Research in Radiobiology

Annual Report of Work in Progress
in the Internal Irradiation Program

MASTER

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

Respectfully Submitted by:
THOMAS F. DOUGHERTY, Director

MARCH 31, 1971

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RESEARCH IN RADIOBIOLOGY

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

Respectfully Submitted by:

Thomas E. Dougherty, Director

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March 31, 1971

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TABLE OF CONTENTS

	Page
Group Leaders and Sections	5
Availability of Previous Reports	6
Current Census of the Beagle Colony	8
Preface to Injection Tables	9
Dosimetry	11
Injection Tables	
I. Toxicity Animals	14
A. Radium-226	14
B. Plutonium-239	24
C. Radium-228 (Mesothorium)	36
D. Thorium-228 (Radiothorium)	42
E. Strontium-90	48
F. Americium-241	54
G. Californium-252	60
H. Californium-249	62
II. Test Animals	64
A. Radium-226	64
B. Plutonium-239	70
C. Radium-228 (Mesothorium)	78
D. Thorium-228 (Radiothorium)	80
E. Strontium-90	82
F. Radium-224 (Quickradium)	86
G. Americium-241	90
H. Lead-210	92
I. Californium-249	94
J. Ancillary	96

TABLE OF CONTENTS (Con't.)

Special Topics	Page
Californium Retention, Excretion and Distribution in Beagles Soon After Injection	102
Early Hematologic Effects of Californium in The Beagle	117
Distribution of Californium-249 and Berkelium-249 in the Soft Tissues of Beagles	126
Intracellular Distribution of ^{249}Cf in Canine Liver	149
The Disappearance of Californium-249 and Californium-252 from the Blood of Beagles	147
Distribution of Injected ^{241}Am in the Beagle Skeleton	151
Subcellular Distribution of $^{241}\text{Am}(\text{III})$ and $^{239}\text{Pu}(\text{IV})$ in Livers Studied Serially	159
Retention of $^{239}\text{Pu}(\text{IV})$ by the Young Adult Beagle	175
Early Skeletal Distribution and Retention of $^{239}\text{Pu}(\text{IV})$ at an Intermediate and Low Dose Level in the Beagle	176
The Interaction of Some Actinide Elements with Blood Constituents	211
Interaction Between Blood Constituents and Some Actinides	212
Accumulation of Macrophages Labelled with Plutonium-239 in Marrow Spaces of Lumbar Vertebral Bodies	228
High Resolution Neutron-Induced Autoradiography of Bone Containing Plutonium-239	240
Electron Microscopy of Radiation Damage in Bone	250
Autoradiographic Study of the Early Distribution of ^{45}Ca in Alveolar Bone of Dogs	270
Effect of Cortisol on Bone Accretion, ^{45}Ca Efflux, and Trabecular Bone Resorption	284
Interrelated Effects of Glucocorticoid and Parathyroid Hormone upon Bone Remodelling	291
Effect of Cortisol on Incorporation of Tritiated-Tetracycline into Bone	311

TABLE OF CONTENTS (Con't.)

	Page
The Distribution of ^3H -Tetracycline in Young-Adult Mouse Bone - A Rapid Method for the Quantitation of New Bone Formation	319
Structural Activity Relationship of Corticosteroids to Growing Bones: Comparison of Activities of Cortisol, Prednisolone, Betamethasone and Dexamethasone	332
Kidney Function in Aging Control Beagle Dogs	344
Genetics of Canine Blood Group "A" and Hypothetical Grouping of the Blood Antigens According to their Serological Properties	350
Growth Dynamics of Osteosarcomas Induced in Beagles by Bone-Seeking Radionuclides	374
Retention and Distribution of Injected ^{210}Pb in Beagles	375
The Association of ^{210}Pb with Constituents of Erythrocytes	376
A Non-linear Interpretation to the "Doubling Dose" Concept	389
Additions to Bibliography (April 1 1970 through March 31 1971)	396
Comparative Effects of ^{239}Pu and ^{241}Am on Biochemical Parameters Effects of Dose and Radionuclide Burden Time	402

GROUP LEADERS AND SECTIONS

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

Section

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Bone	Dr. Webster S.S. Jee
Chemistry	Dr. Walter Stevens
Clinic & Pathology	Dr. Glenn N. Taylor
Hematology	Dr. Jean H. Dougherty
Instrumentation	Mr. Garth D. Westenkow
Physics	Dr. Charles W. Mays
Radiology	Dr. William R. Christensen

AVAILABILITY OF PREVIOUS REPORTS

Copies of our reports may be obtained from the Clearinghouse for Federal Scientific and Technical Information, Springfield, Virginia.

Paper copies \$ 3.00 each; microfiche copies \$ 0.65 each.

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

AVAILABILITY OF PREVIOUS REPORTS (Con't.)

<u>Report</u>	<u>Date</u>	<u>Title</u>
C00-227*	Mar 1963	Research in Radiobiology
C00-228*	Sep 1963	Research in Radiobiology
C00-119-229	Mar 1964	Research in Radiobiology
C00-119-230	Jul 1964	Safety Manual (replace by C00-119-245)
C00-119-231*	Sep 1964	Research in Radiobiology
C00-119-232*	Mar 1965	Research in Radiobiology
C00-119-233*	Sep 1965	Research in Radiobiology
C00-119-234*	Mar 1966	Research in Radiobiology
C00-119-235	Sep 1966	Research in Radiobiology
C00-119-236*	Mar 1967	Research in Radiobiology
C00-119-237*	Mar 1968	Research in Radiobiology
C00-119-238*	Aug 1968	Rb in RBC, Plasma and Urine
C00-119-239	Dec 1968	Cs, Rb and K Metabolism
C00-119-240*	Mar 1969	Research in Radiobiology
C00-119-241*	Mar 1970	Retention and Dosimetry
C00-119-242*	Mar 1970	Research in Radiobiology
C00-119-243*	Jan 1971	Osteosarcoma Growth Dynamics
C00-119-244*	Mar 1971	Research in Radiobiology
C00-119-245*	May 1971	Radiobiology Safety Manual

* Also available on request from this laboratory.

CURRENT CENSUS OF THE BEAGLE COLONY

March 31, 1971

^{252}Cf (californium)	Toxicity dogs	4
	Test dogs	0
^{249}Cf (californium)	Toxicity dogs	4
	Test dogs	1
^{241}Am (americium)	Toxicity dogs	89
	Test dogs	1
^{239}Pu (plutonium)	Toxicity dogs	63
	Test dogs	84
^{228}Th (radiothorium)	Toxicity dogs	29
	Test dogs	0
^{228}Ra (mesothorium)	Toxicity dogs	23
	Test dogs	0
^{226}Ra (radium)	Toxicity dogs	42
	Test dogs	38
^{224}Ra (quickradium)	Test dogs	11
^{210}Pb (lead)	Test dogs	2
^{90}Sr (strontium)	Toxicity dogs	39
	Test dogs	2
X-ray	Test dogs	3
Ancillary (breeding and others)		35
Unassigned dogs		<u>82</u>
TOTAL		552

INJECTION TABLES

Tables I and II list the toxicity and test animals, respectively.

Toxicity animals are those animals which will be 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 i.v. 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 current injection program involves ^{226}Ra and ^{239}Pu at lower dose levels, ^{249}Cf and ^{252}Cf , and test 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" levels of ^{226}Ra , ^{228}Ra (Mesothorium), and ^{90}Sr , and 1.11 times the desired "retained" levels of ^{239}Pu and ^{228}Th (Radiothorium).^{*} The desired "retained activities are the same for all the radionuclides except ^{90}Sr , in which case they are greater by a factor of 10. Injection

^{*}Since radioactive decay and excretion occur continuously, the term "retained" dose is obviously meaningless unless the time after injection is specified. Our present measurements indicate that

average ^{226}Ra retention = 0.25 after 271 days
average ^{239}Pu retention = 0.90 after 6 days
average ^{228}Ra retention = 0.25 after 214 days
average ^{228}Th retention = 0.90 after 6 days
average ^{90}Sr retention = 0.25 after 134 days

level 1 is the basis of the scheme, and is 10 times the maximum permissible concentration of ^{226}Ra in man. Level 1 = $10 \times \frac{0.1 \mu\text{Ci } ^{226}\text{Ra}}{70 \text{ kg man}} = 0.0143$ "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 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 , 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 assignment to serial sacrifice schedule. "E" in the final position is used to denote that the dog listed is not a Beagle from our colony. Any of the above letters denoting a radionuclide may follow the final number, in which case the letter indicates that two radionuclides were given. The injection 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 M1R0 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; these tumors were the subject of a separate report, Research in Radiobiology, September 30, 1963 (C00-228), pp. 95-108. 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 humanely prolonged.

DOSIMETRY

The injection tables include the calculated 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:

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

(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)

$$^{239}\text{Pu} = 0.72 t^{-0.040}$$

$$^{228}\text{Ra} = 0.251e^{-0.982t} + 0.211e^{-0.269t} + 2.10e^{-0.0158t} + 0.177e^{-0.00237t} + 0.151e^{-0.000479t}$$

(pure at $t = 0$)

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

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

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

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

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

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

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

^{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 2.8%, 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.

The careful reader will note that we have extended our toxicity studies to two californium isotopes: ^{249}Cf which decays by α -particle emission and ^{252}Cf which decays both by α -emission and spontaneous fission. To our knowledge, this is the first time that a study has been started to evaluate the cancer-inducing efficiency of fission fragments within tissue. The extremely high linear energy transfer (LET) of this type of radiation makes it especially interesting. Preliminary observations on Cf retention, distribution, binding and hematological effects appear in the first articles of this report, and we anticipate additional new and exciting findings in future years.

TABLE I. TOXICITY ANIMALS (MAR. 31 1971)

A. RADIUM-226

DOG NUMBER	AT INJECTION		INJECTED (μ Ci /KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71	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	2583	
F014R0.0	536	10.80		23	10	64	2350	
M015R0.0	564	12.80		4	2	65	2246	
F016R0.0	469	10.00		7	4	65	2184	
M017R0.0	469	12.50		27	4	66	1799	
F018R0.0	497	12.00		25	5	66	1771	
F019R0.0	533	8.42		13	10	66	1630	
M020R0.0	536	9.70		29	12	66	1553	
F021R0.0	549	9.90		26	1	67	1525	
M022R0.0	533	12.10		22	3	67	1470	
F031R0.0B	536	10.60		23	10	64	2350	
F031R0.0C	536	9.88		23	10	64	2350	
F031R0.0D	542	9.90		21	9	65	2017	
F032R0.0B	542	7.80		21	9	65	2017	
F032R0.0C	532	11.70		21	9	65	2017	
F032R0.0D	532	9.70		21	9	65	2017	
F033R0.0B	532	9.80		21	9	65	2017	
F033R0.0C	496	9.50		25	5	66	1771	
F033R0.0D	496	11.80		25	5	66	1771	
F034R0.0B	525	8.20		26	1	67	1525	
F034R0.0C	520	8.90		22	3	67	1470	
F034R0.0D	484	9.90		22	3	67	1470	
F035R0.0B	502	9.41		1	2	68	1154	
F035R0.0C	502	9.38		1	2	68	1154	
F035R0.0D	552	8.86		9	1	69	811	
F036R0.0B	467	10.10		2	7	68	1002	
F036R0.0C	467	9.17		2	7	68	1002	
F036R0.0D	467	9.08		2	7	68	1002	
F037R0.0B	801	11.10		20	5	69	680	
F037R0.0C	501	10.40		23	9	70	189	
F037R0.0D	501	10.90		23	9	70	189	

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	
F037R0.0B	
F037R0.0C	
F037R0.0D	

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/71	DEATH	
F038R0.0B	501	11.50		23	9	70	189		
F042R0.0B	338	8.00		25	4	69		33	
M013R0.2	529	9.77	0.00577	4	3	64	2583		
F014R0.2	460	8.10	0.00836	23	10	64	2350		
M015R0.2	504	10.80	0.00873	4	2	65	2246		
F016R0.2	486	8.90	0.00665	7	4	65	2184		
M017R0.2	494	11.80	0.00711	27	4	66	1799		
F018R0.2	497	9.30	0.00652	25	5	66	1771		
F019R0.2	533	10.60	0.00785	13	10	66	1630		
M020R0.2	546	11.40	0.00676	29	12	66	1553		
F021R0.2	549	11.50	0.00687	26	1	67	1525		
M022R0.2	533	12.90	0.00961	22	3	67	1470		
M013R0.5	529	11.00	0.0171	4	3	64	2583		
F014R0.5	510	9.75	0.022	23	10	64	2350		
M015R0.5	490	10.40	0.0263	4	2	65	2246		
F016R0.5	500	11.40	0.0205	7	4	65	2184		
M017R0.5	494	9.20	0.0215	27	4	66	1799		
F018R0.5	496	9.10	0.0197	25	5	66	1771		
F019R0.5	533	10.00	0.023	13	10	66	1630		
M020R0.5	536	13.20	0.0206	29	12	66	1553		
F021R0.5	538	8.80	0.0208	26	1	67	1525		
M022R0.5	520	12.30	0.029	22	3	67	1470		
M031R0.5B	508	11.40	0.021	27	4	66	1799		
F031R0.5C	537	9.40	0.0235	22	12	65	1925		
F031R0.5D	537	11.70	0.0238	22	12	65	1925		
M032R0.5B	496	13.40	0.0196	25	5	66	1771		
F032R0.5C	519	10.10	0.0239	22	12	65	1925		
F032R0.5D	509	10.10	0.024	22	12	65	1925		
M033R0.5B	497	12.90	0.0194	25	5	66	1771		
F033R0.5C	527	10.60	0.0212	27	4	66	1799		
F033R0.5D	527	8.70	0.0217	27	4	66	1799		
M034R0.5B	496	10.50	0.0196	25	5	66	1771		
F034R0.5C	524	9.90	0.0215	27	4	66	1799		
F034R0.5D	508	9.70	0.0212	27	4	66	1799		
M035R0.5B	536	10.40	0.0205	29	12	66	1553		
F035R0.5C	532	9.00	0.0201	29	12	66	1553		
F035R0.5D	532	10.20	0.0202	29	12	66	1553		

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

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 AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
M001R1.0	471	8.48	0.0618	20 4 53	5727	170
M002R1.0	627	10.00	0.0876	16 11 53	4054	237
F003R1.0	706	8.68	0.0576	10 3 54	3860	151
M004R1.0	414	8.60	0.0642	7 4 54	2038	108
M005R1.0	490	11.70	0.0436	22 6 54	3780	112
F006R1.0	483	7.23	0.0584	27 7 54	5260	187
M007R1.0	511	11.40	0.0651	24 8 54	3544	167
F008R1.0	861	8.98	0.0559	21 12 54	2988	93
F009R1.0	781	9.88	0.0521	11 4 55	4399	99
M010R1.0	523	11.50	0.0573	27 7 55	4003	157
F011R1.0	511	11.20	0.0522	20 12 55	5580	
F012R1.0	501	9.71	0.0444	17 1 56	3978	124
M013R1.0	529	11.70	0.0527	4 3 64	2583	
F014R1.0	510	10.50	0.0701	23 10 64	1729	148
M015R1.0	490	8.88	0.0797	4 2 65	893	82
F016R1.0	501	8.99	0.0611	7 4 65	2184	
M017R1.0	494	11.40	0.0639	27 4 66	1799	
F018R1.0	496	10.00	0.0589	25 5 66	1771	
F019R1.0	526	11.60	0.0682	13 10 66	1630	
M020R1.0	536	10.00	0.061	29 12 66	1553	
F021R1.0	525	8.10	0.0633	26 1 67	1525	
M022R1.0	484	10.90	0.0861	22 3 67	1470	
F031R1.0B	509	10.40	0.0712	22 12 65	1925	
M001R1.7	523	9.98	0.137	17 1 56	4438	341
M002R1.7	598	7.85	0.163	30 11 56	1273	116
M002R1.7A	493	12.00	0.222	6 3 63	2947	
F003R1.7	473	13.10	0.165	20 12 55	3267	385
M004R1.7	514	6.20	0.163	20 12 55	5495	513
M005R1.7	511	10.10	0.151	20 12 55	4107	488
F006R1.7	491	7.90	0.152	20 12 55	3432	417
M007R1.7	598	7.17	0.163	30 11 56	3142	309
F008R1.7	491	9.50	0.154	20 12 55	2577	378
F009R1.7	598	7.55	0.168	30 11 56	3914	262
M010R1.7	590	9.57	0.167	30 11 56	557	109
M010R1.7A	545	10.60	0.183	7 1 59	4466	
F011R1.7	598	8.17	0.165	30 11 56	5234	
F012R1.7	590	8.95	0.167	30 11 56	2399	210

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	
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	

M001R1.7	OBTURATING ABDOMINAL AORTA AND PULMONARY EMBOLISM
M002R1.7	LYMPHOSARCOMA
M002R1.7A	
F003R1.7	MAMMARY GLAND CARCINOMA
M004R1.7	NEPHRITIS
M005R1.7	OSTEOSARCOMA
F006R1.7	
M007R1.7	BACTERIAL TOXEMIA, INTERSTITIAL CELL ADENOMA
F008R1.7	DRUG ALLERGY
F009R1.7	PYOMETRA
M010R1.7	TRAUMA
M010R1.7A	
F011R1.7	
F012R1.7	UNDETERMINED (NO BONE TUMOR)

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	DEATH	DOSE TO SKELETON (RADS)
M001R2.0	471	8.74	0.382	20 4 53		3440	860
M002R2.0	592	8.21	0.387	16 11 53		3775	625
F003R2.0	541	8.53	0.347	10 3 54		4459	872
M004R2.0	414	10.50	0.361	7 4 54		325	189
M004R2.0A	420	10.60	0.306	11 4 55		4368	1142
M005R2.0	461	11.50	0.267	22 6 54		4703	994
F006R2.0	486	10.60	0.36	27 7 54		4615	1264
M007R2.0	514	11.10	0.413	24 8 54		3425	922
F008R2.0	572	6.95	0.331	21 12 54		4781	996
F009R2.0	592	9.38	0.317	11 4 55		3998	1016
M010R2.0	523	9.95	0.345	27 7 55		3569	1220
F011R2.0	495	9.30	0.31	20 12 55		3297	728
F012R2.0	497	10.30	0.281	17 1 56		2948	742
M001R3.0	473	8.91	1.2	20 4 53		2850	2395
M002R3.0	470	9.02	1.21	16 11 53		2226	1727
F003R3.0	386	7.74	1.11	10 3 54		2497	2323
M004R3.0	412	11.70	1.16	7 4 54		1917	2361
M005R3.0	461	13.00	0.846	22 6 54		2955	2317
F006R3.0	486	9.75	1.14	27 7 54		1932	2246
M007R3.0	514	12.30	1.29	24 8 54		2099	3029
F008R3.0	542	7.76	1.03	21 12 54		2612	1916
F009R3.0	551	8.02	0.987	11 4 55		2487	1839
M010R3.0	525	10.10	1.06	27 7 55		1737	2336
F011R3.0	495	12.90	0.938	20 12 55		1610	1333
F012R3.0	497	11.40	0.883	17 1 56		1897	1639
M001R4.0	471	9.08	3.51	20 4 53		1606	6575
M002R4.0	470	9.53	3.55	16 11 53		1884	6150
F003R4.0	384	8.65	3.33	10 3 54		490	2208
F003R4.0A	598	7.20	3.1	30 11 56		1614	3855
M004R4.0	408	8.83	3.47	7 4 54		1518	6063
M005R4.0	461	13.20	2.42	22 6 54		1659	4505
F006R4.0	486	8.55	3.44	27 7 54		1939	7133
M007R4.0	453	9.55	3.88	24 8 54		1647	5844
F008R4.0	474	8.94	3.14	21 12 54		1324	4615
F009R4.0	542	8.53	3.02	11 4 55		1471	4095
M010R4.0	527	10.80	3.28	27 7 55		1553	7582
F011R4.0	491	10.40	2.84	20 12 55		1469	5273

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
F009R4.0	OSTEOSARCOMA
M010R4.0	OSTEOSARCOMA
F011R4.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
F012R4.0	496	9.61	2.81	17 1 56	1435	3877
M001R5.0	473	9.87	10.5	20 4 53	908	14943
M002R5.0	470	8.85	10.8	16 11 53	1380	18071
F003R5.0	380	7.82	10.1	10 3 54	481	7147
M004R5.0	408	8.90	10.6	7 4 54	1091	16417
M005R5.0	458	10.90	10.1	22 6 54	1220	15433
F006R5.0	486	9.66	10.2	27 7 54	1015	15414
M007R5.0	453	8.85	11.9	24 8 54	1288	16708
F008R5.0	474	7.76	9.68	21 12 54	968	11564
F009R5.0	420	9.16	9.48	11 4 55	1288	15941
M010R5.0	527	10.70	10.2	27 7 55	825	11179

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 WEIGHT (DAYS) (KG)	INJECTED (μ Ci /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 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		4499
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	2583	
F014P0.0	452 9.89		12 5 64	2514	
M015P0.0	527 12.10		23 10 64	2350	
M016P0.0	486 13.90		7 4 65	2184	
M017P0.0	551 12.20		18 11 66	1604	
F018P0.0	536 11.40		29 11 66	1583	
M019P0.0	536 13.10		29 11 66	1583	
F020P0.0	546 8.50		29 12 66	1553	
M021P0.0	549 13.30		26 1 67	1525	
F022P0.0	489 10.60		25 5 67	1406	
M031P0.0B	452 11.80		12 5 64	1763	
M031P0.0C	452 12.60		12 5 64	2514	
M032P0.0B	452 11.20		12 5 64	2514	
M032P0.0C	542 10.30		21 9 65	2017	
M033P0.0B	517 12.10		21 9 65	2017	
M033P0.0C	503 11.70		18 11 65	1959	
M034P0.0B	525 13.50		26 1 67	1525	
M034P0.0C	484 12.70		22 3 67	1470	
M035P0.0B	484 12.50		22 3 67	1470	
M035P0.0C	484 13.10		22 3 67	1470	
M036P0.0B	489 11.00		25 5 67	1406	
M036P0.0C	485 12.20		25 5 67	1406	
M037P0.0B	507 11.70		22 6 67	1378	
M037P0.0C	493 10.40		22 6 67	1378	
M038P0.0B	529 10.70		16 11 67	1231	
M038P0.0C	529 12.20		16 11 67	1231	
M039P0.0B	501 10.70		21 12 67	1196	
M039P0.0C	501 10.10		21 12 67	1196	
M040P0.0B	484 10.30		30 7 68	974	
M040P0.0C	552 11.40		9 1 69	811	
M041P0.0B	560 9.49		17 1 69	803	

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	
M040P0.0B	
M040P0.0C	
M041P0.0B	

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
M042P0.0B	338 11.60		25 4 69	32	
F013P0.1	515 9.46	0.00068	4 3 64	2583	
F014P0.1	452 10.30	0.00055	12 5 64	2514	
M015P0.1	527 9.67	0.00071	23 10 64	2350	
M016P0.1	501 12.00	0.00059	7 4 65	2184	
M017P0.1	551 12.20	0.00057	18 11 66	1604	
F018P0.1	536 9.28	0.0007	29 11 66	1583	
M019P0.1	536 11.60	0.00063	29 11 66	1583	
F020P0.1	536 9.80	0.00075	29 12 66	1553	
M021P0.1	538 11.30	0.00059	26 1 67	1525	
F022P0.1	489 9.80	0.00059	25 5 67	1406	
M031P0.1B	517 12.20	0.00068	4 3 64	2583	
F032P0.1B	549 10.40	0.00059	18 11 65	1959	
M033P0.1B	549 10.80	0.00079	18 11 65	1959	
F034P0.1B	533 11.10	0.00058	18 11 66	1604	
M035P0.1B	489 10.30	0.00059	25 5 67	1406	
F036P0.1B	493 9.79	0.0006	22 6 67	1378	
M037P0.1B	493 11.30	0.00059	22 6 67	1378	
F038P0.1B	513 9.52	0.00057	21 12 67	1196	
M039P0.1B	490 10.50	0.00058	21 12 67	1196	
F044P0.1C	569 8.34	0.00072	2 12 70	119	
F013P0.2	517 9.44	0.00206	4 3 64	2583	
F014P0.2	516 7.44	0.00173	12 5 64	2514	
M015P0.2	505 10.90	0.00201	23 10 64	2350	
M016P0.2	500 11.40	0.00163	7 4 65	2184	
M017P0.2	533 11.80	0.00171	18 11 66	1604	
F018P0.2	530 9.46	0.002	29 11 66	1583	
M019P0.2	530 12.10	0.00198	29 11 66	1583	
F020P0.2	532 8.30	0.00224	29 12 66	1553	
M021P0.2	538 12.10	0.00181	26 1 67	1525	
F022P0.2	485 8.30	0.00176	25 5 67	1406	
M031P0.2B	515 10.70	0.00185	4 3 64	2583	
F031P0.2C	452 11.90	0.00169	12 5 64	2514	
F031P0.2D	429 9.35	0.00186	12 5 64	2514	
M032P0.2B	549 13.60	0.00178	18 11 65	1959	
F032P0.2C	494 10.10	0.00183	4 2 65	2246	
F032P0.2D	490 8.04	0.00193	4 2 65	2246	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M042P0.0B SPECIAL STUDY

F013P0.1
F014P0.1
M015P0.1
M016P0.1
M017P0.1
F018P0.1
M019P0.1
F020P0.1
M021P0.1
F022P0.1
M031P0.1B
F032P0.1B
M033P0.1B
F034P0.1B
M035P0.1B
F036P0.1B
M037P0.1B
F038P0.1B
M039P0.1B
F044P0.1C

F013P0.2
F014P0.2
M015P0.2
M016P0.2
M017P0.2
F018P0.2
M019P0.2
F020P0.2
M021P0.2
F022P0.2
M031P0.2B
F031P0.2C
F031P0.2D
M032P0.2B
F032P0.2C
F032P0.2D

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	DEATH	DOSE TO SKELETON (RADS)
M033P0.2B	513	14.50	0.00178	18 11 65	1959		
F033P0.2C	549	12.50	0.00176	18 11 65	1959		
F033P0.2D	513	12.70	0.00178	18 11 65	1959		
M034P0.2B	533	12.70	0.0017	18 11 66	1604		
F034P0.2C	533	11.50	0.00172	18 11 66	1604		
F034P0.2D	519	9.92	0.00167	18 11 66	1604		
M035P0.2B	489	11.20	0.00173	25 5 67	1406		
F035P0.2C	507	10.50	0.00175	22 6 67	1378		
F035P0.2D	507	9.10	0.00175	22 6 67	1378		
M036P0.2B	479	12.90	0.00177	25 5 67	1406		
F036P0.2C	493	10.40	0.00177	22 6 67	1378		
F036P0.2D	569	8.74	0.00146	16 11 67	1231		
M037P0.2B	529	10.60	0.00149	16 11 67	1231		
F037P0.2C	529	10.10	0.0015	16 11 67	1231		
F037P0.2D	529	7.14	0.00153	16 11 67	1231		
M038P0.2B	517	10.00	0.00152	16 11 67	1231		
F038P0.2C	501	7.95	0.00211	21 12 67	1196		
F038P0.2D	499	9.08	0.00176	21 12 67	1196		
M039P0.2B	542	11.60	0.00214	2 12 70	119		
F039P0.2C	499	9.46	0.00173	21 12 67	1196		
F039P0.2D	499	9.34	0.00176	21 12 67	1196		
F042P0.2C	589	9.55	0.00176	4 9 69	573		
F043P0.2C	542	9.50	0.00239	2 12 70	119		
F013P0.5	517	9.93	0.0054	4 3 64	2388		19
F013P0.5A	501	11.20	0.00495	23 9 70	189		
F014P0.5	516	9.98	0.00493	12 5 64	2514		
M015P0.5	505	8.41	0.00627	23 10 64	2350		
M016P0.5	501	12.60	0.00521	7 4 65	2184		
M017P0.5	533	13.40	0.00506	18 11 66	1604		
F018P0.5	530	8.98	0.00594	29 11 66	1583		
M019P0.5	530	11.90	0.00645	29 11 66	1583		
F020P0.5	532	9.30	0.00553	29 12 66	1553		
M021P0.5	538	9.80	0.00526	26 1 67	1525		
F022P0.5	485	8.10	0.00525	25 5 67	1406		
M031P0.5B	515	10.50	0.00549	4 3 64	1648		14
F031P0.5C	494	8.44	0.00572	4 2 65	2246		
M032P0.5B	549	13.60	0.00546	18 11 65	1959		
F033P0.5B	503	10.10	0.00559	18 11 65	1959		
M034P0.5B	530	12.50	0.00642	29 11 66	1583		
F035P0.5B	501	9.54	0.0052	22 6 67	1378		

