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A STUDY OF THE TRANSLOCATION OF
PLUTONIUM AND AMERICIUM FROM WOUNDS

FOR THE PERIOD

April 1, 1968 to June 30, 1974

SUBMITTED BY

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TABLE OF CONTENTS

	Page
I. INTRODUCTION.....	1
II. AIR OXIDIZED PLUTONIUM.....	3
A. Materials and Methods.....	3
B. Results.....	8
III. PLUTONIUM NITRATE AND HIGH FIRED PLUTONIUM OXIDE EXPERIMENTS.....	31
A. Introduction.....	31
B. Materials and Methods.....	31
C. Results.....	36
D. Summary.....	77
IV. LYMPH NODE CLEARANCE STUDY OF PLUTONIUM FROM SUBCUTANEOUS WOUNDS IN BEAGLES.....	81
A. Materials and Methods.....	81
B. Results.....	93
C. Summary.....	116
V. LYMPH TRANSPORT OF PuO ₂ IN DOGS.....	124
A. Materials and Methods.....	124
B. Results.....	133
C. Summary.....	152
VI. PLUTONIUM LYMPH RELATIONSHIP.....	155
A. Materials and Methods.....	155
B. Results and Discussion.....	164
C. Summary.....	193
VII. PLUTONIUM FLUORIDE CONTAMINATED WOUND STUDY.....	195

VIII.	PLUTONIUM CHLORIDE CONTAMINATED WOUND STUDY.....	207
A.	Experimental Design.....	207
B.	Preliminary Results.....	209
C.	Conclusion.....	209
IX.	LITERATURE CITED.....	215
X.	DEGREES GRANTED.....	216
XI.	LIST OF PUBLICATIONS AND PAPERS.....	217

LIST OF TABLES

Table		Page
1	Particle sized distribution of implant material	4
2	Quantitative chemical and nuclidic composition of implant material	4
3	Ratio factor for major sites of Pu and Am deposition in soft tissues	11
4	Ratio factor for various types of soft tissue	12
5	Ratio factor for minor sites of Pu and Am deposition in skeletal tissue	13
6	Ratio factor for major sites of Pu and Am deposition in skeletal tissue	14
7	Ratio factor for blood, urine, and feces	16
8	Percent of implanted plutonium in tissue	18
9	Percent of implanted americium in tissue	19
10	PuO ₂ long term dogs: summary of clinical pathology	24
11	PuO ₂ for Dow-CSU dog project center	33
12	Fraction of initial implant dose of plutonium in paw with exposure time	38
13	Fraction of initial implant dose of plutonium in lymph node with exposure time	41
14	Fraction of initial implant dose of plutonium in blood with exposure time--Pu(NO ₃) ₄ group	45
15	Fraction of initial implant dose of americium in blood with exposure time--Pu(NO ₃) ₄ group	46
16	Fraction of initial implant dose of plutonium in blood with exposure time--PuO ₂ group	51
17	Fraction of initial implant dose of americium in blood with exposure time--PuO ₂ group	52
18	Fraction of initial implant dose of plutonium in 24 hour urine with exposure time	53

Table		Page
19	Fraction of initial implant dose of americium in 24 hour urine with exposure time	54
20	Fraction of initial implant dose of plutonium per gram of organ tissue	58
21	Fraction of initial implant dose of americium per gram of organ	67
22	Chemical and isotopic properties of plutonium	83
23	Physical properties of plutonium	84
24	Experimental design: utilization of dogs	86
25	Radiochemical analysis: plutonium in paw and popliteal lymph nodes	94
26	Radiochemical analysis: plutonium in external iliac and superficial inguinal lymph nodes	95
27	Lymph node clearance curves on dogs from Group II through V from 1 day after amputation	97
28	Lymph node clearance curves on dogs from Groups IV and V from 100 days after amputation	99
29	Lymph node weights at necropsy	101
30	Histopathologic examination of left popliteal lymph node	102
31	Histopathologic examination of right popliteal lymph node	103
32	Alpha tracks in 6 random microscopic fields of left popliteal lymph node exposed to autoradiographic emulsion for 1 hour	108
33	Electron diffraction crystallographic planes for plutonium oxide	111
34	Histopathologic examination of the left external iliac lymph node	114
35	Histopathologic examination of the right external iliac lymph node	115
36	Lymph sample collection intervals	130
37	Ratio of ^{239}Pu in cellular vs. acellular lymph fractions (mean $\pm$ S.D.)	144

Table		Page
38	Lymph node weights (grams) $\pm$ SD	154
39	Experimental design	157
40	Physical, chemical and isotopic characteristics of $\text{Pu}(\text{NO}_3)_4$ supplied by Rocky Flats	158
41	Physical, chemical and isotopic characteristics of PuO_2 supplied by Rocky Flats	159
42	PuO_2 particle sizes supplied by Inhalation Toxicology Research Institute	160
43	Total lymph volume (ml) collected in 0.5 hour intervals following plutonium implants	165
44	Lymph cell counts	166
45	Experimental design--plutonium fluoride	196
46	PuF_4 for Dow-CSU dog project center	197
47	Experimental design	207
48	Physical, chemical and isotopic characteristics of PuCl_3 supplied by Dow Chemicals	208

LIST OF FIGURES

Figure		Page
1	Particle size distribution of PuO_2 implant material	5
2	Lymph node activity buildup	9
3	Lymph node activity buildup	10
4	Power function curve for fraction of initial dose retained in paw of $\text{Pu}(\text{NO}_3)_4$ dogs	39
5	Exponential function curve for fraction of initial dose retained in paw of PuO_2 dogs	40
6	Fraction of initial dose in superficial cervical lymph node of $\text{Pu}(\text{NO}_3)_4$ dogs	42
7	Fraction of initial dose in superficial cervical lymph node of PuO_2 dogs	43
8	Power function curve for fraction of initial dose in blood of $\text{Pu}(\text{NO}_3)_4$ dogs	47
9	Power function curve for fraction of initial dose in blood of PuO_2 dogs	48
10	Power function curve of fraction of initial dose in urine of $\text{Pu}(\text{NO}_3)_4$ dogs	56
11	Power function curve of fraction of initial dose in urine of PuO_2 dogs	57
12	Radioactivity in right hind paws injected with plutonium as measured by an external wound counter	117
13	Radioactivity in the left popliteal lymph nodes of dogs from Group II as measured by an external wound counter	118
14	Radioactivity in the left popliteal lymph nodes of dogs from Group III as measured by an external wound counter	119
15	Radioactivity in the left popliteal lymph nodes of dogs from Group IV as measured by an external wound counter.	120
16	Radioactivity in the left popliteal lymph nodes of dogs from Group V as measured by an external wound counter	121

Figure		Page
17	Radioactivity in the right popliteal lymph nodes of dogs from Groups II, III, IV, and V as measured by an external wound counter	122
18	Change in the fraction of Pu accumulation in the left superficial cervical lymph node with time. Before and after lymphadenectomy (Group IA)	134
19	Change in the fraction of Pu accumulation in the left superficial cervical lymph node with time. Before and after lymphadenectomy. (Group IB)	135
20	Fraction of ^{239}Pu in selected tissues ($\bar{X} \pm \text{SE}$). Data are from early and delayed lymphadenectomy experiments	136
21	Change in the fraction of Pu accumulation in the left superficial cervical lymph node with time. Before and after lymphadenectomy ($\bar{X} \pm \text{SE}$) (Group II)	139
22	Sketch of polyacrylamide gel showing individual protein bands.	146
23	Percentage of protein bound ^{239}Pu in the protein fractions of dog lymph	146
24	Typical size range of α -stars of $^{239}\text{PuO}_2$ in packed cells of lymph collected 1 hour post implant	148
25	Change in particle size of $^{239}\text{PuO}_2$ particles in the L.S.C.L.N. of beagles with ^{239}Pu accumulation time	150
26	Discontinuous gels	169
27	Discontinuous gels	169
28	Fraction of Pu detected in sections of discontinuous gels at specific times after the proteins entered the resolving gel.	171
29	Fraction of Pu detected in sections of isoelectric focusing gels at specific times after electrophoresis initiated	174
30	Fraction of initial implant dose in afferent lymph (acellular and cellular)	181

Figure		Page
31	Fraction of initial implant dose in efferent lymph (acellular and cellular)	183
32	Fraction of initial implant dose in afferent lymph (acellular and cellular)	185
33	Fraction of initial implant dose in efferent lymph (acellular and cellular)	187
34	Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA	199
35	Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA	200
36	Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA	201
37	Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA	202
38	Clearance of PuF_4 from injection without DTPA treatment. ⁴ All groups averaged	203
39	Clearance of PuF_4 from injection following DTPA treatment. ⁴ All groups averaged	204
40	Buildup and clearance of PuF_4 from the L.S.C.L.N. without DTPA treatment. All ⁴ groups averaged	205
41	Buildup and clearance of PuF_4 from the L.S.C.L.N. following DTPA treatment. All groups averaged	206
42	Clearance of PuCl_3 from the injection site following DTPA treatment. All groups averaged	210
43	Clearance of PuCl_3 from the injection site without DTPA treatment. All groups averaged	211
44	Buildup and translocation of PuCl_3 from the L.S.C.L.N. following DTPA treatment. All groups averaged	212
45	Buildup and translocation of PuCl_3 from the L.S.C.L.N. without DTPA treatment. All groups averaged	213

I. INTRODUCTION

This research project was begun to determine if the ratio of reactor produced plutonium isotopes to the inherent ^{241}Am would be altered during translocation of the material from contaminated wound sites to major points of deposition. Knowledge of any changes is important to the interpretation of ^{241}Am in vivo or external whole body counting data for plutonium body and lung burdens. The assumption implicit in the in vivo method was that plutonium and ^{241}Am remained in fixed ratio in the body. However, urine bioassay data had indicated that ^{241}Am was excreted more rapidly than plutonium which raised doubts about the accuracy of the external counting method. The use of the therapeutic removal agent DTPA (diethylene-triaminepentaacetate) also cast doubt on the constancy of the ratio.

In our studies, we simulated contaminated puncture wounds in beagle dogs by injecting plutonium in chemical forms of the types handled at Rocky Flats. These have included air oxidized plutonium, plutonium nitrate, high fired plutonium oxide (850°C), plutonium fluoride and plutonium chloride, all of which came from Rocky Flats.

In each experiment half of the animals received intravenous injection of DTPA while the other half was untreated. Wound site excision took place only when there was evidence that the injected radionuclide was leaking from the wound site. During the time of the experiment, samples of urine, feces and blood were collected routinely and, as the dogs were serially killed, blood and tissue samples were taken.

Chemical assays for plutonium and ^{241}Am in excreta, blood and

tissues, were performed at the Rocky Flats Plant for PuO_2 air oxidized, and high fired, and for plutonium nitrate. In vivo measurements with scintillation and solid state diode detectors, analysis and correlation of data were made at Colorado State University. Samples for histopathologic study were taken at necropsy from the tissues to be assayed. Because of budgetary considerations, Rocky Flats authorities decided in 1972, to terminate the chemical analysis of our samples. Therefore, no radiochemical assays have been conducted on the necropsy samples for the animals contaminated with plutonium fluoride and plutonium chloride. A proposal has been submitted to the A.E.C. for conducting the radiochemical analysis at Colorado State University.

One of the dogs in the first experiment developed lymphosarcoma and, as a result of this event, ten dogs were implanted for long term observation of lymphsarcoma induction.

In vivo counting of the lymph node proximal to the implants has shown a rapid and relatively high deposition in the node. This has prompted a study of the effect of removal of the node upon the translocation and distribution of the plutonium in different chemical forms.

This report summarizes the results obtained from the beginning of this project on April 1, 1968 until June 30, 1974.

II. AIR OXIDIZED PLUTONIUM EXPERIMENTS

A. Materials and Methods

1. Experimental Design

Forty adult male beagles were obtained from the specific pathogen free (SPF) beagle colony of the Collaborative Radiological Health Laboratory at Colorado State University. These animals were randomly divided into ten study groups, four to a group, and then half of each group was randomly selected to received DTPA treatment.

2. Implanting Procedures

The plutonium oxide (oxidized under ambient conditions) used for the implants was prepared and provided by Rocky Flats. The physical and chemical characteristics of this material are shown in Tables 1 and 2 and in Figure 1. The plutonium was contained in vials (rubber diaphragm stoppered) with about a milligram in each container.

Hypodermic needles and syringes were used for making the implants. The standard site for the contamination puncture wound was the subcutaneous tissue of the left dorsal metacarpal area.

An ultrashort acting anesthetic (sodium thiamylal for injection N.F. 4% solution) was used to prepare the animals for the implant. A hypodermic needle was positioned subcutaneously in the desired spot on the left dorsal metacarpus of the dog. Proper positioning was determined by palpation

TABLE 1. Particle Sized Distribution of Implant Material

<u>Geometrical Mean Diameter, Microns</u>	<u>Fraction</u>
<25	0.79
25-50	0.10
50-75	0.04
>75	0.06

TABLE 2.
Quantitative Chemical and Nuclidic Composition of
Implant Material

<u>Element</u>	<u>Analysis Date</u>	<u>Quantity Present</u>
Am-241	4-29-68	2200 ppm
Pu	3- -67	0.9986 by weight
Fe	3- -67	30 ppm
C	3- -67	99 ppm
U-238	3- -67	78 ppm
Np-237	3- -67	2 ppm

<u>Plutonium Isotope</u>	<u>Percent Present</u>
Pu-238	0.035
Pu-239	86.63
Pu-240	11.69
Pu-241	1.51
Pu-242	0.135

Figure 1. Particle size distribution of PuO_2 implant material.

and by observing that there was no back-flow of blood. This latter observation indicated that intravenous injection had not occurred. By use of a tuberculin syringe and needle, a milliliter of water was injected into the vial containing the plutonium. The vial was shaken to suspend the PuO_2 particles and an aliquot (about 0.25 ml) was then drawn into the syringe. The syringe was attached to the needle previously pre-positioned subcutaneously at the implant site and the injection was made. Upon withdrawal of the needle, an alpha survey meter was used to determine the extent, if any, of surface contamination. The dog's leg was cleaned with hypochlorite solution at least once, surveyed, and the operation repeated until no activity was detected.

3. DTPA Therapy

Following the implant, the dog was placed in a metabolism cage for quantitative excreta collection. About two hours after injection the animals designated to receive therapeutic treatment were given DTPA injections. Subsequent injections were given on the same schedule as the sampling schedule noted in the next section. The DTPA was given at 0.25 grams per injection in 10 ml of physiological saline solution. The usual level of DTPA administration for industrial workers is 1 gram per infusion. The 0.25 grams per injection for the dog was selected by comparing the standard man blood volume to that of the dog (9.5% of body weight). The dogs in this study average 14 kilograms in

weight. Blood volume determinations based on a radio-iodinated serum albumin method have been taken on a few of the dogs. The data verify the above cited number and that the average beagle blood volume is 25% of the standard man volume.

4. Sample Collection

Each dog was maintained in a metabolism cage on a standard schedule where 24 hour urine and feces samples and blood samples were collected. Initial samples were collected the first 7 days, the 9th, 11th and 14th days. (Blood samples were obtained by jugular venepuncture.) The dogs were returned to the outside kennels for two weeks and then placed back in the metabolism unit for three days for sample collections. Each dog was then scheduled for two to three days in a metabolism cage for each month thereafter to procure samples and to administer DTPA, when indicated, until the specified time of sacrifice.

The dogs were killed by overdosage of pentobarbital sodium and exsanguination. The tissue samples collected at necropsy were as follows: left eye, left testis, left kidney, left adrenal gland, trachea (small piece to represent body cartilage), lung (tip of one lobe), spleen, liver (about a fourth of the organ was analyzed), sternum, left 10th rib, two lumbar vertebrae, piece of skull, left femur, left axillary lymph node, right cervical lymph node, left cervical lymph node, and the implant site (left front paw

sectioned at the carpometacarpal joint).

5. Radiochemical Assay

All samples were assayed for Pu and Am radioactivity in Rocky Flats laboratories. The detailed procedures were published in COO-1787-1.

6. In Vivo Counting Equipment and Procedures

In vivo counts on each dog were made on days 1 through 7, 9, 11, 14, 28, 29, and 30 and then monthly following the plutonium implants. Measurements were made with a NaI(Tl) wound counter.

The scintillation counter was used to measure the activity at the wound site and in the left superficial cervical lymph node. This lymph node is the first major node draining the area in the dog's foot and leg which contains the plutonium. The count rate as a function of time was recorded.

B. Results

1. Translocation from the Wound Site

A study of the Pu and Am fractions translocated from the implant site to the various tissues showed no significant time relationship at the 5 percent error level for air oxidized Pu. An exception occurred at the first major lymph node draining the implant site (superficial cervical lymph node). Figure 2 shows the activity buildup detected by in vivo counting, in the lymph node as a function of time

Figure 2.

Figure 3.

Table 3. Ratio factor for major sites of Pu and Am deposition in soft tissues.

Sample	Treatment	Ratio Factor	95% Confidence Level
Liver	None	0.28	0.01 - 0.68
	DTPA	1.48	0.68 - 2.31
Spleen	None	1.03	0.61 - 1.45
	DTPA	1.09	0.67 - 1.51
Kidney	None	0.28	0.11 - 0.45
	DTPA	0.29	0.01 - 1.14
Left Cervical Lymph Node	None	1.40	0.63 - 2.17
	DTPA	1.26	0.78 - 1.74

Table 4. Ratio factor for various types of soft tissue.

Sample	Treatment	Ratio Factor	95% Confidence Interval
Left axillary lymph node	None	1.24	0.50 - 1.98
	DTPA	1.30	0.66 - 1.94
Right cervical lymph node	None	0.59	0.15 - 1.03
	DTPA	0.56	0.01 - 1.22
Lung	None	0.60	0.33 - 0.87
	DTPA	0.68	0.47 - 0.89
Adrenal gland	None	1.98	0.67 - 3.29
	DTPA	0.95	0.42 - 1.48
Thyroid gland	None	0.071	0.049 - 0.093
	DTPA	0.091	0.051 - 0.131
Eye	None	0.39	0.22 - 0.56
	DTPA	0.46	0.23 - 0.69
Trachea	None	0.78	0.01 - 1.69
	DTPA	0.45	0.13 - 0.77
Testis	None	0.34	0.06 - 0.62
	DTPA	0.58	0.18 - 0.98

Table 5. Ratio factor for minor sites of Pu and Am deposition in skeletal tissue.

Sample	Treatment	Ratio Factor	95% Confidence Interval
Rib	None	3.11	1.34 - 4.88
	DTPA	2.30	1.50 - 3.10
Skull	None	1.31	0.63 - 1.99
	DTPA	1.04	0.68 - 1.40
Femur shaft	None	1.56	0.71 - 2.41
	DTPA	1.99	1.19 - 2.79

Table 6. Ratio factor for major sites of Pu and Am deposition in skeletal tissue.

Sample	Treatment	Ratio Factor	95% Confidence Interval
Sternum	None	3.41	1.60 - 5.22
	DTPA	5.62	3.54 - 7.70
Vertebrae	None	5.74	2.83 - 8.65
	DTPA	5.96	1.86 - 10.06
Proximal femur	None	1.93	1.13 - 2.73
	DTPA	6.02	3.60 - 8.44
Distal femur	None	1.48	0.70 - 2.26
	DTPA	2.92	1.68 - 4.16

for dogs not treated with DTPA. A curve not significantly different was obtained for dogs which were treated with DTPA (Figure 3). In both figures the initial increase is rapid with about 40 percent of the first years accumulation occurring in the two weeks after implant. In the untreated animals an apparent equilibrium in all other tissue concentrations of both Pu and Am had been reached at two weeks and did not change significantly for a year.

2. Translocation Ratios of Pu to Am

Radiochemical analysis for all of the amples was completed and ratios of Pu to Am alpha activities were calculated. These ratios were divided by the ratio of Pu to Am in the implant material to produce a ratio factor. This was previously called the mean multiple in reports C00-1787-6, C00-1787-7 and C00-1787-8. A ratio factor greater than one indicates an enhancement of Pu relative to Am and a ratio factor less than one indicates an enhancement of Am relative to Pu.

Ratio factors for each sample type for both untreated and DTPA treated dogs, were computed to measure treatment effect and to compare sample types (Tables 3, 4, 5, and 6). The magnitude of the ratio factor differed for sample type. Enhancement of Am deposition averaged about fourteen-fold in the thyroid while Pu enhancement in the vertebrae averaged about six-fold for untreated dogs (Tables 4 and 6). These two tissues represented the two ratio factor extremes in

Table 7. Ratio factor for blood, urine, and feces.

Sample	Treatment	Ratio Factor	95% Confidence Interval
Blood	None	0.95	0.80 - 1.09
	DTPA	0.80	0.68 - 0.93
Urine	None	0.79	0.71 - 0.87
	DTPA	1.47	1.28 - 1.65
Feces	None	2.11	0.88 - 3.34
	DTPA	2.24	0.89 - 3.59

the data. The general result was Am enhancement in soft tissues and Pu enhancement in skeletal tissue. In the case of skeletal tissue, there seemed to be a direct relationship between hematopoietic activity of bone and its Pu collection enhancement.

A significant DTPA treatment effect was observed in liver and proximal end of the femur (Tables 3 and 6). Enhancement of the relative Pu content was observed in these sample types for dogs treated with DTPA. Samples from DTPA treated dogs showed ratio factor increases of three- and five-fold for proximal femur and liver, respectively. Most of the older tissue samples showed a trend toward Pu enhancement for DTPA treated dogs but the enhancement was not significant at the 95 percent confidence level. Nearly all of this enhancement is, in fact, due to the reduction of the Am concentration which will be described later.

3. Urine Excretion Ratios of Pu and Am

Regression analyses of the ratio factors of urine as a function of post-implant time were completed. These analyses did not show a significant time relationship for urine at the 95 percent confidence level.

The average ratio factor for urine is shown in Table 7 and is somewhat enhanced in Am for untreated dogs. Treatment with DTPA, however, changes the ratio to one of Pu enhancement.

4. Pu and Am Distribution

The average fractions of the implanted Pu translocated to

Table 8. Percent of implanted plutonium in tissue.

Tissue	No Treatment		DTPA Treatment	
	Mean Value	Standard Deviation of Mean	Mean Value	Standard Deviation of Mean
Liver	0.121	0.026	0.113	0.023
Spleen	0.0053	0.0018	0.0073	0.0018
Kidney	0.0014	0.00035	0.00095	0.00030
Lung	0.0057	0.0016	0.0121	0.0058
Thyroid	0.00009	0.00002	0.0002	0.00005
Adrenal	0.0014	0.0005	0.00036	0.00009
Skeleton (femur)	0.111	0.0112	0.157	0.026
Sternum	0.00557	0.00052	0.00522	0.00078
Rib	0.00031 ¹	0.00003	0.00021 ¹	0.00002
Vertebrae	0.00014 ¹	0.00002	0.00013 ¹	0.00003
Skull	0.00006 ¹	0.000008	0.000046 ¹	0.000008

¹ Percent of implanted plutonium per gram of tissue (wet weight).

Table 9. Percent of implanted americium in tissue.

Tissue	No Treatment		DTPA Treatment	
	Mean Value	Standard Deviation of Mean	Mean Value	Standard Deviation of Mean
Liver	0.393 ¹	0.068	0.141 ¹	0.024
Spleen	0.0051	0.001	0.0085	0.0021
Kidney	0.011	0.0025	0.0061	0.0016
Lung	0.0133	0.0026	0.0122	0.0037
Thyroid	0.0028	0.0009	0.0017	0.0004
Adrenal	0.0008	0.0002	0.0016	0.0006
Skeleton (femur)	0.134 ¹	0.027	0.052 ¹	0.0073
Sternum	0.00277 ²	0.00054	0.00122 ²	0.00015
Rib	0.00023 ^{2,3}	0.00005	0.00011 ^{2,3}	0.00002
Vertebrae	0.000047 ³	0.000009	0.000035 ³	0.000004
Skull	0.000095 ³	0.000017	0.000052 ³	0.000009

¹ Mean values significantly different due to treatment at 1% error level.

² Mean values significantly different due to treatment at 5% error level.

³ Percent of implanted americium per gram of tissue (wet weight).

selected tissues were computed and are listed in Table 3 for both untreated and DTPA treated dogs. The Pu concentration in the femur was used to represent the skeletal average for estimating the plutonium burden in the skeleton. Our data show the Pu fraction to be about equal for the liver and skeleton. No evidence of DTPA effect on the Pu concentration was detected in any of the tissues.

The average fractions of the implanted Am in selected tissues are shown in Table 9 for both untreated and DTPA treated dogs. The Am fraction in the liver was about three times that of the skeleton. Significant (1% error level) DTPA treatment effects for both the liver and the skeleton were observed. The Am concentrations in the liver and skeleton were reduced by the factors 2.8 and 2.6 with DTPA therapy. The reductions obtained for sternum and rib tend to confirm the skeletal estimate.

The study of Pu and Am fractions translocated from the implant site to the various tissues showed no significant change with time after implant at the 5 percent error level.

The exception to this statement is the left superficial cervical lymph node which, we previously noted, increased in Pu and Am burden throughout the study period of twelve months. The range of this burden was from 3 percent of the implanted dose at 14 days to 17 percent of the implanted dose at 365 days.

5. Pathological Changes

Four months after receiving 5.8 μCi of PuO_2 , an untreated

dog showed a generalized lymphadenopathy. The condition of the animal deteriorated rapidly thereafter. A radiographic examination was done and showed enlargement of the lymph nodes in the anterior mediastinum and sublumbar regions.

Gross findings at necropsy confirmed the radiographic diagnosis. The anterior mediastinum lymph nodes and the mesenteric and sublumbar lymph nodes were markedly enlarged. Histologic examination of the cervical and prescapular nodes revealed an absence of normal lymph node architecture. The nodes were diffusely infiltrated with lymphoreticular cells varying in appearance from that of mature lymphocytes to lymphoblasts and reticulum cells. Neoplastic lymphocytes were observed infiltrating adjacent adipose and collagenous tissues. The histopathologic diagnosis was lymphosarcoma.

Alpha autoradiography of sections of kidneys, liver, spleen, and prescapular lymph nodes revealed various concentrations of Pu within these tissues. The left prescapular lymph node had the heaviest concentration of radioactive material followed by the liver. The estimated dose to the prescapular lymph node was 7052 rads. The amount of Pu concentrated at this lymph node relative to the total amount injected was estimated to be 5.4 percent of the initial dose.

The authors recognize that a single case of lymphosarcoma following implantation of PuO_2 into the subcutaneous tissues of forty dogs is not proof that the condition was induced by Pu contamination. However, in the colony from which these dogs were obtained, only one other case of lymphosarcoma, in

an 11 year-old dog, has been diagnosed in the last nine years out of a total population numbering in excess of 8000 dogs. The fact that this animal was only 1 1/2 years old and the heavy concentration of Pu detected by alpha autoradiography in the prescapular and cervical lymph nodes must be considered.

Several other animals have had marked destruction of the germinal centers in the prescapular lymph nodes. In several dogs other lymph nodes were enlarged. Microscopically, the enlargement was accounted for by hyperplasia rather than neoplasia. It is quite possible that the dose given to some of these animals may have been too large and have induced destruction of the lymphoid tissue rather than inducing a neoplastic process.

In 1971 ten dogs have been subcutaneously implanted with air oxidized PuO_2 in the left dorsal metacarpus for long term observation as a result of the observed lymphosarcoma. In 1972 eight more dogs were implanted with plutonium nitrate, also for long term observation.

The dogs are housed outside, one or two dogs to a run. They are fed dry dog food (Purina) adlibitum, and observed daily. A complete physical examination, including blood chemistry studies is performed three times a year. Each of the dogs was vaccinated against distemper and rabies. Last year, a thoracic and abdominal radiographic study was performed on each dog.

No physical changes clearly attributable to PuO_2 and

$\text{Pu}(\text{NO}_3)_4$ contamination were found aside from a small firm subcutaneous swelling at the injection site of one dog (#4879). The radiographic examinations were all considered normal except for one dog showing a slight degree of hilar pneumonia.

One dog (#410) was found dead on 10-12-70. Pathologic examination revealed an acute pneumonia unrelated to plutonium treatment. Pathologic examination of routinely processed hematoxylin and eosin stained paraffin sections of spleen, liver, heart, kidney, pancreas, urinary bladder, intestines, stomach, muscle, adrenal, testis, and brain revealed no significant changes.

The clinical pathologic results (see Table 10) were quite variable. Possible trends which will be carefully followed include decreased inorganic serum phosphorus and decreased total white blood cell counts.

Miscellaneous additional changes, unrelated to plutonium administration, included slight hilar pneumonia revealed radiographically in one dog, slight increased respiratory sounds in one dog, mild popliteal lymph node enlargement in one dog, dry hair coats in two dogs, and mild ocular discharges and conjunctivitis in one dog.

Unfortunately, because of budgetary restrictions, the blood analyses of these dogs was discontinued in 1972. The remaining 17 dogs are all in good health and no indication of lymphosarcoma induction has been noted.

Table 10. PuO₂ long term dogs: Summary of clinical pathology.

	Dogs Injected with PuO ₂ -Long Term						Controls			
	9 dogs		8 dogs		9 dogs		22-40 dogs		10 dogs	
	3-16-71		4-22-71		6-1-71		3-16-71		6-1-71	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Biochemistry (Serum)</u>										
Chloride, meq/l	104	2.00	111	7.99	115	2.80	104	2.10	116	1.27
CO ₂ , meq/l	23.1	1.65	22.6	2.66	21.2	2.52	24.2	2.24	23.7	1.70
Potassium, meq/l	4.54	0.15	4.40	0.20	4.16	1.60	4.83	0.33	4.63	0.26
Sodium, meq/l	147	0.88	146	3.15	148	2.07	147	1.06	148	0.99
Blood urea nitrogen, mg %	17.9	3.71	22.2	5.33	21.6	5.73	20.0	3.99	19.8	4.60
Glucose, mg %	103	7.02	97.6	5.85	65.8	9.11	97.9	16.5	78.1	9.01
Total protein, g %	5.89	0.34	6.51	0.21	6.28	0.46	5.84	0.46	5.62	0.36
Albumin, g %	1.67	0.16	1.76	0.28	2.24	0.48	1.54	0.17	2.21	0.54
Calcium, mg %	8.08	0.44	9.35	0.73	10.0	0.53	9.60	0.93	10.4	0.44
Inorganic phosphorus, mg %	4.23	0.56	4.44	0.69	3.59	0.70	5.33	0.78	5.10	0.54
Cholesterol, mg %	164	29.1	166	26.1	187	32.4	179	30.0	170	22.5
Uric acid, mg %	0.40	0.07	0.51	0.11	0.30	0.14	0.45	0.09	0.25	0.05
Creatinine, mg %	1.26	0.19	0.96	0.09	1.57	0.35	1.05	0.15	1.44	0.23
Total bilirubin, mg %	0.32	0.10	0.37	0.21	0.61	0.16	0.37	0.19	0.50	0.12
Alkaline phosphatase, units	31.9	16.3	26.3	13.0	26.4	12.8	51.3	20.1	39.9	10.6
Creatinine phosphokinase, units	213	95.9	187	57.8	265	41.2*	291	202	264	32.2
Lactate dehydrogenase, units	85.7	17.3	115	34.7	273.	39.7*	134	93.8	268	56.2
Serum glutamic-oxaloacetic transaminase, units	58.9	18.7	48.1	9.70	48.5	8.96*	63.3	23.9	52.8	7.86

Table 10, continued.

