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PACIFIC NORTHWEST LABORATORY ANNUAL REPORT FOR 1973 TO THE USAEC DIVISION OF BIOMEDICAL AND ENVIRONMENTAL RESEARCH

PART 1 ~~BIOLOGICAL~~ BIOMEDICAL SCIENCES

Battelle
Pacific Northwest Laboratories
Richland, Washington 99352

AUGUST 1974

Prepared for the U.S. Atomic Energy Commission under Contract AT(45-1):1830

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NOTICE

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PACIFIC NORTHWEST LABORATORY ANNUAL REPORT FOR 1973 TO THE USAEC DIVISION OF BIOMEDICAL AND ENVIRONMENTAL RESEARCH

PART 1 ~~BIOLOGICAL~~ Biomedical SCIENCES

By

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

August 1974

BATTELLE
PACIFIC NORTHWEST LABORATORIES
RICHLAND, WASHINGTON 99352

PREFACE

The Annual Report for 1973 to the U.S. Atomic Energy Commission's Division of Biomedical and Environmental Research represents a change from previous annual reports. For the past 22 years, its composition has reflected our organizational structure--each part of the report was the responsibility of the appropriate research department. In the past several years, research performed for DBER has become more interdisciplinary and more interdepartmental until now only a few projects are conducted wholly within one department. To reflect this change, this report is organized by major program categories according to our schedule-189 submissions. Each part of the Annual Report is comprised of project reports authored by scientists from several research departments. The Annual Report consists of four parts:

Part 1	Biomedical Sciences	Coordinator:	R. C. Thompson
		Editor:	J. L. Simmons
Part 2	Ecological Sciences	Coordinator:	B. E. Vaughan
		Editor:	J. L. Engstrom
Part 3	Atmospheric Sciences	Coordinators:	C. L. Simpson C. E. Elderkin
		Editor:	J. A. Powell
Part 4	Physical and Analytical Sciences	Coordinator:	J. M. Nielsen
		Editor:	L. L. Lahart

Reports previously issued are as follows:

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

W. J. Bair
Program Director, Life Sciences

FOREWORD

The research described in this volume is primarily that conducted in the Biology Department, with the addition, in the sections on Evaluation of Radionuclides in Man and Mechanisms of Radiation Effects, of work conducted in the Physics and Instrumentation Department, and in the section on Inhalation Hazards to Uranium Miners, of work conducted in the Radiological Sciences Department. Biology Department research on blood irradiation and on problems related to the artificial heart program are included in another volume, Part 4 of this Annual Report. The sections of this report correspond to AEC Project Titles (Schedule 189).

The listing of Biology Department staff reflects several organizational changes that occurred during the past year. Two new positions of Associate Department Manager were filled by J. F. Park and W. R. Wiley. Dr. Park was succeeded as Manager of the Inhalation Toxicology Section by D. K. Craig. A new Experimental Pathology Section was created with J. C. Hampton as Manager.

A highlight of the year was the Thirteenth Annual Hanford Biology Symposium on The Cell Cycle in Malignancy and Immunity held October 1-3, 1973, under the chairmanship of J. C. Hampton. The proceedings will be published in the AEC Symposium Series. The Fourteenth Annual Hanford Biology Symposium on Radiation and the Lymphatic System is scheduled for September 30 to October 2, 1974.

A list of 1973 publications relevant to AEC programs, by personnel of the Biology Department, appears at the end of this report. Requests for reprints will be honored as long as the supply lasts.

R. C. Thompson
Biology Department

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TOXICOLOGY OF INGESTED, INJECTED AND TOPICALLY APPLIED RADIONUCLIDES

TOXICOLOGY OF INGESTED, INJECTED AND TOPICALLY APPLIED RADIONUCLIDES

This section contains reports from a wide variety of studies on the metabolism and effects of radionuclides entering the body by routes other than inhalation. A number of reports are concerned with the age factor in the biological disposition of nuclides. Included this year, is not only a progress report on the study of carcinogenesis by ^{239}Pu and by ^{253}Es in the rat relative to age, but reports on the comparison of ^{239}Pu metabolism in the newborn and weanling pig and on the absorption of ^{252}Cf and ^{244}Cm from the gastrointestional tract of adult and neonatal rats. Additional tumor and blood data from the three-generation life-span study of ^{90}Sr effects in miniture swine are presented in another report.

Investigations of the interaction of radionuclides and other factors are illustrated by the reports on the effect of iron deficiency on ^{239}Pu absorption from the gut, and on the embryocidal and teratologic effects of combined administration of Trypan Blue and ^{239}Pu to the pregnant rat. An investigation of the mechanism of enhancement of mammary tumor development by injected ^{239}Pu was initiated as reflected in the report on the distribution of ^{239}Pu in the lactating mammary gland.

Unique among these reports is a study of the mechanism of action of a chemical agent in protecting mice against the intestinal syndrome of acute irradiation.

INFLUENCE OF AGE ON SURVIVAL OF RATS INJECTED WITH ^{239}Pu CITRATE

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

J. O. Hess and J. D. Stearns

Rats were administered ^{239}Pu citrate as fetuses, newborns, weanlings or adults and observed for changes in longevity. Those injected as adults generally showed the greatest decrement in survival times and those treated prenatally showed the least change.

Last year we described the protocol employed to study the long-term response of rats injected at various ages with ^{239}Pu citrate. The doses (μCi/kg) administered (adults and weanlings, 0.3, 1 and 3; newborn, 3, 10 and 30; and prenatal, 6, 20 and 60 to the dam) were chosen to deliver similar radiation doses to the bone of all ages at early times after administration. This report compares the alterations in median survival time produced by plutonium in the different age groups.

Figure 1.1 shows the response of male rats to the varying doses of plutonium in terms of median survival times (MST). The adult and weanling rats showed a progressively shorter survival time as the dose was increased. In the rats exposed neonatally, the MST was decreased only in the group exposed to the highest dose. In contrast to the other groups, this population included a substantial number of animals that died within 90 days after injection. As a result, the MST was only 125 days in comparison to the control value of over 600 days.

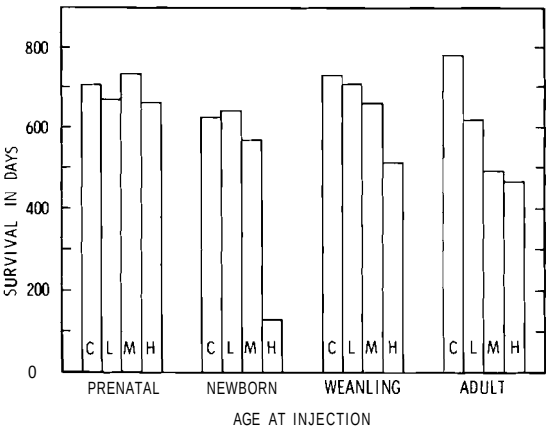

FIGURE 1.1. Influence of Administered Dose and Age at Injection on the Median Life-Spans of Male Rats Treated with ^{239}Pu Citrate (C = Control; L = Low; M = Medium; H = High)

Among the rats exposed prenatally, the MST's were not substantially different from the controls. Estimated cumulative radiation doses to bone for the medium level groups at 18-months postinjection were 22, 355, 165, and 762 rads for the prenatal, newborn, weanling, and adult groups, respectively.

The control females of the postnatal groups lived longer than their male counterparts regardless of whether MST or other measures were used as a criterion (Figure 1.2). In the prenatal group, however, the control females died at a greater rate than the males or the prenatal females injected with low or medium doses of plutonium. The MST for the high dose group was below those for the low and medium groups, but was the same as that for the controls. Among the rats exposed at birth, there was a dose-dependent decrease in MST. However, those exposed at the highest level did not show the high incidence of early deaths seen in the males. Survival was also decreased

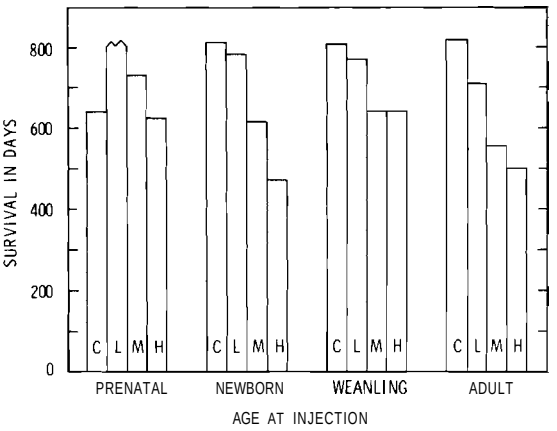

FIGURE 1.2. Influence of Administered Dose and Age at Injection on the Median Life-Spans of Female Rats Treated with ^{239}Pu Citrate (C = Control; L = Low; M = Medium; H = High)

at all levels of exposure in the adults and weanlings. The extent of the decrease was dose-dependent in the adults, but less obviously so in the weanlings. Further evaluation of the results of this experiment awaits the death of surviving animals and the completion of histopathologic studies.

PLUTONIUM METABOLISM IN THE LACTATING RAT

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

J. D. Stearns and J. O. Hess

Pregnant rats of 21 days of gestation and nulliparous rats were injected with ^{239}Pu citrate and killed 1 or 7 days later. The lactating rats had higher levels of Pu in the uterus and mammary glands than did the nonlactating animals. The reverse was true in the case of blood, ovaries, pituitary and pancreas.

In our 1968 Annual Report (BNWL-1050, Part 1) we reported that the administration of ^{239}Pu in the range of 6 to 12 μCi/kg enhanced the rate of mammary tumor development. Although experiments with external radiation have implicated the direct action of radiation on mammary tissue as well as a requirement for intact ovaries in the tumorigenic process, comparable data are not available for plutonium. We have therefore studied the uptake of monomeric ^{239}Pu by the mammary gland and by several other tissues in the normal and lactating female rat.

Plutonium-239 citrate was injected intravenously into four nulliparous rats and four pregnant females at 21 days of gestation. Two animals from each group were killed at 24 hr and the others at 7 days after injection. A number of tissues were removed, weighed and analyzed for ^{239}Pu. Figure 1.3 compares the ^{239}Pu concentration in these tissues. To provide a frame of reference, the concentrations in the femur were 2.0 and 3.0%/g for the nonlactating and and 1.4 and 1.7%/g for the lactating rats after 1 and 7 days, respectively. The total content of plutonium in blood, ovaries, pituitary and pancreas was higher in the nonlactating animals, while plutonium content of mammary gland and uterus was higher in the lactating animals. The higher plutonium content of the mammary gland and uterus is not reflected in the concentration at 24 hr because of the greater mass of these tissues in the lactating animals. However, these tissues in lactating

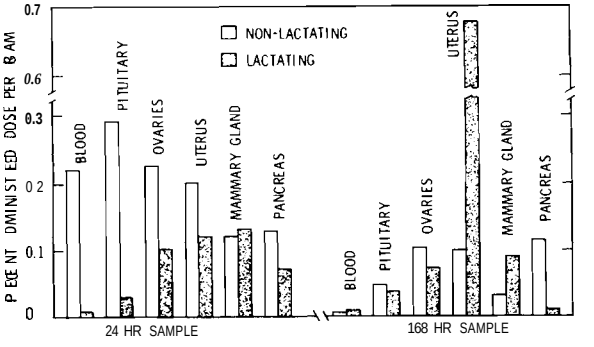

FIGURE 1.3. ^{239}Pu Concentration in Selected Tissues at 1 and 7 Days After Administration to Virgin and Lactating Rats

rats had involuted to some degree by 7 days and the higher content is then reflected in the concentration. The long-term fate of plutonium in the mammary gland and uterus is being investigated.

The calculated radiation dose to nonlactating mammary gland from an injected dose of 1 μCi/kg is approximately 11 rads over a 1-year period, assuming complete retention. Although this magnitude of dose would seem inadequate to explain an increased mammary tumor incidence, the relative effectiveness of neutron exposure has been shown to be high and a similar situation may pertain for alpha irradiation. It is important that the mechanism of mammary tumor induction by plutonium be explored in greater depth.

PLUTONIUM METABOLISM IN IMMATURE SWINE

Investigators:

B. J. McClanahan, H. A. Ragan and D. D. Mahlum

Technical Assistance:

M. L. Greenwell

Newborn and weanling miniature pigs injected intravenously with monomeric ^{239}Pu exhibited a similar distribution and retention pattern with the exception of liver. Initially, the newborn liver had a ^{239}Pu level equivalent to one-third that in the weanling liver. At the end of 28 days it had increased threefold while the weanling liver increased 30%.

Experiments were undertaken to study the metabolism of monomeric ^{239}Pu in young miniature swine. Animals were injected intravenously when either 24 hr or 6 weeks of age. The newborns received doses ranging from 1.30 to 3.0 μCi/kg, while the weanlings received 0.80 to 1.3 μCi/kg. Animals were killed at 1, 7, 14 and 28 days after injection and samples of femur, rib, vertebra and mandible were taken for both radioanalysis and microdosimetry.

Data from selected tissues are presented in Figure 1.4. The most notable difference in the distribution of plutonium between the two age groups was seen in the liver. The liver of the newborn contained only 3.6% of the administered dose compared to 14% for the weanling. During the next 27 days the ^{239}Pu content of the neonatal liver increased threefold to 11%. There was a slight increase to 18% in the Pu level in the weanling liver during the same period. In

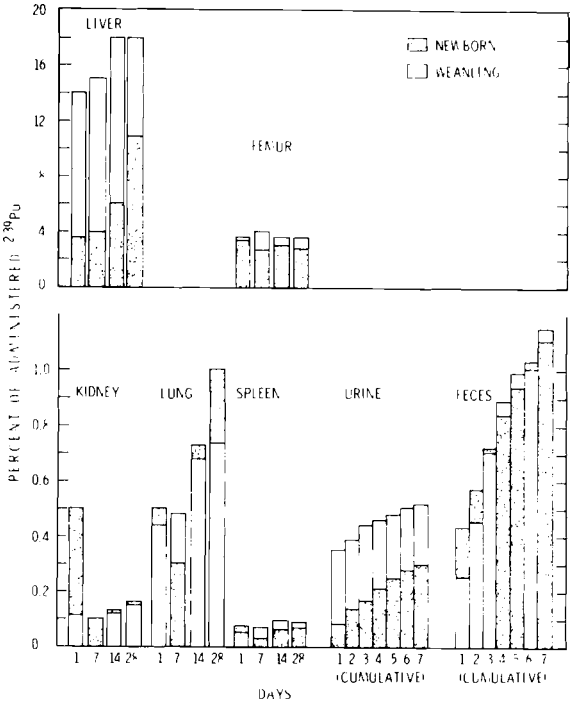

FIGURE 1.4. Distribution of ^{239}Pu in Newborn and Weanling Miniature Pigs

both age groups the largest fraction was found in the skeleton as indicated by the values for the femur. There appeared to be no difference in the skeletal deposition between the two age groups although the number of animals was too small to detect small differences. There also was no significant difference in the cumulative fecal and urinary excretion of the two groups of animals.

It may be speculated that the low initial deposition of plutonium in the newborn liver is a reflection of the immature state of this organ. However, other studies in this laboratory with the neonatal rat showed that plutonium levels in the liver decreased from 55% one day after injection to 45% twenty-nine days later while the femur increased from around 0.4% to 1.9% over the same time period. This is in sharp contrast to what we observed in the neonatal pig. We plan to study both the parenchymal and reticuloendothelial functions of pig liver relative to age to determine the precise physiological basis of the observed Pu deposition.

CROSS PLACENTAL STUDIES WITH ^{238}PU

Investigators:

M. R. Sikov, M. Dedrick, and D. D. Mahlum

Technical Assistance:

J. O. Hess and J. D. Stearns

The partition of ^{238}Pu among the elements of the fetoplacental unit of the rat was different from that for ^{239}Pu. The pattern of embryo mortality produced by injection at 9 days of gestation was also different for the two isotopes.

The distribution of heavy metals within the fetal placental unit (FPU) of the rat has been found to show a general pattern, although differences relating to the specific material, physico-chemical state and stage of gestation are apparent. Doses of ^{239}Pu as low as 3 µCi to the dam have a pronounced embryocidal effect when administered at 9 days of gestation, although 50 µCi is not lethal when given at 15 or 19 days. The present study with ^{238}Pu was undertaken to examine the importance of specific activity in determining the distribution and toxicity of plutonium in the prenatal rat.

Plutonium-238 citrate administered after 9 days of gestation resulted in a sigmoid relationship between dose and embryocidal action, measured at 14 days of gestation (Figure 1.5). The composite curve of the embryocidal action of ^{239}Pu, obtained concurrently and in earlier experiments, is also shown for comparison. Although there were a variety of technical differences between the experiments, it seems clear that the patterns of mortality produced are different.

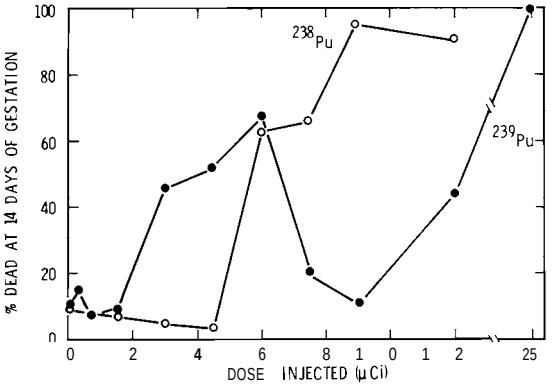

FIGURE 1.5. Embryocidal Effects of Plutonium in the Rat After Administration at 9 Days of Gestation

Neither isotope was teratogenic, although pathologic alterations were produced in the fetoplacental unit. These effects seem to relate to the microscopic distribution of the plutonium. After injection at 9 days of gestation, high levels of activity tended to concentrate along the yolk sac and resulted in a shrunken yolk sac that lacked the characteristic villus appearance. The embryos associated with these shrunken yolk sacs were usually moribund, showing necrosis and general loss of cellular integrity. At the lower lethal doses,

integrity of the placenta was maintained, but at higher doses the forming vascular channels were broken down and the infiltration of cell cores was markedly reduced. At later times of gestation (injection at 15 or 16 days) also, the yolk sac contained the predominant activity. Concentration in the maternal placenta was stage dependent, higher than in the fetal placenta after injection at 15 days while the reverse was true after injection at 19 days.

The concentration of ^{238}Pu and ^{239}Pu in the fetal membranes, placenta, and fetus after injection at 15 or 19 days of gestation are shown in Figure 1.6. Plutonium-239 is more concentrated in the placenta than is ^{238}Pu, while a greater percentage of ^{238}Pu enters the membranes and fetus. These differences suggest that the much smaller mass of ^{238}Pu, and consequent lesser tendency to polymerization, may allow it to pass more freely through the placenta and into the fetal membranes and fetus.

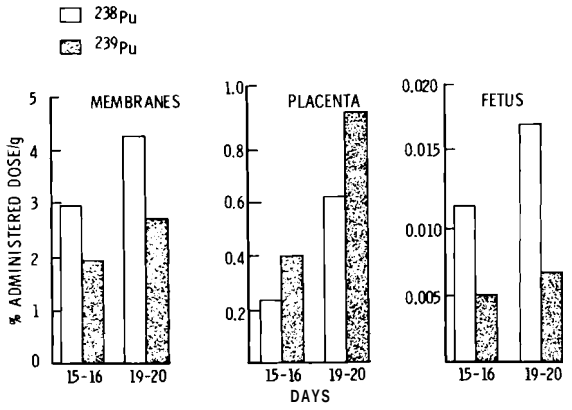

FIGURE 1.6. Concentration of Plutonium in the Fetoplacental Unit of the Rat Injected at 15 to 16 or 19 to 20 Days of Gestation

EFFECTS ON THE RAT EMBRYO OF COMBINED EXPOSURE TO ^{239}Pu AND TRYPAN BLUE

Investigators:

M. R, Sikov and D. D. Mahlum

Technical Assistance:

J. D. Stearns and J. O. Hess

Administration of 1.5 μCi of ^{239}Pu, which in itself was ineffective, to the pregnant rat at either 8 1/2 or 9 days of gestation increased the teratologic as well as the embryocidal effects of Trypan Blue injected at these times of gestation.

In previous studies we showed that injection of ^{239}Pu at 9 days of gestation is embryocidal, but not teratogenic. From the distribution of activity, it was inferred that plutonium interfered with the nutritive functions of the yolk sac. The azo dye, Trypan Blue, has been shown to be both embryocidal and teratogenic (Beck and Loyd, 1966). It appears to be incorporated into the yolk sac lysosomes and acts to disrupt the flow of nutrient to the embryo. Since it has been shown that plutonium localizes in the lysosomes of hepatocytes, it was of interest to determine wheather plutonium and Trypan Blue were acting on common structures or cells.

Eight experimental groups of four or five pregnant rats were injected intraperitoneally with 70 mg/kg Trypan Blue (no anesthesia) and/or intravenously with 1.5 μCi monomeric ^{239}Pu citrate (light ether anesthesia). The timing of these injections is indicated in Figure 1.7. Seven control rats were injected intravenously with a citrate solution (pH = 4), under light ether anesthesia, at 8 1/2 days of gestation. All animals were killed at 16 days of gestation, living and dead fetuses noted, and the former examined for gross anomalies. Some fetuses are being examined for internal malformations, while others are being analyzed with the remainder of the fetoplacental unit to determine whether Trypan Blue alters the distribution of plutonium.

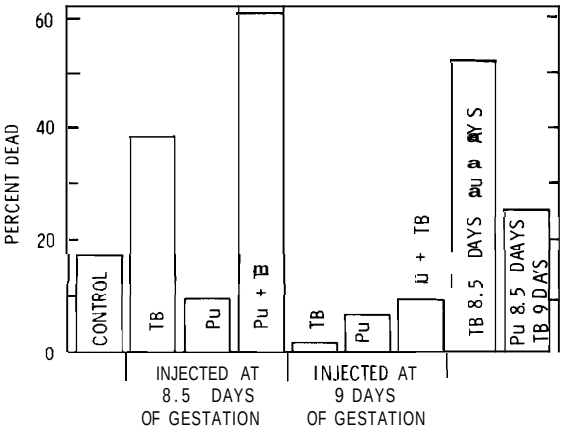

FIGURE 1.7. Embryocidal Effects of ^{239}Pu and Trypan Blue (TB) Injected Singly or in Combination at 8 1/2 or 9 Days of Gestation

Trypan Blue injected at 8 1/2 days increased prenatal mortality; as expected, the plutonium doses produced no such increase (Figure 1.7). Injection of both materials produced a greater lethal effect than either alone. Neither of these materials, separately or together, increased mortality above control level when administered after 9 days of gestation. When either material was given at 9 days, subsequent to administration of the other at 8 1/2 days, mortality was greater than when either was given alone at 8 1/2 or 9 days. Although the quantitative relationships are somewhat obscured by unusually high control mortality, the mortality seen with combined injection was always greater than the sum of the mortalities from individual injection.

No gross malformations were seen among control fetuses or fetuses exposed only to plutonium (Figure 1.8). The fetuses exposed to Trypan Blue had a moderate incidence of anomalies when injected at 8 1/2 days, but substantially less when injected at 9 days. Combined treatment at 8 1/2 days did not affect this incidence, but at 9 days it substantially increased the frequency of malformation. A similarly increased incidence was seen when plutonium preceded the Trypan Blue and the largest increase was seen with plutonium following Trypan Blue.

It appears that a non-lethal dose of ^{239}Pu acted synergistically with the embryocidal agent, Trypan Blue, suggesting that the two act through a common pathway. The dose of plutonium employed produced no teratisms, but increased the teratogenicity of Trypan Blue, further suggesting that they operate via a common pathway.

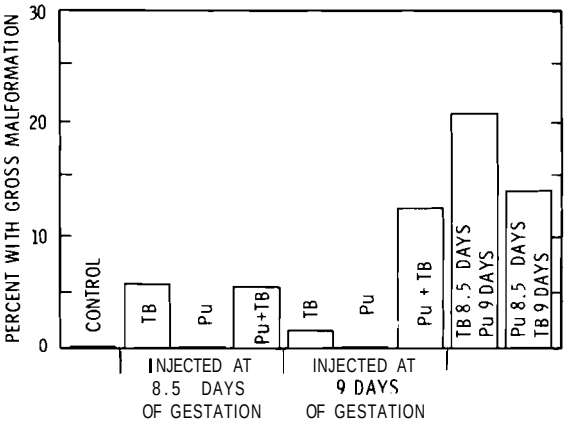

FIGURE 1.8. Teratologic Effects of ^{239}Pu and Trypan Blue (TB) Injected Singly or in Combination at 8 1/2 or 9 Days of Gestation

THE INFLUENCE OF BARBITURATES OR RESTRAINT ON PLUTONIUM-238 EXCRETION OR REMOVAL

Investigator:
M. F. Sullivan
Technical Assistance:
A. L. Crosby

Rats injected with ^{238}Pu and bile-duct-cannulated were subjected to restraint and daily treatment with a long-acting barbiturate, sodium phenobarbital. Restraint had no effect on either excretion or retention of plutonium. Phenobarbital increased bile volume and non-biliary excretion of plutonium. This resulted in decreased retention in both bone and liver, but the effect was not substantial

The bile is a major pathway for excretion of plutonium into the gastrointestinal tract; the monomeric form is excreted in greater amounts than the polymeric (1972 Annual Report). Astley and Sanders, in our 1972 Annual Report, presented data indicating that the barbiturate anesthetic used in cannulating the bile duct and the restraining cages used to hold the animals after cannulation drastically affected plutonium excretion and retention in the liver. Although the barbiturates are known to be liver enzyme inducers, we had not expected their action, nor the chronic restraint, to seriously alter the behavior of plutonium in animals with an external bile fistula.

To more fully reexamine the effect of barbiturates or restraint on plutonium metabolism, rats were bile-duct-cannulated under sodium pentobarbital (Nembutal) anesthesia and injected 1 day later with ^{238}Pu nitrate, intramuscularly. They were given 0.45% NaCl in their drinking water to replace electrolytes lost in the bile during the 5-dsy collection. Some of the rats were injected daily with the long-acting barbiturate, sodium phenobarbital (75 mg/kg). Some were treated with the chelating agent, calcium diethylenetriaminepentaacetic acid (DTPA), and other bile-duct-cannulated rats received both phenobarbital and DTPA. All animals were sacrificed 5 days after plutonium injection. Data from these studies are shown in Table 1.1. Data from Groups A and B indicate that cannulation and the surgical anesthesia used had little effect on either excretion or internal distribution of plutonium. Daily treatment with phenobarbital (Group C) resulted in a 25% increase in the volume of bile secreted, a threefold increase in non-biliary excretion of plutonium via the G.I. tract, and a twofold decrease in excretion via the bile.

Administration of DTPA (Groups D and E) resulted in the expected increase in urinary excretion of plutonium. Removal from both the skeleton and liver was improved by phenobarbital administration.

To evaluate the effect of restraint, rats were injected intravenously with ^{238}Pu and housed in metabolism cages (Group F) or in cylindrical restraining cages (Group G) like those used for bile collections. It is apparent from the data (Table 1.1) that restraint had no effect on either ^{238}Pu excretion or tissue distribution. The control rats injected intramuscularly with ^{238}Pu (Group A) were also housed in metabolism cages and their excretion and distribution patterns did not differ appreciably from the rats of Group B that were restrained and bile-duct-cannulated.

These data demonstrate quite conclusively that restraint does not alter ^{238}Pu excretion. Daily injections of phenobarbital had little effect on the excretion of ^{238}Pu by the bile-duct-cannulated rat.

TABLE 1.1. Plutonium Excretion and Tissue Distribution, 5 Days Following Injection, and After Various Combinations of Treatments. Values are mean percent of injected dose.

Group (number of rats)	A (3)	B (5)	C (4)	D (4)	E (3)	F (3)	G (3)
Treatment		Intramuscular ^{238}Pu(a)			Bile-	Intravenous ^{238}Pu(b)	
		Duct-Cannulated-Restrained				Restrained	
			Phenobarbital(c)	DTPA(d)			
Urine	2.5	2.5	2.3	44	41	2.8	2.6
Feces	1.9	0.9	2.8	2.0	2.4	2.6	2.7
Bile (5-day volume, ml)		0.8 (75)	0.3 (100)	0.8 (96)			
Skeleton	43	38	36	8.0	11	66	71
Liver	8.4	6.4	5.9	0.8	1.5	25	22
Injection Site	40	45	41	33	28		
Total Recovered	95	94	88	89	85	97	98

(a) Intramuscular Pu dose 5 μCi
(b) Intravenous Pu dose 10 μCi
(c) 75 mg/kg/d, for 5 days
(d) 0.05 mg/kg/d Ca DTPA for 5 days

ENHANCED PLUTONIUM ABSORPTION BY IRON-DEFICIENT MICE

Investigator:

H. A. Ragan

Technical Assistance:

D. H. Hunter and
M. C. Perkins

The intestinal absorption and retained body burden of plutonium citrate administered by gastric intubation was significantly greater in adolescent iron-deficient mice than in iron-replete mice.

Plutonium is reported to be avidly associated with the physiologic iron-binding compounds transferrin, ferritin and hemosiderin. Because of this relationship, it is conceivable that gastrointestinal absorption of plutonium might be influenced by tissue-iron status.

Two groups of ICR white mice were fed either an iron-deficient or iron-replete diet between 11 and 40 days of age. Mice on the iron-deficient diet developed a moderately severe microcytic, hypochromic anemia. Animals of both groups were then given 15 μCi of ^{239}Pu citrate by gastric intubation and half of the mice in each group were killed either 24 or 96 hr later.

The percentage of administered plutonium retained in various tissues 24 and 96 hr after gavage is shown in Figure 1.9. The mean plutonium content of soft tissue and bone was higher in iron-deficient mice 24 hr after gavage, although levels in the spleen and kidney were not statistically different between the two groups because of the large individual variations. By 96 hr, the soft tissue content of plutonium in the iron-deficient mice had decreased rather markedly and bone levels had increased, suggesting translocation from soft tissue to bone. In the control group at 96 hr spleen, kidney and blood plutonium content had decreased, the liver plutonium level had increased, and bone content was essentially unchanged, which would indicate some translocation to liver and possibly an increased excretion.

Total absorption as measured after 24 hr was 0.108 ± 0.021% (SEM) in iron-deficient mice and 0.028 ± 0.007% in iron-replete mice. Corresponding values measured after 96 hr were 0.117 ± 0.021% and 0.024 ± 0.005%. Total body burden of plutonium as measured at both time periods was significantly greater ($P < 0.01$) in the iron-deficient mice.

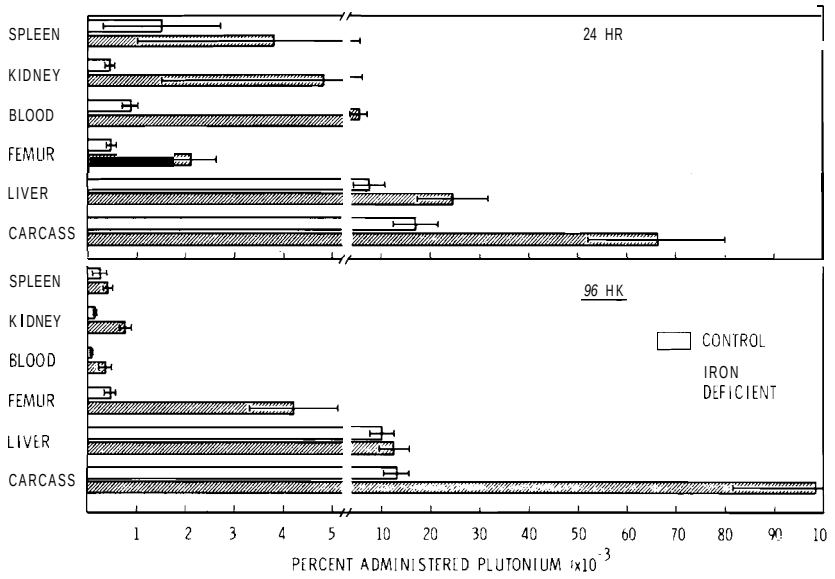

FIGURE 1.9. Percent of Administered ^{239}Pu in Selected Tissues 24 and 96 Hr After Gavage in Iron-Deficient and Iron-Replete Mice

Because of the four-to fivefold increase in plutonium absorption observed in the iron-deficient mice and the prevalence of iron deficiency in certain segments of the human population, further evaluation of these interrelationships is needed. Also, as additional studies of the absorption, subcellular distribution, and plasma binding of plutonium and other radionuclides are conducted, they may ultimately provide information regarding the absorption of iron itself.

ABSORPTION OF CURIUM-244 AND CALIFORNIUM-252 FROM THE GASTROINTESTINAL TRACT OF NEWBORN AND ADULT RATS

Investigator:

M. F. Sullivan

Technical Assistance:

A. L. Crosby

Based on urinary excretion and retention in liver and bone following gavage with $^{244}Cm(NO_3)_3$, $^{244}Cm_2O_3$ or $^{252}Cf(NO_3)_3$, absorption of these compounds from the gastrointestinal tract of adult rats was 0.15, 0.12 and 0.11%, respectively. Gastrointestinal absorption by newborn rats after ^{244}Cm or ^{252}Cf nitrate gavage was 6.3 and 4.4%.

The gastrointestinal absorption of plutonium is much greater in the newborn rat than in the adult. The gastrointestinal absorption of transplutonium elements in adult rats has been shown to be generally greater than plutonium. Therefore, we were interested in comparing the absorption of transplutonium elements in the adult and newborn.

We report here preliminary results from studies with $^{244}Cm(NO_3)_3$, $^{244}Cm_2O_3$ and $^{252}Cf(NO_3)_3$ in neonatal and adult rats. "Soluble" preparations of Cm and Cf were made by diluting a nitric acid solution of the nuclide and adjusting the pH to 3. The $^{244}Cm_2O_3$ employed had been suspended in water for 4 months prior to being mixed with 5% carboxymethylcellulose and administered by gavage. Two-day-old rats were administered 2 μCi in 0.1 ml; adults, 5 μCi in 0.5 ml by stomach tube. Excreta was collected daily for 1 week from the adults but not from the neonates. All animals were sacrificed 7 days after gavage for analysis of tissues.

Excretion and distribution data are shown in Table 1.2. About the same quantity of curium appeared in the urine of adult rats following administration of either the "soluble" nitrate or the "insoluble" oxide. Judging from studies with other high specific activity alpha emitters, it seems likely that the suspension of the $^{244}Cm_2O_3$ for 4 months in water resulted in considerable solubilization, due presumably to radiolytic processes.

Differences were observed, however, in the internal distribution following administration of curium nitrate or oxide. The ratio of skeletal-to-liver deposition was much greater for the nitrate than for the oxide. Summing urinary excretion, bone, and liver retention, total absorption from the gut was 0.12% for the oxide and 0.15% for the nitrate.

Excretion of ^{252}Cf nitrate in the urine of adults was similar to that observed with curium, but much smaller amounts were retained in skeleton and liver. The indication

TABLE 1.2. Distribution of Radioactivity in Adult and Neonatal Rats Given $^{244}Cm(NO_3)_3$, $^{244}Cm_2O_3$, or $^{252}Cf(NO_3)_3$ by Gavage

Tissue or Excreta	Percent of Administered Dose (Average Values for at Least Six Rats)				
	$^{244}Cm(NO_3)_3$		$^{244}Cm_2O_3$	$^{252}Cf(NO_3)_3$	
	Adult	Neonate	Adult	Adult[a]	Neonate
Liver	0.005	0.16	0.02	0.003	0.18
Skeleton[b]	0.07	1.8	0.03	0.002	3.1
Carcass[c]		6.2			4.1
Lungs				0.0	0.006
Skin					0.15
Urine	0.07		0.07	0.1	
Feces	95.8		91.7	94.4	
Total Absorbed	0.15	6.3	0.12	0.11	4.3
Total Recovered	96	6.3	91.8	94.5	4.5

(a) No significant radioactivity was found in the intestine, kidney, spleen, blood, or muscle
(b) Adult skeletal content = single femur content x 23, neonate skeletal content calculated from an analyzed weighed femur on the assumption that total skeleton was 22% of total body weight
(c) Includes skeleton

that about 95% of the absorbed californium was reexcreted in the urine is a surprising observation which requires confirmation.

Absorption from the gastrointestinal tract of newborn rats was particularly reflected in the amount of ^{244}Cm or ^{252}Cf retained by the skeleton. There was also an indication of considerable extraskeletal deposition, particularly in the case of ^{244}Cm, however, there is considerable uncertainty in the extrapolation of total skeletal content from a single femur analysis in the newborn. The relatively low values for lung and skin would seem to rule out the possibility that high carcass values were due to injection into the lungs or to contamination of the skin from excreta. The indicated total absorption of 6.3% for curium and 4.3% for californium are minimum estimates since no account was taken of absorbed radionuclide that may have been reexcreted.

These data show that the gastrointestinal absorption of ^{244}Cm and ^{252}Cf by the adult rat is about 50 times greater than the absorption of ^{239}Pu under similar conditions. As in the case with plutonium, absorption of curium and californium by the newborn is about 50 times greater than is observed for the adult.

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

Investigators:

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

Technical Assistance:

J. O. Hess and J. D. Stearns

Adult Wistar rats, injected with 2.0 μCi/kg or more of ^{253}Es, had shortened median life-spans. A lesser effect of ^{253}Es on life-span was seen in adult Fischer rats.

In previous Annual Reports we have noted differences in the response of adult and weanling Wistar rats to ^{253}Es as measured by early mortality, impaired dentition, and tumor development. In this report we will consider further age-related differences in mortality and tumor development, including data from both Wistar and Fischer rats.

In Experiment 1 male adult and male and female weanling rats of the Wistar line were injected intravenously with ^{253}Es as the chloride (pH 2) at doses ranging from 0.4 to 50 μCi/kg. In Experiment 2 adult, male, Wistar rats were injected with 0.1, 0.4, 2.0, or 5.0 μCi/kg of ^{253}Es as the chloride (pH 2) while Fischer rats of both sexes received 0.4, 2.0, or 5.0 μCi/kg One group of Wistar rats received 2.0 μCi/kg as the citrate for comparison of chemical form; another group was subjected to removal of approximately one-half of their blood on two successive days, 28 days after injection of 2.0 μCi/kg.

The median life-spans of these groups of rats are shown in Figure 1.10. In Experiment 1, animals injected as adults showed shorter survival times than animals injected with a comparable dose as weanlings. The decrement in survival of the weanling females was slightly greater than for the weanling males. Survival times for the Wistar rats in Experiment 2 were similar to those observed for comparable dose groups in Experiment 1. The value for the 5 μCi/kg group was intermediate between that for the 2 μCi groups and that found previously

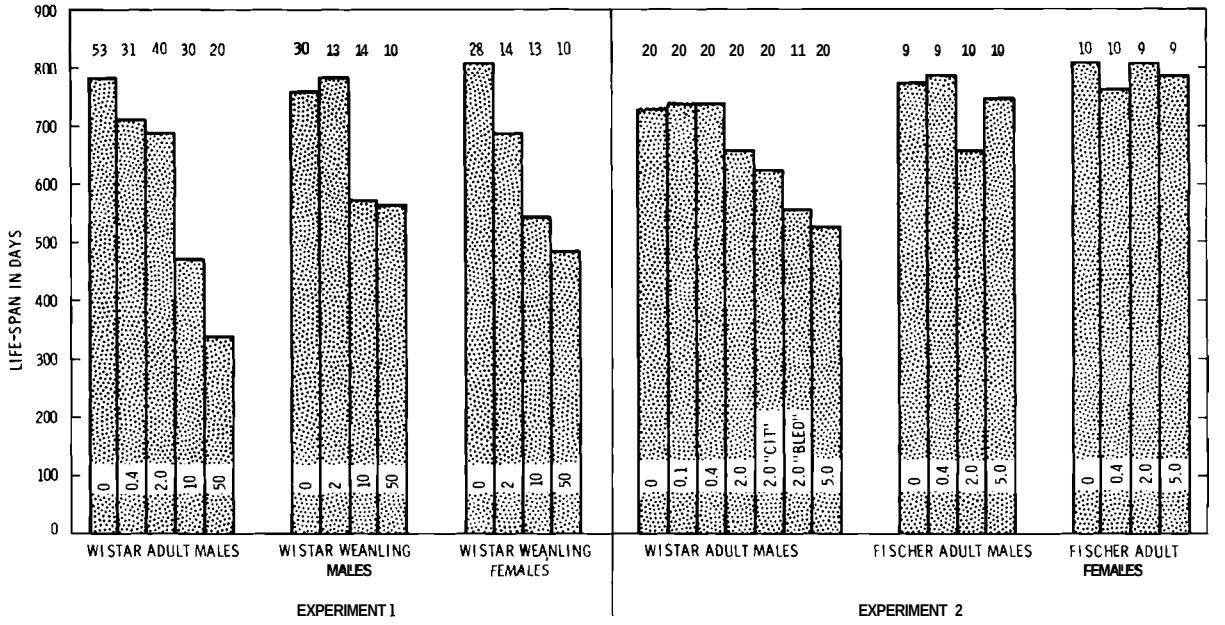

FIGURE 1.10. Median Life-Spans for Rats Injected with ^{253}Es as Adults or Weanlings. [Dose levels (µCi/kg) shown on bar, number of animals shown above bar.]

for the 10 µCi group. The survival of the "bled" 2 µCi/kg group was similar to that of the 5 µCi/kg animals. There was no effect of ^{253}Es on the median life-span of the Fischer females. There was a decrease of about 100 days in the 2 µCi/kg Fischer males, but since no effect was seen at the 5 µCi/kg level, this decrement may be a chance result due to the small number of animals employed. Radiation doses to the femur were estimated for the first 30 days after injection; approximately 65 and 85% of the total dose was delivered during this period for the adult and weanling, respectively. The estimated values were 34 rads for an adult injected with 1 µCi/kg and 24 rads for a weanling receiving the same administered dose.

Microscopic examination of tissues has not been undertaken at this time, but gross observations suggest that the bone tumor incidence is increased in adult, Wistar males that received 2, 5, or 10 µCi/kg and in weanling males that received 2, 10, 50 and 100 µCi/kg. Although the numbers were small, the incidence of bone tumors appears to be elevated in Fischer rats injected with 2 or 5 µCi/kg. The incidence of bone tumors does not appear to exceed approximately 40% for any group. Other pathologic lesions have been found including an increased incidence of hind-limb paralysis in Wistar, adult rats that received 0.4, 2, or 5 µCi/kg, and of enlarged spleens in Fischer rats that received 2 µCi/kg.

BLOOD AND BONE MARROW EFFECTS OF ^{253}Es IN MINIATURE SWINE

Investigator:

H. A. Ragan

Technical Assistance:

D. H. Hunter, M. C. Perkins and G. S. Vogt

Miniature swine given 3 μCi/kg of ^{253}Es citrate intravenously at about 6 weeks old developed an absolute neutropenia and thrombocytopenia of about 3 month's duration. There was a marked depression of the myeloid to erythroid cell ratio of bone marrow during the same 3-month period. The normoblastic population was also affected, but this was not reflected in circulating red cell concentrations.

The purpose of this study was to compare the metabolic and toxic effects of ^{253}Es in a large mammal to those observed during concurrent studies in rodents. Twenty-two miniature swine, approximately 6 weeks of age, were injected intravenously with 3 μCi/kg of ^{253}Es citrate. Urinary and fecal excretion of ^{253}Es were determined, and 10 animals were killed at intervals following injection for measurement of einsteinium tissue distribution and retention. These data were presented in last year's Annual Report. The remaining 12 animals are being maintained for observation of late effects, since hematopoietic and bone neoplasms were observed in rodents exposed to ^{253}Es.