DOG
NUMBER

COMMENTS ON DEAD DOGS

M033P0.2B
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
F042P0.2C
F043P0.2C

F013P0.5 MAMMARY CARCINOMA

F013P0.5A
F014P0.5
M015P0.5
M016P0.5
M017P0.5
F018P0.5
M019P0.5
F020P0.5
M021P0.5
F022P0.5

M031P0.5B STATUS EPILEPTICUS
F031P0.5C
M032P0.5B
F033P0.5B
M034P0.5B
F035P0.5B

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (μ Ci /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
M036P0.5B	479 11.50	0.00527	25 5 67	1406	
F037P0.5B	517 8.39	0.00454	16 11 67	1231	
M038P0.5B	517 10.50	0.00448	16 11 67	1231	
F039P0.5B	490 10.90	0.00528	21 12 67	1196	
M042P0.5B	542 13.10	0.00675	2 12 70	119	
M042P0.5C	542 12.20	0.00668	2 12 70	119	
F042P0.5D	542 9.71	0.00668	2 12 70	119	
F043P0.5B	545 11.60	0.00484	3 10 69	544	
F043P0.5C	535 10.70	0.0048	3 10 69	544	
M044P0.5B	445 11.50	0.0036	3 6 69	99	1
M045P0.5B	472 10.30	0.0035	3 6 69	42	1
M046P0.5B	484 11.80	0.00336	3 6 69	7	1
F014P0.7	533 8.98	0.00947	22 7 69	617	
M015P0.7	533 10.30	0.00941	22 7 69	617	
M016P0.7	516 11.90	0.0102	4 9 69	573	
M017P0.7	540 8.04	0.0103	3 10 69	544	
F018P0.7	521 9.66	0.00942	22 7 69	617	
M019P0.7	501 11.60	0.0104	23 9 70	189	
F020P0.7	521 9.18	0.00926	22 7 69	617	
M021P0.7	499 11.10	0.0104	23 9 70	189	
F022P0.7	538 9.69	0.0108	4 9 69	573	
F023P0.7	538 9.56	0.0108	4 9 69	573	
F024P0.7	516 8.90	0.011	4 9 69	573	
M001P1.0	443 9.41	0.015	1 12 52	4572	98
F002P1.0	422 6.85	0.0163	2 3 53	4810	112
M003P1.0	515 8.00	0.0165	1 6 53	4292	102
M004P1.0	608 9.97	0.0139	16 9 53	4549	91
F005P1.0	620 8.80	0.0142	14 10 53	1539	30
F005P1.0A	472 11.00	0.0168	3 9 58	3764	91
F006P1.0	410 7.38	0.014	12 5 54	4292	86
F007P1.0	510 6.36	0.0167	25 10 54	3981	96
M008P1.0	453 10.60	0.0172	15 3 55	3367	83
F009P1.0	556 7.87	0.0168	9 9 55	2257	60
F010P1.0	641 12.00	0.0152	22 11 55	3649	80
M011P1.0	602 8.90	0.0157	24 4 56	5160	116
M012P1.0	629 9.67	0.0167	29 5 56	2374	60
M013P1.0	504 12.70	0.0153	3 9 58	4592	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M036P0.5B
F037P0.5B
M038P0.5B
F039P0.5B
M042P0.5B
M042P0.5C
F042P0.5D
F043P0.5B
F043P0.5C
M044P0.5B
M045P0.5B
M046P0.5B

SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY

F014P0.7
M015P0.7
M016P0.7
M017P0.7
F018P0.7
M019P0.7
F020P0.7
M021P0.7
F022P0.7
F023P0.7
F024P0.7

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 LYMPHADENOPATHY
M008P1.0 OSTEOSARCOMA
F009P1.0 OSTEOSARCOMA
F010P1.0 MAMMARY CARCINOMA
M011P1.0 THYROID CARCINOMA
M012P1.0 CHRONIC PANCREATITIS
M013P1.0

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
F014P1.0	533	10.40	0.0141	22 7 69	617	
M015P1.0	516	12.80	0.0159	4 9 69	573	
M016P1.0	516	10.60	0.0165	4 9 69	573	
M017P1.0	537	10.90	0.0151	3 10 69	544	
F018P1.0	531	9.89	0.014	22 7 69	617	
M019P1.0	501	9.82	0.0159	23 9 70	189	
F020P1.0	521	10.40	0.0141	22 7 69	617	
M021P1.0	499	10.00	0.0156	23 9 70	189	
F022P1.0	531	9.04	0.0139	22 7 69	617	
F023P1.0	538	11.20	0.0163	4 9 69	573	
F024P1.0	516	10.40	0.0163	4 9 69	573	
M001P1.7	657	8.72	0.0475	26 6 56	3025	210
F002P1.7	527	8.62	0.0431	22 11 55	3430	215
M003P1.7	642	8.63	0.0495	26 6 56	3430	246
M004P1.7	673	8.37	0.0484	10 10 56	3312	233
F005P1.7	642	11.60	0.0493	26 6 56	2659	190
F006P1.7	642	10.30	0.0459	26 6 56	2221	170
F007P1.7	756	9.73	0.0481	10 10 56	3353	234
M008P1.7	673	13.60	0.0479	10 10 56	3282	229
F009P1.7	756	9.72	0.0485	10 10 56	2500	180
F010P1.7	739	10.60	0.0495	10 10 56	467	40
F010P1.7A	472	8.07	0.0457	3 9 58	4214	277
M011P1.7	599	11.60	0.0486	24 4 56	2777	200
M012P1.7	673	9.41	0.0491	10 10 56	2973	213
M013P1.7	504	10.60	0.0473	3 9 58	4375	297
M001P2.0	443	7.61	0.0853	1 12 52	2985	370
F002P2.0	422	7.73	0.112	2 3 53	2780	460
M003P2.0	485	10.50	0.094	1 6 53	3185	420
M004P2.0	608	9.84	0.0862	16 9 53	2948	380
F005P2.0	594	8.12	0.0846	14 10 53	2423	310
F006P2.0	417	7.54	0.0902	12 5 54	2947	370
F007P2.0	485	8.40	0.0996	25 10 54	2093	310
M008P2.0	406	9.73	0.0957	15 3 55	1761	250
F009P2.0	552	9.72	0.101	9 9 55	2014	300
F010P2.0	551	7.94	0.0968	22 11 55	2912	410
M011P2.0	599	10.30	0.0961	24 4 56	1617	230
M012P2.0	622	9.98	0.1	29 5 56	2284	370

DOG
NUMBER

COMMENTS ON DEAD DOGS

F014P1.0
M015P1.0
M016P1.0
M017P1.0
F018P1.0
M019P1.0
F020P1.0
M021P1.0
F022P1.0
F023P1.0
F024P1.0

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
F010P2.0	OSTEOSARCOMA
M011P2.0	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	31/3/71	DEATH	
M001P3.0	443	8.00	0.261	1	12	52		1476	580
F002P3.0	422	6.85	0.312	2	3	53		1947	900
M003P3.0	485	8.74	0.291	1	6	53		1604	700
M004P3.0	608	8.51	0.292	16	9	53		1950	840
F005P3.0	650	8.22	0.288	14	10	53		1504	630
F006P3.0	415	8.38	0.282	12	5	54		1617	670
F007P3.0	485	9.00	0.314	25	10	54		1627	750
M008P3.0	406	9.73	0.3	15	3	55		1771	780
F009P3.0	552	7.67	0.3	9	9	55		1894	840
F010P3.0	533	8.94	0.298	22	11	55		1547	700
M011P3.0	599	10.50	0.309	24	4	56		1198	550
M012P3.0	622	10.20	0.308	29	5	56		1659	760
M001P4.0	443	7.61	0.823	1	12	52		1724	2100
F002P4.0	568	8.65	1.03	2	3	53		1556	2380
M003P4.0	485	9.36	0.929	1	6	53		1198	1680
M004P4.0	566	8.74	0.974	16	9	53		1066	1560
F005P4.0	650	7.05	0.872	14	10	53		1245	1650
F006P4.0	420	9.26	0.811	12	5	54		1357	1660
F007P4.0	485	8.45	0.963	25	10	54		1198	1730
M008P4.0	651	9.22	0.887	15	3	55		1157	1560
F009P4.0	552	8.58	0.96	9	9	55		1343	1920
F010P4.0	527	6.48	0.868	22	11	55		1241	1660
M011P4.0	596	9.56	0.927	24	4	56		1288	1790
M012P4.0	598	11.40	0.838	29	5	56		1463	1840
M001P5.0	418	8.86	2.67	1	12	52		1324	5370
F002P5.0	1151	8.75	3.3	2	3	53		1576	7830
M003P5.0	515	8.10	3.0	1	6	53		499	2340
M004P5.0	566	9.18	3.17	16	9	53		1562	7380
F005P5.0	691	8.77	2.77	14	10	53		2059	8690
F006P5.0	407	7.90	2.57	12	5	54		1194	4620
F007P5.0	482	8.33	2.99	25	10	54		1491	6630
M008P5.0	497	9.55	2.69	15	3	55		1192	4840
F009P5.0	552	9.45	2.73	9	9	55		1145	4750

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

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
M008P5.0	GINGIVITIS
F009P5.0	OSTEOSARCOMA, EPISTAXIS + CIRCULATORY COLLAPSE

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CV/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	DEATH	DOSE TO SKELETON (RADS)
F001M0.0	732	7.33		4 1 54		3451	
F002M0.0	545	6.94		29 11 54	5966		
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	2997		
F008M0.0	654	11.60		9 3 60	4039		
M009M0.0	575	12.40		13 4 60	4004		
M010M0.0	581	13.30		17 7 62		2991	
F011M0.0	475	9.31		18 9 62	3116		
M012M0.0	695	10.00		22 12 60	3751		
F001M0.5	492	9.47	0.0173	17 7 62	3179		
F002M0.5	492	9.15	0.0173	17 7 62	3179		
M003M0.5	493	10.80	0.0199	18 9 62	3116		
M004M0.5	475	12.80	0.0199	18 9 62	3116		
F005M0.5	534	7.83	0.0172	23 10 62	3081		
M006M0.5	510	10.30	0.0171	23 10 62		3019	71
F007M0.5	492	8.87	0.0172	17 7 62	3179		
F008M0.5	654	12.60	0.0159	9 3 60	4039		
M009M0.5	485	11.90	0.017	13 4 60	4004		
M010M0.5	492	10.60	0.0174	17 7 62	3179		
F011M0.5	505	7.82	0.0202	18 9 62	3116		
M012M0.5	510	10.60	0.0165	23 10 62	3081		
F001M1.0	718	7.75	0.0463	4 1 54		2952	196
F001M1.0A	590	8.07	0.0512	23 10 62	3081		
F002M1.0	459	8.25	0.0324	29 11 54		5267	213
M003M1.0	575	13.80	0.0589	13 3 56		3157	306
M004M1.0	601	9.90	0.0481	15 1 57		4260	131
F005M1.0	658	8.80	0.049	5 3 57		4565	192
M006M1.0	521	10.60	0.0468	23 4 57		3402	272
F007M1.0	534	9.89	0.0489	4 6 57		2159	149
F008M1.0	654	12.40	0.0491	9 3 60		3886	240
M009M1.0	485	10.10	0.0504	13 4 60	4004		
M010M1.0	492	9.43	0.0501	17 7 62		2966	213
F011M1.0	505	8.91	0.0613	18 9 62	3116		

DOG
NUMBER

COMMENTS ON DEAD DOGS

F001M0.0	PURULENT MENINGOENCEPHALITIS
F002M0.0	
M003M0.0	BRAIN INFARCTION
M004M0.0	VALVULAR ENDOCARDITIS; MYOCARDIAL INFARCTION
F005M0.0	MAMMARY CARCINOMA
M006M0.0	NEPHRITIS
F007M0.0	STATUS EPILEPTICUS
F007M0.0A	
F008M0.0	
M009M0.0	
M010M0.0	MALIGNANT MELANOMA (ORAL)
F011M0.0	
M012M0.0	

F001M0.5	
F002M0.5	
M003M0.5	
M004M0.5	
F005M0.5	
M006M0.5	MALIGNANT MELANOMA (EYE)
F007M0.5	
F008M0.5	
M009M0.5	
M010M0.5	
F011M0.5	
M012M0.5	

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)
M009M1.0	
M010M1.0	MALIGNANT MELANOMA (EYE)
F011M1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
M012M1.0	528	9.27	0.0498	23 10 62	3081	
F001M1.7	510	7.52	0.151	23 10 62	3081	
F002M1.7	560	9.90	0.183	13 3 56	2383	733
M003M1.7	576	11.00	0.18	13 3 56	2709	723
M004M1.7	601	8.94	0.143	15 1 57	2864	424
F005M1.7	658	12.80	0.141	5 3 57	3234	625
M006M1.7	521	10.00	0.144	23 4 57	3424	393
F007M1.7	534	10.20	0.146	4 6 57	2646	600
F008M1.7	654	10.80	0.148	9 3 60	2486	386
M009M1.7	485	12.60	0.149	13 4 60	2799	672
M010M1.7	492	10.10	0.124	17 7 62	3101	748
F011M1.7	505	10.70	0.179	18 9 62	3116	
M012M1.7	524	9.28	0.153	23 10 62	3017	718
F001M2.0	676	7.60	0.276	4 1 54	1780	870
F002M2.0	517	8.25	0.194	29 11 54	965	198
M003M2.0	576	11.00	0.358	13 3 56	619	355
M004M2.0	601	9.88	0.282	15 1 57	2282	1033
F005M2.0	509	8.30	0.295	5 3 57	2688	928
M006M2.0	502	12.40	0.306	23 4 57	2674	1382
F007M2.0	534	10.10	0.298	4 6 57	2239	1064
F008M2.0	654	12.40	0.3	9 3 60	2386	928
M009M2.0	630	9.99	0.302	13 4 60	1254	561
M010M2.0	430	11.20	0.311	17 7 62	2373	1448
F011M2.0	505	7.03	0.381	18 9 62	2878	1096
M012M2.0	524	9.47	0.306	23 10 62	2471	1494
F001M3.0	519	10.40	0.858	4 1 54	918	1833
F002M3.0	460	6.70	0.612	29 11 54	1856	2075
M003M3.0	579	10.40	0.965	13 3 56	1185	2464
M004M3.0	601	10.20	0.916	15 1 57	1176	1592
F005M3.0	531	8.51	0.94	5 3 57	1869	2148
M006M3.0	502	9.09	0.953	23 4 57	1421	1906
F007M3.0	534	9.94	0.907	4 6 57	1463	3145
F008M3.0	633	11.80	0.95	9 3 60	1447	2158
M009M3.0	630	9.83	0.918	13 4 60	1570	2277

DOG
NUMBER

COMMENTS ON DEAD DOGS

M012M1.0

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	
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
F007M3.0	OSTEOSARCOMA
F008M3.0	OSTEOSARCOMA
M009M3.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M010M3.0	581	10.40	1.0	17	7	62	1575	2318
F011M3.0	499	11.00	1.19	18	9	62	1395	2467
M012M3.0	510	12.90	0.987	23	10	62	1638	2365
F001M4.0	510	7.56	2.6	4	1	54	841	5614
F002M4.0	460	6.95	1.86	29	11	54	778	2272
M003M4.0	579	9.65	3.37	13	3	56	418	1795
M003M4.0A	494	7.34	2.64	4	6	57	1063	5604
M004M4.0	609	7.84	2.47	15	1	57	896	2680
F005M4.0	509	9.63	2.67	5	3	57	1064	4358
M006M4.0	502	9.49	2.66	23	4	57	1121	4784
F007M4.0	544	8.40	2.67	4	6	57	1253	4636
F001M5.0	494	7.77	8.11	4	1	54	232	3854
F002M5.0	460	7.35	5.46	29	11	54	780	7666
M003M5.0	579	8.87	10.4	13	3	56	688	14151
M004M5.0	482	7.29	7.89	15	1	57	561	5662
F005M5.0	658	11.10	8.48	5	3	57	770	9559
M006M5.0	580	7.53	8.67	23	4	57	792	6761
F007M5.0	494	7.35	8.92	4	6	57	966	18094

*(μ Ci²²⁸Th/ μ Ci²²⁸Ra) 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		INJECTED (μ Ci /KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/71	DEATH	
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	4551		
F006T0.0	489	9.64		10	1	61		171	
F006T0.0A	688	8.61		15	12	60	3758		
M007T0.0	517	10.50		7	2	61		1412	
M008T0.0	533	10.80		24	5	61	3598		
F009T0.0	569	8.28		29	6	61	3562		
F010T0.0	536	10.40		28	7	61	3533		
F011T0.0	530	9.45		4	6	63	2857		
F012T0.0	492	9.09		9	7	63	2822		
M001T0.2	682	11.40	0.00164	27	3	62	3291		
M002T0.2	682	10.40	0.00166	27	3	62	3291		
F003T0.2	478	9.86	0.00163	27	3	62	3291		
M004T0.2	478	10.00	0.00166	27	3	62	3291		
M005T0.2	625	13.80	0.00162	9	2	60		889	10
M005T0.2A	530	13.40	0.00173	4	6	63	2857		
F006T0.2	489	8.85	0.00176	10	1	61	3732		
M007T0.2	532	10.50	0.00159	7	2	61	3704		
M008T0.2	494	13.90	0.00189	24	5	61	3598		
F009T0.2	569	7.82	0.00171	29	6	61	3562		
F010T0.2	508	10.50	0.0017	28	7	61	3533		
F011T0.2	530	9.76	0.00171	4	6	63	2857		
F012T0.2	492	7.37	0.0019	9	7	63	2822		
M001T0.5	699	14.30	0.00496	7	9	56		3471	45
M002T0.5	455	10.50	0.0049	28	9	54		1976	41
F003T0.5	659	8.59	0.00485	6	6	55		3032	44
M004T0.5	516	8.58	0.0054	18	10	55		2159	46
M005T0.5	513	8.46	0.00522	14	10	58	4551		
F006T0.5	489	9.66	0.0051	10	1	61	3732		
M007T0.5	532	9.11	0.00491	7	2	61	3704		
M008T0.5	533	9.53	0.00562	24	5	61	3598		
F009T0.5	569	8.62	0.00529	29	6	61	3562		
F010T0.5	508	10.20	0.0051	28	7	61	3533		
F011T0.5	530	7.78	0.00518	4	6	63	2857		

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	
F006T0.0	TRAUMA
F006T0.0A	
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	
M007T0.2	
M008T0.2	
F009T0.2	
F010T0.2	
F011T0.2	
F012T0.2	

M001T0.5	CEREBRAL INFARCTION HEMORRHAGE
M002T0.5	STRANGULATION ON VOMITUS + GRAND MAL
F003T0.5	PYOMETRITIS + SECONDARY PERITONITIS
M004T0.5	STATUS EPILEPTICUS + PNEUMONIA
M005T0.5	
F006T0.5	
M007T0.5	
M008T0.5	
F009T0.5	
F010T0.5	
F011T0.5	

DOG NUMBER	AT INJECTION		INJECTED (μ ci /KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F012T0.5	492	9.94	0.00567	9	7	63	1682	45
M001T1.0	493	9.36	0.0146	8	2	54	3172	132
M002T1.0	699	9.27	0.0146	7	9	56	4570	135
F003T1.0	723	8.84	0.0145	7	9	56	4142	133
M004T1.0	699	8.27	0.0146	7	9	56	3217	132
M005T1.0	513	11.90	0.0146	14	10	58	2886	130
F006T1.0	489	8.81	0.015	10	1	61	3273	136
M007T1.0	532	9.18	0.0147	7	2	61	3538	134
M008T1.0	533	8.69	0.0166	24	5	61	3598	
F009T1.0	527	10.00	0.016	29	6	61	2546	140
F010T1.0	508	10.20	0.015	28	7	61	3420	136
F011T1.0	521	7.55	0.0154	4	6	63	2857	
F012T1.0	472	9.96	0.0167	9	7	63	1263	118
M001T1.5	699	7.95	0.0289	7	9	56	2894	258
M002T1.5	458	10.00	0.0293	28	9	54	2576	257
F003T1.5	609	10.30	0.0303	6	6	55	1921	249
M004T1.5	591	8.59	0.0299	18	10	55	2309	257
M005T1.5	598	9.65	0.0286	9	2	60	1624	223
F006T1.5	489	8.14	0.0292	10	1	61	2373	252
M007T1.5	517	8.83	0.0292	7	2	61	384	95
M007T1.5A	521	9.08	0.0311	4	6	63	2857	
M008T1.5	494	11.60	0.0324	24	5	61	2665	286
F009T1.5	527	8.80	0.0306	29	6	61	2983	274
F010T1.5	508	11.60	0.0296	28	7	61	1859	241
F011T1.5	518	11.40	0.0305	4	6	63	2408	281
F012T1.5	465	7.56	0.0329	9	7	63	2120	278
M001T2.0	491	10.20	0.0976	8	2	54	1282	693
M002T2.0	483	9.16	0.0875	28	9	54	1234	611
F003T2.0	474	7.87	0.0908	6	6	55	1541	695
M004T2.0	553	13.00	0.09	18	10	55	78	70
M004T2.0A	650	10.60	0.0899	7	9	56	1222	625
M005T2.0	598	9.12	0.0848	9	2	60	1085	556
F006T2.0	451	8.65	0.0879	10	1	61	1108	583
M007T2.0	517	8.85	0.0881	7	2	61	1015	558

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
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
M005T2.0 OSTEOSARCOMA
F006T2.0 OSTEOSARCOMA
M007T2.0 OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
M008T2.0	533	10.70	0.0981	24 5 61	1078	641
F009T2.0	527	8.09	0.0979	29 6 61	1209	677
F010T2.0	508	10.70	0.0919	28 7 61	1022	584
F011T2.0	518	10.80	0.0904	4 6 63	1038	579
F012T2.0	464	8.92	0.1	9 7 63	1449	748
M001T3.0	314	9.15	0.301	8 2 54	988	1879
M002T3.0	458	11.90	0.301	28 9 54	859	1736
F003T3.0	471	12.00	0.272	6 6 55	547	1164
M004T3.0	606	9.69	0.285	18 10 55	801	1575
M005T3.0	571	10.70	0.269	9 2 60	890	1584
F006T3.0	451	8.83	0.282	10 1 61	1156	1909
M007T3.0	427	9.90	0.266	7 2 61	861	1536
M008T3.0	494	10.10	0.313	24 5 61	685	1566
F009T3.0	511	11.50	0.298	29 6 61	1062	1933
F010T3.0	508	9.26	0.28	28 7 61	971	1732
F011T3.0	518	10.30	0.29	4 6 63	826	1591
F012T3.0	459	11.50	0.32	9 7 63	804	1773
M001T4.0	480	8.32	0.882	8 2 54	645	4237
M002T4.0	458	8.32	0.916	28 9 54	833	5185
F003T4.0	461	7.25	0.8	6 6 55	763	4290
M004T4.0	606	8.81	0.835	18 10 55	793	4587
M001T5.0	480	9.48	2.76	8 2 54	212	5457
M002T5.0	483	8.22	2.63	28 9 54	97	2535

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 31/3/71 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	4915	
M005S0.0	542	10.60		19	11	57	4158	
M006S0.0	466	9.68		27	5	58	4691	
F007S0.0A	462	9.46		7	1	59	3303	
F008S0.0	483	9.29		19	5	59	4334	
F009S0.0	549	12.40		11	8	59	708	
F009S0.0A	535	11.20		4	6	63	2857	
M010S0.0	522	13.90		29	9	59	4201	
F011S0.0	541	9.60		3	11	59	4166	
M012S0.0	605	8.99		6	1	60	4102	
F001S1.0	1525	6.84	0.573	18	1	55	308	21
F001S1.0A	521	9.38	0.588	14	2	56	5524	
M002S1.0	567	8.81	0.606	14	2	56	5077	90
M003S1.0	493	10.90	0.572	11	9	57	4949	
F004S1.0	525	8.96	0.56	15	10	57	4915	
M005S1.0	555	10.20	0.532	19	11	57	2705	71
M006S1.0	466	9.56	0.581	27	5	58	4691	
F007S1.0	524	9.94	0.517	11	11	58	4523	
F008S1.0	483	10.80	0.697	19	5	59	2783	79
F009S1.0	549	11.60	0.534	11	8	59	3601	87
M010S1.0	522	11.50	0.558	29	9	59	4201	
F011S1.0	543	10.30	0.55	3	11	59	4166	
M012S1.0	607	13.70	0.559	6	1	60	4102	
F001S1.7	526	7.41	1.78	14	2	56	5524	
M002S1.7	567	11.60	1.84	14	2	56	4297	405
M003S1.7	493	9.19	1.69	11	9	57	4846	338
F004S1.7	522	9.60	1.68	15	10	57	4628	279
M005S1.7	560	9.85	1.6	19	11	57	1715	165
M005S1.7A	493	11.40	1.78	6	3	63	2947	
M006S1.7	466	10.60	1.72	27	5	58	4691	
F007S1.7	488	10.20	1.6	11	11	58	3990	248
M008S1.7	472	8.47	2.03	19	5	59	1973	214
F009S1.7	549	10.00	1.62	11	8	59	4250	
M010S1.7	519	13.60	1.66	29	9	59	2947	306

DOG
NUMBER

COMMENTS ON DEAD DOGS

F001S0.0	ADRENAL CORTICAL CARCINOMA; MAMMARY CARCINOMA
M002S0.0	BRONCHOGENIC CARCINOMA
M003S0.0	OBTURATING AORTIC EMBOLISM, NEPHRITIS
F004S0.0	
M005S0.0	TRANSITIONAL CELL CARCINOMA
M006S0.0	
F007S0.0A	DIABETES MELLITUS
F008S0.0	
F009S0.0	TRAUMA
F009S0.0A	
M010S0.0	
F011S0.0	
M012S0.0	

F001S1.0	SACRIFICED -IMPROPER INJECTION AGE-
F001S1.0A	
M002S1.0	AORTIC BODY TUMOR
M003S1.0	
F004S1.0	
M005S1.0	STATUS EPILEPTICUS
M006S1.0	
F007S1.0	
F008S1.0	PANCREATIC ISLET CELL CARCINOMA
F009S1.0	FOREIGN BODY PNEUMONIA; ENTERITIS
M010S1.0	
F011S1.0	
M012S1.0	