	Dogs Injected with PuO ₂ -Long Term						Controls			
	9 dogs		8 dogs		9 dogs		22-40 dogs		10 dogs	
	3-16-71		4-22-71		6-1-71		3-16-71		6-1-71	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Thyroid</u>										
T-3, %	41.9	2.09					40.9	2.16		
T-4, MCG %	2.60	0.84					2.05	0.78		
<u>Lactate Dehydrogenase Isoenzymes</u>										
Band 1	25.1	9.33					27.1	11.2		
Band 2	20.2	7.15					15.7	5.61		
Band 3	13.9	2.23					13.3	4.45		
Band 4	17.0	6.52					16.4	5.19		
Band 5	23.7	6.59					27.1	6.00		
<u>Complete Blood Count (CBC)</u>										
Red blood cells, $1 \times 10^6/\text{mm}^3$	8.16	0.66	8.53	0.27	8.22	0.50	7.60	0.40	7.26	0.37
White blood cells, $1 \times 10^3/\text{mm}^3$	8.66	2.24	8.29	1.57	8.16	2.91	11.8	2.18	9.41	1.60
Hemoglobin, g	19.4	1.72	20.4	0.61	19.5	1.08	18.7	1.38	17.6	0.87
Hematocrit, %	53.8	4.39	57.4	1.59	55.6	3.47	52.3	3.54	49.4	2.10
Mean corpuscular volume	65.6	1.50	66.6	1.40	65.2	1.39	67.1	1.37	64.8	5.57
Mean corpuscular hemoglobin	24.0	0.65	24.9	1.85	23.5	0.91	24.5	0.64	24.5	0.58
Mean corpuscular hemoglobin conc.	36.4	0.57	36.2	1.42	35.6	0.88	36.0	0.72	36.0	0.40

* Hemolyzed serum samples from 5 dogs excluded in calculations.

Table 10, continued.

	Dogs Injected with PuO ₂ -Long Term									
	10 dogs		10 dogs		10 dogs		9 dogs		9 dogs	
	1-30-70		4-29-70		8-13-70		10-20-70		12-29-70	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Biochemistry (Serum)</u>										
Total protein, g %	6.30	0.48	5.91	0.40	6.26	0.56	6.30	0.41	6.37	0.27
<u>Complete Blood Count (CBC)</u>										
White blood cells, $1 \times 10^3/\text{mm}^3$	8.98	2.38	11.2	6.27	10.3	1.66	8.37	1.18	7.90	1.36
Segmented, %	74.3	7.4	79.1	4.84	80.2	6.6	76.3	5.7	78.3	5.9
Lymphocytes, %	22.3	7.2	19.7	4.7	16.4	7.2	18.6	5.05	18.0	6.2
Monocytes, %	2.6	2.9	1.2	1.3	2.6	1.7	4.1	1.3	3.0	1.9
Eosinophils, %	1.0	1.2	0.0	0.0	1.0	1.4	1.0	1.5	0.4	0.5
Hemoglobin, g	18.8	0.93	18.2	1.07	18.0	0.93	18.7	0.67	19.6	0.44
Hematocrit, %	55.1	1.97	55.8	2.82	53.6	2.68	57.2	2.54	59.8	2.49

Table 10, continued.

	PuO ₂ Group 2 (6 months)				Controls					
	4-22-71				3-16-71	4-22-71		6-1-71		
	4 dogs		4 dogs		22-40 dogs	2 dogs-DTPA		10 dogs		
	No Treatment		DTPA				No Plutonium			
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Biochemistry (Serum)</u>										
Chloride, meq/l	112	3.68	112	3.36	104	2.09	114	2.12	116	1.27
CO ₂ , meq/l	26.1	1.75	24.6	2.42	24.2	2.24	24.3	0.35	23.7	1.70
Potassium, meq/l	4.65	0.20	4.77	0.15	4.83	0.33	4.7	0.00	4.63	0.26
Sodium, meq/l	146	2.16	146	0.95	147	1.06	146	0.00	148	0.99
Blood urea nitrogen, mg %	15.5	1.76	14.5	2.38	20.0	3.99	16.0	7.07	19.8	4.60
Glucose, mg %	83.8	6.29	79.3	18.0	97.9	16.5	85.0	85.0	78.1	9.01
Total protein, g %	5.77	0.45	6.07	0.55	5.84	0.46	5.20	0.48	5.62	0.36
Albumin, g %	1.42	0.15	1.42	0.15	1.54	0.17	1.55	0.21	2.21	0.54
Calcium, mg %	10.5	0.54	10.8	0.22	9.60	0.93	10.6	0.56	10.4	0.44
Inorganic phosphorus, mg %	5.50	0.45	5.17	0.61	5.33	0.78	5.80	0.70	5.10	0.54
Cholesterol, mg %	145	23.8	188	10.4	179	30.0	173	10.6	170	22.5
Uric acid, mg %	0.47	0.05	0.45	0.05	0.45	0.09	0.50	0.00	0.25	0.05
Creatinine, mg %	0.92	0.09	0.85	0.12	1.05	0.15	0.95	0.07	1.44	0.23
Total bilirubin, mg %	0.15	0.05	0.17	0.17	0.37	0.19	0.15	0.07	0.50	0.12
Alkaline phosphatase, units	47.3	9.32	36.3	13.1	51.3	20.1	57.5	0.70	39.9	10.6
Creatinine phosphokinase, units	180	61.6	153	37.7	291	202	170	28.3	264	32.2
Lactate dehydrogenase, units	212	26.8	206	136	134	93.8	255	113	268	56.2
Serum glutamic-oxaloacetic transaminase, units	53.8	7.63	43.3	7.22	63.3	23.9	42.0	0.00	52.8	7.86
<u>Thyroid</u>										
T-3, %					40.9	2.16				
T-4, MCG %					2.05	0.78				

Table 10, continued.

	PuO ₂ Group 2 (6 months)				Controls					
	4-22-71				3-16-71	4-22-71		6-1-71		
	4 dogs		4 dogs		22-40 dogs	2 dogs-DTPA		10 dogs		
	No Treatment		DTPA			No Plutonium				
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Lactate Dehydrogenase Isoenzymes</u>										
Band 1					27.1	11.2				
Band 2					15.7	5.61				
Band 3					13.3	4.45				
Band 4					16.4	5.19				
Band 5					27.1	6.00				
<u>Complete Blood Count (CBC)</u>										
Red blood cells, $1 \times 10^6/\text{mm}^3$	6.89	0.55	7.29	0.40	7.60	0.40	6.87	0.29	7.26	0.37
White blood cells, $1 \times 10^3/\text{mm}^3$	10.3	1.65	10.6	0.91	11.8	2.18	10.6	1.13	9.41	1.60
Hemoglobin, g	16.5	1.37	17.7	1.45	18.7	1.38	16.8	0.42	17.6	0.87
Hematocrit, %	47.6	3.68	50.5	3.66	52.3	3.54	48.5	0.21	49.4	2.10
Mean corpuscular volume	69.3	2.06	68.3	1.89	67.1	1.37	68.5	0.70	64.8	5.57
Mean corpuscular hemoglobin	25.5	2.48	24.7	0.78	24.5	0.64	25.0	0.42	24.5	0.58
Mean corpuscular hemoglobin conc.	34.8	0.37	35.5	0.55	36.0	0.72	35.7	0.35	36.0	0.40

Table 10, continued.

	PuO ₂ Group 1 (1 year)							
	3-16-71				4-22-71			
	4 dogs		4 dogs		4 dogs		4 dogs	
	No Treatment		DTPA		No Treatment		DTPA	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Biochemistry (Serum)</u>								
Chloride, meq/l	104	2.16	101	2.06	113	0.96	114	2.88
CO ₂ , meq/l	23.1	2.66	25.2	0.87	23.1	1.49	22.6	2.35
Potassium, meq/l	4.45	0.21	4.62	0.15	4.35	0.29	4.55	0.17
Sodium, meq/l	146	1.41	147	0.96	146	0.96	148	0.50
Blood urea nitrogen, mg %	15.5	2.52	20.0	4.90	14.8	3.77	19.5	7.90
Glucose, mg %	83.5	19.1	84.0	11.6	105	10.8	102	7.25
Total protein, g %	6.17	0.34	5.90	0.22	6.22	0.39	5.95	1.05
Albumin, g %	1.57	0.10	1.65	0.13	1.55	0.06	1.67	0.15
Calcium, mg %	9.12	1.18	9.02	0.83	9.82	0.57	10.4	0.42
Inorganic phosphorus, mg %	4.90	0.69	4.00	0.37	4.52	0.78	4.35	0.58
Cholesterol, mg %	149	14.9	143	19.4	150	14.1	155	17.8
Uric acid, mg %	0.50	0.08	0.52	0.12	0.45	0.10	0.50	0.08
Creatinine, mg %	1.07	0.31	1.27	0.12	0.87	0.09	0.92	0.09
Total bilirubin, mg %	0.65	0.46	0.30	0.14	0.15	0.06	0.20	0.14
Alkaline Phosphatase, units	27.2	16.6	34.5	11.7	32.2	19.9	48.0	15.7
Creatinine phosphokinase, units	364	220	166	50.9	253	153	194	94.3
Lactate dehydrogenase, units	244	145	188	133	214	103	238	34.3
Serum glutamic-oxaloacetic transaminase, units	72.8	37.4	57.3	11.4	53.8	15.9	51.0	6.68
<u>Thyroid</u>								
T-3, %	42.0	0.00	42.3	0.50				
T-4, MCG %	1.80	0.20	1.92	0.48				

Table 10, concluded.

	PuO ₂ Group 1 (1 year)							
	3-16-71				4-22-71			
	4 dogs		4 dogs		4 dogs		4 dogs	
	No Treatment		DTPA		No Treatment		DTPA	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
<u>Lactate Dehydrogenase Isoenzymes</u>								
Band 1	31.2	7.14	24.0	7.62				
Band 2	18.1	6.14	21.0	2.94				
Band 3	14.0	1.76	14.8	4.57				
Band 4	14.2	5.92	15.8	4.99				
Band 5	22.5	5.82	24.5	13.0				
<u>Complete Blood Count (CBC)</u>								
Red blood cells, $1 \times 10^6/\text{mm}^3$	7.96	0.19	8.08	0.41	7.81	0.19	7.55	0.46
White blood cells, $1 \times 10^3/\text{mm}^3$	8.60	1.92	9.27	1.23	8.90	2.16	9.72	1.35
Hemoglobin, g	19.9	0.82	19.6	0.54	19.5	0.59	18.7	1.00
Hematocrit, %	55.8	2.70	54.5	1.70	54.8	1.63	52.4	2.91
Mean corpuscular volume	69.0	2.16	67.0	0.81	69.8	2.22	68.0	1.15
Mean corpuscular hemoglobin	25.1	0.55	24.5	0.43	25.5	0.63	25.8	2.21
Mean corpuscular hemoglobin conc.	36.0	0.25	36.3	0.43	37.7	0.12	35.6	0.29

III. PLUTONIUM NITRATE AND HIGH FIRED PLUTONIUM OXIDE EXPERIMENTS

A. Introduction

The main objectives of this study were to investigate the translocation rates of Pu and Am from simulated puncture wounds contaminated with $\text{Pu}(\text{NO}_3)_4$ and PuO_2 in beagles. The specific objectives were: (a) to determine the rates of Pu translocation from wounds to the various organs in the body, (b) to determine the effect of different physico-chemical form, upon these dynamics, (c) to determine the relative translocation of Pu and Am for these chemical forms to better assess the use of ^{241}Am as a tracer for in vivo Pu determinations and (d) to determine the effectiveness of chelation therapy (DTPA) upon the above parameters.

This report describes the experimental details of the study with emphasis upon the criteria used to plan the experiment. The differences in translocation dynamics between the more soluble $\text{Pu}(\text{NO}_3)_4$ and insoluble "high-fired" PuO_2 were studied. The kinetics of removal of plutonium and its build-up in activity into the L.S.C.L.N., the first node to drain the site of injection, were studied by in vivo measurement techniques and by radio-chemical analysis of selected tissues collected at necropsy. Equations describing the dynamics of the activity were fitted to the data.

B. Materials and Methods

Ninety-six male adult beagles varying in age from 12 to 36

months were used for this study. The dogs were obtained from the barrier-maintained colony of Colorado State University's Collaborative Radiological Health Laboratory (CRHL). The animals were divided randomly into twelve study groups, eight to a group with half of each group randomly selected to receive DTPA treatment. Animals of the same group were housed one or two to a kennel. The dogs were maintained in accordance with provisions of the Laboratory Animal Welfare Act (1967) and under daily veterinary care.

Two chemical forms of Pu were used for this study, PuO_2 (high fired) and $\text{Pu}(\text{NO}_3)_4$. The plutonium nitrate solution was in a 2.0 NHNO_3 solution and the activity concentration was about 15 $\mu\text{Ci/ml}$. The ^{241}Am concentration of the Pu was 750 ppm. The Pu was contained in vials (rubber diaphragm stoppered) which contained 5 ml of solution. A tuberculin syringe and needle was used to inject an aliquot of 0.1 ml in the dog's foot.

The high fired plutonium oxide was prepared from electro-refined unalloyed Pu metal dissolved in HCC and precipitated with oxalic acid. The resulting salt was washed and then calcined to the high fired state of plutonium dioxide for 100 hours at 850° C. The stoichiometry of the oxide so prepared has been accepted as $\text{PuO}_{1.98}$. The oxide was then dried at 80° C. for approximately 100 hours and pulverized by grinding in a tungsten carbide grinding vial employing tungsten carbide grinding balls for 30 minutes using a high speed mechanical shaker. The particle size has been measured by

Table 11. PuO_2 for Dow-CSU dog project center.

Pu	= 0.8826 g/g PuO_2
Cation impurities	= $\approx$ 200 ppm (based on Pu)
^{241}Am	= 360 ppm based on Pu as of Oct. 1, 1970
^{241}Am growth rate	= $\approx$ 15 ppm/month based on Pu
^{238}Pu	= 0.0105 wt %, based on Pu
^{239}Pu	= 93.898 wt %, based on Pu
^{240}Pu	= 5.695 wt %, based on Pu
^{241}Pu	= 0.377 wt %, based on Pu as of June 1970
^{242}Pu	= 0.019 wt %, based on Pu
Atomic wt of Pu	= 239.116 as of June 1970

Particle Size

1 μ or less	68%
1 μ to 3 μ	21%
3 μ to 5 μ	4%
5 μ to 10 μ	6%
> 10 μ	< 1%

Mass Median Diameter approximates 7 μ

optical microscopy. The physical, chemical, and isotopic information for the oxide are shown in Table 11. One mg portion of the dry oxide were packaged in a 5 ml vial. The insoluble PuO_2 particles were suspended in 1.0 ml of physiological saline solution then a 0.1 ml of the solution was injected subcutaneously.

Implant solution calibrations were performed each time a group of dogs was injected by placing three normal 0.1 ml injection aliquots in three separate one liter volumetric flasks of nitric acid solution, mixing and placheting a sample for alpha counting in a gas flow proportional counting system.

Blood, urine and feces sampling followed the same schedule used for the air oxidized PuO_2 experiment and DTPA was administered in the same manner. The in vivo measurements of activity were obtained at the implant site and over the left superficial cervical lymph node (L.S.C.L.N.) in the same manner and according to the same schedule previously described for the air oxidized PuO_2 .

The dogs were anesthetized with pentobarbital sodium and killed by exsanguination at 2 weeks, 1 month, 2 months, 3 months, 6 months and 12 months post-implant time. The tissue samples collected at necropsy were as follows: left eye, left testis, left kidney, left adrenal gland, trachea (small piece to represent body cartilage), lung (tip of one lobe), spleen, liver (about a fourth of the organ was analyzed), sternum,

left 10th rib, two lumbar vertebrae, piece of skull, left femur, left axillary lymph node, right cervical lymph node, left cervical lymph node, thyroid, implant site (left front paw sectioned at the carpometacarpal joint), and bile.

All samples collected are being assayed for plutonium and americium activity in Rocky Flats Laboratories. The general chemical procedures for each sample type are essentially the same as previously reported.

In vivo counts on each dog were made on days 1 through 7, 9, 11, 14, 28, 29 and 30 and then monthly following the plutonium implants. Measurements were made with both a lithium drifted germanium detector and a NaI(Tl) wound counter.

The scintillation counter was used to measure the activity at the wound site and at the left superficial cervical lymph node. This lymph node is the first major node draining the contaminated area of the foot. The count rate was recorded as a function of time.

The Ge (Li) detector was used to measure the 51.6 ke V gamma emission of ^{239}Pu and the 59.6 gamma emission of ^{241}Am . Counts were taken at the wound site, the left cervical lymph node, and the liver area of each dog.

Formalin fixed, hematoxylin and eosin stained paraffin sections were routinely prepared for light microscopy from the right kidney, liver, spleen, heart, right testis, right lobe of the thyroid (and occasionally including parathyroid), right eye, stomach, intestines, urinary bladder, pancreas and right

adrenal. Sections of left and right superficial cervical lymph nodes were prepared for light microscopy from the dogs in group 2 (1 month), group 4 (3 months), and group 5 (6 months). Other organs sectioned for light microscopy from randomly selected dogs included brain, gall bladder, skin, pituitary, prostate, skeletal muscle, and mesenteric lymph nodes. Special stains, including Prussian blue for iron and van Kossa for calcium were occasionally used as indicated under results.

Liver, spleen, and kidney specimens were saved for histopathologic evaluation from the dogs in group 1 (2 weeks).

C. Results

To be able to group the data and compare time, treatment and enhancement effects for different chemical forms, it was necessary to normalize all data to the fraction of the initial implant dose administered to each dog. Monthly values are presented as an average of the 3 daily counts or samples taken on each dog.

The results obtained with the in vivo Na(Tl) wound counter measurements of the 17 ke V L x-rays from the implant site on the left paw are shown in Table 12. The data reflects the average values obtained at the time of sampling or counting for dogs in each treatment group for the chemical form implanted. The animals listed under No Treatment (Table 12) headings were implanted with Pu but received no chelation therapy and those listed under DTPA received DTPA chelation therapy according to the schedule described in the experimental design. Figures

4 and 5 show plots of the best curve fit analysis for this data. It was found that the best curve fit models for the $\text{Pu}(\text{NO}_3)_4$ No Treatment and DTPA groups were the following power functions:

A. No Treatment	$y = AX^B$	where y = time in days, X = fraction of initial implant dose, A = 0.6359, B = -0.1239
B. DTPA	$y = AX^B$	where y = time in days, X = fraction of initial implant dose, A = 0.4436, B = -0.545

For the PuO_2 data the best curve fit analysis found was an exponential curve for both sets of data:

A. No Treatment	$y = Ae^{BX}$	where y and X are the same as above, A = 0.8368, B = -0.006601
B. DTPA	$y = Ae^{BX}$	where y and X are the same as above, A = 0.797, B = -0.005419

The data on the Pu buildup from the wound site to the left superficial cervical lymph node, is presented in Table 13. This data was derived from the in vivo NaI(Tl) wound counter measurements, taken over the L.S.C.L.N. Figures 6 and 7 are plots of the data obtained from the wound counter measurements. Radiochemical analysis of the L.S.C.L.N.'s taken at the 6 necropsy times is shown in Table 13. These data show a similar shaped curve for buildup and regression. These data reinforce the compatibility of the two completely separate measurement techniques. The activity in the L.S.C.L.N. rose to a maximum around 30 to 90 days for both $\text{Pu}(\text{NO}_3)_4$ and PuO_2 forms. This

Table 12. Fraction of Initial Implant Dose of Plutonium in Paw with Exposure Time.

Time Following Implant (days)	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment	DTPA	No Treatment	DTPA
0.0417	0.90 ± 0.13	0.71 ± 0.15	0.762 ± 0.097	0.702 ± 0.105
0.333	0.65 ± 0.08	0.50 ± 0.08	0.865 ± 0.098	0.804 ± 0.108
5 Days	0.57 ± 0.07	0.33 ± 0.06	0.808 ± 0.091	0.788 ± 0.107
11 "	0.63 ± 0.085	0.34 ± 0.06	0.737 ± 0.087	0.758 ± 0.101
14 "	0.51 ± 0.08	0.33 ± 0.07	0.660 ± 0.089	0.745 ± 0.099
31 "	0.35 ± 0.06	0.22 ± 0.06	0.879 ± 0.067	0.930 ± 0.119
60 "	0.31 ± 0.11	0.22 ± 0.06	0.546 ± 0.073	0.552 ± 0.089
90 "	0.31 ± 0.05	0.24 ± 0.05	0.470 ± 0.123	0.516 ± 0.137
125 "	0.49 ± 0.15	0.31 ± 0.08	0.421 ± 0.055	0.370 ± 0.047
170 "	0.37 ± 0.07	0.21 ± 0.09	0.452 ± 0.074	0.380 ± 0.043
221 "	0.33 ± 0.12	0.17 ± 0.03	0.133 ± 0.016	0.160 ± 0.020
248 "	0.36 ± 0.12	0.21 ± 0.02	0.090 ± 0.006	0.147 ± 0.024
304 "	0.38 ± 0.12	0.21 ± 0.02	0.116 ± 0.011	0.164 ± 0.021
357 "	0.18 ± 0.06	0.11 ± 0.03	0.109 ± 0.012	0.163 ± 0.021

Figure 4. Power function curve for fraction of initial dose retained in paw of $\text{Pu}(\text{NO}_3)_4$ dogs. Solid line = No Treatment. Broken line = DTPA Treatment.

Figure 5. Exponential function curve for fraction of initial dose retained in paw of PuO_2 dogs. Solid line = No Treatment.
Broken line = DTPA Treatment.

Table 13 Fraction of Initial Implant Dose of Plutonium in Lymph Node with Exposure Time.

Time Following Implant (days)	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment	DTPA	No Treatment	DTPA
0.0417	0.053 $\pm$ 0.001	0.04 $\pm$ 0.007	0.00125 $\pm$ 0.00071	0.00113 $\pm$ 0.00050
0.333	0.16 $\pm$ 0.029	0.12 $\pm$ 0.022	0.00595 $\pm$ 0.00143	0.00786 $\pm$ 0.00492
5 Days	0.22 $\pm$ 0.036	0.17 $\pm$ 0.030	0.05086 $\pm$ 0.01106	0.02134 $\pm$ 0.00548
11 "	0.24 $\pm$ 0.066	0.20 $\pm$ 0.043	0.08909 $\pm$ 0.01593	0.05050 $\pm$ 0.01435
14 "	0.17 $\pm$ 0.039	0.15 $\pm$ 0.037	0.09292 $\pm$ 0.01363	0.05275 $\pm$ 0.01062
31 "	0.11 $\pm$ 0.02	0.08 $\pm$ 0.02	0.20119 $\pm$ 0.02471	0.08074 $\pm$ 0.00913
60 "	0.31 $\pm$ 0.06	0.35 $\pm$ 0.07	0.13148 $\pm$ 0.02037	0.09331 $\pm$ 0.01554
90 "	0.20 $\pm$ 0.04	0.23 $\pm$ 0.05	0.21260 $\pm$ 0.03606	0.11565 $\pm$ 0.01565
125 "	0.57 $\pm$ 0.12	0.60 $\pm$ 0.10	0.13548 $\pm$ 0.02358	0.07553 $\pm$ 0.00754
170 "	0.10 $\pm$ 0.03	0.09 $\pm$ 0.04	0.14917 $\pm$ 0.05433	0.08157 $\pm$ 0.01568
221 "	0.37 $\pm$ 0.13	0.30 $\pm$ 0.03	0.09288 $\pm$ 0.02640	0.04981 $\pm$ 0.00501
248 "	0.40 $\pm$ 0.13	0.30 $\pm$ 0.03	0.09395 $\pm$ 0.02635	0.04716 $\pm$ 0.00770
304 "	0.43 $\pm$ 0.13	0.43 $\pm$ 0.04	0.16514 $\pm$ 0.09294	0.05842 $\pm$ 0.00361
357 "	0.07 $\pm$ 0.017	0.06 $\pm$ 0.017	0.11203 $\pm$ 0.02736	0.05056 $\pm$ 0.00488

Figure 6, Fraction of initial dose in superficial
cervical lymph node of $\text{Pu}(\text{NO}_3)_4$ dogs.
X = No Treatment.
Square = DTPA Treatment.

FRACTION OF DOSE
3745 907
LOG SCALE

Figure 7. Fraction of initial dose in superficial
cervical lymph node of PuO_2 dogs.
X = No Treatment.
Square = DTPA Treatment.

data was best fitted by a power function model. After reaching this maximum a gradual linear regression in the data occurred throughout the remainder of the 1 year study. The curve fit models for the $\text{Pu}(\text{NO}_3)_4$ data was found to be:

No Treatment

1st component a power function

$$Y = AX^B \quad \begin{array}{l} A = 0.1268 \\ B = 0.1609 \end{array}$$

2nd component a linear function

$$Y = A + BX \quad \begin{array}{l} A = 0.3599 \\ B = -2.7259 \times 10^{-4} \end{array}$$

DTPA

1st component a power function

$$Y = AX^B \quad \begin{array}{l} A = 0.1002 \\ B = 0.1928 \end{array}$$

2nd component a linear function

$$Y = A + BX \quad \begin{array}{l} A = 0.13976 \\ B = -3.0032 \times 10^{-3} \end{array}$$

The curve fit models for the PuO_2 data was:

No Treatment

1st component a power function

$$Y = AX^B \quad \begin{array}{l} A = 0.014006 \\ B = 0.76235 \end{array}$$

2nd component a linear function

$$Y = A + BX \quad \begin{array}{l} A = 0.18134 \\ B = -2.1013 \times 10^{-4} \end{array}$$

DTPA

1st component a power function

$$Y = AX^B \quad \begin{array}{l} A = 0.01020 \\ B = 0.61822 \end{array}$$

Table 14. Fraction of Initial Implant Dose of Plutonium in Blood with Exposure Time -- Pu(NO₃)₄ Group.

Time Following Implant (days)	No Treatment (X10 ⁻⁶)	DTPA (X10 ⁻⁶)
0.021	63.88± 9.90	54.25±10.27
0.042	33.13± 7.13	22.15± 5.15
0.083	54.15± 5.42	36.69± 5.54
0.167	61.07±16.80	40.75± 7.55
0.250	123.20±47.34	70.50±27.56
0.333	67.01±20.24	27.16± 5.87
2	48.25± 4.80	42.01±10.13
3	23.21± 3.23	11.63± 2.02
4	11.09± 0.88	6.09± 1.90
5	9.28± 1.18	2.79± 0.47
6	8.64± 1.87	--
7	--	1.38± 0.21
9	3.11± 0.43	1.95± 0.44
11	2.34± 0.26	1.38± 0.24
14	3.42± 0.73	1.73± 0.28
31	1.50± 0.17	0.95± 0.15
34	1.33± 0.19	0.49± 0.05
59	1.15± 0.13	0.64± 0.15
65	0.61± 0.15	0.31± 0.06
88	0.59± 0.10	0.21± 0.04
93	0.60± 0.14	0.25± 0.05
121	1.09± 0.09	0.53± 0.05
129	1.12± 0.24	0.96± 0.16
157	1.04± 0.14	0.74± 0.07
165	0.78± 0.08	0.38± 0.05
188	1.48± 0.31	1.19± 0.32
221	0.49± 0.07	0.47± 0.15
248	0.65± 0.15	0.24± 0.06
276	0.97± 0.24	0.55± 0.06
304	0.99± 0.17	0.67± 0.12
336	1.06± 0.23	0.64± 0.10
366	0.83± 0.28	0.15± 0.03

Table 15. Fraction of Initial Implant Dose of Americium in Blood with Exposure Time--Pu(NO₃)₄ Group.

Time Following Implant (days)	No Treatment (X10 ⁻⁶)	DTPA (X10 ⁻⁶)
0.021	1.810±0.714	1.804±0.700
0.042	18.472±7.396	4.618±1.715
0.083	7.273±1.782	4.129±0.953
0.167	7.135±1.695	4.041±1.263
0.250	5.508±0.925	2.390±0.669
0.333	6.103±1.887	4.031±1.037
2	2.736±0.537	1.619±0.331
3	1.469±0.523	0.466±0.101
4	2.152±0.705	1.593±0.488
5	0.897±0.202	0.350±0.103
6	0.459±0.091	0.573±0.187
7	0.369±0.085	0.880±0.657
9	0.278±0.083	0.689±0.248
11	0.621±-.322	0.317±0.108
14	0.449±0.172	0.230±0.050
31	0.231±0.049	0.204±0.055
65	0.159±0.074	0.084±0.024
93	0.018±0.003	0.031±0.016
121	0.072±0.037	0.027±-.008
129	3.491±0.826	1.596±0.185
165	0.016±0.003	0.022±0.006
187	4.704±1.912	2.057±0.443
221	0.854±0.148	1.840±0.561
248	2.864±1.405	0.864±0.140
276	1.964±0.573	1.619±0.283
304	1.603±0.371	2.561±0.669
336	1.764±0.612	5.318±1.511

Figure 8. Power function curve for fraction of initial dose in blood of $\text{Pu}(\text{NO}_3)_4$ dogs.
Solid line = No Treatment.
Broken line = DTPA Treatment.

Figure 9. Power function curve for fraction of
initial dose in blood of PuO_2 dogs.
Solid line = No Treatment.
Broken line = DTPA Treatment.

2nd component a linear function

$$A = 0.10098$$

$$B = -1.59127 \times 10^4$$

Johnson (1969) showed a high correlation of coefficient of 0.993 for his PuO_2 (air oxidized) data. Thus, the fact that the power function had a good index of determination for the data in the first components of these curves is strongly supported.