The effects of einsteinium on peripheral blood neutrophils and lymphocytes are shown in Figure 1.11. The decrease in circulating leukocytes after einsteinium administration is due to an absolute depression in segmented and band neutrophils. This effect is evident by day 4 following injection and maximal by day 10. A progressive recovery in neutrophil values then follows, and control and experimental values are indistinguishable by day 100. Mean monocyte and eosinophil values of einsteinium-injected pigs were also maximally depressed by day 10 at about 25% of preinjection levels.

Platelet values in miniature swine normally fall rapidly for several months after weaning, thus it is difficult to assess the effects of treatment during this period. However, at 25 days postinjection mean platelet values from einsteinium pigs were only 56% of controls and remained lower until about 100 days postinjection. There was no apparent effect of einsteinium treatment on the volume of packed red cells or erythrocyte concentrations.

The effects of einsteinium on the myeloid to erythroid (M/E) ratios

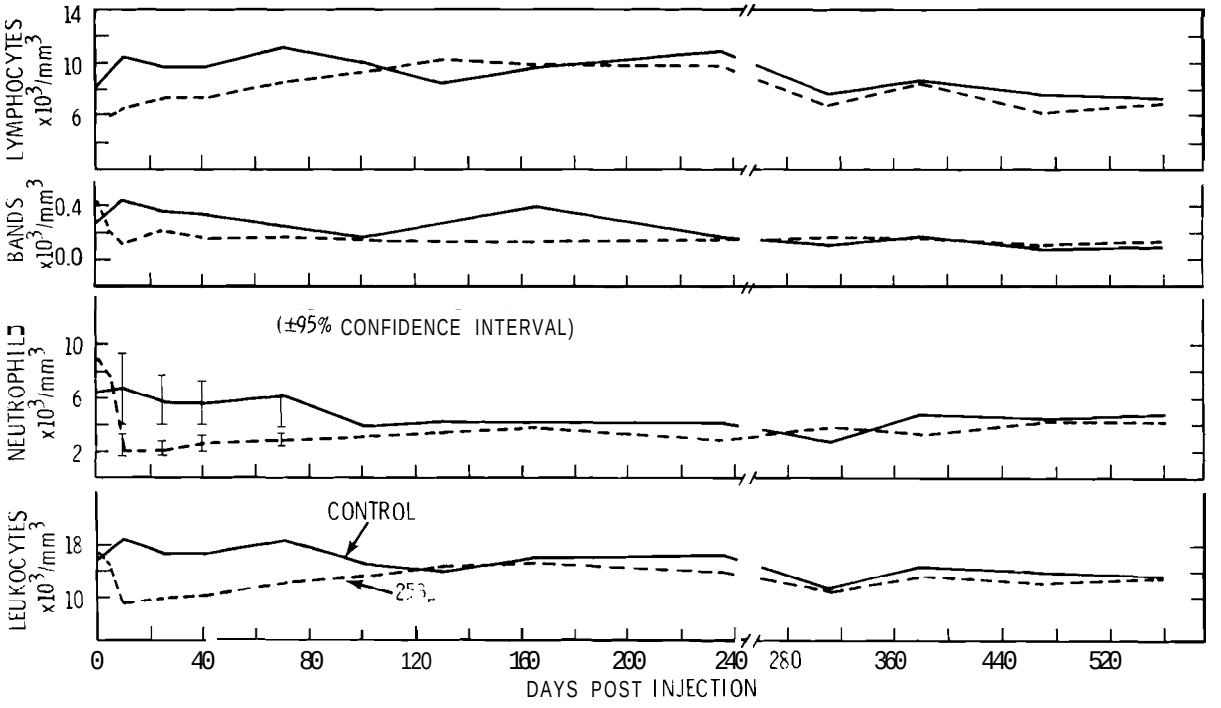

FIGURE 1.11. Effects of Intravenous ^{253}Es Citrate on Peripheral Blood of Miniature Swine

and on mitotic indices in bone marrow at various times after injection are shown in Figure 1.12. The depressed peripheral blood granulocyte values are a result of einsteinium damage to the bone marrow myeloid series as evidenced by the pronounced shift in the M/E ratios. Although not reflected in the peripheral blood or by the M/E ratios, the erythroid series has also been damaged, as indicated by an increase in necrobiotic cells, nuclear abnormalities and naked red cell nuclei. By 102 days postinjection there was no significant difference in the M/E ratio between control and treated animals. However, at two subsequent time periods, 230 and 560 days postinjection, the M/E ratios in einsteinium pigs were significantly less than in controls.

The consistently elevated bone marrow mitotic indices (Figure 1.12) are of particular interest and not readily explained; they may subsequently be of pathogenetic interest if hematopoietic neoplasms develop. It appears that there must be some residual effects of einsteinium irradiation on both erythrocyte and granulocyte precursor cells. Perhaps a clone of defective cells was produced by the initial radiation result and these cells have a shortened half-time in the circulating blood.

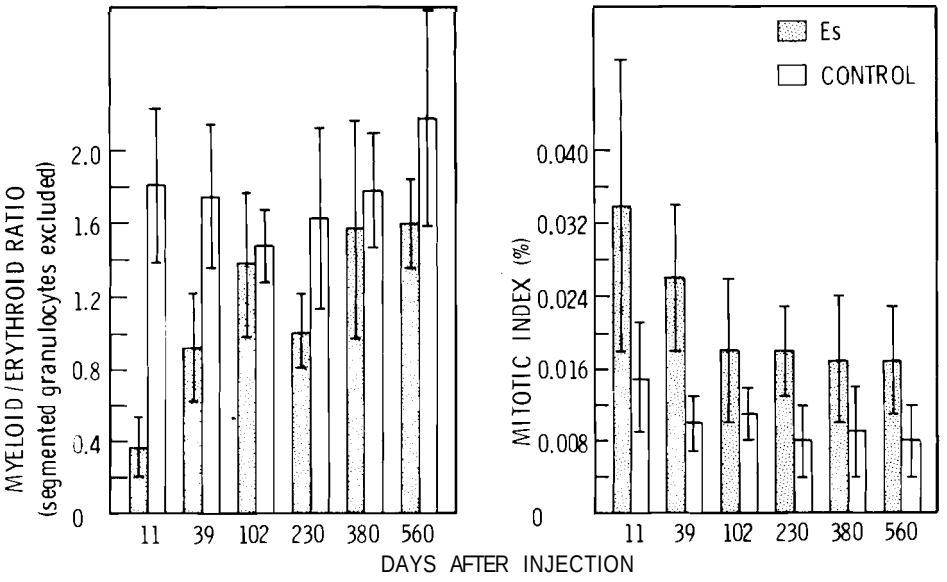

FIGURE 1.12. Bone Marrow Myeloid/Erythroid Ratios and Mitotic Indices of ^{253}Es-Injected and Control Miniature Swine (Mean Values ± S.D.)

SERUM CONSTITUENTS IN ^{253}Es-INJECTED SWINE

Investigator:

P. L. Hackett

Technical Assistance:

E. T. Edmerson

No significant differences were observed between mean values for control and ^{253}Es-injected swine during a period of 19 months following injection of 3 μCi/kg at age 2 months.

Serum consti'tuents in 12 swine injected with 3 μCi ^{253}Es/kg at 2 months of age and 4 control animals have been studied for 19 months. Analyses were made for urea nitrogen, creatinine, glucose and protein levels as well as for alkaline phosphatase, lactic dehydrogenase (LDH), glutamic-oxalacetic-transaminase (SGOT), and glutamic-pyruvic-transaminase (SGPT).

Changes in mean serum values were limited to aging effects; no significant differences between the control and experimental animals were

observed. Protein levels in both groups increased after the fifth month, largely due to increased albumin values; however, after the eleventh month albumin levels decreased and gamma globulins began an appreciable increase (Figure 1.13). During the first 5 experimental months alkaline phosphatase activities declined from 8 to 9 units to 2 units and remained at this level for the rest of the sampling period.

Some recent changes in LDH values and isoenzyme patterns have occurred in both control and experimental swine; however, individual increases in total LDH activity and LDH-5 fraction could not be correlated with hematologic parameters and apparently indicate sporadic muscle or liver damage.

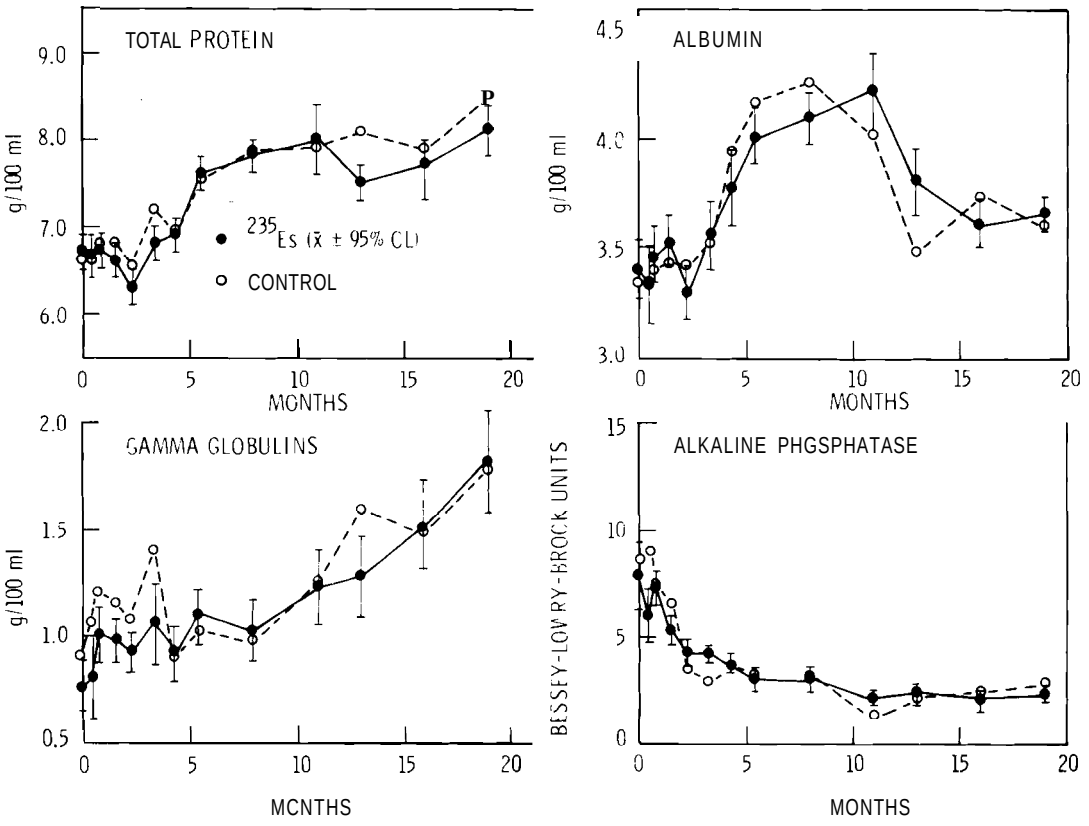

FIGURE 1.13. Serum Constituents in ^{253}Es-Injected Swine

EFFECTS OF ^{90}Sr INGESTION IN MINIATURE SWINE

Investigators:

H. A. Ragan and B. J. McClanahan

Technical Assistance:

D. H. Hunter, M. C. Perkins and G. S. Vogt

Dental lesions, uterine pathology and arthritis continue to be the major causes of death in aged swine fed ^{90}Sr for their lifetime. F_1 generation swine at the 250 µCi/day feeding level showed some recovery in peripheral blood neutrophil and platelet values when removed from ^{90}Sr feeding at 200 days of age; lymphocyte concentrations remained below those of control swine. Leukokinetic studies failed to show differences in the half-life of tagged neutrophils of control or 125 µCi/day swine, except for one ^{90}Sr animal that had extensive myeloid metaplasia.

Progress reports over the past 16 years have detailed the experimental design and objectives of these studies into the effects of chronic ^{90}Sr ingestion in miniature swine. Over 700 pigs, representing three generations, have been exposed by feeding 1, 5, 25, 125, 625, or 3100 µCi ^{90}Sr/day, and an additional 200 animals have served as age-related controls. From the original chronic toxicity study, 44 animals are alive; 18 controls, 18 fed 1 µCi/day, 6 fed 5 µCi/day, and 2 fed 25 µCi/day. Because myeloid neoplasms were observed in the 125 µCi/day group, this feeding level was re-initiated after the animals in the original study had died. Seven animals currently are alive in this group. In an attempt to shorten the induction time for hematopoietic tumors, a group of F_1 generation animals was produced at the 250 µCi ^{90}Sr/day feeding level; seven animals remain in this group.

The animals remaining from the original toxicity study vary in age from 10 to 15 years. In these older swine, uterine pathology, arthritis, and dental defects continue to be the major factors contributing to death (Table 1.3). The most common uterine lesion is leiomyoma, followed by varying degrees of cystic endometritis. Degenerative arthritis is common, but rarely causes severe incapacitation. Ankylosing spondylitis is present to some degree in all old swine and frequently becomes very severe, particularly in the distal thoracic area. Dental lesions consist of perialveolar degeneration with subsequent suppuration, and wearing of the occlusal surfaces to the gum line, resulting in loss of masticatory ability. Uterine pathology and arthritis do not appear to be related to the ^{90}Sr feeding level, but this conclusion is tentative until statistical evaluations are completed. Dental

TABLE 1.3. Deaths in Chronic ^{90}Sr Study During 1973

µCi/Day	Sumber	Age Years	Type[a] Death	Dental Lesions	Arthritis	Uterine Pathology	Other
0	223	14.3	E	++	+++	++++	Peritonitis
	512	13.0	E	++	+++	++++	Peritonitis
	770	12.7	D	++	+++	+	Hepatitis
	778	12.3	E	++++	+++	++	Posterior Paralysis
	1214	10.6	D	++++	++++	++++	Hepatitis
	1270	11.0	D	+	+++	++++	
	1276	10.9	D	--	--	++++	
	1279	10.3	D	++++	--	+++	
	1745	8.7	D	++	++	+++	Peritonitis
	1746	9.0	E	+++	+++	++	Posterior Paralysis
1	301	13.8	D	+++	--	+	Peritonitis
	714	12.3	E	++	++++	++	Hepatomas
	842	12.0	E	+++	+++	++++	Peritonitis
	1038	11.2	E	++++	++	+	Posterior Paralysis
5	465	12.9	E	++++	++	++++	Peritonitis
	808	12.5	E	++++	+++	++++	
25	485	13.8	E				
125	3498	4.2	E	++	--	+	Thrombocytopenia
	3548	4.3	D	--	--	+	Thrombocytopenia
	3552	4.4	D	+	--	Male	Pneumonia
	3573	4.1	E	++	--	+	Anemia
	3578	4.3	E	++	--	--	Anemia
	3606	4.7	D	++	+	--	Anemia
	3987	3.2	E	++	--	--	Chronic Enteritis
	4004	3.4	E				Anemia
250	4132[b]	2.8	E	+++	--	Male	Anemia
	4086[b]	3.6	E	+++	--	--	Anemia
	4136[b]	3.3	D	+	+	--	Peritonitis

(a) E = Euthanasia
D = Died
(b) Removed from ^{90}Sr feeding at 210 days of age

lesions appear more commonly and are more severe in swine ingesting 25 µCi ^{90}Sr/day than in swine at lower feeding levels or in control swine. Strontium-90 analyses on femurs from animals 3000 to 4000 days of age in the 1, 5, 25, and 125 µCi groups tend to be slightly lower than would be predicted from extrapolation of earlier data. However, it does not appear that these differences are statistically significant.

The F_1 generation 250 µCi/day group is of particular interest. With the exception of two animals, ^{90}Sr feeding was discontinued at 200 days of age because 6 of the 21 original animals had died prior to this time from bone marrow depression. One of the animals that continued to

receive ^{90}Sr daily lived for 934 days before developing a myeloproliferative disease; the other lived to 1017 days of age and died of bone marrow hypoplasia. Of 13 pigs removed from ^{9} ' ~feeding, six are dead; two from myeloproliferative disease at 555 and 1330 days of age; three from severe bone marrow depression at 215, 310, or 1210 days; and one because of a traumatic, comminuted, tibial fracture.

The effects of this ^{90}Sr feeding level on peripheral blood values are shown in Figure 1.14. Neutrophils, lymphocytes and platelets were markedly depressed within a few weeks following initiation of ^{90}Sr feeding after weaning at 6 weeks of age. After cessation of ^{90}Sr feeding at 200 days of age, platelet values showed a rather rapid recovery although the mean values remained less than those observed in control pigs. Segmented neutrophils also showed recovery after ^{90}Sr feeding was stopped, but one of the animals continued on ^{90}Sr had a similar recovery pattern. Of particular interest are the lymphocyte concentrations which showed little evidence of recovery after ^{90}Sr feeding was stopped although the mean values did not fall as low as those in either of the pigs that continued on ^{90}Sr.

Femurs of animals from the 250 µCi/day group were analyzed for ^{90}Sr at the time of death (Figure 1.15). Following cessation of ^{90}Sr feeding there was a gradual decline in skeletal ^{90}Sr to a level one-fourth that in the two animals continued on ^{90}Sr feeding. The highest femur concentration was reached at about 150 days of age and had already begun to decline before the animals were removed from ^{90}Sr feeding. This peak concentration was nearly four times that found in the two animals who remained on ^{90}Sr feed until they died at 934 and 1017 days of age.

Since chronic ^{90}Sr feeding results in both a depression in neutrophil values and an increased incidence of myeloproliferative disorders, we were interested in the life-span of peripheral blood leukocytes. Leukocytes from five 125-µCi/day F_1 swine approximately 4 years of age and leukocytes from three age-related controls, were labeled with diisopropylfluorophosphate ($DF^{32}P$) and the clearance pattern determined. Two distinct clearance patterns were observed in the controls and in four of the ^{90}Sr fed animals (Figure 1.16); one pattern showed a single exponential clearance with a half-time of about 8 hr, the other pattern showed a rapid initial half-time of about 2 hr followed by a slower component with a half-time in excess of 10 hr. One of the ^{90}Sr animals (3548) showed a prolonged clearance time. This animal was suspected of being in the early stages of acute myelogenous leukemia 16 weeks prior to the leukokinetic study, and was euthanized 10 days after completion of the study because of a rapidly deteriorating hematologic condition. Blood, bone marrow, and tissue impression smears obtained at necropsy established a diagnosis of myeloid metaplasia rather than myelogenous leukemia.

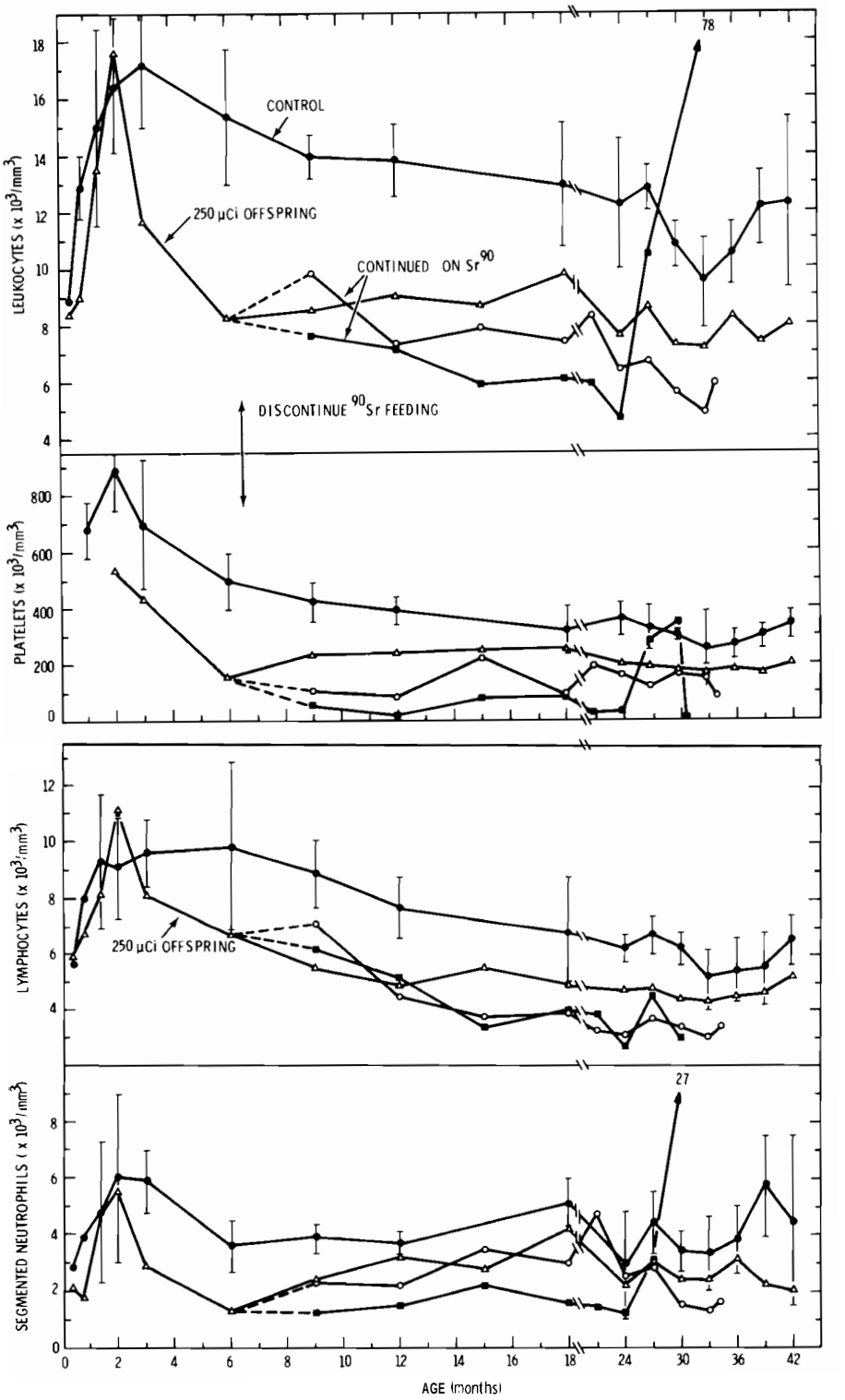

FIGURE 1.14. Peripheral Blood Values in Miniature Swine Ingesting 250 µCi ^{90}Sr Daily (Mean Values ±95% Conf. Int. About Control Means)

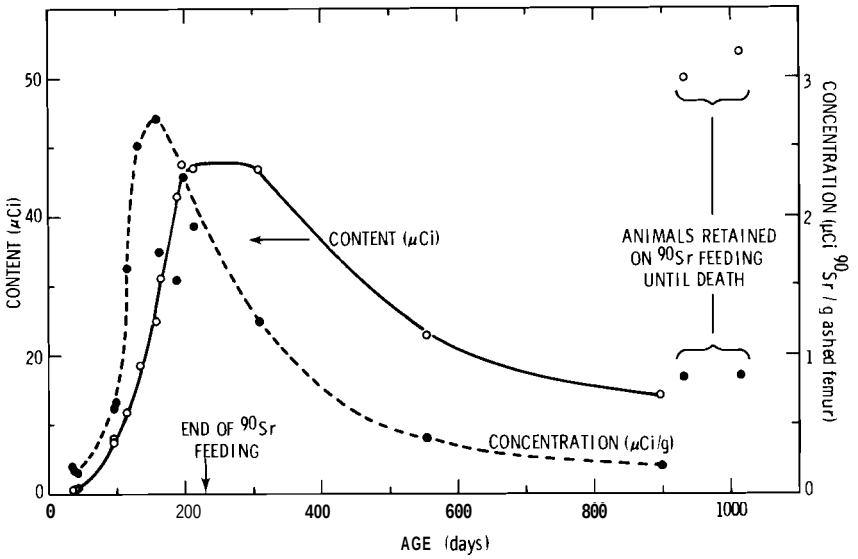

FIGURE 1.15. ^{90}Sr in Femur of Miniature Swine Fed 250 μCi Daily

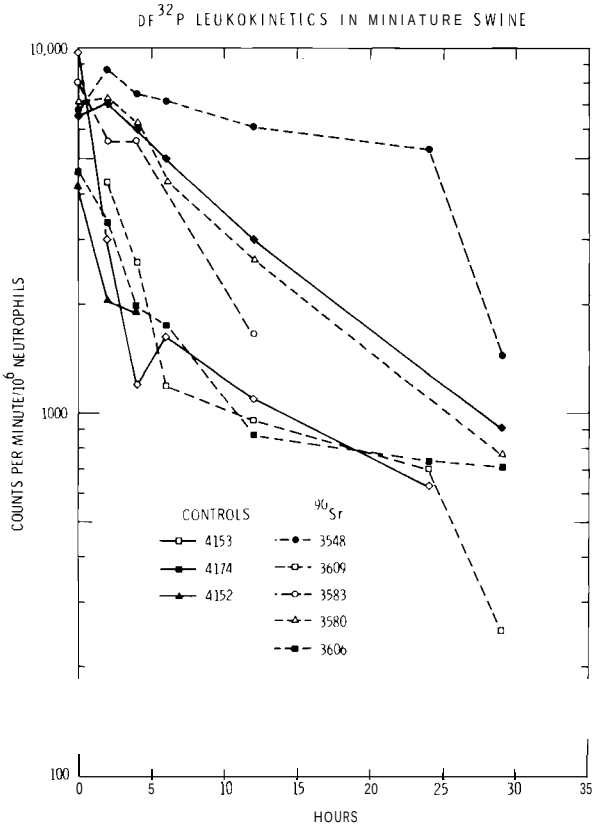

FIGURE 1.16. DF ^{32}P Leukokinetics in Controls and in Miniature Swine Ingesting 125 μCi ^{90}Sr Daily

Platelet adhesiveness was determined in some of the miniature swine from the 125 μCi/day feeding level. This study used Salzman's method of drawing blood, prior to anticoagulant exposure, over a uniform column of glass beads into EDTA, and comparing the platelet count with that obtained from blood collected directly into EDTA. The mean platelet retention obtained from three control swine was 83% with a range of 80 to 87% (Table 1.4). ^{90}Sr-fed swine appeared to show either a normal platelet adhesiveness or a complete failure of platelets to adhere to the glass-bead column. Four had a mean of 77% (range 63 to 92%), and five had 0% retention. The ability of platelets to adhere did not correlate entirely with the degree of hemorrhage noted at necropsy. All animals that failed to manifest platelet retention had control platelet counts $<100 \times 10^3/mm^3$. Platelet retention may also be related to the

TABLE 1.4. Platelet Adhesiveness and Volume of Packed Red Cells in Control and 125 µCi ^{90}Sr/Day Miniature Swine

Animal Number	^{90}Sr (µCi/day)	Platelet Count ($X10^3/mm^3$)	Platelet Retention (%)	Volume of Packed Red Cells (ml/100 ml)	Hemorrhage At Necropsy
961	0	402	80	37.4	
4041	0	255	82	48.7	alive
4095	0	205	87	45.6	alive
3487	125	296	92	48.6	alive
3548	125	235	66	48.4	++
3987	125	220	86	40.3	
4007	125	945	63	27.6	+
3498	125	2	0	51.8	+++
3573	125	61	0	16.1	++
3578	125	38	0	10.3	+
3998	125	6	0	19.2	++
4054	125	96	0	14.9	

degree of anemia, in which case it would be a function of the passage time over the column of glass beads. Regardless of the explanation, the results of this study suggest that the extravasation of blood frequently noted in swine at the 125 µCi/day feeding level is not due to an intrinsic platelet defect as determined by this adhesiveness assay.

BIOCHEMICAL PARAMETERS IN ^{90}Sr-FED SWINE

Investigator:
P. L. Hackett
Technical Assistance:
E. T. Edmerson

Lowered serum globulin levels are observed in offspring of swine fed 250 µCi ^{90}Sr per day.

The F_1 offspring of sows fed 250 µCi ^{90}Sr/day were maintained on a ^{90}Sr feeding regimen until 6 months old. Serum constituents of these animals were examined periodically from 1 to 42 months of age.

Changes in serum constituents with age, in both experimental and

control animals, include decreasing levels of inorganic phosphorus, alkaline phosphatase and lactic dehydrogenase. An early decrease in serum cholesterol and a moderate lowering of glutamic-pyruvic transaminase activity was also observed. Creatinine and protein values, especially gamma globulins, increased, and glutamic-oxalacetic transaminase levels were slightly higher in older animals.

Differences between experimental and control animals were observed in serum protein fractions (Figure 1.17). Total protein values in the two groups were similar, except from 9 to 15 months of age; however, gamma globulins showed a lower trend in the ^{90}Sr-fed swine until they were 36 months old. Beta globulins were also somewhat lower while albumin levels were slightly higher. Globulin patterns similar to those of the group mean were observed in two experimental animals that died at 40 and 43 months of age from an acute leukemoid reaction and leukemia. The investigation of immunoglobulin levels in specific animals of this group has been initiated.

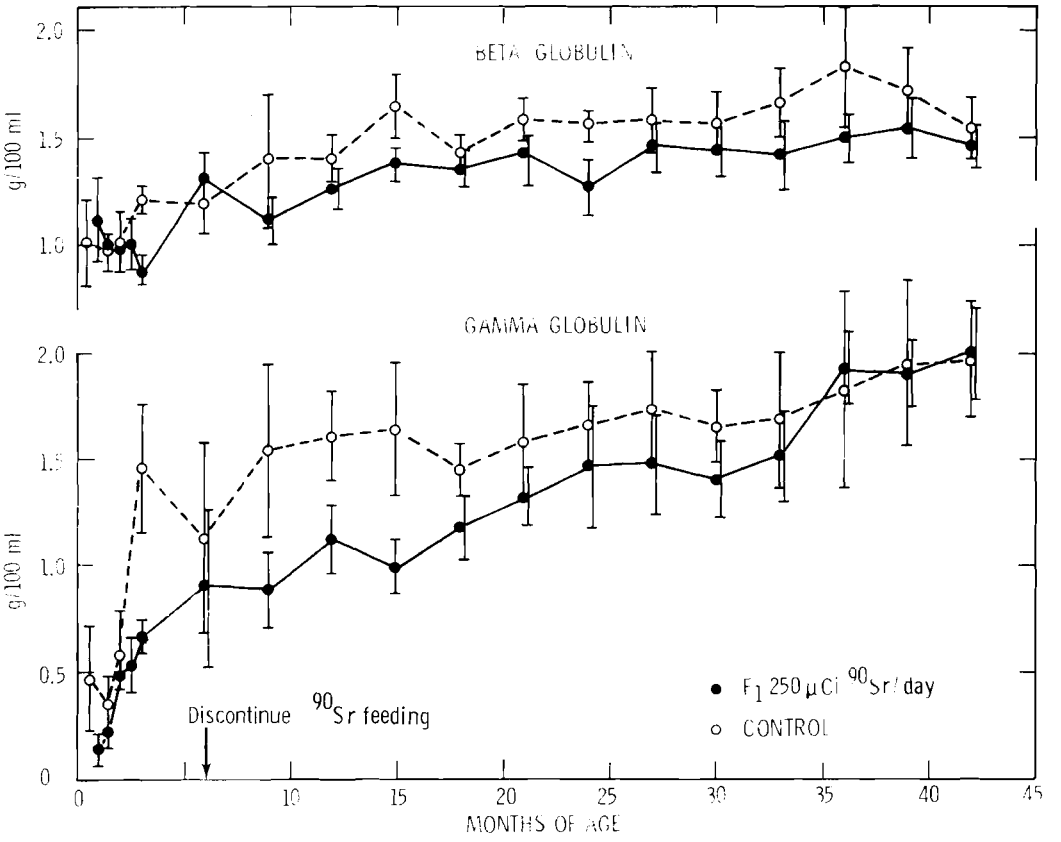

FIGURE 1.17. Changes in Serum Globulins in Offspring of ^{90}Sr-Fed Swine.

ISOENZYMES IN POSTNATAL SWINE

Investigator:
P. L. Hackett
Technical Assistance:
E. T. Edmerson

Isoenzyme values for tissue and serum lactic dehydrogenase changed markedly during the first postnatal month, but alkaline phosphatase levels differed only in total enzyme activity.

Studies of lactic dehydrogenase (LDH) and alkaline phosphatase (AP) isoenzymes in control animals were initiated during the first postnatal month to obtain background data for radiotoxicity studies in neonatal swine. Lactic dehydrogenase isoenzymes were determined by electrophoretic separation and AP isoenzymes by chemical inhibition with urea and L-phenylalanine (LPA) in serum and saline tissue extracts.

Determinations of LDB serum enzymes in groups of 2-day-old, 6-week-old, and adult swine show that total LDH values are higher in the young animals and that the predominant isoenzymes shift from fractions with intermediate mobility (LDH 2, 3, and 4) to the fastest moving component, LDH-1, in the adult (Figure 1.18). Individual values in the young animals were more variable than those in the adult. At least part of this variability appears to be due to the degree of development of the neonate, in that the percentage of LDH-2 is inversely proportional to body weight (Figure 1.19). At necropsy one of the animals was found to have an intestinal obstruction and the values for LDH-2 did not correlate with body weight.

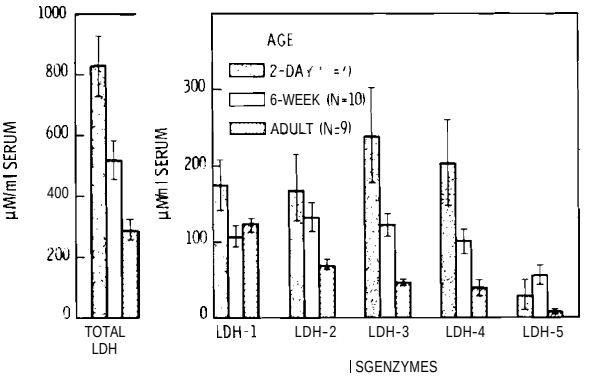

FIGURE 1.18. Serum Lactic Dehydrogenase Values in Young and Adult Swine

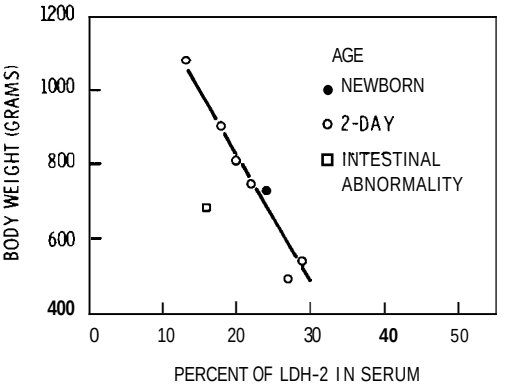

FIGURE 1.19. Serum LDH-2 and Neonatal Body Weight

In order to interpret whether serum LDH isoenzyme patterns in young swine were due to growth and differentiation, or to abnormal events in a toxicity syndrome, LDH serum and organ values were obtained for individual swine from birth to 27 days of age, and in an adult. Predominant serum isoenzyme fractions in these animals were comparable to the previous results.

Of the 15 tissues studied, the highest LDH activity was found in skeletal muscle for all ages except the newborn animal, in which the most active tissue was bone marrow (Figure 1.20). Despite the high activity and large mass of skeletal muscle, it evidently contributes little of its predominant isoenzyme (LDH-5) to the serum of animals at any age. Total cardiac muscle LDH is much higher in the adult than in the young swine and since its major isoenzyme is LDH-1, the adult serum pattern may reflect this activity. Peak liver activity was observed in the 10-day-old animal and the major isoenzyme was LDH-3 in all ages except for the newborn and 2-day-old animals where it was nearly equal to LDH-2. This organ may be the major contributor of these isoenzymes to the serum pool of the young swine. In Figure 1.21, the liver isoenzymes in the 10-day-old animal, the adult control and an adult euthanized for a ^{90}Sr-induced radiation syndrome are compared. The isogram for the ^{90}Sr-fed adult is quite similar to that of the 10-day-old animal. Spleen LDH activity was highest in the adult and showed higher LDH-1 and lower LDH-5 isoenzyme fractions than the newborn, 2- and 10-day-old animals. Marrow isoenzyme patterns were similar to those of the spleen and may indicate less active erythropoiesis in the adult as denoted by lower LDH-5 values.

Alkaline phosphatase activities in postnatal animals were highest in intestine, bone and kidney. In human

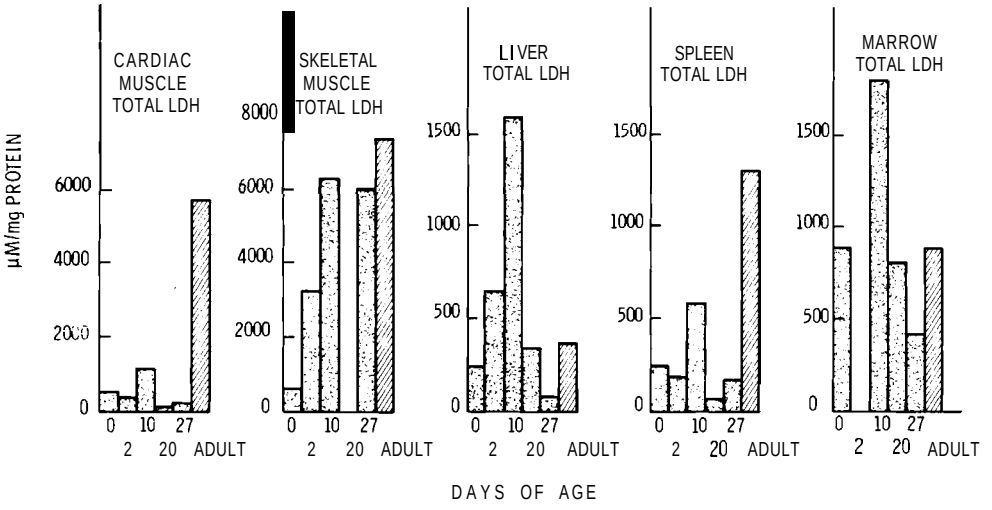

FIGURE 1.20. Lactic Dehydrogenase Activity Patterns in Neonatal and Adult Porcine Tissues

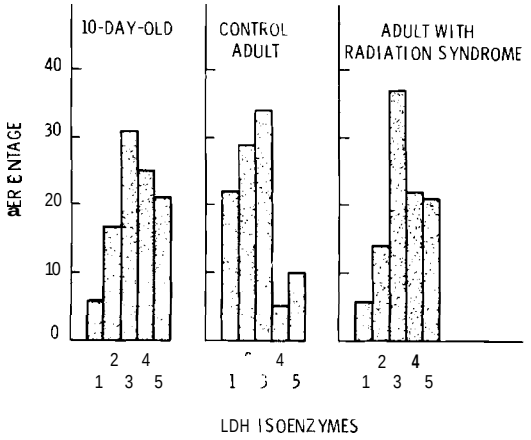

FIGURE 1.21. Comparison of Liver Isoenzymes Showing Similarity of Pattern in 10-Day-Old Swine and an Adult with ^{90}Sr Induced Radiation Syndrome

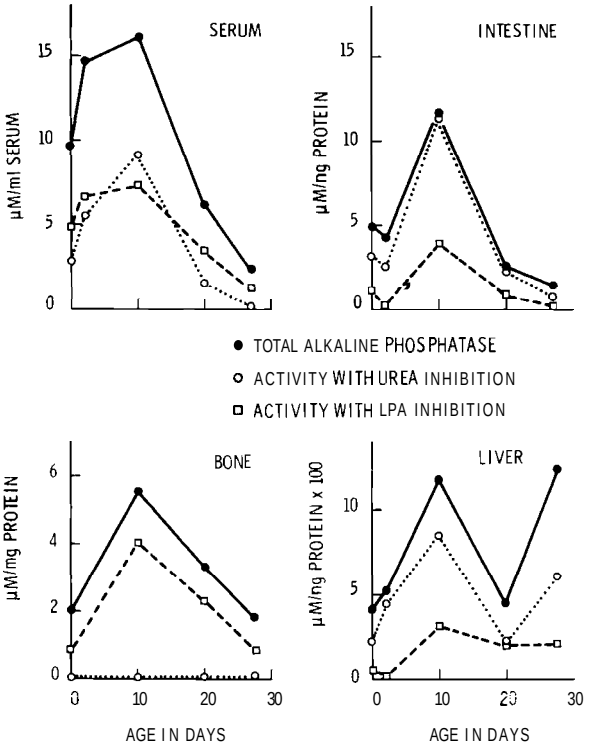

FIGURE 1.22. Alkaline Phosphatase Activity in Postnatal Swine

studies, intestinal AP was found to be most resistant to urea inhibition, with liver and kidney intermediate, and bone least resistant. In swine, this order of urea inhibition is similar, as well as the bone AP resistance to LPA inhibition.

Activity peaks for AP in serum, intestine, bone and liver were observed in the 10-day-old animal, with additional maximum in the 27-day liver (Figure 1.22). Relationships between total activities and activities in the presence of inhibitors appeared to be proportional for all tissues at all ages. The serum AP patterns of the animals suggest a changing rate of release of AP into the serum by the tissues and that contribution of bone AP to the serum pool may be slightly higher than intestinal, kidney or liver AP at all ages except for the 10-day-old animal.

These preliminary studies demonstrate the rapidly changing enzyme levels and isoenzyme patterns of young animals that should be correlated with alterations in cellular morphology to detect functional as well as structural lesions in toxicity syndromes.

STUDIES WITH A PROTECTIVE AGENT

Investigator:
J. C. Hampton
Technical Assistance:
B. Rosario

The protective action of WR2721 in the gastrointestinal tract of irradiated mice appears to be due to its effect on postmitotic cells, which remain on the villus longer than in the case of unprotected mice.

Whole-body exposure of mice to X-irradiation at fairly high doses produces the intestinal syndrome which is characterized by diarrhea, beginning after exposure to 1500 or more rads, followed by death at 3 to 7 days. Animals exposed to 1500 R die between 4 and 7 days whereas those exposed to 3000 R usually die on days 3 to 5. Grossly, the intestine at the time of death appears hemorrhagic and edematous. Microscopically, the crypts of the small intestine show early cell death among the proliferating cells and this becomes extensive at the time the animals are moribund. Concomitantly, villi become shortened as cells are extruded from the villus tip and are not replaced. Cell loss is most severe in the ileum where denuded villi are frequently seen.

Studies described in last year's Annual Report showed that the experimental agent WR2721, S-2 (3 amino-propylamino) ethyl phosphothioic acid hydrate, protects mice exposed to 1500 R of X-ray, but affords no protection following 2500 R. Protected animals exposed to 1500 R show no signs of diarrhea and when sacrificed show no evidence of edema and hemorrhage in the gut. Although there is cell loss in the intestinal epithelium, the vasculature appears to remain intact and the functional capacity of villus epithelial cells is not severely impaired. Only males were used in these early experiments and at least 25% of them were able to produce normal litters when mated with females.

During the past year we have attempted to explain the mode of protection afforded by WR2721, basing our experiments on the assumption that the protective agent acted upon proliferating cells since we did not see denudation of villi. Mice were exposed to 1500 R X-radiation 10 min after receiving intraperitoneal injection of WR2721 at a dose of 600 mg/kg; unprotected irradiated mice of the same age and size were used as controls. ^{3}H-thymidine was administered 30 min prior to sacrifice at 2, 4, 6, 8, 10 and 12 hr and days 1, 2 and 3 postirradiation, to

establish labeling indices auto-radiographically. Preliminary results have shown no significant difference between protected and unprotected mice. Labeling indices of both groups appeared to be essentially the same and both groups showed about the same degree of cell death in the proliferative compartment. Thus, it would appear that WR2721 exerts its effect on postmitotic cells, allowing them to remain on the villus longer and thereby providing the time necessary for repopulation of the proliferative compartment in the crypts and eventual repopulation of the villus epithelium.

