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Research in Radiobiology

**Annual Report of Work in Progress
in the Internal Irradiation Program**

**RADIOBIOLOGY DIVISION OF THE DEPARTMENT OF ANATOMY,
UNIVERSITY OF UTAH COLLEGE OF MEDICINE**

**Respectfully Submitted by:
WEBSTER S. S. JEE, Acting Director**

MARCH 31, 1974

CONTRACT No. AT (11-1)-119

MASTER

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RESEARCH IN RADIOBIOLOGY

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Radiobiology Division of the Department of Anatomy
University of Utah College of Medicine

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fly

"We have lost more than our director.
We have lost a close personal friend."

Professor Thomas F. Dougherty
Director 1955-1974
Radiobiology Division, Anatomy Department
University of Utah

Thomas Francis Dougherty was born in Forman, North Dakota on March 27, 1915. He received a bachelor's of science degree in Chemistry in 1936, a master's of arts degree in Zoology in 1937 and a doctorate of philosophy degree in Anatomy and Pathology under Hal Downey in 1942 at the University of Minnesota. Tom Dougherty was an International Cancer Research Foundation Fellow at Yale University School of Medicine 1942-44 and was Instructor in Anatomy at the same institution 1944-47. In 1947, he was appointed Professor of Anatomy at the University of Utah College of Medicine and two years later became Chairman of that Department. He held this position until his death on February 6, 1974. He is survived by his wife and scientific colleague, Jean, son, Michael, and daughter, Ann.

In a research career that lasted more than thirty years, Tom Dougherty was an energetic and imaginative investigator. His contributions to the scientific literature number well over two hundred articles. His earliest work showed that lymphocytes play an important role in the protection of the body against the action of tubercle bacilli. In collaborative studies with W.U. Gardner, he demonstrated that estrogens are able to induce lymphoid leukemia in certain strains of mice. These studies led him to examine the relationship between endocrine organs and lymphatic tissue.

In classical experiments done in collaboration with Abraham White, Tom conclusively established that growth and maturation of lymphatic tissue are regulated by secretions of the adrenal cortex. This work laid the foundation for all subsequent studies on the role of the adrenal cortical hormones in the control of lymphatic tissue mass and led to the use of cortisol (and synthetic analogues) in the treatment of lymphatic leukemia.

Concomitantly, in 1944, Tom Dougherty and Abe White found that lymphocytes are a major source of gamma-globulin (antibody in immunized animals) and that this protein is released following noxious stimuli--such as inanition, x-irradiation, injection of staphylococci--or injection of adrenal cortical hormones. This evidence provided the link between the regulation of gamma globulin (antibody) release by adrenal cortical hormones and environmental stress, a finding of major biological significance. This work now serves as a foundation for much of modern day immunology.

With Louis Goodman, Alfred Gilman, and his wife Jean, work was performed during the second world war on the possible use of nitrogen mustards as a chemotherapeutic agent in the treatment of lymphosarcoma. As a result of secrecy imposed by war, this work was not published until 1946. It was the basis, however, for all subsequent studies on the use of nitrogen mustards in treating a variety of malignant myeloid diseases.

Another major contribution of Tom Dougherty was the observation that cortisone is able to protect animals against anaphylaxis and ameliorate acute allergic inflammation. Through his insight, he postulated the very important concept that cortisone and adrenal cortical extracts can "minimize the consequences of a wide variety of unrelated inflammation-producing stimuli."* These and subsequent experimental findings provide the basis for a multi-million dollar drug industry.

Subsequent research on the anti-inflammatory actions of glucocorticoids led to the discovery of heparin as an inhibitor of histamine-mediated inflammation. In addition, he and his co-workers found that cortisol, not cortisone, is the biologically active molecule which mediates the responses observed in both lymphatic and connective tissues.

In 1955, Tom Dougherty was appointed director of the Radiobiology Laboratory, which then became a division of the Department of Anatomy. The Radiobiology Laboratory was founded at the University of Utah in 1950 by the U.S. Atomic Energy Commission to study the long-term effects of internally-deposited radioactive elements (^{239}Pu , ^{228}Ra , etc.), which are associated with the nuclear industry. Under his scientific and administrative leadership, the multi-disciplinary laboratory attained its present position of international renown. At the same time, he continued to conduct his basic studies in endocrinology, taught anatomy and nurtured and stimulated developing scientists.

Thomas F. Dougherty was a member of twelve professional societies, was a charter member and served as president of the Reticuloendothelial Society, as editor of its journal, and also served as an editor for the Proceedings of Experimental Biology and Medicine. In addition, he was accorded numerous honors and awards. In 1953-54, he was the Claude Bernard

*Proc. Soc. Exptl. Biol. Med., 75:854, 1950.

Professor of Experimental Medicine and Surgery, University of Montreal; 1954-55 Special Consultant of Surgeon General, U.S. Department of the Army, and member of the faculty of the Army Medical Department, Research and Graduate School; and 1973-74 Distinguished Research Professor, University of Utah.

Those persons who were associated with the Department of Anatomy during Tom Dougherty's chairmanship will recall that he knew when to be a leader as well as how to be a leader. Tom Dougherty was freely available to any person who would seek his advice. He took pleasure in frequent informal sessions and chance encounters with his faculty. Members of his own and other departments will remember him as an investigator who was always well-informed and, even more important, a scholar capable of correlating concepts in apparently unrelated fields in a most provocative manner. His sound scientific judgment allowed him to guide young investigators in a pleasant, but firm manner and to provoke the members of the group to make their best possible efforts. In this way he trained doctoral candidates highly skilled in both the technical and academic aspects of their work.

Whether delivering a medical school lecture or meeting over lunch with faculty and students, Tom always had time for a light story. His exceptionally active sense of humor was known to all. These qualities evoked a remarkable strong devotion to "The Boss" on the part of his faculty and staff. In return, Tom Dougherty strongly supported the members of his department in all of their professional activities.

Tom Dougherty's success in science, indeed in life itself, was the result of an open mind, keen intellect, incisive and intuitive thought, and hard work mixed with a goodly amount of humor. He remains as a fine example to those who knew and worked with him.

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GROUP LEADERS AND SECTIONS

Division of Radiobiology, Department of Anatomy,
University of Utah College of Medicine

Section

Acting Director	Prof. Webster S.S. Jee
Biochemistry	Charles J. Nabors, Jr.
Bone	Webster S.S. Jee
Chemistry	Walter Stevens
Clinic & Pathology	Glenn N. Taylor
Hematology	Jean H. Dougherty
Instrumentation	Garth D. Westenskow
Physics	Charles W. Mays
Radiology	William R. Christensen

AVAILABILITY OF PREVIOUS REPORTS

Copies of our reports may be obtained from the National Technical Information Service, Springfield, Virginia. Paper copies as indicated; microfiche copies \$0.95 each.

<u>Report</u>	<u>Date</u>	<u>Title</u>	<u>Cost</u>
TID-7639	Jun 1954	Consultants' Meeting	\$10.60
AECU-3418	Mar 1955	Annual Report	5.45
AECU-3109	Sep 1955	Semi-Annual Report	5.45
TID-16458	Mar 1956	Annual Report	5.45
TID-16459	Sep 1956	Semi-Annual Report	5.45
AECU-3522	Mar 1957	Annual Report	7.60
AECU-3583	Sep 1957	Semi-Annual Report	5.45
C00-215	Mar 1958	Annual Report	7.60
C00-216*	Mar 1958	Escape of Radon and Thoron	5.45
C00-217	Sep 1958	Semi-Annual Report	7.60
AECU-4112	Feb 1959	Radioactive Fallout	4.00
C00-218*	Mar 1959	Annual Report	7.60
C00-219*	Sep 1959	Semi-Annual Report	5.45
C00-220	Mar 1960	Research in Radiobiology	7.60
C00-221	Aug 1960	Interim Report of ⁹⁰ Sr	4.00
C00-222	Sep 1960	Research in Radiobiology	5.45
C00-223*	Mar 1961	Research in Radiobiology	4.00
C00-224*	Sep 1961	Research in Radiobiology	5.45
C00-225	Mar 1962	Research in Radiobiology	5.45
C00-226	Sep 1962	Research in Radiobiology	5.45

*Also available on request from this laboratory.

AVAILABILITY OF PREVIOUS REPORTS (Con't.)

<u>Report</u>	<u>Date</u>	<u>Title</u>	<u>Cost</u>
C00-227*	Mar 1963	Research in Radiobiology	\$ 7.60
C00-228*	Sep 1963	Research in Radiobiology	7.60
C00-119-229	Mar 1964	Research in Radiobiology	7.60
C00-119-230*	Jul 1964	(Superseded by C00-119-245)	4.00
C00-119-231*	Sep 1964	Research in Radiobiology	7.60
C00-119-232*	Mar 1965	Research in Radiobiology	7.60
C00-119-233*	Sep 1965	Research in Radiobiology	5.45
C00-119-234*	Mar 1966	Research in Radiobiology	10.60
C00-119-235	Sep 1966	Research in Radiobiology	5.45
C00-119-236*	Mar 1967	Research in Radiobiology	7.60
C00-119-237*	Mar 1968	Research in Radiobiology	7.60
C00-119-238*	Aug 1968	Rb in RBC, Plasma, and Urine	5.45
C00-119-239	Dec 1968	Cs, Rb, and K Metabolism	5.45
C00-119-240*	Mar 1969	Research in Radiobiology	7.60
C00-119-241*	Mar 1970	Retention and Dosimetry	7.60
C00-119-242*	Mar 1970	Research in Radiobiology	10.60
C00-119-243*	Jan 1971	Osteosarcoma Growth Dynamics	5.45
C00-119-244*	Mar 1971	Research in Radiobiology	10.60
C00-119-245*	May 1971	Radiobiology Safety Manual	4.00
C00-119-246*	Mar 1972	Research in Radiobiology	10.60
C00-119-247*	Oct 1972	Rb and Cs Metabolism	5.45
C00-119-248*	Mar 1973	Research in Radiobiology	10.60
C00-119-249*	Mar 1974	Research in Radiobiology	8.85

* Also available on request from this laboratory.

STATUS OF THE BEAGLE COLONY

as of 31 March 1974

NUCLIDE	NUMBER OF DOGS
^{253}Es (einsteinium)	7
^{252}Cf (californium)	36
^{249}Cf (californium)	31
$^{243/244}\text{Cm}$ (curium)	1
^{241}Am (americium)	81
^{239}Pu (plutonium)	202
^{228}Th (radiothorium)	16
^{228}Ra (mesothorium)	8
^{226}Ra (radium)	73
^{224}Ra (quickradium)	9
^{90}Sr (strontium)	5
X-Ray	2
Ancillary (breeding and others)	44
Unassigned	<u>157</u>
TOTAL	<u><u>672</u></u>

PREFACE TO THE INJECTION TABLES

Tables I and II list the toxicity and test animals, respectively. Toxicity animals are those animals which are usually maintained until sacrifice becomes a clinical necessity; test animals may be sacrificed as needed for special studies.

Dogs are put into the toxicity study at graded injection levels. At each level, about half the dogs are male and half female. Litter mates are used whenever possible. Each animal receives the designated dose of one radionuclide in a single intravenous injection at approximately 17 months of age. At this age the skeleton is mature with all epiphyses fused except those of the ribs. Twelve such groups have been injected for each of the six radionuclides, ^{226}Ra , ^{239}Pu , ^{228}Ra , ^{228}Th , ^{90}Sr , and ^{241}Am . The present injection program involves ^{226}Ra and ^{239}Pu at lower dose levels, ^{249}Cf and ^{252}Cf , and other animals receiving various radionuclides of current interest.

The five injection levels designated by integers are those specified at the early meetings of the consultants, and those designated by nonintegers have been added by the laboratory staff. Since those injection levels were originally specified in "retained" activities, the actual injections are four times the desired "retained" $\mu\text{Ci/kg}$ of ^{226}Ra , ^{228}Ra (Mesothorium), and ^{90}Sr , and 1.11 times the desired "retained" $\mu\text{Ci/kg}$ of ^{239}Pu , ^{228}Th (Radiothorium), ^{249}Cf , ^{252}Cf , and $^{243/244}\text{Cm}$.* The desired "retained" activities are the same for all the radionuclides except ^{90}Sr , in which case they are greater by a

*Since radioactive decay and excretion occur continuously, the term body retention is obviously meaningless unless the time after injection is specified. Our present measurements indicate that:

average ^{226}Ra retention	= 0.25 at about 271 days
average ^{239}Pu retention	= 0.90 at about 6 days
average ^{228}Ra retention	= 0.25 at about 214 days
average ^{228}Th retention	= 0.90 at about 6 days
average ^{90}Sr retention	= 0.25 at about 134 days
average ^{241}Am retention	= 0.90 at about 6 days
average ^{249}Cf retention	= 0.90 at about 1 day
average ^{252}Cf retention	= 0.90 at about 1 day
av. $^{243/244}\text{Cm}$ retention	= 0.90 at about 1 day

factor of 10. Injection level 1 is the basis of the scheme, and is 10 times the maximum permissible concentration of ^{226}Ra in man. Level 1 =

$$10 \times \frac{0.1 \text{ } \mu\text{Ci } ^{226}\text{Ra}}{70 \text{ kg man}} = 0.0143 \text{ "retained" } \mu\text{Ci/kg. All other injection levels}$$

are simple multiples of level 1 as shown below.

Level 0.1	is	1/27	of level 1
Level 0.2	is	1/9	of level 1
Level 0.5	is	1/3	of level 1
Level 0.7	is	2/3	of level 1
Level 1.5	is	2	times level 1
Level 1.7	is	3	times level 1
Level 2	is	6	times level 1
Level 3	is	18	times level 1
Level 4	is	54	times level 1
Level 4.5	is	94	times level 1
Level 5	is	162	times level 1

The numbering system for the dogs has been built around the injection program and serves as a code to describe each dog's place in the experiment. The first letter tells the sex of toxicity animals (M = male, F = female). When the first letter is T, the dog is a test animal. M, F, or T is followed by a number which denotes chronological order of groups in the case of toxicity dogs and of individual test dogs.

Next comes a code letter for the radionuclide: R = ^{226}Ra , P = ^{239}Pu , M = ^{228}Ra , T = ^{228}Th , S = ^{90}Sr , J = ^{85}Sr , Q = ^{224}Ra , W = ^{241}Am , L = ^{210}Pb , G = ^{249}Cf , F = ^{252}Cf , C = $^{243/244}\text{Cm}$, E = ^{253}Es , and A = Ancillary.

"A" following the regular dog number means that the dog is a replacement, "H" following the regular dog number means that the dog received its dose in more than one injection. "B", "C", or "D" denotes special assignment. "E" in the final position is used to denote that the dog listed is a St. Bernard. "P" in the final position indicates that the nuclide was injected in polymeric form. "Y" in the final position indicates that the animal was injected as a juvenile. Letters denoting a radionuclide may follow the final number, in which case the letter indicates that two radionuclides were given. The in-

jection level refers to the radionuclide appearing first in the identifying code.

Example: MIR5 is a male animal in the first radium group at the highest injection level.

Although MIR5, MIR4, MIR3, MIR2, MIR1, and MIRO constitute a group and were injected at the same time, the tables are arranged according to injection level to facilitate comparison of all the R5 animals, all the R4 animals, etc.

The conditions listed in the injection tables under "Comments on Dead Dogs" present the lesions or factors that had the most prominent effect on the clinical status of the animal. For example, multiple rib fractures, which seldom produced symptoms, are not listed, even though their incidence was usually much higher than the crippling fractures involving the limb bones or mandible. The hematological changes have been omitted unless they were extreme. Increased rate of tooth loss, hepatic changes, eye lesions, and many other factors in the various syndromes have not been included because of space limitations. Over the years many soft tissue tumors have been removed surgically. In many instances, the conditions that have been listed were the reasons for sacrifice of the animal but they were not the immediate cause of death. Most of the animals were euthanized when death appeared imminent or when life could no longer be prolonged humanely.

DOSIMETRY

The injection tables include the calculated average dose in rads to the skeleton at death. ^{226}Ra , ^{228}Ra , and ^{90}Sr doses are calculated for each dog using his individually observed retention values: ^{239}Pu and ^{228}Th doses are from our average skeletal retention equations. For our standard beagle, the following equations were used for the EFFECTIVE* skeletal retention at (t) days after injection:

*Effective retention is decreased both by radioactive decay and biological elimination.

$$^{226}\text{Ra} = 0.412e^{-0.558t} + 0.105e^{-0.0730t} + 0.196e^{-0.00488t} + 0.287e^{-0.000299t}$$

(5-level only)

$$^{226}\text{Ra} = 0.251e^{-0.982t} + 0.211e^{-0.269t} + 0.210e^{-0.0155t} + 0.177e^{-0.00204t} + 0.151e^{-0.000150t}$$

(lower levels)

$$^{222}\text{Rn}/^{226}\text{Ra} = 0.075 (1 - e^{-0.181t}) t^{0.158}$$

(all levels)

$$^{239}\text{Pu} \text{ (dose level 5)} = 0.07e^{-0.0011t} + 0.43$$

$$^{239}\text{Pu} \text{ (dose level 4)} = 0.11e^{-0.0011t} + 0.39$$

$$^{239}\text{Pu} \text{ (dose level 3)} = 0.15e^{-0.0011t} + 0.34$$

$$^{239}\text{Pu} \text{ (dose levels 2 and less)} = 0.29e^{-0.0011t} + 0.21$$

$$^{228}\text{Ra} = 0.251e^{-0.982t} + 0.211e^{-0.269t} + 0.210e^{-0.0158t} + 0.177e^{-0.00237t} + 0.151e^{-0.000479t}$$

(pure at $t = 0$)

84% retention of in vivo produced ^{228}Th and daughters.

$$^{228}\text{Th} = 0.69e^{-0.00113t}$$

$$^{224}\text{Ra}/^{228}\text{Th} = 0.895$$

$$^{212}\text{Pb}/^{228}\text{Th} = 0.866$$

$$^{90}\text{Sr} = 0.36e^{-0.95t} + 0.29e^{-0.12t} + 0.10e^{-0.0092t} + 0.12e^{-0.0020t} + 0.13e^{-0.00022t}$$

Detailed retention data and dosimetric analyses have been presented in a special report C00-119-241 (March 1970).

The revised skeletal doses in this report are based upon a wet skeleton which weighs 7.5% of the body weight at the time of injection. (C00-119-246, pp. 167-192, 1972).

^{228}Ra doses deserve special comment. The dose from "pure" ^{228}Ra and its in vivo produced daughters is based on our best evaluation of 5.77 ± 0.02 yr for the ^{228}Ra half-period. The tabulated total doses include the contributions from ^{228}Th contamination in the injection solutions. For example, ^{228}Th contaminations of 0.6%, 3%, and 15% respectively, account for 2.7%, 13%, and 42% of the total dose in rads at 1000 days. If injected ^{228}Th is 4 times more toxic rad-for-rad than is in vivo produced ^{228}Th , these injected ^{228}Th contaminations would account for 10%, 37%, and 74% of the total biological damage at 1000 days. Therefore, it may be desirable to use only results from the slightly contaminated (0.6% ^{228}Th) dogs in evaluation of ^{228}Ra toxicity.

TABLE I. TOXICITY ANIMALS (MAR. 31 74)

A. RADIUM-226

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	DEATH	
M001R0.0	558	8.03		20	4	53		3116
M002R0.0	487	14.60		16	11	53		3675
F003R0.0	601	11.40		10	3	54		2139
M004R0.0	461	11.00		7	4	54		5284
M005R0.0	460	6.57		22	6	54		4018
F006R0.0	483	8.43		27	7	54		3182
M007R0.0	511	11.00		24	8	54		3360
F008R0.0	638	8.21		21	12	54		3361
F009R0.0	700	11.70		11	4	55		1550
M010R0.0	522	10.90		27	7	55		4698
F011R0.0	544	10.20		20	12	55		4575
F012R0.0	501	8.68		17	1	56		4283
M013R0.0	515	12.30		4	3	64	3679	
F014R0.0	536	10.80		23	10	64	3446	
M015R0.0	564	12.80		4	2	65	3342	
F016R0.0	469	10.00		7	4	65	3280	
M017R0.0	469	12.50		27	4	66	2895	
F018R0.0	497	12.00		25	5	66	2867	
F019R0.0	533	8.42		13	10	66	2726	
M020R0.0	536	9.70		29	12	66	2649	
F021R0.0	549	9.90		26	1	67	2621	
M022R0.0	533	12.10		22	3	67	2566	
F031R0.0B	536	10.60		23	10	64	3446	
F031R0.0C	536	9.88		23	10	64	3446	
F031R0.0D	542	9.90		21	9	65	3113	
F032R0.0B	542	7.80		21	9	65	3113	
F032R0.0C	532	11.70		21	9	65	3113	
F032R0.0D	532	9.70		21	9	65	3113	
F033R0.0B	532	9.80		21	9	65	3113	
F033R0.0C	496	9.50		25	5	66	2867	
F033R0.0D	496	11.80		25	5	66	2867	
F034R0.0B	525	8.20		26	1	67	2621	
F034R0.0C	520	8.90		22	3	67	2566	
F034R0.0D	484	9.90		22	3	67	2566	
F035R0.0B	502	9.41		1	2	68	2250	
F035R0.0C	502	9.38		1	2	68	2250	
F035R0.0D	552	8.86		9	1	69	1907	
F036R0.0B	467	10.10		2	7	68	2098	
F036R0.0C	467	9.17		2	7	68	2098	
F036R0.0D	467	9.08		2	7	68	2098	
F037R0.0B	801	11.10		20	5	69	1776	
F037R0.0C	501	10.40		23	9	70	1285	
F037R0.0D	501	10.90		23	9	70	1285	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001R0.0	SEMINOMA, LYMPHOSARCOMA
M002R0.0	TRANSITIONAL CELL CARCINOMA
F003R0.0	STATUS EPILEPTICUS
M004R0.0	CHRONIC INTERSTITIAL NEPHRITIS; THROMBOSIS
M005R0.0	OBTURATING PULMONARY EMBOLISM
F006R0.0	STATUS EPILEPTICUS
M007R0.0	STATUS EPILEPTICUS, NEPHRITIS
F008R0.0	PANCREATIC ADENOCARCINOMA
F009R0.0	AORTIC BODY TUMOR
M010R0.0	NEPHRITIS
F011R0.0	VAGINAL FIBROMA
F012R0.0	UNDETERMINED
M013R0.0	
F014R0.0	
M015R0.0	
F016R0.0	
M017R0.0	
F018R0.0	
F019R0.0	
M020R0.0	
F021R0.0	
M022R0.0	
F031R0.0B	
F031R0.0C	
F031R0.0D	
F032R0.0B	
F032R0.0C	
F032R0.0D	
F033R0.0B	
F033R0.0C	
F033R0.0D	
F034R0.0B	
F034R0.0C	
F034R0.0D	
F035R0.0B	
F035R0.0C	
F035R0.0D	
F036R0.0B	
F036R0.0C	
F036R0.0D	
037R0.0B	
037R0.0C	
F037R0.0D	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74	DEATH	DOSE TO SKELETON (RADS)
F038R0.0B	501	11.50		23 9 70	1285		
F042R0.0B	338	8.00		25 4 69		33	
M013R0.2	529	9.77	0.00577	4 3 64	3679		
F014R0.2	460	8.10	0.00836	23 10 64	3446		
M015R0.2	504	10.80	0.00873	4 2 65	3342		
F016R0.2	486	8.90	0.00665	7 4 65	3280		
M017R0.2	494	11.80	0.00711	27 4 66	2895		
F018R0.2	497	9.30	0.00652	25 5 66	2867		
F019R0.2	533	10.60	0.00785	13 10 66	2726		
M020R0.2	546	11.40	0.00676	29 12 66	2649		
F021R0.2	549	11.50	0.00687	26 1 67	2621		
M022R0.2	533	12.90	0.00961	22 3 67	2566		
M013R0.5	529	11.00	0.0171	4 3 64		3676	57
F014R0.5	510	9.75	0.022	23 10 64	3446		
M015R0.5	490	10.40	0.0263	4 2 65	3342		
F016R0.5	500	11.40	0.0205	7 4 65	3280		
M017R0.5	494	9.20	0.0215	27 4 66	2895		
F018R0.5	496	9.10	0.0197	25 5 66	2867		
F019R0.5	533	10.00	0.023	13 10 66	2726		
M020R0.5	536	13.20	0.0206	29 12 66	2649		
F021R0.5	538	8.80	0.0208	26 1 67	2621		
M022R0.5	520	12.30	0.029	22 3 67	2566		
M031R0.5B	508	11.40	0.021	27 4 66	2895		
F031R0.5C	537	9.40	0.0235	22 12 65	3021		
F031R0.5D	537	11.70	0.0238	22 12 65	3021		
M032R0.5B	496	13.40	0.0196	25 5 66	2867		
F032R0.5C	519	10.10	0.0239	22 12 65	3021		
F032R0.5D	509	10.10	0.024	22 12 65	3021		
M033R0.5B	497	12.90	0.0194	25 5 66	2867		
F033R0.5C	527	10.60	0.0212	27 4 66	2895		
F033R0.5D	527	8.70	0.0217	27 4 66	2895		
M034R0.5B	496	10.50	0.0196	25 5 66	2867		
F034R0.5C	524	9.90	0.0215	27 4 66	2895		
F034R0.5D	508	9.70	0.0212	27 4 66	2895		
M035R0.5B	536	10.40	0.0205	29 12 66	2649		
F035R0.5C	532	9.00	0.0201	29 12 66	2649		
F035R0.5D	532	10.20	0.0202	29 12 66	2649		

DOG
NUMBER

COMMENTS ON DEAD DOGS

F038R0.0B

F042R0.0B SPECIAL STUDY

M013R0.2

F014R0.2

M015R0.2

F016R0.2

M017R0.2

F018R0.2

F019R0.2

M020R0.2

F021R0.2

M022R0.2

M013R0.5

BILE DUCT CARCINOMA

F014R0.5

M015R0.5

F016R0.5

M017R0.5

F018R0.5

F019R0.5

M020R0.5

F021R0.5

M022R0.5

M031R0.5B

F031R0.5C

F031R0.5D

M032R0.5B

F032R0.5C

F032R0.5D

M033R0.5B

F033R0.5C

F033R0.5D

M034R0.5B

F034R0.5C

F034R0.5D

M035R0.5B

F035R0.5C

F035R0.5D

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M001R1.0	471	8.48	0.0618	20	4	53	5727	227
M002R1.0	627	10.00	0.0876	16	11	53	4054	316
F003R1.0	706	8.68	0.0576	10	3	54	3860	201
M004R1.0	414	8.60	0.0642	7	4	54	2038	144
M005R1.0	490	11.70	0.0436	22	6	54	3780	149
F006R1.0	483	7.23	0.0584	27	7	54	5260	249
M007R1.0	511	11.40	0.0651	24	8	54	3544	223
F008R1.0	861	8.98	0.0559	21	12	54	2988	124
F009R1.0	781	9.88	0.0521	11	4	55	4399	132
M010R1.0	523	11.50	0.0573	27	7	55	4003	209
F011R1.0	511	11.20	0.0522	20	12	55	5462	200
F012R1.0	501	9.71	0.0444	17	1	56	3978	165
M013R1.0	529	11.70	0.0527	4	3	64	3679	
F014R1.0	510	10.50	0.0701	23	10	64	1729	197
M015R1.0	490	8.88	0.0797	4	2	65	893	109
F016R1.0	501	8.99	0.0611	7	4	65	3280	
M017R1.0	494	11.40	0.0639	27	4	66	2895	
F018R1.0	496	10.00	0.0589	25	5	66	2865	
F019R1.0	526	11.60	0.0682	13	10	66	2726	
M020R1.0	536	10.00	0.061	29	12	66	2649	
F021R1.0	525	8.10	0.0633	26	1	67	2621	
M022R1.0	484	10.90	0.0861	22	3	67	2566	
F031R1.0B	509	10.40	0.0712	22	12	65	3009	209
M001R1.7	523	9.98	0.137	17	1	56	4438	455
M002R1.7	598	7.85	0.163	30	11	56	1273	155
M002R1.7A	493	12.00	0.222	6	3	63	3254	973
F003R1.7	473	13.10	0.165	20	12	55	3267	513
M004R1.7	514	6.20	0.163	20	12	55	5495	684
M005R1.7	511	10.10	0.151	20	12	55	4107	651
F006R1.7	491	7.90	0.152	20	12	55	3432	556
M007R1.7	598	7.17	0.163	30	11	56	3142	412
F008R1.7	491	9.50	0.154	20	12	55	2577	504
F009R1.7	598	7.55	0.168	30	11	56	3914	349
M010R1.7	590	9.57	0.167	30	11	56	557	145
M010R1.7A	545	10.60	0.183	7	1	59	4903	681
F011R1.7	598	8.17	0.165	30	11	56	5320	400
F012R1.7	590	8.95	0.167	30	11	56	2399	280

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001R1.0	MELANOMA ORAL CAVITY
M002R1.0	SEMINOMA
F003R1.0	MAMMARY GLAND CARCINOMA
M004R1.0	TRAUMA
M005R1.0	TRANSITIONAL CELL CARCINOMA, HYDRONEPHROSIS
F006R1.0	NEPHRITIS
M007R1.0	STATUS EPILEPTICUS
F008R1.0	LYMPHOSARCOMA
F009R1.0	PNEUMONIA
M010R1.0	FIBROSARCOMA (GINGIVA)
F011R1.0	MELANOMA (EYE); MAMMARY CARCINOMA
F012R1.0	MELANOMA, GINGIVA
M013R1.0	
F014R1.0	UNDETERMINED (NO NEOPLASIA)
M015R1.0	UNDETERMINED (NO NEOPLASIA)
F016R1.0	
M017R1.0	
F018R1.0	
F019R1.0	
M020R1.0	
F021R1.0	
M022R1.0	
F031R1.0B	STATUS EPILEPTICUS

M001R1.7	OBTURATING ABDOMINAL AORTA AND PULMONARY EMBOLISM
M002R1.7	LYMPHOSARCOMA
M002R1.7A	FIBROSARCOMA (GINGIVA)
F003R1.7	MAMMARY GLAND CARCINOMA
M004R1.7	NEPHRITIS
M005R1.7	OSTEOSARCOMA
F006R1.7	PULMONARY CALCIFICATION (NO BONE TUMOR)
M007R1.7	BACTERIAL TOXEMIA, INTERSTITIAL CELL ADENOMA
F008R1.7	DRUG ALLERGY
F009R1.7	PYOMETRA
M010R1.7	TRAUMA
M010R1.7A	UNDIFFERENTIATED MALIGNANCY (NON-SKELETAL); MELANOMA (EYE)
F011R1.7	MELANOMA (ORAL); THYROID CARCINOMA
F012R1.7	UNDETERMINED (NO BONE TUMOR)

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M001R2.0	471	8.74	0.382	20	4	53	3440	1147
M002R2.0	592	8.21	0.387	16	11	53	3775	833
F003R2.0	541	8.53	0.347	10	3	54	4459	1163
M004R2.0	414	10.50	0.361	7	4	54	325	252
M004R2.0A	420	10.60	0.306	11	4	55	4368	1523
M005R2.0	461	11.50	0.267	22	6	54	4703	1325
F006R2.0	486	10.60	0.36	27	7	54	4615	1685
M007R2.0	514	11.10	0.413	24	8	54	3425	1229
F008R2.0	572	6.95	0.331	21	12	54	4781	1328
F009R2.0	592	9.38	0.317	11	4	55	3998	1355
M010R2.0	523	9.95	0.345	27	7	55	3569	1627
F011R2.0	495	9.30	0.31	20	12	55	3297	971
F012R2.0	497	10.30	0.281	17	1	56	2948	989
M001R3.0	473	8.91	1.2	20	4	53	2850	3193
M002R3.0	470	9.02	1.21	16	11	53	2226	2303
F003R3.0	386	7.74	1.11	10	3	54	2497	3097
M004R3.0	412	11.70	1.16	7	4	54	1917	3148
M005R3.0	461	13.00	0.846	22	6	54	2955	3089
F006R3.0	486	9.75	1.14	27	7	54	1932	2995
M007R3.0	514	12.30	1.29	24	8	54	2099	4039
F008R3.0	542	7.76	1.03	21	12	54	2612	2555
F009R3.0	551	8.02	0.987	11	4	55	2487	2452
M010R3.0	525	10.10	1.06	27	7	55	1737	3115
F011R3.0	495	12.90	0.938	20	12	55	1610	1777
F012R3.0	497	11.40	0.883	17	1	56	1897	2185
M001R4.0	471	9.08	3.51	20	4	53	1606	8767
M002R4.0	470	9.53	3.55	16	11	53	1884	8200
F003R4.0	384	8.65	3.33	10	3	54	490	2944
F003R4.0A	598	7.20	3.1	30	11	56	1614	5140
M004R4.0	408	8.83	3.47	7	4	54	1518	8084
M005R4.0	461	13.20	2.42	22	6	54	1659	6007
F006R4.0	486	8.55	3.44	27	7	54	1939	9511
M007R4.0	453	9.55	3.88	24	8	54	1647	7792
F008R4.0	474	8.94	3.14	21	12	54	1324	6153
F009R4.0	542	8.53	3.02	11	4	55	1471	5460
M010R4.0	527	10.80	3.28	27	7	55	1553	10109
F011R4.0	491	10.40	2.84	20	12	55	1469	7031

DOG NUMBER	COMMENTS ON DEAD DOGS
M001R2.0	HEMANGIOSARCOMA (SPLEEN)
M002R2.0	OSTEOSARCOMA
F003R2.0	RETICULUM CELL SARCOMA (NON-SKELETAL)
M004R2.0	PERFORATED ILEUM
M004R2.0A	VALVULAR ENDOCARDITIS
M005R2.0	OSTEOSARCOMA, ADRENAL CORTICAL CARCINOMA
F006R2.0	EPIDERMOID CARCINOMA (TYMPANIC BULLA)
M007R2.0	OSTEOSARCOMA, CUSHING SYNDROME
F008R2.0	UNDIFFERENTIATED MALIGNANCY, SMALL INTESTINE
F009R2.0	MAMMARY CARCINOMA
M010R2.0	OSTEOSARCOMA
F011R2.0	OSTEOSARCOMA
F012R2.0	MAMMARY ADENOCARCINOMA
M001R3.0	OSTEOSARCOMA
M002R3.0	OSTEOSARCOMA
F003R3.0	OSTEOSARCOMA
M004R3.0	OSTEOSARCOMA
M005R3.0	OSTEOSARCOMA
F006R3.0	OSTEOSARCOMA
M007R3.0	OSTEOSARCOMA
F008R3.0	OSTEOSARCOMA
F009R3.0	OSTEOSARCOMA
M010R3.0	OSTEOSARCOMA
F011R3.0	PYOMETRITIS + SECONDARY PERITONITIS
F012R3.0	OSTEOSARCOMA
M001R4.0	OSTEOSARCOMA
M002R4.0	OSTEOSARCOMA
F003R4.0	CANINE DISTEMPER
F003R4.0A	OSTEOSARCOMA
M004R4.0	OSTEOSARCOMA
M005R4.0	OSTEOSARCOMA
F006R4.0	OSTEOSARCOMA
M007R4.0	OSTEOSARCOMA
F008R4.0	OSTEOSARCOMA
009R4.0	OSTEOSARCOMA
010R4.0	OSTEOSARCOMA
F011R4.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F012R4.0	496	9.61	2.81	17	1	56	1435	5169
M001R5.0	473	9.87	10.5	20	4	53	908	19924
M002R5.0	470	8.85	10.8	16	11	53	1380	24095
F003R5.0	380	7.82	10.1	10	3	54	481	9529
M004R5.0	408	8.90	10.6	7	4	54	1091	21889
M005R5.0	458	10.90	10.1	22	6	54	1220	20577
F006R5.0	486	9.66	10.2	27	7	54	1015	20552
M007R5.0	453	8.85	11.9	24	8	54	1288	22277
F008R5.0	474	7.76	9.68	21	12	54	968	15419
F009R5.0	420	9.16	9.48	11	4	55	1288	21255
M010R5.0	527	10.70	10.2	27	7	55	825	14905

DOG NUMBER	COMMENTS ON DEAD DOGS
F012R4.0	OSTEOSARCOMA
M001R5.0	OSTEOSARCOMA
M002R5.0	OSTEOSARCOMA
F003R5.0	CANINE DISTEMPER
M004R5.0	OSTEOSARCOMA
M005R5.0	OSTEOSARCOMA
F006R5.0	OSTEOSARCOMA
M007R5.0	OSTEOSARCOMA
F008R5.0	OSTEOSARCOMA
F009R5.0	OSTEOSARCOMA + ANEMIA
M010R5.0	OSTEOSARCOMA + FRACTURED MANDIBLE

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74	DEATH	DOSE TO SKELETON (RADS)
M001P0.0	443	9.70		1 12 52			4003
F002P0.0	424	6.36		2 3 53			2755
M003P0.0	515	10.80		1 6 53			5362
M004P0.0	426	10.70		16 9 53			5138
F005P0.0	620	9.75		14 10 53			4088
F006P0.0	410	5.59		12 5 54			4490
F007P0.0	515	6.90		25 10 54			5344
M008P0.0	585	10.90		15 3 55			4072
F009P0.0	574	11.00		22 11 55			3032
F010P0.0	658	11.00		22 11 55			3971
M011P0.0	602	10.30		24 4 56			3821
M012P0.0	630	10.90		29 5 56			4143
F013P0.0	517	9.47		4 3 64	3679		
F014P0.0	452	9.89		12 5 64	3610		
M015P0.0	527	12.10		23 10 64	3446		
M016P0.0	486	13.90		7 4 65	3280		
M017P0.0	551	12.20		18 11 66	2700		
F018P0.0	536	11.40		29 11 66	2679		
M019P0.0	536	13.10		29 11 66	2679		
F020P0.0	546	8.50		29 12 66	2649		
M021P0.0	549	13.30		26 1 67	2621		
F022P0.0	489	10.60		25 5 67	2502		
M031P0.0B	452	11.80		12 5 64		1763	
M031P0.0C	452	12.60		12 5 64	3610		
M032P0.0B	452	11.20		12 5 64	3610		
M032P0.0C	542	10.30		21 9 65	3113		
M033P0.0B	517	12.10		21 9 65	3113		
M033P0.0C	503	11.70		18 11 65	3055		
M034P0.0B	525	13.50		26 1 67	2621		
M034P0.0C	484	12.70		22 3 67	2566		
M035P0.0B	484	12.50		22 3 67	2566		
M035P0.0C	484	13.10		22 3 67	2566		
M036P0.0B	489	11.00		25 5 67	2502		
M036P0.0C	485	12.20		25 5 67	2502		
M037P0.0B	507	11.70		22 6 67	2474		
M037P0.0C	493	10.40		22 6 67	2474		
M038P0.0B	529	10.70		16 11 67	2327		
M038P0.0C	529	12.20		16 11 67	2327		
M039P0.0B	501	10.70		21 12 67	2292		
M039P0.0C	501	10.10		21 12 67	2292		
M040P0.0B	484	10.30		30 7 68	2070		
M040P0.0C	552	11.40		9 1 69	1907		
M041P0.0B	560	9.49		17 1 69	1899		

DOG NUMBER	COMMENTS ON DEAD DOGS
M001P0.0	SPLENIC RUPTURE, METASTATIC SEMINOMA
F002P0.0	ANESTHETIC ACCIDENT
M003P0.0	PANCREATIC ADENOCARCINOMA
M004P0.0	THYROID CARCINOMA, NEPHRITIS
F005P0.0	ADRENAL CORTICAL CARCINOMA
F006P0.0	OBTURATING PULMONARY EMBOLISM
F007P0.0	RHABDOMYOSARCOMA
M008P0.0	CIRCULATORY FAILURE
F009P0.0	PULMONARY EMBOLISM, NEPHRITIS
F010P0.0	LEUKEMIA
M011P0.0	FIBROSARCOMA (SPLEEN)
M012P0.0	TESTICULAR CARCINOMA
F013P0.0	
F014P0.0	
M015P0.0	
M016P0.0	
M017P0.0	
F018P0.0	
M019P0.0	
F020P0.0	
M021P0.0	
F022P0.0	
M031P0.0B	STATUS EPILEPTICUS; BILE DUCT OBSTRUCTION
M031P0.0C	
M032P0.0B	
M032P0.0C	
M033P0.0B	
M033P0.0C	
M034P0.0B	
M034P0.0C	
M035P0.0B	
M035P0.0C	
M036P0.0B	
M036P0.0C	
M037P0.0B	
M037P0.0C	
M038P0.0B	
M038P0.0C	
M039P0.0B	
M039P0.0C	
040P0.0B	
040P0.0C	
M041P0.0B	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
M042P0.0B	338	11.60		25 4 69	32	
M081P0.0Y	105	7.10		9 3 72	752	
F082P0.0Y	105	7.30		9 3 72	752	
F083P0.0Y	91	3.97		25 4 72	705	
F084P0.0Y	89	3.67		25 4 72	705	
M086P0.0Y	91	4.37		25 4 72	705	
F013P0.1	515	9.46	0.00068	4 3 64	3679	
F014P0.1	452	10.30	0.00055	12 5 64	3610	
M015P0.1	527	9.67	0.00071	23 10 64	3446	
M016P0.1	501	12.00	0.00059	7 4 65	3280	
M017P0.1	551	12.20	0.00057	18 11 66	2700	
F018P0.1	536	9.28	0.0007	29 11 66	2679	
M019P0.1	536	11.60	0.00063	29 11 66	2679	
F020P0.1	536	9.80	0.00075	29 12 66	2649	
M021P0.1	538	11.30	0.00059	26 1 67	2621	
F022P0.1	489	9.80	0.00059	25 5 67	2502	
M031P0.1B	517	12.20	0.00068	4 3 64	2760	2
F032P0.1B	549	10.40	0.00059	18 11 65	3055	
M033P0.1B	549	10.80	0.00079	18 11 65	3055	
F034P0.1B	533	11.10	0.00058	18 11 66	2700	
M035P0.1B	489	10.30	0.00059	25 5 67	2502	
F036P0.1B	493	9.79	0.0006	22 6 67	2474	
M037P0.1B	493	11.30	0.00059	22 6 67	2474	
F038P0.1B	513	9.52	0.00057	21 12 67	1979	1
M039P0.1B	490	10.50	0.00058	21 12 67	2292	
M044P0.1B	500	10.90	0.00057	8 8 73	235	
F044P0.1C	569	8.34	0.00072	2 12 70	1215	
M045P0.1C	500	9.00	0.00051	8 8 73	235	
F046P0.1C	500	10.20	0.00057	8 8 73	235	
F047P0.1	500	9.34	0.00051	8 8 73	235	
F013P0.2	517	9.44	0.00206	4 3 64	3221	7
F014P0.2	516	7.44	0.00173	12 5 64	3610	
M015P0.2	505	10.90	0.00201	23 10 64	3446	
M016P0.2	500	11.40	0.00163	7 4 65	2841	5
M017P0.2	533	11.80	0.00171	18 11 66	2700	
F018P0.2	530	9.46	0.002	29 11 66	2679	
M019P0.2	530	12.10	0.00198	29 11 66	2679	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M042P0.0B SPECIAL STUDY
M081P0.0Y
F082P0.0Y
F083P0.0Y
F084P0.0Y
M086P0.0Y

F013P0.1
F014P0.1
M015P0.1
M016P0.1
M017P0.1
F018P0.1
M019P0.1
F020P0.1
M021P0.1
F022P0.1
M031P0.1B STATUS EPILEPTICUS
F032P0.1B
M033P0.1B
F034P0.1B
M035P0.1B
F036P0.1B
M037P0.1B
F038P0.1B TRAUMA
M039P0.1B
M044P0.1B
F044P0.1C
M045P0.1C
F046P0.1C
F047P0.1

F013P0.2
F014P0.2
M015P0.2
M016P0.2
017P0.2
018P0.2
M019P0.2

INTESTINAL ILEUS

ENCEPHALITIS

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F020P0.2	532	8.30	0.00224	29 12 66	2649	
M021P0.2	538	12.10	0.00181	26 1 67	2621	
F022P0.2	485	8.30	0.00176	25 5 67	2502	
M031P0.2B	515	10.70	0.00185	4 3 64	2640	5
F031P0.2C	452	11.90	0.00169	12 5 64	3610	
F031P0.2D	429	9.35	0.00186	12 5 64	3610	
M032P0.2B	549	13.60	0.00178	18 11 65	3055	
F032P0.2C	494	10.10	0.00183	4 2 65	3342	
F032P0.2D	490	8.04	0.00193	4 2 65	3342	
M033P0.2B	513	14.50	0.00178	18 11 65	2776	5
F033P0.2C	549	12.50	0.00176	18 11 65	3055	
F033P0.2D	513	12.70	0.00178	18 11 65	3055	
M034P0.2B	533	12.70	0.0017	18 11 66	2700	
F034P0.2C	533	11.50	0.00172	18 11 66	2700	
F034P0.2D	519	9.92	0.00167	18 11 66	2700	
M035P0.2B	489	11.20	0.00173	25 5 67	2502	
F035P0.2C	507	10.50	0.00175	22 6 67	2474	
F035P0.2D	507	9.10	0.00175	22 6 67	2474	
M036P0.2B	479	12.90	0.00177	25 5 67	2502	
F036P0.2C	493	10.40	0.00177	22 6 67	2474	
F036P0.2D	569	8.74	0.00146	16 11 67	2327	
M037P0.2B	529	10.60	0.00149	16 11 67	2327	
F037P0.2C	529	10.10	0.0015	16 11 67	2327	
F037P0.2D	529	7.14	0.00153	16 11 67	2327	
M038P0.2B	517	10.00	0.00152	16 11 67	2327	
F038P0.2C	501	7.95	0.00211	21 12 67	2292	
F038P0.2D	499	9.08	0.00176	21 12 67	2292	
M039P0.2B	542	11.60	0.00214	2 12 70	1215	
F039P0.2C	499	9.46	0.00173	21 12 67	2292	
F039P0.2D	499	9.34	0.00176	21 12 67	2292	
M042P0.2B	504	9.42	0.00182	8 8 73	235	
F042P0.2C	589	9.55	0.00176	4 9 69	1669	
F043P0.2C	542	9.50	0.00239	2 12 70	1215	
M044P0.2B	540	11.60	0.00177	17 11 71	97	<<1
F013P0.5	517	9.93	0.0054	4 3 64	2388	15
F013P0.5A	501	11.20	0.00495	23 9 70	1285	
F014P0.5	516	9.98	0.00493	12 5 64	3610	
M015P0.5	505	8.41	0.00627	23 10 64	3446	
M016P0.5	501	12.60	0.00521	7 4 65	3280	
M017P0.5	533	13.40	0.00506	18 11 66	2700	