Systemic transport of Pu from the lymph or lymph node as well as the wound site occurred in the blood. The results of the radiochemical analysis of 10 ml blood samples are given in Tables 16 and 17. The concentration of Pu in the blood increased with time reaching a peak at 2 to 24 hours. However, in the curve fit analysis plots of Figures 8 and 9, the values for the first components are so near day 0 on the time scale that only the 2nd components of the curves have been plotted. The best curve fit functions for these data was found to be:

A. $\text{Pu}(\text{NO}_3)_4$

- a) No Treatment Group
Plot is a power function:

$$Y = AX^B$$

$$A = 28.7102 \times 10^{-6}$$

$$B = -0.7004$$

- b) DTPA Group
Plot is a power function:

$$Y = AX^B$$

$$A = 13.5395 \times 10^{-6}$$

$$B = -0.68237$$

B. PuO_2

- a) No Treatment Group
Plot is a power function:

$$Y = AX^B$$

$$A = 0.62977 \times 10^{-8}$$

$$B = -0.26896$$

- b) DTPA Group
Plot is a power function:

$$Y = AX^B$$

$$A = 0.47545 \times 10^{-8}$$

$$B = -0.31112$$

The radiochemical analysis of the Am concentration in the blood shows a nearly identical pattern to that of Pu. The data is presented in Tables 14 and 15.

As a fraction of the systemically circulating Pu is excreted through the urine pathway, 24 hour urine samples were collected and analyzed for Pu and Am content. Tables 16 and 17 show the fractions of these isotopes excreted via the urine in 24 hour periods. Curve fit analysis of these data indicate a best fit by using a power function model. The following functions were best fitted to these data:

A. $\text{Pu}(\text{NO}_3)_4$

- a) No Treatment Group:

$$Y = AX^B$$

$$A = 189.07 \times 10^{-5}$$

$$B = -0.5378$$

- b) DTPA Group:

$$Y = AX^B$$

$$A = 1617.18 \times 10^{-5}$$

$$B = -0.7013$$

B. PuO_2

- a) No Treatment Group:

$$Y = AX^B$$

$$A = 0.2915 \times 10^{-5}$$

$$B = -0.4035$$

Table 16. Fraction of Initial Implant Dose of Plutonium in Blood with Exposure Time--PuO₂ Group.

Time Following Implant (days)	No Treatment (X10 ⁻⁸)	DTPA (X10 ⁻⁸)
0.042	0.397±0.088	0.468±0.170
0.083	1.512±0.470	1.107±0.446
0.167	1.439±0.337	0.773±0.215
0.333	1.181±0.384	1.124±0.401
2	1.116±0.470	1.304±0.552
3	0.847±0.207	0.419±0.154
4	0.575±0.113	0.453±0.143
5	0.381±0.079	0.236±0.054
6	0.338±0.076	0.274±0.091
7	0.386±0.092	0.352±0.127
9	0.440±0.075	0.253±0.076
11	0.291±0.064	0.283±0.102
14	0.189±0.085	0.186±0.109
31	0.158±0.048	0.135±0.022
36	0.575±0.413	0.038±0.015
65	0.133±0.025	0.114±0.026
70	0.130±0.034	0.108±0.025
103	0.146±0.032	0.077±0.013
140	0.113±0.020	0.067±0.013
183	0.228±0.109	0.124±0.033
217	0.181±0.045	0.097±0.024
258	0.103±0.035	0.081±0.043
287	0.160±0.091	0.175±0.116

Figure 10. Power function curve of fraction of
initial dose in urine of $\text{Pu}(\text{NO}_3)_4$
dogs.
Solid line = No Treatment.
Broken line = DTPA Treatment.

Table 17. Fraction of Initial Implant Dose of Americium in Blood with Exposure Time--PuO₂ Group.

Time Following Implant (days)	No Treatment (X10 ⁻⁹)	DTPA (X10 ⁻⁹)
0.042	92.507±56.855	89.211± 68.819
0.083	80.015±35.859	60.979± 31.758
0.167	102.297±47.470	151.304±105.912
0.333	24.611± 7.907	9.870± 3.917
2	28.862±11.545	33.980± 19.816
3	32.067±22.996	99.800± 60.990
4	9.800± 2.293	17.540± 7.840
5	8.526± 2.663	18.990± 14.035
6	12.284± 4.428	19.036± 6.852
7	8.548± 2.682	10.285± 3.396
9	6.803± 2.182	7.290± 2.722
11	45.999±15.818	28.708± 13.905
14	28.976± 8.890	4.083± 1.054
31	16.072± 4.911	25.223± 6.909
46	7.043± 1.498	--
65	27.890±24.390	3.602± 0.363
70	8.937± 4.199	19.610± 6.425
78	46.390±27.939	43.383± 20.942
103	31.752±12.866	13.128± 5.961
163	15.307±12.583	40.900± 36.887
183	20.100± 8.278	5.618± 1.314
217	21.496± 8.765	19.096± 7.904

Table 18. Fraction of Initial Implant Dose of Plutonium in 24 hour Urine with Exposure Time.

Time Following Implant (days)	Pu(NO ₃) ₄		Time Following Implant (days)	PuO ₂	
	No Treatment (X10 ⁻⁵)	DTPA (X10 ⁻⁵)		No Treatment (X10 ⁻⁵)	DTPA (X10 ⁻⁵)
2	1233.312±278.032	3676.244±561.894	2	0.344±0.147	6.549±4.616
3	305.322±100.462	2124.812±583.909	3	0.274±0.157	1.355±0.459
4	109.972± 21.183	516.749± 73.452	4	0.981±0.781	0.448±0.226
5	88.234± 15.185	606.613±103.547	5	0.221±0.070	0.432±0.132
6	34.522± 4.418	326.617± 50.231	6	0.034±0.009	0.564±0.216
7	35.746± 5.749	277.699± 45.621	7	0.099±0.029	0.694±0.232
9	22.555± 3.345		9	0.073±0.018	0.280±0.072
11	21.196± 2.921	115.185± 21.525	11	0.071±0.014	0.169±0.066
14	16.294± 2.998	55.544± 9.757	14	0.174±0.075	0.262±0.075
31	11.464± 1.837	49.103± 9.840	33	0.129±0.029	0.414±0.071
59	23.207± 4.817	66.694± 10.089	48	0.006±0.004	0.214±0.161
65	7.251± 1.461	90.448± 22.651	68	0.005±0.004	0.168±0.042
93	9.764± 0.933	73.731± 14.080	78	0.035±0.005	0.050
121	7.004± 0.825	75.246± 19.310	104	0.061±0.011	0.143±0.041
129	30.319± 5.450	104.560± 22.984	137	0.099±0.035	0.168±0.040
157	52.448± 12.389	226.582± 57.475			
165	6.026± 0.843	50.980± 13.441			
187	32.675± 6.022	45.091± 12.423	183	0.110±0.046	0.040±0.021
221	19.376± 5.531	44.865± 25.070	217	0.108±0.031	0.178±0.095
248	16.313± 3.108	51.764± 11.353	258	0.033±0.015	0.068±0.019
276	8.752± 0.993	37.829± 15.709	287	0.030±0.007	0.050±0.027
304	13.843± 2.147	22.041± 5.010			
336	5.540± 1.472	8.978± 4.310			

Table 19, Fraction of Initial Implant Dose of Americium in 24-hour Urine with Exposure Time.

Time Following Implant (days)	$\text{Pu}(\text{NO}_3)_4$		Time Following Implant (days)	PuO_2	
	No Treatment ($\times 10^{-5}$)	DTPA ($\times 10^{-5}$)		No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
2	467.4 $\pm$ 279.7	1214.6 $\pm$ 631.6	2	24.38 $\pm$ 13.82	52.04 $\pm$ 24.33
3	92.5 $\pm$ 32.6	429.6 $\pm$ 123.6	3	10.66 $\pm$ 4.01	87.99 $\pm$ 26.11
4	29.1 $\pm$ 9.5	424.2 $\pm$ 250.2	4	8.61 $\pm$ 1.98	19.13 $\pm$ 4.02
5	49.3 $\pm$ 15.8	115.7 $\pm$ 31.9	5	7.80 $\pm$ 3.71	28.02 $\pm$ 15.49
6	47.6 $\pm$ 19.1	120.9 $\pm$ 43.5	6	19.54 $\pm$ 15.32	12.19 $\pm$ 6.93
7	15.4 $\pm$ 6.9	166.2 $\pm$ 88.3	7	2.35 $\pm$ 1.09	8.74 $\pm$ 3.07
9	15.2 $\pm$ 6.3	69.6 $\pm$ 26.8	9	7.87 $\pm$ 5.22	7.11 $\pm$ 6.26
11	11.4 $\pm$ 4.2	43.4 $\pm$ 12.9	11	4.39 $\pm$ 1.34	11.77 $\pm$ 4.50
14	15.6 $\pm$ 7.3	30.2 $\pm$ 12.3	14	11.73 $\pm$ 2.37	25.65 $\pm$ 9.30
31	6.5 $\pm$ 1.5	15.5 $\pm$ 2.3	33	10.40 $\pm$ 9.26	--
59	--	--	48	3.10 $\pm$ 1.52	6.80 $\pm$ 2.76
65	8.3 $\pm$ 1.9	72.1 $\pm$ 22.3	68	--	--
93	6.8 $\pm$ 1.6	103.6 $\pm$ 42.3	78	--	--
121	9.8 $\pm$ 3.4	59.5 $\pm$ 24.3	104	7.84 $\pm$ 2.03	2.38 $\pm$ 0.75
129	--	--	137	17.94 $\pm$ 10.59	10.01 $\pm$ 3.75
157	--	--	183	0.64 $\pm$ 0.38	0.94 $\pm$ 0.36
165	6.7 $\pm$ 1.4	111.1 $\pm$ 27.9	217	4.74 $\pm$ 4.54	9.02 $\pm$ 4.95
			258	2.32 $\pm$ 1.96	1.87 $\pm$ 0.93
			287	--	1.09 $\pm$ 0.36

b) DTPA Group:

$$Y = AX^B$$

$$A = 1.9581 \times 10^{-5}$$

$$B = -0.6202$$

Plots of these functions are shown in Figures 7 and 8.

Figures 10 and 11 show plots of the data obtained from the radiochemical analysis of the liver. This organ has been recognized as a critical organ and as expected, did show a buildup of activity with time. As the hepatic lymph node drains from the liver, it was analyzed for increased activity above other normal soft tissue. The data of this is shown in Tables 19 and 20.

The liver, spleen, hepatic lymph node, and proximal portion of the femur all indicate a trend toward a peak occurring about 6 months, followed by an apparent decrease from 6 months through 1 year.

Because of translocation occurring from the implant site, small quantities of Pu and Am began to appear in the various organs of the body. Specific organs described in the previous section were removed at prescribed sacrifice times and analyzed for Pu and Am content. Tables 19 and 20 give the normalized concentrations of Pu and Am for the No Treatment and DTPA treatment groups using the two different chemical forms.

Data included in Tables 19 and 20 were derived from the radiochemical analysis for Pu and Am concentration in other organs of interest. These organs included soft tissues such as testes, kidney, adrenal gland, eye, lung, left axillary lymph node, R.S.C.L.N., thyroid, and bile. The kidney was

Figure 11. Power function curve of fraction of
initial dose in urine of PuO_2 dogs.
Solid line = No Treatment.
Broken line = DTPA Treatment.

Table 20. Fraction of Initial Implant Dose of Plutonium per Gram of Organ Tissue.

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment	DTPA	No Treatment	DTPA
	($\times 10^{-6}$)	($\times 10^{-6}$)	($\times 10^{-6}$)	($\times 10^{-6}$)
<u>Left Testis</u>				
2 wks.	0.74 $\pm$ 0.40	0.29 $\pm$ 0.24	0.0020 $\pm$ 0.0005	0.0010 $\pm$ 0.0008
1 mo.	4.84 $\pm$ 1.49	1.26 $\pm$ 0.33	0.0048 $\pm$ 0.0008	0.0035 $\pm$ 0.0008
2 mo.	2.80 $\pm$ 0.47	1.01 $\pm$ 0.19	0.0716 $\pm$ 0.0377	0.0217 $\pm$ 0.0098
3 mo.	--	--	0.0244 $\pm$ 0.0097	0.0521 $\pm$ 0.0265
6 mo.	8.39 $\pm$ 2.66	2.61 $\pm$ 1.18	0.0483 $\pm$ 0.0237	0.0497 $\pm$ 0.0440
1 yr.	4.51 $\pm$ 0.06	1.33 $\pm$ 0.28	0.0316 $\pm$ 0.0193	0.0036 $\pm$ 0.0014
<u>Left Kidney</u>				
2 wks.	7.62 $\pm$ 3.77	0.52 $\pm$ 0.11	0.0156 $\pm$ 0.0047	0.0113 $\pm$ 0.0039
1 mo.	1.99 $\pm$ 0.34	3.49 $\pm$ 1.21	0.0132 $\pm$ 0.0096	0.1985 $\pm$ 0.1886
2 mo.	21.65 $\pm$ 7.27	6.69 $\pm$ 2.79	0.1360 $\pm$ 0.0192	0.0900 $\pm$ 0.0312
3 mo.	--	--	0.0753 $\pm$ 0.0169	0.3073 $\pm$ 0.1757
6 mo.	4.69 $\pm$ 2.35	6.24 $\pm$ 2.00	0.3397 $\pm$ 0.2348	0.0222 $\pm$ 0.0124
1 yr.	8.27 $\pm$ 4.58	25.12 $\pm$ 21.87	0.0235 $\pm$ 0.0060	0.0115 $\pm$ 0.0067
<u>Left Adrenal</u>				
2 wk.	8.00 $\pm$ 3.78	1.42 $\pm$ 0.72	0.1493 $\pm$ 0.1031	0.1729 $\pm$ 0.1298
1 mo.	23.48 $\pm$ 2.86	30.34 $\pm$ 19.92	0.0595 $\pm$ 0.0524	0.1020 $\pm$ 0.0850
2 mo.	17.49 $\pm$ 2.86	6.23 $\pm$ 1.69	0.5104 $\pm$ 0.1713	0.5335 $\pm$ 0.2710

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Left Adrenal (Continued)</u>				
3 mo.	--	--	43.9351 $\pm$ 30.2519	0.6924 $\pm$ 0.3698
6 mo.	24.83 $\pm$ 4.56	8.76 $\pm$ 1.50	3.4660 $\pm$ 2.0249	11.6884 $\pm$ 5.7430
1 yr.	32.38 $\pm$ 11.48	--	0.0765 $\pm$ 0.0332	0.0704 $\pm$ 0.0323
<u>Spleen</u>				
2 wk.	73.67 $\pm$ 40.45	8.30 $\pm$ 3.30	0.0276 $\pm$ 0.0116	0.0608 $\pm$ 0.0540
1 mo.	258.99 $\pm$ 129.02	34.22 $\pm$ 2.66	0.0132 $\pm$ 0.0047	0.0098 $\pm$ 0.0009
2 mo.	30.42 $\pm$ 12.13	18.57 $\pm$ 2.78	0.2577 $\pm$ 0.1124	0.0588 $\pm$ 0.0116
3 mo.	--	--	0.2661 $\pm$ 0.0559	0.0204 $\pm$ 0.0060
6 mo.	68.77 $\pm$ 26.40	21.85 $\pm$ 2.78	0.1195 $\pm$ 0.0802	0.2067 $\pm$ 0.1837
1 yr.	158.49 $\pm$ 102.16	10.67 $\pm$ 3.85	0.0189 $\pm$ 0.0137	0.0582 $\pm$ 0.0512
<u>Liver</u>				
2 wks.	94.04 $\pm$ 35.88	17.13 $\pm$ 10.81	0.0237 $\pm$ 0.0218	0.0012 $\pm$ 0.0006
1 mo.	83.03 $\pm$ 39.68	10.71 $\pm$ 4.01	0.1256 $\pm$ 0.1158	0.0005 $\pm$ 0.0002
2 mo.	80.04 $\pm$ 32.48	2.58 $\pm$ 0.77	0.5221 $\pm$ 0.2852	0.0576 $\pm$ 0.0344
3 mo.	66.31 $\pm$ 13.05	15.77 $\pm$ 4.26	0.0092 $\pm$ 0.0018	0.0419 $\pm$ 0.0267

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Liver (Continued)</u>				
6 mo.	108.82 $\pm$ 58.64	11.56 $\pm$ 4.45	0.0492 $\pm$ 0.0323	0.2873 $\pm$ 0.0421
1 yr.	474.29 $\pm$ 82.75	49.16 $\pm$ 10.24	0.6637 $\pm$ 0.5728	0.5257 $\pm$ 0.3334
<u>Left Eye</u>				
2 wks.	1.42 $\pm$ 0.47	0.41 $\pm$ 0.02	0.0171 $\pm$ 0.0061	0.0021 $\pm$ 0.0010
1 mo.	3.40 $\pm$ 0.72	0.94 $\pm$ 0.31	0.0095 $\pm$ 0.0010	0.0201 $\pm$ 0.0049
2 mo.	1.55 $\pm$ 0.35	1.08 $\pm$ 0.22	0.8271 $\pm$ 0.6884	0.0655 $\pm$ 0.0278
3 mo.	--	--	3.6910 $\pm$ 2.7599	0.1333 $\pm$ 0.0890
6 mo.	7.86 $\pm$ 2.76	2.83 $\pm$ 1.31	0.1035 $\pm$ 0.0498	4.1157 $\pm$ 2.3345
1 yr.	2.21 $\pm$ 0.21	--	0.0594 $\pm$ 0.0383	0.0136 $\pm$ 0.0075
<u>Trachea</u>				
2 wks.	3.53 $\pm$ 2.49	0.26 $\pm$ 0.23	0.0025 $\pm$ 0.0010	0.0006 $\pm$ 0.0004
1 mo.	7.43 $\pm$ 1.18	2.45 $\pm$ 0.94	0.0480 $\pm$ 0.0462	0.0204 $\pm$ 0.0068
2 mo.	3.99 $\pm$ 1.36	16.65 $\pm$ 8.90	0.0841 $\pm$ 0.0329	0.2621 $\pm$ 0.1573
3 mo.	--	--	1.2437 $\pm$ 0.8674	0.1906 $\pm$ 0.1370
6 mo.	10.33 $\pm$ 2.41	4.34 $\pm$ 0.50	0.1003 $\pm$ 0.0347	0.1241 $\pm$ 0.0756
1 yr.	3.82 $\pm$ 0.64	1.13 $\pm$ 0.42	0.0760 $\pm$ 0.0394	0.1039 $\pm$ 0.0391

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Lung</u>				
2 wks.	15.31 $\pm$ 6.22	8.45 $\pm$ 7.66	0.0019 $\pm$ 0.0007	0.0062 $\pm$ 0.0058
1 mo.	6.68 $\pm$ 1.86	2.98 $\pm$ 1.01	0.0306 $\pm$ 0.0297	0.0109 $\pm$ 0.0092
2 mo.	5.37 $\pm$ 1.01	3.14 $\pm$ 1.63	0.0129 $\pm$ 0.0039	0.0293 $\pm$ 0.0121
3 mo.	--	--	0.0791 $\pm$ 0.0351	0.4423 $\pm$ 0.2338
6 mo.	8.43 $\pm$ 0.63	3.43 $\pm$ 0.29	0.0187 $\pm$ 0.0036	0.1467 $\pm$ 0.1037
1 yr.	132.51 $\pm$ 67.96	42.94 $\pm$ 39.23	0.0100 $\pm$ 0.0031	0.0051 $\pm$ 0.0029
<u>Sternum</u>				
2 wks.	158.31 $\pm$ 21.94	14.05 $\pm$ 7.84	0.0113 $\pm$ 0.0030	0.0057 $\pm$ 0.0018
1 mo.	167.17 $\pm$ 67.86	95.04 $\pm$ 46.46	0.0109 $\pm$ 0.0038	0.0072 $\pm$ 0.0016
2 mo.	121.40 $\pm$ 32.65	58.21 $\pm$ 16.02	0.1280 $\pm$ 0.0138	0.0362 $\pm$ 0.0037
3 mo.	133.04 $\pm$ 22.25	24.26 $\pm$ 9.42	0.1596 $\pm$ 0.0199	0.0936 $\pm$ 0.0129
6 mo.	313.77 $\pm$ 53.93	84.73 $\pm$ 10.34	0.0303 $\pm$ 0.0189	0.0281 $\pm$ 0.0047
1 yr.	281.49 $\pm$ 34.66	252.78 $\pm$ 20.59	0.0483 $\pm$ 0.0125	0.0064
<u>Rib</u>				
2 wks.	110.81 $\pm$ 20.63	28.86 $\pm$ 10.99	0.0223 $\pm$ 0.0049	0.0356 $\pm$ 0.0310
1 mo.	255.74 $\pm$ 74.81	133.60 $\pm$ 44.89	0.0628 $\pm$ 0.0164	0.0870 $\pm$ 0.0640
2 mo.	153.54 $\pm$ 32.16	38.55 $\pm$ 9.45	0.2229 $\pm$ 0.0145	0.0686 $\pm$ 0.0101

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Rib (Continued)</u>				
3 mo.	129.60 $\pm$ 35.60	29.72 $\pm$ 3.92	1.1819 $\pm$ 1.0335	0.1090 $\pm$ 0.0399
6 mo.	354.61 $\pm$ 29.01	124.96 $\pm$ 16.71	0.2628 $\pm$ 0.0612	0.0431 $\pm$ 0.0095
1 yr.	280.52 $\pm$ 31.04	58.06 $\pm$ 9.13	0.0613 $\pm$ 0.0145	0.0385 $\pm$ 0.0212
<u>Vertebrae</u>				
2 wks.	46.85 $\pm$ 11.06	17.02 $\pm$ 10.44	0.0062 $\pm$ 0.0009	0.0030 $\pm$ 0.0010
1 mo.	26.29 $\pm$ 15.85	31.56 $\pm$ 9.00	0.0248	0.0065 $\pm$ 0.0009
2 mo.	64.55 $\pm$ 8.25	27.91 $\pm$ 6.98	0.2398 $\pm$ 0.0329	0.0374 $\pm$ 0.0074
3 mo.	95.82 $\pm$ 6.42	24.50 $\pm$ 4.65	0.0928 $\pm$ 0.0122	0.0567 $\pm$ 0.0166
6 mo.	387.22 $\pm$ 40.45	145.17 $\pm$ 30.12	0.1251 $\pm$ 0.0134	0.3613 $\pm$ 0.3190
1 yr.	196.44 $\pm$ 52.42	85.79 $\pm$ 36.59	0.0278 $\pm$ 0.0119	0.0101 $\pm$ 0.0025
<u>Skull</u>				
2 wks.	11.07 $\pm$ 5.43	10.19 $\pm$ 6.06	0.0068 $\pm$ 0.0035	0.0092 $\pm$ 0.0059
1 mo.	5.17 $\pm$ 0.86	11.41 $\pm$ 2.10	0.0104 $\pm$ 0.0078	0.0060 $\pm$ 0.0038
2 mo.	47.66 $\pm$ 13.68	11.98 $\pm$ 2.09	0.0456 $\pm$ 0.0075	0.1748 $\pm$ 0.1600
3 mo.	16.01 $\pm$ 1.72	15.38 $\pm$ 5.25	0.0369 $\pm$ 0.0078	0.0183 $\pm$ 0.0052
6 mo.	58.85 $\pm$ 15.38	57.15 $\pm$ 7.64	0.1977 $\pm$ 0.1617	0.0464 $\pm$ 0.0325
1 yr.	61.94 $\pm$ 12.17	28.10 $\pm$ 1.92	0.0929 $\pm$ 0.0785	0.0155 $\pm$ 0.0089

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Femur (Proximal)</u>				
2 wks.	78.77 $\pm$ 23.59	12.72 $\pm$ 3.39	0.0145 $\pm$ 0.0044	0.0059 $\pm$ 0.0021
1 mo.	51.88 $\pm$ 18.58	61.83 $\pm$ 13.46	0.0734 $\pm$ 0.0457	0.0101 $\pm$ 0.0050
2 mo.	125.99 $\pm$ 26.69	34.21 $\pm$ 19.43	0.2509 $\pm$ 0.1168	0.0676 $\pm$ 0.0227
3 mo.	65.06 $\pm$ 7.75	28.33 $\pm$ 3.90	0.2216 $\pm$ 0.0503	0.1138 $\pm$ 0.0245
6 mo.	380.37 $\pm$ 18.89	121.61 $\pm$ 23.65	0.1832 $\pm$ 0.0159	0.0567 $\pm$ 0.0178
1 yr.	244.93 $\pm$ 12.72	41.28 $\pm$ 8.12	0.0579 $\pm$ 0.0232	0.0238 $\pm$ 0.0047
<u>Femur (Shaft)</u>				
2 wks.	7.89 $\pm$ 1.44	2.55 $\pm$ 0.53	0.0052 $\pm$ 0.0013	0.0038 $\pm$ 0.0028
1 mo.	34.04 $\pm$ 25.72	17.18 $\pm$ 2.97	0.0052 $\pm$ 0.0012	0.0092 $\pm$ 0.0035
2 mo.	28.43 $\pm$ 6.07	9.23 $\pm$ 3.05	0.0928 $\pm$ 0.0237	0.0497 $\pm$ 0.0131
3 mo.	15.53 $\pm$ 2.16	7.44 $\pm$ 1.23	0.0928 $\pm$ 0.0196	0.0430 $\pm$ 0.0218
6 mo.	57.30 $\pm$ 13.56	21.70 $\pm$ 4.69	0.1309 $\pm$ 0.0934	0.0371 $\pm$ 0.0142
1 yr.	71.00 $\pm$ 20.57	39.65 $\pm$ 18.37	0.0254 $\pm$ 0.0150	0.0189 $\pm$ 0.0100
<u>Femur (Distal)</u>				
2 wks.	23.88 $\pm$ 3.06	6.70 $\pm$ 1.75	0.0052 $\pm$ 0.0005	0.0043 $\pm$ 0.0010
1 mo.	79.13 $\pm$ 9.78	32.58 $\pm$ 5.32	0.0239 $\pm$ 0.0061	0.0401 $\pm$ 0.0287
2 mo.	67.65 $\pm$ 14.01	26.44 $\pm$ 7.88	0.1068 $\pm$ 0.0390	0.0894 $\pm$ 0.0488

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Femur (Distal--Continued)</u>				
3 mo.	41.20 $\pm$ 11.82	16.04 $\pm$ 1.98	0.2805 $\pm$ 0.0599	0.1151 $\pm$ 0.0292
6 mo.	138.91 $\pm$ 25.17	58.48 $\pm$ 5.95	0.3560 $\pm$ 0.2212	0.0390 $\pm$ 0.0110
1 yr.	168.88 $\pm$ 40.65	47.10 $\pm$ 28.14	0.0308 $\pm$ 0.0143	0.0149 $\pm$ 0.0008
<u>L. S. C. L. N.</u>				
2 wks.	8,778.73 $\pm$ 5091.12	5,156.25 $\pm$ 1919.42	269.5903 $\pm$ 15.9417	191.3983 $\pm$ 88.7938
1 mo.	23,893.30 $\pm$ 5425.61	17,603.36 $\pm$ 1475.39	541.7794 $\pm$ 111.2964	467.2939 $\pm$ 189.2330
2 mo.	23,829.14 $\pm$ 10081.96	27,849.98 $\pm$ 8383.35	1013.1064 $\pm$ 271.5225	1364.0595 $\pm$ 357.1127
3 mo.	8,106.08 $\pm$ 4221.59	2,582.45 $\pm$ 1507.32	--	614.9849 $\pm$ 298.0859
6 mo.	11,138.89 $\pm$ 1920.26	31,825.02 $\pm$ 3222.00	1092.9112 $\pm$ 619.1151	1538.8583 $\pm$ 235.8470
1 yr.	2,149.79 $\pm$ 1489.54	2,423.96 $\pm$ 763.69	352.6104 $\pm$ 244.2531	222.4972 $\pm$ 60.5927
<u>Left Axillary Lymph Node</u>				
2 wks.			0.0732 $\pm$ 0.0347	0.3762 $\pm$ 0.3532
1 mo.			0.9682 $\pm$ 0.4421	1.0091 $\pm$ 0.9946
2 mo.			0.5719 $\pm$ 0.0986	0.7163 $\pm$ 0.4561
3 mo.			289.2001 $\pm$ 270.8544	2.8866 $\pm$ 1.9800
6 mo.			42.1758 $\pm$ 40.2670	3696.3124 $\pm$ 3657.3957
1 yr.			19.8371 $\pm$ 19.1655	67.0706 $\pm$ 66.9542

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
R. S. C. L. N.				
2 wks.	25.96 $\pm$ 6.96	2.19 $\pm$ 0.88	0.0175 $\pm$ 0.0098	0.0069 $\pm$ 0.0033
1 mo.	31.12 $\pm$ 6.39	20.57 $\pm$ 5.86	0.1382 $\pm$ 0.0816	0.2067 $\pm$ 0.1752
2 mo.	50.01 $\pm$ 10.06	18.28 $\pm$ 5.58	1.9920 $\pm$ 0.5539	0.4929 $\pm$ 0.1930
3 mo.	37.70 $\pm$ 28.28	12.34 $\pm$ 4.41	5.0852 $\pm$ 1.3400	1.5230 $\pm$ 0.7963
6 mo.	43.04 $\pm$ 12.36	11.84 $\pm$ 2.47	1.4010 $\pm$ 1.0649	1.8660 $\pm$ 1.2960
1 yr.	26.56 $\pm$ 7.18	18.70 $\pm$ 3.89	0.6754 $\pm$ 0.3547	0.0629 $\pm$ 0.0403
Thyroid				
2 wks.	2.91 $\pm$ 2.32	2.32 $\pm$ 2.14	0.1606 $\pm$ 0.0497	0.1203 $\pm$ 0.0497
1 mo.	6.97 $\pm$ 1.59	10.69 $\pm$ 2.23	0.0800 $\pm$ 0.0191	0.2788 $\pm$ 0.2057
2 mo.	8.71 $\pm$ 4.20	42.16 $\pm$ 17.34	1.1591 $\pm$ 0.3752	1.8733 $\pm$ 1.4609
3 mo.	15.63 $\pm$ 5.27	139.10 $\pm$ 0.45	2.6771 $\pm$ 1.5360	0.3978 $\pm$ 0.2077
6 mo.	31.93 $\pm$ 21.58	25.77 $\pm$ 16.27	1.5991 $\pm$ 0.9928	3.8689 $\pm$ 0.6434
1 yr.	12.45 $\pm$ 0.78	42.48 $\pm$ 20.99	0.2314 $\pm$ 0.1022	0.1246 $\pm$ 0.1087
Bile				
2 wks.	0.04 $\pm$ 0.02	0.15 $\pm$ 0.03	0.0173 $\pm$ 0.0146	0.0031 $\pm$ 0.0010
1 mo.	1.09 $\pm$ 0.36	0.60 $\pm$ 0.13	0.2508 $\pm$ 0.2444	0.0333 $\pm$ 0.0225
2 mo.	0.26 $\pm$ 0.13	0.08 $\pm$ 0.05	0.0291 $\pm$ 0.0273	0.0835 $\pm$ 0.0587

Table 20. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Bile (Continued)</u>				
3 mo.	0.10 $\pm$ 0.03	0.05 $\pm$ 0.01	0.0186 $\pm$ 0.0085	0.0485
6 mo.	0.09 $\pm$ 0.03	0.05 $\pm$ 0.01	0.0870 $\pm$ 0.0211	0.2739 $\pm$ 0.1511
1 yr.	1.53 $\pm$ 0.81	0.19 $\pm$ 0.12	0.0090	0.0051
<u>Hepatic Lymph Node</u>				
2 wks.		3.06 $\pm$ 0.98	0.1662 $\pm$ 0.1115	0.0162 $\pm$ 0.0080
1 mo.	9.20 $\pm$ 4.18	13.57 $\pm$ 5.66	0.0346 $\pm$ 0.0112	0.1183 $\pm$ 0.0593
2 mo.	31.16 $\pm$ 12.92	13.16 $\pm$ 4.84	0.2399 $\pm$ 0.1177	0.8734 $\pm$ 0.4750
3 mo.	10.35 $\pm$ 7.95	3.85 $\pm$ 1.53	85.8200 $\pm$ 41.7989	2.0331 $\pm$ 1.1349
6 mo.	51.97 $\pm$ 9.55	46.27 $\pm$ 6.55	14.5571 $\pm$ 12.0065	8.9578 $\pm$ 6.0884
1 yr.	123.31 $\pm$ 19.16	25.50 $\pm$ 7.08	1.1012 $\pm$ 1.0501	1.9155 $\pm$ 1.3479

Table 21. Fraction of Initial Implant Dose of Americium per Gram of Organ.