TOXICOLOGY OF INHALED PLUTONIUM AND TRANSPLUTONIUM ELEMENTS

TOXICOLOGY OF INHALED PLUTONIUM AND TRANSPLUTONIUM ELEMENTS

This project seeks to determine the dose-effect-time relationships, together with the biological disposition of inhaled plutonium and transplutonium elements in rodents and dogs to compare with data from other species, chemical forms, particle size distributions, radionuclides and other routes of entry. This information is required for the purpose of developing dose-effect concepts for predicting the dose-effect-time relationships for inhaled Pu and transplutonium materials to which people may be exposed, to determine average radiation dose to various organs and tissues, and to attempt to relate the nonuniform dose from discrete particles to the mechanisms involved in the observed effects (radiation pneumonitis, pulmonary fibrosis and neoplasia, lymphatic necrosis and lymphopenia).

The activities under this project have been subdivided into four categories:

(1) Dose-Effect Relationship Studies - life-span research on rodents and dogs exposed by inhalation to a range of transuranic elements and compounds.

(2) Kinetics and Dosimetry Studies - deposition, retention, translocation and excretion of the inhaled radionuclides.

(3) Biological Effects in Critical Tissues - determination of critical effects, organs and tissues, and investigations of the mechanisms of radiation-induced lymphopenia, pulmonary fibrosis and neoplasia.

(4) Aerosol and Animal Exposure Technique Studies - development of aerosol generation, sampling and characterization procedures, together with animal inhalation exposure techniques.

Data from experiments in all of these categories are included among the reports that follow. Also included, at the end of this section, are two reports of an exploratory nature, which are concerned with the inhalation of krypton-85 and of beryllium oxide.

DOSE-EFFECT STUDIES WITH INHALED PLUTONIUM OXIDE IN DOGS

Investigators:

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

Technical 'Assistance:

J. S. Barnett, E. F. Blanton, E. T. Edrnerson, V. T. Faubert, D. H. Hunter, R. M. Madison, M. C. Perkins, G. L. Webb and E. L. Wierman

Six dose-Zevel groups (3 to 5800 nCi mean initial alveolar burden) of beagle dogs given single exposures to $^{239}PuO_2$ or $^{238}Pu^{16}O_2$ aerosols are being observed for life-span dose-effect relationships. Five dogs in the high-dose-level group exposed to $^{239}PuO_2$ died due to plutonium-induced respiratory insufficiency during the first 2 year's postexposure. The groups with mean initial alveolar burdens higher than 20 nCi show a dose-related Zyrnphopenia which is the most sensitive indication of a biological effect in the dogs to date.

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 pm, 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 pm, mean GSD 1.9) prepared by calcining the oxalate at 700°C and subjecting the product to $H_2{}^{16}O$ steam in Ar 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 2.1). During 1973, 94 dogs were exposed to $^{238}Pu^{16}O_2$. The remainder of the dogs required to complete a 110-dog $^{238}Pu^{16}O_2$ experiment with similar dose-level groups to compare with $^{239}PuO_2$ will be exposed in 1974.

TABLE 2.1. Dose-Effect Studies with Inhaled $^{239}PuO_2$ in Beagles

Dose Level Group	Number of Dogs		Initial Alveolar Deposition(a)	
	Male	Female	nCi(b)	nCi/g Lung(b)
0	10	10	0	0
1	10	10	3.5 ± 1.3	0.04 ± 0.02
2	10	10	22 ± 4	0.3 ± 0.05
3	10	10	79 ± 14	1.1 ± 0.2
4	10	10	303 ± 62	3.9 ± 0.7
5	10	10	1083 ± 167	14.1 ± 2.0
6	3	5	5827 ± 3282	85.6 ± 43.9
	63	65		

(a) Estimated from external thorax counts at 14- and 30-day postexposure and estimated lung weights.
(b) Mean ± 95% confidence interval around the mean.

During the first 2 postexposure years, five dogs in the highest level dose group exposed to $^{239}PuO_2$ were sacrificed when death was imminent due to respiratory insufficiency, and two dogs were euthanized from the low-level sacrifice group for comparison of plutonium tissue distribution. Plutonium analyses have been completed on the tissues of six of the dogs (Table 2.2). Twelve to thirty-four percent of the plutonium was in the thoracic lymph nodes with 64 to 88% in the lungs and less than 3% translocated to other tissues. The fraction of plutonium in the lungs and lymph nodes was similar in the high- and low-dose-level dogs. The skeleton of dog 849 and the liver of dog 817 and 896 contained a larger fraction of the final plutonium body burden compared to the other dogs.

The dogs that were euthanized when death was imminent showed pulmonary fibrosis and fibrosis of the thoracic lymph nodes. Clinical changes included increased respiration rate, anoxemia, hypoxemia and body weight loss associated with the pulmonary fibrosis-induced respiratory insufficiency and lymphopenia.

A dose-related lymphopenia was observed in the $^{239}PuO_2$ dogs (Figure 2.1). The lymphopenia appeared to be related to initial alveolar burden, showing a greater depression in lymphocyte count at the high dose levels; and to time after exposure, the lower dose levels showing lymphopenia later than the high-dose-level dogs. The groups with mean initial alveolar burdens of 80 nCi and higher showed lymphopenia during the first 2 years after exposure, while the

TABLE 2.2. Tissue Distribution of Plutonium in Dogs After Inhalation of $^{239}PuO_2$

Dog Number	Time After Exposure, days	Final Body Burden, nCi	Percent of Final Plutonium Burden				
			Lungs	Thoracic Lymph Nodes(a)	Liver	Skeleton	Cause of Death
747 F	358	5434.4	71.16	28.54	0.07	0.07	Respiratory Insufficiency
906 F	383	6154.2	88.13	11.62	0.03	0.05	Respiratory Insufficiency
849 F	385	0.7	79.21	15.32	0.04	2.60	Periodic Sacrifice
896 F	460	4114.7	81.12	14.79	0.23	0.12	Respiratory Insufficiency
817 M	628	3793.5	63.99	33.86	1.40	0.19	Respiratory Insufficiency
815 M	756	73.8	63.84	32.36	0.08	0.10	Periodic Sacrifice

(a) Tracheobronchial, mediastinal and sternal lymph nodes.

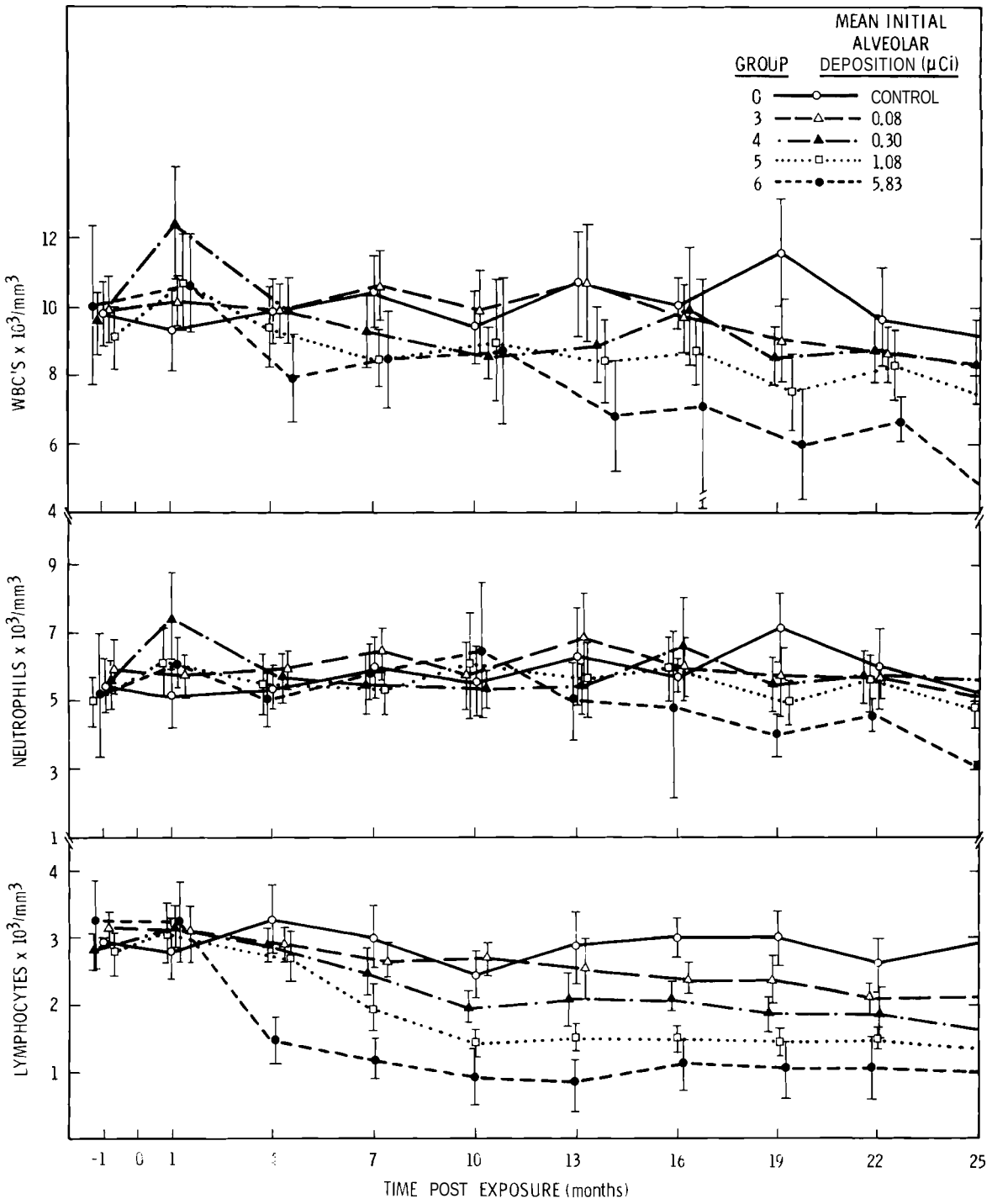

FIGURE 2.1. Leucocyte Values of Dogs After Inhalation of $^{239}PuO_2$

22 nCi and lower dose groups were similar to controls. The higher dose-level groups also showed a leucopenia primarily due to the decrease in lymphocytes. No significant abnormalities were seen in other hematological and clinical chemistry measurements. The lymphopenia in the 80 nCi group at 2 years postexposure is the lowest dose level in which an effect of inhaled plutonium in dogs has been observed, to date. In previous experiments, dogs at the lowest dose level studied died due to pulmonary neoplasia 8 to 11 years after initial alveolar deposition of 200 to 800 nCi $^{239}PuO_2$. These dogs also showed lymphopenia.

Ten dogs were exposed to $^{238}Pu^{16}O_2$ aerosols and sacrificed for plutonium retention and translocation measurements 7, 28, 56, and 91 days after exposure. Table 2.3 shows the tissue distribution of plutonium in these dogs compared to dogs sacrificed 7 to 141 days after inhalation of $^{239}PuO_2$. There appears to be more ^{238}Pu than ^{239}Pu translocated to the thoracic lymph nodes, liver and skeleton during the 28 to 91 day postexposure period.

Table 2.4 shows the dose-level groups for the 94 dogs that have been exposed thus far for the $^{238}Pu^{16}O_2$ life-span dose-effect study. None of the dogs have been sacrificed. The $^{238}Pu^{16}O_2$-exposed dogs also showed a dose-related lymphopenia and leucopenia during the first year postexposure (Figure 2.2). The groups with mean initial alveolar deposition of 140 nCi and higher showed lymphopenia while the lower dose groups were similar to controls. The highest dose-level group also showed a neutropenia.

TABLE 2.3. Tissue Distribution of Plutonium in Dogs After Inhalation of $^{238}Pu^{16}O_2$ or $^{239}PuO_2$

	Mean Percent of Total Plutonium Burden						
	$^{238}Pu^{16}O_2$				$^{239}PuO_2$		
Time After Exposure, days	8	28	56	91	7	29	141
Number of Dogs	1	3	3	3	2	3	4
Lungs	96.9	95.8	91.8	90.6	98.8	95.3	95.5
Thoracic Lymph Nodes[(a)]	0.3	1.1	6.8	6.7	0.1	0.8	4.0
Liver	1.7	0.2	0.2	0.4	0.1	0.1	0.1
Skeleton	0.2	0.9	0.4	1.0	0.1	0.3	0.1
Muscle	0.4	1.0	0.5	0.4	0.3	1.3	0.1
Skin	0.1	0.6	0.3	0.1	0.4	0.3	0.0
All Remaining Tissues	0.4	0.5	0.3	1.2	0.2	0.3	0.4

(a) Tracheobronchial, mediastinal and sternal lymph nodes.

TABLE 2.4. **Dose-Effect Studies with Inhaled $^{238}Pu^{16}O_2$ in Dogs**

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	8	8	0	0
1	9	9	2.3 ± 1.5	0.03 ± 0.02
2	9	10	28 ± 10	0.3 ± 0.1
3	10	10	138 ± 34	1.8 ± 0.4
4	6	4	523 ± 90	6.4 ± 1.0
5	9	6	1618 ± 334	19.4 ± 3.6
6	6	6	5383 ± 1448	72.0 ± 18.4
	57	53		

(a) Estimated from external thorax counts at 14- and 30-day postexposure and estimated lung weights.
(b) Mean ± 95% confidence interval around the mean.

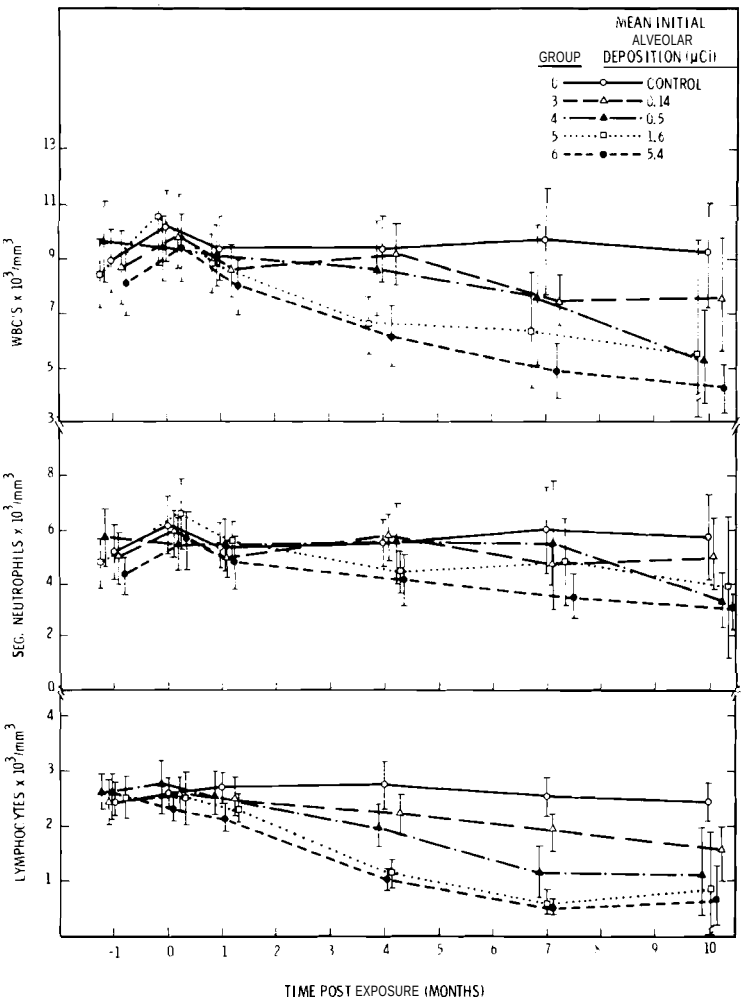

FIGURE 2.2. **Leucocyte Values of Dogs After Inhalation of $^{238}Pu^{16}O_2$**

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

Investigators:

J. F. Park, D. L. Catt, G. E. Dagle, P. L. Hackett, R. J. Olson, G. J. Powers and H. A. Ragan

Technical Assistance:

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

Bone tumors were the primary cause of death in beagle dogs 4 to 6 years after inhalation of $^{238}PuO_2$. The largest fraction of the plutonium retained in the body was in the skeleton followed in descending order by liver, thoracic lymph nodes, and lung.

Beagle dogs were exposed to aerosols of $^{238}PuO_2$ calcined at 350°C (CMD 0.1 μm) or $^{238}PuO_2$ crushed microspheres (CMD 0.1 μm) to determine the long-term disposition and biological effects. Eight of the ten dogs exposed to 350° calcined $^{238}PuO_2$ and eight of the 12 dogs exposed to $^{238}PuO_2$ crushed microspheres were euthanized when death was imminent during the 6-year postexposure period. Table 2.5 shows the cause of death and the ^{238}Pu distribution in the tissues.

Two of the dogs were euthanized because of respiratory insufficiency related to plutonium-induced pulmonary fibrosis. Eight dogs were euthanized because of plutonium-induced bone tumors, one dog had bone and lung tumors, and five dogs died of causes not thought to be related to plutonium exposure. None of the 17 control dogs on the study have died. In addition to the lesions causing death, the dogs had fibrotic tracheobronchial lymph nodes, pulmonary fibrosis, and nodular hyperplasia in the liver. The two high-level dogs that died due to respiratory insufficiency were the only dogs showing clinical pulmonary effects.

The dogs showed a persistent lymphopenia (Figures 2.3 and 2.4), which became apparent in both groups by 200 days after exposure. In the crushed microsphere-exposed dogs, the mean lymphocyte count of the six surviving, exposed dogs remained below that of controls at 6 years postexposure. The total leucocyte count of the exposed dogs was frequently lower than that of controls, primarily due to the low lymphocyte counts and a small but consistent reduction in neutrophils (Figure 2.3). The mean lymphocyte count of the 350" calcined plutonium-exposed dogs was evident until 5 years postexposure when only four of the dogs with the lowest body burdens remained alive (Figure 2.4). The dogs in this group also showed a neutropenia which was apparent 1 month to 2 years after

TABLE 2.5. Mortality and Tissue Distribution of Plutonium in Dogs

Dog Number	Time After Exposure, months	Final Body Burden µCi	% of Final Body Burden				Cause of Death
			Lungs	Thoracic Lymph Nodes(c)	Liver	Skeleton	
	(After Inhalation of $^{238}PuO_2$ Calcined at 350°C)						
492 F	23	3.0	4	4	23	64	Bone Fracture
404 M	36	8.1	32	10	23	32	Respiratory Insufficiency
467 M	38	7.0	15	11	13	57	Respiratory Insufficiency
469 M	54	2.6	34	5	17	41	Bone Tumor
445 M	58	2.5	6	10	23	55	Bone Tumor
438 M	60	2.3	7	11	33	43	Bone Tumor
453 M	62	2.2	17	9	22	47	Bone Tumor
405 M	70	4.0(a)					Bone & Lung Tumor
433 M	74	0.7(a,b)					
459 M	74	0.2(a,b)					
	(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 Wounds
497 F	52	0.2	16	3	31	46	Intestinal Obstruction
481 F	60	0.5	13	23	23	37	Myelogenous Leukemia
489 F	62	2.5	7	26	27	32	Bone Tumor
482 F	70	0.6(a)					Bone Tumor
494 F	75	0.4(a)					Suspect Tumor
488 F	76	1.4(a)					Bone Tumor
480 F	76	0.6(a,b)					
491 M	76	0.6(a,b)					
487 F	76	0.3(a,b)					
496 F	76	0.2(a,b)					

(a) Estimated body burden
(b) Still alive
(c) Tracheobronchial, mediastinal and sternal lymph nodes

exposure, when the higher dose-level dogs were still alive. The dogs also showed a leucopenia due to the neutropenia and lymphopenia. No significant changes were observed in other leucocytes, erythrocytes or hemoglobin. The lower initial mean lymphocyte values in the dogs exposed

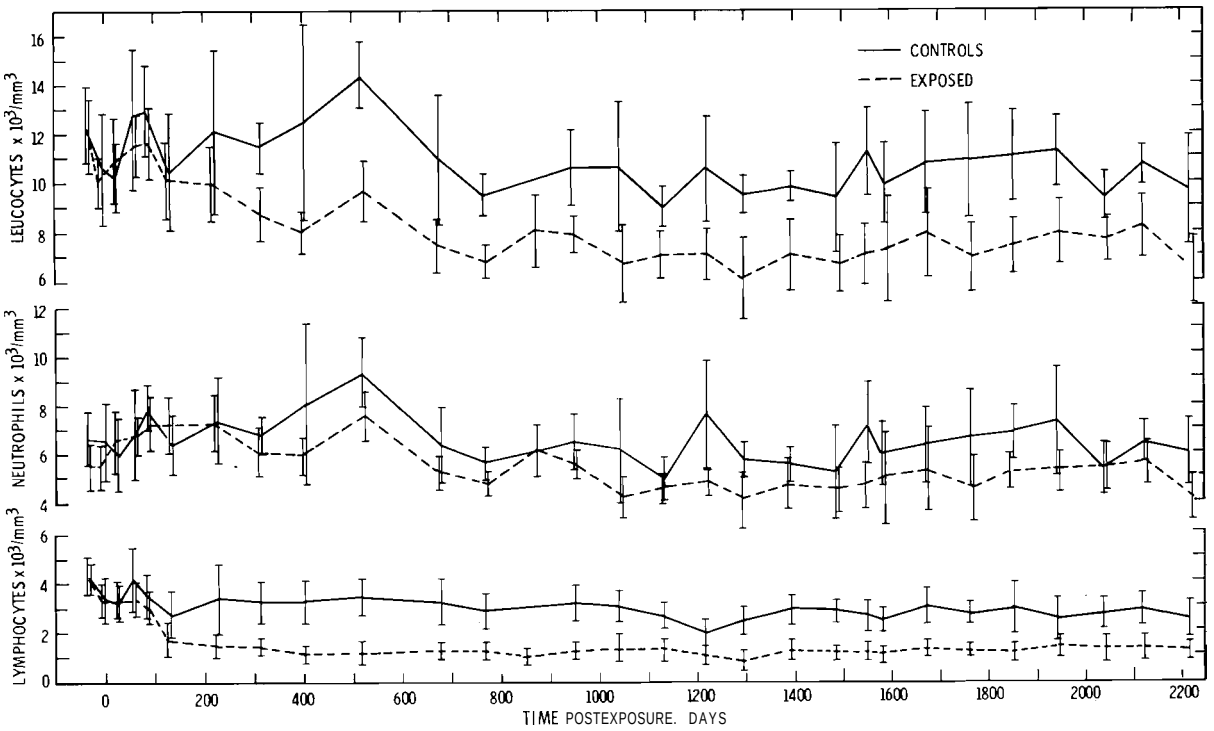

FIGURE 2.3. Leucocyte Values of Dogs After Inhalation of $^{238}PuO_2$ Crushed Microspheres (Means ±95% confidence interval)

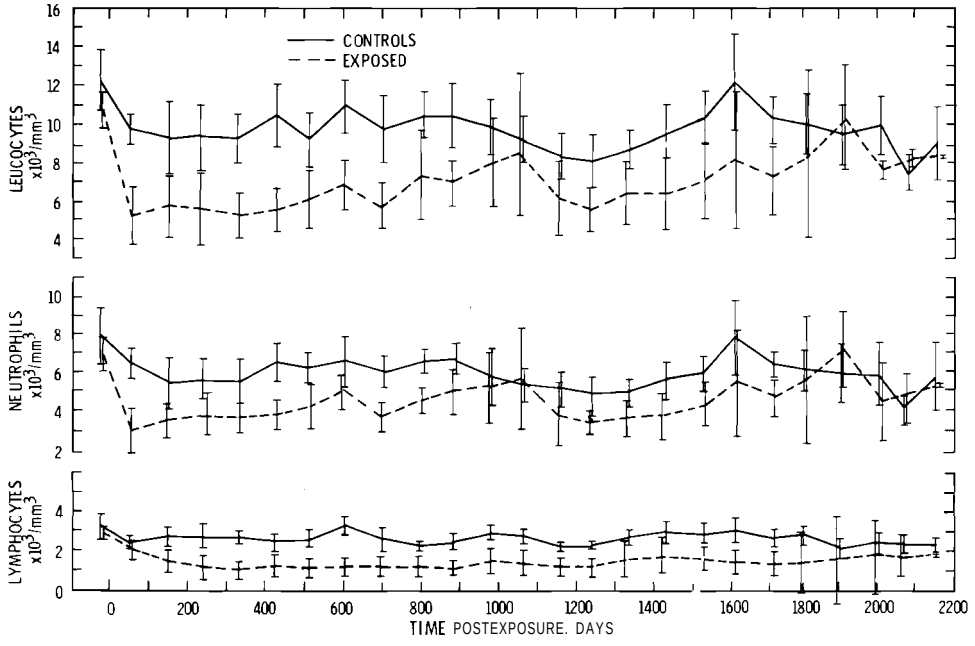

FIGURE 2.4. Leucocyte Values of Dogs After Inhalation of $^{238}PuO_2$ Calcined at 350°C (Means ±95% confidence interval)

to crushed microspheres, compared to the 350° calcined plutonium-exposed group, may be due to an age difference. The dogs were exposed to 350°C calcined $^{238}PuO_2$ when 3 years old and to $^{238}PuO_2$ crushed microspheres when 1 year old. Older dogs generally show lower lymphocyte counts.

No differences were observed in serum blood urea nitrogen, creatinine, glucose, alkaline phosphatase, or glutamic oxalacetic transaminase.

Mean serum glutamic pyruvic transaminase (SGPT) levels were not significantly elevated compared to controls; however, the percentage of elevated SGPT values (>55 units) in dogs receiving ^{238}Pu was higher than the controls during the past year (Figure 2.5). These changes may be related to plutonium-induced lesions in the liver and/or metastatic tumors.

The largest fraction of plutonium retained in the body 5 years after exposure was in the skeleton followed in descending order by liver, thoracic lymph nodes and lung (Table 2.5). The biological effects observed during

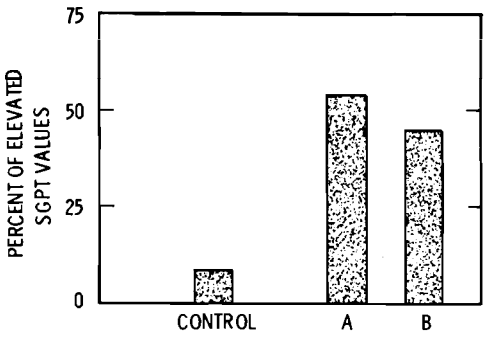

FIGURE 2.5. Percentage of Elevated SGPT Values in ^{238}Pu-Exposed Dogs (A: inhaled crushed microspheres; B: inhaled calcined $^{238}PuO_2$)

the 4 to 6 years following exposure to $^{238}PuO_2$ were primarily related to skeletal deposition; eight dogs died due to bone tumors and one also had a lung tumor. In previous studies with $^{239}PuO_2$, plutonium was retained primarily in the lungs and thoracic lymph nodes; and dogs died 3 to 10 years after inhalation exposure due to lung tumors; no bone tumors were observed. The importance of post-exposure translocation from the lung, and of radiochemical and/or physical state on this translocation, is evident in these results.

TISSUE DISTRIBUTION IN DOGS AFTER INHALATION OF $^{238}Pu^{16}O_2$ (PPO) AND $^{238}Pu^{16}O_2$ Mo CERMET (PMC)

Investigators:

J. F. Park, D. K. Craig, D. L. Catt and R. J. Olson

Technical Assistance:

J. S. Barnett, E. F. Blanton, G. L. Webb and E. L. Wierman

More than 95% of the plutonium was retained in the lungs and thoracic lymph nodes of dogs exposed to PPO and PMC during the 90-day postexposure period with less than 3% in the liver and skeleton. The fraction of plutonium in the liver and skeleton of the PMC dogs was about twice that in the PPO-exposed group.

Nine beagle dogs were exposed to aerosols (mean AMAD 2.1 pm, mean GSD 1.7) nebulized from freshly prepared water suspensions (ultrafilterability 0.25%) of $^{238}Pu^{16}O_2$ Mo cermet (PMC) or to aerosols (mean AMAD 1.9 pm, mean GSD 1.7) nebulized from freshly prepared water suspensions (ultrafilterability 0.02%) of $^{238}Pu^{16}O_2$ (PPO). Three dogs were sacrificed for retention and translocation studies 28, 56, and 91 days after exposure.

The PPO was the normal Savannah River product with 80 ± 2 atom% ^{238}Pu. The $^{238}PuO_2$ was calcined from the oxalate at 700°C and subjected to $H_2{}^{16}O$ steam in Ar exchange at 800°C for 96 hr. The material obtained from Los Alamos Scientific Laboratory was ball-milled to particles with an MMD of 2.8 ym. The PMC, 3% Mo by weight, is representative of space nuclear system fuels from Mound Laboratory production site and was further treated by ball-milling at the Los Alamos Scientific Laboratory to particles with an MMD of 3.6 ym.

Table 2.6 shows the tissue distribution of plutonium 28 to 90 days after inhalation of PPO and PMC. blore than 95% of the plutonium from both materials was retained in the lungs and thoracic lymph nodes during the 90-day postexposure period with less than 3% in the liver and skeleton and 2 to 3% in all other tissues. The fraction of the final body burden in the liver and skeleton of the dogs exposed to PMC was about twice that of those exposed to PPO. These differences were statistically significant at $p < 0.01$ (liver) and $p < 0.05$ (skeleton).

In the PMC exposed group, the fraction of the final body burden in the liver and thoracic lymph nodes increased during the 28- to 90-day postexposure period and the fraction in the lungs decreased. The translocation was significant at $p < 0.05$

TABLE 2.6. Tissue Distribution of Plutonium in Dogs After Inhalation of $^{238}Pu^{16}O_2$ (PPO) and $^{238}Pu^{16}O_2$ Mo Cermet (PMC)

	Mean Percent of Body Burden					
	PPO			PMC		
Time After Exposure, days	28	56	91	28	56	91
Number of Dogs	3	3	3	3	3	3
Lungs	95.8	91.8	90.6	93.9	90.8	86.5
Thoracic Lymph Nodes[(a)]	1.1	6.8	6.7	1.1	6.2	9.8
Liver	0.2	0.2	0.4	0.5	0.7	1.3
Skeleton	0.9	0.4	1.0	1.4	1.5	1.5
Muscle	1.0	0.5	0.4	1.3	0.2	0.7
Skin	0.6	0.3	0.1	1.1	0.1	0.1
All Remaining Tissues	0.5	0.3	1.2	0.6	0.5	0.1

(a) Tracheobronchial, mediastinal and sternal lymph nodes.

for the lungs and $p < 0.01$ for the thoracic lymph nodes and liver as determined by linear regression analyses.

Although only small differences were observed in the fraction of plutonium retained in various tissues of the PMC group compared to the PPO group during the 28- to 90-day postexposure period, the differences may be larger at longer postexposure times if the trend continues toward higher translocation rates in PMC exposed animals.

HISTOPATHOLOGIC EFFECTS OF INHALED ^{239}Pu CITRATE AND ^{239}Pu NITRATE IN DOGS

Investigators:

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Technical Assistance:

W. G. Morrow

Inhaled ^{239}Pu citrate or ^{239}Pu nitrate (1.6 to 11.4 μCi deposited) produced slight to moderate pneumonitis as the major lesion in dogs sacrificed 1 to 100 days after exposure. Controls given 0.27N nitric acid developed a somewhat different lesion. None of the animals exhibited serious pathologic changes that would be expected to alter normal clearance and translocation kinetics.

The disposition of inhaled ^{239}Pu citrate and ^{239}Pu nitrate in dogs was reported in the 1971 Annual Report. Tissues from these animals have since been examined histopathologically to determine if the experimental treatment may have damaged the lung or otherwise altered normal plutonium clearance and translocation kinetics.

A total of 28 dogs (seven males and seven females for each compound) were subjected to a single "nose only" exposure to ^{239}Pu citrate or ^{239}Pu nitrate. Plutonium citrate was generated from a 0.08 M citrate solution buffered at pH 3.5. The plutonium nitrate was generated from a 0.27N nitric acid solution. The aerosol droplet size was 1.45 μm AMAD,[a] GSD[b] 2.75 for citrate; and 0.91 μm AMAD, GSD 3.56 for nitrate. The dogs deposited from 1.6 to 11.4 μCi ^{239}Pu. Animals were sacrificed in groups of two after 1, 3, 7, 14, 60, 62 and 100 days postexposure.

Of the 11 dogs examined after ^{239}Pu citrate exposure, three showed a small amount of pneumonitis (thickened alveolar septa, increased numbers of macrophages, infiltration with inflammatory cells) that is considered to be compatible with a radiation effect. Autoradiographs showed alpha tracks associated with these lesions in the dogs sacrificed 14, 30 and 100 days postexposure. Plutonium was distributed as single tracks or stars throughout the lung sections taken 1 day after exposure. At later time intervals the material was distributed as stars or clusters of tracks mainly in alveolar macrophages, alveolar septal cells and in peribronchiolar areas. Similar lesions were not observed in the control dogs exposed to inhaled 0.08M citrate buffer or in the dogs sacrificed at shorter time intervals after ^{239}Pu citrate exposure. The pneumonitis observed after ^{239}Pu citrate inhalation is believed to be

(a) AMAD = Activity median aerodynamic diameter
(b) GSD = Geometric standard deviation

radiation induced; however, the low degree of severity suggests that plutonium clearance and translocation was probably not seriously influenced.

Similar pathologic changes accompanied inhalation of ^{239}Pu nitrate except that the lesions appeared earlier, within 3 days after exposure, and were observed more consistently in the animals sacrificed at later time intervals. Autoradiographs showed trace to marked amounts of alpha activity in the lungs, tracheobronchial lymph nodes, turbinates, bone and liver. Lung sections showed primarily stars or clusters of tracks over alveolar macrophages, alveolar septa, in peribronchiolar areas and occasionally over macrophages in bronchi. Single tracks were observed in the lung of the dog sacrificed after 1 day. Alpha activity was occasionally correlated with areas of pneumonitis; however, this was not a consistent finding. Two control dogs exposed to 0.27N nitric acid and sacrificed 7 and 100 days later showed what may be a progression in lung damage induced by nitric acid. After 7 days a slight pneumonitis with edema and infiltration of inflammatory cells was observed. After 100 days the lung lesion appeared granulomatous, atypical of the radiation induced changes, but compatible with the earlier inflammatory response seen in the 7-day control dog. In addition, there were moderate diffuse degenerative changes in the liver that may be further indication of nitric acid toxicity. These changes were characterized by large cytoplasmic vacuoles filled with eosinophilic material that compressed the cytoplasm to a narrow rim.

The changes observed in the lung following ^{239}Pu citrate and ^{239}Pu nitrate inhalation were judged to be slight to moderate by histopathologic criteria and in no case did they appear to impair the physiological function of the animals. This is not to say that these levels of plutonium deposition (1.6 to 11.4 μCi) would not eventually produce life shortening or other effects, but only suggests that within the time span of 1 to 100 days postexposure the effect would probably be minimal. Plutonium clearance from the lung was initially more rapid after plutonium citrate inhalation than after plutonium nitrate; however, after 14 days the amount retained in the lung was about the same for the two compounds. While this may be evidence of an effect of plutonium nitrate (or of 0.27N nitric acid) on early clearance kinetics, it is more probably due to the different biological behavior of the citrate and nitrate salts of plutonium. Plutonium citrate is known to be reasonably stable and soluble in biological media, while the nitrate salt would be expected to polymerize and form a relatively insoluble hydroxide in the physiological environment. The citrate anion may also be metabolized which would free the remaining plutonium to bind or polymerize in a manner similar to that observed with plutonium nitrate. These physico-chemical differences could thus account for both the differences in early lung clearance as well as the similarities seen later.

THE ALVEOLAR DEPOSITION OF INHALED PLUTONIUM AEROSOLS IN RODENTS

Investigators:

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Technical Assistance:

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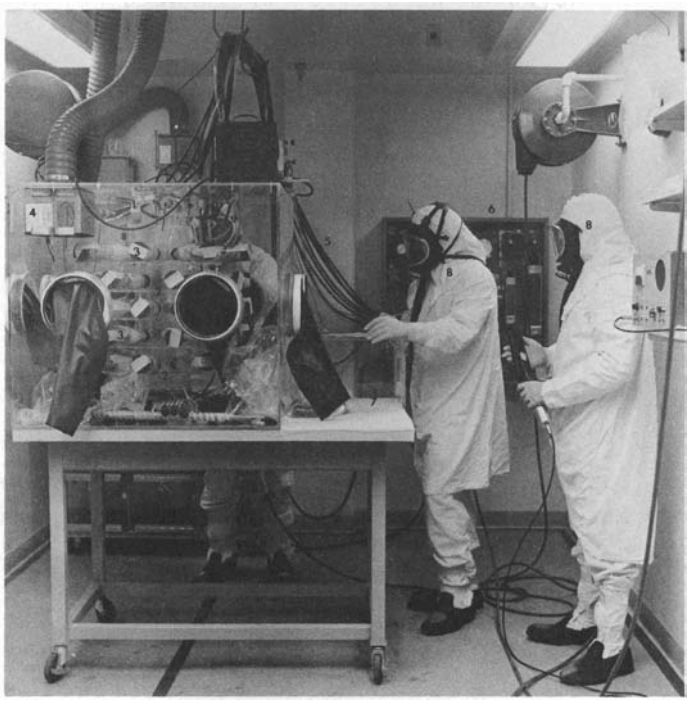
Analysis of the data from a large number of different inhalation studies involving the nose-only aerosol exposure of rodents has failed to yield any consistent significant correlations between percentage alveolar deposition and the various aerosol and exposure condition parameters that were measured. The most useful finding was a very high correlation between alveolar deposition and aerosol concentration or inhaled quantity of material.

Inhalation studies involving the nose-only aerosol exposure of rodents have been conducted in the equipment shown in Figure 2.6. These studies require the deposition of specified alveolar burdens of highly toxic radioactive materials. This quantity deposited (DEP) depends upon the percentage of the inhaled aerosol that is deposited beyond the animal's ciliated epithelium, i.e., percentage alveolar deposition (PCA). The quantity inhaled (INH) is estimated from the product of the aerosol concentration (NCi/l), the exposure time (minutes) and the mean minute volume of the rats (assumed to be 0.1 l/min in every case).

The alveolar deposition in rats of a number of transuranic compounds including PuO_2, $Pu(NO_3)_4$, $^{244}CmO_x$ and various compounds of ^{253}Es, was measured during the course of several inhalation toxicology studies on groups of two to five animals sacrificed within 1, 24 or 48 hr post-exposure. The mean percentage alveolar deposition for each group (defined as the material found in the lung beyond the trachea divided by one-hundredth of the estimated quantity inhaled) was calculated and the results compared with other available parameters, such as the aerodynamic equivalent size distribution of the inhaled aerosol. Significant results are summarized in Table 2.7

For rats exposed to $Pu(NO_3)_4$ and sacrificed within an hour, the mean deposition was 19.2 ± 9.2% for 36 groups of animals for either ^{238}Pu or ^{239}Pu. For these exposures there were no important correlations between any of the measured parameters that were significant at less than the $p = 0.05$ level, aside from that



1 = Aerosol generator
2 = Aerosol chamber
3 = Rats in "Coke" bottles which have bottoms cut off
4 = Filter through which glove box air is exhausted
5 = Compressed air supply and vacuum sampling lines
6 = Control panel
7 = Transfer hatch to glove box
8 = Personnel wearing protective clothing and supplied air masks

FIGURE 2.6. Rodent Exposure Equipment

TABLE 2.7. Summary of Correlations Between Parameters

Compound	PCA Mean ± SD	Correlations and Significance Level(a) CONC Versus AMAD R	N	p	AMAD Versus PCA R	N	p	INH Versus DEP R	N	p
$Pu(NO_3)_4$ (238 and 239)	19.2 ± 9.2	0.326	36	Not	-0.346	36	0.05	0.876	36	0.01
$^{239}PuO_2$	7.6 ± 3.9	0.832	35	0.01	-0.525	32	0.01	0.938	33	0.01
$^{238}PuO_2$	9.8 ± 4.7	-0.584	8	Not	-0.335	8	Not	1.000	8	0.01
$^{244}CmO_x$	15.1 ± 11.8	-0.541	8	Not	0.790	4	Not	0.996	4	0.01
$^{253}Es(X)_3$	18.7 ± 1.5	-0.879	4	Not	-0.196	4	Not	0.929	4	0.1

(a) R = Correlation coefficient
N = Number of comparisons
p = Significance level

between inhaled quantity of material and measured alveolar deposition. For the case of $^{239}PuO_2$ deposition in rats sacrificed 24 or 48 hr postexposure, AMAD was strongly correlated with CONC and less strongly so with percentage deposition (mean = 7.62 ± 3.85% for 32 groups) for AMAD over the range 1.5 to 4.5 μm. The 200-fold lower mass concentration of ^{238}Pu, compared with ^{239}Pu of equivalent activity level, may explain the fact that AMAD was correlated with concentration for ^{239}Pu oxide and nitrate, but not for $^{238}PuO_2$. There was a good correlation between the calculated quantity of material inhaled and the mean quantity deposited in the rat lungs, for all materials studied, although the range of values within each group was about three.

THE RELATIVE QUANTITY OF AIRBORNE PLUTONIUM DEPOSITED IN THE RESPIRATORY TRACT AND ON THE SKIN OF RATS

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Technical Assistance:

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Shaved rats were exposed to a $^{238}Pu(NO_3)_4$ aerosol in a low speed windtunnel. Immediately after exposure, about 40% of the inhaled material was in the lower respiratory tract, about 40% in the head and 20% in the intestines. Plutonium deposited on the skin amounted to about one-fourth of the amount initially deposited in the respiratory tract. Measured values agreed with calculated values within a factor of two.

There are few data on the relative deposition of airborne plutonium within the respiratory tract and on skin. The skin deposit has been usually ignored on the assumption that it would be soon washed off, whereas the pulmonary deposit would have a far longer residence time within the body. However, particulate material deposited on the skin of infants may be of concern because their epidermal layers may be thinner than those of adults, and they are more likely to have tender skin areas exposed.

The quantity of airborne material inhaled by an exposed individual is:

$$\underset{(nCi)}{Q_{INH}} = \underset{(nCi/\ell)}{C} \times \underset{(\ell/min)}{MV} \times \underset{(min)}{t} \quad (1)$$

(a) Ecosystems Department

where:

C = aerosol concentration

MV = volume of air inhaled per unit time

t = exposure time

The quantity of material deposited on an exposed surface is:

$$Q_{surface}\ (nCi) = V\ (cm/sec) \times C \times 60t \times A\ (cm^2) \quad (2)$$

where:

V = the ratio between rate of transfer of material from air to a unit surface and the air concentration (deposition velocity)

A = area of exposed surface

V is a function both of particle size and of windspeed, decreasing as the windspeed decreases and passing through a minimum value which also depends upon windspeed as particle size decreases. For colloidal gold aerosols having AMADs[(a)] in the range 0.7 to 1.0 μm and GSDs[(b)] in the range 1.55 to 1.81, the deposition velocity for the top of a horizontal surface was found to be an order of magnitude greater than that for the bottom surface or onto vertical surfaces facing upstream or downstream.

Some simple animal experiments to measure Q_{INH} and $Q_{surface}$ were conducted in the windtunnel and associated glovebox shown schematically in Figure 2.7. The glovebox is 9 ft long and the experimental section of the windtunnel shown with rats in the filing cabinet-type drawer, is approximately 1 ft^2 in cross-section. A $^{238}Pu(NO_3)_4$ aerosol was introduced into the airstream just downstream from the air intake filter. The flow rate in the windtunnel was approximately 10 cm/sec. Six, 300 g, shaved, female, Wistar rats in individual wire mesh cages, which allowed easy passage of the aerosol, but prevented the animals from turning around or licking themselves, were placed in the exposure drawer, three with noses pointing into the wind and three with noses pointing downwind. Immediately after a 20-min exposure, the rats were sacrificed and plutonium determined in the skin, lungs, stomach and upper third of the small intestine, and head.

Data used to compute the total activity inhaled by the rats during the exposure and the total activity deposited on their skin are summarized in Table 2.8. From Equation (1), the calculated inhaled activity is Q_{INH} = 2750 nCi. From Equation (2), the total activity deposited on the skin is $Q_{surface}$ = 1340 nCi.

