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OFFICE OF VICE PRESIDENT FOR RESEARCH
PROPOSAL SECTION

July 12, 1971

Mr. Harold N. Miller
Director of Contracts Division
U. S. Atomic Energy Commission
Chicago Operations Office
9800 South Cass Avenue
Argonne, Illinois 60439

Dear Mr. Miller,

Enclosed are six (6) copies of a progress report (Renewal of Contract No. AT(11-1)-1787)entitled "A Study of the Translocation of Plutonium and Americium from Puncture Wounds" with Robert L. Watters and Jacques L. Lebel as Principal Investigators.

Six copies of this report were sent to your office on June 29, 1971, but Dr. Watters has discovered that Table 14 (pages 43 and 44) was inadvertently omitted from the previous progress report submitted. Therefore, would you kindly destroy the copies of the report previously submitted?

Sincerely yours,

James F. Brown

James F. Brown
Contracts and Grants Administrator

JFB/rv

Enclosure: 6 copies progress report

cc: Robert L. Watters

DISCLAIMER

COO 1787-12

Summary Report

of the

Department of Radiology and Radiation Biology
Colorado State University
Fort Collins, Colorado

TO

U. S. Atomic Energy Commission
Chicago Operations Office
Argonne, Illinois

ON

Contract No. AT(11-1)-1787

A STUDY OF THE TRANSLOCATION OF PLUTONIUM AND AMERICIUM FROM WOUNDS

FOR THE PERIOD

April 1, 1968 to June 15, 1971

SUBMITTED BY

R. L. Watters and J. L. Lebel
Principal Investigators

June 25, 1971

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 (diethylenetriaminepentaacetate) 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 and high fired plutonium oxide (850°C) 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. *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.

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 lymphosarcoma 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 15, 1971.

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 receive 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 Table 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 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

TABLE 1. Particle Sized Distribution of Implant Material

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

TABLE 2.

Quantitative Chemical and Nuclidic Composition of Implant Material

Element	Analysis Date	Quantity Present
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

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

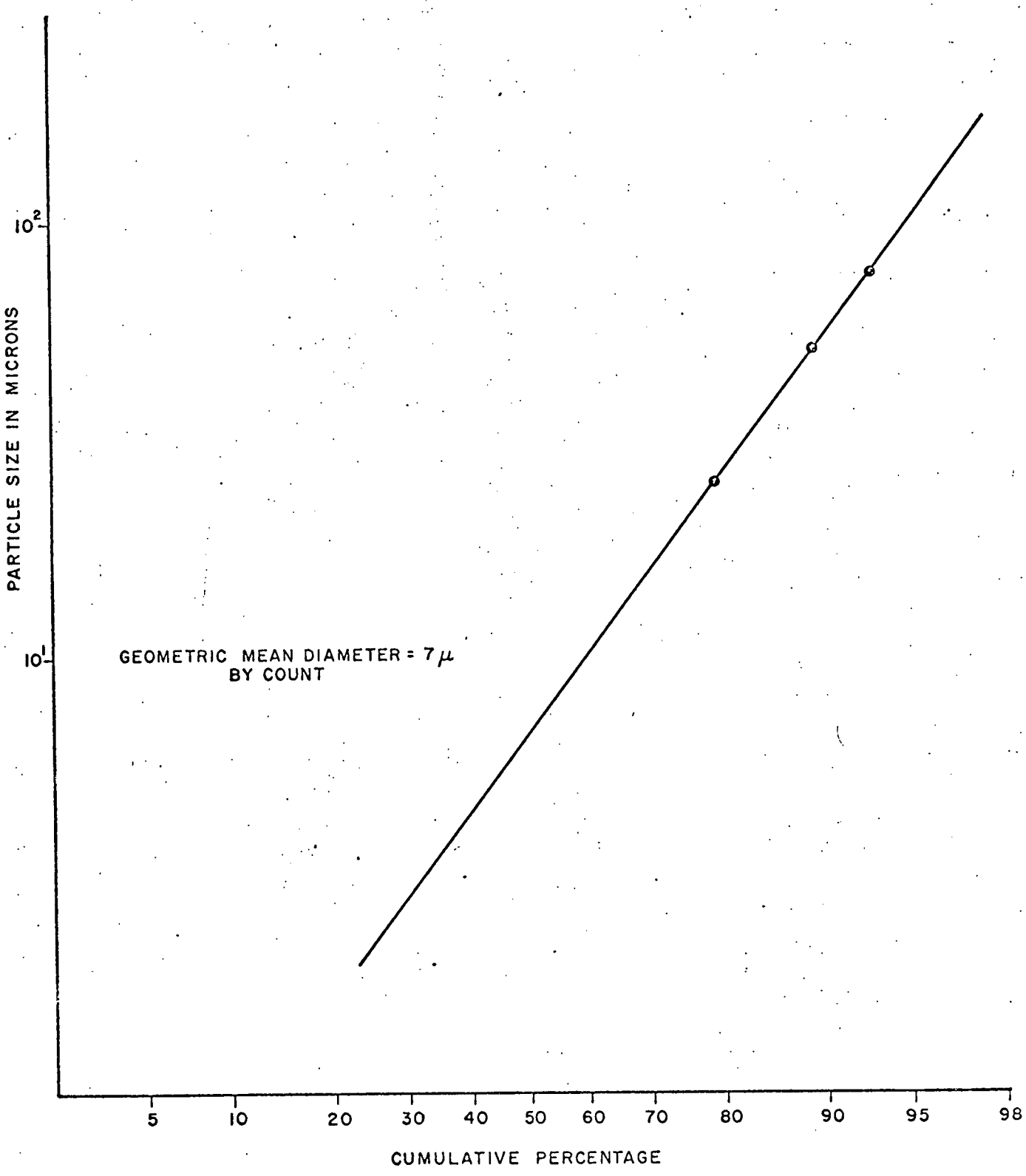

Figure 1. Particle size distribution of PuO_2 implant material.

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 radioiodinated 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 for dogs not treated with DTPA. A curve not significantly different was obtained for dogs which were treated with DTPA (Fig. 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 samples 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 COO-1787-6, COO-1787-7 and COO-1787-8. A ratio factor greater than one indicates a relative 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.

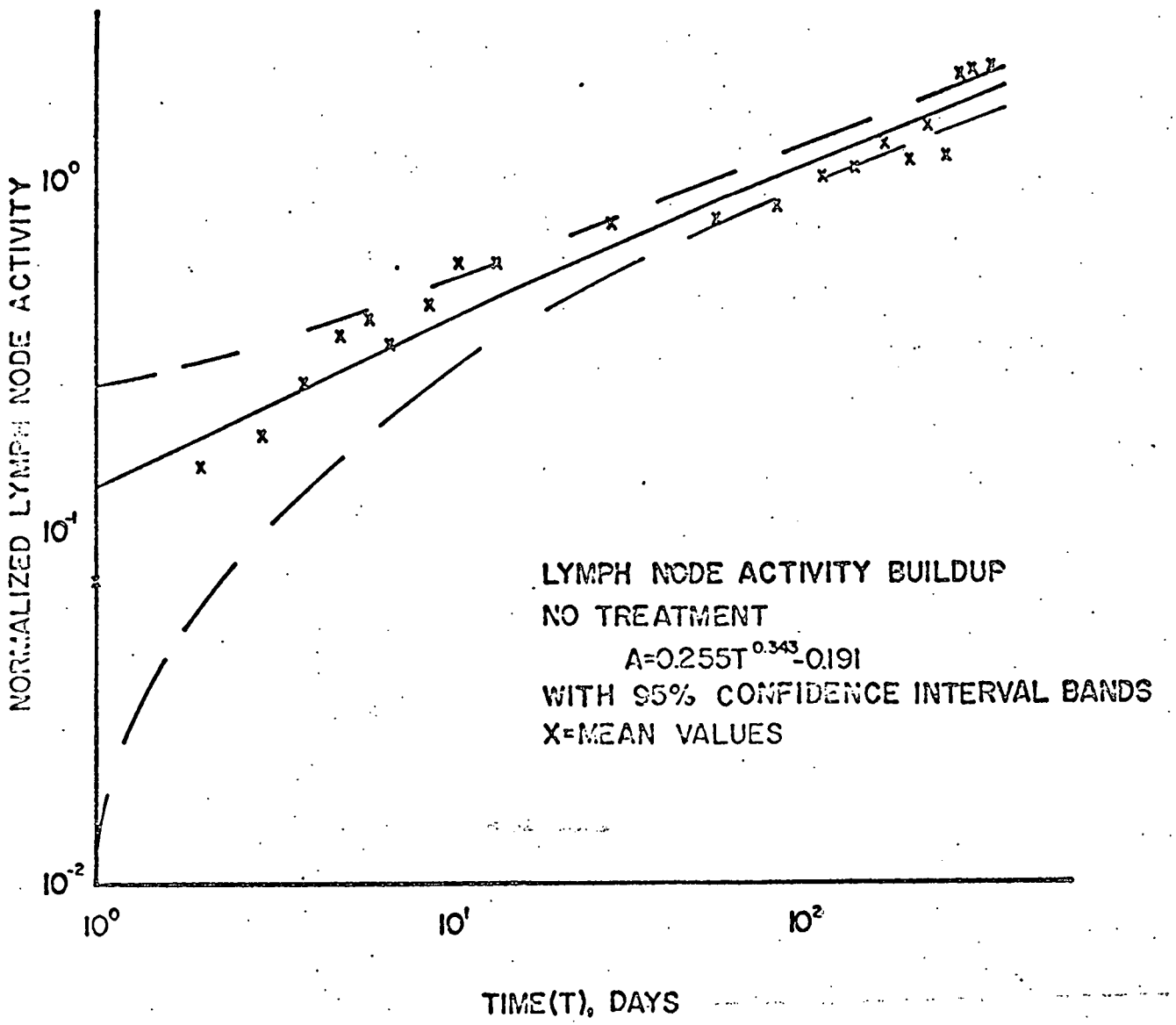
NORMALIZED LYMPH NODE ACTIVITY
10⁰
10⁻¹
10⁻²
10⁰
10¹
10²
TIME(T), DAYS
LYMPH NODE ACTIVITY BUILDUP
NO TREATMENT
A=0.255T^0.343-0.191
WITH 95% CONFIDENCE INTERVAL BANDS
X=MEAN VALUES

Figure 2.