F001S1.7	
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	
F007S1.7	ARTHRITIS; MAMMARY CARCINOMA
F008S1.7	STATUS EPILEPTICUS, CHRONIC PANCREATITIS
F009S1.7	
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 31/3/71 DEATH	DOSE TO SKELETON (RADS)
F011S1.7	543	11.00	1.68	3 11 59	3180	239
M012S1.7	607	11.90	1.68	6 1 60	4102	
F001S2.0	502	5.59	3.7	18 1 55	3269	477
M002S2.0	567	8.97	3.42	14 2 56	3768	631
M003S2.0	494	7.82	3.39	11 9 57	4295	670
F004S2.0	522	9.68	3.41	15 10 57	4775	736
M005S2.0	560	8.72	3.24	19 11 57	3253	531
M006S2.0	466	9.19	3.5	27 5 58	4691	
F007S2.0	488	11.20	3.19	11 11 58	3421	457
F008S2.0	465	9.49	4.14	19 5 59	3955	685
F009S2.0	473	14.10	3.28	11 8 59	2467	566
M010S2.0	508	10.70	3.34	29 9 59	3436	593
F011S2.0	543	10.40	3.41	3 11 59	4166	
M012S2.0	607	11.60	3.49	6 1 60	4102	
F001S3.0	468	7.36	11.6	18 1 55	5149	3289
M002S3.0	565	9.62	11.6	14 2 56	4263	2605
M003S3.0	494	11.40	10.8	11 9 57	4947	2398
F004S3.0	527	9.17	10.6	15 10 57	3101	1490
M005S3.0	557	8.90	10.1	19 11 57	4640	2137
M006S3.0	466	9.44	10.9	27 5 58	4691	
F007S3.0	486	9.80	10.1	11 11 58	4018	1816
F008S3.0	465	12.50	12.9	19 5 59	4334	
F009S3.0	468	10.00	10.1	11 8 59	4250	
M010S3.0	519	12.50	10.3	29 9 59	2898	2099
F011S3.0	541	9.00	10.8	3 11 59	4166	
M012S3.0	605	8.43	10.2	6 1 60	4102	
F001S4.0	468	8.74	33.3	18 1 55	3682	6613
M002S4.0	567	11.20	32.6	14 2 56	2093	5688
M003S4.0	593	9.83	32.1	11 9 57	2781	4139
F004S4.0	528	8.24	32.1	15 10 57	4844	7780
M005S4.0	562	9.65	30.6	19 11 57	4427	6277
M006S4.0	504	16.00	32.7	3 9 58	3530	6899
F007S4.0	478	10.90	30.9	11 11 58	4523	
F008S4.0	465	10.90	40.6	19 5 59	2206	7766

DOG
NUMBER

COMMENTS ON DEAD DOGS

F011S1.7 AORTIC THROMBUS; METASTATIC CALCIFICATION OF LUNGS
M012S1.7

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
F007S2.0 PANCREATIC ISLET CELL ADENOMA
F008S2.0 PNEUMONIA
F009S2.0 UNDETERMINED (NO BONE TUMOR)
M010S2.0 BACTERIAL VALVULAR ENDOCARDITIS
F011S2.0
M012S2.0

F001S3.0 UNDETERMINED (NO BONE TUMOR)
M002S3.0 NEPHRITIS
M003S3.0 SEMINOMA; HYDROCEPHALUS
F004S3.0 MAMMARY CARCINOMA
M005S3.0 SERTOLI CELL TUMOR
M006S3.0
F007S3.0 MAMMARY CARCINOMA; THYROID CARCINOMA
F008S3.0
F009S3.0
M010S3.0 FIBROSARCOMA (GINGIVA)
F011S3.0
M012S3.0

F001S4.0 NOT DETERMINED (NO OSTEOSARCOMA)
M002S4.0 SQUAMOUS CELL CARCINOMA -GINGIVA-
M003S4.0 OBSTRUCTING PULMONARY EMBOLISM
F004S4.0 SQUAMOUS CELL CARCINOMA; ECTOPIC THYROID CARCINOMA
M005S4.0 HEMANGIOSARCOMA (SPLEEN)
M006S4.0 SEMINOMA
F007S4.0
F008S4.0 UNDETERMINED (NO BONE TUMOR)

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	DEATH	DOSE TO SKELETON (RADS)
F009S4.0	468	9.56	30.6	11 8 59	4250		
M010S4.0	517	8.20	31.3	29 9 59	4201		
F011S4.0	542	8.86	32.7	3 11 59		2114	3324
M012S4.0	605	10.90	32.3	6 1 60	4102		
F001S4.5	530	9.00	64.2	16 3 66	1841		
M002S4.5	530	12.20	63.6	16 3 66	1841		
M003S4.5	530	11.90	63.8	16 3 66		1493	5949
F004S4.5	530	9.80	64.5	16 3 66	1841		
M005S4.5	496	13.30	61.3	16 3 66		993	5424
M006S4.5	496	12.00	63.8	16 3 66	1841		
F007S4.5	511	9.90	64.5	16 3 66	1841		
F008S4.5	511	9.90	64.5	16 3 66	1841		
F009S4.5	511	10.30	64.0	16 3 66		1028	5201
M010S4.5	496	14.00	60.9	16 3 66	1841		
F011S4.5	496	11.90	63.8	16 3 66		1758	6434
M012S4.5	485	11.40	63.7	16 3 66	1841		
F001S5.0	434	9.38	103.0	18 1 55		960	8384
M002S5.0	551	12.20	102.0	14 2 56		255	3213
M002S5.0A	545	11.40	96.6	7 1 59		1740	12295
M003S5.0	507	10.30	102.0	15 10 57		2256	16559
F004S5.0	528	11.40	105.0	15 10 57		1448	9527
M005S5.0	621	8.53	95.2	19 11 57		1285	10243
M006S5.0	504	9.33	98.8	3 9 58		35	652
M006S5.0A	462	11.20	94.2	7 1 59		1021	11538
F007S5.0	478	10.20	92.7	11 11 58		1129	10899
F008S5.0	535	11.20	90.5	7 1 59		1469	11132
F009S5.0	459	8.82	93.5	11 8 59		1982	13607
M010S5.0	517	8.55	95.9	29 9 59		990	7657
F011S5.0	542	8.97	102.0	3 11 59		1667	10016
M012S5.0	606	12.50	99.2	6 1 60		1165	8128

DOG
NUMBER

COMMENTS ON DEAD DOGS

F009S4.0
M010S4.0
F011S4.0
M012S4.0

BLOOD DYSCRASIA, PYOMETRA

F001S4.5
M002S4.5
M003S4.5
F004S4.5
M005S4.5
M006S4.5
F007S4.5
F008S4.5
F009S4.5
M010S4.5
F011S4.5
M012S4.5

SEVERE ANEMIA, INFARCTION, MYELOID METAPLASIA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

F001S5.0
M002S5.0
M002S5.0A
M003S5.0
F004S5.0
M005S5.0
M006S5.0
M006S5.0A
F007S5.0
F008S5.0
F009S5.0
M010S5.0
F011S5.0
M012S5.0

OSTEOSARCOMA

STRANGULATED INGUINAL HERNIA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

SEVERE ANEMIA, AUTOAGGLUTINATION, INFARCTION, SPLENOMEGALY
INTESTINAL HEMORRHAGE

OSTEOSARCOMA, INFARCTION + THROMBOCYTOPENIA

STATUS EPILEPTICUS

OSTEOSARCOMA

SQUAMOUS CELL CARCINOMA ARISING FROM FRONTAL SINUS

SEVERE ANEMIA + THROMBOCYTOPENIA

HEMANGIOSARCOMA (LEFT MANDIBLE)

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 31/3/71 DEATH	DOSE TO SKELETON (RADS)
F013W0.2	533	10.60	0.00179	13 10 66	1630	
M014W0.2	533	14.90	0.00178	13 10 66	1630	
F024W0.2	477	11.30	0.00181	21 3 68	1105	
F031W0.2	472	10.90	0.0018	8 5 68	1057	
M041W0.2	467	11.90	0.0018	2 7 68	1002	
M048W0.2	484	11.00	0.00174	30 7 68	974	
M049W0.2	498	10.70	0.00175	25 11 69	491	
F058W0.2	496	10.40	0.00168	26 1 70	429	
M062W0.2	486	13.30	0.00175	24 2 70	400	
F069W0.2	542	10.80	0.0018	22 4 70	343	
M078W0.2	501	13.50	0.00178	16 7 70	258	
M079W0.2	501	10.00	0.00179	16 7 70	258	
F088W0.2	531	8.36	0.00177	25 8 70	218	
M095W0.2	526	13.10	0.00173	25 8 70	218	
F011W0.5	533	8.17	0.00532	13 10 66	1630	
M012W0.5	533	11.90	0.00539	13 10 66	1630	
M023W0.5	487	12.20	0.0053	21 3 68	1105	
M029W0.5	472	10.40	0.00548	8 5 68	1057	
F030W0.5	472	10.60	0.00538	8 5 68	1057	
F040W0.5	467	9.40	0.00528	2 7 68	1002	
M050W0.5	552	11.80	0.00526	25 11 69	491	
M059W0.5	496	11.50	0.00503	26 1 70	429	
M063W0.5	486	11.40	0.00524	24 2 70	400	
M070W0.5	497	12.80	0.00531	22 4 70	343	
F080W0.5	501	11.90	0.00545	16 7 70	258	
F081W0.5	501	12.10	0.00548	16 7 70	258	
F089W0.5	531	9.66	0.00527	25 8 70	218	
M096W0.5	490	12.00	0.00533	25 8 70	218	
F009W1.0	517	8.60	0.016	15 9 66	1658	
F010W1.0	517	9.90	0.0162	15 9 66	1658	
M020W1.0	513	10.80	0.0161	21 3 68	1105	
F021W1.0	513	9.36	0.0166	21 3 68		
F021W1.0A	552	11.40	0.0159	25 11 69	491	
M022W1.0	487	11.60	0.0164	21 3 68	1105	
M028W1.0	472	12.10	0.0158	8 5 68	1057	
F051W1.0	552	8.25	0.0163	25 11 69	491	
M060W1.0	496	10.00	0.0157	26 1 70	429	

232

5

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

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
M028W1.0
F051W1.0
M060W1.0

ACCIDENTAL STRANGULATION

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M064W1.0	486	10.40	0.0158	24	2	70	400	
F071W1.0	485	12.10	0.0157	22	4	70	343	
M082W1.0	501	12.40	0.0163	16	7	70	258	
F090W1.0	526	10.90	0.016	25	8	70	218	
M097W1.0	490	10.40	0.016	25	8	70	218	
F042W1.7	495	9.26	0.0484	30	7	68	974	
F043W1.7	492	10.40	0.0481	30	7	68	974	
F044W1.7	492	7.46	0.0473	30	7	68	974	
M045W1.7	492	11.90	0.0486	30	7	68	974	
M046W1.7	484	8.42	0.0479	30	7	68	974	
M047W1.7	484	11.10	0.0486	30	7	68	974	
F052W1.7	552	9.57	0.0493	25	11	69	491	
M061W1.7	496	10.70	0.0458	26	1	70	429	
M065W1.7	486	11.20	0.0471	24	2	70	400	
F072W1.7	500	11.10	0.0479	22	4	70	343	
M083W1.7	501	12.60	0.0493	16	7	70	258	
F091W1.7	490	13.30	0.048	25	8	70	218	
M098W1.7	490	13.30	0.048	25	8	70	218	
F007W2.0	561	12.60	0.0952	15	9	66	1658	
F008W2.0	561	11.70	0.0957	15	9	66	1658	
M019W2.0	513	13.40	0.097	21	3	68	1105	
M027W2.0	473	12.70	0.0961	8	5	68	1057	
M038W2.0	477	9.88	0.0945	2	7	68	1002	
F039W2.0	468	9.21	0.0948	2	7	68	1002	
M053W2.0	498	9.24	0.096	25	11	69	491	
F066W2.0	486	9.12	0.0935	24	2	70	400	
M073W2.0	552	14.30	0.0965	17	6	70	287	
F084W2.0	493	10.60	0.0984	16	7	70	258	
M085W2.0	493	10.80	0.0987	16	7	70	258	
F092W2.0	490	10.60	0.0962	25	8	70	218	
M005W3.0	561	15.00	0.305	15	9	66	1658	
F006W3.0	561	11.90	0.31	15	9	66	1510	547
F018W3.0	523	8.60	0.307	21	3	68	1105	
M026W3.0	473	12.40	0.31	8	5	68	1057	

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

F008W2.0

M019W2.0

M027W2.0

M038W2.0

F039W2.0

M053W2.0

F066W2.0

M073W2.0

F084W2.0

M085W2.0

F092W2.0

M005W3.0

F006W3.0

F018W3.0

M026W3.0

OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ ci /KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M036W3.0	477	11.00	0.305	2	7	68	1002	
F037W3.0	468	8.44	0.294	2	7	68	1002	
M054W3.0	498	10.50	0.306	25	11	69	491	
M067W3.0	485	11.80	0.295	24	2	70	400	
F074W3.0	542	10.00	0.302	22	4	70	343	
F075W3.0	555	9.42	0.308	17	6	70	287	
M086W3.0	493	11.30	0.312	16	7	70	258	
F093W3.0	490	11.20	0.301	25	8	70	218	
M100W3.0	542	11.00	0.304	2	12	70	119	
M003W4.0	517	12.60	0.897	28	6	66	1737	
F004W4.0	517	9.40	0.911	28	6	66		1533 1976
M017W4.0	523	9.87	0.924	21	3	68	1105	
F025W4.0	473	10.50	0.927	8	5	68	1057	
M034W4.0	477	10.70	0.893	2	7	68	1002	
F035W4.0	477	8.87	0.902	2	7	68	1002	
F055W4.0	498	8.37	0.914	25	11	69	491	
M068W4.0	485	11.80	0.89	24	2	70	400	
F076W4.0	485	9.37	0.899	22	4	70	343	
M077W4.0	500	10.50	0.906	22	4	70	343	
M087W4.0	501	13.10	0.916	16	7	70	258	
F094W4.0	490	11.30	0.912	25	8	70	218	
M001W5.0	517	10.40	2.78	28	6	66	401	1636
M002W5.0	517	12.70	2.83	28	6	66	448	1734

* Preliminary measurements indicate the liver dose to be approximately 4 times that to the skeleton.

²⁴¹Am dogs shown in this table will be maintained until sacrifice becomes a clinical necessity. Their original "T" (test) designation 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
F037W3.0
M054W3.0
M067W3.0
F074W3.0
F075W3.0
M086W3.0
F093W3.0
M100W3.0

M003W4.0
F004W4.0
M017W4.0
F025W4.0
M034W4.0
F035W4.0
F055W4.0
M068W4.0
F076W4.0
M077W4.0
M087W4.0
F094W4.0

KIDNEY, THYROID AND LIVER DEGENERATION

M001W5.0 LIVER DEGENERATION; KIDNEY DEGENERATION
M002W5.0 LIVER DEGENERATION; KIDNEY DEGENERATION

G. CALIFORNIUM-252

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M001F3.0	583	11.60	0.31	3	3	71	28	
F002F3.0	583	10.60	0.31	3	3	71	28	
F003F3.0	583	8.66	0.313	3	3	71	28	
F004F3.0	583	9.69	0.316	3	3	71	28	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001F3.0
F002F3.0
F003F3.0
F004F3.0

H. CALIFORNIUM-249

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F001G3.0	584	11.60	0.29	24	2	71	35	
M002G3.0	580	13.20	0.282	24	2	71	35	
M003G3.0	580	13.70	0.284	24	2	71	35	
F004G3.0	580	8.79	0.283	24	2	71	35	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F001G3.0
M002G3.0
M003G3.0
F004G3.0

TABLE II. TEST ANIMALS (MAR. 31, 1971)

A. RADIUM-226*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	DEATH	DOSE TO SKELETON (RADS)
T001R5.0	996	11.10	10.3	1 12 52	1074		9469
T002R5.0	920	8.40	4.39	12 1 53	1368		4724
T003R5.0	1467	8.29	4.76	12 1 53	428		1316
T004R5.0	459	10.00	10.6	6 7 53	1		24
T005R5.0	126	6.14	11.7	6 10 53	1		25
T006R5.0	126	6.14	11.4	6 10 53	1		24
T007R5.0	126	6.14	11.8	6 10 53	1		25
T008R5.0	290	5.52	1.92	10 5 55	58		488
T009R5.0	2275	10.40	1.94	10 5 55	58		585
T010R5.0	43	1.02	1.98	10 5 55	49		293
T011R5.0	43	1.58	1.91	10 5 55	49		363
T012R5.0	397	12.30	9.72	9 5 56	225		2707
T013R5.0	397	7.59	9.76	9 5 56	188		2319
T014R4.0	674	8.12	3.17	11 7 56	72		380
T015R4.0	672	9.03	3.11	11 7 56	2127		5117
T016R5.0	604	12.40	9.68	11 7 57	12		183
T017R5.0H	383	12.20	9.87	28 10 58	1147		13655
T018R5.0H	383	11.10	10.8	28 10 58	1226		12628
T019R5.0H	383	11.30	10.7	28 10 58	1219		11580
T020R5.0H	393	11.40	10.6	28 10 58	1340		13981
T021R5.0H	381	11.80	10.1	28 10 58	386		3710
T022R5.0H	381	11.90	10.1	28 10 58	587		5911
T023R4.0H	384	9.50	4.05	25 11 58	1471		4340
T024R4.0H	384	11.90	3.24	25 11 58	1505		5593
T025R4.0H	379	11.30	3.42	25 11 58	1309		4672
T026R4.0H	379	11.00	3.48	25 11 58	1780		4719
T027R4.0H	372	11.50	3.34	25 11 58	1414		3382
T028R3.0H	372	11.70	1.11	25 11 58	387		357

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 31/3/71 DEATH	DOSE TO SKELETON (RADS)
T029R5.0	474	13.50	10.4	3 3 59	216	3404
T030R5.0	474	11.50	10.4	3 3 59	178	2851
T031R5.0	471	10.50	10.4	3 3 59	303	4758
T032R3.0	471	11.40	1.13	3 3 59	2249	1864
T033R3.0	471	10.60	1.15	3 3 59	1822	1921
T034R3.0	470	15.70	1.12	3 3 59	1737	1603
T035R3.0J	670	9.44	0.951	5 5 59	8	14
T036R4.0	695	10.20	2.99	22 12 60	1154	3647
T037R4.0	695	9.53	3.0	22 12 60	1627	3234
T038R4.0	695	10.10	3.02	22 12 60	1503	3657
T040R1.0	899	13.00	0.0483	3 4 62	7	1
T041R1.0	899	12.70	0.0487	3 4 62	63	3
T042R1.7	967	14.00	0.146	4 4 62	7	2
T043R1.7	963	13.20	0.145	4 4 62	64	11
T044R3.0	938	11.10	0.937	4 4 62	68	71
T045R3.0	939	13.60	0.941	5 4 62	7	12
T046R3.0	810	12.50	0.928	5 4 62	69	103
T047R6.0	99	5.27	29.4	11 6 62	4	352
T048R6.0	2842	11.20	25.1	27 12 62	49	1597
T049R5.0	485	10.60	7.54	2 5 63	5	111
T050R5.0	485	13.70	7.46	2 5 63	15	274
T051R5.0	418	13.30	8.48	8 5 63	92	1689

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

T049R5.0 SPECIAL STUDY
T050R5.0 SPECIAL STUDY
T051R5.0 SPECIAL STUDY

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T052R5.0	418	10.70	8.57	8	5	63	15	258
T053R5.0	418	12.00	8.5	8	5	63	33	611
T054R5.0	417	11.40	8.76	22	5	63	5	88
T055R5.0	417	11.60	8.61	22	5	63	33	534
T056R5.0	417	11.60	8.61	22	5	63	90	1461
T057R4.0	501	12.10	2.72	15	8	63	14	54
T058R4.0	496	11.70	2.41	15	8	63	61	286
T059R4.0	496	9.64	2.57	15	8	63	63	272
T060R4.0	490	12.10	2.33	15	8	63	117	415
T061R4.0	490	9.48	2.7	15	8	63	371	1567
T062R4.0	490	8.63	2.68	15	8	63	460	1586
T063R3.0	559	8.72	0.899	29	1	64	36	51
T064R3.0	551	8.42	0.919	29	1	64	63	73
T065R3.0	551	11.60	0.922	29	1	64	70	99
T066R3.0	549	10.10	0.904	29	1	64	132	140
T067R3.0	549	12.70	0.898	29	1	64	134	168
T068R3.0	549	12.10	0.917	29	1	64	1667	1033
T069R3.0	499	8.84	0.919	29	1	64	622	651
T070R3.0	499	14.20	0.922	29	1	64	1996	2114
T071R5.0	4025	13.80	9.23	28	1	69	42	698

* 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 - T27R4H; 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
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B. PLUTONIUM-239*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	DEATH	DOSE TO SKELETON (RADS)
T000P5.0	647	11.40	3.05	24 6 52		1	10
T001P5.0	1581	12.70	3.04	13 10 52		29	160
T002P5.0	914	11.90	6.85	15 9 52		44	520
T003P5.0	942	9.65	3.22	13 10 52		610	3040
T004P5.0	1016	8.78	3.02	13 10 52		365	1750
T005P5.0	474	10.40	2.69	14 12 54		400	1720
T006P5.0	527	6.16	2.73	14 12 54		406	1750
T007P5.0	475	7.40	2.68	14 12 54		777	3230
T008P5.0	527	8.32	2.67	14 12 54		863	3540
T009P5.0	551	10.30	2.8	22 11 55		15	80
T010P5.0	534	11.90	2.74	23 11 55		15	70
T011P5.0	516	12.10	2.76	22 11 55		28	140
T012P5.0	487	9.23	2.74	23 11 55		28	140
T013P5.0	587	8.27	3.16	24 4 56		3	20
T014P5.0	587	9.38	2.43	24 4 56		7	30
T015P5.0	737	8.32	2.79	15 10 56		1	10
T016P5.0	673	10.70	2.85	10 10 56		92	440
T017P5.0	739	11.10	3.01	12 2 57		210	990
T018P5.0	739	8.16	2.83	12 2 57		217	960
T019P5.0	688	8.86	2.91	15 12 60		1400	6129
T020P5.0	688	13.00	2.68	15 12 60		474	1970
T021P5.0	688	10.30	2.72	15 12 60		939	3940
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	10
T026P1.0	556	12.00	0.016	28 7 61		647	20
T027P3.0	556	11.50	0.332	28 7 61		755	390
T028P1.0	552	10.50	0.015	9 8 61		559	10
T029P3.0	552	12.10	0.296	9 8 61		560	260

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
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T028P1.0	SPECIAL STUDY
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T029P3.0	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 31/3/71 DEATH	DOSE TO SKELETON (RADS)
T030P1.0	548	12.40	0.0148	9 8 61	35	1
T031P3.0	520	13.00	0.305	9 8 61	40	20
T032P1.0	520	8.47	0.0162	9 8 61	274	10
T033P1.0	550	10.70	0.0153	15 9 61	375	10
T034P1.0	550	9.68	0.0154	15 9 61	746	20
T035P3.0	550	11.90	0.303	15 9 61	362	180
T036P1.0	544	10.40	0.0158	15 9 61	5	
T037P1.0	542	8.59	0.0148	15 9 61	186	4
T038P3.0	489	7.96	0.304	15 9 61	187	90
T039P1.0	1534	10.70	0.0151	15 9 61	376	10
T040P1.0	1534	9.92	0.0177	15 9 61	769	20
T041P5.0	543	8.50	3.01	30 11 64	1227	5586
T042P5.0	510	11.40	2.4	10 2 65	13	57
T043P5.0H	600	14.00	2.86	15 7 65	40	200
T044P5.0H	517	12.00	2.72	21 9 65	35	168
T045P5.0H	420	12.30	2.98	28 10 65	5/24	1
T046P5.0	420	11.90	3.01	28 10 65	732	3402
T047P5.0	806	12.40	3.02	30 11 65	69	353
T048P5.0	554	8.50	2.61	11 3 66	1327	5222

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 31/3/71 DEATH	DOSE TO SKELETON (RADS)
T049P1.0	103	5.00	0.0162	5 7 66	1730	
T050P3.0	103	5.30	0.296	5 7 66	1730	
T051P5.0	104	4.80	2.73	6 7 66	1055	4382
T052P4.0	437	11.80	0.949	7 7 67	14	24
T053P5.0	1517	13.90	2.82	11 3 69	750	
T054P5.0	906	11.30	2.77	11 3 69	404	1769
T055P4.0	445	10.60	0.785	3 6 69	14	20
T056P5.5	501	11.20	3.73	29 7 69	7	48
T057P2.0E	618	49.40	0.0961	10 9 69	567	
T058P3.0E	573	52.30	0.291	10 9 69	567	
T059P3.0E	591	44.50	0.29	5 11 69	511	
T060P3.0E	567	45.20	0.314	6 1 70	449	
T061P2.0E	581	47.20	0.0983	6 1 70	449	
T062P2.0E	583	52.50	0.156	22 1 70	433	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T049P1.0

T050P3.0

T051P5.00 OSTEOSARCOMA

T052P4.0 SPECIAL STUDY

T053P5.0
T054P5.0 SPECIAL STUDY

T055P4.0 SPECIAL STUDY

T056P5.5 SPECIAL STUDY

T057P2.0E

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

T061P2.0E
T062P2.0E

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T063P5.0	581	9.13	2.77	14	12	70	107	
T064P0.0	595	8.60		28	12	70	93	

- * 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 T57 through T62 are St. Bernards.

DOG
NUMBER

COMMENTS ON DEAD DOGS

T063P5.0

T064P0.0

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001M4.5	529	9.13	4.23	8	9	54	314	1598
T002M4.5	463	8.93	4.27	8	9	54	755	5097
T003M5.0	579	9.15	10.6	13	3	56	700	15867

* (μ Ci²²⁸Th/ μ Ci²²⁸Ra) 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 (RADIOTHORIUM)*

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
T001T5.0	607 9.30	4.88	1 12 53	23	1161
T002T5.0	501 8.48	2.56	8 2 54	77	1979
T003T4.0	429 10.40	0.87	8 2 54	820	4878
T004T5.0	455 8.92	2.59	28 9 54	113	2883
T005T5.0	455 10.10	2.32	28 9 54	65	1524
T006T4.0	591 7.01	0.884	18 10 55	651	4273
T007T3.0	591 9.23	0.298	18 10 55	910	1777
T008T3.0	606 9.23	0.293	14 10 58	1043	1883
T009T3.0	447 11.00	0.285	4 2 59	1	3
T010T3.0	447 14.20	0.289	4 2 59	8	24
T011T3.0	500 8.62	0.335	16 6 59	22	76
T012T3.0	514 10.60	0.302	7 7 59	22	69
T013T3.0	754 13.10	0.298	28 7 59	22	68

* T11, 12, 13T3 received 40,4, and 0.4 mg²³²Th, respectively.