The animal data are summarized in Figure 2.8, only the means for each

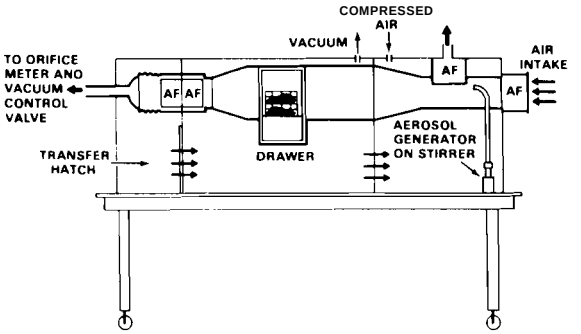

FIGURE 2.7. Transverse Section of Glove Box and Aerosol Exposure Wind Tunnel

(a) AMAD - activity median aerodynamic diameter

(b) GSD - geometric standard deviation

TABLE 2.8. Values Used to Compute $^{238}Pu(NO_3)_4$ Inhaled by Rats and Deposited on Their Skin

Item	Quantity
Aerosol Concentration	740 nCi/ℓ
Minute volume of rats	0.185 ℓ/min
Exposure time	20 min
Calculated inhaled activity	2750 nCi
Size Distribution of Aerosol	
AMAD	1.03 μm
GSD	1.81
Deposition velocity	
Onto horizontal surface (V_1)	7×10^{-3} cm/sec
Onto other surfaces (V_2)	8×10^{-4} cm/sec
Mean Body Weight	286 g
Mean Skin Area	460 cm^2
Percentage of surface of rats	
Horizontal facing upwards	40%
Other	60%
Calculated deposition on skin of rats	1340 nCi

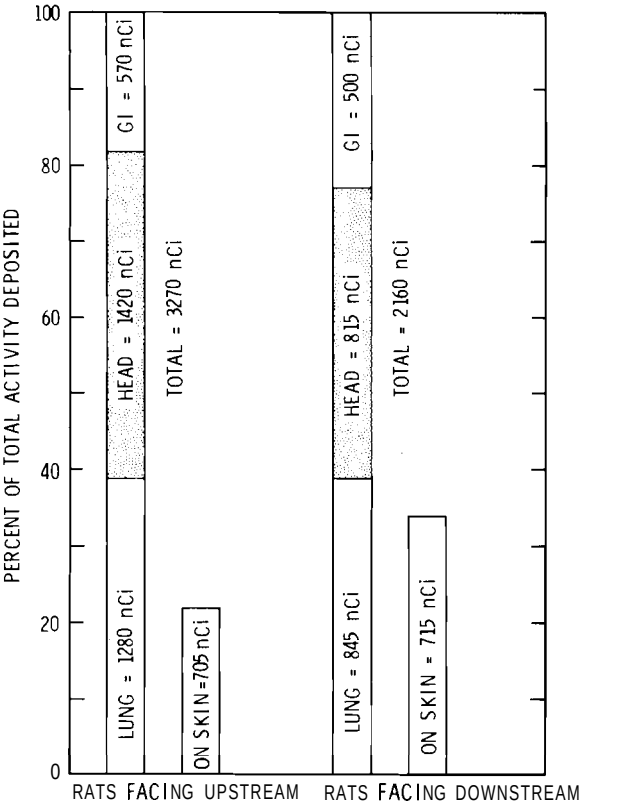

FIGURE 2.8. Distribution of $^{238}Pu(NO_3)_4$ in Exposed Rats

of the two groups being given. It is apparent that there is little difference between the activity deposited on the skins of the two groups. However, the total initial deposition of $^{238}Pu(NO_3)_4$ in the rats facing downstream was only 66% of that in the rats facing the other way. The measured quantity of material deposited on the skin of these rats is about half the quantity $Q_{surface}$ calculated on the basis of the stated assumptions The relative importance of the material deposited on the skin will increase with increasing particle size and wind velocity. However, it seems unlikely that the skin deposition would ever greatly exceed the total initial deposition in the respiratory tract, since larger particles will settle out close to the point of emission. The good agreement between calculated and experimentally determined values in this study suggest that deposition on the skin and in the respiratory tract can be reasonably estimated for varying conditions.

EARLY FATE OF INHALED $^{238}PuO_2$, $^{239}PuO_2$ AND $^{244}CmO_x$ IN RATS

Investigators:

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Technical Assistance:

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Inhaled $^{239}PuO_2$ was cleared from the lung and excreted in the feces, while inhaled "aged" $^{238}PuO_2$ or $^{244}CmO_x$ showed much greater translocation to liver and bone. Fresh $^{238}PuO_2$ showed greater in vivo solubilization than did $^{239}PuO_2$, while fresh $^{244}CmO_x$ was highly soluble in the lung.

The in vivo solubility of inhaled transuranic oxides plays an important role in determining their tissue distribution and resultant pathologic effects.

Female rats were exposed to $^{238}PuO_2$, $^{239}PuO_2$ or $^{244}CmO_x$ within 2 days after calcination at 750°C and suspension in water, or to an aerosol of $^{238}PuO_2$ that had been in water suspension for 15 months prior to its aerosolization, referred to as "aged" $^{238}PuO_2$. Following exposure, ten rats per exposure group were placed in individual metabolism cages and sacrificed 30 days after exposure. Their tissues were analyzed for alpha activity by scintillation counting. Several rats were sacrificed 1 day after exposure and autoradiograms prepared of the lung.

The $^{239}PuO_2$ aerosol exhibited the greatest activity median aerodynamic diameters (AMAD), with $^{238}PuO_2$ being intermediate and "aged" $^{238}PuO_2$ and $^{244}CmO_x$ the smallest. Autoradiograms of lung taken 1 day after exposure demonstrated the overwhelming particulate nature of deposited $^{238}PuO_2$ and $^{239}PuO_2$. "Aged" $^{238}PuO_2$ and $^{244}CmO_x$ in the lung showed abundant single tracks, interspersed with "stars", indicative of polymeric or particulate material.

The ultrafilterability of $^{239}PuO_2$ water suspensions was about 0.2%; $^{239}PuO_2$ showed no increased ultrafilterability after 2 years in suspension. The ultrafilterability of $^{238}PuO_2$ increased from 0.2% after 1 day in water suspension to about 30% after 15 months in suspension. The ultrafilterability of $^{244}CmO_x$ was 1.3% after 1 day in suspension.

Initial total respiratory tract deposition was taken as the amount of plutonium or curium in excreta plus that found in the rat at 30 days after exposure. Upper respiratory tract deposition was taken as the amount of plutonium or curium excreted in the feces the first 3 days after exposure. Initial alveolar deposition was taken as the amount of plutonium or curium excreted in the feces from 4 to 30 days plus the amount excreted in the urine from 0 to 30 days plus the amount in the

body at 30 days after exposure. Of the initial total respiratory tract deposition, about 82% of $^{239}PuO_2$, 78% of $^{238}PuO_2$, 52% of "aged" $^{238}PuO_2$ and 24% of $^{244}CmO_x$ was deposited on the ciliated epithelium of the upper respiratory tract, the remainder being initially deposited in the alveolar region.

Urinary excretion of transuranics was greatest for $^{244}CmO_x$ and "aged" $^{238}PuO_2$ (Table 2.9). The lung, liver and skeleton concentrated the greatest amount of inhaled transuranics (Table 2.10). The lung retained much less "aged" $^{238}PuO_2$ than fresh $^{238}PuO_2$ or $^{239}PuO_2$, and even less $^{244}CmO_x$. The liver and skeletal burdens were greatest for rats exposed to ^{244}Cm and "aged" ^{238}Pu; liver and skeletal burdens for $^{238}PuO_2$ rats were slightly greater than for $^{239}PuO_2$ rats (Tables 2.9 and 2.10).

About 60% of the initial alveolar lung burden of $^{239}PuO_2$ a s cleared at 30 days, as compared to 48% of $^{238}PuO_2$, 79% of "aged" $^{238}PuO_2$ and 91% of $^{244}CmO_x$.

TABLE 2.9. Ratio of Excretion in Urine, Feces and Tissue Distributions for Inhaled Transuranic Oxides at 30 Days After Exposure

Ratio	$^{239}PuO_2$	$^{238}PuO_2$	"Aged" $^{238}PuO_2$	$^{244}CmO_x$
Excreted:				
Feces/Urine	38	26	15	5.3
Tissues:				
Lung/Bone	80	43	1.2	0.4
Lung/Liver	1300	260	6.1	1.1

TABLE 2.10. Tissue Distribution of Inhaled Transuranic Oxides at 30 Days After Exposure

Tissue	Percent Terminal Body Burden			
	$^{239}PuO_2$	$^{238}PuO_2$	"Aged" $^{238}PuO_2$	$^{244}CmO_x$
Lung	89	93	42	19
Liver	0.07	0.35	6.9	18
Bone	1.1	2.1	35	46
Pulmonary Lymph Nodes	1.2	1.8	0.53	0.89
Spleen	0.08	0.08	0.34	0.25
G.I. Tract	1.6	0.64	2.2	1.9
Kidney	0.06	0.06	0.40	0.47
Skin	0.78	0.86	1.2	2.2
Remaining Tissues	6.3	1.6	11	12

Insoluble, particulate $^{238}PuO_2$ and $^{239}PuO_2$ were more readily excreted in the feces, probably being cleared via alveolar macrophages; while "aged" $^{238}PuO_2$ and $^{244}CmO_x$ were more readily absorbed into the blood and translocated to liver and skeleton or excreted in urine. Freshly prepared $^{238}PuO_2$ showed somewhat greater translocation to liver and skeleton than did $^{239}PuO_2$. Inhaled $^{244}CmO_x$ was highly soluble in the lung, irrespective of its age in water suspensions.

TOXICOLOGY OF INHALED $^{238}PuO_2$, $^{239}PuO_2$ AND $^{244}CmO_x$ IN THE RAT HIGH DOSE EFFECTS

Investigator:

C. L. Sanders

Technical Assistance:

D. M. Meier and J. D. Burruss

Groups of rats exposed to aerosols of $^{238}PuO_2$, $^{239}PuO_2$ or $^{244}CmO_x$ have been observed for distribution of radionuclides in tissues and for pulmonary pathology for periods up to a year postexposure. Plutonium-238 dioxide showed greater in vivo mobility than $^{239}PuO_2$; $^{244}CmO_x$ was highly mobile. Of the rats dying at the highest exposure levels, about 33% exhibited mostly squamous cell metaplasia and 20% mostly squamous cell neoplasia. Metaplasia developed from regions of severe alveolar scarring associated with high PuO_2 particle concentrations; neoplasms infiltrated areas of lower PuO_2 particle concentration.

The potential carcinogenicity of inhaled transuranic elements in the lung of man is a problem of recognized importance. The oxides of these elements constitute the most common chemical forms to which man may be exposed.

Five groups of 70 female. SPF rats were exposed to aerosols of $^{238}PuO_2$, $^{239}PuO_2$, or $^{244}CmO_x$. Deposition in the lung ranged over three to four orders of magnitude (Table 2.11). Initially, both $^{238}PuO_2$ and $^{239}PuO_2$ were randomly distributed throughout the lung parenchyma in particulate form, as evidenced by formation of alpha stars on autoradiograms. Autoradiograms of lungs following inhalation of $^{244}CmO_x$ showed nearly all the activity distributed as single tracks or loose aggregates of single tracks.

High mortality has occurred at the highest exposure level of each transuranic oxide. All rats exposed to

TABLE 2.11. Survival and Lung Burden of Rats Following Inhalation of Transuranic Oxides

Transuranic Oxide	Number Rats Alive	Number Rats Dead	Time Postexposure Days	Deposition in Lung at 30 Days, nCi
$^{239}PuO_2$	55	5	505	0.08
	50	10	469	0.19
	52	8	446	3.3
	82	8	428	20
	16	14	379	180(a)
$^{238}PuO_2$	59	1	328	0.013
	61	4	325	0.035
	58	2	391	3.3
	58	2	384	7.5
	34	26	296	140
$^{244}CmO_x$	65	0	230	0.022
	63	2	217	0.18
	64	1	96	4.5
	32	0	243	36
	0	23	83	380
Unexposed Controls	144	6	83-505(b)	

(a) One day after inhalation.
(b) Controls placed on experiment at staggered intervals.

$^{244}CmO_x$, with an initial alveolar burden of about 1500 nCi, were dead by 83 days after exposure. Most of the rats with initial alveolar burdens of 200 to 250 nCi $^{238}PuO_2$ or $^{239}PuO_2$ were dead at 300 days after inhalation (Table 2.11).

Plutonium-239 dioxide was more tenaciously retained in the lung than was $^{238}PuO_2$. Both $^{238}PuO_2$ and $^{239}PuO_2$ were translocated to pulmonary lymph nodes, amounting to 8% of terminal body burdens. About fivefold more ^{238}Pu than ^{239}Pu was translocated to skeleton; somewhat more ^{238}Pu than ^{239}Pu was translocated to liver (Table 2.12). These metabolic differences between the two plutonium isotopes indicated a greater in vivo mobility for $^{238}PuO_2$ than for $^{239}PuO_2$. Only about 20% of the terminal body burden of ^{244}Cm remained in the lung as compared to over 90% of Pu at comparable times after inhalation. Over 50% of the terminal body burden of ^{244}Cm was found in the skeleton, 5 to 10% in the liver, and less than 1% in the pulmonary lymph nodes, indicative of the extreme mobility of the inhaled $^{244}CmO_x$ (Table 2.12).

Pulmonary metaplasia or neoplasia was not observed in the high exposure level ^{244}Cm rats, due to their limited survival (Table 2.11). Of all rats dying from the highest doses of either $^{238}PuO_2$ or $^{239}PuO_2$, 36% exhibited metaplastic growth in the

TABLE 2.12. Distribution of Transuranic Elements Following Inhalation of the Oxide

Oxide Inhaled	Days Postexposure	Number of Samples	% Terminal Body Burden			
			Lung	Pulmonary Lymph Nodes	Skeleton	Liver
$^{244}CmO_x$	35-83	19	15	0.7	59	8.4
$^{238}PuO_2$	35-88	7	96	1.7	1.1	0.3
$^{238}PuO_2$	106-296	13	83	8.9	5.9	0.9
$^{239}PuO_2$	185-346	13	90	8.1	0.4	0.3

lung parenchyma, and about 20% exhibited pulmonary tumors. Metaplasia appeared earlier than neoplasia. About 70% of animals with metaplasia or neoplasia exhibited the squamous cell type, the remainder exhibited the bronchiolo-alveolar type (adenocarcinoma). Two rats exhibited mixed squamous cell carcinoma and bronchiolo-alveolar adenocarcinoma. Nearly all the animals at the highest dose levels died of radiation pneumonitis with severe alveolar septal fibrosis (Figure 2.9) or from lung tumors.

Squamous cell metaplasia occurred as early as 130 days after exposure, usually within scarred, high-dose areas of the lung parenchyma (Figure 2.10). At later time periods (or at higher doses) the incidence and severity of metaplasia increased, leading to carcinoma in situ, and ultimately to highly invasive squamous cell carcinoma (Figures 2.11 and 2.12).

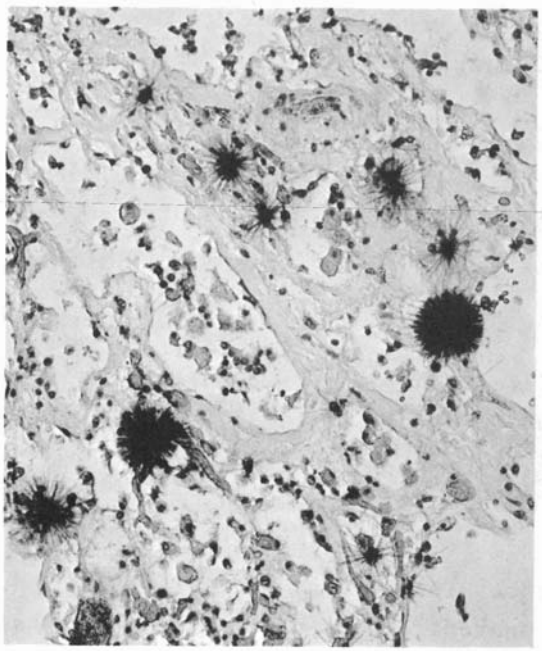

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FIGURE 2.9. Severe Alveolar Septal Fibrosis at 6 Months After Inhalation

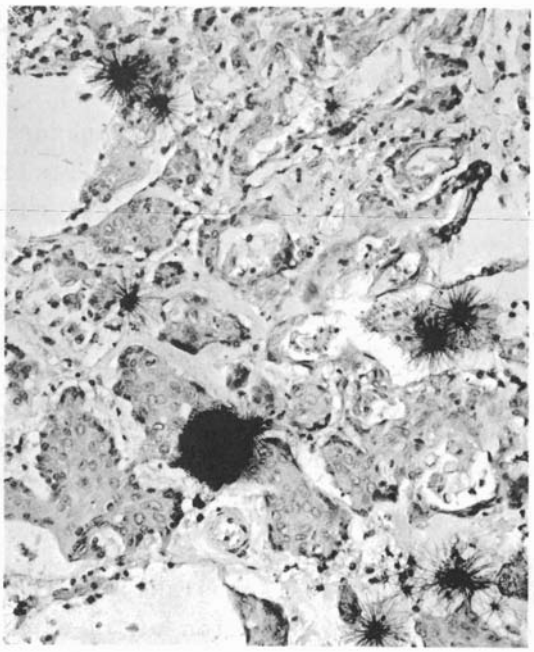

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FIGURE 2.10. Early Squamous Cell Metaplasia in Scarred Area at 5 Months After Inhalation

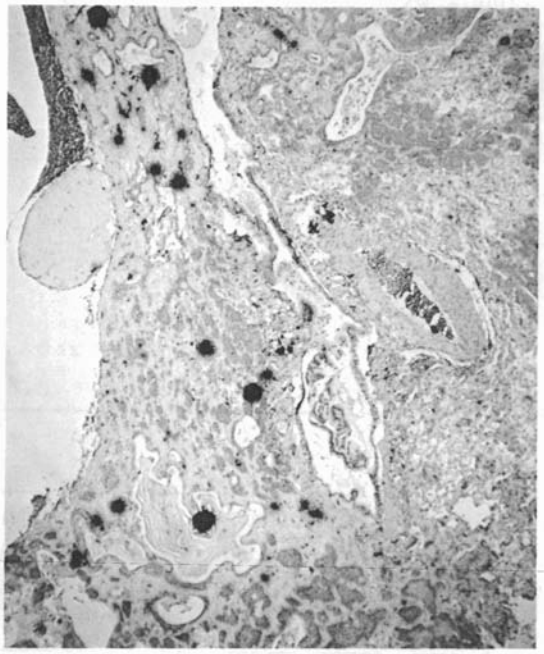

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FIGURE 2.11. Squamous Cell Metaplasia and Neoplasia Originating from Scarred Subpleural Region of Lung at 8 Months After Inhalation

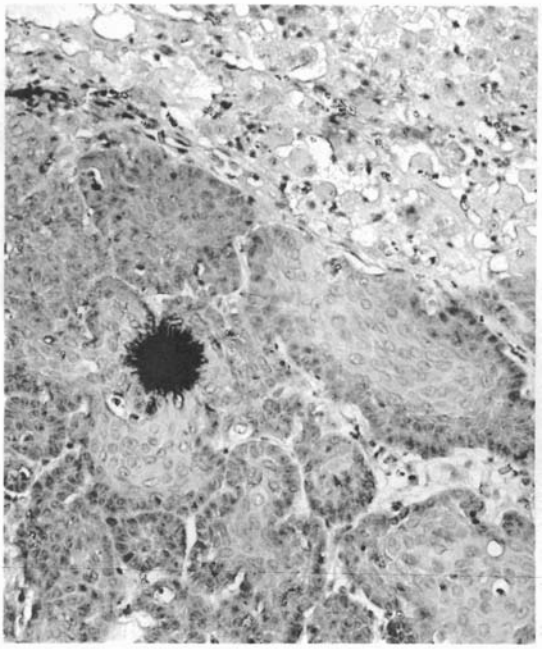

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FIGURE 2.12. Same Animal as in Figure 2.11 Showing Squamous Cell Carcinoma Surrounding a PuO_2 Particle

Autoradiograms of Lung Following Inhalation of $^{238}PuO_2$. Two week exposure, all show pulmonary regions of high PuO_2 concentration.

PULMONARY CLEARANCE AND CARCINOGENICITY OF INTRATRACHEALLY INSTILLED $^{239}PuO_2$ AND ASBESTOS

Investigator:
C. L. Sanders
Technical Assistance:
D. M. Meier and J. D. Burruss

Asbestos intratracheally instilled with $^{239}PuO_2$ causes increased pulmonary retention of plutonium within asbestiotic lesions. The early incidence of lung tumors is decreased by concurrent instillation of asbestos.

In last year's Annual Report we described the initiation of an experiment in which four groups of 70-day old, female, Wistar, SPF rats were given by intratracheal instillation: 1) physiological saline, 2) 1 mgm chrysotile asbestos, 3) 30 nCi $^{239}PuO_2$, or 4) 1 mgm chrysotile asbestos plus 50 nCi $^{239}PuO_2$. In this report we describe some preliminary results from this continuing experiment.

The retention of $^{239}PuO_2$ in the lungs of rats also given asbestos was considerably greater than that seen in rats given only $^{239}PuO_2$ (Table 2.13). Translocation of ^{239}Pu to pulmonary lymph nodes at times long after instillation was greater in animals given PuO_2 plus asbestos than in those given only PuO_2. The average radiation dose delivered to the lungs of rats given PuO_2 plus asbestos was about seven times greater than the dose to the lungs of rats given only $^{239}PuO_2$.

Most of the animals in all groups are still alive and no great differences in survival times are as yet apparent (Table 2.14). However, the

TABLE 2.13. Retention of ^{239}Pu in Lung and Pulmonary Lymph Nodes Following Intratracheal Instillation of $^{239}PuO_2$ With and Without Asbestos. (Values are percent of initial alveolar deposition ± standard deviation.)

	$^{239}PuO_2$ Only	$^{239}PuO_2$ + Asbestos
Lung		
30 days	48212	66k4.5
300-700 days	4.6 ± 4.0	26 ± 7.9
Lymph Nodes		
30 days	2.5 ± 1.3	1.1 ± 0.3
300-700 days	1.2 ± 0.6	7.3 ± 2.8

TABLE 2.14. Mortality and Lung Tumor Incidence After 700 Days

Treatment	Mortality Dead/Total	Number of Dead Rats with Lung Tumors
Saline	14/26	0
Asbestos	7/22	1
$^{239}PuO_2$	9/22	4
$^{239}PuO_2$ + Asbestos	9/27	

incidence of lung tumors in dead rats given only $^{239}PuO_2$ is about four times that seen in animals given either asbestos or asbestos plus $^{239}PuO_2$ (Table 2.14). Both bronchiolo alveolar adenocarcinomas and,squamous cell carcinomas were induced by PuO_2 alone. Single, undifferentiated carcinomas were seen in groups given asbestos or asbestos plus PuO_2. All but one of the observed pulmonary tumors was highly metastatic throughout the lung, two showing growths in the pleural cavity and one in the peritoneal cavity and liver.

PuO_2 particles given with asbestos were mostly confined within small asbestos-induced, scar tissues in peribronchiolar regions of the lung parenchyma (Figure 2.13), markedly limiting the number of epithelial "target" cells within range of the alpha emissions, when compared to the numbers of cells that may be "hit" when PuO_2 is given by itself. These early results clearly demonstrate the importance of dose distribution in alpha radiation-induced carcinogenesis in the lung.

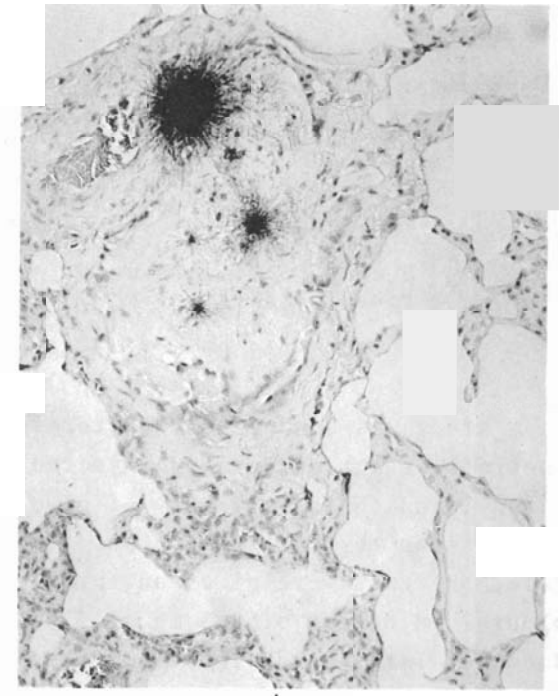

FIGURE 2.13. Autoradiogram Demonstrating the Localization of $^{239}PuO_2$ Particles in Asbestos-Induced Scar Tissue in Peribronchiolar Region of Lung, at 653 Days After Intratracheal Instillation of $^{239}PuO_2$ Plus Asbestos

DISTRIBUTION AND CARCINOGENICITY OF INTRAPLEURALLY INJECTED $^{239}PuO_2$

Investigator:
C. L. Sanders
Technical Assistance:
D. M. Meier and J. D. Burruss

Intrapleurally injected $^{239}PuO_2$ was rapidly cleared into peritoneal and thoracic lymphatics. As compared with controls, tumor incidence was increased and median survival time decreased. Effects were less marked than following intraperitoneal injection.

A study was conducted to determine the fate of intrapleurally injected $^{239}PuO_2$ and the sensitivity of pleural tissues to tumor induction. Female, SPF rats were given an intrapleural injection of 0.5 mg, 1.3 µm diameter latex beads in 0.5 ml physiologic saline, or an intrapleural injection of 800 nCi $^{239}PuO_2$, approximately 0.2 µm diameter particles in 0.5 ml physiologic saline.

Injected $^{239}PuO_2$ was initially concentrated in subpleural lymphatics of the lung and in lymph nodes in the thoracic cavity. The largest amount of activity at 30 days after injection was found in the liver (Table 2.15), indicating a rapid removal of PuO_2 particles from the pleural cavity, possibly by thoracic lymphatics. The amount of ^{239}Pu in lung and liver decreased by 1 to 2 years after injection, while that found in other thoracic tissues, principally lymph nodes, stayed the same (Table 2.15).

TABLE 2.15. Distribution of ^{239}Pu Following Intrapleural Injection of $^{239}PuO_2$

Tissue	% Initial ^{239}Pu Deposition	
	30 Days[a]	300-700 Days[a]
Lung	8.2	4.8
Thoracic Tissues[b]	10.8	12.0
Liver	29.8	10.3
G.I. Tract	0.3	1.2
Spleen	1.7	2.1
Skeleton	0.8	1.7

(a) Time after injection.
(b) Includes trachea, main stem bronchi, heart, thymus and thoracic lymph nodes.

All rats injected with PuO_2 have died, with a median survival time of 575 days after injection; half the rats given latex beads have died, with a median survival time of 717 days (Table 2.16). No tumors in the thoracic cavity were seen in animals given latex beads, as compared to two tumors in the thoracic cavity

TABLE 2.16. Survival and Tumor Induction Following Intrapleural Injection of Latex Beads or $^{239}PuO_2$

Treatment	% Dead Rats	Median Survival Time, Days	Tumor Incidence, % Thorax	Tumor Incidence, % Total[a]
Latex Beads	50 (14/28)[b]	717	0	7.1
$^{239}PuO_2$	100 (27/27)[b]	575	7.4	30

(a) Dead rats, all but mammary tumors
(b) Number dead rats/total number of rats

in rats given PuO_2, including one pericardial mesothelioma and one sarcoma; the mesothelioma was associated with an area of high PuO_2 concentration (Figure 2.14).

A peritoneal fibrosarcoma was seen in the rats given latex beads as compared to six abdominal tumors (pheochromocytoma, leiomyosarcoma of urinary bladder, two peritoneal carcinomas of unknown origin, subcutaneous melanoma and adrenal cortical carcinoma) in PuO_2 injected rats.

These results indicate that mesothelioma and sarcoma can be induced by PuO_2 deposited in the pleural cavity. However, the incidence of these tumors from intrapleurally injected $^{239}PuO_2$ is much lower than that seen following injection of comparable amounts of $^{239}PuO_2$ into the peritoneal cavity. The differences may be due to the limited concentration of PuO_2 into "hot spots" in the pleural cavity, or to the rapid clearance of PuO_2 from the pleural cavity by thoracic lymphatics, as well as to possible differences in tissue sensitivity.

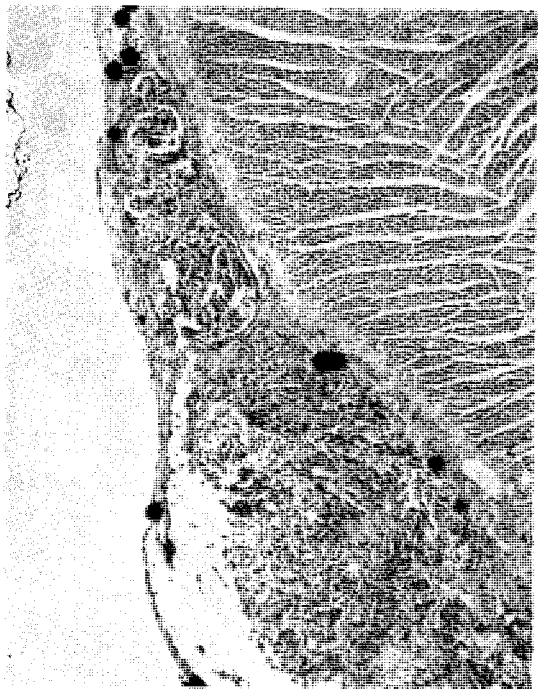

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FIGURE 2.14. Autoradiogram of Pericardial Region Demonstrating Induction of Mesothelioma in Region of $^{239}PuO_2$ Particle Localization, 391 Days After Intrapleural Injection.

CLEARANCE OF PuO_2 FROM MEDIASTINAL LYMPH NODES

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

Plutonium dioxide was translocated from the peritoneal cavity to the mediastinal lymph nodes of rats. Significant clearance of PuO_2 was observed from these lymph nodes with an estimated half-life of about 1 year. No tumors developed from these PuO_2-laden lymph nodes.

Inhaled PuO_2 particles are slowly translocated from lung to pulmonary lymph nodes. The resulting concentration of plutonium in pulmonary lymph nodes is often much greater than that in lung, which raises the question of whether pulmonary lymph nodes might properly be considered the "critical tissue" following PuO_2 inhalation. An uncertainty in this picture is the retention of the plutonium in lymph nodes. To study this problem, we have injected $^{239}PuO_2$ into the peritoneal cavity of rats. A significant fraction of this PuO_2 will be transported to mediastinal lymph nodes, in which its retention may be followed by serial sacrifice.

Female, Sprague-Dawley CD and Wistar SPF, 65- to 90-day-old rats were given a single, intraperitoneal injection of 360, 2000 or 2900 nCi $^{239}PuO_2$, suspended in 1 ml of isotonic saline; mean particle diameters were about 0.2 μm. The thymus gland and closely associated mediastinal lymph nodes were analyzed for plutonium content by liquid scintillation counting, and qualitatively by autoradiography, at intervals up to 600 days after injection.

The autoradiograms demonstrated a selective uptake of PuO_2 into mediastinal lymph nodes, with very little plutonium appearing in other extrapulmonary tissues of the thoracic cavity. The mediastinal lymph nodes showed severe fibrosis and destruction of nearly all lymphoid elements within a few months after injection of PuO_2.

Accumulation of PuO_2 by mediastinal lymph nodes was completed within 50 days after injection; a maximum of about 5% of injected plutonium being attained (Figure 2.15). Subsequently, there was a trend of decreasing lymph node burden with time, the amount present in individual samples being quite variable. Accumulation and retention of PuO_2 by mediastinal lymph nodes was apparently not influenced by the amount injected over the eightfold range studied. Lymph node plutonium content decreased to about 1.0% of injected dose by 600 days after injection. Regression analysis showed that clearance was significantly

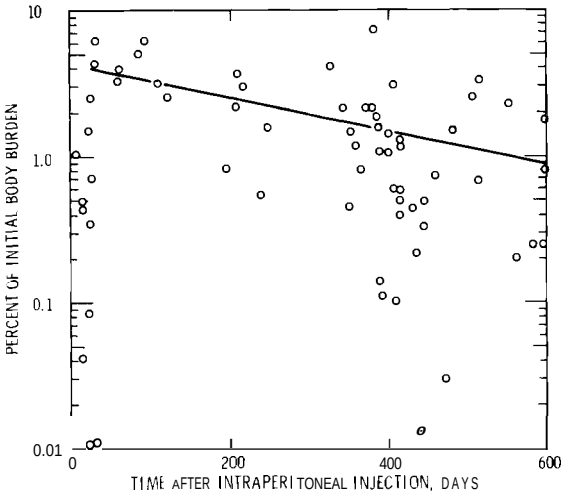

FIGURE 2.15. Uptake and Clearance of Intraperitoneally Injected $^{239}PuO_2$ Particles by Mediastinal Lymph Nodes in the Rat. (Each point represents data from one animal.)

greater than zero at the 0.01 probability level. The half-life of PuO_2 in the lymph nodes was estimated at about 1 year.

No tumors derived from mediastinal lymph nodes were seen in about 250 rats observed for their life-span following intraperitoneal injection of $^{239}PuO_2$, indicating that these nodes are not "critical" for tumor induction despite their heavy concentration of $^{239}PuO_2$. Clearance of PuO_2 from these lymph nodes was more rapid than had been expected. Further studies, in progress, with inhaled transuranic oxides in the rat and hamster should help to clarify this problem.

EARLY DISPOSITION OF INHALED PLUTONIUM AND EINSTEINIUM IN THE RAT

Investigator:

J. E. Ballou

Technical Assistance:

R. A. Gies, W. G. Morrow and E. F. Blanton

The early postexposure urinary excretion of inhaled plutonium and einsteinium compounds appears to be influenced by the radiation dose to the lung. The rate of lung clearance, on the other hand, appears to be related to the mass of material deposited. Lung burdens of less than 1 nCi plutonium were cleared most rapidly with relatively large amounts excreted in urine during the first week after inhalation exposure.

Fifteen hundred male, Wistar rats were exposed, nose only, for 30 min to aerosols of $^{239}Pu(NO_3)_4$, $^{238}Pu(NO_3)_4$, $^{253}Es(NO_3)_3$, $^{253}Es(OH)_3$ as a preliminary to long-term biological effects studies in beagle dogs. The initial lung burdens ranged from

0.06-920, 0.02-1500, 325-520 and 200-650 nCi, respectively, for the various compounds. Lung burdens were determined by sacrificing animals immediately after exposure.

The fraction of the lung burden excreted in urine during the first postexposure week was significantly higher in those animals with the lowest lung burdens of ^{239}Pu and ^{238}Pu (0.058 and 0.0203 nCi, respectively). This relationship also held for daily excretion on the first and seventh days after inhalation exposure. The explanation for the differences in urinary excretion is not readily apparent; however, it seems to be related to the radiation dose rather than to the mass of material inhaled. That is to say, comparable nCi amounts of inhaled ^{238}Pu, ^{239}Pu and ^{253}Es, representing several orders of magnitude difference in mass, showed similar patterns of urinary excretion. This effect of the magnitude of the initial lung burden on the fraction excreted the first week after exposure is shown in Figure 2.16. Values for $^{239}PuO_2$, taken from the work of Sanders, show a similar relationship to that seen with the more soluble compounds used in this study.

The rate of early lung clearance (Figure 2.17), in contrast to the urinary excretion pattern, is clearly influenced by the mass of material initially deposited in the lung. Plutonium-238 was translocated more rapidly than ^{239}Pu when lung burdens exceeded 1 nCi (~16 ng ^{239}Pu). Lower doses of the plutonium isotopes were

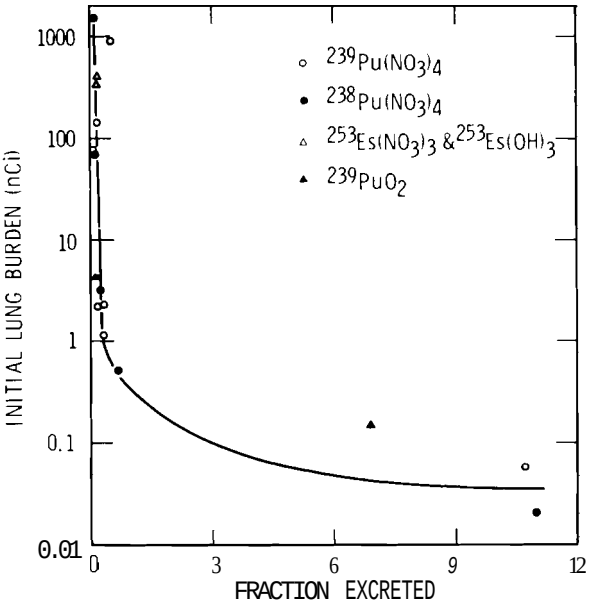

FIGURE 2.16. Fraction of Initial Lung Burden Excreted in First Week

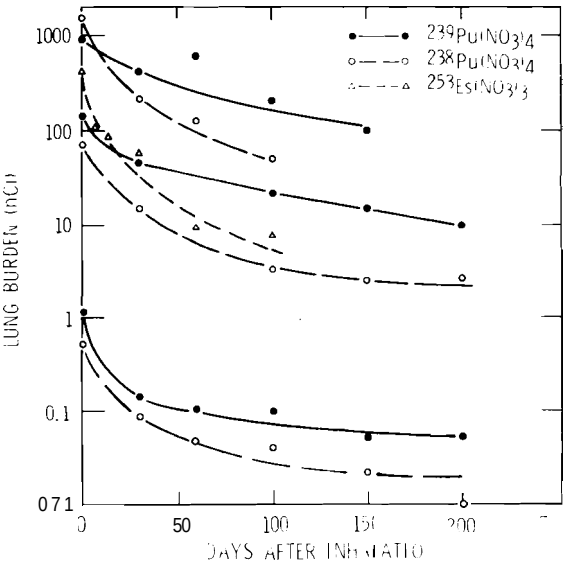

FIGURE 2.17. Effect of Lung Burden on Lung Clearance

cleared at about the same rate. The most rapid lung clearance was observed with ^{253}Es, a trivalent transuranic element with a comparatively high specific activity.

If low plutonium burdens in the range of 0.1 to 1 maximum permissible lung burdens are somehow more readily excreted or translocated from the lung, one should expect greater relative deposition in the nonpulmonary tissues of these animals. This was found to be the case when tissues were analyzed radiochemically. The values for plutonium in the skinned and eviscerated carcass (mostly muscle and skeleton) either equalled or exceeded the lung burden in the two low dose groups. With higher initial burdens the deposition in carcass was relatively lower in keeping with the lower fraction removed by urinary excretion. These results suggest that inhaled plutonium lung burdens estimated from early urinary excretion data may be too high, particularly in the dose ranges of practical interest. In point of fact, human exposure cases frequently show unexpected high values for urinary plutonium excretion during the first few days after exposure. The plutonium values in urine collected 10 to 14 days after exposure are generally applied to the Langham excretion model since these latter data are thought to be more predictive of the plutonium body burden.

Tissues from all exposure groups analyzed immediately after the 30-min aerosol exposure showed the highest radionuclide deposition in pelt, GI tract and lung. Retention in the upper respiratory tract (represented by trachea, nose and head) was surprisingly low apparently because the radionuclides were cleared from these tissues to the GI tract, blood or to the pelt during the inhalation exposure. The particle size distribution appeared to have little influence on the site of deposition. This was observed despite a fivefold range in AMAD during the 42 separate aerosol exposures. The only suggestion of a particle size effect was seen with the highest $^{239}Pu(NO_3)_4$ level where the AMAD was >2. An exceptionally large amount of ^{239}Pu was retained in the nose as might be predicted because of the large particle size.

DISPOSITION OF INHALED AND INTRATRACHEALLY INSTILLED ^{253}Es IN RATS

Investigator:
J. E. Ballou
Technical Assistance:
R. A. Gies and W. G. Morrow

The pattern of lung clearance and translocation to bone was similar for intratracheally instilled $^{253}EsCl_3$, inhaled $^{253}Es(NO_3)_3$ and inhaled $^{253}Es(OH)_3$. Instilled Es(OH)3, given at pH 12, was more rapidly cleared and deposited less in bone. The long-term biological effects of inhaled and instilled ^{253}Es are expected to be about the same in bone, but may be different in lung because of differences in Sung dose distribution.

Preliminary biological effects studies with ^{253}Es employed intratracheal administration rather than inhalation exposure because of the limited quantity of ^{253}Es available. The long-term effects of the intratracheally instilled radionuclide (principally radiation pneumonitis and bone tumors) were reported in the previous Annual Report. The observation that bone tumors were the major neoplastic lesion, rather than lung tumors that might have been expected after incorporation via the lung, suggested the need for further investigations with inhaled einsteinium compounds.

Studies reported here compare the retention and distribution of intratracheally instilled $^{253}EsCl_3$ (pH 2) and $^{253}Es(OH)_3$ (pH 12) with that after inhalation of aerosolized $^{253}Es(NO_3)_3$ (0.27N HNO_3) and $^{253}Es(OH)_3$ (pH 7). Retention of the inhaled and instilled compounds in lung is shown in Figure 2.18. With the exception of the instilled $^{253}Es(OH)_3$ all compounds and methods of administration showed similar lung retention kinetics. The basicity (pH 12) of the instilled

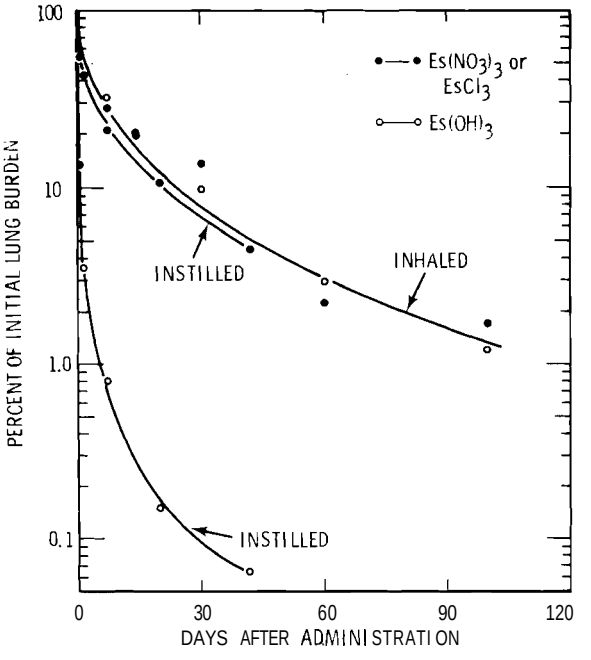

FIGURE 2.18. Clearance of Intratracheally Instilled and Inhaled Einsteinium Compounds from Lung. (Data are corrected for physical decay.)

$^{253}Es(OH)_3$ suggests that lung damage may have been a factor in the accelerated lung clearance observed with these animals.

The build-up and retention of einsteinium in the skeleton (Figure 2.19) was also closely similar for all groups except those given the more basic $Es(OH)_3$ by intratracheal instillation. The values for ^{253}Es in bone were two orders of magnitude lower in the $^{253}Es(OH)_3$ group although translocation from the lung was more extensive, as seen in Figure 2.18. The more basic material was apparently excreted rather than deposited after being cleared from the lung; however, a material balance study was not made to confirm this.

