticuloendothelial system prior to utilization of the iron. This may account for the greatly increased concentration of plutonium in spleen following iron dextran administration, the assumption being that plutonium is, in this case, associated with the iron colloid rather than with transferrin, since colloidal iron is not transferrin-bound.

GUT-RELATED STUDIES OF RADIONUCLIDE TOXICITY

Person in Charge: M. F. Sullivan

This project is concerned with the fate of radioactive materials that are ingested or translocated to the intestine after clearance from the lung. It is also concerned with the effects of radiation, either from an internally deposited radionuclide or from an external source, on the gastrointestinal tract and its functions.

Present emphasis in this project is placed on measurement of the gastrointestinal absorption of the actinide elements. It has long been known that the absorption of plutonium is increased in the very young rat. We are studying this phenomenon for several actinides and find not only increased absorption, but an even more markedly increased retention in the intestinal mucosa. Studies involve both insoluble oxide forms and more soluble nitrate and organically complexed forms. Of particular interest are current experiments in which metabolically incorporated actinides from plant and animal sources are being fed to determine their absorbability. Information from these studies will be of critical importance in evaluating the food-chain hazard from environmentally dispersed actinides over long periods of time.

Also included in this project are studies of the effects of neutrons from spontaneously fissioning californium-252, on normal tissues in both rats and miniature swine. The effects on the gastrointestinal tract and urinary tract are of particular concern in radiation therapy where clinical treatment of cancer of the uterine cervix may result in exposure of these normal tissues adjacent to the genital tract.

PUBLICATIONS SINCE JANUARY, 1973

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.

Sullivan, M. F., A. L. Crosby, and B. W. Wachholz. Measurement of plasma protein loss into the irradiated gastrointestinal tracts of swine, dogs, rabbits, rats, and mice. Clinical Invest. (Submitted for publication)

ABSORPTION OF TRANSURANIC ELEMENTS FROM RAT GUT

Investigators:
M. F. Sullivan and A. L. Crosby

Absorption of ^{237}Np was decreased tenfold when fed to adult rats in a form that was bound to animal tissue. Plutonium-238 absorption, on the other hand, was about doubled by binding to tissue before administration. Absorption of ^{238}Pu, ^{239}Pu and ^{241}Pu, as nitrates, was essentially the same for all isotopes; the more insoluble oxides were less well absorbed. Retention of ^{237}Np nitrate by newborn rats decreased threefold if they were gavaged at eight days instead of two days after birth, principally due to the lower amounts stored in the mucosa of the small intestine. More soluble forms of administered plutonium showed an increased absorption and retention in the mucosal epithelium of the newborn rat.

Observations on the absorption of ^{233}U, ^{237}Np, ^{238}Pu, ^{241}Am, ^{244}Cm and ^{253}Es from the gastrointestinal tracts of adult and neonatal rats were reported in the 1974 Annual Report. The newborn rat, as compared with the adult, shows enhanced absorption of many of these radionuclides and enhanced incorporation in the lower small intestine. In this report we will discuss additional results on the absorption of these radionuclides in various chemical states, including metabolically incorporated forms.

"Soluble" forms of the radionuclides were prepared by adjusting the acidity of nitric acid solutions to a pH of 2 and diluting to a concentration of 20 μCi/ml. The oxides were administered as water suspensions of equivalent radioactivity. Two-day-old neonates received 0.05 to 0.1 ml; 8-day-old animals, 0.1-0.2 ml; and adults, 0.5 ml of these solutions or suspensions. Higher doses were administered to a few animals whose tissues were prepared for autoradiographs.

Higher doses were also administered, intragastrically or intraperitoneally, to 2- or 8-day-old rats, which were killed 5 hrs later and fed in toto, or lower small intestine only, to fasted adult rats. The dose to the "cannibal" rats was determined by analysis of other injected newborns. Uneaten bone fragments were analyzed and their radioactivity subtracted to arrive at the dose ingested.

Excreta was collected from individual adult rats, daily for 4 days, then pooled for 3 days. One group of adult animals was maintained in restraining cages after dosing, to prevent coprophagy and the possibility of cross-contamination of urine with feces. No excreta was collected from animals that cannibalized the neonates, since it was thought that urine might be contaminated by the high activity in the feces and uneaten bone fragments. All animals were killed at 7 days after gavage and their tissues analyzed radiochemically.

Distribution and excretion data for adult rats are shown in Table 15.1. The liver and skeleton contained the majority of absorbed radioactivity. Biologically incorporated neptunium was absorbed to a substantially lower degree (10- to 20-fold) than inorganic neptunium. Biologically incorporated plutonium, on the other hand, seemed to be absorbed to a greater degree than inorganic plutonium (1- to 10-fold, depending upon the groups compared). Variation in the isotope (and consequently in the specific activity) apparently had little effect on the

TABLE 15.1. Distribution of Radionuclides in Adult Rats Seven Days After Administration by Gavage or Feeding.

RADIONUCLIDE	FORM ADMINISTERED	NUMBER OF RATS AND SEX	PERCENT OF ADMINISTERED DOSE				
			SKELETON[a]	LIVER	SKELETON + LIVER	URINE	SKELETON + LIVER + URINE
^{237}Np	NITRATE	15 F	0.63	0.07	0.7	0.45	1.15
^{237}Np	BIOLOGICALLY INCORPORATED[b]	6 F	0.03	0.003	0.03		
^{237}Np	NITRATE	7 M	0.54	0.02	0.56		
^{237}Np	BIOLOGICALLY INCORPORATED[b]	7 M	0.05	0.003	0.05		
^{238}Pu	NITRATE	15 F	0.007	0.003	0.01	0.02	0.03
^{238}Pu	NITRATE[c]	3 F	0.003	0.0003	0.003	0.02	0.02
^{238}Pu	NITRATE[d]	3 F	0.003	0.0004	0.003	0.01	0.01
^{238}Pu μ	BIOLOGICALLY INCORPORATED[e]	4 M	0.03	0.0007	0.03		
^{238}Pu	BIOLOGICALLY INCORPORATED[f]	3 M	0.01	0.004	0.01	0.03	0.04
^{239}Pu	NITRATE	6 F	0.016	0.0007	0.017	0.005	0.022
^{241}Pu	NITRATE	6 F	0.018	0.009	0.027	0.005	0.032
^{238}Pu	OXIDE	6 F	0.004	0.0006	0.0046	0.007	0.012
^{238}Pu	BIOLOGICALLY INCORPORATED[g]	5 F	0.009	0.0003	0.009		
^{238}Pu	OXIDE[h]	5 F	0.01	0.0	0.01	0.015	0.025

[a] SKELETON ESTIMATED AS 23 x SINGLE FEMUR CONTENT

[b] RADIONUCLIDE AS NITRATE ADMINISTERED BY GAVAGE TO 8-DAY-OLD RAT THAT WAS KILLED AND FED IN TOTO TO ADULT

[c] RATS MAINTAINED IN RESTRAINING CAGES AFTER GAVAGE

[d] CONTROL RATS MAINTAINED IN METABOLISM CAGES FOR COMPARISON WITH (g)

[e] RADIONUCLIDE AS NITRATE INJECTED INTRAPERITONEALLY INTO 2-DAY-OLD RAT THAT WAS KILLED AND FED IN TOTO TO ADULT

[f] RADIONUCLIDE AS NITRATE ADMINISTERED BY GAVAGE TO 2-DAY-OLD RAT; THREE DAYS LATER THE ILEUM WAS FED TO ADULT

[g] RADIONUCLIDE AS OXIDE INJECTED INTRAPERITONEALLY TO 8-DAY-OLD RAT THAT WAS KILLED AND FED IN TOTO TO ADULT

[h] SNS-GRADE $^{238}PuO_2$

amount of plutonium absorbed from the GI tract. About 0.02 percent of the gavaged dose of ^{238}Pu nitrate, 0.02 percent of ^{239}Pu nitrate, and 0.03 percent of ^{241}Pu nitrate were absorbed. These values are substantially higher than some values previously reported. To show that the urinary radionuclide output of gavaged animals was not due to contamination from the feces, rats in one experiment were restrained to eliminate the possibility of such contamination. The urine of restrained rats was more radioactive than that of the unrestrained controls, suggesting that neither fecal contamination nor coprophagy is a complicating factor in these experiments.

Our earlier report on the absorption of actinides by the newborn indicated high concentrations in the skeleton, in the mucosa of the small intestine, and in its contents (Annual Report, 1975). The data shown in Table 15.2 suggest that the levels of radionuclides retained in the skeleton are greater, and the amount in the mucosa or recovered from the intestinal contents is decreased, with increasing age, probably due to maturation of the epithelium of the gut. It should be noted that skeletal estimates for the young animals are quite uncertain and more significance attaches to the figures given for "Total Absorbed", which reflect a total carcass analysis. Both absorption and retention within the intestine are lower for the oxides than for the nitrates. This is consistent with results reported in last year's Annual Report for americium and curium.

TABLE 15.2. Distribution of Radionuclides in Young Rats Seven Days After Administration by Gavage.

RADIO-NUCLIDE	FORM ADMINISTERED	NUMBER OF RATS	AGE, days	PERCENT OF ADMINISTERED DOSE				
				SKELETON[a]	LIVER	INTESTINE WALL	INTESTINE CONTENTS	TOTAL ABSORBED[b]
^{237}Np	NITRATE	5	2	1.0	0.02	18.9	13.7	0.8
^{237}Np	NITRATE	6	4	0.7	0.01	11.3	9.8	0.5
^{237}Np	NITRATE	10	8	3.6	0.10	5.5	7.5	1.2
^{238}Pu	NITRATE	7	2	2.7	0.2	0.7	0.5	2.2
^{239}Pu	NITRATE	5	2	0.6	0.06	4.6	3.0	0.4
^{238}Pu	OXIDE[c]	10	2	0.0	0.03	0.4	0.3	0.05
^{239}Pu	OXIDE[d]	11	3	0.01	0.002	0.4	0.1	0.02

(a) SKELETON ESTIMATED 0.22 x BODY WT (g) x CONCENTRATION IN FEMUR (%/g)

(b) TOTAL ABSORBED ESTIMATED AS SUM OF SEPARATE ANALYSES ON FEMUR, LIVER, LUNG, AND RESIDUAL CARCASS (NOT INCLUDING INTESTINAL WALL OR CONTENTS)

(c) SNS-GRADE $^{238}PuO_2$

(d) WEAPONS-GRADE $^{239}PuO_2$

ACUTE TOXICITY OF REACTOR-CATASTROPHE EFFLUENT

Person in Charge: M. F. Sullivan

This project, initiated late in FY 1974, has the specific and limited objective of providing data on the acute toxicity of ingested, nonabsorbed radionuclides such as might result from a catastrophic reactor accident. The need for this information became apparent in the preparation of the Rasmussen Report (WASH-1400), and the early data from this project were recently employed in the revision of Appendix VI of that report.

The experimental approach has been to determine acutely lethal doses in rats and dogs, using a high-energy beta emitter, ^{106}Ru-^{106}Rh, and a low-energy beta emitter, ^{147}Pm. Radiation dose to the radiosensitive cells at the base of the crypts was measured by surgically implanted thermoluminescent dosimeters in the dog, and calculated from measurements of passage time in the rat. From these measurements and a knowledge of the distances from the intestinal contents to the sensitive target cells in man, it will be possible to estimate the lethal dose for man.

Initial studies with ^{106}Ru-^{106}Rh and with ^{147}Pm have been completed. In future experiments we will study the effect of combined exposures to weak and strong beta emitters with a superimposed external gamma irradiation to more closely simulate a reactor accident. In all of these studies careful observations are made of histopathology in all affected tissues, and animals not dying acutely are observed for longer-term effects. Such long-term observation in ^{106}Ru-^{106}Rh-exposed dogs has identified a previously unreported gastrointestinal radiation syndrome in which the animal survives the acute effects but succumbs from prolonged diarrhea resulting from an inability to repair the mucosal damage to the large intestine.

ACUTE TOXICITY IN RATS AND DOGS OF INGESTED PROMETHIUM-147 AND RUTHENIUM-106

Investigators:

M. F. Sullivan, T. D. Mahony and F. T. Cross

Technical Assistance:

P. S. Ruemmler, J. L. Beamer, M. T. Karagianes, S. Bayer and G. W. R. Endres

The oral LD_{50} in rats for the weak beta-emitter ^{147}Pm was about 6 Ci/kg. A combination of one-half that dose with one-half the LD_{50} (9 mCi/kg) of the high energy beta-emitter, ^{106}Ru-^{106}Rh, caused similar mortality. Death was due to large bowel damage as a consequence of the long residence time in that segment. Dogs fed doses in excess of 3.2 mCi/kg of ^{106}Ru-^{106}Rh died of extensive large bowel injury. Dogs given doses between 2.75 and 3.2 mCi/kg often survived for several weeks with persistent anorexia and diarrhea before dying of irreparable injury to the large bowel.

Both high-energy beta-emitting radionuclides such as ^{106}Ru-^{106}Rh and low-energy emitters such as ^{103}Ru might be released to the environment in the event of a reactor catastrophe. Information on the acute toxicity of these compounds was needed for the Rasmussen Study Report (Appendix VI, WASH-1400). In last year's Annual Report we published toxicity data on rats and limited data on beagle dogs for an isotope at the high end of the energy spectrum, ^{106}Ru-^{106}Rh. The dog mortality and hematologic data are now complete, as is the dosimetric information (discussed in another volume of this Annual Report). The beta-emitter ^{147}Pm (average energy, 0.0621 Mev) is not an abundant product of nuclear fission; it was used in these studies as a stand-in for more prominent low-energy beta emitters, such as ^{103}Ru (average energy, 0.0653 Mev). Promethium-147 is poorly absorbed from the GI tract, has a more convenient half-life than ^{103}Ru (2.6 years versus 40 days), and is much less expensive.

The ^{106}Ru and ^{147}Pm were obtained as chlorides in acid solution. These were adjusted with NaOH to a pH of 1.0 and diluted to provide an administered dose of about 1.0 ml. Dogs received ^{106}Ru mixed in a 100-g meat bolus eaten ad libitum under visual observation. Passage time of ^{106}Ru through the dog was monitored with appropriate beta-gamma detection instruments. Passage time of the intestinal contents of rats was measured

by administering 10 μCi of ^{106}Ru per rat to 200-g rats and killing them in groups of three at 0, 3, 6, 9, 12, 15, 18, 21 and 24 hours. Ruthenium-106 activity was determined in segments of the upper and lower colon and cecum. The data from this study are shown in Figure 16.1 along with data previously obtained in this laboratory by Thompson and Hollis (Annual Report, 1955) and by Sikov and Mahlum (Annual Report, 1967) under somewhat different conditions. An 18-hour fast before ^{106}Ru administration was responsible for the more rapid emptying of the bowel in the Thompson and Hollis study. Sikov and Mahlum quantified the content at only three time periods and considered the colon as a single segment. On the basis of the passage time reported here it was possible to calculate mucosal surface doses of 4200 rads to the cecum, 2000 rads to the upper colon and 1500 rads to the lower colon from an LD_{50} (9 mCi/kg) of ^{106}Ru-^{106}Rh.

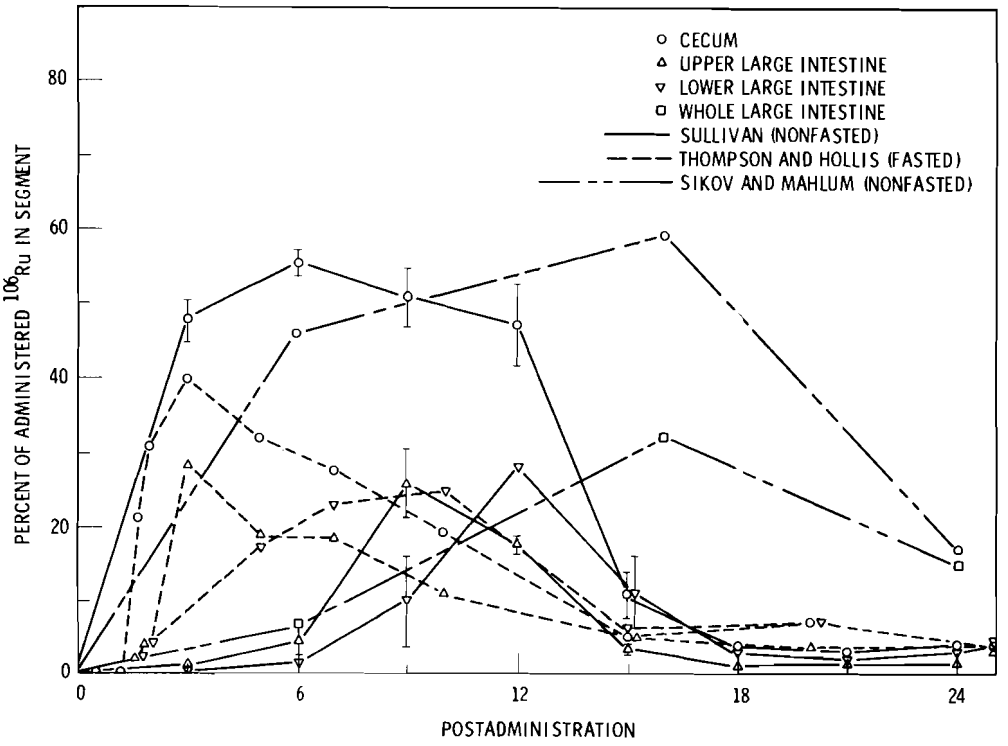

FIGURE 16.1. Passage Time of Intestinal Contents Labeled with ^{106}Ru-^{106}Rh Through the Large Bowel of Fasted and Nonfasted Rats.

Mortality data for ^{147}Pm in rats was obtained in only a few animals because of the very large quantities of this isotope necessary to cause death (Table 16.1). The LD_{50} of ^{106}Ru-^{106}Rh was approximately 9 mCi/kg; the median lethal dose for the limited number of ^{147}Pm-gavaged rats was near 6 Ci/kg. Doses of about one-half the LD_{50} for both ruthenium and promethium were administered to measure their combined toxicity. Death did result from this mixture, suggesting that the mechanisms for causing death from gut injury were similar and additive for the two radionuclides (Table 16.1).

Hematology data obtained on ^{147}Pm-exposed rats at 5 days after gavage with 7.2 Ci/kg indicated a 30% decrease in the lymphocyte count and 140% increase in the neutrophil count. Serial bleeding after ^{106}Ru ingestion (1975 Annual Report) indicated that lymphocytes were lowest 3 days after

TABLE 16.1. Mortality and Survival Data in Rats for Injected Promethium-147 and Ruthenium-106.

RADIONUCLIDE	DOSE, mCi/kg	MORTALITY	AVERAGE SURVIVAL TIME, days
^{106}Ru-^{106}Rh	<7.5	0/16	
	7.5	1/4	9
	8	4/10	9
	9	5/14	7
	10	5/9	8
^{147}Pm	<4000	0/7	
	4200	1/2	7
	5200	0/1	
	5200>	3/3	6
	6250	1/2	
$^{106}Ru + ^{147}Pm$	4.7 + 2500	0/6	
	5.0 + 3125	3/5	9

gavage and that a return to near-normal values was reached by 6 days; neutrophil levels continued to rise for 2 weeks after treatment.

Segments of the GI tract examined for histopathologic damage indicated that the large bowel was the most severely injured segment. The calculated dose to the surface of that segment from 7.2 Ci/kg of ^{147}Pm was 11.5×10^4 rads for the lower colon and 1.7×10^5 rads for the cecum; the estimated dose to target cells was 3360 and 4900 rads, respectively. Histopathologic changes in the colon were not appreciably different from those seen following ^{106}Ru-^{106}Rh administration, but damage to the stomach was dissimilar. Promethium-147 caused most severe damage to the squamous stomach (Figure 16.2), whereas ^{106}Ru-^{106}Rh had its major effect on the glandular stomach. This may have been due to the shallow penetration of the weak beta radiation emitted by ^{147}Pm.

The dog mortality and survival data obtained after ingestion of ^{106}Ru-^{106}Rh are shown in Table 16.2. All dogs receiving 3.5 mCi/kg, or more, died from acute intestinal injury. Dogs receiving between 2.25 and 3.2 mCi/kg also eventually died or were sacrificed in a moribund condition. A few with implanted dosimeters were killed to follow the progression of injury or repair. Animals surviving the acute mortality period were anorectic; however, two dogs that survived for 60 and 116 days after ingestion resumed eating after 3 weeks. Diarrhea persisted in all animals until death. Hematology data on four young dogs that received 2.9 mCi/kg are shown in Figure 16.3. Lymphocyte counts were reduced to about 20 percent of their pretreatment values between 5 and 11 days after dosing; neutrophil counts rose continuously.

Four dogs that had received between 2.9 and 4.0 mCi/kg, and later died or were killed in a moribund condition, developed duodenal ulcers. The ulcers perforated the serosal surface and

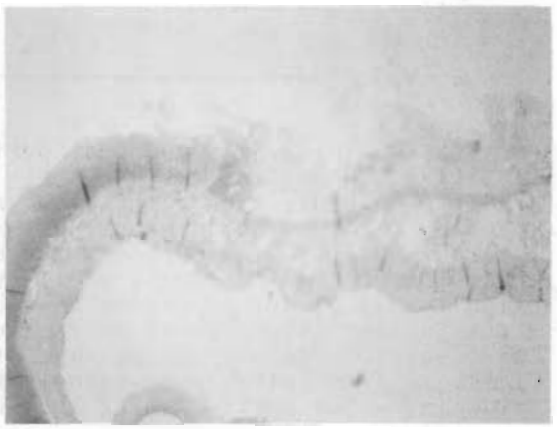

FIGURE 16.2. Damage to Rat Stomach After Gavage with ^{147}Pm. The relatively normal glandular stomach is seen on the left; the severely damaged squamous stomach on the right has been almost completely separated from the underlying mucosa muscularis while the glandular stomach shows only slight damage. (7.2 Ci/kg, ^{147}Pm).

TABLE 16.2. Mortality and Survival Data for Ingested ^{106}Ru-^{106}Rh in Dogs.

DOG NUMBER	SEX	AGE AT DOSING, months	DOSE mCi/kg	SURVIVAL TIME, days	WT AT DEATH, % OF WT AT DOSING
640	FEMALE	69	2.25	(8)[b]	
987	MALE	43	2.25	(8)[b]	93
1015	FEMALE	43	2.25	25	67
988	MALE	40	2.6	(63)[b]	97
1017	FEMALE	42	2.8	(8)[b]	89
963	FEMALE	44	2.9	(4)[b]	100
1016	FEMALE	40	2.9	60	71
985	FEMALE	44	2.9	116	78
1477	MALE	7	2.9	26	54
1497	MALE	7	2.9	19	42
1479	MALE	9	2.9	25	57
1482	MALE	9	2.9	24	59
1021	MALE	40	3.0	25	64
984	FEMALE	41	3.2	25	89
967	MALE	42	3.5	12	70
969	MALE	43	3.5	9	77
968	MALE	42	3.7	25	57
986	FEMALE	42	3.7	15	68
1019	MALE	39	3.9	12	76
965	MALE	42	4.0	18	65

(a) TIME FOLLOWING ADMINISTRATION OF ^{106}Ru TO DEATH OR SACRIFICE WHEN MORIBUND

(b) PLANNED SACRIFICE OF NON-MORIBUND ANIMAL

were accompanied by a chemical peritonitis. The minimal evidence of radiation injury around the site and the low readings on adjacent dosimeters suggest that ulceration may have been induced by the stress of starvation (about 14 days), rather than by irradiation. The segment of the bowel most severely damaged by passage of ^{106}Ru was the colon, particularly the midcolon. The epithelium of that area was often totally destroyed and replaced by a lamina propria consisting of large non-nucleated cells covered by surface exudate of leukocytes (Figure 16.4).

In continuing studies with rats, an external gamma dose of 50 rads from a ^{60}Co source is being delivered two hours prior to an LD_{50} dose of ^{106}Ru-^{106}Rh. This will more closely simulate the kind of exposure that might be received from a reactor

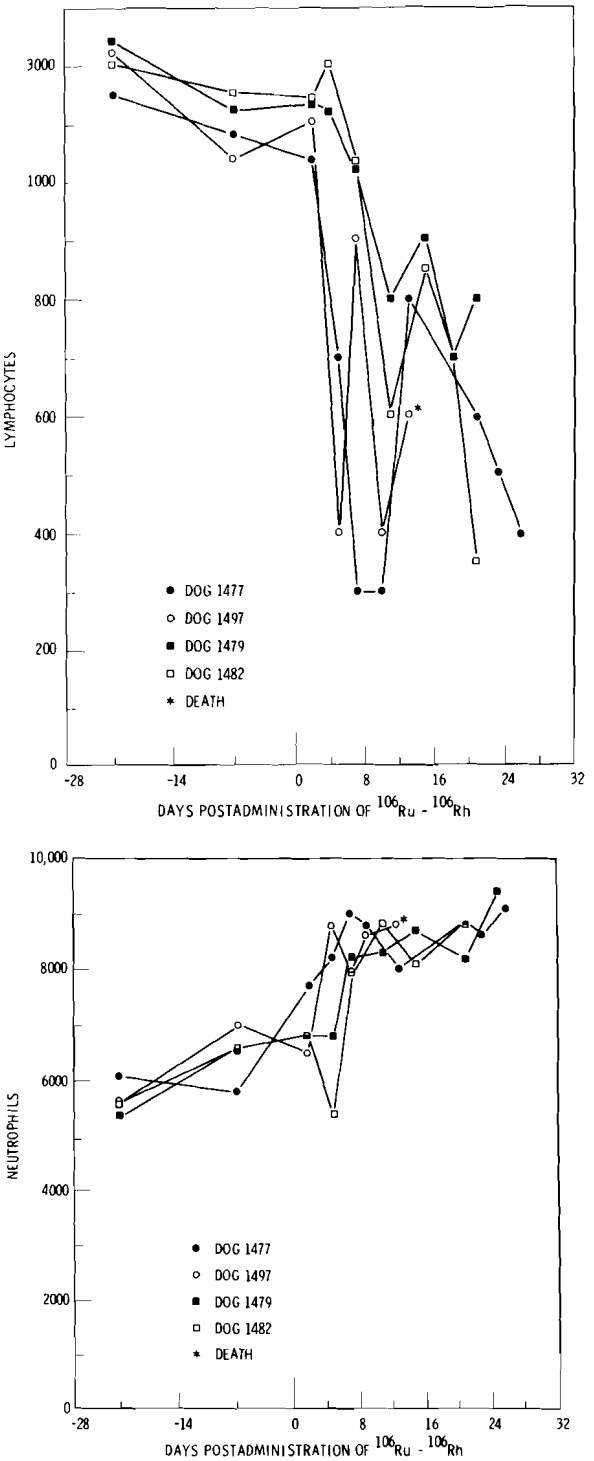

FIGURE 16.3. Lymphocyte and Neutrophil Counts of Beagle Dogs Administered 2.9 mCi/kg of ^{106}Ru-^{106}Rh.