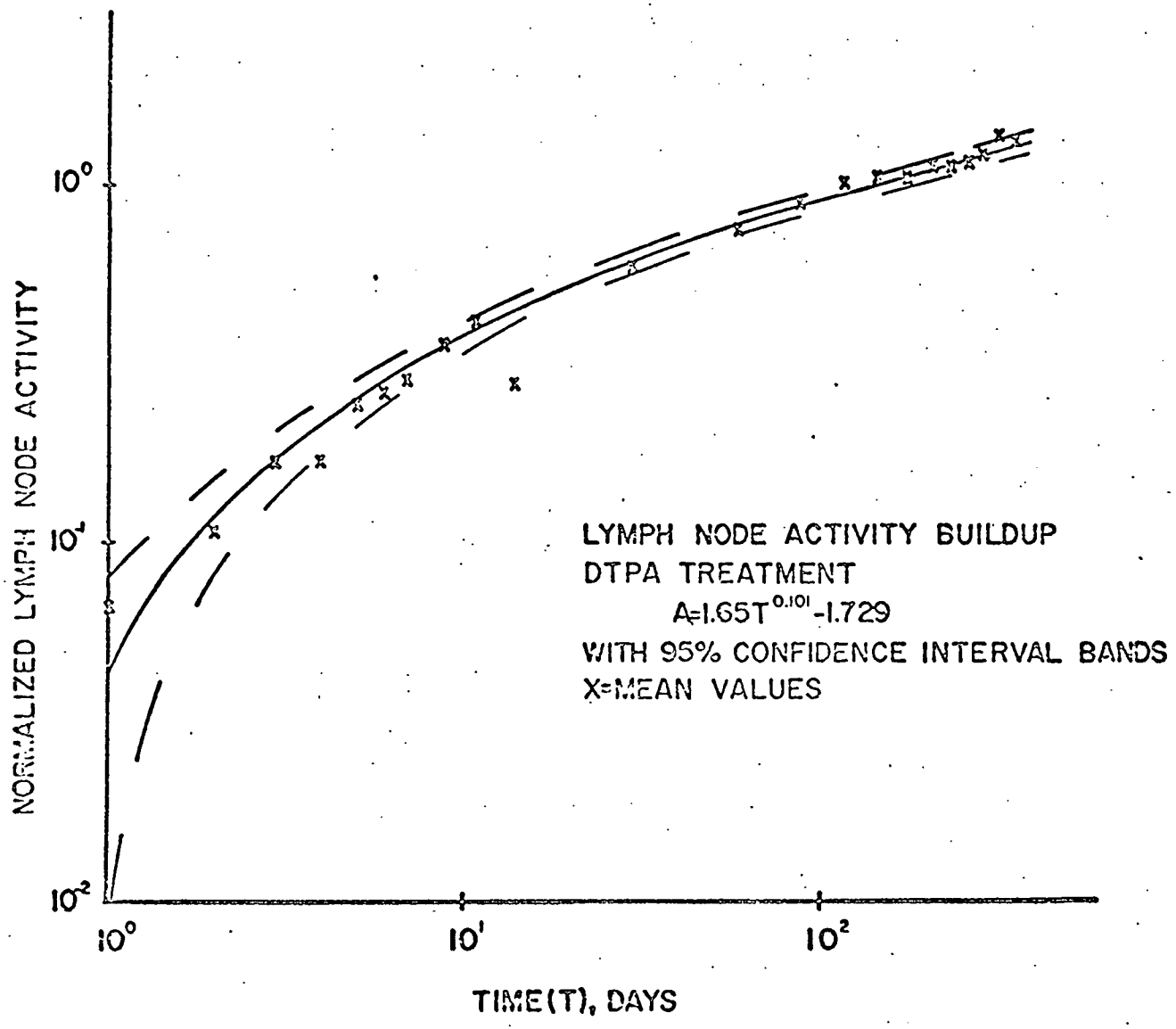
LYMPH NODE ACTIVITY BUILDUP
DTPA TREATMENT
A=1.65T^0.101-1.729
WITH 95% CONFIDENCE INTERVAL BANDS
X=MEAN VALUES
NORMALIZED LYMPH NODE ACTIVITY
10^0
10^1
10^2
10^0
10^1
10^2
TIME(T), DAYS

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

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 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 other 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.

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

4. Pu and Am Distribution

The average fractions of the implanted Pu translocated to 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

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).

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.

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. The description of the dogs follows:

Dog No.	Date Born	Implanted	Estimated Amount Implanted*
397	12-67	6-23-69	150 μg
412	12-67	6-23-69	140 μg
4737	5-9-68	6-23-69	160 μg
410	12-67	6-23-69	60 μg
4873	6-3-68	12-16-69	150 μg
4879	6-6-68	12-16-69	140 μg
4961	7-30-68	12-16-69	150 μg
4962	7-30-68	12-16-69	150 μg
4963	7-30-68	12-16-69	140 μg
4965	7-30-68	12-16-69	150 μg

* Final amounts to be determined after necropsy.

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 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.

Table 10. PuO_2 long term dogs: Summary of clinical pathology.

	Dogs Injected with PuO_2-Long Term						Controls			
	9 dogs 3-16-71		8 dogs 4-22-71		9 dogs 6-1-71		22-40 dogs 3-16-71		10 dogs 6-1-71	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Biochemistry (Serum)										
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 3-16-71		8 dogs 4-22-71		9 dogs 6-1-71		22-40 dogs 3-16-71		10 dogs 6-1-71	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Thyroid										
T-3, %	41.9	2.09					40.9	2.16		
T-4, MCG %	2.60	0.84					2.05	0.78		
Lactate Dehydrogenase Isoenzymes										
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		
Complete Blood Count (CBC)										
Red blood cells, 1 x $10^6/mm^3$	8.16	0.66	8.53	0.27	8.22	0.50	7.60	0.40	7.26	0.37
White blood cells, 1 x $10^3/mm^3$	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 1-30-70		10 dogs 4-29-70		10 dogs 8-13-70		9 dogs 10-20-70		9 dogs 12-29-70	
	Mean	S. D.	Mean	S. D.	Mean	S D.	Mean	S. D.	Mean	S. D.
Biochemistry (Serum)										
Total protein, g %	6.30	0.48	5.91	0.40	6.26	0.56	6.30	0.41	6.37	0.27
Complete Blood Count (CBC)										
White blood cells, 1 x $10^3/mm^3$	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 No Treatment		4 dogs DTPA		22-40 dogs		2 dogs-DTPA No Plutonium		10 dogs	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Biochemistry (Serum)										
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
Thyroid										
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 No Treatment		4 dogs DTPA		22-40 dogs		2 dogs-DTPA No Plutonium		10 dogs	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Lactate Dehydrogenase Isoenzymes										
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				
Complete Blood Count (CBC)										
Red blood cells, 1 x 10^6/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 x 10^3/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 No Treatment		4 dogs DTPA		4 dogs No Treatment		4 dogs DTPA	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Biochemistry (Serum)								
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
Thyroid								
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 No Treatment		4 dogs DTPA		4 dogs No Treatment		4 dogs DTPA	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Lactate Dehydrogenase Isoenzymes								
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				
Complete Blood Count (CBC)								
Red blood cells, $1 \times 10^6/mm^3$	7.96	0.19	8.08	0.41	7.81	0.19	7.55	0.46
White blood cells, $1 \times 10^3/mm^3$	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 EXPERIMENT

A. Materials and Methods

The experiment used 48 adult 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, 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 plutonium nitrate solution was in a 2.0 N HNO_3 solution and the activity concentration was about 15 μCi/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. Solution calibration was performed each time a group of dogs was injected. By use of a tuberculin syringe and needle, an aliquot of the plutonium nitrate solution (0.10 ml) was drawn into the syringe. The syringe was attached to the needle previously positioned in the dog's foot and the injection was made.

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 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 keV 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).

Microscopic examination was made of routinely prepared hematoxylin and eosin stained sections.

Autoradiographs were prepared from unstained paraffin sections of right and left superficial cervical lymph nodes from each dog in groups 4 and 5 and of the spleens from each dog in group 4. The sections were exposed to Kodak AR-10 stripping film for 18 days in light-tight boxes and then developed with Kodak D-19 using standard procedures.

The specimens prepared for electron microscopy consisted of left and right cervical lymph nodes and the spleen from each dog in groups 3 (2 months) and 6 (12 months). The tissues were removed from the anesthetized animals and immediately fixed in cold 2% gluteraldehyde, post-fixed in osmium tetroxide, routinely processed, and embedded in Epon.

B. Results

1. Radiochemical Assay

The Rocky Flats Bioassay Laboratory has not completed the radiochemical assays for this experiment because of the increased sample load after the May 1969 fire and because of a strike which occurred later. More laboratory technicians were hired to relieve the back-log of samples but the analyses for Am and the more difficult tissue samples are still not finished.

The results of Pu assays for liver and proximal end of the femur have been received for several groups of dogs (Table 11). Although the data are not complete the effect of DTPA therapy upon Pu deposition in the liver is obvious. DTPA treatment dogs have liver burdens lower by a factor 8 than in the untreated dogs. The proximal end of the femur also shows a trend toward lower concentrations with DTPA therapy but this has not been tested statistically because of incomplete data. The effect of DTPA therapy upon Pu

Table 11. Plutonium in the liver and proximal end of the femur after $Pu(NO_3)_4$ implant.

Group	Treatment	Liver (fraction of implant)	Proximal End of Femur (fraction of implant/gm)
2 weeks	None	0.0407 ± 0.0350	$7.88 \times 10^{-5} \pm 4.72 \times 10^{-5}$
	DTPA	0.0054 ± 0.0050	$1.25 \times 10^{-5} \pm 4.5 \times 10^{-6}$
1 month	None	0.0289 ± 0.0257	$5.19 \times 10^{-5} \pm 2.63 \times 10^{-5}$
	DTPA	0.0038 ± 0.0028	$6.18 \times 10^{-5} \pm 2.33 \times 10^{-5}$
2 months	None	0.0267 ± 0.0202	$12.6 \times 10^{-5} \pm 5.34 \times 10^{-5}$
	DTPA	0.00093 ± 0.00049	$3.43 \times 10^{-5} \pm 3.37 \times 10^{-5}$
3 months	None	Not	$7.11 \times 10^{-5} \pm 1.21 \times 10^{-5}$
	DTPA	Completed	$2.64 \times 10^{-5} \pm 3.43 \times 10^{-6}$
6 months	None	0.0355 ± 0.0383	$15.2 \times 10^{-5} \pm 1.5 \times 10^{-5}$
	DTPA	0.0042 ± 0.0040	$4.86 \times 10^{-5} \pm 1.89 \times 10^{-5}$
12 months	None	Not	Not
	DTPA	Completed	Completed

deposition after $Pu(NO_3)_4$ implant is different than we observed with air oxidized Pu. No treatment effect for Pu was noted in that earlier experiment although a treatment effect was seen for Am in the liver and skeleton.

Untreated dogs in this experiment show higher depositions of Pu in the liver and in the proximal end of the femur by one order of magnitude compared to the data in the air oxidized PuO_2 experiment (Table 12). This is not surprising considering the more soluble nature of $Pu(NO_3)_4$ which allows for easier and, therefore, increased translocation from the wound site. This increased amount translocated will be shown later in the discussion of in vivo counting.

Assays for Pu concentrations in blood are not completed but the data received so far are plotted in Figure 4 for untreated dogs and Figure 5 for DTPA treated dogs. The two sets of data appear to follow essentially the same excretion curve. The DTPA treatment curve appears to be consistently lower but more assay data must be accumulated before a statistical analysis can be made.

2. In vivo Counting

Results of the NaI (Tl) counting are displayed in the next four Figures. Figure 6 shows the average fraction of material remaining in the paws of untreated dogs as a function of time. Figure 7 shows the results for dogs treated with DTPA. Figures 8 and 9 show the average fraction of material in the left superficial cervical lymph node as a function of time for untreated and DTPA treated dogs.