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001T5.0 DIED, SPECIAL STUDY
T002T5.0 SPECIAL STUDY

T003T4.0 CRIPPLING FRACTURES + NEPHRITIS

T004T5.0 THROMBOCYTOPENIA + PURPURA
T005T5.0 NEPHRITIS, THROMBOCYTOPENIA + PURPURA

T006T4.0 CRIPPLING FRACTURES

T007T3.0 SPECIAL STUDY
T008T3.0 OSTEOSARCOMA
T009T3.0 SPECIAL STUDY
T010T3.0 SPECIAL STUDY
T011T3.0 SPECIAL STUDY
T012T3.0 SPECIAL STUDY
T013T3.0 SPECIAL STUDY

E. STRONTIUM-90*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71	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	626
T003S5.0	144	6.19	148.0	5 3 54		28	957
T004S5.0	151	7.05	148.0	5 3 54		41	1945
T005S5.0	144	5.25	148.0	5 3 54		116	4863
T006S5.0	155	7.01	87.0	16 3 54		1/24	2
T007S5.0	155	6.74	87.0	16 3 54		2	84
T008S0.0	243	7.00					
T008S2.0H	67	3.69	2.74	27 9 55		66	31
T009S2.0H	67	2.79	3.62	27 9 55		66	40
T010S2.0H	67	3.11	3.25	27 9 55		132	93
T011S2.0H	67	3.85	2.62	27 9 55		132	75
T012S3.0	593	10.60	10.5	11 9 57		5	15
T013S4.0	324	10.50	19.1	8 7 60		8	50
T014S5.0	542	10.00	96.1	7 11 61		9	175
T015S5.0	595	9.43	98.4	7 11 61		30	423
T016S2.0	604	9.71	3.27	8 11 61		9	6
T017S6.0	670	7.18	295.0	19 1 62		14	689
T018S6.0	670	5.94	302.0	19 1 62		1369	18913

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001S0.0 SPECIAL STUDY

T002S5.0 SPECIAL STUDY

T003S5.0 SPECIAL STUDY

T004S5.0 SPECIAL STUDY

T005S5.0 SPECIAL STUDY

T006S5.0 SPECIAL STUDY

T007S5.0 SPECIAL STUDY

T008S0.0 SPECIAL STUDY

T008S2.0H SPECIAL STUDY

T009S2.0H SPECIAL STUDY

T010S2.0H SPECIAL STUDY

T011S2.0H SPECIAL STUDY

T012S3.0 BREMSSTRAHLUNG PHANTOM

T013S4.0 BREMSSTRAHLUNG PHANTOM SAM MCGEE

T014S5.0 SPECIAL STUDY

T015S5.0 SPECIAL STUDY

T016S2.0 SPECIAL STUDY

T017S6.0 LEUKOPENIA, THROMBOCYTOPENIA + PURPURA

T018S6.0 HEMANGIOSARCOMA (ISCHIUM)

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
T019S6.0	670	5.43	284.0	19 1 62	23	637
T020S4.0J	440	8.54	28.9	2 10 63	13	78
T021S2.5J	363	7.20	8.3	2 10 63	13	143
T022S5.0	545	9.01	99.0	1 4 69	729	
T023S5.0	545	11.60	100.0	1 4 69	729	

* 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	
T023S5.0	

F. RADIUM-224*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/71 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	100
T003Q4.0	466	13.10	2.91	27 3 63	2708	100
T004Q5.0	480	9.55	9.71	24 4 63	1462	400
T005Q5.0	455	9.67	9.59	24 4 63	1638	400
T006Q6.0	455	8.29	21.4	17 10 63	13	800
T007Q5.0	465	11.80	8.56	6 11 63	2053	400
T008Q5.0	475	9.77	8.62	6 11 63	16	300
T009Q4.0	503	9.80	2.57	4 12 63	1451	100
T010Q4.0	503	10.30	2.57	4 12 63	262	100
T011Q3.0	495	9.10	0.885	4 12 63	2674	
T012Q3.0	495	13.50	0.889	4 12 63	2674	
T013Q3.0	495	11.30	0.912	4 12 63	2674	
T014Q3.0	438	10.30	0.87	4 12 63	2674	
T015Q4.0	515	12.70	2.73	1 2 68	1154	
T016Q2.0	515	9.36	0.31	1 2 68	1154	
T017Q2.0	515	10.20	0.311	1 2 68	1154	
T018Q2.0	502	9.68	0.306	1 2 68	1154	

DOG NUMBER	COMMENTS ON DEAD DOGS
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T001Q3.00	SPECIAL STUDY
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T002Q4.00	OSTEOSARCOMA
T003Q4.0	HEMANGIOSARCOMA

T004Q5.0	OSTEOSARCOMA, EPIDERMOID CARCINOMA (FRONTAL SINUS)
T005Q5.0	OSTEOSARCOMA

T006Q6.0	PURPURA HEMORRHAGICA
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T007Q5.0	OSTEOSARCOMA
T008Q5.0	PURPURA HEMORRHAGICA

T009Q4.0	STRANGULATION ON VOMITUS AND GRAND MAL
T010Q4.0	STATUS EPILEPTICUS

T011Q3.0	
T012Q3.0	
T013Q3.0	
T014Q3.0	

T015Q4.0	
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T016Q2.0	
T017Q2.0	
T018Q2.0	

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T019Q1.0	515	11.80	0.0475	1	2	68	1154	
T020Q1.0	515	10.40	0.0472	1	2	68	1154	
T021Q1.0	502	9.08	0.0447	1	2	68	1154	

* 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		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T015W5.5	858	11.50	4.53	23	10	67	1	10
T016W5.0	461	10.70	2.78	29	1	68	22	80
T032W5.5	553	11.00	4.46	30	4	68	7	40
T033W5.5	393	10.50	4.47	30	4	68	8	46
T056W5.0	552	11.30	2.9	25	11	69	15	50
T057W5.0	496	7.01	2.77	26	1	70	15	47
T099W5.0	547	11.30	2.67	10	11	70	141	

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T015W5.5 SPECIAL STUDY

T016W5.0 SPECIAL STUDY

T032W5.5 SPECIAL STUDY
T033W5.5 SPECIAL STUDY

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

H. LEAD-210*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/71	DEATH	
T001L5.0	522	9.78	10.7	24	6	69	645		
T002L5.0	522	9.16	10.7	24	6	69		28	180
T003L5.0	522	9.78	10.7	24	6	69	645		

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001L5.0

T002L5.0

T003L5.0

SPECIAL STUDY

I. CALIFORNIUM-249

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/71 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001G5.0	597	12.20	2.84	24	2	71	35	
T002G5.0	584	10.70	2.77	24	2	71		7
T003G5.0	584	9.89	2.8	24	2	71		21

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001G5.0

T002G5.0

T003G5.0

SPECIAL STUDY

SPECIAL STUDY

J. ANCILLARY

DOG NUMBER	DAYS AGE AS OF 31/3/71 DEATH	DOSE TO SKELETON (RADS)
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	4415	
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)
M042A	STATUS EPILEPTICUS
F043A	SPECIAL STUDY
F044A	ADRENAL CORTICAL CARCINOMA

DOG NUMBER	DAYS AGE AS OF 31/3/71 DEATH	DOSE TO SKELETON (RADS)
F045A	5858	
F047A		1732
F048A		5075
F049A	5246	
M050A		2264
F051A		1089
F052A		509
F053A	5010	
F054A		3190
F055A		4563
M056A		701
F057A		4322
M058A		767
M059A		567
M060A		4025
M061A	4775	
F062A	4720	
F063A		4530
F068A		4521
F070A	4131	
M071A		1472
M073A		5695
F074A		5553
M075A		5284
F076A	4041	
F077A	4026	
F078A	3760	
F079A	3671	
F080A	3615	
F081A	3615	
M082A	3378	
F083A	3302	
M084A	3302	
M085A	3572	
M086A		499
F087A	2810	
F088A	2810	
F089A	2966	
F090A	2750	
F091A	2740	
F092A	2740	
M093A	2685	
F094A	2685	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F045A	
F047A	SPECIAL STUDY
F048A	CHRONIC PANCREATITIS
F049A	
M050A	SPECIAL STUDY
F051A	SPECIAL STUDY
F052A	SPECIAL STUDY
F053A	
F054A	SPECIAL STUDY
F055A	NEPHRITIS; METASTATIC CALCIFICATION
M056A	VOLVULUS + PERITONITIS
F057A	UNDIFFERENTIATED MALIGNANCY
M058A	SPECIAL STUDY
M059A	SPECIAL STUDY
M060A	TRANSFERRED TO EXPERIMENTAL GROUP (SEE T071R50).
M061A	
F062A	
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	
F080A	
F081A	
M082A	
F083A	
M084A	
M085A	
M086A	SPECIAL STUDY
F087A	
F088A	
F089A	
F090A	
F091A	
F092A	
M093A	
F094A	

DOG NUMBER	DAYS AGE AS OF 31/3/71 DEATH	DOSE TO SKELETON (RADS)
F095A	2670	
F096A	2653	
F097A	2653	
F098A	2559	
F099A		479
M100A		407
M101A		290
F102A		243
M103A		217
M104A		188
F105A		157
F106A	2254	
F107A	2163	
F108A		1969
F109A	1990	
F110A	1760	
F111A	1748	
F112A	1479	
F113A	615	
F114A	619	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F095A
F096A
F097A
F098A
F099A
M100A
M101A
F102A
M103A
M104A
F105A
F106A
F107A
F108A
F109A
F110A
F111A
F112A
F113A
F114A

SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY

ENCEPHALOMALACIA (BACTERIAL)

CALIFORNIUM RETENTION, EXCRETION AND
DISTRIBUTION IN BEAGLES SOON AFTER INJECTION

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Glenn N. Taylor, and Jerry L. Williams

Abstract: The metabolism of californium has been studied in 11 beagles from 0 to 82 days after intravenous injection with Cf-249 or Cf-252 as Cf(III) in citrate buffer. Total excretion during the first 21 days after injection was mainly urinary. Of the Cf eliminated in this 3 week period, about 3/5 appeared in the first day's collection. Nearly twice as much californium was excreted in 3 weeks after injection as has been observed with beagles given Am-241 or Pu-239. A combination of total-body and partial-body gamma-ray counting of the living dogs indicated that at 1 week about 20% of the injected Cf was deposited in the liver and that nearly 60% remained in non-liver tissue (mainly skeleton). This retention pattern persisted until at least 82 days after injection. In contrast, the retention of Am-241 during a corresponding period averaged about 50% of the injected activity in liver and 40% in skeleton, while values for Pu-239 averaged about 30% in liver and 60% in skeleton. Two dogs were sacrificed for distribution studies 7 and 21 days after the injection of Cf-249. Tissues exhibiting relatively high Cf concentrations were liver, skeleton, kidney, dura, connective tissue, and, in the 7 day dog, the thyroid. Although liver concentration of Cf was lower relative to that of Pu-239 or especially Am-241, kidney concentration of Cf was significantly higher. Skeletal Cf concentration was similar to that for Pu-239 but was about double that for Am-241 at corresponding burden times. Average thyroid concentrations of these 3 elements were highest for americium and californium but lowest for plutonium. Tissues at risk from incorporated californium appear to be liver, skeleton, kidney, and thyroid.

INTRODUCTION

Californium-252 decays by spontaneous fission in about 3% of its radioactive disintegrations.⁽¹⁾ Because of the accompanying emission of fission neutrons, Cf-252 has been proposed as a portable neutron source of very small size with virtually unlimited applications.⁽²⁾ The U. S. Atomic Energy Commission has made Cf-252 available in microgram and milligram quantities for research and development in such varied applications

as cancer therapy, neutron radiography, clinical laboratory tests; pollution control; prospecting for minerals or underground water; non-destructive testing or quality control in manufacturing; short-lived radionuclide production; studies of neutron effects, neutron shielding, or neutron scattering; fissile materials detection; soil moisture determination; chemical or metallurgical studies; in the manufacture of electronic components; civil defense planning; startup of nuclear reactors or sustaining operation of subcritical facilities, and reactor design studies.^(2,3) Estimates have been made of Cf-252 production requirements by 1980 of up to several hundred grams per year.^(2,3) As the use of Cf-252 increases, an accurate assessment of its biological hazard when accidentally incorporated into humans becomes imperative.

The purpose of our present studies is to compare metabolism and effects of Cf-252 in beagles with those of previously studied emitters so that an evaluation of the Cf-252 hazards to man can be made.

Cf-249 was also included in the experiment. Because its radioactive decay is virtually all by alpha emission, comparison of the biological effects of Cf-249 with those of Cf-252 can provide a method of identifying the separate effects of alpha and fission fragment emission by Cf-252. In addition, the discrete 333 and 388 keV gamma-rays of Cf-249⁽¹⁾ make this isotope much more convenient to use in tracer and metabolic experiments than does the continuous spectrum of fission gamma-rays from Cf-252.

CALIFORNIUM DECAY ENERGIES

Cf-249 releases 5.83 MeV of alpha⁽¹⁾ plus 0.10 MeV of recoil energy per disintegration (5.93 MeV total).

Each radioactive disintegration of Cf-252 by fission (3.1%) releases

185 MeV, ⁽⁴⁾ and each disintegration by alpha emission (96.9%) releases 6.11 MeV alpha ⁽¹⁾ plus 0.10 MeV recoil energy (6.21 MeV total). Approximately half of the total alpha-plus-fission dose-rate is accounted for by each of these two modes of Cf-252 decay:

$$\text{fission} = 185 \text{ MeV} \times 3.1\% = 5.74 \text{ MeV}$$

$$\text{alpha} = 6.21 \text{ MeV} \times 96.9\% = \underline{6.02 \text{ MeV}}$$

$$\text{total energy/Cf-252 decay} = 11.76 \text{ MeV}$$

The dose-rate per retained μCi of Cf-252 is therefore nearly twice that of Cf-249.

METHODS

The Cf-252 and Cf-249 used in these studies were both obtained from the Isotopes Sales Division, Oak Ridge National Laboratory. Injection solutions were subsequently prepared by putting Cf(III) into a sodium citrate-citric acid buffer solution of pH 3.5. Eleven young adult beagles were injected intravenously with either Cf-249 or Cf-252 (Table 1). Animals were housed in separate stainless steel metabolism cages for the first 21 days after injection. All excreta produced during this period were collected and assayed for Cf content by comparing the gamma-ray counting rate of each sample with that of a standard in an identical container.

Two of the dogs which had been given Cf-249 were sacrificed for distribution studies, T2G5 at 7 days and T3G5 at 21 days after injection. Individual bones, organs or tissues were assayed for their Cf-249 content by gamma-ray counting. Californium retention in the total-body and in the liver was determined in the living animal by a combination of total-body counting and partial-body counting. Details of this procedure can be found in Refer-

Table 1. Beagles injected with Cf-249 or Cf-252

Isotope	Dog	Sex	Age (days)	Weight (kg)	Injected μCi	$\mu\text{Ci/kg}$
Cf-249*	T1G5	M	597	12.2	34.6	2.84
	T2G5	M	584	10.7	29.6	2.77
	T3G5	F	584	9.89	27.7	2.80
	F1G3	F	584	11.6	3.37	0.29
	M2G3	M	580	13.2	3.72	0.28
	M3G3	M	580	13.7	3.89	0.28
	F4G3	F	580	8.79	2.49	0.28
Cf-252**	M1F3	M	583	11.6	3.5	0.3
	F2F3	F	583	10.6	3.2	0.3
	F3F3	F	583	8.66	2.6	0.3
	F4F3	F	583	9.69	2.9	0.3

*The "G" in each dog's experimental number designates Gamma-emitting californium-249.

**The "F" in each dog's experimental number designates Fissioning californium-252.

ence 5 and a description of the counting system is given in Reference 6. The anesthetized animal was placed for total-body counting in a curved lucite box which bent his midline into an arc having a 33-cm radius. The box was positioned such that the dog's midline was 33 cm from the face of an 8 x 4 inch NaI (Tl) crystal. This counting arrangement was designed so that detector response would be relatively independent of the location of an emitter along the length of a dog. The lucite container molded the animals to a constant 15 cm thickness to provide similar photon absorption from dog to dog, regardless of size, within the size limits of our adult beagles. Both the animal and the detector were housed during the counting period in a low-background iron room constructed of 12 inch thick battleship steel.

A lead (Pb) shield 20 cm high, 18 cm wide, and 2.54 cm thick was interposed between the crystal and the dog's liver during partial-body counting. A shield of this size is large enough to adequately cover the liver. Its 2.54 cm thickness attenuated the 333 and 388 keV gamma-rays of Cf-249 to less than 0.3%.⁽⁷⁾ Calculation of the Cf-249 activity in the liver of the intact dog was based on (a) the calibration of total-body counting using the californium burden computed as injected activity minus the activity which had been excreted for the first 5 days after injection; and (b) the calibration provided by two Cf-249 dogs which were counted with and without the Pb shield immediately following sacrifice and liver excision. Livers were placed in polyethylene bottles, and their gamma-ray counting rates were compared with that of a known activity of Cf-249 in an identical bottle. The radioactivity in this standard was diluted with distilled

water to the approximate volume of the livers so that effects of geometry and self absorption in the standard and in the livers would be similar.

In these 2 dogs, the fraction of non-liver californium covered by the Pb shield during partial-body counting averaged about 0.38. Each dog's counting rate with the Pb shield in place was therefore divided by $(1-0.38) = 0.62$ to estimate the counting rate of the total non-liver californium. The counting rate of the activity in the liver was found by subtracting the calculated counting rate of the non-liver activity from the counting rate of the unshielded dog. Percent retained Cf values were then obtained by comparing the counting rates of (a) non-liver Cf, (b) liver Cf, and (c) total dog Cf with that of a duplicate of the dog's Cf injection inside of a suitable phantom.⁽⁶⁾

Although the total-body counting of dogs injected with Cf-252 was calibrated adequately by use of the excreta subtraction technique described for the Cf-249 dogs, determination of the liver vs. non-liver partitioning for Cf-252 by total-body and partial-body counting results was not as satisfactory. It was found that the fission gamma-ray spectrum of Cf-252 was attenuated to only 33.6% by 2.54 cm of Pb, to 23.8% by 5.08 cm of Pb, and to 18.6% by 7.62 cm of Pb. This is at least partly because of the high average energy of the fission gamma-rays and their continuous energy distribution in the spectrum. Since the liver retention values for Cf-252 obtained by counting the living dog are so much more uncertain than those for Cf-249, only the total-body retention values for Cf-252 are reported here. As dogs injected with Cf-252 are sacrificed and excised livers become available, the calibration for Cf-252 liver retention can be completed.

Until then, it will be assumed that partitioning of Cf-252 between liver and non-liver tissue is identical to that for Cf-249.

RESULTS AND DISCUSSION

Excretion. Table 2 summarizes the average measured californium excretion during the first 3 weeks after injection for all 11 dogs.

Table 2. Average excretion of Cf-249 or Cf-252 by 11 Beagles during the first 3 weeks after injection.

Collection Interval (days)	Percent of Injected Californium Excreted			
	Urine	Cage Washings	Feces	Total
0-1	10.297%	0.958%	3.223%	14.478%
1-2	0.905	0.326	1.415	2.646
2-3	1.267	0.231	1.148	2.646
3-7	0.683	0.457	1.071	2.211
7-14	0.393	0.364	0.408	1.165
14-21	0.248	0.232	0.222	0.702
0-21	13.793%	2.568%	7.487%	23.848%

The excretion of Cf-249 did not differ significantly from that of Cf-252.

Of the total excretion during this 3 week period, about 43% appeared in the first day's urine collection, and nearly 14% in the first day's fecal collection. After the first week, urine collections contained about as much of the injected californium as the fecal collections during the same time period. In contrast to other radionuclide studies we have made in beagles, cage washings contained significant amounts of activity. The

cages were washed periodically with an acid solution that might have picked up californium which had plated from the urine onto the stainless steel cage floor. Nearly all the californium in cage washings was therefore tentatively assigned to the urine. Further studies are planned to determine what fraction of the activity in cage washings originates from urine and from feces.

A comparison of Cf excretion with that of Pu and Am (Table 3) reveals that about twice as much of the injected Cf as Pu or Am was eliminated during this initial 3 week period. Both Am and Cf were excreted mainly in the urine while Pu excretion was predominantly fecal.

Table 3. Excretion of Pu, Am, and Cf by Beagles during the first 3 weeks after intravenous injection

Number of dogs	Element	Reference	% in Urine	% in Feces	Total % Excreted
19	Pu	(8)	3.9	8.2	12.1
4	Am	(5)	9.7	1.4	11.1
11	Cf	(Table 2)	16.4	7.5	23.9

Retention. The measured total-body retention and partitioning of retained californium activity between liver and non-liver tissue is shown in Table 4. For comparison, the total-body retention calculated by excreta subtraction at corresponding times is also given. At 1 week following injection, nearly 60% of the injected californium was found in non-liver tissue (mainly skeleton) and about 20% remained in the liver. Total-body retention of Cf-249 and Cf-252 at corresponding times soon after injection appeared to be similar.

Table 4. Biological retention of injected californium in beagles.

Dog	Days after injection	% of Injected Californium in			% Retention via excreta subtraction
		Liver	Non-liver	Total-body	
(a) Cf-249					
T1G5	1	28.4	57.0	85.4	92.4
	14	16.1	62.8	78.9	85.2
	47	12.1	62.4	74.5	--
T2G5	2	26.0	60.0	86.0	85.2
	5	19.8	58.6	78.4	82.5
	7	19.2	59.1	78.3	81.6
T3G5	2	23.5	54.1	77.6	82.2
	5	19.4	54.2	73.6	77.0
	7	17.9	54.4	72.3	76.4
	14	18.9	54.2	73.1	75.6
	21	18.3	53.9	72.3	75.1
F1G3	1	29.4	56.6	86.0	85.4
	5	20.1	59.5	79.6	79.7
	7	18.6	58.5	77.1	78.8
	14	18.9	60.8	79.7	77.3
	21	19.3	58.3	77.6	76.6
	42	19.9	56.8	76.7	--
	61	19.2	55.4	74.6	--
	82	19.8	55.4	75.2	--
M2G3	1	28.0	59.4	87.4	84.7
	5	18.1	61.1	79.2	80.7
	7	17.6	60.8	78.4	79.8
	14	18.8	60.7	79.5	78.6
	21	18.1	60.2	78.3	78.0
	42	18.0	58.0	76.0	--
	61	18.5	58.2	76.7	--
	82	17.0	60.6	77.6	--
M3G3	1	22.4	61.4	83.8	87.6
	5	14.4	64.1	78.5	82.2
	7	12.2	65.1	77.3	81.4
	14	12.4	64.2	76.6	80.3
	21	13.3	63.0	76.3	79.7
	42	10.7	63.6	74.3	--
	61	13.5	59.2	72.7	--
	82	14.1	59.9	74.0	--
F4G3	1	31.7	59.4	91.1	83.6
	5	24.2	60.4	84.6	77.8
	7	23.1	60.2	83.3	77.0
	14	21.8	60.7	82.5	75.8
	21	22.9	59.8	82.7	75.1
	42	24.8	58.9	83.7	--
	61	22.0	58.0	80.0	--
	82	23.0	59.6	82.6	--

Table 4 (continued)

Dog	Days after injection	% of Injected Californium in Total-body	% Retention via excreta subtraction
(b) Cf-252			
MIF3	7	79.4	78.5
	36	76.2	--
	54	75.1	--
	75	73.9	--
F2F3	7	77.2	77.3
	36	73.4	--
	54	73.0	--
	75	69.8	--
F3F3	7	75.0	73.4
	36	69.7	--
	54	68.8	--
	75	66.2	--
F4F3	7	76.5	79.0
	36	72.1	--
	54	72.8	--
	75	69.6	--

One week following the injection of Am-241 in beagles, about 50% of the injected activity was deposited in liver and about 40% in non-liver tissue (mainly skeleton).⁽⁵⁾ Injected Pu-239 deposits about 60% in the skeleton and 30% in the liver.⁽⁸⁾ Retention values for americium and plutonium are compared with those for californium in Table 5.

Table 5. Retention of Pu, Am and Cf by beagles one week after intravenous injection in citrate solution

Reference	Element	Percent of Injected Radioactivity		
		Liver	Non-liver	Total
(8)	Plutonium	30	60	90
(5)	Americium	50	40	90
(Table 4)	Californium	20	60	80

Distribution. Cf-249 was found in every tissue which was counted (Table 6). At 1 and 3 weeks after injection, about 50% of the injected californium was in the skeleton, about 20% was in the liver and about 10% was in other tissues. Some organs and tissues exhibiting a relatively high concentration of Cf-249 were the liver, skeleton, kidney, connective tissue, dura, and, in one dog, the thyroid. Californium compares with Am-241 in tissue distribution,⁽⁵⁾ except that the average concentration of Cf was greater in the skeleton and relatively less in the liver and thyroid. Also, Cf seems to exhibit a more ubiquitous distribution. For example, while the brain and thymus of these two dogs definitely contained Cf, these two organs were below the limit of detection in the four Am-241 dogs sacrificed 1 to 22 days after injection.⁽⁵⁾ The concentration (% injected activity per kg) in the skeleton was about 0.64 of that in the liver in T2G5 and 0.69 in T3G5, while it averaged only 0.15 in four dogs given Am-241 and sacrificed 1 to 22 days after injection.⁽⁵⁾ This compares with an average of about 0.70 in two dogs reported in Reference 8 which were injected with Pu-239 and sacrificed 29 to 44 days later. Relative concentrations of californium in skeleton and liver are more like plutonium than americium (Table 7).

The kidney concentration of Cf was higher than for either Am or Pu at early times. Table 7 shows that the average kidney concentration of Cf-249 in two dogs sacrificed at 7 and 21 days was 22.8*, while the average of four dogs given Am-241 and sacrificed 1 to 22 days later was 15.6*.⁽⁵⁾ The average kidney concentration in four dogs sacrificed 7, 14, 35, and 40 days after the injection of Pu-239 was 14.6*.

* Percent of total injected activity retained within organ divided by organ weight in kilograms.

Table 6. Distribution of injected Cf-249 in the tissues of two beagles.

Tissue	Male Dog T2G5 7 days after injection			Female Dog T3G5 21 days after injection		
	Wet weight (g)	% of Injected Cf-249	% kg	Wet weight (g)	% of Injected Cf-249	% kg
Liver	301.6	19.2	63.7	235.5	18.3	77.7
Skeleton	1070.	44.13	41.2	989.	53.13	53.7
Thyroid	0.853	0.0491	57.6	0.561	0.00908	16.2
Kidneys	42.5	0.927	21.8	37.32	0.886	23.7
Dura	1.021	0.020	19.6	0.802	0.016	20.0
Connective Tissue*	9.81*	0.185	18.9	-----	-----	-----
Lymphatic Tissue*	8.87*	0.123	13.9	6.35*	0.075	11.8
Lungs and Trachea	83.39	1.21	14.5	67.71	0.559	8.3
Spleen	24.01	0.224	9.3	18.37	0.117	6.4
Aorta	3.82	0.037	9.7	4.90	0.025	5.1
Salivary Glands	-----	-----	-----	11.75	0.074	6.3
Adrenals	1.31	0.008	6.1	0.992	0.006	6.0
Prostate	7.11	0.030	4.2	-----	-----	-----
Pituitary	0.070	0.00027	3.9	0.060	0.00023	3.8
Ovaries	-----	-----	-----	0.595	0.002	3.4
GI Tract	440.5	1.685	3.8	375.0	0.525	1.4
Pelt	1600.	4.132	2.6	1650.	2.095	1.3
Heart	102.88	0.207	2.0	80.62	0.118	1.5
Eyes	12.9	0.026	2.0	11.25	0.017	1.5
Thymus	5.611	0.0095	1.7	19.19	0.030	1.6
Pancreas	18.59	0.044	2.3	16.87	0.015	0.9
Testes	23.9	0.033	1.4	-----	-----	-----
Muscle*	4500.*	3.400	0.8	5000.*	2.657	0.5
Brain	82.86	0.013	0.2	76.9	0.017	0.2
Omentum	-----	-----	-----	139.5	0.027	0.2
Total Cf-249		75.693			78.700	
Ret. by body counting		78.3			72.2	
Ret. by excreta subtraction		81.6			75.1	

* Incomplete sample

-- Not measured

Table 7. Concentration of Cf, Am, and Pu in skeleton, liver, kidney, and thyroid of beagles soon after injection.

Days after Injection	Dog	% of Injected Activity per kg			
		Skeleton	Liver	Kidney	Thyroid
7	T2G5	41.2	63.7	21.8	57.6
21	<u>T3G5</u>	<u>53.7</u>	<u>77.7</u>	<u>23.7</u>	<u>16.2</u>
	Average Cf	47.4	70.7	22.8	36.9
1	T15W5.5	24.6	175.2	24.7	114.6
7	T32W5.5	27.5	191.5	13.5	43.2
8	T33W5.5	28.6	153.2	11.8	42.0
22	<u>T16W5</u>	<u>27.5</u>	<u>194.2</u>	<u>9.7</u>	<u>20.4</u>
	Average Am	27.0	178.5	15.6	55.1
29	T1P5	49.2	73.0		
44	T2P5	55.8	76.6		
7	T56P5		106.1		
7	T14P5		68.7	18.8	
14	T55P5		110.6	13.4	
35	T30P1		159.7	18.7	
40	T31P3		102.9	7.4	
13	T42P5				5.3
35	T44P5				6.8
(404)*	(T54P5)*	—	—	—	<u>(6.6)*</u>
	Average Pu	52.5	99.7	14.6	6.1

*Although the thyroid concentration for this 404 day dog was not included in the average for short-term animals, it is shown for comparative purposes.