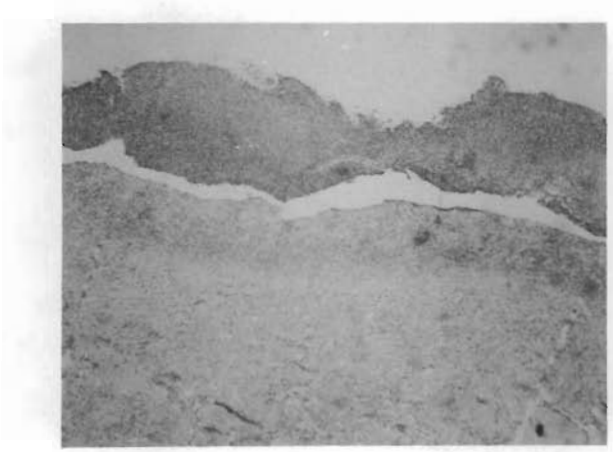

FIGURE 16.4. A Section of the Midcolon of a Dog Fed 2.9 mCi/kg of ^{106}Ru-^{106}Rh 60 Days Before Necropsy, Showing Mucosa That Has Been Replaced by a Surface Exudate of Leukocytes.

catastrophe. We will also study the effect of repeated sublethal doses of ingested ^{106}Ru-^{106}Rh, a type of exposure that might result following widespread environmental contamination. The failure of the large bowel to regenerate following midlethal exposures suggests that its resistance to repeated sublethal exposures may be less than generally inferred from previous observations on the small intestine.

TOXICOLOGY OF CHRONICALLY FED ^{90}Sr IN MINIATURE SWINE

Person in Charge: H. A. Ragan

This now-terminated project was first funded in FY 1958. It has sought to establish the dose-effect relationships following daily exposure to ingested ^{90}Sr through the life spans of three generations of miniature swine. In summary, at daily ingested dose levels of 1 and 5 μCi/day, no life shortening occurred but a chronic neutropenia was manifested. At 25, 125, 625 and 3100 μCi/day, dose-related life shortening and hematopoietic dyscrasias were evident. No effects on reproductive performance or teratogenicity occurred in 1- through 625-μCi/day feeding levels. A high incidence of nephritis, arthritis, cystic endometritis, and benign uterine tumors occurred in all aged swine regardless of dose level, including control animals. Bone tumors were infrequently observed, but aged swine fed 25 μCi/day had an increased incidence of both benign and malignant neoplasms of soft tissue.

All remaining swine on this project have been killed and the total dose-effect data are being subjected to statistical analysis. A major publication effort is envisioned during the course of the next year.

PUBLICATIONS SINCE JANUARY, 1973

Ragan, H. A., R. L. Buschbom, W. L. Clarke, P. L. Hackett, and B. J. McClanahan. 1973. Late Effects of Chronic ^{90}Sr Ingestion in Miniature Swine, pp. 145-154. In: Second International Conference on Strontium Metabolism. (CONF-720818). NTIS, Springfield, VA.

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BIOCHEMICAL INDICES OF POLLUTANT CARCINOGENICITY

Person in Charge: J. E. Morris

This relatively new project seeks to analyze, in an integrated model system, the problem of the etiology of tumor induction by energy-related carcinogens in terms of physiological, immunological, and virological events.

Studies at this laboratory have shown that plutonium, in the form of inhaled particles of $^{239}PuO_2$, produces a dose-related lymphopenia in the beagle dog, and pulmonary neoplasia as a primary cause of death in the beagle dog and rat. It is this system of tumorigenesis which we are using as our model to define, identify, and evaluate basic changes occurring during the oncogenic process in terms of the immune competence, genetic expression and biochemical potential of the exposed animal.

Studies of the effects of inhaled plutonium on immune competence will include an examination of the ability of the immune system of the exposed animal to recognize foreign antigens and thus respond to them; and to survey its own antigens and thus, perhaps, to reject neoplastic cells and maintain normal tissue antigenicity. Studies relating to genetic factors will investigate the expression of quiescent genes (viral genomes, synthesis of new cell-surface antigens, for example) in lung tissue and in cultured cells from animals that have inhaled plutonium.

It is hoped that these studies, proceeding in a correlated fashion, will lead to the determination of those events occurring early in the course of inhaled Pu toxicity; from initiation, perhaps at the cell genome, to expression in the form of changed biochemical potential and altered tissue antigenicity. This should lead to the development and understanding of the mechanism of tumorigenesis and provide methodologies and information essential to the determination of the early effects for a wide variety of energy-related carcinogens.

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HEMATOLOGIC EFFECTS OF $^{239}PuO_2$ INHALATION IN RATS

Investigator:
H. A. Ragan
Technical Assistance:
S. L. English, D. H. Hunter, M. C. Perkins,
E. D. Blanton, and D. Meier

In rats, mean lung burdens of about 800 nCi of ^{239}Pu resulted in a significant lymphocyte depression by 30 days postexposure. This was followed by a marked and progressive neutrophilia over the next 60 days. Extrapulmonary ^{239}Pu concentrations were <5% of the lung burden.

Twenty female Wistar rats 80 days of age were exposed (nose-only) to plutonium dioxide. The $^{239}PuO_2$ aerosol had an activity mean aerodynamic diameter (AMAD) of 2.46 μm with a geometric standard deviation of 1.6 μm and a concentration of 0.976 μCi/ℓ. The exposure period was 90 minutes. Fifteen age-related controls were sham-exposed for a comparable period. Blood samples were obtained periodically from the tail vein for hematologic evaluations. Both groups of rats were housed and handled under identical conditions throughout the study.

Five rats were killed 30 days following exposure to determine ^{239}Pu retention. Of the remaining 15 exposed rats, 11 died or were euthanized between 46 and 84 days postexposure, and four sacrificed on Day 86.

The mean ^{239}Pu lung burden of the five rats killed 30 days after exposure was 960 nCi (range 450-1600); for the remaining 15 rats the mean lung burden at death was 740 μCi (range 300-1600). The total extrapulmonary burden of ^{239}Pu was less than 5% of the lung burden at any time period (Table 18.1).

Leukocyte values for the exposed and control groups are shown in Figure 18.1. By 30 days after $^{239}PuO_2$ inhalation, mean lymphocyte

TABLE 18.1. Tissue Burdens in Rats Following $^{239}PuO_2$ Inhalation (Mean ± SD).

TISSUE	^{239}Pu, nCi 30 DAYS (N = 5)	46-86 DAYS (N = 15)
LUNG	960 ± 460	740 ± 400
TRACHEOBRONCHIAL LYMPH NODES	5 ± 7	6 ± 8
LIVER	0.9 ± 0.6	1.9 ± 1.6
SPLEEN	0.3 ± 0.2	0.4 ± 0.4
CARCASS	30 ± 12	29 ± 51

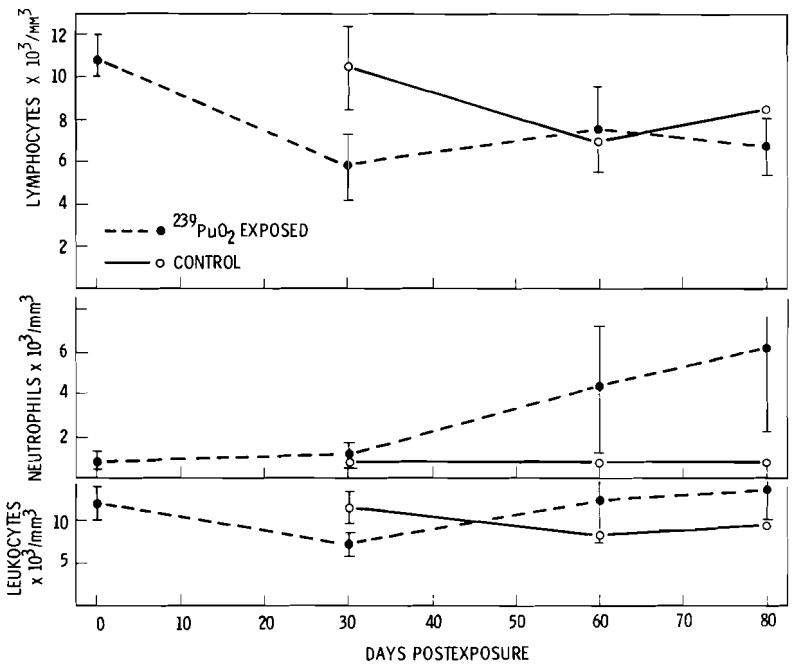

FIGURE 18.1. Leukocyte Evaluations in Rats Exposed to $^{239}PuO_2$ by Inhalation and Control Rats (Mean ± SD).

values were ∿50% of pre-exposure values, whereas those from the control group were comparable to pre-exposure levels. By 60 days post-exposure there was evidence of some recovery in lymphocyte values, which corresponded in time to a marked fall in control values, so that control and experimental groups did not differ markedly. A pronounced neutrophilia was evident in the exposed rats by Day 60 postexposure and this continued through the remainder of the study. A modest increase in monocyte concentrations was evident by Day 80 post-exposure, but not previous to that time. Eosinophil values of the plutonium-exposed rats were always comparable to those of the control groups.

All exposed rats lost weight and exhibited an elevated respiratory rate prior to death. At necropsy, the lungs had varying degrees of consolidation and edema, but neither was extreme. The tracheobronchial lymph nodes were usually much more prominent than normal, but this may have been a relative change. It was of interest, and possibly significant, that the thymus was not discernible in rats with marked polypnea and weight loss. While it is known that starvation results in thymic involution, it is not possible to determine from this study whether the loss of thymic tissue preceded or followed the weight loss, or to know the status of the thymus at the nadir of the lymphopenia. Future studies will investigate these interrelationships.

DENSITY GRADIENT SEPARATION AND SIZE DISTRIBUTION OF BEAGLE LYMPHOCYTES

Investigator:
H. A. Ragan
Technical Assistance:
K. Debban and D. Hunter

Density gradient techniques for obtaining pure, single-cell suspensions of peripheral blood mononuclear cells were applied to beagle dogs that had inhaled plutonium. There was a tendency toward a loss of intermediate-size lymphocytes in dogs with ^{239}Pu lung burdens, and a shift to larger cells when compared with control dogs.

Beagle dogs exposed to $^{239}PuO_2$ by inhalation develop a selective dose-related lymphopenia and, at certain exposure levels, a high incidence of pulmonary neoplasms. In order to study specific characteristics and immunologic functions of the lymphocyte populations affected by plutonium inhalation, efficient separation procedures are required to isolate these lymphocytes from other blood cells. The method selected was a Ficoll-Isopaque density gradient, since this appeared to have the least possibility of altering the lymphocytes, or of selecting a specific population.

The procedure used is summarized in Table 18.2. The choice of anticoagulation method is important depending on the use intended for the isolated cells: Defibrination removes platelets but results in an initial leukocyte loss of about 20% and a lymphocyte loss of about 30% when compared with blood collected in EDTA. These losses are undoubtedly due to trapping in fibrin masses. Fibrin agregates also interfere with size distribution studies and with determination of IgG binding sites. Heparin results in an irreversible aggregation of leukocytes when they are concentrated, and the final lymphocyte suspension is heavily contaminated with platelets. Blood collected in acid-citrate-dextrose (ACD) and sedimented in dextran prior to gradient separation resulted in heavy platelet contamination, loss of leukocytes, and reduced viability of the lymphocytes. EDTA, the anticoagulant ultimately selected, resulted in good lymphocyte separation except for marked platelet contamination.

TABLE 18.2. Method for Density Gradient Separation of Beagle Lymphocytes.

1) DEFIBRINATE OR ANTICOAGULATE
2) MIX 2 ml OF BLOOD AND 2 ml OF SALINE
3) LAYER ON 3 ml FICOLL-ISOPAQUE IN 12x100 mm GLASS TUBE
4) SEDIMENT 45 MINUTES IN COLD ROOM
5) CENTRIFUGE 60 MINUTES AT 400-1000 X g
6) CAREFULLY ASPIRATE MONONUCLEAR BAND
7) WASH CELLS 3X IN TRIS/BSS OR SALINE
8) RESUSPEND IN AUTOLOGOUS SERUM

The EDTA/Ficoll-Isopaque preparations resulted in a consistently high proportion of lymphocytes, and contaminants were usually restricted to other mononuclear cells, i.e., monocytes. However, if there is a tendency to eosinophilia in the peripheral blood, these cells are concentrated and the final suspension may contain 20-60% eosinophils. Other granulocytes are rarely present.

Viability of isolated lymphocytes, as evaluated by trypan blue exclusion, was 99.5 ± 0.8% (mean ± SD) in defibrinated blood, 96.5 ± 1% in EDTA blood, and 76.5 ± 2.1% in ACD/dextran-sedimented blood. Recovery of lymphocytes from the peripheral blood was 33 ± 11% using defibrinated blood, and 52.8 ± 12.1% when the blood was collected in EDTA.

Isolated lymphocytes were distributed by volume using a Coulter counter and multichannel analyzer, and the results plotted on an X-Y recorder.

Initial efforts to eliminate any contribution from occasional red cells or platelet aggregates, using a commercial lysing agent, were unsuccessful because the treatment also lysed the cytoplasm of the lymphocytes so that only the nucleus remained. With subsequent experience in using the gradient, erythrocytes were absent from the suspension, and platelets were eliminated from the plots by proper instrument settings. Lymphocyte size distributions in dogs with ^{239}Pu lung burdens of 500-1500 nCi, as compared with their age-related controls, are shown in Figure 18.2. There was considerable individual variation in the lymphocyte size distribution of both groups. However, it is clear that the mean size distribution curve of ^{239}Pu-exposed dogs is flatter than that of controls; the skewness, however, is more apparent to the right, i.e., to larger cell types.

We will continue to follow these changes in dogs at various intervals following plutonium inhalation. They will also be correlated with other characterizations of the lymphocyte populations influenced by these exposures. The density gradient separation is also effective in the isolation of cells from pulmonary lavage specimens, and size distribution data may indicate early changes in pulmonary cells not detectable by other means.

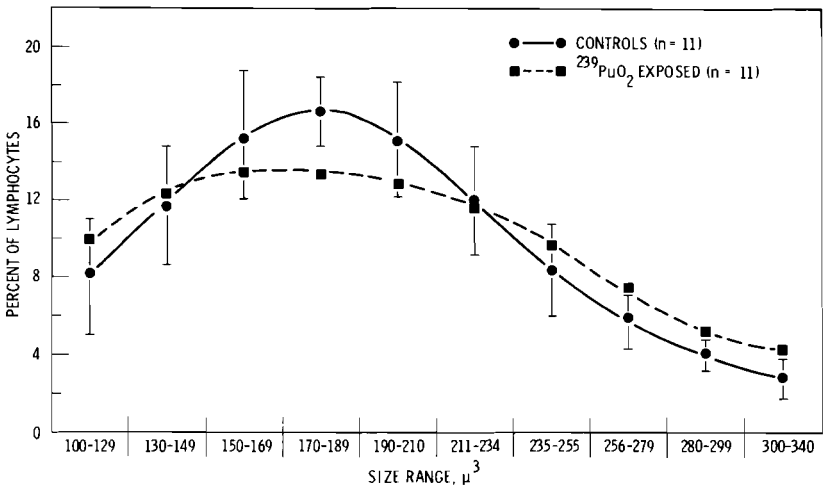

FIGURE 18.2. Lymphocyte Size Distribution in Control Dogs and Dogs Exposed to $^{239}PuO_2$ by Inhalation (mean ± SD about control mean)

EFFECTS OF PLUTONIUM ON THE IMMUNE SYSTEM: IMMUNOGLOBULIN-M IN BEAGLE DOGS EXPOSED TO PLUTONIUM AND IN UNEXPOSED CONTROLS

Investigator:
J. E. Morris
Technical Assistance:
R. S. Moore

Serum Immunoglobulin-M (IgM) levels in plutonium-oxide-exposed and unexposed beagle dogs were measured with a Mancini assay. With this assay, there was no apparent difference in IgM levels between the control group and groups of dogs with differing amounts of $^{238}PuO_2$ or $^{239}PuO_2$ deposited in the lung.

In a continuing study to ascertain the effects of inhaled plutonium oxide on the immune system, the serum IgM levels of beagle dogs were measured. These measurements will provide baseline data necessary to the understanding of effects of inhaled plutonium oxide on the humoral components of the immune mechanism.

During the past year over 4000 canine serum samples from exposed and unexposed female beagle dogs were assayed with a Mancini assay. The animals ranged in age from 1 to 6 years. The IgM levels in unexposed male and female beagle dogs are essentially the same, and show a slight increase during this time (Figure 18.3).

The IgM levels in beagle dogs, 1 to 48 months after exposure to $^{238}PuO_2$, were not statistically different from those of unexposed controls. The range of $^{238}PuO_2$ deposition in the lung was from <0.0001 to 6 μCi. Neither was there any significant difference in IgM levels of dogs receiving an initial deep lung deposition of <0.001 to 5 μCi of $^{239}PuO_2$. However, preliminary data obtained from litter mates exposed to different levels of plutonium suggest that there may be a genetic component to plutonium oxide effect on circulating immunoglobulin levels which will require additional study for final analysis.

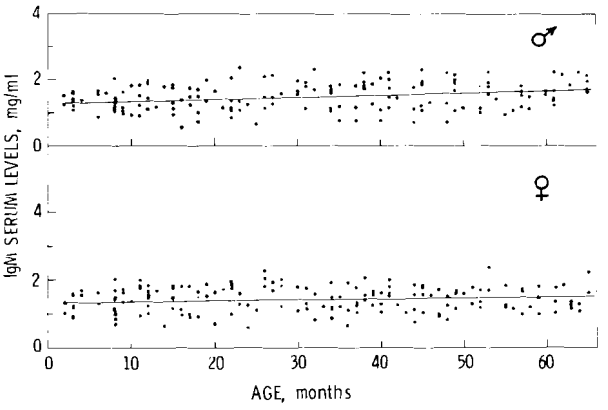

FIGURE 18.3. Age-related Serum IgM Levels in Male and Female Beagle Dogs.

DNA POLYMERASE ACTIVITY IN RADIATION-INDUCED OSTEOSARCOMAS

Investigator:
M. E. Frazier
Technical Assistance:
J. Cresto and T. K. Andrews

Viruses of the RNA tumor virus group are etiological agents of spontaneously occurring neoplastic diseases in a number of animal species. Biochemical assay of cells for viral RNA-instructed DNA polymerase (RIDP) in a cytoplasmic particulate fraction has increased the sensitivity of detection of RNA-tumor virus infection. Using these techniques we have been able to identify RIDP in tissues from beagles with radiation-induced osteosarcomas.

A limited number of radiation-induced malignancies appear to be associated with C-type virus particles similar to the RNA tumor viruses. For example, radiation-induced leukemia in swine and mice are both associated with intact C-type viruses. The porcine isolate is capable of effecting transformation of tissue culture cells while the Kaplan agent will serve as an etiologic agent for murine leukemia. These systems suggested that oncornavirus might be present in other radiation-induced tumors; we therefore examined radiation-induced tumors in the beagle dog.

Attempts with electron microscopy to demonstrate C-type viruses in tumors from beagles with either spontaneous or radiation-induced malignant diseases have been unsuccessful, as have attempts to isolate infectious virus from tumor extracts using in vivo and tissue-culture monitoring systems. Failure to isolate virus from these tumors could be due to: 1) the presence of incompetent viruses capable of inducing transformation but not capable of total transcription of the virus genome; 2) incomplete or noninfectious virus particles; 3) use of cell lines or animal systems incapable of supporting virus replication; 4) the inability to detect limited virus replication by conventional techniques. The demonstration of RIDP (RNA-instructed DNA polymerase)--reverse transcriptase--in the virions of Rous sarcoma virus provided the first exploitable biochemical marker for detecting infection by these agents. RIDP has also been found in viruses of unknown oncogenic potential. These viruses, which are apparently not oncogenic but contain RIDP and RNA (and replicate via a DNA intermediate), are classified as retraviruses.

The role of the RIDP in these viruses is to catalyze the synthesis

of a complementary DNA copy (cDNA) from the virion's 70S RNA template. The steps in this reaction are:

RNA → RNA:cDNA hybrid → DNA:cDNA duplex,

with a portion of the cDNA eventually being incorporated into the cell genome. Earlier studies that described the presence of RIDP in normal cells utilized synthetic templates. These templates are now known to be substrates for both RIDP and cellular polymerases, and thus do not allow resolution of the two activities. A subsequent observation that RIDP-like activity could be detected in normal chicken embryos seemed to support Temin's hypothesis that RIDP may have functions in normal cells. However, these experiments did not rule out the possibility that the RIDP was the result of expression of an endogenous C-type chicken virus. In spite of these controversial findings relating to soluble RIDP, the presence of reverse transcriptase in a cytoplasmic particulate of the same size and density as known oncornaviruses is considered indicative of either retravirus or oncornavirus infection. Therefore, utilizing available biochemical techniques, we have sought to determine whether RIDP is present in beagles with radiation-induced bone or lung tumors.

A schematic outline for the fractionation of tumor cells, and for the synthesis and analysis of the endogenous reverse transcriptase (RIDP) product from the cytoplasmic particulate fraction is shown in Figure 18.4.

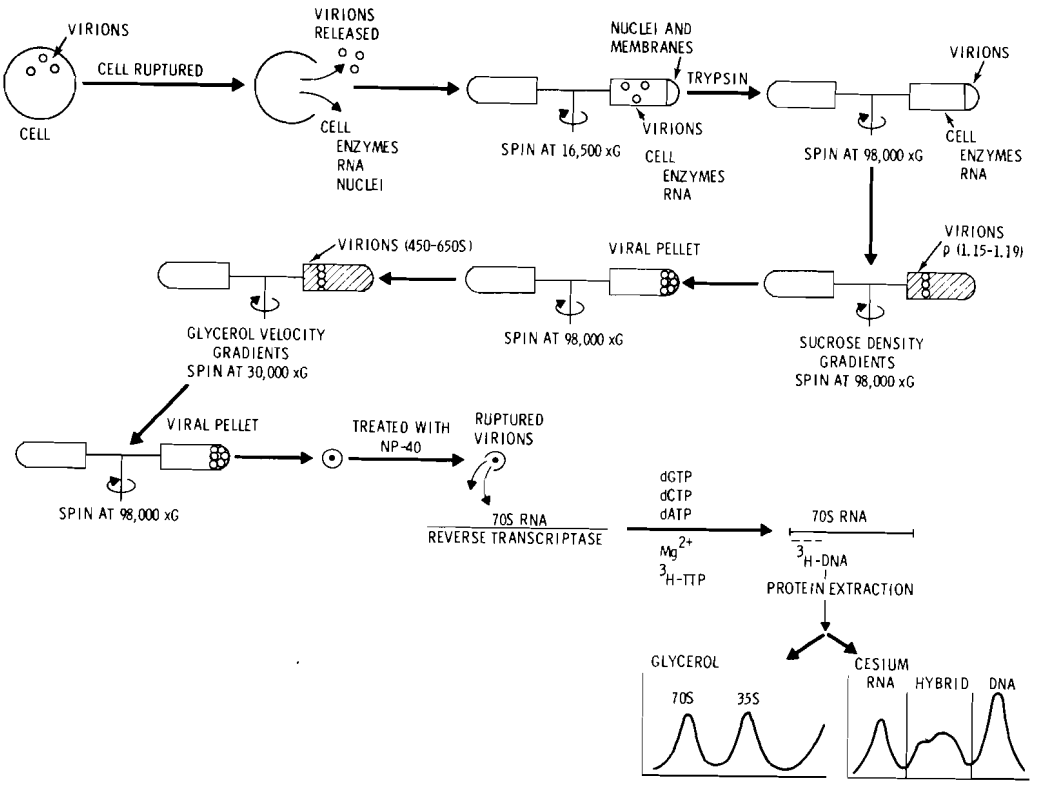

FIGURE 18.4. Detection of Particulate Reverse Transcriptase in Cells.

DNA synthesis was monitored by following the incorporation of ^{3}H-labeled thymidine triphosphate (TTP) into acid-insoluble products. Figure 18.5 shows the kinetics of DNA synthesis in a pellet prepared from a canine radiation-induced osteosarcoma. The kinetic curve of DNA polymerase activity showed a peak of [^{3}H] DNA synthesis at 15-30 min which was ∿78% RNase sensitive, indicating that RNA served as a template for the DNA synthesis.