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Left Testis</u>				
2 wks.	1.059 $\pm$ 0.558	0.412 $\pm$ 0.826	0.433 $\pm$ 0.335	0.046 $\pm$ 0.041
1 mo.	1.161 $\pm$ 0.669	3.311 $\pm$ 2.692	0.058 $\pm$ 0.029	0.284 $\pm$ 0.056
2 mo.	0.949 $\pm$ 0.255	0.728 $\pm$ 0.114	0.057 $\pm$ 0.030	1.608 $\pm$ 0.839
3 mo.	1.420 $\pm$ 1.040	1.086	0.066	1.886 $\pm$ 1.530
6 mo.	1.793 $\pm$ 0.536	2.097 $\pm$ 1.460	0.351 $\pm$ 0.203	0.090 $\pm$ 0.042
1 yr.	--	--	0.460	0.072
<u>Left Kidney</u>				
2 wks.	32.484 $\pm$ 14.979	2.795 $\pm$ 0.972	0.127 $\pm$ 0.065	0.050 $\pm$ 0.009
1 mo.	--	--	0.291	0.310 $\pm$ 0.304
2 mo.	47.429 $\pm$ 10.270	68.793 $\pm$ 19.373	0.365 $\pm$ 0.105	0.537 $\pm$ 0.230
3 mo.	45.790 $\pm$ 9.719	2.920 $\pm$ 1.270	0.549	4.442 $\pm$ 2.911
6 mo.	82.835 $\pm$ 55.083	7.410 $\pm$ 2.278	0.062 $\pm$ 0.013	0.040 $\pm$ 0.034
1 yr.	--	--	0.045	0.130 $\pm$ 0.102
<u>Left Adrenal Gland</u>				
2 wks.	6.698 $\pm$ 3.608	3.353 $\pm$ 1.218	0.969 $\pm$ 0.348	0.494 $\pm$ 0.080
1 mo.	6.088 $\pm$ 6.032	3.774 $\pm$ 0.739		3.918 $\pm$ 1.946
2 mo.	12.241 $\pm$ 1.315	1.820 $\pm$ 0.581	0.695 $\pm$ 0.271	7.141 $\pm$ 1.087

Table 21. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Left Adrenal Gland (Continued)</u>				
3 mo.	--	1.136 $\pm$ 0.256	5.596	6.977 $\pm$ 3.629
6 mo.	13.270 $\pm$ 1.116	3.758 $\pm$ 0.744	0.181 $\pm$ 0.092	2.254 $\pm$ 2.218
1 yr.	--	--	0.87	--
<u>Spleen</u>				
2 wks.	26.329 $\pm$ 8.010	3.638 $\pm$ 1.380	0.091 $\pm$ 0.030	0.039 $\pm$ 0.033
1 mo.	63.998 $\pm$ 26.388	--	0.214	0.120
2 mo.	28.748 $\pm$ 26.324	7.769 $\pm$ 1.797	0.282 $\pm$ 0.109	0.108 $\pm$ 0.031
3 mo.	1.910	5.004 $\pm$ 4.925	1.420	0.296 $\pm$ 0.132
6 mo.	27.647 $\pm$ 9.337	9.060 $\pm$ 2.325	0.285 $\pm$ 0.131	0.346 $\pm$ 0.318
1 yr.	37.780 $\pm$ 4.430	--	0.020	0.014
<u>Liver</u>				
2 wks.	37.690 $\pm$ 14.177	8.154 $\pm$ 3.642	0.308 $\pm$ 0.119	0.215 $\pm$ 0.156
1 mo.	40.658 $\pm$ 8.182	1.593 $\pm$ 1.031	--	--
2 mo.	118.979 $\pm$ 57.314	2.542 $\pm$ 1.962	2.065 $\pm$ 1.079	0.521 $\pm$ 0.298
3 mo.	57.078 $\pm$ 53.823	9.405 $\pm$ 5.533	2.075 $\pm$ 0.902	1.182 $\pm$ 0.715
6 mo.	142.303 $\pm$ 97.392	16.883 $\pm$ 6.249	0.869 $\pm$ 0.367	0.548 $\pm$ 0.191
1 yr.	--	--	0.034	0.481 $\pm$ 0.469

Table 21. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Left Eye</u>				
2 wks.	2.300 $\pm$ 1.101	0.842 $\pm$ 0.331	1.158 $\pm$ 0.562	0.346 $\pm$ 0.172
1 mo.	3.388 $\pm$ 0.791	2.348 $\pm$ 1.304	1.906 $\pm$ 0.466	9.518 $\pm$ 5.599
2 mo.	1.202 $\pm$ 0.174	0.754 $\pm$ 0.422	2.344 $\pm$ 1.602	24.195 $\pm$ 19.109
3 mo.	--	--	6.180	3.918 $\pm$ 0.632
6 mo.	2.345 $\pm$ 0.394	0.90 $\pm$	0.598 $\pm$ 0.334	2.911 $\pm$ 2.906
1 yr.	113.210 $\pm$ 8.642	--	4.740	0.452 $\pm$ 0.194
<u>Trachea</u>				
2 wks.	2.675 $\pm$ 0.695	5.989 $\pm$ 5.697	0.204 $\pm$ 0.106	0.175 $\pm$ 0.119
1 mo.	7.706 $\pm$ 1.966	1.211 $\pm$ 0.466	0.974 $\pm$ 0.579	1.880
2 mo.	6.463 $\pm$ 0.973	15.562 $\pm$ 13.066	0.542 $\pm$ 0.298	2.031 $\pm$ 1.244
3 mo.	--	--	6.180	0.098 $\pm$ 0.052
6 mo.	18.260 $\pm$ 3.484	3.433 $\pm$ 0.915	0.297 $\pm$ 0.110	0.303 $\pm$ 0.160
1 yr.	--	--	3.325 $\pm$ 0.785	1.794 $\pm$ 1.620
<u>Lung</u>				
2 wks.	2.592 $\pm$ 0.589	1.232 $\pm$ 0.929	0.037 $\pm$ 0.012	
1 mo.	6.367 $\pm$ 3.616		0.397 $\pm$ 0.009	0.906 $\pm$ 0.190
2 mo.	3.146 $\pm$ 1.136	4.873 $\pm$ 2.316	1.139 $\pm$ 0.418	0.849 $\pm$ 0.361

Table 21. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Lung (Continued)</u>				
3 mo.	2.437 $\pm$ 0.497	5.949 $\pm$ 1.171	1.028 $\pm$ 0.159	1.490 $\pm$ 0.269
6 mo.	10.220 $\pm$ 3.062	2.380 $\pm$ 0.739	0.303 $\pm$ 0.216	0.030 $\pm$ 0.028
1 yr.	--	--	1.352 $\pm$ 1.200	--
<u>Sternum</u>				
2 wks.	7.547 $\pm$ 2.012	2.980 $\pm$ 0.996	0.058 $\pm$ 0.029	0.036 $\pm$ 0.018
1 mo.	1.531 $\pm$ 1.056	17.953 $\pm$ 8.122	0.348	0.296
2 mo.	42.647 $\pm$ 15.459	20.621 $\pm$ 11.171	1.688 $\pm$ 0.254	0.907 $\pm$ 0.330
3 mo.	--	2.771 $\pm$ 1.613	0.693 $\pm$ 0.179	0.880 $\pm$ 0.289
6 mo.	54.940 $\pm$ 41.353	27.583 $\pm$ 10.645	0.333 $\pm$ 0.039	0.432 $\pm$ 0.317
1 yr.	--	--	1.080 $\pm$ 0.518	0.026
<u>Rib</u>				
2 wks.	4.427 $\pm$ 1.221	2.423 $\pm$ 1.116	0.127 $\pm$ 0.091	0.132 $\pm$ 0.070
1 mo.	2.106 $\pm$ 1.120	14.081 $\pm$ 9.443	0.424	1.583 $\pm$ 0.690
2 mo.	25.418 $\pm$ 6.119	15.487 $\pm$ 4.927	4.591 $\pm$ 0.826	4.083 $\pm$ 0.850
3 mo.	49.400 $\pm$ 21.202	6.490 $\pm$ 3.288	2.421 $\pm$ 1.764	5.940 $\pm$ 3.393
6 mo.	199.093 $\pm$ 27.455	52.003 $\pm$ 13.938	1.619 $\pm$ 0.506	1.880 $\pm$ 1.406
1 yr.	--	--	3.566 $\pm$ 3.262	0.830

Table 21. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Vertebrae</u>				
2 wks.	7.021 $\pm$ 2.481	3.576 $\pm$ 1.985	0.002 $\pm$ 0.001	0.013 $\pm$ 0.007
1 mo.	0.874 $\pm$ 0.181	5.391 $\pm$ 1.602	0.435 $\pm$ 0.275	0.225 $\pm$ 0.218
2 mo.	11.725 $\pm$ 8.998	10.370 $\pm$ 6.436	1.890 $\pm$ 0.352	0.881 $\pm$ 0.463
3 mo.	39.995 $\pm$ 14.988		0.851 $\pm$ 0.318	0.345 $\pm$ 0.280
6 mo.	178.520 $\pm$ 99.431	33.520 $\pm$ 30.353	0.288 $\pm$ 0.134	0.023 $\pm$ 0.017
1 yr.	--	--	1.260 $\pm$ 1.233	0.028
<u>Skull</u>				
2 wks.	1.693 $\pm$ 0.395	1.903 $\pm$ 0.571	0.020 $\pm$ 0.011	0.123 $\pm$ 0.032
1 mo.	2.132 $\pm$ 0.553	1.870 $\pm$ 1.015	0.757 $\pm$ 0.227	0.912 $\pm$ 0.909
2 mo.	38.258 $\pm$ 8.695	11.892 $\pm$ 5.437	0.313 $\pm$ 0.029	0.246 $\pm$ 0.097
3 mo.	9.513 $\pm$ 3.909	5.800 $\pm$ 3.708	0.518 $\pm$ 0.107	0.578 $\pm$ 0.230
6 mo.	33.335 $\pm$ 21.065	22.958 $\pm$ 11.870	0.425 $\pm$ 0.312	0.826 $\pm$ 0.649
1 yr.	--	--	0.567 $\pm$ 0.530	0.209 $\pm$ 0.200
<u>Femur (Proximal)</u>				
2 wks.	5.210 $\pm$ 1.835	2.498 $\pm$ 1.426	0.181 $\pm$ 0.165	0.030 $\pm$ 0.020
1 mo.	7.568 $\pm$ 6.994	25.550 $\pm$ 23.912	0.328 $\pm$ 0.221	0.257 $\pm$ 0.148
2 mo.	38.700 $\pm$ 16.855	13.329 $\pm$ 9.975	0.593 $\pm$ 0.175	1.505 $\pm$ 1.404

Table 21. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Femur (Proximal--Continued)</u>				
3 mo.	22.187 $\pm$ 15.033	19.607 $\pm$ 19.506	0.736 $\pm$ 0.322	0.758 $\pm$ 0.282
6 mo.	121.943 $\pm$ 59.877	45.513 $\pm$ 23.413	0.413 $\pm$ 0.075	0.133 $\pm$ 0.047
1 yr.	327.240 $\pm$ 296.080	132.50	0.099	0.166 $\pm$ 0.100
<u>Femur (Shaft)</u>				
2 wks.	2.272 $\pm$ 0.950	0.833 $\pm$ 0.218	0.113 $\pm$ 0.098	0.392 $\pm$ 0.334
1 mo.	1.254 $\pm$ 0.222	4.800 $\pm$ 4.292	0.347 $\pm$ 0.293	0.21
2 mo.	42.706 $\pm$ 25.376	8.417 $\pm$ 4.075	1.142 $\pm$ 0.760	0.494 $\pm$ 0.231
3 mo.	20.590 $\pm$ 15.330	6.903 $\pm$ 3.457	1.241 $\pm$ 0.416	0.922 $\pm$ 0.679
6 mo.	32.278 $\pm$ 18.061	60.625 $\pm$ 52.035	1.002 $\pm$ 0.557	0.097 $\pm$ 0.037
1 yr.	--	--	0.292	1.993 $\pm$ 1.801
<u>Femur (Distal)</u>				
2 wks.	3.815 $\pm$ 0.998	2.390 $\pm$ 1.201	0.021 $\pm$ 0.007	0.030 $\pm$ 0.021
1 mo.	3.167 $\pm$ 1.463	10.544 $\pm$ 3.980	0.575 $\pm$ 0.042	0.787 $\pm$ 0.513
2 mo.	2.185 $\pm$ 0.929	16.093 $\pm$ 0.950	0.412 $\pm$ 0.140	0.316 $\pm$ 0.107
3 mo.	18.203 $\pm$ 7.541	0.473 $\pm$ 0.237	0.855 $\pm$ 0.069	0.327 $\pm$ 0.063
6 mo.	37.635 $\pm$ 14.421	20.635 $\pm$ 10.733	0.604 $\pm$ 0.519	0.108 $\pm$ 0.074
1 yr.	--	--	0.167 $\pm$ 0.124	0.199 $\pm$ 0.162

Table 21. Continued

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>L. S. C. L. N.</u>				
2 wks.	3686.372 $\pm$ 1924.405	2275.064 $\pm$ 886.907	419.776 $\pm$ 145.236	307.717 $\pm$ 216.101
1 mo.	--	6766.855 $\pm$ 1906.181	--	1776.254 $\pm$ 1418.584
2 mo.	6740.956 $\pm$ 3888.250	12072.526 $\pm$ 6313.369	805.420 $\pm$ 787.942	6548.312 $\pm$ 3118.764
3 mo.	3368.305 $\pm$ 1808.409	270.715 $\pm$ 136.678	--	1061.665 $\pm$ 358.728
6 mo.	4230.410 $\pm$ 784.746	5264.473 $\pm$ 3152.922	2364.987 $\pm$ 466.102	1036.924 $\pm$ 123.582
1 yr.	--	3531.700 $\pm$ 1100.780	1165.875 $\pm$ 800.683	1252.100
<u>Left Axillary Lymph Node</u>				
2 wks.	251.981 $\pm$ 180.149	957.458 $\pm$ 669.127	2.538 $\pm$ 1.944	6.446 $\pm$ 5.599
1 mo.	--	8.644 $\pm$ 7.062	12.904	16.875 $\pm$ 0.207
2 mo.	422.072 $\pm$ 248.129	333.401 $\pm$ 328.960	248.050 $\pm$ 245.885	31.914 $\pm$ 20.859
3 mo.	574.453 $\pm$ 429.619	2.990 $\pm$ 0.780	7.989 $\pm$ 3.370	5.769 $\pm$ 3.391
6 mo.	215.553 $\pm$ 202.161	190.883 $\pm$ 155.448	3.601 $\pm$ 1.704	0.114 $\pm$ 0.068
1 yr.	93.745 $\pm$ 77.055	--	59.779 $\pm$ 6.049	3.057 $\pm$ 1.663
<u>Left Paw</u>				
2 wks.	--	--	1662.442 $\pm$ 1052.418	1793.902 $\pm$ 334.544
1 mo.	--	--	1266.892	1131.261

Table 21. Continued

Time Following Implant	Pu(NO ₃) ₄		PuO ₂	
	No Treatment (X10 ⁻⁶)	DTPA (X10 ⁻⁶)	No Treatment (X10 ⁻⁶)	DTPA (X10 ⁻⁶)
<u>Left Paw (Continued)</u>				
2 mo.	--	--	21.284±12.073	8.540± 5.752
3 mo.	--	--	36.273± 5.553	44.204±11.091
6 mo.	--	2343.230±1885.940	58.295±38.420	39.486± 9.837
1 yr.	--	--	17.561± 9.232	18.115
<u>R. S. C. L. N.</u>				
2 wks.	21.728±13.971	9.655± 4.783	0.829± 0.261	2.900± 1.814
1 mo.	--	7.324± 2.880	107.242±102.126	--
2 mo.	126.882±93.74	24.528±10.444	4.625± 1.475	8.461± 4.608
3 mo.	--	12.780± 6.772	--	1.265± 0.976
6 mo.	12.003± 3.917	10.908± 4.970	2.808± 1.982	2.856± 1.629
1 yr.	97.750±71.610	--	41.174±36.412	0.634± 0.536
<u>Thyroid</u>				
2 wks.	6.617± 3.925	3.898± 1.337	1.168± 0.182	4.906± 4.241
1 mo.	16.189± 0.967	35.318±35.093	4.711± 4.391	1.146± 0.537
2 mo.	60.006±43.344	18.044± 8.802	8.745± 2.936	2.633± 0.371
3 mo.	10.455± 2.641	15.410± 9.913	8.644± 5.390	2.153± 0.898

Table 21. Continued.

Time Following Implant	$\text{Pu}(\text{NO}_3)_4$		PuO_2	
	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)	No Treatment ($\times 10^{-6}$)	DTPA ($\times 10^{-6}$)
<u>Thyroid (Continued)</u>				
6 mo.	25.750 $\pm$ 8.545	40.280 $\pm$ 20.708	1.290 $\pm$ 0.668	2.529 $\pm$ 2.432
1 yr.	--	--	19.005 $\pm$ 4.088	15.552 $\pm$ 14.647
<u>Bile</u>				
2 wks.	--	--	0.146 $\pm$ 0.040	0.226 $\pm$ 0.111
1 mo.	1.115 $\pm$ 0.548	3.581 $\pm$ 1.343	0.037	0.038 $\pm$ 0.032
2 mo.	2.283 $\pm$ 1.943	0.780 $\pm$ 0.303	0.530 $\pm$ 0.304	0.770 $\pm$ 0.359
3 mo.	0.379 $\pm$ 0.241	0.500 $\pm$ 0.211	0.430 $\pm$ 0.235	--
6 mo.	0.238 $\pm$ 0.061	0.275 $\pm$ 0.105	0.810 $\pm$ 0.413	0.988 $\pm$ 0.532
1 yr.	13.403 $\pm$ 10.968	3.670 $\pm$ 2.648	0.923	0.153
<u>Hepatic Lymph Node</u>				
2 wks.	--	9.720 $\pm$ 5.770	0.675 $\pm$ 0.547	0.625 $\pm$ 0.279
1 mo.	--	--	13.199 $\pm$ 11.058	7.301 $\pm$ 6.333
2 mo.	40.984 $\pm$ 14.977	7.653 $\pm$ 2.211	2.147 $\pm$ 1.278	1.664 $\pm$ 0.995
3 mo.	4.095 $\pm$ 1.765	3.568 $\pm$ 0.719	--	6.421 $\pm$ 2.446
6 mo.	19.478 $\pm$ 8.946	19.145 $\pm$ 3.436	1.479 $\pm$ 1.236	7.146 $\pm$ 5.661
1 yr.	--	--	--	18.249 $\pm$ 6.381

of interest because of the amount of urine filtered through this organ. The kidney contained high activity of Pu and Am with preferential levels of Am over Pu. The lung was radiochemically analyzed to find out if significant levels were deposited in this organ through blood exchange.

There are evidences in the literature which suggest that the left axillary lymph node can become the first major lymph node draining the paw if the implant is deposited slightly off the medial area of the metacarpal region. There is also suggestion that there could be variation among dogs in the distribution of lymphatic channels which would account for larger intake in the axillary lymph node observed in some dogs. The data (Tables 20 and 21) show some dogs with higher concentrations of Pu in the left axillary lymph node than in the L.S.C.L.N. and some dogs with essentially background activity in the axillary node. Based on work done at the University of Utah and by Johnson (1969) high concentration of Am in the thyroid has been reported. Tables 20 and 21 show an increased Am concentration of 4-10 folds in the PuO_2 experiment but no significant difference in the $\text{Pu}(\text{NO}_3)_4$ study. Bile is of interest because this is a pathway for excretion of Pu through the liver and intestinal tract. Data in Tables 19 and 20 indicate no significant differences from that of non-concentrating soft tissues. A section of trachea was taken as a representative cartilage as a comparison to that of soft tissue and bone. The data (Tables 20 and 21) are likewise similar to that of non-concentrating soft tissues.

Additional bone samples consisting of sternum, rib, vertebrae, skull, femur (shaft), and femur (distal) were analyzed and the data shown in Tables 19 and 20. These selections of bone represent various bone types. The data show differences in deposition with different types. The trabecular bone represented by the femur (proximal) showed greater concentrations than the flat bones represented by the femur (shaft).

Because of the continuing decay of ^{239}Pu to ^{241}Am described in the previous section all Am data and the wound count data were corrected for growth of Am from the time of implant until the count time or the time of chemical separation of the Pu and Am.

Because overlying tissue over the L.S.C.L.N. and over the paw produced a significant absorption of the 17 ke V x-rays, as was demonstrated by Ramsden and Speight (1967), tissue phantoms were constructed from masonite and from tissue and absorption curves were constructed. Measurements were performed on several dogs to determine the approximate average tissue overlay and all wound count data was corrected for tissue absorption.

D. Summary

The dynamics of translocation and deposition in various organs of Pu and Am from $\text{Pu}(\text{NO}_3)_4$ and "high fired" PuO_2 implanted in the paw of beagle dogs was studied. In vivo measurements of activity were taken over the paw wound site and the left superficial cervical lymph node (L.S.C.L.N.).

Very rapid movement of activity was observed from the paw to the L.S.C.L.N. Activity was seen in the L.S.C.L.N. within minutes in nearly all dogs with both chemical forms. The data show at 1 year a 70% reduction in Pu from this implant site for the $\text{Pu}(\text{NO}_3)_4$ experiment and a 90% reduction for the high fired PuO_2 . The L.S.C.L.N. for both forms of Pu injected showed buildup dynamics reaching peaks at approximately 30 to 60 days followed by a linear regression in activity. The buildups are best described kinetically up to 1 year by power function curves, and linear functions fitted to the data. The activity in the blood rose rapidly following implant to a peak reached at 2 to 24 hours following implant. The time of maximum accumulation varied according to the chemical form and the treatment group. This peak in activity was followed by a power function reduction through the 1 year studies.

The major pathway for removal of Pu from the body is by urine elimination. This data showed a power function model for urine excretion. The urine did show a higher excretion of Am by about ten-fold over the ratio of Pu to Am in the implant material. A buildup pattern similar to the L.S.C.L.N. was demonstrated for liver, spleen, hepatic lymph node, and proximal portion of the femur.

Nearly all tissues analyzed showed a significantly lower concentration of activity when chelation treatment was used. Only the solid or compact bone samples represented by femur shaft and skull failed to show treatment effects.

There was a significantly greater concentration of Pu in

bone than Am especially observed in the $\text{Pu}(\text{NO}_3)_4$ experiment. The thyroid data did show a greater Am concentration in the PuO_2 experiment consistent with previous reports.

This study raised several important questions which need to be answered through further experimentation. The following are some of the questions needed to be answered: 1) What effect does particle size have on translocation of Pu from the wound site? This study showed great differences in translocation dynamics differences. Because of the ranges in particle sizes and agglomeration characteristics of Pu in solution it was impossible to determine if the very small particles were ionized or transported or if any selective processes existed. A study using mono-dispersed particles would be more helpful in investigating this question. 2) All the data obtained so far on the various chemical forms should be selectively analyzed to see if a particular bone can be used as a model for total skeletal deposition. 3) Many questions remain unanswered regarding transport mechanisms, transport forms (ionic or particulate), and routes from the wound site to the systemic system. From previous experiments it is known that the lymphatic system and lymph node are major routes of translocation. However, certainly some Pu gets directly into the blood stream from the wound site while some may get into the blood through lympho-veous shunts along the lymph channels draining the wound site before reaching the S.C.L.N. In addition, some Pu is probably leaving the lymph node via blood circulation, although most often Pu must leave the lymph node through

the efferent ducts leading to the thoracic duct. Thus considerably more study of this system is indicated.

Our data suggest a very rapid movement of Pu from a wound site, an event which should be considered seriously by medical and health physics people when dealing with such cases. Our data also show an active lymph system having an ability to build up Pu in the lymph node and the capability to clear it gradually into the circulation.

IV. LYMPH NODE CLEARANCE STUDY OF PLUTONIUM FROM SUBCUTANEOUS WOUNDS IN BEAGLES

A. Materials and Methods

1. Experimental Animals

A total of 19 adult male beagles was obtained from the barrier maintained beagle colony of the Collaborative Radiological Health Laboratory at Colorado State University. The beagles were housed outdoors at the Foothills Research Campus of Colorado State University in kennels designed by the Collaborative Radiological Health Laboratory. The beagles were fed ad libitum with dry Purina dog chow* and watered with pans or an automatic watering system. The beagles were observed daily and regular veterinary medical care was provided, including vaccination for rabies, canine distemper, and infectious canine hepatitis.

2. Plutonium Oxide

The high fired plutonium oxide was supplied by the Rocky Flats Division of the Dow Chemical Company in 5 ml pharmaceutical ampules fitted with airtight rubber diaphragms. Each vial contained approximately 1 mg. The high fired oxide was prepared from electrefined unalloyed plutonium metal dissolved in HCl and precipitated with oxalic acid. The washed salt was calcined to the high fired state of plutonium dioxide for 100 hours at 850 degrees Celsius,

*Lab Dog Chow, Ralston Purina Co., St. Louis, Mo.

dried at 80 degrees Celsius for approximately 100 hours, and pulverized by grinding in a tungsten carbide grinding vial. The stoichiometry of the oxide formed was accepted as $\text{PuO}_{1.98}$. The particle size was measured by optical microscopy. The physical, chemical, and isotopic properties of the oxide are listed in Tables 22 and 23.

The amount of plutonium in lymph nodes at necropsy was determined by extraction and liquid scintillation procedures similar to those described by Keough and Powers (1970). The lymph node specimens were reduced to carbon-free ash in new 100 ml beakers by digestion with hot concentrated nitric acid and muffling at 450 degrees Celsius. The ash was soaked with 6 M nitric-6 M hydrofluoric acid and twice evaporated to dryness. Then the residue was evaporated to dryness in 8 M nitric acid and dissolved in hot 2 M nitric acid containing 0.2 M boric acid to make a final solution containing 1 to 15% dissolved salts. A 0.1 ml aliquot of this solution was added to 10 ml of 2 M nitric acid, and a drop of 4 M urea, in 20 ml counting vials. The extractant-scintillator solution (4 ml) was added, the vials shaken vigorously for 30 seconds, and the shaking repeated 5 times at 5 minute intervals. The alpha activity in the vials was then determined by liquid scintillation. The disintegrations per minute were multiplied by 100 to compensate for the dilution made with 2 M nitric acid.

3. Experimental Procedures

The beagles were assigned to experimental groups.

Table 22, Chemical and Isotopic Properties of Plutonium

Pu	0.8826 g/g PuO ₂
Cation impurities	≈200 ppm (based on Pu)
²⁴¹ Am	360 ppm based on Pu as of Oct. 1, 1970
²⁴¹ Am growth rate	≈15 ppm/month based on Pu
²³⁸ Pu	0.0105 wt %, based on Pu
²³⁹ Pu	93.898 wt %, based on Pu
²⁴⁰ Pu	5.695 wt %, based on Pu
²⁴¹ Pu	0.377 wt %, based on Pu as of June, 1970
²⁴² Pu	0.019 wt %, based on Pu
Atomic wt of Pu	239.116 as of June, 1970

Table 23. Physical Properties of Plutonium

<u>Particle Size</u>	
1 μ or less	68%
1 μ to 3 μ	21%
3 μ to 5 μ	4%
5 μ to 10 μ	6%
>10 μ	<1%
Mass Median Diameter Approximates .7 μ	

(Table 24) and were brought indoors for all experimental procedures. The laboratory was prepared for the injection of plutonium by covering the walls and floors with plastic and waxed paper. During the implanting procedures, personnel-protective equipment, including respirators, was used and surveillance was made by radiation monitoring personnel from the health physics group of the Rocky Flats Division of the Dow Chemical Company. The dogs were kept in metabolism cages to collect urine and feces for a few days after implantation; these wastes were delivered to the Rocky Flats installation for disposal.

The plutonium oxide was injected subcutaneously into the dorsal surface of the left hind paw of 15 dogs (Groups I through V), and the right hind paw of one dog from each of Groups II through V. The dogs were given a preanesthetic injection of propiopromazine hydrochloride* and anesthetized with intravenously administered pentobarbital sodium. The injection site was clipped of hair, cleaned with 70% ethyl alcohol, and isolated with paper towels. The insoluble plutonium oxide particles in 5 ml vials were suspended in 1.0 ml of physiological saline solution and 0.25 ml of this suspension was withdrawn into a 1 ml tuberculin glass syringe. The glass syringe was attached to a 21 gauge needle prepositioned subcutaneously. Proper positioning of the needle was determined by aspiration to ascertain that no back flow

*Tranvet, Diamond Laboratories, Des Moines, Iowa,

Table 24. Experimental Design: Utilization of Dogs

Group & Dog No.	Inject PuO ₂	Rear Paw	Amputate Tarsus	DTPA	Necropsy
	Left	Right			
<u>Group I</u>					
2398	yes	no	no	no	4 weeks
2515	yes	no	no	no	"
2546	yes	no	no	no	"
<u>Group II</u>					
2396	yes	no	4th week	no	8 weeks
2509	yes	no	" "	no	"
2405	yes	yes	" "	no	"
<u>Group III</u>					
2426	yes	no	" "	no	16 weeks
2536	yes	no	" "	no	"
2386	yes	yes	" "	no	"
<u>Group IV</u>					
2403	yes	no	" "	no	32 weeks
2535	yes	no	" "	no	"
2527	yes	yes	" "	no	"
<u>Group V</u>					
2406	yes	no	" "	yes	32 weeks
2526	yes	no	" "	yes	"
2397	yes	yes	" "	yes	"
<u>Controls:</u>					
2540	no	no	" "	no	8 weeks
2519	no	no	" "	no	16 weeks
2541	no	no	" "	no	32 weeks
2534	no	no	" "	yes	32 weeks

of blood occurred that would indicate the accidental intravenous injection of the plutonium. The plutonium was then injected into the subcutaneous tissues.