Thyroid concentrations of Cf, Am, and Pu at comparable times after injection are also shown in Table 7. It was much less for Pu-239 than Am or Cf. Although the average for the two Cf-249 dogs was somewhat lower than that for the four Am-241 dogs, one of the Cf dogs (T2G5) exhibited a thyroid concentration (57.6% per kg) as high as the Am average of 55.1. It is hoped that the sacrifice of additional animals after Cf injection will help explain the wide variation in thyroid concentration between the two dogs already studied (Table 6). Based on the tissue distribution of Cf and the observed effects in beagles of injected Pu-239^(9,10) and Am-241,⁽¹¹⁾ we expect the skeleton, kidney, liver and thyroid to be among the organs at risk after californium injection.

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REFERENCES

1. C.M. Lederer, J.M. Hollander and I. Perlman; Table of Isotopes, Sixth Edition (John Wiley and Sons, Inc., New York); pp 149, 442, 443 (1967).
2. Glenn T. Seaborg; Californium-252: radioisotope with a future; in CONF-681032 (U.S.A.E.C. Div. of Tech. Info.) 1-9 (1968).
3. J.L. Crandall; Survey of applications for Cf-252; Ibid.; 225-256 (1968).
4. A. Prince; Nuclear and physical properties of Cf-252; Ibid.; p. 37 (1968).

5. R. D. Lloyd, C. W. Mays, G. N. Taylor, and D. R. Atherton; Americium-241 studies in beagles; Health Physics 18: 149-156 (1970).
6. R. D. Lloyd, D. H. Taysum, and C. W. Mays; Design and calibration of a total-body counting system for measuring radioactivity in beagles; in Retention and Dosimetry of Some Injected Radionuclides in Beagles (Univ. of Utah Report C00-119-241); 2-24 (1970).
7. Robley D. Evans; The Atomic Nucleus; McGraw-Hill Book Co., New York; p. 716 (1962).
8. Betsy J. Stover, D. R. Atherton, F. W. Bruenger, and Dawn S. Buster; $^{239}\text{PuIV}$: its distribution in the beagle; in Delayed Effects of Bone-Seeking Radionuclides, C. W. Mays et al., editors; University of Utah Press, Salt Lake City; 109-123 (1969).
9. C. W. Mays et al.; Radiation-induced bone cancer in beagles; Ibid; 387-408 (1969).
10. G. N. Taylor, T. F. Dougherty, L. Shabestari, and J. H. Dougherty; Soft tissue tumors in internally-irradiated beagles; Ibid., 323-336.
11. Ray D. Lloyd, Webster S. S. Jee, David R. Atherton, Glenn N. Taylor, and Charles W. Mays; Am-241 in beagles: biological effects and skeletal distribution; in The Radiobiology of Plutonium, (Betsy J. Stover and W. S. S. Jee, editors); in press.

EARLY HEMATOLOGIC EFFECTS OF CALIFORNIUM IN THE BEAGLE

Jean H. Dougherty

ABSTRACT: The hematologic changes following intravenous injection of ^{249}Cf or ^{252}Cf into 11 young adult beagles are reported for the first 8 weeks post-injection. Three dogs which received $\sim 2.8 \mu\text{Ci/kg}$ of ^{249}Cf developed a severe depression of granular leukocytes and platelets which was maximal 2 to 3 weeks following injection with little recovery by 8 weeks. The lymphocytes were decreased below normal by 2 weeks and continued to fall thereafter to one-third of pre-injection values. Changes in granular leukocyte values on 4 dogs injected with $\sim 0.28 \mu\text{Ci/kg}$ of ^{249}Cf were compared to those of 4 dogs injected with $\sim 0.28 \mu\text{Ci/kg}$ of ^{252}Cf . There was a greater and more prolonged depression in the ^{252}Cf injected dogs probably due to the added bone dose rate from fission fragments on bone surfaces which would cause a greater irradiation of bone marrow. There have been no changes thus far in red cells, platelets or lymphocytes in dogs receiving $0.28 \mu\text{Ci/kg}$ of either ^{249}Cf or ^{252}Cf .

INTRODUCTION

Study of the effects of ^{249}Cf and ^{252}Cf in beagles has been underway in the Radiobiology Division for several months. The following report gives the hematologic changes in these initial animals over the first 8 weeks post-injection. Since the retention, excretion and distribution of californium in beagles differs from ^{241}Am and ^{239}Pu ,⁽¹⁾ it is of interest to compare the early hematologic changes of these transuranic elements at similar injection levels. Also, since ^{249}Cf decays mainly by alpha emission and ^{252}Cf by fission fragment and alpha emission, a comparison of the hematologic changes at similar injected activities will give an indication of the relative toxicity to the hematopoietic system of alpha particles vs. fission fragments.

METHODS

The injection levels and related data on the 11 dogs injected with

^{249}Cf or ^{252}Cf are given in Table 1 of the previous reference.⁽¹⁾ Hematologic methods are the same as performed on the other radionuclides in our study and have been reported previously.⁽²⁾ Since no californium dogs have been injected at the 0-level as yet, the hematologic changes noted on each dog at each dose level with time will be related back to the dog's own pre-injection control counts.

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

Blood counts on 3-level dogs injected with ^{249}Cf (G3) or ^{252}Cf (F3) have been made at 5 days post-injection and then at weekly intervals for the 8 week period that has elapsed since the time of injection. Only one 5-level dog (T1G5) has been on the experiment this period of time. Dog T2G5 was sacrificed 1 week and T3G5 at 3 weeks after injection for distribution studies.

RESULTS AND DISCUSSION

5-level ^{249}Cf Dogs. Blood cell changes on the 3 dogs at the highest level of ^{249}Cf (dogs T1G5, T2G5 and T3G5) are given in Figures 1 and 2.

The leukocytes (Fig. 1) are decreased in numbers as early as 5 days after injection due mainly to a pronounced fall in pmns. The pmns reach their lowest levels by 2 to 3 weeks (mean of 450 cells per cu mm) and by 8 weeks in the one surviving dog there may be a beginning recovery. The lymphocytes fall more slowly and in T1G5 are one-third of pre-injection values at 8 weeks. The platelets are maximally depressed by 3 to 4 weeks

Fig. 1. Leukocyte and platelet responses following injection of approximately 2.8 $\mu\text{Ci/kg}$ of ^{249}Cf (5-level) for 8 weeks post-injection.

Fig. 2. Red cell values (VPRC, Hgb, reticulocytes) and sedimentation rate of same dogs as Fig. 1.

and thereafter show a slight rise (T1G5). Monocytes and eosinophils respond similarly to pmns.

The red cell picture as shown by VPRC, Hgb, reticulocytes and sedimentation rates is given in Fig. 2. There is a disparity in the effects among these 3 dogs. The more severe changes in T1G5 may possibly be due to repeated liver biopsies which were performed after injection. The dog sacrificed at 1 week (T2G5) had a slight fall in VPRC, Hgb and sedimentation rate and a drop in reticulocytes from a pre-injection mean of 0.8% to 0.1%. Dog T3G5 sacrificed at 3 weeks showed essentially no changes in any of the above values at any time interval studied with the exception of a decrease in reticulocytes. Dog T1G5, on the other hand, had a fall in red cell values beginning at 5 days and becoming maximal between 4 and 5 weeks post-injection at which time the VPRC was 30 ml/100 ml, Hgb 9.8 gm% and the sedimentation rate was 56 mm/hr. The reticulocytes fell to 0.2% at 2 weeks and rose to a high of 1.7% at 6 weeks. At 8 weeks, VPRC and Hgb were not back to pre-injection levels.

The above early changes in leukocytes and platelet levels following injection of $\sim 2.8 \mu\text{Ci/kg}$ of ^{249}Cf are similar to those seen after injection of $\sim 2.8 \mu\text{Ci/kg}$ of ^{241}Am (5-level) into 4 adult beagles,⁽³⁾ and in dogs injected with $\sim 2.8 \mu\text{Ci/kg}$ of ^{239}Pu .⁽⁴⁾ The leukocyte recovery rate seems to be a little slower in the 5-level californium dogs but this is based on only one dog at 8 weeks post-injection. It is obviously not possible to make comparisons of the red cell response with any other radio-nuclides at this time.

3-Level ^{249}Cf and ^{252}Cf Dogs. The leukocyte and platelet values of the 4 dogs injected with ^{249}Cf are given in Fig. 3 and those of the 4 dogs

Fig. 3. Leukocyte responses following injection of approximately 0.28 $\mu\text{Ci/kg}$ of ^{249}Cf (3-level) for 8 weeks post-injection.

Fig. 4. Leukocyte responses following injection of approximately 0.28 $\mu\text{Ci/kg}$ of ^{252}Cf (3-level) for 8 weeks post-injection.

injected with ^{252}Cf in Fig. 4. There were no significant changes in red cell values in any of the 3-level dogs so these values were not graphed.

The leukocytes of dogs at the 3-level of ^{249}Cf (Fig. 3) did not show the sharp decrease seen in the G5 dogs (dose dependency). There was a slight decline in pmns at 2 to 3 weeks with recovery to normal values thereafter, but no decrease in lymphocytes. There was very little if any effect on platelets at this level as all post-injection counts were within normal limits.

The leukocyte depression was a little greater in the dogs injected with ^{252}Cf . In this group the pmns reached values below 4,000 cells/mm³ whereas the ^{249}Cf injected dogs remained with two exceptions (dogs M3G3 and F4G3 at 3 weeks) above 4,000 cells. There was also less recovery of pmns in the ^{252}Cf dogs. Again, as with ^{249}Cf , there were no significant changes in lymphocytes or platelets from control values.

The more pronounced depressive effect on the pmns of the dogs injected with ^{252}Cf is probably due to the added bone dose rate from fission fragments on the bone surfaces which would cause a greater irradiation of the hematopoietic bone marrow tissue.

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REFERENCES

1. R. D. Lloyd, C. W. Mays, D. R. Atherton, G. N. Taylor and J. L. Williams; Californium retention, excretion and distribution in beagles soon after injection. (This report.)

2. Jean H. Dougherty and L. S. Rosenblatt; Changes in the hemogram of the beagle with age. J. Gerontol., 20: 131-138 (1965).
3. Jean H. Dougherty; Preliminary report on hematological effects of ^{241}Am in the beagle. C00-119-242, pp. 354-362 (1970).
4. Jean H. Dougherty; Hematologic changes induced by ^{239}Pu in beagles. In: The Radiobiology of Plutonium, ed. by W. S. S. Jee and Betsy J. Stover (in Press, 1971).

DISTRIBUTION OF CALIFORNIUM-249 AND BERKELIUM-249 IN THE SOFT TISSUES OF BEAGLES

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Abstract: The microscopic distribution pattern of ^{249}Cf and ^{249}Bk in the soft tissues of beagles, at one to three weeks following a single intravenous injection of a citrate solution, was very similar to ^{241}Am . Relatively high concentrations occurred in the hepatic cells of the liver, the glomeruli of the kidney, the interfollicular region of the thyroid, the cartilaginous tissues of the lung, and media of the smaller arterioles of most organs. Very intense, but sparsely scattered "hot spots" were also present in the renal papillae and in the submucosa of the bronchioles. Lesser sites of localization were the endocardium of the AV heart valves, the glassy membranes of the larger hairs of the coat, the zona pellucida of the Graafian follicles and the zona arcuata of the adrenal cortex. With the exception of the liver, where the radionuclide was principally within the hepatic cells, most of the deposition sites were extracellular, within or adjacent to connective tissue which gave a positive periodic acid-Schiff reaction.

INTRODUCTION

Two well known similarities in the biological behavior of elements in the lanthanide and actinide series is their tendency to localize in the skeleton and liver⁽¹⁻¹³⁾. In the rat the skeletal concentration has been observed to vary inversely with that initially in the liver and the degree of localization to be somewhat related to the ionic radius⁽⁹⁾. As would be expected, the similarities in tissue retention extend beyond these two organs, at least with respect to some of the transuranics.

It is the purpose of this study to summarize some of the more obvious soft tissue sites of early ^{249}Cf and ^{249}Bk localization in the dog.

METHODS

The dogs used in these studies were purebred beagles from a moderately

inbred beagle colony which received the radionuclide via a single intravenous injection of a citrate solution at ~ 19 months of age⁽¹⁴⁾. The two dogs were sacrificed at 7 and 21 days post-injection. In addition to the ^{249}Cf , tracer amounts of ^{249}Bk were also present in the injection solution. However, this relatively small amount of ^{249}Bk on an atom basis resulted in an appreciable number of beta tracks because of its relatively short half-life (314 days). Because of the low energy (0.125 MeV maximum) of the β -particles from ^{249}Bk , its dose in rads is insignificant compared to the α -particle dose from ^{249}Cf (5.83 MeV, average).

The autoradiograms were from acetone fixed tissues, processed according to the methods of Arnold and Jee⁽¹⁵⁾.

RESULTS

The early deposition of ^{249}Cf in the soft tissues occurred in both intracellular and interstitial sites. The principal intracellular retention was in the hepatic cells of the liver with little if any activity in the reticuloendothelial (RE) cells or the portal structures, at these relatively short post-injection times (Fig. 1). Within the hepatic parenchyma the distribution was uniform without any apparent "hot spots".

Much lesser sites of intracellular deposition were the zona arcuata cells of the adrenal cortex and RE cells in the spleen and lymph nodes.

Most of the soft tissue extracellular deposition of californium was retained in or on connective tissue which was generally PAS positive; however, retention did not occur in all PAS positive tissues.

The most widely distributed deposition site was the media of the small arterioles, principally those with only 1 - 2 layers of muscle. The nuclide appeared to be localized in the connective tissue and not

the smooth muscle component (Fig. 2). Significant retention in this portion of the arterial system was found in all of the organs examined.

A second arterial site in which above average retention occurred was the aorta and the coronary arteries, with the concentration being significantly higher in the coronaries (Fig. 3). Relatively intense "hot spots" were present in the circumflex branch. As in the various other arterial sites, the ^{249}Cf appeared to be retained in the connective tissue component.

In addition to the coronary arteries, selective cardiac localization also occurred in the connective tissue of the endocardium on the ventricular aspect of the mitral valve. The most intense activity was at a point very near the valvular attachment to the cardiac skeleton.

In the lungs most of the ^{249}Cf was in or immediately adjacent to the perichondrium of the cartilaginous plates. However, a lesser but preferential deposition also occurred in the lamina propria of the bronchioles (Figs. 4-5). Occasional, very intense, "hot spots" were present in the submucosa of the bronchioles, but these were relatively infrequent.

The most generalized interstitial deposition of ^{249}Cf within any organ occurred in the thyroid where retention was almost exclusively in the interfollicular connective tissue (Fig. 6). Although some activity was present in all of the interstitial areas, significant regional variations in concentration occurred, including the presence of some relatively focal "hot spots". Activity in the colloid or epithelial components was very low.

Another organ with a very significant ^{249}Cf connective tissue retention

was the kidney. High concentrations were present in the glomeruli and the renal papillae (Fig. 7). Lesser amounts were retained in the inter-tubular tissue, principally in the cortical areas (Fig. 8). Occasional, very intense, "hot spots" were present in the medulla, usually fairly close to the renal papillae (Fig. 9). The precise location of californium in the glomerular tuft could not be unequivocally determined by autoradiography; however, the high activity of the capsular basement membrane was very suggestive that its homologue, the glomerular basement membrane, was the principal site of deposition in the region of the glomerular capillaries.

A rather unique site of relatively high extracellular localization was in the glassy membrane of the hair follicles (Figs. 10-11). Deposition was restricted to a short segment peripheral to the neck of the papilla and deep to the point of attachment of the erector pili muscle. Such deposition was found only in the primary hairs. Retention in the secondary follicles and the various other adnexa was not observed.

Another extracellular site of focal ^{249}Cf retention was the region of the zona pellucida of some of the larger and more mature Graafian follicles (Figs. 12-13). The activity was not high but could have significant consequences because of its proximity to the ovum. Selective localization in the smaller follicles did not occur.

The general distribution of ^{249}Bk , which was injected concurrently with ^{249}Cf , in the amount of perhaps 1% on an atom basis was almost identical to that of ^{249}Cf . However, we have not determined, as yet, the relative tissue concentration factors and quantitative differences that may exist. Autoradiographic distinction between the two radionuclides was readily achieved because of the differences in the types of energy

released, beta for the ^{249}Bk and alpha for ^{249}Cf . There were no sites found in which either one of these radionuclides was deposited unilaterally.

DISCUSSION

Our clinical experience with ^{249}Cf presently covers only about 4 months; however, its qualitative resemblance to ^{241}Am in the local soft tissue deposition pattern indicates that the toxicity syndromes of the two radionuclides should have some similarities⁽¹¹⁻¹²⁾. Any major variation will probably be related to differences in the degree of localization and retention and not to differences in sites.

Evaluation of the ultimate consequences of the soft tissue ^{249}Cf burden will require relatively long latent periods, especially at the lower dose levels. However, at the highest level, $\sim 2.8 \mu\text{Ci } ^{249}\text{Cf/kg}$, it is reasonably safe at this time to predict that renal failure will be a major end-point and probably the primary cause of death because of the relatively marked localization in the glomeruli. Our experience with ^{241}Am suggests that such deaths from renal failure should occur beginning at ~ 400 days post-injection. ^{249}Cf induced thyroid lesions, similar to those observed in ^{241}Am toxicity are also expected.

Thus far, ^{241}Am soft tissue neoplasms have not been observed, such that we do not have a good basis for predicting the carcinogenicity of ^{249}Cf . However, the liver, thyroid, kidney and possibly the hair follicles are certainly potential sites. Nevertheless, these possible tumor sites are in competition with the skeleton as neoplastic end-points and may never undergo neoplasia because of earlier death from bone cancer. We know this competitive factor is at least partly responsible for the

relatively low incidence of liver tumors in ^{239}Pu toxicity in the dog.

The potential hazard of the moderate localization of ^{249}Cf adjacent to the ovum, in the Graafian follicle, is as yet undetermined. However, with the deposition occurring principally in the relatively mature follicles, the radiation would be delivered during its more radioresistant stage of development, at least in comparison to the oogonia, but this would not be the case in the adjacent granulosa cells which are actively dividing at this time.⁽¹⁶⁻¹⁹⁾

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REFERENCES

1. J. Carritt, R. Fryxell, J. Kleinschmidt, R. Kleinschmidt, W. Langham, A. San Pietro, R. Schaffer, and B. Schnap; The distribution and excretion of plutonium administered intravenously to the rat; J. Biol. Chem. 171: 273-283 (1947).
2. K. G. Scott, D. J. Axelrod, H. Fisher, J. F. Crowley, and J. G. Hamilton; The metabolism of plutonium in rats following intramuscular injection; J. Biol. Chem. 176: 283-293 (1948).
3. J. Schubert, M. P. Finkel, M. R. White, and G. M. Hirsch; Plutonium and yttrium content of the blood, liver, and the skeleton of the rat at different times after intravenous administration; J. Biol. Chem. 182: 635-642 (1950).
4. H. G. Parker, A. G. Low-Beer, and E. L. Isaac; Comparison of retention and organ distribution of ^{241}Am and ^{252}Cf in mice; Health Physics 8: 679-684 (1962).

5. B. J. Stover, D. R. Atherton, and H. Keller; Metabolism of ^{239}Pu in adult beagle dogs; Rad. Res. 10: 2, 130-47 (1959).
6. W. H. Langham; Physiology and toxicology of plutonium-239 and its industrial medical control; Health Phys. 2: 172-185 (1959).
7. D. M. Taylor, F. D. Lowby, and M. F. Kemper; The metabolism of americium and plutonium in the rat; Phys. Med. Biol. 6: 73-86 (1961).
8. W. J. Bair, D. H. Willard, J. P. Herring, and L. A. George II; Retention, translocation, and excretion of inhaled ^{239}Pu ; Health Phys. 8: 6, 639-649 (1962).
9. P. W. Durbin; Distribution of the transuranic elements in mammals; Health Phys. 8: 665-671 (1962).
10. J. E. Ballou, W. J. Bair, A. C. Case, and R. C. Thompson; Studies of neptunium in the rat; Health Phys. 8: 685-688 (1962).
11. G. N. Taylor, W. S. S. Jee, J. L. Williams, B. Burggraaf, and W. Angus; Microscopic distribution of ^{241}Am in the beagle; Research in Radiobiology, C00-119-240, 97-118 (1969).
12. G. N. Taylor, W. S. S. Jee, N. Dockum, and E. Hromyk; Microscopic distribution of ^{241}Am in the beagle thyroid gland; Health Physics 17: 5, 723-725 (1969).
13. P. J. Magno, P. E. Kauffman and P. R. Groulx; Plutonium-239 in human tissues and bone; Rad. Health Data 10: 4, 47-50 (1969).
14. T. F. Dougherty, B. J. Stover, J. H. Dougherty, W. S. S. Jee, C. W. Mays, C. E. Rehfeld, W. R. Christensen, and H. C. Goldthrope; Studies of the biological effects of ^{226}Ra , ^{239}Pu , ^{228}Ra (MsTh), ^{228}Th (RdTh), and ^{90}Sr in adult beagles; Radiat. Res. 17: 625-681 (1962).
15. J. S. Arnold and W. S. S. Jee; Embedding and sectioning of undecalcified bone and its application to radioautography; Stain Techn. 29:

225-239 (1954).

16. P. Rubin and G. W. Casarett; Clinical Radiat. Pathology; W. B. Saunders Co., Philadelphia, 401-402 (1968).
17. A. M. Mandl; The radiosensitivity of germ cells; Biol. Rev. 39: 288-371 (1964).
18. B. H. Erickson; Radioresponse of the prepuberal porcine ovary; Int. J. Radiat. Biol. 13: 1, 57-67 (1967).
19. H. Peters; Radiation sensitivity of oocytes at different stages of development in the immature mouse; Rad. Res. 15: 582-593 (1961).

Fig. 1. Autoradiogram of liver 7 days following injection of $\sim 2.8 \mu\text{Ci/kg}$ of α -emitting ^{249}Cf plus tracer amounts of β -emitting ^{249}Bk showing focal deposition of both ^{249}Bk and ^{249}Cf in the hepatic parenchyma of beagle T2G5. 21 day exposure. The α particles from ^{249}Cf show as line tracks, whereas the β -particles from ^{249}Bk show as point dots.

Fig. 2. Autoradiogram of arteriole in beagle kidney 7 days following injection showing focal deposition of ^{249}Cf and ^{249}Bk in the media (M) of the vessel wall. Same dog as Fig. 1. 35 day exposure. x 550.

Fig. 3. Autoradiogram of coronary artery 7 days post-injection showing localization of ^{249}Cf and ^{249}Bk in the media (M). No preferential deposition occurred in the intima (I) or the adventitia (A). Same dog as Fig. 1. 21 days exposure. x 550.

Fig. 4. Autoradiogram of the wall of small bronchiole 21 days following injection of $\sim 2.8 \mu\text{Ci } ^{249}\text{Cf/kg}$ in beagle T3G5 showing relatively heavy localization of ^{249}Cf and ^{249}Bk in the perichondrium and/or periphery of a cartilaginous plate (P). A moderate increase in activity is also apparent in the lamina propria and muscle layer (M). 14 day exposure. $\times 550$.

Fig. 5. Autoradiogram of wall of bronchiole 21 days following injection of ^{249}Cf and ^{249}Bk showing the intense "hot spots" occasionally seen in the lamina propria. Same dog as Fig. 4. 14 day exposure. x 550.

Fig. 6. Autoradiogram of thyroid 7 days following injection, showing the relatively high focal depositions of ^{249}Cf and ^{249}Bk in the inter-follicular region. Retention in the colloid (C) was low or absent. Same dog as Fig. 1. 35 day exposure. x 550.

Fig. 7. Autoradiogram of kidney 7 days following injection showing selective deposition of ^{249}Cf and ^{249}Bk in the glomerular tuft (G), the capsular basement membrane (arrow) and the intertubular region. Same dog as Fig. 1. 21 days exposure x 550.

Fig. 8. Autoradiogram of kidney cortex 7 days following injection showing focal deposition of ^{249}Cf and ^{249}Bk in the intertubular region.

Same dog as Fig. 1. 5 week exposure. x 550.

Fig. 9. Autoradiogram of medullary region in kidney 7 days following injection showing the "hot spots" of ^{249}Cf and ^{249}Bk which occurred in moderate numbers near the pelvis. These tentatively appeared to be in the interstitial areas, but this could not be unequivocally established. Same dog as Fig. 1. 21 day exposure. x 550.

Fig. 10. Autoradiogram of skin 7 days following injection showing selective deposition of ^{249}Cf and ^{249}Bk in the region of the glassy membrane of a primary hair follicle (P). There is no significant activity in the secondary follicles or the adnexa. Same dog as Fig. 1. 35 day exposure. x 200.

Fig. 11. Autoradiogram of primary hair follicle 21 days following injection showing focal deposition of ^{249}Cf and ^{249}Bk in the region of the glassy membrane. There is no significant activity in the other regions of the follicle. Same dog as Fig. 4. 14 day exposure. x 550.

Fig. 12. Autoradiogram of Graafian follicle 7 days following injection showing moderate focal deposition of ^{249}Cf and ^{249}Bk in the region of zona pellucidum (arrow). There is no significant localization in the granulosa cells (G) or the region of follicular liquid (L). Same dog as Fig. 1. 56 day exposure. x 200.

Fig. 13. Higher magnification of same autoradiogram shown in Fig. 12.

× 550.

THE DISAPPEARANCE OF CALIFORNIUM-249 AND CALIFORNIUM-252
FROM THE BLOOD OF BEAGLES*

Walter Stevens and F. W. Bruenger

Beagles were injected with ^{249}Cf and ^{252}Cf in citrate buffer, pH 3.5. The concentration of the nuclide in the plasma and whole blood was measured at increasing time intervals from 5 minutes to 48 hours post-injection. Small quantities of californium were found with the cellular component. The concentration in plasma decreased very rapidly during the first few hours after injection. At 24 hr, less than 1% of the injected dose was still circulating. These data are similar to the disappearance of americium from the blood of dogs. ^{249}Cf and ^{252}Cf disappeared from the circulation at the same rate. Plasma constituents were separated by gel filtration and ion exchange chromatography. Because of the rapid removal of the nuclide from the blood, meaningful data were obtained only at short times after injection. Californium was found to be associated with high molecular weight proteins and those of a molecular weight of approximately 70,000. Ion exchange chromatography on DEAE Sephadex of this fraction showed that transferrin was involved in this association. Data obtained in vivo from canines were extended by in vitro experiments with human blood. ^{249}Cf was incubated in vitro at 37°C with human blood for 20 minutes. At the end of this time, cells were separated from the plasma and the plasma was subjected to gel chromatography on double columns of Sephadex G-100-G-200. This chromatography showed that californium-protein distribution pattern was nearly identical

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to that seen in dogs. Re-chromatography of the G-100-G-200 fractions on DEAE-Sephadex columns indicated that the peaks of transferrin and californium did not coincide exactly which provides evidence for the presence of another yet unidentified protein component capable of associating with or binding californium. Appreciable quantities of the nuclide appeared in the low molecular weight region indicating that protein does not bind californium tenaciously and that complexes with inorganic or other molecules of low molecular weight found in plasma were formed. The low stability of the californium-protein complex is probably responsible for the high rate of its disappearance from the blood stream.