On the basis of these results it is expected that instilled and inhaled $^{253}Es(NO_3)_3$ will produce about the same radiation dose in bone, but that lung will receive a more uniform distribution after inhalation exposure. The potential for bone tumor induction should be about the same after either method of administration, but the potential for lung tumor induction may be quite different Studies are now in progress to investigate these possibilities.

The distribution and translocation of ^{253}Es was similar after inhalation of both $^{253}Es(NO_3)_3$ and $^{253}Es(OH)_3$ aerosols. The material initially deposited in lung was rapidly translocated to skeleton which was the major reservoir of ^{253}Es after about

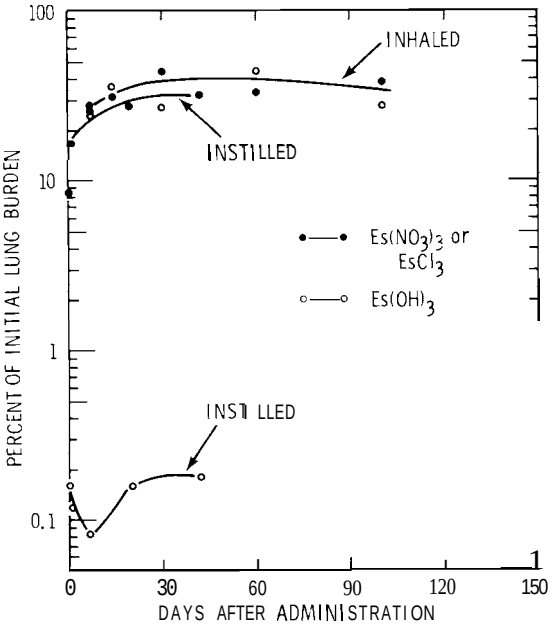

FIGURE 2.19. Clearance of Intratracheally Instilled and Inhaled Einsteinium Compounds from the Skeleton. (Data are corrected for physical decay.)

2 weeks. The concentration of ^{253}Es in lung and thus the radiation dose rate, exceeded that in other tissues for the first 30 days after exposure. The total absorbed radiation dose was also highest in lung because the short physical half-life of ^{253}Es (20.5 days) precludes the long-term accumulation of dose in tissues such as bone which retain the translocated radionuclide. Although the concentration of ^{253}Es in skeleton eventually exceeded that in lung, by this time physical decay had reduced the ^{253}Es level by a factor of eight and the cumulative radiation dose to bone amounted to only one-fifth that in lung.

HISTOPATHOLOGIC EFFECT OF INTRATRACHEALLY INSTILLED $^{253}EsCl_3$ IN RATS

Investigators:

J. E. Ballou, R. H. Busch and G. E. Dagle

Technical Assistance:

R. A. Gies and W. G. Morrow

The major pathologic lesions produced by intratracheally instilled $^{253}EsCl_3$ were: 1) radiation pneumonitis at a dose of 47.2 µCi/kg, 2) osteosarcomas at a dose of 10.7 µCi/kg, and 3) soft tissue tumors, including lung tumors, at a dose of 0.214 µCi/kg.

Male Wistar rats were administered $^{253}EsCl_3$ by intratracheal instillation and the biological effects were followed for the animal's life-span or 880 days. The disposition of ^{253}Es and the early biological effects were reported in the 1972 Annual Report. This report presents the results of studies still in progress to determine the long-term biological effects in these animals.

Table 2.17 summarizes the experimental protocol and lists the estimated radiation dose to major organs.

The most frequent lesion and usual cause of death in the high level animals was radiation pneumonitis. Two tumors were found in this group; an osteogenic sarcoma 180 days after instillation and a benign tumor of the adrenal cortex. Approximately one-third of the rats survived beyond 180 days so they are presumed to have been subject to bone tumor induction but did not develop this lesion.

TABLE 2.17. Experimental Protocol[a]

Administered Dose, µCi/kg	Number of Rats	Estimated Cumulative Radiation Dose, rads			
		Lung	Kidney	Liver	Skeleton
47.2	29	15,000	1,000	250	3,000
10.7	48	3,000	200	50	600
0.214	48	60	5	1	15
HCl pH 2	43	---	---	---	---

(a) The experiment was terminated after 880 days. At this time nine rats were sacrificed; four that received 0.214 µCi/kg and five HCl control rats.

At least 30 malignant tumors and 10 benign tumors were found in the middle exposure group (10.7 μCi/kg). Twenty of the malignancies were identified as osteosarcomas, four were leukemias and three tumors were primary to the lung (two adenocarcinomas and one hemangiosarcoma). The first bone tumor was found in a rat that died 240 days after ^{253}Es administration. All but four of the rats in this group lived 240 days or longer. The three primary lung tumors occurred later in the experiment, about 1 year after the initial bone tumor was observed. Ten rats lived at least this long and were considered potential candidates for lung tumors.

The limited information now available for rats given 0.214 μCi/kg indicates that bone tumors will not be a major lesion at this level; however, soft tissue tumors including primary lung tumors have been identified. The significance of these lesions cannot be established until the examination of control tissues is completed.

EFFECT OF INHALED $^{239}PuO_2$ ON LUNG LIPIDS

Investigators:

E. G. Tombropoulos, J. G. Hadley, J. M. Thomas and D. K. Craig

Technical Assistance:

E. F. Blanton

Rat Lung mitochondria showed a significant increase in rates of lipid biosynthesis (as measured by palmitate incorporation) 1 day following exposure to $^{239}PuO_2$ aerosol at average lung depositions above 23 to 40 nCi.

In previous studies we demonstrated that increased lung mitochondrial lipid biosynthesis occurred as the earliest observed effect of 800 R thoracic X-irradiation in rats. We have now performed experiments to assess the effects of the alpha-irradiation associated with plutonium on lung lipid biosynthesis. These experiments were based on the premise that both the uniformly distributed X-irradiation and the randomly distributed alpha-irradiation from plutonium particles would cause similar changes to the lung's biochemical potential.

Since we wished to determine not only if an effect occurred, but also the approximate threshold level of effect, rats were exposed by inhalation to $^{239}PuO_2$ to obtain initial lung doses between 1 and 500 nCi.

Serial sacrifices were made of exposed and sham-exposed animals, usually six to ten at any sacrifice time. Deposition of $^{239}PuO_2$ was measured in the excised tissue at the time of death and a mitochondria1 fraction was prepared from the excised lung. Lung mitochondria from both sham-exposed and experimentally treated animals were incubated with ^{3}H-palmitate and the extent of incorporation of the palmitate into various lipid classes measured at the end of 40 min of incubation.

Because of the small number of replicate rats used at each time in individual experiments, we evaluated the results using Scheffe's randomization test; we calculated one-tailed probabilities since our initial experiments suggested a numerical increase in lipid incorporation by mitochondria from treated animals. These probabilities are exact and involve only the single assumption that both treated and control animals are from the same unknown distribution. Table 2.18 presents the results from these experiments. Lung depositions of 22 to 400 nCi appear to be associated with significantly increased lipid biosynthesis by the isolated lung mitochondria. Examination of the various lipid classes (monoglycerides, diglycerides, triglycerides, cholesterol esters, lecithin, and phosphatidylethanolamine) revealed no preferential stimulation of incorporation for any particular lipid class. This stimulated palmitate incorporation is similar to that observed following thoracic X-irradiation.

TABLE 2.18. Lipid Biosynthesis by Rat Lung Mitochondria as Affected by Inhalation of ^{239}Pu

^{239}Pu Deposited, nCi	Time Postexposure, days	Means (n = 6 to 10) nmoles ^{3}H-palmitate Incorporated/mg Protein		Signed Difference	P Value
		Sham-Exposed	$^{239}PuO_2$-Exposed		
Low Level					
1.2	1	9.7	9.5	-0.2	0.47
0.42	14	8.6	10.3	+1.7	0.27
0.347	71	3.7	4.1	+0.4	0.23
1.7	157	4.1	8.9	+4.8	0.16
Medium Level					
23	1	36.9	119.8	+83	0.05
42	1	9.7	16.9	+7.2	0.06
High Level					
154.2		36.9	108.3	+71	0.05
419.4		43.6	61.6	+18	0.05

The increased lipid synthesis by lung mitochondria isolated from animals exposed to either medium or high doses of $^{239}PuO_2$ is statistically significant ($P < 0.05$ or $P < 0.06$). A similar conclusion (exposure-dependent increase in palmitate incorporation by lung mitochondria) may be warranted at lower depositions (less than 2 nCi) since smaller P values are related to increased post-exposure times, even though no individual study exhibits the usually accepted level ($P < 0.05$) for rejection of the hypothesis of no difference. The low dose data presented here are highly equivocal and more experimentation is needed to establish a threshold level. Biochemical effects at the 23 nCi level of plutonium deposition, as early as 1 day postexposure, have not been previously reported.

As the biochemical potential of lung mitochondria probably results in the synthesis of some numerical fraction of total lung lipid synthesized, regardless of class, we investigated the effects of PuO_2 on the ability of lung to incorporate and turn over palmitate in the physiologically important lipid: lecithin (the lipid component of lung surfactant). Following injection with ^{3}H-palmitate, animals from the eight deposition groups shown in Table 2.18 were sacrificed at various time intervals (0 to 72 hr), and lecithin isolated from lung lavage fluid. The time intervals of sacrifice covered both uptake and disappearance of the injected palmitate into the lecithin fraction. As a limited number of animals and timepoints were employed in this study, the data were analyzed by simply calculating the signed difference between the means of control and treated animals at common times and statistically assessing all eight experiments at once using the binomial distribution.

The overall analysis of the metabolism of lung lavage lecithin implied that there were statistically significant differences ($P < 0.05$) between the experimentally treated and control group animals. After 45 hours these differences were positive with respect to the control, and may be the result of one or any combination of the following factors:

1) Differences in rate of transfer of palmitate from blood to lung.
2) Differences in initial and/or final lecithin pool size.
3) Differences in rates of degradation of lung lavage lecithin.

Further experimentation is needed to differentiate between these possibilities.

TRANSMISSION OF RADIATION-INDUCED TUMORS IN BEAGLE DOGS

Investigators:

M. E. Frazier and R. H. Busch

Technical Assistance:

T. K. Andrews

Tumors induced in dogs by inhaled plutonium were cultured and transmitted to 31-to 33-day-old dog fetuses in utero. Tumors developed in the pups showing similarities to the donor tumor. Studies are continuing to characterize the transmitted tumor and identify the sources, i.e., whether of graft or host origin.

Cell cultures have been prepared from lung and bone tumors arising in beagle dogs following exposure to inhaled plutonium. Evaluation of the cultured cells by all commonly applied criteria (i.e., cell morphology, lack of contact-inhibitory mechanisms, cloning efficiency, and growth in soft agar) indicated that tumor cells were being grown in culture. However, even though all lines of evidence indicated that these cells were indeed tumor cells the final test required that they be transmitted in vivo. Therefore, the following experiments were initiated to test the oncogenic potential of these cell cultures.

Primary cell cultures were prepared from radiation-induced osteogenic sarcomas of beagle dogs utilizing standard tissue culture procedures. Following the in vitro examination of these cultures for characteristics of tumor cells, the cells were inoculated into canine fetuses in utero. Approximately 2×10^6 cells, from fifth passage cell cultures, were inoculated into each 31- to 33-day fetus. Of the seven pups born to two bitches, three were born dead. Shortly after birth (within 2 to 7 days) palpable growths developed in all of the living pups. The two dogs injected with tumor cells from dog 445 were sacrificed, due to extensive metastasis and impending death, 14 days after birth. The two pups that received cells from dog 469 also developed tumors; however, it was not necessary to sacrifice these animals until they were 1 to 2 months old.

All of the pups developed large tumor masses at the site of inoculation. Similarly, they all had widespread metastases. These metastatic tumors involved muscle, lymph nodes, mandible, scapula, and various long bones. Histopathology reports indicated that the tumors bore a striking resemblance to the tumors from the

donor dogs. There was osteoid formation in the transmitted tumors and they were diagnosed as osteogenic sarcomas.

The tumors from the inoculated pups were again grown in cell culture and investigations have begun to compare their growth characteristics with those of the original implanted cells. Also, since the sex of some of the recipient dogs is different from the sex of the donors, we can determine, by chromosome analysis, whether the tumors in the pups were host or graft in origin. Finally, both original and transmitted tumors are being examined by electron microscopy for evidence of viruses.

While there are some reports in the literature of canine tumors in cell culture and transmission, to our knowledge there are no reports of radiation-induced bone or lung tumors that have been grown in culture and subsequently transmitted in vivo. Several cell cultures from lung and bone tumors, in addition to the one described, have been established in this laboratory and we have now begun transmission of these tumors. Future efforts will center around attempted cell-free transmission as well as the whole cell transmission of these tumors in an effort to establish model systems for investigating the mechanisms of radiation-induced lung and bone tumors. Such transmissible tumors would allow us to examine and compare the pathology and development of these tumors in animals not exposed to radiation and thus, allow the separation of the pathology of the radiation effects from the pathology of the tumor. Finally, if viruses are involved in the development of these tumors, we will utilize these model systems to investigate how viruses and radiation act in concert to produce the malignancies observed in the plutonium-exposed animals.

PRELIMINARY INVESTIGATIONS WITH INHALED ^{85}Kr IN THE RAT AND BEAGLE DOG

Investigators:

J. E. Ballou and W. C. Cannon

This report summarizes preliminary efforts to develop techniques for studying the inhalation toxicology of radioactive gases. Results are shown for rats and dogs exposed "nose only" to an atmosphere containing ^{85}Kr.

An aerosol exposure chamber was assembled which permitted simultaneous "nose only" exposure of several rats and one dog to a recirculating airstream containing ^{85}Kr. Respiratory moisture and CO_2 were removed by adsorption and oxygen was added as required to maintain physiological conditions.

Six adult rats (three male and three female) and one 11-year-old female dog were exposed for 3 hours to an airstream containing 160 μCi ^{85}Kr/l. The rats were restrained during exposure and subsequent total body counting. The dog was tranquilized using acetylpromazine to facilitate handling during exposure and later chest counting using two gamma detectors placed on the lateral surfaces of the thorax. The total body retention of ^{85}Kr in rats and thorax retention in the dog is shown in Figure 2.20. Retention in the rats can be reasonably described by a single exponential function with a clearance half-time of 20 min. This compares favorably with values that

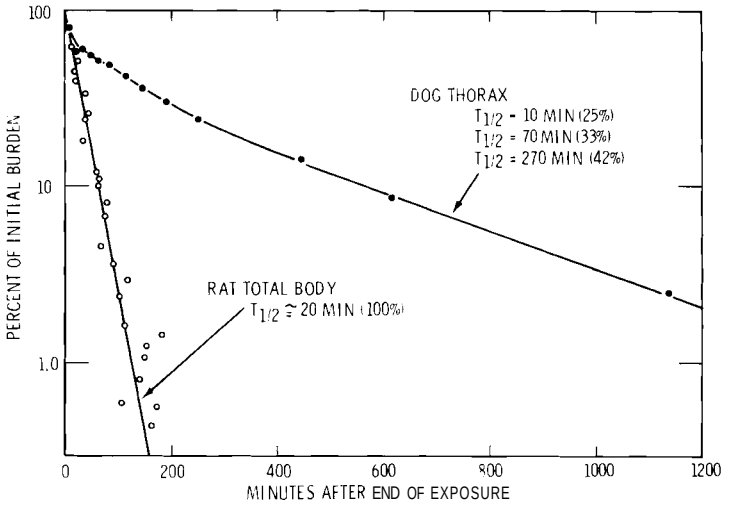

FIGURE 2.20. Clearance of Inhaled ^{85}Kr from a Rat and a Dog

have been estimated for man. The whole body partition coefficient (concentration ^{85}Kr in rat/concentration ^{85}Kr in airstream) was 0.08 for 345-g female rats and 0.05 for 527-g males.

Krypton retention in the dog thorax was more prolonged than in the total rat except for about 25% of the thorax burden which cleared with a 10-min half-time. The longer retention components in the dog may reflect a species difference, an effect of age or other physiological factors, or may be due to the use of the immobilizing drug acetylpromazine. Clearance from another dog that died shortly after exposure was considerably more prolonged than for the live dog illustrated in Figure 2.20. The loss of ^{85}Kr by diffusion from the dog carcass was clearly a much slower process than loss occurring via the breath.

FATE OF INHALED HIGH-FIRED BERYLLIUM OXIDE IN RATS AND HAMSTERS

Investigators:

C. L. Sanders, W. C. Cannon, G. J. Powers and R. R. Adee

Technical Assistance

J. D. Burrus and D. M. Meier

Rats and hamsters were exposed to aerosols of high-fired BeO. The BeO particles were rapidly phagocytized by alveolar macrophages which showed marked structural alterations; other pulmonary cells were not affected. BeO was tenaciously retained in the Lung, less than 20% of the alveolar deposit being cleared in 63 days.

Male and female rats and hamsters were exposed to aerosols of BeO, prepared by heating at 1000°C. Animals were sacrificed at intervals during the first 63 days after exposure for light and electron microscopic examination of tissues, for enumeration of cells in lung lavages, and for analyses of beryllium contents by atomic absorption spectroscopy.

Significant biological effects were limited to the alveolar macrophage in the lung. Macrophages exhibited a somewhat vacuolated appearance within a few weeks after exposure, BeO particles being seen within many of these vacuoles. Within a few more weeks, these macrophages had become greatly enlarged, with a highly vacuolated, foamy cytoplasm containing lysosomal

material, BeO particles and various cellular debris (Figure 2.21). Surprisingly, there was no significant reduction in numbers of cells found in lung lavages. Only from 2 to 5% of the lung Be content could be removed by 2 × 10 ml saline lavages. Yet BeO particles were seen with the

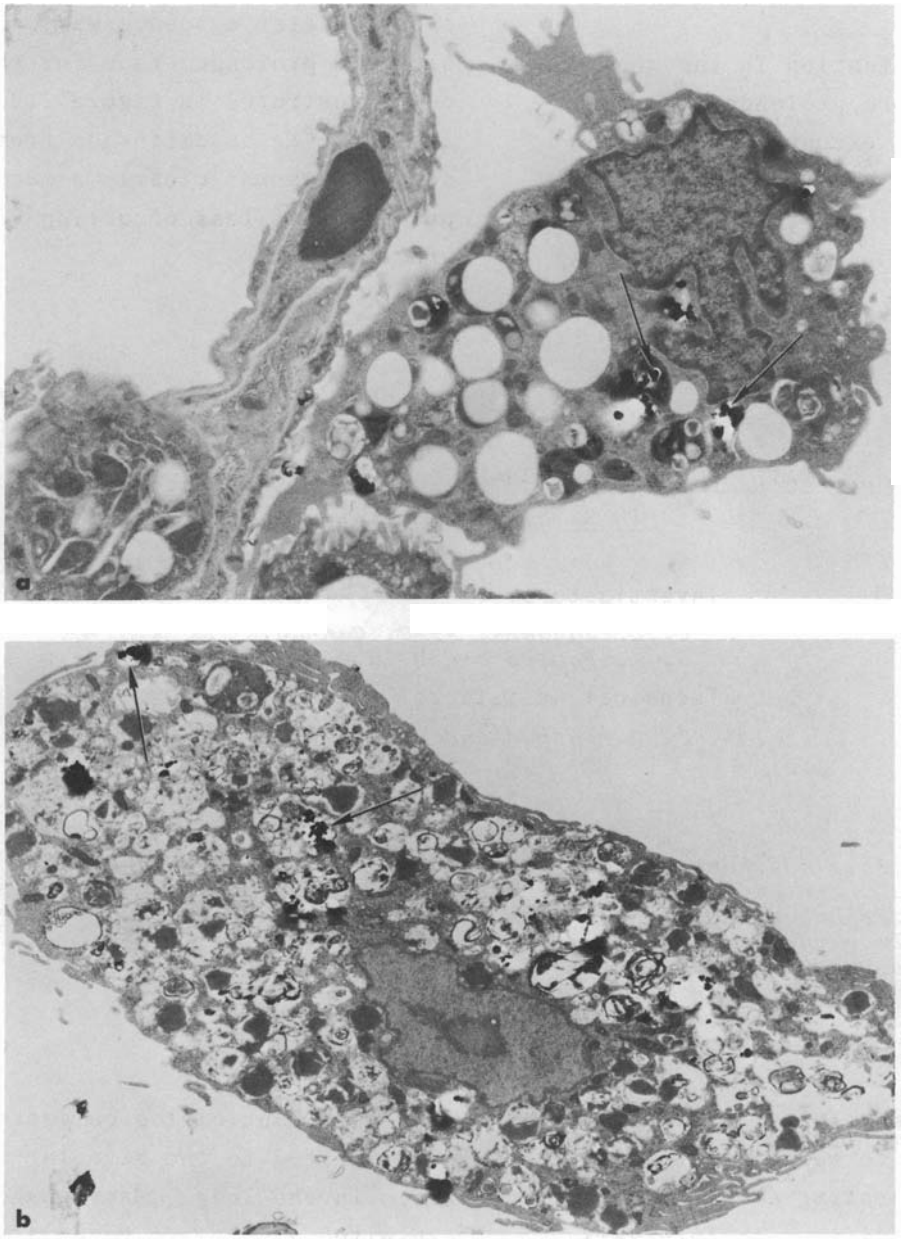

FIGURE 2.21. Electron Micrographs of Alveolar Macrophages Following Inhalation of BeO Particles. (Arrows point to subcellular location of BeO particles within phagolysosomal structures.)
a. 21 days after exposure. 7,950X
b. 49 days after exposure. 7,950X

electron microscope only within alveolar macrophages and occasionally in alveolar air spaces. Thus damage to macrophages by BeO interferred with their removal by lavage.

About 20% of inhaled BeO (mass median aerodynamic diameter, 1.0 μm) was deposited in the alveoli. In rats, only 16% of initial alveolar beryllium burden was cleared from the lung by 63 days after exposure, as determined by excreta analyses. Most lung clearance in both rats and hamsters occurred during the first week after exposure (Table 2.19).

Small amounts of beryllium were translocated to pulmonary lymph nodes of rats, amounting to 1 to 2 μg beryllium. No detectable amounts of beryllium were found in liver or bone of hamsters or rats. No significant differences were seen in the behavior of BeO in the lungs of either male or female, or of either rats or hamster.

TABLE 2.19. Retention of Inhaled BeO in the Rat and Hamster Lung. (Values are means from five rats per group.)

Time After Exposure, days	Amount of Beryllium in the Lung, μg			
	Rat		Hamster	
	Female	Male	Female	Male
1	150	140	20	15
7	91	110	11	14
14	110	88	-	-
21	88	110	5.0	8.1
35	88	110	8.5	7.2
49	92	-	7.0	9.2
63	120	120	10	8.5

Studies with high-fired BeO are continuing with life-span studies in 560 rats given initial alveolar depositions of approximately 0, 1, 10, and 100 μg beryllium. Animals will be examined for beryllium tissue distribution and pathologic-carcinogenic reactions in the lung and other tissues.

INHALATION HAZARDS TO URANIUM MINERS

INHALATION HAZARDS TO URANIUM MINERS

The sixfold increased incidence of lung cancer among uranium ore miners of the Colorado plateau has been strongly correlated with the daily exposures of these men to pathogenic mine air contaminants including radon, radon daughters, uranium ore dust, diesel engine exhaust, and cigarette smoking. These inhalation hazards may interact physically to alter the attached-unattached fractions and particle attachment sizes of airborne radon daughters, to cause marked differences in respiratory tract deposition patterns. Degenerative or proliferative pathogenic changes in the lungs caused by daily inhalation of uranium ore dust and/or cigarette smoking may interact with radiological damage produced by inhaled radon daughters. The rate of absorbed dose may also play a significant role in developing pulmonary pathology.

The objectives of this project are to determine fundamental cause and effect relationships of prolonged inhalation of controlled levels of uranium mine air pollutants using large and small experimental animals for comparison with similar observations in uranium miners, and to determine the mechanism of action of these agents or combination of agents in the development of respiratory tract and systemic organ pathology. An understanding of these relationships will aid in determining whether the current reductions in radon daughter exposure levels are sufficient to reduce the risk of lung cancer in uranium miners, or whether reduced exposure to other pathogenic or carcinogenic mine air pollutants must be considered in establishing safe levels.

RADIOLOGICAL CHARACTERIZATION OF URANIUM MINE AEROSOLS

Investigator:
P. O. Jackson[a]

Measurement of aerosols generated in operating uranium mines and in simulated uranium mine atmospheres indicates four aerosol component distributions. Relative attachment sizes of radon daughters depend upon the nature and time sequence of mining operations.

The particle size distributions of radon daughter aerosols in the experimental animal inhalation exposure chambers and in two operating uranium mines were measured. The inhalation exposure chambers contained defined combinations of room air, diesel exhaust, uranium ore and radon daughters for daily exposures of experimental animals to characterized levels of uranium mine air contaminants. The uranium mine samples were collected in the Uravan Belt and Ambrosia Lake areas of Colorado and New Mexico. The mine sites and operations studied included drifts, stopes, grisslies, electrostatic precipitators, blasting, mucking, slushing, drilling, dumping and loading.

Radon daughters are primarily attached to particles with diameters less than a few tenths of a micron, which is below the range of conventional impaction aerosol samplers. This difficulty has in the past limited the information concerning these distributions. A new, low-pressure cascade impactor having a last stage cutoff diameter of 0.06 μm for unit density spheres was used to study these radon daughter aerosols. Scintillation flasks and wire screens were used to determine the Rn concentration and unattached fraction of RaA, respectively.

The radon daughter activity distributions were seldom log-normal, but appeared instead to be combinations of distributions. Measurements in the inhalation exposure chambers under controlled aerosol conditions suggest that each activity distribution can be explained (assuming that attachment rate depends on total aerosol particulate surface area) by a composite aerosol substrate consisting of three separate aerosol components, each having a different particle size distribution, plus the unattached radon daughter component. These components appear to be:

1. Unattached radon daughters which diffuse to the first impactor.

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stages (apparent diameters from about 2 μm to about 6 μm).

2. Small condensation nuclei (diameters of about 0.01 to 0.05 μm in the inhalation chambers).
3. Large condensation nuclei (smoke and fumes, diameters of about 0.05 to 0.5 μm in the inhalation chambers).
4. Uranium ore dust (diameters of about 0.2 to 3 μm in the inhalation chambers).

These four components may also be used to interpret the observed uranium mine aerosol distributions, which are quite sensitive to particular mine operations and conditions. Significant shifts in the shapes of the radioactivity (radon daughter) distributions occurred with the onset of different mining operations, causing one or more of the four components to dominate the spectrum.

The usual log-normal parameters of activity median aerodynamic diameter (AMAD) and geometric standard deviation were found not to adequately describe the radioactivity distribution. All of the measured apparent AMADs fell between 0.10 and 0.36 μm, with the majority exhibiting apparent AMADs between 0.2 and 0.3 μm. The largest AMADs were measured in heavily worked areas having large ore dust contributions. Distinctly biomodal activity distributions were occasionally observed. For example, up to 50% of the total radon daughter activity in one inhalation chamber was attached to particles smaller than 0.06 μm, while the remainder was attached to a much larger aerosol with a median aerodynamic diameter of 0.34 μm.

The activity distributions of the individual radon daughters, RaA, RaB, RaC, were similar in most cases. In the inhalation chambers there was a tendency for more of the RaC to be associated with larger sized particles than RaA, probably because of greater coagulation during the longer lifetime of RaC. In the uranium mines, such coagulation processes were usually obscured by larger effects from short term changes in the airborne particle size distributions, associated with active mining (Figure 3.1).

A significant increase in radon daughter concentrations was observed in mine air following blasting. An hour after blasting, the radioactivity concentrations were still twofold higher than before blasting, measured at a short distance down the ventilation path from the blast site (Figure 3.2).

It is evident that the time-sequence of mining processes is an important determinant of radon distributions. Differences in the relative contribution of any of the three postulated aerosol component distributions affects the peak attachment size and the subsequent physical and biological behavior of inhaled radon daughters.

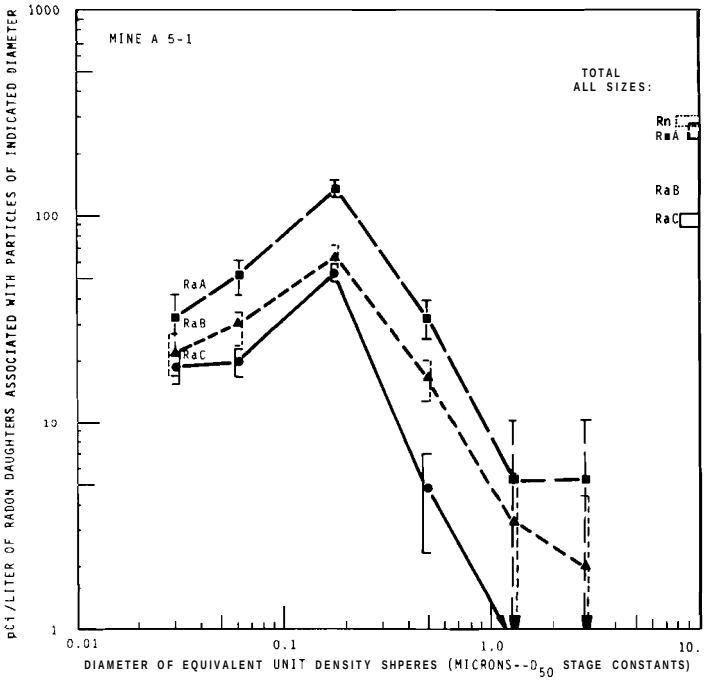

FIGURE 3.1. Radon Daughter Activity Distributions Obtained in a Drift During a Period of Low Mining Activity and Relatively Clean Air

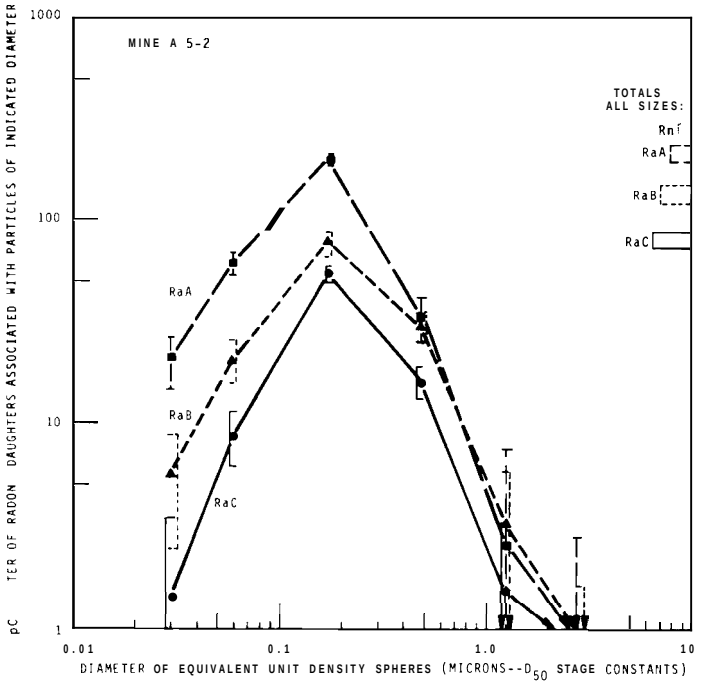

FIGURE 3.2. Radon Daughter Activity Distributions in the Same Drift During a Period of Heavy Mining, Showing a Shift of the RaB and RaC to Larger Particles Caused by Aerosolized Ore Dust

ELEMENTAL CHARACTERIZATION OF ANIMAL TEST CHAMBERS AND URANIUM MINE AEROSOLS

Investigator:
J. A. Cooper[a]

The elemental compositions of air samples from mines and from experimental animal inhalation chambers were determined as a function of particle size.

Particulate samples were collected in Colorado plateau uranium mines and in experimental animal exposure chambers. Neutron activation, energy dispersive X-ray fluorescence and atomic absorption spectrophotometric analyses of the cascade impactor stages provided elemental mass distributions for various locations and operations in the mines and in the exposure chambers.

Uranium ore handling, blasting, and welding were the only significant sources of inorganic elements in uranium mine aerosols. Diesel engine exhaust did not significantly contribute to the elemental content of either the mine or exposure chamber atmospheres. The uranium ore dust in mines reached concentrations of several mg/m^3 and showed mass median aerodynamic diameters (MMAD) greater than 1 µm, while aerosols generated by blasting and welding had average MMADs of about 0.5 pm.

The inorganic elements Na, Al, Si, K, Ca, Ti, V, Fe and U were measured in most samples and had MMADs between 1 and 7 µm (Figure 3.3). The relative concentrations of these elements were not significantly enriched in the aerosol compared to that found in the parent ore when normalized to Fe content, and are therefore thought to be generated by the dispersion of ore dust.

The concentrations of S, Zn and Pb were greatly enhanced in the mine aerosols over that found in the ore and showed an average MMAD of about 0.5 µm. Concentrations of Zn and Pb ranged up to a few $µg/m^3$, about equal to those found in typical urban aerosols, and should not constitute a serious health hazard. However, the S concentrations were as high as about 50 $µg/m^3$, about five times greater than some of the highest values reported for urban aerosols, and might constitute a health hazard depending on molecular form. The high concentrations of these elements probably arise from the black powder fuses (S) and the detonating caps (Zn and Pb) used in blasting. The high S, Zn and Pb concentrations remained for several hours after blasting.

Cd were measured in a few selected aerosol and ore samples from both mines. The Cd values ranged from less than 36 ng/m^3 to 160 ± 130 ng/m^3.

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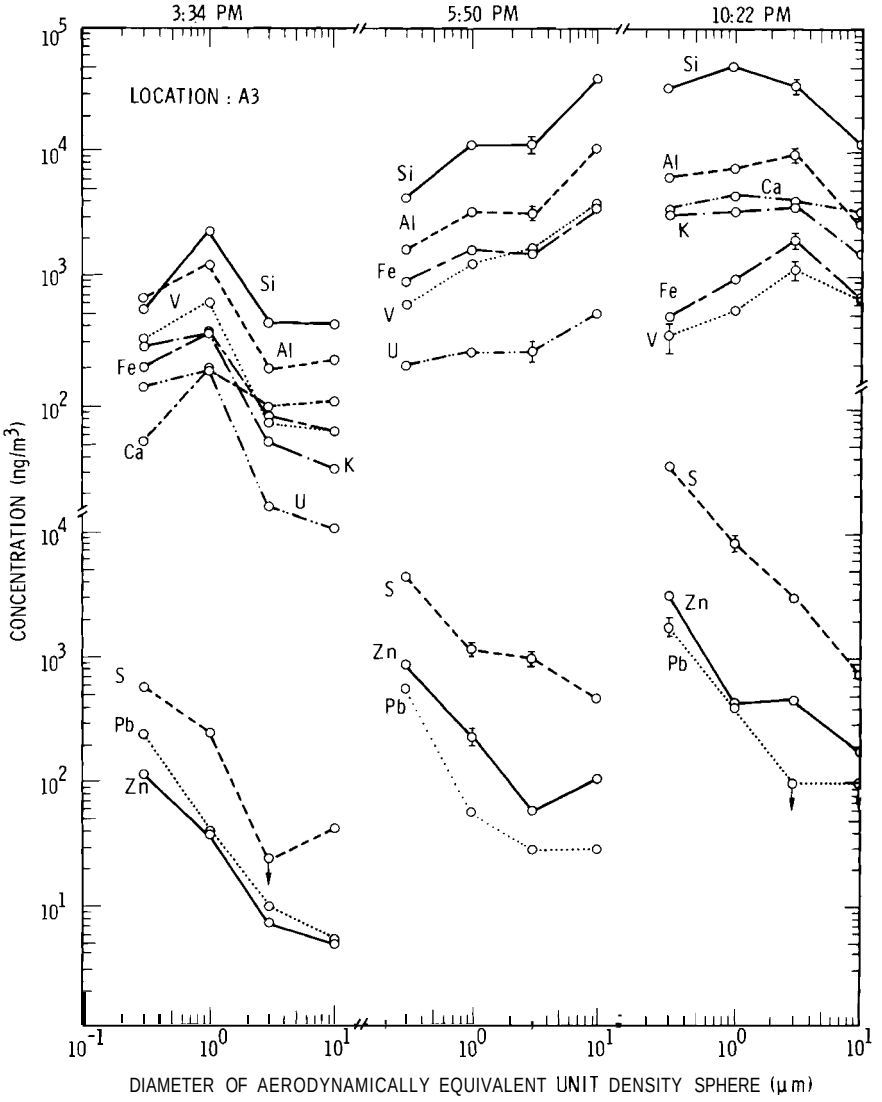

FIGURE 3.3. Mass Distributions of the More Abundant Elements Measured in a Working Stope of the Trackless Mine. The shape of the distributions for the ore related elements (top curves) reflect varying contributions of ore dust aerosols while the lower S, Zn and Pb curves show that the high concentrations of these elements are not a rapidly passing condition, but are more a general condition for long periods after a blast.

This value represents an enrichment of about 100-fold over the ore, but is only slightly greater than the highest reported value for an urban aerosol. Measurements of Be ranged from 27 to 95 ng/m^3 with errors of about 50%. These values range from 2 to 10 times greater than the highest urban aerosol measurements and are enriched by about 100-fold.

The measured levels of the elements Cl, Sc, Cr, Mn, Co, Ni, Cu, Ga, Ge, As, Se, Br, Sr, Y, Zr, Mo, Hg and Th were also measured and are

consistent with a possible origin from the uranium ore. These results suggest that uranium ore and blasting materials are the primary sources of the inorganic elements found in uranium mine aerosols. Particles with MMAD greater than 1 μm are associated primarily with ore dust and exhibit a similar elemental composition. Those with MMADs between 1 and 2 μm have probably been generated by blasting or possibly drilling. Those with MMAD >2 μm have most likely been generated by normal abrasive action encountered in the movement of ore such as mucking, slushing, hauling and dumping. Particles with MMAD between 0.1 and 1.0 μm contain the elements generated by blasting. The inhalation hazards of the elements S, Cd, and Be, as well as the long-lived alpha-emitting members of the uranium decay series present in the ore dust, should be further evaluated for potential damaging effects to the respiratory tract and systemic organs.

CHARACTERIZATION OF THE ORGANIC COMPONENTS IN ANIMAL TEST CHAMBER AND URANIUM MINE AEROSOLS

Investigator:
M. R. Petersen[a]

Diesel exhaust scot may contribute to the health problems posed by radon daughters in uranium mine air. The levels and size distributions of benzo(a)pyrene, and the non-volatile n-alkanes absorbed on mine air particulates were determined.

Particulate samples of diesel engine exhaust were collected in experimental animal exposure chambers and in two uranium mines by drawing air through glass-fiber filters, followed by extraction with benzene to remove the organic components. Size-fractionated samples were collected with Andersen cascade air samplers and similarly extracted. The n-alkanes were determined with a gas chromatograph equipped with a flame ionization detector. This gas chromatographic technique was not sufficiently sensitive for benzo(a)-pyrene determinations, so thin layer chromatography and ultraviolet fluorimetry were employed.

The concentrations of benzo(a)pyrene, a known carcinogen, in both operating uranium mines ranged from 26-57 ng/m^3 of air. In the exposure chamber concen'trations up to 262 ng/m^3 were found. The n-alkanes

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found in the mine particulates had longer carbon chain length (carbon numbers ranged from C_{14} to C_{26}, peaking at C_{21}, Figure 3.4) than the n-alkanes in diesel fuel (C_{11} to C_{24}, peaking at C_{14}). Concentrations of n-alkanes in mine air ranged up to 3 $\mu g/m^3$ and in the exposure chambers up to 30 $\mu g/m^3$.

The carbon chain length of the absorbed n-alkanes is correlated with the particle size in mines as well as in the exposure chamber (Figure 3.5). For example, hexadecane ($C_{16}H_{34}$) predominates on particles having a mass median diameter of 4.0 μm. The heavier n-alkanes occur on decreasingly smaller particles with docosane ($C_{22}H_{46}$) predominating on 0.4 μm diameter particles. This size fractionation is thought to be related to the mechanisms of soot formation during combustion of diesel fuel.

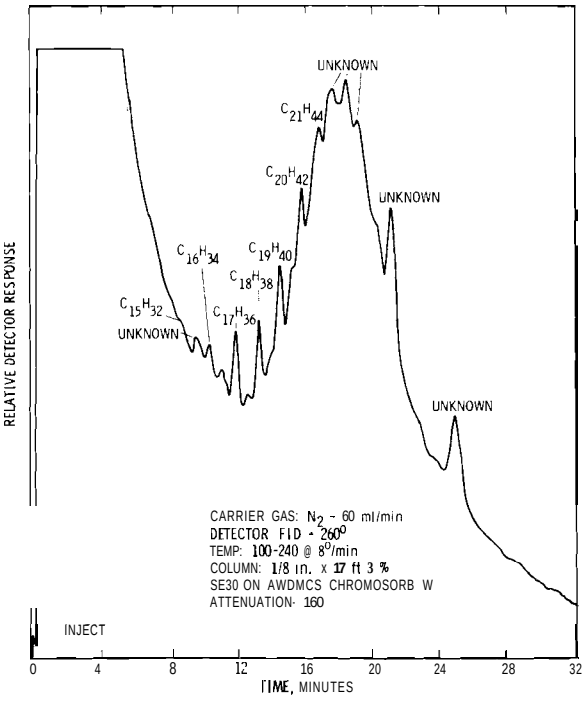

FIGURE 3.4. Gas Chromatogram of Benzene Extract of Aerosols Collected in a Uranium Mine with Diesel Equipment. Only nonvolatile n-alkanes were identified. The gross hump is thought to consist of isometric alkanes.

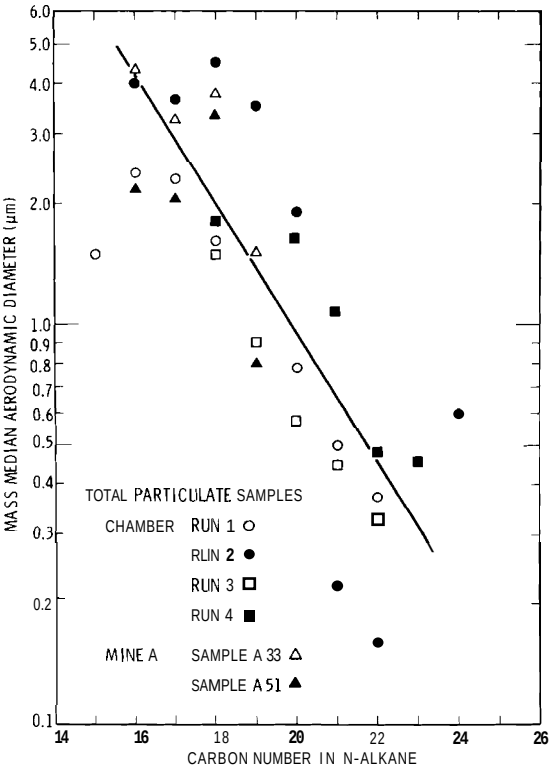

FIGURE 3.5. Relationship Showing Increasing Chain Length of n-Alkanes with Progressively Smaller Particle Size Distribution.

The measured benzo(a)pyrene contents of particulates in the mines and also in the animal chambers are in the ranges reported for European cities during certain periods of inversion and thus appear to pose no unusual health hazard, per se. The measured n-alkane concentrations in mine aerosols are about tenfold higher than in the average U.S. urban

sample. These compounds by themselves appear to pose no health hazards, but might potentiate the toxicity of adsorbed benzo(a)pyrene. The measured soot levels in the mines are about five times higher than the total particulate levels in the average American urban atmosphere.

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

Investigators:

R. F. Palmer, B. O. Stuart and R. E. Filipy

Technical Assistance:

H. G. Steele and J. C. Gaven

Hamsters, rats, and mice were chronically exposed to 2000 to 8500 WL of 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 90 hours/week to aerosols consisting of radon plus 2000 to 8500 working levels of radon daughters with and without 18 mg/m^3 carnotite uranium ore dust. Condensation nuclei concentrations ranged from 2000 to 4000/ml in the chamber without uranium ore dust, and from 90,000 to 120,000/ml in the chamber with ore dust.