DOG NUMBER	COMMENTS ON DEAD DOGS
F020P0.2	
M021P0.2	
F022P0.2	
M031P0.2B	MELANOMA (ORAL)
F031P0.2C	
F031P0.2D	
M032P0.2B	
F032P0.2C	
F032P0.2D	
M033P0.2B	PNEUMONIA
F033P0.2C	
F033P0.2D	
M034P0.2B	
F034P0.2C	
F034P0.2D	
M035P0.2B	
F035P0.2C	
F035P0.2D	
M036P0.2B	
F036P0.2C	
F036P0.2D	
M037P0.2B	
F037P0.2C	
F037P0.2D	
M038P0.2B	
F038P0.2C	
F038P0.2D	
M039P0.2B	
F039P0.2C	
F039P0.2D	
M042P0.2B	
F042P0.2C	
F043P0.2C	
M044P0.2B	SPECIAL STUDY
F013P0.5	MAMMARY CARCINOMA
F013P0.5A	
F014P0.5	
015P0.5	
016P0.5	
M017P0.5	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F018P0.5	530	8.98	0.00594	29 11 66	2679	
M019P0.5	530	11.90	0.00645	29 11 66	2679	
F020P0.5	532	9.30	0.00553	29 12 66	2649	
M021P0.5	538	9.80	0.00526	26 1 67	2621	
F022P0.5	485	8.10	0.00525	25 5 67	2502	
M025P0.5	509	9.70	0.00539	30 1 74	60	
M026P0.5	509	9.96	0.00536	30 1 74	60	
M031P0.5B	515	10.50	0.00549	4 3 64	1648	15
F031P0.5C	494	8.44	0.00572	4 2 65	2546	16
M032P0.5B	549	13.60	0.00546	18 11 65	2270	14
F033P0.5B	503	10.10	0.00559	18 11 65	3055	
M034P0.5B	530	12.50	0.00642	29 11 66	1920	14
F035P0.5B	501	9.54	0.0052	22 6 67	2474	
M036P0.5B	479	11.50	0.00527	25 5 67	2502	
F037P0.5B	517	8.39	0.00454	16 11 67	2327	
M038P0.5B	517	10.50	0.00448	16 11 67	2327	
F039P0.5B	490	10.90	0.00528	21 12 67	2292	
M042P0.5B	542	13.10	0.00675	2 12 70	1215	
M042P0.5C	542	12.20	0.00668	2 12 70	1215	
F042P0.5D	542	9.71	0.00668	2 12 70	1215	
F043P0.5B	545	11.60	0.00484	3 10 69	1640	
F043P0.5C	535	10.70	0.0048	3 10 69	1640	
M043P0.5D	500	12.00	0.00546	8 8 73	235	
M044P0.5B	445	11.50	0.0036	3 6 69	99	<11
M044P0.5C	504	8.48	0.00604	8 8 73	235	
M045P0.5B	472	10.30	0.0035	3 6 69	42	+68
F045P0.5C	540	10.00	0.00516	17 11 71	35	<<1
M046P0.5B	484	11.80	0.00336	3 6 69	7	<<1
F046P0.5C	540	9.15	0.00516	17 11 71	7	<<1
F014P0.7	533	8.98	0.00947	22 7 69	1705	
M015P0.7	533	10.30	0.00941	22 7 69	1713	
M016P0.7	516	11.90	0.0102	4 9 69	1669	
M017P0.7	540	8.04	0.0103	3 10 69	1640	
F018P0.7	521	9.66	0.00942	22 7 69	1713	
M019P0.7	501	11.60	0.0104	23 9 70	1285	
F020P0.7	521	9.18	0.00926	22 7 69	1713	
M021P0.7	499	11.10	0.0104	23 9 70	1285	
F022P0.7	538	9.69	0.0108	4 9 69	1669	
F023P0.7	538	9.56	0.0108	4 9 69	1669	
F024P0.7	516	8.90	0.011	4 9 69	1669	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F018P0.5	
M019P0.5	
F020P0.5	
M021P0.5	
F022P0.5	
M025P0.5	
M026P0.5	
M031P0.5B	STATUS EPILEPTICUS
F031P0.5C	SPECIAL STUDY
M032P0.5B	SPECIAL STUDY
F033P0.5B	
M034P0.5B	SPECIAL STUDY
F035P0.5B	
M036P0.5B	
F037P0.5B	
M038P0.5B	
F039P0.5B	
M042P0.5B	
M042P0.5C	
F042P0.5D	
F043P0.5B	
F043P0.5C	
M043P0.5D	
M044P0.5B	SPECIAL STUDY
M044P0.5C	
M045P0.5B	SPECIAL STUDY
F045P0.5C	SPECIAL STUDY
M046P0.5B	SPECIAL STUDY
F046P0.5C	SPECIAL STUDY

F014P0.7
M015P0.7
M016P0.7
M017P0.7
F018P0.7
M019P0.7
F020P0.7
M021P0.7
022P0.7
023P0.7
F024P0.7

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M025P0.7	506	10.90	0.0117	8	8	73	235	
M026P0.7	494	10.20	0.0112	20	9	73	192	
M027P0.7	494	11.90	0.0116	20	9	73	192	
F028P0.7	494	10.60	0.011	20	9	73	192	
F029P0.7	493	8.63	0.0113	20	9	73	192	
F030P0.7	487	9.91	0.0113	20	9	73	192	
M031P0.7	508	13.20	0.00956	4	12	73	117	
M032P0.7	508	9.46	0.00967	4	12	73	117	
M033P0.7	509	10.70	0.0103	30	1	74	60	
M034P0.7	508	10.10	0.00979	4	12	73	117	
F035P0.7	508	10.60	0.00981	4	12	73	117	
F036P0.7	508	11.10	0.00999	4	12	73	117	
M037P0.7	507	11.60	0.00999	4	12	73	117	
F038P0.7	507	11.40	0.00995	4	12	73	117	
F039P0.7	499	8.24	0.00969	4	12	73	117	
M040P0.7	499	10.70	0.0099	4	12	73	117	
M041P0.7	533	11.90	0.0105	30	1	74	60	
M042P0.7	533	11.60	0.0106	30	1	74	60	
M043P0.7	533	10.10	0.0106	30	1	74	60	
M044P0.7	533	10.20	0.0105	30	1	74	60	
M045P0.7	509	10.80	0.0104	30	1	74	60	
M046P0.7	509	10.20	0.0105	30	1	74	60	
F047P0.7	508	8.39	0.0103	30	1	74	60	
M048P0.7	502	10.30		5	3	74	26	
M049P0.7	471	12.30		5	3	74	26	
M001P1.0	443	9.41	0.015	1	12	52	4572	66
F002P1.0	422	6.85	0.0163	2	3	53	4810	74
M003P1.0	515	8.00	0.0165	1	6	53	4292	68
M004P1.0	608	9.97	0.0139	16	9	53	4549	60
F005P1.0	620	8.80	0.0142	14	10	53	1539	27
F005P1.0A	472	11.00	0.0168	3	9	58	3764	63
F006P1.0	410	7.38	0.014	12	5	54	4292	58
F007P1.0	510	6.36	0.0167	25	10	54	3981	65
M008P1.0	453	10.60	0.0172	15	3	55	3367	50
F009P1.0	556	7.87	0.0168	9	9	55	2257	43
F010P1.0	641	12.00	0.0152	22	11	55	3649	56
M011P1.0	602	8.90	0.0157	24	4	56	5160	76
M012P1.0	629	9.67	0.0167	29	5	56	2374	44
M013P1.0	504	12.70	0.0153	3	9	58	5277	75
F014P1.0	533	10.40	0.0141	22	7	69	1713	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M025P0.7
M026P0.7
M027P0.7
F028P0.7
F029P0.7
F030P0.7
M031P0.7
M032P0.7
M033P0.7
M034P0.7
F035P0.7
F036P0.7
M037P0.7
F038P0.7
F039P0.7
M040P0.7
M041P0.7
M042P0.7
M043P0.7
M044P0.7
M045P0.7
M046P0.7
F047P0.7
M048P0.7
M049P0.7

M001P1.0	OSTEOSARCOMA
F002P1.0	CIRCULATORY FAILURE
M003P1.0	OSTEOSARCOMA
M004P1.0	BILE DUCT CARCINOMA
F005P1.0	COLITIS, ENTERITIS + SECONDARY HEPATIC NECROSIS
F005P1.0A	THYROID CARCINOMA
F006P1.0	CARCINOMA OF COLON
F007P1.0	TRAUMA LYMPHADENOPHATHY
M008P1.0	OSTEOSARCOMA
F009P1.0	OSTEOSARCOMA
F010P1.0	MAMMARY CARCINOMA
M011P1.0	THYROID CARCINOMA
012P1.0	CHRONIC PANCREATITIS
013P1.0	SENILITY, HYDROCEPHALUS
F014P1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
M015P1.0	516	12.80	0.0159	4 9 69	1669	
M016P1.0	516	10.60	0.0165	4 9 69	1669	
M017P1.0	537	10.90	0.0151	3 10 69	1640	
F018P1.0	531	9.89	0.014	22 7 69	1713	
M019P1.0	501	9.82	0.0159	23 9 70	1285	
F020P1.0	521	10.40	0.0141	22 7 69	1713	
M021P1.0	499	10.00	0.0156	23 9 70	1285	
F022P1.0	531	9.04	0.0139	22 7 69	1713	
F023P1.0	538	11.20	0.0163	4 9 69	1669	
F024P1.0	516	10.40	0.0163	4 9 69	1669	
M025P1.0	504	11.00	0.0168	8 8 73	235	
M001P1.7	657	8.72	0.0475	26 6 56	3025	151
F002P1.7	527	8.62	0.0431	22 11 55	3430	150
M003P1.7	642	8.63	0.0495	26 6 56	3430	173
M004P1.7	673	8.37	0.0484	10 10 56	3312	165
F005P1.7	642	11.60	0.0493	26 6 56	2659	142
F006P1.7	642	10.30	0.0459	26 6 56	2221	116
F007P1.7	756	9.73	0.0481	10 10 56	3353	165
M008P1.7	673	13.60	0.0479	10 10 56	3282	162
F009P1.7	756	9.72	0.0485	10 10 56	2500	134
F010P1.7	739	10.60	0.0495	10 10 56	467	36
F010P1.7A	472	8.07	0.0457	3 9 58	4214	187
M011P1.7	599	11.60	0.0486	24 4 56	2777	145
M012P1.7	673	9.41	0.0491	10 10 56	2973	154
M013P1.7	504	10.60	0.0473	3 9 58	4375	200
M001P2.0	443	7.61	0.0853	1 12 52	2985	268
F002P2.0	422	7.73	0.112	2 3 53	2780	334
M003P2.0	485	10.50	0.094	1 6 53	3185	310
M004P2.0	608	9.84	0.0862	16 9 53	2948	268
F005P2.0	594	8.12	0.0846	14 10 53	2423	228
F006P2.0	417	7.54	0.0902	12 5 54	2947	281
F007P2.0	485	8.40	0.0996	25 10 54	2093	240
M008P2.0	406	9.73	0.0957	15 3 55	1761	203
F009P2.0	552	9.72	0.101	9 9 55	2014	237
F010P2.0	551	7.94	0.0968	22 11 55	2912	299
M011P2.0	599	10.30	0.0961	24 4 56	1617	191
M012P2.0	622	9.98	0.1	29 5 56	2284	258

DOG
NUMBER

COMMENTS ON DEAD DOGS

M015P1.0
M016P1.0
M017P1.0
F018P1.0
M019P1.0
F020P1.0
M021P1.0
F022P1.0
F023P1.0
F024P1.0
M025P1.0

M001P1.7	OSTEOSARCOMA
F002P1.7	OSTEOSARCOMA
M003P1.7	CHROMOPHOBE ADENOMA OF PITUITARY, PROSTATE CARCINOMA
M004P1.7	OSTEOSARCOMA
F005P1.7	OSTEOSARCOMA
F006P1.7	OSTEOSARCOMA
F007P1.7	OSTEOSARCOMA
M008P1.7	OSTEOSARCOMA
F009P1.7	OSTEOSARCOMA
F010P1.7	ACUTE ENTERITIS
F010P1.7A	OSTEOSARCOMA
M011P1.7	BILE DUCT CARCINOMA
M012P1.7	LEUKEMIA
M013P1.7	CHONDROSARCOMA

M001P2.0	OSTEOSARCOMA
F002P2.0	OSTEOSARCOMA
M003P2.0	OSTEOSARCOMA
M004P2.0	OSTEOSARCOMA
F005P2.0	OSTEOSARCOMA
F006P2.0	OSTEOSARCOMA
F007P2.0	SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
M008P2.0	ASPIRATION PNEUMONIA
F009P2.0	OSTEOSARCOMA
010P2.0	OSTEOSARCOMA
011P2.0	OSTEOSARCOMA
M012P2.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ ci/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/74	DEATH	
M001P3.0	443	8.00	0.261	1	12	52		1476	579
F002P3.0	422	6.85	0.312	2	3	53		1947	886
M003P3.0	485	8.74	0.291	1	6	53		1604	695
M004P3.0	608	8.51	0.292	16	9	53		1950	830
F005P3.0	650	8.22	0.288	14	10	53		1504	650
F006P3.0	415	8.38	0.282	12	5	54		1617	678
F007P3.0	485	9.00	0.314	25	10	54		1627	760
M008P3.0	406	9.73	0.3	15	3	55		1770	782
F009P3.0	552	7.67	0.3	9	9	55		1894	831
F010P3.0	533	8.94	0.298	22	11	55		1547	689
M011P3.0	599	10.50	0.309	24	4	56		1198	569
M012P3.0	622	10.20	0.308	29	5	56		1659	758
M081P3.0Y	91	4.03	0.32	25	4	72	705		
M086P3.0Y	89	3.29	0.319	25	4	72	705		
M089P3.0Y	89	4.23	0.312	25	4	72	705		
M001P4.0	443	7.61	0.823	1	12	52		1724	2215
F002P4.0	568	8.65	1.03	2	3	53		1556	2521
M003P4.0	485	9.36	0.929	1	6	53		1198	1784
M004P4.0	566	8.74	0.974	16	9	53		1066	1678
F005P4.0	650	7.05	0.872	14	10	53		1245	1736
F006P4.0	420	9.26	0.811	12	5	54		1357	1749
F007P4.0	485	8.45	0.963	25	10	54		1198	1850
M008P4.0	651	9.22	0.887	15	3	55		1157	1650
F009P4.0	552	8.58	0.96	9	9	55		1343	2050
F010P4.0	527	6.48	0.868	22	11	55		1241	1723
M011P4.0	596	9.56	0.927	24	4	56		1288	1904
M012P4.0	598	11.40	0.838	29	5	56		1463	1938
M001P5.0	418	8.86	2.67	1	12	52		1324	5873
F002P5.0	1151	8.75	3.3	2	3	53		1576	8575
M003P5.0	515	8.10	3.0	1	6	53		499	2577
M004P5.0	566	9.18	3.17	16	9	53		1562	8167
F005P5.0	691	8.77	2.77	14	10	53		2059	9292
F006P5.0	407	7.90	2.57	12	5	54		1194	5121
F007P5.0	482	8.33	2.99	25	10	54		1491	7368
M008P5.0	497	9.55	2.69	15	3	55		1192	5351
F009P5.0	552	9.45	2.73	9	9	55		1145	5226
M081P5.0Y	94	4.60	2.68	1	3	72	760		

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001P3.0 OSTEOSARCOMA
F002P3.0 OSTEOSARCOMA
M003P3.0 OSTEOSARCOMA
M004P3.0 OSTEOSARCOMA
F005P3.0 OSTEOSARCOMA
F006P3.0 OSTEOSARCOMA
F007P3.0 OSTEOSARCOMA
M008P3.0 OSTEOSARCOMA
F009P3.0 OSTEOSARCOMA
F010P3.0 OSTEOSARCOMA
M011P3.0 OSTEOSARCOMA
M012P3.0 OSTEOSARCOMA
M081P3.0Y
M086P3.0Y
M089P3.0Y

M001P4.0 OSTEOSARCOMA
F002P4.0 OSTEOSARCOMA
M003P4.0 OSTEOSARCOMA
M004P4.0 OSTEOSARCOMA
F005P4.0 OSTEOSARCOMA
F006P4.0 OSTEOSARCOMA
F007P4.0 OSTEOSARCOMA
M008P4.0 OSTEOSARCOMA
F009P4.0 OSTEOSARCOMA
F010P4.0 OSTEOSARCOMA
M011P4.0 OSTEOSARCOMA
M012P4.0 OSTEOSARCOMA

M001P5.0 OSTEOSARCOMA
F002P5.0 OSTEOSARCOMA + FRACTURED MANDIBLE
M003P5.0 LIVER DEGENERATION + ASCITES
M004P5.0 OSTEOSARCOMA
F005P5.0 OSTEOSARCOMA, LIVER DEGENERATION + HEPATIC HEMORRHAGE
F006P5.0 OSTEOSARCOMA
F007P5.0 OSTEOSARCOMA + CRIPPLING FRACTURE
008P5.0 GINGIVITIS
009P5.0 OSTEOSARCOMA, EPISTAXIS + CIRCULATORY COLLAPSE
M081P5.0Y

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F082P5.0Y	94	4.80	2.66	1	3	72	760	
F083P5.0Y	94	4.00	2.66	1	3	72	760	
F084P5.0Y	94	3.55	2.68	1	3	72	760	
F085P5.0Y	94	4.15	2.64	1	3	72	760	
M086P5.0Y	93	3.75	2.95	25	4	72	705	
F087P5.0Y	93	4.15	2.93	25	4	72	705	
F088P5.0Y	93	3.65	2.96	25	4	72	705	
M089P5.0Y	93	3.79	2.92	25	4	72	705	
M090P5.0Y	93	4.38	2.97	25	4	72	705	
M091P5.0Y	91	3.78	2.9	25	4	72	705	
M092P5.0Y	91	3.82	2.87	25	4	72	705	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F082P5.0Y
F083P5.0Y
F084P5.0Y
F085P5.0Y
M086P5.0Y
F087P5.0Y
F088P5.0Y
M089P5.0Y
M090P5.0Y
M091P5.0Y
M092P5.0Y

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74	DEATH	DOSE TO SKELETON (RADS)
F001M0.0	732	7.33		4 1 54		3451	
F002M0.0	545	6.94		29 11 54		6155	
M003M0.0	579	13.00		13 3 56		5056	
M004M0.0	601	10.30		15 1 57		4816	
F005M0.0	671	11.20		5 3 57		4581	
M006M0.0	492	7.56		23 4 57		4934	
F007M0.0	395	8.71		4 6 57		1414	
F007M0.0A	594	10.90		15 1 63		3624	
F008M0.0	654	11.60		9 3 60		5009	
M009M0.0	575	12.40		13 4 60		4130	
M010M0.0	581	13.30		17 7 62		2991	
F011M0.0	475	9.31		18 9 62		3248	
M012M0.0	695	10.00		22 12 60		4810	
F001M0.5	492	9.47	0.0173	17 7 62	4275		
F002M0.5	492	9.15	0.0173	17 7 62		3689	124
M003M0.5	493	10.80	0.0199	18 9 62	4212		
M004M0.5	475	12.80	0.0199	18 9 62		4193	159
F005M0.5	534	7.83	0.0172	23 10 62		3958	107
M006M0.5	510	10.30	0.0171	23 10 62		3019	95
F007M0.5	492	8.87	0.0172	17 7 62	4275		
F008M0.5	654	12.60	0.0159	9 3 60		4208	93
M009M0.5	485	11.90	0.017	13 4 60	5100		
M010M0.5	492	10.60	0.0174	17 7 62	4275		
F011M0.5	505	7.82	0.0202	18 9 62		4033	140
M012M0.5	510	10.60	0.0165	23 10 62		3920	111
F001M1.0	718	7.75	0.0463	4 1 54		2950	261
F001M1.0A	590	8.07	0.0512	23 10 62	4177		
F002M1.0	459	8.25	0.0324	29 11 54		5267	284
M003M1.0	575	13.80	0.0589	13 3 56		3157	408
M004M1.0	601	9.90	0.0481	15 1 57		4260	175
F005M1.0	658	8.80	0.049	5 3 57		4565	256
M006M1.0	521	10.60	0.0468	23 4 57		3402	363
F007M1.0	534	9.89	0.0489	4 6 57		2159	199
F008M1.0	654	12.40	0.0491	9 3 60		3886	320
M009M1.0	485	10.10	0.0504	13 4 60		4670	429
M010M1.0	492	9.43	0.0501	17 7 62		2966	284
F011M1.0	505	8.91	0.0613	18 9 62	4212		

DOG NUMBER	COMMENTS ON DEAD DOGS
F001M0.0	PURULENT MENINGOENCEPHALITIS
F002M0.0	UNDETERMINED; SENILITY
M003M0.0	BRAIN INFARCTION
M004M0.0	VALVULAR ENDOCARDITIS; MYOCARDIAL INFARCTION
F005M0.0	MAMMARY CARCINOMA
M006M0.0	NEPHRITIS
F007M0.0	STATUS EPILEPTICUS
F007M0.0A	PNEUMONIA
F008M0.0	AORTIC THROMBOEMBOLISM
M009M0.0	VIRUS PNEUMONIA
M010M0.0	MALIGNANT MELANOMA (ORAL)
F011M0.0	ASPIRATION PNEUMONIA
M012M0.0	PULMONARY THROMBOLISM TRANSITIONAL CELL CARCINOMA
F001M0.5	
F002M0.5	CHRONIC PANCREATITIS
M003M0.5	
M004M0.5	UNDIFFERENTIATED SARCOMA (NON-SKELETAL)
M005M0.5	UNDETERMINED
M006M0.5	MALIGNANT MELANOMA (EYE)
F007M0.5	
F008M0.5	BACTERIAL ENTERITIS
M009M0.5	
M010M0.5	
F011M0.5	BILE DUCT OBSTRUCTION; EYE MELANOMA
M012M0.5	STATUS EPILEPTICUS
F001M1.0	SARCOMA (SPLEEN)
F001M1.0A	
F002M1.0	OSTEOSARCOMA
M003M1.0	OSTEOSARCOMA
M004M1.0	PNEUMONIA; PANCREATITIS
F005M1.0	MALIGNANT MELANOMA (EYE)
M006M1.0	EPIDERMOID CARCINOMA (PENIS)
F007M1.0	SARCOMA (HEART)
F008M1.0	MALIGNANT MELANOMA (EYE)
009M1.0	VALVULAR ENDOCARDITIS
010M1.0	MALIGNANT MELANOMA (EYE)
F011M1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
M012M1.0	528	9.27	0.0498	23 10 62	3786	308
F001M1.7	510	7.52	0.151	23 10 62	4177	
F002M1.7	560	9.90	0.183	13 3 56	2383	977
M003M1.7	576	11.00	0.18	13 3 56	2709	964
M004M1.7	601	8.94	0.143	15 1 57	2864	565
F005M1.7	658	12.80	0.141	5 3 57	3234	833
M006M1.7	521	10.00	0.144	23 4 57	3424	524
F007M1.7	534	10.20	0.146	4 6 57	2646	800
F008M1.7	654	10.80	0.148	9 3 60	2486	515
M009M1.7	485	12.60	0.149	13 4 60	2799	896
M010M1.7	492	10.10	0.124	17 7 62	3101	997
F011M1.7	505	10.70	0.179	18 9 62	3325	1347
M012M1.7	524	9.28	0.153	23 10 62	3017	957
F001M2.0	676	7.60	0.276	4 1 54	1780	1160
F002M2.0	517	8.25	0.194	29 11 54	965	264
M003M2.0	576	11.00	0.358	13 3 56	619	473
M004M2.0	601	9.88	0.282	15 1 57	2282	1377
F005M2.0	509	8.30	0.295	5 3 57	2688	1237
M006M2.0	502	12.40	0.306	23 4 57	2674	1843
F007M2.0	534	10.10	0.298	4 6 57	2239	1419
F008M2.0	654	12.40	0.3	9 3 60	2386	1237
M009M2.0	630	9.99	0.302	13 4 60	1254	748
M010M2.0	430	11.20	0.311	17 7 62	2373	1931
F011M2.0	505	7.03	0.381	18 9 62	2878	1461
M012M2.0	524	9.47	0.306	23 10 62	2471	1992
F001M3.0	519	10.40	0.858	4 1 54	918	2444
F002M3.0	460	6.70	0.612	29 11 54	1856	2767
M003M3.0	579	10.40	0.965	13 3 56	1185	3285
M004M3.0	601	10.20	0.916	15 1 57	1176	2123
F005M3.0	531	8.51	0.94	5 3 57	1869	2864
M006M3.0	502	9.09	0.953	23 4 57	1421	2541
F007M3.0	534	9.94	0.907	4 6 57	1463	4193
F008M3.0	633	11.80	0.95	9 3 60	1447	2877
M009M3.0	630	9.83	0.918	13 4 60	1570	3036

DOG NUMBER	COMMENTS ON DEAD DOGS
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M012M1.0	LYMPHATIC LEUKEMIA
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F001M1.7	
F002M1.7	OSTEOSARCOMA
M003M1.7	OSTEOSARCOMA
M004M1.7	CARCINOMA SMALL INTESTINE
F005M1.7	OSTEOSARCOMA
M006M1.7	OSTEOSARCOMA
F007M1.7	OSTEOSARCOMA
F008M1.7	OSTEOSARCOMA
M009M1.7	OSTEOSARCOMA
M010M1.7	OSTEOSARCOMA
F011M1.7	OSTEOSARCOMA
M012M1.7	UNKNOWN

F001M2.0	OSTEOSARCOMA
F002M2.0	INTESTINAL HEMORRHAGE
M003M2.0	PNEUMONIA
M004M2.0	OSTEOSARCOMA
F005M2.0	OSTEOSARCOMA
M006M2.0	OSTEOSARCOMA
F007M2.0	CHRONIC PANCREATITIS
F008M2.0	OSTEOSARCOMA
M009M2.0	OSTEOSARCOMA
M010M2.0	OSTEOSARCOMA
F011M2.0	OSTEOSARCOMA
M012M2.0	OSTEOSARCOMA

F001M3.0	OSTEOSARCOMA
F002M3.0	OSTEOSARCOMA
M003M3.0	OSTEOSARCOMA
M004M3.0	OSTEOSARCOMA
F005M3.0	OSTEOSARCOMA
M006M3.0	OSTEOSARCOMA
007M3.0	OSTEOSARCOMA
008M3.0	OSTEOSARCOMA
M009M3.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M010M3.0	581	10.40	1.0	17	7	62	1575	3091
F011M3.0	499	11.00	1.19	18	9	62	1395	3289
M012M3.0	510	12.90	0.987	23	10	62	1638	3153
F001M4.0	510	7.56	2.6	4	1	54	841	7485
F002M4.0	460	6.95	1.86	29	11	54	770	3029
M003M4.0	579	9.65	3.37	13	3	56	418	2393
M003M4.0A	494	7.34	2.64	4	6	57	1063	7472
M004M4.0	609	7.84	2.47	15	1	57	896	3573
F005M4.0	509	9.63	2.67	5	3	57	1064	5811
M006M4.0	502	9.49	2.66	23	4	57	1121	6379
F007M4.0	544	8.40	2.67	4	6	57	1253	6181
F001M5.0	494	7.77	8.11	4	1	54	232	5139
F002M5.0	460	7.35	5.46	29	11	54	780	10221
M003M5.0	579	8.87	10.4	13	3	56	688	18868
M004M5.0	482	7.29	7.89	15	1	57	561	7549
F005M5.0	658	11.10	8.48	5	3	57	770	12745
M006M5.0	580	7.53	8.67	23	4	57	792	9015
F007M5.0	494	7.35	8.92	4	6	57	966	24125

* (μ Ci $^{228}\text{Th}/\mu$ Ci ^{228}Ra) injected = 0.15 for F1M1.0, 2.0, 3.0, 4.0, 5.0.
= 0.03 for F2M1.0, 1.7, 2.0, 3.0, 4.0, 5.0,
M3M1.0, 1.7, 2.0, 3.0, 4.0, 5.0.
= 0.006 for groups 4, 5, 6, 7, 8, 9, 10, 11, 12,
and dogs F1M0.5, F2M0.5, M3M0.5, F1M1A,
F1M1.7, and M3M4.0A.

DOG NUMBER	COMMENTS ON DEAD DOGS
M010M3.0	OSTEOSARCOMA
F011M3.0	OSTEOSARCOMA
M012M3.0	OSTEOSARCOMA
F001M4.0	OSTEOSARCOMA + CRIPPLING FRACTURE
F002M4.0	OSTEOSARCOMA
M003M4.0	STRANGULATED INGUINAL HERNIA
M003M4.0A	OSTEOSARCOMA, NEPHRITIS, ULCERATIVE GINGIVITIS + PNEUMONIA
M004M4.0	FRACTURED MANDIBLE + ULCERATIVE GINGIVITIS
F005M4.0	OSTEOSARCOMA
M006M4.0	OSTEOSARCOMA
F007M4.0	OSTEOSARCOMA
F001M5.0	NEPHRITIS + SEVERE ANEMIA
F002M5.0	CRIPPLING FRACTURES
M003M5.0	ULCERATIVE GINGIVITIS
M004M5.0	CRIPPLING FRACTURE
F005M5.0	ULCERATIVE GINGIVITIS
M006M5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
F007M5.0	ULCERATIVE GINGIVITIS, MYOCARDIAL INFARCTION + GLAUCOMA

D. THORIUM-228 (RADIOTHORIUM)

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
M001T0.0	493 8.24		8 2 54	4895	
M002T0.0	488 7.28		28 9 54	5510	
F003T0.0	797 11.60		6 6 55	2592	
M004T0.0	591 8.10		18 10 55	3072	
M005T0.0	458 10.40		14 10 58	5306	
F006T0.0	489 9.64		10 1 61	171	
F006T0.0A	688 8.61		15 12 60	4549	
M007T0.0	517 10.50		7 2 61	1412	
M008T0.0	533 10.80		24 5 61	4694	
F009T0.0	569 8.28		29 6 61	4658	
F010T0.0	536 10.40		28 7 61	4629	
F011T0.0	530 9.45		4 6 63	3953	
F012T0.0	492 9.09		9 7 63	3918	
M001T0.2	682 11.40	0.00164	27 3 62	4387	
M002T0.2	682 10.40	0.00166	27 3 62	4387	
F003T0.2	478 9.86	0.00163	27 3 62	4387	
M004T0.2	478 10.00	0.00166	27 3 62	4387	
M005T0.2	625 13.80	0.00162	9 2 60	889	13
M005T0.2A	530 13.40	0.00173	4 6 63	3953	
F006T0.2	489 8.85	0.00176	10 1 61	4767	18
M007T0.2	532 10.50	0.00159	7 2 61	3897	20
M008T0.2	494 13.90	0.00189	24 5 61	4694	
F009T0.2	569 7.82	0.00171	29 6 61	3897	21
F010T0.2	508 10.50	0.0017	28 7 61	4217	21
F011T0.2	530 9.76	0.00171	4 6 63	3953	
F012T0.2	492 7.37	0.0019	9 7 63	3350	23
M001T0.5	699 14.30	0.00496	7 9 56	3471	60
M002T0.5	455 10.50	0.0049	28 9 54	1976	55
F003T0.5	659 8.59	0.00485	6 6 55	3032	59
M004T0.5	516 8.58	0.0054	18 10 55	2159	61
M005T0.5	513 8.46	0.00522	14 10 58	4856	67
F006T0.5	489 9.66	0.0051	10 1 61	4518	63
M007T0.5	532 9.11	0.00491	7 2 61	4800	
M008T0.5	533 9.53	0.00562	24 5 61	4599	69
F009T0.5	569 8.62	0.00529	29 6 61	4149	65
F010T0.5	508 10.20	0.0051	28 7 61	4629	
F011T0.5	530 7.78	0.00518	4 6 63	3952	63

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001T0.0	RETICULUM CELL SARCOMA (SOFT TISSUE)
M002T0.0	NEPHRITIS
F003T0.0	BRAIN HEMORRHAGE
M004T0.0	LYMPHOSARCOMA
M005T0.0	LYMPHOSARCOMA
F006T0.0	TRAUMA
F006T0.0A	PERICARDITIS
M007T0.0	BRAIN HEMORRHAGE
M008T0.0	
F009T0.0	
F010T0.0	
F011T0.0	
F012T0.0	

M001T0.2	
M002T0.2	
F003T0.2	
M004T0.2	
M005T0.2	STRANGULATION ON VOMITUS + GRAND MAL
M005T0.2A	
F006T0.2	ISLET CELL TUMOR
M007T0.2	UNDIFFERENTIATED MALIGNANCY (INTESTINE)
M008T0.2	
F009T0.2	PULMONARY THROMBO EMBOLISM
F010T0.2	BILE DUCT OBSTRUCTION; MAMMARY CARCINOMA
F011T0.2	
F012T0.2	HEPATIC CELL CARCINOMA

M001T0.5	CEREBRAL INFARCTION HEMORRHAGE
M002T0.5	STRANGULATION ON VOMITUS + GRAND MAL
F003T0.5	PYOMETRITIS + SECONDARY PERITONITIS
M004T0.5	STATUS EPILEPTICUS + PNEUMONIA
M005T0.5	PROSTATITIS
F006T0.5	ISLET CELL TUMOR
M007T0.5	
M008T0.5	OSTEOSARCOMA
009T0.5	MAMMARY CARCINOMA
010T0.5	
F011T0.5	PULMONARY THROMBOEMBOLISM

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F012T0.5	492	9.94	0.00567	9 7 63	1682	60
M001T1.0	493	9.36	0.0146	8 2 54	3172	176
M002T1.0	699	9.27	0.0146	7 9 56	4570	180
F003T1.0	723	8.84	0.0145	7 9 56	4142	177
M004T1.0	699	8.27	0.0146	7 9 56	3217	176
M005T1.0	513	11.90	0.0146	14 10 58	2886	173
F006T1.0	489	8.81	0.015	10 1 61	3273	181
M007T1.0	532	9.18	0.0147	7 2 61	3538	179
M008T1.0	533	8.69	0.0166	24 5 61	4694	
F009T1.0	527	10.00	0.016	29 6 61	2546	187
F010T1.0	508	10.20	0.015	28 7 61	3420	181
F011T1.0	521	7.55	0.0154	4 6 63	3953	
F012T1.0	472	9.96	0.0167	9 7 63	1263	157
M001T1.5	699	7.95	0.0289	7 9 56	2894	344
M002T1.5	458	10.00	0.0293	28 9 54	2576	343
F003T1.5	609	10.30	0.0303	6 6 55	1921	332
M004T1.5	591	8.59	0.0299	18 10 55	2309	343
M005T1.5	598	9.65	0.0286	9 2 60	1624	297
F006T1.5	489	8.14	0.0292	10 1 61	2373	336
M007T1.5	517	8.83	0.0292	7 2 61	380	127
M007T1.5A	521	9.08	0.0311	4 6 63	3110	373
M008T1.5	494	11.60	0.0324	24 5 61	2665	381
F009T1.5	527	8.80	0.0306	29 6 61	2983	365
F010T1.5	508	11.60	0.0296	28 7 61	1859	321
F011T1.5	518	11.40	0.0305	4 6 63	2408	375
F012T1.5	465	7.56	0.0329	9 7 63	2120	371
M001T2.0	491	10.20	0.0976	8 2 54	1282	924
M002T2.0	483	9.16	0.0875	28 9 54	1234	815
F003T2.0	474	7.87	0.0908	6 6 55	1541	927
M004T2.0	553	13.00	0.09	18 10 55	78	93
M004T2.0A	650	10.60	0.0899	7 9 56	1222	833
M005T2.0	598	9.12	0.0848	9 2 60	1085	741
F006T2.0	451	8.65	0.0879	10 1 61	1108	777
M007T2.0	517	8.85	0.0881	7 2 61	1015	744

DOG
NUMBER

COMMENTS ON DEAD DOGS

F012T0.5 LIVER DEGENERATION, ANESTHETIC REACTION

M001T1.0 OSTEOSARCOMA
M002T1.0 PERIANAL GLAND ADENOMA
F003T1.0 UNDIFFERENTIATED MALIGNANCY (SOFT TISSUE)
M004T1.0 OSTEOSARCOMA THYROID CARCINOMA PERIANAL ADENOCARCINOMA
M005T1.0 STATUS EPILEPTICUS
F006T1.0 STOMACH PERFORATION
M007T1.0 OSTEOSARCOMA
M008T1.0
F009T1.0 LEIOMYOSARCOMA
F010T1.0 OSTEOSARCOMA
F011T1.0
F012T1.0 PNEUMONIA

M001T1.5 OSTEOSARCOMA
M002T1.5 OSTEOSARCOMA
F003T1.5 COMA OF UNKNOWN ETIOLOGY (NO BONE TUMOR)
M004T1.5 OSTEOSARCOMA
M005T1.5 OSTEOSARCOMA
F006T1.5 OSTEOSARCOMA
M007T1.5 LEPTOSPIROSIS
M007T1.5A OSTEOSARCOMA; (PNEUMONIA)
M008T1.5 OSTEOSARCOMA
F009T1.5 OSTEOSARCOMA
F010T1.5 OSTEOSARCOMA
F011T1.5 OSTEOSARCOMA
F012T1.5 OSTEOSARCOMA

M001T2.0 OSTEOSARCOMA
M002T2.0 OSTEOSARCOMA
F003T2.0 OSTEOSARCOMA
M004T2.0 TRAUMA
M004T2.0A OSTEOSARCOMA
D005T2.0 OSTEOSARCOMA
D006T2.0 OSTEOSARCOMA
M007T2.0 OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M008T2.0	533	10.70	0.0981	24	5	61	1078	855
F009T2.0	527	8.09	0.0979	29	6	61	1209	903
F010T2.0	508	10.70	0.0919	28	7	61	1022	779
F011T2.0	518	10.80	0.0904	4	6	63	1038	772
F012T2.0	464	8.92	0.1	9	7	63	1449	997
M001T3.0	314	9.15	0.301	8	2	54	988	2505
M002T3.0	458	11.90	0.301	28	9	54	859	2315
F003T3.0	471	12.00	0.272	6	6	55	547	1552
M004T3.0	606	9.69	0.285	18	10	55	801	2100
M005T3.0	571	10.70	0.269	9	2	60	890	2112
F006T3.0	451	8.83	0.282	10	1	61	1156	2545
M007T3.0	427	9.90	0.266	7	2	61	861	2048
M008T3.0	494	10.10	0.313	24	5	61	685	2088
F009T3.0	511	11.50	0.298	29	6	61	1062	2577
F010T3.0	508	9.26	0.28	28	7	61	971	2309
F011T3.0	518	10.30	0.29	4	6	63	826	2121
F012T3.0	459	11.50	0.32	9	7	63	804	2364
M001T4.0	480	8.32	0.882	8	2	54	645	5649
M002T4.0	458	8.32	0.916	28	9	54	833	6913
F003T4.0	461	7.25	0.8	6	6	55	763	5720
M004T4.0	606	8.81	0.835	18	10	55	793	6116
M001T5.0	480	9.48	2.76	8	2	54	212	7276
M002T5.0	483	8.22	2.63	28	9	54	97	3380

DOG
NUMBER

COMMENTS ON DEAD DOGS

M008T2.0 OSTEOSARCOMA
F009T2.0 OSTEOSARCOMA
F010T2.0 OSTEOSARCOMA
F011T2.0 OSTEOSARCOMA
F012T2.0 OSTEOSARCOMA

M001T3.0 OSTEOSARCOMA + SEVERE ANEMIA
M002T3.0 OSTEOSARCOMA + TRAUMA
F003T3.0 OSTEOSARCOMA
M004T3.0 OSTEOSARCOMA
M005T3.0 OSTEOSARCOMA
F006T3.0 OSTEOSARCOMA
M007T3.0 OSTEOSARCOMA
M008T3.0 OSTEOSARCOMA
F009T3.0 OSTEOSARCOMA
F010T3.0 OSTEOSARCOMA
F011T3.0 OSTEOSARCOMA
F012T3.0 HEMANGIOSARCOMA (HUMERUS)

M001T4.0 OSTEOSARCOMA + CRIPPLING FRACTURE
M002T4.0 OSTEOSARCOMA, CRIPPLING FRACTURE + NEPHRITIS
F003T4.0 ULCERATIVE GINGIVITIS + NEPHRITIS
M004T4.0 ULCERATIVE GINGIVITIS

M001T5.0 NEPHRITIS
M002T5.0 PANCYTOPENIA

E. STRONTIUM-90

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F001S0.0	502	8.48		18	1	55	5484	
M002S0.0	600	11.10		14	2	56	3838	
M003S0.0	493	9.03		11	9	57	3516	
F004S0.0	520	8.19		15	10	57	5755	
M005S0.0	542	10.60		19	11	57	4158	
M006S0.0	466	9.68		27	5	58	4726	
F007S0.0A	462	9.46		7	1	59	3303	
F008S0.0	483	9.29		19	5	59	4482	
F009S0.0	549	12.40		11	8	59	708	
F009S0.0A	535	11.20		4	6	63	3425	
M010S0.0	522	13.90		29	9	59	4977	
F011S0.0	541	9.60		3	11	59	4831	
M012S0.0	605	8.99		6	1	60	5198	
F001S1.0	1525	6.84	0.573	18	1	55	308	28
F001S1.0A	521	9.38	0.588	14	2	56	5219	90
M002S1.0	567	8.81	0.606	14	2	56	5077	120
M003S1.0	493	10.90	0.572	11	9	57	5363	189
F004S1.0	525	8.96	0.56	15	10	57	5902	198
M005S1.0	555	10.20	0.532	19	11	57	2705	95
M006S1.0	466	9.56	0.581	27	5	58	5739	292
F007S1.0	524	9.94	0.517	11	11	58	5619	
F008S1.0	483	10.80	0.697	19	5	59	2783	105
F009S1.0	549	11.60	0.534	11	8	59	3601	116
M010S1.0	522	11.50	0.558	29	9	59	5297	
F011S1.0	543	10.30	0.55	3	11	59	4944	141
M012S1.0	607	13.70	0.559	6	1	60	4184	133
F001S1.7	526	7.41	1.78	14	2	56	5624	627
M002S1.7	567	11.60	1.84	14	2	56	4297	540
M003S1.7	493	9.19	1.69	11	9	57	4846	451
F004S1.7	522	9.60	1.68	15	10	57	4628	372
M005S1.7	560	9.85	1.6	19	11	57	1715	220
M005S1.7A	493	11.40	1.78	6	3	63	4043	
M006S1.7	466	10.60	1.72	27	5	58	5581	725
F007S1.7	488	10.20	1.6	11	11	58	3990	331
M008S1.7	472	8.47	2.03	19	5	59	1973	285
F009S1.7	549	10.00	1.62	11	8	59	4803	552
M010S1.7	519	13.60	1.66	29	9	59	2947	408

DOG NUMBER	COMMENTS ON DEAD DOGS
F001S0.0	CARCINOMA PANCREAS
M002S0.0	BRONCHOGENIC CARCINOMA
M003S0.0	OBTURATING AORTIC EMBOLISM, NEPHRITIS
F004S0.0	NEPHRITIS; SENILITY
M005S0.0	TRANSITIONAL CELL CARCINOMA
M006S0.0	MELANOMA (ORAL)
F007S0.0A	DIABETES MELLITUS
F008S0.0	DIABETES MELLITUS
F009S0.0	TRAUMA
F009S0.0A	MAMMARY CARCINOMA
M010S0.0	FIBROSARCOMA (NON-SKELETAL)
F011S0.0	MAMMARY CARCINOMA
M012S0.0	
F001S1.0	SACRIFICED -IMPROPER INJECTION AGE-
F001S1.0A	PULMONARY THROMBO-EMBOLISM
M002S1.0	AORTIC BODY TUMOR
M003S1.0	SQUAMOUS CELL CARCINOMA
F004S1.0	NEPHRITIS; MAMMARY CARCINOMA
M005S1.0	STATUS EPILEPTICUS
M006S1.0	LYMPHOSARCOMA
F007S1.0	
F008S1.0	PANCREATIC ISLET CELL CARCINOMA
F009S1.0	FOREIGN BODY PNEUMONIA; ENTERITIS
M010S1.0	
F011S1.0	TRANSITIONAL CELL CARCINOMA
M012S1.0	BILIARY OBSTRUCTION
F001S1.7	HEMANGIOENDOTHELIAL SARCOMA (LIVER)
M002S1.7	HEMANGIOSARCOMA (SOFT TISSUE ORIGIN)
M003S1.7	OBTURATING THROMBOEMBOLISM, AORTA
F004S1.7	PANCREATIC CARCINOMA
M005S1.7	COMA OF UNKNOWN ETIOLOGY (NO BONE TUMOR)
M005S1.7A	
M006S1.7	TRANSITIONAL CELL CARCINOMA; HYDRONEPHROSIS
F007S1.7	ARTHRITIS; MAMMARY CARCINOMA
008S1.7	STATUS EPILEPTICUS, CHRONIC PANCREATITIS
009S1.7	LYMPHOSARCOMA; NEPHRITIS
M010S1.7	OBTURATING PULMONARY EMBOLISM, NEPHRITIS

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F011S1.7	543	11.00	1.68	3 11 59	3180	319
M012S1.7	607	11.90	1.68	6 1 60	4717	473
F001S2.0	502	5.59	3.7	18 1 55	3269	636
M002S2.0	567	8.97	3.42	14 2 56	3768	841
M003S2.0	494	7.82	3.39	11 9 57	4295	893
F004S2.0	522	9.68	3.41	15 10 57	4775	981
M005S2.0	560	8.72	3.24	19 11 57	3253	708
M006S2.0	466	9.19	3.5	27 5 58	5193	1131
F007S2.0	488	11.20	3.19	11 11 58	3421	609
F008S2.0	465	9.49	4.14	19 5 59	3955	913
F009S2.0	473	14.10	3.28	11 8 59	2467	755
M010S2.0	508	10.70	3.34	29 9 59	3430	791
F011S2.0	543	10.40	3.41	3 11 59	4880	879
M012S2.0	607	11.60	3.49	6 1 60	4584	943
F001S3.0	468	7.36	11.6	18 1 55	5149	4385
M002S3.0	565	9.62	11.6	14 2 56	4263	3473
M003S3.0	494	11.40	10.8	11 9 57	4947	3197
F004S3.0	527	9.17	10.6	15 10 57	3101	1987
M005S3.0	557	8.90	10.1	19 11 57	4640	2849
M006S3.0	466	9.44	10.9	27 5 58	5667	4090
F007S3.0	486	9.80	10.1	11 11 58	4018	2421
F008S3.0	465	12.50	12.9	19 5 59	4832	3749
F009S3.0	468	10.00	10.1	11 8 59	4599	3547
M010S3.0	519	12.50	10.3	29 9 59	2898	2799
F011S3.0	541	9.00	10.8	3 11 59	4831	1273
M012S3.0	605	8.43	10.2	6 1 60	4831	2176
F001S4.0	468	8.74	33.3	18 1 55	3682	8817
M002S4.0	567	11.20	32.6	14 2 56	2093	7584
M003S4.0	593	9.83	32.1	11 9 57	2781	5519
F004S4.0	528	8.24	32.1	15 10 57	4844	10373
M005S4.0	562	9.65	30.6	19 11 57	4427	8369
M006S4.0	504	16.00	32.7	3 9 58	3530	9199
F007S4.0	478	10.90	30.9	11 11 58	4664	10560
F008S4.0	465	10.90	40.6	19 5 59	2206	10355