A calibration technique was devised (Bistline, 1972) to determine the amount of plutonium implanted into each dog. A Germanium (Li) detector was used to measure the amount of material contained in each vial prior to implanting 2 dogs from each vial. Following implantation, the activity was measured in the injected paws and what remained in the vial. A ratio of activity in the paws could then be correlated to an accurate measurement of the amount used from each vial.

The injected paws, and corresponding popliteal lymph nodes, were monitored in situ approximately twice a week for radioactivity with a NaI wound counter. The wound counter, a 2-inch diameter by 4 mm thick NaI(Tl) scintillation detector with a thin beryllium window, measured 17 kev L X rays of uranium and neptunium, which follow the decay of plutonium and americium. The wound counter data were calibrated with a source having a similar ratio of uranium and neptunium L X rays as the injected material.

The amount of plutonium in the popliteal lymph nodes during the antemortem phase of the study was calculated from the counts obtained with the wound counter. Since the efficiency of the wound counter varied during the course of the study, the counts per minute were normalized to 1000 counts per minute obtained with a 1 microcurie

source of plutonium. The normalized counts on the day of necropsy were then related to the disintegrations per minute found in the lymph node with liquid scintillation procedures. The conversion factor obtained on the day of necropsy was used retrospectively to estimate the amount of plutonium in the lymph node from the day of implantation. Regression curves were determined with a Hewlett-Packard Calculator* using a polynomial regression program.

Approximately 4 weeks after implantation of the plutonium, the left hind leg was amputated at the tarsus for the dogs in Groups II through V and for the control groups. Each dog was given a preanesthetic injection of propiopromazine hydrochloride and anesthetized with an intravenous injection of pentobarbital sodium. The hair from the left hind leg was clipped and the leg was washed with soap and water. The surgical site was cleaned with 70% ethyl alcohol and isolated with sterile drapes. A skin incision was made around the circumference of the leg about 2 cm below the tarsus and extended proximally to the tarsus on the anterior and posterior surfaces. The skin was reflected proximally and a tourniquet was tightened around the leg to stop circulation. A scalpel was then used to amputate through the tarsus at a location chosen to leave the tibial and fibular tarsal bones intact. The

*Model 9810, Hewlett-Packard, 815 14 S.W., Loveland, Colo.

tourniquet was released and the major arteries ligated with chromatic 00 catgut. The skin was trimmed with scissors to fit the stump and sutured with No. 1 silk. Bandages were applied and changed daily for several days. Each dog received 2 cc of a penicillin and streptomycin mixture daily, injected into muscles of the forelimb, for 5 days. Skin sutures were removed in 10 to 14 days.

One dog in Group II (No. 2398) did not recover from deep anesthesia following surgery and the next day was shifted into Group I and killed. Another dog originally intended for Group I was subsequently transferred into Group II as a replacement. The remaining dogs recovered promptly from the amputation surgery and rapidly became accustomed to walking on 3 legs. After a few weeks there seemed to be very little diminution of mobility.

The trisodium calcium salt of diethylenetriaminepentaacetic acid (DTPA) was administered intravenously to dogs in Group V and to one control dog. Injections of 0.25 gm of DTPA dissolved in 10 ml of physiological saline were made into the jugular vein twice weekly from the time of amputation to necropsy.

4. Necropsy and Histopathology

At the time of sacrifice the dogs were anesthetized with pentobarbital sodium and the following lymph nodes were bilaterally removed and fixed in 10% buffered formalin: popliteal, external iliac, superficial inguinal and deep inguinal (when present). The lymph nodes were later

weighed and monitored for radioactivity with a NaI wound counter. After the lymph nodes were removed, the dogs were killed by exsanguination. The carcasses were frozen for later disposal supervised by the Radiation Health Officer at Colorado State University.

Specimens for histopathologic evaluation were routinely processed, embedded in paraffin, cut at 6 microns, and stained with hematoxylin and eosin. Each dog had 2 sections prepared from different areas of the left and right popliteal lymph nodes, and 1 section prepared from each left and right external iliac lymph node. In addition, Dog 2397 (Group V) had sections prepared from the superficial inguinal lymph nodes. Special stains applied to selected slides were Prussian blue for iron and Masson's trichrome for connective tissues.

Autoradiographs were prepared by dipping paraffin sections of lymph nodes in Ilford K-5* emulsion and exposing the sections for 24 hours before developing with Dektol.** Similar autoradiographs exposed for 1 hour were prepared from contaminated popliteal lymph nodes, randomly sorted, and coded to hide their identity. Six random fields from the 1 hour exposed autoradiographs were selected for examination with a 40X objective on a Zeiss*** microscope

*Ilford Nuclear Research Emulsion, Ilford Limited, Ilford, Essex.

**Eastman Kodak Co., Rochester, N.Y.

***Carl Zeiss, 7082 Oberkochen, West Germany.

(8X eye-pieces and 2X Optovar). In each field the total number of alpha track sources and the total number of tracks per source were tabulated. Since it was usually not possible to distinguish the point source of an alpha star, a source was considered to approximate the size of a cell 8 microns in diameter.

5. Electron Microscopy

Specimens for electron microscopy, selected from the left and right popliteal and external iliac lymph nodes, were immediately immersed in cold 2% gluteraldehyde buffered in Tyrode's solution. After 4 hours fixation the specimens were washed and held in cold Tyrode's buffer overnight, post-fixed in osmium tetroxide, processed in a routine manner, and embedded in epoxy resin (Epon 812^{*}). A total of 10 to 20 random blocks were prepared from each lymph node.

Autoradiographs were prepared from 1 micron thick Epon sections of popliteal lymph nodes that were cut with glass knives on a Porter-Blum^{**} ultramicrotome. These sections were dipped in Ilford K-5 emulsion, held in refrigerated light-tight boxes for 1 to 3 days, developed with Dektol, and examined for autoradiographic localization of plutonium particles.

Thin sections, cut at approximately 600 Angstroms thick with a diamond knife on an LKB^{***} Ultratome, were prepared

^{*}Ladd Research Industries, Inc., Burlington, Vt.

^{**}Sorvall MT-2, Ivan Sorvall, Inc. Norwalk, Conn.

^{***}LKB Instruments, Inc., Rockville, Md.

from blocks shown by autoradiography to contain plutonium particles. The thin sections were mounted on uncoated 150 or 300 mesh copper grids, stained with uranyl acetate and lead citrate, and examined with 50 KV on an RCA EMU-3G* electron microscope located at the Collaborative Radiological Health Laboratory, Colorado State University. Additional specimens were examined with 100 KV on an RCA EMU-4* electron microscope at Battelle Pacific Northwest Laboratories.

Electron microscopic autoradiography was performed on one specimen from the left popliteal lymph node of Dog 2536 from Group III (16 weeks) that had alpha activity with light microscopy. The loop method (Caro, 1962) was used with Ilford L-4 emulsion on collodion coated copper grids. The grids were developed with D-19** after 2 weeks exposure.

Electron diffraction patterns of plutonium particles were obtained from popliteal lymph node specimens examined from Dog 2386 with a Phillips 200*** electron microscope at Rocky Flats, Colorado. Bright field pictures, and selected area diffraction patterns of particles were taken at 100 KV accelerating voltage. An aluminum standard was used to calculate the camera constant used for indexing the diffraction patterns ($d = \frac{L \lambda}{r}$; where d = spacing in crystal, L = camera constant, and r = radius).¹ Electron

*Radio Corporation of America, Camden, New Jersey.

**Eastman Kodak Co., Rochester, New York.

***N. V. Phillips, Eindhoven, Netherlands.

¹ASTM Powder Diffraction File Card No. 6-360 (PuO_2) was used as a diffraction reference.

diffraction patterns from plutonium particles were also searched for on a JOEL* 1 megavolt electron microscope at the University of Colorado in Boulder; no diffraction patterns were obtained with this scope.

B. Results

1. Plutonium Analysis

The percent of the implanted dose recovered in the popliteal lymph node specimens at necropsy ranged from 1% to 12% with a mean of 5% (Table 25). It is noteworthy that the right popliteal lymph node, which drained plutonium injection sites for a longer period of time, contained a higher mean percent of the implant dose than did each of the contralateral left popliteal lymph nodes from amputated sides.

The external iliac lymph nodes showed a significant amount of plutonium that drained from the popliteal lymph node in several dogs (Table 26). The amount of plutonium present, however, was generally small compared to the amount present in the popliteal lymph node.

2. External (in situ) Counting Data

The radioactivity of paws injected with plutonium, as measured by an external wound counter, decreased for approximately 3 to 4 weeks (Figure 12). The radioactivity seemed to stabilize at that time, with the paws retaining

*Japan Electron Optics Laboratory, Akishima, Tokyo, Japan.

Table 25. Radiochemical Analysis: Plutonium in Paw and Popliteal Lymph Nodes

Group & Dog No.	Implant left paw μ Ci	Left popliteal lymph node μ Ci (% implant)	right paw μ Ci	Right popliteal lymph node μ Ci (% implant)
<u>Group I (4 weeks)</u>				
2398	15.6	1.0 (6)		
2515	39.4	1.0 (3)		
2546	14.6	0.8 (5)		
<u>Group II (8 weeks)</u>				
2396	11.9	0.3 (2)		
2509	21.0	1.4 (7)		
2405	22.6	0.4 (2)	9.2	0.5 (5)
<u>Group III (16 weeks)</u>				
2426	27.5	0.8 (3)		
2536	16.9	0.7 (4)		
2386	18.8	0.4 (2)	15.6	0.7 (4)
<u>Group IV (32 weeks)</u>				
2403	19.1	0.3 (2)		
2535	24.3	2.0 (8)		
2527	19.2	0.5 (3)	13.9	1.6 (12)
<u>Group V (32 weeks)</u>				
2406	24.0	0.2 (1)		
2526	10.6	1.1 (10)		
2397	36.0	2.9 (8)	17.7	1.6 (9)

Table 26. Radiochemical Analysis: Plutonium in External Iliac and Superficial Inguinal Lymph Nodes

Group & Dog No.	Left External Iliac Lymph Node dpm/gram	Right External Iliac Lymph Node dpm/gram	Left Superficial Inguinal Lymph Node dpm/gram	Right Superficial Inguinal Lymph Node dpm/gram
<u>Group I</u> (4 weeks)				
2398	758	435		
2515	873	662		
2546	4, 682	N. S.		
<u>Group II</u> (8 weeks)				
2396	343	496		
2509	13, 466	700		
2405*	1, 209	6, 054		
<u>Group III</u> (16 weeks)				
2426	4, 683	1, 261		
2536	566, 642			
2386*	404, 312			
<u>Group IV</u> (32 weeks)				
2403	796	502		
2535	7, 055	608		
2527*	59, 010	52, 420		
<u>Group V</u> (32 weeks)				
2406	44, 177	174		
2526	6, 894	291		
2397*	15, 424	2, 764, 678	1, 845	403, 134

*Plutonium was injected into the left and right paw.
N. S. Not significantly greater than background.

about 70% of their initial implant radioactivity.

Plutonium rapidly reached the popliteal lymph nodes. In several dogs plutonium was detected with the wound counter a few minutes after injection. After about 4 weeks, when the level of radioactivity detectable in the popliteal lymph nodes with the wound counter had stabilized, the left hind paw was amputated. The day of amputation was considered as "day 1" for purposes of determining clearance of plutonium from popliteal lymph nodes, since at this time there could be no continued movement of plutonium to the popliteal lymph node from the amputated paw.

The in situ wound counting data showed no clearance of plutonium from the popliteal lymph nodes from dogs in Group II (Figure 13, Table 27). Dog 2405 (Group II), however, had a greater increase in radioactivity in the right popliteal lymph node, draining the non-amputated right paw injected with plutonium, than occurred in the left popliteal lymph node, draining the amputated left paw injected with plutonium. The data from Group II dogs showed a slight clearance of plutonium from the left popliteal lymph node, as evidenced by a negative linear regression slope in 1 dog (No. 2426), but positive linear regression slopes in the other 2 dogs (Figure 14, Table 27) indicating that there was no clearance of plutonium. There was no significant difference between the left and right popliteal lymph node regression slopes of dog 2386

Table 27. Lymph Node Clearance Curves on Dogs from Groups II through V from 1 Day after Amputation

Group, Dog Number, & Lymph Node*	Y Intercept	Slope
<u>Group II</u>		
2396L	0.031	+0.000047
2509L	0.067	+0.000041
2405L	0.015	+0.0000030
2405R	0.040	+0.00031
<u>Group III</u>		
2426L	0.028	-0.000027
2536L	0.033	+0.000040
2386L	0.016	+0.000042
2386R	0.043	+0.000022
<u>Group IV</u>		
2403L	0.027	-0.0000013
2535L	0.085	-0.000041
2527L	0.026	+0.000024
2527R	0.12	+0.000039
<u>Group V</u>		
2406L	0.006	+0.0000099
2526L	0.107	+0.000032
2397L	0.087	+0.000011
2397R	0.152	-0.00033

* L = Left Popliteal lymph node
R = Right Popliteal lymph node

(Group III) injected with plutonium in both the left and right hind paws.

The left popliteal lymph node external counting data from dogs in Groups IV and V (Figures 15 and 16, Tables 27 and 28) was plotted from day 100 to provide information on plutonium concentration after dogs in Groups II and III were sacrificed. No significant differences were found (using Student's t test) between the linear regression coefficients of the left popliteal lymph nodes from the dogs in Groups IV (no DTPA) and V (DTPA). The slopes were negative in 5 out of 6 dogs, and the slope was essentially flat in the remaining dog (No. 2527).

The right popliteal lymph node external counting data showed positive regression slopes (Figure 17, Table 27) in each dog except dog 2397. The right popliteal lymph node of dog 2397 had markedly reduced activity with the wound counter for several days at approximately 3 months and again 5 months after amputation. At these times perinodal tissues became firm, enlarged, and warm. The decreased counts were clearly due, in part, to increased tissue absorption of the 17 kev x-rays. During these times the dog was febrile and was treated with penicillin. Several syringes of brownish fluid were aspirated from a fluctuant subcutaneous area over the right popliteal lymph node; over 100 counts per 40 seconds were counted in one of these syringes containing brown fluid. After necropsy, plutonium was detected in the superficial inguinal lymph

Table 28, Lymph Node Clearance Curves on Dogs from Groups IV and V from 100 Days after Amputation

Group, Dog Number, & Lymph Node*	Y Intercept	Slope
<u>Group IV</u>		
2403L	0.045	-0.00012
2535L	0.116	-0.00024
2527L	0.028	+0.0000092
<u>Group V</u>		
2406L	0.011	-0.000017
2526L	0.116	-0.000018
2397L	0.134	-0.00029

* L = Left Popliteal lymph node

nodes with the wound counter, further suggesting some spillage into the extranodal tissues of the leg. This was the only animal with detectable amounts of wound counter activity in the superficial inguinal lymph nodes.

3. Weights of Popliteal Lymph Nodes

The weights of the popliteal lymph nodes are tabulated in Table 29. Since the weights of the popliteal lymph nodes were quite variable among dogs, it seems appropriate to compare the right and left popliteal lymph nodes in individual dogs. In each control dog having the left hind paw amputated longer than four weeks before necropsy, the left popliteal lymph node was lighter than the right popliteal lymph node.

The earlier effects of plutonium, observed in dogs from Group I, tended to increase lymph node weights. At later time intervals, popliteal lymph nodes having plutonium generally weighed less than contralateral popliteal lymph nodes not containing plutonium. It must be pointed out also that the weights recorded were actually more scar tissue than functioning lymph node parenchyma.

4. Histopathology of Popliteal Lymph Nodes (Tables 30 and 31)

The right popliteal lymph nodes in the control dogs were not significantly different in morphology from the right popliteal lymph nodes in groups of dogs injected with plutonium in the left paw. The cortex was composed of lymphoid nodules with variable sized germinal centers. The germinal centers generally had a crescent of small

Table 29. Lymph Node Weights at Necropsy

Group & Dog Number	Left Popliteal Lymph Node, g.	Right Popliteal Lymph Node, g.
<u>Group I (4 weeks)</u>		
2398	1.07	0.56
2515	0.55	0.48
2546	0.48	0.43
<u>Group II (8 weeks)</u>		
2396	0.73	0.58
2509	0.55	0.63
2405	1.08	0.89
<u>Group III (16 weeks)</u>		
2426	0.54	0.48
2536	0.28	0.63
2386	0.46	0.42
<u>Group IV (32 weeks)</u>		
2403	1.11	1.28
2535	0.56	0.78
2527	0.43	0.32
<u>Group V (32 weeks)</u>		
2406	0.89	0.88
2526	0.34	0.55
2397	0.65	0.44
<u>Controls:</u>		
2540 (8 weeks)	0.53	0.49
2519 (16 weeks)	0.28	0.48
2541 (32 weeks)	0.41	0.84
2534 (32 weeks)	0.44	1.63

Table 30 Histopathologic examination of left popliteal lymph node.

Table 3

Histopathologic examination of left popliteal lymph node.

Dosage Group & Animal Number	Inflammatory Changes								autoradiographic activity	Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis	cavitations		number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																							
2398	±	2	1	2	1	0	0	0	3	2	2	0	2	2	1	1	1	1	1	±	2	2	1
2515	±	2	2	2	1	±	0	0	3	1	±		1	2	1	1	0	2	1	1	±	±	1
2546	1	2	1	2	1	±	0	0	3	2	1	0	2	2	1	1	±	3	1	1	0	0	1
Group II (8 weeks)																							
2396	1	1	1	1	1	0	0	0	3	1-2	2	0	2	2	1	±	1	2	1	±	±	0	1
2509	3	1	±	1	2	2	2	0	3	1	0	0	1	1	1	±	±	2	1	±	0	0	±
2405	2	2	1	1	1	0	0	0	3	1-2	2	0	2	2	1	±	1	1	1	±	±	±	±
Group III (16 weeks)																							
2426	2-3	1	1	1	1-2	1	2	0	3	2	2	0	2	2	1	1	1	2	1	1	1	1	1
2536	2	1-2	±	1	1	±	1	0	3	1	1	0	2	2	1	1	1	3	1	±	±	±	±
2386	2	2	1	2	1	±	1	0	3	1	2	0	2	1	1	1	1	2	1	1	1	1	1
Group IV (32 weeks)																							
2403	1	1	1	1	1	1	0	0	2	1	1	0	2	2	1	1	±	2	1	1	1	0	1
2535	3	2	1	1	2	±	1	0	3	1	2	0	2	2	1	±	1	2	1	1	±	0	1
2527	3	1	1	2	1	1	2	0	3	1	1	0	2	2	1	1	1	2	1	1	1	0	1
Group V (32 weeks)																							
2406	3	2	1	1	1	±	0	0	3	1	±	0	0	0	0	0	0	0	0	0	0	0	0
2526	3	1	1	2	1	±	2	0	3	1	±	0	2	2	±	1	1	2	±	1	1	0	±
2397	3	2	1	2	2	1	2	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Controls (8 weeks)																							
2540	0	0	0	0	0	0	0	0	0	2	2	1	1-2	1	1	±	1	2	1	0	±	±	0
(16 weeks)																							
2519	0	0	0	0	0	0	0	0	0	1	1	2	1	±	1	0	2	2	1	0	0	0	0
(32 weeks)																							
2541	0	0	0	0	0	0	0	0	0	2	1	±	1	1	1	±	±	2	1	±	±	±	0
(32 weeks)																							
2534	0	0	0	0	0	0	0	0	0	2	2	±	2	2	1	±	±	1	1	±	±	±	±

Key =
 0=none
 0+=trace
 ±=very slight (or very small amount)
 ±-1=very slight-to-slight (or very small-to-small amount)

1=slight (or small amount)
 1-2= slight-to-moderate (or small-to-moderate amount)
 2=moderate
 2-3=moderate-to-marked
 3-4=marked-to-extreme
 4=extreme

Table 3 Histopathologic examination of right popliteal lymph node.

Dosage Group & Animal Number	Inflammatory Changes							autoradiographic activity	Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis		number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																						
2398	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	2	1	±	1	2	1	
2515	0	0	0	0	0	0	0	0	2	2	1	2	2	1	0	1	1	±	0	0	±	
2546	0	0	0	0	0	0	0	0	2	2	0	2	2	1	±	2	1	1	1	±	1	
Group II (8 weeks)																						
2396	0	0	0	0	0	0	0	0 ⁺	2	2	0	1	1	1	±	0	2	1	±	±	0	0
2509	0	0	0	0	0	0	0	0	2	2	±	1	1	1	1	0	3	1	±	±	0	0
2405	2	3	1	1	1	0	0	3	1	1	0	2	2	1	±	±	2	1	±	±	1	1
Group III (16 weeks)																						
2426	0	0	0	0	0	0	0	0	2	1-2	0	1-2	2	1	1	1	2	1	1	±	1	1
2536	0	0	0	0	0	0	0	0 ⁺	2-3	2	±	2	2	1	±	0	2	1	±	0	±	±
2386	4	0	1	2	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
Group IV (32 weeks)																						
2403	0	0	0	0	0	0	0	0	2	2	0	2	2	1	1	0	2	1	±	±	0	0
2535	0	0	0	0	0	0	0	0	2	2	±	2	2	1	±	1	2	1	±	±	±	1
2527	2	2	1	1	1	±		2-3	1	2	0	1	1	1	1	±	2	1	1	1	1	1
Group V (32 weeks)																						
2406	0	0	0	0	0	0	0	0	2	2	0	2	2	1	±	±	1	1	±	±	±	±
2526	0	0	0	0	0	0	0	0	2	2	0	2	2	1	±	0	2	1	±	0	0	0
2397	3-4	1	1	2	2	±	2	3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	
Controls (8 weeks)																						
2540	0	0	0	0	0	0	0	0	2	2	1	1-2	1	1	±	1	2	1	0	±	±	0
(16 weeks)																						
2519	0	0	0	0	0	0	0	0	2	2	0	2	1	1	1	±	2-3	1	1	±	±	0
(32 weeks)																						
2541	0	0	0	0	0	0	0	0	2	1	±	2	2	1	1	±	2	1	1	±	±	1
(32 weeks)																						
2534	0	0	0	0	0	0	0	0	3	2-3	0	2-3	2	1	±	1	1	1	±	1	±	1

Key =

0 = none

0⁺ = trace

± = very slight (or very small amount)

±1 = very slight-to-slight (or very small-to-small amount)

1 = slight (or small amount)

1-2 = slight-to-moderate (or small-to-moderate amount)

2 = moderate

2-3 = moderate-to-marked

3-4 = marked-to-extreme

4 = extreme

lymphocytes on the capsular side. Scattered foci of phagocytosed nuclear debris were present in germinal centers in direct proportion to the size of the germinal centers. Foci of adipose tissue were occasionally seen between the nodules in various areas of the cortex. The paracortical areas consisted of a rather uniform population of lymphocytes that blended into the medullary cords which varied in thickness and were composed of plasma cells, lymphocytes, and neutrophils on a supporting framework of reticular cells. The medullary cords had scattered fragments of nuclear debris, and Russell bodies that were present in direct proportion to the number of plasma cells. The medullary sinuses had numerous diffusely distributed macrophages and lymphocytes. Areas of hemorrhage, erythrophagocytosis, and neutrophilic infiltration were common in the medullary sinuses. Eosinophils, mast cells, and megakaryocytes were occasionally present. Small amounts of hemosiderin were common in both the medullary cords and medullary sinuses. The cellular content of the subcapsular sinuses generally reflected the content of the medullary sinuses.

The effects of amputation on popliteal lymph node morphology were observed in the control dogs. Aside from dog 2540, in which the left paw was amputated 4 weeks before necropsy, the general trend in morphology of the left popliteal lymph node after amputation was atrophy. The left popliteal lymph node from dog 2519, removed approximately

3 months after amputation, had fewer cortical nodules, smaller germinal centers, thinner medullary cords, and fewer cells in the medullary sinuses than the contralateral right popliteal lymph node. Seven months after amputation, the left popliteal lymph node from dog 2541 had thinner cords with reduced numbers of plasma cells, and the left popliteal lymph node from dog 2534 had fewer cortical nodules, smaller germinal centers, and thinner medullary cords.

The effects of plutonium were present in the left popliteal lymph nodes from dogs in Group I (4 weeks). Moderate sized, irregularly shaped areas of cortex were placed by increased numbers of reticular cells mixed with macrophages, fibrocytic proliferation, a few collagen fibers, and diffuse infiltration with neutrophils. The mixture of inflammatory cells extended in areas through the capsule, generally obscured the subcapsular sinus, and blended with remnants of lymphoid tissue in the cortex and medulla. The areas replaced by inflammation had moderate numbers of pyknotic and karyorrhexic nuclei diffusely distributed among the reticular cells and adjacent lymphoid tissues, small amounts of hemosiderin phagocytized by macrophages, and very small amounts of hemorrhage.

Inflammatory changes occupied relatively large areas of the left popliteal lymph nodes of dogs in Group I (4 weeks), but the normal morphology was intact in most areas of these

lymph nodes. Although some cortical nodules were largely replaced with reticular cells, other normal appearing nodules had germinal centers. The medullary sinuses had marked numbers of macrophages in one dog, which was probably a reflection of the inflammatory reaction in other areas of the node. A moderate amount of hemorrhage in the medullary sinuses of dog 2398 was probably from the amputation surgery the previous day; this dog was not originally intended for Group I, but was necropsied since it never recovered from anesthesia.

Small amounts of scar tissue replaced areas of cortex in the left popliteal lymph nodes from dog 2396 (Group II) and dog 2403 (Group IV) injected with plutonium into the left paw. The scar tissue consisted primarily of fibroblasts and collagen mixed with small numbers of reticular cells, macrophages, neutrophils, necrotic debris, and, in dog 2403, hemorrhage. Although the cellular infiltrate extended into the medulla and the capsule, the general morphology of these lymph nodes remained intact with large areas of essentially normal cortex and medulla.

The right popliteal lymph node from dog 2405 (Group II) had large areas of cortex replaced by reticular cells that were diffusely mixed with fibroplasia, macrophages, neutrophils, scattered necrotic debris, and hemosiderin. The cellular infiltration extended into the capsule. The cortex still had small numbers of remaining lymphoid germinal centers. The medulla was relatively normal.

Moderate to extreme amounts of scar tissue replaced large areas of the popliteal lymph nodes draining plutonium injection sites in the remaining dogs from Groups II (8 weeks) through V (32 weeks). The scar tissue in these lymph nodes was generally composed of dense fibrous tissue encapsulating areas up to 0.5 cm in diameter composed of strands of collagen in an eosinophilic matrix that became progressively more acellular toward the center. The eosinophilic matrix contained a few fibroblasts and collagen fibers, a few widely scattered neutrophils and macrophages, a small amount of diffusely distributed pyknotic and karyorrhexic debris, and hemosiderin. The hemosiderin was generally phagocytosed by macrophages but toward the center of the scar tissue often appeared extracellular. In several lymph nodes from dogs in Groups II through V, moderate sized foci of necrotic debris were mixed with the golden brown pigment. Foci of hemorrhage were frequently present. Medium sized muscular arteries in the center of the scar tissue frequently had smudgy, necrotic walls. Small areas of cavitation were present in the left popliteal lymph node of dog 2397; these cavities appeared largely void of contents and were lined with scar tissue.

Adjacent to the scar tissue, most of the heavily involved popliteal lymph nodes had increased numbers of reticular cells and macrophages replacing further areas of the cortex and medulla. Occasional mitotic figures

Table 32. Alpha Tracks in 6 Random Microscopic Fields of Left Popliteal Lymph Node Exposed to Autoradiographic Emulsion for 1 Hour

Group	Dog	Tracks/Source ($\bar{X} \pm \text{S. E.}$)
I (4 weeks)	2398	2.7 ± 0.4
	2515	1.9 ± 0.9
	<u>2546</u>	<u>5.9 ± 2.3</u>
	mean	3.5 ± 1.3
II (8 weeks)	2396	1.0 ± 0.0
	2509	6.8 ± 1.2
	<u>2405</u>	<u>2.5 ± 0.4</u>
	mean	3.4 ± 1.0
III (16 weeks)	2426	5.0 ± 0.7
	2536	4.8 ± 0.9
	<u>2386</u>	<u>3.9 ± 0.5</u>
	mean	4.5 ± 0.6
IV (32 weeks)	2403	1.9 ± 0.4
	2535	5.5 ± 0.6
	<u>2527</u>	<u>9.4 ± 2.3</u>
	mean	5.5 ± 0.8
V (32 weeks)	2406	6.3 ± 0.8
	2526	10.8 ± 2.1
	<u>2397</u>	<u>11.9 ± 3.2</u>
	mean	9.7 ± 1.1

were present among the reticular cells. Small foci of plasma cells and lymphocytes were frequently in the capsule or surrounding perinodal tissue. The popliteal lymph nodes from several dogs in Groups II through V had no recognizable cortex and/or medulla.

The autoradiographs showed the distribution of plutonium particles was related to lymph flow through the popliteal lymph node. In the dogs from Group I the plutonium particles were found primarily in the subcapsular areas and the areas between the cortical nodules corresponding to Zone 1 of Nopajaroonsri (1971). Although the morphology of the lymph nodes was severely altered, in the groups of dogs exposed to plutonium for longer periods of time, the plutonium particles were more concentrated in the medullary areas nearer the efferent lymphatics. The lymph flow was probably severely altered, however, by the formation of scar tissue.

Autoradiographs of the left popliteal lymph nodes exposed for 24 hours had marked numbers of large alpha stars and alpha clusters aggregated in areas of scar tissue and necrosis. The alpha activity was more pronounced over regions where hemosiderin was deposited, but alpha tracks occasionally originated from macrophages lacking any discernible pigment. There was scattered alpha activity in areas with increased numbers of reticular cells. The right popliteal lymph nodes from dogs injected with plutonium into the right paw had similar amounts of alpha activity, although

the right popliteal lymph node of dog 2397 had overlapping large alpha stars denser than any other section. Traces of alpha activity, composed of single tracks, were present in several popliteal lymph nodes not draining injection sites; alpha stars, however, were only present in lymph nodes draining injections sites.

In autoradiographs exposed for 1 hour, alpha tracks counted from different random microscopic fields (Table 32) showed that the average numbers of tracks per plutonium source increased as a function of time. There were other very concentrated areas of alpha activity in the popliteal lymph nodes of dogs from Groups IV and V that further indicated an aggregation of alpha tracks.