These data have not been analyzed statistically but some general observations will be made for this report.

The time relationship for the reduction of the Pu in the implant site appear to be similar for the untreated and DTPA

Table 12. Comparison of Pu deposition for $Pu(NO_3)_4$ with air oxidized PuO_2.

	$Pu(NO_3)_4$	Air Oxidized PuO_2
Liver (average fraction of implant)	0.033	0.00121
Proximal end of femur (fraction of implant/gm)	9.6×10^{-5}	4×10^{-6}

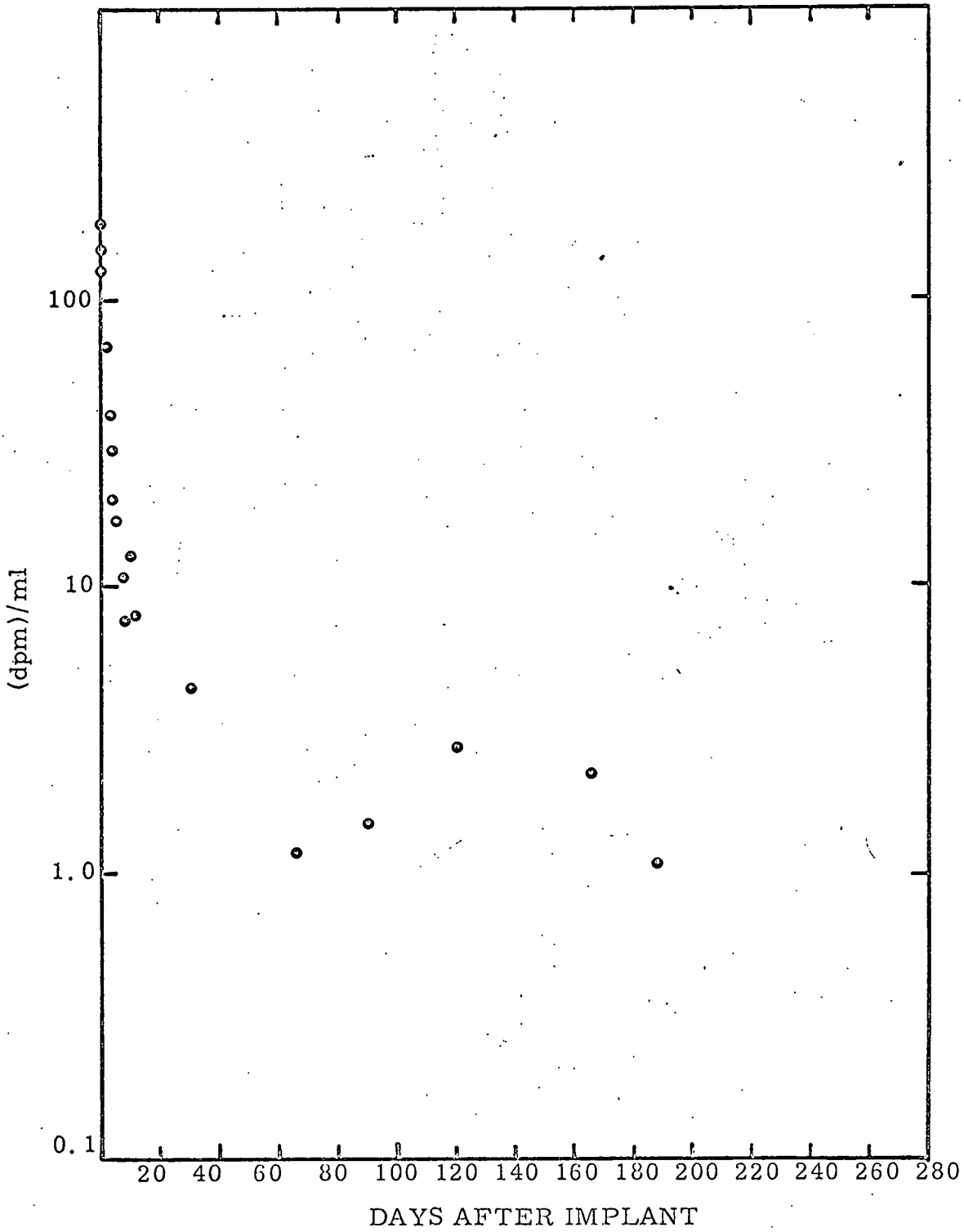

Figure 4. Variation with time of the average blood concentration of Pu in dogs implanted with $Pu(NO_3)_4$. No DTPA was given.

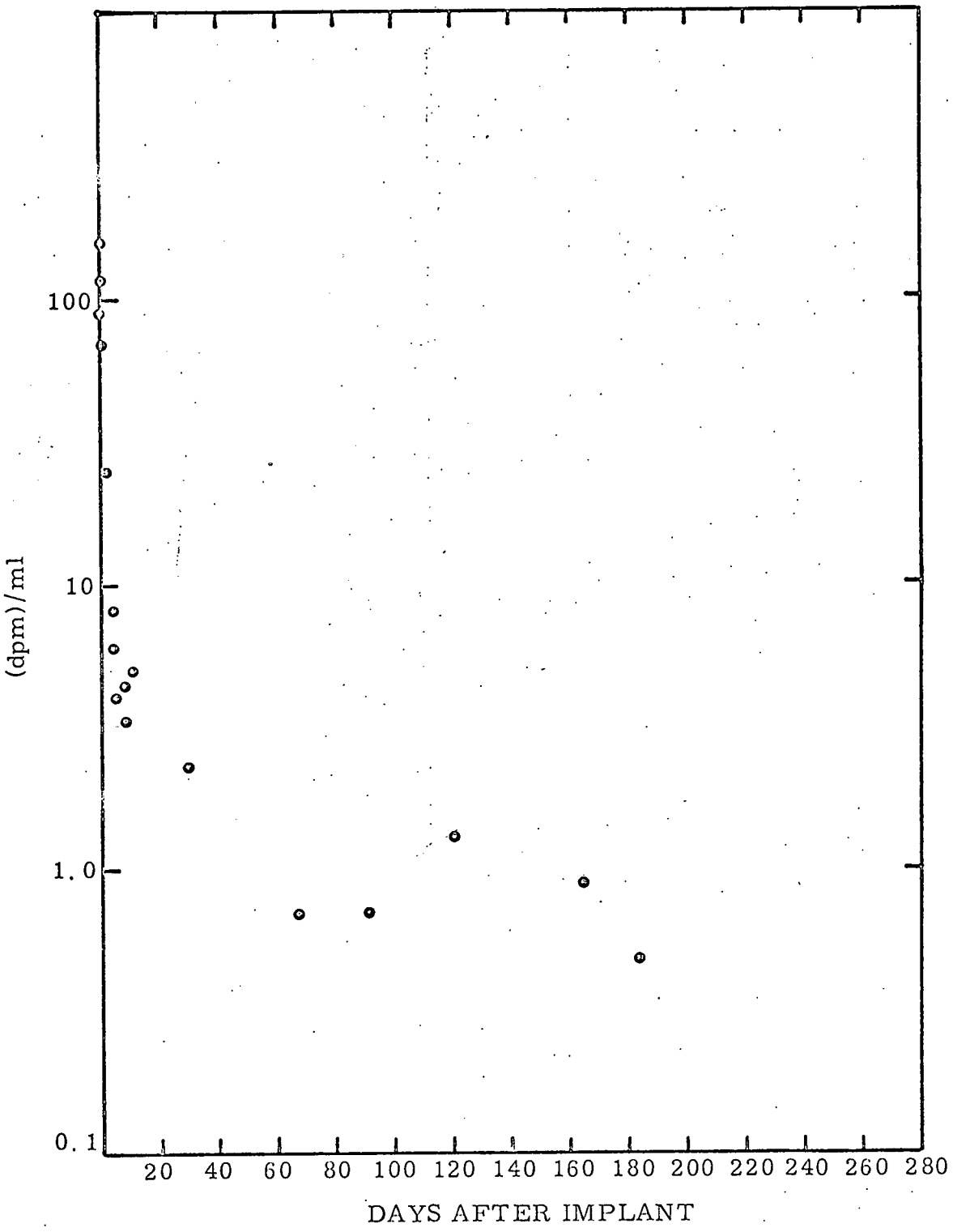

Figure 5. Variation with time of the average blood concentration of Pu in dogs implanted with $Pu(NO_3)_4$. DTPA treatment was given.

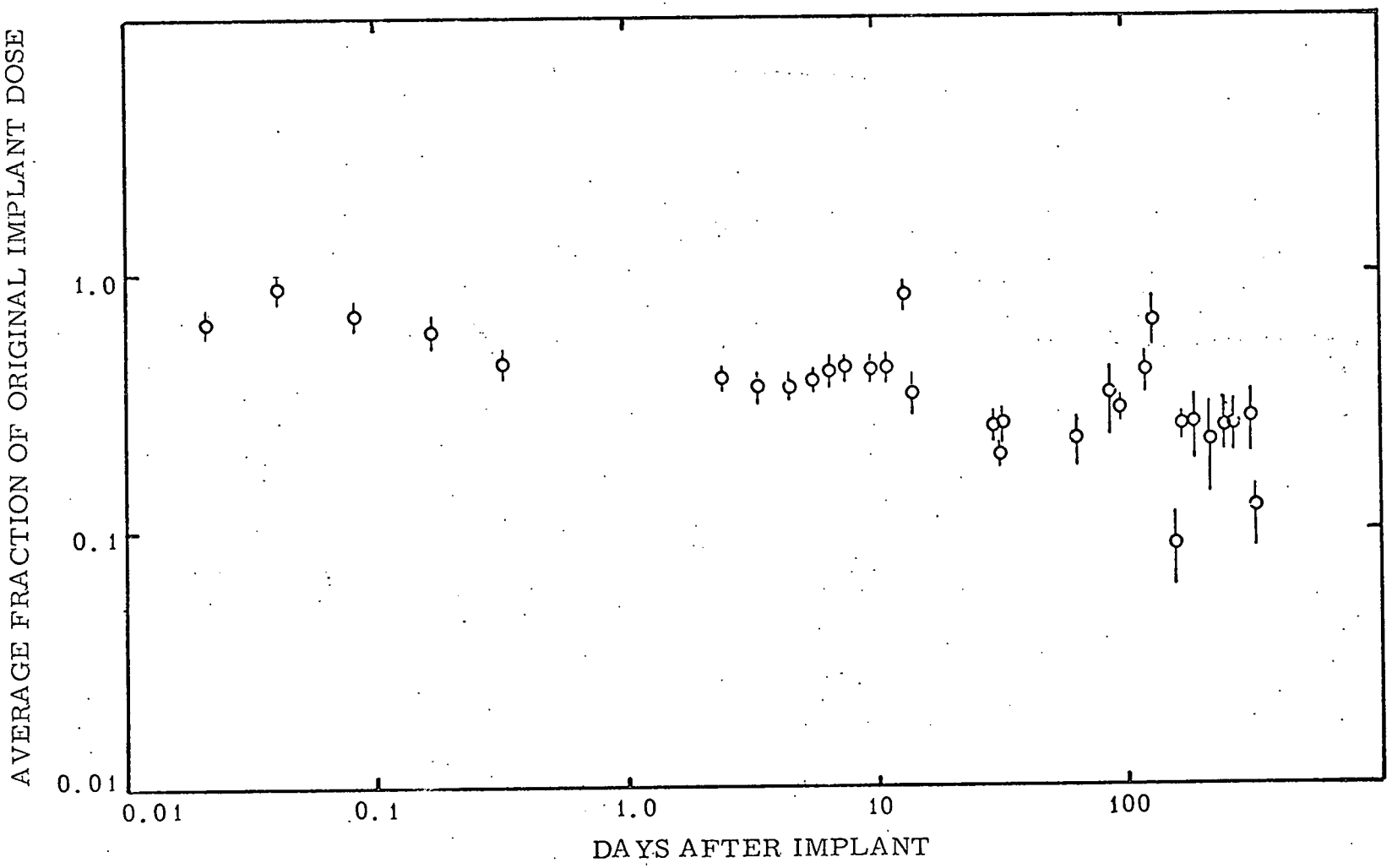

Figure 6. The reduction in fraction of Pu in the implant site with time for dogs not treated with DTPA.