DOG NUMBER	COMMENTS ON DEAD DOGS
F011S1.7	AORTIC THROMBUS; METASTATIC CALCIFICATION OF LUNGS
M012S1.7	ISLET CELL TUMOR
F001S2.0	BACTERIAL PNEUMONIA
M002S2.0	UNDETERMINED SOFT TISSUE SARCOMA +BRONCHIOGENIC CARCINOMA
M003S2.0	STATUS EPILEPTICUS; THYROID CARCINOMA
F004S2.0	MAMMARY CARCINOMA
M005S2.0	ULCERATIVE STOMATITIS
M006S2.0	UNDIFFERENTIATED SARCOMA (LIVER); NEPHRITIS
F007S2.0	PANCREATIC ISLET CELL ADENOMA
F008S2.0	PNEUMONIA
F009S2.0	UNDETERMINED (NO BONE TUMOR)
M010S2.0	BACTERIAL VALVULAR ENDOCARDITIS
F011S2.0	MAMMARY SARCOMA
M012S2.0	HEPATIC CELL CARCINOMA
F001S3.0	UNDETERMINED (NO BONE TUMOR)
M002S3.0	NEPHRITIS
M003S3.0	SEMINOMA; HYDROCEPHALUS
F004S3.0	MAMMARY CARCINOMA
M005S3.0	SERTOLI CELL TUMOR
M006S3.0	NEPHRITIS; TESTICULAR MALIGNANCY
F007S3.0	MAMMARY CARCINOMA; THYROID CARCINOMA
F008S3.0	UNDETERMINED
F009S3.0	CHROMAPHOBE ADENOMA
M010S3.0	FIBROSARCOMA (GINGIVA)
F011S3.0	PYELONEPHRITIS
M012S3.0	TRANSITIONAL CELL CARCINOMA
F001S4.0	NOT DETERMINED (NO OSTEOSARCOMA)
M002S4.0	SQUAMOUS CELL CARCINOMA -GINGIVA-
M003S4.0	OBTURATING PULMONARY EMBOLISM
F004S4.0	OSTEOSARCOMA;SQUAMOUS CELL CARCINOMA; THYROID CARCINOMA
M005S4.0	HEMANGIOSARCOMA (SPLEEN)
06S4.0	SEMINOMA
07S4.0	OSTEOSARCOMA
F008S4.0	UNDETERMINED (NO BONE TUMOR)

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F009S4.0	468	9.56	30.6	11	8	59	4942	10132
M010S4.0	517	8.20	31.3	29	9	59	4242	8493
F011S4.0	542	8.86	32.7	3	11	59	2114	4432
M012S4.0	605	10.90	32.3	6	1	60	4226	7053
F001S4.5	530	9.00	64.2	16	3	66	2937	
M002S4.5	530	12.20	63.6	16	3	66	2707	9660
M003S4.5	530	11.90	63.8	16	3	66	1493	7932
F004S4.5	530	9.80	64.5	16	3	66	2197	12133
M005S4.5	496	13.30	61.3	16	3	66	993	7232
M006S4.5	496	12.00	63.8	16	3	66	2843	9990
F007S4.5	511	9.90	64.5	16	3	66	2813	13480
F008S4.5	511	9.90	64.5	16	3	66	2325	12725
F009S4.5	511	10.30	64.0	16	3	66	1028	6935
M010S4.5	496	14.00	60.9	16	3	66	2064	14240
F011S4.5	496	11.90	65.8	16	3	66	1758	8579
M012S4.5	485	11.40	63.7	16	3	66	2253	13116
F001S5.0	434	9.38	103.0	18	1	55	960	11179
M002S5.0	551	12.20	102.0	14	2	56	255	4284
M002S5.0A	545	11.40	96.6	7	1	59	1740	16393
M003S5.0	507	10.30	102.0	15	10	57	2256	22079
F004S5.0	528	11.40	105.0	15	10	57	1448	12703
M005S5.0	621	8.53	95.2	19	11	57	1285	13657
M006S5.0	504	9.33	98.8	3	9	58	35	869
M006S5.0A	462	11.20	94.2	7	1	59	1021	15384
F007S5.0	478	10.20	92.7	11	11	58	1129	14532
F008S5.0	535	11.20	90.5	7	1	59	1469	14843
F009S5.0	459	8.82	93.5	11	8	59	1982	18143
M010S5.0	517	8.55	95.9	29	9	59	990	10209
F011S5.0	542	8.97	102.0	3	11	59	1667	13355
M012S5.0	606	12.50	99.2	6	1	60	1165	10837

DOG
NUMBER

COMMENTS ON DEAD DOGS

F009S4.0	UNDETERMINED
M010S4.0	MENINGIOMA; PERIANAL GLAND CARCINOMA
F011S4.0	BLOOD DYSCRASIA, PYOMETRA
M012S4.0	ADENOCARCINOMA NASAL CAVITY
F001S4.5	
M002S4.5	OSTEOSARCOMA
M003S4.5	SEVERE ANEMIA, INFARCTION, MYELOID METAPLASIA
F004S4.5	HEMANGIOSARCOMA (SPLEEN)
M005S4.5	OSTEOSARCOMA
M006S4.5	OSTEOSARCOMA
F007S4.5	OSTEOSARCOMA; SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
F008S4.5	HEMANGIOSARCOMA (BONE)
F009S4.5	OSTEOSARCOMA
M010S4.5	SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
F011S4.5	OSTEOSARCOMA
M012S4.5	OSTEOSARCOMA; HEMANGIOSARCOMA (BONE)
F001S5.0	OSTEOSARCOMA
M002S5.0	STRANGULATED INGUINAL HERNIA
M002S5.0A	OSTEOSARCOMA
M003S5.0	OSTEOSARCOMA
F004S5.0	OSTEOSARCOMA
M005S5.0	SEVERE ANEMIA, AUTOAGGLUTINATION, INFARCTION, SPLENOMEGALY
M006S5.0	INTESTINAL HEMORRHAGE
M006S5.0A	OSTEOSARCOMA, INFARCTION + THROMBOCYTOPENIA
F007S5.0	STATUS EPILEPTICUS
F008S5.0	OSTEOSARCOMA
F009S5.0	SQUAMOUS CELL CARCINOMA ARISING FROM FRONTAL SINUS
M010S5.0	SEVERE ANEMIA + THROMBOCYTOPENIA
F011S5.0	HEMANGIOSARCOMA (LEFT MANDIBLE)
M012S5.0	HEMANGIOSARCOMA (RIB)

F. AMERICIUM-241*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F013W0.2	533	10.60	0.00179	13 10 66	2726	
M014W0.2	533	14.90	0.00178	13 10 66	2726	
F024W0.2	477	11.30	0.00181	21 3 68	2201	
F031W0.2	472	10.90	0.0018	8 5 68	1478	4
M041W0.2	467	11.90	0.0018	2 7 68	2098	
M048W0.2	484	11.00	0.00174	30 7 68	2070	
M049W0.2	498	10.70	0.00175	25 11 69	1587	
F058W0.2	496	10.40	0.00168	26 1 70	1525	
M062W0.2	486	13.30	0.00175	24 2 70	1496	
F069W0.2	542	10.80	0.0018	22 4 70	1439	
M078W0.2	501	13.50	0.00178	16 7 70	1354	
M079W0.2	501	10.00	0.00179	16 7 70	1354	
F088W0.2	531	8.36	0.00177	25 8 70	1314	
M095W0.2	526	13.10	0.00173	25 8 70	1314	
F011W0.5	533	8.17	0.00532	13 10 66	2726	
M012W0.5	533	11.90	0.00539	13 10 66	2726	
M023W0.5	487	12.20	0.0053	21 3 68	2201	
M029W0.5	472	10.40	0.00548	8 5 68	2153	
F030W0.5	472	10.60	0.00538	8 5 68	2153	
F040W0.5	467	9.40	0.00528	2 7 68	2098	
M050W0.5	552	11.80	0.00526	25 11 69	1587	
M059W0.5	496	11.50	0.00503	26 1 70	1525	
M063W0.5	486	11.40	0.00524	24 2 70	1496	
M070W0.5	497	12.80	0.00531	22 4 70	1439	
F080W0.5	501	11.90	0.00545	16 7 70	1354	
F081W0.5	501	12.10	0.00548	16 7 70	1354	
F089W0.5	531	9.66	0.00527	25 8 70	1314	
M096W0.5	490	12.00	0.00533	25 8 70	1314	
F009W1.0	517	8.60	0.016	15 9 66	2745	
F010W1.0	517	9.90	0.0162	15 9 66	2750	53
M020W1.0	513	10.80	0.0161	21 3 68	2201	
F021W1.0	513	9.36	0.0166	21 3 68	232	7
F021W1.0A	552	11.40	0.0159	25 11 69	1587	
M022W1.0	487	11.60	0.0164	21 3 68	2201	
M028W1.0	472	12.10	0.0158	8 5 68	2145	
F051W1.0	552	8.25	0.0163	25 11 69	1587	
M060W1.0	496	10.00	0.0157	26 1 70	1525	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F013W0.2
M014W0.2
F024W0.2
F031W0.2
M041W0.2
M048W0.2
M049W0.2
F058W0.2
M062W0.2
F069W0.2
M078W0.2
M079W0.2
F088W0.2
M095W0.2

BRONCHOGENIC CARCINOMA

F011W0.5
M012W0.5
M023W0.5
M029W0.5
F030W0.5
F040W0.5
M050W0.5
M059W0.5
M063W0.5
M070W0.5
F080W0.5
F081W0.5
F089W0.5
M096W0.5

F009W1.0
F010W1.0
M020W1.0
F021W1.0
F021W1.0A
M022W1.0
J28W1.0
J51W1.0
M060W1.0

BRONCHOGENIC CARCINOMA

ACCIDENTAL STRANGULATION

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M064W1.0	486	10.40	0.0158	24	2	70	1496	
F071W1.0	485	12.10	0.0157	22	4	70	1439	
M082W1.0	501	12.40	0.0163	16	7	70	1354	
F090W1.0	526	10.90	0.016	25	8	70	1314	
M097W1.0	490	10.40	0.016	25	8	70	1314	
F042W1.7	495	9.26	0.0484	30	7	68	2070	
F043W1.7	492	10.40	0.0481	30	7	68	2070	
F044W1.7	492	7.46	0.0473	30	7	68	2070	
M045W1.7	492	11.90	0.0486	30	7	68	2070	
M046W1.7	484	8.42	0.0479	30	7	68	2070	
M047W1.7	484	11.10	0.0486	30	7	68	2070	
F052W1.7	552	9.57	0.0493	25	11	69	1587	
M061W1.7	496	10.70	0.0458	26	1	70	1525	
M065W1.7	486	11.20	0.0471	24	2	70	1496	
F072W1.7	500	11.10	0.0479	22	4	70	1439	
M083W1.7	501	12.60	0.0493	16	7	70	1354	
F091W1.7	490	13.30	0.048	25	8	70	1314	
M098W1.7	490	13.30	0.048	25	8	70	1314	
F007W2.0	561	12.60	0.0952	15	9	66	1847	253
F008W2.0	561	11.70	0.0957	15	9	66	2754	
M019W2.0	513	13.40	0.097	21	3	68	2201	
M027W2.0	473	12.70	0.0961	8	5	68	2153	
M038W2.0	477	9.88	0.0945	2	7	68	2098	
F039W2.0	468	9.21	0.0948	2	7	68	2098	
M053W2.0	498	9.24	0.096	25	11	69	1587	
F066W2.0	486	9.12	0.0935	24	2	70	1496	
M073W2.0	552	14.30	0.0965	17	6	70	1383	
F084W2.0	493	10.60	0.0984	16	7	70	1354	
M085W2.0	493	10.80	0.0987	16	7	70	1354	
F092W2.0	490	10.60	0.0962	25	8	70	1314	
M005W3.0	561	15.00	0.305	15	9	66	1917	880
F006W3.0	561	11.90	0.31	15	9	66	1510	729
F018W3.0	523	8.60	0.307	21	3	68	1756	760
M026W3.0	473	12.40	0.31	8	5	68	2127	825

DOG
NUMBER

COMMENTS ON DEAD DOGS

M064W1.0
F071W1.0
M082W1.0
F090W1.0
M097W1.0

F042W1.7
F043W1.7
F044W1.7
M045W1.7
M046W1.7
M047W1.7
F052W1.7
M061W1.7
M065W1.7
F072W1.7
M083W1.7
F091W1.7
M098W1.7

F007W2.0 OSTEOSARCOMA
F008W2.0
M019W2.0
M027W2.0
M038W2.0
F039W2.0
M053W2.0
F066W2.0
M073W2.0
F084W2.0
M085W2.0
F092W2.0

M005W3.0 OSTEOSARCOMA
 J06W3.0 OSTEOSARCOMA
 J18W3.0 OSTEOSARCOMA
M026W3.0 OSTEOSARCOMA; FIBROSARCOMA (BONE); HEPATOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
M036W3.0	477	11.00	0.305	2 7 68	1696	653
F037W3.0	468	8.44	0.294	2 7 68	1764	671
M054W3.0	498	10.50	0.306	25 11 69	1587	
M067W3.0	485	11.80	0.295	24 2 70	1496	
F074W3.0	542	10.00	0.302	22 4 70	1439	
F075W3.0	555	9.42	0.308	17 6 70	1383	
M086W3.0	493	11.30	0.312	16 7 70	1354	
F093W3.0	490	11.20	0.301	25 8 70	1314	
M100W3.0	542	11.00	0.304	2 12 70	1198	496
M003W4.0	517	12.60	0.897	28 6 66	1779	2960
F004W4.0	517	9.40	0.911	28 6 66	1533	2635
M017W4.0	523	9.87	0.924	21 3 68	1132	1813
F025W4.0	473	10.50	0.927	8 5 68	1527	2095
M034W4.0	477	10.70	0.893	2 7 68	1566	1616
F035W4.0	477	8.87	0.902	2 7 68	1323	2213
F055W4.0	498	8.37	0.914	25 11 69	1388	1696
M068W4.0	485	11.80	0.89	24 2 70	1415	1680
F076W4.0	485	9.37	0.899	22 4 70	1439	
M077W4.0	500	10.50	0.906	22 4 70	633	1000
M087W4.0	501	13.10	0.916	16 7 70	1300	1605
F094W4.0	490	11.30	0.912	25 8 70	1314	
M001W5.0	517	10.40	2.78	28 6 66	401	2181
M002W5.0	517	12.70	2.83	28 6 66	448	2312

* Measurements made to date indicate the liver dose to be approximately 4 times that to the skeleton.

The original "T" (test) designation for the above animals has been changed to "M or F" (male or female toxicity) designations. For example, the male dog originally injected as T001W5.0 is now redesignated as M001W5.0.

DOG NUMBER	COMMENTS ON DEAD DOGS
M036W3.0	OSTEOSARCOMA
F037W3.0	OSTEOSARCOMA
M054W3.0	
M067W3.0	
F074W3.0	
F075W3.0	
M086W3.0	
F093W3.0	
M100W3.0	OSTEOSARCOMA
M003W4.0	OSTEOSARCOMA; NEPHRITIS
F004W4.0	KIDNEY, THYROID AND LIVER DEGENERATION
M017W4.0	OSTEOSARCOMA; THROMBOSIS
F025W4.0	MESOTHELIOMA; KIDNEY DEGENERATION
M034W4.0	OSTEOSARCOMA
F035W4.0	PANCREATIC ADENOCARCINOMA
F055W4.0	OSTEOSARCOMA
M068W4.0	OSTEOSARCOMA
F076W4.0	
M077W4.0	LIVER DEGENERATION
M087W4.0	OSTEOSARCOMA
F094W4.0	
M001W5.0	LIVER DEGENERATION; KIDNEY DEGENERATION
M002W5.0	LIVER DEGENERATION; KIDNEY DEGENERATION

G. CALIFORNIUM-252

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
M001F0.0	562	12.00		1 2 72	789	
F002F0.0	545	10.60		3 1 73	452	
F003F0.0	545	9.43		3 1 73	452	
F004F0.0	492	10.60		27 2 73	397	
M005F0.0	562	10.40		1 2 72	789	
M006F0.0	509	10.90		28 11 72	488	
M001F0.1	498	13.00	0.0006	26 7 72	613	
F002F0.1	524	9.15	0.00064	2 11 72	514	
F003F0.1	545	10.10	0.00075	3 1 73	452	
F004F0.1	492	9.44	0.0006	27 2 73	397	
M005F0.1	498	10.40	0.0006	26 7 72	613	
M006F0.1	524	10.30	0.00062	2 11 72	514	
M001F0.5	498	12.20	0.00525	26 7 72	613	
F002F0.5	513	11.00	0.00529	2 11 72	514	
F003F0.5	511	8.89	0.00525	27 2 73	397	
F004F0.5	585	11.20	0.00518	27 2 73	397	
M005F0.5	494	9.44	0.0053	26 7 72	613	
M006F0.5	524	11.00	0.00529	2 11 72	514	
M001F1.0	586	9.69	0.0163	8 9 71	935	
F002F1.0	586	8.28	0.0167	8 9 71	935	
F003F1.0	539	8.89	0.0167	8 9 71	935	
F004F1.0	539	10.00	0.0165	8 9 71	935	
M005F1.0	539	12.90	0.0165	8 9 71	935	
M006F1.0	513	9.67	0.0165	2 11 72	514	
M001F2.0	498	11.50	0.0922	26 7 72	613	
F002F2.0	545	9.85	0.0905	3 1 73	452	
F003F2.0	511	9.16	0.0907	27 2 73	397	
F004F2.0	573	9.33	0.091	27 2 73	397	
M005F2.0	494	10.20	0.0905	26 7 72	613	
M006F2.0	513	11.40	0.0912	2 11 72	514	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001F0.0
F002F0.0
F003F0.0
F004F0.0
M005F0.0
M006F0.0

M001F0.1
F002F0.1
F003F0.1
F004F0.1
M005F0.1
M006F0.1

M001F0.5
F002F0.5
F003F0.5
F004F0.5
M005F0.5
M006F0.5

M001F1.0
F002F1.0
F003F1.0
F004F1.0
M005F1.0
M006F1.0

M001F2.0
F002F2.0
F003F2.0
F004F2.0
M005F2.0
M006F2.0

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M001F3.0	583	11.60	0.289	3	3	71	1124	
F002F3.0	583	10.60	0.289	3	3	71	1124	
F003F3.0	583	8.66	0.292	3	3	71	1124	
F004F3.0	583	9.69	0.295	3	3	71	1115	
F005F3.0	524	11.10	0.284	28	11	72	488	
M006F3.0	513	10.20	0.293	2	11	72	514	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001F3.0
F002F3.0
F003F3.0
F004F3.0
F005F3.0
M006F3.0

H. CALIFORNIUM-249

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F001G0.0	499	7.77		23 10 73	159	
M002G0.0	509	10.50		28 11 72	488	
M003G0.0	509	10.10		28 11 72	488	
F004G0.0	502	11.10		5 3 74	26	
F006G0.0	499	11.40		23 10 73	159	
F001G0.1	499	9.91	0.00061	23 10 73	159	
M002G0.1	486	13.10	0.00063	5 7 72	634	
M003G0.1	486	10.10	0.00063	5 7 72	634	
M006G0.1	486	11.60	0.00064	5 7 72	634	
F001G0.5	499	9.20	0.00485	23 10 73	159	
M002G0.5	514	12.00	0.00514	29 2 72	761	
M003G0.5	514	12.60	0.00518	29 2 72	761	
F004G0.5	471	10.90	0.00516	5 3 74	26	
M006G0.5	514	11.80	0.00511	29 2 72	761	
F001G1.0	555	8.58	0.0154	16 12 71	836	
M002G1.0	486	11.40	0.0152	5 7 72	634	
M003G1.0	486	11.50	0.0154	5 7 72	634	
F004G1.0	555	10.50	0.0154	16 12 71	836	
F005G1.0	471	9.29	0.0153	5 3 74	26	
M006G1.0	524	10.60	0.016	28 11 72	488	
F001G2.0	558	9.32	0.0905	16 12 71	836	
M002G2.0	555	11.00	0.0916	16 12 71	836	
M003G2.0	486	10.80	0.0935	5 7 72	625	
F004G2.0	558	10.30	0.0915	16 12 71	836	
F005G2.0	555	9.44	0.0913	16 12 71	836	
M006G2.0	524	10.00	0.0963	28 11 72	488	
F001G3.0	584	11.60	0.29	24 2 71	1131	
M002G3.0	580	13.20	0.282	24 2 71	1131	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F001G0.0
M002G0.0
M003G0.0
F004G0.0
F006G0.0

F001G0.1
M002G0.1
M003G0.1
M006G0.1

F001G0.5
M002G0.5
M003G0.5
F004G0.5
M006G0.5

F001G1.0
M002G1.0
M003G1.0
F004G1.0
F005G1.0
M006G1.0

F001G2.0
M002G2.0
M003G2.0
F004G2.0
F005G2.0
M006G2.0

. 001G3.0
M002G3.0

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M003G3.0	580	13.70	0.284	24	2	71	1131	
F004G3.0	580	8.79	0.283	24	2	71	1131	
M006G3.0	524	10.10	0.293	28	11	72	488	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M003G3.0
F004G3.0
M006G3.0

I. EINSTEINIUM-253

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F001E3.0	470	1.12	0.284	5	6	73	299	
M003E3.0	470	1.13	0.288	5	6	73	299	
M004E3.0	470	7.93	0.294	5	6	73	299	
F001E5.0	495	8.70	2.85	5	6	73	299	
F002E5.0	483	9.21	2.81	5	6	73	299	
M003E5.0	470	1.04	2.84	5	6	73	299	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F001E3.0
M003E3.0
M004E3.0

F001E5.0
F002E5.0
M003E5.0

TABLE II. TEST ANIMALS (MAR. 31 1974)

A. RADIUM-226*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001R5.0	996	11.10	10.3	1	12	52	1074	12625
T002R5.0	920	8.40	4.39	12	1	53	1368	6299
T003R5.0	1467	8.29	4.76	12	1	53	428	1755
T004R5.0	459	10.00	10.6	6	7	53	1	32
T005R5.0	126	6.14	11.7	6	10	53	1	33
T006R5.0	126	6.14	11.4	6	10	53	1	32
T007R5.0	126	6.14	11.8	6	10	53	1	33
T008R5.0	290	5.52	1.92	10	5	55	58	651
T009R5.0	2275	10.40	1.94	10	5	55	58	780
T010R5.0	43	1.02	1.98	10	5	55	49	391
T011R5.0	43	1.58	1.91	10	5	55	49	484
T012R5.0	397	12.30	9.72	9	5	56	220	3609
T013R5.0	397	7.59	9.76	9	5	56	188	3092
T014R4.0	674	8.12	3.17	11	7	56	72	507
T015R4.0	672	9.03	3.11	11	7	56	2127	6823
T016R5.0	604	12.40	9.68	11	7	57	12	244
T017R5.0H	383	12.20	9.87	28	10	58	1140	18207
T018R5.0H	383	11.10	10.8	28	10	58	1226	16837
T019R5.0H	383	11.30	10.7	28	10	58	1219	15440
T020R5.0H	393	11.40	10.6	28	10	58	1340	18641
T021R5.0H	381	11.80	10.1	28	10	58	386	4947
T022R5.0H	381	11.90	10.1	28	10	58	587	7881
T023R4.0H	384	9.50	4.05	25	11	58	1471	5787
T024R4.0H	384	11.90	3.24	25	11	58	1505	7457
T025R4.0H	379	11.30	3.42	25	11	58	1309	6229
T026R4.0H	379	11.00	3.48	25	11	58	1780	6292
T027R4.0H	372	11.50	3.34	25	11	58	1414	4509
T028R3.0H	372	11.70	1.11	25	11	58	387	476

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001R5.0	OSTEOSARCOMA
T002R5.0	OSTEOSARCOMA
T003R5.0	SPECIAL STUDY
T004R5.0	SPECIAL STUDY
T005R5.0	SPECIAL STUDY
T006R5.0	SPECIAL STUDY
T007R5.0	SPECIAL STUDY
T008R5.0	SPECIAL STUDY
T009R5.0	SPECIAL STUDY
T010R5.0	SPECIAL STUDY
T011R5.0	SPECIAL STUDY
T012R5.0	SPECIAL STUDY
T013R5.0	SPECIAL STUDY

T014R4.0	SPECIAL STUDY
T015R4.0	OSTEOSARCOMA

T016R5.0	SPECIAL STUDY
T017R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T018R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T019R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T020R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T021R5.0H	NEPHRITIS
T022R5.0H	CRIPPLING FRACTURES

T023R4.0H	OSTEOSARCOMA
T024R4.0H	OSTEOSARCOMA
T025R4.0H	OSTEOSARCOMA
T026R4.0H	OSTEOSARCOMA
T027R4.0H	OSTEOSARCOMA

T028R3.0H	SPECIAL STUDY
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DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T029R5.0	474	13.50	10.4	3 3 59	216	4539
T030R5.0	474	11.50	10.4	3 3 59	178	3801
T031R5.0	471	10.50	10.4	3 3 59	303	6344
T032R3.0	471	11.40	1.13	3 3 59	2249	2485
T033R3.0	471	10.60	1.15	3 3 59	1822	2561
T034R3.0	470	15.70	1.12	3 3 59	1737	2137
T035R3.0J	670	9.44	0.951	5 5 59	8	19
T036R4.0	695	10.20	2.99	22 12 60	1154	4863
T037R4.0	695	9.53	3.0	22 12 60	1627	4312
T038R4.0	695	10.10	3.02	22 12 60	1503	4876
T040R1.0	899	13.00	0.0483	3 4 62	7	1
T041R1.0	899	12.70	0.0487	3 4 62	63	4
T042R1.7	967	14.00	0.146	4 4 62	7	3
T043R1.7	963	13.20	0.145	4 4 62	64	15
T044R3.0	938	11.10	0.937	4 4 62	68	95
T045R3.0	939	13.60	0.941	5 4 62	7	16
T046R3.0	810	12.50	0.928	5 4 62	69	137
T047R6.0	99	5.27	29.4	11 6 62		469
T048R6.0	2842	11.20	25.1	27 12 62	49	2129
T049R5.0	485	10.60	7.54	2 5 63	5	148
T050R5.0	485	13.70	7.46	2 5 63	15	365
T051R5.0	418	13.30	8.48	8 5 63	92	2252

DOG NUMBER	COMMENTS ON DEAD DOGS
T029R5.0	NEPHRITIS
T030R5.0	NEPHRITIS
T031R5.0	NEPHRITIS
T032R3.0	OSTEOSARCOMA
T033R3.0	OSTEOSARCOMA, NEPHRITIS
T034R3.0	OSTEOSARCOMA
T035R3.0J	SPECIAL STUDY
T036R4.0	OSTEOSARCOMA
T037R4.0	OSTEOSARCOMA
T038R4.0	OSTEOSARCOMA
T040R1.0	SPECIAL STUDY
T041R1.0	SPECIAL STUDY
T042R1.7	SPECIAL STUDY
T043R1.7	SPECIAL STUDY
T044R3.0	SPECIAL STUDY
T045R3.0	SPECIAL STUDY
T046R3.0	SPECIAL STUDY
T047R6.0	SPECIAL STUDY
T048R6.0	LEUKOPENIA, PNEUMONIA + SPECIAL MELANOMA STUDY
049R5.0	SPECIAL STUDY
050R5.0	SPECIAL STUDY
T051R5.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YP		
T052R5.0	418	10.70	8.57	8	5	63	15	344
T053R5.0	418	12.00	8.5	8	5	63	33	815
T054R5.0	417	11.40	8.76	22	5	63	5	117
T055R5.0	417	11.60	8.61	22	5	63	33	712
T056R5.0	417	11.60	8.61	22	5	63	90	1948
T057R4.0	501	12.10	2.72	15	8	63	14	72
T058R4.0	496	11.70	2.41	15	8	63	61	381
T059R4.0	496	9.64	2.57	15	8	63	60	363
T060R4.0	490	12.10	2.33	15	8	63	117	553
T061R4.0	490	9.48	2.7	15	8	63	371	2089
T062R4.0	490	8.63	2.68	15	8	63	460	2115
T063R3.0	559	8.72	0.899	29	1	64	36	68
T064R3.0	551	8.42	0.919	29	1	64	63	97
T065R3.0	551	11.60	0.922	29	1	64	70	132
T066R3.0	549	10.10	0.904	29	1	64	132	187
T067R3.0	549	12.70	0.898	29	1	64	134	224
T068R3.0	549	12.10	0.917	29	1	64	1667	1377
T069R3.0	499	8.84	0.919	29	1	64	622	868
T070R3.0	499	14.20	0.922	29	1	64	1996	2819
T071R5.0	4025	13.80	9.23	28	1	69	42	931
T072R5.0	4776	9.45	12.4	17	8	72	54	1593

* The multiple injection dogs were male beagles born in Davis, California but injected in our laboratory. Each was injected 6 times over a 280 day period with 56 days between each injection. Each ^{226}Ra injection was 20.0 μCi for the dogs T17R5H - T22R5H; 6.41 μCi for T23R4H - T27R5H; and 2.16 μCi for T28R3H. Tabulated for each dog are his age at 1st injection, his average weight during the injection period, total μCi /average weight, the date of 1st injection, the time from 1st injection to death, and sum of the skeletal doses computed from each injection to death.

T35R3J also received 99 μCi ^{85}Sr .

T39R0.0 has been reassigned and is now M12M0.0.

DOG
NUMBER

COMMENTS ON DEAD DOGS

T052R5.0	SPECIAL STUDY
T053R5.0	SPECIAL STUDY
T054R5.0	SPECIAL STUDY
T055R5.0	SPECIAL STUDY
T056R5.0	SPECIAL STUDY

T057R4.0	SPECIAL STUDY
T058R4.0	SPECIAL STUDY
T059R4.0	SPECIAL STUDY
T060R4.0	SPECIAL STUDY
T061R4.0	SPECIAL STUDY
T062R4.0	SPECIAL STUDY

T063R3.0	SPECIAL STUDY
T064R3.0	SPECIAL STUDY
T065R3.0	SPECIAL STUDY
T066R3.0	SPECIAL STUDY
T067R3.0	SPECIAL STUDY
T068R3.0	OSTEOSARCOMA
T069R3.0	SPECIAL STUDY
T070R3.0	OSTEOSARCOMA

T071R5.0	MELANOMA ORAL CAVITY
T072R5.0	SPECIAL STUDY

B. PLUTONIUM-239*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T000P5.0	647	11.40	3.05	24	6	52	1	5
T001P5.0	1581	12.70	3.04	13	10	52	29	156
T002P5.0	914	11.90	6.85	15	9	52	44	534
T003P5.0	942	9.65	3.22	13	10	52	610	3361
T004P5.0	1016	8.78	3.02	13	10	52	365	1912
T005P5.0	474	10.40	2.69	14	12	54	400	1862
T006P5.0	527	6.16	2.73	14	12	54	406	1918
T007P5.0	475	7.40	2.68	14	12	54	777	3534
T008P5.0	527	8.32	2.67	14	12	54	863	3896
T009P5.0	551	10.30	2.8	22	11	55	15	75
T010P5.0	534	11.90	2.74	23	11	55	15	73
T011P5.0	516	12.10	2.76	22	11	55	28	137
T012P5.0	487	9.23	2.74	23	11	55	28	136
T013P5.0	587	8.27	3.16	24	4	56	3	17
T014P5.0	587	9.38	2.43	24	4	56	7	30
T015P5.0	737	8.32	2.79	15	10	56	1	5
T016P5.0	673	10.70	2.85	10	10	56	92	463
T017P5.0	739	11.10	3.01	12	2	57	210	1107
T018P5.0	739	8.16	2.83	12	2	57	217	1075
T019P5.0	688	8.86	2.91	15	12	60	1400	6752
T020P5.0	688	13.00	2.68	15	12	60	474	2189
T021P5.0	688	10.30	2.72	15	12	60	939	4304
T023P1.0	1485	13.10	0.0172	28	7	61	96	3
T024P1.0	559	13.10	0.0172	28	7	61	97	3
T025P1.0	559	13.80	0.0167	28	7	61	467	12
T026P1.0	556	12.00	0.016	28	7	61	647	15
T027P3.0	556	11.50	0.332	28	7	61	755	402
T028P1.0	552	10.50	0.015	9	8	61	559	13
T029P3.0	552	12.10	0.296	9	8	61	560	272

DOG
NUMBER

COMMENTS ON DEAD DOGS

T000P5.0	SPECIAL STUDY
T001P5.0	SPECIAL STUDY
T002P5.0	SPECIAL STUDY
T003P5.0	SPECIAL STUDY
T004P5.0	SPECIAL STUDY
T005P5.0	SPECIAL STUDY
T006P5.0	SPECIAL STUDY
T007P5.0	SPECIAL STUDY
T008P5.0	SPECIAL STUDY
T009P5.0	SPECIAL STUDY
T010P5.0	SPECIAL STUDY
T011P5.0	SPECIAL STUDY
T012P5.0	SPECIAL STUDY
T013P5.0	SPECIAL STUDY
T014P5.0	SPECIAL STUDY
T015P5.0	SPECIAL STUDY
T016P5.0	SPECIAL STUDY
T017P5.0	SPECIAL STUDY
T018P5.0	SPECIAL STUDY
T019P5.0	OSTEOSARCOMA, BLOOD DYSCRASIA, LIVER DEGENERATION
T020P5.0	LIVER DEGENERATION, ASCITES + THROMBOCYTOPENIA
T021P5.0	TOXIC NEPHRITIS + LIVER DEGENERATION
T023P1.0	SPECIAL STUDY
T024P1.0	SPECIAL STUDY
T025P1.0	SPECIAL STUDY
T026P1.0	SPECIAL STUDY
T027P3.0	SPECIAL STUDY
T028P1.0	SPECIAL STUDY
029P3.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T030P1.0	548	12.40	0.0148	9 8 61	30	<1
T031P3.0	520	13.00	0.305	9 8 61	40	21
T032P1.0	520	8.47	0.0162	9 8 61	274	7
T033P1.0	550	10.70	0.0153	15 9 61	375	9
T034P1.0	550	9.68	0.0154	15 9 61	746	17
T035P3.0	550	11.90	0.303	15 9 61	362	184
T036P1.0	544	10.40	0.0158	15 9 61	5	<<1
T037P1.0	542	8.59	0.0148	15 9 61	186	5
T038P3.0	489	7.96	0.304	15 9 61	187	98
T039P1.0	1534	10.70	0.0151	15 9 61	376	9
T040P1.0	1534	9.92	0.0177	15 9 61	769	20
T041P5.0	543	8.50	3.01	30 11 64	1227	6156
T042P5.0	510	11.40	2.4	10 2 65	13	55
T043P5.0H	600	14.00	2.86	15 7 65	40	203
T044P5.0H	517	12.00	2.72	21 9 65	35	169
T045P5.0H	420	12.30	2.98	28 10 65	5/24	1
T046P5.0	420	11.90	3.01	28 10 65	732	3747
T047P5.0	806	12.40	3.02	30 11 65	69	368
T048P5.0	554	8.50	2.61	11 3 66	1327	5754

DOG NUMBER	COMMENTS ON DEAD DOGS
T030P1.0	SPECIAL STUDY
T031P3.0	SPECIAL STUDY
T032P1.0	SPECIAL STUDY
T033P1.0	SPECIAL STUDY
T034P1.0	SPECIAL STUDY
T035P3.0	SPECIAL STUDY
T036P1.0	SPECIAL STUDY
T037P1.0	SPECIAL STUDY
T038P3.0	SPECIAL STUDY
T039P1.0	SPECIAL STUDY
T040P1.0	SPECIAL STUDY
T041P5.0	PURPURA HEMORRHAGICA; AUTOHEMAGGLUTINATION; LIVER DEGENERATION
T042P5.0	SPECIAL STUDY
T043P5.0H	SPECIAL STUDY
T044P5.0H	SPECIAL STUDY
T045P5.0H	SPECIAL STUDY
T046P5.0	LIVER DEGENERATION
T047P5.0	SPECIAL STUDY
T048P5.0	UNDIFFERENTIATED SARCOMA (BONE)

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T049P1.0	103	5.00	0.0162	5 7 66	2826	
T050P3.0	103	5.30	0.296	5 7 66	2826	
T051P5.0	104	4.80	2.73	6 7 66	1055	4831
T052P4.0	437	11.80	0.949	7 7 67	14	24
T053P5.0	1517	13.90	2.82	11 3 69	1559	7016
T054P5.0	906	11.30	2.77	11 3 69	404	1936
T055P4.0	445	10.60	0.785	3 6 69	14	20
T056P5.5	501	11.20	3.73	29 7 69	7	46
T057P2.0E	618	49.40	0.0961	10 9 69	1506	181
T058P3.0E	573	52.30	0.291	10 9 69	917	421
T059P3.0E	591	44.50	0.29	5 11 69	973	442
T060P3.0E	567	45.20	0.314	6 1 70	784	393
T061P2.0E	581	47.20	0.0983	6 1 70	1545	
T062P2.0E	583	52.50	0.156	22 1 70	1223	251

DOG
NUMBER

COMMENTS ON DEAD DOGS

T049P1.0

T050P3.0

T051P5.00 OSTEOSARCOMA

T052P4.0 SPECIAL STUDY

T053P5.0 LIVER DEGENERATION; ADRENALECTOMY
T054P5.0 SPECIAL STUDY

T055P4.0 SPECIAL STUDY

T056P5.5 SPECIAL STUDY

T057P2.0E OSTEOSARCOMA

T058P3.0E OSTEOSARCOMA
T059P3.0E OSTEOSARCOMA
T060P3.0E OSTEOSARCOMA

T061P2.0E
062P2.0E OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T063P5.0	581	9.13	2.77	14 12 70	490	2337
T064P0.0	595	8.60		28 12 70	1189	
T065P4.0P	542	11.50	0.904	30 11 71	14	2
T066P4.0P	542	10.60	0.913	30 11 71	865	
T067P4.0P	542	10.40	0.907	30 11 71	865	
T068P1.0E	569	44.20	0.0158	20 4 72	710	
T069P1.0E	569	32.00	0.016	20 4 72	710	
T070P1.0E	588	40.60	0.0152	9 5 72	691	
T071P0.5E	588	48.90	0.00521	9 5 72	691	
T072P0.5E	611	44.50	0.00512	1 6 72	668	
T073P0.5E	611	38.80	0.00507	1 6 72	668	
T074P4.0	3694	7.73	0.937	28 3 73	368	
T075P4.0	3478	8.47	0.897	28 3 73	368	
T076P4.0	3413	10.70	0.894	28 3 73	368	
T077P3.0	3413	9.95	0.31	28 3 73	368	
T078P3.0	2488	8.21	0.32	28 3 73	368	
T079P0.5	483	11.10	0.00523	5 6 73	134	2
T080P0.2E	569	48.70	0.00153	25 7 73	249	
T081P0.2E	569	44.30	0.00157	25 7 73	249	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T063P5.0 SPECIAL STUDY

T064P0.0

T065P4.0P SPECIAL STUDY

T066P4.0P

T067P4.0P

T068P1.0E

T069P1.0E

T070P1.0E

T071P0.5E

T072P0.5E

T073P0.5E

T074P4.0

T075P4.0

T076P4.0

T077P3.0

T078P3.0

T079P0.5 SPECIAL STUDY

060P0.2E

T081P0.2E

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T082P0.2E	597	47.20	0.00191	22	8	73	221	
T083P0.1E	575	51.30	0.00061	22	8	73	221	

* T22P0.0 has been reassigned and is now F06T0.0A.

T043P5.0H was also given 1.01 μ Ci ^{239}Pu /kg one day prior to sacrifice.

T044P5.0H was given 0.833 μ Ci ^{239}Pu /kg and about 9.17 μ Ci ^{59}Fe /kg one day prior to sacrifice.

Dogs in the above tabulation having the letter E as the final entry in the "Dog number" column are St. Bernards. Those having the letter P in that position were given plutonium in polymeric form.

DOG
NUMBER

COMMENTS ON DEAD DOGS

T082P0.2E

T083P0.1E

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001M4.5	529	9.13	4.23	8	9	54	314	2131
T002M4.5	463	8.93	4.27	8	9	54	755	6796
T003M5.0	579	9.15	10.6	13	3	56	700	21156

* (μ Ci $^{228}\text{Th}/\mu$ Ci ^{228}Ra) injected = 0.03.

DOG NUMBER	COMMENTS ON DEAD DOGS
T001M4.5	CANINE DISTEMPER
T002M4.5	SPECIAL STUDY
T003M5.0	ULCERATIVE GINGIVITIS, SEVERE ANEMIA + CRIPPLING FRACTURE

D. THORIUM-228 (RADIO THORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T001T5.0	607	9.30	4.88	1 12 53	23	1548
T002T5.0	501	8.48	2.56	8 2 54	77	2639
T003T4.0	429	10.40	0.87	8 2 54	820	6504
T004T5.0	455	8.92	2.59	28 9 54	113	3844
T005T5.0	455	10.10	2.32	28 9 54	65	2032
T006T4.0	591	7.01	0.884	18 10 55	651	5697
T007T3.0	591	9.23	0.298	18 10 55	910	2369
T008T3.0	606	9.23	0.293	14 10 58	1043	2511
T009T3.0	447	11.00	0.285	4 2 59	1	4
T010T3.0	447	14.20	0.289	4 2 59	8	32
T011T3.0	500	8.62	0.335	16 6 59	22	101
T012T3.0	514	10.60	0.302	7 7 59	22	92
T013T3.0	754	13.10	0.298	28 7 59	22	91

* T11, 12, 13T3 received 40, 4, and 0.4 mg ^{232}Th , respectively.

2

DOG NUMBER	COMMENTS ON DEAD DOGS
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T001T5.0	DIED, SPECIAL STUDY
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T002T5.0	SPECIAL STUDY
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T003T4.0	CRIPPLING FRACTURES + NEPHRITIS
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T004T5.0	THROMBOCYTOPENIA + PURPURA
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T005T5.0	NEPHRITIS, THROMBOCYTOPENIA + PURPURA
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T006T4.0	CRIPPLING FRACTURES
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T007T3.0	SPECIAL STUDY
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T008T3.0	OSTEOSARCOMA
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T009T3.0	SPECIAL STUDY
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T010T3.0	SPECIAL STUDY
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T011T3.0	SPECIAL STUDY
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T012T3.0	SPECIAL STUDY
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T013T3.0	SPECIAL STUDY
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E. STRONTIUM-90*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T001S0.0	151	7.71		5 3 54	112	
T002S5.0	149	6.85	148.0	5 3 54	18	835
T003S5.0	144	6.19	148.0	5 3 54	28	1276
T004S5.0	151	7.05	148.0	5 3 54	41	2593
T005S5.0	144	5.25	148.0	5 3 54	116	6484
T006S5.0	155	7.01	87.0	16 3 54	1/24	3
T007S5.0	155	6.74	87.0	16 3 54	2	112
T008S0.0	243	7.00				
T008S2.0H	67	3.69	2.74	27 9 55	66	41
T009S2.0H	67	2.79	3.62	27 9 55	66	53
T010S2.0H	67	3.11	3.25	27 9 55	132	124
T011S2.0H	67	3.85	2.62	27 9 55	132	100
T012S3.0	593	10.60	10.5	11 9 57	5	20
T013S4.0	324	10.50	19.1	8 7 60	8	67
T014S5.0	542	10.00	96.1	7 11 61	9	233
T015S5.0	595	9.43	98.4	7 11 61	30	564
T016S2.0	604	9.71	3.27	8 11 61	9	8
T017S6.0	670	7.18	295.0	19 1 62	14	919
T018S6.0	670	5.94	302.0	19 1 62	1369	25217

DOG NUMBER	COMMENTS ON DEAD DOGS
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T001S0.0	SPECIAL STUDY
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T002S5.0	SPECIAL STUDY
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T003S5.0	SPECIAL STUDY
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T004S5.0	SPECIAL STUDY
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T005S5.0	SPECIAL STUDY
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T006S5.0	SPECIAL STUDY
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T007S5.0	SPECIAL STUDY
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T008S0.0	SPECIAL STUDY
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T008S2.0H	SPECIAL STUDY
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T009S2.0H	SPECIAL STUDY
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T010S2.0H	SPECIAL STUDY
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T011S2.0H	SPECIAL STUDY
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T012S3.0	BREMSSTRAHLUNG PHANTOM
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T013S4.0	BREMSSTRAHLUNG PHANTOM SAM MCGEE
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T014S5.0	SPECIAL STUDY
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T015S5.0	SPECIAL STUDY
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T016S2.0	SPECIAL STUDY
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17S6.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
T018S6.0	HEMANGIOSARCOMA (ISCHIUM)

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T019S6.0	670	5.43	284.0	19	1	62	23	849
T020S4.0J	440	8.54	28.9	2	10	63	13	104
T021S2.5J	363	7.20	8.3	2	10	63	13	191
T022S5.0	545	9.01	99.0	1	4	69	1525	11690
T023S5.0	545	11.60	100.0	1	4	69	1379	14121

* T08 . . . 11S2.0H were given 10 injections, 1 μ Ci each at weekly intervals.
Age at first injection, wt. is average during the injection period, μ Ci/kg
is total ^{90}Sr /average weight, date is at first injection, days are from first
injection to death, and dose is computed from mid-injection to death.

T20S4.0J received 0.5 μ Ci ^{85}Sr .

T21S2.5J received 0.5 μ Ci ^{85}Sr and 600 μ Ci ^{89}Sr .

DOG NUMBER	COMMENTS ON DEAD DOGS
T019S6.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
T020S4.0J	SPECIAL STUDY
T021S2.5J	SPECIAL STUDY
T022S5.0	HEMANGIOSARCOMA (BONE)
T023S5.0	OSTEOSARCOMA

F. RADIUM-224*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T001Q3.0J	460	9.55	0.875	26 3 63	4/24	
T002Q4.0	466	12.00	2.91	27 3 63	2317	133
T003Q4.0	466	13.10	2.91	27 3 63	2708	133
T004Q5.0	480	9.55	9.71	24 4 63	1462	533
T005Q5.0	455	9.67	9.59	24 4 63	1638	533
T006Q6.0	455	8.29	21.4	17 10 63	13	1067
T007Q5.0	465	11.80	8.56	6 11 63	2053	533
T008Q5.0	475	9.77	8.62	6 11 63	16	400
T009Q4.0	503	9.80	2.57	4 12 63	1451	133
T010Q4.0	503	10.30	2.57	4 12 63	262	133
T011Q3.0	495	9.10	0.885	4 12 63	3668	40
T012Q3.0	495	13.50	0.889	4 12 63	3770	
T013Q3.0	495	11.30	0.912	4 12 63	3770	
T014Q3.0	438	10.30	0.87	4 12 63	3770	
T015Q4.0	515	12.70	2.73	1 2 68	1692	133
T016Q2.0	515	9.36	0.31	1 2 68	2250	
T017Q2.0	515	10.20	0.311	1 2 68	2250	
T018Q2.0	502	9.68	0.306	1 2 68	2250	

DOG NUMBER	COMMENTS ON DEAD DOGS
T001Q3.0J	SPECIAL STUDY
T002Q4.00	OSTEOSARCOMA
T003Q4.0	HEMANGIOSARCOMA
T004Q5.0	OSTEOSARCOMA, EPIDERMOID CARCINOMA (FRONTAL SINUS)
T005Q5.0	OSTEOSARCOMA
T006Q6.0	PURPURA HEMORRHAGICA
T007Q5.0	OSTEOSARCOMA
T008Q5.0	PURPURA HEMORRHAGICA
T009Q4.0	STRANGULATION ON VOMITUS AND GRAND MAL
T010Q4.0	STATUS EPILEPTICUS
T011Q3.0	AORTIC BODY TUMOR
T012Q3.0	
T013Q3.0	
T014Q3.0	
T015Q4.0	OSTEOSARCOMA
T016Q2.0	
17Q2.0	
18Q2.0	

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T019Q1.0	515	11.80	0.0475	1	2	68	2250	
T020Q1.0	515	10.40	0.0472	1	2	68	2250	
T021Q1.0	502	9.08	0.0447	1	2	68	2250	

* The skeletal doses in rads are only from ^{224}Ra and its daughters. In at least some of these dogs, appreciable ^{210}Pb and ^{228}Th contamination was injected. The dose from the ^{210}Pb and ^{228}Th has not been included. (See the article ^{210}Pb contamination of ^{228}Th : Its Contribution to Dose in Beagles in Our ^{228}Th Toxicity Studies in C00-119-237.)