5. Electron Microscopy of Popliteal Lymph Nodes

The electron microscopic identification of plutonium particles in popliteal lymph nodes was accomplished in 3 ways. First, electron dense structures having the described appearance of plutonium (Adee and Sanders, 1968) were identified in specimens of popliteal lymph nodes that had alpha activity demonstrated autoradiographically with light microscopy. Since these structures were not seen in control popliteal lymph nodes from the uninjected sides, it is reasonable to identify these structures as plutonium. The two more direct ways of identification were with electron diffraction and by electron microscopic autoradiograph. Particles identified by each of the 3 different ways had a similar appearance and were associated

Table 33. Electron Diffraction Crystallographic Planes for Plutonium Oxide

ASTM Reported		Observed Values*		
d	hkl	d ¹	d ²	d ³
3.097	111	3.17	3.02	3.08
2.695	200	2.64	2.68	2.59
1.908	220	1.92	1.92	
1.627	311	1.62	1.68	1.58
1.558	222		1.58	1.50
1.349	400	1.36	1.35	1.35
1.238	331	1.24	1.25	1.22
1.207	420			
1.1015	422	1.10	1.10	1.12
1.0379	333	1.04	1.04	1.04
.95359	440		.966	.91
.91185	531			

*observed d-spacings calculated from $L\lambda$ (3.17) which has measured from diffraction patterns of pure aluminum standard.

d¹ as received (standard)

d² radioactive particles in stained popliteal lymph tissue (Fig. 22)

d³ radioactive particles in unstained popliteal lymph tissue (Fig. 21)

with similar types of cellular structures.

The plutonium particles were aggregated in round to oval clear spaces within phagolysosomes of macrophages and degenerate cells that could not be clearly identified. The plutonium particles were polygonal, had smooth or slightly serrated surfaces, and, although they were generally electron opaque, occasionally less dense appearing peripheral areas were present. They were distinct and never intimately surrounded with adhering membrane structures or other electron dense material. The plutonium particles frequently appeared partially dislodged from the clear space containing them, possibly due to dislodgement during cutting. The clear spaces containing plutonium particles were not clearly lined with a membrane and the edges may have been spread apart by the electron beam. Myelin whorls were common in the cytoplasm of cells showing severe damage due to plutonium.

The surrounding scar tissue contained fibroblasts, and collagen fibers with the usual periodicity. A portion of a macrophage containing electron dense material resembling hemosiderin is shown. Phagocytosed hemosiderin was a common histologic feature. The capillaries were unremarkable.

6. External Iliac (Tables 34 and 35) and Superficial Inguinal Lymph Nodes

Compared to the popliteal lymph nodes, the external iliac lymph nodes from control dogs tended to have fewer

lymphocytes in the cortical nodules and smaller germinal centers. A moderate amount of adipose tissue was present in dog 2515. The external iliac lymph nodes did not show any appreciable effects of amputation. A moderate arteritis involved one muscular artery in the right external iliac lymph node of dog 2509; this was probably an incidental finding and unrelated to plutonium deposition.

There were no changes attributable to plutonium in the external iliac lymph nodes from dogs in Groups I or II. External iliac lymph nodes from 2 dogs (Numbers 2536 and 2386) in Group III, dog 2527 in Group IV, and dog 2397 in Group V had areas of cortex replaced with foci of reticular cells and macrophages mixed with scattered necrotic debris. These foci were associated with fibrosis, neutrophilic infiltration, and hemosiderin deposition. The general morphology of the lymph nodes was intact and the uninvolved areas were essentially normal.

Autoradiographs exposed for 24 hours had single tracks in several left and right external iliac lymph nodes from Groups I and II. Alpha activity, generally composed of randomly distributed small stars in medullary cords, was present in several dogs from Groups II through V. The plutonium particles present in the external iliac lymph nodes were clearly smaller and less numerous than present in the popliteal lymph nodes.

The right superficial inguinal lymph node from dog

Table 34 Histopathologic examination of left external iliac lymph node.

Dosage Group & Animal Number	Inflammatory Changes							autoradiographic activity	Cortex			Medullary Cords					Medullary Sinusoids				
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis		number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis
Group I (4 weeks)																					
2398	0	0	0	0	0	0	0	0+	1-2	1	0	2	1	2	±	1	1	1	3	0	0
2515	0	0	0	0	0	0	0	0	2	2	2	2	1	1	1	2	1	1	0	0	1
2548	0	0	0	0	0	0	0	0	1	0	0	2	1	1	±	0	2	1	1	0	0
Group II (8 weeks)																					
2396	0	0	0	0	0	0	0	0+	1-2	1	0	2	2	1	0	±	1	1	±	±	±
2509	0	0	0	0	0	0	0	1	2	2	±	1-2	1	1	1	±	1	1	±	1	0
2405	0	0	0	0	0	0	0	0+	1-2	1	0	2	2	±	1	1	2	1	±	±	±
Group III (16 weeks)																					
2426	0	0	0	0	0	0	0	±	1	1	1	2	2	1	1	1	2	1	±	1	0
2536	0	1	0	±	1	0	0	2	2	2	0	1	1	1	1	1	2	1	1	1	±
2386	1	±	±	±	±	0	0	2	2	2	0	2	2	1	1	±	1	1	±	±	1
Group IV (32 weeks)																					
2403	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	±	2	1	1	±	±
2535	0	0	0	0	0	0	0	±	2	1	0	2	2	1	±	1	1	1	±	1	0
2527	0	0	0	0	0	0	0	1	2	1	±	2	1	±	1	±	2	1	1	1	0
Group V (32 weeks)																					
2406	0	0	0	0	0	0	0	1	1	±	0	2	1	1	1	±	2	1	±	±	±
2526	0	0	0	0	0	0	0	±	2	1-2	0	2	2	±	±	±	2	1	±	1	±
2397	±	1	1	1	±	0	0	±-1	1-2	2	0	2	2	1	1	1	2	1	±	±	±
Controls (8 weeks)																					
2540	0	0	0	0	0	0	0	0	2	±	0	1	1	1	±	1	2	1	±	±	±
(16 weeks)																					
2519	0	0	0	0	0	0	0	0	2	1-2	1	2	2	2	1	1	2	2	±	0	±
(32 weeks)																					
2541	0	0	0	0	0	0	0	0	2	±	1	2	2	1	1	±	2	1	±	0	1
(32 weeks)																					
2534	0	0	0	0	0	0	0	0	2	2	±	1-2	2	1	±	1	1-2	1	±	0	0

Key =

0 = none
 0+ = trace
 ± = very slight (or very small amount)
 ±-1 = very slight-to-slight (or very small-to-small amount)
 1 = slight (or small amount)

1-2 = slight-to-moderate (or small-to-moderate amount)
 2 = moderate
 2-3 = moderate-to-marked
 3-4 = marked-to-extreme
 4 = extreme

Table 35 Histopathologic examination of right external iliac lymph node.

Dosage Group & Animal Number	Inflammatory Changes							autoradiographic activity	Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis		number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																						
2398	0	0	0	0	0	0	0	0 ⁺	2	1	0	2	1	2	±	1	2	1	1	0	0	0
2515	0	0	0	0	0	0	0	0 ⁺	2	1	1	2	1	1	1	1	2	1	1	±	0	1
2546	0	0	0	0	0	0	0	0	2	2	0	1	1	1	±	±	1	1	±	1	0	±
Group II (8 weeks)																						
2396	0	0	0	0	0	0	0	0	2	1	±	1	1	1	±	±	1	1	±	1	0	0
2509	0	0	0	0	0	0	2	0	2	1	0	1	1	1	1	0	1-2	1	±	0	0	0
2405	0	0	0	0	0	0	0	±	1	±	0	1-2	1	1	1	±	2-3	1	±	1	0	±
Group III (16 weeks)																						
2426	0	0	0	0	0	0	0	0	1	±	0	1	1	1	1	±	2	2	±	2	0	0
2536																						
2386																						
Group IV (32 weeks)																						
2403	0	0	0	0	0	0	0	0	1	±	0	1	1	1	1	0	2	1	1	±	±	0
2535	0	0	0	0	0	0	0	0	2	1-2	0	2	2	1	±	±	1	1	±	1	0	0
2527	0	1	±	1	0	0	0	1	1	±	0	1	0	1	0	0	3	1	0	±	0	0
Group V (32 weeks)																						
2406	0	0	0	0	0	0	0	0	1	±	1	2	1	1	1	±	2	1	±	±	0	0
2526	0	0	0	0	0	0	0	0	2	2	0	1-2	2	1	±	±	2	1	±	±	±	0
2397	0	0	0	0	0	0	0	±	2	2	0	2	2	1	1	±	2	1	±	±	±	±
Controls (8 weeks)																						
2540	0	0	0	0	0	0	0	0	2	2	0	1	1	1	±	0	2-3	1	±	0	0	0
(16 weeks)																						
2519	0	0	0	0	0	0	0	0	2	±	0	1-2	1	1	±	0	2	2	±	0	0	0
(32 weeks)																						
2541	0	0	0	0	0	0	0	0	2	1	1	2	2	1	1	±	2	1	±	0	±	±
(32 weeks)																						
2534	0	0	0	0	0	0	0	0	1-2	2	0	2	2	1	±	0	2-3	1	±	0	0	0

Key =

0 = none

0⁺ = trace

± = very slight (or very small amount)

±-1 = very slight-to-slight (or very small-to-small amount)

1 = slight (or small amount)

1-2 = slight-to-moderate (or small-to-moderate amount)

2 = moderate

2-3 = moderate-to-marked

3-4 = marked-to-extreme

4 = extreme

2397 in Group V (32 weeks) had moderate sized areas of cortex replaced by increased numbers of reticular cells and macrophages that were associated with fibrosis, neutrophilic infiltration, scattered necrotic debris, and hemosiderin deposition. The 24 hour autoradiograph had a small to moderate amount of alpha activity composed of stars in the area of cortex replacement.

C. Summary

The lymph node clearance of plutonium oxide subcutaneously implanted into the hind paws of beagles was studied.

External in situ scintillation data were collected from the popliteal lymph nodes of each dog. The left hind paw was amputated 4 weeks after implantation to prevent continued deposition of plutonium oxide particles in the left popliteal lymph node. Groups of beagles were sacrificed 4, 8, 16 and 32 weeks after implantation for histopathologic, electron microscopic, and radiochemical analysis of regional lymph nodes.

Plutonium rapidly accumulated in the popliteal lymph nodes. Histopathologic changes in the popliteal lymph nodes with plutonium particles were characterized primarily by reticular cell hyperplasia, increased numbers of macrophages, necrosis, and fibroplasia. Eventually the plutonium particles became sequestered by scar tissue that often replaced the entire architecture of the lymph node. Light microscopic autoradiographs of the popliteal lymph nodes showed a time

Figure 12. Radioactivity in right hind paws
injected with plutonium as measured
by an external wound counter.

Figure 13. Radioactivity in the left popliteal lymph nodes of dogs from Group II as measured by an external wound counter.

Figure 14. Radioactivity in the left popliteal lymph nodes of dogs from Group III as measured by an external wound counter.

Figure 15. Radioactivity in the left popliteal lymph nodes of dogs from Group IV as measured by an external wound counter.

Figure 16. Radioactivity in the left popliteal lymph nodes of dogs from Group V as measured by an external wound counter.

Figure 17. Radioactivity in the right popliteal lymph nodes of dogs from Groups II, III, IV, and V as measured by an external wound counter.

related increase in number of alpha tracks per plutonium source. Electron microscopy showed that plutonium particles were aggregated in phagolysosomes of macrophages.

There was slight clearance of plutonium from the popliteal lymph nodes of dogs monitored for 32 weeks. The clearance of plutonium particles from the popliteal lymph nodes was associated with necrosis of macrophages. The external iliac lymph nodes contained fewer plutonium particles and had less severe histopathologic changes than the popliteal lymph nodes. The superficial inguinal lymph nodes of one dog contained appreciable amounts of plutonium. Treatment with diethylenetriaminepentaacetic acid (DTPA) did not influence the clearance of plutonium from the popliteal lymph nodes.

V. LYMPH TRANSPORT OF PuO_2 IN DOGS

It has been shown that the superficial cervical lymph nodes (S.C.L.N.) are the first major nodes draining plutonium from implants made in the subcutaneous fascia over the dorsal metacarpus in beagle dogs.¹ Since lymphosarcoma associated with high nodal concentration of plutonium has been reported in dogs,² the possibility of Pu-induced lymphosarcoma in humans must be considered. Data from pilot studies in this laboratory³ suggest that surgical excision of a contaminated lymph node may be a useful therapeutic procedure in the management of accidental plutonium contamination in humans.

The lymphadenectomies in the pilot experiments were performed on the same day that the Pu implant was made. The results obtained in the pilot experiments concerning translocation of Pu beyond the left superficial cervical lymph node (L.S.C.L.N.), combined with data concerning the long term accumulation of Pu in the L.S.C.L.N., suggested the design of the present experiment.

A. Materials and Methods

1. Dog Assignments

Twenty-six adult beagles and seven mongrel dogs were used in this study. The beagles were obtained from the barrier-maintained colony of the Collaborative Radiological Health Laboratory and the mongrel dogs were obtained from

the Surgical-Metabolic Laboratory on the Foothills Campus at Colorado State University. The implant procedure was as previously described. The high fired $^{239}\text{PuO}_2$ was prepared and provided by Rocky Flats.

2. Implant Procedures

The geometric mean count diameter was reported to be 0.7 microns. The plutonium was packaged in rubber diaphragm stoppered vials which contained an average of 1 mg of PuO_2 . The PuO_2 was diluted with 1 cc of sterile, deionized water before implant.

The dogs were given a pre-anesthetic (25 mg propiopromazine hydrochloride) intramuscularly 1 hour before the anesthetic (pentobarbital sodium) was injected intravenously to effect. No side effects to the drugs were seen.

3. Lymphadenectomy Procedures

a) Early Lymphadenectomy (Groups IA and IB)

Lymphadenectomy was performed on two groups of dogs at 1, 2, 4, and 8 hours after Pu implant. The time schedule for lymphadenectomy was based on the data of Johnson et al. (1970) and Bistline et al. (1972 a), which showed that a high percentage of Pu accumulated in the L.S.C.L.N. in the first 24 hours after implant. Group IA consisted of eight male beagles and Group IB consisted of eight female beagles. In each group two contaminated dogs were left intact and two uncontaminated controls underwent lymphadenectomy.

The superficial cervical lymph nodes are described as commonly consisting of three or more nodes, but in this

study only a single, large node was excised. The other nodes are very small and very difficult to identify. Plutonium build-up with time over the L.S.C.L.N. region was determined by in vivo counting of the 17 ke V x-ray complex with a thin NaI(Tl) detector.

The dogs in Group IA were killed 13 days after implant and the dogs in Group IB were killed 11 days after implant. Samples of liver, spleen, hepatic lymph node, axillary lymph node and right and left superficial cervical lymph nodes were taken at necropsy. Activity was determined by placing the NaI detector on the surface of the tissue and counting the 17 ke V x-rays. A more accurate determination of the ^{239}Pu activity in the tissues was made by chemical extraction and liquid scintillation α -counting using the method of Keough and Powers (1970). The total tissue weight was used for all tissues except for liver samples. About 10 g of liver tissue were analyzed.

b) Delayed Lymphadenectomy (Group II)

The results obtained in the early lymphadenectomy studies concerning translocation of Pu beyond the L.S.C.L.N. combined with data concerning the long term Pu accumulation in the L.S.C.L.N. (Bistline et al. 1972 a), suggested the design of the delayed lymphadenectomy study.

Ten male beagles ranging in age from 3 1/2 to 5 years were used in this study. Each of the ten dogs was injected with 0.25 cc of a high-fired PuO_2 suspension. Implant procedures used were the same as those described for the early

lymphadenectomy studies.

The activity in the left paw and in the L.S.C.L.N. was determined by counting the 17 ke V x-ray complex with a thin NaI detector. The dogs were monitored for 14 days after implant. During this time in vivo accumulation of Pu in the L.S.C.L.N. was recorded with the NaI detector. After equilibrium was reached on the 14th day after implant the L.S.C.L.N.s were surgically excised from 5 of the 10 dogs and the activity in the region of the excised node was determined. Daily counts over the implant site in the left paw and in the L.S.C.L.N. region were made for four successive days after lymphadenectomy. Thereafter, the dogs were counted twice a week. Dogs in Group II were killed 35 days after surgery and were necropsied. The tissues sampled at necropsy were the same as those sampled in the early experiments. Tissue samples were analyzed for ^{239}Pu according to the method of Keough and Powers (1970), to determine whether or not the Pu burdens in the tissues were higher in the lymphadenectomized dogs than in the intact dogs.

4. Study of Rapid Lymph Transport of Pu

Two methods were investigated for the collection of lymph from a dog's limb. In both methods the afferent lymphatic vessels to the popliteal lymph node were isolated in the femoral region of a dog's hind limb along the dorsal and plantar branches of the lateral saphenous vein. One vessel was chosen for lymph collection and the others were

ligated, so that the unrestricted vessel might carry a greater volume of lymph.

One method consisted of placing a plastic trough under the isolated lymphatic vessel and allowing the lymph to drain into a collecting vial. This procedure was used for one dog. The more suitable method (used in the second dog) consisted of cannulating a lymphatic vessel with medical grade polyvinyl tubing using the method of Nelson and Swan. For the implant and collecting procedure the dogs were anesthetized. The lymph vessels were surgically exposed, isolated and cannulated in one dog. In both dogs, lymph flow was stimulated by gently massaging the dog's paw and by intravenously infusing a saline solution. When adequate lymph flow was obtained, a 22 gauge hyperdermic needle was positioned subcutaneously in the dorsal metatarsus for PuO_2 implant. A 0.1 cc implant of the PuO_2 suspension was made and lymph was collected for 2 hours. Collection intervals for the cannulated lymph vessel are listed in Table 36.

Thirty minutes after implant 4 microscope slides of lymph were prepared for autoradiography by placing 3 drops of lymph on each slide. The slides were stained by Wright's stain before being autoradiographed with Kodak AR-10 stripping film. The slides were developed after 3, 5, 7, and 14 days exposure.

Lymph samples were centrifuged to separate cells from the cell-free fraction. The packed cells were formalin-

fixed, paraffin-embedded, and hematoxylin-and eosin-stained before being autoradiographed. Cellular and acellular fractions were prepared for liquid scintillation α -counting using the method of Keough and Powers (1970).

Acellular fractions were prepared for disc electrophoresis.

5. Discontinuous (Disc) Electrophoresis

Disc electrophoresis is a special form of polyacrylamide gel electrophoresis, in which the sample passes through two gels of different pore size and pH. In the first gel, ions migrate into a region of discontinuous pH's and become tightly concentrated into a stack of contiguous bands. As the migration advances across the boundary into the smaller-pore separating gel, different pH conditions are encountered. Salts pass through the protein layers, and separation in a constant current gradient then proceeds under the combined influence of electrophoretic mobility and molecular sieving by the gel. The double action of first concentrating and stacking, then separating is what gives disc electrophoresis increased sharpness of resolution over single element polyacrylamide or starch systems.

The gel system used covers proteins ranging from 10,000-1,000,000 molecular weight, with maximum resolution from 30,000-300,000 molecular weight.

Preliminary tests showed that 150 λ of lymph could be easily separated by the gel and contained sufficient ^{239}Pu to be counted in a liquid scintillation counter. This volume was used for the final analyses.

TABLE 36

Lymph Sample Collection Intervals

Tube Number	Collection Interval
1	0-15 min
2	15-30 min
3	30-60 min
4	60-120 min

After the gels were prepared and placed in the electrophoresis apparatus, 200 λ (150 λ lymph + 50 λ of 50% glucose) were layered on the surface of the stacking gel. A current of 0.5 ma per column was applied to the gel until the proteins reached the separating gel. The current was then increased to 1.5 ma per column. Protein separation was completed in 6 hours.

The gel columns were then removed from the glass tubes, were stained with Coomassie Blue dye, and sectioned to isolate the following protein groups: a) Albumin and α -globulins, b) β -globulins, including transferrin, c) γ -globulins. These groups were identified by comparison with published electrophoretic patterns of serum proteins. The gel sections were prepared for liquid scintillation α -counting using the method of Keough and Powers.

6. Autoradiography of Lymph Node Sections

Autoradiographs were obtained from lymph node sections of dogs in Groups I and II using Kodak AR-10 stripping film. The lymph nodes had been excised at the following times after Pu implant: 1 hour, 8 hours, 2 weeks, and 1 year. The film was exposed to the α -radiation for 72 hours. The autoradiographic slides were developed, hematoxlin-and eosin-stained, and viewed through a light microscope at a magnification of 160 X. Photomicrographs were made of the sections, and the size of the α -stars seen in each preparation was compared with those seen in the other lymph node preparations. In addition, the size of α -stars seen in the packed cells of

lymph, which had been prepared for autoradiography in the same way as the lymph node sections and were developed after 72 hours exposure.

7. Contrast Studies of Lymphatic Vessels

The anatomy of the lymphatic system in the dog's forelimbs and hindlimbs was studied because observations made in previous experiments at this laboratory have shown that individual dogs may have elevated Pu burdens in the axillary lymph node while other dogs contain a very small burden in this node. No explanation of this difference in Pu translocation was made in the past.

Subcutaneous injections of India ink were made in the dog's forepaws and hindpaws in the dorsal metacarpal and metatarsal areas. The dogs were killed with an overdose of pentobarbital sodium about 10 min. after the ink was injected. An incision was made through the skin of the dog's leg, and lymphatic vessels were easily followed, since they were filled with ink, colored latex was then injected directly into the lymphatic vessels. The skin was reflected and gross observations were made while tracing the latex-filled vessels to a lymph node.

To ascertain the number of lymph nodes in the L.S.C.L.N. chain of a beagle, 10 cc of a 5% solution of Niagara Sky Blue 6B (J. T. Baker Chemical Company, Phillipsburg, New Jersey), a relatively non-toxic vital dye, was injected intravenously. The 10 kg dog was killed 14 days after injection. During that time the dye was absorbed and then largely removed from the

reticulo-endothelial (RE) system. At necropsy selected lymph nodes were excised and weighed.

B. Results and Discussion

The present experimental work was conducted to identify the mechanism by which high-fired $^{239}\text{PuO}_2$ is rapidly translocated from a subcutaneous wound via the lymphatic system, and to determine the effect of regional lymph node excision on subsequent Pu translocation. The first area of research was chosen because investigators at Colorado State University have observed, but have not explained, rapid Pu translocation from subcutaneous wounds to regional lymph nodes. The second area was chosen in order to evaluate lymphadenectomy as a possible therapeutic procedure in Pu wound cases.

The principal experimental model used for these studies was the beagle. The criteria which make the beagle a useful model for systemic studies which may be applied to man have been reviewed by Andersen (1970) and Bustad et al. (1972). Mongrel dogs were used when beagles were not available.

The data generated in this study were obtained by measurement of the 17 ke V uranium L x-rays by in vivo counting, by liquid scintillation α -counting, by autoradiography of tissue samples and by disc electrophoresis assays of lymph.

1. Early Lymphadenectomy Experiments

Accumulation of Pu in the L.S.C.L.N. of the lymphadenectomized dogs indicated that the residual lymph nodes assumed the filtering function of the excised node within

Figure 18. Change in the fraction of Pu accumulation in the left superficial cervical lymph node with time. Before and after lymphadenectomy (Group IA)

Figure 19, Change in the fraction of Pu accumulation
in the left superficial cervical lymph node
with time. Before and after lymphadenectomy
(Group IB)

Figure 20. Fraction of ^{239}Pu in selected tissues
($\bar{X} \pm \text{SE}$). Data are from early and
delayed lymphadenectomy experiments.
L = Liver
S = Spleen
HLN = Hepatic Lymph Node

2 days after excision. The build-up curves from the two early lymphadenectomy experiments (Groups I and II) are presented in Figures 18 and 19, respectively. The low, erratic activity detected in the L.S.C.L.N. region of the lymphadenectomized dogs for a few days after surgery was due to the fact that the residual lymph nodes were not yet functioning efficiently, and lymph was translocated beyond the L.S.C.L.N. region. The slopes of the build-up curves for lymphadenectomized dogs parallels the slopes of the curves for the intact dogs 4 to 6 days after surgery. At necropsy L.S.C.L.N. samples with detectable Pu were obtained from all lymphadenectomized dogs. Between the time of excision of the primary node and time of necropsy (11 to 13 days), the residual lymph nodes had enlarged to the size of the original lymph node, Table 38.

The liver, spleen and hepatic lymph nodes in the lymphadenectomized dogs accumulated greater burdens of Pu than did the same organs in the intact dogs. The data from the early experiments are shown in Figure 20. These data suggest that much of the Pu translocation beyond the L.S.C.L.N. occurred before the residual lymph nodes began to function efficiently. The rate at which the residual lymph nodes begin to accumulate Pu may be a major factor in determining how much Pu translocates to other organs. The greater and more rapid lymph node build-up of Pu in Group IB might be explained by improved surgical techniques which caused less trauma to tissues in the L.S.C.L.N. region.

Since Group IB consisted entirely of female beagles and Group IA consisted entirely of male beagles, the question of sex differences in Pu build-up arose. However, the male dogs in the delayed lymphadenectomy study, Group II, also showed rapid Pu build-up in the L.S.C.L.N. region after surgery. These results tend to support the idea that improved surgical techniques and not sex differences are the reason for the more rapid Pu build-up in the L.S.C.L.N. region after surgery.

The early experiments suggested that there may be an optimal time range (10-20 days after implant) during which the L.S.C.L.N. might be excised, to remove a maximal amount of Pu, while permitting a minimal amount of Pu to translocate beyond the lymph node. The results from the early lymphadenectomy experiments combined with data concerning the long-term accumulation of Pu in the L.S.C.L.N. (Bistline et al., 1972 a.) suggested the design of the delayed lymphadenectomy experiment.

2. Delayed Lymphadenectomy Experiment

The build-up of Pu in the L.S.C.L.N. region was determined by taking daily counts with a NaI(Tl) detector before and after lymphadenectomy. These data are shown in Figure 21. The rapid translocation of a large amount of Pu in dog #1440 could have been due to accidental implantation of the PuO_2 either directly into a lymphatic vessel or in very close proximity to a lymphatic vessel. The built-up data from dog #1440 were included with the lymphadenectomized dogs

Figure 21. Change in the fraction of Pu accumulation in the left superficial cervical lymph node with time. Before and after lymphadenectomy ($\bar{X} \pm SE$) (Group II)

Square = Dog #1440

Circle = Dogs with L.S.C.L.N. intact

Diamond = Lymphadenectomized dogs

immediately after surgery, since build-up in the residual lymph nodes paralleled that seen in other lymphadenectomized dogs. The build-up of Pu in the L.S.C.L.N. seen in this experiment is consistent with that seen in the long-term, high-fired PuO_2 experiments conducted by Bistline et al., (1972). No equation was determined for the build-up curves seen in Figure 21, since Bistline et al. (1972 a) show that after day 50 the Pu build-up curves level off and then exhibit a negative slope. The curves in the delayed lymphadenectomy experiment were determined only to day 49. They leveled off by day 10 but did not develop a negative slope. The dogs were killed 35 days after surgery and were necropsied. The tissues sampled underwent radiochemical analysis for ^{239}Pu by liquid scintillation α -counting. The overall recovery of the procedure was determined to be 90% using tissues spiked with known amount of $^{239}\text{PuO}_2$.

It was hypothesized that the leveling-off of the Pu build-up in the L.S.C.L.N. was due to a decreased translocation rate of Pu from the implant site to the L.S.C.L.N. The build-up curves (Figure 21) show that this is the case. The residual L.S.C.L.N. accumulated Pu 10 to 100 times more slowly than did the original L.S.C.L.N. The leveling-off of the L.S.C.L.N. build-up may also be due to Pu translocating beyond the L.S.C.L.N. Translocation from the L.S.C.L.N. in intact dogs has been demonstrated by Johnson (1969) and Bistline (1972).

The relationship between tissue burdens in intact versus

lymphadenectomized dogs parallels that seen in Groups IA and IB (Figure 20). It was hypothesized that delayed lymphadenectomy would allow for maximal Pu removal with the excised node, while allowing minimal Pu translocation beyond the L.S.C.L.N. chain.

According to Thompson, the spleen, under certain conditions, may accumulate Pu concentrations as high or higher than those in liver or bone. Conditions that favor deposition in liver seem also to favor deposition in spleen. However, spleen Pu concentrations are usually lower than those in liver if retention in the liver is high as in man, dog and pig. The data in Figure 20 parallel the Pu deposition described by Thompson (1967). The hepatic lymph node also shows significant Pu accumulation.

The therapeutic value of lymphadenectomy for management of Pu-contaminated wounds in humans may be improved by considering it in conjunction with chelation therapy and/or excision of the contaminated wound site which should minimize the Pu reservoir.

Since the concept of "dose commitment" (rem to critical organ of reference man per μCi) rather than "organ burden" is expected to become the basic standard for internal dose, it becomes important to consider the total dose an individual will receive during his lifetime. Morgan points out the importance of removing as much ^{239}Pu as possible from the body of a contaminated worker. He provides the example of a worker accumulating a bone burden equal to one-half of the maximum

permissible organ burden, $q/2$, (sic) which would correspond to an average dose rate to the skeleton of 30 rem/yr. This would lead to an excessive skeletal dose during the following 40 years and would restrict all his future employment as a radiation worker. Morgan also states that a bone burden of $q/2$ would yield a 7% chance that the worker may die of radiation-induced cancer.

Since translocation of $^{239}\text{PuO}_2$ beyond intact lymph nodes to more radiosensitive tissues has been shown to occur (Johnson 1969; Bistline 1972), a long-term build-up and concentration of ^{239}Pu in the skeleton and liver may occur from Pu initially concentrated in lymph nodes. The total "dose commitment" to these organs would then be in excess of that allowed by the recommendations of the ICRP (1966). Lymphadenectomy, chelation therapy and wound excision should decrease the individual's "dose commitment".

3. Rapid Lymph Transport of Pu

Lymph was collected from the hind limb of two dogs. In the first dog the lymph was collected in two clear fractions before excessive contamination with blood occurred. In the second dog lymph was collected in four fractions. All samples were allowed to clot before being centrifuged to separate the cellular and acellular fractions. No Pu contamination was detected with a portable α -survey meter during any of the procedures.

After centrifugation the cellular and acellular fractions of lymph from the tubes were separated. Both fractions were

prepared for α -counting. The ration of ^{239}Pu in the cellular fraction versus the acellular fraction in both dogs was approximately 2:1 (Table 37). This result suggests that a major process of PuO_2 translocation in lymph is by phagocytosis of PuO_2 particles which enter the lymphatic system. Sanders (1972) and Sanders and Jackson (1972) have described phagocytosis of PuO_2 particles by macrophages. The acellular lymph fraction, which contained about one-third of the rapidly translocated Pu, was analyzed by disc electrophoresis to determine the transport mechanism of PuO_2 in lymph.