Both normal and tumor tissue from several beagles was processed and assayed by the simultaneous detection test. As can be seen in Figure 18.6, an osteosarcoma sample yielded a distinct peak of radioactivity in the form of acid-precipitable ^{3}H-TTP in the 60-70S region of the gradient. To demonstrate that the tritiated peak in this region was due to the synthesis of DNA on a heteropolymeric 60-70S RNA molecule, two parallel reactions were run. First, the addition of RNase to the reaction mixture decreased the radioactivity peak in the 70S region from 408 cpm to 35 cpm; second, omission of one of the deoxyribonucleoside triphosphates from the reaction mixture reduced the 70S peak to an undetectable level. This indicated that we were not measuring an end-addition enzyme or the restricted complementary copying of polyadenylic acid regions of an RNA molecule.

The results of our experiments to date are summarized in Table 18.3. Tissues from dogs with radiation-induced tumors and spleens from exposed and control dogs without lung or bone tumors were negative by the kinetics and simultaneous detection tests. However, five samples from beagles with osteosarcomas gave positive values, indicating the presence of a cytoplasmic particulate fraction (of the size and density of known oncornaviruses) that contains RIDP.

Our results indicate that RIDP is present in at least some radiation-induced malignancies in beagles, and that the presence of this enzyme can at least be considered indicative of retravirus infection. However, because of the widespread occurrence of

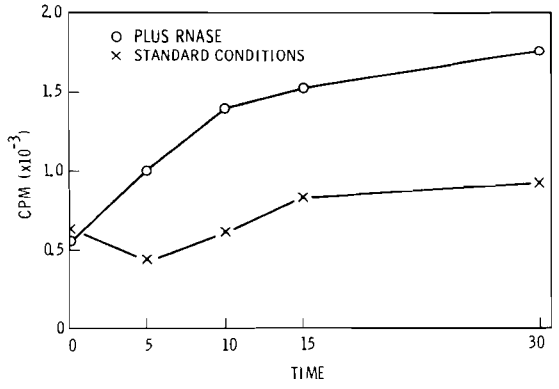

FIGURE 18.5. Kinetics of an Endogenous Reverse Transcriptase Reaction in a Cytoplasmic Particulate Fraction Isolated from the Tumor Tissue of a Beagle with an Osteosarcoma. Two 100-µl reactions were performed. A standard endogenous reverse transcriptase reaction was allowed to proceed in one aliquot (0-0) while the remaining sample was treated with RNase A to destroy any available RNA template.

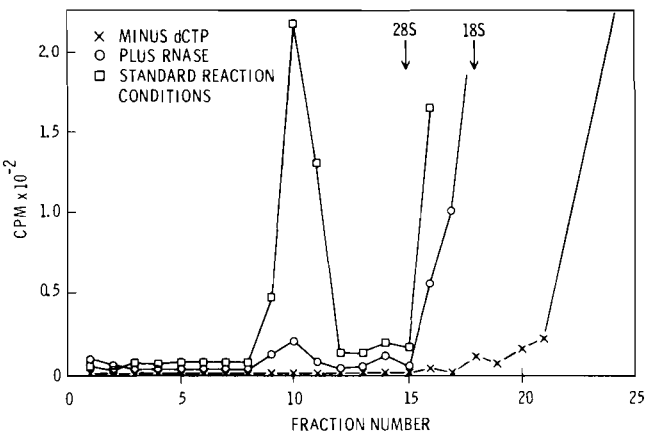

FIGURE 18.6. Simultaneous Detection of ^{3}H-DNA:70S RNA Complex as the Product of an Endogenous Reverse Transcriptase Reaction Carried out with Tumor Tissues from a Beagle with Radiation-Induced Osteosarcoma. The external markers used were ^{3}H-labeled 28S and 18S RNA from NC-37 cells.

these agents, their presence in diseased tissues (e.g. tumors) does not by itself indicate they are etiologic agents in these diseases. For example, the radiation may be activating an endogenous nononcogenic retravirus in vivo in a manner analogous to the in vitro activation of retraviruses resulting from the treatment of cell cultures with compounds such as 5-bromo-deoxyuridine.

Experiments are currently underway to confirm and extend these findings. Specifically, we are involved in attempts to isolate and purify reverse transcriptase from radiation-induced tumors; and we are employing molecular hybridization studies to examine the degree of homology between known oncornaviruses and nucleic acid components isolated from these osteosarcomas.

TABLE 18.3. Detection of Reverse Transcriptase in Radiation Induced Tumors.

	DOG #	ISOTOPE	DOSE nCi	ROUTE	DIAGNOSIS	KINETICS OF ^{3}H-TTP INCORPORATION (%RNASE SENSITIVE)	SIMULTANEOUS DETECTION *** + RNASE	STANDARD CONDITION
1	M3G3	^{249}Cf	3896*	INJECTION	OSTEOSARCOMA	55	35	408
2	T74W3	^{241}Am	3020*	INJECTION	OSTEOSARCOMA	70	10	312
3	M54W3	^{241}Am	3213*	INJECTION	OSTEOSARCOMA	45	18	180
4	482	^{238}Pu	800**	INHALATION	OSTEOSARCOMA	75	<10	320
5	469	^{238}Pu	2600**	INHALATION	OSTEOSARCOMA	78	<10	227
6	273	^{239}Pu	1500**	INHALATION	BRONCHIOLOALVEOLAR CARCINOMA	<10	<10	<10
7	278	^{239}Pu	800**	INHALATION	BRONCHIOLOALVEOLAR CARCINOMA	<10	<10	<10
8	252	^{239}Pu	460**	INHALATION	BRONCHIOLOALVEOLAR CARCINOMA	<10	<10	<10
9	1241	---	---	CONTROL	---	<10	<10	<10
10	1102	---	---	CONTROL	---	<10	<10	<10
11	1223	---	---	CONTROL	---	<10	<10	<10
12	1214	^{238}Pu	12**	INHALATION	---	<10	<10	<10

* INJECTED DOSE
** BODY BURDEN AT DEATH
*** COUNTS PER MINUTE IN THE 70S REGION OF THE GRADIENT. THESE ASSAYS WERE RUN ON 10% OF THE TOTAL MATERIAL AVAILABLE

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EFFECTS OF POLLUTANT METALS IN NUTRITIONALLY DEFICIENT POPULATIONS

Person in Charge: H. A. Ragan

This project was first funded in FY 1976 and, as originally formulated in the Schedule 189, stressed the influences of protein deficiency. With the concurrence of ERDA Division of Biomedical and Environmental Research, the scope was subsequently altered to emphasize the influences of iron deficiency.

Iron deficiency is one of the most common nutritional diseases of young children and women of child-bearing age. Despite the paucity of data on absorption of metal pollutants in iron-deficient subjects, it is known that some elements compete for iron-binding proteins in plasma and in cells, and that some interfere with iron metabolism. Iron deficiency may enhance the absorption, translocation or transplacental passage of heavy metals, increasing the risk of toxic effects in the young, teratogenic effects in the fetus, or interference with iron metabolism in subjects already compromised in regard to their iron status. The effects of combined iron deficiency and exposure to heavy metal pollutants on immune competence are of particular concern in regard to carcinogenesis.

Initial studies under this project will determine the influence of iron deficiency on the gastrointestinal absorption of cadmium, nickel, arsenic and vanadium in rats. Metals whose absorption is enhanced by iron deficiency will be further studied to determine their effects on hematopoiesis and immune competence when chronically administered to control and iron-deficient rats. Reportable results from these studies are not yet available.

DELAYED EFFECTS OF INHALED OIL SHALE AND SPENT SHALE PARTICLES IN EXPERIMENTAL ANIMALS

Person in Charge: J. E. Lund

This new project was designed to investigate the possible health hazard of dusts that may be produced in the processing of oil shale. Shale oil may be expected to contribute significantly to the U.S. energy supply in the years ahead. Its production will become an important industry. Dust generation from crushing and related operations, and from the transportation of ore to crushing facilities has been estimated to involve as much as one percent of the total mass of shale ore handled. Newer equipment and methods could reduce this loss of airborne particles to the atmosphere, and the extent to which such control measures are required may become an important factor in the design of processes and facilities.

Initial intratracheal instillation experiments will be designed to define the toxic potential of oil shale materials. Two rodent species will be given intratracheal doses of oil shale, spent shale, quartz (fibrosis control), benzpyrene and iron oxide (carcinogen positive control), and vehicle. Acute toxicity and repeated dose studies will be initiated to determine optimum dose levels for the long-term study. The long-term study will be initiated before the end of FY 1976. The results of these studies will be examined to determine possible adverse health effects and to determine if a more extensive inhalation study is needed.

Pulmonary diseases associated with coal mining (pneumonoconiosis) and uranium minimg (lung cancer) are well established. Since the development of shale oil as an energy source represents a "new" industry in terms of large-scale development, it is important to investigate possible adverse health effects prior to exposure of large populations to the process effluents. This study represents a small part of the total effort required, which includes source-term characterization of emissions (particulate, vapor, liquid, etc.), toxicologic studies, and environmental studies.

This project was first funded in FY 1976. Initial experiments are underway but reportable results are not yet available.

EVALUATION OF MUTAGENICITY AND CARCINOGENICITY FOR COMPOUNDS DERIVED FROM OIL SHALE PROCESSES

Person in Charge: R. A. Pelroy

Utilization of oil shale as a fuel source will release to the atmosphere many organic and inorganic materials. Many of these materials, especially the polyaromatic compounds and aromatic amines, are potentially carcinogenic. This project will test oil shale chemicals for potential carcinogenicity, using microbial and mammalian tissue-culture bioassays.

The microbial assays, which are low-cost and technically simple to perform, will be used for initial screening. In these tests, the capacity of chemicals to revert frame-shift and point mutations in special auxotrophic strains of Salmonella typhimurium is taken as an index of carcinogenicity. Chemicals which are positive in the microbial system will progress to the second phase of testing involving exposure of fibroblastic tissue culture cells from the syngeneic mouse strain, Balb/c3T3. Plating efficiency, survival, and especially cellular transformation of the clones, will be used as indicators of chemical-cell interaction. Cellular transformation assays will be carried out by plating samples of treated cells in soft agar. The final stage in testing will involve transplantation of transformed cells into recipient animals; development of tumors will be indicative of high carcinogenic potential.

This project was first funded in FY 1976. Six chemicals have thus far been screened in the S. typhimurium system: anthanthrene benzo[ghi]perylene, fluoranthene, perylene, phenanthrene and pyrene. Anthanthrene, perylene and fluoranthene are mutagenic, suggesting carcinogenic potential. In particular, fluoranthene was a very strong frame-shift mutagen; this chemical is not currently designated as a carcinogen. Benzo[ghi]perylene is weakly mutagenic but only with point mutants. All of the chemicals which increase the rate of mutation in S. typhimurium require metabolic activation with microsomal enzymes.

CHARACTERIZATION OF PATHWAYS AND KINETICS FOR LUNG CLEARANCE OF POLYCYCLIC HYDROCARBONS IN RATS AND DOGS

Person in Charge: J. T. Veal

Polycyclic hydrocarbons are primarily introduced to man through the respiratory tract by cigarette smoking and by breathing fumes resulting from combustion of fossil fuels. The potential health hazard resulting from inhalation of these chemicals is well recognized, but the dose-response relationships for these compounds, when inhaled from the environment, are the subject of much debate.

This project will characterize the role of the respiratory tract in the introduction of polycyclic hydrocarbons and their metabolites into the body. It will investigate, in animals exposed via aerosol inhalation, the kinetics and the translocation pathways from the lung of environmentally significant polycyclic hydrocarbons such as benzo(a)pyrene, anthracene, fluoranthene, chrysene, 3-methylcholanthrene and pyrene. The influence on deposition, translocation and metabolism, of such aerosol properties as particle size, aerosol density and chemical form, will be studied.

The experimental procedure will involve the generation and delivery of well characterized aerosols of radioactively labeled polycyclic hydrocarbons to the respiratory tracts of dogs and rats. The translocation of these materials from the lung will be determined by monitoring blood flow from the lung for the polycyclic hydrocarbons and their metabolites, and by subsequent analysis of respiratory tract tissues.

This project was first funded in FY 1976 and reportable results have not yet been obtained.

EVALUATION OF RADIONUCLIDES IN MAN

Person in Charge: I. C. Nelson

This project is concerned with the development and application of methods for evaluating the radiological impact of the nuclear industry on its workers and on residents in the environs. Presently the project emphasizes post-mortem studies in which the quantities of radionuclides, particularly plutonium, are measured in samples of tissues obtained at autopsy from former workers and residents in the local environs of the Hanford project and from individuals at more distant locations. In addition, this project supports the U.S. Transuranium Registry through radiochemical analyses of samples obtained by the Registry from exposed workers and unexposed controls throughout the country. The development of an adequately sensitive and accurate technique for assessing the internal deposition of promethium oxide has also been an important part of this project. Recently, a program of whole-body counting measurements on residents and control groups in the Hanford environs was initiated in order to quantify the environmental impact of fission product waste management programs at Hanford.

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EVALUATION OF POSTMORTEM TISSUE SAMPLES

Investigators:
I. C. Nelson[a], L. J. Kirby[b] and
V. W. Thomas, Jr.[b]
Technical Assistance:
D. T. Harless[b], R. M. Bernard[a] and
F. N. Eichner[a]

Collection and radiochemical analysis of postmortem tissue samples (lung, liver, bone and tracheobronchial lymph nodes) from individuals formerly residing in the vicinity of the Hanford project continued during the past year. Postmortem tissue samples and blood samples were also analyzed for the U.S Transuranium Registry (USTR). During the year commencing November 1, 1974, 85 analyses for plutonium-238 and plutonium-239+240 were performed on samples from the Hanford locality, and 41 analyses for plutonium-238 and plutonium-239+240 on samples obtained from the USTR. In addition, about 60 internal cross-check samples were processed in duplicate. Currently in process are 29 samples from the Hanford locality and 30 USTR tissue samples. About 80 samples from the Hanford environs are awaiting processing.

Discussions on the results of past plutonium analyses of interlaboratory comparison samples were held with investigators from other participating laboratories (HASL and LASL) in conjunction with the 21st Annual Bioassay and Analytical Chemistry Meeting. Differences observed in results were discussed and variations in techniques were noted. It was concluded that an additional set of calibration samples at lower levels of plutonium activity would be prepared by LASL and distributed to HASL, PNL and other interested laboratories.

Plutonium-242 is our tracer of choice for yield determination in the alpha energy analysis of tissues for ^{238}Pu and ^{239}Pu. If the ^{239}Pu content of a sample is high, the yield information may be masked and, consequently, the plutonium results would be lost. That problem is overcome by processing a second, smaller aliquot (usually retained for such contingencies) for ^{239}Pu, using ^{236}Pu as a yield tracer. From the results of that processing, the amount of ^{239}Pu in the original sample may be estimated with adequate sensitivity. The ^{238}Pu content of the original aliquot may then be estimated using the calculated ^{239}Pu result as a basis for ^{238}Pu radiochemical yield.

A summary of results obtained prior to November 1970 was given in last year's annual report. Complete results are available on 17 additional local residents, 8 of whom

(a) Physics and Instrumentation Department
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had worked on the Hanford project but with little probability of exposure to plutonium. Other cases for whom analytical processing is complete are awaiting completion of a search for work histories. Among the newly completed cases, none had a concentration of plutonium in lungs exceeding 2 fCi Pu/g; about two-thirds had less than 0.1 fCi Pu/g. In the liver, the largest concentration was 3 fCi Pu/g, with 70% of the results lower than 0.5 fCi Pu/g, and 40% less than 0.1 fCi Pu/g. Few positive results were noted for bone or tracheobronchial lymph nodes; all were less than 3 fCi Pu/g.

DEPOSITION OF RADON DAUGHTERS IN THE TRACHEOBRONCHIAL TREE

Investigator:
I. C. Nelson[a]
Technical Assistance:
R. M. Bernard[a]

Studies of the deposition of radon daughters on the mucosa of the proximal bronchi of the tracheobronchial tree centered on completing a comparison of deposition in the various airways using a diffusion equation and the Weibel Regular and Irregular Dichotomous Lung Models. It was concluded from this study that proximal to the subsegmental bronchi, the difference in calculated area deposition in the variously sized tubes was not sufficient to permit identification of preferential sites of deposition, which presumably would imply preferential sites of tumorigenesis. Although particle removal times, depth of sensitive tissues, etc., were not taken into account, it was concluded that the less complicated Weibel Regular Lung Model would suffice for most applications in uranium miner lung dosimetry.

(a) Physics and Instrumentation Department

CHEMICAL RADIATION PROTECTION

Person in Charge: J. C. Hampton

Damaging effects of radiation, ranging from acute radiation sickness to chronic debilitating disease and tumor induction, have been described for nearly every source of radiation and route of administration. Relatively little progress has been made, however, in developing practical protective and treatment measures. The compound WR2721 [S-2(3-aminopropylamine) ethyl phosphorothioic acid hydrate] protects mice exposed to 1500 R X-rays and might be particularly useful in modulating the acute response of lung or gastrointestinal tissue, for example, following inhalation of radionuclides, thus buying time and reducing injury pending institution of normal therapeutic removal procedures or medical intervention.

The immediate aims of this project are to determine the intracellular localization of ^{35}S-labeled WR2721 through cell fractionation and autoradiography, to determine major organs and tissues that take up the compound, and to evaluate the efficacy of WR2721 in modulating damage to tissues acutely exposed to both internal and external radiation sources.

Initial cell fractionation studies on liver, kidney and small intestine have been completed. The results show that the major part of the ^{35}S label was contained in the soluble, microsome-rich fraction. The activity was highest in liver and lowest in kidney, and this is consistent with known ribosome content of liver, intestinal epithelial, and kidney tubule cells. These observations are useful in interpreting our previous work which showed that the protective effect of WR2721 was related to postmitotic cell survival rather than to the protection of cells in the proliferative compartment.

PUBLICATIONS SINCE JANUARY 1973:

Hampton, J. C. and P. H. Grundy. 1974. The Fine Structure of Intestinal Epithelial Cells of Mice Irradiated after Treatment with WR2721. Proceedings of the 8th International Congress of Electron Microscopy, Canberra, Australia, August 25-31 (In Press)

LOCALIZATION OF ^{35}S-WR2721 IN CELLS

Investigator:
J. C. Hampton
Technical Assistance:
R. R. Adee and J. T. Cresto

Compound WR2721 protects mice exposed to 1500 R X-rays, the protective effect being exerted on postmitotic cells. Evidence to date shows the compound to be localized mainly in the cytoplasm, probably associated with the microsome fraction.

Earlier work from this laboratory showed that mice protected with WR2721 [S-2(3-aminopropylamino) ethyl phosphorothioic acid hydrate], administered 10 min prior to exposure to 1500 R X-rays never developed the acute intestinal syndrome, failed to become hemorrhagic and did not succumb to marrow failure. Studies of the intestinal epithelium showed that the compound had little effect on proliferative cells but, rather, protected postmitotic cells, permitting them to survive long enough for the proliferative compartment to recover and to restore a normal epithelial covering on the intestinal villi.

This report deals with experiments intended to localize intracellular binding sites of ^{35}S-WR2721, in order to bring about a more precise understanding of the mode of action of the drug in its role as a radioprotective agent.

Twenty-four C57 black mice, 18-20 g body weight, were used in a single experiment to determine intracellular localization of ^{35}S-WR2721 in liver, kidney, gonads, and intestine. The mice were divided into three groups, each comprised of four males and four females. One group received only WR2721; another group, WR2721 10 minutes prior to exposure to 1500 R X-rays; and a third group, 1500 R X-rays immediately prior to treatment with WR2721. Half of each group, two males and two females, was sacrificed at 2 hr and the other half was sacrificed at 24 hr posttreatment. Specimens of liver, kidney and small intestine were subjected to cell fractionation procedures and to autoradiography. Gonads were used for autoradiography only.

Pooled specimens of liver, of kidney and of intestine were used for

cell fractionation studies, and from these, after homogenization, a low-speed (960 X g) soluble fraction and a pellet fraction were obtained. The pellet was resuspended in fresh media, analyzed for ^{35}S, sedimented again and analyzed a second time. The bar graphs shown in Figures 24.1 and 24.2 summarize the results; data for liver in subgroup 3b are not available.

The soluble fraction contains microsomes and lighter elements of the cells and the activity is highest in liver and lowest in kidney (Figure 24.1); this correlates

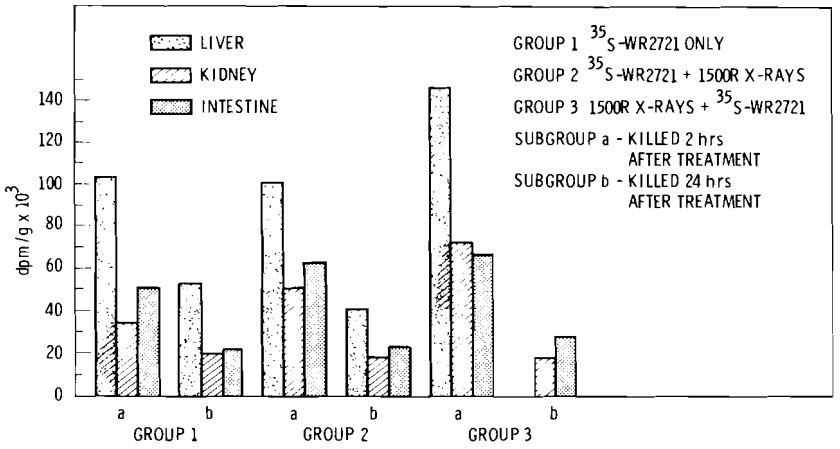

FIGURE 24.1. ^{35}S-WR 2721 in Tissue: Low-speed Soluble Fraction.

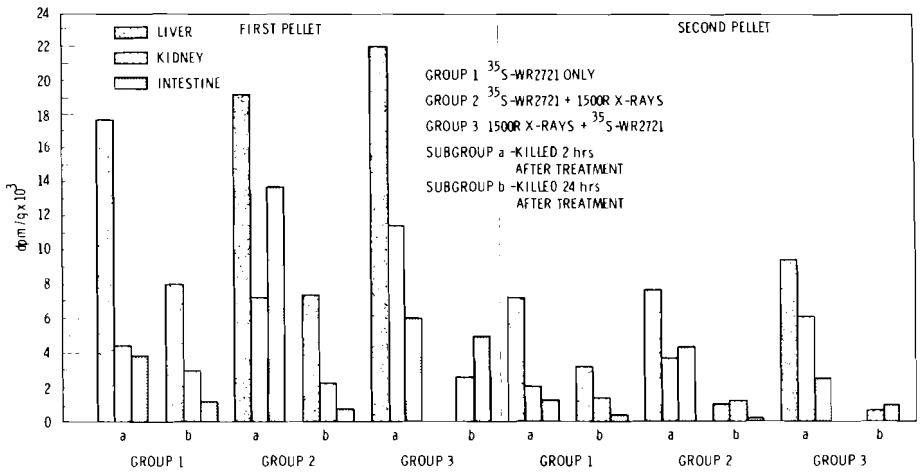

FIGURE 24.2. ^{35}S-WR 2721 in Tissue: Low-speed Pellet.

directly with RNA content of cells comprising the three tissues. Note that there is no difference between liver uptake in mice receiving only WR2721 and irradiated mice injected with WR2721 prior to exposure; but uptake by liver in mice injected after radiation exposure is considerably higher. Activity of ^{35}S in the first pellet fraction was much less than in the soluble fraction and this was substantially reduced by washing and further centrifugation, indicating that the pellet fractions still contained appreciable amounts of lighter elements. Further separations and characterization of the fractions have not yet been carried out.

The results to date are in agreement with the concept that WR2721 exerts its protective effect on postmitotic cells rather than on proliferating cells, permitting cells already on the villi to survive long enough for the generative compartment to recover and repopulate the villus epithelium. In some respects, the morphologic appearance of the gut epithelium in protected, irradiated mice is similar to that observed when the generative compartment was held in mitotic arrest up to four days by injections of colchicine.

Although the crypt cell populations were severely reduced in number, the villi remained covered, indicating that villus epithelial cells can remain on the villus longer than would be predicted on the basis of cell turnover data. Accordingly, it appears that exposure to radiation not only interferes with cell proliferation but that it also has a life-shortening effect on maturing and mature cells. It is the latter population that WR2721 appears to protect, but the mechanisms involved are unknown.

Localization of ^{35}S-WR2721 in the microsome fraction does not necessarily imply that the protective effect derives from reactions that serve to maintain viable pathways for protein synthesis, although it could be a correct assumption. The compound is toxic, so the microsome fraction could also be the site of detoxification, particularly in liver. Neither of these considerations is mutually exclusive and the intent of current studies is to determine, if possible, the mode of protection and to be able to make informed judgments concerning protection against radiation sources other than X-rays.

TREATMENT FOR RADIONUCLIDE INCORPORATION

Person in Charge: V. H. Smith

The objective of this project is to decrease the damage potential from inhaled, skin- or wound-absorbed, or ingested radionuclides. While primarily addressed to the needs for worker protection in the nuclear industries and laboratories, it also looks to the possible treatment needs arising from exposure of larger segments of the population. The approach is to develop methods that will prevent absorption, hasten excretion, improve decontamination, or alter translocation of the radionuclides -- all for the purpose of minimizing radiation dose to sensitive tissues. Necessarily associated studies include mechanisms of radionuclide absorption, transport, mobilization and tissue distribution, preparation of new treatment agents and methodology, and the toxicology, pharmacology, pharmaceutics, and pharmacodynamics of treatment agents.

Current research includes an intensive effort to obtain the information required in support of an IND to legally permit the use of inhaled Ca-DTPA, and possibly Zn-DTPA in humans. This includes studies on the comparative effectiveness of the two salts when given by inhalation or injection, and on the possibly toxic effects of inhalation treatment with these agents. Insoluable radionuclides in lung and liver are often retained in phagocytic cells of the reticuloendothelial system. Attempts are being made to prepare suitable pharmaceutical vectors, e. g. microencapsulated chelating agents, that will be able to enter these cells and possibly remove an additional fraction of the radionuclide not available by current methods. Cultured lung macrophages are being studied to better understand the processes of radionuclide release from such cells and to serve as an economical test system for optimizing new treatments before testing in animals. Other studies are aimed at finding new treatment agents, preventing absorption of radionuclides, and establishing the effectiveness of therapy for multiple radionuclide insults.