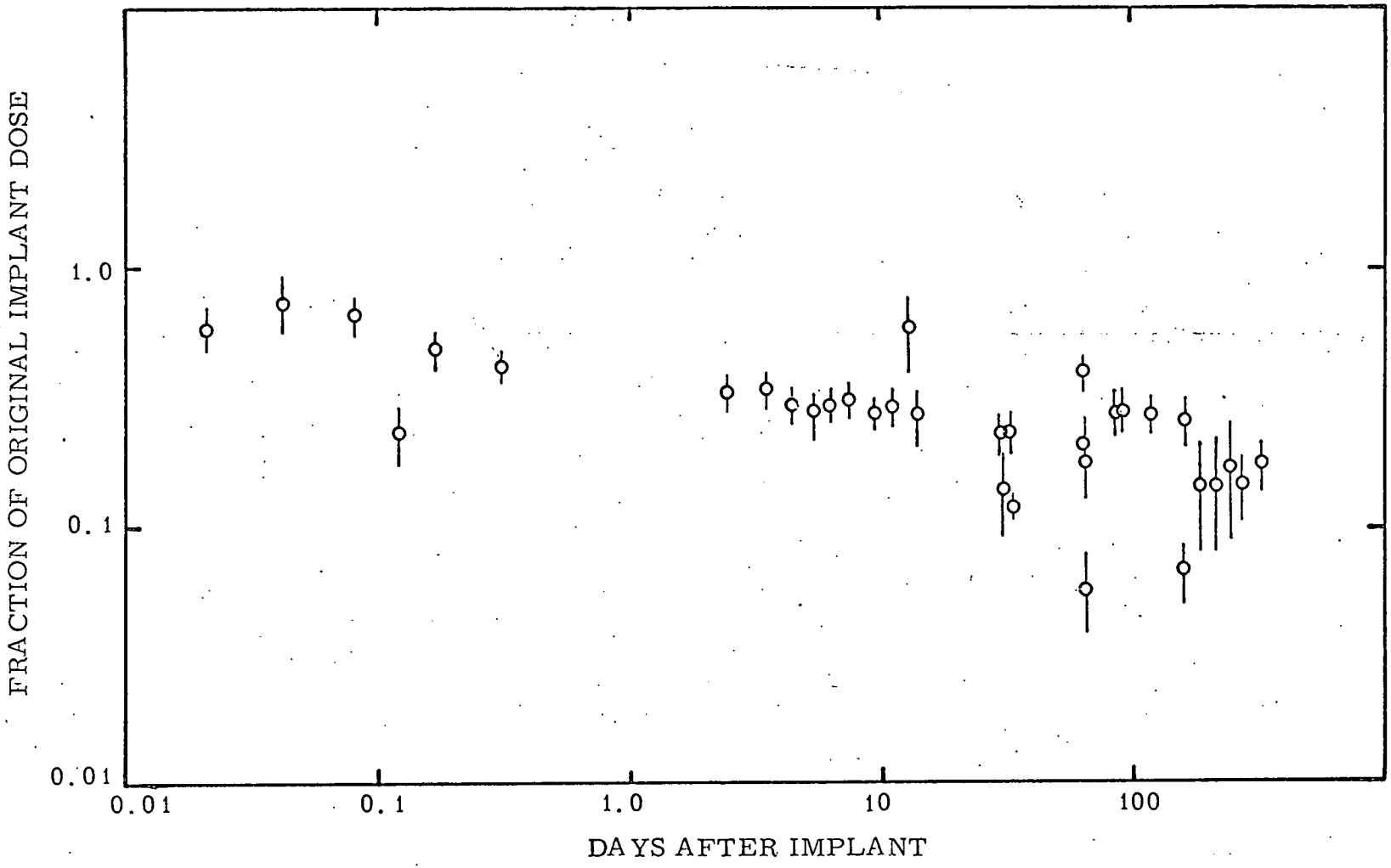

Figure 7. The reduction in the fraction of Pu in the implant site with time for dogs treated with DTPA.

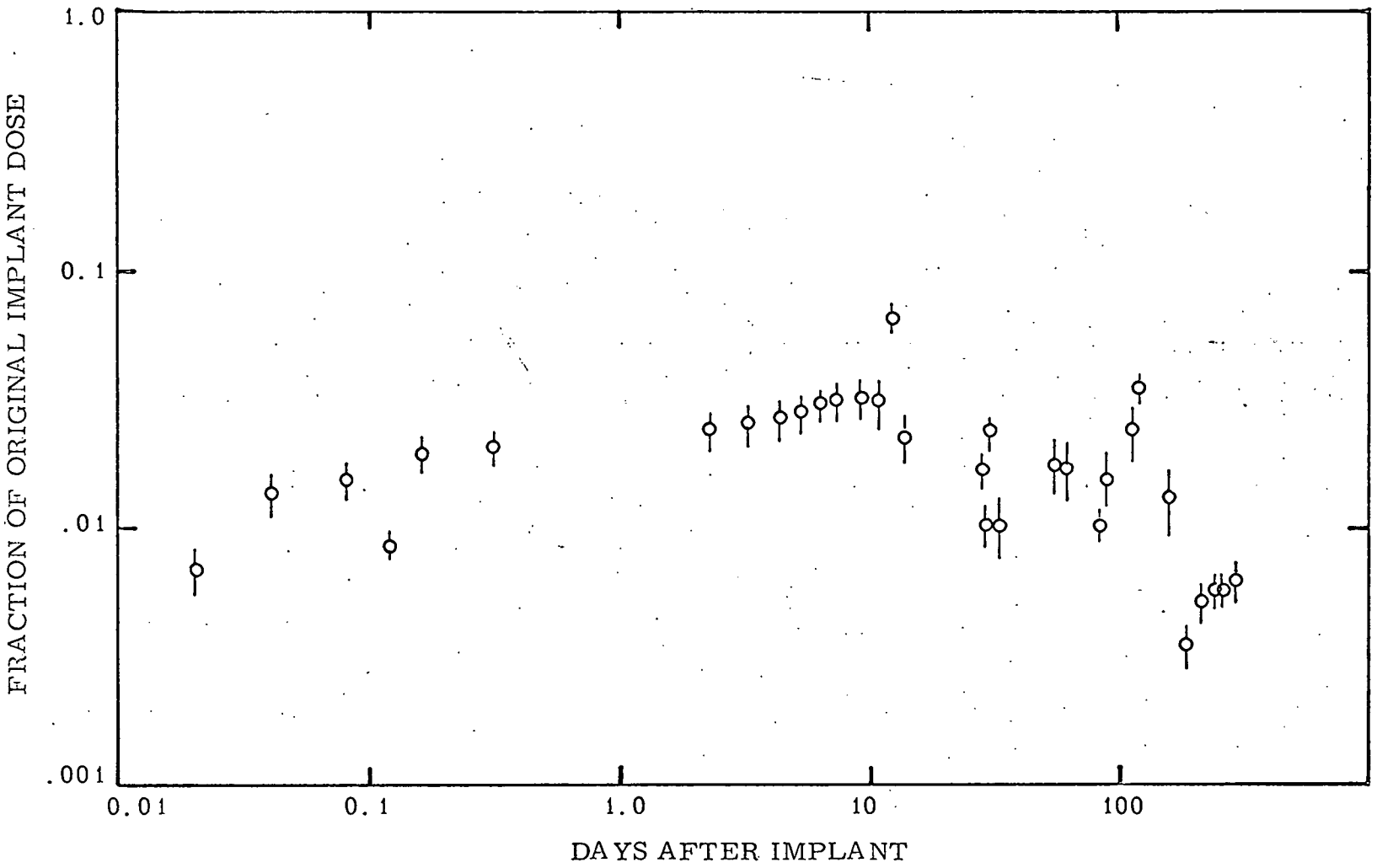

Figure 8. The change in the fraction of Pu in the left cervical lymph node with time for dogs not treated with DTPA.