INTRACELLULAR DISTRIBUTION OF ^{249}Cf IN CANINE LIVER*

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

Three beagles were injected intravenously with ^{249}Cf in citrate buffer. Two of these dogs were sacrificed at 7 days and 21 days respectively. Liver biopsies were performed on the third dog starting at one day after injection. Liver specimens from all dogs were homogenized in sucrose - Ca^{++} and subjected to differential centrifugation. The concentrations of ^{249}Cf and protein were determined in each fraction. A redistribution of the nuclide in the various fractions as a function of time was observed. Most of the ^{249}Cf was initially bound by soluble proteins, probably ferritin. At later times increasing quantities were associated with intracellular organelles. In addition, homogenate fractions free of nuclei and debris (homogenate after 6000 g-min) were subjected to centrifugation for 220,000 g-min in a continuous linear sucrose gradient of 10-50%. All fractions were collected and analyzed for: ^{249}Cf , protein, acid phosphatase (lysosomal marker) and cytochrome c-oxidase (mitochondrial marker). At one day after injection more than 50% of the nuclide was found in the three lightest fractions and approximately 10% in the three heaviest fractions. Their relation was reversed with time and at 21 days 10% was found in the lightest fractions and 43% in the heaviest fractions. At this time more than 50% of the nuclide in the liver was associated with the fraction collected at 60,000 g-min, designated as mitochondria. This fraction showed high cytochrome oxidase

* Abstract Submitted to the Eleventh Hanford Biology Symposium: The Biological Implications Of The Transuranium Elements

activity and also acid phosphatase activity. It is presently assumed that redistribution occurs from soluble proteins into mitochondria and then into lysosomes. Similar data have been obtained from kidney and spleen homogenates.

DISTRIBUTION OF INJECTED ^{241}Am IN THE BEAGLE SKELETON

R. D. Lloyd, D. R. Atherton, Susan S. Gaufin
and C. W. Mays

Abstract: The distribution of ^{241}Am in the skeletons of 10 beagles injected with 0.31 to 4.53 $\mu\text{Ci/kg}$ at 1 to 1533 days prior to sacrifice was determined by gamma-ray counting. Comparison of percent retained skeletal ^{241}Am values with percent skeletal ash values for each bone or bone group showed that bones containing much trabecular bone had a higher ratio of skeletal Am to skeletal ash than the average for the total skeleton, while bones containing much cortical bone had a lower ratio than the skeletal average.

INTRODUCTION

Studies of the metabolism and radiotoxicity of ^{241}Am in beagles have been in progress at this laboratory since 1966. Preliminary results of the investigation and the experimental design have been reported previously.⁽¹⁻¹⁵⁾

METHODS

Individual bones or bone groups were obtained at autopsy from ten of the ^{241}Am -burdened beagles. Virtually the entire skeleton of each animal was made available for this study. Each bone was placed midway between the opposed faces of two 8 x 4 inch NaI(Tl) crystals⁽¹⁶⁾ so that its ^{241}Am content could be determined via the 60 keV gamma-ray. Summed data from the two crystals were recorded by a multi-channel pulse-height analyser. The counting rate of each whole bone or bone group at 60 keV was compared with that of a duplicate of the dog's ^{241}Am injection solution. These injection standards were counted in such a configuration as to duplicate

the photon self-absorption and geometry of the bone.

RESULTS AND DISCUSSION

The percent of retained skeletal ^{241}Am in each bone or bone group is shown for all ten dogs in Table 1. There was striking uniformity in relative skeletal distribution among all dogs but T6W3. No obvious reason for the difference in fractional retention between T6W3 and the other animals is clearly obvious at the present time. Two other dogs (T57W5 and T4W4) were females, and one other dog (T4W4) lived at least as long after injection. Examination of post-mortem skeletal radiographs revealed no important difference in bone size or cortical thickness between T6W3 and other dogs in Table 1. A similar variation in relative distribution was observed by Stover et al., among beagles injected with ^{239}Pu . They reported that in one of three dogs studied in detail, there was a greater proportion of retained plutonium in the extremities and less in the ribs and vertebrae.⁽¹⁷⁾

It is of interest to compare the distribution of ^{241}Am with the distribution of mineral in the beagle skeleton. The first column in Table 2 lists the average percent skeletal ^{241}Am values for individual bones or bone groups from Table 1. In the second column is an estimate of the percent skeletal ash contained in each of the corresponding bones or bone groups. These data represent an average of 22 dogs from our colony for which skeletal ash weights have been reported.^(18,19) The third column in Table 2 is a ratio of percent skeletal ^{241}Am and percent skeletal ash for each bone.

Since injected ^{241}Am deposits on bone surfaces,⁽¹⁵⁾ it is not surprising that the bones for which this ratio is significantly higher than the average of 1.0 for the total skeleton (such as the sternum, thoracic vertebrae, lumbar vertebrae, and caudal vertebrae) are those which contain a significantly higher fraction of trabecular bone than the average for the total skeleton. This is probably due to the greater area of bone surfaces per gram in trabecular as opposed to cortical bone. Moreover, the ratios shown in Table 2 for bones containing much cortical bone (lower leg bones, paws, skull, and mandible) are significantly lower than the average for the total skeleton of 1.0, while the ratio is close to the skeletal average for bones such as the cervical vertebrae, humerus, femur, pelvis, and scapula, which contain a more representative mixture of trabecular and cortical bone.

REFERENCES

1. R. D. Lloyd, C. W. Mays, G. N. Taylor, D. R. Atherton, and L. R. Shabestari; Retention of injected ^{241}Am in beagles; Health Physics 13: 938 (1967).
2. R. D. Lloyd, C. W. Mays, G. N. Taylor, D. R. Atherton, and L. R. Shabestari; Americium metabolism in beagles; Research in Radiobiology, Univ. of Utah Report C00-119-236, 197-206 (1967).
3. R. D. Lloyd, D. R. Atherton, G. N. Taylor, and C. W. Mays; Soft-tissue deposition of injected ^{241}Am ; Health Physics 15: 175 (1968).

4. D. R. Atherton, R. D. Lloyd, G. N. Taylor, B. J. Stover, and C. W. Mays; Distribution of ^{241}Am in the beagle; Radiation Research 33: 506 (1968).
5. F. W. Bruenger, W. Stevens, and B. J. Stover; ^{241}Am in the blood; in vivo and in vitro observations; Radiation Research 37: 349-360 (1969); and Research in Radiobiology, Univ. of Utah Report C00-119-237, 135-152 (1968).
6. W. Stevens, Betsy J. Stover, F. W. Bruenger, and G. N. Taylor; Some observations on the deposition of Americium-241 in the thyroid gland of the beagle; Radiation Research 39: 201-206 (1969); and Research in Radiobiology, Univ. of Utah Report C00-119-237, 133-134 (1968).
7. R. D. Lloyd, C. W. Mays, and G. N. Taylor; Strontium, radium, and americium metabolism in beagles; Health Physics 17: 384 (1969).
8. D. R. Atherton, R. D. Lloyd, G. N. Taylor, B. J. Stover, and C. W. Mays; Distribution of ^{241}Am in the beagle; Research in Radiobiology, Univ. of Utah Report C00-119-237, 117-123 (1968).
9. David R. Atherton, Betsy J. Stover, R. D. Lloyd, and F. W. Bruenger; A comparison of the macro-distribution of ^{241}Am and ^{59}Fe in the young adult beagle; Radiation Research 39: 478 (1969); and Research in Radiobiology, Univ. of Utah Report C00-119-240, 129-138 (1969).
10. R. D. Lloyd, C. W. Mays, D. R. Atherton, and G. N. Taylor; Am-241 studies in beagles; Health Physics 18: 149-156 (1970).
11. R. D. Lloyd, C. W. Mays, D. R. Atherton, and G. N. Taylor;

- Am-241 retention in beagles; Retention and Dosimetry of Some Injected Radionuclides in Beagles, Univ. of Utah Report C00-119-241, 123-143 (1970).
12. G. N. Taylor, W. S. S. Jee, N. Dockum, and E. Hromyk; Microscopic distribution of Am-241 in the beagle thyroid gland; Health Physics 17: 723-725 (1969); and Research in Radiobiology, Univ. of Utah Report C00-119-237, 124-132 (1968).
 13. Betsy J. Stover, F. W. Bruenger, and W. Stevens; The subcellular distribution of ^{239}Pu and ^{241}Am in the canine liver; Research in Radiobiology, Univ. of Utah Report C00-119-242, 202 (1970).
 14. Jean H. Dougherty; Preliminary report on the hematological effects of ^{241}Am in the beagle; Research in Radiobiology, Univ. of Utah Report C00-119-242, 354-362 (1970).
 15. Ray D. Lloyd, Webster S. S. Jee, David R. Atherton, Glenn N. Taylor, and Charles W. Mays; Am-241 in beagles; biological effects and skeletal distribution; in The Radiobiology of Plutonium (W. S. S. Jee and Betsy J. Stover, editors). In press.
 16. R. D. Lloyd, R. L. Aamodt, W. W. Wagner, and R. C. Pendleton; Calibration of a gamma-ray spectrometer for soils counting; International Journal of Applied Radiation and Isotopes 19: 773-775 (1968).
 17. Betsy J. Stover, D. R. Atherton, F. W. Bruenger and Dawn S. Buster; $^{239}\text{Pu}(\text{IV})$: its distribution in the beagle; in Delayed Effects of Bone-Seeking Radionuclides (C. W. Mays et al., editors) University of Utah Press, Salt Lake City, 109-123

(1969) .

18. D. R. Atherton, C. W. Mays, and Betsy J. Stover; Radionuclide distribution in adult beagle bones; Radiobiology Laboratory Semi-Annual Progress Report C00-217, 118-125 (1958).
19. David R. Atherton, Betsy J. Stover, and F. W. Bruenger; Fractional weight of the beagle skeleton; Research in Radiobiology, University of Utah Report C00-119-240, 162-171 (1969).

Table 1. Distribution of Injected ^{241}Am in the Beagle Skeleton

Days after injection	1	7	8	15	15	22	401	448	1510	1533		
Inj $\mu\text{Ci/kg}$	4.53	4.46	4.47	2.90	2.77	2.78	2.78	2.83	0.31	0.91		
Sex	M	M	M	M	F	M	M	M	F	F		
Dog	T15W5.5	T32W5.5	T33W5.5	T56W5	T57W5	T16W5	T1W5	T2W5	T6W3	T4W4	Mean	S.D.
	PERCENT OF <u>RETAINED</u> SKELETAL ^{241}Am											
Calvarium	2.61%	1.63%	1.25%	1.80%	3.13%	2.12%	2.31%	3.24%	2.54%	3.93%	2.46	± 0.81
Maxillae	4.53	5.35	6.83	5.72	5.40	6.24	5.92	6.64	8.10	5.10	5.98	± 1.02
Mandibles	2.00	2.51	3.33	2.81	2.27	2.66	2.65	2.60	4.14	2.22	2.72	± 0.62
Cerv. Vert	8.44	8.01	5.69	8.54	8.23	8.54	9.11	7.91	6.18	8.71	7.94	± 1.11
Thor. Vert	18.29	18.19	17.56	17.88	18.41	18.51	16.96	16.47	9.03	18.20	16.95	± 2.86
Lumb. Vert*	20.41	19.03	17.56	18.74	19.35	18.89	16.61	17.65	8.76	18.26	17.53	± 3.26
Caud. Vert	1.55	1.98	1.91	1.85	1.68	1.87	1.80	1.75	1.63	1.72	1.77	± 0.13
Paws	1.63	2.09	2.41	1.83	1.92	2.21	2.32	2.42	8.35	2.55	2.77	± 1.98
Radii	0.91	1.05	1.36	1.15	1.01	1.16	1.14	1.28	1.85	0.99	1.19	± 0.27
Ulnae	1.07	1.30	1.54	1.48	1.30	1.35	1.38	1.42	2.31	1.32	1.45	± 0.33
Humeri	6.29	6.56	6.40	6.05	6.16	5.69	6.80	6.10	7.37	5.94	6.34	± 0.48
Tib+Fib ⁺	2.81	3.74	3.74	3.22	3.21	3.32	3.94	3.70	5.76	3.04	3.65	± 0.82
Femora	5.31	6.53	5.86	6.04	5.83	5.31	5.97	5.90	7.93	5.87	6.06	± 0.75
Pelves	5.10	4.84	5.38	4.61	4.82	4.54	5.85	5.13	7.26	5.06	5.26	± 0.80
Scapulae	2.86	3.00	3.99	3.50	4.02	3.61	3.93	3.62	4.79	3.77	3.71	± 0.55
Ribs	8.61	8.11	9.03	8.68	8.02	8.69	8.74	8.44	10.45	7.18	8.60	± 0.83
Sternum	7.21	5.88	5.96	6.10	5.24	5.29	4.57	5.33	3.55	6.14	5.53	± 0.99
Os Penis ⁺	0.37	0.20	0.20	--	--	--	--	0.40			0.29	± 0.11
TOTAL	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		
% of Inj ^{241}Am in Skeleton	19.6%	19.0%	18.8%	24.3%	23.6%	18.6%	29.2%	33.3%	33.4%	31.5%		

*Including sacrum

⁺Including patellae⁺Males only -- Not Measured

Table 2. ^{241}Am in the Beagle Skeleton

Bone	% of Skeletal ^{241}Am (Table 1)	% of Skeletal Ash (Ref. 18 & 19)	<u>Skeletal Am</u> Skeletal Ash
Skull	8.44	16.7	0.5
Mandibles	2.72	8.6	0.3
Cerv. Vert.	7.94	7.0	1.1
Thor. Vert.	16.95	6.3	2.7
Lumb. Vert.	17.53	7.5	2.3
Caud. Vert.	1.77	0.7	2.5
Paws	2.77	9.7	0.3
Radii	1.19	2.7	0.4
Ulnae	1.45	2.9	0.5
Humeri	6.34	6.3	1.0
Tib. + Fib.	3.65	6.3	0.6
Femora	6.06	6.7	0.9
Pelves	5.26	5.3	1.0
Scapulae	3.71	3.8	1.0
Ribs	8.60	8.0	1.1
Sternum	5.53	1.2	4.6
Os Penis	0.29	0.3	1.0

SUBCELLULAR DISTRIBUTION OF $^{241}\text{Am}(\text{III})$ AND $^{239}\text{Pu}(\text{IV})$

IN LIVERS STUDIED SERIALY

W. Stevens, F.W. Bruenger, D.R. Atherton,
and Betsy J. Stover

Abstract. Two beagles were injected with $2.67 \mu\text{Ci}$ of $^{241}\text{AmIII}$ and $2.77 \mu\text{Ci}$ of $^{239}\text{PuIV/kg}$ respectively. Liver specimens were obtained by serial surgical biopsies. Samples were homogenized, subjected to differential and sucrose density gradient centrifugation. Specific enzyme markers were used for locating mitochondria and lysosomes. The density distribution of these organelles in the dog did not allow a clear separation of these particles. At short times after injection, both nuclides were associated mostly with soluble liver proteins (ferritin). Later, increasing quantities migrated into the mitochondrial-lysosomal fraction. The rate of redistribution was faster for plutonium than for americium. In the dog injected with plutonium the percent dose in the cytosol changed from 44% to 3% and the percent dose in the mitochondrial-lysosomal fraction from 28% to 62% during a period of 70 days. Gradient separation of 6,000 g-min supernatants showed a shift in the americium content of heavy fractions from 3% to 50% and a concomitant shift from 76% to 29% in the light fractions during the same period. The respective values for plutonium are 8% to 32% in the heavy fraction and 66% to 21% in the light fraction during a period of 30 days with a second redistribution at time ≥ 30 days. On the basis of protein concentration, the highest concentration of both nuclides was associated with fractions of high acid phosphatase activity, but no linear relationship between acid phosphatase and total radioactivity was observed.

INTRODUCTION

Liver is one of the principal target organs for ^{241}Am and ^{239}Pu when monomeric forms of these nuclides are injected into canines. The uptake, distribution, retention and chemical association of these nuclides in the liver have long been of interest in this laboratory. Previous reports from other laboratories have described the localization of plutonium in lysosomal fractions (Rahman and Lindenbaum, Boocock, et al. ^(1,2)) in rodent livers. Some preliminary data on the relative concentration of ^{239}Pu and ^{241}Am and its association with ferritin in the cytosol were presented earlier. ^(3,4,5)

In liver fractionated by differential centrifugation, the largest percentage of plutonium was in the fraction designated as the mitochondrial-lysosomal fraction (10,000 x g and 25,000 x g) followed by the nuclei-cell debris fraction (1,000 x g pellet). The cytosol contained 1/2 to 1/3 of that seen in the other two fractions. In the cytosol the majority of either nuclide was bound to ferritin. These and other data ^(6,7) indicated also that a redistribution of nuclide in the liver takes place between short times (0-2 weeks) and long times (5 years) after injection. In this communication the redistribution of the nuclides at the molecular and subcellular level is described for times between 0 and 70 days post-injection.

MATERIALS AND METHODS

A male beagle weighing 11.3 kg received an intravenous injection of 2.67 μCi of $^{241}\text{Am}/\text{kg}$ in citrate buffer of pH 3.5. At one hour after injection less than 10% of the injected dose was circulating and a section of liver was removed by surgical biopsy. Successive biopsies were performed at 7, 29, and 69 days post-injection. Similarly, a female beagle weighing 9.13 kg received an intravenous injection of 2.77 μCi of $^{239}\text{Pu}/\text{kg}$ in the medium described above. Since plutonium is cleared from the circulation at a much slower rate, the first biopsy was not performed until 1 day after injection when about 24% of the nuclide was still circulating. Further biopsies were performed at 4, 8, 30, and 71 days post-injection.

Liver specimens were immediately cooled in ice, weighed, minced, and homogenized 2 times in two volumes of 0.25 molar sucrose which was also three millimolar in calcium ion and 0.1 molar in sodium chloride. The homogenate was then separated by differential centrifugation and by density gradient centrifugation. Fractions were analyzed for protein content,

acid phosphatase, cytochrome C oxidase, and radioactivity. A flow sheet outlining the differential centrifugation procedure is presented in Fig. 1. For comparison the schema of previous differential centrifugation procedures is included and appears in the upper right hand corner of Fig. 1. Changes in the procedure were made to bring this technique of analyzing canine livers in line with that of others in order to facilitate comparisons with work done on animals other than the beagle.

Gradient centrifugation was carried out with the 600 x g supernatant and the resuspended 3,000 x g, and 40,000 x g pellets. Gradients were prepared in an automatic gradient maker (Beckman). One ml of 82% sucrose was placed in the bottom of each tube to avoid the formation of a pellet. The gradients covered the range of sucrose concentration from 52 to 12%. An aliquot of the 600 x g supernatant was layered on the top of the gradient. This was then centrifuged for 120 minutes at 1,850 x g after which the bottoms of the tubes were punctured and fractions were collected. On each of these fractions protein was determined according to the method of Lowry⁽⁸⁾. Total acid phosphatase was done according to technical bulletin 104 of Sigma Chemical Company and cytochrome oxidase was determined according to the method of Cooperstein and Lazarow.⁽⁹⁾ Americium was quantitated by counting its 60 keV gamma radiation. ²³⁹Pu alpha particles were counted by a scintillation counting technique.

RESULTS

The distribution of ²³⁹Pu in canine livers, 7 days post-injection, is shown in Table 1. These data were obtained and reported in last year's Progress Report.⁽¹⁰⁾ The liver was fractionated by differential centrifugation as shown in the upper right hand corner of Fig. 1. The largest

percentage of plutonium in the liver was found in the fraction designated as mitochondrial-lysosomal followed by the nuclei and debris fraction. The majority of the ^{239}Pu found in the cytosol was bound to ferritin. The large concentration of plutonium found in the nuclei and debris fraction may be due to the presence of whole cells that would also sediment at 6,000 g-min.

The relative distribution of acid phosphatase, cytochrome C oxidase, americium expressed as counts per minute per mg of protein and the percent of the total americium in each fraction are shown in Fig. 2. These data were obtained by sucrose gradient centrifugation of 600 x g supernatants from a dog injected with americium. The acid phosphatase activity is a marker for lysosomes, and the cytochrome C oxidase represents mitochondria. No change in distribution of acid phosphatase was observed. Beginning at 7 days post-injection, the peaks of acid phosphatase activity (Sigma units/mg of protein) and the concentration of americium (CPM/mg of protein) coincided. However, the amount of nuclide, that is the percent of total americium in these fractions, was small. At short times after injection, americium was found mostly in the light fractions; later it became associated with the heavier fractions. The heavy portion represents mostly mitochondria; the lighter one (top) light particles and soluble material.

Similar data were obtained from the animal injected with ^{239}Pu and subjected to serial liver biopsies as shown in Fig. 3. The data obtained are similar to those observed in the dog injected with americium. Beginning at 1 day after injection the concentration of plutonium (counts per minute/mg of protein) showed a peak occurring at intermediate densities, representing lysosomes. This was maintained throughout the length of the experiment. The distribution (percent of the total activity in each fraction),

however, shifted from light fractions at one day to mostly heavy fractions at 30 days, and by 71 days this distribution had changed once again so that the plutonium was now equally distributed throughout the entire density range studied. This is in contrast to the americium where at this time the largest portion of the material was found in the heavy fraction. The two areas of cytochrome C oxidase activity represent a heavy fraction containing intact mitochondria and a light fraction representing cytochrome C oxidase that has been solubilized due to the rupture and destruction of mitochondria.

Table 2 summarizes the density distributions of plutonium and americium in specimens obtained by liver biopsy at various times after injection. The heavy fraction represents the large mitochondria and some of the larger microsomes (first 3 tubes of sucrose gradient were pooled). The light fraction represents the last 3 tubes of sucrose gradient fractionation and contains the very small cellular organelles and soluble material. The data are presented as the percent of the total nuclide placed on the gradient. As the percent of the total radioactivity in the light fraction decreased a concomittant increase was seen in the heavy fractions in both animals.

The percent of the injected plutonium in the whole liver found in the mitochondrial fraction (3,000 x g pellet) and the ratio of the mitochondrial fraction to that of the whole liver are shown in Table 3. The amount of plutonium in the whole liver remained constant throughout the experiment with the exception of the values seen at 4 days which probably represents a sampling artifact. The relative concentration of the plutonium in the mitochondrial pellet increased gradually from 1 day to 70 days and mitochondria and other heavy organelles contained 62% of the total plutonium

found in the whole liver. Sucrose density fractionation of this mitochondrial pellet indicated that the vast majority was associated with the heaviest particles. Similar treatment of the 40,000 x g pellet (lysosomes) showed a distribution of radioactivity which was more nearly uniform throughout the gradient.

In livers from animals injected with americium, the mitochondria showed a similar pattern, and at the end of the experimental period (69 days) the mitochondrial fraction contained 50% of the americium found in the liver, representing more than 30% of the total nuclide retained by the animal. Thus, the total amount of nuclide found in the mitochondrial fraction of the liver was greater for the dog injected with americium than for that injected with plutonium. Similar data for liver cytosols obtained from both the plutonium and the americium dog are given in Table 4 and the ratio of the respective amounts in cytosol and whole liver is given. In dogs injected with americium the percent of the injected dose in the cytosol decreased from 20% to 5% and the ratio of concentration from 0.37 to 0.11, thus, both the amount and the concentration in the cytosol changed at approximately the same rate. Similar trends were observed in cytosols from dogs injected with plutonium although the decrease was more marked, going from ~ 7% to 0.6% in the total amount and from 0.44 to 0.3 in the ratio of concentration. This is a rather distinct change in plutonium distribution and correlates with the increases seen in the mitochondrial fractions.

DISCUSSION

Monomeric americium and plutonium when injected as the citrate were bound to ferritin in canine livers. At short times after injection, a

redistribution occurred and the nuclides became associated with subcellular organelles. A similar redistribution was observed earlier in rodents injected with Pu.⁽²⁾ In this case, evidence was obtained that the nuclide was associated with lysosomes. In canines the highest concentration of both nuclides (on the basis of radioactivity/mg of protein) also coincided with the highest concentration of acid phosphatase (measured as enzyme-activity/mg of protein) but in terms of total radioactivity present, the amount of nuclide in the regions of acid phosphatase activity was very small and no linear relationship between phosphatase activity and radioactivity existed at any time during the period of observation. From this we conclude that in the canine (whose metabolism of the two nuclides differs from that in the rodent) there is little or no direct transfer of americium and plutonium from the soluble form into the lysosomes.

Figure 4 illustrates what we propose happens to monomeric ^{239}Pu when injected intravenously into dogs. First the nuclide is picked up by transferrin and transported both to the liver and the bone marrow. In each of these organs it is released from the transferrin at the cell membrane (erythroblasts in bone marrow, parenchymal cell in liver) and is bound by the glycoprotein matrix of bone and by ferritin in the liver cell. In the liver cell the plutonium ferritin can be found free in the cytosol or within mitochondria. Plutonium ferritin in mitochondria is probably in equilibrium with the soluble plutonium in the cytosol and/or membrane bound plutonium. Evidence for the binding of plutonium to subcellular membranes was obtained by rupture of these membranes with sonic shock or treatment of the mitochondrial pellet with sodium deoxycholate. Neither of these treatments caused the release of more than 30% of plutonium or americium from this fraction, the membrane fraction contained more than

70% of the initial radioactivity. Finally, upon death of mitochondria from irradiation or other causes, plutonium is released and is subsequently picked up by the lysosomes where the dead mitochondria are broken down and metabolized into component parts. Death of cells, which contain large amounts of plutonium, result in the release of this nuclide into the blood stream as large particles which are then taken up by macrophages and concentrated in the periportal areas. A similar mechanism may exist for americium but it is too early to describe its details. This schema would exist only for plutonium or americium in the completely monomeric form. Polymeric plutonium would be handled in a different fashion and would initially be phagocytized by the reticuloendothelial cells of the liver. In the reticuloendothelial cells then the plutonium polymer may be broken down and transported in a soluble form to the hepatocytes.

REFERENCES

1. Y. E. Rahman and A. Lindenbaum; Lysosome particles and subcellular distributions of polymeric tetravalent plutonium-239; *Radiat. Res.* 21: 575 (1964).
2. G. Boocock, C. J. Danpure, D. S. Popplewell, and D. M. Taylor; The subcellular distribution of plutonium in rat liver; *Radiat. Res.* 42: 381 (1970).
3. F. W. Bruenger, Betsy J. Stover, W. Stevens, and D. R. Atherton; Exchange of $^{239}\text{PuIV}$ between transferrin and ferritin in vitro; *Health Phys.* 16: 339 (1969).
4. Betsy J. Stover, F. W. Bruenger, and W. Stevens; Association of americium with ferritin in the canine liver; *Radiat. Res.* 43: 173 (1970).
5. F. W. Bruenger, Betsy J. Stover, and W. Stevens; $^{239}\text{Pu(IV)}$: Its

subcellular distribution and association with ferritin in canine livers; Health Phys. (in Press).

6. G. N. Taylor, W. S. S. Jee, N. L. Dockum, and E. Hromyk; Translocation of Pu and Am in beagle livers; (Abstract) Rad. Res. Soc. Annual Meeting (1967).
7. G. N. Taylor, T. F. Dougherty, and W. R. Christensen; Some toxicity aspects of internally deposited plutonium-239; Pathology of Irradiation, Edited by Charles C. Perdjis, Williams and Wilkins (1971).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall; Protein measurement with the Folin phenol reagent; J. Biol. Chem. 193: 265 (1951).
9. S. J. Cooperstein and A. Lazarow; A microspectrophotometric method for the determination of cytochrome oxidase; J. Biol. Chem., 189: 665 (1951).
10. Betsy J. Stover, F. W. Bruenger, and W. Stevens; The subcellular distribution of plutonium in the liver and its association with ferritin; Res. in Radiobiology, Univ. of Utah Report C00-119-242, 131 (1970).