PUBLICATIONS SINCE 1973

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IN VITRO TESTING OF AGENTS TO REMOVE INTRACELLULAR TRANSURANIC ELEMENTS

Investigators:
R. P. Schneider and A. V. Robinson
Technical Assistance:
L. M. Butcher

An in vitro test system to study biological mechanisms of particulate radionuclide uptake, intracellular solubilization and mobilization of these particulates is being developed. In vitro cultures of essentially pure rabbit-lung macrophages have been maintained for 2 weeks without cell turnover or alteration. Suspensions of freshly harvested cells have been shown to phagocytize $^{239}PuO_2$ particles.

Particulate forms of Pu reaching the lung are rapidly phagocytized. The particles are located within phagolysosomes of macrophages where they are not available for removal by chelators, which cannot penetrate cell membranes. Effective therapeutic procedures must either remove the intact particles from the lung, or cause their conversion to forms which can be complexed and excreted in the urine. To better study the mechanisms of particulate radionuclide uptake and release from lung macrophages, an in vitro cell culture system was investigated. Since the reticuloendothelial system and its macrophages are involved in the handling of particulate radionuclides in many organs, information from lung macrophages might have wide application to the understanding of these processes.

Lung macrophages are harvested from rabbits by lung lavage. The yield is about 10^7 cells per rabbit. The cells, after concentration by centrifugation, attach to the surface of plastic tissue culture flasks to a density of 0.2×10^6 cells. This adherence of macrophages to the plastic surface allows selective removal of contaminating lymphocytes and erythrocytes by washing. The culture medium finally selected was standard medium M-199 with Earle's salts, supplemented with 20% rabbit serum. In this system, the condition and number of cells can be directly examined through the transparent walls of the flasks. Attachment of about 90% of the macrophages occurs within 1 hour. Loss of cells from cultures averages only about 5% per day, and the cultures are morphologically stable for 2 weeks.

$^{239}PuO_2$ particles (average diameter, about 0.1 μm) were incubated with cells in suspension at 37°C. Subsequent autoradiography of smears of the cells demonstrated that Pu particles were actively phagocytized in this in vitro system. Attachment of cells to the flasks was apparently not inhibited by the radionuclide. Continuing studies will measure effects of various treatment agents on the solubility of Pu in the macrophages.

LATE EFFECTS OF INHALED Ca-DTPA IN RATS PREVIOUSLY EXPOSED TO $^{239}Pu(NO_3)_4$ AEROSOLS

Investigators:
J. E. Ballou, G. E. Dagle, K. E. McDonald and R. L. Buschbom
Technical Assistance:
R. A. Gies

Inhaled Ca-DTPA administered at six weekly intervals, 1-hour exposures, 3 mg/exposure, commencing 20 days after $Pu(NO_3)_4$ inhalation, reduced Pu deposition in skeleton and liver by 20% from sham-treated control values but did not influence bone and lung tumor incidence. A single lung tumor developed in a rat treated only with Ca-DTPA, however this lesion cannot be attributed with certainty to the drug treatment.

The acute toxicity of inhaled Ca-DTPA was presented in previous Annual Reports. Information on the long-term effects following a typical treatment sequence were sought in this study.

Rats were exposed to $Pu(NO_3)_4$ aerosols and treated 20 days afterwards with six weekly inhaled doses of Ca-DTPA (10 mg/kg). The absorbed chelate dose was about 3 mg/exposure, as determined by measuring DTPA excreted in urine during the first three days after Ca-DTPA inhalation. The experimental protocol is summarized in Table 25.1.

Changes in weight gain and survival, as well as Ca-DTPA decorporation efficacy, were reported in previous Annual Reports. Briefly, there was no effect of Ca-DTPA administration on weight gain and survival, although the chelating agent reduced skeletal and hepatic Pu levels by 20% ($p = 0.05$) from the nontreated control level. There was no apparent effect of treatment on Pu retention in the lung.

As rats died during the long-term phase of this study, the major tissues were examined histopathologically and the significant lesions were recorded. Lung, liver, skeleton, tracheobronchial lymph nodes, pelt and residual soft tissues were analyzed for Pu content to calculate cumulative life-time radiation dose.

In Table 25.2, the malignant tumor incidence in lung and bone is listed for the various treatment

TABLE 25.1. Experimental Protocol

TREATMENT	NUMBER OF RATS
$Pu(NO_3)_4$[(a)] + SHAM Ca-DTPA	20
$Pu(NO_3)_4$ + Ca-DTPA[(b)]	46
SHAM Ca-DTPA[(c)]	30
Ca-DTPA	70
NONTREATED CONTROLS	100

(a) INITIAL LUNG BURDEN AVERAGED 78 nCi; RANGE 51-105 nCi MEASURED IMMEDIATELY AFTER EXPOSURE.

(b) ESTIMATED DEPOSITION WAS 3 mg/RAT FOR EACH OF SIX WEEKLY ONE-HOUR EXPOSURES.

(c) SHAM-EXPOSED ANIMALS WERE SUBJECTED TO THE SAME TREATMENT AS THE EXPOSED GROUPS EXCEPT THAT NO AEROSOL WAS DELIVERED.

TABLE 25.2. Number of Malignant Lung and Bone in Rats Administered $Pu(NO_3)_4$ and Ca-DTPA by Inhalation.

	LUNG				SKELETON
TREATMENT	TOTAL	ADENOCARCINOMAS	SQUAMOUS CELL CARCINOMAS	ADENOSQUAMOUS CARCINOMAS	OSTEOSARCOMAS
$Pu(NO_3)_4$ + SHAM Ca-DTPA	9/20	7/20	1/20	1/20	1/20
$Pu(NO_3)_4$ + Ca-DTPA	18/46	16/46	2/46	0/46	1/46
SHAM Ca-DTPA	0/30	0/30	0/30	0/30	0/30
Ca-DTPA	1/70	1/70	0/70	0/70	0/70
NONTREATED CONTROLS	0/100	0/100	0/100	0/100	0/100

groups. The lung tumors included 23 adenocarcinomas, four squamous cell carcinomas, and one mixed type adenosquamous carcinoma. Only two osteosarcomas were found: one in the group exposed to $Pu(NO_3)_4$ + Ca-DTPA, and one in the $Pu(NO_3)_4$ + sham Ca-DTPA group. No significant difference in tumor incidence was shown between these two groups. The single lung tumor observed with Ca-DTPA alone cannot be attributed with any degree of certainty to the chelate treatment. Although the normal incidence of lung tumors in the Wistar strain is quite low (on the order of 0.1%), lung tumors have been reported by others in control rats exposed to acidic nitrate aerosols.

The accumulated radiation dose to lung and skeleton was calculated for each rat on the basis of the amount of plutonium in these tissues at death and the plutonium retention kinetics, determined from analyses of animals that were serially sacrificed or that died during the course of this study. The accumulated radiation dose to lung and corresponding numbers of lung tumors are listed in Table 25.3 for both Ca-DTPA-treated and sham-treated rats. Similar data are shown for bone tumors in Table 25.4. The cumulative lung dose varied over a 50-fold range, due mostly to the variation in initial deposition (average 78 nCi; range 51-105 nCi) and differences in life span ($\sim$ 500-1000 days). Lung tumors were found in rats accumulating from 50 to 2000 rads to the lung. Only two bone tumors were observed (Table 25.4), one in each $Pu(NO_3)_4$ group, after cumulative doses of 10 and 25 rads. The results indicate that inhaled Ca-DTPA neither augmented nor repressed the tumorigenicity of inhaled $Pu(NO_3)_4$ in these animals.

TABLE 25.3. Lung Tumors and Cumulative Radiation Dose in Rats Administered Inhaled Ca-DTPA or Sham Ca-DTPA After $Pu(NO_3)_4$ Inhalation.

LIFETIME ACCUMULATED DOSE TO LUNG, rads	NUMBER OF RATS IN DOSE RANGE		AVERAGE DAYS AT RISK		NUMBER OF RATS WITH LUNG TUMORS	
	Ca-DTPA	SHAM Ca-DTPA	Ca-DTPA	SHAM Ca-DTPA	Ca-DTPA	SHAM Ca-DTPA
36-100	5	1	603	888*	1	1
101-300	13	3	555	546	5	1
301-500	16	7	657	608	5	3
501-1000	8	5	714	699	6	2
1001-2000	1	3	975*	641	1	2
Ca-DTPA	70	30	604	591	1	0
NONTREATED CONTROL	99	-	677	---	0	-

* VALUE FOR ONE RAT

TABLE 25.4. Bone Tumors and Cumulative Radiation Dose in Rats Administered Inhaled Ca-DTPA or Sham Ca-DTPA After $Pu(NO_3)_4$ Inhalation.

LIFETIME ACCUMULATED DOSE TO BONE, rads	NUMBER OF RATS IN DOSE RANGE		AVERAGE DAYS AT RISK		NUMBER OF RATS WITH OSTEOSARCOMAS	
	Ca-DTPA	SHAM Ca-DTPA	Ca-DTPA	SHAM Ca-DTPA	Ca-DTPA	SHAM Ca-DTPA
2-10	18	0	496	0	1	0
11-20	11	6	586	593	0	0
21-30	5	2	692	680	0	1
31-78	10	10	792	653	0	0
Ca-DTPA	70	30	604	591	0	0
CONTROL	99	--	677	---	0	0

REMOVAL OF INTRATRACHEALLY INTUBATED $^{253}Es(NO_3)_3$ FROM RATS BY INHALED OR INJECTED Ca- OR Zn-DTPA

Principal Investigator:
V. H. Smith
Technical Assistance:
K. A. Allen and D. W. Willard

Twenty-seven days after the intratracheal instillation of $^{253}Es(NO_3)_3$ to rats, Ca-DTPA, Zn-DTPA, or mixtures of the two, were given by inhalation or intraperitoneal injection for a series of nine treatments spaced over 3 weeks. The cation had no effect on treatment effectiveness but administration by injection may give better removal of Es from the liver than does inhalation. High doses of the chelating agent remove more Es, though the magnitude of this effect decreases with the number of treatments. Increased retention of Es was observed in the lungs of most treated rats.

It would appear that a biologically mobile actinide such as einsteinium might be more readily removed by chelating agents from sites of tissue deposit than less mobile actinides such as plutonium. We have examined the effect of Ca- and/or Zn-DTPA on the removal of Es, as influenced by dose and route of administration.

Adult, female Wistar rats under light ether anesthesia were intubated intratracheally with 17.4 μCi $^{253}Es(NO_3)_3$ in 0.4 ml of 0.1 M HNO_3. After 27 days, 30-min inhalation treatments or 1-ml intraperitoneal injections of DTPA were started. Treatments were given three times a week on alternative days for 3 weeks. Animals were sacrificed and tissues taken for radioanalysis 21 days after treatments were initiated. The inhaled dose of DTPA was estimated from ^{14}C label excreted in urine of rats exposed to ^{14}C-DTPA at the same time as the treated animals. The inhaled DTPA dose was about 0.03 mmol/kg and this same dose was given intraperitoneally; a higher dose of 1 mmol/kg was also given to compare dose effects. The low dose was about the same as the human intravenous dose, 0.2 mmol/kg. All Es measurements were corrected for decay.

The high radiation dose from 17.4 μCi ^{253}Es deposited in the lungs produced no effects on weight gain, and no gross evidence of pneumonitis or other lesions in the lungs. During the treatment period, control rats excreted 2.6% of the initial Es dose; those treated with the low DTPA dose excreted six times more than the controls. The difference due to treatment dose appeared to diminish with the number of treatments as does the effectiveness per treatment (Figure 25.1). There were no differences in Es excretion due to the cation associated with DTPA, or to the mode of delivery.

The Es content of lung, liver, and skeleton as measured in the various treatment groups are shown in Table 25.5. Regardless of dose, there appeared to be little or no effect on Es deposition as the cationic component of DTPA was varied. With three exceptions, treatment with DTPA appeared to increase the concentration of Es in the lung, an observa-

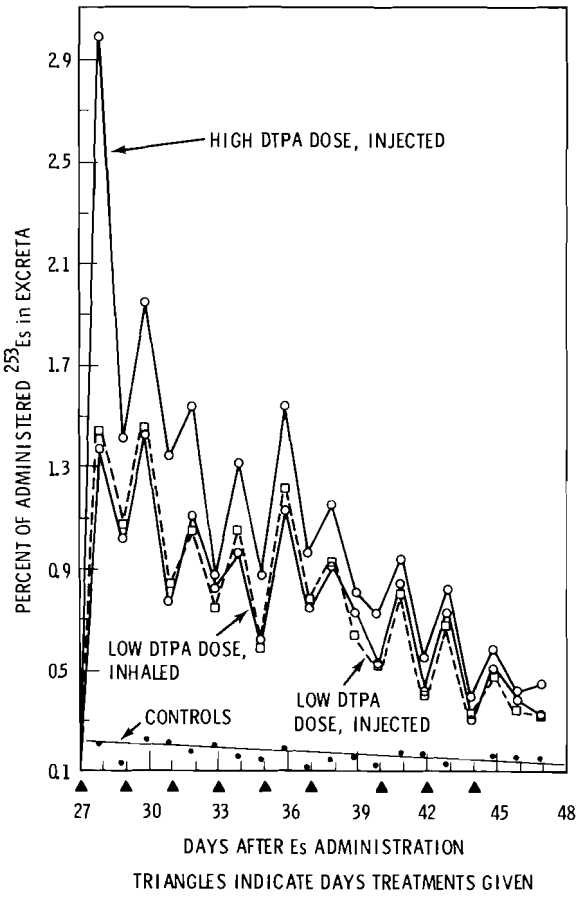

FIGURE 25.1. Excretion of Intratracheally Administered ^{253}Es by the Rat and the Effect of DTPA on That Excretion. Zn- and Ca-DTPA results at each level were combined to show the effect of route and dose level.

TABLE 25.5. Einsteinium Content of Organs of Rats Treated With Inhaled or Injected Ca- or Zn-DTPA.

TREATMENT ROUTE	NUMBER OF ANIMALS	DPTA/TREATMENT, mol/kg	RATIO OF Ca: ZN DPTA SALT	EINSTEINIUM RETENTION, MEAN % OF ADMINISTERED DOSE		
				LUNG	LIVER	SKELETON
INHALED	8	0.03	1:0	9.8[b]	0.77[a]	37[a], [b]
	8	0.03	2:3	8.8[b]	0.66	36[b]
	8	0.03	1:9	12.0[b]	0.61	33[b]
	8	0.03	0:1	9.3[b]	0.70	41[a]
INJECTED, INTRAPERITONEALLY	8	0.03	1:0	9.9[b]	0.47[a]	34[b]
	8	0.03	2:3	13.0[b]	0.52	36[b]
	7	0.03	1:9	10.0[b]	0.86	35[b]
	8	0.03	0:1	11.0[b]	0.54	41[a]
	7	0.10	1:0	5.9[a]	0.30[b]	28[b]
	7	0.10	0:1	10.0[b]	0.36[a], [b]	32[b]
	5	0.10	1:9	12.0[b]	0.35[b]	32[b]
CONTROL	8	---	---	6.8[a]	1.30	42[a]
BEFORE TREATMENT[b]	9	---	---	9.0[b]	2.30	43[a]

[a] MEANS WITH SAME LETTER ARE NOT DIFFERENT FROM EACH OTHER AT THE 0.05 SIGNIFICANCE LEVEL

[b] ANIMALS SACRIFICED AT BEGINNING OF TREATMENT PERIOD

tion inconsistent with previous experience and presently being further investigated. Es removal from liver showed a marked dose effect; injected DTPA appeared to be more efficient at removing Es from liver than did inhaled DTPA. There was little or no effect of dose or route of administration on concentrations of Es in bone. Both dose regimens appeared to decrease bone levels of Es by approximately 10%. Comparison of tissue Es levels in control animals held 48 days postexposure versus animals held 21 days postexposure suggests that, with the exception of skeleton, there is considerable natural loss of this element. It would appear that the effectiveness of DTPA as a removal agent is not well correlated with the relative biologic mobility of Es.

PREPARATION OF ALBUMIN MICROSPHERIODS CONTAINING ZN-DTPA

Investigator:
D. H. Willard and V. H. Smith

Zn-DTPA was encapsulated in materials presenting both a lipid surface (liposomes) and a protein surface. The amount of Zn-DTPA incorporated was on the order of 10^{-18} to 10^{-20} moles for a 1-μm diameter capsule; barely enough to complex the Pu contained in a moderate-size Pu polymer. Neither type of microcapsule caused distress or gross lung lesions when inhaled by, or intracheally administered to rats.

The chelating agent DTPA, when administered as the Ca or Zn salt, does not appear to cross cell membranes. Since inhaled Pu is quickly incorporated in lung macrophages, a microencapsulated system for delivery of DTPA to the lung might allow the DTPA to enter the cells and release Pu not available to conventional intravenous therapy.

Using the procedures of Rahman et al. (J. Lab. Clin. Med. 83: 640, 1974), liposomes containing ^{14}C-DTPA were prepared. An additional ultrasonication step was used to obtain liposomes in the respirable size range (< 2 μm). In our hands, the prepared liposomes contained disappointingly small amounts of DTPA, $\sim 10^{-20}$ mole (± an order of magnitude) as estimated by ^{14}C measurements. Despite their fragile appearance and sensitivity to osmotic changes, the liposomes appeared to aerosolize from a Retec nebulizer essentially unchanged, as judged from microscopic examination. No gross lesions of the lungs were noted in rats that inhaled or were intratracheally intubated with liposomal DTPA at doses of 1-9 mg/kg body weight.

The poor yields experienced in the preparation of liposomes led us to try incorporating Zn-DTPA into beef serum microcapsules, using essentially the procedures of Kramer (J. Pharm. Sci. 63: 1647, 1974). In a typical preparation, the particle-size distribution was bimodal, ranging from 10 to 20 μm and from 0.2 to 5 μm. The larger particles were separated by centrifugation and contained about 85% of the encapsulated DTPA; the smaller particles contained 15%. The particles were washed with ethanol and diethyl ether to give a readily storable dry powder. The smaller-sized albumin microcapsules (CMD 1.2 μm) were stable when nebulized, but the DTPA started to leach out as soon as they were put into water to produce aerosols for inhalation by the rats. No toxic reactions were noted after inhalation of 3 mg DTPA/kg body weight or after intratracheal intubation of up to 4 times higher doses.

More DTPA was taken up by the albumin microcapsules than by the lysosomes, but the amount was still small. This would be roughly equimolar to a Pu polymer of about 400[-O-Pu-O-] units.

The binding of DTPA to albumin without microencapsulation was also studied in an effort to obtain higher DTPA-loading. In Figure 25.2, the results of testing for effects of possible binding on the diffusion of ^{14}C-labeled Zn-DTPA are compared for Zn-DTPA, albumin + Zn-DTPA, and Zn-DTPA in albumin microscapsules. The presence of albumin does retard the appearance of label in the diffusate, and Zn-DTPA from microcapsules showed an even slower appearance.

The diffusion was solution-limited, so that differences in the curves represent differences in availability of Zn-DTPA to diffuse across the membrane. Despite these in vitro differences, urine collected from two rats intratracheally injected with Zn-DTPA (^{14}C) microcapsules showed 50% of the DTPA in the urine in 2 hours, the same as for unencapsulated Zn-DTPA.

Some alterations in the preparation procedure are being tried to increase loading and yield of DTPA-albumin microcapsules in the respiratory size range. In vitro tests for uptake in macrophages will be followed by in vivo tests for effectiveness of removal.

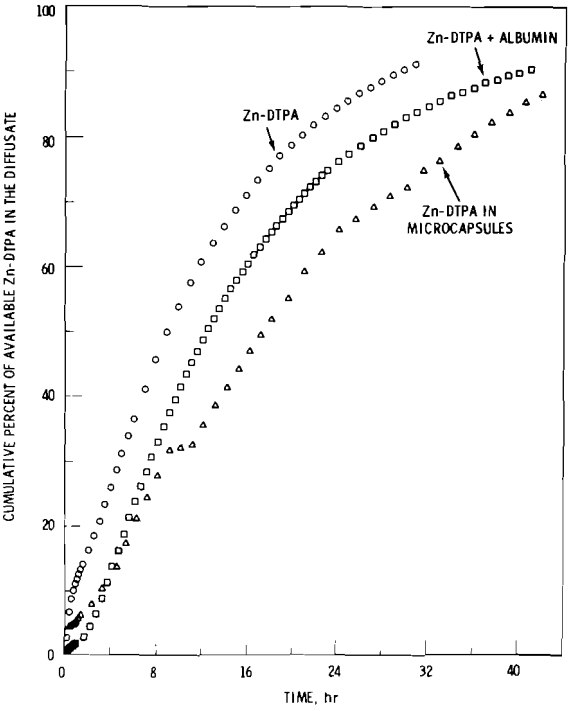

FIGURE 25.2. Relative Diffusion Through a Spectropore #2 Membrane of ^{14}C-DTPA as Zn-DTPA, Zn-DTPA + Albumin and Zn-DTPA in Albumin Microcapsules.

TREATMENT FOR TOXIC METAL EXPOSURES ASSOCIATED WITH NON-NUCLEAR ENERGY TECHNOLOGIES

Person in Charge: V. H. Smith

This project has the broad objective of developing treatments for over-exposure to toxicants encountered in some of the new non-nuclear energy technologies. Necessarily associated with this effort is the identification of toxic agents, their effects and the mechanisms of their toxicity, the development of suitable test models and exposure procedures, and the synthesis of this information in the formulation of effective treatment procedures.

Initial efforts are being directed toward the toxic metals: cadmium, vanadium, nickel, cobalt, chromium, and arsenic, which are associated with catalytic processes used in shale oil production, and are products of fossil fuel combustion, generally. In the hope of finding complexing agents combining high selectivity for metals with low toxicity in mammals, biologically derived chelons will be tested. The siderochromes produced by some bacteria chelate several metals with a high degree of specificity, transport them across bacterial membranes, and may be capable of removing toxic metals from within cells and from the circulatory system. They have the advantage of small molecular size, which should make them poor antigens. The technique of inducing organisms to produce siderochromes against specific metals will be investigated.

This project was first funded in FY 1976 and no reportable results are as yet available. Several catechol-producing microorganisms have been obtained and isolation of siderochromes is underway.

BLOOD IRRADIATION FOR MEDICAL APPLICATIONS

Person in Charge: F. P. Hungate

Extracorporeal irradiation of blood, using repeated brief exposures, has been shown to suppress rejection of tissue transplants and to inhibit progression of chronic lymphocytic leukemia. There remains a need to establish the conditions of dose administration which optimize therapeutic effect, to study the basic processes by which blood irradiation produces such effects, and to improve the technique of blood irradiation through the development of improved and portable blood irradiators. Irradiators now in use are large, expensive and consequently available to relatively few patients. It also appears probable that continuous irradiation of blood during critical periods, with lower dose rates, may constitute a more effective treatment.

Tests since FY 1969 on a number of photon-, alpha-, and beta-emitting isotopes, for their ability to deliver effective doses to blood, have resulted in the choice of ^{170}Tm, encapsulated in vitreous carbon, as the source material. The ^{170}Tm beta irradiation passes through the carbon layer and effectively irradiates blood; the direct and indirect (bremsstrahlung) radiation is readily shielded; the containment and blood interface material is unaffected by radiation doses in excess of 10^{10} rads and is relatively nonthrombogenic. An additional advantage is the fact that irradiation units can be totally fabricated with stable ^{169}Tm, which is converted to radioactive ^{170}Tm by a final neutron activation. Total weight of shielded prototype units is about 500 g.

Current and projected future efforts include: (1) continued materials and design research leading to improved portable irradiators, and their preclinical evaluation in animals; (2) basic hematologic and immunologic studies relating to blood irradiation and its effects on cellular and humoral immune response; (3) liaison with regulatory bodies to provide them with information pertinent to ultimate licensing; and (4) continued and expanded support of, and collaborative research with, clinical groups interested in using blood irradiation as an adjuvant in the suppression of transplant rejection and the treatment of certain lymphocytic neoplasms.

PUBLICATIONS SINCE 1973

Hungate, F. P., B. I. Griffin, F. T. Cross, and W. F. Riemath. 1973. Improvements in design of a ^{90}Sr-^{90}Y portable blood irradiator, p. 380. In: Proceedings of the 26th Annual Conference on Engineering in Medicine and Biology, vol. 15. The Alliance for Engineering in Medicine and Biology, Arlington, VA

Hungate, F. P., W. R. Riemath, L. R. Bunnell, and M. F. Gillis. Chronic blood irradiation: A new approach. Proceedings of the Hanford Biology Symposium on Radiation and the Lymphatic System. AEC Symposium Series. (In Press)

DEVELOPMENT OF A PORTABLE BLOOD IRRADIATOR FOR MEDICAL APPLICATIONS

Investigators:
F. P. Hungate, L. R. Bunnell,
and W. F. Riemath

A new design for the fully portable blood irradiator was completed and fabrication methods are being finalized. Initial tests indicate that this design overcomes thrombogenesis problems previously encountered; its use in A-V shunts is significantly simplified relative to previous models. Animal data are now being acquired.