T001Q3.0J also received 18.0 μ Ci ^{85}Sr .

DOG
NUMBER

COMMENTS ON DEAD DOGS

T019Q1.0

T020Q1.0

T021Q1.0

G. AMERICIUM-241*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
T015W5.5	858	11.50	4.53	23 10 67	1	13
T016W5.0	461	10.70	2.78	29 1 68	20	107
T032W5.5	553	11.00	4.46	30 4 68	7	53
T033W5.5	393	10.50	4.47	30 4 68	8	61
T056W5.0	552	11.30	2.9	25 11 69	15	67
T057W5.0	496	7.01	2.77	26 1 70	15	63
T099W5.0	547	11.30	2.67	10 11 70	252	1213
T101W5.0	399	10.40	2.98	17 8 72	1	4
T102W3.0	515	11.60	0.28	10 10 72	17	7
T103W3.0	501	10.60	0.283	10 10 72	537	
T104W3.0	2658	7.67	0.305	28 11 72	488	
T105W3.0	2224	7.78	0.301	28 11 72	488	
T106W3.0	2224	13.80	0.308	28 11 72	488	
T107W5.0	3543	9.24	2.34	2 4 73	36	128
T108W3.0	507	12.60	0.304	8 8 73	235	
T109W3.0	506	9.90	0.306	8 8 73	235	
T110W3.0	506	9.30	0.306	8 8 73	235	
T111W3.0	506	9.81	0.303	8 8 73	235	
T112W3.0	506	6.92	0.333	23 10 73	44	23
T113W3.0	499	9.35	0.333	23 10 73	159	

* ^{241}Am dogs missing from the sequence for "test" animals have been redesignated as "Toxicity" animals and appear earlier in Table 1.

DOG NUMBER	COMMENTS ON DEAD DOGS
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T015W5.5	SPECIAL STUDY
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T016W5.0	SPECIAL STUDY
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T032W5.5	SPECIAL STUDY
T033W5.5	SPECIAL STUDY

T056W5.0	SPECIAL STUDY
T057W5.0	SPECIAL STUDY
T099W5.0	SPECIAL STUDY
T101W5.0	SPECIAL STUDY

T102W3.0	SPECIAL STUDY
T103W3.0	
T104W3.0	
T105W3.0	
T106W3.0	

T107W5.0	MELANOMA (MOUTH)
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T108W3.0	
T109W3.0	
T110W3.0	
T111W3.0	
T112W3.0	INTUSSUSCEPTION
T113W3.0	

H. LEAD-210*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001L5.0	522	9.78	10.7	24	6	69	1497	18160
T002L5.0	522	9.16	10.7	24	6	69	28	30
T003L5.0	522	9.78	10.7	24	6	69	1100	16930

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001L5.0	OSTEOSARCOMA
T002L5.0	SPECIAL STUDY
T003L5.0	OSTEOSARCOMA

I. CALIFORNIUM-252

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001F5.0	586	11.40	2.81	8	9	71	36	453
T002F5.0	540	10.70	2.87	17	11	71	13	147

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001F5.0	SPECIAL STUDY
T002F5.0	SPECIAL STUDY

J. CALIFORNIUM-249

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/74	DEATH	
T001G5.0	597	12.20	2.84	24	2	71		500	3131
T002G5.0	584	10.70	2.77	24	2	71		7	26
T003G5.0	584	9.89	2.8	24	2	71		21	95

DOG

NUMBER

COMMENTS ON DEAD DOGS

T001G5.0 NEPHRITIS; MYOCARDIAL INFARCTION

T002G5.0 SPECIAL STUDY

T003G5.0 SPECIAL STUDY

K. CURIUM-243/244

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001C5.0	511	10.40	2.6	27	2	73	397	
T002C5.0	485	12.20	2.64	27	2	73	6	32
T003C5.0	485	11.40	2.64	27	2	73	13	69
T004C5.0	485	12.50	2.64	27	2	73	20	107
T005C5.0	485	12.80	2.63	27	2	73	384	2613
T006C5.0	498	10.70	2.9	22	10	73	87	463

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001C5.0	
T002C5.0	SPECIAL STUDY
T003C5.0	SPECIAL STUDY
T004C5.0	SPECIAL STUDY
T005C5.0	LIVER DEGENERATION
T006C5.0	SPECIAL STUDY

L. EINSTEINIUM-253

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/74	DEATH	
T001E5.0	470	9.82	2.87	5	6	73		7	33
T002E5.0	483	12.20	2.89	5	6	73		21	171
T003E5.0	483	11.00	2.84	5	6	73		55	201
T004E5.0	484	1.23	2.97	6	6	73	298		
T005E5.0	484	11.20	2.93	10	9	73		7	57

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001E5.0	SPECIAL STUDY
T002E5.0	SPECIAL STUDY
T003E5.0	SPECIAL STUDY
T004E5.0	
T005E5.0	SPECIAL STUDY

M. ANCILLARY*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED		DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO YR		
F001A						1383	
F002A						2492	
M003A						1451	
M004A						3346	
M005A						3747	
M006A						5266	
M007A						3896	
M008A						3746	
F009A						3719	
F010A						2605	
F011A						4198	
F012A						4219	
F013A						4527	
F014A						3777	
F015A						4874	
F016A						4410	
F017A						2145	
F018A						5921	
F019A						4166	
F020A						2464	
F021A						5508	
F022A						4350	
M023A						1741	
M024A						3074	
F025A						5646	
M026A						4133	
M027A						2130	
M028A						3114	
M029A						5017	
F031A						5266	
F032A						1990	
F033A						3282	
F034A						2584	
M035A						529	
M036A						1971	
M037A						4091	
F038A						3802	
M039A						4406	
M040A						4666	
F041A						4704	
M042A						1265	
F043A						3883	
F044A						5016	

DOG NUMBER	COMMENTS ON DEAD DOGS
F001A	SPECIAL STUDY
F002A	SPECIAL STUDY
M003A	SPECIAL STUDY
M004A	NOT DETERMINED
M005A	TRANSITIONAL CELL CARCINOMA, NEPHRITIS, PNEUMONIA
M006A	BRAIN HEMORRHAGE
M007A	LYMPHOSARCOMA
M008A	PROGRESSIVE PARALYSIS, CAUSE UNKNOWN
F009A	VAGINAL FIBROMA
F010A	SPECIAL STUDY
F011A	MAMMARY CARCINOMA
F012A	SEVERE OSTEOARTHRITIS
F013A	SPECIAL STUDY
F014A	OBTURATING EMBOLISM OF PORTAL VEIN
F015A	TRANSITIONAL CELL CARCINOMA URINARY BLADDER
F016A	SPECIAL STUDY
F017A	TRAUMA
F018A	NEPHRITIS
F019A	MAMMARY GLAND CARCINOMA
F020A	SPECIAL STUDY
F021A	MAMMARY CARCINOMA; THYROID CARCINOMA
F022A	LYMPHOSARCOMA
M023A	OBTURATING PULMONARY EMBOLISM
M024A	SPECIAL STUDY
F025A	ISLET CELL TUMOR; PNEUMONIA
M026A	SEMINOMA
M027A	SPECIAL STUDY
M028A	SPECIAL STUDY
M029A	MELANOMA ORAL CAVITY
F031A	UNDETERMINED
F032A	LYMPHOSARCOMA
F033A	OBTURATING PULMONARY EMBOLISM
F034A	SPECIAL STUDY
M035A	SPECIAL STUDY
M036A	SPECIAL STUDY
M037A	SPECIAL STUDY
F038A	MAMMARY CARCINOMA
M039A	OBTURATING PULMONARY THROMBO EMBOLISM
M040A	EPIDERMOID CARCINOMA (GINGIVA), PNEUMONIA
F041A	LEIOMYOSARCOMA (SPLEEN)
42A	STATUS EPILEPTICUS
43A	SPECIAL STUDY
F044A	ADRENAL CORTICAL CARCINOMA

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F045A				6182	
F047A				1732	
F048A				5075	
F049A				4773	
M050A				2264	
F051A				1089	
F052A				509	
F053A				5520	
F054A				3190	
F055A				4563	
M056A				701	
F057A				4322	
M058A				767	
M059A				567	
M061A				5511	
F062A				5348	
F063A				4530	
F068A				4521	
F070A				5227	
M071A				1472	
M073A				5695	
F074A				5553	
M075A				5284	
F076A				5137	
F077A				5122	
F078A				4856	
F079A				4359	
F080A				4711	
F081A				4711	
M082A				3627	
F083A				4398	
M084A				4398	
M085A				4668	
M086A				499	
F087A				3906	
F088A				3906	
F089A					
F090A					
F091A				3836	
F092A				3836	
M093A					
F094A					
F095A				3766	

DOG NUMBER	COMMENTS ON DEAD DOGS
F045A	PNEUMONIA; SENILITY
F047A	SPECIAL STUDY
F048A	CHRONIC PANCREATITIS
F049A	ISLET CELL TUMOR; BRAIN HEMORRHAGE
M050A	SPECIAL STUDY
F051A	SPECIAL STUDY
F052A	SPECIAL STUDY
F053A	THYROID CARCINOMA
F054A	SPECIAL STUDY
F055A	NEPHRITIS; METASTATIC CALCIFICATION
M056A	VOLVULUS + PERITONITIS
F057A	UNDIFFERENTIATED MALIGNANCY
M058A	SPECIAL STUDY
M059A	SPECIAL STUDY
M061A	FIBROSARCOMA (ABDOMEN)
F062A	AORTA THROMBO-EMBOLISM
F063A	MAMMARY CARCINOMA, TRANSITIONAL CELL CARCINOMA
F068A	UNDIFFERENTIATED CARCINOMA
F070A	
M071A	SPECIAL STUDY
M073A	DEGENERATION OF ADRENAL GLAND + DIABETES MELLITUS
F074A	LYMPHO SARCOMA
M075A	AORTIC THROMBUS
F076A	
F077A	
F078A	
F079A	PNEUMONIA
F080A	
F081A	
M082A	BRONCHIOGENIC CARCINOMA
F083A	
M084A	
M085A	
M086A	SPECIAL STUDY
F087A	
F088A	
F089A	REASSIGNED, SEE T074P40
F090A	REASSIGNED, SEE T075P40
F091A	
F092A	
93A	REASSIGNED, SEE T076P40
94A	REASSIGNED, SEE T077P30
F095A	

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/74 DEATH	DOSE TO SKELETON (RADS)
F096A				3749	
F097A				3749	
F098A				3655	
F099A					479
M100A					407
M101A					290
F102A					243
M103A					217
M104A					188
F105A					157
F106A				3350	
F107A				3259	
F108A					1969
F109A					2253
F110A					
F111A				2844	
F112A				2575	
F113A				1711	
F114A				1715	
F115A				1864	
F116A				1351	
F117A				1351	
F118A				1351	
F119A				1275	
F120A				1275	
F121A				1351	
F122A				1107	
F123A					375
F124A				1107	
F125A				1057	
F126A				1057	
F128A				1027	
F129A				1024	
F130A				638	
F131A				637	
F132A				593	
F133A				497	
F134A				497	
F135A				473	
M136A				593	
F137A				354	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F096A

F097A

F098A

F099A

SPECIAL STUDY

M100A

SPECIAL STUDY

M101A

SPECIAL STUDY

F102A

SPECIAL STUDY

M103A

SPECIAL STUDY

M104A

SPECIAL STUDY

F105A

SPECIAL STUDY

F106A

F107A

F108A

ENCEPHALOMALACIA (BACTERIAL)

F109A

METRITIS

F110A

REASSIGNED, SEE T078P30

F111A

F112A

F113A

F114A

F115A

F116A

F117A

F118A

F119A

F120A

F121A

F122A

F123A

ACCIDENTAL STRANGULATION

F124A

F125A

F126A

F128A

F129A

F130A

F131A

F132A

F133A

F134A

F135A

F136A

137A

99,881

EVALUATION OF LONG-TERM ZnDTPA THERAPY IN BEAGLES

G. N. Taylor, L. Roberts, J. L. Williams,
and Lorraine Shabestari

ABSTRACT: ZnNa_3DTPA given subcutaneously to beagles, either daily or five times daily, in daily doses of up to about 0.36 m Moles/kg (10 times the recommended daily human dosage of DTPA) has not produced any serious symptoms during daily therapy 7 days per week extending over a period of eight months. The only detectable change observed, thus far, was an occasional, slight melena, which was detectable only by chemical procedures. Clinical illness was not produced. Increased toxicity was not observed when the daily dose was divided into five equally spaced injections.

INTRODUCTION

The effectiveness of ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) therapy in the partial removal of certain types of internal radionuclide contamination is well established. However, deaths and illness have been reported in man and animals from EDTA therapy and in animals from DTPA treatment (Ahrens and Aronson, 1968; Foreman et al., 1956; Reuber and Bradley, 1960; Reuber, 1967; Schwartz et al., 1966; and Taylor et al., 1973). These agents were variously described as enterotoxins, nephrotoxins and hepatotoxins. Thus, considerable caution is exercised in their medicinal use and the recommended dose regimen in man is presently very conservative, at least in comparison to some of the animal studies (Norwood and Fuqua, 1969).

Nearly all of the untoward effects described for these chelating agents have been associated with their calcium salts administered by continuous infusion or frequent injections or high dose levels. Much of the toxicity of the calcium salts of EDTA or DTPA appears to be related to the depletion of zinc or other essential metals which are involved in DNA metabolism (Bohne, 1972; Catsch et al., 1965; Lieberman et al., 1963; Weber, 1969; Weber et al.,

1970; and Taylor and Jones, 1972). Thus, the therapeutic safety of DTPA can be significantly increased by the use of the zinc rather than the calcium salt of DTPA and without any major reduction of the chelation efficiency, at least with respect to ^{239}Pu , ^{241}Am , ^{242}Cm , and ^{253}Es (Seidel and Volf, 1972; and Smith, 1972).

It is the purpose of this report to summarize some of the clinical observations involving six dogs which have received daily injections of ZnDTPA continually for up to 8 months. These are interim results and the study will continue for an indefinite period in an effort to determine the radionuclide removal by prolonged ZnDTPA decorporation therapy and to indicate any toxicological effects, which may be produced under somewhat maximal conditions.

METHODS

All of the animals used were young adult female purebred beagles which were born and reared at this laboratory. They received approximately 0.3 $\mu\text{Ci/kg}$ of ^{241}Am citrate via a single intravenous injection. ZnDTPA therapy was begun two weeks later. The chelate was given subcutaneously according to the following schedule:

Dog No.	Body wt. (kg)	Volume of each chelate injection (cm^3)	Amount ZnDTPA per injection (m Mole)	Frequency of injections
T108W3	12.62	10	0.338*	Once daily
T109W3	9.90	10	0.338*	Once daily
T110W3	9.30	2	0.068*	Five times daily
T111W3	9.81	2	0.068*	Five times daily
T112W3	6.80	20	6.76	Five times daily
T113W3	9.45	20	0.676	Five times daily

* Approximately equivalent, on a body weight basis, to the dosage of DTPA recommended in any single day for human therapy. (1 g DTPA/70 kg = 0.036 m Mole DTPA/kg.)

All of the ZnDTPA was compounded at this laboratory by combining equimolar quantities of ZnCl_2 and DTPA, using NaOH for the source of sodium and in sufficient quantity to produce a solution with a pH of 7. The injection solutions were isotonic, except for the material used in dog number T112W3. In this instance, the solution was hypertonic because of the high concentration of DTPA and NaCl necessary to achieve a practical injection volume.

Occult blood in the urine and feces was determined by Hematest Reagent Tablets.* Urinary proteins were determined by the methods of Savory, et al.

RESULTS

The most significant lesions observed thus far, occurred in the animal receiving the highest dose (approximately 135 x the amount per kg of DTPA recommended for humans in any single day). In this instance, severe local irritation was induced in the subcutis of the dorsal thorax at the site of injection, and secondary edema in the more ventral regions became extensive. The inflammatory response appeared to be related to the hypertonicity of the injection solution, rather than to any toxicity related specifically to the ZnDTPA. This dog died after 31 days of chelate therapy, with the immediate cause of death due to an intussusception of the distal small intestine (the prolapse into the intestinal lumen of an immediately adjacent part). Tentatively, this did not appear related to the chelate treatment since the remainder of the intestinal tract was normal, and since this condition occurs occasionally in control dogs. The autopsy findings did not reveal any intestinal lesions similar to those observed with protracted CaDTPA therapy in beagles (Taylor et al., 1973).

The other five dogs, which received the isotonic ZnDTPA solutions, are

* Ames Company, Division Miles Laboratories, Inc., Elkhart, Indiana.

alive and healthy 8 months after the beginning of treatment and have not developed any observable inflammatory changes at the injection site. Subcutaneous injection of the isotonic solution did not appear to produce serious discomfort. Their urinalyses, blood urea nitrogen, blood creatinine, bromsulphalein dye retention and Van den Bergh values all remained within normal limits. Appetite was normal and no significant changes in body weight occurred. The only abnormality noted was the occurrence of a slight melena (traces of blood in feces) occurring 1 or 2 weeks after the initiation of ZnDTPA treatment and involving each of the six dogs studied. The presence of trace amounts of blood in the feces was not a daily condition and was frequently observed only once or twice a week. It was apparent only by chemical methods, and grossly visible blood in the feces was not seen.

Except for the very slight melena, which did not produce any clinical illness, ZnDTPA administered subcutaneously to beagles, in daily doses of up to 3.38 mM/day, thus far appears to be relatively non-toxic, even when the total daily dose is fractionated and given by five injections, equally spaced over the 24 hour period. The minimal toxicity associated with ZnDTPA and its comparable effectiveness as a chelating agent suggests that it should supersede CaDTPA in human decorporation therapy.

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DECORPORATION OF ^{241}Am IN BEAGLES BY DTPA

Ray D. Lloyd, Susan S. McFarland, G. N. Taylor,
J. L. Williams and C. W. Mays

ABSTRACT: Chelation treatments using daily subcutaneous injections of ZnNa_3DTPA were begun two weeks after the intravenous administration of ^{241}Am -citrate to 6 beagles. Retention of americium in the liver and in non-liver tissue (mainly skeleton) was followed serially in the living dogs by a combination of total-body and partial-body counting. During the first 7 months of DTPA therapy, roughly the same fraction of deposited ^{241}Am was removed from 2 dogs by 1 injection each day of 0.030 m moles of DTPA per kg body mass as from 2 other dogs by 0.036 m moles/kg body given in 5 injections per day. Increasing the daily amount by factors of 11 and 150 in the 2 remaining dogs only slightly increased the removal of ^{241}Am . By 2 months of chelation therapy, americium retention in non-liver tissue was about 1/2 and in liver was less than 1/10 of the respective pretreatment values, while liver ^{241}Am had correspondingly decreased to about 1/100 of pretreatment retention by 5 months of DTPA administration. Non-liver retention at 5 months averaged about 40% of pretreatment values and had decreased to 34% by 7 months of chelation therapy.

INTRODUCTION

The use of Diethylene Triamine Penta Acetic Acid (DTPA) for removal of incorporated transuranium elements in humans has been recommended for a number of years (1-7), and the calcium-trisodium salt of this chelating agent (Ca-DTPA) has been employed in human decorporation therapy with some success in radioisotope removal (2-5, 8-15). It was previously assumed (2, 3, 16) that some form of continuous administration, such as frequent injections or slow intravenous drip, would prove to be an optimum treatment schedule for the most efficient safe removal of the transuranium elements. However, subsequent studies in beagles have shown that the injection of about 0.007 m moles of Ca-DTPA/kg body mass every 5 hours was fatal at 4 to 9 days of administration to all dogs so treated (17). Single daily injections of the same total amount (about 0.035 m moles/kg each day) were found to be much less toxic, and dogs given an equal molar quantity of the zinc-trisodium salt of this

chelating agent (Zn-DTPA) on the same 5 hour schedule were found to be practically symptom-free for as long as 19 days of treatment. This much lower toxicity of Zn-DTPA as compared with Ca-DTPA has also been reported by other investigators (6, 18-20). The purposes of our present study were, first, to determine whether for a comparatively fixed body burden there is any advantage in chelation therapy of multiple injections during each day over a single daily injection of the same quantity of chelating agent, and, second, to evaluate the effects of increasing the total daily amount of DTPA upon radionuclide removal. Because of the extreme toxicity previously found for the frequent administration of Ca-DTPA in beagles, we elected to use Zn-DTPA in this investigation.

METHODS

Six young adult female beagles were each given a single intravenous injection of about 0.3 μCi ^{241}Am citrate per kg body mass. Fourteen days later, when the ^{241}Am was rather firmly deposited in liver and skeleton (21), chelation therapy was initiated on a continuing daily schedule as shown in Table 1. The total quantity of 0.027 to 0.037 m moles DTPA/kg given each day to the first 4 dogs listed in the table corresponds closely to the recommended 1 g DTPA/day for a 70 kg human = 0.036 m moles/kg/day. The daily DTPA injections of the remaining 2 animals correspond to about 150 and 11 times this recommendation, respectively.

All urine and feces which were excreted by these 6 animals were collected during the first 6 weeks after ^{241}Am injection, and 7 day collections were made on a monthly or bimonthly schedule thereafter for about an additional 16 weeks. The ^{241}Am content of each excreta sample was determined by gamma-ray counting, and the retention of ^{241}Am in the liver and in non-liver tissue

Table 1. Beagles given Zn-DTPA for removal of incorporated ^{241}Am

<u>Dog</u>	<u>m moles DTPA/kg per injection</u>	<u>No. of injections per day</u>	<u>m moles DTPA/kg per day</u>
T108W3	0.027	1	0.027
T109W3	0.034	1	0.034
T110W3	0.0073	5	0.037
T111W3	0.0069	5	0.035
T112W3	1.014	5	5.0
T113W3	0.072	5	0.36

DTPA given at 0800 hrs or 0800, 1200, 1700, 2200, and 0300 hrs.

of the living dogs was followed periodically by a combination of total-body and partial-body counting. Our methods of measuring ^{241}Am in excreta and in beagles are described in detail in reference 21.

RESULTS AND DISCUSSION

Tables 2 through 8 exhibit the results for retention and excretion measurements. It is evident from these data that roughly the same fraction of deposited ^{241}Am was removed from the 2 dogs given 0.030 m moles of DTPA/kg per day in one daily injection as from the 2 dogs given about the same total amount in 5 injections per day. Similarity of retention in liver and in non-liver tissue (mainly skeleton) among all 4 of these animals extended to the most recent measurements at nearly 7 months of chelation therapy.

Retention in both liver and non-liver tissue was less in the 2 animals given 0.36 and especially 5.0 m moles DTPA/kg each day beginning soon after the initiation of chelation treatments and extending to about 7 weeks after radionuclide injection. From about 7 weeks to 5 months after injection, however,

the liver and non-liver retention of Tl13W3, given 0.36 m moles/kg each day, resembled that of the 4 dogs which averaged 0.033 m moles/kg per day. Similar data during the same period for Tl12W3, given 5.0 m moles/kg each day, are not available, as this animal died 45 days after radionuclide injection with the immediate cause of death resulting from intussusception, an infolding of one segment of the intestine within another, resulting in necrosis, which appears unrelated to DTPA therapy despite the extremely high chelate level. Other dogs in our colony have developed this condition in the absence of DTPA treatments, and the intestine on either side of the intussusception in Tl12W3 was found to be healthy and normal upon microscopic examination, not resembling at all the intestinal syndrome observed in animals dying of Ca-DTPA toxicity (17). At death, however, the animal showed severe local inflammation at the site of DTPA injections. This subcutaneous inflammation extended over most of the thorax with gravitation of exudate into the ventral regions and forelegs.

As shown in Tables 2-7, the excretion of ^{241}Am in the first day's urine amounted to nearly $3/4$ of the total excretion during the entire first week after injection. In the second week, total excretion was much less, and the fraction accounted for by the feces was roughly $1/3$ of the total. Following the initiation of DTPA treatments at 2 weeks after injection, the fraction of total excretion represented by the fecal output of ^{241}Am increased relative to that observed within the pretreatment period. Because a substantial amount of the liver burden was being removed by the DTPA at the time when fecal excretion was highest, these data may suggest that a sizable fraction of the ^{241}Am chelated from the liver was excreted via the gut, perhaps by way of the bile duct. After most of the liver ^{241}Am had been eliminated, the fecal to total excretion ratio began to diminish to an approximately constant value

for the dogs shown in Tables 2-5 of about 0.15 from 65 to 155 days after injection or 51 to 141 days of chelation therapy. These relationships are exhibited in Figure 1 for the dogs given about 0.033 m moles/kg per day.

SUMMARY AND CONCLUSIONS

The administration of about 0.033 m moles Zn-DTPA/kg/day beginning 2 weeks after radionuclide injection reduced the ^{241}Am body burden of beagles by about the same amount regardless if the chelating agent was given in a single daily injection or in 5 injections per day. Under these conditions, therefore, there appears to be no advantage in chelation therapy of multiple over single daily injections of DTPA for the removal of a firmly deposited body burden. These treatments reduced the non-liver ^{241}Am to about 1/3 of the pretreatment retention by 203 days of chelation therapy and the liver burden to less than 1/200 of the retention determined prior to the initiation of DTPA administration. In contrast to the average retention of these 4 DTPA-treated animals at 217 days after injection in the liver and non-liver tissue of about 0.2% and 16% of the injected ^{241}Am , respectively, the corresponding retention of injected ^{241}Am by untreated beagles from our colony at the same time after injection averaged about 50% in the liver and 40% in non-liver tissue (see Figures 2 and 3 of reference 21). James and Taylor (22) concluded from their studies of DTPA treatments in ^{239}Pu -injected rats that DTPA therapy was unable to remove a significant fraction of plutonium which is deposited in bone, and that removal would be even less in adult mammals such as the dog or human in which bone remodelling rates are much smaller than in the continuously growing rat. In our studies, however, the DTPA treatments removed nearly 2/3 of the ^{241}Am which was in non-liver tissue (mainly skeleton) just before the initiation of therapy (Table 8, Figures 2 and 3).

Although the retention of ^{241}Am in dogs given 0.36 and especially 5.0 m moles Zn-DTPA/kg each day was noticeably lower during the first 5 weeks of chelation therapy than that of the animals averaging 0.033 m moles/kg each day, the retention of the animal receiving 0.36 m moles/kg per day was similar thereafter to the dogs at 0.09 this dosage. Unfortunately, the dog given 5.0 m moles/kg per day died at 31 days of treatment from causes perhaps unrelated to the experiment. We conclude that there is little advantage in chelation therapy of increasing the daily administration of Zn-DTPA to more than the recommended amount for humans of about 0.036 m moles/kg per day, which is equivalent to approximately 1 g DTPA daily in a 70 kg man. This conclusion is supported by data of other studies (2, 23).

It is of some interest to note that both dogs in this experiment which were given 5 injections per day each of 0.007 m moles/kg of Zn-DTPA, were still alive and healthy after nearly 7 months of treatment, whereas all dogs so treated with the corresponding dosage of Ca-DTPA died after 4 to 9 days.

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Figure 1. Time course of the fraction of total weekly excretion of ^{241}Am that was eliminated in the feces by 4 dogs given DTPA each day beginning 2 weeks after radionuclide injection (T108W3, T109W3, T110W3, T111W3). The first week's excretion was dominated by the ^{241}Am output in just the first day's urine, which amounted to an average of 72% of the total excretion for the entire week. During the second 7 day period, the feces accounted for roughly 1/3 of the total. Following the initiation of DTPA treatments at 2 weeks after injection, the relative fecal excretion increased as a substantial amount of the liver ^{241}Am activity began to be removed, indicating that a considerable fraction of the ^{241}Am chelated from the liver was being excreted via the gut. After most of the liver ^{241}Am had been eliminated, the fecal to total excretion ratio began to diminish to an approximately constant value of about 0.15 from 9 to 22 weeks after injection or 7 to 20 weeks of chelation therapy. (Plotted points represent weekly means for all 4 dogs, and vertical bars indicate $\pm$ one standard deviation).

Figure 2. The effect of Zn-DTPA treatments upon ^{241}Am retention in liver. For the animals at about 0.033 m moles/kg/day, the figure shows the mean (open circles) and range (vertical bars). Note that the retention in the 4 dogs given about 0.033 in moles DTPA/kg each day was similar to the 2 dogs given about 11 and 150 times as much (see Table 8).

Figure 3. The effect of Zn-DTPA treatments upon ^{241}Am retention in non-liver tissue (mainly skeleton). For the animals at about 0.033 m moles/kg/day, the figure shows the mean (open circles) and range (vertical bars). Although the retention in the 4 dogs given about 0.033 m moles/kg each day was similar to the 2 dogs given about 11 and 150 times as much (Table 8), lower values of these 2 animals from the beginning of chelation therapy until about 80 days after radio-nuclide injection may indicate a more rapid removal of available ^{241}Am from soft tissue by the higher levels of DTPA which were administered. James and Taylor (22) have proposed that chelation therapy for ^{239}Pu fixed in the skeleton can only remove radioactivity that is released from bone by the action of osteoclasts. Perhaps, in our dogs, the loss of ^{241}Am in non-liver tissue after a few weeks of chelation therapy mainly reflects the average rate of remodelling in regions of residual ^{241}Am in bone. The rate of bone remodelling slows down with normal aging, and is slower deep within the bone volume than on bone surfaces.

Table 2. Excretion and retention of injected ^{241}Am by female beagle T108W3 given a daily total of 0.027 m moles Zn-DTPA/kg in a single injection each day.

Days after injection	Excretion: % of injected Am			Retention: % of injected Am			
	Urine	Feces	Total	injection minus excretion total body	by <u>in vivo</u> counting		
					total body	liver	non liver
1	3.821	0.916	4.737	95.263	96.8	47.6	49.2
2	0.229	0.541	0.770	94.493			
3	0.232	0.230	0.462	94.031			
4	0.116	0.116	0.232	93.799			
5	1.082	0.157	1.239	92.560	90.2	41.1	49.1
6	0.168	0.100	0.268	92.292			
7	0.292	0.046	0.338	91.954			
8	0.040	0.107	0.147	91.807	90.5	42.0	48.5
9	0.114	0.040	0.154	91.653			
10	0.126	0.020	0.146	91.507			
11	0.212	0.021	0.233	91.274			
12	0.033	0.128	0.161	91.113	93.2	43.8	49.4
13	0.070	0.074	0.144	90.969			
14	0.031	0.035	0.066	90.903			
begin DTPA treatment							
15	2.994	0.364	3.358	87.545	83.7	38.7	45.0
16	3.252	1.585	4.837	82.708			
17	2.780	4.012	6.792	75.916			
18	2.216	2.707	4.923	70.993			
19	1.949	0.938	2.887	68.106			
20	1.334	1.079	2.413	65.693			
21	0.992	0.412	1.404	64.289			
22	0.306	0.782	1.088	63.201	62.8	16.5	46.3
23	1.544	1.870	3.414	59.787			
24	0.772	0.615	1.387	58.400			
25	0.782	0.279	1.061	57.339			
26	0.488	0.722	1.210	56.129			
27	0.820	0.248	1.068	55.061			
28	0.613	0.169	0.782	54.279			
29	0.338	0.122	0.460	53.819	52.2	8.5	43.7
30	0.498	0.591	1.089	52.730			
31	0.499	0.212	0.711	52.019			
32	0.418	0.278	0.696	51.323			
33	0.436	0.152	0.588	50.735			
34	0.343	0.180	0.523	50.212			
35	0.419	0.145	0.564	49.648			
36	0.345	0.104	0.449	49.199	44.2	4.2	40.0
37	0.310	0.068	0.378	48.821			
38	0.302	0.289	0.591	48.230			
39	0.312	0.143	0.455	47.775			
40	0.314	0.134	0.448	47.327			
41	0.319	0.058	0.377	46.950			

Table 2. (T108W3, Cont'd.)

<u>Days after injection.</u>	<u>Excretion: % of injected Am</u>			<u>Retention: % of injected Am</u>			
	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	injection minus excretion <u>total body</u>	by <u>in vivo</u> counting		
					<u>total body</u>	<u>liver</u>	<u>non liver</u>
42	0.276	0.116	0.392	46.558			
43	0.312	0.183	0.495	46.063	42.7	4.1	38.6
65-71	1.129	0.195	1.324		27.5	1.5	26.0
99					23.7	0.4	23.3
94-100	0.876	0.177	1.053				
127					21.2	0.1	21.1
156					19.0	0.3	18.7
149-155	0.358	0.057	0.415				
217					15.7	0.1	15.6

Table 3. Excretion and retention of injected ^{241}Am by female beagle T109W3 given a daily total of 0.034 m moles Zn-DTPA/kg in a single injection each day.

Days after injection	Excretion: % of injected Am			Retention: % of injected Am			
	Urine	Feces	Total	injection minus excretion total body	by <u>in vivo</u> counting		
					total body	liver	non liver
1	10.359	1.254	11.613	88.387	91.3	46.0	45.3
2	0.470	0.275	0.745	87.642			
3	0.332	0.042	0.374	87.268			
4	0.060	0.140	0.200	87.068			
5	0.342	0.162	0.504	86.564	86.1	40.7	45.4
6	0.070	0.029	0.099	86.465			
7	0.078	0.028	0.106	86.359			
8	0.084	0.082	0.166	86.193	86.1	40.4	45.7
9	0.076	0.007	0.083	86.110			
10	0.126	0.020	0.146	85.964			
11	0.032	0.031	0.063	85.901			
12	0.034	0.164	0.198	85.703	87.8	43.2	44.6
13	0.003	0.000	0.003	85.700			
14	0.025	0.018	0.043	85.657			
begin DTPA treatment							
15	2.310	0.000	2.310	83.347	85.1	39.4	45.7
16	4.792	3.458	8.250	75.097			
17	3.975	1.084	5.059	70.038			
18	3.756	4.521	8.277	61.761			
19	2.958	0.443	3.401	58.360			
20	2.599	1.978	4.577	53.783			
21	2.068	0.253	2.321	51.462			
22	1.556	1.065	2.621	48.841	53.6	12.8	40.8
23	0.978	1.051	2.029	46.812			
24	0.674	0.606	1.280	45.532			
25	1.057	0.473	1.530	44.002			
26	0.944	0.403	1.347	42.655			
27	0.681	0.338	1.019	41.636			
28	0.644	0.189	0.833	40.803			
29	0.510	0.118	0.628	40.175	44.5	5.6	38.9
30	0.224	0.350	0.574	39.601			
31	0.513	0.207	0.720	38.881			
32	0.522	0.106	0.628	38.253			
33	0.338	0.198	0.536	37.717			
34	0.414	0.064	0.478	37.239			
35	0.373	0.179	0.552	36.687			
36	0.368	0.076	0.444	36.243	36.2	3.2	33.0
37	0.255	0.073	0.328	35.915			
38	0.251	0.204	0.455	35.460			
39	0.317	0.066	0.383	35.077			
40	0.337	0.079	0.416	34.661			
41	0.264	0.078	0.342	34.319			

Table 3. (T109W3, Cont'd.)

<u>Days after injection</u>	<u>Excretion: % of injected Am</u>			<u>Retention: % of injected Am</u>			
	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	injection minus excretion <u>total body</u>	by <u>in vivo</u> counting		
					<u>total body</u>	<u>liver</u>	<u>non liver</u>
42	0.276	0.018	0.294	34.025			
43	0.217	0.100	0.317	33.708	35.6	2.1	33.5
65-71	1.008	0.146	1.154		25.7	0.7	25.0
99					21.4	0.2	21.2
94-100	0.674	0.090	0.764				
127					18.9	0.6	18.3
156					18.0	0.4	17.6
149-155	0.350	0.077	0.427				
217					17.4	0.1	17.3

Table 4. Excretion and retention of injected ^{241}Am by female beagle T110W3 given a daily total of 0.037 m moles Zn-DTPA/kg in 5 injections per day.

Days after injection	Excretion: % of injected Am			Retention: % of injected Am			
	Urine	Feces	Total	injection minus excretion total body	by <u>in vivo</u> counting		
					total body	liver	non liver
1	10.437	1.022	11.459	88.541	90.7	49.6	41.1
2	0.274	0.162	0.436	88.105			
3	0.328	0.300	0.628	87.477			
4	0.124	0.226	0.350	87.127			
5	0.186	0.099	0.285	86.842	87.3	46.8	40.5
6	0.109	0.051	0.160	86.682			
7	0.092	0.066	0.158	86.524			
8	0.075	0.029	0.104	86.420	87.3	47.3	40.0
9	0.145	0.050	0.195	86.225			
10	0.069	0.030	0.099	86.126			
11	0.040	0.033	0.073	86.053			
12	0.029	0.037	0.066	85.987	89.2	48.9	40.3
13	0.018	0.000	0.018	85.969			
14	0.045	0.021	0.066	85.903			
		begin DTPA treatment					
15	1.640	0.101	1.741	84.162	81.9	44.0	37.9
16	3.628	3.092	6.720	77.442			
17	3.668	4.446	8.114	69.328			
18	3.982	3.330	7.312	62.016			
19	2.165	1.033	3.198	58.818			
20	2.370	1.539	3.909	54.909			
21	0.931	1.452	2.383	52.526			
22	1.712	0.851	2.563	49.963	52.3	13.8	38.5
23	0.888	1.616	2.504	47.459			
24	0.757	0.702	1.459	46.000			
25	1.253	0.544	1.797	44.203			
26	0.629	0.872	1.501	42.702			
27	0.616	0.063	0.679	42.023			
28	0.266	0.381	0.647	41.376			
29	0.805	0.396	1.201	40.175	42.8	7.8	35.0
30	0.155	0.372	0.527	39.648			
31	0.466	0.175	0.641	39.007			
32	0.440	0.138	0.578	38.429			
33	0.284	0.042	0.326	38.103			
34	0.374	0.212	0.586	37.517			
35	0.286	0.000	0.286	37.231			
36	0.433	0.165	0.598	36.633	34.8	4.8	30.0
37	0.197	0.000	0.197	36.436			
38	0.208	0.158	0.366	36.070			
39	0.378	0.136	0.514	35.556			
40	0.162	0.014	0.176	35.380			
41	0.211	0.082	0.293	35.087			

Table 4. (T110W3, Cont'd.)

<u>Days after injection</u>	<u>Excretion: % of injected Am</u>			<u>Retention: % of injected Am</u>			
	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	injection minus excretion <u>total body</u>	<u>by in vivo counting</u>		
					<u>total body</u>	<u>liver</u>	<u>non liver</u>
42	0.055	0.102	0.157	34.930			
43	0.242	0.088	0.330	34.600	32.8	2.5	30.3
65-71	1.790	0.147	1.937		20.8	0.7	20.1
99					17.3	0.7	16.6
94-100	0.679	0.343	1.022				
127					15.4	0.1	15.3
156					13.8	0.3	13.5
149-155	0.293	0.054	0.347				
217					12.4	0.1	12.3

Table 5. Excretion and retention of injected ^{241}Am by female beagle T111W3 given a daily total of 0.035 m moles Zn-DTPA/kg in 5 injections per day.

Days after injection	Excretion: % of injected Am			Retention: % of injected Am			
	Urine	Feces	Total	injection minus excretion total body	by <u>in vivo</u> counting		
					total body	liver	non liver
1	9.894	1.108	11.002	88.998	90.7	41.8	48.9
2	0.226	0.035	0.261	88.737			
3	0.205	0.018	0.223	88.514			
4	0.167	0.099	0.266	88.248			
5	0.189	0.211	0.400	87.848	87.6	40.3	47.3
6	0.117	0.074	0.191	87.657			
7	0.091	0.052	0.143	87.514			
8	0.086	0.067	0.153	87.361	87.6	40.7	46.9
9	0.076	0.028	0.104	87.257			
10	0.073	0.019	0.092	87.165			
11	0.037	0.020	0.057	87.108			
12	0.030	0.012	0.042	87.066	88.2	41.4	46.8
13	0.018	0.000	0.018	87.048			
14	0.018	0.004	0.022	87.026			
		begin DTPA treatment					
15	1.703	0.419	2.122	84.904	87.4	39.7	47.7
16	1.273	0.392	1.665	83.239			
17	3.743	5.243	8.986	74.253			
18	1.659	0.145	1.804	72.449			
19	3.168	3.000	6.168	66.281			
20	1.060	0.573	1.633	64.648			
21	1.972	0.319	2.291	62.357			
22	1.984	0.807	2.791	59.566	60.5	18.2	42.3
23	0.724	1.993	2.717	56.849			
24	0.937	0.790	1.727	55.122			
25	1.123	0.795	1.918	53.204			
26	0.310	0.283	0.593	52.611			
27	0.086	0.169	0.255	52.356			
28	0.463	0.219	0.682	51.674			
29	0.498	0.332	0.830	50.844	52.3	10.9	41.4
30	0.634	0.620	1.254	49.590			
31	0.517	0.297	0.814	48.776			
32	0.580	0.164	0.744	48.032			
33	0.403	0.036	0.439	47.593			
34	0.412	0.197	0.609	46.984			
35	0.494	0.210	0.704	46.280			
36	0.290	0.125	0.415	45.865	44.7	6.3	38.4
37	0.276	0.000	0.276	45.589			
38	0.279	0.166	0.445	45.144			
39	0.268	0.158	0.426	44.718			
40	0.279	0.129	0.408	44.310			
41	0.323	0.088	0.411	43.899			

Table 5. (T111W3, Cont'd.)

<u>Days after injection</u>	<u>Excretion: % of injected Am</u>			<u>Retention: % of injected Am</u>			
	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	injection minus excretion <u>total body</u>	by <u>in vivo</u> counting		
					<u>total body</u>	<u>liver</u>	<u>non liver</u>
42	0.244	0.102	0.346	43.553			
43	0.282	0.114	0.396	43.157	42.4	4.5	37.9
65-71	1.221	0.208	1.429		25.6	0.7	24.9
99					23.5	0.6	22.9
94-100	0.609	0.238	0.847				
127					21.2	0.3	20.9
156					19.8	0.2	19.6
149-155	0.299	0.048	0.347				
217					17.7	0.4	17.3

Table 6. Excretion and retention of injected ^{241}Am by female beagle T112W3 given a daily total of 5.0 m moles Zn-DTPA/kg in 5 injections each day.

Days after injection	Excretion: % of injected Am			Retention: % of injected Am			
	Urine	Feces	Total	injection minus excretion total body	by <u>in vivo</u> counting		
					total body	liver	non liver
1	5.304	1.354	6.663	93.337	94.9	49.0	45.9
2	0.422	0.417	0.839	92.498			
3	0.256	0.298	0.554	91.944	89.6	43.4	46.2
4	0.184	0.136	0.320	91.624			
5	0.117	0.188	0.305	91.319			
6	0.415	0.061	0.476	90.843	94.0	45.4	48.6
7	0.112	0.082	0.194	90.649			
8	0.137	0.102	0.239	90.410			
9	0.162	0.030	0.192	90.218	89.5	43.1	46.4
10	0.761	0.056	0.817	89.401			
11	0.211	0.049	0.260	89.141			
12	0.033	0.036	0.066	89.075			
13	0.668	0.014	0.682	88.393	92.6	43.6	49.0
14	0.212	0.040	0.252	88.141			
begin DTPA treatments							
15	5.350	1.239	6.589	81.552			
16	5.136	4.518	9.655	71.897	70.0	27.9	42.1
17	4.923	3.543	8.466	63.431			
18	4.709	3.443	8.152	55.279			
19	2.486	2.458	4.944	50.335			
20	2.321	0.553	2.874	47.461			
21	1.542	1.392	2.934	44.527			
22	1.528	0.751	2.279	42.248			
23	1.146	0.690	1.836	40.412	39.8	8.2	31.6
24	1.151	0.370	1.521	38.891			
25	0.842	0.355	1.197	38.694			
26	0.862	0.286	1.148	36.546			
27	0.720	0.197	0.917	35.629			
28	0.716	0.231	0.947	34.682			
29	0.684	0.098	0.782	33.900	31.6	3.6	28.0
30	0.618	0.107	0.725	33.175			
31	0.562	0.136	0.698	32.477			
32	0.483	0.075	0.558	31.919			
33	0.368	0.062	0.430	31.489			
34	0.526	0.093	0.619	30.870			
35	0.471	0.108	0.579	30.291			
36	0.450	0.472	0.922	29.369			
37	0.299	0.072	0.371	28.998	28.9	2.8	26.1
38	0.416	0.059	0.475	28.523			
39	0.318	0.060	0.378	28.145			
40	0.313	0.038	0.351	27.794			
41	0.552	0.047	0.599	27.195			

Table 6. (T112W3, Cont'd.)

Days after injection	<u>Excretion: % of injected Am</u>			<u>Retention: % of injected Am</u>			
	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	injection minus <u>excretion</u> <u>total</u> <u>body</u>	by <u>in vivo</u> counting		
					<u>total</u> <u>body</u>	<u>liver</u>	<u>non</u> <u>liver</u>
42	0.473	0.060	0.533	26.662			
43	0.364	0.060	0.424	26.238			
44	0.284	0.046	0.330	25.908	26.5	2.1	24.4
45					25.0	1.5	23.5
					Retention by tissue counting		
					<u>total</u> <u>body</u>	<u>liver</u>	<u>non</u> <u>liver</u>
45					21.6	0.8	20.8

Table 7. Excretion and retention of injected ^{241}Am by female beagle T113W3 given a daily total of 0.36 m moles Zn-DTPA/kg in 5 injections each day.

Days after injection	Excretion: % of injected Am			Retention: % of injected Am			
	Urine	Feces	Total	injection minus excretion total body	by <u>in vivo</u> counting		
					total body	liver	non liver
1	13.205	0.778	13.983	86.017	86.7	41.0	45.7
2	0.409	0.380	0.789	85.228			
3	0.305	0.008	0.313	84.915	87.7	41.9	45.8
4	0.154	0.257	0.411	84.504			
5	0.229	0.194	0.423	84.081			
6	0.134	0.072	0.206	83.875	85.0	40.5	44.5
7	0.121	0.065	0.186	83.689			
8	0.118	0.057	0.175	83.514			
9	0.113	0.069	0.182	83.332	85.7	40.9	44.8
10	0.074	0.010	0.084	83.248			
11	0.050	0.026	0.076	83.172			
12	0.046	0.018	0.064	83.108			
13	0.045	0.022	0.067	83.041	85.1	39.6	45.5
14	0.059	0.011	0.070	82.971			
begin DTPA treatment							
15	2.866	0.698	3.564	79.407			
16	2.852	2.812	5.664	73.743	75.3	30.0	45.3
17	2.838	0.568	3.406	70.337			
18	2.823	2.490	5.313	65.024			
19	2.110	3.339	5.449	59.575			
20	2.403	2.112	4.515	55.060			
21	2.295	1.044	3.339	51.721			
22	1.976	0.011	1.987	49.734			
23	1.658	0.000	1.658	48.076	44.7	9.0	35.7
24	0.815	1.929	2.744	45.332			
25	0.917	0.363	1.280	44.052			
26	1.011	0.650	1.661	42.391			
27	0.773	0.633	1.406	40.985			
28	0.686	0.236	0.922	40.063			
29	0.482	0.153	0.635	39.428	36.3	4.7	31.6
30	0.815	0.274	1.089	38.339			
31	0.246	0.093	0.339	38.000			
32	0.736	0.154	0.890	37.110			
33	0.245	0.181	0.426	36.684			
34	0.412	0.097	0.509	36.175			
35	0.516	0.180	0.696	35.479			
36	0.181	0.062	0.243	35.236			
37	0.601	0.095	0.696	34.540	33.3	2.2	31.1
38	0.161	0.061	0.222	34.318			
39	0.277	0.046	0.323	33.995			
40	0.350	0.115	0.465	33.530			
41	0.291	0.024	0.315	33.215			

Table 7. (T113W3, Cont'd.)