Figure 3.6 shows the survival curves for the three species exposed to radon daughters without uranium ore dust, together with a curve showing the cummulative working level hours (CWLH) of radon daughters to which they were exposed. The increase in the slope of the CWLH curve is a result of the radon daughter concentrations increasing as the number of animals in the chamber decreased. The radon level in the chamber remained at $\sim$ 4.8 $\mu Ci/\ell$ throughout the exposure period.

Figure 3.7 shows the survival curves for each of the species exposed to radon daughters with uranium ore dust. The nearly constant slope of the CWLH curve shows that the concentrations of radon daughters in the chamber were little affected by the number of animals. The radon level in the chamber was maintained at 2.5 $\mu Ci/\ell$ throughout the exposure period. Comparison of survival curves under the two exposure conditions shows greater mortality rates

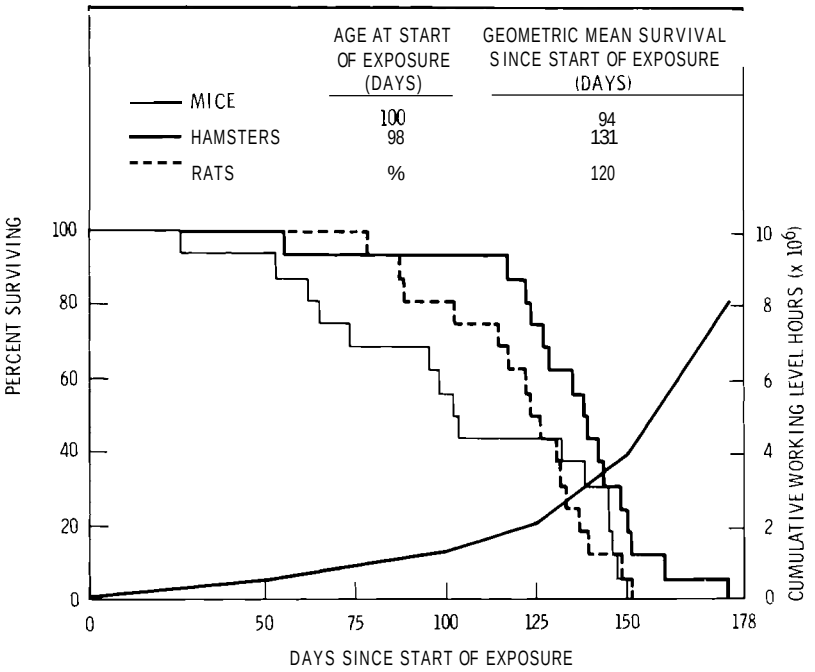

FIGURE 3.6. Survival Curves of Rats, Hamsters, and Mice Exposed to Radon Daughters Without Concomitant Uranium Ore Dust, Together with a Curve Showing the Cumulative Working Level Hours of Radon Daughters to Which They Were Exposed

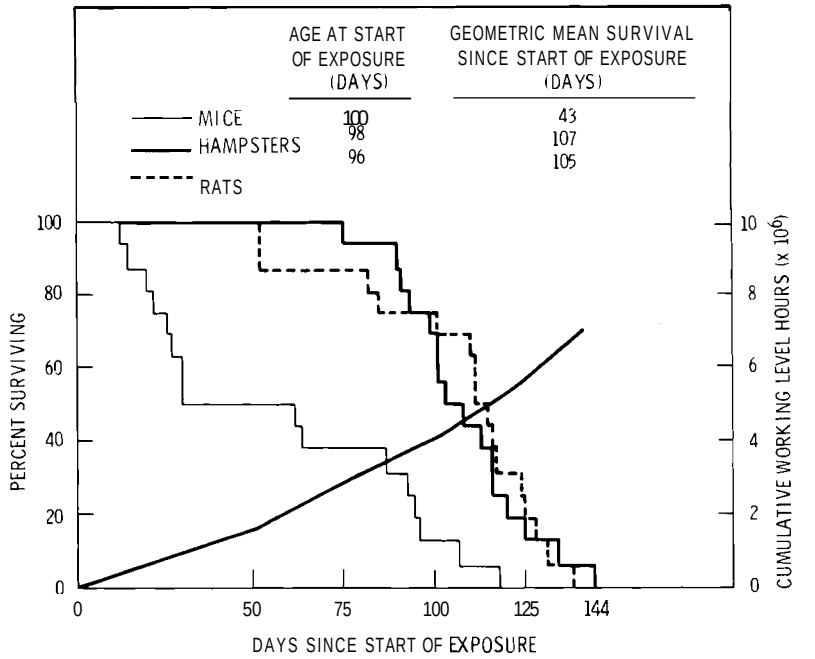

FIGURE 3.7. Survival Curves of Rats, Hamsters, and Mice Exposed to Radon Daughters with Concomitant Uranium Ore Dust, Together with a Curve Showing the Cumulative Working Level Hours of Radon Uaughters to Which They Were Exposed

for all species inhaling radon daughters with uranium ore dust, rather than radon daughters alone; mice show a 50% reduction in mean survival time. Weight losses compared to controls were also greater in all species inhaling radon daughters with ore dust rather than radon daughters alone.

Histopathology of radon daughter-exposed mice included acute interstitial pneumonitis, severe pulmonary congestion, and suppurative rhinitis; mice inhaling radon daughters with ore showed these lesions plus macrophage proliferation, alveolar septal cell hyperplasia, and bronchial epithelial hyperplasia.

Hamsters inhaling radon daughters showed proliferating lesions characterized by alveolar septal thickening, bronchiolar epithelial hyperplasia, septal fibrosis, and occasionally adenomatoid metaplasia and squamous metaplasia; hamsters inhaling radon daughters with ore dust showed similar effects plus granulomatous response and intense septal fibrosis.

Rats inhaling radon daughters showed lesions similar to those of hamsters but more focalized; rats exposed to radon daughters with ore showed similar lesions, with greater consolidation and pneumoconiosis.

Classical radiation pneumonitis with alveolar septal fibrosis and occasional bronchiolar epithelial hyperplasia were the predominant deep lung lesions seen in all species. In contrast to hamsters exposed 30 hr/week to 1200 WL of radon daughters and uranium ore dust in a previous study, proportionately more of the pathology was seen in the upper respiratory tracts of the hamsters in the present study. The contrast between markedly affected trachea and major bronchi versus relatively little effects in deep lung was most evident in rats. Findings of severe suppurative laryngitis and bronchitis were frequent in rats, and may have been an important contributing factor to their death. These findings dictate further studies involving sacrifice and radioactivity analyses of tracheal and lung tissues to determine relative absorbed radiation doses at these sites for correlation with developing degenerative and proliferative changes in the respiratory tract in each species.

We are presently exposing rats, hamsters and mice to the above levels of uranium mine contaminants during five 6-hr periods per week. It is hoped this lowered exposure rate will allow the animals to live long enough for proliferative epithelial changes to progress beyond the stage of squamous metaplasia to invasive tumor formation.

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

Investigators:

B. O. Stuart, R. E. Filipy, P. L. Hackett, H. A. Ragan and E. G. Tombropoulous

Technical Assistance:

J. C. Gaven, W. Skinner, K. C. Upton, L. R. Peters, G. M. Louis, C. R. Petty, W. A. Zimmerman and R. B. Greenfield

After 4 years of daily inhalation exposures to 600 WL radon daughters plus uranium ore dust and/or cigarette smoking, observed pulmonary lesions include macrophage proliferation, septal fibrosis, epithelial hyperplasia, emphysema, endothelial proliferation, and bronchiolar-alveolar epithelial changes involving multiple foci of squamous metaplasia with atypia.

Three groups of beagle dogs are continuing to receive daily inhalation exposures to cigarette smoke (ten cigarettes/day) with and without concomitant exposure to 600 WL of radon daughters and uranium ore dust, as described in Table 3.1; control dogs receive identical daily periods of sham smoking. After 4 years of daily exposures, group mean respirations per minute were 31 ± 14 and 28 ± 8 for Group 1 and Group 2 dogs, respectively, compared to 20 ± 3 for controls. Tidal volumes for Group 1 and Group 2 dogs have decreased to 0.08 ± 0.04ℓ and 0.09 ± 0.03ℓ, respectively, compared to 0.14 ± 0.02ℓ for controls. Mean body weights of all experimental groups continue to be not significantly different from controls.

TABLE 3.1. Experimental Design

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

(a) Two dogs in each group receive periodic lung washing for exfoliative cytology studies.
(b) Each dog receives ten cigarettes daily over two shifts, 7 days per week.

Group 1 and Group 2 dogs continue to show significantly higher neutrophil levels than controls. This neutrophilia in Group 2 dogs appears to be the result of chronic irritation and increased pulmonary cell death from the combined effects or radon daughters with ore and the cigarette smoke. Although mean lymphocyte

levels are not statistically different from controls, most of the dogs in Group 1 and Group 2 have absolute lymphocyte values below those found in the comparably aged colony control dogs of this laboratory. Quarterly analyses of serum enzymes in all dogs are beginning to show lower values for glutamic pyruvic transaminase in several Group 1 and Group 2 dogs compared to mean control levels. Alkaline phosphatase levels in all experimental groups (Groups 1, 2 and 3) are somewhat lower than controls, and Group 2 dogs showed slightly higher mean thyroxine levels than controls, although group means in both cases are not statistically different from controls.

Histopathologic examinations were completed on the lungs of dogs that died or were sacrificed, and replaced in the experiment, during the first 4 years of this continuing project. This involves seven dogs from Group 1, six dogs from Group 2, two dogs from Group 3, and three dogs from Group 4 (sham-smoking controls). Lesions observed in the lungs of dogs that died or were sacrificed before March of 1973 were described in the previous Annual Report.

Since March 1973, eight dogs from Groups 1 and 2 required sacrifice because of rapidly developing pulmonary insufficiency; one dog from Group 3 was sacrificed for comparison with the Group 1 and 2 dogs. All of these dogs had received at least 3 1/2 years of daily exposures.

All sacrificed Group 1 dogs (radon daughters with ore dust plus sham smoking) showed large numbers of alveolar macrophages containing uranium ore dust, with accumulation of these macrophages around bronchi, bronchioles, and smaller blood vessels. In alveoli adjacent to these areas, pronounced septal fibrosis and extensive epithelial hyperplasia had occurred. There was marked pleural thickening and septal fibrosis of subpleural alveoli (Figure 3.8). The septal fibrosis was accompanied by extensive alveolar epithelial hyperplasia. Fibrosis of vascular walls occluding small blood vessels of the periphery of the lungs, and endothelial proliferation were present. Areas of slight to moderate vesicular emphysema were found. Four of the five dogs in this group showed numerous foci of squamous metaplasia with cells showing atypical nuclei (Figure 3.9 a and b); these epithelial changes are very similar to the squamous metaplasia occurring just prior to invasive squamous carcinoma, as

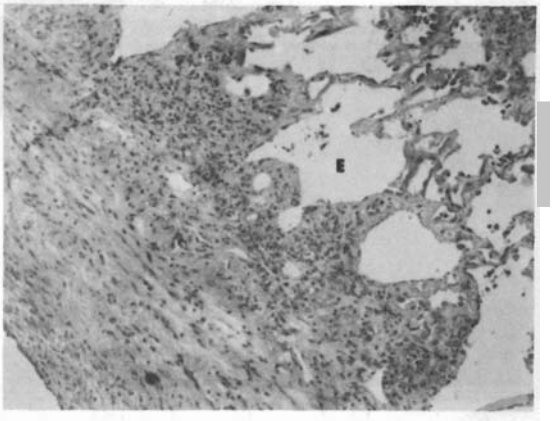

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FIGURE 3.8. Pleural Thickening and Peripheral Alveolar Septal Fibrosis from the Lung of a Dog After 47 Months Exposure to Radon Daughters with Uranium Ore Dust. Also shown are emphysematous vesicles (E).

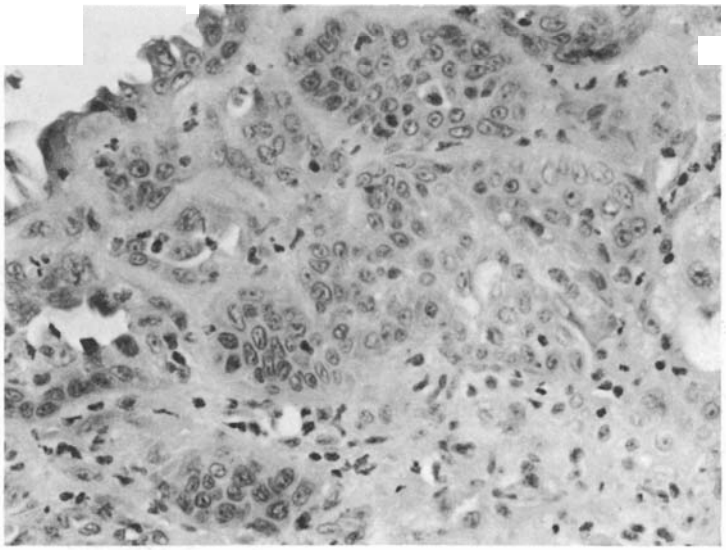

FIGURE 3.9a. Squamous Metaplasia of Alveolar Epithelium from the Lung of a Dog After 46 Months Exposure to Radon Daughters with Uranium Ore Dust.

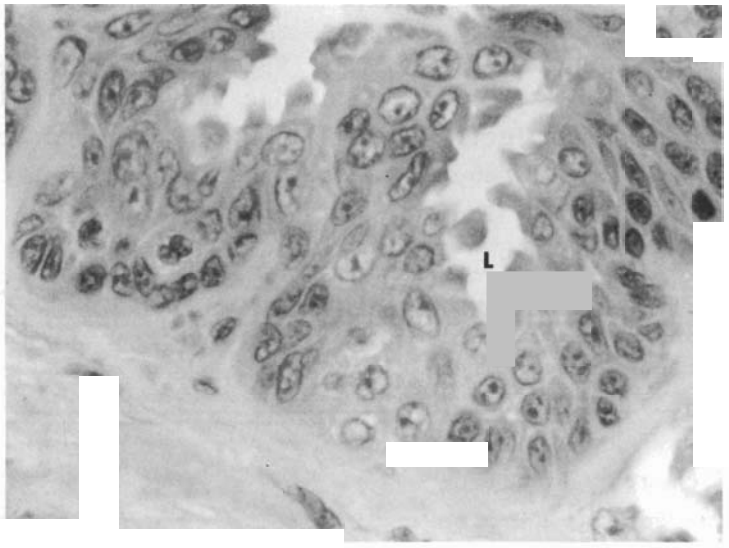

FIGURE 3.9b. Higher Magnification of the Lesion Shown in Figure 3.9a. Atypical nuclei are present in cells lining the alveolar lumen (L).

seen in parallel studies with hamsters in this laboratory.

All sacrificed dogs of Group 2 (daily cigarette smoking plus inhalation of radon daughters with ore dust) had developed the peribronchiolar and perivascular accumulation of macrophages with focal infiltration of mononuclear cells described for the Group 1 dogs, together with large numbers of alveolar macrophages. Lungs had slight to moderate septal fibrosis and epithelial hyperplasia of adjacent alveoli, as well as pleural thickening and fibrosis of subpleural alveolar septa. Vascular occlusion due to fibrosis of the vessel wall was found in peripheral regions of the lungs of two of the three dogs sacrificed in this group. Subpleural interstitial fibrosis with associated alveolar epithelial metaplasia was observed, involving extensive areas of the lungs in one case. Progression from cuboidal metaplasia to squamous metaplasia of the alveolar epithelium was evident (Figure 5.10). A striking pathological change in the lungs of Group 2 dogs was advanced pulmonary emphysema. In two dogs, slight to moderate vesicular emphysema was observed; large emphysematous bulli were found in the lungs of a third dog (Figure 3.11). These bulli were evident grossly as large elevations on the pleural surface. Foci of cuboidal to squamous metaplasia of alveolar epithelium were also found in this dog. Another dog from this group was very recently sacrificed and showed large subpleural bulli.

The one sacrificed dog that had received 49 months of daily cigarette

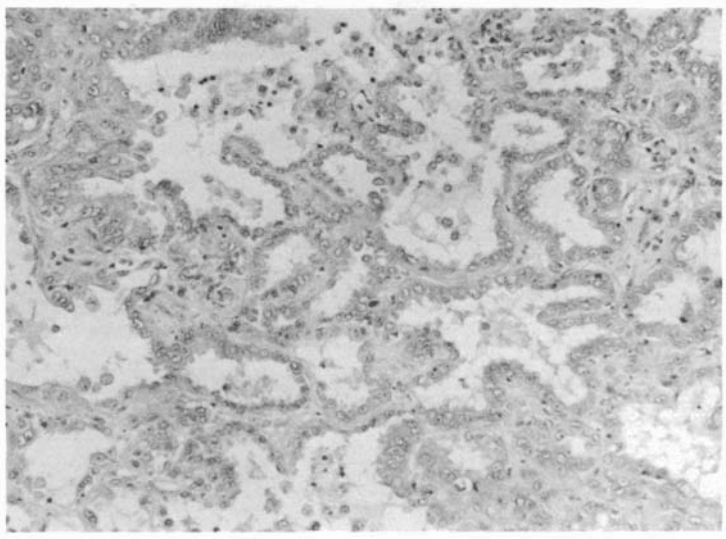

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FIGURE 3.10, Cuboidal Metaplasia of Alveolar Epithelium and Associated Alveolar Septal Fibrosis from the Lung of a Dog after Exposure to Radon Daughters with Uranium Ore Dust and Cigarette Smoke for 48 Months. The lesion has progressed to squamous metaplasia as shown in the upper left portion of the photomicrograph.

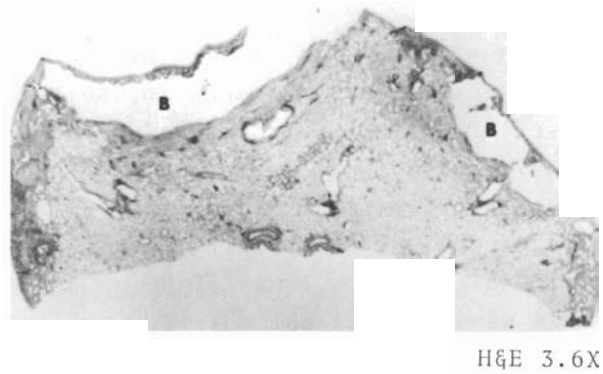

FIGURE 3.11. Photomicrograph of a Lung Section from a Dog After 48 Months' Exposure to Radon Daughters with Uranium Ore Dust and Cigarette Smoke. The emphysematous bulli (B) are a prominent feature of the lungs from this dog.

smoking only (Group 3) showed pulmonary lesions including vesicular emphysema and occasional foci of subpleural interstitial fibrosis with associated alveolar epithelial metaplasia. This lesion was a frequent observation in the lungs of Group 2 dogs. This cigarette-smoking dog also showed foci of tracheal epithelial squamous metaplasia.

In lung lavage samples the percentages of unsaturated fatty acids in phosphatidylcholine rose from 25 to 28% in control dogs to 37% in cigarette-smoking dogs and 40% in dogs inhaling radon daughters with ore alone. This increase of total unsaturated fatty acids usually involved an increase in arachidonic acid which has been reported to be increased in lung washings from humans with pulmonary adenomas.

DISPOSITION OF LONG-LIVED URANIUM CHAIN ALPHA EMITTERS FOLLOWING REPEATED INHALATION EXPOSURES OF LABORATORY ANIMALS TO URANIUM ORE

Investigators:
B. O. Stuart and P. O. Jackson[a]
Technical Assistance:
J. C. Gaven, H. G. Steele and K. C. Upton

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

Whether retention of ^{230}Th inhaled as a constituent of airborne uranium ore dust is governed by the characteristics of the parent particles, is a question we have been studying for more than 10 years. In 1963 preliminary studies with rats inhaling aerosols of crushed pitchblende ore in secular equilibrium showed a rapid in vivo separation of ^{230}Th from parent uranium in all tissues, including lungs and tracheobronchial lymph nodes. Subsequent studies with beagle dogs inhaling pitchblende ore dust have given a similar indication of independent biological behavior of the uranium and thorium components.

Current life-span studies involving daily inhalation of carnotite uranium ore (4% U_3O_8) by hamsters show retained $^{230}Th/^{234}U$ ratios as high as 2.0, though the ore dust inhaled daily was at secular equilibrium. Beagle dogs inhaling this carnotite uranium ore together with radon daughters, after 6 months of daily 4-hr exposures, showed marked non-equilibrium ratios of $^{230}Th/^{234}U$, ranging from 4.2 to 6.8 in lungs and 2.9 to 23 in thoracic lymph nodes.

These experiments have all shown a rapid in vivo separation of thorium from uranium in lungs, tracheobronchial lymph nodes, and systemic organs, when these elements are inhaled as constituents of uranium ore. This pattern has been found with two types of ore (pitchblende and carnotite) in three species of mammals (rats, hamsters and dogs), and with a variety of concentrations and exposure schedules. It thus seems clear that ^{230}Th should be considered as a separate radionuclide in evaluating exposure hazards in uranium mining and milling operations.

(a) Radiological Sciences Department

EVALUATION OF RADIONUCLIDES IN MAN

EVALUATION OF RADIONUCLIDES IN MAN

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

Analyses of postmortem tissue samples from Hanford plant workers and local residents have been conducted on a very limited scale sine 1949. The purpose has been to establish baseline quantities of significant radionuclides as distributed within the body as related to age, occupation, geographic residence, and point in time. Postmortem studies are required because present methods of in vivo measurement do not give adequate data on such important radionuclides as ^{239}Pu. Another important aspect of this project is concerned with the development of more sensitive and more accurate techniques for in vivo assessment of internal deposition of radionuclides.

The effort devoted to this project during the past year was again curtailed due to the diversion of personnel to the preparation of environmental impact statements.

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]

Present studies evaluating postmortem tissue samples are an outgrowth of studies conducted on a limited scale since 1949. Collection of postmortem tissue samples (lung, liver, bone and tracheobronchial lymph nodes) from individuals residing or formerly residing in the vicinity of the Hanford complex continued during the past year. Emphasis on this work has been increasing and the region of interest in sample collection has been enlarged, as have our processing capabilities. The purpose of this work is to establish baseline quantities of significant radionuclides as distributed within the body and as related to age, occupation, geographical residence and point in time, in conjunction with establishing the environmental impact of nuclear facilities. Postmortem studies are required because present methods of in vivo measurement do not give adequate data on such important radionuclides as ^{239}Pu.

Analyses of postmortem tissue samples and blood samples are also performed for the U.S. Transuranium Registry. By the year's end, 128 Registry samples had been processed to completion, 23 Registry samples and 64 environmental samples were in process, and 3 Registry samples and 84 environmental samples were awaiting processing

During 1973 a four-detector alpha spectrometer system (Figure 4.1) was installed, which will permit isotopic analysis of alpha-emitting plutonium isotopes in tissue samples. In addition, this apparatus allows use of plutonium tracers such as ^{236}Pu and ^{242}Pu with which chemical yield of the analytical process can be checked in the processing of each sample. In the previous system a blank sample to which ^{239}Pu had been added was processed with the tissue samples and the chemical yield of the latter assumed. The new technique can assure that the correct chemical yield is used. Although scintillation counting techniques will continue to be used in those cases where large amounts of plutonium are expected and the isotopic composition is not required, this technique is not compatible with the use of plutonium tracers for chemical yield. Similarly, the alpha track counting technique will continue to be used where very small quantities of plutonium are expected in tissue samples and the isotopic composition of plutonium is not required.

(a) Physics and Instrumentation Department
(b) Radiological Science Department

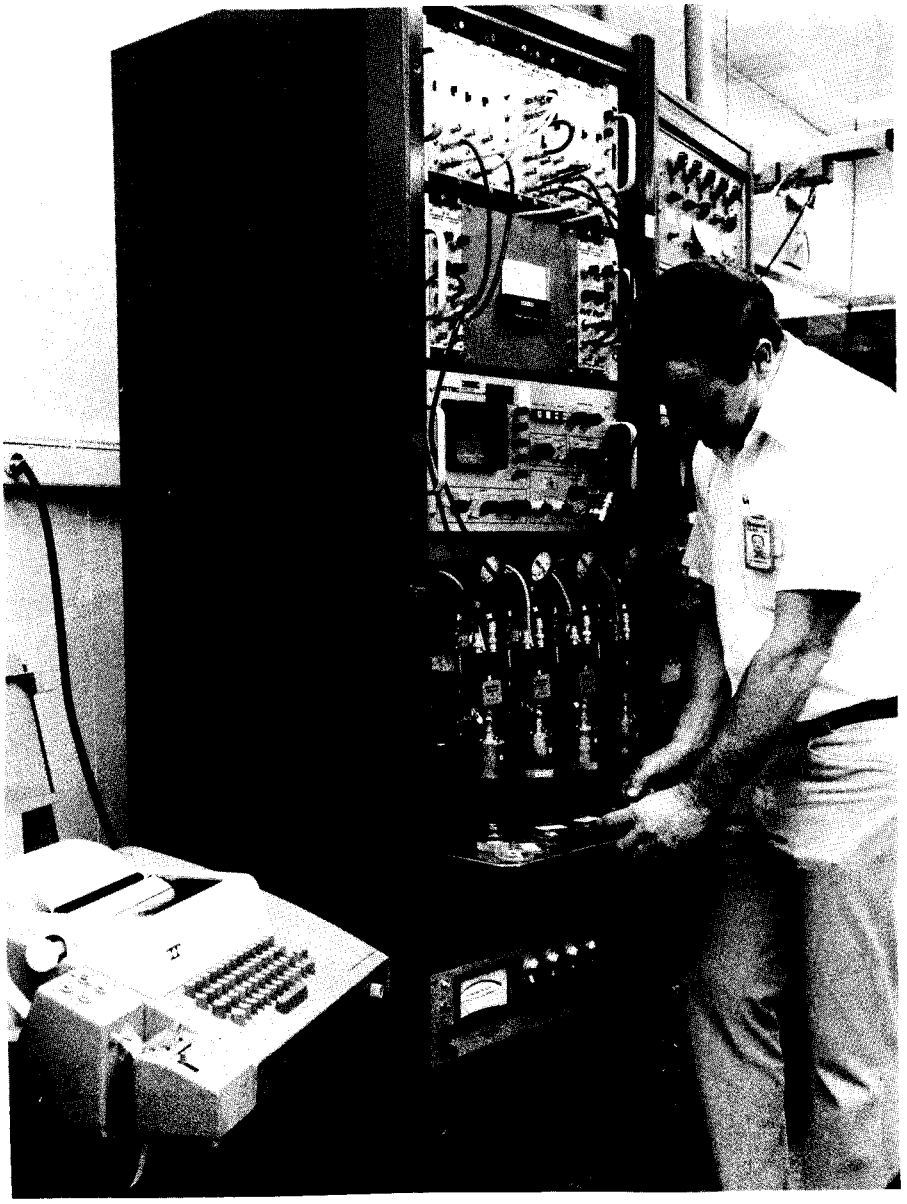

FIGURE 4.1. Four-Detector Alpha Spectrometer System Employed for Plutonium Isotopic Analysis of Tissue Samples

QUANTIFICATION OF INHALED PROMETHIUM

Investigators:
I. C. Nelson,[a] *J. E. Ballou, B. I. Griffin*[a] *and D. K. Craig*
Technical Assistance:
F. N. Eichner[a]

Human data on the retention of inhaled promethium are badly needed for diagnosing the retention of $^{147}Pm_2O_3$ in accidentally exposed workers. Exposure of human volunteers to the readily detectable ^{143}Pm seems feasible, providing a suitable carrier can be found that will provide the necessary mass and that will behave like promethium in the human body. Samarium has been proposed as such a carrier and experiments comparing the behavior of intravenously injected and inhaled samarium and promethium in rats were described in last year's Annual Report. The results of these rat studies indicated that, for a reasonable time after exposure, samarium is suitable as a carrier for promethium.

A similar experiment has been performed in which a mixed promethium-samarium oxide aerosol was inhaled by beagle dogs. Evaluation of tissue distribution and excretion data from this experiment has not been completed. If these data confirm the earlier results obtained with rats, plans will be made for exposure of human volunteers to a ^{143}Pm, samarium oxide-carried aerosol.

EFFECTIVE HALF-LIFE OF ^{137}Cs IN BONE

Investigator:
H. E. Palmer[a]

Since the amount of stable cesium in bone is 10 to 20 times the total amount present in other body tissues, it is possible that there may be a heretofore undetected long-lived component of ^{137}Cs in bone. About 0.01% of ingested ^{22}Na reaches the bone and remains there for a very long time. There is also an essentially nonexchangeable pool of potassium in bone. If a long-lived cesium component exists, it would be important in the evaluation of environmental exposure. In addition to ^{137}Cs from fallout,

(a) Physics and Instrumentation Department

small amounts of ^{137}Cs are expected to be released to the biosphere from nuclear power reactors.

Measurements were made at about 2-month intervals on an Eskimo student attending school at Chemawa, Oregon to test the hypothesis concerning a long-lived component of ^{137}Cs. The measurements indicated that if the phenomenon exists, it is such a small factor compared to total body cesium as to be of no significance in dosimetry considerations.

REMOVAL OF RADIONUCLIDES

REMOVAL OF RADIONUCLIDES

This project seeks to develop methods for decreasing the damage from ingested, absorbed or inhaled radionuclides by hastening their removal or otherwise decreasing the radiation dose to sensitive tissues. Associated with this effort is the development of information on the behavior and effects of radionuclides as a basis for establishing and recommending therapeutic procedures. This project seeks to be responsive to the practical needs of persons responsible for the treatment of external or internal radionuclide poisoning, to act in a supportive role in examining hazards of applications of radionuclides, and to try to anticipate problems associated with human involvement with radionuclides in the nuclear industry and defense.

Much of the past year's effort was concerned with evaluating the relative effectiveness and safety of inhaled chelating agents, as compared with intravenous administration. Of particular concern was the observation of acutely lethal effects when DTPA concentrations were continuously maintained at relatively low levels. Studies of the effect of treatment with DTPA in altering mortality and pathology over the life-span of rats that received $^{239}Pu(NO_3)_4$ by inhalation are continuing, with no significant differences in mortality between treated and untreated groups during the past year.

COMPARATIVE EFFECTIVENESS OF INHALED AND INJECTED Ca- OR Zn-DTPA FOR REMOVING INTRAMUSCULARLY DEPOSITED $^{238}Pu(NO_3)_4$ FROM THE RAT

Investigator:
V. H. Smith
Technical Assistance:
M. D. Snyder

Under conditions simulating those encountered by man in industrial accidents, inhaled DTPA was as effective as intraperitoneally injected DTPA in removing intramuscularly deposited plutonium from the rat. The zinc salt, under the prompt treatment conditions tested, was not as effective as the calcium salt.

The inhalation route for the administration of decorporating agents has obvious advantages over the intravenous route in terms of patient acceptance and treatment convenience. However, the therapeutic effectiveness of the inhalation route under various conditions of radionuclide incorporation remains to be established. A series of experiments are therefore being run to compare the injection and inhalation routes for DTPA administration. The variables to be studied include the radionuclide and its compound form, route of radionuclide incorporation, time of treatment after incorporation, and chelate used. The first experiment, described here, considers an intramuscularly deposited, relatively soluble form of plutonium and its removal by inhaled or injected Ca- or Zn-DTPA.

Adult female rats were injected intramuscularly with 1.2 μCi $^{238}Pu(NO_3)_4$ in 25 μl of pH 2 HNO_3. One hour later (prompt treatment conditions) the rats, in groups of eight, were treated with 0.5 or 0.035 mmol/kg Ca- or Zn-DTPA intraperitoneally. The lower dose was also given by inhalation over a 30-min period using a nebulizer representative of those used in human inhalation therapy. Plutonium-injected control rats were exposed to aerosols of, or injected with, 0.9% saline.

Animals were sacrificed after 4 days. Controls retained an average of 23% of the plutonium dose in the liver, 41% in the skeleton and 22% remained at the injection site. The effectiveness of the treatment procedures is summarized in Table 5.1. Best results were achieved with the

TABLE 5.1. Retention of Intramuscularly Deposited $^{238}Pu(NO_3)_4$ in the Rat 4 Days After Treatment with Inhaled or Injected Ca- or Zn-DTPA

Treatment Route→	Percent of Control Retention[a]					
	Intraperitoneal				Inhaled	
Treatment Level→	0.5 mmol/kg		0.035 mmol/kg		0.035 mmol/kg	
Agent→	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA
Tissue						
Liver	9	20a	30b	57c	22ab	45c
Femur	17	57	40a	88b	31a	73b
Injection Site	55a	8Zb	79b	92c	64a	84bc

(a) Numbers in the same row with the same letter (a, b or c) are not statistically different at 5% significance level according to Duncan's multiple range test.

0.5 mmol/kg level, and under these prompt treatment conditions the Ca salt is superior to the Zn salt. Inhalation was slightly, though not significantly, superior to injection at the lower dose of DTPA; again the Ca salt was more effective than the Zn salt although not to the same degree as at the higher dose.

The quantity of chelate absorbed from the lung of man will be about one-third of the 1 g of Ca-DTPA inhaled, or about 0.009 mmol/kg. This is about one-fourth of the lower dose level employed on the present study. The mass of Pu given the rats is comparable to that encountered in human accidental incorporations, being equivalent to less than an occupationally permissible body burden of ^{239}Pu. If one can assume similar effects in man and rat, the inhalation treatment should be as effective as treatment via the intravenous route. This conclusion should be considered valid for the conditions of this experiment *only,* until further comparisons are made.

DISTRIBUTION AND RETENTION OF INHALED DTPA

Investigator:
V. H. Smith
Technical Assistance:
M. D. Snyder

As with $CaNa_2EDTA$, the rate of appearance in rat urine of inhaled $CaNa_3DTPA$ and $ZnNa_3DTPA$ is retarded compared with the intravenously administered material. No difference between the Zn and Ca salt was statistically verified. Assuming that urinary excretion mimics blood levels, inhaled DTPA should be available for metal decorporation over longer time periods than DTPA intravenously injected.

As part of a general evaluation of the safety and effectiveness of inhaled DTPA for the removal of responsive radionuclides, the metabolism of ^{14}C-labeled DTPA was studied in a manner analogous to previous studies with EDTA, as described in last year's Annual Report.

Ten female rats, average weight 240 g, fitted with indwelling urethral catheters, were injected with, or exposed total-body, to aerosols generated from $CaNa_3DTPA$ or $ZnNa_3DTPA$ tagged with ^{14}C-DTPA (obtained from Amersham/Searle Corp.). Inhalation exposures were for 30 min to an aerosol concentraiton of about 15 mg/liter; particle size was about 2.2 μm MMAD, σg = 2.0. The absorbed dose, as deduced from urinary excretion, averaged 2.8 mg with a range of 1.9 to 11 mg. The feces, collected over the same period, contained 2 to 4 times the amount found in urine. Based on a lung weight of 1.5 g/rat, the average absorbed dose of Ca-DTPA was equivalent to 1.8 g in a human lung, or about four times the lung dose delivered in a typical human inhalation treatment.

For comparison, labeled chelate was administered intravenously at a dose of 5 mg/rat. Recovery of label in this case was 91 to 99%, averaging 95%, with the feces accounting for about 1% of this (range 0.5 to 3%) and the remainder found in urine. The expired air and the skin were not included in the assay to be consistent with methods used for animals receiving DTPA by inhalation.

As one would expect, the excretion of DTPA (Figure 5.1) is very similar to that previously observed for EDTA. In both cases the inhaled chelate persists in the body, as reflected in urinary output, for longer periods of time than does the intravenous dose, indicating that absorption across the lung to blood slows down the rate of appearance in urine. Whether DTPA is cleared from lung to urine more slowly than EDTA, as suggested in Figure 5.1, cannot be established from these data because

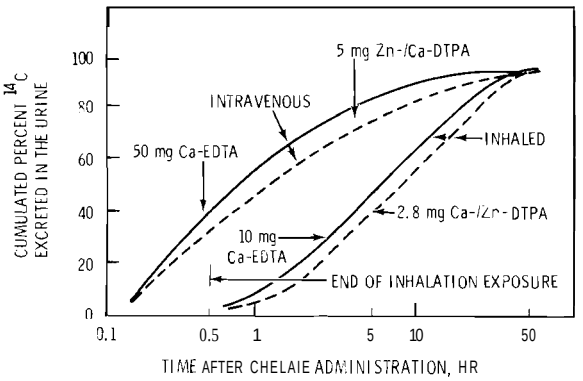

FIGURE 5.1. Appearance of ^{14}C in Rat Urine Following Inhalation or Injection of ^{14}C-Labeled Chelating Agents

elimination across the lung, like elimination by the kidney, is influenced by concentration. Thus, the mass of DTPA employed was about one-fifth the mass of EDTA, which may have retarded its clearance relative to EDTA. No significant differences in results were detected in these experiments between the Zn- or Ca-salt of DTPA.

The distribution of Ca-EDTA from the rat lung to other tissues is shown in Figure 5.2. Some 4% of the chelate is still in the lung 4 hr after inhalation. The half-time in urine indicated in Figure 5.2 for serially sacrificed rats is about half that shown in Figure 5.1 for rats with urinary catheters. In Figure 5.1 the 100% includes all chelate collected in 3 days urine but at least 10% of that represents absorption from the gut which would make the urine output a lesser fraction of the data base of Figure 5.1. The remainder of the difference is likely experimental variation; following a single aerosol exposure four rats were sacrificed per time period compared to 14 exposures for Figure 5.1.

Two-thirds to three-fourths of the total chelate inhaled is excreted via the gastrointestinal tract with a distribution during the first 24 hr as shown in Figure 5.3. The chelate appears to move with the food and at 24 hr about 25% is still in the large gut. Inhalation of a chelatable radionuclide, followed by inhalation treatment with a chelating agent, might well result in significant production of the radionuclide-chelate complex in the stomach and small intestine, which might result in increased absorption into the body. However, any such chelated material absorbed would likely be excreted via the urine with little tissue deposition of the radionuclide, especially in the presence of an excess of the chelating agent.

Administration of chelating agents by inhalation provides a longer presence of the agent in the body than does intravenous administration. Concentration of the chelating agent in the blood after 24 hr is higher following inhalation than following intravenous injection of a tenfold larger dose. This persistence may provide the inhalation route with sufficient removal efficiency to offset the higher concentrations achieved intravenously. The pattern of release from the lung may be important, too, since the initial high levels of inhaled chelating agent are absorbed from lung to blood dependent on initial concentration.

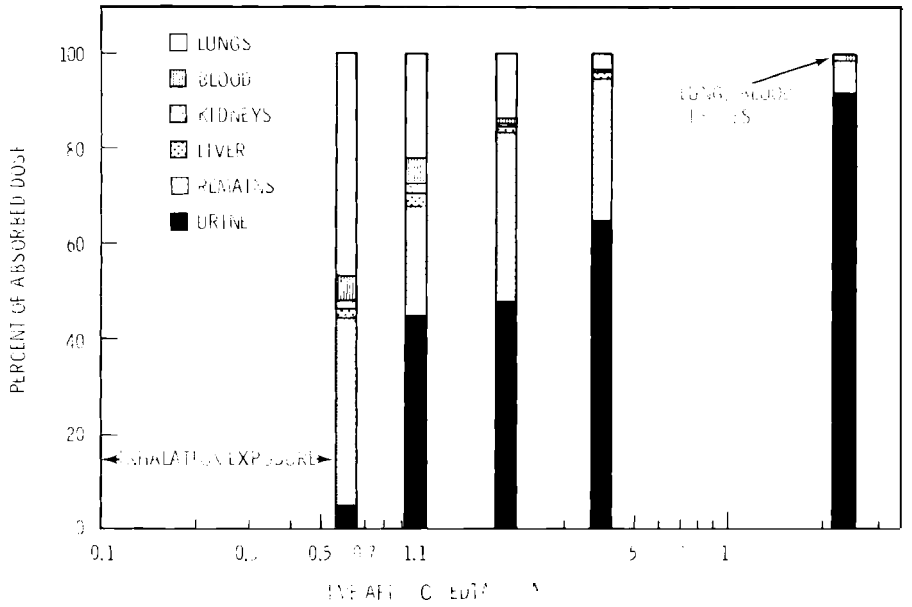

FIGURE 5.2. Distribution of the Absorbed Fraction of Inhaled Ca-EDTA in Rats

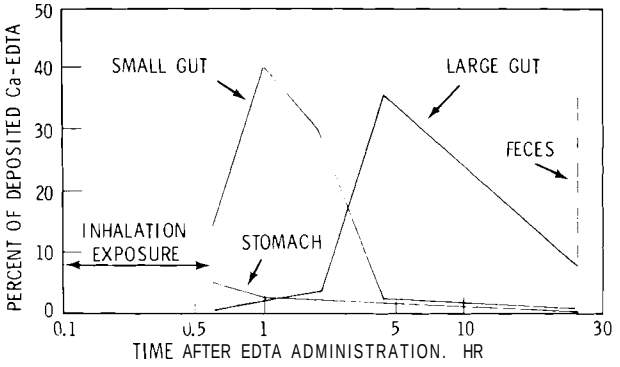

FIGURE 5.3. Percent of Deposited Ca-EDTA in the Gastrointestinal Tract of Rats Following Aerosol Exposure

Urinary excretion following injection or inhalation of Ca- or Zn-DTPA was so similar, the greater utility of one over the other based on persistence in the body was not demonstrated in this test system.

TOXICITY OF INHALED DTPA

Investigator:
V. H. Smith
Technical Assistance:
M. D. Snyder

No significant gastrointestinal pathology was seen in rats, during or following inhalation of Ca-DTPA at daily dose levels up to 20 times those normally taken by man and for up to 20 consecutive days. Even at these high levels the inhalation route seems unable to maintain systemic levels of DTPA sufficiently high to produce the gastrointestinal syndrome seen following continuous infusion. As previously noted, a transient vesicular emphysema was produced that generally was not present 3 weeks after cessation of treatment.

Since inhaled Ca-DTPA persists for a longer time in the rat than intravenously injected Ca-DTPA there was some concern that the gastrointestinal syndrome, seen following continuous infusion of Ca-DTPA, might be produced upon repeated inhalation treatment. Two aspects of this problem were studied in the experiments described below: (1) would repetitive high doses produce the syndrome, and (2) what is the rate of recovery from any effects noted?

Female rats weighing 260 to 320 g were exposed to very dense Ca-DTPA aerosols (∿7 mg/ℓ; MMAD 2.3 to 3.8 μm, σg 2.1 to 2.6) for 15, 30, 60 or 120 min, twice daily. The exposure chamber was cooled to maintain internal temperatures below 25° over the longer exposure times. The average Ca-DTPA dose to the rat was 7.3 mg/exposure hour; daily doses were about 0.025, 0.05, 0.1 and 0.2 mmol $kg^{-1}d^{-1}$; the human dose is 0.028 mmol kg^{-1}. Four animals were sacrificed from each dose level after 1, 10 and 20 days of treatment, and after 1, 7 and 21 days of recovery following treatment. Control rats were treated with aerosolized 5% saline solution.

No pathology was observed following the 1-day exposures. In the animals studied after longer exposure there was a slightly higher incidence and severity of vesicular emphysema in the DTPA-treated rats than in the saline-treated animals. After 7 or more days of treatment there was some emphysema in all groups; but except for the very lowest dose, there was no clear-cut correlation of incidence or severity with DTPA dose or recovery period out to 10 days. After 21 days recovery, only one of four rats from the highest DTPA-exposure group, and one of four rats from the saline group, showed emphysema. At the two highest doses a slight softening of the feces was noted but this was not confirmed as a gastrointestinal syndrome at the histopathological level,

and may have simply reflected the amount of chelate going through the gut following the inhalation.