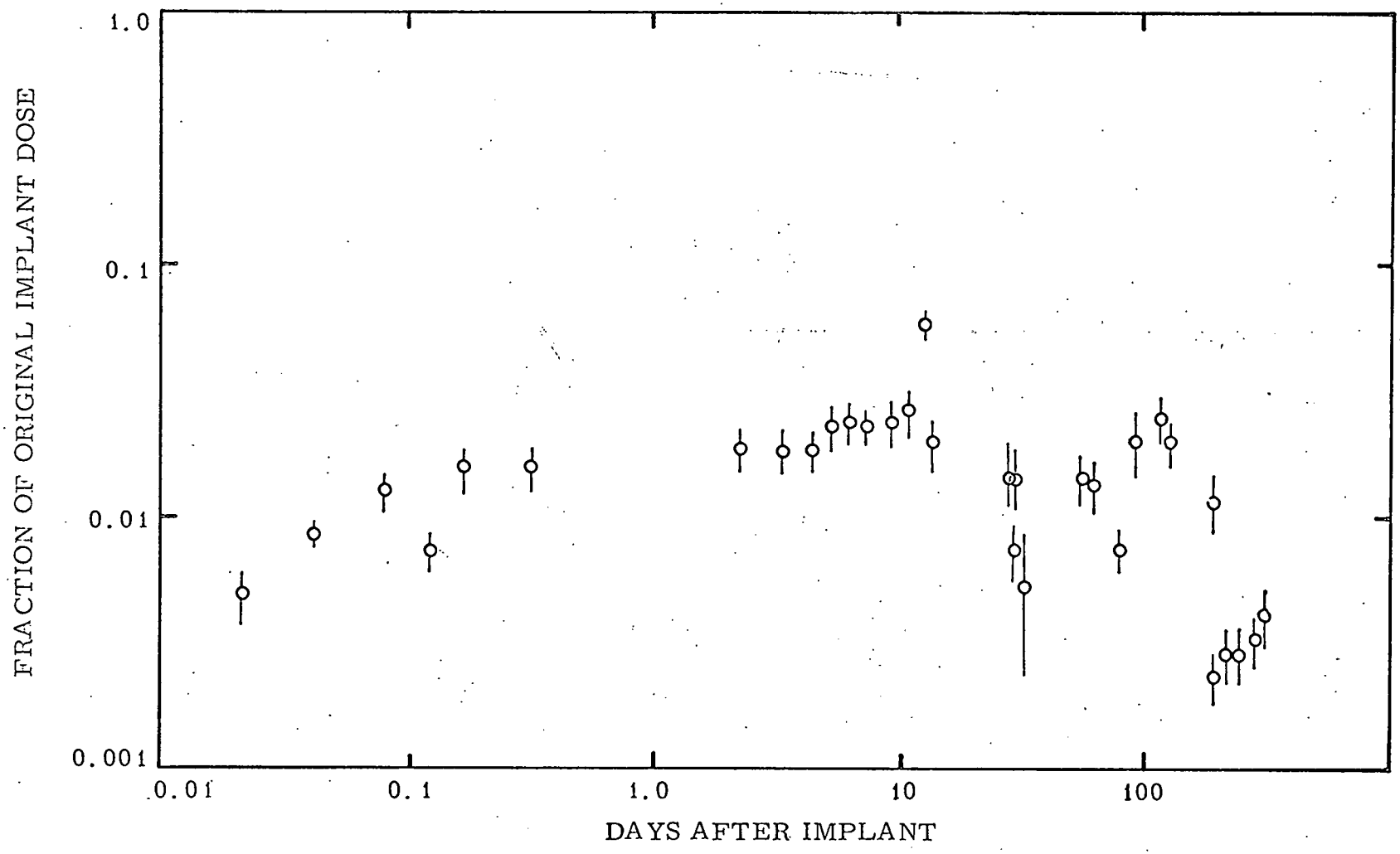

Figure 9. The change in the fraction of Pu in the left cervical lymph node with time for dogs treated with DTPA.

treated dogs. In both sets of data, however, the reduction at the end of one year was greater than for the previous experiment of air oxidized Pu. The data show that the amount of Pu at the implant site has been reduced to about 30 percent of the original, whereas in the first experiment with the air oxidized Pu the reduction was to about 80 percent of the original. At this point we attribute this reduction to the more soluble nature of the plutonium nitrate form.

The kinetics of the Pu deposition at the cervical lymph node appear to be different for the nitrate implant as compared to the air oxidized implants. The nitrate experimental results indicate that the Pu burden goes through a maximum value in five to ten days and then slowly decreases. In the oxide case the burden continued to increase in the lymph node throughout the year of the study. Our data also show that the burden approaches the maximum within the first 24 hours for the nitrate form. In fact, we were able to detect the 17 keV X-ray at the lymph node within five minutes of implant.

Table 13 shows the ratio of the 51.6 keV ^{239}Pu gamma to the 59.6 keV ^{241}Am gamma for the measurements with the Ge (Li) detector which have been taken over the left superficial lymph node and the liver. The data accumulated thus far indicate an enhancement of Pu over Am at the left superficial cervical lymph node and in the liver. Radiochemical assay data will be needed to confirm this observation.

3. Histopathology Studies

The only changes clearly related to the administration of plutonium occurred in the left superficial cervical lymph nodes from 7 of 8 dogs in group 5 (6 months). These changes (see Table 14) consisted of lymphoid atrophy, scar tissue formation and granulomatous inflammation. The atrophy was

Table 13. Pu enhancement determined by in vivo Ge (Li) detector measurements [$Pu(NO_3)_4$ dogs].

Dog Number	Time After Implant	Organ Monitored	Treatment	$\frac{(Pu/Am)_{organ}}{(Pu/Am)_{implant}}$ = Ratio Factor
5147	8 weeks	L.S.C.L.N.*	None	8.5
	8 weeks	Liver		9.4
5024	6 weeks	L.S.C.L.N.	None	6.5
	6 weeks	Liver		5.3
5064	8 weeks	L.S.C.L.N.	DTPA	11.2
	8 weeks	Liver		9.7

* Left superficial cervical lymph node.

Table 14.

Plutonium Nitrate: 1 Year Subcutaneous Implant Study in Beagles
Summary of Histopathology

Group (Duration)	Treatment	Animal Number	Left Cervical Lymph Node: Increased Macrophages	Left Cervical Lymph Node: Atrophy	Left Cervical Lymph Node: Scar	Left Cervical Lymph Node: Granulomatous Inflammation	Left Cervical Lymph Node: Autoradiographic Activity	Right Cervical Lymph Node: Autoradiographic Activity	Spleen: Hemopoiesis	Spleen: Subcapsular Siderosis	Spleen: Disseminated Siderosis	Spleen: Autoradiographic Activity	Kidney: Cellular Infiltration	Kidney: Tubular Degeneration	Kidney: Glomerular Lipidosis	Liver: Cellular Infiltration	Liver: Siderosis	Lung: Subpleural Fibrosis	Lung: Cellular Infiltration	Lung: Osseous Metaplasia	Brain: Focal eosinophilic Degeneration	Brain: Focal meningeal Hyperplasia
Group 1 (2 weeks)	None	4157							N						±	N						
		4199							N				N			N						
		4156							N					±	±	±						
		4008							N				N			N						
	DTPA	4085							N				N			N						
		4112							N				N			±						
		4155							N				N			N						
		4087							1		1				±	±	1					
Group 2 (1 month)	None	4285	N					N	N				N			N		1			N	
		4091	N					N			1			1		N		2			N	
		4184		1						1			N			N						1
		4904		2					N					1		N					N	
	DTPA	4024	N					N	N				N			N		1			N	
		4031	N					N	N				N			N		2			N	
		4192	N					N			1			2	1	N					N	
		4009	N					N	N				N			N						1	
Group 3 (2 month)	None	5097							N				N				±		±			
		5176							N				N			N		1				
		5024							N				N			N			±			
		5147							N				N			N		N				
	DTPA	5102							N				N			N			1			
		5179							N				N			N		1				
		5064							N				N			N		N				
		5143							N				N				±	1				
Group 4 (3 month)	None	4198	N				2	N	0				0		±	N		2			1	
		4100	N				2	N	0	N			0	N		N		1			1	
		4190	N				2	N	0	N			0	N		N		N			±	
		4976	N				1-2	N	0	N			0	N		N						
	DTPA	4940	N				0	N	0	N			0	N			±		1			
		5034	N				2	N	0	N			0_+	N							T	
		4189	N				0	N	0_+	N			0_+		±	N		N	±		±	
		4973	N				1-2	N	0^+	N			0_+	N		N						
Group 5 (6 month)	None	4662		1			0^+	N	0^+_+	N			N			N	±		2			
		4454		2	3		3	N	0^+_+	N			N			N			1			
		4825			1		2	N	0^+_+	N			N			N			1			
		4783			3	2	3	N	0	N			N			N			1		N	
	DTPA	4781			2	3	2	N	0_+	N				1		N		N				
		4337		1			0^+	N	0^+_+	N			N			N			1			
		4750	N				1	N	0	N			N			N	±	N				
		4870		1			0^+	N	0	N			N			N		N			1	
Group 6 (12 month)	None	4944							N						±	N		2				
		4928							N				N			N		2				
		4284							N						±	N		1				
		4459									1			±		N		N				
	DTPA	4822								1			N			N			+	+			
		4663							N				N			N			T	±			
		4320							N				N			N	±			+			
		4373							N				N			N		N					

Key:

N	no visible lesions (normal, or essentially so)	1-2	small-to-moderate (or slight-to-moderate)
0_+	no activity seen	2	moderate
0^+	trace (occasional tracks but no alpha stars)	3	marked
+	very small (or very slight)	4	extreme
T	small (or slight)		

Table 14, Continued

Plutonium Nitrate: 1 Year Subcutaneous Implant Study in Beagles
Summary of Histopathology

Group (Duration)	Treatment	Animal Number	Testis: Tubular Degeneration	Testis: Cellular Infiltration	Thyroid: Thyroiditis	Eye: Cellular Infiltration	Eye: Mineralization	Heart: Cellular Infiltration	Heart: Coronary Intimal Hyperplasia	Gall Bladder: Lymphoid Hyperplasia	Skin: Dermatitis	Pituitary: Cysts	Prostate: Cellular Infiltration	Stomach	Intestine	Urinary Bladder	Pancreas	Adrenal	Skeletal Muscle	Parathyroid	Mesenteric Lymph Node
Group 1 (2 weeks)	None	4157																			
		4199																			
		4156																			
		4008																			
	DTPA	4085																			
		4112																			
		4155																			
		4087																			
Group 2 (1 month)	None	4285	N		N	N		N		N				N	N	N		N	N		
		4091		2	1	1-2		N						N	N	N	N	N			
		4184	N			1	1	N		1				N	N	N	N	N	N		
		4904	N		N		N	N						N	N	N	N	N		N	
	DTPA	4024		1	+	N	N	N				1		N	N	N	N	N	N		
		4031			+	1	N	N						N	N	N	N	N	N	N	
		4192		2	2	N	N	N					N	N	N	N	N	N		N	
		4009			1	2	N	N				1		N	N	N	N	N	N	N	
Group 3 (2 month)	None	5097	N			2	N	N						N	N	N	N	N			
		5176	N		N		N	N						N	N	N	N	N		N	
		5024	N			1	N	N						N	N	N	N	N		N	
		5147	N				N	N						N	N	N	N	N			
	DTPA	5102	N		N		N	N						N	N	N	N	N			
		5179		3		3	N	N						N	N	N	N	N			
		5064	N		N		N	N						N	N	N	N	N		N	
		5143	N		N		N	N						N	N	N	N	N			
Group 4 (3 month)	None	4198	N			+		N		N				N	N	N	N	N			
		4100	N		N		N	N		N		N		N	N	N	N	N			
		4190	N		N			N		N		N		N	N	N	N	N		N	N
		4976	N			+	+	N		N				N	N	N	N	N		N	N
	DTPA	4940	N		N		N	N		N		N		N	N	N	N	N		N	N
		5034		1		3	N	N		N		N		N	N	N	N	N		N	N
		4189		1	N			N		N		N	1		N	N	N	N			N
		4973												N	N	N	N	N		N	N
Group 5 (6 month)	None	4662		1	N		N	N						N	N	N	N			N	
		4454			N		N	N						N	N	N	N	N	N	N	
		4825	N		N		N	N						N	N		N	N	N	N	
		4783	N				N	N						N	N	N	N	N	N		
	DTPA	4781	N				N	N						N	N	N	N	N	N		N
		4337	N		N			N						N	N	N	N				
		4750	N		N		N	N						N	N	N		N	N		
		4870	N			1	N	N						N	N	N	N	N	N		
Group 6 (12 month)	None	4944	N		N		N	N						N	N	N	N	N	N	N	N
		4928	N			1		N						N	N	N	N	N		N	
		4284	N				+	N	+					N	N	N	N	N			
		4459	N		N		N	N						N	N	N	N	N		N	
	DTPA	4822	N		N			N							N	N	N	N			
		4663	N			2	+		+					N	N	N	N	N		cyst	
		4320		1	N		N	N						N	N	N	N	N		N	
		4373	N		N		N	N						N	N	N	N	N			

Key:

N	no visible lesions (normal, or essentially so)	1-2	small-to-moderate (or slight-to-moderate)
0	no activity seen	2	moderate
0+	trace (occasional tracks but no alpha stars)	3	marked
+	very small (or very slight)	4	extreme
T	small (or slight)		

manifest by decreased size of cortical follicles and medullary cords. The scar tissue, generally replacing areas of medulla, was composed of loosely arranged eosinophilic fibrillar material with fibroblasts and macrophages. The granulomatous reaction, composed of a mixture of macrophages, cells resembling reticulum cells, and epithelioid cells, occurred diffusely around the areas of scar tissue and replaced areas of cortex. In dog #4781, a diffuse granulomatous proliferative reaction occurred in the perinodal tissues; the cellular infiltration, containing a few mitotic figures, included lymphocytes, macrophages, epithelioid cells, and eosinophils. The areas of scar tissue contained a moderate amount of phagocytized black pigment, and a moderate amount of golden-brown, iron positive pigment interpreted as hemosiderin. The black pigment, which resembled India ink used in these dogs for ear tattooing, also occurred in the right superifical cervical lymph nodes and was regularly present in lymph nodes from dogs not given plutonium. Autoradiographs contained numerous alpha stars in the areas of scar tissue and in occasional apparently normal medullary areas. The region of diffuse perinodal granulomatous reaction in dog #4781 lacked significant numbers of alpha stars.