A significant advance in concept for the portable irradiator was achieved by bringing together ^{170}Tm as the radiation source and vitreous carbon (VC) as the containment and blood interface material. Very pure, nonradioactive $^{169}Tm_2O_3$ is cast as a thin layer in a cylinder of polymerized polyfurfuryl alcohol. A thin, pour-through layer of resin provides containment and a very smooth blood interface, while an outer, thick (4 mm) layer provides strength and adsorption of the 0.96 MeV betas in a low-Z material. Following casting and machining (if needed) the unit is vitrified in a controlled atmosphere furnace. Neutron activation then converts the ^{169}Tm to ^{170}Tm. The VC is neither activated nor damaged by the high radiation received during activation and subsequent decay of the ^{170}Tm. The 129-day half-life of ^{170}Tm gives a useful life for the units without presenting serious long-term disposal problems.

The primary objective of the past year was to design a unit which could be readily adapted for use by clinicians, and to obtain animal data relating daily radiation dose to lymphocyte response.

The initial units were constructed with an inserted tube of 0.25-mm thick pyrolytic carbon through which the blood flowed. These units had two features which appeared to be undesirable for clinical application. First, attachment of shunt tubing to the insert tube was not sufficiently positive and would result in radiation exposure if disassembled. Second, the thickness of the insert tube necessitated more ^{170}Tm to achieve a given blood dose, thus requiring more exterior shielding to reduce the attendant bremsstrahlung radiation. The second problem we hoped to solve by substituting a flow-through VC inner coating for the pyrolytic carbon tube. To solve the first problem,

a new connector design was developed using teflon inserts fitted to slide into an enlarged portion at each end of the VC-Tm units. The enlargement was specially drilled to give a square shoulder against which the [illegible]

human patients. Figure 27.2 shows such a unit with silastic tubing of the shunt attached.

To fabricate the units shown in

IRRADIATION OF BLOOD BY ^{238}PU ALPHA PARTICLES

Investigators:

F. P. Hungate, W. F. Riemath, G. G. Culver, M. F. Gillis, and H. A. Ragan

PUBLICATIONS SINCE JANUARY, 1973

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NUCLEIC ACID COMPONENTS FROM STRONTIUM-90 EXPOSED MINIATURE SWINE

Investigator:
M. E. Frazier
Technical Assistance:
T. K. Andrews and J. T. Cresto

The reverse transcriptase associated with porcine type C virus particles was used to generate a tritium-labeled DNA product complementary to the viral RNA template. Results of nucleic acid hybridization experiments indicate this ^{3}H-DNA (probe) was copied from heteropolymeric regions of the porcine viral RNA. Probe prepared from this porcine type C virus contains sequences that possess some homology in sequence to RNA isolated from viruses known to cause similar diseases in other animals.

The methodology for preparation of a ^{3}H-DNA complementary copy of an oncornavirus RNA was described in last year's annual report. This procedure involves the isolation from leukemic cells of the fraction enriched for virus particles containing the 70S RNA and RNA-directed DNA polymerase. This fraction is used to generate a ^{3}H-DNA, endogenously synthesized in the presence of high concentrations of actinomycin D (to inhibit host and viral DNA-directed DNA synthesis). The ^{3}H-DNA is subsequently purified, using hydroxyapatite and Sephadex chromatography. The material is self-annealed to remove all self-complementary material and the resultant ^{3}H-DNA is used to detect complementary sequences in nucleic acids from a variety of sources.

Information on the nature of the complexes between a ^{3}H-DNA complementary to porcine type C virus RNA (PV-^{3}H-DNA) and the various DNA and RNA's was obtained by examining the thermal stability of the hybrid structures. In these experiments the PV-^{3}H-DNA is annealed to excess DNA or RNA and the resultant hybrids melted on hydroxyapatite. In this system, unpaired single strands elute with 0.15 M phosphate buffer at 60°C, imperfectly paired duplexes dissociated between 65° and 80°C, and properly paired duplexes elute above 80°C.

The PV-^{3}H-DNA product was initially checked by hybridization with RNA isolated from C-type particles produced in tissue cultures originating from leukemic swine, with synthetic polyadenylic acid, or against RNA from four known oncornaviruses (Table 28.1). Under stringent conditions nearly 85% of the ^{3}H-DNA hybridized to the porcine RNA. The mean elution temperature [Tm(e)] of the

TABLE 28.1. Hybridization of Porcine C-Type Virus ^{3}H-DNA With RNA.

RNA	% HYBRIDIZATION >60°C	$Tm_{(e)}$, °C
AVIAN MYELOBLASTOSIS VIRUS	1.4	<65.0
RAUSCHER LEUKEMIA VIRUS	24.1	70.9
MOLONEY SARCOMA VIRUS	26.1	69.5
POLYADENYLIC ACID	5.3	<65.0
PORCINE TYPE C VIRUS	84.8	84.0
FELINE LEUKEMIA VIRUS	15.4	71.0

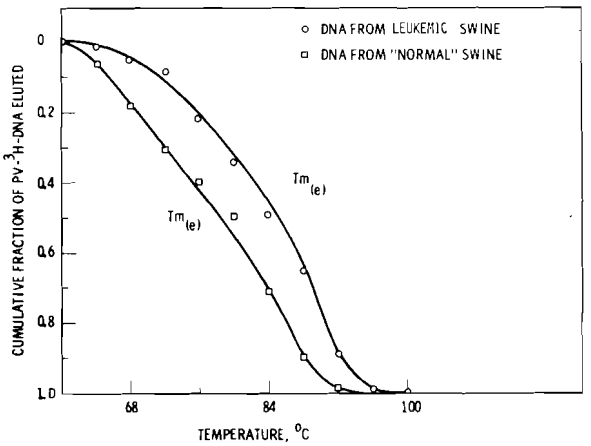

FIGURE 28.1. Melting Curve of PV-^{3}H-DNA-cellular DNA hybrids from Hydroxyapatite. The extent of hybridization was 60-76% for normal swine DNA and 88-90% for leukemic swine DNA. The cellular DNA's renaturation was followed optically, and 90% of the normal swine DNA reannealed with a $Tm_{(e)}$ of about 85°C, while a similar percent of the leukemic swine DNA reannealed with a $Tm_{(e)}$ of about 84.3°C.

hybrids from hydroxyapatite was 84°C, indicating that a properly paired duplex (RNA:DNA) was formed in the annealing reaction with these two species. Synthetic polyadenylic acid and avian myeloblastosis RNA hybridized poorly to the PV-^{3}H-DNA, indicating that hybridizations due to large stretches of ^{3}H-labeled thymidine were insignificant. Finally, the PV-^{3}H-DNA hybridized to some degree with several mammalian oncornaviruses (Rauscher, Moloney, and feline leukemia viruses), indicating some degree of homology; but at the same time, the percent of hybridization (<27%) and the relatively low $Tm_{(e)}$ indicate a high degree of mismatching. Therefore, while these oncornaviruses may contain some related sequences, they are not identical to the porcine isolates.

Using the PV-^{3}H-DNA product as a molecular probe, we have looked at the DNA isolated from both leukemic and nonleukemic swine to determine if virus-related sequences were present (Figure 28.1). These experiments have revealed a high percent of hybridization (65-76%) of normal porcine DNA to the PV-^{3}H-DNA probe, and a relatively high $Tm_{(e)}$ (~79°C). While this indicates considerable sequence homology between the putative swine leukemia virus genome and a DNA component of normal swine, a higher $Tm_{(e)}$ (~84.5°C) was consistently obtained with DNA from leukemic swine.

The higher $Tm_{(e)}$ obtained with DNA from leukemic swine may represent increased sequence homology with the PV-^{3}H-DNA probe. The thermal stability of nucleic acid duplexes depends on their length, base composition, and the complementarity of base pairs. Since the size of the cell DNA's was similar, the higher thermal stability of duplexes formed between PV-^{3}H-DNA and DNA from leukemic swine means that any one of the following possibilities or combinations could exist:

1) There is a more precise pairing of base nucleotides,
2) There is a higher G:C content in these leukemic DNA preparations,
3) The leukemic cells contain additional DNA sequences not present in nonleukemic cells.

While the reason for the increased percent of hybridization and $Tm_{(e)}$ of the PV-^{3}H-DNA probe to DNA from leukemic swine is not currently known, future investigations will be directed at resolving this issue.

Based on the data available from this study we can make the following statements:

1) The $Tm_{(e)}$ and percent hybridizations observed between PV-^{3}H-DNA and PV-RNA indicate that PV-^{3}H-DNA is a specific probe.
2) There is some sequence homology with murine oncornaviruses.
3) The swine isolate is not the result of contamination or infection with a murine virus.
4) The PV-^{3}H-DNA probe contains a considerable amount of sequence homology with normal swine DNA.

RAPID-MIXING/PULSE RADIOLYSIS TECHNIQUE FOR FAST REACTION STUDIES

Investigators:
W. D. Felix and L. A. Braby

A rapid-mixing/pulse radiolysis technique was developed for the study of specific radical reactants. An irradiated solution is rapidly driven into a mixing chamber and the resultant products are investigated spectrophotometrically.

Although certain radicals may be relatively stable in a given environment, it is quite probable that in most biological systems radiation-induced radicals will react within the range of microseconds to seconds after exposure. The study of free radical effects, therefore, requires the techniques of fast reaction kinetics, i.e., pulse radiolysis and flash photolysis. Both of these techniques, share a common problem: irradiation of a complex mixture introduces a wide range of radicals within that mixture, dependent upon the complexity of the initial system. An effective kinetic study of pertinent reactions can only be made if specific reactions can be identified and the reactants or products of these reactions followed directly.

To circumvent this problem we have developed a method to selectively follow the reactions of a specific radical with a known mixture of compounds. In this technique, which combines rapid-mixing and pulse radiolysis techniques, an irradiated solution is rapidly driven into a mixing chamber where the radicals react with a substrate of interest. This mixture is then driven into a chamber where flow is stopped and products of the reaction, or the reactants themselves, are observed spectrophotometrically. The system, as shown in Figure 28.2, consists of a modified Durham fast-mixing assembly, 1/4-meter monochromator, ultraviolet spectrophotometer, high-intensity lamp, and a Van de Graaf accelerator as the radiation source. The original Durham fast-mixing unit

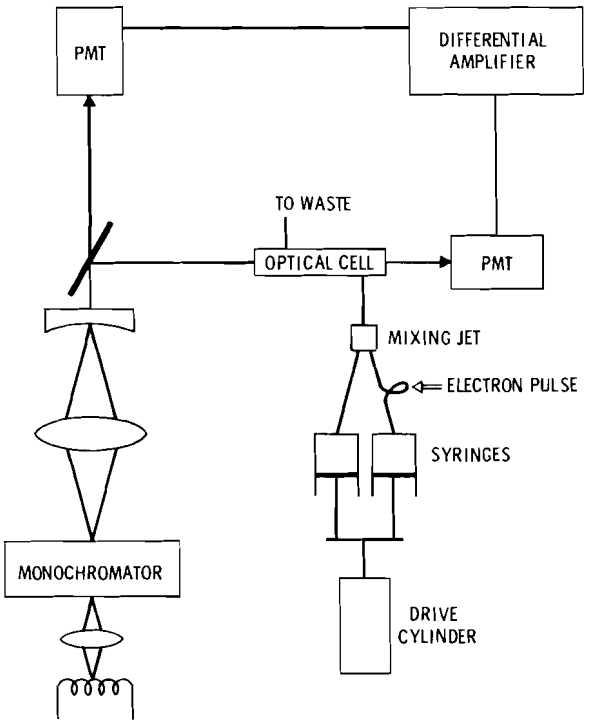

FIGURE 28.2. Schematic Drawing of Fast-Mixing Pulse Radiolysis System.

was modified by replacement of pneumatic valves with faster acting valves, and by replacement of the electronic system to allow control of the drive response as a function of the accelerator pulse. Availability of stable DC components allowed the use of a balanced photomultiplier system without the interjection of a rotating sector or other AC conversion device. Because of the stability of the DC electronics and the well matched light paths, the nulled response from the balanced photomultiplier tubes showed significant reduction of noise associated with the light source.

Tests using flash-photolyzed dye solutions have shown that free radicals may be examined when their lifetimes are greater than a few milliseconds. This limitation is determined by the speed of the pneumatic drive system. Faster speeds should undoubtedly be obtained with the addition of faster valve assemblies and reduction of the liquid path length prior to mixing.

DAMAGE TO PHOSPHOLIPID MEMBRANES BY ATTACK OF RADIATION-INDUCED FREE RADICALS

Investigator:
D. R. Kalkwarf(a)

Spherical vesicles, bounded by a single phospholipid bilayer and containing an aqueous solution of chromophore, were used as model systems to assess the sensitivity of cell-membrane components to free-radical attack. Vesicles prepared from either stearoyl sphingomyelin or dipalmitoyl phosphatidylcholine were lysed by radicals derived from irradiated galactose. The data suggest that phospholipid components of membranes could be sensitive sites for radiation damage.

Membrane damage would appear to be a particularly effective mechanism for amplifying radiobiological effects. In past years we have used washed suspensions of mammalian erythrocytes as model systems to gain insight into the general problem of free-radical attack on living-cell membranes. In order to separate damage caused by radicals from other radiation effects, a technique was developed to expose erythrocyte surfaces to radiation-induced radicals outside of the radiation field. The radicals were formed by gamma irradiation of pure biochemical crystals, assayed by electron spin resonance measurements and then released into the erythrocyte suspensions by dissolution of the crystalline matrix. Radicals derived from irradiated galactose, lactose and serine were found to lyse the cells, whereas radicals derived from irradiated glucose, glycine and alanine were without effect at similar concentrations.

The same technique has now been used to investigate the sensitivity of individual membrane components to radical attack. Phospholipids are major constituents of most living-cell membranes and comprise 20 wt% of the erythrocyte ghost. Two of these, stearoyl sphingomyelin and dipalmitoyl phosphatidylcholine, were tested. Using the method of Huang and Thompson, each of these compounds was formed into suspensions of spherical vesicles, approximately 200 Å in diameter and bounded by a single phospholipid bilayer to simulate their arrangement in living-cell membranes. This procedure involved preparation of aqueous emulsions of the pure phospholipids, sonication at 70°C to create the vesicles, and separation of the single-bilayer vesicles from multilayered liposomes and external solutes by gel chromatography on Sepharose 4B. During sonication, the internal volume of each vesicle was loaded with 1.8×10^{-2} M N-2, 4-dinitrophenylglycine (DNP-glycine) to indicate changes in permeability of the vesicles. Both this internal solution and the solution outside the vesicles was buffered at pH 7.4 with μ = 0.1 M phosphate.

Vesicles were exposed to galactose radicals by rapid dissolution of irradiated galactose crystals in the aqueous suspensions. Similar suspensions were exposed to nonirradiated crystals of galactose as controls. Periodically, the release of DNP-glycine was determined by passing a sample of each suspension through a gel chromatographic column of Sephadex G-25 and analyzing the effluent with a UV-monitor set at 360 nm. Figure 28.3 shows the results of such assays. Intact vesicles passed rapidly through the voids in the column and appeared first in the effluent. DNP-glycine appeared later and was

(a) Physics & Instrumentation Dept.

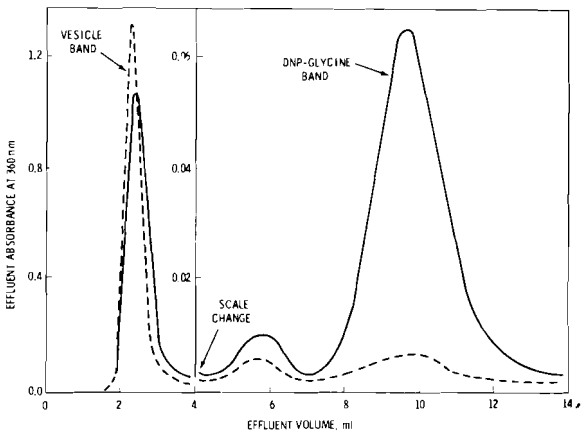

FIGURE 28.3. Separation of DNP-Glycine from Sphingomyelin Vesicles Exposed to either 50 mg of Irradiated Galactose Containing 5 x 10^{17} Radicals (——) or an Equal Quantity of Unirradiated Galactose (----).

well separated from the first peak. Total DNP-glycine in each suspension was evaluated by resonicating the vesicles in fresh buffer, repassing them through the gel chromatographic column and measuring the amount of dinitrophenylglycine outside the vesicles. This quantity represented essentially all of the dinitrophenylglycine in the sample since the vesicles occupied only a small fraction of the suspension's volume.

DNP-glycine was released much more rapidly from vesicles exposed to radicals. Data taken 24 hours after exposure (Figure 28.3) indicate that 39% of the DNP-glycine was released from stearoyl sphingomyelin vesicles exposed to 5 x 10^{17} galactose radicals, while only 3% of the DNP-glycine was released in the control suspension. Similar results were obtained with vesicles prepared from dipalmitoyl phosphatidylcholine.

It is important to note that the phospholipids tested thus far contain only saturated fatty acid residues. Phospholipids containing unsaturated residues would be expected to be even more reactive. In order to obtain a quantitative evaluation of these reactivities, however, factors such as vesicle/radical ratio and the kinetics of DNP-glycine release must be analyzed in more detail. When that is accomplished, a method may be available for clearly identifying radical-sensitive sites on membranes and those radicals most capable of causing damage.

RADIATION-INDUCED CELL DAMAGE

Investigators:
W. D. Felix and M. H. Schneiderman

The addition of irradiated crystals of galactose to Chinese hamster ovary cells resulted in mitotic delay, whereas exposure to nonirradiated crystals resulted in no detectable delay. The inference from this preliminary data is that free radicals or other transient irradiation products have reacted with external cellular components.

Previous annual reports have described our studies of free radical damage to model biochemical systems in which solid irradiated crystalline material was used as a medium for damage induction. We have now extended these studies to the investigation of free radical damage to Chinese hamster ovary (CHO) cells. This cellular system is of particular interest for use with irradiated crystals since it is known that very small doses from conventional radiation sources delay progression into mitosis from the G2 phase. This mitotic behavior of CHO cells is readily evaluated and therefore provides a very sensitive and precise measure of cellular delay.

Cells grown as a monolayer in 75-cm square plastic dishes were mechanically shaken and the cells entering the late metaphase were washed off. Repeated shaking at 10-min intervals resulted in a reproducible release rate of mitotic cells. Known amounts of irradiated galactose crystals were released into the flasks via an air stream. The amounts added to this monolayer of CHO cells were sufficient to make the surrounding media slightly hypertonic. Hypertonicity was corrected shortly after dissolution of the crystals by dilution and washing. Subsequent mitotic delay was determined as a function of the cell yield in the washings. A modified Coulter counter was used to make all cell counts.

Control exposures were made using similar weights of unirradiated galactose and the freeze-dried residue from an aqueous solution of irradiated galactose. Neither of the control exposures resulted in mitotic delay, whereas exposure to irradiated crystals resulted in measurable delay. The implication is that free radicals or other active transient irradiation products, such as peroxides, are reacting with external cellular components with resultant mitotic delay. However, this conclusion is based on results from preliminary experiments. For future experiments, the delivery system will be improved to guarantee a more uniform dispersion of crystals.

INTERACTION OF FOSSIL-FUEL-DERIVED TOXIC METALS WITH BIOLOGICAL MEMBRANES

Person in Charge: R. P. Schneider

The ionic forms of most toxic metals penetrate cell membranes slowly and it seems likely that their primary effects are exerted at the membrane level. Information on the interaction of metals with membrane functions may therefore be expected to aid in predicting potential effects of trace metals from fossil-fuel combustion.

Research being conducted under this project centers around four functions of cell membranes which may be linked to possible mechanisms of trace metal pathogenesis. These functions and the approaches we are using to study them are considered below.

Immune surveillance of cancerous cells may play an important role in metal carcinogenesis. Lymphocyte functions important in the detection and killing of foreign cells are known to be affected by heavy metals. To examine these processes we are studying the synthesis and distribution of dog lymphocyte membrane markers which bind anti-canine IgG, as well as the changes in these markers which may occur as a result of antigen and/or metal binding.

Membranes may be directly involved in regulation of cell growth and division by sensing cell-cell contact and relaying this information to the nucleus. We are examining the role of the membrane in these processes using cultured glial (brain) cells, which simultaneously stop cell division and synthesize a specific protein (S-100 protein).

Control of cell activity and division is, to a large extent, dependent on the transmission of information contained in external impermeant signal molecules through the cell membrane to the nucleus. The induction of exocellular protease synthesis by external protein inducers in Neurospora crassa is being studied as a model system.

The envelope of RNA tumor viruses is derived, in part, from the host cell membrane. The ATPase of avian myeloblastosis virus is being used as a marker for studying the origin of virus envelope proteins and the extent of viral control over host cell membrane composition.

Increased understanding of the biochemical basis of metal toxicity will provide a rational basis for predicting effects, and influence the design of experiments to assess risk. Since the effects being examined are primary effects occurring on the periphery of the cell, they may provide critical information for the detection and early treatment of cancer.

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SYNTHESIS OF S100 PROTEIN IN GLIAL CELLS: SELECTION OF A CONTACT-SENSITIVE CELL LINE

Investigators:
R. G. Rupp and W. R. Wiley
Technical Assistance:
L. S. Winn

A contact-sensitive line of C_6 glial cells was obtained by treatment with FUdR. These C_6F cells have a saturation density half that of the C_6 cells. The confluent density is obtained with daily medium changes and is therefore not due to suboptimal medium conditions.

As cultures of growing mammalian cells reach a high density, their growth rate decreases. This decrease in cellular proliferation requires cell-cell contact and, perhaps, the presence or absence of certain soluble factors in the medium. This decreased growth phenomenon has been termed "contact inhibition" or, perhaps more accurately, "density-dependent inhibition" of growth.

Biochemical events specific to density-inhibited cells are of interest because cancer may be caused, in part, by a loss of this density-dependent control. In 1970, Pfeiffer et al. (J. Cell Physiol. 75, 329-340, 1970) reported that cultured rat glial cells (C_6 cells) produced increased amounts of S100 protein, a nervous-system-specific protein, during density-dependent inhibition of growth. This protein was produced at much lower rates by exponentially growing cells. Our laboratory is investigating the effect of density-dependent inhibition of growth on macromolecular synthesis, in particular on the synthesis of S100 protein. The basic question to be answered is: What event(s) occur in the cell membrane due to cell-cell contact to effect the increase in S100 protein synthesis?

Our data raise questions about the conditions of cell culture under which Pfeiffer et al. obtained the observed S100 protein synthesis. We have found that the confluent cell density (that at which cell-cell contact is maximal) varies with the number of medium changes. Figure 29.1 shows that with daily medium changes, cells reach a density of 1.5×10^8 cells/flask. This is eight times the cell density reported by Pfeiffer et al., using the same culture conditions except for the frequent medium changes. Furthermore, cells never completely cease dividing in order to fill up spaces vacated by cells detaching from the flask. The graph also shows that, confronted by infrequent medium changes, cells can be made to exhibit apparent states of density-dependent inhibition.

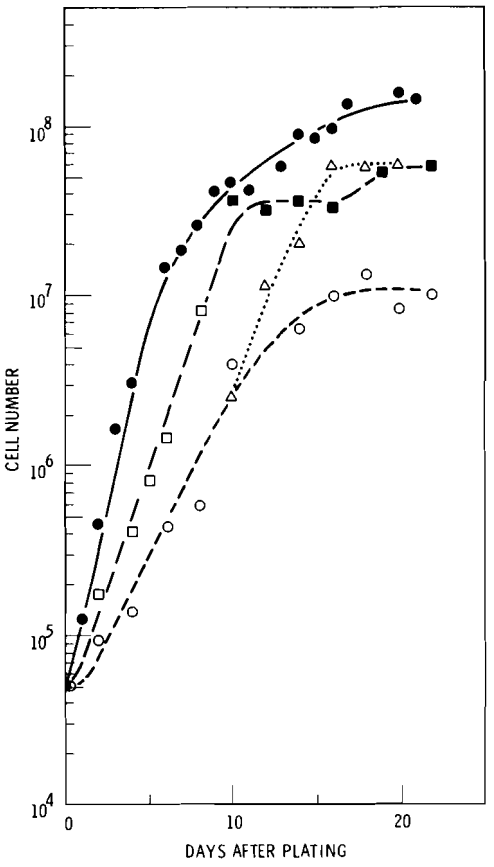

FIGURE 29.1. Growth of C_6 Cells under Various Medium-Changing Regimens. (●——●) Daily changes; (■——■) changes every third day; (△...△) daily changes beginning on the tenth day; (○---○) no changes.

From these experiments it is apparent that a degree of caution is required when attributing biochemical events to cell-cell contact; these events may actually be due to sub-optimal medium conditions. In order to circumvent this problem we have attempted to select for a population of C_6 cells that exhibits density-dependent inhibition of growth due to cell-cell contact under optimal medium conditions, i.e. contact-sensitive cells. Exponentially growing C_6 cells were treated with fluorodeoxyuridine (FUdR) which kills actively dividing cells. Those cells not dividing because of cell-cell contact would be unaffected by the drug. After three, three-day exposures to FUdR the cells were cloned. Of the eight clones obtained, one exhibited the characteristics of contact sensitivity. Figure 29.2 shows that these cells, designated C_6F, grow to confluent density of about 7×10^7 cells/flask with daily medium changes. This maximum population is 50% of the number of C_6 cells obtained under the same conditions. In addition, labeling index experiments indicate that the C_6F cells apparently stopped dividing more completely at confluency than do the C_6 cells. The labeling index after a 20-minute pulse with ^{3}H-TdR was 5-6% in the C_6 cells and 0-1% in the C_6F cells.