By the end of each 30 min or longer exposure, the nasal areas of the muzzle appeared red and slightly inflamed. This went away within about 10 min after removal from the chamber. The rats treated with DTPA rarely blinked or closed their eyes; this was more common among rats treated with saline. No damage to the eyes was noted.

No pathology of any consequence was found in the kidneys, ovaries, nares, pancreas, adrenals, urinary bladder, trachea, esophagus, cerebrum, cerebellum, or generally in the liver and gastrointestinal tract. In three animals the liver showed fatty change, i.e., small foci of hepatic parenchymal cells containing lipid vacuoles, after 10 days exposure at the 0.1 and 0.2 mmol $kg^{-1}d^{-1}$ Ca-DTPA exposure levels. Two of four rats killed 1 day following the highest level of Ca-DTPA exposure showed a small increase in the number of goblet cells in the epithelial layer of the intestine. None of the fibrotic changes previously observed with intracheally injected Ca-DTPA at high dose levels were observed under the conditions of inhalation.

REMOVAL OF INTRAMUSCULARLY INJECTED ^{238}Pu-NITRATE FROM THE RAT BY CONTINUOUS INFUSION OF CHELATING AGENTS

Investigators:
M. F. Sullivan and V. H. Smith
Technical Assistance:
A. L. Crosby

Under prompt treatment conditions continuous infusion of EDTA or DTPA showed little therapeutic advantage over rapidly administered, single, daily treatments. Due to the greater toxicity of continuously infused Ca-DTPA, procedures sustaining that agent in the system overly long should be considered potentially hazardous.

Ninety percent of intravenously injected EDTA or DTPA is excreted in the urine within about 6 hr. Sustained maintenance of low, but useful, concentrations of chelating agent in the blood might be more effective in removing or preventing the translocation of radionuclides susceptible to chelation therapy. To evaluate the possible usefulness of slow release formulations of chelating agents, Ca-EDTA, Ca-DTPA and Zn-DTPA were

administered by continuous infusion to rats injected intramuscularly, 1 hr previously, with $^{238}Pu(NO_3)_4$ in pH_2 HNO_3. The appropriate amount of chelating agent in 7.4 ml of pH 7.2 solution was delivered, over every 24-hr period, by Harvard infusion pumps, via polyethylene catheters implanted in the peritoneal cavity, or under the skin in the nuchal region, of partially restrained rats. Effectiveness of plutonium removal was compared with that following daily injection of the same amount of chelating agent to similarly housed animals. The pathologic consequences of continuous infusion are described in another report in this volume.

Results are summarized in Figure 5.4. It is apparent that Ca-DTPA is more effective than Zn-DTPA and that even under the conditions of continuous infusion the more medically acceptable Ca-EDTA is still too inefficient to be considered as a substitute for DTPA. In these experiments the subcutaneous route was essentially equivalent to the intraperitoneal route. Constantly infused Ca-DTPA, at the lower dose given subcutaneously, appears more efficient than the single daily injections, decreasing bone retention by about an additional 4%. Consistent with dose-effect experience, the 10-fold higher dose removes more plutonium than the lower Ca-DTPA dose; but, due to toxicity of the constant infusion administration, one is not able to say that one mode of administration is superior to the other.

Although continuously infused Ca- or Zn-DTPA was slightly more effective under prompt treatment conditions

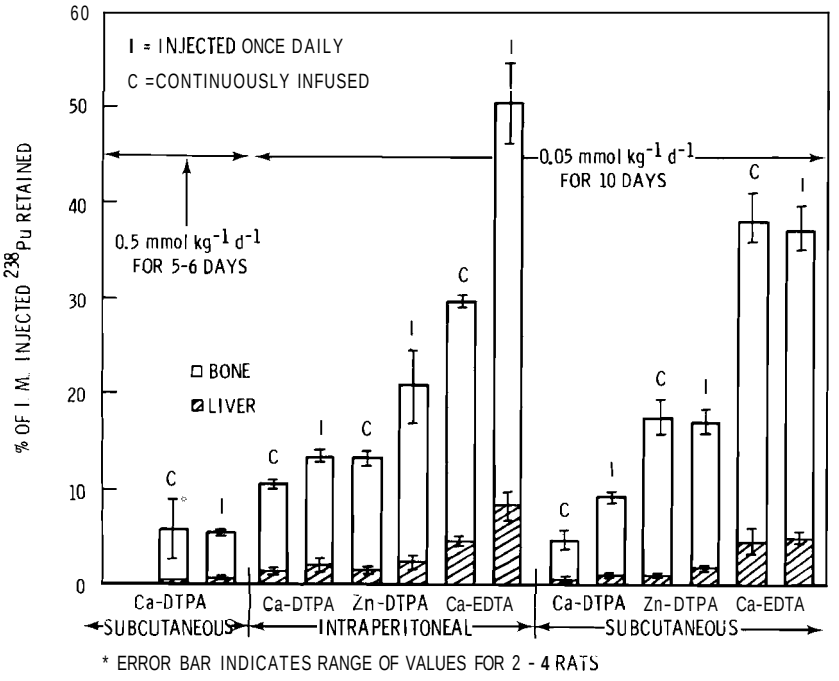

FIGURE 5.4. Percent of Intramuscularly Injected $^{238}Pu(NO_3)_4$ Retained in Rats After Prompt Treatment with Infused or Injected DTPA or EDTA

in certain instances than single daily injection, the toxicity associated with continuous infusion would contraindicate such dosage. The use of higher dose levels would seem more important than the maintenance of sustained blood levels. Still to be determined are the effects on more mobile actinides and on firmly bound plutonium burdens.

TOXICITY OF CONTINUOUSLY MAINTAINED LEVELS OF CHELATING AGENTS IN THE RAT AND MINIATURE SWINE

Investigators:

M. F. Sullivan, T. D. Mahony, H. A. Ragan, J. E. Lund, P. L. Hackett, J. L. Beamer and V. H. Smith

Technical Assistance:

A. L. Crosby, K. A. Strikwerda and M. D. Snyder

At dose levels employed in humans and under the special conditions of maintaining constant levels of the chelating agents, Ca-DTPA and Ca-EDTA were toxic in both the rat and miniature swine. Effects were particularly evident in the gastrointestinal tract and bone marrow. These effects are not seen when the chelate is administered over a short time span. Zn-DTPA was much less toxic than the Ca salt.

In studies of the efficacy of continuously maintained levels of chelating agents in removing plutonium from rats, it was observed that treatments at the level used in man, 0.028 mmol/kg/day, produced marked gastrointestinal pathology and even death. This syndrome was further studied in the rat and in the pig to determine those conditions which must be avoided if similar pathology is to be avoided in the treatment of humans.

Lightly restrained, female rats were individually caged and the appropriate amount of the chelating agent in about 7 ml of pH 7.2 solution was infused by Harvard infusion pumps, continuously, 24 hr a day, subcutaneously, intraperitoneally or intravenously via implanted catheters. The subcutaneous route produced the more consistent pathology and was the only route used during continuous infusion studies in 60 to 90 kg, female Hanford Miniature Swine. Controls received equal doses, once daily, of chelating agent administered intraperitoneally by rapid injection. Table 5.2 summarizes the mortality data on rats and pigs for various

TABLE 5.2. Toxicity of Continuously Infused DTPA

Chelate	Daily Dose mmol kg^{-1}	Fraction of Human Dose	Days Surviving on Treatment	
			Rats[a]	Pigs[b]
Ca-DTPA	1	36	3	
	0.5	18	5-6	
	0.05	1.8	7-12	5-7
	0.025	0.9	>14	8-14
	0.012	0.4		>14
Zn-DTPA	0.05	1.8	>14	>14
	0.025	0.9	>14	>14

(a) Combined results from intraperitoneal, intravenous, or subcutaneous infusion. four to eight rats/dose level.
(b) Subcutaneous infusion. Four pigs/dose level except for Zn-DTPA where there were two pigs/dose level.

dosage levels of Ca-DTPA and Zn-DTPA. At lethal dose levels anorexia was the earliest symptom noted, usually followed by profuse diarrhea including excretion of mucus; 1 to 2 days later the animals were moribund or dead. Damage to the gastrointestinal tract was similar to that previously reported for much higher doses of EDTA or DTPA.

Distribution and degree of pathology in various portions of the gastrointestinal tract of rats is shown in Table 5.3 for 0.05 mmol $kg^{-1}d^{-1}$ doses of the chelates. These effects are characterized by complete growth arrest of the columnar cells. The distended crypts are filled with leukocytes, much as seen after X-irradiation, except the cells do not show the same degree of hyperchromatism and nuclear atypia. The squamous stomach is severely damaged, the outer keratinized epithelium being separated by fluid-filled vesicles from the underlying squamous mucosa; the normally thin, lightly populated, basal cell layer of the squamous epithelium is thickened with proliferating squamous cells. The glandular stomach is affected to a greater degree than the squamous stomach by many toxic agents, but is relatively spared by these chelating agents, as is also the epithelial tissue of the esophagus.

Histopathology observed in the liver may be an inflammatory response to the gut damage. Contrary to the usual expectation from chelating agents, there was no observed kidney damage.

Bone marrow preparations were examined at 1, 3, 5, 7 and 10 days after subcutaneous infusion of 0.05 mmol Ca-DTPA $kg^{-1}d^{-1}$ into pairs of rats. Results are summarized in Table 5.4. Damage was moderate in 5 days and severe after 7 days of treatment. Effects were most pronounced in the red cell series as evidenced by increased myeloid to erythroid ratios resulting from marked erythroid hypoplasia, the presence of many necrobiotic normoblasts, frequent naked normoblast nuclei, and varying degrees of increased immaturity in the myeloid series. The marrow damage indicated in Table 5.4 due to single daily injections of Zn-DTPA was not seen in subsequent experiments, nor in pigs, even following infusion, and is not consistent with the general lack of pathology associated with animals treated with Zn-DTPA. Other experiments checking this observation will be reported subsequently.

TABLE 5.3. Comparative Pathology from Administration of Chelates to Rats

Chelate Dose and Route of Administration	No. of Rats	Gastrointestinal Tract Segment								
		Stomach								
		Squamous	Glandular	Uuodenum	Jejunum	Ileum	Cecum	Colon	Liver	Kidney
0.05 mmol $kg^{-1}d^{-1}$ Ca-DTPA										
Infusion, s.c.	3	+++	o	++	++	++	+++	++	+	o
Infusion, i.p.	1	++++		+++	++	+++	++++	+++		o
Injection, i.p.	4	o	o	o	o	o	o	o	o	o
0.05 mmol $kg^{-1}d^{-1}$ Zn-DTPA										
Infusion, i.p.	2	+	o	o	o	o		++		o
Injection, i.p.	2	o	o	o	o	o	o		+	o
0.005 mmol $kg^{-1}d^{-1}$ Ca-EDTA										
Infusion, i.p.	2	o	o	o	o	o	o			o
Injection, i.p.	2	o	o	o	o	o	o		o	o

o = No damage
+ → ++++ = Increasing damage
s.c. = Subcutaneous
i.p. = Intraperitoneal

TABLE 5.4. Chelate-Produced Bone Marrow Damage in Rats

Dose mmol $kg^{-1}d^{-1}$	Chelate	Dosage Days	Severity of Changes[a]	
			Subcutaneous Infusion	Intraperitoneal Daily
0.025	Ca-DTPA	10	+	±
0.05	Ca-DTPA	1	·	+
		3	+	o
		5	++	o
		7	+++	o
		10	++++	o
0.025	Zn-DTPA	10	++	++
0.05	Zn-DTPA	10	+	+++
0.05	Ca-EDTA	10	++++	+

(a) o = No damage
· → ++++ = Increasing severity of damage

Three of six miniature swine receiving 0.05 mmol Ca-DTPA $kg^{-1}d^{-1}$ showed a pronounced neutropenia and lymphopenia. Platelet values in these three animals were also depressed to less than 30% of normal by day 7 of the treatment, but this degree of thrombocytopenia did not compromise blood coagulation and there was no effect noted on the prothrombin times of any animals. Depending upon the severity of diarrhea and/or emesis, varying degrees of hemoconcentration were noted. The bone marrow and peripheral blood response to Zn-DTPA was much less severe, even after 14 days, than to similar doses of Ca-DTPA.

Based on the above, and data in the literature, efforts to obtain approval of Zn-DTPA by the FDA would seem warranted, to provide a greater safety margin in repetitive treatments; Ca-DTPA would still be preferred for prompt treatment because of its more efficient radionuclide removal. Attempts to prolong the presence of DTPA must be carefully evaluated to assure the absence of conditions capable of eliciting the gastrointestinal or marrow syndrome observed in the present studies.

In view of the substantially lesser toxicity of Zn-DTPA, zinc depletion by Ca-DTPA is an attractive explanation for the higher toxicity

of the calcium salt. Figure 5.5 shows the effect of continuous infusion of Ca-DTPA in miniature swine on the concentration of Zn in blood; the dose-related decrease was not observed during infusion with Zn-DTPA. During the first 4 days following the infusion of Ca-DTPA there was a 10-fold increased rate of urinary zinc excretion. Zinc excretion in the urine during treatment with Zn-DTPA increased to about 100 times pretreatment levels. No significant changes in calcium, copper, manganese, iron, or cobalt levels in blood were observed during DTPA treatments. Using ^{14}C-labeled chelating agents, the daily excretion of label was usually >90% of the amount administered, indicating that toxicity is probably not due to an accumulation of the chelating agent.

Rats receiving 0.5 mmol $kg^{-1}d^{-1}$ Ca-DTPA by constant infusion were given daily injections of 0.5 mmol zinc acetate kg^{-1} intraperitoneally in an attempt to prevent the toxic effects. The zinc-treated rats lived 66% longer than rats without the supplemental zinc. Failure to completely negate the toxicity of Ca-DTPA may have been due to biological unavailability of the injected zinc, or to the rapid secretion of the Zn. In both rats and pigs, an interruption in the constant infusion of Ca-DTPA seemed always to be followed by a rapid recovery of the animal, though recovery processes have not been quantitatively examined.

In considering these data in relation to current human treatment practices, it must be noted that single, rapidly administered doses did not cause pathology to the degree observed with constant infusion. It seems unlikely, therefore that the effects noted here would occur under normal conditions of human treatment.

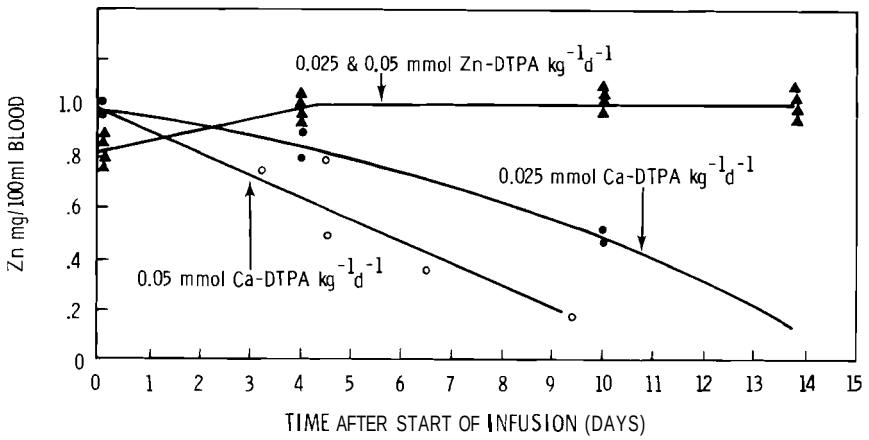

FIGURE 5.5. Zn Concentration in Whole Blood Following Continuous Infusion of DTPA in Swine

THE CHELATABILITY OF PLUTONIUM AND EINSTEINIUM IN BLOOD

Investigator:

J. E. Ballou

Technical Assistance:

R. A. Gies and W. G. Morrow

Compared to trivalent actinides, tetravalent plutonium citrate was relatively slowly cleared from blood, whether given intravenously or by intratracheal instillation; 50 to 80% of the Pu in blood, as long as 3 hr after administration, was ultrafilterable in the presence of added DTPA. Einsteinium, a trivalent actinide, was even more readily chelatable in blood. However, due to rapid translocation, only a small fraction of the dose was available in blood after 3 hr. These results indicate the importance of blood clearance kinetics to the success of DTPA therapy.

The advantages of prompt DTPA therapy in radionuclide decorporation are well recognized; however, the time span when efficient early removal can be achieved has not been precisely defined. In order to develop an effective treatment regimen it is of interest to know more about 1) the kinetics of early clearance of the radionuclide from blood, a major target of prompt DTPA therapy, and 2) the chelatability of the radionuclide in the circulating blood after different modes of exposure and at various times after incorporation. This report summarizes results from a continuing study to determine the clearance kinetics and chelatability of the transuranic elements in the circulating blood.

Blood samples were withdrawn at intervals from 5 to 240 min after administration of ^{239}Pu citrate or ^{253}Es nitrate, intravenously or intratracheally, to a dog anesthetized with pentobarbital. Radioactivity was determined in whole blood samples to give the blood clearance curves shown in Figure 5.6. At each sampling time two samples of blood plasma, one made 7×10^{-3} M in Ca-DTPA, were ultrafiltered to obtain a measure of the availability of the radionuclide for chelation by DTPA. Since most of the radionuclide is attached to plasma proteins, the same results are obtained whether plasma or whole blood is used in these experiments.

After intravenous injection of the citrate, plutonium was cleared quite slowly from the blood (Figure 5.6a). As long as 3 hr after plutonium injection, DTPA chelated about 42% of the administered dose (about 80% of the amount then present in blood). Only a small fraction of the plutonium was ultrafilterable without DTPA.

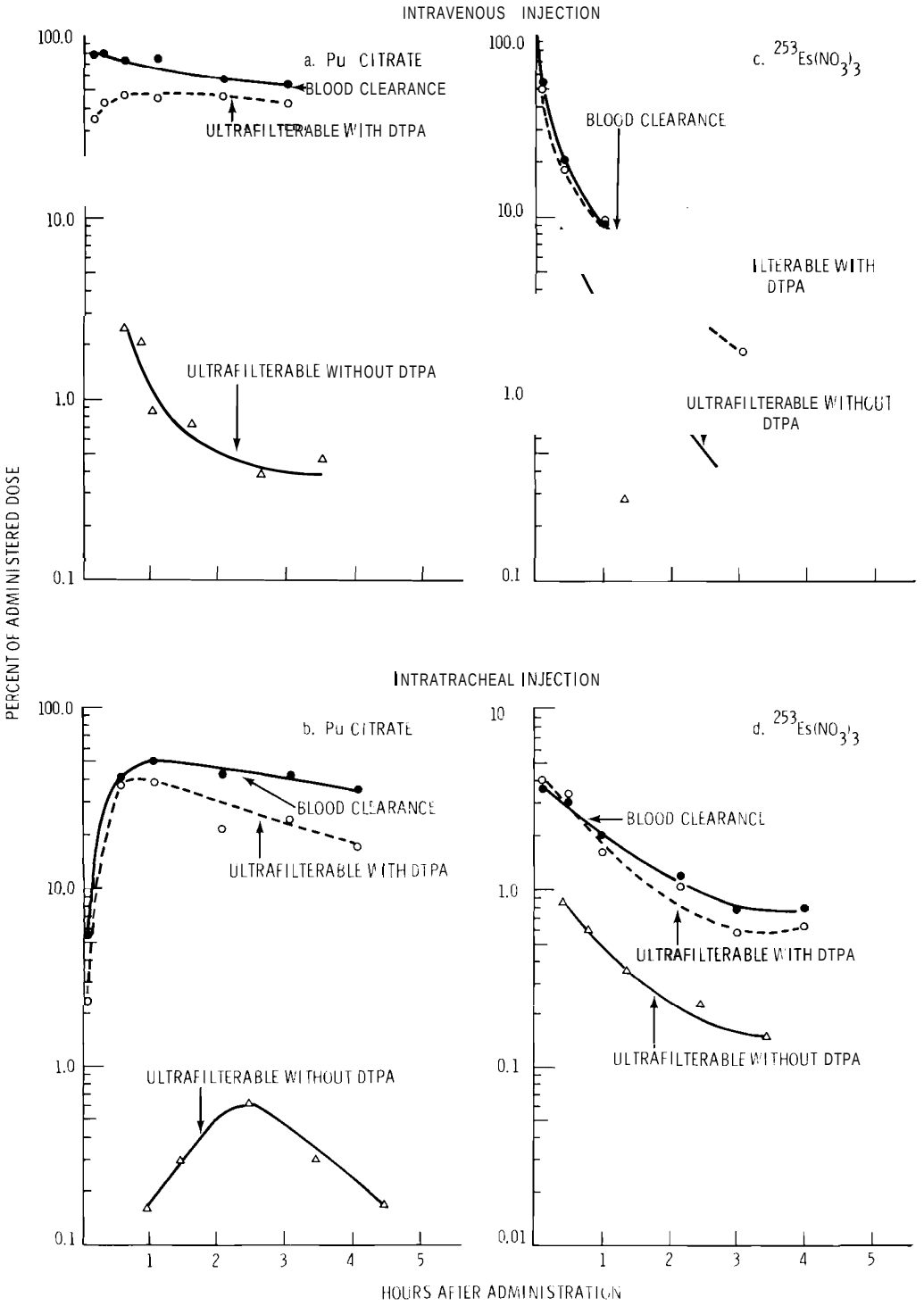

FIGURE 5.6. Chelatability of Pu Citrate and $^{253}Es(NO_3)_3$ in Dog Blood. DTPA added to blood samples withdrawn at indicated time.

After intratracheal instillation (Figure 5.6b), blood plutonium was more readily chelated during the early phase of translocation from lung to blood than at later times. After 4 hr, about 20% of the dose, or 50 to 55% of the plutonium then present in blood, was chelatable. Again, the ultrafilterability without DTPA was negligible.

The behavior of ^{253}Es, a trivalent actinide of high specific activity, differed from that of plutonium both in blood clearance kinetics and in ultrafilterability. Intravenous $^{253}Es(NO_3)_3$ was more quickly cleared from the blood than plutonium and essentially all of the einsteinium in blood was chelatable (Figure 5.6c). A significant fraction of the blood einsteinium was ultrafilterable without DTPA, perhaps indicative of differences in blood binding or transport mechanisms.

The results after intratracheal instillation of $^{253}Es(NO_3)_3$ (Figure 5.6d) are puzzling in that a build-up phase was not seen in the blood clearance curve and the amount present in blood never reached very high levels. This may reflect a rapid clearance from blood, perhaps accentuated by the very small radionuclide mass involved. Other studies, with rats exposed to $^{253}Es(NO_3)_3$ by inhalation, show a high rate of lung clearance, and early translocation of about 40% of the lung burden to other tissues. It is clear from the present studies that einsteinium is more available for chelation by DTPA than is plutonium. A large fraction of the einsteinium reaching the blood was also ultrafilterable without DTPA, in agreement with the results obtained after intravenous injection.

The results of these studies emphasize differences in radionuclide metabolism that may influence the success of early DTPA therapy. Radionuclides with relatively long retention times in blood, e.g. plutonium, provide the greatest margin for delay in treatment while still promising some degree of successful removal. Other radioelements, other compounds or other modes of incorporation exhibit different blood clearance kinetics, calling for different DTPA treatment regimes. The period during which greater decorporation can be achieved by "prompt" chelation therapy is thus dependent on many factors, and while it may be as long as 4 hr after the radionuclide enters the blood, it is often less.

LONG-TERM EFFECTS OF INHALED DTPA IN RATS PREVIOUSLY EXPOSED TO $^{239}Pu(NO_3)_4$ AEROSOL

Investigator:
J. E. Ballou
Technical Assistance:
R. A. Gies and W. G. Morrow

The acute toxicity of inhaled DTPA in rats and hamsters was reported in last year's Annual Report. DTPA doses up to ten times the level used in humans induced only transient pathologic lesions including a mild form of emphysema. Studies are now in progress to determine the late effects of inhaled DTPA and inhaled $Pu(NO_3)_4$ in 519 male rats.

Plutonium retention was reduced about 50% by six, weekly DTPA inhalations starting 20 days after plutonium inhalation. The survival, weight gain and incidence of pathologic lesions is being followed for the life-span of these animals and their appropriate treatment control groups. After 500 days there were no statistically significant differences in survival, weight change or incidence of gross pathological lesions among rats receiving Pu with no treatment, sham treated, or treated with DTPA, despite differences in cumulative lung dose of 1200, 600 and 320 rads, respectively. The DTPA treatments did not decrease the number of early deaths nor the number of lung lesions observed grossly at sacrifice, despite the lower plutonium burden. Whether DTPA treatment will increase survival or alter the severity or type of late effects will be determined as these animals are observed over their life-span.

MECHANISMS OF RADIATION EFFECTS

MECHANISMS OF RADIATION EFFECTS

The measurement of overt pathologic changes induced by ionizing radiation has produced data vital to the establishment of radiation protection standards. Such gross changes, however, as with most clinical measures, are the observed terminal effects of events occurring early in the growth, development, and differentiation of the cell. The studies included in this project are concerned with basic biologic and chemical indices that may aid in understanding the primary effects of the radiation insult and also serve in the diagnosis of such early effects.

Several reports are concerned with the cell membrane of the eukaryotic microorganism Neurospora crassa, the regulation and biochemistry of enzymes located on the organelle, and the nature of the organization of proteins in its structure. Information is presented on the role of virus in ^{90}Sr-induced leukemia and on the potential interaction of host membrane-virus coat in a leukemia virus. The damage effected by the interaction of radiation-produced free radicals with enzyme molecules and with the membrane of the red blood cell is discussed.

REGULATION OF A PLASMA MEMBRANE INVERTASE IN A CELL WALL-LESS MUTANT OF NEUROSPORA CRASSA

Investigators:
W. R. Wiley and K. Winn

Invertase activity in the slime-mold variant of Neurospora crassa appears to be associated with the membrane of the cells. The enzyme is regulated by repression; cells suspended in the absence of carbon source actively synthesize invertase, cells grown in the presence of sucrose have constitutive levels of the enzyme associated with their membrane.

In wild type cells of Neurospora the enzyme invertase has been shown to be localized between the cell wall and cell membrane. Chang and Trevithick (1972) recently reported that the exo-invertase was covalently linked to the cell wall. Enzyme activity in the cytoplasm has never been observed.

Using a cell wall-less mutant of N. crassa ("slime"), data were accumulated in our laboratory which suggest that the exo-invertase is intimately associated with the external surface of the cell membrane. According to this model invertase is synthesized and inserted into the plasma membrane; growth of the cells and synthesis of the exo-invertase results in a release of the high molecular weight invertase into the space between the cell wall and plasma membrane (peripheral space). Since the enzyme is large relative to the pores of the cell wall, invertase accumulates in the peripheral spaces of the cells, thereby accounting for the results obtained by other investigators for wild-type cells. Evidence in support of this hypothesis was obtained by showing that 76% of the cell-bound invertase in the slime-mutant was intimately associated with purified plasma membrane vesicles prepared from slime.

The enzymic and nucleic acid content characteristic of nuclei, mitochondria, microsomes and the cell sap are virtually absent in the purified vesicle preparations. Table 6.1 shows the percent of some cellular components associated with the membrane preparations. The absence of cytochrome C, succinic dehydrogenase and Mg^{+2}-dependent ATPase suggest that the vesicle preparations are free of mitochondria. They are also free of nuclei and microsomes as judged by the virtual absence of DNA and RNA. The presence of 5 to 10% of

TABLE 6.1. Enzymic and Nucleic Acid Contents of Purified Membrane Vesicles

	Percent of Activity Present in Intact Cells
DNA	<0.05 (a)
RNA	<8
Succinic dehydrogenase	0.02
Cytochrome C	N.D. (b)
µg ATPase	0.2
5' Nucleotidase	0.05
Repressible alkaline phosphatase	35
Glucose-6 phosphatase	8
Repressible invertase	76

(a) Near the limits of detection
(b) Not detectable in vesicle preparation, typical cytochrome C spectra observed in cell extracts at comparable protein concentration

the RNA of the cell in the cytoplasmic membrane vesicles has been observed by others. The function of this membrane-bound nucleic acid is presently under intensive investigation in other laboratories. The high percentage of the cellular invertase and alkaline phosphatase in the membrane vesicle fraction indicates that these enzymes are intimately associated with the cytoplasmic membrane.

The plasma membrane vesicles were very heterogeneous in size (0.1µm to 0.7µm) and density (1.17 to 1.22 gm/cm^3). Figure 6.1 shows the distribution of membrane protein and invertase activity from a continuous gradient after centrifugation of purified vesicle preparations at 90,000 × g for 3 hr. Invertase and protein were determined in 0.5-ml fractions taken from the bottom of the centrifuge tube. The variation in invertase activity in vesicles of differing density follows closely variations in membrane proteins.

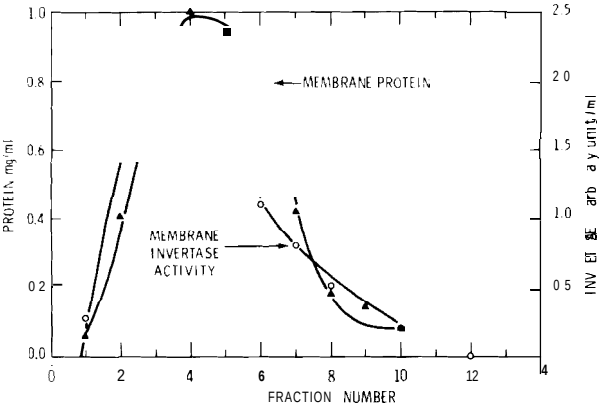

FIGURE 6.1. Centrifugation of Membrane Vesicles Derived from N. crassa, Slime Mold Mutant on a Discontinuous Sucrose Gradient at 90,000 × G for 3 Hr. Membrane protein and invertase activity was measured on vesicles. Note the absence of soluble invertase activity (material at the top of the gradient) in the centrifuged material.

Metzenberg (1962) showed that invertase in periplasmic space in Neurospora was derepressable by growth in certain sugars. The membrane-bound invertase described above is regulated in a manner very similar to that of the periplasmic enzyme in wild-type cells examined by Metzenberg.

Figure 6.2 shows the time course of derepression for invertase synthesis following growth for 24 hr (doubling time of these cells is 6 hr) in medium containing 2% sucrose. The cells were harvested, washed and resuspended in medium without a carbon source. As illustrated in Figure 6.1, there is a 20- to 30-min delay in the initiation of synthesis followed by a linear increase in invertase specific activity.

The linear increase in invertase activity continues for approximately 90 min. Increase in invertase specific activity (from 0.4 to 16) is inhibited by glucose and sucrose. Synthesis of the enzyme requires de novo protein synthesis since cycloheximide completely inhibited enzyme synthesis.

After 90 min of linear enzyme synthesis there was a linear decrease of specific activity of membrane-bound invertase. The decay in activity is consistent with the hypothesis that the accumulation of invertase in the peripheral space in wild-type cells is a result of membrane turnover of this enzyme.

An alternative and certainly a less interesting model is that invertase is nonspecifically degraded due to the carbon starvation conditions used to derepress the cells, which would invalidate these results in terms of their physiological significance. By examining invertase activity in the derepression medium and by growing cells under nonstarvation conditions we should be able to distinguish between these two models.

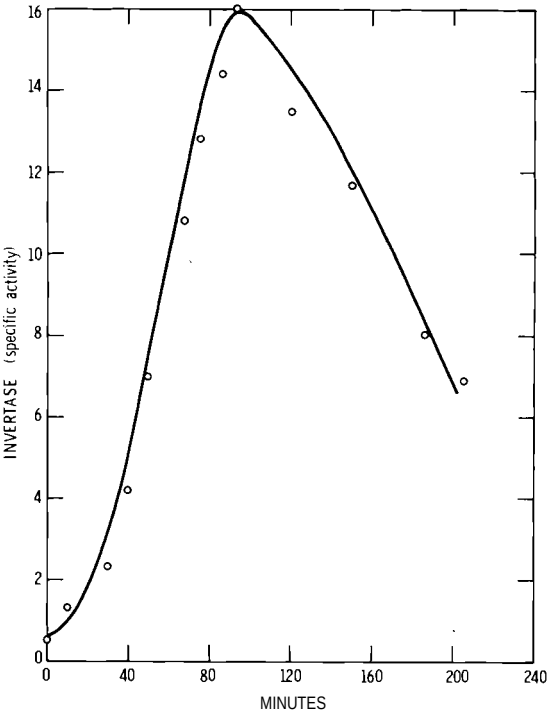

FIGURE 6.2. Time Course of Invertase Biosynthesis in N. crassa, Slime Mold Mutant. Cells were grown for 24 hr in a medium containing sucrose and then transferred to a medium containing no carbon source. Invertase activity associated with the cells was measured at the intervals shown.

In FY 1975 we will use the membranes from slime variants of Neurospora and the invertase derepression data to answer questions concerned with the synthesis, insertion, and topography of specific enzymes in cell membranes of Neurospora. The fact that invertase is derepressible in the mutant should permit us to use immunological techniques for these studies.

THE EFFECTS OF SUGARS ON PROTEASE BIOSYNTHESIS IN NEUROSPORA CRASSA

Investigator:
H. *Drucker*
Technical Assistance:
L. C. Neal

Over the concentration range 10^{-5}-10^{-3}M, sugars fell into four groups: those causing no effect, e.g., fructose; those capable of accelerating protease biosynthesis, e.g., glucose; those effecting a weak catabolite repression, e.g., lactose; and one sugar, cellobiose, which appeared to cause extreme catabolite repression at very low concentrations.

Previous reports have presented evidence supporting a model for the regulation of exocellular protease biosynthesis in the fungi Neurospora crassa. In this model the first step in the induction of protease from N. crassa involves the autocatalytic activation by limited proteolysis of an inactive protease zymogen located in the cells' periphytic space. Two products are generated by the autocatalytic cleavage: an active proteolytic enzyme which is released from the cell into the milieu, and a zymopeptide. In the presence of a protein substrate, this zymopeptide enters the cell and serves as the "message" to the cells' genetic equipment that a metabolizable protein incapable of entering the cell lies in the medium. As this model appeared to explain the events occurring early in protease biosynthesis (taking place at the level of the cell membrane), we began studies to determine what effects small molecular weight nutrilites, located in the cell cytoplasm, would have on protease biosynthesis. In a previous report, we discussed the effects of commonly occurring amino acids on the induction process. In this report, we will consider the effects of common mono- and disaccharides on the biosynthesis of exocellular protease.

A typical experiment consisted of growing cells of N. crassa strain 74A on 1% sucrose, minimal salts medium for 12 hr, harvesting the cells and suspending them in a medium containing salts alone (no carbon source) for 30 min to effect starvation and depletion of endogenous sugar pools, and then resuspending them in a medium containing protein (1% bovine serum albumin, BSA), desired concentration of sugar, and activating protease (thermolysin). In earlier work, using cells grown from conidia on protein as principal carbon source, we had examined the effects of sugars at high substrate concentration levels (0.06-0.1M), where the sugar behaved as a more

easily metabolizable carbon and energy source than did the protein, and had observed either catabolite repression, mild stimulation or no effect on protease biosynthesis. Since we were interested now in determining the effect of sugars on the regulation of exocellular protease early in induction, low substrate levels of added nutrilite (10^{-3}-10^{-5}M) were employed in the studies with log phase cells.

In the initial screening study, no sugar [with the exception of D(+) cellobiose] affected the rate of protease production when present at concentrations between 10^{-5}-5 × 10^{-4}M. At sugar concentrations of 10^{-3}M, three types of response were observed in terms of protease biosynthesis.
1) Accelerated protease production: Sucrose, glucose, D(-) mannitol, L(-) sorbose, and D(+) arabitol affected an increase in rate of protease production of from 29 to 39% over control with no sugar added. Sucrose, glucose, and maltose are good carbon and energy sources for N. crassa and, at high concentrations effect catabolite repression of protease biosynthesis. Mannitol and D(+) arabitol are poor carbon and energy sources which, in cells grown from conidia on a protein-containing medium, cause low levels of catabolite repression (mannitol) or mildly stimulate protease production [D(+) arabitol]. L(-) sorbose does not appear to serve as either a carbon or an energy source for N. crassa and previous work has shown that in a medium containing substrate quantities of protein and L(-) sorbose, conidiospores do not germinate and thus protease is not produced.
2) No effect on protease biosynthesis: Fructose, alpha-melibiose, d-ribose, L-arabitol, D-a-galactose cause no changes in rates of protease biosynthesis as compared to controls with no sugars added. With the exception of fructose, these are sugars which are poorly metabolized by N. crassa and have little or no effects on protease biosynthesis in cultures grown from conidia on medium containing BSA and the test sugars. Fructose, in cultures grown from conidia, is half again as good a catabolite repressor as glucose or sucrose.
3) Weak catabolite repression of protease biosynthesis: D(+) lactose, alpha-lactose, L(-) fucose, D(-) xylose, L-fucose lowered rates of protease biosynthesis from 20 to 30% as compared to controls with no sugars added. These are all sugars that are poorly metabolized by N. crassa (as compared to glucose or sucrose) and which effect catabolite repression of protease biosynthesis in cells grown from conidia on protein plus substrate quantities of sugar.

Only one sugar, D(+) cellobiose, appeared to be capable of almost complete repression of protease biosynthesis. At concentrations of 10^{-4}M, protease biosynthesis was repressed 10%. At 10^{-3}M cellobiose, the rate of protease biosynthesis was 26% that of the control containing no added sugar. Higher concentrations of cellobiose did not increase the extent of repression. Thus, it would appear that cellobiose is a most

efficient catabolite repressor. Earlier work had shown that cellobiose was a good carbon source for growth of N. crassa and an excellent catabolite repressor of protease biosynthesis by cells grown from conidia on medium-containing sugar and BSA.

This initial survey, along with the results in last year's Annual Report on the effects of amino acids upon protease biosynthesis, has resulted in two molecular "tools" for analyzing the regulation of exoenzyme biosynthesis within the cell cytoplasm and, hopefully, at the level of the cells' genetic material. The phenomena of "turning on" protease genes may be analyzed by employing the specific repressor tryptophan (functioning by way of its conversion to the metabolite kynurenine) which, when present, completely blocks the synthesis of enzyme. Regulation of the rates of protease biosynthesis may be examined by the use of the catabolite repressor D(+) cellobiose, a poorly metabolized sugar which drastically lowers the rates of protease biosynthesis.

STUDIES ON THE ACCELERATION AND CATABOLITE REPRESSION OF PROTEASE BIOSYNTHESIS IN NEUROSPORA CRASSA BY SUCROSE, GLUCOSE, AND FRUCTOSE

Investigator:
H. *Drucker*
Technical Assistance:
L. C. Neil

Glucose and sucrose, at low concentrations (10^{-5}-10^{-3}M), appear to accelerate synthesis of exocellular protease in Neurospora crassa. At higher concentrations, these sugars effect catabolite repression of protease biosynthesis. The concentration dependence of acceleration and repression of protease biosynthesis by sucrose was identical to that of glucose when compared on a mole monosaccharide basis. Over the same concentration range, fructose was without effect. The data suggest that in starved cells of N. crassa invertase activity and monosaccharide transport and utilization may be coupled in some fashion.

As discussed in the preceding report, low concentrations (10^{-5} -10^{-3}M) of the sugars glucose and sucrose appeared to accelerate the rates of protease biosynthesis in the fungi N. crassa. Earlier work, however, had demonstrated that these sugars at a concentration of 0.06 to 0.1M would effect catabolite repression of the synthesis of Neurospora

exocellular protease. We therefore examined the effects of these sugars, both singly and in combination, over the concentration range 1 to 40 mM. These studies resulted in an interesting paradox, as will be seen.

Starved cells of N. crassa were suspended in a medium containing the given sugar (or sugars) at the desired concentration, plus protein substrate and activating protease (thermolysin). Both the initial (slow) rate of protease production and the terminal (fast) rate of protease induction were measured and will be expressed here as percent of control rate (no sugar added). As both rates appear to have the same dependence on sugar concentration, we will discuss here only the terminal (fast) rate of induction.

When glucose was employed as an added sugar during protease induction, the rate of protease biosynthesis appeared to increase over the concentration range 0 to 5 mM glucose, reaching a maximum at approximately 8 mM glucose, decreasing to control levels at 17 mM glucose, and then reaching a terminal rate, 30% of control, at 30 mM glucose. This result is consistent with the following interpretation: At low glucose levels, the sugar behaves as a supplementary carbon and energy source for the starved cells, but is not capable of effecting catabolite repression; at higher glucose levels, the sugar is used as the sole carbon and energy source and catabolite repression is effected.

When sucrose is substituted for glucose in experiments identical to those described above, a qualitatively similar pattern of results is obtained, but with a displaced concentration dependence. Maximum acceleration of protease synthesis is observed at 8 mM glucose; at 4 mM sucrose. Control levels of induction rate are reached at 17 mM glucose; at 9 mM sucrose. Maximum repression of exocellular protease is observed at 30 mM glucose; at 15 mM sucrose. If 1 mole of the disaccharide acts like 2 moles of monosaccharide, the concentration discrepancy is resolved.

The literature on sucrose utilization by Neurospora would suggest, however, that sucrose cannot be treated as if both the fructosyl and glucosyl moities were metabolically equivalent. Under normal conditions of growth where cells are not starved, Metzenberg and Marzlaff have demonstrated that the first step in sucrose metabolism by N. crassa consists of hydrolysis of the disaccharide to component monosaccharides by an exocellular invertase. Intact sucrose is not transported into the cell. Glucose released by the exocellular invertase activity is transported into the cell and utilized; fructose is only transported and utilized after all available glucose is depleted from the media. If this were the case for sucrose utilization in the protease induction system, then both the acceleration of protease biosynthesis and the repression of synthesis should be equivalent, mole-for-mole, for sucrose and glucose. Instead of this, as previously stated, sucrose appears to be experimentally equivalent to two moles of glucose.

One must consider, however, that the cells in our experiments were starved cells and thus not "normal". Levels of invertase activity in the induction medium must have been quite low, if the enzyme was present at all. In order to explain the sucrose effect on protease biosynthesis in terms of data and interpretations available in the literature, one would have to postulate that a low level of exocellular invertase is produced very early in starvation and/or protease induction, resulting in sucrose hydrolysis. Further, the cells must be able to transport both the glucosyl and fructyl residues simultaneously (or nearly simultaneously). If this were the case, fructose should stimulate or repress protease biosynthesis in this system to the same degree and with the same concentration dependence as glucose. Fructose, however, was shown to be without effect on the protease induction system, implying that free fructose is either not transported and utilized, or is transported and utilized in a fashion considerably different from that of the fructose released from sucrose by invertase.

If free fructose in this experimental system is transported by the same membrane proteins that carry the fructose released from sucrose by invertase, then added fructose should result in increased stimulation of protease induction at low levels of fructose and sucrose and increased repression at high concentrations. In experiments where protease induction was measured in cultures containing both fructose and sucrose, the extent of stimulation or repression was a simple function of sucrose concentration; again, added fructose had no effect on the induction process.

At this point in our research, we suggest the following speculative working model. Neurospora might possess a constitutive membrane-bound invertase activity. Hydrolysis of sucrose in our experimental conditions occurs at the cell membrane, and the monosaccharides released are immediately transported by transport proteins coupled in some fashion to the invertase activity. These monosaccharide transport sites function only in the utilization of the fructyl and glucosyl moities released by the cell-bound invertase and do not function for the transport of free glucose or fructose present in the media. This model would explain most of our experimental observations. Free fructose would not effect protease biosynthesis because it would never enter the cell. Sucrose would behave as two moles of glucose because both monosaccharide moities enter by way of the postulated invertase-transport complex.