TABLE 1
DISTRIBUTION OF ^{239}Pu IN LIVER FRACTIONS

	% of Total Retained ^{239}Pu	% of Total in the Liver
Nuclei and debris	6.5	24.0
Mitochondria	8.5	31.5
Cytosol	2.8	10.5
Liver retention was 27% of total body retention.		

TABLE 2
DISTRIBUTION OF ^{241}Am AND ^{239}Pu IN HEAVY
AND LIGHT FRACTIONS AFTER SUCROSE
GRADIENT SEPARATION OF 600g SUPERNATANTS†

Time after injection	Heavy*		Light**	
	Am	Pu	Am	Pu
1 hour	3.4		76.2	
1 day		8.1		66.0
4 days		4.3		34.4
7/8 days	12.1	22.6	45.0	34.8
29/30 days	16.9	31.9	49.3	13.2
69/70 days	50.4	17.6	29.4	20.8

† % of total activity in gradient.

* Sum of first three fractions.

** Sum of last three tubes.

TABLE 3

PERCENT DOSE OF ^{239}Pu IN WHOLE LIVER AND
LIVER MITOCHONDRIAL FRACTION (3000 x g)

Sample	Time after injection				
	1 day	4 days	8 days	30 days	70 days
% D in whole liver	15.1	25.2	16.8	17.0	18.8
% D in mitochondria*	4.2	9.1	6.5	11.0	11.6
$\left[\frac{\% \text{ D in mitochondria}}{\% \text{ D in whole liver}} \right]$	0.278	0.361	0.387	0.647	0.617

* Assume mitochondria comprise 20% of total liver protein.

TABLE 4

PERCENT DOSE OF NUCLIDE IN LIVER CYTOSOL*

Nuclide	Sample	Time after injection					
		1 hour	1 day	4 days	8 days	29/30 days	69/70 days
	% dose in cytosol	19.5	--	--	--	6.1	5.3
^{241}Am	$\left[\frac{\% \text{ dose in cytosol}}{\% \text{ dose in liver}} \right]$	0.370	--	--	--	0.110	0.115
	% dose in cytosol	--	6.7	3.6	2.2	0.5	0.6
^{239}Pu	$\left[\frac{\% \text{ dose in cytosol}}{\% \text{ dose in liver}} \right]$	--	0.440	0.140	0.130	0.30	0.30

* Assume cytosol comprises 40% of total liver protein.

Fig. 1. Schema outlining the present procedure used in the separation of liver tissue. The insert shows the separation procedure used previously.

Fig. 2. Density distribution of acid phosphatase, cytochrome C oxidase and americium found in 600 x g supernatant of liver biopsies following centrifugation in 12 to 52% sucrose gradients.

Fig. 3. Density distribution of acid phosphatase, cytochrome C oxidase and Pu found in 600 x g supernatant of liver biopsies following centrifugation in 12 to 52% sucrose gradients.

Fig. 4. A hypothetical schema of the fate of monomeric plutonium in liver following i.v. injection.

RETENTION OF $^{239}\text{Pu}(\text{IV})$ BY THE YOUNG ADULT BEAGLE*

Betsy J. Stover, D. R. Atherton, D. S. Buster,
and F. W. Bruenger

When $^{239}\text{Pu}(\text{IV})$ is given intravenously in 0.08M citrate buffer, pH 3.5, the important deposition sites are liver and skeleton. Metabolism of plutonium over six dose levels, (0.016 to 2.8 $\mu\text{Ci } ^{239}\text{Pu}/\text{kg}$ injected), is characterized by very slow rates of decrease, and by considerable variation between dogs which may mask effects of time and dose level. No effect of dose level on excretion rate nor of time on skeletal retention at the 2.8 $\mu\text{Ci}/\text{kg}$ level was observed. In contrast plutonium is lost from liver at a rate that increases with increasing dose level. Skeletal retention at lower dose levels during the first two years has been determined from measurements of 16 skeletons, from four dogs at 0.30 $\mu\text{Ci}/\text{kg}$ and 12 at 0.016 $\mu\text{Ci}/\text{kg}$, and is given by

$$B = 48.3 e^{-0.000458t}$$

in which B is percent injected plutonium in the skeleton and time is expressed in days. Retention in the humerus and third lumbar vertebra at subsequent times covering the normal life-span of the beagle is higher than predicted by this equation. These results are consistent with a higher rate of bone remodelling, which leads to removal from and burial of plutonium initially on bone surfaces, during early adult years, followed by a decrease in bone remodelling during which the skeletal retention of plutonium decreases more slowly.

* Abstract of presentation at the Radiation Research Society Meeting, Boston, May 1971.

EARLY SKELETAL DISTRIBUTION AND RETENTION OF $^{239}\text{Pu(IV)}$
AT AN INTERMEDIATE AND LOW DOSE LEVEL IN THE BEAGLE

Betsy J. Stover, David R. Atherton, and Dawn S. Buster

Abstract: Four dogs were injected intravenously with $0.30 \mu\text{Ci } ^{239}\text{Pu/kg}$ and twelve with $0.016 \mu\text{Ci } ^{239}\text{Pu/kg}$ and sacrificed at times ranging from 5 to 769 days after injection. Tetravalent, non-colloidal plutonium in about 9 ml 0.08 M citrate buffer solution of pH 3.5 was given in each case. Representative portions of all parts of the skeleton (except the sacrum) were analyzed for ^{239}Pu . Both the amount and the concentration in wet bone were measured so that total skeletal retention and average radiation dose rate for a particular bone could be estimated. The calculated skeletal retention is given by

$$B = 48.9 e^{-0.00050t} \quad \text{for } t < 1000\text{ds.}$$

which is not valid beyond about 1000 days. To illustrate the variation in skeletal deposition and retention from dog to dog and also bone to bone, data for each of the 16 are presented in this report.

INTRODUCTION

Our initial measurements of the distribution and retention of plutonium in the beagle were limited to high dose levels and the first four years after injection (1). A skeletal retention equation was derived from measurements of excretion and hepatic retention which was consistent with the limited skeletal measurements. However, since it was obtained mainly by difference, it was, in a sense, an upper limit for retention in the skeleton.

Extension of the measurements of excretion rate through eight years gave mostly values consistent with extrapolation of the equation for four years for urinary excretion and somewhat higher values for urinary plus fecal excretion (2). A few samples from dogs at the P2-level ($0.095 \mu\text{Ci/kg}$) were somewhat higher than anticipated. There was also evidence that retention in the liver at the 5-level ($2.9 \mu\text{Ci/kg}$) decreased more rapidly.

Investigation of the dose level (i.e., radiation damage) effect on hepatic retention was extended over six dose levels (0.016 $\mu\text{Ci/kg}$ to 2.9 $\mu\text{Ci/kg}$), and the data on excretion were reanalyzed for effect of dose level (3,4,5). A set of four equations for hepatic retention of ^{239}Pu was developed from the experimental results. Dose levels P1, P1.7, and P2 did not show a significant dose level effect and one equation suffices. Separate equations were calculated for the P3, P4, and P5 dose levels. Initial depositions did not differ significantly but the rate of loss of plutonium from the liver increased with increasing dose level. Interim excretion measurements from six months to four years exhibited no dose level effect for dose levels P2, P3, P4, and P5. However, this was not conclusive since the fluctuations in the excretion could mask a small dose level effect.

Measurement of the retention of ^{239}Pu in the humerus and third lumbar vertebra of 22 dogs at the highest dose level over 3 to 2059 days showed no significant decrease with time. However, the mean of the long term vertebral retention at a mean time of 1338 days exceeded that for the P4 dose level at 1320 days ($0.01 < p < 0.02$), and the long term retentions in both bones from the P5 dose level at 1338 days exceeded those of the P3 dose level at a mean time of 1650 days ($p < 0.01$).

These results suggested that, in the absence of a gross effect of dose level on excretion rate, the effect of dose level on skeletal retention was opposite to that on hepatic retention. If so, then determination of this effect was necessary in order to calculate skeletal dose rates at the all important low dose levels. It was

clear that the original skeletal retention equation yielded an over-estimation of the long term dose rate at low dose levels, and that additional data must be obtained.

MATERIALS AND METHODS

Young adult beagles were given intravenous injections of ^{239}Pu (IV) in 0.08 M citrate buffer solution of pH 3.5. Four received 0.30 $\mu\text{Ci/kg}$ (P3-level) and twelve received 0.016 $\mu\text{Ci/kg}$ (P1-level). The dogs were sacrificed at times ranging from 5 to 769 days after injection. Death for each animal was by exsanguination following sodium pentothal anesthesia.

Skeletal parts of each animal available for distributional analysis amounted to about 40% of the entire skeleton, the actual fraction varying from dog to dog. Each bone was carefully defleshed and, whenever possible, an attempt was made to obtain wet as well as ashed weights of all parts. Some of the skeletons had been inadvertently stored in acetone,* and could not be rehydrated satisfactorily, however, these wet weights are included in the results section. Ashed weights were obtained on all skeletal parts and, of course, are the more accurate of the two weights.

Teeth were not separated from bone in which they were set, thus upper teeth are included in skull specimens and lower teeth in mandibular samples.

The analysis of these specimens for their ^{239}Pu content was done by extracting the plutonium from an appropriate aliquot into

*Skeletons of T35P3, T24,30,39P1 were stored in acetone.

scintillator solution with EHPA (di-2-ethyl-hexyl phosphoric acid) according to the method reported previously. (6).

Samples were done in duplicate and a pair of samples from a bone solution containing a known concentration of ^{239}Pu were run with each extraction set (up to 20 samples). Counting gear was monitored by counting a known quantity of ^{239}Pu (contained in a solution of the same constitution as the bone and control samples) several times during the counting of each set of samples.

The yield of the extraction system, as determined on 96 control samples, averaged 89.4% with a fractional standard deviation of 0.063. These control samples contained from about 500 to 1500 pCi of ^{239}Pu each.

The agreement between pairs of samples was appraised by relating the range between the members of a sample pair to their mean value ($r/\bar{R}$). The average such value for 415 pairs of samples was 0.044 ± 0.014 . The amounts of plutonium in these samples ranged from a few tens to a few thousand pCi. Yield did not appear to be affected by the amount of plutonium in the sample.

The counting equipment was nicely consistent, giving 1.02 ± 0.011 counts/disintegration in a series of 553 observations of the ^{239}Pu -EHPA-scintillator standard (4077 pCi).

RESULTS

The results of the skeletal analyses are presented in Tables 1 through 16. The animals at the P3-level are listed first in order of increasing time after injection and those at the P1-level follow in

the same order. The distribution and estimated total retention* are given for each dog. Since only portions of the skeletons were analyzed, some assumptions had to be made in order to estimate the total retention. The fraction of a specific skeletal part that was actually analyzed is given in the second column of each of the Tables 1-16. In the case of the symmetrical skeleton, deposition was assumed to be symmetrical and one member of the pair was analyzed and the result was multiplied by two to give the % dose/part shown in the third column. The results obtained on portions of the asymmetric skeleton were treated similarly but required a more drastic assumption. For example, if the sample consisted of four of the seven cervical vertebrae, the total was obtained by multiplying the measured amount of plutonium in the sample of four by $7/4$ to obtain an estimate of the total amount in the cervical vertebrae. This has the serious limitation that the implicit assumptions that the concentration of plutonium in, and the size of the three missing vertebrae are equal to the average values of these quantities for the four that were measured. A further short-coming is that the fraction analyzed was not the same throughout the series. For example, samples of thoracic vertebrae contained 5, 6, 7, 8, 9, 10, or 11 vertebrae.

The sacrum was missing from each skeleton, and cervical vertebrae humeri, and sternum, were missing in one, two, and three, skeletons. respectively. "Paper sacra", etc. were "constructed" as follows: In a prior report the sacrum was shown from measurements on eleven dogs to comprise 1.14% of the skeleton on an ashed weight basis (7). Using this value and the measured skeletal ash, a corrected value for total skeletal ash is obtained. Then from the observed average ashed weight/

*Retention is in % of injected dose.

wet weight for each dog a wet weight is calculated for the sacrum and a corrected value for total wet skeletal weight is obtained. Similarly the average fraction of total skeletal plutonium in the sacrum is used to estimate the plutonium in the sacrum (or other missing bone). From this fraction and the measured total a corrected value for total skeletal retention is obtained.

The paws were not completely defleshed in this series of measurements and thus the wet weight was not obtained. It was assumed to be equal to the average value of the ashed weight/wet weight ratio of the samples that were actually measured for the particular dog.

The final column in Tables 1-16 gives the relative concentration in each skeletal part. If 10% of the ^{239}Pu in the skeleton is in a set of bones which has a weight equal to 10% of the total skeleton then the ratio is unity. The advantage of this quantity is that if only one skeletal part is available for analysis, but the relative concentration and the fraction of the skeleton comprised by this part are known, then a rough estimate of total ^{239}Pu in the skeleton can be made.

The means and standard deviations of the percent dose per skeletal part, percent dose per gram of wet bone, the ratio of ashed weight to wet weight of the skeletal part* and the relative concentration are given in Tables 17, 18 and 19 for four dogs at the P3 dose level, eleven at the P1 level, and for the combined fifteen animals respectively. One dog at the P1 dose level was sacrificed at just 5 days after injection and was not included. The values of percent dose per

*The average ratio of total skeletal ash to its wet weight for the 16 dogs in this study is 0.422 ± 0.025 .

gram of wet bone show that the average skeletal dose rates for these skeletal parts vary considerably from the average for the entire skeleton. (Note that the value for the tail is most likely too low since complete defleshing was difficult.) The relative concentration is lower than average in the bones of the head, neck, tail, and distal halves of the extremities, and higher than average in the bones of the chest, pelvis, and proximal halves of the extremities.

Another aspect of the variability of skeletal retention is shown in Tables 20, 21, 22 for the four P3, eleven P1, and fifteen P3 + P1 dogs, respectively. (The dog sacrificed at 5 days has been omitted again.) A single exponential has been fit to each set of data for retention in a given skeletal part. The calculated values of the intercept, a , and the coefficient of t (days), b , in ae^{bt} are given as well as the probability, p , that $|b|$ differs significantly from zero. The apparent increases with time are not significant but a number of the decreases are, including the estimated total skeletal retention.

DISCUSSION

The distribution of ^{239}Pu in the skeleton during the first two years after injection has been determined for beagles at the P1 level ($0.016 \mu\text{Ci/kg}$). At this dose level in the beagle there is no significant life-shortening but incidence of osteosarcoma is about 36%. Supporting data were also obtained from dogs at the P3 level ($0.30 \mu\text{Ci/kg}$). There is considerable variation in the macroscopic distribution in the various skeletal parts. In some parts the retention appeared constant, which probably means that the rate of decrease was

masked by the variation between dogs. In other skeletal parts, retention decreased during the two years as did the estimated total retention.

The latter is given by

$$B = (48.9 \pm 3.4) e^{-(5.01 \pm 1.45) 10^{-4} t} \quad (1)$$

for $t < 1000$ days.

Hepatic retention was also measured in the two groups of dogs.

The results have been reported in conjunction with long term data at the six dose levels ranging from 0.016 to 2.9 $\mu\text{Ci/kg}$ (2,3,4,5). Single exponentials for retention in the liver were calculated for each dose level, and for groups of dose levels. In all cases the value of the intercept (at $t = 0$) fell between 30 and 35% of the injected dose, and the rate of decrease of hepatic retention decreased with decreasing dose level, i.e., the coefficients of t became smaller. However, when the values for the P1 dogs which were sacrificed during the first two years are analyzed in the absence of the long term data, there is no significant decrease with time, and the mean is 32.5% of the injected dose.

In Table 23 we combine this result, and Eq. (1), for bone with results on excretion and distribution in other soft tissues (4) to obtain a "balance sheet" for the early disposition of ^{239}Pu at the time when the amount of circulating plutonium has become quite low, i.e., several weeks after injection. Most of the injected plutonium is accounted for, and, if the same fractional loss is assigned to each of the four categories, the corrected values are obtained. As noted above, values for "initial" deposition in the liver are 30 to 35%. Values for skeletal retention at 22 days, which were obtained by

difference, and therefore are probably a little too high, are, for the P5 level $B = 57 \pm 10$, and for the P3 level $B = 55 \pm 9$ (4). Thus, when the young adult beagle is given an intravenous injection of $^{239}\text{Pu}(\text{IV})$ in 0.08 M citrate buffer of pH 3.5 over the injected dose level range 0.016 to 2.9 $\mu\text{Ci}/\text{kg}$, about 1/2 goes to the skeleton, 1/3 goes to the liver, and 1/6 is either excreted or is deposited in other soft tissue.

In Figure 1 are shown the data for hepatic and skeletal retention in the P1 dogs during the first two years after injection. During these two years, skeletal retention decreases so that at 2 years and at low dose levels, approximately 1/3 of the injected ^{239}Pu is in the skeleton, 1/3 in the liver, and 1/3 has been excreted. (The previous report gave $30 \pm 4\%$ excreted at four years for dose levels P2 (0.095 $\mu\text{Ci}/\text{kg}$) to P5 (4).

At higher dose levels, decrease in skeletal retention is slower than at the P1 level (4). At longer times at the P1 level the decrease is slower than that given by Eq (1). (8). Both are consistent with the observation that the normal rate of bone remodeling in the dog is still rapid at 1.5 years of age and decreases to a low value at three years, and that at the higher dose levels radiation damage impedes the remodeling processes (9).

Fig. 1. Skeletal and hepatic retention by the beagle for 2 years following intravenous injection of 0.016 $\mu\text{Ci}/\text{kg}$ of ^{239}Pu .

Table 1

T31P3

Wt. at Injection: 13000 (g) Wt. of Wet Skeleton: 853 (g)
 Inj. to Death: 40 (days) Wt. of Ashed Skeleton: 355 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.08	0.0245	0.463	0.45
Mandibles	1/2	0.99	0.0176	0.552	0.32
Cervical Vertebrae	4/7	2.26	0.0390	0.468	0.72
Thoracic Vertebrae	6/13	6.67	0.110	0.380	2.02
Lumbar Vertebrae	2/7	6.80	0.117	0.367	2.14
Caudal Vertebrae	12/15	0.32	0.0118	0.139	0.22
Paws*	2/4	1.21	0.0147	0.416	0.27
Radii	1/2	0.48	0.0236	0.451	0.44
Ulnae	1/2	0.47	0.0214	0.481	0.39
Humeri	1/2	4.16	0.0662	0.357	1.21
Tibiae-Fibulae	1/2	2.18	0.0455	0.483	0.83
Femora	1/2	4.06	0.0750	0.448	1.37
Pelvis	1/2	3.64	0.0829	0.439	1.52
Scapulae	1/2	2.56	0.0818	0.418	1.50
Ribs	13/26	5.32	0.0653	0.347	1.20
Sternum	4/7	1.16	0.101	0.179	1.86
Sacrum	0	1.24	0.127	0.416	2.34
Total		46.5	0.0545	0.416	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** $\frac{\text{Fraction of } ^{239}\text{Pu retained by skeletal part}}{\text{Fraction of skeletal wet weight in part}}$

Table 2

T38P3

Wt. at Injection: 7960 (g) Wt. of Wet Skeleton: 608 (g)
 Inj. to Death: 187 (days) Wt. of Ashed Skeleton: 249 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.60	0.0419	0.482	0.58
Mandibles	1/2	1.23	0.0329	0.535	0.45
Cervical Vertebrae	4/7	2.33	0.0514	0.458	0.70
Thoracic Vertebrae	6/13	6.50	0.157	0.384	2.16
Lumbar Vertebrae	3/7	4.85	0.114	0.360	1.56
Caudal Vertebrae	12/15	0.24	0.0129	0.152	0.18
Paws*	2/4	1.42	0.0298	0.410	0.33
Radii	1/2	0.40	0.0306	0.480	0.42
Ulnae	1/2	0.38	0.0271	0.492	0.37
Humeri	1/2	3.47	0.0806	0.356	1.10
Tibiae-Fibulae	1/2	1.56	0.0434	0.423	0.60
Femora	1/2	3.68	0.0902	0.395	1.24
Pelvis	1/2	3.18	0.113	0.452	1.55
Scapulae	1/2	2.84	0.135	0.451	1.85
Ribs	12/26	6.35	0.101	0.345	1.38
Sternum	1/1	1.16	0.100	0.159	1.37
Sacrum	0	1.18	0.170	0.410	2.33
Total		44.4	0.0730	0.410	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** $\frac{\text{Fraction of } ^{239}\text{Pu retained by skeletal part}}{\text{Fraction of skeletal wet weight in part}}$

Table 3

T35P3

Wt. at Injection: 11900(g) Wt. of Wet Skeleton: 947 (g)
 Inj. to Death: 362(days) Wt. of Ashed Skeleton: 442 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.28	0.0235	0.499	0.50
Mandibles	1/2	1.17	0.0183	0.564	0.39
Cervical					
Vertebrae	4/7	2.22	0.0304	0.540	0.65
Thoracic					
Vertebrae	6/13	8.06	0.120	0.467	2.57
Lumbar					
Vertebrae	3/7	4.24	0.0731	0.443	1.57
Caudal					
Vertebrae	12/15	0.31	0.0111	0.162	0.24
Paws*	2/4	1.73	0.0230	0.466	0.49
Radii	1/2	0.42	0.0180	0.527	0.39
Ulnae	1/2	0.41	0.0154	0.514	0.33
Humeri	1/2	3.15	0.0536	0.396	1.15
Tibiae-Fibulae	1/2	1.65	0.0284	0.499	0.61
Femora	1/2	4.18	0.0629	0.484	1.35
Pelvis	1/2	3.50	0.0663	0.510	1.42
Scapulae	1/2	2.24	0.0590	0.475	1.27
Ribs	13/26	4.56	0.0522	0.408	1.12
Sternum	1/1	1.84	0.0911	0.199	1.96
Sacrum	0	1.17	0.108	0.466	2.34
Total		44.1	0.0466	0.466	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
 Fraction of skeletal wet weight in part

Table 4

T29P3

Wt. at Injection: 12100 (g)
Inj. to Death: 560 (days)

Wt. of Wet Skeleton: 832 (g)
Wt. of Ashed Skeleton: 363 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.58	0.0265	0.428	0.56
Mandibles	1/2	1.34	0.0205	0.565	0.43
Cervical					
Vertebrae	4/7	1.86	0.0385	0.494	0.80
Thoracic					
Vertebrae	9/13	5.67	0.101	0.384	2.11
Lumbar					
Vertebrae	2/7	4.90	0.106	0.428	2.22
Caudal					
Vertebrae	12/15	0.27	0.0118	0.152	0.25
Paws*	2/4	1.22	0.0118	0.436	0.25
Radii	1/2	0.45	0.0245	0.536	0.51
Ulnae	1/2	0.55	0.0273	0.512	0.57
Humeri	0	3.66	0.0699	0.436	1.46
Tibiae-Fibulae	1/2	1.46	0.0314	0.496	0.66
Femora	1/2	2.92	0.0595	0.476	1.24
Pelvis	1/2	3.09	0.0826	0.491	1.73
Scapulae	1/2	1.95	0.0651	0.446	1.36
Ribs	13/26	4.82	0.0607	0.341	1.27
Sternum	4/7	0.97	0.0762	0.191	1.63
Sacrum	0	1.06	0.112	0.436	2.35
Total		39.8	0.0478	0.436	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
Fraction of skeletal wet weight in part

Table 5

T36Pl

Wt. at Injection: 10400 (g) Wt. of Wet Skeleton: 773 (g)
 Inj. to Death: 5(days) Wt. of Ashed Skeleton: 322 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	2.44	0.020	0.473	0.34
Mandibles	1/2	1.16	0.0196	0.551	0.33
Cervical					
Vertebrae	4/7	2.25	0.0458	0.479	0.76
Thoracic					
Vertebrae	6/13	7.73	0.157	0.378	2.61
Lumbar					
Vertebrae	2/7	6.32	0.130	0.370	2.16
Caudal					
Vertebrae	15/15	0.16	0.0070	0.124	0.12
Paws*	2/4	1.11	0.0144	0.417	0.24
Radii	1/2	0.33	0.0156	0.453	0.26
Ulnae	1/2	0.30	0.0140	0.452	0.23
Humeri	1/2	4.94	0.0978	0.426	1.62
Tibiae-Fibulae	1/2	1.59	0.0344	0.445	0.57
Femora	1/2	4.36	0.0868	0.436	1.44
Pelvis	1/2	3.47	0.0909	0.452	1.51
Scapulae	1/2	2.93	0.0977	0.437	1.67
Ribs	13/26	5.44	0.0728	0.345	1.21
Sternum	1/1	0.86	0.137	0.184	2.26
Sacrum	0	1.18	0.134	0.417	2.22
Total		46.6	0.0603	0.417	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt. for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
 Fraction of skeletal wet weight in part

Table 6

T30P1

Wt. at Injection: 12400 (g)
Inj. to Death: 35 (days)

Wt. of Wet Skeleton: 722 (g)
Wt. of Ashed Skeleton: 328 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	2.54	0.0228	0.469	0.37
Mandibles	1/2	0.80	0.0164	0.561	0.26
Cervical					
Vertebrae	4/7	2.35	0.0448	0.495	0.74
Thoracic					
Vertebrae	11/13	7.98	0.161	0.425	2.59
Lumbar					
Vertebrae	3/7	5.69	0.123	0.440	1.99
Caudal					
Vertebrae	15/15	0.18	0.0187	0.255	0.30
Paws*	2/4	1.53	0.0215	0.449	0.35
Radii	1/2	0.35	0.0194	0.480	0.31
Ulnae	1/2	0.40	0.0209	0.506	0.34
Humeri	1/2	5.17	0.111	0.466	1.79
Tibiae-Fibulae	1/2	1.81	0.0406	0.486	0.65
Femora	1/2	3.30	0.0670	0.442	1.08
Pelvis	1/2	3.41	0.0874	0.449	1.41
Scapulae	1/2	2.32	0.0789	0.436	1.27
Ribs	13/26	5.26	0.0671	0.354	1.08
Sternum	0	0.64	0.0729	0.171	1.18
Sacrum	0	1.14	0.136	0.449	2.20
Total		44.9	0.0621	0.449	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
Fraction of skeletal wet weight in part

Table 7

- 192 -

T24P1

Wt. at Injection: 13100 (g) Wt. of Wet Skeleton: 793 (g)
 Inj. to Death: 97 (days) Wt. of Ashed Skeleton: 357 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.83	0.0313	0.496	0.50
Mandibles	1/2	1.23	0.0248	0.565	0.40
Cervical					
Vertebrae	4/7	2.39	0.0424	0.503	0.68
Thoracic					
Vertebrae	8/13	5.70	0.134	0.425	2.14
Lumbar					
Vertebrae	2/7	5.48	0.103	0.445	1.74
Caudal					
Vertebrae	15/15	0.30	0.0220	0.231	0.34
Paws*	2/4	2.09	0.0248	0.450	0.40
Radii	1/2	0.39	0.0197	0.477	0.31
Ulnae	1/2	0.49	0.0220	0.490	0.35
Humeri	1/2	5.34	0.108	0.460	1.73
Tibiae-fibulae	1/2	2.28	0.0437	0.482	0.70
Femora	1/2	4.72	0.0821	0.437	1.31
Pelvis	1/2	3.49	0.0861	0.487	1.38
Scapulae	1/2	3.09	0.100	0.432	1.60
Ribs	13/26	6.48	0.0771	0.359	1.23
Sternum	1/1	1.03	0.146	0.210	2.33
Sacrum	0	1.26	0.139	0.450	2.22
Total		49.6	0.0626	0.450	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ^{239}Pu retained by skeletal part
Fraction of skeletal wet weight in part