<u>Days after injection</u>	<u>Excretion: % of injected Am</u>			<u>Retention: % of injected Am</u>			
	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	injection minus excretion <u>total body</u>	<u>by in vivo counting</u>		
					<u>total body</u>	<u>liver</u>	<u>non liver</u>
42	0.156	0.023	0.179	33.036			
43	0.309	0.063	0.372	32.664			
44	0.412	0.028	0.440	32.224	31.6	2.6	29.0
73-79 80	0.995	0.134	1.129		24.3	0.9	23.4
101-107	0.581	0.083	0.664		21.9	0.0	21.9
129-135	0.596	0.102	0.698				
141					20.1	0.3	19.8

Table 8. Percent of pretreatment retention in 6 beagles given daily DTPA therapy (as determined by total-body and partial-body counting in vivo).

days after injection	T108W3		T109W3		T110W3		T111W3		T112W3		T113W3	
	non liver	liver	non liver	liver	non liver	liver	non liver	liver	non liver	liver	non liver	liver
1	99.6	108.7	101.6	106.5	102.0	101.4	104.5	101.0	93.7	112.4	100.4	103.5
3									94.3	99.5	100.7	105.8
5	99.4	93.8	101.8	94.2	100.5	95.7	101.1	97.3				
6									99.2	104.1	97.8	102.3
8	98.2	95.9	102.5	93.5	99.2	96.7	100.2	98.3				
9									94.7	98.9	98.5	103.3
12	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0				
13									100.0	100.0	100.0	100.0
15					begin DTPA therapy							
16	91.1	88.4	102.5	91.2	98.5	90.0	101.9	95.9				
22	93.7	37.7	91.5	29.6	95.6	28.2	90.4	44.0	85.9	64.0	99.6	75.8
23									64.5	18.8	78.5	22.7
29	88.5	19.4	87.2	13.0	86.8	16.0	88.5	26.3	57.1	8.3	69.5	11.9
36	81.0	9.6	74.0	7.4	74.4	9.8	82.1	15.2				
37									53.3	6.4	68.4	5.6
43	78.1	9.4	75.1	4.9	75.2	5.1	81.0	10.9				
44									49.8	4.8	63.7	6.6
71	52.6	3.4	56.1	1.6	49.9	1.4	53.2	1.7				
80											51.4	2.3
99	47.2	0.9	47.5	0.5	41.2	1.4	48.9	1.5				
107											48.1	0.0
127	42.7	0.2	41.0	1.4	38.0	0.2	44.7	0.7				
141											43.5	0.8
156	37.8	0.7	39.5	0.9	33.5	0.6	41.9	0.5				
217	31.6	0.2	38.8	0.2	30.5	0.2	37.0	1.0				

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ALTERNATE METHODS FOR ADMINISTERING CHELATING AGENTS IN DECORPORATION THERAPY

W. Stevens, D. R. Atherton, B. J. Grube, and F. W. Bruenger

INTRODUCTION

During the last year studies on the removal of americium and other actinides from beagles were begun. Since this is a relatively new field of research for the laboratory, a number of experiments were started to study the effect of some alterations of the molecular structure of the chelating agents and the effect of the mode of administration by using physical vehicles to transport the chelating agents to specific sites in the body. This is a preliminary report on the results of these experiments. Chelating agents were of the polyaminocarboxylic acid (PACA) type except for Desferrioxamine B.*

METHODS

Male Sprague-Dawley rats of 240-260 g weight were injected intraperitoneally with approximately 1 μ Ci of ^{241}Am in citrate buffer of pH $\approx$ 3.5. ^{241}Am was chosen because its 60 keV γ -radiation made it possible to measure the whole body retention as a function of time. It is recognized that this nuclide

* The following abbreviations are used:

DTPA = diethylenetriamine pentaacetic acid

EGTA = ethylenebis glycol(α -aminoethylether) tetraacetic acid

EDDA = ethylenediaminediacetic acid

HEDTA = N-hydroxy ethylenediaminetriacetic acid

DFOA = Desferal (methanesulfonate of desferrioxamine B)

(valence III and ionic radius $0.98 \text{ \AA}$) will probably yield results somewhat different from those obtained with ^{239}Pu (valence IV and ionic radius $0.9 \text{ \AA}$). Animals were counted on the first day after injection and the body burden at that time was set equal to unity. All rats were counted twice a week. Animals received treatment on 12 and 20 days after injection. Aqueous solutions of chelators were given intraperitoneally as the Zn-complexes. Microencapsulated chelators (liposomes) were prepared from the Zn-chelates either according to a method given by Batzri⁽¹⁾ and given via the saphenous vein or they were prepared by a method developed in this laboratory and given intraperitoneally or orally. In the latter case, the chelators were microencapsulated (under a nitrogen pressure of 3-4 pounds/in²) by spray injecting 20 ml of a 25% solution of the Zn-chelate into 10 ml of chloroform containing 20 mg of lecithin and 4 mg of dicetylphosphate. The spray injection was done at 75°C . The resulting suspension was stirred for 1 minute and then sonicated until all the CHCl_3 had evaporated. Liposomes were separated by centrifugation for 15 min at $48,000 \times g$, washed with 5 ml of physiological saline, again separated by centrifugation and rehomogenized in saline.

Esters of the aminocarboxylic acids were prepared by the reaction of crystalline acid with absolute EtOH in a stream of dry $\text{HCl}_{(\text{g})}$ at 75°C . After evaporation of excess EtOH, the ester was extracted with $(\text{Et})_2\text{O}$. This procedure produced an ethyl ester of EDTA and DTPA in good yield, but yields of EGTA-esters were consistently low.

Stable emulsions of esters were prepared by mixing the ester (1 part) with Crisco* oil (5 parts) and suspending this mixture in 15 parts of an aqueous solution of Triton WR 1339* (10 mg/ml). Emulsification was

* Proctor and Gamble (Crisco Oil) and Ruger Chemical Co., Inc. (Triton)

accomplished by sonication in an ice bath. This oil emulsion of the PACA-esters was given orally.

Aqueous solutions of chelating agents were given in equimolar quantities (0.038 mmol). Exact amounts of Zn-chelators encapsulated in liposomes or as oil emulsions are not easily prepared but, as shown elsewhere,⁽²⁾ varying the concentration of the agent by a factor or more than two orders of magnitude had little or no effect on the total quantity of ^{241}Am removed from beagles. Differences in quantities given caused by the preparation were therefore disregarded.

RESULTS

Results of the present study were summarized in Table 1 and Fig. 1. In Group A, four different modes of administering the chelating agent are compared. Zn-DTPA was given (13.3 mg DTPA per rat/administration) either as (1) an aqueous solution, ZnDTPA(aq), (2) as microencapsulated material prepared according to the method of Batzri (liposome 1), (3) as DTPA ethyl ester in an oil emulsion, DTPA(em), or (4) in the combination (1 + 2) or (1 + 3). When given as a single preparation, Zn-DTPA(aq) was the most effective. When the same total amount of Zn-DTPA(aq) was given in combination with either the microencapsulated material or the oil emulsion, both combinations were more effective than the aqueous material alone while the combination of Zn-DTPA(aq) with DTPA(em) was the most effective. The effectiveness of the latter was quite pronounced in the decorporation from the skeleton as shown by the % of injected americium (% D) retained by femori, humeri and lumbar vertebrae.

In Group B, four modes of administration of EGTA are compared, (1) Zn-EDTA(aq) in an amount equimolar to the dose of DTPA used, (2) microencapsulated ZnEGTA prepared by the method developed in this laboratory and given orally

Fig. 1
Comparison between treated rats and controls (dashed line)

Fig. 1 (continued)
Comparison between treated rats and controls (dashed line).

Fig. 2
Comparison between treated rats and controls (dashed line)

Fig. 2 (continued)
Comparison between treated rats and controls (dashed line)

Fig. 3
Comparison between treated rats and controls (dashed line)

TABLE I
Summary of Decorporation Studies in the Rat
(Average of 3 Rats)

Treatment	ΔT (days)	% D* Retained ($\pm$ S.D.)	% D Liver	% D Kidney	% D Femuri Humeri 2 Lumbar Vertebrae
A CONTROL	29	51.4 $\pm$ 10.4	8.1	1.6	7.17
Zn-DTPA(aq)		33.5 $\pm$ 2.3	0.57	0.97	5.53
Zn-DTPA-Lipos. I**		37.6 $\pm$ 1.0	1.13	1.73	6.03
Zn-DTPA + Zn-DTPA- Lipos. I		32.0 $\pm$ 2.9	0.53	1.1	5.50
DTPA-ester-emulsion		37.0 $\pm$ 4.2	1.77	1.2	5.90
Zn-DTPA + DTPA-ester emulsion		28.3 $\pm$ 4.0	0.55	0.65	4.55
B Zn-EGTA(aq)	28	41.2 $\pm$ 0.7	2.0	1.10	5.04
Zn-EGTA-Lipos. II***		50.8 $\pm$ 0.5	3.6	1.16	6.11
Zn-EGTA + EGTA-ester- Lipos. II		39.8 $\pm$ 3.3	1.73	0.93	7.26
EGTA-ester-emulsion		48.2 $\pm$ 0.3	1.05	1.46	6.35
Zn-EGTA + Egta-ester emulsion		33.4	1.0	1.86	4.1
C Zn-EDDA(aq)	35	49.6 $\pm$ 1.3	3.93	1.03	6.4
Zn-HEDTA(aq)		35.9 $\pm$ 1.6	0.87	0.92	4.23
DFOA(aq)		42.0 $\pm$ 4.3	1.53	1.02	6.06
Zn-DTPA + DFOA(aq)		25.2 $\pm$ 3.4	0.18	0.51	4.05

* D = Injected dose of ^{241}Am .

** Lipos. I = Liposomes prepared according to the method of Batzry and given intravenously.

*** Lipos. II = Liposomes prepared by spray injection and given orally.

(liposome II), (3) EGTA(em) and (4) as a combination of (1 + 2) or (1 + 3). EGTA was less effective in the removal of ^{241}Am than DTPA. Again the combination of two different preparations of EGTA was more effective than Zn-EGTA alone and the combination of Zn-EGTA(aq) and the oil emulsion, EGTA(em), yielded the highest degree of decorporation in this series.

In Group C, the effectiveness of EDDA, HEDTA and one other chelating agent which does not belong to the class of polyaminocarboxylic acids was tested. This was Desferrioxamine B. All of these agents were given as an aqueous solution. Although the individual agents were less effective than DTPA, the combination of DTPA and DFOA was the most effective treatment tested in these three groups.

DISCUSSION

The effectiveness of DTPA (given as the Zn-chelate because of its very low toxicity)⁽³⁾ has been known for a long time and was again demonstrated by this laboratory. DTPA, however, is rapidly excreted from the body and the entrance of DTPA into cells is limited (in the liver it is approximately 2%)⁽⁴⁾ A more lipophilic DTPA-preparation given in the form of microencapsulated material or as an oil emulsion should enhance the intracellular effectiveness in organs like liver, spleen, lung, etc..

Administration of microencapsulated chelators has recently been explored,⁽⁵⁻⁸⁾ an improvement in the preparation of the lipid-vehicle, however, seems desirable. Both techniques, the method of Batzry and our own, provide a simple method for microencapsulation of chelates contained in an aqueous phase. However, some problem exists due to slight aggregation of particles following preparation by spray injection.

Ethyl esters of polyaminocarboxylic acids were first tested successfully

by Markley.⁽⁹⁾ At that time, the esters were given by injection in aqueous solutions and appeared quite toxic. Oil emulsions of those ethyl esters seem to have a lower toxicity and can be given either orally or, by adding a greater percentage of water to the emulsion, they can be given intravenously. If given intravenously, these oil emulsions are partially deposited in the lung. Thus, this type of administration may later prove to become the treatment of choice after inhalation exposure. Experiments to prepare different esters with other, less toxic, (fatty) alcohol groups are presently underway.

The differences in the diminished effectiveness of the other agents when compared to DTPA may reflect the weaker ligand field, the spatial arrangement of ligands and the smaller number of available ligands which these compounds possess, i.e., DTPA-8, EDDA-4, HEDTA-5 (or 6), EDTA-6. DFOA, of course, does not belong to the polyaminocarboxylic acids, but it is a hydroxamic acid derivative. It was interesting to see that in spite of the low effectiveness of DFOA alone, the combination of Zn-DTPA and DFOA was the most effective treatment tested with respect to both liver and skeletal retention.

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DECORPORATION OF ^{241}Am FROM BONE USING DTPA AND OSTEOPOROTIC AGENTS

Darrell R. Fisher, David H. Taysum, Charles W. Mays, Donald Kimmel,
Douglas Brammer, Thomas Brammer, and Susan S. McFarland

ABSTRACT: Total-body retention of ^{241}Am was evaluated sequentially in thirty C57 black mice (male and female) each injected intraperitoneally with 0.08 μCi ^{241}Am in citrate solution at 132 days of age. The objective was to determine whether decorporation by the zinc trisodium chelate of diethylenetriaminepentaacetic acid (Zn-DTPA) could be enhanced by osteoporotic agents.

In the first experiment, 3-24 days after ^{241}Am administration, the daily subcutaneous injections of heparin (1600 U.S.P. units/kg/day) plus ZnDTPA (0.036 m mole/kg/day) had no detectable effect on decorporation compared to the action of physiological saline plus ZnDTPA.

In a second experiment continuing with mice previously treated with saline plus ZnDTPA in the first experiment, the daily subcutaneous injections of parathyroid extract (400 U.S.P. units/kg/day) plus ZnDTPA during 24-45 days after ^{241}Am administration removed about 11% more ^{241}Am than was removed by saline plus ZnDTPA during this period.

INTRODUCTION

At present, the most effective treatment for increasing the removal of systemically distributed transuranium elements from the body is the prolonged administration of the chelating agent diethylenetriaminepentaacetic acid (DTPA). The DTPA forms a stable chelate complex with the unbound heavy metal atoms, which is then excreted by the host.

Prolonged ZnDTPA therapy begun 14 days after i.v. injection of ^{241}Am citrate and continuing daily for seven months was effective in removing 99% of the ^{241}Am deposited in the liver and about 60% of the nuclide deposited in the skeleton of beagles, ⁽¹⁾ the two major deposition sites of monomeric ^{241}Am . ⁽²⁾ Herring et al. ⁽³⁾ showed that ^{241}Am concentrated much more on resorbing bone surfaces than on actively calcifying surfaces. The ability of ^{241}Am to deposit on endosteal surfaces where its short-range α -particles irradiate sensitive osteogenic cells makes it highly carcinogenic. Thus, it would be of

much value to remove a greater portion of the radionuclide from bone than is currently possible with DTPA treatment alone. The idea occurred to us that the bone surface could possibly be "etched" by agents which induce osteoporosis to free the nuclide for chelation by DTPA.

Several agents have been reported to motivate osteoporosis. Among these is heparin, which is derived from animal tissue and standardized for use as an anticoagulant. Osteoporosis has been reported in man following long-term administration of more than 10,000 U.S.P. units of heparin daily,^(4,5) and also in experimental rats.⁽⁶⁾ However, a contradictory report has been published⁽⁷⁾ and the exact effects of heparin remain unresolved. It has been suggested that heparin stimulates the parathyroid gland, and stimulates the production and activity of collagenase enzyme.⁽⁶⁾

Parathyroid extract (PTE) is prepared from beef parathyroid glands, and is used clinically in the treatment of hypoparathyroidism. It is generally agreed that the parathyroid hormone has three main physiological actions: (a) inhibition of tubular reabsorption of phosphate, (b) stimulation of bone resorption, and (c) facilitation of intestinal absorption of calcium.⁽⁸⁾

In two separate experiments, we injected heparin and parathyroid extract into mice in an attempt to increase the effectiveness of ZnDTPA in the skeletal decorporation of ^{241}Am .

MATERIALS AND METHODS

Experiment 1: Heparin Treatment

Thirty C57 black mice 132 days old grown on water and Purina Lab Chow were distributed six mice per cage into five plastic cages (Table 1). Each mouse (average weight 25 g) was given an intraperitoneal injection of about 0.08 μCi ^{241}Am (ICN Pharmaceuticals) and 0.01 μCi ^{85}Sr in sodium citrate

solution buffered to pH 3.5. Americium was chosen for this study because of its increasing production in nuclear power reactors. ^{241}Am emits a 60 keV γ -ray (36% abundance) and is a possible model for other actinides. Strontium-85 was included as an index of bone mineral volume being removed during treatment.

Three days after injection, to conform with previous work done by Rosenthal and Lindenbaum,⁽⁹⁾ decorporation treatment was begun. Mice in cages 1 and 2 (odd number = male mice) each received daily 0.2 ml of the zinc tri-sodium chelate of diethylenetriaminepentaacetic acid (0.036 m mole/kg/day), and 0.2 ml (1600 U.S.P. units/kg/day) sodium heparin (Panheprin-Abbott) by subcutaneous injection. The ZnDTPA dose is equivalent to the recommended 1 g DTPA/day treatment for a 70 kg human. Mice in cages 3 and 4 were given daily injections of 0.2 ml ZnDTPA and 0.2 ml isotonic saline. Cage 7 was left untreated as a test control (Table 1).

Table 1. Daily Treatment Schedule

<u>Days 3-18 (Expt. 1)</u>			<u>Days 24-45 (Expt. 2)</u>		
<u>Cage No.</u>	<u>Sex</u>	<u>Treatment</u>	<u>Cage No.</u>	<u>Sex</u>	<u>Treatment</u>
1	Male	Heparin, ZnDTPA	3A	Male	PTE, ZnDTPA
2	Female	Heparin, ZnDTPA	4A	Female	PTE, ZnDTPA
3	Male	Saline, ZnDTPA	3B	Male	Saline, ZnDTPA
4	Female	Saline, ZnDTPA	4B	Female	Saline, ZnDTPA
7	Male	No treatment			

Radioactivity was measured by total-body γ -ray counting of each mouse positioned between two 8-inch by 4-inch NaI(Tl) scintillation crystals inside a low-background iron room. Total-body counts were made on days 3, 5, 7, 10,

and 17 to measure the percent biological retention. As an additional check, the cages and excreta were also counted.

Experiment 2: Parathyroid extract treatment

On day 24, cages 3 and 4 (saline and ZnDTPA in experiment 1) were divided into cages 3A, 3B, 4A, and 4B of three mice each. The mice in the A cages received daily subcutaneous injections of 0.1 ml (400 U.S.P. units/kg/day) parathyroid extract (Lilly) and 0.2 ml ZnDTPA. Those in the B cages received daily injections of 0.1 ml saline and 0.2 ml ZnDTPA (Table 1). Total-body retention was measured on days 24, 31, 38, and 45. The animals were sacrificed after the final count and prepared for autoradiography and histology studies.

RESULTS AND DISCUSSION

Experiment 1: Heparin plus ZnDTPA

The decorporation of ^{241}Am by ZnDTPA was not significantly enhanced by simultaneous heparin treatment. Heparin plus ZnDTPA therapy removed 74.44% (average in male and female mice) of the ^{241}Am present at the start of ZnDTPA therapy, three days after injection of ^{241}Am (Table 2).

Table 2. Average percent ^{241}Am retention, and percent of day 3 retention excreted by day 17.

<u>Treatment</u>	<u>Sex</u>	<u>Cage</u>	<u>Percent retention</u>					<u>% of day 3 burden excreted</u>	<u>Av.</u>
			<u>day 3</u>	<u>day 5</u>	<u>day 7</u>	<u>day 10</u>	<u>day 17</u>		
Heparin, ZnDTPA	M	1	76.66	37.65	26.43	22.36	19.06	75.14	74.44
Heparin, ZnDTPA	F	2	55.45	36.80	25.80	18.27	14.56	73.74	
ZnDTPA	M	3	78.23	41.33	27.71	22.86	19.90	74.56	73.98
ZnDTPA	F	4	69.40	44.46	29.15	22.75	18.46	73.40	
Untreated	M	7	76.18	74.32	73.34	71.70	67.48	11.42	

The percentage decorporation of ^{241}Am by ZnDTPA alone averaged 73.98%. (An analysis of individual mice revealed the same trends as cage averages with little variability. Therefore, only the cage averages are presented.) If a heparin effect on decorporation exists, it appears insignificant relative to the decorporation by ZnDTPA alone (Fig. 1).

The continual loss of strontium was nearly equivalent in each group throughout the first experiment (Table 3). Apparently neither the heparin nor DTPA therapy affected the relative amount of bone resorption.

Table 3. Average percent ^{85}Sr retention, and percent of day 3 retention excreted by day 17.

Treatment	Sex	Cage	Percent retention.					% of day 3 burden excreted	Av.
			day 3	day 5	day 7	day 10	day 17		
Heparin, ZnDTPA	M	1	28.78	24.53	22.43	21.08	19.10	33.64	32.78
Heparin, ZnDTPA	F	2	24.63	21.88	20.43	18.65	16.77	31.91	
ZnDTPA	M	3	32.56	28.73	25.81	23.75	21.43	34.18	34.12
ZnDTPA	F	4	29.50	25.83	24.55	21.91	19.45	34.07	
Untreated	M	7	34.96	30.76	27.86	25.98	23.34	33.24	

In mice treated with heparin, some external bleeding was noticed as well as subcutaneous "blisters" filled with blood near the site of heparin injection. Eight of twelve mice injected daily with heparin died. Autopsy showed the probable cause to be enteritis, a severe intestinal inflammation. The infection could have been unrelated to the treatment. If it was associated with the treatment, infection might have been caused by bacteria in the heparin solution, or by interaction between heparin and the ZnDTPA solution. However, the heparin and ZnDTPA injections were made separately on opposite sides of

Figure 1. Average total-body retention of injected ^{241}Am in male and female mice (Expt. 1). Pre-therapy retention was by coincidence initially lower in heparinized mice. The heparin plus DTPA slope is equivalent to the saline plus DTPA slope, indicating equivalent therapeutic effects.

the animals. Correction was made for mice which died by comparing their previous radionuclide retention to patterns established by living mice.

In evaluating retention data, the question arises as to why the heparin therapy failed to initiate an increase in ^{241}Am decorporation. Osteoporosis may result from either a decrease in new bone formation or a process which increases bone destruction, since bone is both formed and destroyed during normal metabolism. Thompson⁽⁶⁾ reported that heparin decreased new bone formation with an unknown effect upon resorption. Our results show that heparin had no significant effect on ^{241}Am or ^{85}Sr decorporation during the therapy period.

Experiment 2: PTE plus ZnDTPA

Mice from cages 3 and 4 were used again in a second experiment to examine the possible effects of parathyroid extract used with ZnDTPA treatment in decorporation.

After twenty-one days of therapy with PTE and ZnDTPA, an average 18.44% of the day 24 ^{241}Am burden was removed, as compared with 16.57% nuclide removed from mice injected with ZnDTPA only (Table 4).

Table 4. Average percent ^{241}Am retention, and percent of day 24 retention excreted by day 45.

<u>Treatment</u>	<u>Sex</u>	<u>Cage</u>	<u>Percent retention</u>				<u>% of day 24 burden excreted</u>	<u>Av.</u>
			<u>day 24</u>	<u>day 31</u>	<u>day 38</u>	<u>day 45</u>		
PTE, ZnDTPA	M	3A	18.28	17.16	15.97	15.30	16.30	18.44
PTE, ZnDTPA	F	4A	17.65	16.41	15.22	14.02	20.57	
ZnDTPA	M	3B	19.26	18.12	17.22	16.66	13.50	16.57
ZnDTPA	F	4B	17.92	16.40	15.52	14.40	19.64	

The difference was greatest among male mice where 16.30% ^{241}Am was removed by PTE plus ZnDTPA treatment, and 13.50% by ZnDTPA alone. The observed effect (although not greatly pronounced) of PTE to increase ^{241}Am decorporation by ZnDTPA prompts further investigation.

The removal of ^{85}Sr from the skeleton was greater in PTE-treated mice than in non PTE-treated mice, although the difference here again was not large (Table 5). Presumably some ^{85}Sr involved in active hormone-induced bone resorption was made available for excretion. Bingham et al.⁽¹⁰⁾ confirmed

Table 5. Average percent ^{85}Sr retention, and percent of day 24 retention excreted by day 45.

Treatment	Sex	Cage	Percent retention				% of day 24 burden excreted	Av.
			day 24	day 31	day 38	day 45		
PTE, ZnDTPA	M	3A	18.75	16.90	15.52	14.06	25.02	26.18
PTE, ZnDTPA	F	4A	15.18	13.43	12.88	11.03	27.34	
ZnDTPA	M	3B	19.94	18.52	17.29	15.61	21.72	22.95
ZnDTPA	F	4B	20.71	10.03	17.29	15.70	24.19	

earlier studies that PTE increased osteoclastic activity, lessened collagen synthesis by osteoblasts, and stimulated the activity of the osteocytes. We suggest that early ZnDTPA plus PTE therapy might enhance ^{241}Am decorporation. Autoradiography and histological studies on mouse femurs (in progress at this printing) may indicate the regions in the bone where ^{241}Am remained.

An ideal method of increasing decorporation of bone-seeking radionuclides would be one which gently dissolved the bone surface without seriously injuring other body functions in the host. Further investigation of parathyroid extract and other osteoporotic agents seems desirable.

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THE EFFECTS OF PHYTOHEMAGGLUTININ-P (PHA-P) ON BONE CELLS OF THE TIBIAL AND FEMORAL METAPHYSIS OF THE RATS

T.M. Wang and W.S.S. Jee

ABSTRACT: In animals (i.e. rats or mice, etc.) treated with PHA, past observations such as blast cell transformation and increased DNA synthesis have been confirmed. However, no work has been done concerning PHA effects on bone and bone cells. The current studies found the labeling index of osteoprogenitor cells was increased by higher doses (25 and 50 mg/kg/day) of PHA. In addition, the number of osteoblasts was decreased at all doses, and the number of osteoclasts was increased at all doses.

INTRODUCTION

The Phytohemagglutinin (PHA) is of special interest to us because of the possibility that it may effect the bone and bone cells and provide insight into the development of new endpoints of radiation damage in the growing skeleton. PHA is extracted from the Red-kidney beans of the genus Phaseolus, is mucoprotein in nature, and has a molecular weight 140,000.⁽¹⁻⁷⁾ It is known to be a powerful mitogenic agent in lymphocyte tissue culture and causes a great deal of blast cell transformation and increased DNA synthesis.⁽⁸⁻¹⁴⁾

The purpose of this work is to define dose-response relationships of the PHA upon bone quantity and bone cells, with ultimate goal of relating its findings.

MATERIALS AND METHODS

Male Sprague-Dawley rats initially weighing 100 grams were used (Hormone Assay, Chicago, Illinois) in this study. The animals were housed in plastic cages in a 75°F animal room on a 12 hour light-dark cycle, and

maintained on a normal diet of laboratory chow (Wayne Lab. Blox, Allied Mills Inc., Chicago, Illinois).

The PHA was supplied in the desicated powdered form (PHA-P, Difco Laboratories, Detroit, Michigan) in which 5 ml sterilized normal saline were added just prior to injection of the animals.

The purpose and protocol of experiment was as follows:

EXPERIMENT:

This experiment was designed to determine the effects of the PHA on osteoprogenitor cell DNA synthetic activity, endochondral growth and number of functional bone cells as well as the quantity of hard tissue (metaphyseal bone).

Fifteen rats were given daily subcutaneous injections of 0, 25 and 50 mg/kg/day for 7 days of PHA. The animals were weighed daily and the dosage given in a volume of 0.15 ml/100 g animal weight.

Three days prior to sacrifice the rats were given 15 mg tetracycline (Acromycin, Lederle) per kg body weight intravenously. One hour prior to sacrifice, the animals were given 1 μ Ci tritiated thymidine (3 HTdR) (methyl- 3 H, ICN, SA=14.1 Ci/mM) per gram body weight subcutaneously.

At autopsy proximal tibias and distal femurs were collected. Tibias from each animal were fixed in 3% phosphate-buffered formalin, decalcified in 14% EDTA, and embedded in a modified methyl methacrylate.^(15,16) Consistent 3 micron thick sections of both frontal and cross sections of the proximal tibia were cut on the Jung microtome. Histological sections

were stained with hematoxylin and eosin (H&E). Sections for autoradiography were coated with NTB liquid emulsion (Eastman Kodak, Rochester, New York), and allowed to expose for 3 weeks in a dry, cold atmosphere. The autoradiographs were developed in Kodak D-19 for 4 minutes, fixed in Kodak fixer for 10 minutes, and rinsed in tap water (27°C) for 2 hours. They were then stained with H&E.⁽¹⁷⁾ The femur was defatted and dehydrated in a series of acetone and ether changes. It was embedded in bioplastic and sawed in 250 μ thick sections which were then ground to 100 μ . The 100 μ sections were microradiographed at 12 kV and 30 mA for 2 minutes. Following microradiography of the 100 micron ground sections of the distal femoral metaphysis they were further ground to 50 microns and mounted on glass slides for ultraviolet microscopy (tetracycline analysis).

Labeling Index of Osteoprogenitor Cells. The percentage of ³HTdR-labeled nuclei was determined collectively for the mesenchymal cells, in an 0.29 mm² area of the proximal tibial metaphyseal primary spongiosa. This type of cell comprises the osteoprogenitor cell pool in this area.

Mesenchymal cells have inconspicuous cytoplasm and generally dark staining, oval or fusiform nuclei. A labeled nucleus was one having at least three silver grains directly over it.

Quantity of Hard Tissue (metaphyseal bone). The percent bone in the microradiographs in a 6 mm² area of the distal femoral metaphysis was determined using the quantitative television microscope (QTM, Metal Research, Cambridge, England), image-analyzing computer.

Endochondral Growth. The distance from the resting zone of the growth cartilage to the center of the tetracycline label was measured on the 50 micron ground sections, using the ultraviolet microscope to determine longitudinal bone growth.

Number of Functional Bone Cells. The number of osteoblasts, osteoclasts, and bone surface intersections on a horizontal grid were counted in five 0.94 mm^2 areas of the metaphysis. Bone surface perimeter was estimated by applying the following formula: $\text{Perimeter} = \pi/2 \times H \times D$ where H = number of intersections with bone, D = distance between the lines on the grid, and $\pi/2$ is a shape correction factor.

RESULTS

The labeling index of the osteoprogenitor cells in the tibial primary spongiosa increased in the 25 and 50 mg/kg/day of PHA when compared to controls. (Table I)

The percent metaphyseal area occupied by hard tissue was significantly decreased in all doses of PHA when compared to controls and each other. (Table II)

The labeling index of the proliferating chondroblasts and the width of the growth plate was slightly decreased in the rats receiving 25 and 50 mg/kg/day of PHA. (Table III) Longitudinal bone growth was similarly decreased at these doses. (Table IV)

There were fewer osteoblasts present in 25 and 50 mg/kg/day doses of PHA. Conversely, there were more osteoclasts in all dose levels of PHA.

The total number of osteoblasts was decreased in both 25 and 50 mg/kg/day doses of PHA which was only significant in the treatment of 50 mg/kg/day of PHA. The number of osteoblasts/mm bone surface was significantly decreased in both 25 and 50 mg/kg/day doses of PHA when compared to controls, while the total number of osteoclasts and the number of osteoclasts/mm bone surface were increased in all dose levels of PHA but only the number of osteoclasts/mm bone surface was significantly increased in both dose levels of PHA. (Table V)

DISCUSSION

Much of the previously published work with PHA has been done in vitro or in vivo in lymphoid tissues and hematopoietic tissues. (8,11,12,13,14) However no work has been done concerning PHA effects on bone and bone cells.

PHA when injected into growing rats, the findings in this study are:

- (1) Increased the labeling index of osteoprogenitor cells in the primary spongiosa of the proximal tibial metaphysis.
- (2) Increased the total number of osteoclasts and the number of osteoclasts per mm of bone surface in the tibial metaphysis.
- (3) Decreased the total number of osteoblasts and the number of osteoblasts per mm of bone surface in the tibial metaphysis.
- (4) Decreased the percent trabecular bone in the tibial metaphysis.
- (5) Decreased the labeling index of chondroblasts in the growth plate.
- (6) Decreased the width of the growth plate.
- (7) Decreased the endochondral growth.

The decrease in longitudinal bone growth of the tibia might be explained by depressed proliferation of chondroblasts in the growth plate.

The decrease in the number of osteoblasts/mm bone surface and the total number of osteoblasts certainly can not be explained on the basis of labeling index, which was increased at all doses where the number of osteoblasts was decreased.

The three explanations which seem possible are:

(1) An effect inhibiting the differentiation of cells from osteoprogenitor cells to osteoblasts, or (2) a diminution of osteoblast lifespan, or (3) a combination of both.

The increase in the number of osteoclasts/mm bone surface and the total number of osteoclasts in the proximal tibia can be explained in that there is most likely an increase in the rate of differentiation from osteoprogenitor cells to osteoclasts in this site.

The decrease in bone volume of the tibial metaphysis can be explained that there is a decrease in the number of osteoblasts/mm bone surface and the total number of osteoblasts and an increase in the number of osteoclasts/mm bone surface and the total number of osteoclasts and decreased the endochondral growth.

Further studies into the mechanism by which PHA produces these changes are continuing.

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Table I. Labeling Index of Osteoprogenitor
Cells of the Tibial Primary Spongiosa

PHA TREATMENT	CONTROL ±S.E.	PHA 25mg/kg/day ±S.E.	PHA 50mg/kg/day ±S.E.
7 DAYS	10.17% ±0.37	13.14%*** ±0.35	13.22%*** ±0.15

p<0.005

Table II. Effects of PHA on Percent Bone
of The Proximal Tibial Metaphysis

PHA TREATMENT	CONTROL ±S.E.	PHA 25mg/kg/day ±S.E.	PHA 50mg/kg/day ±S.E.
7 DAYS	13.90% ±0.40	11.97%* ±0.42	10.29%*** ±0.37

* p <0.05; *** p<0.005

Table III. Labeling Index and Width of the
Growth Plate of the Tibia

Dose mg PHA per kg per day	Labeling index of growth plate of tibia ± S.E.	Width of growth plate of tibia ±S.E.
CONTROL	12.15% ±0.51	4.91μ ±0.01
25mg/kg/day	10.18%* ±0.40	4.44μ** ±0.05
50mg/kg/day	10.08%* ±0.34	4.29μ** ±0.16

*p<0.05; **p<0.01 ***p<0.005

Table IV. Effects of PHA on the Endochondral Growth

PHA TREATMENT	CONTROL ±S.E.	PHA 25mg/kg/day ±S.E.	PHA 50mg/kg/day ±S.E.
7 DAYS	957±11μ	901±6μ ^{**}	880±25μ ^{**}

^{**}
p<0.01

Table V. Bone Cell Population Analysis

Dose mg PHA per kg per day	Total Osteoblasts ±S.E.	Total Osteoclasts ±S.E.	Osteoblasts per mm bone surface ±S.E.	Osteoclasts per mm bone surface ±S.E.
CONTROL	138.20 ±13.98	21.62 ±1.55	11.56 ±0.57	1.48 ±0.08
25mg/kg/day	107.20 ±6.29	27.62 ±2.51	7.70 ^{**} ±0.65	1.95 [*] ±0.10
50mg/kg/day	87.00 ^{**} ±5.86	26.62 ±2.30	7.41 ^{***} ±0.32	1.87 [*] ±0.15

^{*}
p<0.05;

^{**}
p<0.01;

^{***}
p<0.005

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PLUTONIUM, DIPHOSPHONATES, AND BONE

W. S. S. Jee, D. B. Kimmel, S. C. Miller, J. A. Yee,
L. A. Woodbury, and Rebecca Dell

ABSTRACT: Ethane-1-hydroxy-1, 1-diphosphonate (EHDP) inhibited the uptake of Pu-239 at the bone surface, when it was given for as little as five days before injection of Pu-239. In evaluating its other effects, it was deduced that EHDP reduced the rate of bone resorption. It was also shown that dichloromethylene diphosphonate (Cl₂MDP) was five to ten times more effective in inhibiting bone resorption than EHDP.

Administration of EHDP increased dramatically the rate of osteoclast nuclei production while also increasing somewhat the rate of osteoclast nuclei attrition. Studies on osteoblasts indicated that EHDP inhibited the differentiation of osteoblasts by inhibiting DNA synthesis in osteoprogenitor cells from which osteoblast nuclei are derived.

This story has two participants. One is plutonium (Pu-239), one of the most studied elements, radioactive or otherwise, in history. The in vivo distribution of Pu-239 citrate injected intravenously is approximately 50% to the liver and 50% to the skeleton. It is a bone surface seeker (Arnold and Jee, 1957).

The second is the diphosphonates. These are simple chemical compounds containing phosphorus-carbon-phosphorus bonds. Interest in them dates back to the 1930's when it was demonstrated that low concentrations of condensed phosphates (P-O-P-O-P units) (Fig. 1) were able to prevent the precipitation of calcium carbonate from solution. Condensed phosphate is used in many household products as a water softener (Russell and Smith, 1973). The diphosphonates have these same properties with the additional property of being resistant to all known naturally-occurring enzymes. (Russell and Smith, 1973).

The two diphosphonates which have been best studied are disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP) and disodium dichloromethylene diphosphonate

where n is equal to or greater than 1.

Figure 1. This is the chemical structure of condensed phosphate, where N is a large number.

Figure 2. These are the chemical structures of two diphosphonates, EHDP and Cl₂MDP. Note the substitution in the main portion of the compound of "P-C-P" for "P-O-P".

(Cl₂MDP) (Fig. 2). Most of their physico-chemical-biologic effects are probably related to the strong affinity which the diphosphonates have for calcium ions and for amorphous calcium phosphate crystals. Diphosphonates also bind strongly on the surface of crystals of hydroxyapatite, progressively displacing orthophosphate from the crystal surface as more diphosphonate becomes available in relation to the amount of orthophosphate (Jung, Bisaz, and Fleisch, 1973).

In general, diphosphonates can prevent various types of experimentally-induced soft tissue calcification such as aortic and kidney calcification (Fleisch et al., 1970), calcium deposition in skin (Casey et al., 1972), peri-articular calcification (Francis et al., 1972), and bladder calculi (Fraser et al., 1972). Higher doses of EHDP inhibit the calcification of bone leading to the appearance of excess osteoid tissue in bone (Fleisch, Bisaz, et al., 1970), (Jowsey et al., 1970), (King et al., 1971), (Russell et al., 1973). Many studies show that diphosphonates can inhibit bone resorption both in vitro (Reynolds et al., 1973), (Fleisch et al., 1969), and in vivo (Michael et al., 1971), (Muhlbauer et al., 1971).

EHDP is absorbed in the gastro-intestinal tract, to an extent which varies in different species -- from one to ten percent in rats, rabbits, monkeys and man, but more in dogs. Approximately half of the absorbed compound is deposited in bone, while the rest is excreted in the urine. It, like Pu-239, is primarily a bone surface seeker (Fig. 3), a result of its affinity for crystals of hydroxyapatite and amorphous calcium phosphate. Only minimal amounts go to soft tissues. There is no significant breakdown in the body (Michael, King, et al., 1972).

There are several clinical situations in which EHDP has shown good results. It has been used quite successfully in cases of ectopic bone tissue

Figure 3. This detailed autoradiograph shows the distribution of ^3H -EHDP in rabbit cortical bone 24 hours after injection. Note the burial beneath osteoid (O) on the endosteal (E) surface and surface deposits on the periosteum (P) (500X).

formation, as in myositis ossificans progressiva (Bassett et al., 1969) (Geho, 1973), myositis ossificans after paraplegia, and calcinosis after dermatomyositis (Cram, 1971). It has also been used in cases of Paget's disease of bone (Smith, 1973).

There remains a great deal of work to be done in investigating the diphosphonates. They are only partly successful in the treatment of ectopic mineralization. Their marked effects when used in Paget's disease are produced by a dose (20 mg/kg/day) which is too high for routine long-term use. Further animal and clinical experimental work will probably elucidate the mode of action and proper clinical therapeutic regimen for the use of the diphosphonates.

The striking thing to the radiobiologist is that there are two characters with a similar affinity for the bone surface. The question is asked in this laboratory, "is it possible to load the bone surface with EHDP to subsequently reduce the possible sites for the uptake of Pu-239 in bone?"

1. UPTAKE OF PLUTONIUM IN RATS PRE-TREATED WITH EHDP

A. Use of 5 or 25 days pre-treatment.

In an initial experiment, 300-gram male Sprague-Dawley rats were treated for 5 or 25 days with 0.4 or 10 mg EHDP/kg/day. After the respective periods of pre-treatment they were injected with 3.34 μ Ci Pu-239/kg and autopsied three days later.

Table 1 summarizes the data from that experiment. There was no difference from controls in the uptake of Pu-239 in the rats pretreated for either 5 or 25 days with only 0.4 mg EHDP/kg/day. However, there was a marked suppression (17% and 30%) in the uptake of Pu-239 in the tibiae of rats pre-treated

with 10 mg EHDP/kg/day for 5 and 25 days (Fig. 4) (Table 1).

Autoradiographic analysis of the distal femurs showed grossly diminished uptake of Pu-239 at 10 mg EHDP/kg/day for both pre-treatment periods (Fig. 5). After 25 days, nearly all bone surfaces showed decreased uptake of Pu-239, while after 5 days, only the epiphyseal surfaces showed less uptake. The percent of epiphyseal bone surfaces which are not covered with plutonium was surveyed using linear intercept sampling procedures. In this limited study, there was five times as much surface free of plutonium in rats treated with 10 mg EHDP/kg/day for 25 days as there was in plutonium-injected control animals. Furthermore, in animals treated for 5 days with either 0.4 or 10 mg EHDP/kg/day, there was twice as much epiphyseal surface free of plutonium when compared to the plutonium-injected controls (Fig. 6).

B. Use of 150 to 180 days Pre-treatment.

A study is in progress involving the daily injection of 300-gram rats with 0.4, 2.0, 4.0, or 10.0 mg EHDP/kg/day for 150 days. After this time, one group was given 2.0 μ Ci of Pu-239/kg and sacrificed three days later. The other group received 2.0 μ Ci Pu-239/kg and was sacrificed 30 days later during which time EHDP treatment continued. The only portions of this study completed to date are the microradiography and contact autoradiography.

Figure 7 compares the uptake of Pu-239 in the bones of rats allowed to go only three days post-injection. Note again the diminished uptake, most notably in the epiphysis as compared to the epiphysis of the control (a). Note also, the diminution of uptake of Pu-239 at surfaces throughout the entire bone in animals treated with 4.0 (d), and 10.0 (e) mg EHDP/kg/day. Figure 8 is a series of representative microradiographs of the proximal tibiae from the control and four dose levels of diphosphonates. The control contained

Table 1. Plutonium-239 uptake in rat tibiae

<u>EHDP Dose (mg/kg/day)</u>	<u>Period of Treat- ment (days)</u>	<u>Ash Wt. (g) ± S.E.</u>	<u>%-Diff From Controls</u>	<u>Counts per min. per gram ash ± Sample S.E.</u>	<u>%-Diff From Controls</u>
0	25	0.2887 ± 0.0171	--	181.9 ± 12.2	--
0.4	5	0.2906 ± 0.0114	--	182.2 ± 8.3	--
0.4	25	0.3056 ± 0.0049	--	187.3 ± 11.8	--
10.0	5	0.2953 ± 0.0189	--	149.7 ± 15.9	-17%
10.0	25	0.2412 ± 0.0217	-17%	124.9 ± 10.9	-30%

Table 11. Effect of EHDP (10 & 30 days) upon growth plate parameters

<u>EHDP mg/kg/Day</u>	<u>Endochondral Growth/Day (μ±S.E.)</u>	<u>Growth Plate Thick. (μ±S.E.)</u>	<u>Hypertrophic Cell Size (μ±S.E.)</u>	<u>Chondrocyte Label. Index (%±S.E.)</u>	<u>Calc. Rate⁺ Cell Prod./Day (±S.E.)</u>
<u>10 Days</u>					
Control	219±6	448±6	35.5±1.0	15.8±0.3	6.2±0.2
0.2	221±8	475±14	35.0±0.4	15.5±0.3	6.4±0.3
0.4	226±3	473±6	34.7±0.6	15.4±0.2	6.4±0.1
2.0	197±8	444±26	34.4±1.4	15.2±0.1	6.0±0.3
10.0	184±5***	452±16	30.7±0.8**	13.4±0.7	5.8±0.1
<u>30 Days</u>					
Control	149±4	331±10	28.7±0.8	13.7±0.4	5.2±0.2
0.2	148±5	369±10	28.8±0.2	12.4±0.5	5.1±0.2
0.4	147±11	367±20	28.0±0.9	13.3±0.4**	5.2±0.4
2.0	141±7***	357±11***	27.8±0.7***	12.2±0.4**	5.2±0.3**
10.0	43±14	581±54	20.3±1.1	12.0±0.5	3.4±0.7

* p<0.05; ** p<0.025; *** p<0.005

⁺ Calculated Rate of Cell Production/Day (Thorngren and Hansson, 1972)

Figure 4. These contact autoradiographs show the three-day uptake of Pu-239 in the distal femoral metaphysis of 300 gram rats pretreated with 0 mg EHDP/kg/day for 25 days (a), 10 mg EHDP/kg/day for 5 days (b), or 10 mg EHDP/kg/day for 25 days (c). Note the reduced uptake after five days pretreatment in the epiphysis and the generalized decrease after 25 days pretreatment (X 8).

Figure 5. These nuclear emulsion autoradiographs of seven microns thick sections of the proximal tibial epiphysis show the marked localized reduction in Pu-239 uptake. The autoradiographs are from controls (a), rats treated with 10 mg EHDP/kg/day for 5 days (b), or rats treated with 10 mg EHDP/kg/day for 25 days.

Figure 6. After 25 days of pre-treatment with 10 mg EHDP/kg/day, more than 50% of the surfaces in the proximal tibial epiphysis were not covered with Pu-239.

16% (a), the 0.4 mg EHDP/kg/day group 30% (b), and the 2.0 mg EHDP/kg/day 41% (c) metaphyseal hard tissue after 150 days of treatment. The 4.0 and 10.0 (d and e) mg EHDP/kg/day dose levels contained large amounts of osteoid with widened epiphyseal plates, while the 2.0 mg EHDP/kg/day group contained smaller, but significant amounts of osteoid on various bone surfaces.

Figure 9 compares the autoradiographs of bones from animals treated with four different dosages of EHDP for 150 days prior to and 30 days after injection of Pu-239. Note again, the decrease in deposition with increasing dose, particularly in the epiphysis.

In Figure 10, the microradiographs of these animals are presented. Again, the increase in trabecular bone in the proximal tibial metaphysis with diphosphate treatment is evident. The microradiograph of the control (a) probably demonstrates the reaction of an animal to the deposition of toxic amounts of Pu-239 at the bone surface ... rapid bone resorption of the affected bone. Once again, the two highest doses (d and e) resulted in widened epiphyseal plates with a condition of rickets.