4. Disc Electrophoresis Results

Since Pu in blood plasma has been shown to be protein-bound, the Pu present in the acellular lymph fraction, which is qualitatively identical with plasma, was assumed to be protein-bound. Therefore, disc electrophoresis, a technique capable of separating proteins, was used to determine whether or not the Pu carried in lymph was protein-bound.

Five replicate lymph samples from each of the two dogs were run in order to assess the reproducibility of the procedure. All 10 gels contained the same protein bands. A sketch of the protein separation in the gels is presented in Figure 22. Three protein bands are indicated along with the grouped globulin fractions.

It has been reported that at least 15 major protein groups of blood plasma have been identified. Only 11 protein bands were seen in the lymph samples analyzed. The fact that lymph

TABLE 37

Ratio of ^{239}Pu in Cellular vs.
Acellular Lymph Fractions
(Mean $\pm$ S.D.)

Cellular Fraction	Acellular Fraction
1.98 $\pm$ 0.193	1.00 $\pm$ 0.048

contains only 50% as much protein as plasma may explain the smaller number of protein bands seen in the lymph samples analyzed. It was also reported that the proteins seen in polyacrylamide gels are dependent on the origin of the sample.

After the gels were removed from the glass tubes they were sectioned for liquid scintillation α -counting. The results of the α -counting are shown in Figure 23. These results show that the greater percentage of Pu is associated with the β -globulin fraction (including transferrin). These results, which are similar to those seen for Pu binding by plasma proteins, were not unexpected, since the protein composition of lymph is qualitatively identical to the protein composition of plasma. Therefore, the binding properties of proteins, such as transferrin, should be similar in lymph or plasma.

5. Autoradiography Results

The autoradiographs made on drops of whole lymph applied directly to a microscope slide yielded no detectable α -tracks. The preparation of the slides for staining and autoradiography may have removed the Pu, whether it was free, protein-bound or cellularly trapped.

The autoradiographs of the cellular fraction of lymph, developed after 72 hours exposure, showed a wide range of $^{239}\text{PuO}_2$ particle sizes. The size range in the packed cells was estimated to be 0.1 to 1.0 μ . This is illustrated in Figure 24. Whether the larger particles were single particles

Figure 22. Sketch of polyacrylamide gel showing individual protein bands. The gel was sectioned into three globulin fractions for liquid scintillation α -counting.

Figure 23. Percentage of protein bound ^{239}Pu in the protein fractions of dog lymph.

or agglomerations of small particles was not determined. Particle agglomeration in the C.S.U. Pu-wound project is being studied by Dagle (1973) using x-ray diffraction techniques. Particle sizes were estimated optically from the autoradiographs using the methods of Leary and Moss. These results indicate that the phagocytic cells in lymph are capable of transporting $^{239}\text{PuO}_2$ particles of varying size.

The autoradiographic study of lymph nodes excised at different times after implant (1 hour, 8 hours, 2 weeks, and 1 year) was used to determine whether there was more rapid transport of large or small $^{239}\text{PuO}_2$ particles via lymph.

The results of this study are shown in Figure 25. The α -stars seen are typical of all fields examined on the slides. The 1 hour and 8 hour lymph node sections contained only small α -stars, while the 2 week and 1 year sections contained a wide range of α -star sizes. The number of α -stars per field was low on the 1 hour and 8 hour specimens, and high on the 2 week and 1 year specimens.

These results suggest that the $^{239}\text{PuO}_2$ which is most rapidly translocated to the L.S.C.L.N. is protein-bound. If the cellular fraction of lymph were to move as rapidly as the acellular fraction, larger $^{239}\text{PuO}_2$ particles would undoubtedly be found in lymph node sections excised shortly after Pu implant. No large α -stars were seen in the lymph node sections excised on the day of Pu implant, but a very wide size range

Figure 24. Typical size range of α -stars of $^{239}\text{PuO}_2$ in packed cells of lymph collected 1 hour post-implant. 72 hour exposure. (1066 X magnification).

of α -stars was seen in all lymph node sections excised after two weeks.

The conclusion made from these results is that lymphatic translocation of $^{239}\text{PuO}_2$ is divided into at least two phases. During the first phase small protein-bound $^{239}\text{PuO}_2$ is transported to the regional lymph node within minutes. Enough $^{239}\text{PuO}_2$ is translocated via this mode that the low energy U L x-rays can be detected, in vivo. The larger $^{239}\text{PuO}_2$ particles may be phagocytized by cells in the lymph and may be transported to the lymph node more slowly. By 2 weeks, however, the size range of α -stars is similar to that seen at 1 year.

The lymphatic system obviously has the capacity to rapidly carry foreign matter from the site of entry to a lymph node, where it can be retained. The rapid translocation of Pu via the lymph is surprising when one considers that all implants were made while the dogs were under pentobarbital sodium anesthesia. This anesthetic has been shown to markedly reduce lymph flow.

6. Lymphatic System Anatomy

The India ink and latex injections in the dog's left forepaw out-lined a lymph vessel which loops around the distal portion of the first metacarpus. Afferent vessels to this looping vessel come from the other metacarpals. The gross anatomical study attempted yielded the following results. The portion of the looping vessel which is dorsal to the first metacarpal bone was followed to the L.S.C.L.N., while the por-

Figure 25. Change in particle size of $^{239}\text{PuO}_2$ particles in the L.S.C.L.N. of beagles with Pu accumulation time.

- a. 1 hour $^{239}\text{PuO}_2$ accumulation
- b. 8 hour $^{239}\text{PuO}_2$ accumulation
- c. 2 week $^{239}\text{PuO}_2$ accumulation
- d. 1 year $^{239}\text{PuO}_2$ accumulation

All samples autoradiographed for 72 hours
500 X magnification.

tion of the lymph vessel which is ventral to the first metacarpal bone was followed to the left axillary lymph node.

The elevated axillary lymph node burdens may, therefore, be explained by these findings. Any Pu which is picked up by the ventral lymph vessels may go to the axillary lymph node instead of the L.S.C.L.N.

The gross studies of the anatomy of the hind paw out-lined lymph vessels which all went to the popliteal lymph node. Since only one pathway is seen in the hind leg, it may be the preferable site for Pu implant in future studies of lymph transport in beagles.

Reports indicate that the number and size of lymph nodes in the superficial cervical lymph node chain in dogs may vary. The dog used to ascertain the size and number of L.S.C.L.N.'s was killed 14 days after injection of the Niagara Sky Blue dye. All lymph nodes were stained dark blue, while all surrounding tissues were stained light blue. The lymph nodes could be easily seen.

The right superficial cervical lymph node chain consisted of three lymph nodes, and the L.S.C.L.N. chain consisted of two lymph nodes. These findings are in agreement with descriptions in the literature concerning the number of lymph nodes in the superficial cervical lymph node chain.

The weights of the lymph nodes sampled are shown in Table 38. These are consistent with those seen in all lymph-adenectomy experiments and in long-term experiments conducted

at this laboratory.

These results explain why only one L.S.C.L.N. was seen in the lymphadenectomy experiments. The smaller nodes in the chain were not distinguishable from the surrounding tissues. Therefore, only a single, large node was excised.

C. Summary

The major goals of this study were twofold: a) to study the effect of lymphadenectomy in PuO_2 translocation, and b) to gain new information concerning the mechanisms of rapid lymph transport of PuO_2 to regional lymph nodes. Minor anatomical studies supplied answers to questions which arose during the course of the study.

The potential for long-term bone and liver accumulation of Pu, and the serious consequences of such accumulation, make it imperative to remove as much Pu from the body as possible as soon as possible. The lymphadenectomy studies suggest that lymph node excision may be an effective therapeutic procedure in Pu wound management. Chelation therapy and/or wound site excision may increase the therapeutic value of excision of Pu-contaminated lymph nodes.

The transport of PuO_2 in lymph appears to be primarily by way of the cellular fraction. The range of particle sizes seen in the cellular fraction was estimated to be from 0.1 to 1.0 μ . The fraction of PuO_2 transported by the acellular lymph fraction was shown by disc electrophoresis to be

primarily associated with the β -globulin fraction, which includes transferrin, the major Pu-transport protein in blood plasma.

Autoradiographic studies on sections of lymph nodes, which were excised at 1 hour, 8 hours, 2 weeks and 1 year, showed that the translocation of very small PuO_2 "particles" from the wound site to the lymph node occurred during the first day after PuO_2 implant. By 2 weeks, large PuO_2 particles could be found in the lymph node. The range of particle sizes seen at 2 weeks was the same as that seen in the lymph node sections taken 1 year after implant.

Since large PuO_2 particles were present in the lymph 1 hour after implant, but no large PuO_2 particles were seen in the lymph node sections excised 1 hour after implant, it is felt that the rapid Pu translocation to the lymph node occurs via the proteins of lymph, rather than by phagocytosis of the PuO_2 particles. The PuO_2 which is phagocytized probably reaches the lymph node much later than the protein-bound Pu.

The minor anatomical studies showed that the high axillary lymph node burdens of Pu seen in earlier experiments were due to implant being made near the medial portion of the dog's paw. The PuO_2 was then translocated to the axillary lymph node rather than to the superficial cervical lymph node.

The lymphatic system has been shown to be a significant route by which PuO_2 can be translocated from simulated puncture wounds. Further studies are needed to assess the long-term effect of Pu in lymph nodes.

TABLE 38
Lymph Node Weights (grams) $\pm$ SD

Group	Excised LSCLN	LSCLN at Necropsy
Group IA	0.453 ± 0.138	1.020 ± 0.305
Group IB	1.091 ± 0.027	1.057 ± 0.494
Group II	1.135 ± 0.213	1.508 ± 0.445
Three-month PuO ₂ Experiment	--	1.637 ± 0.780
Dye Study		
Ventral		
Right	--	0.783
Left	--	1.105
Dorsal		
Right	--	0.145, 0.068
Left	--	0.117

VI. PLUTONIUM LYMPH RELATIONSHIP

This study was initiated to answer the following questions:

1. How is Pu being transported in the lymph? How is the protein molecule transferrin actually involved in this movement?
2. What differences occur in the binding of plutonium in the afferent and efferent lymph channels to and from the popliteal lymph node?
3. How does the binding of soluble and insoluble chemical forms of plutonium in the lymph compare?
4. What effect does particle size have on the movement of Pu from a wound site? How do particles found in afferent and efferent lymph differ in size?

A. Materials and Methods

1. Experimental Design

Table 39 summarizes the experimental design used for this study. Two different chemical forms of plutonium were used in these experiments -- plutonium nitrate and plutonium oxide. Reactorproduced polydispersed plutonium oxide and plutonium nitrate were supplied by Rocky Flats Plant, Dow Chemical Company, Denver, Colorado. Monodispersed pure $^{239}\text{PuO}_2$ was supplied by the Inhalation Toxicology Research Institute, Albuquerque, New Mexico. Relevant characteristics of the plutonium compounds used are listed in Tables 40, 41, and 42.

2. Experimental Animals

A total of 20 adult dogs, 8 mongrels and 12 beagles, were

Table 39

Experimental Design

Dog No.	Chemical Form of Pu	Plutonium Characteristics	Lymph Vessel Collected
1	$\text{Pu}(\text{NO}_3)_4$	soluble	afferent
2	$\text{Pu}(\text{NO}_3)_4$	soluble	efferent
3	PuO_2	polydispersed	afferent
4	PuO_2	polydispersed	efferent
5	PuO_2	monodispersed .16 μ particles	afferent
6	PuO_2	monodispersed .16 μ particles	efferent
7	PuO_2	monodispersed .54 μ particles	afferent
8	PuO_2	monodispersed .54 μ particles	efferent
9	PuO_2	monodispersed 1.05 μ particles	afferent
10	PuO_2	monodispersed 1.05 μ particles	efferent

Table 40

Physical, Chemical and Isotopic Characteristics
of $\text{Pu}(\text{NO}_3)_4$ Supplied by Rocky Flats

$\text{Pu}(\text{NO}_3)_4$	Characteristics
Pu	= 269 g/ml
Total Alpha activity	= 20.2 MCi/ml
^{238}Pu	= 0.0098 wt %
^{239}Pu	= 93.810 wt %
^{240}Pu	= 5.796 wt %
^{241}Pu	= 0.309 wt % (9/73)
^{242}Pu	= 0.019 wt %
^{241}Pu	= 590 ppm based on Pu (9/73)

Table 41

Physical, Chemical and Isotopic Characteristics
of PuO₂ Supplied by Rocky Flats

PuO ₂	Characteristics
Pu	= 0.8826 g/g
Cation impurities	= 200 ppm (based on Pu)
An ²⁴¹	= 360 ppm based on Pu as of 10/1/70
An ²⁴¹ growth rate	= 15 ppm/month based on Pu
Pu ²³⁸	= 0.0105 wt %, based on Pu
Pu ²³⁹	= 93.898 wt %, based on Pu
Pu ²⁴⁰	= 5.695 wt %, based on Pu
Pu ²⁴¹	= 0.377 wt %, based on Pu as of 6/70
Pu ²⁴²	= 0.019 wt %, based on Pu
Atomic wt. of Pu	= 239.116 as of June, 1970
<u>Particle size (μ)</u>	
1 or less	68%
1 to 3	21%
3 to 5	4%
5 to 10	6%
10	1%

Mass Median Diameter Approximates 0.7

Table 42

PuO₂ Particle Sizes Supplied by Inhalation
Toxicology Research Institute

Segment [*] Number	Particle Size (μm)
2	1.05 ± 0.1 (SD)
7	0.54 ± 0.05
18	0.16 ± 0.02

* Segment number from Lovelace Aerosol Particle Separator System, Inhalation Toxicology Research Institute, Albuquerque, New Mexico.

obtained for use in this study. The dogs chosen weighed from 35-40 pounds. The beagles came from the barrier-maintained colony of the Collaborative Radiological Health Laboratory. The mongrels were obtained as needed from the Colorado State University Experimental Animal Service and placed in quarantine for three weeks prior to inclusion in an experiment. The beagles were housed in the cage facilities of the Collaborative Radiological Health Laboratory. They were fed ad libitum and watered with pans or an automatic watering system (licks) depending on the season. The dogs were observed daily and given regular veterinary care.

Six New Zealand white rabbits were used in the immunological studies. They were kept in temperature and air-controlled facilities at the Colorado State University Foothills Research Campus. They were fed ad libitum with rabbit pellets supplemented by fresh greens. Daily observation and care was maintained during the course of the experiments.

3. Lymph Analysis

Initially a 1.0-1.5 ml sample of lymph was collected from each dog to be used for control values.

Cell counts were obtained using the Unipet¹ system and a Brightline² hemocytometer. An average of 3-4 counts were

¹Becton-Dickinson and Co., Rutherford, N.J.

²AO Instrument Co., Buffalo, N.Y.

performed on each sample to obtain an accurate estimate for each dog. Smears were made from each sample, stained with modified Wrights-Giensa stain, and examined for morphology.

Protein determinations were made on each sample using the Lowry method. Normal lymph samples were also run through each of the electrophoretic procedures (to be described in the section on plutonium-contaminated lymph) to give a basis for comparison of plutonium-induced changes.

Lymph samples from plutonium-exposed dogs were initially broken down into cellular and acellular fractions by centrifugation. Sample size was measured for calculation of plutonium content. Liquid scintillation procedures were used to count cellular fractions. Some samples were designated for fission track analysis. A 25 λ sample of acellular lymph was taken from each collected fraction for later analysis for plutonium by liquid scintillation techniques. The remaining acellular fraction was retained for biochemical analysis.

Several different electrophoretic techniques were used to analyze the lymph. Discontinuous gel electrophoresis, isoelectric focusing gel electrophoresis, cellulose acetate electrophoresis and immunoelectrophoresis techniques were used. Disc gel, isoelectric focusing gel, and cellulose acetate sectioned samples were counted by liquid scintillation α -counting using a Nuclear Chicago Mark II¹ Liquid

¹Nuclear Chicago, Des Plains, Ill.

Scintillation α -Counter. Plutonium contaminated and other protein samples were processed by the Keough and Powers (1970) procedure for liquid scintillation α -counting.

Time-studies using lymph protein contaminated with $\text{Pu}(\text{NO}_3)_4$ were conducted to determine changes during electrophoresis in plutonium-protein relationships. Several known protein samples were compared with lymph proteins. The proteins used for comparison included canine albumin¹, canine α , β , and γ -globulin², canine transferrin³, equine transferrin⁴, and ovatransferrin⁵. They were run through both disc and isoelectric focusing electrophoresis. The information was used for comparison as to location and identification of lymph protein groups which were also run through the disc and isoelectric focusing gel systems.

A preparative polyacrylamide gel column⁶ was used to separate 40 mg of pooled lymph proteins. Fifteen ml of stacking gel and 40 ml of resolving gel were used in the column. Peak samples were pooled and dialysed, using dialysis membranes with a molecular weight cut off of 12,000

¹Sigma Chemical Co., St. Louis, Mo.

²Mann Research Labs., Orangeburg, N.Y.

³Miles Laboratories, Kankakee, Wis.

⁴National Biochemical Corp., Cleveland, Ohio

⁵Dr. P. Azari, Colorado State University, Ft. Collins, Colo.

⁶Shandon Scientific Company Limited, London, England

against distilled water. The peak samples were freeze-dried and analyzed with discontinuous and isoelectric focusing gel systems.

Lymph cells selected for analysis by the fission track method were collected by passage through Millipore filter¹ with a pore size of 0.45 μ . The filters were dried and taken to the Rocky Flats Plant of Dow Chemical Company, Denver, Colorado, for analysis.

B. Results and Discussion

1. Lymph Constituents

a. Lymph Flow

The volume of lymph collected following plutonium implants is shown in Table 43. For afferent lymph the total volume ranged from 4.5 ml to 7.5 ml, while the total volume for efferent lymph ranged from 4.5 ml to 8.5 ml for the 4.0 hour collection period.

No large differences are seen between afferent and efferent lymph flow. The lymph flow in each dog was consistent during each 0.5 hour collection interval, although there was variability from dog to dog. Thus, implantation of plutonium did not increase lymph flow over the 4.0 hour collection period.

b. Lymph Cells

The lymph cell counts for the different dogs used.

¹Millipore Corporation, Bedford, Mass.

Table 43

Total Lymph Volume (ml) Collected in 0.5 Hour
Intervals Following Plutonium Implants

Dog No.	Elapsed Hours After Implant								Total for 4.0 Hours
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	
<u>Afferent</u>									
1	1.2	1.1	0.9	0.6	0.4	1.0	1.0	1.0	7.2
3	0.8	0.8	0.6	1.0	0.9	0.9	0.6	0.5	5.9
5	0.7	0.6	0.8	0.4	0.5	0.9	0.7	1.3	5.9
7	0.7	0.5	0.5	0.4	0.5	0.5	0.6	0.6	4.5
9	0.7	0.6	0.6	0.6	0.5	0.7	0.4	0.8	4.9
<u>Efferent</u>									
2	1.1	0.9	1.0	1.0	0.8	1.2	1.4	1.1	8.5
4	0.7	0.6	0.8	0.5	0.6	0.6	0.8	0.8	5.4
6	0.7	1.3	1.0	1.2	0.5	1.1	0.8	0.7	7.3
8	0.5	0.6	0.6	0.6	0.7	0.7	0.6	0.9	4.5
10	0.6	0.6	0.4	0.7	0.6	0.7	0.5	0.8	4.9

Table 44
Lymph Cell Counts

Dog No.	Plutonium implanted	Cells per mm ³
<u>Afferent pre-implant</u>		
1	Pu(NO ₃) ₄	110
3	polydispersed PuO ₂	220
5	0.16 μ particles PuO ₂	220
7	0.54 μ particles PuO ₂	110
9	1.05 μ particles PuO ₂	440
	Average	220
<u>Efferent pre-implant</u>		
2	Pu(NO ₃) ₄	525
4	polydispersed PuO ₂	23,595
6	0.16 μ particles PuO ₂	2,640
8	0.54 μ particles PuO ₂	1,320
10	1.05 μ particles PuO ₂	8,910
	Average	7,398

in the plutonium implant experiments are shown in Table 44. All cell counts were made before implantation. The average cell count for afferent lymph was 220 cells per mm^3 , while the average cell count for efferent lymph was 7398 cells per mm^3 . These results indicate that the lymph cell counts obtained from the dogs in these experiments were within the normal range.

c. Lymph Protein Concentration

Lymph protein concentrations prior to plutonium implant, 1.0 hour post-implant and 4.0 hours post-implant were determined. No significant difference was seen between the protein concentration means before implantation, 1.0 hour post-implant, and 4.0 hours post-implant in both afferent and efferent lymph. Absence of a significant change indicates either that the plutonium insult was not injurious enough to cause a change in lymph proteins or that a delayed reaction occurred which was not detectable in the short 4.0 hour collection period.

The pre-implant protein concentration for efferent lymph was 13.81 ± 4.02 mg/ml, while the pre-implant protein concentration for afferent lymph was 21.43 ± 2.80 mg/ml. Using a student's t-test the means were found to be significantly different at the 0.05 level. The data obtained in this experiment for the afferent and efferent lymph vessels of the popliteal lymph node showed a slightly higher protein concentration on the efferent side of the lymph node.

Protein concentration is an important factor to consider when comparing plutonium binding in serum and lymph. In the lymph there is a reduced number of binding sites due to a lower concentration of proteins.

2. Biochemical Studies of Normal Lymph

a. Standard Proteins

Commercially available standard proteins were compared, by discontinuous gel electrophoresis, with normal lymph proteins to determine the location of specific protein groups in lymph. The location of transferrins from different species, along with canine afferent lymph, are illustrated in Figure 26. This comparison allows the transferrin bands in canine afferent lymph to be identified after being electrophoresed on discontinuous gels. Four known canine serum protein groups (albumin, α -globulin, β -globulin, and γ -globulin) are matched against canine afferent lymph. Excess afferent lymph protein was electrophoresed on gel D to allow a visualization of even the weakest band (Figure 27).

b. Afferent versus Efferent Lymph

To investigate whether a qualitative difference occurs, afferent and efferent lymph samples were electrophoresed on both discontinuous and isoelectric focusing gel systems. Little difference was seen in the afferent and efferent lymph protein bands using these two systems.

c. Preparative Gel Column

A preparative gel column was used to isolate proteins

Figure 26. Discontinuous gels

- A. Normal canine afferent lymph with arrow indicating transferrin.
- B. Purified canine transferrin (arrow).
- C. Purified equine transferrin (arrow).
- D. Purified ovatransferrin (arrow).

Figure 27. Discontinuous gels

- A. Canine albumin (bottom arrow) and α -globulins (upper two arrows).
- B. Canine β -globulins (between arrows).
- C. Canine γ -globulins (between arrows).
- D. Normal canine afferent lymph (top arrow indicating γ -globulins, middle arrow indicating canine transferrin, and bottom arrow indicating canine albumin).

into groups. Optical density readings (280 mμ) were made on fractions collected from the preparative gel column to determine protein content. The peaks contained different lymph protein groups that could be used in the comparison of the gel systems. Fractions making up each peak were pooled and electrophoresed on both discontinuous and isoelectric focusing gel systems. Both gel systems produced the same results for the identification of the specific proteins in these two peaks; therefore, it is reasonable to use the two gel systems for comparison of specific lymph proteins.

d. Time Studies

The plutonium-protein bond has been described by Taylor as very weak. To evaluate the meaning of plutonium peaks found on both discontinuous and isoelectric focusing gels, an experiment, using paired samples, was performed with each gel system.

1. Discontinuous Gel Electrophoresis

The sections of discontinuous gels containing a fraction of plutonium in each section, are plotted in Figure 28. Two major Pu peaks were seen during the protein separation in the resolving gel. Both peaks spread over more sections and diminish in height with time. At 90 minutes peak 1 spreads from section 8 to section 12, while peak 2 spreads from section 29 to section 36. Previous studies with standard proteins indicated that peak 1 corresponded with the location

Figure 28. Fraction of Pu detected in sections of discontinuous gels at specific times after the proteins entered the resolving gel.

Section No.

Section No.

of transferrin and peak 2 with albumin.

Diminution of the plutonium peak is the result of a stripping effect whereby plutonium molecules are removed from transferrin by the electrical field that exists during electrophoresis. Also the slow movement of transferrin molecules in the gel may allow for more competitive binding of other ions to Pu, causing the plutonium-transferrin bond to break. This would cause an even further reduction in the height of the plutonium peak.

Albumin makes up a large percentage of the total lymph proteins (60-70%); thus, the mass of albumin alone caused spreading of albumin molecules over several sections. Part of the ion front in the gel overlaps albumin and may contribute some plutonium molecules to the albumin peak. The decrease in the Pu peak associated with the albumin bands is undoubtedly due to a stripping effect such as was observed with transferrin molecules. However, because albumin moves much faster on the gel the stripping effect is minimized.

This time study indicated that plutonium peaks, associated with specific proteins, can be determined but that accurate estimates of the percentage of Pu binding to a particular protein would be difficult due to the stripping effect and to the competitive binding of other ions to plutonium occurring at the pH of this gel system.

2. Isoelectric Focusing Gel Electrophoresis

Sections of isoelectric focusing gels containing a fraction of Pu in each section are plotted in Figure 29. Three major Pu peaks are consistently seen as the proteins are separated in the resolving gel. Again the peaks spread and diminish in height with time, especially the first two peaks. At 6.0 hours peak 1 spreads from section 1 to 4, peak 2 spreads from section 8 to 11, and peak 3 spreads from section 17 to 25.

Peak 1 did not correspond to the location of any protein and was found to be a plutonium precipitate that occurred when the plutonium contaminated lymph was applied to the gels. Previous studies with standard proteins indicated that peak 1 corresponded with the location of albumin and peak 2 with transferrin.

Each of the transferrin states has a different isoelectric point and causes a spreading effect on the gels since they contain a pH gradient. The height of the Pu peak associated with transferrin decreases slightly with time but the stripping effect is not as severe as with the disc gels.

The mass of the albumin fraction again causes a spreading effect. The plutonium peak associated with albumin quickly reaches its isoelectric point in the gels and remains stationary. This causes a much greater stripping effect than was seen with the disc gels.

Figure 29. Fraction of Pu detected in sections of isoelectric focusing gels at specific times after electrophoresis initiated.

Section No.

Section No.

The isoelectric focusing gel time study indicates that the same problems exist as with the disc electrophoresis system. Identification of specific proteins involved in plutonium binding can be achieved but the problems outlined preclude an accurate estimate of the percentage of plutonium binding to an individual protein by the methods used in this study.

3. Plutonium Contaminated Lymph

a. Acellular versus Cellular Lymph Fractions

Two processes are involved in the transport of plutonium in lymph -- the cellular process which involves the phagocytosis of plutonium particles and the acellular process which binds plutonium to proteins or other biological macromolecules. Investigation of the amounts of plutonium moving in these two processes is important in devising therapeutic regimens for plutonium contaminated wound incidents.

Using polydispersed PuO_2 , Gomez (1973) showed that 65% of the plutonium moved in the cellular fraction. However, the ratio of acellular to cellular plutonium may change with time as dissolution of Pu particles occurs. Gomez did not report the time post-implant of his estimate.

1. Soluble versus Insoluble Plutonium

Soluble and insoluble forms of plutonium are initially handled very differently by the body when

they gain entry through contaminated wounds. To investigate the differences, a soluble form of plutonium ($\text{Pu}(\text{NO}_3)_3$) and an insoluble form (PuO_2) were chosen. No attempt was made to fit data to regression curves because only one dog was used for each set of conditions. For $\text{Pu}(\text{NO}_3)_4$ it was found that the fraction of implant dose was a factor of a 1000 greater in the acellular fraction than in the cellular fraction for the afferent lymph. This is consistent with what is expected with ionic forms of plutonium. No significant change in the acellular or cellular fraction of plutonium was seen with time.

For $\text{Pu}(\text{NO}_3)_4$ in efferent lymph our results showed that the acellular fraction contained 50 times as much plutonium as the cellular fraction as judged from the fraction of implant dose parameter. This is again consistent with what is expected with ionic forms of plutonium. As with afferent lymph, significant changes in cellular and acellular plutonium concentration were not seen with time.

As plutonium is translocated in lymph it eventually reaches a lymph node, where it can be phagocytosed by macrophages, pass into the vascular system via lymph-venous communications, or leave the node through efferent lymph vessels. Comparison of acellular and cellular lymph fractions is important in determining Pu movement in afferent and efferent

lymph. Contrasting plutonium transport in afferent and efferent lymph enables one to determine how the lymph node functions in removing plutonium from lymph in the early hours after a wound exposure.

Soluble plutonium can be considered to be in an ionic form when it enters the body. Two different reactions may take place when entry occurs via a wound -- polymeric or colloidal forms of plutonium may result from hydrolysis or it can be complexed by proteins and other types of biological macromolecules. The first reaction is predominant. Thus, most of the plutonium becomes fixed at the wound site initially. Over a period of a year, up to 70% of a soluble form of plutonium, such as $\text{Pu}(\text{NO}_3)_4$, may be removed from a wound site. This removal occurs either by phagocytosis of Pu particles or by dissolution.

It was found that the acellular Pu fraction for the $\text{Pu}(\text{NO}_3)_4$ dogs was ten times higher in afferent lymph than in efferent lymph. This fact indicates that either Pu was moving into the vascular system via lymph-venous communications or that it was being picked up and retained by cells within the lymph node. From the data reported by Bistline (1973) it is known that high activity is found in the lymph node within the first few hours following $\text{Pu}(\text{NO}_3)_4$ implants. This would point to the retention of plutonium within the

lymph node although it does not preclude the movement of some plutonium into the vascular system via lymph-venous communications.

The cellular Pu fraction was consistently less in efferent lymph although the difference was slight. This would indicate that plutonium was being retained by cells in the lymph node and was not passing through the node into efferent lymph channels.

Insoluble plutonium was handled differently by the body. When entering the body, insoluble Pu was in a particulate form and therefore, the large excesses of ionic Pu did not exist. With time, phagocytosis or dissolution of the Pu particles takes place, where upon the same processes that moved soluble Pu occurred.

In both afferent and efferent lymph Pu concentration in the acellular fraction was found to be 5 to 10 times higher than in the cellular fraction until three hours post-implant when the positions reversed. The initially higher concentration of Pu in acellular lymph may be due to a particle surface phenomenon whereby dissolution initially occurs at a much greater rate on the outermost surface of the particle. This assumption would explain the initial peak in ionic Pu available for movement by the acellular fraction that was seen.