The left superficial cervical lymph nodes from 2 dogs in group 2 (1 month) had small to moderately increased numbers of diffusely distributed macrophages in the subcapsular and medullary sinuses. These macrophages may have been responding to materials from the implant site. The left superficial cervical lymph nodes from the dogs in group 4 (3 months) were free of pathologic alteration, but autoradiographs contained significant numbers of alpha stars in the medullary cords.

Kidney sections from 2 dogs in group 1 (2 weeks) had small areas of tubular degeneration, composed primarily of cellular casts in distal convoluted tubules. Although these changes could have been due to plutonium, dogs held for longer times did not show such changes. The small amount of tubular degeneration in dog #4192 from group 2 (1 month) was associated with a focal interstitial nephritis similar in appearance to inflammatory lesions occasionally seen in control dogs not given plutonium.

The liver from dog #4087 in group 1 (2 weeks) had small numbers of disseminated small foci of golden-brown pigment associated with focal sinusoidal cell hyperplasia and minimal amounts of cellular infiltration. Although this is an unusual lesion, it was probably unrelated to plutonium administration since the dogs held for longer durations of treatment did not show similar changes. Furthermore, this dog also had mild disseminated siderosis and hemopoiesis in the spleen, suggesting a hemolytic anemia. Autoradiographs of spleens from the dogs in group 4 (3 months) contained occasional tracks but no alpha stars.

The remaining changes observed were unrelated to the plutonium administration. These changes were of a type commonly seen in control beagles or were of an incidental nature.

The lungs from 20 out of 40 dogs had small to moderate sized foci of subpleural interstitial fibrosis. These foci were associated with cuboidal-shaped alveolar epithelial cells and, occasionally, squamous metaplasia. The fibrosis was associated with a very small focus of osseous metaplasis in one dog from group 6 (12 months).

Kidney sections had occasional foci of glomerular lipidosis in 1 dog from group 1 (2 weeks) and 2 dogs from

group 6 (12 months). This lesion consisted of large foamy cells and scar tissue occupying portions of widely scattered glomeruli.

In the brains of several dogs small eosinophilic granular structures, approximately the size of neurons, were in the nuclei gracilis and nuclei cuneatus. These structures were not accompanied by surrounding inflammation or degeneration. The brain from one dog in group 2 (1 month) had a small focus of meningeal hyperplasia dorsal to the corpus callosum. The meningeal hyperplasia consisted of a polypoid arrangement of cuboidal epithelial cells and stroma containing adipose tissue and a single site of perivascular lymphocytic infiltration.

Small to marked amounts of tubular degeneration and atrophy occurred in the testes from 6 dogs (3 from group 2 and 1 each from groups 3, 5 and 6). This lesion was characterized by seminiferous tubules lined with only Sertoli cells, a few spermatocytes, and a few spermatids occasionally forming multinucleated cells. The tubular degeneration was frequently associated with peritubular lymphocytic infiltration. Due to the decreased size of the tubules, the interstitial cells occasionally seemed more prominent.

A small amount of focal subcapsular siderosis of the spleen occurred in 1 dog from group 2 (1 month) and 1 dog from group 6 (12 months). The subcapsular siderosis was associated with a thickened capsule and some mineralization. A small amount of disseminated siderosis occurred in the red pulp of 1 dog from group 1, 2 dogs from group 2, and 1 dog from group 6.

Slight to marked degrees of thyroiditis were present in several dogs. The thyroiditis varied from occasional very small foci of lymphocytes to marked diffuse plasmocytic

and lymphocytic infiltration forming germinal centers.

Eye sections from 4 dogs in group 4, 1 dog in group 5, and 4 dogs in group 6 had minimal amounts of mineralization in the retina. This mineralization, positive with the van Kossa reaction for calcium, consisted of basophilic strands in the layer of optic nerve fibers near the optic disc. The periphery of the retina from these dogs contained a few foci of mineralization the size of optic nerve cells.

The skin from 3 dogs in group 4 (3 months), sectioned from the ventral abdomen, had small areas of mild dermatitis composed of a thickened epidermis and a mixed inflammatory reaction in the dermis.

The pituitary gland from 2 dogs in group 2 (1 month) had small multiloculated cysts in the pars distalis.

Miscellaneous lesions included a small amount of lymphoid hyperplasia in the gall bladder of 1 dog, a small multiloculated cyst in the parathyroid of 1 dog, and mild intimal hyperplasia in a coronary artery from 1 dog.

The specimens processed for electron microscopy have not yet been examined.

IV. High Fired Plutonium Oxide Experiments

A. Materials and Methods

The experimental protocol for the high fired plutonium oxide study is essentially the same as for the previous study. The technique for the implant is identical to that for the air oxidized experiment.

The high fired oxide was prepared from electrorefined unalloyed Pu metal dissolved in HCl 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 $PuO_{1.98}$. 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 optical microscopy.

The physical, chemical, and isotopic information for the oxide are shown in Table 15.

One mg portions of the dry oxide were packaged in the 5 ml vials. The insoluble PuO_2 particles are suspended in 1.0 ml of physiological saline solution then 0.1 ml of the solution is injected subcutaneously. Sample collection, DTPA treatment, *in vivo* counting and necropsy schedules are the same as the $Pu(NO_3)_4$ experiment. At this time the dogs for the two month study have not been injected.

No radiochemical assay data have been produced at this time but some *in vivo* counting data are available.

Implant area and superficial cervical lymph node monitoring with the NaI (Tl) wound detector revealed small transport from the implant site to the lymph node.

Table 15. PuO_2 for Dow-CSU dog project center.

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

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

The reduction in Pu was not detectable at the implant site by the wound counting technique but Pu accumulation was detected in the lymph node. Figure 10 shows the fraction of implant dose accumulated in the lymph node with time for dogs not treated with DTPA. The fraction of implant dose accumulated in the lymph node for dogs treated with DTPA is shown in Figure 11. The untreated dogs exhibit a trend to accumulate more Pu in the superficial cervical lymph node than do the DTPA treated dogs but there are no significant differences in the data according to a preliminary Student t test. A comparison of the slopes for these data with those obtained for air oxidized PuO_2 indicates that they are similar. A complete statistical analysis will be performed at the end of the high fired oxide experiment to confirm the tentative observation.

Ge (Li) detector measurements over the superficial cervical lymph nodes show a tendency for the concentration of Pu over Am. Data accumulated five to six hours after the PuO_2 implant show two-fold to three-fold enhancement of Pu relative to ^{241}Am (Table 16) and data at 15 days show even higher average enhancement. The variance of the data is large, however, and this increase over the time interval cannot be tested until better data are accumulated during the remainder of the experiment.

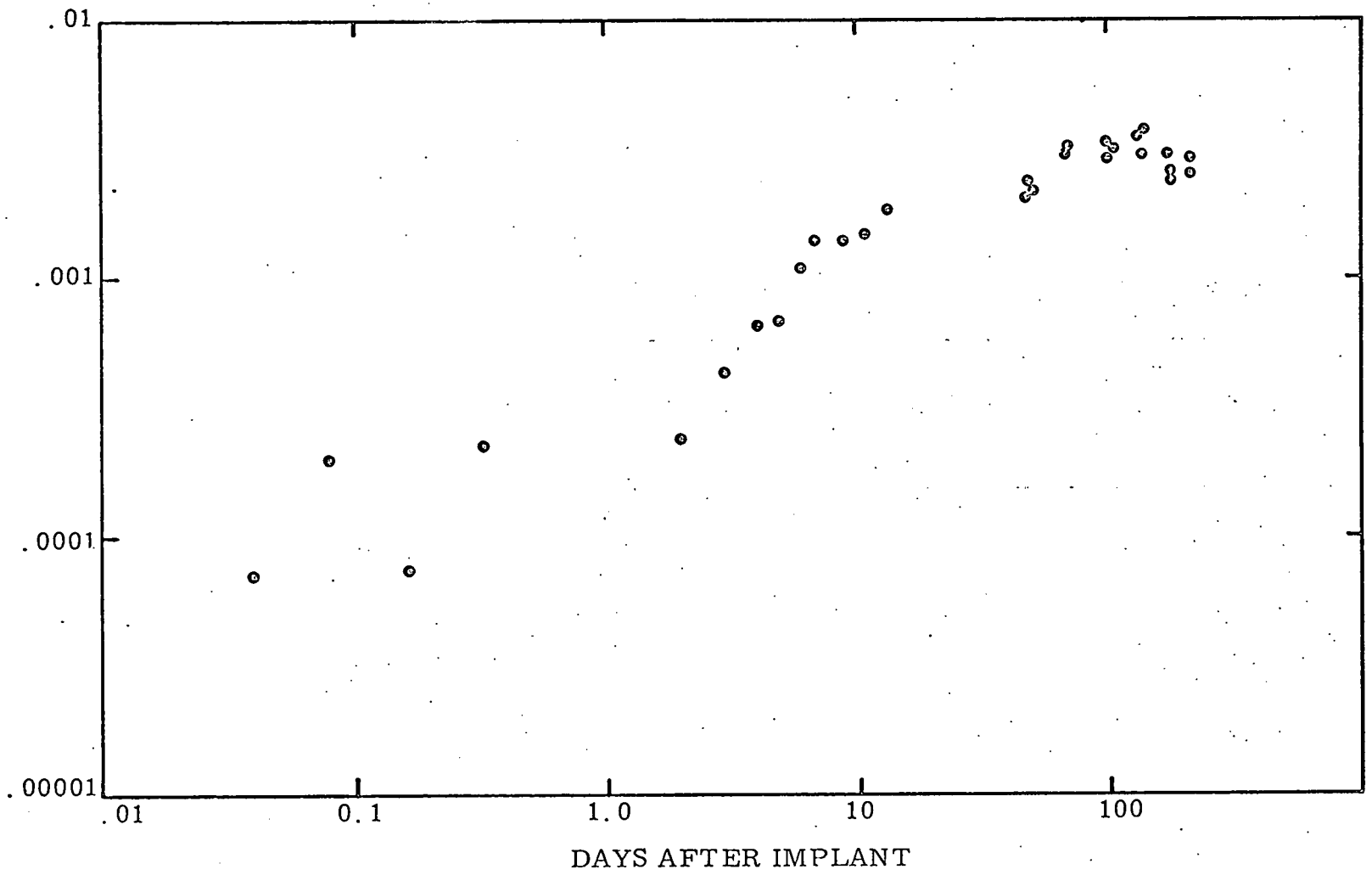

Figure 10. The change in the fraction of Pu in the left superficial cervical lymph node with time for dogs not treated with DTPA.