Comparison of the microradiographs and autoradiographs shows that there is a dichotomy to the use of diphosphonates in preventing the uptake of Pu-239. In this particular animal, the 300 gram rat, treatment with 0.4 and 2.0 mg EHDP/kg/day drastically increased the amount of bone surface in the proximal tibial metaphysis. At the same time, there was a decrease in the amount of Pu-239 uptake per unit surface. These contributing factors could not have been accounted for in ashing-counting data alone (which may have shown no change), but required a histologic detailed-autoradiographic analysis. In this way, the effectiveness of the diphosphonates at the bone surface is truly shown. It is suspected that the character of trabecular bone in higher animals is such that the administration of the diphosphonates would result

Figure 7. These contact autoradiographs show the pattern of Pu-239 uptake in the proximal tibial metaphysis of 300 gram rats pre-treated with 0 (a), 0.4 (b), 2.0 (c), 4.0 (d), or 10.0 (e) mg EHDP/kg/day for 150 days, in which the Pu-239 was allowed to remain for 3 days. Note once again the deposition pattern in the epiphysis, less with increasing dose. Note also the generalized decreased uptake after pre-treatment with 4 (d) or 10 (e) mg EHDP/kg/day (X 4).

Figure 8. These microradiographs are from the proximal tibial metaphysis of 300 gram rats treated with 0 (a), 0.4 (b), 2.0 (c), 4.0 (d), or 10.0 (e) mg EHDP/kg/day. Note the greatly increased bone mass after 0.4 (b) or 2.0 (c) mg EHDP/kg/day. Note also, the greatly widened growth plates in animals receiving 4 and 10 mg EHDP/kg/day (X 8).

Figure 9. These contact autoradiographs are from 300 gram rats which were injected for 180 days with (left to right) 0 (a), 0.4 (b), 2.0 (c), 4.0 (d), or 10.0 (e) mg EHDP/kg/day, in which Pu-239 was allowed to remain 30 days after injection. Note decreased uptake in the epiphysis and generalized lighter uptake in animals receiving over 4 (d and e) mg EHDP/kg/day (X 4).

Figure 10. These microradiographs are from 300 gram rats treated with 0 (a), 0.4 (b), 2.0 (c), 4.0 (d), or 10.0 (e) mg EHDP/kg/day (left to right, top to bottom) for 180 days. The drastic reduction in the bone mass in the control is probably due to bone resorption in response to Pu-239 after 30 days exposure. Note again the greater bone mass with 0.4 (b) or 2.0 (c) mg EHDP/kg/day and the widened growth plates with 4 (d) or 10 (e) mg EHDP/kg/day for this length of time (X 8).

in a greater mass of bone without a significant increase in the amount of bone surface. Thus, not only would EHDP be useful in inhibiting Pu-239 uptake at individual bone surfaces, but also would be effective in inhibiting the total Pu-239 uptake in an area of spongy bone, a critical area in tumor production.

With 4.0 and 10.0 mg EHDP/kg/day, for 150-180 days, there is partially diminished uptake of Pu-239. This is not as ideal as it might sound, because there are large amounts of osteoid present on nearly every bone surface. While it could be conceived that a thick layer of protection (between the cell and the alpha-emitting nuclide) would be beneficial, it is more likely that this sort of skeleton is not functional from the aspects of fracture repair, bone modeling, and bone remodeling.

In summary, if the bone surface is loaded with proper amounts of EHDP, bone mass of a skeleton is increased, while the uptake of Pu-239 upon the bone surface is decreased.

II. DOSE RESPONSE-TIME STUDIES OF EHDP

The above EHDP pre-treatment resulting in the diminution of Pu-239 uptake also produced some most interesting bone tissue responses. These changes involved both the hard tissues and the cells of bone, which prompted us to conduct dose-time response studies for 10 or 30 days with 0.2, 0.4, 2.0, or 10 mg EHDP/kg/day in 100 gram Sprague-Dawley rats.

In general, the factors analyzed can be broken down as follows:

- I. Rate of Endochondral Growth
- II. Growth Cartilage
 - Chondrocyte labeling index
 - Size of Degenerative Cell lacunae
 - Thickness of growth plate

- III. Bone Formation (^3H -Proline)
 - Percent epiphyseal trabeculae labeling
 - Burial in epiphyseal trabeculae
 - Perichondrial plate burial
 - Endosteal diaphysis burial
- IV. Bone Cells
 - Total metaphyseal osteoblasts
 - Total metaphyseal osteoclasts
 - Average nuclei/osteoclast
 - Osteoblasts/mm hard tissue
 - Osteoclasts/mm hard tissue
- V. Amount of Metaphyseal Hard Tissues
 - Percent Bone
 - Percent Cartilage Cores

For a short period (10 days) the amount of hard tissues (M) in the proximal tibial metaphysis of the normal growing rat is constant. There is an input of new hard tissues from formation (F) and a loss of hard tissues from resorption (R), the processes occurring simultaneously in this area, a total process known as bone modeling. A good approximation, thus, is that F is approximately equal to R. Since there is no good direct way to measure the rate of bone resorption only, an indirect attempt may be made to deduce the rate of bone resorption in this region. It is also possible that in some situations the osteoclast population size and characteristics may serve as an index of resorption.

Tables II to V summarize the dose response studies to EHDP for 10 and 30 days. Table II lists the effects upon the growth plate. After 10 days, 10 mg/kg/day depresses the hypertrophic cells' size and labeling index of the chondrocytes, thus, resulting in a significant depression in endochondral growth rate. After 30 days, all growth plate parameters were decreased at 10 mg/kg/day, while only the labeling index of chondrocytes was depressed at 2.0 mg/kg/day (Figs. 11 and 12).

Table III lists the effects of various dose levels upon four different

Table III. Effect of EHDP (10 & 30 days) upon bone apposition (³H-proline studies)

<u>EHDP mg/kg/Day</u>	<u>Labeled Bone Surfaces (%±S.E.)</u>	<u>Epi. Trab. Burial (μ±S.E.)</u>	<u>Supra-Epi. Bone Burial (μ±S.E.)</u>	<u>Periosteal Shaft Burial (μ±S.E.)</u>
<u>10 Days</u>				
Control	50.3±1.8	2.4±0.1	2.2±0.2	6.2±0.2
0.2	51.0±1.8	2.5±0.3	2.1±0.2	6.3±0.2
0.4	45.4±2.9	2.4±0.2	2.0±0.1	6.3±0.2
2.0	43.0±1.7***	1.7±0.1***	1.3±0.1***	5.9±0.1
10.0	34.0±3.0***	1.5±0.2***	1.2±0.1	5.0±0.3***
<u>30 Days</u>				
Control	51.5±2.5	1.8±0.1	1.6±0.1	5.2±0.1
0.2	51.9±2.2	1.5±0.1***	1.5±0.1	5.0±0.2
0.4	46.9±3.4	1.6±0.1*	1.7±0.1	5.1±0.2
2.0	39.6±4.1*	1.5±0.1**	1.7±0.2	4.6±0.1***
10.0	38.0±4.8*	1.7±0.1	1.6±0.1	4.2±0.2***

* p<0.05; ** p<0.025; *** p<0.005

Table IV. Effect of EHDP (10 & 30 days) upon bone cell population-1

<u>EHDP mg/kg/Day</u>	<u>Labeling Index Osteoprogenitor Cells (%±S.E.)</u>	<u>Total Osteoblasts (±S.E.)</u>	<u>Osteoblasts/mm Bone Surface (±S.E.)</u>
<u>10 Days</u>			
Control	15.9±0.4	305±7	5.9±0.2
0.2	15.7±0.4	312±22	5.7±0.5
0.4	16.2±0.4	309±5	6.2±0.2
2.0	15.3±0.9	281±13	5.0±0.4
10.0	12.9±0.6***	175±17***	2.5±0.3
<u>30 Days</u>			
Control	10.4±0.5	234±8	6.1±0.5
0.2	9.9±0.4	238±15	6.2±0.3
0.4	9.9±0.3	226±18	5.9±0.7
2.0	9.8±0.4	185±10**	3.7±0.2***
10.0	6.6±0.5***	135±14***	1.7±0.1***

* p<0.05; ** p<0.025; *** p<0.005

Table V. Effect of EHDP (10 & 30 days) upon bone cell population-11

<u>EHDP mg/kg/Day</u>	<u>Total Osteoclasts ($\pm$S.E.)</u>	<u>Osteoclasts/mm Bone Surface ($\pm$S.E.)</u>	<u>Average Number of Nuclei/Osteoclasts ($\pm$S.E.)</u>
<u>10 Days</u>			
Control	62 $\pm$ 2	1.2 $\pm$ 0.1	4.1 $\pm$ -.1
0.2	77 $\pm$ 4*	1.5 $\pm$ 0.1*	5.2 $\pm$ 0.2***
0.4	82 $\pm$ 5***	1.7 $\pm$ 0.2*	5.3 $\pm$ 0.3***
2.0	101 $\pm$ 9***	1.8 $\pm$ 0.2**	5.5 $\pm$ 0.4***
10.0	128 $\pm$ 6***	1.8 $\pm$ 0.2***	5.8 $\pm$ 0.1***
<u>30 Days</u>			
Control	70 $\pm$ 2	1.8 $\pm$ 0.2	1.8 $\pm$ 0.2
0.2	84 $\pm$ 4**	2.2 $\pm$ 0.1	2.2 $\pm$ 0.1
0.4	85 $\pm$ 7**	2.1 $\pm$ 0.2	2.1 $\pm$ 0.2
2.0	120 $\pm$ 3***	2.4 $\pm$ 0.1**	2.4 $\pm$ 0.1**
10.0	85 $\pm$ 5**	1.1 $\pm$ 0.1**	1.1 $\pm$ 0.1**
* p<0.05; ** p<0.025; *** p<0.005			

Table VI. Effect of EHDP (10 & 30 days) upon metaphyseal area occupied by mineralized tissues

<u>EHDP mg/kg/Day</u>	<u>Hard Tissues ($\pm$S.E.)</u>	<u>Bony Tissue ($\pm$S.E.)</u>	<u>Calcified Cart. Core ($\pm$S.E.)</u>
<u>10 Days</u>			
Control	24.5 $\pm$ 0.6	16.7 $\pm$ 1.2	5.5 $\pm$ 0.1
0.2	24.5 $\pm$ 0.8	16.5 $\pm$ 0.9	5.4 $\pm$ 1.1
0.4	26.7 $\pm$ 0.8	15.4 $\pm$ 0.7	4.9 $\pm$ 0.5
2.0	29.0 $\pm$ 0.9***	16.6 $\pm$ 1.2	5.7 $\pm$ 0.6
10.0	34.7 $\pm$ 1.1***	22.2 $\pm$ 0.5***	7.5 $\pm$ 0.2***
<u>30 Days</u>			
Control	19.8 $\pm$ 1.2	13.9 $\pm$ 1.0	4.5 $\pm$ 0.3
0.2	23.6 $\pm$ 0.8*	13.8 $\pm$ 0.8	4.4 $\pm$ 0.2
0.4	26.4 $\pm$ 1.5***	13.5 $\pm$ 1.3	4.3 $\pm$ 0.6
2.0	27.5 $\pm$ 1.5***	16.5 $\pm$ 0.7	6.2 $\pm$ 0.3***
10.0	39.6 $\pm$ 1.2***	32.8 $\pm$ 1.2***	14.6 $\pm$ 0.9***
* p<0.05; ** p<0.025; *** p<0.005

Figure 11. Normal histology of the zone of cell degeneration of the growth cartilage of a growing rat is shown (180 X).

Figure 12. Appearance of same zone in rat treated with 20 mg EHDP/kg/day for 10 days is shown. Note the smaller size of the lacunae in this region when compared to the control. Note, also, the increased height of the growth cartilage (180X).

bone forming sites following 10 and 30 days treatment. The epiphyseal trabeculae proved to be the most sensitive sampling site. In general, though, the rate of bone apposition is most significantly depressed by 2.0 and 10.0 mg EHDP/kg/day for both 10 and 30 days (Figs. 13 and 14).

Tables IV and V list the effects upon the metaphyseal bone cell populations, the osteoblasts, osteoclasts, and osteoprogenitor cells. The labeling index of osteoprogenitor cells located within 220μ of the zone of cell hypertrophy of the growth cartilage was determined (Figs. 15 and 16). All other cell numbers were counted in sixty-four $220 \times 220\mu$ areas located within a 2mm x 3mm area of the metaphysis of the proximal tibia, just distal to the growth cartilage. Table IV demonstrates that the effects of EHDP on osteoblasts are cumulative and depressive, 2.0 mg EHDP/kg/day having no effect after 10 days, but a depressive effect after 30 days (Figs. 13 and 14). The effects of EHDP are constant over this time period in relation to the osteoclast parameters (Table V). It is suggested that the effect of EHDP that increases the number of osteoclasts and osteoclast nuclei is a transient phenomenon (Figs. 17 and 18).

Lastly, Table VI demonstrates the dose response-time effect of EHDP upon the hard tissues content of the proximal tibial metaphysis. The most sensitive parameter is the percent metaphyseal hard tissue in the 2 x 3 mm area of the proximal tibial metaphysis measured from microradiographs by the image-analyzing microscope. It demonstrates again that treatment with the diphosphonates is cumulative, a lower dose eventually creating more hard tissues. After 10 days increase in the percent of hard tissues was observed only as low as 2.0 mg/kg/day. However, after 30 days, the increase in the percent of hard tissues occurred as low as 2.0 mg EHDP/kg/day dose level.

Figure 13. The depth of burial of ^3H -Proline in the perichondrial ring of bone is shown in a control animal. Note the lining of osteoblasts (white arrow) on the bone surface (1800 X).

Figure 14. The depth of burial of ^3H -Proline in the perichondrial ring of bone is shown in an animal treated for 10 days with 10 mg EHDP/kg/day. Note the flattened lining of osteoblasts (black arrow) at the bone surface (1800 X).

Fig. 15. The large number of labeled osteoprogenitor cells in the proximal tibial metaphysis in a control animal is shown (2000 X).

Fig. 16. The number of labeled osteoprogenitor cells in the proximal tibial metaphysis in an animal treated with 20 mg EHDP/kg/day for 10 days is shown. There is only one well-labeled cell (arrow) (2000 X).

Fig. 17. A single osteoclast with four nuclei, found in the proximal tibia metaphysis is shown (2000 X).

Fig. 18. A single osteoclast containing over 40 nuclei is seen in an animal treated for 10 days with 20 mg EHDP/kg/day (2000 X).

The results of the time-dose response to EHDP of the proximal tibia can be summarized as follows:

Cumulative Effects of EHDP

- increased percent hard tissues
- decreased bone formation
- decreased osteoblast numbers

Non-cumulative Effects of EHDP

- endochondral growth rate
- osteoprogenitor cell labeling index
- osteoclast numbers

After 10 days of a high (10 mg EHDP/kg/day) dose, there is a decrease in bone formation ($F\downarrow$), but an increase in hard tissues ($M\uparrow$). The increase is, by inference, from the suppression in bone resorption ($R\downarrow\downarrow$), by an amount greater than the depression of formation. After 30 days of this same dose, there is a more drastic reduction in bone formation ($F\downarrow\downarrow$) coupled with a more extensive increase in the amount of hard tissues ($M\uparrow\uparrow$). This is again, attributed to a decrease in the rate of resorption ($R\downarrow\downarrow\downarrow$). The situation which prevailed with 10 mg EHDP/kg/day after 10 days, prevails with 2 mg EHDP/kg/day after 30 days. Perhaps most significant is the case occurring with a very low dose of EHDP. After ten days, there was no effect (NE) on the rate of bone formation (F-NE) or in the quantity of hard tissues (M-NE), implying no change in the rate of bone resorption (R-NE). However, after thirty days, continued treatment resulted in a situation of (F-NE) with ($M\uparrow$), implying that resorption was impeded ($R\downarrow$). One can conclude that there was a decrease in the rate of bone resorption even with this very low dose of EHDP; one knows, also, for a fact that there was no effect on the rate of bone formation with this dose. Clearly the effects of EHDP on hard tissue are cumulative. There seems to be a dose of EHDP which will eventually result in an increase in bone mass solely by decreasing the rate of bone resorption, having no effect

Table VII. Temporal response to EHDP

Differences in 10 and 30 Days Treatment:

1. No change in magnitude of response at 10-30 days:
 Endochondral growth rate
 Labeling index, osteoprogenitor cells
 Osteoclast numbers
2. Same changes at 30 days with "lower dose of" EHDP as at 10 days:
 Increase percent hard tissues
 Decrease bone formation
 Decrease osteoblast numbers

At 30 days - more hard tissues with "lower dose of" EHDP

<u>10 Days</u>	<u>30 Days with "lower dose of" EHDP</u>
Formation (NE) F	Formation (NE) F
Resorption (↓↓) R	Resorption (↓↓) R
Hard tissues (↑) M	Hard tissues (↑) M
<u>10 Days (0.2 mg EHDP)</u>	<u>30 Days (0.2 mg EHDP)</u>
Formation (NE) F	Formation (NE) F
Resorption (NE) R	Resorption (↓) R
Hard tissues (NE) M	Hard tissues (↑) M

↑ increased rate
↓ decreased rate
NE no drug effect

on the rate of bone formation, a most desirable situation. Table VII summarizes this situation.

III. STRUCTURAL ACTIVITY RELATIONSHIP OF TWO DIPHOSPHONATES

The fruitful approach to the possible clinical use of the diphosphonates is to develop a compound without any side effects. Currently, Procter and Gamble has two diphosphonates, EHDP and Cl_2MDP , which have been tested extensively. In the past, it has been shown that high doses of EHDP inhibit the mineralization of bone, while the same relative doses of Cl_2MDP do not (Schenk et al., 1973).

The present experiment studied the same endpoints listed under part II in this report, using 3 equivalent doses of EHDP and Cl_2MDP , evaluated on a mg phosphorus level. They were 0.1, or 5.0 mg P of either compound/kg/day, which is 0.4, 4.0, or 20 mg EHDP, as compared to 0.47, 4.68, or 23.4 mg Cl_2MDP /kg/day.

Tables VIII through XII summarize the effects upon the growth plate parameters, bone formation rates, and bone cell populations.

Table VIII shows that the response of the growth cartilage parameters and endochondral growth was quite similar, except at the doses of EHDP which caused mineralization defects in calcifying cartilage and bone. This mineralization effect is probably reflected in the reduced chondrocyte labeling index and hypertrophic zone lacunar size for EHDP. The fact that Cl_2MDP has no mineralization effect at these doses means that there is only a drug toxicity effect to the cells by Cl_2MDP . Table IX shows that Cl_2MDP was more effective in depressing bone formation in the epiphyseal trabeculae, while at other sites, the results were variable.

Table X shows that Cl_2MDP has less effect on the labeling index of

Table VIII. Effects of diphosphonates upon growth plate parameters

Dose mg/kg/Day	Endochondral Growth/Day ($\mu\pm$ S.E.)	Growth Plate Thick. ($\mu\pm$ S.E.)	Hypertrophic Cell Size ($\mu\pm$ S.E.)	Chondrocyte Label. Index ($\%\pm$ S.E.)	Calc. Rate ⁺ Cell Prod./Day ($\pm$ S.E.)
Control	236 $\pm$ 5	503 $\pm$ 6	35.8 $\pm$ 0.5	15.4 $\pm$ 0.4	6.5 $\pm$ 0.1
<u>EHDP</u>					
0.4	238 $\pm$ 9	490 $\pm$ 7	34.8 $\pm$ 0.5	14.8 $\pm$ 0.9	6.9 $\pm$ 0.3
4.0	211 $\pm$ 4***	516 $\pm$ 7	33.7 $\pm$ 0.6**	13.6 $\pm$ 1.8	6.3 $\pm$ 0.1
20.0	0***	855 $\pm$ 30***	25.1 $\pm$ 1.1***	11.1 $\pm$ 0.8***	---
<u>Cl₂MDP</u>					
0.47	239 $\pm$ 10	507 $\pm$ 8	34.9 $\pm$ 0.5	15.1 $\pm$ 1.0	6.6 $\pm$ 0.4
4.68	215 $\pm$ 6**	508 $\pm$ 7	34.6 $\pm$ 0.4*	14.5 $\pm$ 0.5	6.2 $\pm$ 0.2
23.4	157 $\pm$ 6***	479 $\pm$ 6**	31.2 $\pm$ 0.6***	13.4 $\pm$ 1.2*	5.0 $\pm$ 0.3***

* p<0.05; ** p<0.025; *** p<0.005

+ calculated rate cell production/day

Table IX. Effect of EHDP upon bone apposition (³H-proline studies)

Dose mg/kg/Day	Labeled Bone Surfaces ($\%\pm$ S.E.)	Epi. Trab. Burial ($\mu\pm$ S.E.)	Supra-Epi. Bone Burial ($\mu\pm$ S.E.)	Endosteal Shaft Burial ($\mu\pm$ S.E.)
Control	57.1 $\pm$ 2.2	2.1 $\pm$ 0.2	3.9 $\pm$ 0.1	5.0 $\pm$ 0.2
<u>EHDP</u>				
0.4	51.6 $\pm$ 1.5	2.1 $\pm$ 0.2	3.7 $\pm$ 0.1	5.1 $\pm$ 0.2
2.0	54.0 $\pm$ 1.7	2.0 $\pm$ 0.2	3.6 $\pm$ 0.1	4.6 $\pm$ 0.3
10.0	47.9 $\pm$ 1.5*	1.7 $\pm$ 0.1*	3.3 $\pm$ 0.1***	4.2 $\pm$ 0.2**
20.0	46.8 $\pm$ 2.2**	1.2 $\pm$ 0.1***	3.0 $\pm$ 0.2***	2.4 $\pm$ 0.5***
<u>CP₂MDP</u>				
0.47	52.8 $\pm$ 2.6	2.1 $\pm$ 0.2	3.6 $\pm$ 0.2	5.0 $\pm$ 0.2
2.34	53.8 $\pm$ 1.5	1.7 $\pm$ 0.1*	3.3 $\pm$ 0.2***	4.6 $\pm$ 0.2
11.6	48.0 $\pm$ 2.6*	1.5 $\pm$ 0.1**	3.1 $\pm$ 0.3***	4.5 $\pm$ 0.2
23.4	44.3 $\pm$ 1.8***	1.3 $\pm$ 0.1***	2.9 $\pm$ 0.2***	4.7 $\pm$ 0.3

* p<0.05; ** p<0.025; *** p<0.005

Table X. Effect of diphosphonates upon bone cell population-I

<u>Dose mg/kg/Day</u>	<u>Labeling Index Osteoprogenitor Cells (%±S.E.)</u>	<u>Total Osteoblasts (±S.E.)</u>	<u>Osteoblasts/mm Bone Surface (±S.E.)</u>
Control	15.7±0.7	284±8	6.3±0.2
<u>EHDP</u>			
0.4	13.6±0.6	271±20	5.9±0.4
4.0	11.5±0.5***	267±14	4.7±0.2***
20.0	6.1±0.4***	131±5***	2.6±0.1***
<u>Cl₂MDP</u>			
0.47	15.0±1.3	252±10	4.5±0.3***
4.68	13.4±0.6**	189±22***	2.6±0.4***
23.4	12.5±0.2***	131±6***	1.5±0.1***

* p<0.05; ** p<0.025; *** p<0.005

Table XI. Effect of diphosphonates upon bone cell population-II

<u>Dose mg/kg/Day</u>	<u>Total Osteoclasts (±S.E.)</u>	<u>Osteoclasts/mm Bone Surface (±S.E.)</u>	<u>Average Numbers of Nuclei/Osteoclast (±S.E.)</u>
Control	71±3	1.6±0.1	3.8±0.1
<u>EHDP</u>			
0.4	94±6***	2.0±0.1	4.9±0.2***
4.0	137±6***	2.5±0.2	6.1±0.2***
20.0	84±5***	1.7±0.1	5.4±0.1***
<u>Cl₂MDP</u>			
0.47	116±3***	2.1±0.1***	5.4±0.4***
4.68	165±4***	2.2±0.1***	6.1±0.2***
23.4	161±6***	1.9±0.2***	5.8±0.4***

* p<0.05; ** p<0.025; *** p<0.005

osteoprogenitor cells, while having a greater depressive effect on osteoblasts. Table XI shows that Cl_2MDP also increases the total number of osteoclasts more than equivalent doses of EHDP. However, when the comparison is based on the amount of hard tissue surface there is no significant difference.

Again, the net result of the bone formation and resorption is the increase in metaphyseal hard tissue. Table XII shows the increase in hard tissues at 4.0 mg EHDP/kg/day which is equal to that produced by 0.47 mg Cl_2MDP . Cl_2MDP is 5 to 10 times more effective than EHDP is increasing the amount of hard tissue in the proximal tibia of the 100 gram rat.

Table XIII summarizes the relationship of EHDP to Cl_2MDP for the parameters studied. By inference, Cl_2MDP is more effective at inhibiting bone resorption at equivalent doses. In view of these factors, Cl_2MDP also merits further study in the prevention of Pu-239 uptake by bone.

IV. EHDP AND THE DYNAMICS OF THE OSTEOCLAST NUCLEI

A most intriguing part of these diphosphonate studies was the appearance of an increased number of osteoclasts coupled with apparent decreased hard tissue resorption. We postulated that the piling up of nuclei in osteoclasts was due to a lack of osteoclastic metabolic activity which in turn decreased the outflow of nuclei. To prove the above postulate, rapidly growing rats were treated with 0, 0.4, or 4.0 mg EHDP/kg/day for seven days. They were then injected with 0.5 μCi tritiated thymidine ($^3\text{HTdR}$) every 8 hours for 40 hours (six injections). EHDP treatment continued throughout the experiment. Autopsies were conducted at one hour after the sixth injection, 24, 48, 72, 120, 192, 264, and 336 hours after the final injection of $^3\text{HTdR}$. The use of the eight hour interval for $^3\text{HTdR}$ injection was based on the unpublished

Table XII. Influence of diphosphonates upon quantity of hard tissues in proximal tibia metaphysis

Dose mg/kg/Day	EHDP Hard Tissues (%±S.E.)	Cl ₂ MDP Hard Tissues (%±S.E.)
Control	23.7±0.5	23.7±0.5
0.4	25.8±0.6*	29.7±2.6***
2.0	28.4±1.5***	37.1±1.1***
4.0	30.9±0.7***	40.1±1.0***
10.0	35.6±1.1***	44.1±3.0***
20.0	29.3±0.9***	51.5±0.5***

* p<0.05; ** p<0.025; *** p<0.005

Table XIII. Comparative Dose Response Studies

EHDP = Cl₂MDP

Decrease endochondral growth
Decrease labeling index, OP cells
Increase osteoclasts numbers

Cl₂MPD > EHDP

Increase quantity of hard tissues
Decrease bone formation
Decrease osteoblasts numbers

10 mg EHDP

Formation (↓) F
Resorption (↓↓) R
Hard tissues (↑) M

10 mg Cl₂MPD

Formation (↓↓) F
Resorption (↓↓↓) R
Hard tissues (↑↑) M

0.4 mg EHDP

Formation (NE) F
Resorption (↓) R
Hard tissues (↑) M

0.47 mg Cl₂MPD

Formation (NE) F
Resorption (↓↓) R
Hard tissues (↑↑) M

studies of Kimmel and Jee in the proximal tibial metaphysis which showed that T_s (duration of DNA synthesis phase) for proliferating osteoprogenitor cells is 5.5-6 hours.

The following parameters were studied in an $880\mu \times 1980\mu$ area beginning at the zone of cell hypertrophy of the growth cartilage in the center of the proximal tibial metaphysis as a function of time and EHDP dose:

- total number of osteoclasts
- total number of osteoclast nuclei
- average number of nuclei per osteoclast
- percent of labeled osteoclast nuclei

The following results were noted: 1) Between 120 and 336 hours, the number of osteoclasts increased from 39 to 47 in control animals, from 48 to 54 in low dose animals, and from 51 to 75 in high dose animals (see Fig. 19); 2) The total number of osteoclast nuclei went from 150 to 190 in controls, 225 to 270 in low dose animals, and 310 to 435 in high dose animals (see Fig. 20); 3) The average number of nuclei per osteoclast was 3.9 in control, 5.0 in low dose, and 6.0 in high dose animals (see Fig. 21); 4) The maximum number of labeled nuclei was 55 at 144 hours after the last injection of $^3\text{HTdR}$ in control animals; these same numbers were 102 after 120 hours and 154 after 96 hours in low and high dose animals, respectively (see Fig. 22).

The data concerning the numbered of labeled osteoclast nuclei were treated statistically to determine a possible model for generation and disappearance of nuclei of osteoclasts in the proximal tibial metaphysis of the growing rat.

The data was consistent with a three compartment model in which cells become labeled in Compartment I and migrate into Compartment II at a linear rate A . The labeled cells then exit from Compartment II into Compartment III exponentially at a rate proportional to e^{-kt} .

Figure 19. The total number of osteoclasts as a function of time and continued EHDP treatment is shown. There is an increase in the number of osteoclasts with 4.0 mg EHDP/kg/day treatment for 23 days.

Figure 20. The total number of nuclei within osteoclasts as a function of time and continued EHDP treatment is shown. There is an increase in the number of nuclei within osteoclasts with 4.0 mg EHDP/kg/day for 23 days.

Figure 21. The average number of nuclei/osteoclast is shown as a function of EHDP dose and time. Though this number does not change with time, there are more nuclei/osteoclast with each increasing dose of EHDP.

Figure 22. The number of osteoclasts' nuclei labeled at various times following six injections of $^3\text{HTdR}$ is shown. More labeled nuclei appear more rapidly with increasing doses of EHDP, when compared to controls.

In the controls, the rate of labeled nuclei production was 24.85 nuclei/day while the rate of exit was 15.12%/day, or about 23 nuclei/day; these data, bearing in mind the errors of counting, are probably consistent with the steady state of osteoclast nuclei number observed in the data. In the 0.4 mg EHDP/kg/day group, the rate of nuclei production was 56.61 nuclei/day, while the rate of exit was 18.4%/day or 45 nuclei/day. In the 4.0 mg EHDP/kg/day group, the rate of nuclei production was 107 nuclei/day, while the rate of exit was 22.59%/day, or 80 nuclei/day. Both treated groups reveal data which suggest an increase in the number of osteoclast nuclei over time (i.e., a more rapid rate of entry than exit). This is definitely reflected in the 4.0 mg EHDP/kg/day group data on total osteoclast nuclei over the three weeks of the experiment.

In summary, there was: 1) an increased number of osteoclasts with time and dose; 2: an increase in total osteoclast nuclei with time and dose; 3) an increase in average numbers of nuclei/osteoclast with dose; and 4) an increase in both the rate of influx and efflux of nuclei of osteoclasts with increasing dose, the rate of influx being much more increased than that of efflux.

The approach of multiple labeling of precursor cells with $^3\text{HTdR}$ in an attempt to study the kinetics of differentiated cells is novel, at least for bone cells. In this case, the multiple label was found necessary because of the rather small numbers of precursor cells labeled with one injection of tritiated thymidine (10-15% of the population). Osteoclast nuclei do not become labeled while part of an osteoclast; rather they are labeled as either osteoprogenitor cells or monocyte-macrophages, both of which cell types are popularly implicated as precursors. The data generated in this experiment are not compatible with the contributing of significant numbers of liberated osteocytes to the pool of osteoclast nuclei (Figs. 23 and 24).

Figure 23. A single osteoclast from a control animal with 6 of 7 nuclei labeled with $^3\text{HTdR}$ 120 hrs after a series of six injections of $^3\text{HTdR}$, (2000X).

Figure 24. Three large osteoclasts from an animal treated with 4.0 mg EHDP/kg/day are shown. There is multiple labeling in all three at 120 hrs post multiple-labeling (2000X).

During the period of inflow of labeled osteoclast nuclei in this experiment (approximately the first 72 hours), it is likely that the constant rate of cell nucleus differentiation that is detected is rather accurate. During the period of outflow, several factors complicate the interpretation of the data. The first is the possible inability to detect labeled nuclei (using three silver grains as a confidence level) due to the fact that they have been through a number of divisions which would dilute the radioactivity in the nucleus. At longer and longer time periods, this becomes more and more likely as the osteoprogenitor cells which were originally labeled continue to divide and give rise to osteoclast nuclei. The second is the coefficient of variation of the average lifespan of osteoclast nuclei. If it is large, it would result in a highly variable time when the labeled nuclei, which flowed in as a near-pulse, would exit from osteoclasts. These problems undoubtedly are reflected in the logarithmic model for exit of nuclei which is presented.

However, it is extremely inviting to believe that the size of a tissue's functional cell populations is determined in some way by the state of the tissue, when compared to a normal state. In the case of the diphosphonates, one has created an excess amount of bone tissue, thus possibly stimulating the need for extra bone resorptive cells. With the larger functional population, a more rapid rate of nuclear loss is predicted and found.

It is clear that the original assumption of osteoclast nuclei persisting longer is not true. The actual case is a very much accelerated rate of entry with an accelerated rate of exit, resulting in a net inflow of nuclei for a period of time long enough to increase the size of the pool in response to the apparent needs of the tissue.

V. EFFECT OF DIPHOSPHONATES ON DIFFERENTIATION OF OSTEOBLASTS

An integral part of the diphosphonate effects is the depression of osteoblasts numbers and activity with increasing doses of EHDP. It was postulated that the osteoblasts' response was the result of the indirect effects of parathyroid hormone secretion. We chose to study a region of stimulated bone formation in the orthodontically-stimulated periodontal ligament (PDL) model of Roberts and Jee (Roberts and Jee, 1974a, 1974b) using intact and parathyroidectomized (PTX) rats. Rapidly growing 100-gram rats were injected with 0, 0.2, 0.4, 2.0, or 10.0 mg EHDP/kg/day for 10 days. A light orthodontic elastic was placed in the contact area between the first and second left maxillary molars. Four hours later, a series of seven injections of ($^3\text{HTdR}$) was begun at six hour intervals. Animals were sacrificed after 80 and 120 hours of stimulation. Studies of the percent labeled PDL fibroblasts (Fig. 25) and osteoblasts (Fig. 26) were carried out.

The following items were observed: 1) In the intact rat, there was a significant depression in the fibroblast labeling index at both time periods with 10 mg EHDP/kg/day. 2) In the intact rat, there was a mild depression in percent labeled osteoblasts at 2.0 mg EHDP/kg/day, and a significant depression in the percent labeled osteoblasts with 10 mg EHDP/kg/day (Table XIV). 3) In the PTX rats, there was no suppression in percent labeled fibroblasts at any dose of diphosphonates, when compared to PTX controls; however, all PTX animals had significantly lower percent labeled fibroblasts, when compared to their intact counterparts. 4) There was only a depression in percent labeled osteoblasts at 10 mg EHDP/kg/day. There was no difference in percent labeled osteoblasts when PTX is compared to intact controls (Table XV).

10 mg EHDP/kg/day reduces the percent labeled fibroblasts in intact animals. this it may do by reducing parathyroid gland secretion, as the percent labeled

Figure 25. This figure shows the large number of periodontal ligament fibroblasts which become labeled in a multiple-labeling study within 80 hrs of stimulation by orthodontic elastic placement between the first and second left maxillary molars. Bone (b) and cementum (c) are clearly shown (500 X).

Figure 26. This picture shows the number of osteoblasts (a) and osteocytes (b) which are labeled with tritiated thymidine 80 hrs after a series of injections of $^3\text{HTdr}$ and 120 hrs after stimulation (2000 X).

Table XIV. Percent labeled cells in stimulated PDL cells of intact rats following EHDP treatment

EHDP dose (mg/kg/day)	PERCENT LABELED CELLS			
	Fibroblasts		Osteoblasts	
	80 hrs.	120 hrs.	80 hrs.	120 hrs.
0	53% ± 2%	51% ± 4%	68% ± 2%	72% ± 8%
0.2	49% ± 2%	47% ± 3%	73% ± 8%	74% ± 9%
0.4	50% ± 3%	49% ± 2%	75% ± 6%	74% ± 2%
2.0	49% ± 2%	49% ± 2%	63% ± 4%	63% ± 2%
10.0	38% ± 2%	39% ± 2%	50% ± 1%	51% ± 3%

Table XV. Percent labeled cells in stimulated PDL of parathyroid-ectomized rats following EHDP treatment

EHDP dose (mg/kg/day)	PERCENT LABELED CELLS			
	Fibroblasts		Osteoblasts	
	80 hrs.	120 hrs.	80 hrs.	120 hrs.
0	37% ± 4%	44% ± 3%	70% ± 3%	71% ± 2%
0.2	36% ± 1%	37% ± 4%	63% ± 3%	66% ± 3%
0.4	39% ± 3%	38% ± 2%	72% ± 5%	60% ± 10%
2.0	36% ± 1%	38% ± 2%	66% ± 3%	69% ± 2%
10.0	42% ± 2%	42% ± 2%	34% ± 2%	35% ± 2%

fibroblasts drops to the level of PTX animals. Furthermore, in PTX animals, this level of EHDP does not depress the percent labeled fibroblasts.

10 mg EHDP/kg/day in intact animals depresses the percent of osteoblasts which differentiate from labeled fibroblasts. In PTX animals, 10 mg EHDP/kg/day also reduced the percent of osteoblasts which differentiate from labeled fibroblasts, while not affecting the percent of osteoblasts which differentiate from unlabeled fibroblasts. This portion of the response is not mediated by the parathyroid glands. This is further born out by the fact that parathyroidectomy does not affect the differentiation of labeled fibroblasts to osteoblasts.

In summary, parathyroidectomy depresses the size of the PDL fibroblast population which synthesizes DNA in response to an orthodontic stimulus by 40%. 10 mg EHDP/kg/day to intact rats does the same. Parathyroidectomy does not affect the differentiation of labeled fibroblasts to osteoblasts, but 10 mg EHDP/kg/day does, in both intact and PTX animals, and to a greater extent in PTX animals.

CONCLUDING REMARKS

The most dangerous aspect of long-term plutonium toxicity is its residence for long periods in the skeleton. The most significant part of this is the fact that, following initial deposition at all bone surfaces, it can only leave the surfaces through processes of bone modeling and remodeling. In areas where these processes proceed slowly, removal and/or burial takes correspondingly longer; this places bone lining cells in those regions at risk to alpha-irradiation for extended periods of time.

This report deals first with a compound, EHDP, which can inhibit the uptake of Pu-239 in either or both of two separate ways. First, in rather high

doses (10 mg/kg/day or greater) given for as little as five days before Pu-239 injection, it decreases the surface uptake of Pu-239, while inhibiting the proper mineralization of bone. Second, at lower doses (0.4 mg/kg/day) for 150 days, it decreases the surface uptake of Pu-239 without inhibiting the proper mineralization of bone. It is very significant to note that EHDP was very effective at decreasing Pu-239 uptake in an area of comparatively slow bone surface modeling (in this case, the long bone epiphysis of the 500 gram rat), the very type of area whose lining cells would be most at risk to Pu-239 irradiation. It is suspected that, at surfaces which are rather static, EHDP will adhere to the surfaces if given in repeated small doses, thus decreasing the number of possible sites for Pu-239 deposition.

A great problem with the aging skeleton of humans is the net loss of bone mass over time --- a condition known in its extreme symptomatic cases as osteoporosis. The diphosphonates, EHDP and Cl_2MDP , show great promise in inhibiting bone resorption with no other side effects when administered in small doses for long periods of time; this would have the effect of increasing the bone mass of a skeleton, thus decreasing the likelihood of osteoporosis. Cl_2MDP appears to be five to ten times more effective in stopping the loss of bone than equivalent doses of EHDP.

Persons interested in cell kinetics have been intrigued by the osteoclast and its many nuclei. In the course of these studies, it was established that treatment with the diphosphonates causes a rise in the number of osteoclasts, number of nuclei/osteoclast, and total number of nuclei inside osteoclasts in a confined area. A study employing multiple injections of $^3\text{HTdR}$ showed that EHDP increases the rate of inflow of osteoclast nuclei to osteoclasts, while speeding the outflow to a lesser degree. This finding was especially interesting, in the light of decreased amounts of bone resorption ---

that there should be a greater number of osteoclasts. It is further proof that the direct action of EHDP is on the bone itself and osteoclast cellular findings are secondary to that factor.

However, further studies on osteoblasts have shown that there may be a direct effect on both their differentiation and function. First, the rate of bone matrix formation is lowered by 4.0 mg EHDP/kg/day and more, with no defective mineralization, and, second, 10 mg EHDP/kg/day decreases the rate of osteoblast differentiation in a stimulated system.

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EINSTEINIUM STUDIES IN BEAGLES

Ray D. Lloyd, John G. Dockum, David R. Atherton, Charles W. Mays,
and Jerry L. Williams

ABSTRACT: The early excretion, retention, and tissue distribution of einsteinium has been studied following the intravenous injection of ^{253}Es citrate into 6 young adult beagles. Although the first day's urinary excretion by two of these animals was about 3 and 6 times the average of the other 4 dogs, excretion of the ^{252}Cf contaminant in the injection solution by these same individuals was also much higher. The ratio of excreted Cf to excreted Es in the first day's urine averaged 0.68 for all six dogs and was 0.66 and 0.72 in the 2 animals which excreted an abnormally high fraction of their injected Es. We have never seen previously such anomalous excretion within any group of beagles injected with Cf, Cm, Am, or Pu. Of the other transuranium elements which we have studied, Es appears to most closely resemble Cf in its excretion, retention, and tissue distribution. The ^{253}Es concentration of exsanguinated tissues was about equal in skeleton and liver, the two tissues of highest concentration. Thyroid concentration was about 1/4 to 1/3 that of the skeleton.

INTRODUCTION

Element number 99, einsteinium, was first identified in the radioactive debris of a 1952 nuclear explosion (Ghiorso, 1973). The two Es isotopes which were recovered from the fallout of this first thermonuclear test were the 20.5 day ^{253}Es and the 38.3 day ^{255}Es , both formed as a result of multiple neutron captures by ^{238}U . The retention, excretion, and tissue distribution of the transuranium elements has been studied in our beagles for plutonium (Stover et al., 1969, 1972), americium (Lloyd et al., 1970), californium (Lloyd et al., 1972a,b), and curium (Lloyd et al., 1974). This communication reports the results of a similar investigation using ^{253}Es .

METHODS

Six young adult beagles, averaging 10.2 kg, were injected intravenously with 2.9 μCi ^{253}Es /kg in citrate buffer. Their retention of the injected einsteinium was followed for about one month by total-body counting using

techniques corresponding to those reported previously (Lloyd et al., 1970). Excreta were collected for the first three weeks after injection and their ^{253}Es content was determined by gamma-ray counting. The ^{253}Es within individual bones, organs, and tissues was evaluated for the 3 dogs which were sacrificed during the first 8 weeks of the experiment.

Einsteinium-253 decays with a 20.5 day half-period by alpha emission, accompanied in only 0.06% of the disintegrations by gamma-rays of 0.39 to 0.43 MeV in energy (Lederer et al., 1967). Contamination of our ^{253}Es stock (which we obtained from Oak Ridge National Laboratory) with about 9×10^{-4} $\mu\text{Ci } ^{252}\text{Cf}/\mu\text{Ci } ^{253}\text{Es}$ (as of 6 June 1973) further complicated the gamma-ray counting of dogs, excreta, and tissues (Figure 1). All spectra were consequently corrected in the region of the ^{253}Es photopeak for the contribution of the continuous gamma-ray spectrum of ^{252}Cf . As the ^{253}Es activity continued to diminish by radioactive decay, this correction represented a larger and larger fraction of the total counting-rate at 0.34-0.44 MeV, and the determination of ^{253}Es content of dogs and other samples by gamma-ray counting, therefore, became more and more uncertain with increasing time.

A small photopeak at about 1 MeV was also evident in the photon spectrum of our ^{253}Es . Serial counting of a sample of the stock solution indicated that this peak exhibited a physical half-period of roughly 250 ± 30 days. It was tentatively identified by half-period and gamma-ray energy as resulting from the decay of ^{250}Bk , the short-lived daughter of 276 day ^{254}Es , a likely contaminant of ^{253}Es . The prominent gamma-rays associated with the β -decay of 3.2 hour ^{250}Bk are 0.99 and 1.03 MeV in 47% and 39% of the disintegrations, respectively (Lederer et al., 1967).

RESULTS AND DISCUSSION

Excretion. Urinary and fecal excretion of injected ^{253}Es citrate for each of these six animals are shown in Tables 1-7. Einsteinium excretion during days 4 through 7, 8 through 14, and 15 through 21 has been combined in the tables. Although most of the total excretion was urinary and was accounted for mainly by the first day's urine, variability in einsteinium excretion from dog to dog appeared to be much greater than the variability previously observed for americium (Lloyd et al., 1970), californium (Lloyd et al., 1972a) or curium (Lloyd et al., 1974).

The ^{252}Cf in the first day's urine sample of all Es-injected dogs, except T1E5 and F2E5, ranged between 4.7% and 12.6% of the injected ^{252}Cf contamination averaging 9.2%. This compares with the Cf excretion of about 10% in the first days urine of the Cf-injected dogs reported in Lloyd et al. (1972a), while the ^{252}Cf in the first day's urine of T1E5 and F2E5 represented 27.5% and 61.0% of the injected ^{252}Cf contamination, respectively. Both the Es and Cf excretion were very much larger in these 2 animals than that seen in any of the many other dogs injected with transuranium elements at this project. However, the ratio of excreted Cf to excreted Es in the first day's urine averaged 0.68 for all six dogs and was 0.66 and 0.72 for T1E5 and F2E5, respectively, indicating an abnormal excretion for Cf as well as Es by these 2 animals (Table 8).

Both of the dogs which exhibited unusually high excretion during the first day (T1E5 and F2E5) were females, but the retention of female dog F1E5 more closely resembled that of the male dogs. None of the 3 females was in estrous at the time of injection. T1E5 and F2E5 had started estrous 66 and 74 days, respectively, before injection while F1E5 had started estrous 160 days before injection. Since dogs of this age enter estrous about every 220 days, T1E5

Table 1. Excretion and retention of injected ^{253}Es by female beagle TIE5 (9.82 kg at injection, 28.2 μCi injected, sacrificed 7 days after injection).

days after injection	Excretion			Retention		
	Urine	Feces	Total	injection minus excretion	by total body count	by tissue counting
1	0.418	0.034	0.452	0.548	0.451	
2	0.015	0.014	0.029	0.519		
3	0.009	0.002	0.011	0.508		
4-7	0.009	0.007	0.016	0.492	0.497	0.472

Table 2. Excretion and retention of injected ^{253}Es by male beagle T2E5 (12.2 kg at injection, 35.3 μCi injected, sacrificed 21 days after injection).

days after injection	Excretion			Retention		
	Urine	Feces	Total	injection minus excretion	by total body count	by tissue counting
1	0.132	0.013	0.145	0.855	0.821	
2	0.017	0.026	0.043	0.812		
3	0.003	0.010	0.013	0.799		
4-7	0.009	0.015	0.024	0.775		
8					0.776	
8-14	0.005	0.008	0.013	0.762		
15					0.856	
15-21	0.008	0.009	0.017	0.745	0.723	0.719

Table 3. Excretion and retention of injected ^{253}Es by male beagle T3E5 (11.0 kg at injection, 31.2 μCi injected, sacrificed 55 days after injection).

days after injection	Excretion			Retention		
	Urine	Feces	Total	Injection minus excretion	by total body count	by tissue counting
1	0.075	0.025	0.100	0.900	0.913	
2	0.011	0.014	0.025	0.875		
3	0.010	0.002	0.012	0.863		
4-7	0.010	0.021	0.031	0.832		
8					0.892	
8-14	0.009	0.010	0.019	0.813		
15					0.851	
15-21	0.009	0.011	0.020	0.793		
22					0.891	
30					0.811	
34					0.841	
55						0.742

and F2E5 were in the early part of the cycle when injected and F1E5 was in a late part of the cycle. There is to our knowledge, however, no evidence that the stage of estrous affects radionuclide excretion.