The suggested model is operationally identical to a more trivial model. Starved cells have transport sites for sucrose and glucose, but not fructose, and possess an intracellular invertase activity. At this time, such a model cannot be rejected out of hand. However, Metzenberg and Marzlaff in normally grown cells did not observe a sucrose transport system nor an intracellular invertase. Spheroplasts (membrane sacs) of N. crassa are stabilized by sucrose,

which would not be the case if the sugar were transported efficiently by the cells. The application of these last two observations is teleological in the extreme, and only further experimentation can establish either of these suggested models.

CHARACTERIZATION AND PURIFICATION OF N. CRASSA EXOCELLULAR PROTEASES

Investigators:
K. Miyata and H. Drucker
Technical Assistance:
L. C. Neil

An exocellular alkaline protease from Neurospora crassa was purified to crystallinity and partially characterized. The enzyme was characterized as an alkaline protease by its pH optima, and its inactivation by di-isopropylfluorophosphate and dimethylsulfonyl fluoride. The enzyme appears to be unstable to autodigestion and requires calcium and pH 7.0 buffers for optimal stabilization.

To understand the regulation of exocellular protease biosynthesis in Neurospora crassa in terms of factors controlling the synthesis of individual proteases and peptidases, it is necessary to purify and characterize these enzymes. In this report, we will discuss the properties of three proteases found in N. crassa cell-free growth medium, and the purification of one of these.

A crude preparation of N. crassa exocellular protease was obtained from cell-free media by precipitation with 60% saturation ammonium sulfate. The supernatant from the precipitation had little or no enzyme activity. An analysis of the kinds of proteolytic activities produced by N. crassa and present in this crude enzyme preparation was then performed.

Enzyme activity was measured as a function of pH on the substrates hemoglobin, serum albumin, and casein. There appears to be an acid protease with a pH optimum of 4, a neutral protease with a pH optimum of approximately 7, and an alkaline protease with a pH optimum of 10. Effects of a number of known inhibitors of protease activity were determined. Diisopropylphosphoryl fluoride appeared to inhibit all protease activity at pH 10, with 12% and 21% of control activity remaining at pH 4 and 7. These results again suggest that

three types of exocellular protease are produced by N. crassa, with perhaps 70% of the activity associated with alkaline protease. Dimethylsulfonyl fluoride, another inhibitor of alkaline protease activity, was incubated with crude enzyme preparation and, again, results were obtained suggesting that approximately 70% of N. crassa exocellular protease is alkaline protease. The inhibitor para-chloromercuribenzoate, an inhibitor of enzymes requiring sulfhydryl groups for activity had no effect on the crude enzyme preparation, suggesting that papain-like enzymes are not produced by N. crassa. Although EDTA and o-phenanthroline had previously been demonstrated to inhibit approximately 30% of the enzyme present in cell-free media, they effected no inhibition of activity in the crude enzyme preparation. This may be due to the high levels of bovine serum albumin present in the crude preparation.

On the basis of the above work, it appeared that a large part of the exocellular protease activity secreted by N. crassa consisted of alkaline protease activity. In addition, Marzlaff had shown that under conditions where protein was used as a source of sulfur for Neurospora, only one enzyme, alkaline protease, appeared to be produced by the cells, implying that this enzyme may play a singularly important role in the metabolism of protein. For these two reasons, we began our purification studies with the alkaline protease. Figure 6.3 summarizes the procedures used.

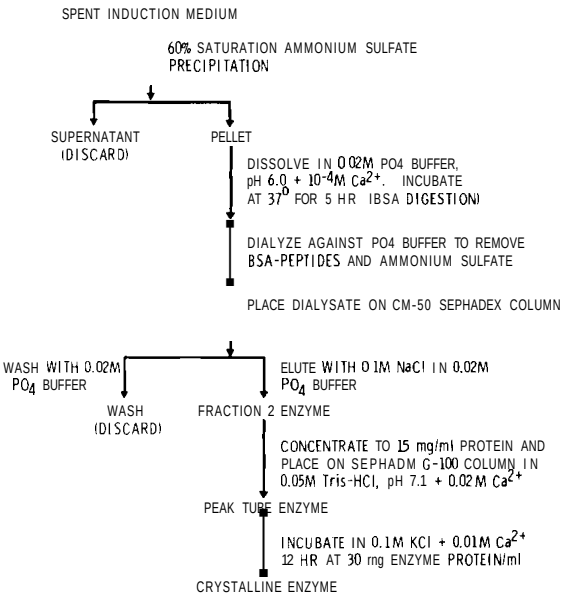

FIGURE 6.3. Flow Diagram for Purification of N. crassa Alkaline Protease

After the purification step involving CM-50 Sephadex, an approximate 100X purification over the crude enzyme preparation had been achieved (Fraction 2). Fold purification based on units of activity/mg protein are somewhat misleading in this system, as crude enzyme contains large quantities of the substrate protein (BSA) used in induction. On nonprotein substrates, pure enzyme preparations from other microbiota are sometimes observed with a five- to tenfold purification. Obviously, this would not be observed in our system. Fraction 2 lost activity upon storage and rechromatography, suggesting that it might be prone to autodigestion Attempts were therefore made to stabilize and partially characterize this fraction.

Fraction 2 appears to contain exclusively alkaline protease activity;

it is 100% inhibited by di-isopropyl-fluorophosphate and dimethylsulfonyl fluoride, but not inhibited by EDTA or parachloromercuribenzoate. Incubation of enzyme and substrate with a number of metals (Mg, Ca, Sr, Mn, Co, Zn, Cd, Cu, and Hg) resulted in no inactivation or increase in activity. However, Ca^{2+} and Sr^{2+} at 10^{-2}M appeared to stabilize the enzyme against thermal denaturation.

Addition of casein and bovine serum albumin to dilute purified enzyme solutions markedly increased thermal stability and prevented activity loss. pH stability curves demonstrated maximal stabilization of the enzymic activity at pH 7.0 at temperatures of either 4" or 37°C.

The above findings allowed us to resume purification, working in buffers at a pH of 7.0, calcium concentrations of 10^{-2}M, and in highly concentrated enzyme solutions The additional purification procedures as outlined in Figure 6.3 resulted in the isolation of crystalline enzyme. This crystalline enzyme was subjected to electrophoresis at pH 8.7 and appeared to be homogenous both in protein and enzymatic activity. Further studies with the crystalline enzyme are now in progress.

REGULATION OF ION TRANSPORT ACROSS MAMMALIAN CELL MEMBRANES

Investigator:
R. P. Schneider
Technical Assistance:
L. M. Butcher

The sigmoidicity of the response of Na^+ efflux in intact cells to Naf concentration is dependent on the K^+ concentration inside the cells but indifferent to the external K^+ concentration. The data are explained by a model in which intracellular K^+ regulates Na^+ efflux by acting as an allosteric or feedback inhibitor creating a threshold effect. The model has proven to be useful in understanding cell volume regulation and inhibition by the pesticide DDT.

(Na-K)-ATPase AND SODIUM TRANSPORT

Animal cells maintain a low internal concentration of Na^+ (5-15 mM) by means of an active transport [(Na+K)-ATPase] system in the cell membrane. Work on the kinetics of the membrane (Na+K)-ATPase and Na^+ transport in pig erythrocytes led to the formulation of a model which explained the regulation of Na^+ transport in mammalian cells. In this

model, intracellular K^+ plays an important role in the regulation of Na^+ efflux by acting as a feedback or allosteric inhibitor. A brief description of the model based on the allosteric model of regulation of Monod, Wyman and Changeux follows.

The system exists in an equilibrium between a form in which three Na^+-binding sites have high affinity and one in which they have low affinity. In the presence of high K^+ and absence of Na^+, the K^+ site is occupied and the low affinity form predominates. Addition of Na^+ activates and shifts the equilibrium toward the high affinity form displacing K^+. The equilibrium shift and the multiple Na^+ sites generate sigmoidal Na^+ saturation curves since binding of the first Na^+ ion tends to stabilize the other sites in the high affinity form.

The model was tested during 1973 by measuring the response of active (ATP-driven) Na^+ transport to varying Na^+ concentration in swine erythrocytes containing reduced levels of K^+. The results of these experiments conformed exactly to predictions based on the model. Cells, which contained normal levels of K^+, i.e., 130 mM minus the Na^+ concentration, showed a sigmoidal response to Na^+ concentration; the apparent order of the dependence on Na^+ was 2.4. Cells containing intermediate and low concentrations of K^+ were prepared by replacing K^+ with Li^+. The response to Na^+ of these cells was less sigmoidal; the order was 1.8 and 1.2 in cells containing intermediate and low levels of K^+, respectively. Other experiments showed that the inhibitory effects of K^+ was restricted to the inside. The relationship between Na^+ efflux and Na^+ concentration of high K^+ cells incubated in high Li^+ solutions was sigmoidal, whereas that of high Li cells in high K^+ solutions was hyperbolic (Figure 6.4). The regulatory properties of the transport system exhibit pronounced asymmetry.

The allosteric-like kinetics of the system provide fine control of the steady state, since the efflux rate is proportional to Na^+ concentration to the 2.4 power. Further, at low concentrations, plots of Na^+ efflux and concentration show a threshold effect at the steady state Na^+ concentration.

The utility of the regulation model has been demonstrated by its ability to explain volume regulation in red cells, and inhibition of (Na+K)-ATPase by an environmental pollutant, DDT.

REGULATION OF CELL VOLUME

It has been known for several years that the high internal concentration of protein in cells, relative to the outside, generates an osmotic gradient which tends to move water into the cell. The cell's volume is maintained by the Na^+ transport system, which increases the efflux of Na^+ and erases the osmotic gradient. During 1973 the response of efflux to Na^+ concentration was measured in cells which were swollen by suspending them in dilute solutions. This

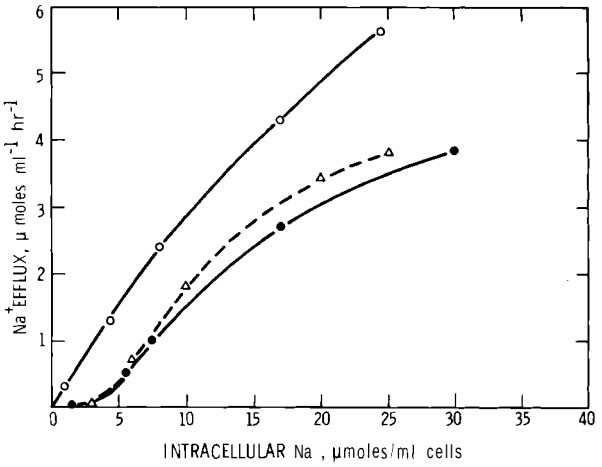

FIGURE 6.4. The Response of the Pig Red Cell Na^+ Transport System to Intracellular Na^+ Concentration. Two kinds of cells were used in this experiment: those containing the normal high concentration of K^+, which were suspended in medium containing 145 mM LiCl (solid circles) or 145 mM NaCl (triangles); and cells containing low K^+ (15 μmoles/ml cells) suspended in medium containing 145 mM KC1 (open circles). This figure demonstrates that the sigmoid response to intracellular Na^+ is caused by intracellular and not extracellular K^+.

experiment demonstrated that the affinity of the transport system for Na was increased by increased cell volume. Further, the order of dependence decreased from 2.3 to 1.8, suggesting a release from K^+ inhibition which, in turn, implies that the increased cell volume altered the allosteric properties of the system. Two alternative explanations are possible: 1) the increased cell volume physically stressed the membrane in such a way as to shift the equilibrium to the high affinity form of the enzyme, 2) the movement of water into the cell decreased the concentration of K^+, which resulted in decreased allosteric inhibition and increased affinity for Na^+.

During 1973, a method was developed which permits one to vary the total cation content of red cells without changing cell volume. Cells containing reduced levels of K^+ and Na^+ can be prepared by incubating them in p-chloromercuripheny1 sulfonate in solutions with one-half the normal NaCl and KC1 concentrations, the osmotic strength being maintained with sucrose. Similarly, cell volumes can be altered without changing the internal concentrations of Na^+ and K^+. Experiments can now be designed to examine the effects of altered K^+ levels and volume changes independently.

DDT INHIBITION

Previous work on DDT inhibition of the (Na+K)-ATPase showed that the level of inhibition was dependent on the concentrations of Na^+ and K^+; i.e., the enzyme was protected by Na^+ and inhibition was potentiated by K^+. Work during 1973 showed that a large amount of DDT was bound to membranes from rat brain containing the (Na+K)-ATPase. The concentration of bound DDT at saturation was 350 pmoles/μg membrane protein; this is 12% (w/w) of the total protein in the membrane. Since the DDT molecule is exceedingly hydrophobic, it probably accumulates in the lipid phase of the membrane. The binding was not dependent on the concentrations of Na^+ and K^+, suggesting that the effect of the cations on inhibition was on the enzyme and not on the availability of DDT.

The high membrane concentration of DDT required to block enzyme activity indicates that DDT accumulation in the lipid phase alters the molecular arrangements of the lipid and interferes indirectly with the allosteric transitions of the system, possibly by stabilizing the enzyme in the K^+ inhibited form.

STUDIES OF TYPE C CANCER VIRUS ENVELOPE

Investigator:
R. P. Schneider

An ATPase on the Type C chicken leukemia virus was partially characterized and the virus produced by host cells other than myeloblasts was found to lack the enzyme, suggesting that its origin is the myeloblast membrane. Attempts to separate the enzyme from the virus particle have been unsuccessful to date.

A study of avian myeloblastosis virus (AMV) which causes myeloblastic leukemia in chickens was initiated in 1973. Preliminary work was conducted in the laboratory of Dr. George Beaudreau at Oregon State University in order to learn techniques and to ascertain the feasibility of committing effort in 1974 to a study of the interaction of this virus with the cell membranes of infected cells. The virus, like the one associated with leukemia in ^{90}Sr-treated swine, is a Type C virus, which consists of an electron-dense core of RNA and protein enveloped by a lipid-protein structure. These particles are released from the host cell by budding and the virus envelope may be derived from host cell membrane. The virus is released into the blood of infected chickens, which contain up to 2×10^{12} AMV particles per milliliter of plasma. The AMV then, can be easily harvested and purified in relatively large quantities from either infected chickens or cultured myeloblasts.

The AMV particles present in the blood of leukemic chickens contain an ATPase of high specific activity, 60 $\mu moles\ min^{-1}\ mg^{-1}$ viral protein. Since an ATPase purified from bacteria has a specific activity of only 50 $\mu moles\ min^{-1}\ mg^{-1}$, the enzyme is apparently a major constituent of the virus envelope. The initial studies conducted in collaboration with Dr. Beaudreau were concerned with characterizing the ATPase. The enzyme activity is stimulated 30% by 0.1M Na^+ or K^+, but the addition of the ions

together produces no additional effect and the activity is not inhibited by ouabain. Therefore, the enzyme is not, as was originally suspected, a (Na+K)-ATPase derived from the host cell since this enzyme requires Na^+ and K^+ and is specifically blocked by ouabain.

The AMV is able to cause kidney tumors and infect fibroblasts in vitro. Our work has shown that, unlike the virus derived from circulating leukemia cells, the virus from these sources lacks measurable ATPase activity. This suggests that the enzyme is derived from the host cell membrane during the budding process; it follows then that either the kidney tumor and fibroblast cells lack the membrane ATPase or the budding occurs at specific sites which are dependent on the host cell type. These possibilities will be explored further.

An important objective of the study is to isolate the ATPase enzyme from the virus. Purification will permit detailed characterization of the enzyme and the preparation of specific antibodies to it. The antibodies can then be used to assay for the enzyme in pre-leukemic cells and to isolate its specific messenger RNA in infected cells for the study of regulation of synthesis of the viral envelope. To this end, several methods which have proven useful for the solubilization of other membrane-bound enzymes were used in an unsuccessful attempt to remove the enzyme in active form from the virus. These included treatment of the virus with NaI, detergents and osmotic shock. Further attempts will be made and we anticipate that a successful method will be found.

Since the virus particle is a source of host cell plasma membrane it is potentially an exciting new tool for the study of membrane function, synthesis, and changes during transformation to the neoplastic state. The source of exterior proteins is of interest since a coat of protein derived from the host cell may mask the virus from the immune system of the infected animal.

SEARCH FOR ADDITIONAL EVIDENCE OF ONCORNAVIRUS INFECTION IN THE STRONTIUM-90 RADIATION-INDUCED LEUKEMIA OF MINIATURE SWINE

Investigators:
M. E. Frazier and J. E. Morris
Technical Assistance:
T. K. Andrews

Two more activities considered to be diagnostic for the presence of oncornavirus have been found in cases of ^{90}Sr-induced porcine Leukemia. These are reverse transcriptase activity and 70S RNA core material present in plasma.

We have demonstrated that there are viruses associated with the ^{90}Sr-induced leukemia in miniature swine. These viruses isolated from leukemic swine are similar to the RNA tumor viruses (oncornaviruses) in size, density, chemical composition, morphology, and site of maturation. It remained to be determined whether or not the virus isolated from these swine were true oncornaviruses.

The RNA tumor viruses exhibit two biochemical properties unique to them as a group. They possess a large single stranded RNA molecule with a sedimentation coefficient of 60-70S, often referred to as a high molecular weight RNA. They also contain reverse transcriptase, an enzyme capable of using the viral RNA as a template to generate a DNA complementary copy. In addition, leukemia viruses contain an interspecies specific (gs-3) antigen that is common to all mammalian oncornaviruses. If our porcine isolates have these three characteristics, considered diagnostic features of the RNA tumor viruses, they must be classified as oncornaviruses.

Complexes containing 70S RNA and RNA-directed DNA polymerase have been detected in four of six plasmas from leukemic swine. Further, we have shown that this reverse transcriptase activity and high molecular weight RNA are associated with a particle of the same density as our porcine virus isolates (1.13 to 1.18 g/cc). Four plasmas from normal swine did not exhibit these two biochemical properties. However, these findings as well as previous experience with human plasma, indicate that plasma is not the best source for the simultaneous detection of these two unique biochemical properties. We therefore plan to test virus-producing cell cultures, and various fresh and frozen tissues from leukemic swine, to determine a better source of the putative porcine leukemia virus. The virus will be purified and a radioactively labelled DNA will be synthesized by the reverse transcriptase

on its own endogenous template. The labelled DNA will then be used as a probe in molecular hybridization studies to: 1) determine the degree of homology between the porcine virus and the known leukemia viruses, 2) determine the taxonomic position of the natural host of the virus isolated from leukemic swine, and 3) examine on a molecular basis how virus and radiation interact to produce the observed leukemia.

Antisera to the following viruses: Moloney Leukemia Virus, Moloney Sarcoma Virus, Rauscher Leukemia Virus, and three isolates from swine with myelogenous leukemia are being prepared in goats. These antisera will be used to characterize and ascertain the relationship of the antigenic components of these porcine viruses to the known RNA tumor viruses. The antisera will then be used to assay serum and tissue extracts for viral antigens. The findings will be correlated with the molecular hybridization experiments.

A comparison of properties of the viruses isolated from leukemic swine and the RNA tumor viruses (Table 6.2) indicate that the porcine isolates possess most of the properties of the known oncornaviruses. Based on these findings the porcine Type C virus should be tentatively classified as an oncornavirus, and investigated for its involvement in the induction of leukemia in swine exposed to radiation by the chronic ingestion of ^{90}Sr.

TABLE 6.2. Comparison of Properties of the Virus Isolated from Leukemic Swine and the RNA Tumor Viruses

Property	Porcine Virus	RNA Tumor Viruses
Particle Size	80-100 nm	80-100 nm
Morphology: Spherical Particle Having A Dense Internal Component Surrounded By An Envelope	Yes	Yes
Virus Density	1.13-1.18 Sucrose	1.16-1.18 Sucrose
RNA Size	60-70S	60-70S
Reverse Transcriptase	Yes	Yes
Oncogenic In Vivo	?	Yes
Transform Cultures In Vitro	Yes	Yes
Site of Maturation Budding From Cell Membrane	Yes	Yes
Leukemia Virus (gs-3) Interspecies Specific Antigen	?	Yes

EFFECTS OF RADIATION-INDUCED RADICALS ON ENZYME ACTIVITY

Investigators:
W. D. Felix[(a)] *and D. R. Kalkwarf*[(a)]

Reactions between the enzyme aldolase and irradiated crystals of galactose, malonic acid, glycine, and succinic acid were studied in aqueous solution. After addition of the irradiated crystals, enzymatic activity was decreased, with galactose and succinic acid showing greater effectiveness of inactivation than malonic acid or glycine. Enzyme added to solutions of predissolved crystals showed normal or slightly decreased activity levels. The use of irradiated crystals in radiation chemical studies is a convenient technique for the study of specific radical-substrate reactions without the complication of competitive radical reactions.

Enzymes play a crucial role in the control of cellular functions; therefore, it is important to understand the mechanisms of enzyme reactions following radiation insult. From reported studies on enzymes in aqueous solution, it is apparent that radiation-induced radicals are highly selective in reacting with amino acids present in the protein chain. Generally, the sulfur-containing and ring-structured amino acids are the most radio-labile. In this study, we have investigated the reactions between the enzyme aldolase and various radiation-generated free radicals. By using irradiated crystals of selected compounds, it was possible to examine the inactivation efficiency of specific free radicals without interference from competing radicals.

Radicals were formed within crystalline materials by y-irradiation. Following release of the irradiated crystals in an aqueous solution of aldolase, intensity of radical attack was determined by analysis of the remaining enzyme activity. To show that the loss of activity was indeed due to radical attack, a control solution was prepared in which enzyme was added to a predissolved solution of irradiated crystals. By predissolving the crystals, the free radicals had completely reacted with themselves or with nonradical substrates before the enzyme was added.

Enzymatic activity decreased in direct proportion to the quantity of irradiated crystals of galactose, succinic acid, malonic acid or glycine added to a solution. Evidence for the dependence of inactivation of radical concentration is shown in Figure 6.5, where enzyme activity is plotted against the number of radicals added per enzyme activity unit for the addition of irradiated crystals of galactose or succinic acid.

Clearly, the technique of using irradiated crystals for studies of radical reactions is a convenient method for isolation of the reactions of specific radical entities with complex protein structures.

(a) Physics and Instrumentation Department

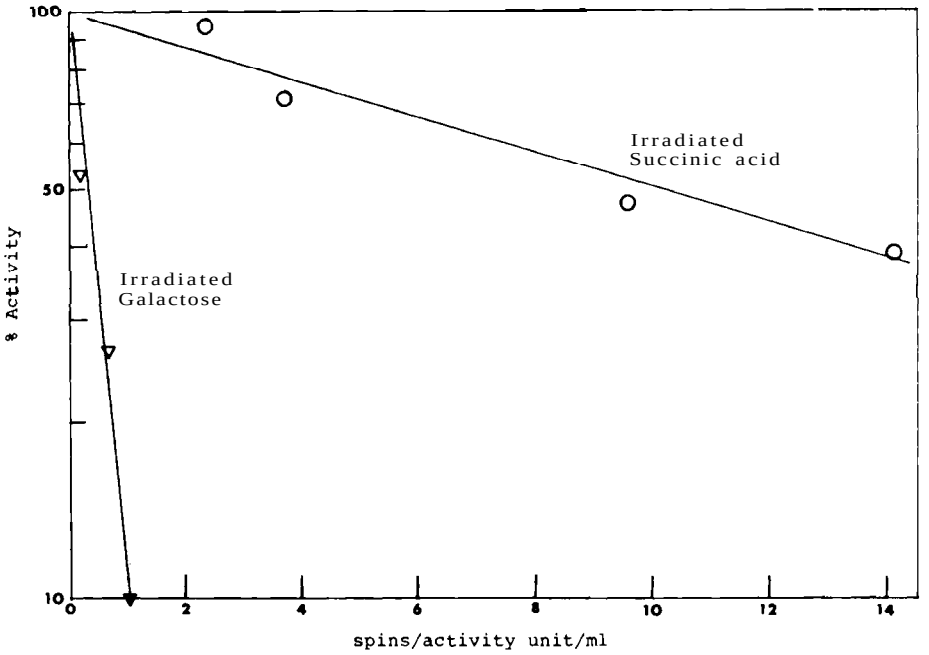

FIGURE 6.5. Aldolase Deactivation as a Function of a Radical Concentration

FREE RADICAL DAMAGE TO MAMMALIAN ERYTHROCYTES

Investigators:

D. R. Kalkwarf[(a)] *and W. D. Felix*[(a)]

Technical Assistance:

S. A. Frazer[(a)]

Free radicals derived from irradiated crystals of galactose and lactose hemolyzed porcine erythrocytes while radicals derived from irradiated glycine, α-alanine and fructose did not. The extent of hemolysis was found to be proportional to the initial radical/cell ratios. The data indicated that the radical-cell reactions occurred much faster than release of radicals from the dissolving crystals.

In previous studies we demonstrated the hemolytic effect of free radicals released from irradiated crystals of biochemicals following dissolution in an erythrocyte suspension. We have now extended this investigation to other radiation-induced radicals and sought to determine the mechanism of the process.

(a) Physics and Instrumentation Department

Erythrocyte suspensions were prepared from freshly drawn porcine blood. The red cells were washed free of plasma and resuspended in isotonic saline solution (0.9% NAC1) adjusted to pH 7.4 with 0.137g Na_2HPO_4, 7 H_2O per liter.

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

Free radicals derived from irradiated crystals of galactose and lactose increased the rate of hemolysis over that observed in controls, while radicals derived from glycine, a-alanine and fructose had no effect. In each case, crystals were added to give initial radical concentrations of approximately 5×10^{17} radicals/ml. In previous studies, radicals derived from galactose and lactose were found to reduce flavin mononucleotide while those derived from the other substances did not. Hemolysis of cells with radicals derived from irradiated lactose could only be followed qualitatively since the radicals also chemically altered the hemoglobin.

Variation in the radical/cell ratio was used to investigate the mechanism of the reaction. Radicals derived from irradiated galactose were used for this study; and hemoglobin release was evaluated 10 hr after exposure, when the hemolysis curves had reached a plateau. The results illustrated in Figure 6.6 show that the percentage of hemolyzed cells increased linearly with the radical/cell ratio. This implies that the extent of cell damage, eventually expressed as hemolysis, is proportional to the average concentration of radicals around each cell. This further supports the thesis that free radicals are the damaging agents.

At high radical/cell ratios, the extent of hemolysis would be expected to reach a maximum value due to radical-radical reactions. The rate of this recombination should be second order in radical concentration and thus compete more effectively with radical-cell reactions as the

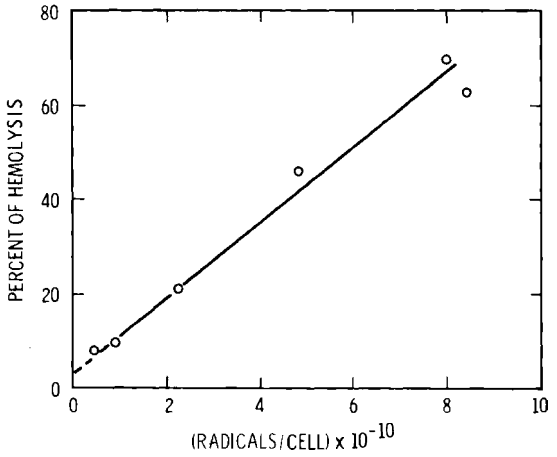

FIGURE 6.6. Radical-Induced Hemolysis as a Function of Galactose-Radical/Cell Ratio

radical concentration increased. The lack of evidence for this process suggests that the radical/cell ratios actually achieved in solution are probably a very small fraction of those calculated from the number of radicals added in crystalline form. This would be particularly true if release of radicals from the dissolving crystals was much slower than reactions of the radicals in solution.

PUBLICATIONS AND PRESENTATIONS

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PUBLICATIONS

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Adee, R. R., "Temperature Dependent Variations in Membrane Synthesis in the Eye of the Rainbow Trout," Annual Meeting of Society for Experimental Biology and Medicine, Vancouver, B.C., October 27.

Bair, W. J., "The Transuranium Technical Group: Origins and Objectives," Health Physics Society Meeting, Miami Beach, FL, June 17-21.

Bair, W. J., "Statement to the NAS-NRC Low-Dose Study Group," National Academy of Sciences/National Research Council Committee on Nuclear Science Ad Hoc Panel on Research Needs in the Field of Low-Dose Radiation Exposure, Snowmass, CO, July 23-25.

J. E. Ballou, "Breathing Variables and the Breath Thoron Measurements," Noble Gases Symposium, Las Vegas, NV, September 24-28.

Beamer, J. L., "A Slide Tour of Hanford and Battelle Life Sciences," Meeting of the Biological Photographic Association, Richland, WA, May 11.

Brown, M. G., "Facility Design for Beagle Dogs on Life-Span Radionuclide Studies," Canadian Association for Laboratory Animal Science Convention, Ottawa, Ontario, Canada, November 5.

Busch, R. H., "Myoclonus Body Epilepsy in Beagle Dogs - An Animal Model for Human Disease," Seminar, University of Minnesota, Minneapolis, MN, April 12.

Craig, D. K., J. R. Decker, and J. P. Herring, "A System for Separating Expired from Inspired Aerosol During the Inhalation Exposure of Beagle Dogs," Lung Morphology Conference, Albuquerque, NM, May 7-8.

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Dionne, P. J., D. K. Craig, and J. R. Decker, "Minicomputer Control of Aerosol Inhalation Exposures," IEEE Region 6 Conference on Minicomputers and Their Applications, Honolulu, HI, May 2-4.

Gruber, H. E., "Variation in Eyespot Ultrastructure in Chlamydomonas reinhardi," Annual Meeting of Society for Experimental Biology and Medicine, Vancouver, B.C., October 27.

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Hampton, J. C., "Observations on the Protective Effect of WR2721 in Mice Exposed to X-rays," Annual Meeting of Society for Experimental Biology and Medicine, Vancouver, B.C., October 27.

Horstman, V. G., "Pigs, Man and Radionuclides," Meeting of Instrument Society of America, Richland, WA, November 13.

Horstman, V. G., "Facilities for Production and Maintenance of Beagle Dogs for Life-Span Studies with Radionuclides," Oregon Branch of the American Association for Laboratory Animal Science, Corvallis, OR, May 9.

Horstman, V. G., "Biomedical Research," Umatilla Biology High School Students, Richland, WA, April 18.

Hungate, F. P., B. I. Griffin, F. T. Cross, and W. F. Riemath, "Improvements in Design of a ^{90}Sr-^{90}Y Portable Blood Irradiator," 26th Annual Conference on Engineering in Medicine and Biology, Minneapolis, MI, September 30-October 4.

Hungate, F. P., "Biological Behavior of Transplutonium Elements and Isotopes," U.S.A.E.C. Transplutonium Program Committee Meeting, Berkeley, CA, November 13-14.

Mahlum, D. D., "Age: A Consideration in the Biological Disposition and Effects of Radionuclides," Seminar, Radiological Sciences Department, University of Washington, Seattle, WA, February 6.

Palmer, R. F. and B. O. Stuart, "Biological Effects of Daily Inhalation of Radon and Its Short-lived Daughters in Experimental Animals," Noble Gases Symposium, Las Vegas, NV, September 24-28.

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Ragan, H. A., "Biomedical Research in Battelle-Northwest Laboratory," Regional Medical Technologists' Monthly Meeting, Richland, WA, January 25.

Ragan, H. A., "Diagnostic Hematology," Seminar, Washington State University, Pullman, WA, April 25.

Ragan, H. A., "Clinical Hematology," Southeastern Washington Veterinary Medical Association Meeting, Pasco, WA, January 16.

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ORGANIZATION CHARTS

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(1) Terminated
(2) Transferred
(3) Science and Engineering Program

Park, J. F., "The Critical Organ Concept for Inhaled Plutonium," Health Physics Society Meeting, Miami, FL, June 17-21.

Park, J. F., D. K. Craig, and V. H. Smith, "Solubility Changes of Pu Oxides in Suspension and Effect on Biological Behavior Alfter Inhalation by Beagle Dogs," Health Physics Society Meeting, Miami Beach, FL, June 17-21; IRPA International Conference, Washington, D.C., September 9-14.

Ragan, H. A., "Biomedical Research in Battelle-Northwest Laboratory," Regional Medical Technologists' Monthly Meeting, Richland, WA, January 25.

Ragan, H. A., "Diagnostic Hematology," Seminar, Washington State University, Pullman, WA, April 25.

Ragan, H. A., "Clinical Hematology," Southeastern Washington Veterinary Medical Association Meeting, Pasco, WA, January 16.

Sanders, C. L., "Metabolism and Carcinogenicity of Inhaled ^{238}Pu in Rats," Health Physics Society Meeting, Miami Beach, FL, June 17-21.

Schneider, R. P., "Regulation of Sodium Transport," Biology Seminar, Battelle, Pacific Northwest Laboratories, Richland, WA, September 25.

Sikov, M. R. and D. D. Mahlum, "Influence of Age on the Lake Effects of Monomeric ^{239}Pu in the Rat," Radiation Research Society Meeting, St. Louis, MO, April 29-May 3.

Sikov, M. R. and D. D. Mahlum, "Response of the Fetoplacental Unit of the Rat to Alpha Irradiation," 13th Annual Meeting of the Teratology Society, St. Jovite, Quebec, Canada, June 13-16.

Sikov, M. R. and D. D. Mahlum, "Movement of Heavy Radionuclides from the Maternal Circulation to the FPU of the Rat," 13th Annual Meeting of the Teratology Society, St. Jovite, Quebec, Canada, June 13-16.

Sikov, M. R., D. D. Mahlum, and F. P. Hungate, "Late Effects of ^{253}Es in Adult and Weanling Rats," Health Physics Society Meeting, Miami Beach, FL, June 17-21.

Smith, V. H., "The Biological Disposition of Es $(NO_3)_3$ in Rats After Intravenous, Intramuscular and Subcutaneous Administration," IRPA Third International Conference, Washington, D.C., September 9-14.

Smith, V. H., "Research Development in Chelation Therapy for Incorporated Radionuclides," International Workshop on Recent Advances in Medical Management in Radiation Accidents, Washington, D.C., September 17-19.

Smith, V. H., "Decorporation of ^{253}Es from Rats with DTPA," Health Physics Society Meeting, Miami Beach, FL, June 17-21.

Smith, V. H., "The Distribution of Inhaled Chelating Agents and the Rate of Transfer From Lung to Urine," 1973 American Industrial Hygiene Conference, Boston, MA, May 21-25.

Stuart, B. O., "The Use of a Perfused Lung as a Model for Aerosol Deposition," Lung Morphology Conference, Albuquerque, NM, May 7-8.

Stuart, B. O. and P. O. Jackson, "In Vivo Separation of Long-lived Uranium Chain Alpha-emitters in Experimental Animals During and After Chronic Inhalation," Health Physics Society Meeting, Miami Beach, FL, June 17-21.

Sullivan, M. F., J. L. Beamer, and T. D. Mahony, "Effects of Intracavitary Irradiation by Californium-252," Conference on Fast Neutrons in Radio therapy, The Hague, Holland, October 4; IAEA Meeting, Munich, Germany, October 22-26; Center for Study of Nuclear Energy, Mol, Belgium, October 29.

Sullivan, M. F., "^{252}Cf in Radiotherapy?" Biology Seminar, Battelle, Pacific Northwest Laboratories, Richland, WA, December 11.

Thompson, R. C., "Plutonium Hazard Concerns - The Developing Picture," Biology Seminar, Battelle, Pacific Northwest Laboratories, Richland, WA, March 6.

Thompson, R. C., "The Transuranium Technical Group: A Progress Report," Health Physics Society Meeting, Miami Beach, FL, June 17-21.

Thompson, R. C., "Biology of Plutonium and Transplutonium Elements," Louisiana State University in New Orleans, New Orleans, LA, June 22.

Thwaites, W., "Arginine Synthesis and Amino Acid Transport in Neurospora," Biology Seminar, Battelle, Pacific Northwest Laboratories, Richland, WA, April 24.

Tombropoulos, E. G. and J. G. Hadley, "Lipid Synthesis by Perfused Lung," American Oil Chemists Society, Chicago, IL, September 16-20.

Wildung, R. E., T. R. Garland, and H. Drucker, "The Potential Role of the Soil Microbiota in Increasing Plutonium Solubility in Soil and Plutonium Uptake by Plants," American Society of Agronomy Soil Science Society of America, Las Vegas, NV, November 11.

Wiley, W. R., "Induction of a Plasma Membrane-bound Co^{+2} Activated Amino-pe'ptidase in Neurospora," Sickle Cell Anemia Symposium, Baton Rouge, LA, April 26.

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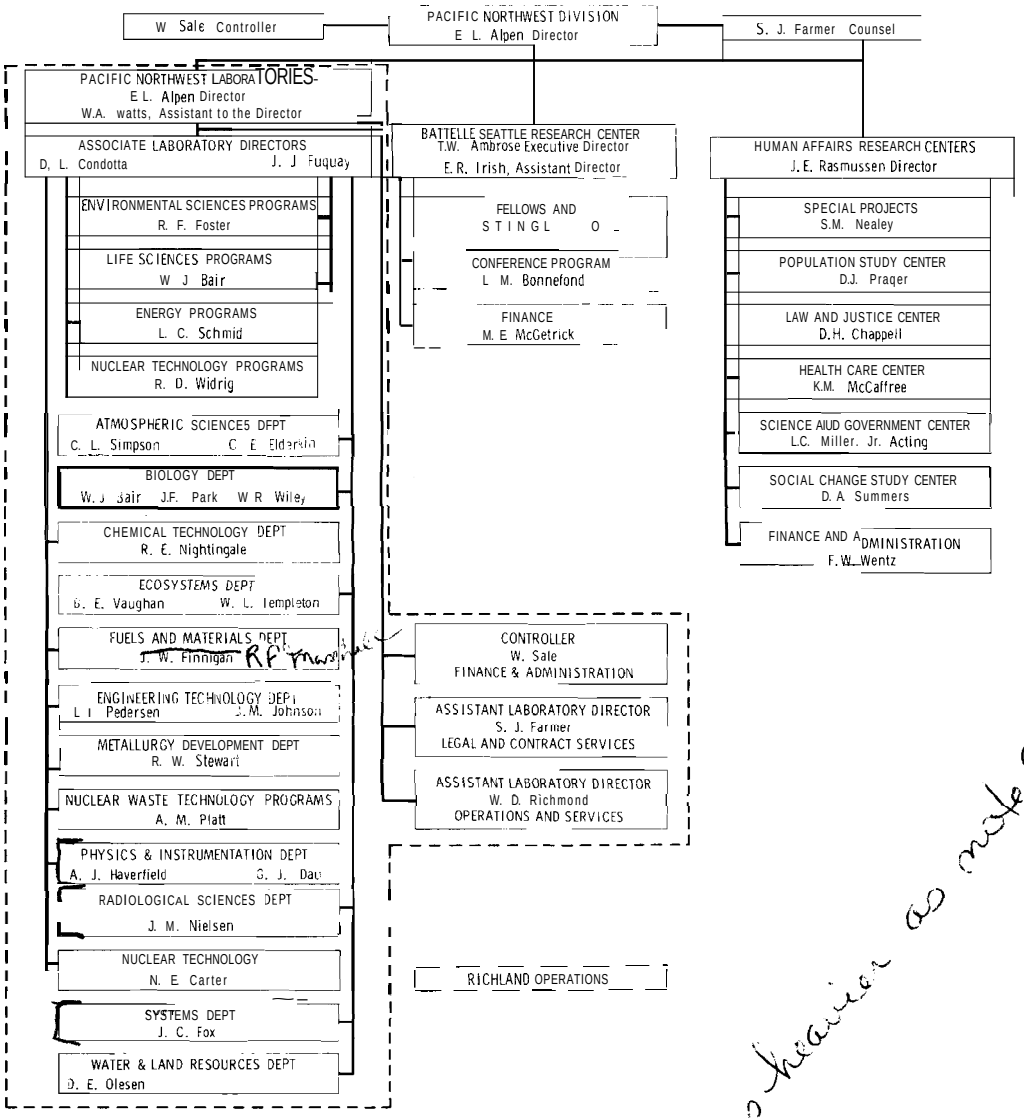

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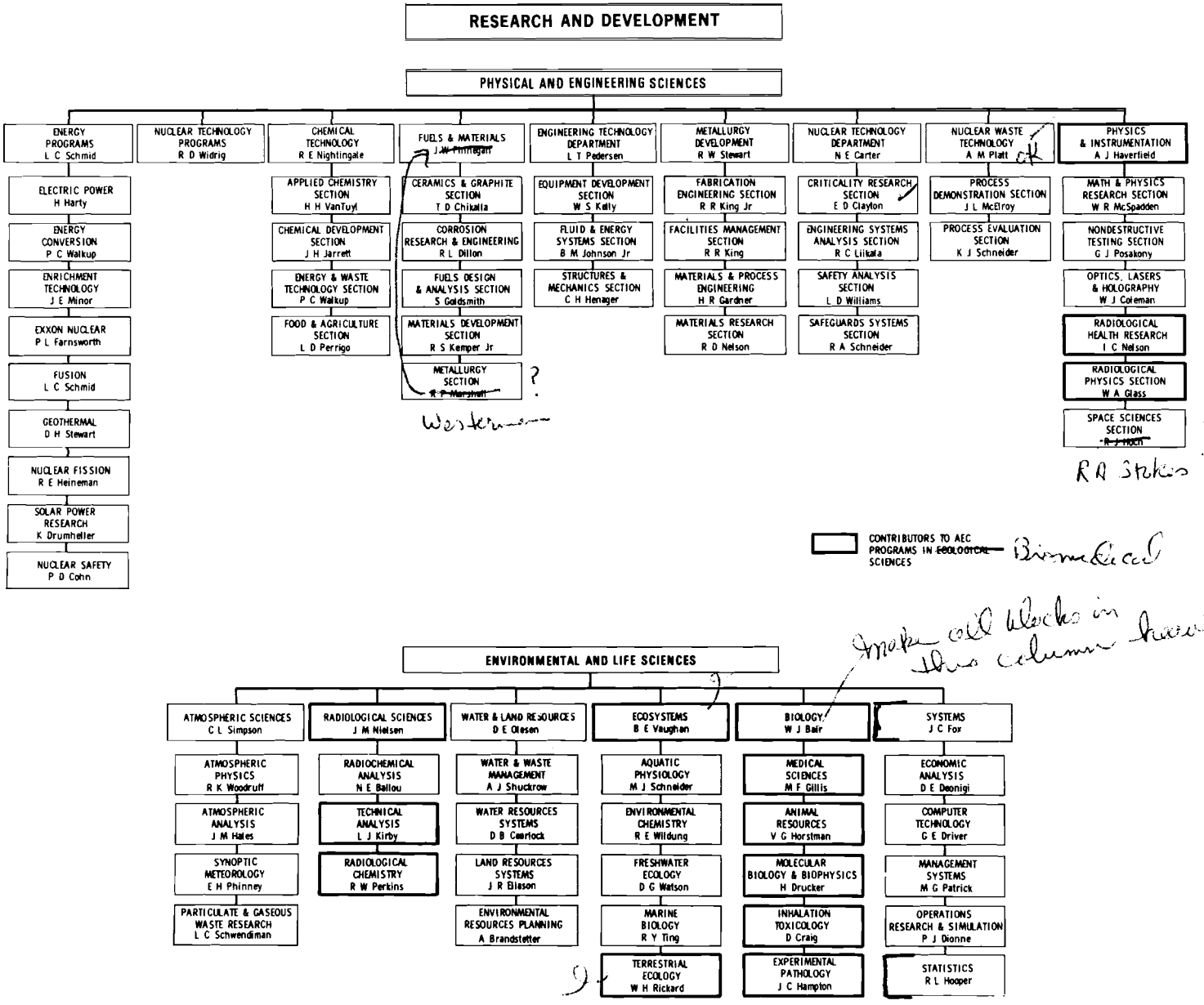
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