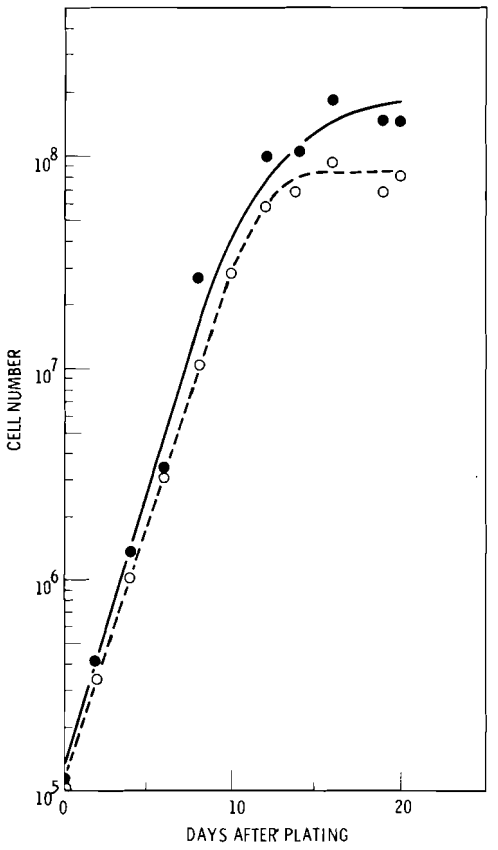

FIGURE 29.2. Growth of C_6 and C_6F Cells with Daily Medium Changes. (●——●) C_6 cells; (○---○) C_6F cells.

The cells are not merely FUdR-resistant cells because we have shown them to be as sensitive to FUdR as are the C_6 cells.

The C_6F cells are more flattened and somewhat larger than the C_6 cells. In addition, a small number of large, flattened cells can be seen in the C_6 cultures. This may indicate that the FUdR treatments of the C_6 cells have selected for a population of cells within the C_6 culture.

Changes in macromolecular (particularly S100 protein) synthesis which occur as a result of contact sensitivity will be studied in the C_6F cells.

SYNTHESIS OF S100 PROTEIN IN GLIAL CELLS: PURIFICATION OF BEEF BRAIN S100 PROTEIN FOR IMMUNOLOGICAL STUDIES

Investigators:

R. G. Rupp, J. E. Morris, and W. R. Wiley

Technical Assistance:

L. S. Winn

A rapid method for extracting electrophoretically pure S100 protein from beef brains is described.

Methods previously employed in other laboratories to extract S100 protein from beef brains yield a heterogeneous population of proteins as judged by polyacrylamide electrophoresis. The methods are also time-consuming. We have developed a rapid method for the isolation of electrophoretically pure S100 protein.

The method consists of homogenizing 1000 g of beef brains in 4000 ml of 0.01 M potassium phosphate, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, pH 7.2, at 4°C. All subsequent steps are performed at 4°C. The homogenate was centrifuged at 18,000 x g for 60 min. The supernatant was brought to 80% saturation with $(NH_4)_2SO_4$. After centrifuging at 18,000 x g for 60 min, the supernatant was adjusted to pH 4.0 with 1 N HCl. The resulting precipitate was collected by centrifugation and was dissolved in 150 ml of 0.075 M NaCl, 0.01 M potassium phosphate, 0.01 M 2-mercaptoethanol, 0.001 M EDTA, pH 7.2, and dialyzed exhaustively against the same buffer. After a clarifying spin, the sample was loaded onto a 2.5 x 35-cm diethylaminoethyl (DEAE)-cellulose column. Nonabsorbed material was eluted with 200 ml of starting buffer (0.075 M NaCl, 0.01 M potassium phosphate, 0.01 M 2-mercaptoethanol, 0.001 M EDTA, pH 7.2). Absorbed material was eluted with a linear gradient of 500 ml of starting buffer to 500 ml of the same buffer with 0.5 N NaCl. Three-ml fractions were collected and their absorbance was measured at 280 nm. The last peak of material, which eluted at 0.3 M NaCl, was

pooled and concentrated by adding $(NH_4)_2SO_4$ to 100% concentration and adjusting the pH to 4.0 with 1 N HCl. The precipitate was collected by centrifugation and dissolved in 10 ml of 0.1 M potassium phosphate, 0.4 M $(NH_4)_2SO_4$, 0.01 M 2-mercaptoethanol, 0.001 M EDTA. After exhaustive dialysis the sample was applied to a 2.5 x 60-cm Sephadex G-100 column. Fifty-drop fractions were collected and the absorbance at 280 nm was determined. The material whose peak was at fraction 70 was pooled and determined to be S100 protein.

Electrophoresis of this last fraction on ten-percent polyacrylamide-charged gels revealed only one band of protein under conditions which would have detected 1% contamination. Our purified material coelectrophoresed with S100 protein (generously provided by Dr. Thomas Vaneman). In addition, it reacted with identity on double diffusion gels with Vaneman's antibody directed against a sample of S100 protein.

This protein preparation will now be used to obtain anti-S100 antibodies in goats, which will be employed in immunoassays for the quantitation of S100 protein in glial cells.

REGULATION OF EXOCELLULAR PROTEASES IN NEUROSPORA CRASSA: ROLE OF CELLULAR AMINO ACID POOLS IN REGULATION

Investigators:
H. Drucker and B. L. Cohen
Technical Assistance:
L. Neil

Neurospora crassa, when grown on a medium where protein is sole source of nitrogen, develops intracellular amino acid pools which are qualitatively and quantitatively distinct from those found in nonstarved cells or in cells starved for nitrogen in the absence of protein substrate. The nature of the change in amino acid pools suggests that they may play a role in the repression of protease biosynthesis.

In previous work, the effect of the 20 commonly occurring amino acids on protease biosynthesis from *N. crassa* grown on a protein as sole source of carbon, nitrogen, or sulfur was examined. The amino acids which effect a greater than 50% decrease in protease synthesis at concentrations of 10^{-4} to 10^{-3} M under various regimens are shown in Table 29.1. If the data on amino acid repression have any physiologic meaning, they should correlate in some fashion with the kinetics of accumulation and the size of amino acid pools in organisms grown on protein as sole N-,C- or

TABLE 29.1. Amino Acids Effecting 50% Inhibition of Protease Synthesis.

PROTEIN AS N-SOURCE	PROTEIN AS S-SOURCE	PROTEIN AS C-SOURCE
ARGININE	CYSTEINE	CYSTEINE
TRYPTOPHAN	CYSTINE	CYSTINE
THREONINE	METHIONINE	HISTIDINE
		ISOLEUCINE
		LEUCINE
		LYSINE
		METHIONINE
		PHENYLALANINE
		TRYPTOPHAN
		VALINE
		THREONINE

S-source and without added amino acids. This study examines changes in amino acid pool sizes under conditions where protein is sole nitrogen source.

The 20 commonly occurring amino acids may be separated into four groups based upon our measurements of pool size kinetics (Table 29.2):

1) The amino acid pool size follows the kinetics of exocellular protease biosynthesis, i.e., the pool shows small increases in the interval 0-1.5 hr when protease is being made at a low rate, and large increases in the interval 1.5-4.5 hours when protease is being secreted at a maximal rate.

2) The amino acid pool shows a monotonic increase throughout the period of induction.

3) The amino acid pool remains essentially constant throughout the period of induction.

4) The amino acid pool increases and decreases as a function of incubation time, but these changes do not parallel changes in levels of exocellular protease.

Of the seven amino acids showing kinetics of pool accumulation similar to the kinetics of protease secretion, all except tyrosine are excellent repressors of protease biosynthesis when cells are grown under conditions where protein is sole carbon source (see Table 29.2). The amino acids (other than threonine) which repress protease biosynthesis under

TABLE 29.2. Kinetics of Amino Acid Accumulation: Protein as Sole N-Source for N. crassa.

	FOLLOWS PROTEASE KINETICS	MONOTONIC INCREASE	CONSTANT	NO CORRELATION
ALA	-	-	-	+
ARG	-	+	-	-
ASP	-	-	-	+
CYS	-	-	+	-
GLU	-	-	+	-
GLY	-	-	+	-
HIS	+	-	-	-
ISOLEU	-	-	-	+
LEU	+	-	-	-
LYS	+	-	-	-
METH	-	+	-	-
PHE	+	-	-	-
PRO	-	-	+	-
SER	-	-	+	-
THRE	+	-	-	-
TYR	+	-	-	-
VAL	+	-	-	-
ASPX	-	+	-	-
GLU	-	-	+	-

[a] BASED UPON DATA DERIVED FROM EXPERIMENTAL PROTOCOL IN LEGEND FIG. 7

conditions where protein is sole nitrogen source were either too low to be measured in the cells (tryptophan) or changed monotonically (arginine).

The ratio of maximal amino acid pool size to pool size at 0 time was calculated. When the amino acids were grouped on the basis of this concentration ratio (ratio value 1-1.5, 1.5-3, greater than 3), we found that, with the exception of tyrosine and threonine, all of the amino acids which effect specific and substantial repression of protease biosynthesis under conditions of carbon starvation show concentration ratios of 3 and above (Table 29.3). Those amino acids which demonstrate specific repression under conditions of nitrogen starvation were (except for threonine) not accumulated to any substantial degree.

If one compares maximum amino acid concentrations in nitrogen-starved cells (no protein substrate)

TABLE 29.3. A Comparison of Amino Acid Concentration Ratios[a] in Cells of N. crassa Induced for Protease Synthesis under Conditions of Nitrogen Starvation.

AMINO ACID CONCENTRATION RATIO = 1-1.5	AMINO ACID CONCENTRATION RATIO = 1.5-3	AMINO ACID CONCENTRATION RATIO > 3
ALANINE	ASPARTIC ACID	HISTIDINE
ARGININE	GLUTAMIC ACID	LYSINE
CYSTINE	ISOLEUCINE	METHIONINE
GLYCINE		LEUCINE
PROLINE		PHENYLALANINE
SERINE		THREONINE
ASPARAGINE		TYROSINE
GLUTAMINE		VALINE

[a] $\text{CONCENTRATION RATIO} = \frac{\text{MAXIMAL POOL SIZE OF AMINO ACIDS}}{\text{MINIMAL (0 TIME OF INDUCTION) POOL SIZE OF AMINO ACIDS}}$

with maximum concentrations of amino acids in cells induced for protease in the presence of protein as sole nitrogen source, the increased pool size for those amino acids repressing protease biosynthesis under conditions where protein is sole carbon source is still apparent.

The above comparisons show clearly that nitrogen-starved cells (no protein substrate) bear little or no resemblance in terms of amino acid pool behavior to cells induced to make protease by the presence of protein as sole nitrogen source.

Two trivial explanations of the nature of amino acid pools in protease-induced cells of N. crassa can be discarded:

1) The pool size is a reflection of the amino acid composition of the protein substrate employed. Analyses of the amino acid composition of the substrate (bovine serum albumin) versus the amino acid pools show no such correlation.

2) Pool size is a reflection of the relative rates of transport for individual amino acids released by the exocellular proteases from the protein substrate. Analyses of the affinity constants and rates for amino acid transport do not support this explanation of the data.

We postulate that cells grown on protein as a sole source of nitrogen, sulfur, or carbon are altered in their metabolism in such a way that they tend to form pools of amino acids, and products of amino acid metabolism (as has been shown previously for tryptophan), which are capable of effecting repression of protease biosynthesis. These pools are large relative to their size in non-starved cells, but relatively small compared to total amino acid pool size, and thus do not involve the expenditure of large amounts of energy and nutrilite. How these amino acids effect repression of protease biosynthesis under differing nutrilite regimens is not clear. The experiments described here (and previous work) would suggest that there may be regulatory cross links in the synthesis of exocellular protease under conditions of nitrogen, sulfur, and carbon deprivation which are not amenable to straightforward analyses of single nutrilite and metabolite elements within the cell.

REGULATION OF EXOCELLULAR PROTEASES IN NEUROSPORA CRASSA: OBSERVATION OF A CONSTITUTIVE ZYMOPROTEASE AND ITS POTENTIAL ROLE IN INDUCTION

Principal Investigators:
H. Drucker and J. E. Morris
Technical Assistance:
L. Neil

Cells of N. crassa grown in complete medium appear to possess low levels of a protease zymogen. The protease zymogen increases intracellularly when exocellular protease is induced by growth on a protein substrate as sole carbon source. The kinetics of its induction are consonant with a role for this enzyme in regulation of exocellular protease biosynthesis.

We have previously shown that cultures of Neurospora crassa grown from conidia on a medium where protein is primary carbon source show constant levels of intracellular protease during the interval of time when induction of exocellular protease occurs. The same appears to be the case in exponential-phase cells of N. crassa (Figure 29.3). These data would seem to indicate that there was no accumulation of exocellular enzyme inside the cell during the time course of protease biosynthesis.

In order to determine whether protein with immunologic characteristics similar to one of the exocellular proteases (the alkaline protease) might accumulate within the cell in amounts too low to be detected by the standard assay of protease activity, further tests were conducted. Using an immunoprecipitin ring test we found significant quantities of material cross-reacting with antibody directed against exocellular alkaline protease in the crude extract of the induced cells (Table 29.4). A comparison of immunologically reactive material in the cell versus amounts present in the cell filtrate (presumably exocellular alkaline protease), as a function of induction time, suggested that immunologically reactive material was present early in induction, i.e. before secretion of detectable levels of exocellular protease.

Given the high level of protein immunologically related to exocellular alkaline protease inside the cell, and the low and constant levels of

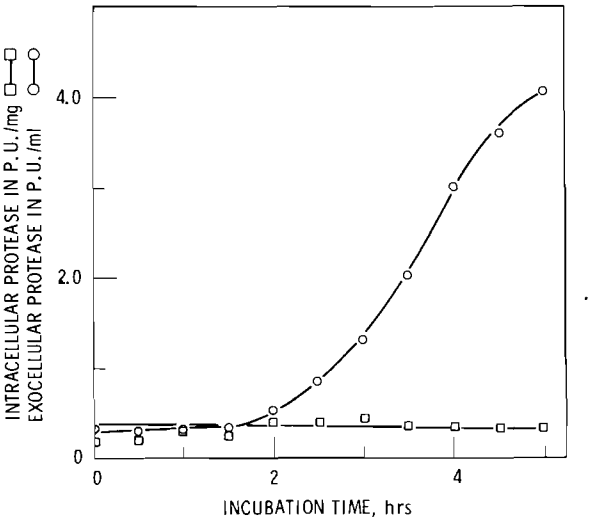

FIGURE 29.3. Induction of Exocellular Protease from N. crassa and Levels of Intracellular Protease during Induction. Cells grown 12 hrs in medium containing sucrose as carbon source were transferred to medium where the protein bovine serum albumin was sole carbon source. At the given intervals, samples of cell-free filtrate and cells were removed. Protease levels in the cell filtrate and in cell extracts were determined.

TABLE 29.4. Immunologic Reactivity Versus Anti-Exocellular Alkaline Protease of Induced Cell Extracts and Exocellular Filtrate

INDUCTION TIME, hr	IMMUNOLOGIC REACTIVITY OF EXOCELLULAR FILTRATE	IMMUNOLOGIC REACTIVITY OF CELL EXTRACT
0	-	-
0.5	-	-
1.0	-	-
1.5	-	+
2.0	(±)	++
2.5	++	+++
3.0	++++	++++
3.5	++++	++++
4.0	++++	++++
4.5	++++	++++
5.0	++++	++++

RESULTS OF IMMUNOPRECIPITIN RING TEST IN WHICH ANTISERA AGAINST EXOCELLULAR CRYSTALLIZED ALKALINE PROTEASE IS OVERLAID, IN A CAPILLARY TUBE, WITH THE TEST MATERIALS. A PRECIPITIN BAND AT THE INTERFACE IMPLIES IMMUNOLOGIC REACTIVITY. EXTENT OF REACTIVITY WAS JUDGED BY EYE-EXAMINATION OF SIZE OF PRECIPITIN ZONE.

protease as determined by measures of protease activity in cell cytoplasm, we performed electrophoretic analyses of crude extracts of N. crassa induced to make protease over time intervals of 0-5 hr. To resolve exocellular enzyme species from intracellular enzyme species, a control of exocellular enzyme was run at the same time and on the same plate with the crude extract. In the system employed, protease is detected by its activity on a milk-protein-agar overlay of a cellulose acetate electropherogram.

Exocellular enzyme (Figure 29.4, column C) consisted of three electrophoretically distinct components. The lower bond (most electropositive material) can be identified as the activating protease thermolysin, which is employed (as previously described) in the induction of protease from carbon-starved cells of N. crassa. The upper band (most electronegative) is similar to purified exocellular alkaline protease in electrophoretic behavior. Little or no electrophoretically resolvable protease activity could be seen in the crude extracts from cells induced for 0 time, or for up to 5 hr (Figures 29.4i and 29.4ii, column B).

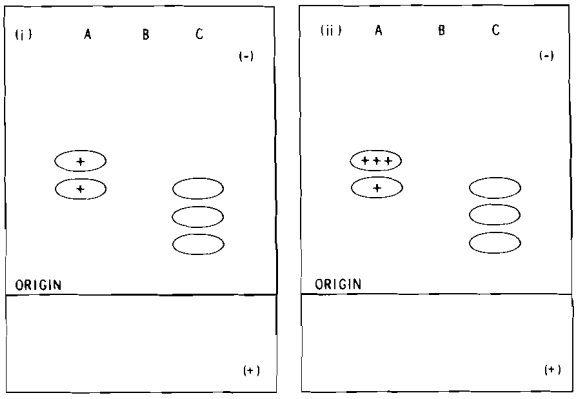

FIGURE 29.4. Electrophoresis of intracellular and exocellular protease activities of N. crassa. Cell filtrate and cell extracts derived from the experiment described in Figure 29.3 were electrophoresed on strips of cellulose acetate (Gelman Sepraphore III) in 0.5 M Tris-borate buffer, pH 9.1 for 45 min. Visualization of protease activity was effected by placing electropherogram against a thin layer of agar containing milk protein. Zones not staining for protein with Naphthol Black are shown. i. Zero-time sample. Column A: 1:1 mix of intracellular and exocellular enzyme; 3.0 P.U./ml. Column B: Intracellular protease, 1.48 P.U./ml extract; Column C: exocellular protease after 5 hrs of induction, 4.5 P.U./ml. ii. Same as i, except extract made from cells induced for exocellular protease by 1 hr of incubation in medium where protein is sole carbon source.

However, when the exocellular protease and crude extract were mixed (1:1; total units of mixed enzyme, 3.5), a new band of protease activity was observed (Figures 29.4i and 29.4ii, column A) which was electrophoretically distinct from all three of the exocellular enzymes and from the alkaline protease activity clearly visible in the mixture. This material, activated by the exocellular protease, was also detectable in the cell cytoplasm of uninduced cells. It appeared, in a qualitative sense as judged by electrophoresis, to accumulate in the cells during the course of induction. Further, thermolysin, the activating protease in induction

of exocellular protease from cells grown on protein as sole carbon source, effected the same phenomena when added to the crude extracts of either induced or uninduced cells of N. crassa. It caused the appearance of a new protease species more electronegative than those found either exocellularly or intracellularly. It would thus appear that N. crassa possesses a constitutive protease in zymogen form, increasing in level during the course of exocellular protease induction.

Direct measurements were made of protease activity activated in the cell cytoplasm by either exocellular protease or thermolysin, as a function of induction time for exocellular protease during growth on protein as sole carbon source (Figure 29.5). It can be seen that the levels of activatable material are highest at the approximate time when exocellular protease begins to be observed in culture filtrates (after 2 hrs of incubation); the levels decrease thereafter, but never reach levels as low as those present in the uninduced cells. The time course for increase and decrease of zymogen is almost identical to that observed for the previously reported increase and decrease of cytoplasmic protease-inducing material.

These data suggest that alkaline protease may be present in zymogen form even in uninduced cells of N. crassa. Because previous work has implied a role in the induction process for a zymoprotease bound to cells of N. crassa (perhaps between wall and membrane), it is tempting to postulate that the zymoprotease described in this report may be the one involved in the initial phase of induction. Efforts presently in progress are examining this possibility.

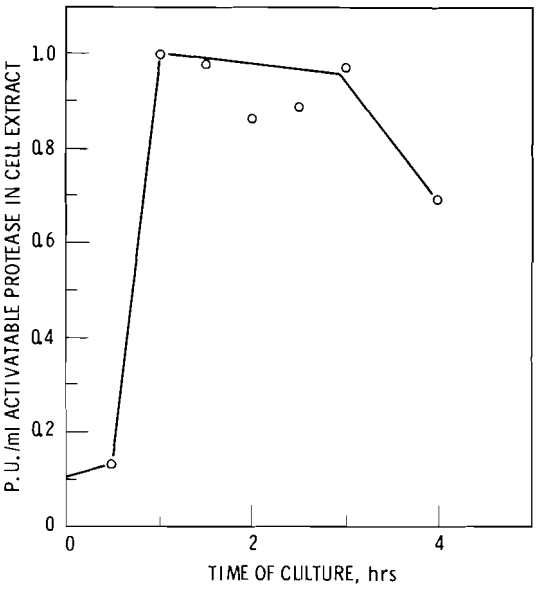

FIGURE 29.5. Levels of Protease in Zymogen Form in Crude Extracts of N. crassa Induced for Exocellular Protease Synthesis. Induced cells as in Figure 29.3 were extracted and a sample of exocellular protease from a 5-hr induced culture was added (1:1 by volume) to the extract. A value was calculated for protease present, based upon the sum of intracellular and exocellular protease activity, measured separately. "Activatable" protease is the difference between this calculated activity and that measured in the mixtures.

EXTRACTION AND CHARACTERIZATION OF THE ATPASE OF AVIAN MYELOBLASTOSIS VIRUS

Investigator:
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Technical Assistance:
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The ATPase of avian myeloblastosis virus is inhibited by treatment with phospholipase C; activity is restored upon the subsequent addition of lecithin to the treated virus, demonstrating that the ATPase requires lecithin for activity. A particle which has a tenfold increase in specific activity can be isolated from viruses which have been treated with a detergent in the presence of added lecithin and subsequently dialysed. The data suggest that the enzyme is an integral component of the lipid portion of the viral envelope.

During maturation and release of animal RNA tumor viruses from host cells, the virus "buds" through the cell membrane. Due to this process, viruses are enveloped by a lipid and protein membrane which superficially resembles the cell membrane. Little is known of the maturation sites, viral control of the composition of the host cell membrane, or the contribution of cell membrane to the structure of the viral envelope. The ATPase of avian myeloblastosis virus (AMV) provides a good opportunity to study these problems since it appears to be derived from the host myeloblast; it is part of or external to the viral envelope; and ATPases are ubiquitous components of cell membranes.

Last year we reported that attempts to extract the ATPase from AMV were unsuccessful and that the ATPase was inhibited by phospholipase C. This enzyme degrades lecithin (phosphatidyl choline) into diglyceride and phosphocholine. Further studies have now shown that the action of phospholipase C is to degrade viral lecithin which is required for activity of the ATPase. AMV was treated with phospholipase C for 1 hour, destroying 90% of the ATPase activity. The virus was then concentrated and washed free of added enzyme by centrifugation, suspended in sonicated dispersions of various phospholipid and assayed for ATPase. Synthetic dipalmitoyl lecithin increased activity sixfold while other lipids stimulated activity from two to fourfold (Table 29.5). The data suggest that the enzyme is associated with phospholipid and thus appears to be an

TABLE 29.5. Effect of Phospholipids on Phospholipase-Treated AMV

PHOSPHOLIPID ADDED (2.5 mg/ml)	RELATIVE ATPase ACTIVITY
NONE	1.0
L-α-LECITHIN (SYNTHETIC)	4.1
L-α-LECITHIN (DIPALMITOYL)	5.8
LECITHIN (EGG)	2.3
CEREBROSIDES	1.5
L-α-CEPHALIN (DIPALMITOYL)	2.8
LYSOCEPHALIN	2.2
PHOSPHATIDYL SERINE	2.5
DIPALMITOYL LECITHIN AND LYSOCEPHALIN	2.6

AMV WAS TREATED WITH 1.0 mg/ml PHOSPHOLIPASE C FOR 1 HOUR AT 20° WASHED BY CENTRIFUGATION AND ASSAYED FOR ATPase AFTER ADDITION OF SONICATED SUSPENSIONS OF THE PHOSPHOLIPIDS

integral part of the lipid bilayer of the envelope. Further study of lecithins containing fatty acid moieties of varying chain lengths showed that dipalmitoyl lecithin (DPL) provided the most complete restoration of activity.

The demonstration that the ATPase required lecithin provided the information necessary for removal of the active enzyme from the virus. The virus was disrupted in a detergent (sodium cholate) in the presence of added DPL; the mixture was then dialyzed to remove the detergent. Subsequent centrifugation of the dialysate on sucrose density gradients showed that no protein was detectable at the density of the intact virus (1.16), and that the ATPase was associated with a particle which had a density of about 1.08. Further experiments revealed, however, that the density of the ATPase particle varied, depending on the relative concentration of DPL and virus protein. At low protein concentrations relative to DPL, the density was about 1.08; at intermediate ratios the density was increased to 1.10; and at higher viral protein concentrations it was 1.12. Optimal separation of activity from other virus components by centrifugation is possible when the density of the ATPase-containing particle is 1.10. A relatively small fraction of the total protein applied to the gradient is associated with virtually all of the ATPase (Figure 29.6). The specific activity (enzyme units/mg protein) of the enzyme is about tenfold higher in this peak than in the dialyzed material prior to the separation step. We are presently attempting to isolate the ATPase in pure form for characterization and preparation of antibodies. These will enable us to localize the enzyme in infected and noninfected cells and, perhaps, to eventually purify its messenger-RNA.