Retention. Total-body retention measurements are also summarized in Tables 1-6. All retention (and excretion) values in these tables have been corrected for radioactive decay. Therefore, they reflect biological rather than effective retention. Agreement between retention values calculated from

Table 4. Excretion and retention of injected ^{253}Es by female beagle FIE5 (8.70 kg at injection, 24.8 μCi injected).

days after injection	Excretion			Retention	
	Urine	Feces	Total	Injection minus excretion	by total body count
1	0.146	0.026	0.172	0.828	
2	0.016	0.008	0.024	0.804	0.812
3	0.008	0.001	0.009	0.795	
4-7	0.008	0.014	0.022	0.773	
8					0.836
8-14	0.010	0.011	0.021	0.752	
17					0.808
15-21	0.006	0.008	0.014	0.738	
22					0.773
31					0.659
35					0.669

Table 5. Excretion and retention of injected ^{253}Es by female beagle F2E5 (9.21 kg at injection, 25.9 μCi injected).

days after injection	Excretion			Retention	
	Urine	Feces	Total	Injection minus excretion	by total body count
1	0.848	0.008	0.856	0.144	
2	0.006	0.009	0.015	0.129	0.110
3	0.010	0.001	0.011	0.118	
4-7	0.007	0.010	0.017	0.101	
8					0.152
8-14	0.012	0.011	0.023	0.078	
17					0.058
15-21	0.006	0.004	0.010	0.068	

Table 6. Excretion and retention of injected ^{253}Es by male beagle M3E5 (10.4 kg at injection, 29.5 μCi injected).

days after injection	Excretion			Retention	
	Urine	Feces	Total	Injection minus excretion	by total body count
1	0.194	0.001	0.195	0.805	
2	0.015	0.034	0.049	0.756	0.763
3	0.007	0.007	0.014	0.742	
4-7	0.013	0.014	0.027	0.715	
9					0.849
8-14	0.006	0.004	0.010	0.705	
18					0.633
15-21	0.007	0.004	0.011	0.694	
23					0.654
30					0.846
34					0.665

the measured excretion and those obtained on corresponding days by total-body counting or by counting of individual organs and tissues appears to be reasonable.

Table 7 compares the total excretion by beagles in the first 3 weeks after injection of the transuranium elements plutonium (Stover et al., 1969), americium (Lloyd et al., 1970), curium (Lloyd et al., 1974), californium (Lloyd et al., 1972a), and einsteinium (Tables 2 to 6). If F2E5 is considered anomalous and is excluded from the average, the urinary, fecal, and total excretion of einsteinium appear similar to those of californium.

Table 7. Excretion of Pu, Am, Cm, Cf, and Es by beagles during the first 3 weeks after intravenous injection of citrate solution.

<u>Number of Dogs</u>	<u>Element Name & No.</u>	<u>% in Urine</u>	<u>% in Feces</u>	<u>Total % Excreted</u>
19	Pu (94)	4	8	12
4	Am (95)	10	1	11
5	Cm (96)	8	3	11
11	Cf (98)	16	8	24
4 [*]	Es (99)	18	7	25
5 ^{**}	Es (99)	32	7	39

* Excluding T1E5, sacrificed before 3 weeks, and excluding F2E5.

** Excluding T1E5, but including F2E5.

Table 9 compares the retention of injected transuranium elements by beagles. These data were taken from the same sources as were those in Table 7. If the values for T1E5 and F2E5 are excluded from the averages for ²⁵³Es, the retention of einsteinium appears to be similar to that of californium.

Table 8. Urinary excretion of ^{253}Es and ^{252}Cf during the first day after injection

<u>Dog</u>	<u>Sex</u>	<u>^{252}Cf</u>	<u>^{253}Es</u>	<u>Cf/Es</u>
T1E5	F	27.5%	41.8%	0.66
T2E5	M	8.7	13.2	0.66
T3E5	M	4.7	7.5	0.63
F1E5	F	10.8	14.6	0.74
F2E5*	F	61.0	84.8	0.72
M3E5	M	12.6	19.4	0.65
Average				0.68
± S. D.				± 0.04

*In an attempt to determine whether the anomalously high urinary excretion by this dog during the first day of the original study might occur again, F2E5 was also injected with about 0.3 μCi $^{249}\text{Cf}/\text{kg}$ at 357 days after the ^{253}Es + ^{252}Cf injection. The ^{249}Cf injection occurred 78 days after the beginning of an estrous cycle. Excretion of ^{249}Cf in the urine during the first day of this subsequent study was only 12.2%, a value which was similar to the average ^{252}Cf urinary excretion by all the other ^{253}Es -injected dogs except T1E5 and F2E5, and to the average first day's urinary excretion of about 10% for the Cf-injected dogs reported by Lloyd et al. (1972a). Therefore, the high excretion of ^{253}Es and ^{252}Cf by F2E5 in the first experiment cannot be explained as a consistent characteristic of this particular dog or by the stage of estrous at injection. The records indicate that new syringes and needles were used for all injections of the dogs in this study and that no studies which involved the chelating agent, DTPA, were in progress at the time of the experiment or within more than a month before. In addition, the cage in which F2E5 was kept for 1 week prior to ^{253}Es injection and for 3 weeks after had never been used previously to house any dog given DTPA treatments. Furthermore, F2E5 and F1E5 were injected with ^{253}Es solution from the same bottle, and T1E5 was injected with solution from the same bottle as was M3E5. Based on this evidence, it appears unlikely that the excessive excretion of ^{253}Es and ^{252}Cf by F2E5 could have resulted from the accidental injection or ingestion of DTPA.

Table 9. Biological retention of Pu, Am, Cm, Cf, and Es by beagles one week after intravenous injection in citrate solution

Element Name & No.	Percent of Injected Radioactivity		
	Liver	Non-liver	Total
Pu (94)	30	60	90
Am (95)	50	40	90
Cm (96)	35	53	88
Cf (98)	20	60	80
Es (99)	← see Table 10 →		77*
Es (99)	← see Table 10 →		62**

* Average of 4 dogs, excluding T1E5 and F2E5.

** Average of all 6 dogs.

Table 10. Distribution and concentration of injected ^{253}Es in selected exanguinated tissues of 3 beagles, 7 to 54 days after injection.

	T1E5 (7 days)		T2E5 (21 days)		T3E5 (55 days)	
	% of injected Es	%/kg	% of injected Es	%/kg	% of injected Es	%/kg
Skeleton	30.819	41.8*	53.460	58.4*	51.717	62.7*
Liver	10.690	49.0	11.677	52.1	13.016	60.5
Thyroid	0.026	42.5	0.015	15.4	0.011	14.9
Kidney	0.656	24.0	0.638	14.3	0.194	5.2
Spleen	0.165	7.5	0.126	5.8	0.114	5.0
Muscle	4.190	0.9	3.687	0.7	7.252	1.4
Pelt	1.470	1.2	3.089	1.9	2.190	1.7

* Assuming that the skeleton was 7.5% of body weight at injection.

Distribution. Table 10 shows the ^{253}Es content and concentration in some organs and tissues of 3 dogs sacrificed at 7, 21, and 55 days after injection. Like americium, curium, and californium, einsteinium appears to concentrate in the skeleton, liver, and thyroid. Of the transuranium elements we have studied, Am deposits to a greater extent in liver, while Cm, Pu, and especially Cf and Es deposit mainly in the skeleton. Concentrations in liver (relative* to the skeleton = 1.0) were 5.0 for Am, 2.8 for Cm, 1.9 for Pu, 0.99 for Es, and 0.98 for Cf. Concentrations in thyroid (relative* to the skeleton = 1.0) were 1.5 for Am, 0.74 for Cm, 0.53 for Cf, 0.45 for Es, and 0.11 for Pu (see Table 2 of Lloyd et al., 1972b, Table 5 of Lloyd et al., 1974, Stover et al., 1972, and Table 10 of this report for data from which the preceding ratios were calculated).

CONCLUSIONS

We observed an abnormally high urinary excretion of both ^{253}Es and ^{252}Cf in 2 of the 6 dogs in our limited study, but so far we can offer no satisfactory explanation for it. Comparison of the disposition of the five transuranium elements Pu, Am, Cm, Cf, and Es in beagles indicates that whereas Am is concentrated to a relatively greater extent in the liver and thyroid than the others, Cm, Cf, and Es may be more nearly similar to one another than they are to either Am or Pu. Excretion, retention, and tissue distribution of einsteinium appears to closely resemble that of californium when injected into beagles as the citrate. Further studies are required to elucidate the observed variability in Es and Cf excretion and its effect upon comparisons with the other transuranium elements.

* Assuming the skeletal mass to be 7.5% of injection weight.

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Figure 1. Photon spectrum of a sample of our ^{253}Es stock solution recorded on 6 June 1973, with an 8" x 4" NaI(Tl) crystal having an outer envelope of 0.02" thick stainless steel. As indicated in the figure, the continuous photon spectrum was due mainly to the presence of ^{252}Cf . Superimposed upon this can be seen the primary photopeaks of ^{253}Es at about 0.39 MeV and what we tentatively identified as $^{254}\text{Es} + ^{250}\text{Bk}$ at about 1.0 MeV.

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ON THE EARLY RETENTION AND DISTRIBUTION OF ^{253}Es BY THE BEAGLE

D. R. Atherton, F. W. Bruenger and W. Stevens

Abstract: ^{253}Es in 0.08 M citric acid-sodium citrate buffer at pH 3.5 was given by intravenous injection to 10 beagles. The concentration of the einsteinium in plasma for the first 48 hours following injection are adequately described by a 3 component exponential equation. The circulating nuclide diminishes to insignificance in less than 6 days. The major deposition sites are liver and skeleton. Less of the injected einsteinium is retained by the liver than has been observed for any of the actinides except ^{228}Th . Skeletal retention and urinary excretion are highly variable and appear to be at least somewhat related.

INTRODUCTION

In May 1973 a supply of ^{253}Es was obtained. With it the laboratory's experience with the actinide elements in the beagle was expanded. The short half-life of this α -emitting nuclide (20.5 days) precludes all but very early retention and distribution studies. Its use does provide an opportunity to obtain some information on the effects of high intensity internal α -radiation of short duration. Early retention and excretion data are reported here while the long term results of brief intense α -irradiation will not be available for several years.

MATERIALS AND METHODS

^{253}Es was supplied by Oak Ridge National Laboratories. Their pulse height analysis indicated that the α -particles emitted by this material had an energy of 6.63 Mev, characteristic of the decay of the nuclide. This analysis was confirmed in our laboratory. However serial pulse height analysis terminating after 13 half-lives of ^{253}Es indicated that in 1100 α 's of the injected material there was about 1 α resulting from the decay of ^{252}Cf , i.e. as of 6 June 1973.

Ten beagles from the Radiobiology colony were injected intravenously with ^{253}Es in 0.08 M citrate buffer of pH ≈ 3.5 .⁽¹⁾ Four animals were injected with $\sim 2.9 \mu\text{Ci/kg}$ (5-level) and used for distribution and retention studies with exposure times from 7 to 55 days. Three of the remaining animals were injected at the 5 level and three with $\sim 0.29 \mu\text{Ci/kg}$ (3-level). These dogs will be observed throughout their lives. Four months after injecting nine of these animals, the remaining ^{253}Es was used for the injection of the last 5 level dog which was sacrificed at 7 days post-injection. In the solution used for this injection the ^{253}Cf to ^{253}Es activity ratio had changed to about 1 to 20.

The amount of ^{253}Es circulating in plasma was measured at times ranging from 5 minutes through 48 hours. Aliquots of plasma were digested in a 1:1 mixture of 30% H_2O_2 and concentrated HClO_4 .⁽²⁾ After a colorless solution was obtained, Instagel* was added and α -activity was quantified by comparing a standard ^{253}Es source (prepared with the same H_2O_2 - HClO_4 medium and suspended in Instagel) to the samples. Counting was done using a 2" photomultiplier tube, appropriate amplifier and a 512 channel analyzer.

Soft tissues were assayed for their ^{253}Es content as described above. Aliquots of such samples weighing up to about a gram were processed in scintillation vials and were counted without having to be transferred.

Bone specimen were ashed in a muffle furnace at about 500°C , dissolved in 2 N HNO_3 , placed in as small a volume as practical, and aliquots (usually 1.0 ml) were then counted as described above.

* Packard Instrument Co.

Figure 1 % of injected ^{253}Es in circulating blood from 5 minutes through 2 days.

RESULTS

The level of circulating ^{253}Es is presented in Figure 1. The data appeared to be reasonably well described by three exponentials representing about 35, 17, and 0.8% of the injected nuclide remaining in circulating plasma with half times of about 15 minutes, 2 and 20 hours, respectively. The analagous curve for circulating curium is included for comparison; data for it were reported previously.⁽³⁾ All reported retentions are "biological" retentions, i.e., corrected for radioactive decay.

The distribution of einsteinium in selected tissues of the four 5-level beagles is presented in Tables 1 and 2. Table 1 shows the total nuclide retained by a variety of tissues and Table 2 shows the concentration in the tissues listed in Table 1 along with several other tissues whose total mass is not known.

TABLE 1
Retention (% of Injected ^{253}Es)

PART	T1E5 7d	T5E5 7d	T2E5 21d	T3E5 55d
Liver	11.1	16.2	10.9	13.0
Kidney	0.656	0.966	0.638	0.194
Spleen	0.165	0.209	0.126	0.114
Thyroids	0.0261	0.0204	0.0149	0.0106
Adrenals		0.0062	0.0061	0.0105
Eyes	0.0187	0.0167		
Humerus	0.904	3.74	1.94	2.14
3rd Lumbar Vertebra	0.435	0.930	0.875	0.924

Measurements of whole body retention, excretion and detailed distribution

on T1E5, T2E5, and T3E5 were done by the Physics Group and are reported elsewhere.⁽⁴⁾ Due to the complications arising from the relative increase in γ -ray radiation contributed by ^{252}Cf , the Physics Group did not participate in the analysis of T5E5. Therefore the analytical work was done by the Chemistry Group and results of excretion studies and evaluation of soft tissue and skeletal retention done by α -analysis are shown in Tables 3, 4, and 5 respectively.

TABLE 2
Concentration of Retained ^{253}Es (% inj'd/gram)

PART	T1E5 7d	T5E5 7d	T2E5 21d	T3E5 55d
Liver	0.0510	0.0594	0.0485	0.0318
Kidneys	0.0240	0.0236	0.0143	0.0052
Spleen	0.0075	0.0106	0.0058	0.0050
Thyroids	0.0426	0.0361	0.0154	0.0149
Adrenals		0.0048	0.0042	0.0084
Eyes	0.0018	0.0014		
Thymus	0.0013	0.0024	0.0014	0.0014
Salivary G.		0.0085	0.0042	0.0029
Loose Conn. Tissue		0.00077	0.00076	0.0012
Dura	0.0149	0.0205	0.0125	0.0050
Muscle		0.00035	0.00015	0.00061
Humerus	0.0426	0.118	0.0533	0.0608
3rd Lumbar Vertebra	0.0688	0.0893	0.0802	0.0914

DISCUSSION

The concentration of circulating ^{253}Es initially diminishes at a somewhat slower rate than that observed with curium. However after a short time their concentrations appear to decline at about equal rates. Both nuclides have been essentially removed from the circulation in less than 1 week.

As has been observed with other actinides,⁽³⁾ there are two principal sites for einsteinium deposition: the liver and the skeleton. However the initial deposition in the liver is the lowest for any actinide studied except ^{228}Th . The concentrations found in thyroid, dura and kidney are all several times higher than that seen in the other soft tissue and, in fact, approach the concentrations observed in hepatic tissue and bone.

The total einsteinium deposited in bone ranged from 30 to 60% of the injected dose, the concentration being the highest of any tissue studied.

The Physics Group reported skeletal retention of 31, 53, and 52% for T1E5, T2E5, and T3E5 respectively.⁽⁴⁾ If the skeletal material made available and analyzed by the Chemistry Group is representative of the entire skeleton and if the skeletal mass is taken to be 7.5% of the dog's weight, the skeletal retention of T5E5 was 62% of the injected dose.

TABLE 3
T5E5 Excretion of ^{253}Es

ΔT (inj'n to sample in days)	% of Injected		Cumulative Total	F/U
	Urine	Feces Total Day		
1	11.03	----	11.03	----
2	2.15	0.79	13.97	0.37
3	0.81	2.29	17.07	2.82
4	0.49	0.53	18.10	1.08
5	0.35	0.34	18.78	0.96
6 & 7	0.45	0.29	19.52	0.65
Total	15.28	4.24	19.52	0.28

TABLE 4
Retention of ^{253}Es in T5E5 Soft Tissues

	% of Injected	% of inj'd/gram
Liver	16.2	0.0594
Kidney	0.966	0.0236
Spleen	0.209	0.0106
Thyroid	0.0204	0.0361
Adrenal	0.0062	0.0048
Eye	0.0167	0.0014
Thymus		0.0024
Salivary Gland		0.0085
Loose Connective Tissue		0.00077
Dura		0.0205
Muscle		0.00035
Lung	0.433	0.0053
Pancreas	0.0255	0.0014
Trachea		0.0130
Skin		0.0014

TABLE 5
T5E5 Skeletal Retention of ^{253}Es

	% of Injected	$\left(\frac{\% \text{ of Inj'd}}{\text{g wet bone}} \right)$
Humerus	3.74	0.118
3rd Lumbar Vertebra	0.930	0.0893
3rd Cervical Vertebra	0.489	0.0633
2nd Thoracic Vertebra	0.580	0.105
3rd Thoracic Vertebra	0.532	0.107
Scapula	1.10	0.0616
Ulna	0.372	0.0311
Mandible	0.717	0.0308
Femur	1.70	0.0552

The most predictable feature of the biological response by the beagle to the intravenous administration of ^{253}Es appears to be the rate at which it leaves the circulating blood. Next is the amount that one can expect to find deposited in the liver. Variation in the amounts deposited in the skeleton correlate with urinary excretion of the nuclide,⁽⁴⁾ and, in these studies, was highly variable. The observed variation in skeletal retention of einsteinium cannot be explained at present.

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THE EARLY SUBCELLULAR DISTRIBUTION OF EINSTEINIUM IN CANINE LIVERS

F. W. Bruenger, B. J. Grube, D. R. Atherton, D. S. Buster and W. Stevens

Abstract: Studies of the subcellular distribution of actinide elements were enlarged to include ^{253}Es . Beagles were injected with $3\ \mu\text{Ci}$ of the nuclide in $0.08\ \text{M}$ citrate buffer of pH 3.5 per kg of body weight. Liver specimen were obtained either by serial surgical liver biopsies or at time of sacrifice of the animal. The nuclide was first associated with soluble liver proteins (mainly ferritin). With increasing time after injection, einsteinium became associated with subcellular liver organelles. The concentration of einsteinium in the crude mitochondrial fraction was usually higher than it was in the crude lysosomal fraction. In the mitochondrial fraction 41% was either membrane bound or associated with sedimentable granular material and 59% was present in soluble form at 7 days. A considerable but variable fraction of the nuclide remained bound to ferritin. Lysosomes and heavier organelles are involved in the binding. Less einsteinium deposits in the liver than do other actinides except ^{228}Th and biological variation is greater.

INTRODUCTION

The subcellular distribution of ^{239}Pu in the liver has been of interest to many research workers for a number of years. (1,2,3,4) As part of an effort to investigate the mechanism by which elements having the 5f electron configuration are deposited in various organs, as bone, liver, thyroid, etc., our laboratory has been engaged in a study of their subcellular distribution as a function of time. Results of studies with ^{239}Pu , ^{241}Am , ^{249}Fm , ^{252}Cf and ^{243}Am , ^{244}Cm have been presented. During the last year, these studies were extended to include ^{253}Es . It is projected that the amount of einsteinium produced in the U. S. will be relatively small. The short half-life of ^{253}Es , however, makes it possible to study the long term biological effect of short term exposure to α -radiation.

A clearer understanding of the basic behavior of all the actinides in a model biological system will facilitate the correlation of these element's

biological associations with known physical and chemical properties. Knowledge of the molecular and cellular events leading to deposition of the actinides in the liver will aid in finding a more rational basis for their decorporation. Work presented here describes the distribution of ^{253}Es in the beagle liver up to 55 days after injection. The variation in retention, distribution and excretion of ^{253}Es (and its ^{252}Cf contaminant which was present at a concentration of 1% of the ^{253}Es concentration on an atom for atom basis) was greater than previously observed in any other beagles injected with actinide elements. No explanation for this unusual behavior has been found. However the extremely small number of atoms of the nuclide present makes it more likely to be affected by small biological variations.

METHODS

The number of beagles injected with einsteinium, the dose level, the characterization of the nuclide and other pertinent data are described elsewhere in this Progress Report.⁽⁵⁾ The distribution of ^{253}Es in the liver was measured over a time from 2 hours to 55 days after injection. At 13, 21, and 55 days after injection liver specimen were obtained at time of sacrifice and at 2 hours, 2, 7, and 29 days after injection, by surgical biopsy. Liver samples were immediately cooled on ice, minced and homogenized in 0.25 M sucrose containing 3 mM Ca^{++} . All subsequent operations were performed at 0-4°C. Differential centrifugation, isopycnic sucrose density gradients, protein analyses, and enzyme determinations were carried out as described before.⁽⁶⁾ Membranes of the mitochondrial homogenate obtained at 7 days after injection were ruptured with 3% Na-desoxycholate. The resulting lysate was centrifuged and both the pellet and supernatant were analyzed for einsteinium. A cytosol sample was concentrated by pervaporation and

then subjected to chromatography on a double column of Sephadex G-200 (2.5 x 50 cm), as described before.⁽⁶⁾ Resulting fractions were analyzed for protein and einsteinium content.

Einsteinium was quantified by liquid scintillation counting using the method of Seidel.⁽⁷⁾

RESULTS

The concentration of einsteinium in all liver fractions was expressed as Es-activity/mg protein. In data obtained by differential centrifugation, the activity of ^{253}Es in the original homogenate was arbitrarily set equal to 100. Results of the differential centrifugation are presented in Table 1. The activity in the supernatant after removal of nuclei and debris (6000 g-min) did not change significantly over the period of observation. Variations can easily be explained by the heterogeneity of the specimen. At 2 hours after injection, most of the nuclide was associated with soluble liver proteins of the cytosol. As time after injection increased, einsteinium concentrations in the subcellular fractions increased to a greater extent than observed with any other actinide. Most of the differences can be attributed to biological variation, since data obtained from T4E5 by serial liver biopsies showed considerably less variation than that seen between different animals. The fractions designated as "mitochondrial" and "lysosomal" should not be considered as pure fractions. They are simply enriched in the respective organelles.

The concentrations of ^{253}Es per unit of acid phosphatase were compared in the mitochondrial fraction, the lysosomal fraction, and in the 6000 g-min fraction, i.e. the fraction from which neither mitochondria nor lysosomes had been removed. The data were normalized so that the phosphatase activity

of the original homogenate was equal to 10 and the einsteinium activity was equal to 100 (Table 2). The concentration of einsteinium in the mitochondrial fraction was considerably higher than that of the lysosomal fraction.

An aliquot of the mitochondrial preparation was lysed and separated into a fraction containing membranes and possibly some granular material and into a soluble fraction. Ninety six percent of the original nuclide was recovered and of this 59% was associated with soluble material and 41% was found in the pellet.

Isopycnic density gradients were performed on all samples of the homogenate after removal of nuclei and debris (6000 g-min), and on rehomogenized mitochondrial pellets. Since biopsy specimen yielded only a small lysosomal fraction, lysosomal gradients were performed only when larger tissue samples were available after sacrifice of the animals. Profiles from lysosomal homogenates were therefore omitted. The resulting distribution of Es-activity/mg protein, phosphatase/mg protein, Es-activity/phosphatase unit and % of total nuclide as a function of density in each fraction are given in Figs. 1 and 2. The correlation between acid phosphatase concentration and nuclide concentration increased as a function of time. Also presented in Figs. 3 and 4 is the Es-activity/phosphatase unit versus density for 2 hours, 7 days and 21 days of the 6000 g-min gradients and the mitochondrial gradient. All of the curves show a nonlinear relation between einsteinium content and phosphatase activity.

Chromatography of a cytosol sample obtained at 7 days after injection revealed that 15.5% of the nuclide in the cytosol at that time was associated with high M. W. or granular material, 75.6% was associated with ferritin and 8.8% with some component of M. W. $\approx$ 5000. The elution profile is given in Fig. 5.

DISCUSSION

After intravenous injection of ^{253}Es in citrate buffer the deposition of the nuclide in liver cells follows a pattern qualitatively similar to that observed with other actinide elements. There were however quantitative differences. The variations seen in total retention and cellular distribution from dog to dog were much greater than observed with other actinides.

Initially all of the nuclide was bound to soluble protein (most of it to ferritin) in the cytosol. With increasing time after injection, however, the concentration of the nuclide in subcellular particles increased and that in the cytosol declined. In the mitochondrial fraction, more nuclide was associated with soluble proteins than has been observed with other actinides. The concentration of einsteinium in the cytosol was similar to that observed with ^{243}Cm , ^{244}Cm , less than that observed with ^{241}Am , but greater than that seen with ^{239}Pu . If the nuclide were associated with a single subcellular entity whose identity is determined by measuring a respective marker enzyme, then the ratio of nuclide concentration to enzyme concentration should be constant throughout the gradient. Deviations from constancy of the ratio of nuclide activity/phosphatase unit were observed. Therefore ^{253}Es is bound by more than one subcellular organelle as was also observed with other actinide elements. However in the mitochondrial fraction, these deviations were less pronounced than in the case of curium. (8)

Even though there is a significant correlation between phosphatase activity (units/mg protein) and einsteinium activity (CPM/mg protein), the total amount of einsteinium in the region in which this correlation is most pronounced, represents a small fraction of the total einsteinium in the gradient. The greatest amount was found in the fractions of higher density which are predominantly mitochondria. Association of einsteinium with

lysosomes, however, seems somewhat greater than with other actinides.

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TABLE 1
Concentration of Einsteinium in Liver Fractions
Obtained by Differential Centrifugation*

Time After Injection (Days)	T4E5 0.083	T4E5 2	T5E5 7	T4E5 13	T2E5 21	T4E5 29	T3E5 55
Original Homogenate	100.	100.	100.	100.	100.	100.	100.
Homogenate Free of Nuclei and Debris	118.	122.	130.	118.	153.	138.	97.
Homogenate Free of Mitochondria	106.	72.	81.	56.	55.	57.	44.
Cytosol	98.	31.	23.	13.	25.	14.	34.
Mitochondrial Fraction	120.	317.	241.	536.	525.	824.	267.
Lysosomal Fraction	103.	198.	356.	321.	277.	338.	503.

* Data expressed as Es-activity/mg of protein with the activity of the original homogenate arbitrarily set to 100.

TABLE 2
Concentration of ^{253}Es per Unit of Lysosomal Marker*

Time after Injection (Days)	T4E5 0.083	T4E5 2	T5E5 7	T4E5 13	T2E5 21	T4E5 29	T3E5 55
Homogenate Free of Nuclei and Debris	11.8	12.2	13.	11.8	15.3	13.8	9.7
Mitochondrial Fraction (M)	6.6	26.6	27.3	30.7	38.6	27.3	24.5
Lysosomal Fraction (L)	6.1	13.9	16.2	14.9	18.6	14.3	31.2
M/L	1.1	1.9	1.7	2.1	2.1	1.9	0.8

* $\frac{\text{CPM/mg protein}}{\text{Acid Phosphatase/mg Protein}}$

Acid phosphatase activity of 6000 g-min homogenate fraction free of nuclei and debris normalized to 10 Sigma Units and Einsteinium activity (CPM/mg protein) of the original homogenate normalized to 100.

Fig. 1 Results of isopycnic density gradient centrifugation of the original liver homogenate after removal of nuclei and debris. Curves for CPM/phosphatase unit, CPM/mg protein and phosphatase/mg protein are shown on an arbitrary scale. Also shown is the percent of total einsteinium found in each fraction. Except at 2 hours and at 2 days, good correlation between acid phosphatase activity and einsteinium activity exists but this correlation is not in the area in which the greatest amount of einsteinium in the gradient is found.

Fig. 2 Results of isopycnic density gradient centrifugation of the rehomogenized mitochondrial pellets.

Fig. 3 The ratio of nuclide concentration to acid phosphatase concentration in the original homogenate after removal of nuclei and debris is plotted against density of the sucrose gradient. If the nuclide were only associated with lysosomes, the above ratio would be constant. Strong deviation from constancy proves the involvement of other subcellular organelles.

Fig. 4 The ratio of nuclide concentration to acid phosphatase concentration in the rehomogenized mitochondrial pellet.

Fig. 5 Elution profile of liver cytosol after gel chromatography on a double column of Sephadex G-200. Time after injection of ^{253}Es was 7 days. At this time more than 75% of the nuclide in the liver was bound to ferritin.

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EARLY HEMATOLOGIC EFFECTS OF CURIUM AND EINSTEINIUM IN BEAGLES

Jean H. Dougherty

ABSTRACT: The hematologic changes following a single injection of curium ($^{244}\text{Cm} + ^{243}\text{Cm}$) at the 5-dose level, or einsteinium (^{253}Es) at the 3 and 5-dose levels into a small number of adult beagles have been summarized for the first 9 to 12 months following injection. These actinide elements are surface-seeking alpha emitters with the einsteinium having a very short half-life (20.5 days). The early hematologic effects follow the typical patterns of early granular leukocyte and erythrocyte depression as seen after injection of high dose levels of other transuranic elements. However, the rates and magnitude of recovery of the various cells are much more rapid in dogs injected with the short lived ^{253}Es .

INTRODUCTION

A pilot study was begun in early 1973 to investigate the biologic effects of curium (^{244}Cm highly enriched with ^{243}Cm) in the adult beagle. Early excretion, retention, and distribution studies were summarized by the chemistry and physics groups in a previous annual report (1-3).

Also, in mid-1973, a pilot study was begun on einsteinium (^{253}Es) using beagles, which was also mainly set up using short term animals for early retention and distribution studies, due to its very short half-life of 20.5 days (4, 5).

The following report is a summary of the hematologic changes for the first year following curium injection and for 9 months after ^{253}Es injection. No previous reports of hematological effects of these actinide elements in a large mammal have been published.

METHODS

Curium: Five male beagles were injected i.v. with approximately 2.6 $\mu\text{Ci Cm}$ citrate/kg. Details of injection procedures are given in a previous report (1). Three dogs were sacrificed at 6, 13, and 20 days post-injection

for early retention and distribution studies. One dog (T1C5) was included to provide a series of liver biopsies and one (T5C5) was to be maintained for its lifetime.

Einsteinium: Of the 10 dogs which served as the experimental animals for ^{253}Es , 4 were injected with approximately 2.9 $\mu\text{Ci/kg}$ (5-level) for sacrifice from 7 to 55 days post-injection, and 3 each at the 3-level (0.29 $\mu\text{Ci/kg}$) and 5-level (2.9 $\mu\text{Ci/kg}$) were designated for long-term studies.

The hematologic determinations were done similarly for dogs receiving either nuclide. The dogs were bled from the jugular vein at the same time in the morning prior to feeding. Three control counts were performed on each animal at weekly intervals during the month prior to injection. These control determinations represent the pre-injection values which are compared to post-injection values in determining the effect of the nuclide on blood cells since there were no sham-injected (0-level) controls in these pilot studies.

The effects on red blood cells are evaluated by determining the volume of packed red cells (VPRC), hemoglobin (Hgb) and reticulocytes. White cell values obtained are total leukocyte counts (WBC) and absolute numbers of polymorphonuclear leukocytes (PMNS), lymphocytes, monocytes and eosinophils. Blood platelets and sedimentation rates are also determined.

Post-injection blood counts are determined weekly for the first month after injection and then monthly on 5-level dogs and every three months on dogs at lower injection levels. The experimental time reported for curium is for 12 months and for ^{253}Es for 9 months post-injection since this is the time lapsed after injection as of the writing of this report.

Figure 1. Leukocyte responses following injection of 2.6 µCi/kg of Curium (5-level). In this and subsequent figures the dotted lines represent ± 1.0 standard deviation about the average values obtained from a large series of normal adult beagles.

Figure 2. Eosinophils, platelets, hemoglobin and VPRC for the same dogs as in Figure 1.

RESULTS

A. Effects on curium 5-level dogs (Fig. 1 and Fig. 2).

1. Leukocytes: There was a drop in WBC beginning at one week which was most severe at 3 weeks (pre-injection mean ~ 12.0 thousand cells/mm³ down to 2.5 thousand cells/mm³) with beginning recovery at 1 month then slowly leveling off in the two remaining dogs to levels of about one-third to one-half of pre-injection values. The PMN response paralleled the WBC response with a decline to ~ 750 cells/mm³ at 3 weeks. At 11 and 12 months there seemed to be a further decline of PMNS. This pattern of cell loss and recovery was also seen to a lesser extent in monocytes (Fig. 1) and eosinophils (Fig. 2). Blood lymphocytes (Fig. 1) dropped to one-half of normal values during the first month post-injection and there was a further steady decline over the first year post-injection so that the lowest lymphocyte levels were seen at one year in the two surviving dogs (75% of pre-injection values).
2. Platelets (Fig. 2): The platelets fell in the same manner as leukocytes with low values at 3-4 weeks of between 50,000-150,000/mm³ (pre-injection range was from 250,000-500,000/mm³). Again, there was a rise in numbers at two months to slightly below pre-injection levels and a dip again at 11-12 months to levels comparable to those of the early period of decline.
3. VPRC and Hgb (Fig. 2): The fall in red cells as seen by VPRC and hemoglobin values began at variable times post-injection. Two of the dogs had normal values at 2 and 3 weeks; 1 dog was below normal levels at 1 week, and the remaining 2 dogs first showed an anemia at 3 weeks. By 1 month all of the surviving dogs were anemic (VPRC of 41-43) (pre-injection range of 49-54) with correspondingly low hemoglobin values.

The dog which had a further drop of VPRC and hemoglobin at 2 months was the dog which had several liver biopsies post-injection. There was a leveling off of VPRC and Hgb in the two surviving dogs after 3 months, but the red cell values did not completely return to pre-injection levels in either dog during the first year post-injection.

B. Effects on einsteinium 5-level dogs (Figs. 3 & 4).

1. Leukocytes (Fig. 3): The drop in WBC values was very rapid down to one-fourth of pre-injection values at 1 week with lowest values at 2 weeks. In the remaining dogs there was beginning a recovery at 3 weeks or 1 month with an accelerating rate of recovery during subsequent months in the 3 surviving dogs. WBC values from the 6-9 months were again in the low normal range. The PMNS showed a very sharp post-injection drop with minimal low mean values at 2 and 3 weeks with values of about 600 cells/mm³. The PMNS (Fig. 3) began to rise at 1 month, but values below the control period persisted throughout the first year. The monocytes (Fig. 3) declined at the same time as the PMNS with a recovery to normal or low normal values by 4 months post-injection in two of the dogs (Fig. 4). Eosinophils dropped to almost zero at 2-3 weeks and then recovered in a similar manner as the monocytes.

The lymphocytes (Fig. 3) showed the most variable response of any of the various leukocytes to einsteinium injection. There was a decline of lymphocytes beginning at 1 week in three of the 5-level dogs with values of one-third of pre-injection levels through 3 weeks. One dog with post-injection values of 2,000 cells/mm³ had very high normal values. Of the three survivors, one animal remained lymphopenic and the other two had normal values for the rest of the 9 month period reported.

Figure 3. Leukocyte responses following injection of 2.9 $\mu\text{Ci/kg}$ einsteinium (5-level).

Figure 4. Eosinophils, platelets, hemoglobin and VPRC for same dogs as Fig. 3.

2. Platelet response: In Fig. 4, the platelets are shown for 6 months post-injection. There was a drop down to 20,000-140,000/mm³ from 3 weeks to 1 month post-injection. The recovery in platelets was very rapid with normal values again 2 months after injection. These cells remained within normal limits subsequently.

3. Red cell response dogs: The effect on red cells was quite variable in this group of dogs as to time and extent of the anemia. The time of maximal drop in most of the dogs was between 3 weeks to 1 month. No change was seen in dogs sacrificed at early times post-injection. Recovery was evident at 2 months with only 1 dog showing any further period of anemia during the first 9 months.

C. Effects on einsteinium 3-level dogs (Fig. 5 and Fig. 6).

1. Leukocytes: There were only three dogs injected at this level and as may be seen from Fig. 5, the leukocyte response varied widely. The most consistent finding after injection was a drop in PMNS in all of the dogs which was maximal at 2 to 4 weeks. Two of the dogs showed subsequent return of PMNs to normal values whereas the dog with the lowest control values stayed below the normal range for 6 months. There was no significant effect on eosinophils and lymphocytes except for a slight depression below normal at 2 weeks post-injection. Platelet levels remained normal in these dogs.

2. VPRC and Hgb: These values are presented in Fig. 6 to show again the wide variability in these animals. Two of the dogs had values on the low side of normal prior to injection and remained so during the next 9 months. There was no evidence of any depressive effects on the red cell values at this dose level.

Figure 5. Leukocyte responses following injection of 0.29 $\mu\text{Ci/kg}$ of einsteinium (3-level).

Figure 6. Eosinophils, hemoglobin, and VPRC for the same dogs as Fig. 5.

DISCUSSION

The early hematological effects of curium and einsteinium were similar to those seen after injection of comparable activities of other transuranic elements in beagles (6, 7, 8). For example, after injection of the 5-level of ^{239}Pu , ^{241}Am , ^{252}Cf , ^{249}Cf and the 3-level of ^{228}Th , there was a drop in WBC of 80-85% of preinjection values during the first month post-injection. The granular leukocytes (segmented PMNS and eosinophils) and monocytes were depressed to 90-95% of preinjection values at 2-3 weeks after injection. Blood lymphocytes exhibited a slower downward trend with the minimal values reached between 6 and 12 months after injection (9).

The severity of the acute depressive effects on blood cells and rate of recovery of these cells were related to the intensity and duration of the α -radiation as well as to the site of deposition. This is well illustrated in relation to curium and einsteinium of which the latter has an extremely short half-life. The initial effect on the granular leukocytes was the same, i.e., a 90% drop at 3 weeks. However, the pattern of recovery was quite different. The recovery of the cells in the dogs injected with einsteinium was much more rapid and complete than with curium. In fact, the granular leukocytes of the curium dogs did not show recovery to pre-injection levels at any time during the first year. The platelets of 5-level curium dogs were also below normal levels throughout the first year. There was also an indication of a further depression of granular leukocytes and platelets at 12 months on the 5-level curium dogs.

The depressive effects on VPRC and Hgb after curium and einsteinium was less than on granular leukocytes and appeared at different times (Fig. 2 and Fig. 4). There was an earlier period of anemia in the einsteinium dogs, the rate of recovery was much more rapid in these animals whereas 5-level curium dogs showed low values throughout the first year post-injection.

The 3-level einsteinium dogs did show a moderate depression of WBC and PMNS but with the exception of one dog, there was prompt recovery to normal values. There was only a slight effect on monocytes, eosinophils and lymphocytes and no effect on red cells.

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²¹⁰Pb RETENTION, EXCRETION AND DISTRIBUTION IN BEAGLES FOLLOWING INTRAVENOUS INJECTION

R. D. Lloyd, C. W. Mays, D. R. Atherton,
and F. W. Bruenger

ABSTRACT: Carrier-free ²¹⁰Pb was administered intravenously to 10 young adult beagles in a sodium citrate citric acid solution at pH 3.5. Retention was determined by total-body counting of the 47 keV γ-ray. Total excreta collections from 3 dogs for the first 3 weeks after injection were assayed intact for their ²¹⁰Pb content by γ-ray counting. About 3/4 of the excreted ²¹⁰Pb appeared in the feces. The fraction of the injected activity still in the circulating blood was 0.4 at 1 day, 0.14 at 1 week, and 0.03 at 1 month. By 1 hour following injection, red cells had more than 99% of the ²¹⁰Pb that was in blood. During the first 1600 days after administration the retention of injected Pb in our dogs could be described by the equation,

$$R = 69.7e^{-0.0555t} + 16.9e^{-0.00376t} + 13.4e^{-0.000140t},$$

where R = percent retention at *t* days after injection. One dog sacrificed 28 days after administration had about 63% of the retained ²¹⁰Pb in the skeleton, 20% in the liver, 8% in the blood, and 9% in other soft tissues. Assuming a skeletal mass equal to 7.5% of body weight at injection, the skeleton had the highest concentration of any tissue measured. Liver concentration was about 0.8 times that of the skeleton. Relative liver concentration was much lower in 2 dogs sacrificed 1100 and 1497 days after injection, 0.03 and 0.005 times that in the skeleton, which contained 97% and 99%, respectively, of the ²¹⁰Pb retained in the total-body at death. The effectiveness, rad for rad, of injected ²¹⁰Pb for osteosarcoma production in beagles appeared to be not greatly different from that of ²²⁶Ra. At long times after injection, the ²¹⁰Po/²¹⁰Pb ratio in the skeleton was close to 1.0.

INTRODUCTION

Lead (Pb) contamination is becoming increasingly important in the pollution of the environment (Beasley and Palmer, 1966, Holtzman, 1966, 1968, Jaworowski, 1967, Scientist and Citizen, 1968, Kauranen and Miettinen, 1969, Blanchard and Moore, 1970, Langford, 1971). Moreover, the residual body content of radioactive lead (²¹⁰Pb) has been investigated as a method of assessing the past exposure of uranium miners to ²²²Rn and its radioactive daughters (Black, 1961, 1962, Black et al., 1968, Cohen and Kneip, 1968, Eisenbud et al.,

1969, Rosen et al., 1969, Blanchard 1969, Blanchard et al., 1969, 1973, Fisher, 1969, 1970, Raabe, 1970, Holtzman, 1970, Wagner et al., 1972). Metabolic studies of Pb in large mammals are necessary so that these problems can be understood more clearly.

Previous reports (Atherton et al., 1968, Atherton and Stover, 1969) have described the complications inadvertently introduced into our ^{224}Ra studies by the ^{210}Pb contamination in some ^{228}Th solutions from which our ^{224}Ra was produced. The radiotoxicity of ^{210}Pb must be established so that the biological effects of ^{210}Pb radiation can be distinguished from those of the ^{224}Ra radiation. This is particularly important because of the German experience with ^{224}Ra -injected humans (Spiess, 1969, Spiess and Mays, 1970) and the consequent necessity for evaluating the dosimetry of ^{224}Ra to determine its toxicity relative to ^{226}Ra and other internally-deposited emitters.

METHODS

Three male beagles aged 522 days (T1L5, T2L5, and T3L5) were each given an intravenous injection of carrier-free ^{210}Pb in an 0.08 molar sodium citrate-citric acid buffer solution (pH 3.5). The ^{210}Po daughter had been removed from the ^{210}Pb shortly before injection. ^{210}Pb was administered similarly to seven other beagles (2 males and 5 females), aged 502 to 515 days, as an inadvertent contaminant in their ^{224}Ra injection solution as summarized in Table 1. The $^{210}\text{Po}/^{210}\text{Pb}$ ratio in this injection solution is unknown.

Blood samples were obtained from T1L5, T2L5, and T3L5 by venipuncture on 4 occasions during the first day, beginning at 30 minutes after injection, and at various intervals thereafter for the first 72 days. Whole blood samples were separated into plasma and red cell fractions, and their ^{210}Pb content was determined using Knothole, a sidewell NaI(Tl) detector (Lloyd et al., 1967).

Table 1. Beagles injected with ^{210}Pb

<u>Dog</u>	<u>Sex</u>	<u>Age Days</u>	<u>Weight kg</u>	<u>Injected $\mu\text{Ci } ^{210}\text{Pb}$</u>	<u>Injected $\mu\text{Ci } ^{210}\text{Pb/kg}$</u>
T1L5	M	522	9.78	104.6	10.7
T2L5	M	522	9.16	98.0	10.7
T3L5	M	522	9.78	104.6	10.7
T15Q4	M	515	12.7	46.1	3.63
T16Q2	F	515	9.36	3.86	0.412
T17Q2	F	515	10.2	4.22	0.414
T18Q2	M	502	9.68	3.94	0.407
T19Q1	F	515	11.8	0.746	0.0632
T20Q1	F	515	10.4	0.653	0.0628
T21Q1	F	502	9.08	0.540	0.0595

All urine and feces were collected from T1L5, T2L5, and T3L5 for the first 3 weeks after injection. Urine samples in polyethylene bottles were treated with 40 ml of concentrated HCl to ensure that the ^{210}Pb would remain in solution. Volumes were adjusted to 1 liter with distilled water. Feces were sealed in #2 cans after the addition of enough formalin and distilled water to fill the can. Counting rates of urine samples were compared with the counting rate of a known activity of ^{210}Pb dissolved in 960 ml of distilled water in a polyethylene bottle, identical to those containing the samples, to which 40 ml concentrated HCl had been added. The counting rates of fecal samples were compared with that of a known ^{210}Pb activity dispersed in 480 ml of solution in a #2 can. Samples were placed between two 8 x 4 inch NaI(Tl) crystals for counting (Lloyd et al., 1968). Total-body retention was determined by gamma-ray counting using the 47 keV photon associated with the radioactive decay of ^{210}Pb . The dog was placed in a low-background

counting chamber with 12 inch thick steel walls, and radioactivity measurements were made with a NaI(Tl) crystal 8 inches in diameter and 4 inches thick. The anesthetized dog was placed on its back in a lucite box which curved its body along an arc of 33 cm radius and which positioned its midline at a distance of 33 cm from the center of the crystal face. This box molded the animals to a uniform maximum lateral thickness of 15 cm so that photon self-absorption would be relatively uniform from dog to dog. A ^{210}Pb standard in 10 ml of solution in a glass ampoule was counted inside of a lucite and polyethylene cylinder 13.25 cm in diameter to simulate the average absorption of gamma-radiation by a dog's body (Lloyd, 1970).

One animal, T2L5, was sacrificed 28 days after injection. The dog was exsanguinated and then perfused with physiological saline solution. This procedure eliminated from the tissues much of the ^{210}Pb which was associated with blood. Individual bones and soft tissues were assayed for their ^{210}Pb content by gamma-ray counting. The detectors used were either Knothole or a pair of 8 x 4 inch NaI(Tl) crystals. Corrections were made for the differences in counting rates between ampoule standards and samples of differing photon self-absorption. As a check on the accuracy of our γ -ray analysis, two bones from T2L5, the left humerus and the third lumbar vertebra, were γ -counted using the 8 x 4 inch NaI(Tl) crystals, and then they were subjected to wet ashing and radiochemical analysis using techniques described by Atherton and Stover (1969). By γ -counting, with correction for photon absorption, the percent of the injected ^{210}Pb found in the left humerus and in the third lumbar vertebra appeared to be 0.721% and 0.299%, respectively. Values obtained by radiochemical analysis were 0.719% and 0.296%, in excellent agreement with the values for the intact bones. Two other dogs, T3L5, and T1L5, developed

A

bone sarcomas and were sacrificed at 1100 and 1497 days after injection, respectively. The bones and soft tissues were assayed similarly for their ^{210}Pb content.