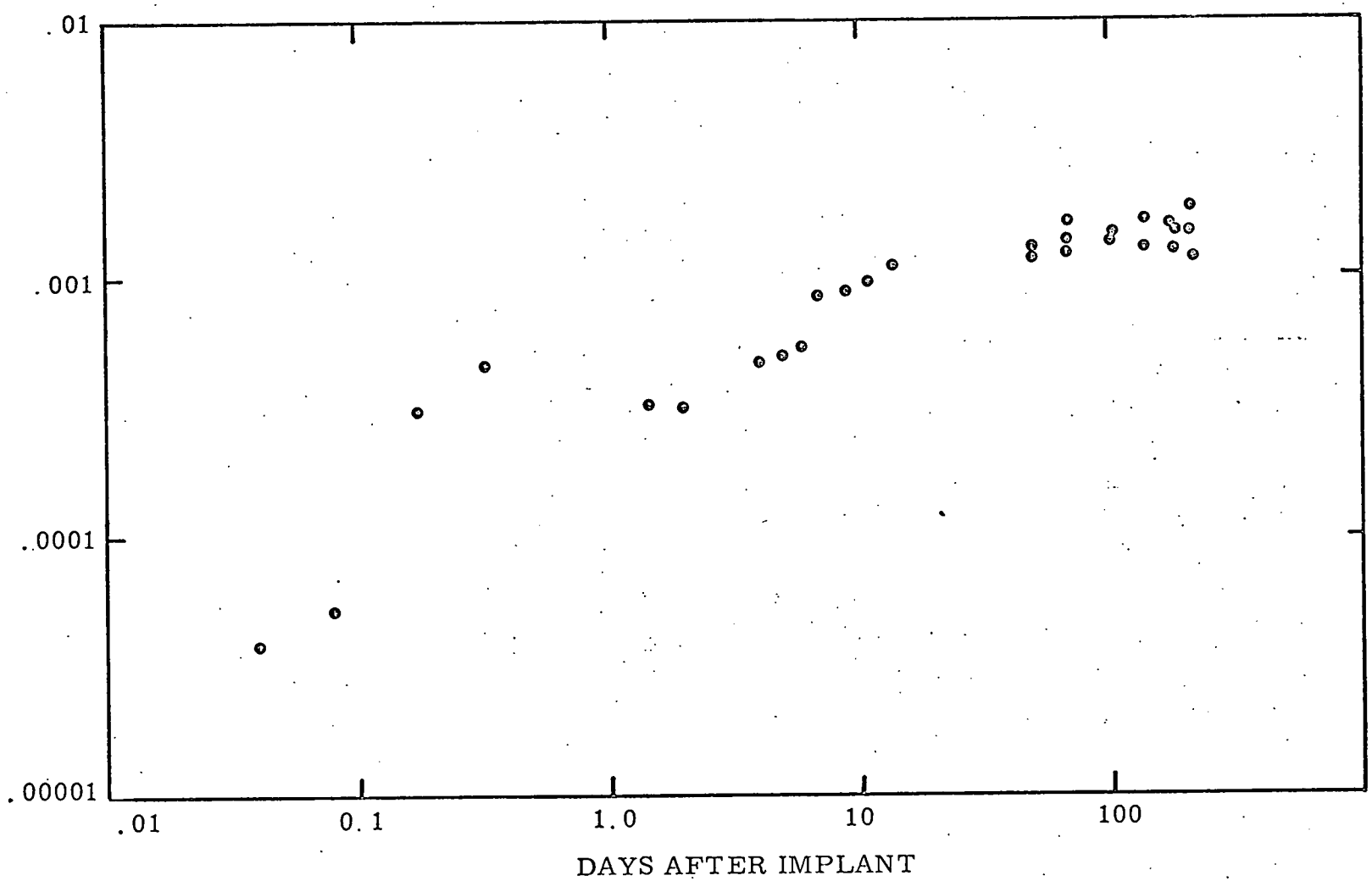

Figure 11. The change in the fraction of Pu in the left superficial cervical lymph node with time for dogs treated with DTPA.

Table 16. Pu enhancement in the left superficial cervical lymph node determined by in vivo Ge (Li) detector measurements. (High fired PuO_2 dogs).

Post Implant Time	Treatment	$\frac{(Pu/Am)_{organ}}{(Pu/Am)_{implant}}$ = Ratio Factor
6 hours	None	2.2 ± 0.3
	DTPA	2.1 ± 0.7
15 days	None	5.3 ± 2.0
	DTPA	8.5 ± 2.7

V. Effect of Lymph Node Removal on PuO_2 Translocation

Experiments involving beagle dogs at Colorado State University in which plutonium implants were made in the subcutaneous fascia over the dorsal metacarpus have shown the superficial cervical lymph nodes to be the first major nodes draining the implant area.[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. Surgical excision of a contaminated lymph node may be a useful therapeutic procedure in the management of accidental plutonium contamination in humans.

The purpose of this pilot study was to investigate the effects of lymphadenectomy on the translocation of high fired PuO_2 from a simulated puncture wound in the left dorsal metacarpus of the beagle dog.

Eight male beagle dogs were used in this experiment. Six dogs were contaminated with PuO_2 and two dogs were used as controls. Comparisons were made between dogs from which the left superifical cervical lymph node was excised and dogs in which the lymph node was left intact. The left superficial cervical lymph nodes were removed from the dogs at one, two, four or eight hours after the PuO_2 implant. Two lymphadenectomized dogs received no plutonium.

The dogs were maintained in metabolism cages for 13 days and blood and excreta samples were collected. Accumulation of ^{239}Pu in

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(1) Johnson, L. J., E. H. Bull, J. L. Lebel and R. L. Watters. 1970. Kinetics of lymph node activity accumulation from subcutaneous PuO_2 implants. Health Physics, 18, pp. 416-418.

(2) Lebel, J. L., E. H. Bull, L. J. Johnson, and R. L. Watters. 1970. Lymphosarcoma associated with nodal concentration of Pu in dogs: A preliminary report. J. Vet. Res., 31, pp. 1513-1516.

the left superficial cervical lymph node was determined by counting the low-energy photon complex with a thin NaI (Tl) detector. Table 17 shows the count rates in the region of the left superficial cervical lymph nodes obtained prior to lymphadenectomy. Accumulation of ^{239}Pu in the lymphadenectomized dogs indicated that the entire left superficial cervical lymph node chain had not been removed. In each case one large lymph node was excised, but Miller et al., described the superficial cervical lymph nodes as commonly consisting of three or more nodes.[3] At necropsy left superficial cervical lymph node samples with detectable plutonium were obtained from all the lymphadenectomized dogs. This indicates that small, undetected nodes continued to accumulate plutonium and began to enlarge.

All dogs were killed 13 days after implant, and normal necropsy procedures were followed. The only deviation from the standard procedure was that the samples of liver, spleen, hepatic lymph node, axillary lymph node, and right and left superficial cervical lymph node were not sent to the Rocky Flats Division of the Dow Chemical Company for chemical analysis.

At necropsy counts were taken from these six tissue samples using the thin NaI (Tl) detector. These data are shown in Figure 12. The activity in the left superficial cervical lymph node area of the lymphadenectomized dogs may be due to the fact that residual lymph node tissue remained intact. The erratic axillary lymph node activity may be due to the fact that the implants varied somewhat to each side of the midline of the paw. It may be that lymph from the dorsal surface of the dog's front paw may drain to either the superficial cervical lymph node or to the axillary lymph node depending on the area of implant. Radiographic studies are planned in an attempt to study the lymph drainage from the paw.

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(3) Miller, M. E., G. C. Christensen and H. E. Evans. Anatomy of the Dog, W. B. Saunders Co., Philadelphia, London. (1964)

Table 17. Plutonium accumulation in left superficial cervical lymph node. (Low-energy photon counts from left superficial cervical lymph node area.)

Time (days)	Control	Control	Lymphadenectomy			
			1 hour	2 hours	4 hours	8 hours
0	41	3	0	0	0	6
0.042	699	52	3	80	6	7
0.084	844	67	--	--	8	6
0.167	1253	51	--	--	277	2
0.332	1045	67	--	--	--	--
1.0	2439	92	12	66	92	9
2.0	2839	106	34	71	225	9
3.0	2902	194	49	65	225	19
4.0	2643	110	157	94	345	36
5.0	2698	111	89	86	160	18
6.0	2948	108	329	103	202	39
8.0	3564	149	386	244	344	171
10.0	3914	170	423	272	930	122