General properties of the extracted enzyme differ from those of the enzyme associated with intact AMV. The viral enzyme is sensitive to NaCl concentration; activity is

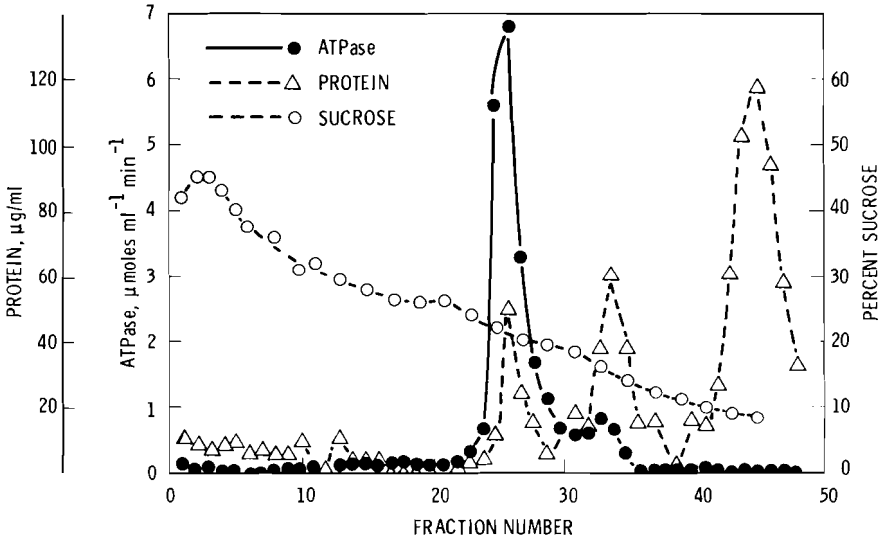

FIGURE 29.6. Sucrose Density Gradient Fractionation of AMV Treated with Detergent. Viruses were disrupted in 1% sodium cholate and 4 mg/ml dipalmitoyl lecithin, dialyzed to remove cholate and allow lecithin vesicle formation and centrifuged at 200,000 x g for 16 hours. The fractions start at the bottom of the tube and progress to the top.

maximal in 0.05 mM NaCl, and is reduced to one-third of maximal in 0.5 M. However, activity of the extracted enzyme varies by only 13% throughout a NaCl concentration range of 0.0 M to 0.6 M. Unlike the viral ATPase, the extracted enzyme is insensitive to changes in pH from 7 to 10, alternate freezing and thawing, and sonication. When held at room temperature in the presence of NaN_3 to inhibit microbial growth, the activity of extracted ATPase was stable for 14 days. These properties are typical of enzymes fixed to a nonbiological solid support, and suggest that the local environment of an enzyme is different in the extracted particle than in the virus.

Several lines of evidence suggest that the ATPase is an integral component of the lipid bilayer of the viral envelope. The enzyme is highly resistant to removal from the virus by methods commonly used to extract membrane-bound enzymes. It is accessible to proteases and substrate external to the envelope. The demonstrated requirement for lecithin suggests that it normally resides in a lipid milieu. It seems unlikely that the enzyme is merely a contaminant from the host cell which is accidently absorbed to the virus prior to maturation, since it is apparently part of a membrane structure. Further studies will examine the characteristics of the ATPase in infected myeloblasts. The enzyme may be part of a subunit of the cell membrane which is used as a site for virus maturation.

BIOLOGICAL EFFECTS OF INTRACORPOREAL RADIOISOTOPE HEAT SOURCES

Person in Charge: M. F. Gillis

The completely implanted, radioisotope-powered artificial heart will subject surrounding tissues to heat and radiation. The major objective of this project is to determine the long-term local and systemic effects of prototypical $^{238}Pu^{16}O_2$ heat sources implanted in the abdominal region of miniature swine.

Earlier work at this laboratory and elsewhere has shown that dogs and swine are relatively tolerant of added endogenous heat, so long as local tissue damage is avoided. Heat fluxes up to 4.7 watt/cm^2 are tolerated for years by aortic blood flowing through a tubular heat exchanger in the thorax of swine. Soft tissue surrounding a heat source appears to have a heat flux limit at least 100-fold lower than this. Experiments using dogs abdominally implanted with strontium-yttrium mock radiation sources have indicated that radiation from prototypical $^{238}Pu^{16}O_2$ sources will not produce detectable effects over periods of a few years. Nothing is known, however, concerning long-term effects, or possibly synergistic effects, of exposure to combined heat and radiation.

The major effort of this project has been directed toward the development of a method for implanting 29-watt $^{238}Pu^{16}O_2$ sources in the abdominal region of miniature swine for long-term study. The source must be contained in an implantable holder of sufficient surface area to keep the heat flux within limits tolerated by tissue adjacent to the surface of the implant. This requires definition of tissue heat flux tolerance limits and knowledge of their variation with implant size and with time following implantation. This project has also included fundamental experiments designed to characterize heat transfer properties of traumatized and healing tissues in vivo, a difficult area of investigation and one which is critical to many design considerations of the ERDA Artificial Heart Program.

PUBLICATIONS SINCE 1973

Decker, J. R. and M. F. Gillis. 1973. A completely implantable three channel temperature biotelemetry system. ISA Trans. 12(2), 97-102.

BIOLOGICAL EFFECTS OF INTRACORPOREAL RADIOISOTOPE HEAT SOURCES

Investigators:
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Technical Assistance:
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A surface heat flux of 0.04 watts/cm^2 from a retroperitoneal implant with healthy surface ingrowth of tissue prior to generation of heat is intolerable, producing gross tissue necrosis. Percutaneous cooling of hot implants during the postoperative healing period is a feasible technique, but our current plutonium heat source implant design has been proven of inadequate size and a new design is described. Rough calculations based on tissue conductivity and conductance values suggest that even with this larger device, added heat to proximate tissues may produce long-term changes even though the heat burden may be tolerable over relatively short periods.

Our previous report described an experiment in which a discoid (8-in diameter, 3-in center thickness, 810-cm^2 surface area) having a central electrical heater was implanted retroperitoneally in a swine and, three weeks later, the heater surgically replaced with a 29-watt $^{238}Pu^{16}O_2$ heat source. Thirty days later, postmortem examination revealed that most of the scar capsule around the device was grossly healthy in appearance with necrosis of only the basal layer of tissue immediately against the surface of the heater. Ingrowth to the dacron velour covering was good except over the cap removed for insertion of the heat source at the second operation, where ingrowth was apparently prevented by the high surface temperature.

To decide whether this discoid design would suffice, and to obtain additional information on tolerable heat flux, a discoid was prepared identical to the one above but with an insulating cap and an insulating annulus around the periphery of that cap. The discoid was implanted retroperitoneally in a swine and allowed to heal for two months. By means of a small bar magnet in the discoid cap and a stud finder, the area of the cap was exposed at a second operation and a 29-watt heat source inserted. The scar capsule in the operation area was grossly healthy and well attached to the dacron velour surface.

(a) Engineering Technology Department

Surface temperature was monitored telemetrically over the next 23 days (Figure 30.1). It increased to about 115°F in a few days, tended to plateau for the next 10-11 days, then began to increase slowly, reaching about 125°F 23 days after source insertion. This clearly indicated tissue necrosis around the device, so the animal was sacrificed. As expected, all tissue in the vicinity of the device was baked and necrotic. Because the insulating cap and annulus reduced the conducting surface area of the discoid by about 100 cm^2, the resulting average heat flux was 0.041 watts/cm^2, supporting our earlier findings which set the maximum tolerable flux at 0.03 watts/cm^2 or less. The discoid, therefore, is inadequate for long-term implantation of 29-watt sources.

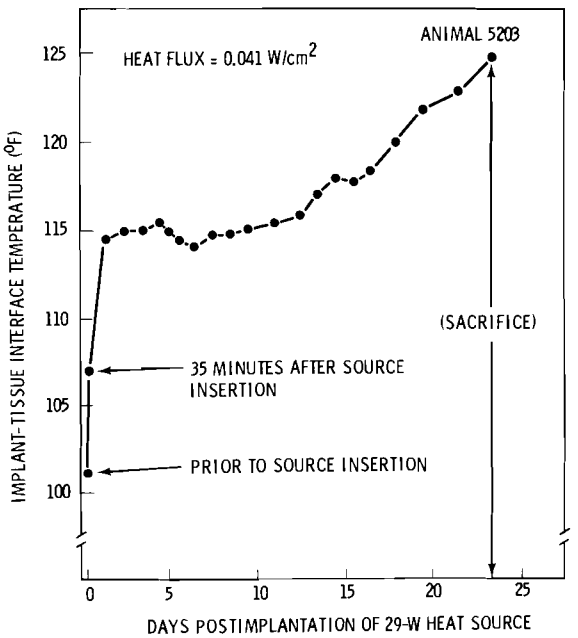

FIGURE 30.1. Implant-tissue Interface Temperature Variation with Time after Insertion of a 29-watt $^{238}Pu^{16}O_2$ Heat Source. The discoid source holder was implanted in the retroperitoneal region of a swine and allowed to heal in place prior to inserting the source at a second operation.

In another attempt to solve the problem, we surgically created a retroperitoneal space on one side of a swine which represented the maximum space attainable and, based on measurements of this space, designed an irregular source holder having a total surface area of about 1200 cm^2 (Figure 30.2). This is currently being equipped with temperature telemetry, after which it will be covered with dacron velour, sterilized by gamma irradiation and implanted in a swine. When a 29-watt source is inserted, average surface heat flux will be about 0.024 watts/cm^2. If this proves to be an acceptable design, it will be used for long-term implantation of 29-watt sources.

Our previous report described the beginning of an experiment in which a discoid similar to those described above was equipped with temperature telemetry and coolant tubing coils around the source cavity. A 29-watt plutonium heat source was inserted in the discoid and the discoid implanted retroperitoneally in a swine with coolant tubing exiting percutaneously. Water flow through the tubing was controlled automatically to maintain the device surface temperature at a preset value. The intent was to determine the feasibility of percutaneous source cooling during the early postoperative period and see if, after a sufficient period of time, coolant flow would decrease to zero as healing in the presence of elevated temperature progressed. The surface temperature was maintained at 104°F for three weeks, then deliberately increased to 106°F.

This preparation functioned for two months and demonstrated the feasibility of percutaneous source cooling. Unfortunately, because of one power interruption and several interruptions in coolant flow due to the animal mutilating the cooling tubing, there were several excursions in device temperature which resulted in a loss of tissue attachment and the formation of a large seroma. Thus we did not attain the second objective of this experiment. Work described to us by F. A. Kallfelz, Cornell University suggests that coolant flow can indeed be gradually reduced in such preparations, but the

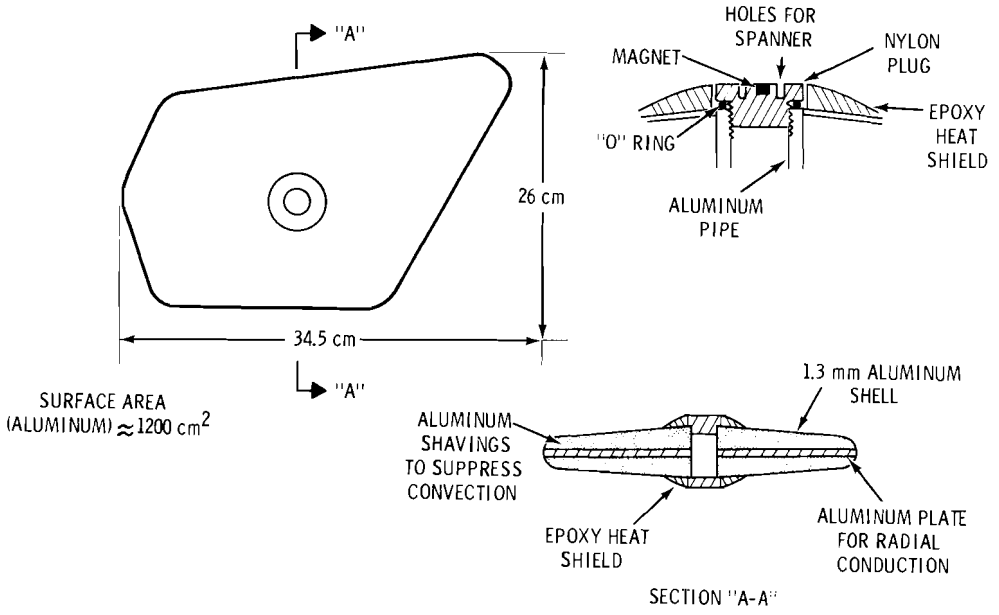

FIGURE 30.2. Sketch of the New Implant Design for Holding a $^{238}Pu^{16}O_2$ Heat Source. This will occupy virtually all of the available retroperitoneal space in one side of a miniature swine. The magnet in the source cavity cap is for locating the center of the cap during subsequent surgery (see text).

surface temperature must at all times be tissue-tolerable if the preparation is to endure.

One alternative method of cooling an abdominally located heat source, proposed some time ago, was to transect the thoracic aorta and reroute flow through an arterial prosthesis connected to a source-containing heat exchanger in the abdomen, continuing on the other side of the exchanger with arterial prosthesis to an end-to-side anastomosis with the post-renal aorta. We performed such a bypass operation on a swine, minus the heat exchanger, to assess the feasibility of such an approach for a long-term preparation (years). The animal was sacrificed 18 months after the bypass operation.

The bypass was patent throughout, but the tissue which had built up on the inner surface of the prosthesis was very thick (3 mm minimum) and in two locations, one where the prosthesis passed through the diaphragm, the lumen was reduced to a diameter of ca. 1 mm. There is little doubt that total occlusion would eventually have resulted. The development in swine of such thick "pseudointimal" linings in prostheses has been reported by others, and we have concluded that such a preparation could not be relied upon for long-term studies.

It is of interest to estimate how much tissue surrounding a hot implant is involved in carrying away the heat. Reported tissue conductivities vary

widely due to animal used, tissue type, degree of trauma inflicted, tissue vascularity and perfusion rate, vasoactivity in response to local heating and autonomic factors, and experimental technique. As an example, however, the average conductivity of rabbit subcutaneous tissues has been reported by one group[1] to be about 0.01 W/(cm·°C).

Using implants with volumes ranging from 71 to over 1500 cm^3, we have measured conductance in the swine. Our values ranged from 0.010 to 0.035 W/(cm^2·°C). Combining these results with the above conductivity value, we conclude that the effective sink distance is 1-4 cm, indicating that a relatively small amount of tissue near the implant surface is responsible for transfer of heat from the implant. This is what one would expect, and it means that to minimize temperature one must (1) maximize device surface area and (2) encourage the formation of highly vascular tissue around the implant.

If we assume that a shell of tissue about 1 cm thick around the new source holder discussed earlier (surface area 1200 cm^2) must carry away the heat from a 29-watt source in that holder, the average rate of heat transfer per unit of volume of tissue in the shell is about 0.02 watts/cm^3, to which must be added the relatively small contribution of metabolic heat production in the shell tissue. This is comparable to the rate of heat production in highly metabolic tissues, e.g. malignancies[1], which raises the real possibility that changes in tissue surrounding a hot implant may result over the long term even though the surface heat flux (and temperature) is tolerable over relatively short periods. One objective of our project is to resolve that question.

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1. Nilsson, S. K. and Gustafsson, S. E., Surface temperature over an implanted artificial heat source. Phys. Med. Biol. 19: 677-691, 1974.

ARTIFICIAL HEART PROGRAM - RECIPIENT RADIATION EXPOSURE

Person in Charge: F. T. Cross

The objective of this project is to determine the dose from radioisotopic heat sources proposed for use in circulatory support systems. Involved are considerations of heat sources and radioisotope systems design, materials, radiation dosimetry to various tissues, and the estimation of biological effects.

Dose measurements are made with prototype heat sources placed in various tissue-equivalent phantoms. Computer codes are developed or adapted to simulate the results of these measurements. The codes are then used in parametric dose rate studies, such as studies of shielding requirements, and for the calculation of dose rates to various organs and tissues that are not easily measured in vivo.

Currently, experiments are continuing to determine the dose to bystanders resulting from their exposure to a recipient of an artificial heart. Dosimetry assistance will also be provided for animal experiments designed to determine the combined effects of heat and radiation in swine.

PUBLICATIONS SINCE JANUARY, 1973

Cross, F. T. 1975. Dose Rates to a Bystander from an Artificial Heart Power Source. Health Phys. 29, 350-352.

ARTIFICIAL HEART PROGRAM - POPULATION RADIATION EXPOSURE

Person in Charge: R. W. McKee

Successful development and routine use of ^{238}Pu-fueled artificial heart devices would expose persons in the vicinity of these devices to small amounts of radiation. The objective of this project is to develop comprehensive estimates of these low-level radiation doses so that the physiological implications to persons other than the device users can be evaluated.

To develop these estimates, the potential artificial heart population is subdivided into smaller groups whose daily activities can be identified by sample survey techniques. Dose calculations for all of the activities are summed for all user groups to estimate the population dose. Essential components of the study include (1) projections of the potential artificial heart users by age, sex, household characteristics, etc., (2) surveys to develop time-distance relationships between potential recipients and other persons, (3) calculations of radiation dose factors, and (4) development of a computer program to sum and classify the population dose.

Using this approach, the population dose has been estimated over a wide range of possible conditions. For the year 2000 the estimated range is from 60,000 to 500,000 rem. A detailed topical report describing these results is in the process of final editing and review prior to publication.

A major uncertainty in determining population dose, and the principal reason for the wide range of estimates, is the projected demand for artificial hearts. Additional work has been proposed to identify this requirement, employing a panel of cardiologists and surgeons to define criteria for artificial heart eligibility.

PUBLICATIONS SINCE JANUARY, 1973

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ARTIFICIAL HEART PROGRAM - MEDICAL GRADE ^{238}Pu FROM ^{241}Am

Person in Charge: R. W. McKee

For applications in medical devices such as pacemakers and artificial hearts, a supply of ^{238}Pu with less than 0.3 ppm of ^{236}Pu is needed because of the disproportionately high radiation dose produced by the daughters of ^{236}Pu. The past and current principal source of ^{238}Pu has been through neutron irradiation of ^{237}Np in ERDA reactors, with subsequent chemical separation of the ^{238}Pu formed by neutron capture. Unfortunately, a small amount of ^{236}Pu is also formed in this process. Power reactors will be discharging spent fuel containing significant quantities of ^{241}Am which, if separated, could be irradiated to produce a ^{238}Pu product free of ^{236}Pu.

The objective of this project is to evaluate the economics and technical practicability of producing medical grade ^{238}Pu by irradiating ^{241}Am recovered from spent power reactor fuel. The ^{241}Am target irradiation could be performed in power reactors.

The scope of the project includes: (1) projecting the availability of ^{241}Am, (2) identifying the facilities and cost for americium recovery, and (3) identifying the optimum target irradiation and processing cycle and cost of producing ^{238}Pu.

MEDICAL GRADE ^{238}Pu FROM ^{241}Am - PROGRESS REPORT

Investigator:
R. W. McKee(a)

Shielding requirements for fabrication of americium targets were evaluated, a preliminary flowsheet for processing the irradiated Am targets was developed, and the AMRAD computer program, which models the irradiation cycle, was improved.

During the previous year the physical constraints in the ALTHAEA computer code were calibrated to produce irradiation calculations that matched experimental data for americium and curium compositions; irradiation calculations for spent power-reactor fuel and americium target compositions were computed; development of the AMRAD computer program for analysis of the ^{241}Am target irradiation cycle was begun; and preliminary estimates of the cost of recovering ^{241}Am from spent power reactor fuel were made. The past year's work included determination of shielding requirements for fabrication of americium targets, development of a preliminary flowsheet for processing the irradiated ^{241}Am-^{243}Am mixture, and further development of the AMRAD computer program to provide a more detailed description of the production cycle.

Analyses of shielding requirements for americium target fabrication indicate that estimated photon dose rates from $^{243}AmO_2$ in the processed material and in thin dust films will probably preclude target fabrication in gloveboxes. Table 33.1 indicates dose rates through 60-ml lead gloves from the surface of a container holding 114 g of various ^{241}Am-^{243}Am mixtures. Table 33.2 shows estimated dose rates from thin dust layers inside gloveboxes and the time available for using gloves before a 75 rad/yr extremity exposure limit is reached. It appears that it will not be practical to process AmO_2 in

TABLE 33.1. Dose Rates from the Surface of a Container, through 60-mil Heavy Lead Gloves for Various ^{241}Am-^{243}Am Mixtures Totalling 114 g.

ISOTOPIC MIXTURES	DOSE RATE, rads/yr
100% ^{241}Am	18.7
67% ^{241}Am - 33% ^{243}Am	555
50% ^{241}Am - 50% ^{243}Am	832
33% ^{241}Am - 67% ^{243}Am	1110
100% ^{243}Am	1640

(a) Systems Engineering Department

TABLE 33.2. Estimated Dose Rates to Hands from Thin Dust Layers on the Surface of 60-mil Gloves and on the Inside of the Gloveboxes.

ISOTOPIC MIXTURE	DOSE RATE mrads/hr	TIME REQUIRED TO ATTAIN 75 R/yr EXPOSURE LIMIT, hours
100% ^{241}Am	94	800
67% ^{241}Am - 33% ^{243}Am	2800	27
50% ^{241}Am - 50% ^{243}Am	4200	18
33% ^{241}Am - 67% ^{243}Am	5600	13.5
100% ^{243}Am	8200	9.1

any glovebox operation in which dust is generated. Calculations for remote-processing shielding requirements for kilogram quantities of AmO_2 indicate that a 2.54-cm-thick lead photon shield and 15.2-cm-thick neutron shield should be adequate.

In developing the preliminary flowsheet for reprocessing of the irradiated ^{241}Am-^{243}Am targets, three areas were identified on which more work is needed: (a) the nature of the target diluent, (b) the choice between solvent extraction or ion exchange for plutonium removal, and (c) radioiodine control. Both ZrO_2 and aluminum cermet target diluents were found to be objectionable--the ZrO_2 because dissolution is very difficult and the Al cermet because of its leachability, resulting in possible contamination of power reactor coolant. A possible solution, which needs further investigation, may be a graphite diluent.

The technology of both ion exchange and solvent extraction systems for Pu separations is well developed, but the presence of ^{242}Cm in the system under consideration presents problems. Because of the large amount of heat generated (~120 watts/gram) by the ^{242}Cm, the diameter of ion-exchange columns must be limited to 4 inches to prevent runaway exothermic resin oxidation by nitric acid. Because of this limit on throughput, a single ion exchange line for Pu separation would probably be feasible only for an annual production of 500 kg ^{238}Pu or less, using a continuous processing facility. This is more than adequate, however, for projected medical grade ^{238}Pu requirements during the early years of production.

Solvent extraction systems do not have the throughput limitations of the ion exchange system, but present other problems--principally degradation of the TBP solvent by ^{242}Cm radioactivity, and consequent loss of Pu in the unstrippable reaction product. This can be minimized by either aqueous feed dilution or use of centrifugal contactors having short residence times. If processing of the irradiated Am mixture is done on a campaign basis in an existing facility, it would undoubtedly use a solvent extraction process. Overall, solvent extraction appears to be the better choice for Pu separation.

The yield of high-quality ^{238}Pu from a second separation process is maximized by allowing a minimum cooling period before the first plutonium separation. However, with a short cooling period ^{131}I levels are significant. Based on calculated ^{131}I generation rates, and assuming a cooling time of 30 days before processing, approximately 100 curies of ^{131}I will be generated per kg of ^{238}Pu separated. At a production level of 500 kg of ^{238}Pu per year, a decontamination factor (DF) of 5×10^4 would be necessary to limit release to one curie per year. Further work will be needed to achieve this high DF.

Work has continued on improving the AMRAD computer code, which models the entire production cycle for purposes of production and cost analysis. This work has been directed towards developing a more detailed description of the production cycle to identify operating conditions that would maximize production of high purity ^{238}Pu.

ALVEOLAR CLEARANCE OF INHALED METAL OXIDES

Person in Charge: C. L. Sanders

Metal oxides produced during the combustion of fossil fuels for energy production constitute a potential health hazard to human populations living near, or working in, such facilities. Available information on the cellular fate of inhaled metal oxides indicates that the alveolar macrophage and the type I alveolar epithelial cell play prime roles in determining the early spatial-temporal distribution of metal oxides, and in their subsequent clearance from the lung. Metal oxides that damage the cells in which they are phagocytized may have a longer retention time in the lung, and constitute a greater health hazard, than metal oxides that produce little damage and are more rapidly cleared.

This project will examine the role of the alveolar macrophage, alveolar epithelial cells, and other pulmonary cells in influencing the distribution within the lung, and the clearance from the lung, of several inhaled metal oxides, initially those of lead, cadmium and mercury. Electron microscopy will be employed to examine quantitatively the distribution of metal oxides in lung cells and the damage which they cause. Other studies will assess the degree of inhibition of clearance of a test aerosol given after metal oxide inhalation. We will also examine the degree of inhibition of methylcholanthrene-induced aryl hydrocarbon hydroxylase activity in a stimulated population of macrophages previously loaded with metal oxide.

This project received EPA "Pass Through" funding in FY 1976. Reportable results have not yet been obtained.

EFFECTS OF SULFUR POLLUTANTS ON LUNG PHYSIOLOGY AND BIOCHEMISTRY

Person in Charge: S. M. Loscutoff

This project seeks to define the vasoactive agents responsible for changes in pulmonary function during inhalation of sulfur pollutants. The guinea pig is known to be highly sensitive to inhaled sulfur compounds, responding with an increased pulmonary resistance and a decreased pulmonary compliance. This response of the guinea pig is similar to that of humans with pre-existing pulmonary or cardiovascular disease. It is hoped that the definition of agents responsible for pulmonary changes in laboratory animals may suggest means of protecting sensitive individuals in the human population from air pollution episodes.

Guinea pigs will be exposed to respirable mists of sulfuric acid at concentrations sufficient to produce rapid increases in pulmonary resistance. Pulmonary resistance and compliance will be monitored continuously from simultaneous measurements of pleural pressure, tidal volume and air flow. The influence of different vasoactive agents on the response to sulfuric acid will be assessed by pharmacologically blocking the action of each agent with specific inhibitory drugs.