RESULTS

^{210}Pb in the blood. By 1 hour following injection, more than 99% of the ^{210}Pb in the blood was associated with the red cell fraction, a condition maintained for at least 72 days. Table 2 lists the calculated percent of the injected ^{210}Pb in the circulation at various times after injection. During the first day, nearly half the ^{210}Pb retained in the total-body was associated with erythrocytes. The total blood volume of each animal used in these calculations was based upon the relationship of blood volume and body weight for beagles in our colony (94.4 ml blood/kg body) determined by Parkinson and Dougherty (1958).

Table 2. Persistence of ^{210}Pb in the circulating blood of 3 beagles following ^{210}Pb injection.

Time after injection	Percent of injected ^{210}Pb in the circulating blood		
	<u>T1L5</u>	<u>T2L5</u>	<u>T3L5</u>
0.5h	50.3%	47.1%	53.5%
1.0h	51.3	49.0	52.1
3.0h	53.1	51.0	56.7
6.5h	52.6	53.8	57.7
1d	38.4	39.6	43.0
2d	28.2	31.8	35.0
3d	22.6	24.8	27.3
7d	12.6	13.7	15.0
13d	6.8	7.5	8.6
21d	4.66	5.72	5.69
27d	3.04	3.19	3.67
36d	2.05		2.01
55d	0.80		1.11
72d	0.480		0.625

^{210}Pb distribution. At 28 days after injection, the skeleton of T2L5 contained 63% of the retained ^{210}Pb . Liver had 20%, the blood had 8% and the other soft tissues had 9% of the retained ^{210}Pb (Table 3). The concentration of ^{210}Pb activity in the liver was the highest of any soft tissue, about 3 times higher than in the kidneys, about 16 times higher than in the spleen, about 8 times higher than in the blood, about 15 times higher than in the combined soft tissue and about 7 times higher than in the whole dog, but was only about 0.8 times that calculated for a 687 gram skeleton (7.5% of body weight at injection). Long after injection, the retained ^{210}Pb was found to be mostly in the skeleton, 97% in T3L5 at 1100 days, and 99% in T1L5 at 1497 days (Table 4). Relative liver concentration, based on a skeletal mass equal to 7.5% of body weight at injection, was only 0.03 and 0.005 times that of the skeleton for these 2 dogs.

^{210}Pb excretion. Results of excreta analysis are shown in Table 5. Also exhibited are retention values obtained by subtracting the cumulative excretion (and removal of activity in blood samples) from 100%. The excretion of ^{210}Pb in these animals was predominantly fecal. About 75% of the ^{210}Pb excreted in the first 3 weeks after injection appeared in the feces. Except for day 1 when 66% of the total excretion was in the feces and for days 4, 14, 15, 16, 20, and 21 when the fecal excretion was between 82% and 89% of the total, values for individual days fell between 70% and 77%.

Measurement of total-body ^{210}Pb . The net counting rates of T1L5, T2L5, and T3L5, soon enough after injection that no loss of ^{210}Pb had occurred, averaged about 3% higher than the corresponding net counting rates of ampoule standards containing duplicates of their respective ^{210}Pb injections. Subsequently, it appeared as if the total-body counting rate per retained μCi was progressively decreasing during the first 3 weeks after injection. Such an effect could be accounted for by our assumption that as ^{210}Pb left the blood

Table 3. Distribution (% of injected ^{210}Pb) and concentration (% of injected ^{210}Pb per gram of tissue) of ^{210}Pb in 3 beagles at death.

Part	T2L5, *† 28 days after injection		T3L5, * 1100 days after injection		T1L5, * 1497 days after injection	
	% inj. Pb	%/g	% inj. Pb	%/g	% inj. Pb	%/g
Skeleton	23.7	0.0345	11.0	0.0150	8.3	0.0113
Liver	7.540	0.0279	0.107	0.00042	0.027	0.00006
Kidney	0.445	0.0091	0.006	0.00014	<0.003	—
Spleen	0.041	0.0017	0.0007	0.00005	<0.003	—
Muscle	0.533	0.0001	0.071	0.00002	0.083	0.00002
Pelt	1.629	0.0010	0.067	0.00004	0.011	0.00001
G. I. Tract	0.296	0.0006	0.009	0.00002	<0.003	—
Blood	3.190	0.0035	<0.003	—	—	—
Testes	0.005	0.0002	<0.003	—	<0.003	—
Brain	0.040	0.0005	<0.003	—	<0.003	—
Heart	0.007	0.0001	<0.003	—	<0.003	—
Lungs & Trachea	0.073	0.0009	0.005	0.00005	0.005	0.00002
Prostate	<0.003	—	<0.003	—	<0.003	—
Eyes	<0.003	—	0.004	0.00035	0.003	0.00028
Pancreas	0.068	0.0025	0.005	0.00033	<0.003	—
Salivary	0.003	0.0003	0.00004	0.00001	0.00006	0.00001
Aorta	<0.003	—	<0.003	—	<0.003	—
Bladder	<0.003	—	—	—	—	—
Thymus	0.0004	0.0002	0.000007	0.000003	0.000065	0.000027
Adrenal	0.0003	0.0004	—	—	0.000016	0.00001
Pituitary	0.00003	0.0008	—	—	—	—
Parathyroids	0.00002	0.0040	—	—	—	—
Tonsil	0.00035	0.0004	—	—	—	—
Lymph Nodes	0.000968	0.0008	0.00046	0.000072	0.00010	0.000012
Thyroid	0.00071	0.0010	0.000053	0.000080	—	—
Total Soft Tissue	13.9	0.00183	0.275	0.000034	0.129	0.000016
Total Dog	37.6	0.0042	11.3	0.0012	8.4	0.0009

* Animal sacrificed by exsanguination.

† About 2500 ml saline solution was used to flush the circulatory system after death.

Table 4. Skeletal distribution: percent of retained skeletal ^{210}Pb .

<u>Bone</u>	<u>T2L5</u> 28 days after injection	<u>T3L5</u> 1100 days after injection	<u>T1L5</u> 1497 days after injection
Skull	14.41%	16.64%	11.25%
Mandibles	4.24	6.07	4.66
Cerv. Vert.	5.55	5.50	5.23
Thor. Vert.	17.13	11.41	8.71
Lumb. Vert.*	10.68	6.98	8.12
Tail	1.16	1.76	1.14
Paws	3.83	5.82	6.24
Radii	1.22	2.15	1.90
Ulnae	1.48	2.36	2.25
Humeri	6.15	7.21	7.67
Tib. + Fib.**	3.88	5.37	5.88
Femurs	5.30	6.52	6.92
Pelvis	5.83	5.99	7.18
Scapulae	4.43	4.44	5.07
Ribs	11.37	9.08	13.67
Sternum	3.34	2.70	4.11
Total	100.0 %	100.0 %	100.0 %

* including sacrum

** including patellae

Table 5. Average urinary and fecal excretion by T1L5, and T3L5 for the first 21 days after ^{210}Pb injection. Retention calculated via loss of ^{210}Pb by excretion and blood samples is shown in the last column.

Days after injection	% Injected ^{210}Pb in urine	% Cumulative urinary excretion	% Injected ^{210}Pb in feces	% Cumulative fecal excretion	% Cumulative total excretion	% Injected ^{210}Pb removed in blood samples	% Cumulative removed in blood	% Total cumulative loss	100% Minus loss (retention)
1	2.19	2.19	4.22	4.22	6.41	2.84	2.84	9.25	90.75
2	1.10	3.29	3.68	7.90	11.19	0.36	3.20	14.39	
3	1.63	4.92	3.97	11.87	16.79	0.27	3.47	20.26	79.74
4	0.51	5.43	3.29	15.16	20.59			24.06	
5	0.93	6.36	2.17	17.33	23.69			27.16	
6	0.68	7.04	2.01	19.34	26.38			29.85	
7	0.52	7.56	1.30	20.64	28.20	0.16	3.63	31.83	68.17
8	0.51	8.07	1.68	22.32	30.39			34.02	
9	0.52	8.59	1.65	23.97	32.56			36.19	
10	0.45	9.04	1.21	25.18	34.22			37.85	
11	0.37	9.41	1.06	26.24	35.65			39.28	
12	0.32	9.73	0.74	26.98	36.71			40.34	
13	0.31	10.04	0.86	27.84	37.88	0.09	3.72	41.60	
14	0.24	10.28	1.41	29.25	39.53			43.25	56.75
15	0.20	10.48	1.47	30.72	41.20			44.92	
16	0.19	10.67	0.88	31.60	42.27			45.99	
17	0.24	10.91	0.72	32.32	43.23			46.95	
18	0.21	11.12	0.62	32.94	44.06			47.78	
19	0.24	11.36	0.60	33.54	44.90			48.62	
20	0.21	11.57	1.65	35.19	46.76			50.48	
21	0.16	11.73	0.78	35.97	47.70	0.06	3.78	51.48	48.52
Total		11.73		35.97	47.70				
% of total excretion		25%		75%	100%				

and soft tissue to become deposited in bone, the attenuation of the low energy, 47 keV photons was increased. Clearly, a calibration factor was required which, (a) matched the total-body counting results to the excretion results for the first 21 days after injection, (b) matched the total-body counting results of T2L5 on day 28 to the total ^{210}Pb content of the bones and soft tissues of T2L5 at sacrifice, and (c) accounted for the changing photon attenuation postulated for the first few weeks after injection without also predicting incredible values at long times after injection. The expression which was chosen to represent such a calibration factor and which gave a reasonable fit to the data was:

$$\text{CF} = 0.97 + 0.2068 (1 - e^{-0.2266t}) \quad (1)$$

where CF = the calibration factor to be multiplied by the dog's total-body net counting rate at t days after injection.

Retention values calculated using results of total-body counting and this calibration factor were slightly lower than those based on excreta analysis. The retention value for T2L5 on day 28 calculated similarly was slightly higher than the retention value based on the analysis of individual bones, organs, and tissues of T2L5. Overall agreement is reasonable, however, and if some excreted ^{210}Pb had not been measured because of inadvertent losses of excreta, or if some retained ^{210}Pb had not been measured in the tissues of T2L5 because of the omission of some tissues from the counting schedule, the agreement would have been even better. Our experience has been that the true retention is characteristically never underestimated by excreta measurements and is characteristically never overestimated by the analysis of individual bones and soft tissues.

The results of total-body counting for all 10 animals included in this study, corrected by the appropriate calibration factor, are reported in Table 6

Table 6. Retention of injected ^{210}Pb in beagles as determined by total-body counting.

<u>DOG</u>	<u>DAYS OF AGE AT INJECTION</u>	<u>INJECTED $\mu\text{Ci } ^{210}\text{Pb/kg}$</u>	<u>DAYS AFTER INJECTION</u>	<u>PERCENT BIOLOGICAL RETENTION</u>
T1L5	522	10.7	1	85.0
			3	76.2
			7	64.8
			14	50.9
			21	44.6
			28	37.8
			73	24.2
			218	14.6
			657	12.6
			1119	11.0
			1497	9.7
T2L5	522	10.7	1	90.4
			3	75.9
			7	67.9
			14	53.6
			21	45.9
			28	40.1
T3L5	522	10.7	1	88.6
			3	78.6
			7	69.7
			14	55.0
			21	47.2
			28	41.3
			73	27.7
			218	17.0
			657	13.1
			1100	10.5
T15Q4	515	3.63	35	43.0
			67	33.0
			99	27.1
			187	22.6
			357	18.3
			503	16.1
			585	16.1
			727	14.8
			1166	13.7
			1628	12.7
T16Q2	515	0.412	67	25.9
			99	22.6
			187	19.8
			357	17.0
			503	13.2

Table 6. (Cont'd.)

<u>DOG</u>	<u>DAYS OF AGE AT INJECTION</u>	<u>INJECTED $\mu\text{Ci } ^{210}\text{Pb/kg}$</u>	<u>DAYS AFTER INJECTION</u>	<u>PERCENT BIOLOGICAL RETENTION</u>
			585	12.2
			727	10.9
			1166	10.0
			1628	9.8
T17Q2	515	0.414	67	32.5
			99	26.3
			187	22.3
			357	20.8
			503	15.4
			585	14.9
			727	13.4
			1166	12.8
			1628	11.5
T18Q2	502	0.407	67	32.2
			99	27.8
			187	23.4
			357	20.4
			503	14.8
			585	15.7
			727	11.2
			1166	10.8
			1628	9.8
T19Q1	515	0.0632	67	31.8
			99	26.2
			187	21.2
			357	19.4
			503	18.0
			585	16.9
			733	15.2
			1628	11.5
T20Q1	515	0.0628	67	31.5
			99	25.4
			187	23.6
			357	16.2
			503	15.5
			585	14.3
			733	12.1
			1628	10.4
T21Q1	502	0.0595	67	32.1
			99	28.3
			187	28.1
			357	20.4
			503	18.7
			585	18.8
			733	16.2
			1628	9.2

and are displayed in Figure 1. We have elected to represent the total-body retention of injected ^{210}Pb in these dogs by a sum of exponentials. The expression found by the method of least squares to describe the biological retention of ^{210}Pb in a typical beagle was:

$$\% \text{ } ^{210}\text{Pb} \text{ retention} = 69.7e^{-0.0555t} + 16.9e^{-0.00376t} + 13.4e^{-0.000140t} \quad (2)$$

as shown in Figure 1. This equation indicates that 69.7% of the injected ^{210}Pb atoms were retained with a biological half-time of 12 days, 16.9% with 184 days, and 13.4% with 4951 days.

DISCUSSION

^{210}Pb in the blood. We found that by 1 hour after injection, more than 99% of the ^{210}Pb in the blood was associated with red cells. Booker et al. (1969) also found that nearly all the Pb in the blood was in the red cell fraction. It has been determined (Bruenger et al., 1973) that about 90% of the ^{210}Pb in erythrocytes is in the cytoplasm, probably bound to hemoglobin. Our results indicated that during the first day, nearly half of the retained total-body lead was in the blood. Similarly, Booker et al. (1969) and Hursh et al. (1969) have reported that in humans, up to a maximum of about 50% of administered ^{212}Pb was fixed in circulating red cells.

^{210}Pb distribution. Concentration measurements in beagles at 840 days after exposure which were reported in Table 1 of Black (1962) resembled those of T3L5 and T1L5 at 1100 and 1497 days after injection in that there was a somewhat uniform soft tissue distribution as well as a greater fraction of the retained activity in the skeleton (80%-90%). The studies of Cohen et al., (1973) indicated that most of the retained Pb in humans is in bone. Blanchard and Moore (1971) also reported that the skeleton was the most important site

Figure 1. Biological retention of injected ^{210}Pb in beagles. Measured retention values are indicated by circles, and serial measurements of the same dog are connected by lines. The curve representing the average retention for all dogs was fit through the data by the method of least squares, parallel to the slopes of the lines representing the retention of individual animals.

Figure 2. Comparison of Pb, Sr, and Ra retention equations for beagles in our colony for about the first 1600 days after injection.

of long-term ^{210}Pb deposition in humans, containing about 79% of the total-body burden. Data of Torvik et al. (1974) showed that the principal site of Pb deposition in the rat is the skeleton.

^{210}Pb excretion and retention. Hursh (1973) also found that the predominant excretion of intravenously injected ^{210}Pb by beagles to be fecal, and reported that after the first 10 days following injection, the feces accounted for 56% to 71% of the total ^{210}Pb excreted. However, the administered ^{210}Pb was not carrier-free and was, perhaps, mainly lead formate. The excreted ^{210}Pb content in the first day's urine produced by the dogs in his study was "... very high ..." and ranged between 7.3% and 16.7% of the injected activity, averaging 10.7%, while the corresponding average of our dogs reported in Table 3 was 2.19%, only about 1/3 of the total ^{210}Pb output during the first day. Excretion of both inhaled and injected ^{212}Pb by the human subjects of Booker et al. (1969), however, was mostly urinary, perhaps at least partly accounted for by the differences in human and canine hemoglobin (Bruenger et al., 1973).

In an earlier study by Bell and Gilliland (1964), four 1.5 year old beagles and three adult mongrel dogs were injected intravenously with ^{210}Pb in "tracer quantities" (chemical form not stated). Total-body retention values were calculated from the measurement of ^{210}Pb in the excreta. The authors stated that "faecal excretion accounted for less than 1% of the total urinary plus faecal ^{210}Pb excretion." Indicated retention of the injected Pb (their Table 1) averaged more than 92% at one week. The cumulative excretion was claimed to have been 8% at the end of the first year and 10% after two years, making the calculated retention at these two points in time 92% and 90%, respectively. However, it seems probable that appreciable ^{210}Pb may have been lost from the feces during sample processing.

Black (1962) exposed 6 female beagles aged 4 to 6 years to ^{210}Pb via inhalation in a radon-enriched atmosphere. Retention was determined by excreta analysis. The proportion of the total ^{210}Pb elimination which was excreted in the feces ranged between 67% at 21 days and 90% at 630 days post exposure. Calculated total-body retention was about 26%-32% at 1 year and about 12%-15% at 2 years after exposure. Following an initial rapid loss of activity, an effective half-time of about 330 days was determined for the period between about 300 and 800 days after exposure.

The striking differences in excretion and calculated retention between the study of Black (1962) and that of Bell and Gilliland (1964) was initially attributed by Cohen and Howells (1968) to the difference in mode of administration. However, comparison of data from other studies with our results shows that the retention and excretion of ^{210}Pb by our Pb-injected beagles were quite similar to the dogs of Black (1962) which were exposed by inhalation, reasonably similar to the dogs of Hursh (1973) which were exposed by intravenous injection, but remarkably different from the reported retention and excretion by dogs of Bell and Gilliland (1964), which were given ^{210}Pb by injection.

Because of the similarities in retention and excretion of our dogs injected with Pb-citrate and both Black's (1962) dogs exposed by inhalation plus Hursh's (1973) dogs injected with Pb-formate, we believe that not all ^{210}Pb excreted in the feces was accounted for in the studies reported by Bell and Gilliland (1964), resulting in both an inaccurate estimate of the ratio of fecal-to-total excretion and incorrectly high estimates of total-body retention. Moreover, retention values derived from excreta measurements for animals in our studies were monitored independently by total-body counting and by tissue analysis. Retention of Black's animals, calculated from

excretion data, agreed with those obtained at autopsy. In the studies of Bell and Gilliland, however, tissue analysis at sacrifice failed to verify the retention calculated from excreta measurements. Determinations of stable Pb excretion for human subjects, cited by Black (1962), agree with his dog data and with our dog data. Aub (1956) reported the retention of 50%-60% at 120 days with a subsequent biological half-time of about 346 days for dogs injected with ^{210}Pb (chemical form not stated). These results also agree closely with those of Black (1962) (Table 7).

Table 7. Retention of ^{210}Pb in dogs at 1 year and 2 years after administration as reported by various authors.

<u>Author</u>	<u>Mode of Administration</u>	<u>Retention</u>	
		<u>1 yr</u>	<u>2 yr</u>
Bell & Gilliland (1964)	injection	92%	90%
Aub (1956)	injection	~34%	~16%
Black (1962)	inhalation	~27%	~14%
Hursh (1973)	injection	22%	18%
This study	injection	17%	13%

Table 4 of Hursh (1973) compares the biological half-times of ^{210}Pb as determined in dogs and baboons by various investigators. The half-time of the longest-lived exponential term in the various retention equations ranged from 120 to 1940 days, averaging about 800 days. Our preliminary data, as of 1970, were included in his table; over the first 700 days after injection, the half-time of the longest lived exponential term in the retention equation for our animals appeared to be about 990 days, but when all our present data for the entire 1600 day period were included, the corresponding longest-lived component had a 4951 day half-time. Perhaps if the studies of Hursh

(1973) were extended in time, the retention half-times might resemble our values more closely.

Using data of other investigators, Fisher (1969) calculated the biological half-time of stable lead in humans to be about 3000 days. He compared his result with the estimates of Holtzman (1962) which were 2085 days in the total-body and 6350 days in the skeleton. Studies also in humans discussed by Kehoe (1961) indicated half-times for lead in the total-body of something like 2000 days.

Hursh (1973) commented on the similarity of ^{210}Pb retention in his beagles to that of ^{226}Ra and ^{90}Sr . Between about 60 and 1600 days, the ^{210}Pb retention equation resulting from our studies (Figure 1) yields similar values to the equations that describe the retention of ^{226}Ra and especially ^{90}Sr in our beagles injected as young adults (Lloyd, 1970). In general, between about 60 and 1600 days after injection, the retention in our beagles of $^{226}\text{Ra} > ^{90}\text{Sr} > ^{210}\text{Pb}$ (Figure 2). Of particular interest is the striking similarity of the final term in each of the 3 equations which indicate that between 13% and 15% of the injected radionuclide was retained with a biological half-time of between 4621 and 4951 days. Of course, the ^{210}Pb retention measurements do not extend as long in time after injection as do those for Ra and Sr, but our data seem to support the suggestion of Hursh (1973) that the similarity among Pb, Ra, and Sr retention may be more closely related to long-term skeletal remodelling rates (and skeletal radionuclide recycling rates) than to differences in chemical behavior of elements that are all incorporated into bone mineral.

Figure 3 compares the toxicity of ^{210}Pb relative to ^{226}Ra for the induction of bone sarcomas in our dogs. The figure shows for these 2 nuclides the average time from injection to death with osteosarcoma as a function of

Figure 3. Comparison of the average time from injection to death with osteosarcoma as a function of average skeletal dose at 1 year before death for beagles injected with 1.07 $\mu\text{Ci } ^{226}\text{Ra/kg}$, 3.2 $\mu\text{Ci } ^{226}\text{Ra/kg}$, 10.4 $\mu\text{Ci } ^{226}\text{Ra/kg}$, and 3.63 or 10.7 $\mu\text{Ci } ^{210}\text{Pb/kg}$ (Mays et al., 1969, Atherton et al., 1968). The values for the three dogs injected with ^{210}Pb (rectangles) fall within or near the envelope produced by connecting the $\pm$ one standard deviation points for the 3 groups of dogs injected with ^{226}Ra (average skeletal doses calculated on the assumption that skeletal weight equals 7.5% of the total-body weight at injection). The elongated rectangle representing T15Q4 has a lower limit equal to the calculated skeletal dose of 2981 rads from ^{210}Pb plus 133 rads from the injected ^{224}Ra and an upper limit that indicates the equivalent total dose if 133 rads of ^{224}Ra radiation is as effective for osteosarcoma induction as 1330 rads from ^{210}Pb and its daughters (see Mays et al., 1969, Spiess and Mays, 1970, Mays and Lloyd, 1972, for discussions of Relative Biological Effectiveness in osteosarcoma production by internal emitters, wherein the RBE of ^{224}Ra relative to ^{226}Ra appears to lie between 1 and 10). An average of these 3 dogs injected with ^{210}Pb is shown by the triangle; the average for an assumption of a ^{224}Ra RBE of 1 for T15Q4 and the average for an RBE of 10 both lie within the symbol. Based on these limited results for only 3 dogs given ^{210}Pb , the hypothesis cannot be rejected that the radiation from ^{210}Pb and its daughters in dogs is somewhat equal, rad for rad, to the radiation from ^{226}Ra and its daughters in producing osteosarcoma.

average skeletal dose at 1 year before death to allow for a reasonable tumor growth period (Thurman et al., 1970). Assumptions used in ^{210}Pb dosimetry were a skeletal mass equal to 7.5% of the total-body weight at injection for all dogs, a skeletal retention described by the final 2 terms of our retention equation (to allow for early soft-tissue ^{210}Pb deposition), the ratio of the retention measured for the individual dog to the retention of the "typical" dog (equation 2), and the decay in bone of 1 atom of ^{210}Bi and 1 atom of ^{210}Po for every atom of ^{210}Pb that decays in the skeleton, since the $^{210}\text{Po}/^{210}\text{Pb}$ ratio is less than 1.0 soon after injection, but should attain a value slightly greater than 1.0 at long times after injection (see equation 2.9, page 478 of Evans, 1955). Since the individual values for T1L5, T3L5, and T15Q4 as well as their average value fall within or near the envelope produced by connecting the points which represent ± 1 S.D. in time to death with osteosarcoma for the 11 dogs injected with $1.07 \mu\text{Ci } ^{226}\text{Ra/kg}$, the 12 dogs injected with $3.2 \mu\text{Ci } ^{226}\text{Ra/kg}$, and the 9 dogs injected with $10.4 \mu\text{Ci } ^{226}\text{Ra/kg}$, the hypothesis cannot be rejected that the radiation from ^{210}Pb and its daughters in dogs is somewhat equal, rad for rad, to the radiation from ^{226}Ra and its daughters in producing osteosarcoma.

Determination of the ^{210}Po by electrochemical displacement, and the ^{210}Pb using methods described by Atherton and Stover (1969) in the left humeri and 3rd lumbar vertebrae of T3L5 at 1100 days after ^{210}Pb injection and T1L5 at 1497 days after ^{210}Pb injection indicated that despite assumptions to the contrary (ICRP, 1969) the ratio of ^{210}Po to ^{210}Pb in the skeleton is about 1.0. Values for the humerus and vertebra of T3L5 were 1.06 and 1.03, respectively, while corresponding ratios for T1L5 were 0.995 and 0.929. These data are in accord with reports of Holtzman (1963), Jaworowski (1964), and Blanchard (1967) that the $^{210}\text{Po}/^{210}\text{Pb}$ ratio in the skeleton approximates

unity. Therefore, the calculation of skeletal doses for ^{210}Pb deposition should include the α -ray contribution from ^{210}Po that is produced in the skeleton from the radioactive decay of ^{210}Pb and subsequently remains in the bone.

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TRANSURANIUM ELEMENTS IN GONADAL TISSUE

Charles W. Mays, Ray D. Lloyd, and David R. Atherton

ABSTRACT: Young adult dogs were injected intravenously with transuranium elements in citrate solution. All of the dogs were beagles, except one female St. Bernard given ^{239}Pu . At sacrifice 1-2127 days after injection, the gonadal retention and concentration relative to that in the total body at injection were:

<u>SEX</u>	<u>NUCLIDES</u>	<u>NUMBER OF DOGS</u>	<u>GONAD RETENTION (% of inj.)</u>	<u>RELATIVE CONC. [gonad $\mu\text{Ci/kg}$ injected $\mu\text{Ci/kg}$]</u>
MALE	^{239}Pu	3	0.0461	0.34
	^{241}Am	12	0.0120	0.08
	^{243}Am & ^{244}Cm	5	0.0122	0.07
	^{249}Bk & ^{252}Cf	3	0.0204	0.11
	Combined Male	23	0.0176	0.11
FEMALE	^{239}Pu	1	0.0013	0.18
	^{241}Am	3	0.0012	0.14
	^{249}Bk & ^{252}Cf	2	0.0038	0.43
	Combined Female	6	0.0021	0.24

Present data indicate very long retention times for transuranium elements in gonadal tissue.

The deposition of transuranium elements in gonadal tissue is very low (Table 1). The average retention in the ovaries (0.002% of the injected nuclide) was almost 10-fold lower than that in the testes (0.02%), reflecting the much smaller size of the ovaries. In adult beagles, the ovaries are about 0.01% of body weight (0.9 g/10,420 g) while the testes are about 0.2% or (20 g/11,690 g). For comparison, the report of the ICRP task group on reference man (now in press) indicates that in adult humans the ovaries are about 0.02% of body weight (11 g/58,000 g) which is a greater fraction than for beagles, while the testes are about 0.05% or (35 g/70,000 g) which is less than for beagles.

In all cases, the canine gonadal concentration was lower than the initial average body concentration, reflecting a competitive high uptake by the skeleton and liver. For ^{239}Pu , the liver concentration in dogs is typically about 10 times higher than body average (30% uptake in the liver which constitutes about 3% of body weight).

Although the gonadal uptake is small, the retention times are long, characteristic of the retention of transuranium elements in other tissues. Autoradiography is in progress to evaluate the local dose received by the reproductive cells.

Table 1. Gonadal retention of transuranium elements

Sex	Nuclides	Days inj. to death	Dog Number	Injected ($\mu\text{Ci/kg}$)	Body wt. at inj. (kg)	Gonad wt. at death (g)	Gonad retention (% of inj.)	Rel. concn gonad $\mu\text{Ci/kg}$ inj. $\mu\text{Ci/kg}$
Male	^{239}Pu	1	T0P5	3.05	11.40	14.80	0.0789	0.608
		134	T79P0.5	0.0523	11.10	20.55	0.0341	0.184
		362	T35P3	0.303	11.90	12.84	0.0254	0.235
			Av. Pu	Male	11.47	16.06	0.0461	0.342
	^{241}Am	8	T33W5.5	4.47	10.50	19.89	0.0060	0.032
		401	M1W5	2.78	10.40	18.00	0.0140	0.081
		448	M2W5	2.83	12.70	12.12	0.0145	0.152
		633	M77W4	0.906	10.50	12.10	0.0106	0.092
		1132	M17W4	0.924	9.87	14.96	0.0165	0.109
		1198	M100W3	0.304	11.00	29.15	0.0140	0.053
		1300	M87W4	0.916	13.10	21.12	0.0099	0.061
		1415	M68W4	0.890	11.80	21.01	0.0080	0.045
		1566	M34W4	0.893	10.70	17.73	0.0108	0.065
		1779	M3W4	0.897	12.60	15.98	0.0096	0.076
		1917	M5W3	0.305	15.00	29.12	0.0131	0.067
		2127	M26W3	0.310	12.40	23.45	0.0169	0.089
			Av. Am	Male	11.71	19.55	0.0120	0.077
	^{243}Pu & ^{244}Cm	6	T2C5	2.64	12.20	24.13	0.0130	0.066
		13	T3C5	2.64	11.40	24.41	0.0170	0.079
		20	T4C5	2.64	12.50	24.46	0.0100	0.051
		87	T6C5	2.90	10.70	23.79	0.0090	0.040
		384	T5C5	2.63	12.80	14.22	0.0119	0.107
			Av. Cm	Male	11.92	22.20	0.0122	0.069
	^{249}Pu & ^{252}Cf	7	T2G5	2.77	10.70	23.90	0.0330	0.148
		36	T1F5	2.81	11.40	20.31	0.0171	0.096
		500	T1G5	2.84	12.20	18.15	0.0111	0.075
			Av. Cf	Male	11.43	20.79	0.0204	0.106
	Av. Pu + Am + Cm + Cf (23 Male)				11.69	19.83	0.0176	0.114
Female	$^{239}\text{Pu}^*$	1506*	T57P2E*	0.0961*	49.40*	3.59*	0.00128*	0.176*
	^{241}Am	15	T57W5	2.77	7.01	0.61	0.00139	0.160
		1510	F6W3	0.310	11.90	0.86	0.00097	0.134
		1847	F7W2	0.0952	12.60	1.25	0.00125	0.126
			Av. Am	Female	10.50	0.91	0.00120	0.140
	^{249}Pu & ^{252}Cf	13	T3F5	2.87	10.70	1.16	0.00566	0.522
		21	T3G5	2.80	9.89	0.60	0.00200	0.330
			Av. Cf	Female	10.29	0.88	0.00383	0.426
	Av. Pu + Am + Cf (5* or 6 Female)				10.42*	0.90*	0.00209	0.241

* Dog T57P2E was a St. Bernard: All other dogs were beagles. The body and ovarian weights for T57P2E were omitted from the averages, although her gonad retention and relative concentration were used.

CORTISOL METABOLISM IN SKIN OF BEAGLES BEARING ^{241}Am :
A POSSIBLE PRE-NEOPLASTIC CHANGE IN STEROID METABOLISM

C.J. Nabors, Jr., and J.S. Hinckley

ABSTRACT: Skin from beagles bearing ^{241}Am with and without tumors and controls was studied. The metabolism of cortisol-4- ^{14}C was significantly elevated over controls in both ^{241}Am groups. This metabolic alteration may indicate a pre-neoplastic change in ^{241}Am injected beagles.

INTRODUCTION

Ionizing radiation decreases the production of corticosteroids from the adrenal glands and alters corticosteroid conjugation and biotransformation by other tissues (1-3). Ungar *et al.*, found that hydroxylations at C-11, C-17 and C-21 were inhibited when perfused calf adrenals were exposed to ionizing radiation from a Co^{60} source (4). *In vivo* studies indicate adrenal steroidogenesis is altered by ionizing radiation in a wide range of doses (5,6). These responses have been observed in studies using both sources of external radiation, and with radionuclides injected into various species of experimental animals. Radiation effects not only the production of steroids by the adrenal cortex, it also effects steroid conjugation and biotransformation by liver, kidney and skin (7,8).

The deposition of actinide elements in experimental animals has been studied extensively (9). It has been found that internally deposited ^{241}Am localizes in connective tissue in addition to its well-known deposition in bone and liver (10). The major effect of ^{241}Am in beagle dogs thus far identified is the production of osteosarcoma. The biotransformation of adrenal steroids by the skin is well known (11-14). Cortisol metabolism in skin wounds varies with the stage of healing (14). The purpose of this study was to observe the effect of ^{241}Am on the metabolism of cortisol by the skin in both injected animals and animals with identifiable carcinoma.

METHODS AND MATERIALS

The animals used were pure bred beagle dogs born and reared in our laboratory. Full thickness skin biopsies were obtained from the backs of beagle dogs under general anesthesia (sodium pentathol, I.V.). The three groups of beagles used in this study were either control animals injected with a radionuclide carrier solution or animals injected with ^{241}Am . All animals injected with ^{241}Am received $0.3 \mu\text{Ci/kg}$, I.V. in a single dose, and were injected in young adulthood at about 17 months of age. The hair back of the dogs was clipped prior to biopsy and each biopsy was weighed. The specimens were finely minced in iced Krebs phosphate buffer, pH 7.4. Cortisol-4- ^{14}C (S.A. $0.15 \mu\text{Ci}/\mu\text{g}$) and cortisone-1,2- ^3H (New England Nuclear, S.A. $250 \mu\text{Ci}/1.7 \mu\text{g}$) were purified by paper chromatography immediately before use (15). An equimolar mixture containing 0.27 nmoles of (15,000 dpm) ^{14}C -cortisol and ^3H -cortisone (150,000 dpm) was added to each incubation flask in 50 λ of propylene glycol. Samples of 0.4 gm tissue mince and 20 ml buffer were incubated with the mixture for 3 hours at 37°C in air in a water bath shaker. Incubations were terminated by addition of an equal volume of warm acetone. Controls containing buffer and steroid were incubated and boiled tissue controls containing minced skin, steroid, buffer and substrate were prepared. All samples (including controls) were extracted three times with warm acetone, then filtered through a double layer of shark skin filter paper and dried under vacuum until all acetone was removed (16). The remaining aqueous residues were extracted four times with ethyl acetate and dried completely. The steroid samples were then suspended in chloroform-methanol (1:1) and authentic reference cortisol and cortisone added to the samples which were

then dried under nitrogen and chromatographed on 8 cm wide Whatman #1 paper in the hexane-formamide system of Zaffaroni (15). The chromatograms were developed for 3 hours to separate any lipid impurities from the steroids. The chromatograms were air dried for 5 minutes and placed in the chloroform-formamide system and further developed for 3 hours. The chromatograms were dried overnight and counted on a Packard Model 7201 gas flow radiochromatogram scanner. Areas of radioactivity corresponding to the authentic cortisol and cortisone were eluted with methanol and aliquots taken for liquid scintillation counting. The remainder of the eluates were then dried and acetylated with acetic-anhydride:pyridine (2:1) overnight. Authentic substance U of Reichstein was added to the eluates containing cortisol. The acetylated samples were then purified by chromatography in benzene-formamide. The chromatograms were developed for 4 hours and counted on the radiochromatogram scanner. The radioactive unknowns and carriers were eluted with methanol and aliquots were taken for liquid scintillation counting. A modification of the method of Mays et al., was used to determine the amounts of ^{14}C and ^3H in each sample (17). The net metabolism of cortisol was then calculated by adding cortisol metabolites and subtracting the amount of cortisone reduced to cortisol in the reverse reaction. The identity of the radioactive steroid metabolites was further confirmed by recrystallization to constant specific activity with the appropriate reference compound.

RESULTS

The beagle dogs used in this study were male and female animals of approximately the same age. The tumor-bearing animals were biopsied at sacrifice, except M26W3 which was biopsied after X-ray tumor diagnosis

and again prior to sacrifice. Serum glutamic-pyruvic transaminase (SGPT) levels were measured at various times, since this enzyme is known to be altered because of hepatic ^{241}Am deposition (18). No SGPT measurements greater than the 95% confidence limit (52 units) were observed in the control animals (Table I). The number of elevated SGPT levels in animals bearing ^{241}Am (designated by W3 following the animal number) were greater in males than in females (Table I).

Table II is a summary of the metabolism of cortisol and cortisone. The conversion of cortisol to cortisone was greater in the ^{241}Am non-tumor animals than in the control animals. The cortisol to cortisone transformation in the tumor-bearing animals, however, was somewhat less than that observed in control dogs. However, the tumor-bearing animals exhibited significant transformation of cortisol to substance U of Reichstein. The total catabolism of cortisol was found to be greater in the tumor animals than in the non-tumor ^{241}Am injected dogs (33% vs 20%). Cortisol metabolism in non-tumor ^{241}Am injected animals was greater than cortisol catabolism in control animals (20 vs 15%). The data in Table II show that steroid biotransformation by dog skin was altered in the direction of increased cortisol catabolism after exposure to ionizing radiation. An analysis of variance between the three groups indicated the presence of significant differences ($p < .05$). Dunnet's t test was used to compare the ^{241}Am injected and ^{241}Am tumor animals with the control group. Both radionuclide injected groups differed significantly from controls ($p < .05$).

DISCUSSION

Previous studies have shown that adrenal steroid biosynthesis, conjugation and metabolism are altered by ionizing radiation (1-8). The preser

study shows increased cortisol metabolism in skin of beagle dogs bearing doses of ^{241}Am . Similarly, there is an increase in cortisol metabolism in skin of ^{241}Am injected dogs that have developed tumors. Since both tumor bearing and non-tumor bearing animals injected with ^{241}Am exhibited significantly increased destruction of cortisol, this altered metabolism may first take place in a preneoplastic stage. All of the ^{241}Am injected beagles with this dose have thus far died with tumors. A similar observation has recently been reported in preneoplastic and tumor bearing AKR mice (19). In the mouse, adenyl cyclase activity was elevated in both stages.

The cortisol and cortisone metabolites that were isolated in this study have been found in prior studies of skin (12-14). One remarkable transformation was the large conversion of cortisol to Reichstein's U in the tumor bearing animals. This metabolic pathway is otherwise inactive (Table II). Similar transformation has been found by our laboratory in skin of a beagle pup (Nabors and Hinckley, unpublished observation), and may be related to the presence of rapidly proliferating tissue. Unusual metabolic pathways have previously been demonstrated in malignant tissue (20).

The alterations in SGPT found in the present study are consistent with previous reports (18). Female beagles of the same age appear to be less resistant to radiation induced increases in SGPT (Table I). Recently, another sex-related radiation effect was described. Renal failure is higher in male beagles receiving ^{60}Co gamma radiation than in females (20).

Cortisol is the most potent endogenously produced anti-inflammatory and tumor inhibitory steroid (20,22). A reduction of the tissue concentrations of this hormone could promote the development of a pre-neoplastic state. With less cortisol present, the organism's ability to inhibit rapid cellular proliferation is diminished. The alteration of cortisol metabolism in skin of ²⁴¹Am bearing animals may be related to stress.

Previous studies on stress effects on steroid metabolism have demonstrated decreased, rather than increased steroid metabolism (20). Further study will be needed to more clearly relate cortisol metabolism to a radiation induced pre-neoplastic state.

TABLE I

Age, length of burden time and elevated serum glutamic pyruvic transaminase levels in control and ^{241}Am injected beagles.

Dog	Sex	Treatment	Age (days)	months post injection	# elevated SGPT measurements
1	F	Control	2701	53.6	--
2	F		1649	35.7	--
3	F		1650	35.7	--
4	M		2472	67.3	--
5	M		2378	60.6	--
6	F	^{241}Am	1816	42.5	--
7	F		1828	42.4	1
8	F		1694	40.1	--
9	M		1921	47.4	17
10	M		1817	44.4	13
11	M	^{241}Am w/tumor*	2547	69.1	13
12	M		2600	70.9	1
13	M		1728	39.5	13

* These samples were obtained from the same animal

Dog	Treatment	% $F_k-4^{14}C \rightarrow E_k-4^{14}C$	% $E_k-1,2-^3H \rightarrow F_k-1,2-^3H$	% $E_k-1,2-^3H \rightarrow U_R-1,2-^3H$	% $F_k-4-^{14}C \rightarrow U_R-4-^{14}C$	Net Cortisol Metabolism	p vs. Control
1-5	Control n=7	17.66 (11.14-18.52)	4.71 (2.84-6.28)	3.63 (2.77-4.51)	-- --	$\bar{x} = 14.97 \pm 2.3 \text{ S.D.}$	
6-10	^{241}Am n=7	21.78 (16.63-27.07)	2.03 (1.08-3.38)	4.49 (1.89-8.86)	-- --	$\bar{x} = 20.62 \pm 4.25 \text{ S.D.}$	<0.05
11-13	^{241}Am w/tumor n=4	12.48 (7.7-14.7)	4.66 (1.38-6.45)	1.97 (1.3-2.45)	16.84 (4.0-25.97)	$\bar{x} = 24.43 \pm 4.94 \text{ S.D.}$	<0.05

Table II. Metabolism of 0.27 nmoles each of cortisol $-4-^{14}C$ and cortisone $-1,2-^3H$. Cortisol (F_k), cortisone (E_k) and substance U of Reichstein (U_R) were isolated. Net metabolism of cortisol was corrected for cortisone transformed to cortisol.

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EFFECTS OF DTPA ON TISSUE CULTURED FIBROBLASTS

Charles J. Nabors, Jr.

ABSTRACT: An in vitro method for evaluating relative toxicities of various chelating agents is being evaluated in our laboratory. Preliminary studies have shown that Ca-DTPA is toxic to L-929 fibroblasts in concentrations ranging from 2.3×10^{-9} to 2.3×10^{-5} M. In contrast, Zn-DTPA was not toxic below 2.2×10^{-5} M. This system may provide a rapid means of screening proposed decorporation agents for potential toxicity.

INTRODUCTION

The possibility of environmental contamination with ^{239}Pu or other actinide elements has increased with the need for nuclear energy as a fuel source. Therefore, it is of prime importance to understand ways in which internally deposited radionuclides can be removed from humans in the event of an accident. Studies on various decorporation agents in this laboratory and others have shown that ZnDTPA is much safer, and only slightly less effective as a decorporation agent than CaDTPA (1-3). When CaDTPA and ZnDTPA were administered to beagle dogs it was found that CaDTPA was far more toxic. (3)

The present studies are an attempt to develop an in vitro method for evaluating toxicity of various decorporation agents. Previous studies in this laboratory and others have shown that for evaluating the potency of anti-inflammatory agents, the inhibition of fibroblast proliferation in tissue culture provides a reliable biological assay which relates to clinical evaluation of the potency of these drugs in humans (4). This assay has been applied to study the relative toxicity of CaDTPA and ZnDTPA.

METHODS

Murine fibroblasts (L-929) were grown as monolayers in Eagle's minimum essential medium with Earle's balanced salt solution containing 2mM glutamine, 10% calf serum, 100U/ml penicillin and 0.1 mg/ml streptomycin. This medium contains $1.8 \times 10^{-3}M$ Ca^{++} but no added Zn^{++} . Culture tubes (18 X 150 mm) containing 5 ml of medium were inoculated on day zero of the experimental period with 100,000 cells. Following a 24 hour stationary phase to allow cell attachment to the glass surface, the tubes were rotated 4 days at 1/5 revolution per min. On day five, cells were scraped free of the glass with a rubber policeman, mixed 10 times in an automatic syringe and diluted to give cell suspensions of a convenient concentration for cell counting. Counts were made in a Coulter model B cell counter. Details of the methods are described in a previous publication (4). The derivatives of DTPA were prepared by mixing equimolar quantities of DTPA and either Zinc chloride or calcium chloride in saline. The saline solutions were neutralized with NaOH and aliquots not larger than 1 μ l/ml added to tissue culture medium. Five replicate tubes of each concentration of DTPA cortisol or control cultures were utilized to determine each value.

RESULTS

Initial experiments in this series were made with cortisol as a reference since this is a well studied inhibitor of fibroblast proliferation. $CaDTPA$ in concentrations ranging from $2.3 \times 10^{-9}M$ to $2.3 \times 10^{-5}M$ caused an average of 50% inhibition of fibroblast proliferation in tissue culture. This effect took place in a tissue culture medium which contained $1.8 \times 10^{-3}M$ $CaCl_2$ as a normal constituent. In the same incubations, an equivalent inhibition of fibroblast proliferation was obtained with $1.4 \times 10^{-4}mM$ cortisol. $ZnDTPA$ did not inhibit fibroblast proliferation over a range of concentration from

2.2×10^{-9} M to 2.2×10^{-6} M. High concentrations of ZnDTPA (2.2×10^{-5}) did however, inhibit fibroblast proliferation.

DISCUSSION

The results of these experiments suggest that it will be possible to utilize the inhibition of fibroblast proliferation in tissue culture as a bioassay for toxicity studies on at least some decorporation agents. Studies now underway are designed to explore further the relative toxicities of various derivatives of DTPA as well as the relationship of this toxicity to the in vitro decorporation of deposited actinides in beagle dogs. Since previous studies have shown that the fibroblast may be used to evaluate anti-inflammatory drugs, the possibility exists that toxicities of decorporation agents may also be screened in vitro. These preliminary studies and other data from our laboratory (3) indicate that in vitro toxicity of DTPA derivatives is similar to in vivo observations in beagles. This difference in toxicity is similar to the difference in the therapeutic index for CaDTPA and ZnDTPA (2). This assay system may provide a rapid and sensitive means for evaluating toxicity of potential decorporation agents.

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STEROID 11 β -HYDROXYDEHYDROGENASE ACTIVITY IN BEAGLES
BEARING $^{241}\text{Am}^*$

C. J. Nabors, Jr., J. S. Hinckley, and K. L. Fowkes

Department of Anatomy,
Radiobiology Division,
University of Utah,
Salt Lake City, Utah 84132, U.S.A.

ABSTRACT: Skin of beagles bearing injected burdens of ^{241}Am (0.3 $\mu\text{Ci/kg}$) and control, vehicle-injected dogs was incubated in vitro with an equimolar mixture (0.27 n moles) of ^{14}C -cortisol and ^3H -cortisone. Biopsies of 5 ^{241}Am and 6 control animals obtained under pentothal anesthesia were minced and incubated in Krebs phosphate buffer, pH 7.4 for 3 hr at 37°C. The metabolites of these two steroids were isolated by extraction and paper chromatography. The conversion of cortisol to cortisone was higher in skin of ^{241}Am injected animals (8 incubations) than in controls (10 incubations, $p < .05$). The reverse reaction, cortisone conversion to cortisol appeared to be higher in controls although there was no statistical difference. This metabolic picture represents increased destruction of cortisol, the most potent anti-inflammatory, lymphocytolytic glucocorticoid produced in the beagle. Cortisone has no ability to inhibit fibroblast proliferation in a test for anti-inflammatory activity. Preliminary measurements of serum cortisol show that this steroid is decreased in blood of ^{241}Am injected animals. Radioimmunoassay measurements of plasma cortisol and cortisone in these animals will also be presented.

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