The plutonium concentration in the acellular fraction of afferent lymph was found to be 50 times higher than in the acellular fraction of efferent lymph. On the other hand, the cellular fraction in afferent lymph was a 1000 times higher than the cellular fraction of efferent lymph at 0.5 hour post-implant but was reduced to a factor of 10 at the end of 4.0 hours. Both of these findings indicate that a large amount of PuO_2 is being retained in the popliteal lymph node. These findings parallel the data obtained by Bistline (1973) for PuO_2 . Dagle (1973) showed that PuO_2 particles were being phagocytosed by macrophages within the lymph node. This mechanism would account for the retention of PuO_2 in the lymph node.

Overall, it can be stated that for dogs implanted with $\text{Pu}(\text{NO}_3)_4$, the fraction of the initial implant dose was found to be higher by two to three orders of magnitude in the acellular fraction of both afferent and efferent lymph than in the respective cellular fractions. This contrasts with the lymph of dogs implanted with polydispersed PuO_2 where the acellular fraction of initial implant dose is only slightly higher until 3.0 hours post-implant, when the trend reverses itself. The higher concentration of Pu in the acellular fractions of lymph from dogs implanted with $\text{Pu}(\text{NO}_3)_4$ gives further evidence for the

theory that most of the initial plutonium transport of soluble forms of plutonium is via a protein bound mechanism.

The fraction of the initial implant dose in afferent lymph (acellular and cellular fractions) in two different dogs, one implanted with $\text{Pu}(\text{NO}_3)_4$ and the other with polydispersed PuO_2 , is plotted in Figure 30. The data shows that the initial implant dose moving in afferent lymph was approximately two orders of magnitude higher in the dog implanted with $\text{Pu}(\text{NO}_3)_4$ than in dog implanted with polydispersed PuO_2 during the 4.0 hour collection period. This indicates that the soluble $\text{Pu}(\text{NO}_3)_4$ was leaving the wound site much faster than the insoluble PuO_2 which is in agreement with the conclusions reached by Bistline.

The fractions of the initial implant dose in efferent lymph (acellular and cellular fractions) in two different dogs, one implanted with $\text{Pu}(\text{NO}_3)_4$ and the other with polydispersed PuO_2 , are presented in Figure 31. The fraction of the initial implant dose moving in efferent lymph was found to be approximately ten times higher in the dog implanted with $\text{Pu}(\text{NO}_3)_4$ during the 4.0 hour collection period implying that a greater amount of the particular PuO_2 was retained within the popliteal lymph node.

Figure 30. Fraction of initial implant dose in
afferent lymph (acellular and cellular).

X = $\text{Pu}(\text{NO}_3)_4$

Square = polydispersed PuO_2

2. Monodispersed Plutonium Oxide Particles

Particle size effects have often been used as an explanation for the protracted movement of polydispersed PuO_2 from a wound site (Dagle, 1973; Gomez, 1973; Bistline, 1973). Three sizes (0.16 μ , 0.54 μ , and 1.05 μ) of monodispersed plutonium oxide particles were used to investigate the particle size problem in the lymphatic transport of PuO_2 .

This experiment compared the transport of 0.16 μ monodispersed PuO_2 particles in afferent and efferent lymph. The cellular fraction is consistently higher in Pu content in afferent lymph but varies for efferent lymph. The lower plutonium content found in efferent lymph results from the retention of PuO_2 particles in the lymph node. This observation was further emphasized by the fact that the plutonium concentration was 100 times higher in afferent lymph than efferent lymph. No data for the acellular fraction in efferent lymph is reported after 1.5 hours post-implant because it was below minimal detectable activity (0.4 pico curies).

Overall, the data obtained for all three particle sizes demonstrates that the cellular fraction of lymph, whether afferent or efferent, transports a greater amount of Pu than the acellular fraction. It was also found that the range of particle sizes studied in these experiments indicates that the phagocytic

Figure 31. Fraction of initial implant dose in efferent lymph (acellular and cellular).

X = $\text{Pu}(\text{NO}_3)_4$

Square = polydispersed PuO_2

cells in lymph can transport varying particle sizes of PuO_2 ; thus, particle size does not prohibit cellular transport of PuO_2 in particles up to 1.0μ .

A composite graph of all three particle sizes (0.16μ , 0.54μ , and 1.05μ) of PuO_2 in total afferent lymph (cellular and acellular fractions) is presented in Figure 32. As can be observed, it is difficult to discern differences in the transport of the specific particle sizes, although the trend suggests that the larger particles are transported more slowly.

In Figure 33, the data obtained for the same three particle sizes of PuO_2 were plotted to compare differences in total efferent lymph. However, no definite particle size differences can be distinguished.

Data in Figure 32 suggests that particle size, below 1.0μ , is not the significant factor in the total lymph transport of PuO_2 from a wound site that other researchers have suggested (Johnson, 1969; Gomez, 1973; Bistline, 1973). This assumption was based on the fact that the various sizes of PuO_2 particles were undergoing dissolution at the wound site and were being transported in the acellular fraction in the first few hours following implant. This data represents, however, only ten individual dogs; thus, precluding extensive statistical inference.

Information obtained for all particle sizes

Figure 32. Fraction of initial implant dose in afferent lymph (acellular and cellular).

X = monodispersed (0.16 μ) PuO₂

Square = monodispersed (0.54 μ) PuO₂

Diamond = monodispersed (1.05 μ) PuO₂

indicated that the lymph node is an effective filtering organ for PuO_2 particles of varying sizes transported in lymph. It is significant that smaller Pu particles (0.16μ) are moving beyond the lymph node via the cellular fraction of efferent lymph after only 0.5 hours but that the larger particles (1.05μ) do not pass through until 2.0 hours post-implant. Therefore, for plutonium actually transported in particulate form, it can be concluded the lymph node is much more effective in stopping the larger particles. This observation implies that the size of the particle reaching the lymph node will influence whether the particle is retained in the lymph node or passes through it.

b. Discontinuous Gel Electrophoresis

Discontinuous gel electrophoresis was used to assess whether there was a qualitative change in the protein binding of plutonium in lymph as it passes through a lymph node and to evaluate any possible effect that the chemical form of Pu may have on the protein binding mechanism. Experiments were conducted to compare plutonium binding in afferent and efferent lymph using two different chemical forms of plutonium, $\text{Pu}(\text{NO}_3)_4$ and polydispersed PuO_2 .

It was observed in both afferent and efferent lymph that when $\text{Pu}(\text{NO}_3)_4$ is introduced into the body through a wound site, it travels primarily with the lymph proteins, albumin and transferrin. Transferrin has been cited as

Figure 33. Fraction of initial implant dose in efferent lymph (acellular and cellular).

X = monodispersed (0.16 μ) PuO₂

Square = monodispersed (0.54 μ) PuO₂

Diamond = monodispersed (1.05 μ) PuO₂

the chief protein binding Pu in serum. All of these studies involved plutonium incubated in large amounts of serum or following injection of plutonium intravenously; thus, the plutonium was exposed to large amounts of protein which included transferrin. This contrasts to the situation in lymph where protein concentration is much lower and the lymph at the wound site may only amount to one or two ml; thus, the amount of transferrin available for the binding of plutonium molecules is significantly less. Norwood outlined the problems of comparing plutonium binding when the plutonium is injected intravenously as opposed to the industrial wound case. It is believed that in the latter case the excess plutonium will bind to other proteins such as albumin. In comparing plutonium binding to different proteins, Turner and Taylor indicated that a small amount of plutonium may be bound to serum albumin. This means that albumin could be used as a secondary site for plutonium binding. According to Wasserman and Mayerson albumin concentration is higher in lymph than in serum. Data presented in this study supports this contention. Thus, it is not unlikely to find plutonium bound to albumin in lymph.

c. Isoelectric Focusing Gel Electrophoresis

To get a comparison with the results of the discontinuous gel system, isoelectric focusing gel electrophoresis was performed on the same lymph samples.

Plutonium concentrations in sections of isoelectric focusing gels on which electrophoresis was carried out to separate $\text{Pu}(\text{NO}_3)_4$ contaminated afferent and efferent lymph proteins were obtained. It confirmed the results obtained on polydispersed PuO_2 movement in afferent and efferent lymph proteins determined with discontinuous gel electrophoresis. Both electrophoretic techniques indicated that plutonium, soluble or insoluble, is handled the same way in afferent and efferent lymph; thus, no change in the binding of plutonium takes place after it has moved through a lymph node.

d. Cellulose Acetate

To compare distribution of particular protein groups (albumin, α -globulins, β -globulins, and γ -globulins) in Pu contaminated afferent and efferent lymph with time, electrophoresis was performed with cellulose acetate as the support medium. The means for the different time periods (control pre-implant, 1.0 hour post-implant, and 4.0 hours post-implant) were compared and found not to be significantly different. This indicates that plutonium does not elicit a qualitative change in the lymph proteins during the early hours following implant. The lack of a shift in protein groups indicates that plutonium was constantly being picked up from the wound site and transported by the same lymph protein groups.

Since cellulose acetate strip electrophoresis has often been used as a technique in grossly determining the protein group which binds serum plutonium. An experiment comparing cellulose acetate electrophoresis with the two gel techniques (discontinuous and isoelectric focusing) was of interest. Afferent and efferent lymph proteins, contaminated with $\text{Pu}(\text{NO}_3)_4$ or polydispersed PuO_2 , were again electrophoresed.

It was found that the percentage of Pu was highest in β -globulins followed by albumin, γ -globulins and α -globulins. This was true for both afferent and efferent lymph proteins contaminated with either soluble or insoluble forms of plutonium. One of the principal proteins in β -globulins is transferrin. From this experiment it can be concluded that albumin and transferrin are the principal lymph proteins carrying plutonium. This agrees with the results obtained with discontinuous and isoelectric focusing gel electrophoresis.

e. Immuno-electrophoresis

Since immuno-electrophoresis has been used to study the binding of small molecules to proteins, it was felt that this technique might be of value in investigating the plutonium-lymph relationship.

Autoradiography of the immuno-electrophoresis slides failed to produce any concentrations of alpha tracks along the protein arcs. In fact, alpha tracks were

scattered quite randomly throughout the immunoelectrophoresis slides. Several factors could account for this failure to demonstrate the alpha tracks in the transferrin and albumin protein arcs as expected. It is possible that the plutonium could have dissociated because of the high ma used during the electrophoretic process. It is also possible that an agar-plutonium interaction could have occurred whereby the plutonium became bound by the agar. The antibody could have interacted with the plutonium. Most likely, however, the problem resided in the time necessary for diffusion (24-48 hours) so that the antigen-antibody interaction could occur. This long diffusion process could have allowed the weak plutonium-protein bands to break and create a situation favorable to the influx of other ions found on the slide that could bind plutonium. These results lead to the conclusion that immunoelectrophoresis is not a good technique for studying the plutonium-lymph relationship, at least under the conditions set forth in this experiment.

4. Fission Track Analysis

Research conducted at Rocky Flats Plant (Dow Chemical Company, Denver, Colorado) indicated that fission track analysis was a good technique for measuring particle size of PuO_2 . Since lymph cells can be collected on a filter, it was thought that fission track analysis might be used to determine the size of PuO_2 particles that they were

transporting. Monodispersed PuO_2 particles were used for implantation so that a comparison could be made with the particles in the lymph cells.

It was found that in afferent lymph all three particle sizes implanted were slightly reduced in size. This observation would point to dissolution or selective transport of smaller particles, since the particles were not purely monodispersed. It is believed that dissolution was at least partially responsible for the size reduction as indicated by the plutonium content in the acellular fraction of monodispersed PuO_2 . Because of the low activity in the efferent lymph of the dogs implanted with 0.54 μ and 1.05 μ monodispersed PuO_2 , only two and three particles were detected on the filters respectively; thus, it is difficult to ascertain whether the trend showing an even greater reduction in the particle sizes in efferent lymph was true. If this trend is in fact correct, then the lymph node is selectively removing larger particles. Gomez (1973) reported that only small alpha stars (no size given) were seen in afferent lymph on the first day of implant. This contrasts with the fission track data in that all three sizes of PuO_2 particles were seen in efferent lymph.

The particle size information showed that particles in afferent lymph are smaller than at the implant site and that after passing through the lymph node the average is reduced even further.

This was consistently seen with all three particle sizes.

C. Summary

These experiments were undertaken to study the uptake and transport mechanism of soluble ($\text{Pu}(\text{NO}_3)_4$) and insoluble (poly-dispersed PuO_2) plutonium in afferent and efferent lymph from the popliteal lymph node of dogs after simulated wound exposures.

Normal values for some of the constituents of afferent and efferent lymph were determined. The normal cell count was 220 cells/mm³ in afferent lymph and 7398 cell/mm³ in efferent lymph. Protein concentration in afferent lymph was 13.81 ± 4.02 mg/ml, while efferent lymph contained 21.43 ± 2.80 mg/ml.

The protein concentration in afferent and efferent lymph did not change during the 4.0 hour period, post-implant, that lymph samples were collected. Qualitative changes in specific lymph protein groups (albumin, α -globulins, β -globulins, and γ -globulins) were not observed during the 4.0 hour collection period.

The validity of using electrophoretic techniques in studying the Pu-lymph relationship was substantiated. Discontinuous and isoelectric focusing gel electrophoretic systems were useful in determining which proteins bind Pu, but were inaccurate in determining percentages of Pu binding to specific proteins because Pu atoms were being stripped off of the proteins during electrophoresis.

Lymph was partitioned into acellular and cellular fractions. Plutonium nitrate concentration was higher in the acellular fraction of both afferent and efferent lymph than in the cellular

fraction, while the polydispersed PuO_2 concentration was higher in the cellular fraction.

Monodispersed PuO_2 particles (0.16 μ , 0.54 μ , and 1.05 μ) were compared on the basis of their movement in lymph from a simulated wound exposure. Particle size was not found to be an important factor in the total movement (acellular and cellular lymph fractions) of Pu in afferent and efferent lymph. Regardless of particle size, more Pu moved in the cellular fraction of both afferent and efferent lymph than in the acellular fraction.

Discontinuous gel, isoelectric focusing gel, and cellulose acetate strip electrophoresis indicated that transferrin and albumin were the two principal proteins transporting plutonium in the acellular fraction of both afferent and efferent lymph.

Immuno-electrophoresis proved to be an inaccurate technique to study protein-bound plutonium.

Fission track analysis indicated that dissolution of the monodispersed Pu particles takes place at the wound site. Plutonium oxide particles at the implant site were larger than in afferent lymph and larger in afferent lymph than in efferent lymph. The dissolution of PuO_2 accounts for its rapid movement in acellular lymph following implant.

VII. PLUTONIUM FLUORIDE CONTAMINATED WOUND STUDY

The purpose of this research project which started in February 1972, is to study the movement of Pu and ^{241}Am from subcutaneous injections of PuF_4 . The experiment used 36 male beagles from the beagle colony of the Collaborative Radiological Health Laboratory at Colorado State University. The animals were divided randomly into six study groups, six to a group, with half of each group randomly selected to receive chelation therapy with the trisodium salt of diethylenetriaminepentaacetic acid (DTPA). The treated animals were given a chelation therapy schedule commonly followed for human use in the nuclear industry. Single intravenous (I.V.) injections of 5×10^{-4} moles of DTPA were given two hours after implant, then daily for 10 days, and then for three consecutive days once a month. Animals of the same group were housed one or two to a kennel. These groups were divided as shown in Table 45.

The PuF_4 used for this experiment was provided by the Rocky Flats Division of the Dow Chemical Company. The fluoride was prepared by normal production methods. The salt was dried at 80 degrees C for approximately 100 hours and pulverized by grinding in a tungsten carbide grinding vial employing WC_2 grinding balls for 30 minutes using a high speed mechanical shaker. The particle size has been measured by optical microscopy. The physical, chemical and isotopic information for the fluoride are shown in Table 46.

The plutonium fluoride salt as received from the Dow Chemical Plant was contained in 5 cc vials (rubber diaphragm stoppered). The PuF_4 salt was suspended in 1 ml of doubly distilled sterile deionized water. The activity concentration of the suspension was about 63 $\mu\text{Ci/ml}$. The ^{241}Am concentration of Pu was 505 ppm as of January 24, 1972, with

Table 45. Experimental Design -- Plutonium Fluoride.

Group	Duration of Experiment	Number of Dogs	Implant Date	Necropsy Date
I	2 weeks	6	2/15/72	2/29/72
II	1 month	6	3/ 2/72	3/30/72
III	2 months	6	6/ 8/72	8/ 8/72
IV	3 months	6	8/ 1/72	11/ 1/72
V	6 months	6	9/ 5/72	3/ 5/73
VI	12 months	6	3/22/72	3/22/73

Table 46. PuF_4 for Dow-CSU Dog Project Center.

Pu	= 0.76 g/g
Cation Impurities	= 2000 PPM (based on Pu)
^{241}Am	= 505 PPM (based on Pu as of 01/24/72)
^{241}Am Growth Rate	= 15 PPM/Month (based on Pu)
^{238}Pu	= 0.015 Wt % (based on Pu)
^{239}Pu	= 93.730 Wt % (based on Pu)
^{240}Pu	= 5.849 Wt % (based on Pu)
^{241}Pu	= 0.346 Wt % (based on Pu as of 1/24/72)
^{242}Pu	= 0.020 Wt % (based on Pu)
Atomic Wt of Pu	= 239.11

Particle Size Data

μ Range	Number of Particles	Percent	Median Mass Diameter
- 5 μ	1480	47.43	1.60
5 - 10 μ	1010	32.37	8.74
10 - 20 μ	500	16.03	34.82
20 - 30 μ	100	3.20	28.61
30 - 40 μ	20	.65	11.21
40 - 50 μ	5	.16	5.40
50 - 60 μ	4	.12	7.02
60 - 70 μ	<u>1</u>	<u>.03</u>	2.79
	3120	99.99	

The particle sizes were obtained with light microscopy techniques.

a growth rate of 15 ppm per month. The injection and monitoring techniques as well as collection of excrements and blood were done according to procedures previously described. The animals were maintained in accordance with provisions of the Laboratory Animal Welfare Act. The kennels are located on the Foothills Research Campus of Colorado State University.

The tissue samples taken at necropsy, as well as the blood and excreta samples, were sent to the Rocky Flats Division of the Dow Chemical Company, Golden, Colorado, for chemical analysis. No results have as yet been received from the Dow Chemical group. It is hoped that the radiochemical analysis will be done in our laboratory at Colorado State University next year.

The determination of the amount of plutonium implanted in each dog must await the chemical analysis of the left paw (implant site) for each dog. Therefore, it was not possible to plot build-up curves for the accumulation of plutonium in the left superficial cervical lymph node (L.S.C.L.N.).

The in vivo counting data indicate a very gradual build up of plutonium in the L.S.C.L.N. of all dogs implanted. These are similar to data obtained with $^{239}\text{PuO}_2$ (air oxidized), $^{239}\text{PuO}_2$ (high fired) and $\text{Pu}(\text{NO}_3)_4$ contaminated dogs experiment previously conducted in this laboratory (Figures 34, 35, 36, 37, 38, 39, 40, and 41).

Figure 34. Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA.
Group I.

NET COUNTS / 40
ONDS

Figure 35. Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA.
Group II.

Figure 36. Change in the accumulation of Pu in the left superficial cervical lymph node with time for dogs treated and not treated with DTPA.
Group III.

Figure 37. Change in the Accumulation of Pu in the
left superficial cervical lymph node with
time for dogs treated and not treated with
DTPA.
Group IV.

Figure 38. Clearance of PuF_4 from injection without DTPA treatment. All groups averaged.

Figure 39. Clearance of PuF_4 from injection following DTPA treatment. All groups averaged.

Figure 40. Build-up and clearance of PuF_4 from
the L.S.C.L.N. without DTPA treatment.
All groups averaged.

Figure 41. Build-up and clearance of PuF_4 from the L.S.C.L.N. following DTPA^{4-} treatment. All groups averaged.

VIII. PLUTONIUM CHLORIDE CONTAMINATED WOUND STUDY

The purpose of this research project which started in April 1972, is to study the movement of ^{239}Pu and ^{241}Am from subcutaneous injections of PuCl_3 .

A. Experimental Design

For this study, the chemical form of plutonium used was PuCl_3 . Thirty-two male beagle dogs were used for this study. They were divided into four groups according to the following experimental design:

Table 47

Experimental Design

Time from Implant to Necropsy	Number of Dogs Contaminated	Number of Dogs Contaminated and Receiving DTPA
2 weeks (Group I)	4	3
2 months (Group II)	4	4
6 months (Group III)	5	4
12 months (Group IV)	4	4

Blood, urine and fecal samples were collected at scheduled intervals and according to the procedures described for the other chemical forms.

The dogs were obtained from the barrier-maintained colony of the Collaborative Radiological Health Laboratory of Colorado State University. They were maintained in accordance with the provisions of the Laboratory Animal Welfare Act (1967). The dogs were kept

in metabolic cages during sampling periods. They remained in outside runs otherwise. Implant procedures were as previously described and were performed under the same surveillance of a radiation monitoring officer from the Health Physic Group of Dow Chemical Division, Rocky Flats Plant. The detection procedures were as previously described.

The plutonium chloride used in this experiment was supplied by the Dow Chemical-Rocky Flats Plant with the following characteristics.

Table 48

Physical, Chemical and Isotopic Characteristics
of PuCl_3 Supplied by Dow Chemicals

Pu	2.80 $\mu\text{g/ml}$
Total alpha activity	20.7 $\mu\text{Ci/ml}$
^{238}Pu	0.098% wt
^{239}Pu	93.853 wt
^{240}Pu	5.770 wt
^{241}Pu	0.344 wt (12-18-72)
^{242}Pu	0.019 wt
^{241}Am	518 ppm bosidon Pu (12-18-72)
pH	1.3

0.25 ml of this solution was injected into the left metacarpal area of each dog used in this experiment.

B. Preliminary Results

While the actual experiment has been completed, the analysis of the tissue samples collected at necropsies as well as blood and excrement samples have not been performed for reasons previously mentioned. The only usable data was obtained from the thin-window NaI wound counter used to detect the activity at the injection site and at the L.S.C.L.N. The averaged results for the two treatment groups are shown in Figures 42, 43, 44, and 45. The plot of the data revealed that the curves parallel closely the results of the previous studies. However, statistical analysis has not yet been performed to determine whether a better fit can be made with an exponential model or a power function model. There are some indications that DTPA treatment does not speed the movement of plutonium. However, this will have to be investigated through careful statistical analysis. More precise computer analyses are being developed to investigate various hypothesis and determine least square fit regression curves for the data.

C. Conclusion

It is still premature to draw conclusions from the results of this experiment. While it may be expected that the PuCl_3 will closely parallel the soluble $\text{Pu}(\text{NO}_3)_4$ results, statistical analysis will be needed to demonstrate such similar effects. In anticipation of the PuCl_3 data from the tissue sample analysis, possible models derived from the $\text{Pu}(\text{NO}_3)_4$ data are being prepared. An important part of the conclusions to be drawn from this experiment will be the comparison of the PuCl_3 results to each of the other four

Figure 42. Clearance of PuCl_3 from the injection site following DTPA treatment. All groups averaged.

Figure 43. Clearance of PuCl_3 from the injection site without DTPA³ treatment. All groups averaged.

TREATMENT GROUP - NO DTPA

Figure 44. Build-up and translocation of PuCl_3 from the L.S.C.L.N. following DTPA^3 treatment. All groups averaged.

Figure 45. Build-up and translocation of PuCl_3
from the L.S.C.L.N. without DTPA
treatment. All groups averaged

chemical forms tested.

Only this kind of comparative analysis will provide insight into the various parameters and mechanisms involved. In addition, a closer look at the relative translocation of plutonium and americium will be possible. The data obtained for this experiment was derived from a two channel energy discrimination of the 60 ke V americium x-ray and the 17 ke V x-rays of both plutonium and americium. This should allow the analysis of the relative translocation of the two elements in the short time period immediately following implant.

LITERATURE CITED

- Johnson, L. J., E. H. Bull, J. L. Lebel and R. L. Watters. Kinetics of Lymph Nodes Activity Accumulation from Subcutaneous PuO_2 Implants. Health Physics 18, 416-418. 1970.
- Lebel, J. L., E. H. Bull, L. J. Johnson and R. L. Watters. Lymphosarcoma Associated with Nodal Concentration of Pu in Dogs: A Preliminary Study. Am J. Vet. Res. 31, 1513-1516. 1970.
- Watters, R. L., J. L. Lebel, L. J. Johnson and E. H. Bull. A Study of the Translocation of Plutonium and Americium from Wounds: First Technical Progress Report. COO-1787-1. 1968.
- Johnson, L. J. Relative Translocation and Distribution of Pu and Am from Experimental PuO_2 Subcutaneous Implants in Beagles: Special Report to U.S.A.E.C. COO-1787-6. 1969.
- Watters, R. L., J. L. Lebel, L. J. Johnson and E. H. Bull. A Study of the Translocation of Plutonium and Americium from Wounds: Second Technical Progress Report. COO-1787-8. 1969.
- Watters, R. L., J. L. Lebel, R. W. Bistline, G. E. Dagle and L. S. Gomez. A Study of the Translocation of Plutonium and Americium from Puncture Wounds: Third Technical Progress Report. COO-1787-11. 1971.
- Gomez, L. S. Lymph Transport of $^{239}\text{PuO}_2$ in Dogs: Special Report to U.S.A.E.C. COO-1787-17. 1973.
- Dagle, G. E. Lymph Node Clearance of Plutonium from Subcutaneous Wounds in Beagles: Special Report to U.S.A.E.C. COO-1787-18. 1973.
- Bistline, R. L. Translocation Dynamics of $^{239}\text{-Plutonium}$: Special Report to U.S.A.E.C. COO-1787-20. 1973.
- Dagle, G. E., J. L. Lebel, R. D. Phemister, R. L. Watters and L. S. Gomez. Translocation Kinetics of Plutonium in the Popliteal Lymph Nodes of Beagles. Health Physics J. (accepted for publication) 1974.
- Dagle, G. E., R. D. Phemister, J. L. Lebel, R. S. Jaenke and R. L. Watters. Plutonium Lymphadenitis in Beagles. Rad. Research J. (accepted for publication) 1974.
- Schallberger, J. A. The Plutonium-Lymph Relationship: Special Report to U.S.A.E.C. COO-1787-24. 1974.
- Lebel, J. L. and R. L. Watters. A Study of the Translocation of Plutonium and Americium from Puncture Wounds: Fourth Technical Progress Report. COO-1787-16. 1972.

DEGREES GRANTED

1. Johnson, Lamar J.	Ph.D.	1969
2. Bull, Emory H.	M.S.	1970
3. Gomez, Leo G.	Ph.D.	1973
4. Dagle, Gerald E.	Ph.D.	1973
5. Bistline, Robert L.	Ph.D.	1973
6. Schallberger, Joseph A.	Ph.D.	1974

LIST OF PUBLICATIONS AND PAPERS

- C00-1787-1 A Study of the Translocation of Plutonium and Americium from Puncture Wounds. First Technical Progress Report.
- C00-1787-2 The Movement of Plutonium and Americium from Wound Sites. Paper
- C00-1787-3 Relative Tissue Distribution of Pu and Am from Experimental PuO₂ Contaminated Puncture Wounds.
- C00-1787-4 Kinetics of Lymph Node Activity Accumulation from Subcutaneous PuO₂ Implants.
- C00-1787-5 Lymphosarcoma Associated with Nodal Concentration of Plutonium in Dogs. A Preliminary Report.
- C00-1787-6 Relative Translocation and Distribution of Pu and Am from Experimental PuO₂ Subcutaneous Implants in Beagles. Lamar Johnson.
- C00-1787-7 Relative Distribution of Plutonium and Americium Following Experimental PuO₂ Implants. L. J. Johnson, R. L. Watters, C. R. Lagerquist, and S. E. Hammond.
- C00-1787-8 A Study of the Translocation of Plutonium and Americium from Puncture Wounds. Second Technical Progress Report.
- C00-1787-9 Retention of Subcutaneously Placed PuO₂ in the Beagle. L. J. Johnson, R. L. Watters, C. R. Lagerquist, and S. E. Hammond. Paper. H. P. Society 15th Annual Meeting.
- C00-1787-10 The Distribution of Pu and Am: Subcutaneous Administration of PuO₂ and the Effect of Chelation Therapy. L. J. Johnson, R. L. Watters, J. L. Lebel, C. R. Lagerquist, and S. E. Hammond.
- C00-1787-11 A Study of the Translocation of Plutonium and Americium from Puncture Wounds. Third Technical Progress Report. June 25, 1971.
- C00-1787-12 A Study of the Translocation of Plutonium and Americium from Puncture Wounds. Summary Report, 1968 to June 1971.
- C00-1787-13 Progress in the Beagle Studies at Colorado State University, R. L. Watters and J. L. Lebel. Health Physics: 22-534-537. 1972.
- C00-1787-14 A Study of Translocation Dynamics of Plutonium and Americium from Simulated Puncture Wounds in Beagle Dogs. R. W. Bistline, R. L. Watters, and J. L. Lebel. Health Physics: 22-629-634. 1972.

- COO-1787-15 The Effect of Lymph Node Removal on PuO_2 Translocation. L. S. Gomez, J. L. Lebel, and R. L. Watters. Health Physics: 22-833-836. 1972.
- COO-1787-16 A Study of the Translocation of Plutonium and Americium from Puncture Wounds. Fourth Technical Progress Report. June 30, 1972.
- COO-1787-17 Lymph Transport of $^{239}\text{PuO}_2$ in Dogs. L. S. Gomez and J. L. Lebel. Special Report to W.S.A.E.C. 1973.
- COO-1787-18 Lymph Node Clearance of Plutonium from Subcutaneous Wounds in Beagles. G. E. Dagle and J. L. Lebel. Special Report to U.S.A.E.C. 1973.
- COO-1787-19 Effects of Lymphadenectomy on $^{239}\text{PuO}_2$ Translocation in Dogs. L.G. Gomez, R. W. Bistline, G. E. Dagle, J. L. Lebel and R. W. Watters. Health Physics J. Vol. 26. 1974.
- COO-1787-20 Translocation Dynamics of $^{239}\text{Plutonium}$. R. W. Bistline, and J. L. Lebel. Special Report to U.S.A.E.C. 1973.
- COO-1787-22 Translocation Kinetics of Plutonium in the Popliteal Lymph Nodes of Beagles. G. E. Dagle, J. L. Lebel, R. D. Phemister, R. L. Watters and L. G. Gomez. Accepted for publication, Health Physics J. 1974.
- COO-1787-23 Plutonium Induced Lymphadenitis in Beagles. G. E. Dagle, R. D. Phemister, J. L. Lebel, R. S. Jaenke and R. L. Watters. Accepted for publication, Rad. Research J. 1974.
- COO-1787-24 The Plutonium-Lymph Relationship. J. A. Schallberger and J. L. Lebel. Special Report to U.S.A.E.C. 1974.