Upon completion of these initial studies, it will be important to determine whether other animal species respond in a manner similar to the guinea pig and whether other sulfur pollutants produce responses similar to those of sulfuric acid mists. These studies will provide a better understanding of events responsible for changes in pulmonary function during exposure to sulfur pollutants. Based on this information, the most effective means of protecting sensitive humans from air pollution crises may be suggested.

This project received EPA "Pass Through" funding in FY 1976.
Reportable results are not yet available.

COMBINED EFFECTS OF ACUTE AND CHRONIC EXPOSURE TO CO, NO_2, SO_2 AND FLY ASH

Person in Charge: S. M. Loscutoff/A. J. Gandolfi

This project seeks to define in an animal model the biological effects of exposure to pollutant atmospheres which could realistically develop around large-scale fossil-fuel-burning power plants. Most previous studies related to the health effects of air pollutants have focused on one pollutant material administered at levels higher than actual environmental concentrations. This study is concerned with health effects of combinations of pollutants administered at ambient concentrations.

Three exposure groups and a control group of rats will be evaluated during the initial phase of this study. One group will be exposed six hours per day, five days per week, to an atmosphere containing NO_x, SO_2, CO and fly ash in concentrations simulating the atmosphere surrounding a fossil-fuel plant during a plume-trapping episode. A second group of rats will be exposed on the same schedule to pollutant concentrations at ten times the concentrations administered to the first group. A third group will be exposed to the same atmosphere as group two, but with 20-minute morning and afternoon exposures simulating exposure to a looping plume. Group three animals will be exposed to the same total dose of pollutant material as group one, but dose rates in the two groups will be very different.

Effects of pollutant exposure will be assessed from physiological, biochemical and anatomical evaluations. Physiological measurements will evaluate flow-resistive and elastic properties of the lung. Biochemical measurements will assess acute damage to lung function, adaptive changes making the lung resistant to repeated insults, and chronic changes in lung metabolism. Anatomical changes will be assessed from periodic histopathological evaluation of lung sections from exposed animals.

Following completion of initial studies, the beneficial effects of removing either NO_x, SO_2, CO or fly ash from the pollutant mixture will be evaluated to determine which component contributes most to the harmful effects of pollutant exposure. Results of these studies should suggest the most effective means of reducing the health risks to human populations living near fossil-fuel plants.

This project received EPA "Pass Through" funding in FY 1976. Reportable results are not yet available.

FACTORS INFLUENCING THE CROSS-PLACENTAL TRANSFER AND TERATOGENICITY OF METALLIC POLLUTANTS

Person in Charge: M. R. Sikov

This project will seek to define the specific influences of factors affecting the cross-placental transfer of heavy metals and their distribution throughout the fetoplacental unit as a function of time after exposure. These data will define the tissues at risk and provide a quantitative estimate of dose. As such, they will provide a rational basis on which to interpret and interrelate the results of teratologic studies.

The precise design of studies to be performed with specific pollutants will vary with the amount and credibility of the information presently available, or which may become available during the course of the study. In general, however, cross-placental transfer and distribution will be evaluated at four different gestational ages selected to represent stages in the continuum of embryonic and placental development. The intravenous, oral, and inhalation routes of administration, which will provide differences in the rate at which the metals are presented to the placenta, as well as possible differences in their chemical binding in blood, will be compared. Since metabolism may be influenced by the mass administered, a low dose level, as well as one in the teratogenic range, will be studied.

This project will provide some of the toxicity data required to assess the teratogenic hazard of heavy metal pollutants. More importantly, it will provide the distribution and retention data required to integrate the results of past and future studies on these materials and to more readily extrapolate them to the practical situation. Initial studies are being performed with lead, which is known to be fetotoxic and teratogenic. Subsequent studies will investigate arsenic, cadmium, and selenium, which have been shown to be embryocidal and/or teratogenic, as well as nickel, which has yielded equivocal results, and vanadium, which apparently has not been evaluated.

This project received EPA "Pass Through" funding in FY 1976. Reportable results are not yet available.

GENETIC EFFECTS FROM ELECTRIC FIELDS AT THE CHROMOSOMAL LEVEL OF DROSOPHILA

Person in Charge: F. P. Hungate

As electrical power generation facilities become more concentrated, more of the public will be exposed to electric fields associated with transmission lines, and more workmen will be entering fields of the sort found in electric switch yards. The United States has no exposure limits relative to human exposure to electric fields. Russian workers have reported effects on humans, and in that country there are controls for exposures in excess of 5 kV/m. While there is no present evidence relating electric fields to genetic effects, it would seem prudent to evaluate this possibility. Since the charge distribution along the DNA chain is not uniform, it is not unreasonable that AC fields might increase the frequency of chromosome breakage, or that any electric fields might adversely affect the restitution of breaks occurring for other reasons while in the presence of an electric field.

This project will employ genetic and cytologic techniques to identify frequencies of chromosome aberrations following exposures in electric fields up to 2 MV/m. Initial studies will involve DC fields. Drosophila strains bearing appropriate markers will be used to detect frequencies of offspring bearing new linkage relationships, and for scoring of aberrations in squash preparations of F_1 salivary glands. To study effects on restitution of breaks, a higher frequency of breaks than is normally present will be obtained by exposing the flies to ionizing radiation during exposure in the electric field. Strains of Salmonella typhimurium, auxotrophic for histidine, will also be exposed to the individual and combined electric and ionizing radiation fields to obtain evidence of effects at the locus level.

This project received EPA "Pass Through" funding in FY 1976 and is still in the stage of material acquisition and technique development.

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PUBLICATIONS AND PRESENTATIONS

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

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Sanders, C. L. 1975. Dose distribution and neoplasia in the lung following intratracheal instillation of $^{239}PuO_2$ and asbestos. Health Phys. 28, 383-386.

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Sanders, C. L. and G. E. Dagle. 1974. Studies of pulmonary carcinogenesis in rodents following inhalation of transuranic compounds, pp. 422-429. In: Experimental Lung Cancer. Carcinogenesis and Bioassays, E. Karbe and J. F. Park (eds.). Springer-Verlag, NY.

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*Schneiderman, G. S., T. R. Garland, H. Drucker, and R. E. Wildung. 1975. Growth and chromium transport characteristics of microorganisms isolated from chromium enriched soil. Agronomy Abstracts, 130-131.

*Sikov, M. R. and D. D. Mahlum. 1975. Toxicity of ^{241}Am and ^{244}Cm after administration at nine days of gestation in the rat. Radiat. Res. 62, 565. (Abstract)

*Sikov, M. R., V. H. Smith, and D. D. Mahlum. 1975. Embryotoxicity of the calcium and zinc salts of Diethylenetriaminepentaacetic acid (DTPA) in Wistar rats. Teratology 11, 34A. (Abstract)

Stuart, B. O., R. E. Filipy, R. F. Palmer, H. A. Ragan, and P. L. Hackett. 1974. The biological effects of inhaled radon daughters with uranium ore and cigarette smoking in beagle dogs. Health Phys. 27, 628. (Abstract)

*Sullivan, M. F., P. S. Ruemmler, J. L. Beamer, and T. D. Mahony. 1975. Intestinal radiation injury: The lower bowel syndrome. Radiat. Res. 62, 579. (Abstract)

Thompson, R. C. 1975. Transuranium element toxicity - dose response relationships at low exposure levels. Summary and speculative interpretation relative to exposure limits, pp. 1278-1284. In: Radiation Research, Biomedical, Chemical and Physical Perspectives, O. F. Nygaard, H. I. Adler and W. K. Sinclair (eds.). Academic Press, New York.

Thompson, R. C. 1975. Animal data on plutonium toxicity. Health Phys. 29, 511-519.

*Thompson, R. C. 1974. Implications with respect to protection criteria, pp. 271-289. In: Plutonium and Other Transuranium Elements: Sources, Environmental Distribution and Biomedical Effects, USAEC Doc. WASH-1359.

*Thompson, R. C. 1974. Effects of plutonium in animals, pp. 56-63. In: Plutonium Information Meeting for an Ad Hoc Subcommittee of the Advisory Committee. (CONF-740115). NTIS, Springfield, VA.

Viola, M. V., M. E. Frazier, L. White, J. Brody, and S. Spiegelman. 1975. RNA-instructed DNA polymerase activity in a cytoplasmic particulate fraction in brains from Guamanian patients. J. Exp. Med. 142, 483-494.

Young, J. R., B. F. Gore, D. D. Mahlum, J. A. Strand, R. C. Thompson, and J. K. Soldat. 1975. Information Requirements for Controlled Thermonuclear Reactor Environmental Impact Statements, BNWL-1883, Battelle-Northwest, Richland, WA.

*Zwicker, G. M., D. D. Mahlum, and M. R. Sikov. 1975. Influence of age on the tumorigenicity of plutonium-239 in rats. Proc. Amer. Assoc. for Cancer Research, 16, 161. (Abstract)

1975 BIOLOGY DEPARTMENT PRESENTATIONS

Adee, R. R. and C. L. Sanders. Identification, localization and cytotoxic effects of particulates in alveolar macrophage and epithelium. Symposium of Cytotoxic Effects of Environmental Pollutants, University of California, Berkeley, April 18.

Bair, W. J. Recent results of animal studies on the deposition, retention and translocation of plutonium compounds. IAEA Seminar on the Diagnosis and Treatment of Incorporated Radionuclides, Vienna, Austria, December 8-12.

Bair, W. J. and J. M. Thomas. Prediction of the health effects of inhaled transuranium elements from experimental animal data. IAEA Symposium on Transuranium Nuclides in the Environment, San Francisco, CA, November 17-21.

Bair, W. J. Biomedical aspects of plutonium. Seminar at the University of Washington, Department of Nuclear Engineering, Seattle, WA, November 12.

Bair, W. J. Review of plutonium induced lung cancer in experimental animals. Seminar, National Radiological Protection Board, Harwell, England, April 4.

Bair, W. J. The hot particle issue. Meeting of Columbia Chapter of Health Physics Society, Richland, WA, January 28.

Ballou, J. E., G. E. Dagle, K. E. McDonald, and R. L. Buschbom. Influence of inhaled Ca-DTPA on the long-term effects of inhaled Pu nitrate. Annual Health Physics Society Meeting, Buffalo, NY, July 13-17.

Cannon, W. C. Deposition of inhaled aerosols in beagle dogs. Eighth Aerosol Technology Meeting, Chapel Hill, NC, October 6-8.

Craig, D. K., J. F. Park, and J. L. Ryan. Effect of physicochemical properties on metabolism of transuranium oxide aerosols inhaled by beagle dogs. Association for Aerosol Research, Bad Soden, West Germany, October 16-18.

Craig, D. K., J. R. Decker, and R. L. Buschbom. Disposition of highly toxic radioactive aerosols inhaled by beagle dogs. Annual Health Physics Society Meeting, Buffalo, NY, July 13-17.

Craig, D. K. and R. L. Buschbom. The aerodynamic equivalent size distribution of inhaled and exhaled polydispersed aerosols in beagle dogs. American Industrial Hygiene Conference, Minneapolis, MN, May.

Dagle, G. E. and J. F. Park. Pulmonary pathology induced by inhaled plutonium in beagles. Workshop on the Biological Effects and Toxicity of Pu-239 and Ra-226, Sun Valley, ID, October 6-9.

Frazier, M. E. Virologic and molecular aspects of leukemic patients in remission. Seminar, Biology Department, Battelle-Northwest, Richland, WA, December 16.

Frazier, M. E. Reverse transcriptase in leukocytes of leukemic patients in remission. Seminar, Washington State University, Pullman, WA, December 12.

Frazier, M. E., J. F. Park, W. S. S. Jee, and G. Taylor. Investigation of the role of oncornavirus in radiation-induced osteosarcomas. Workshop on the Biological Effects of Pu-239 and Ra-226, Sun Valley, ID, October 6-9.

Frazier, M. E. Evidence for RNA tumor virus in leukemic miniature swine. American Society for Microbiology, New York, NY, April 27-May 2.

Frazier, M. E. In vitro interactions of lymphocytes and cultured cells from beagles with plutonium-induced bone tumors. Seminar, University of Connecticut Medical Center, Farmington, CT, April 24.

Horstman, V. G. Radiation biology at Hanford. Animal Resources Branch, Washington State University, Pullman, WA, May 2; Tri-City Transportation Club, Pasco, WA, May 1; Kiwanis Club Farm-City Day, Richland, WA, January 29.

Hungate, F. P., L. R. Bunnell, W. F. Riemath, and M. T. Karagianes. Extracorporeal irradiations of blood in goats with fully portable irradiators. Radiation Research Society 23rd Annual Meeting, Miami Beach, FL, May 11-15.

Hungate, F. P. Extracorporeal blood irradiation. Seminar, University of Washington, February 19.

Mahlum, D. D., M. R. Sikov, and F. P. Hungate. The influence of temporal distribution of alpha dose in bone tumor incidence. Workshop on the Biological Effects and Toxicity of Pu-239 and Ra-226, Sun Valley, ID, October 6-9.

Mahlum, D. D. and M. R. Sikov. Metabolism of ^{241}Am citrate relative to age. Radiation Research Society 23rd Annual Meeting, Miami Beach, FL, May 11-15.

Miyata, K. and H. Drucker. Purification and properties of an exocellular alkaline protease produced by Neurospora crassa. American Society for Microbiology, New York, April 27-May 2.

Park, J. F. Biological effects of inhaled plutonium in beagles. Seminar, Electrical Power Research Institute, Palo Alto, CA, August 29.

Pelroy, R. A. Regulation of the reductive pentose cycle in a unicellular blue-green alga. Seminar, Washington State University, Pullman, WA, April 16.

Pelroy, R. A. Regulation of photosynthesis in blue-green algae. Seminar, Biology Department, Battelle-Northwest, Richland, WA, April 8.

Ragan, H. A. Body-iron stores and plutonium metabolism. 15th Annual Hanford Life Sciences Symposium, Richland, WA, September 29-October 1.

Ragan, H. A. Density gradient separation and size distribution of beagle lymphocytes. Annual Meeting of the American Society of Veterinary Clinical Pathologists, Anaheim, CA, July 13.

Sanders, C. L. Health implications of plutonium: Inhalation carcinogenesis in rodents. IPHA Annual Meeting, Chicago, IL, November 16-20.

Sanders, C. L. Inhalation carcinogenesis of high-fired $^{238}PuO_2$ and $^{239}PuO_2$. Workshop on the Biological Effects and Toxicity of Pu-239 and Ra-226, Sun Valley, ID, October 6-9.

Schneider, R. P. Apparent allosteric control of sodium transport in red cells. Seminar, Botany Department, Washington State University, Pullman, WA, February 26.

Schneiderman, G. S. Plutonium-resistant fungi and actinomycetes in soil. I. Mechanism of Pu toxicity. Amer. Soc. Agron. Meeting, Knoxville, TN, August 24-28.

Shipler, D. B., J. E. Ballou, B. I. Griffin, and I. C. Nelson. Development of a Diagnostic Model for Inhaled Promethium-147 Oxide - Animal Studies. IAEA Meeting, Vienna, Austria, December 8-12.

Sikov, M. R. and D. D. Mahlum. Age and other factors influencing the metabolism and effects of ^{131}I in the rat. IAEA International Symposium on Biological Effects of Low Level Radiation Pertinent to Protection of Man and His Environment, Chicago, IL, November 3-7.

Sikov, M. R. and D. D. Mahlum. Influence of age and physicochemical form on the effects of ^{239}Pu on the skeleton of the rat. Workshop on the Biological Effects and Toxicity of Pu-239 and Ra-226, Sun Valley, ID, October 6-9.

Sikov, M. R. and D. D. Mahlum. Toxicity of ^{241}Am and ^{244}Cm after administration at nine days of gestation in the rat. Radiation Research Society 23rd Annual Meeting, Miama Beach, FL, May 11-15.

Sikov, M. R., V. H. Smith, and D. D. Mahlum, Embryotoxicity of the calcium and zinc salts of Diethylenetriaminepentaacetic acid (DTPA) in Wistar rats. 15th Annual Meeting, Teratology Society, Pocono Manor, PA, May 11-14.

Smith, V. H., J. E. Ballou, J. E. Lund, H. A. Ragan, R. H. Busch, P. L. Hackett, and D. H. Willard. Aspects of inhaled DTPA toxicity in the rat, hamster and beagle dog and treatment effectiveness for excorporation of Pu from the rat. IAEA Meeting, Vienna, Austria, December 8-12.

Smith, V. H., H. A. Ragan, J. E. Lund, P. L. Hackett, and D. H. Willard. The toxicity of inhaled Ca-DTPA in the beagle dog. Health Physics Society Meeting, Buffalo, NY, July 13-17.

Stuart, B. O., R. F. Palmer, and R. E. Filipy. Long-term pulmonary effects of combined chemical and radionuclide exposures in experimental animals. IAEA Symposium, Chicago, IL, November 3-7.

Stuart, B. O. Deposition and clearance of inhaled particles. Symposium on "Target Organ Toxicity: Lung," Cincinnati, OH, September 16-17.

Sullivan, M. F., P. S. Ruemmler, J. L. Beamer, and T. D. Mahony. Intestinal radiation injury: The lower bowel syndrome. Radiation Research Society 23rd Annual Meeting, Miami Beach, FL, May 11-15.

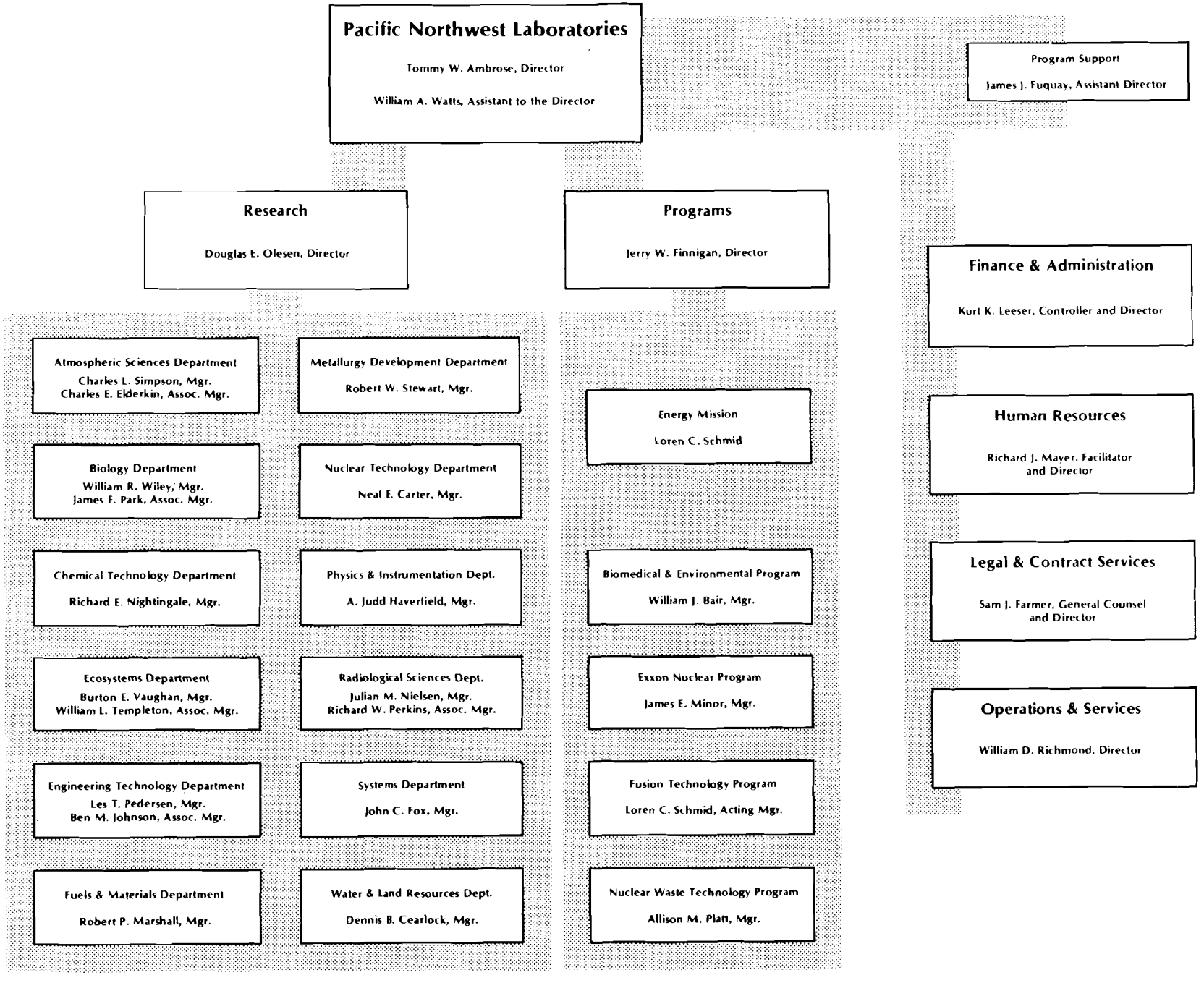
Thompson, R. C. Health effects from low-level exposure to transuranic elements. International Symposium on Transuranium Nuclides in the Environment, San Francisco, CA, November 17-21.

Wiley, W. R. Biomedical research at Hanford. Rotary Club, Richland, WA, June 17.

Wiley, W. R. Future of science and technology: An optimistic view. For the Black Executive Exchange Program (BEEP), Norfolk State College, Norfolk, VA, April 16; Student Fair, Richland, WA, March 22.

Zwicker, G. M., D. D. Mahlum, and M. R. Sikov. Influence of age on the tumorigenicity of plutonium-239 in rats. Meeting of the American Association for Cancer Research, San Diego, CA, May 8-10.

ORGANIZATION CHARTS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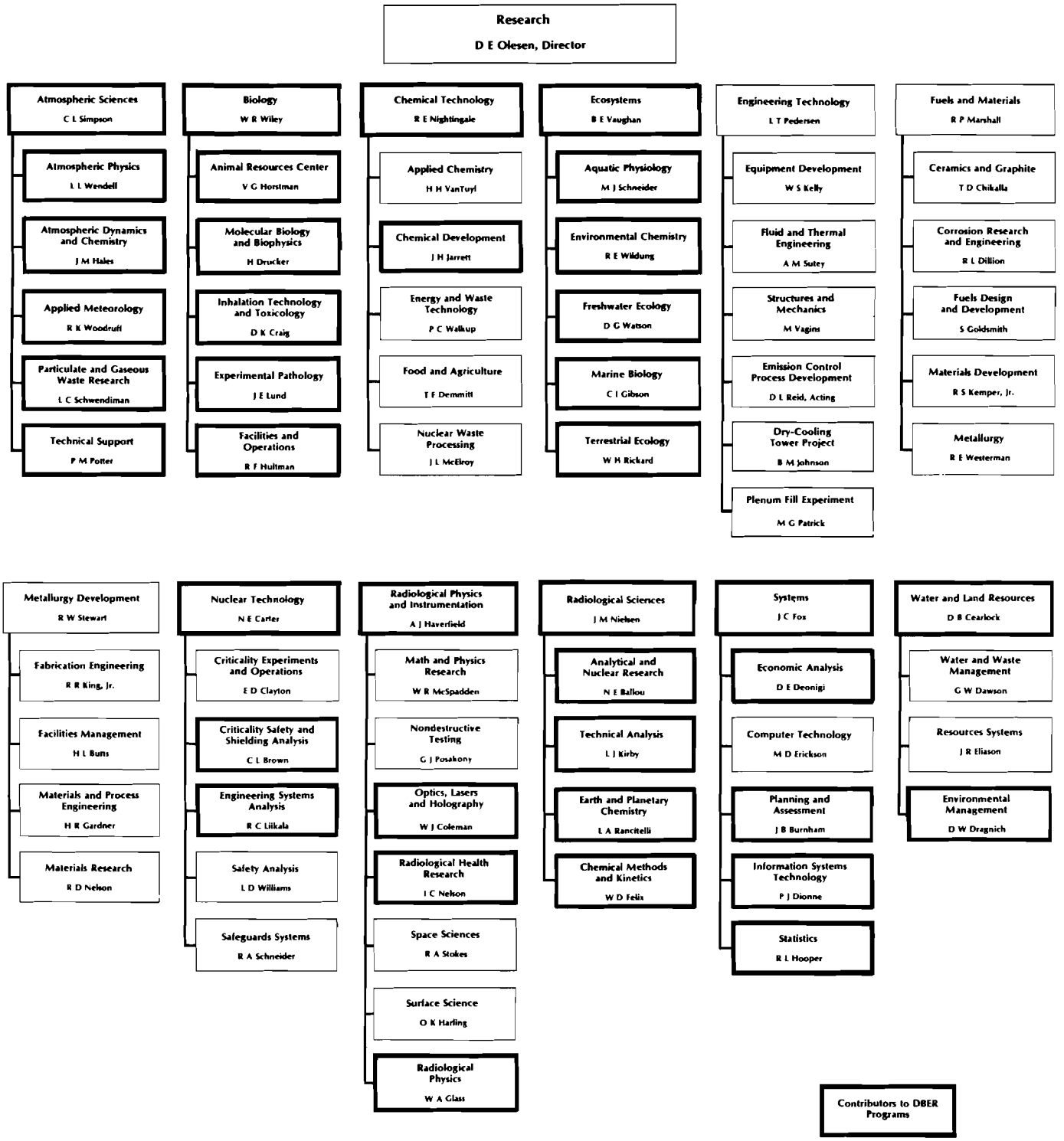

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(2) Youth Opportunity Program (YOP)
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(4) Inquiry Into Science Program (IIS)

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---

(1) Cooperative Office Education Program (COE)
(2) Terminated
(3) Visiting Scientist from the National Institute of Radiological Sciences, Chiba-shi, Japan
(4) Temporary Assignment to USERDA, Washington, D.C.
(5) Inquiry Into Science Program (IIS)
(6) Hourly Call-In
(7) Illness Leave of Absence
(8) Summer Student
(9) Deceased

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(4) Summer Student
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