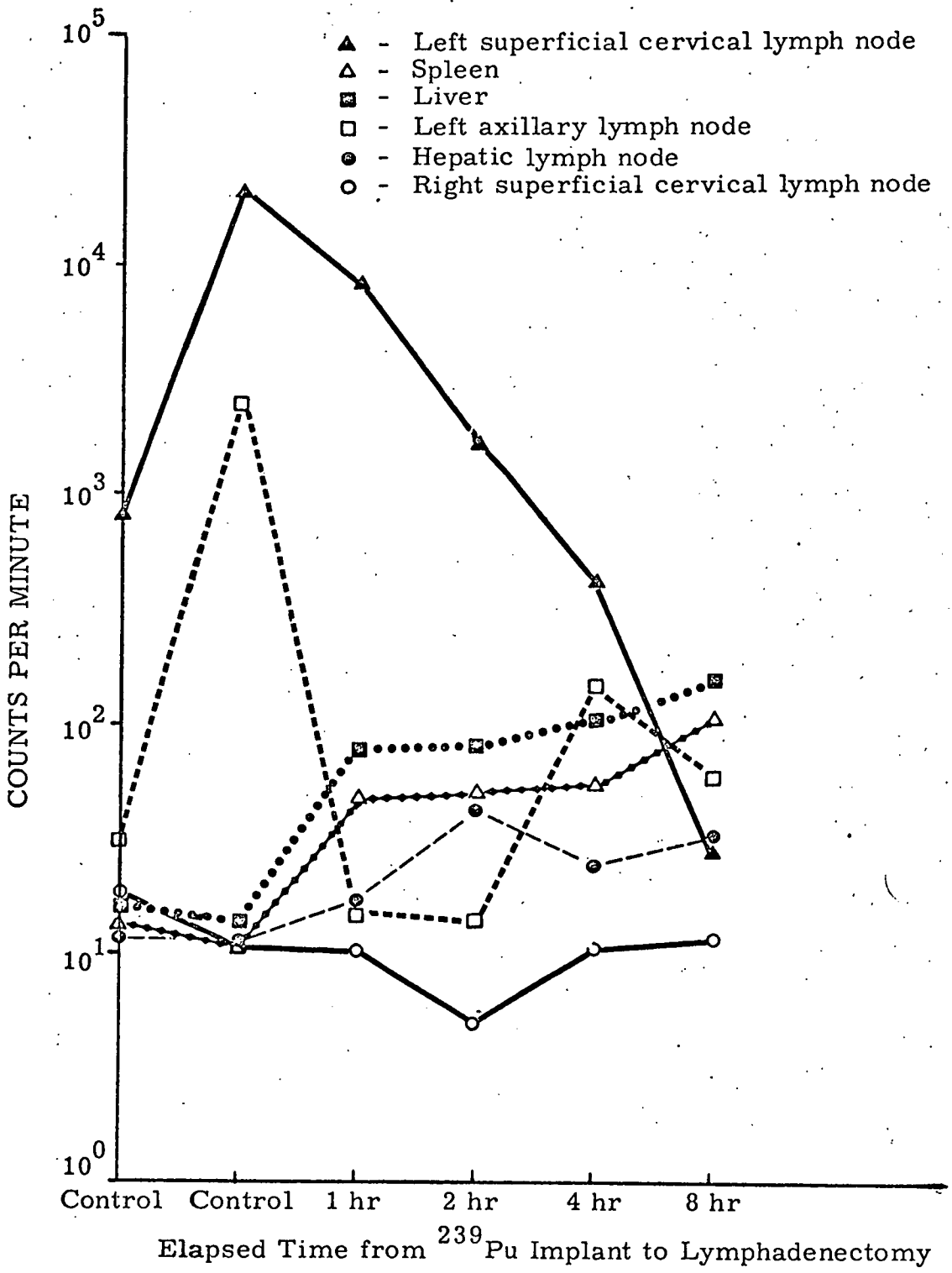

Figure 12. Low-energy photon counts of dog tissues using NaI (Tl) probe.

Radiochemical analyses were performed on the tissue samples using the liquid scintillation method of Keough and Powers.[4] Difficulties in sample preparation resulted in valid data for only the hepatic lymph nodes. These data are shown in Figure 13. Corrections to the procedure have now been made and a second experiment is planned.

The results of this study suggest that there may be an optimal time range during which the left superficial cervical lymph node may be excised in order to remove a maximal amount of plutonium, while allowing a minimal amount to translocate beyond the lymph node. The data in Table 17 suggest that the greatest rate of plutonium accumulation in the left superficial cervical lymph node occurred during the first 8 hours. The accumulation continued to increase with time, but at a lesser rate. The optimal time range for excision may extend beyond 8 hours, therefore, future experiments will include lymphadenectomies at later time periods.

The determination of an optimal time range for excision of the lymph node should be aided by the liquid scintillation α-counting data, since low-energy photon counting is limited by tissue self-absorption and by the variable geometry presented by the various tissue samples. The efficiency of counting ^{239}Pu by the liquid scintillation method approaches 100 percent, while the efficiency of the NaI (Tl) detector is much lower. The efficiency differences in these two counting systems can be seen by comparing the hepatic lymph node counts on Figure 12 and Figure 13. The liquid scintillation counts are about one order of magnitude greater than the sodium iodide counts.

Since the superficial cervical lymph node chain contains three or more nodes, careful excision of the larger contaminated nodes may allow the remaining nodes to increase their filtering function.

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(4) Keough, R. F. and G. J. Powers. 1970. Determination of plutonium in biological materials by extraction and liquid scintillation counting. Analytical Chemistry, 42, pp. 419-421.

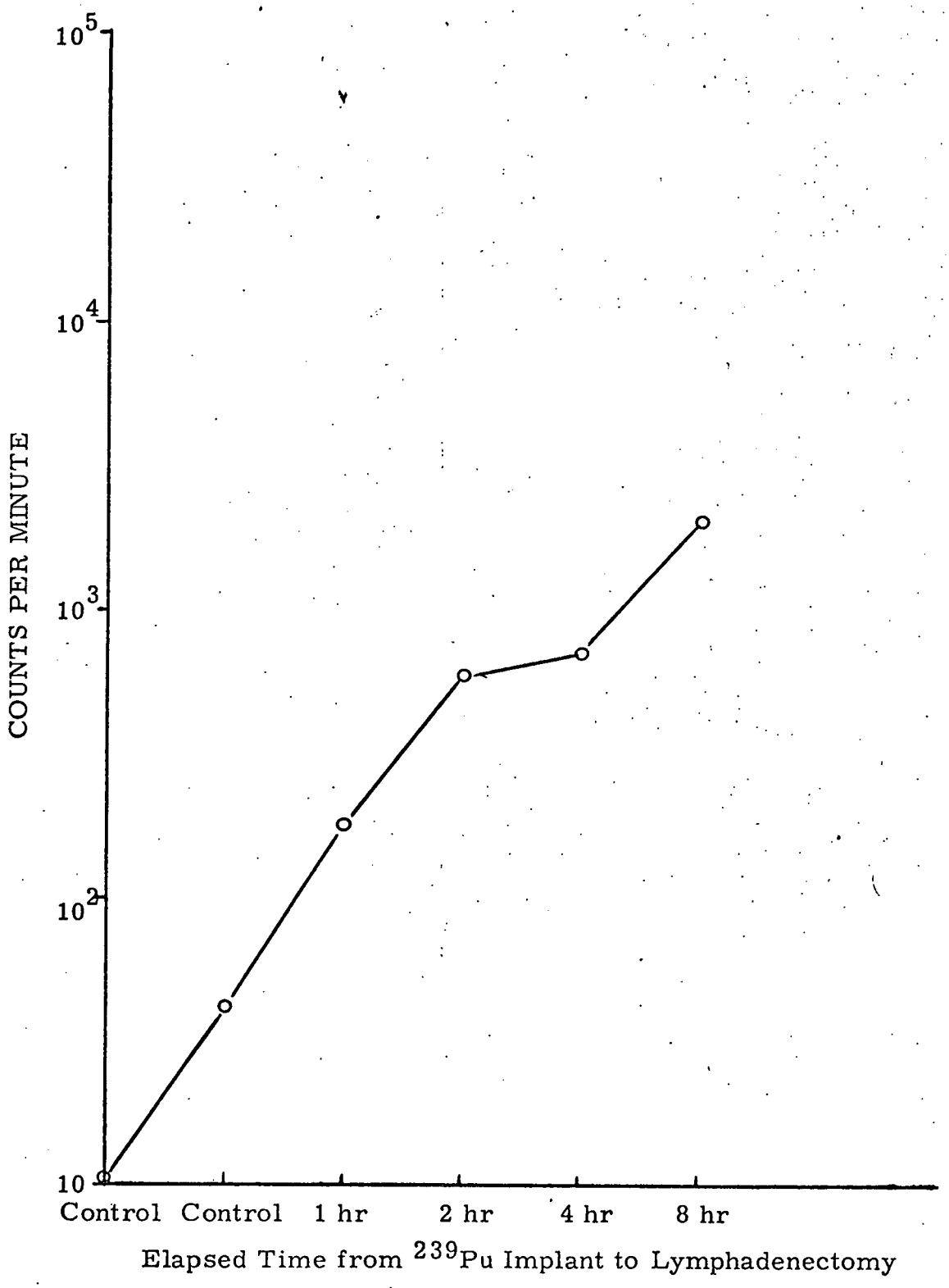

Figure 13. Liquid scintillation α-counts of hepatic lymph nodes.

Continued integrity of the filtering function may decrease the amount of plutonium which will be translocated beyond the nodes. Figure 12 shows that as the capacity of the left superficial cervical lymph node to accumulate plutonium decreased, the amount of plutonium translocated to the liver, spleen and hepatic lymph node increased.

The information acquired in this experiment suggest that lymphadenectomy might be considered in the wound management of humans contaminated with plutonium. Even when excision of the wound site is performed, the lymph nodes which drain the wound area should be monitored. If an accumulation of plutonium in the lymph nodes is observed, lymphadenectomy could then be considered.

VI. List of Publications and Papers

COO-1787-1 Watters, R. L., J. L. Lebel, L. J. Johnson and E. H. Bull. 1968. A study of the translocation of plutonium and americium from wounds. First Technical Progress Report.

COO-1787-2 Watters, R. L. and L. J. Johnson. The movement of plutonium and americium from wound sites. Presented January 31, 1969 at the Midyear Topical Symposium of the Health Physics Society, Los Angeles, California.

COO-1787-3 Johnson, L. J., R. L. Watters, C. R. Lagerquist and S. E. Hammond. 1969. Relative tissue distribution of Pu and Am from experimental PuO_2 contaminated puncture wounds. (Abst.) Health Physics 17: 383.

COO-1787-4 Johnson, L. J., E. H. Bull, J. L. Lebel and R. L. Watters. 1970. Kinetics of lymph node activity accumulation from subcutaneous PuO_2 implants. Health Physics 18:416-418.

COO-1787-5 Lebel, J. L., E. H. Bull, L. J. Johnson and R. L. Watters. 1970. Lymphosarcoma associated with nodal concentration of plutonium in dogs: A preliminary report. Am. J. Vet. Res. 31: 1513-1516.

COO-1787-6 Johnson, L. J. 1969. Relative translocation and distribution of Pu and Am from experimental PuO_2 subcutaneous implants in beagles. Special Report to U. S. Atomic Energy Commission (Dissertation).

COO-1787-7 Johnson, L. J., R. L. Watters, C. R. Lagerquist and S. E. Hammond. 1970. Relative distribution of plutonium and americium following experimental PuO_2 implants. Health Physics 19: 743-749.

COO-1787-8 Watters, R. L., J. L. Lebel, L. J. Johnson and E. H. Bull. 1969. A study of the translocation of plutonium and americium from wounds. Second Technical Progress Report.

COO-1787-9 Johnson, L. J., R. L. Watters, C. R. Lagerquist and S. E. Hammond. 1970. Retention of subcutaneously placed PuO_2 in the beagle. (Abst.) Health Physics 19: 337.

COO-1787-10 Johnson, L. J., R. L. Watters, J L. Lebel, C. R. Lagerquist, and S. E. Hammond. The distribution of Pu and Am: Subcutaneous administration of PuO_2 and the effect of chelation therapy. In Pu as an Environmental Hazard, W. S. S. Jee and B. J. Stover, Eds. University of Utah Press. (In press.)

COO-1787-11 Watters, R. L., J. L. Lebel, R. W. Bistline, G. E. Dagle and L. S. Gomez. 1971. A study of the translocation of plutonium and americium from puncture wounds. Third Technical Progress Report.

COO-1787-12 Watters, R. L. and J. L. Lebel. 1971. A study of the translocation of plutonium and americium from puncture wounds. Summary Report for the period April 1968 to June 1971.