Table 8

T37P1

Wt. at Injection: 8590 (g) Wt. of Wet Skeleton: 695 (g)
 Inj. to Death: 186 (days) Wt. of Ashed Skeleton: 294 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.52	0.0318	0.441	0.49
Mandibles	1/2	1.02	0.0230	0.534	0.35
Cervical					
Vertebrae	4/7	1.96	0.0430	0.488	0.66
Thoracic					
Vertebrae	10/13	6.41	0.137	0.395	2.12
Lumbar					
Vertebrae	4/7	5.41	0.127	0.454	1.96
Caudal					
Vertebrae	15/15	0.24	0.0175	0.186	0.27
Paws*	2/4	1.94	0.0266	0.424	0.41
Radii	1/2	0.50	0.0296	0.484	0.46
Ulnae	1/2	0.47	0.0262	0.465	0.40
Humeri	1/2	4.53	0.101	0.406	1.56
Tibiae-Fibulae	1/2	2.14	0.0496	0.471	0.77
Femora	1/2	3.80	0.0867	0.467	1.34
Pelvis	1/2	3.24	0.0856	0.440	1.32
Scapulae	1/2	2.19	0.0852	0.423	1.32
Ribs	13/26	5.87	0.0775	0.339	1.20
Sternum	1/1	0.65	0.136	0.178	2.12
Sacrum	0	1.14	0.144	0.424	2.22
Total		45.0	0.0648	0.424	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** $\frac{\text{Fraction of } ^{239}\text{Pu retained by skeletal part}}{\text{Fraction of skeletal wet weight in part}}$

Table 9

T32P1

Wt. at Injection: 8470 (g) Wt. of Wet Skeleton: 569 (g)
 Inj. to Death: 274 (days) Wt. of Ashed Skeleton: 212 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	5.62	0.0505	0.412	0.61
Mandibles	1/2	2.50	0.0546	0.528	0.66
Cervical					
Vertebrae	0	2.10	0.0505	0.373	0.71
Thoracic					
Vertebrae	7/13	5.61	0.131	0.310	1.58
Lumbar					
Vertebrae	2/7	5.48	0.170	0.312	2.05
Caudal					
Vertebrae	15/15	0.24	0.0178	0.125	0.21
Paws*	2/4	1.81	0.0363	0.373	0.44
Radii	1/2	0.49	0.0408	0.445	0.49
Ulnae	1/2	0.54	0.0467	0.399	0.56
Humeri	1/2	7.45	0.247	0.431	2.98
Tibiae-Fibulae	1/2	1.40	0.0537	0.411	0.65
Femora	1/2	3.42	0.110	0.418	1.33
Pelvis	1/2	1.88	0.0824	0.401	1.00
Scapulae	1/2	1.98	0.0767	0.316	0.93
Ribs	12/26	4.96	0.0811	0.306	1.07
Sternum	1/1	0.39	0.113	0.194	1.37
Sacrum	0	1.19	0.184	0.373	2.22
Total		47.1	0.0827	0.373	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
 Fraction of skeletal wet weight in part

Table 10

T33P1

Wt. at Injection: 10700 (g)
Inj. to Death: 375 (days)

Wt. of Wet Skeleton: 969 (g)
Wt. of Ashed Skeleton: 374 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	4.25	0.0248	0.407	0.57
Mandibles	1/2	1.25	0.0176	0.540	0.41
Cervical					
Vertebrae	4/7	2.02	0.0316	0.428	0.73
Thoracic					
Vertebrae	6/13	6.00	0.0902	0.332	2.07
Lumbar					
Vertebrae	3/7	4.82	0.0822	0.375	1.89
Caudal					
Vertebrae	15/15	0.22	0.0080	0.121	0.18
Paws*	2/4	1.44	0.0136	0.386	0.31
Radii	1/2	0.39	0.0193	0.485	0.45
Ulnae	1/2	0.41	0.0184	0.483	0.43
Humeri	1/2	5.06	0.0936	0.435	2.15
Tibiae-Fibulae	1/2	1.21	0.0236	0.452	0.54
Femora	1/2	3.30	0.0604	0.425	1.39
Pelvis	1/2	2.93	0.0661	0.420	1.52
Scapulae	1/2	2.01	0.0540	0.406	1.24
Ribs	13/26	5.14	0.0525	0.301	1.24
Sternum	1/1	0.61	0.0556	0.126	1.27
Sacrum	0	1.07	0.0966	0.386	2.22
Total		42.1	0.0435	0.386	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
Fraction of skeletal wet weight in part

Table 11

T39Pl

Wt. at Injection: 10700 (g) Wt. of Wet Skeleton: 822 (g)
 Inj. to Death: 376 (days) Wt. of Ashed Skeleton: 371 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	2.83	0.0210	0.462	0.55
Mandibles	1/2	0.67	0.0128	0.544	0.33
Cervical					
Vertebrae	4/7	2.81	0.0625	0.638	1.64
Thoracic					
Vertebrae	6/13	5.49	0.0764	0.320	2.00
Lumbar					
Vertebrae	3/7	4.29	0.0806	0.461	2.11
Caudal					
Vertebrae	15/15	0.36	0.0124	0.188	0.32
Paws*	2/4	1.22	0.0156	0.451	0.41
Radii	1/2	0.21	0.0116	0.506	0.30
Ulnae	1/2	0.27	0.0132	0.526	0.35
Humeri	1/2	3.42	0.0705	0.463	1.85
Tibiae-Fibulae	1/2	0.91	0.0185	0.491	0.48
Femora	1/2	3.04	0.0594	0.463	1.56
Pelvis	1/2	2.52	0.0632	0.486	1.65
Scapulae	1/2	1.56	0.0510	0.445	1.34
Ribs	13/26	4.01	0.0485	0.366	1.27
Sternum	0	0.64	0.0836	0.172	2.20
Sacrum	0	0.80	0.0850	0.451	2.22
Total		31.4	0.0382	0.451	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
 Fraction of skeletal wet weight in part

Table 12

T25P1

Wt. at Injection: 13800 (g)
Inj. to Death: 467 (days)

Wt. of Wet Skeleton: 953 (g)
Wt. of Ashed Skeleton: 398 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.90	0.0244	0.442	0.55
Mandibles	1/2	1.19	0.0193	0.538	0.44
Cervical					
Vertebrae	4/7	1.94	0.0305	0.493	0.69
Thoracic					
Vertebrae	7/13	5.91	0.0816	0.371	1.85
Lumbar					
Vertebrae ^a	3/7	4.41	0.0721	0.433	1.63
Caudal					
Vertebrae	15/15	0.61	0.0717	0.165	1.63
Paws	2/4	2.50	0.0246	0.418	0.56
Radii	1/2	0.86	0.0372	0.476	0.84
Ulnae	1/2	0.54	0.0211	0.477	0.48
Humeri ^a	1/2	3.84	0.0713	0.473	1.62
Tibiae-Fibulae	1/2	1.64	0.0291	0.462	0.66
Femora	1/2	3.30	0.0564	0.439	1.28
Pelvis	1/2	2.82	0.0561	0.442	1.27
Scapulae	1/2	1.77	0.0465	0.416	1.06
Ribs	13/26	5.10	0.0527	0.338	1.20
Sternum	0	0.64	0.0648	0.172	1.47
Sacrum	0	1.05	0.0962	0.418	2.19
Total		42.0	0.0441	0.418	1.00

^a Wet weights obtained on only these two parts. Other wet weights are estimated from ashed wt/wet weight average of the other animals in this series.

** Fraction of ²³⁹Pu retained by skeletal part
 Fraction of skeletal wet weight in part

Table 13

T28Pl.

Wt. at Injection: 10500 (g) Wt. of Wet Skeleton: 799 (g)
Inj. to Death: 559 (days) Wt. of Ashed Skeleton: 318 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	3.62	0.0261	0.428	0.52
Mandibles	1/2	1.35	0.0237	0.519	0.47
Cervical					
Vertebrae	4/7	2.17	0.0390	0.468	0.77
Thoracic					
Vertebrae	6/13	5.70	0.110	0.398	2.17
Lumbar					
Vertebrae	3/7	4.00	0.0858	0.408	1.70
Caudal					
Vertebrae	15/15	0.27	0.0153	0.135	0.35
Paws*	2/4	2.17	0.0264	0.398	0.52
Radii	1/2	0.48	0.0268	0.459	0.53
Ulnae	1/2	0.53	0.0287	0.476	0.57
Humeri	1/2	3.83	0.0947	0.431	1.88
Tibiae-Fibulae	1/2	1.51	0.0349	0.467	0.69
Femora	1/2	3.33	0.0664	0.402	1.31
Pelvis	1/2	2.88	0.0720	0.396	1.42
Scapulae	1/2	2.18	0.0692	0.390	1.37
Ribs	11/26	4.78	0.0540	0.299	1.07
Sternum	1/1	0.54	0.0545	0.114	1.08
Sacrum	0	1.02	0.112	0.398	2.22
Total		40.4	0.0505	0.398	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
Fraction of skeletal wet weight in part

Table 14

T26P1

Wt. at Injection: 12000 (g)
Inj. to Death: 647 (days)

Wt. of Wet Skeleton: 793 (g)
Wt. of Ashed Skeleton: 325 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative ** Conc.
Skull	1/2	3.87	0.0271	0.419	0.66
Mandibles	1/2	1.35	0.0235	0.531	0.57
Cervical					
Vertebrae	4/7	1.76	0.0343	0.460	0.83
Thoracic					
Vertebrae	6/13	3.88	0.0778	0.378	1.88
Lumbar					
Vertebrae	3/7	3.16	0.0673	0.402	1.63
Caudal					
Vertebrae	15/15	0.16	0.0108	0.154	0.26
Paws*	2/4	1.49	0.0189	0.410	0.46
Radii	1/2	0.34	0.0186	0.483	0.45
Ulnae	1/2	0.44	0.0212	0.472	0.51
Humeri	1/2	2.59	0.0544	0.431	1.32
Tibiae-Fibulae	1/2	1.25	0.0270	0.446	0.65
Femora	1/2	3.59	0.0724	0.440	1.75
Pelvis	1/2	2.44	0.0584	0.431	1.41
Scapulae	1/2	1.59	0.0522	0.440	1.26
Ribs	13/26	3.54	0.0461	0.344	1.12
Sternum	1/1	0.51	0.0480	0.164	1.16
Sacrum	0	0.83	0.0919	0.410	2.23
Total		32.8	0.0413	0.41	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** $\frac{\text{Fraction of } ^{239}\text{Pu retained by skeletal part}}{\text{Fraction of skeletal wet weight in part}}$

Table 15

T34P1

Wt. at Injection: 9680 (g) Wt. of Wet Skeleton: 707 (g)
 Inj. to Death: 746 (days) Wt. of Ashed Skeleton: 296 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	4.13	0.0327	0.428	0.56
Mandibles	1/2	1.43	0.0270	0.530	0.52
Cervical					
Vertebrae	3/7	1.84	0.0393	0.496	0.75
Thoracic					
Vertebrae	5/13	4.48	0.102	0.419	1.96
Lumbar					
Vertebrae	1/7	3.26	0.0869	0.426	1.66
Caudal					
Vertebrae	15/15	0.26	0.0154	0.131	0.30
Paws*	2/4	1.90	0.0266	0.419	0.51
Radii	1/2	0.52	0.0305	0.485	0.58
Ulnae	1/2	0.60	0.0317	0.484	0.61
Humeri	0	4.37	0.104	0.419	2.00
Tibiae-Fibulae	1/2	1.59	0.0379	0.476	0.72
Femora	1/2	2.70	0.0619	0.448	0.77
Pelvis	1/2	2.45	0.0704	0.444	1.35
Scapulae	1/2	1.92	0.0716	0.428	1.37
Ribs	13/26	4.10	0.0574	0.352	1.10
Sternum	1/1	0.38	0.0680	0.185	1.30
Sacrum	0	0.94	0.116	0.419	2.23
Total		36.9	0.0522	0.419	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
 Fraction of skeletal wet weight in part

Table 16

T40Pl

Wt. at Injection: 9920 (g) Wt. of Wet Skeleton: 848 (g)
 Inj. to Death: 769(days) Wt. of Ashed Skeleton: 357 (g)

Skeletal Part	Fraction Analyzed	% Dose Part	% Dose g Wet Bone	Ashed Wt. Wet Wt.	Relative** Conc.
Skull	1/2	2.90	0.0224	0.430	0.62
Mandibles	1/2	0.99	0.0180	0.520	0.50
Cervical					
Vertebrae	4/7	1.62	0.0272	0.477	0.75
Thoracic					
Vertebrae	6/13	5.33	0.0818	0.373	2.25
Lumbar					
Vertebrae	3/7	3.96	0.0705	0.420	1.94
Caudal					
Vertebrae	15/15	0.20	0.0130	0.166	0.37
Paws*	2/4	1.44	0.0175	0.421	0.48
Radii	1/2	0.38	0.0190	0.484	0.52
Ulnae	1/2	0.35	0.0159	0.492	0.44
Humeri	1/2	2.81	0.0550	0.451	1.51
Tibiae-Fibulae	1/2	1.05	0.0200	0.451	0.55
Femora	1/2	1.80	0.0324	0.448	0.89
Pelvis	1/2	2.60	0.0571	0.460	1.57
Scapulae	1/2	1.73	0.0511	0.426	1.40
Ribs	13/26	2.19	0.0255	0.357	0.70
Sternum	1/1	0.75	0.0882	0.196	2.42
Sacrum	0	0.78	0.0810	0.421	2.22
Total		30.9	0.0364	0.421	1.00

* Wet bone not weighed - Estimated using average ashed wt/wet wt for this dog.

** Fraction of ²³⁹Pu retained by skeletal part
Fraction of skeletal wet weight in part

Table 17

Average Skeletal Retention and Distribution for Four 3-Level
Plutonium Beagles 40 to 560 Days after Injection

Skeletal Part	<u>% Dose</u> Part		<u>% Dose</u> g Wet Bone		<u>Ashed Wt.</u> Wet Wt.		Relative Concentration	
	Av.	FSD*	Av.	FSD*	Av.	FSD*	Av.	FSD*
Skull	3.38	0.074	0.0291	0.30	0.468	0.065	0.52	0.11
Mandibles	1.18	0.12	0.0223	0.32	0.554	0.025	0.40	0.14
Cervical								
Vertebrae	2.17	0.098	0.0398	0.22	0.490	0.075	0.72	0.088
Thoracic								
Vertebrae	6.72	0.15	0.122	0.20	0.404	0.10	2.22	0.11
Lumbar								
Vertebrae	5.20	0.21	0.114	0.20	0.400	0.11	1.87	0.19
Caudal								
Vertebrae	0.28	0.14	0.0119	0.062	0.151	0.062	0.22	0.14
Paws	1.40	0.17	0.0183	0.33	0.429	0.059	0.33	0.33
Radii	0.44	0.083	0.0242	0.21	0.498	0.081	0.44	0.12
Ulnae	0.45	0.17	0.0228	0.25	0.470	0.11	0.42	0.26
Humeri	3.61	0.12	0.0676	0.16	0.386	0.099	1.23	0.13
Tibiae-								
Fibulae	1.71	0.19	0.0372	0.23	0.475	0.075	0.68	0.16
Femora	3.71	0.15	0.0719	0.19	0.451	0.089	1.30	0.055
Pelvis	3.35	0.077	0.0862	0.23	0.473	0.070	1.56	0.082
Scapulae	2.40	0.16	0.0762	0.59	0.448	0.052	1.49	0.17
Ribs	5.26	0.15	0.0698	0.31	0.360	0.089	1.24	0.089
Sternum	1.28	0.30	0.0921	0.12	0.182	0.095	1.70	0.16

* Fractional Standard Deviation

Table 18

Average Skeletal Retention and Distribution for Eleven 1-Level
Plutonium Beagles 35 to 769 Days after Injection

Skeletal Part	<u>% Dose</u> <u>Part</u>		<u>% Dose</u> <u>g Wet Bone</u>		<u>Ashed Wt.</u> <u>Wet Wt.</u>		Relative Concentration	
	Av.	FSD*	Av.	FSD*	Av.	FSD*	Av.	FSD*
Skull	3.73	0.22	0.0286	0.29	0.440	0.061	0.55	0.14
Mandibles	1.25	0.38	0.0237	0.47	0.537	0.027	0.45	0.25
Cervical								
Vertebrae	2.09	0.16	0.0405	0.25	0.484	0.132	0.81	0.34
Thoracic								
Vertebrae	5.68	0.18	0.108	0.27	0.377	0.109	2.06	0.12
Lumbar								
Vertebrae	4.67	0.20	0.0971	0.32	0.416	0.102	1.84	0.096
Caudal								
Vertebrae	0.28	0.44	0.0202	0.87	0.169	0.259	0.41	0.99
Paws	1.78	0.22	0.0229	0.28	0.418	0.062	0.44	0.17
Radii	0.45	0.36	0.0248	0.36	0.472	0.073	0.48	0.32
Ulnae	0.46	0.21	0.0242	0.38	0.479	0.066	0.46	0.21
Humeri	4.40	0.31	0.101	0.52	0.442	0.049	1.85	0.24
Tibiae-								
Fibulae	1.53	0.28	0.0348	0.34	0.463	0.049	0.67	0.20
Femora	3.30	0.22	0.0686	0.29	0.439	0.043	1.33	0.17
Pelvis	2.79	0.17	0.0713	0.17	0.442	0.067	1.39	0.12
Scapulae	2.03	0.21	0.0669	0.26	0.414	0.087	1.29	0.14
Ribs	4.68	0.25	0.0581	0.28	0.338	0.072	1.12	0.14
Sternum	0.61	0.29	0.0846	0.40	0.171	0.168	1.61	0.31

* Fractional Standard Deviation

Table 19

Average Skeletal Retention and Distribution for Fifteen One
and Three Level Plutonium Beagles 35 to 769 Days after Injection

Skeletal Part	% Dose Part		% Dose g Wet Bone		Ashed Wt. Wet Wt.		Relative Concentration	
	Av.	FSD*	Av.	FSD*	Av.	FSD*	Av.	FSD*
Skull	3.64	0.20	0.0286	0.29	0.447	0.067	0.54	0.13
Mandibles	1.23	0.33	0.0233	0.43	0.542	0.029	0.43	0.23
Cervical								
Vertebrae	2.11	0.14	0.403	0.23	0.485	0.12	0.79	0.30
Thoracic								
Vertebrae	5.96	0.18	0.111	0.26	0.384	0.11	2.10	0.12
Lumbar								
Vertebrae	4.72	0.20	0.0986	0.29	0.412	0.10	1.85	0.12
Caudal								
Vertebrae	0.28	0.38	0.0180	0.85	0.164	0.38	0.36	0.99
Paws	1.67	0.23	0.0217	0.30	0.421	0.060	0.41	0.23
Radii	0.44	0.31	0.0246	0.32	0.479	0.076	0.47	0.29
Ulnae	0.46	0.20	0.0238	0.34	0.485	0.060	0.45	0.22
Humeri	4.19	0.29	0.0920	0.51	0.428	0.085	1.69	0.28
Tibiae-								
Fibulae	1.58	0.25	0.0351	0.31	0.466	0.056	0.67	0.19
Femora	3.41	0.20	0.0695	0.26	0.442	0.056	1.32	0.14
Pelvis	2.94	0.17	0.0753	0.20	0.450	0.073	1.44	0.12
Scapulae	2.13	0.21	0.0694	0.37	0.423	0.084	1.34	0.16
Ribs	4.83	0.23	0.0612	0.29	0.344	0.080	1.15	0.13
Sternum	0.79	0.48	0.0866	0.33	0.174	0.15	1.64	0.27

* Fractional Standard Deviation

Table 20

Early Retention and Distribution of ^{239}Pu by
Four 3-Level Beagles^a Described by $R = a.e^{bt}$

Skeletal Part	$a \pm \sigma_a$	$b \pm \sigma_b$ $\times 10^4$	P^b
Skull	3.15 $\pm$ 0.22	+ 2.26 $\pm$ 2.01	0.5 > P > 0.4
Mandibles	1.02 $\pm$ 0.07	+ 4.87 $\pm$ 2.04	0.2 > P > 0.1
Cervical			
Vertebrae	2.41 $\pm$ 0.15	- 3.15 $\pm$ 1.42	0.2 > P > 0.1
Thoracic			
Vertebrae	7.03 $\pm$ 1.05	- 1.84 $\pm$ 4.38	0.8 > P > 0.7
Lumbar			
Vertebrae	6.08 $\pm$ 0.97	- 6.01 $\pm$ 4.69	0.4 > P > 0.3
Caudal			
Vertebrae	0.294 $\pm$ 0.04	- 1.33 $\pm$ 4.46	0.8 > P > 0.7
Paws	1.35 $\pm$ 0.24	+ 0.86 $\pm$ 5.28	0.9 > P > 0.8
Radii	0.445 $\pm$ 0.04	- 0.71 $\pm$ 2.55	0.9 > P > 0.8
Ulnae	0.403 $\pm$ 0.06	+ 3.53 $\pm$ 4.48	0.6 > P > 0.5
Humeri	3.86 $\pm$ 0.42	- 2.42 $\pm$ 3.07	0.6 > P > 0.5
Tibiae-			
Fibulae	2.03 $\pm$ 0.22	- 6.42 $\pm$ 3.21	0.2 > P > 0.1
Femora	4.26 $\pm$ 0.51	- 5.15 $\pm$ 3.60	0.3 > P > 0.2
Pelvis	3.57 $\pm$ 0.23	- 2.25 $\pm$ 1.84	0.4 > P > 0.3
Scapulae	2.84 $\pm$ 0.26	- 6.26 $\pm$ 2.63	0.2 > P > 0.1
Ribs	5.81 $\pm$ 0.76	- 3.72 $\pm$ 3.76	0.5 > P > 0.4
Sternum	1.28 $\pm$ 0.38	- 0.93 $\pm$ 8.60	P > 0.9
Total ^c	47.3 $\pm$ 1.14	- 2.78 $\pm$ 0.70	0.1 > P > 0.05

^a Interval from injection to death ranged from 40 to 560 days.

^b Probability that calculated slope, b, does not differ from zero.

^c An estimate of the retention by the sacrum is included in this value.

Table 21

Early Retention and Distribution of ^{239}Pu by
Eleven 1-Level Beagles^a Described by $R = ae^{bt}$

Skeletal Part	$a \pm \sigma_a$	$b \pm \sigma_b$ $\times 10^4$	P^b
Skull	3.53 $\pm$ 0.49	+ 0.78 $\pm$ 2.91	0.8 > P > 0.7
Mandibles	1.08 $\pm$ 0.23	+ 2.12 $\pm$ 4.45	0.7 > P > 0.6
Cervical			
Vertebrae	2.43 $\pm$ 0.18	- 3.99 $\pm$ 1.56	0.1 > P > 0.05
Thoracic			
Vertebrae	7.00 $\pm$ 0.55	- 5.45 $\pm$ 1.64	P < 0.01
Lumbar			
Vertebrae	6.07 $\pm$ 0.35	- 7.51 $\pm$ 1.13	P < 0.01
Caudal			
Vertebrae	0.263 $\pm$ 0.06	- 0.50 $\pm$ 4.76	P > 0.9
Paws	1.78 $\pm$ 0.25	- 0.62 $\pm$ 2.84	0.8 > P > 0.7
Radii	0.396 $\pm$ 0.09	+ 1.61 $\pm$ 4.54	0.8 > P > 0.7
Ulnae	0.462 $\pm$ 0.06	- 0.78 $\pm$ 2.81	0.8 > P > 0.7
Humeri	5.89 $\pm$ 0.82	- 8.09 $\pm$ 2.99	0.05 > P > 0.02
Tibiae-			
Fibulae	1.90 $\pm$ 0.28	- 6.22 $\pm$ 3.11	0.1 > P > 0.05
Femora	4.20 $\pm$ 0.46	- 6.46 $\pm$ 2.32	0.05 > P > 0.02
Pelvis	3.17 $\pm$ 0.31	- 3.42 $\pm$ 2.09	0.2 > P > 0.1
Scapulae	2.46 $\pm$ 0.23	- 5.03 $\pm$ 1.96	0.1 > P > 0.05
Ribs	6.57 $\pm$ 0.79	- 9.12 $\pm$ 2.46	P < 0.01
Sternum	0.708 $\pm$ 0.11	- 4.32 $\pm$ 3.41	0.3 > P > 0.2
Total ^c	48.9 $\pm$ 3.37	- 5.00 $\pm$ 1.45	P < 0.01

^a Interval from injection to death ranged from 35 to 790 days.

^b Probability that calculated slope, b, does not differ from zero.

^c An estimate of the retention by the sacrum is included in this value.

Table 22

Early Retention and Distribution of ^{239}Pu by Four 3-Level and Eleven 1-Level Beagles^a Described by $R = ae^{bt}$

Skeletal Part	$a \pm \sigma_a$	$b \pm \sigma_b$ $\times 10^4$	P^b
Skull	3.39 $\pm$ 0.32	+ 1.36 $\pm$ 2.16	0.6 > P > 0.5
Mandibles	1.07 $\pm$ 0.16	+ 2.53 $\pm$ 3.26	0.5 > P > 0.4
Cervical			
Vertebrae	2.43 $\pm$ 0.13	- 3.90 $\pm$ 1.17	P < 0.01
Thoracic			
Vertebrae	7.16 $\pm$ 0.50	- 5.27 $\pm$ 1.58	P < 0.01
Lumbar			
Vertebrae	6.12 $\pm$ 0.32	- 7.43 $\pm$ 1.19	P < 0.01
Caudal			
Vertebrae	0.275 $\pm$ 0.04	- 1.03 $\pm$ 3.60	0.8 > P > 0.7
Paws	1.59 $\pm$ 0.17	- 0.70 $\pm$ 2.56	0.8 > P > 0.7
Radii	0.411 $\pm$ 0.06	+ 0.96 $\pm$ 3.35	0.8 > P > 0.7
Ulnae	0.449 $\pm$ 0.04	- 0.005 $\pm$ 0.23	P > 0.9
Humeri	5.06 $\pm$ 0.61	- 5.91 $\pm$ 2.60	0.05 > P > 0.02
Tibiae-			
Fibulae	1.96 $\pm$ 0.20	- 6.52 $\pm$ 2.35	0.02 > P > 0.01
Femora	4.26 $\pm$ 0.34	- 6.45 $\pm$ 1.81	P < 0.01
Pelvis	3.36 $\pm$ 0.26	- 3.88 $\pm$ 1.75	0.05 > P > 0.02
Scapulae	2.60 $\pm$ 0.18	- 5.74 $\pm$ 1.61	P < 0.01
Ribs	6.42 $\pm$ 0.57	- 8.27 $\pm$ 1.98	P < 0.01
Sternum	0.932 $\pm$ 0.19	- 6.74 $\pm$ 4.58	0.2 > P > 0.1
Total ^c	48.8 $\pm$ 2.39	- 4.72 $\pm$ 1.09	P < 0.01

^a Interval from injection to death ranged from 35 to 769 days.

^b Probability that calculated slope, b, does not differ from zero.

^c An estimate of the retention by the sacrum is included in this value.

Table 23
Distribution of ^{239}Pu at $t \approx 22$ days

	Observed (% dose)	Corrected (% dose)
Skeleton	48.9 ± 3.4	51
Liver	32.5 ± 5.2	34
Excreta	12.1 ± 2.1	13
Other	<u>2.0</u>	<u>2</u>
Total	95.6	100

REFERENCES

1. Betsy J. Stover, D.R. Atherton, and N. Keller: Metabolism of ^{239}Pu in adult beagle dogs. *Radiat. Res.* 10:2, 130-147 (1959).
2. Betsy J. Stover, D.R. Atherton, F.W. Bruenger, and D.S. Buster: Further studies of the metabolism of ^{239}Pu in adult beagles. *Health Physics* 8:6, 589-597 (1962).
3. Betsy J. Stover, David R. Atherton, Friedrich W. Bruenger, and Dawn S. Buster: ^{239}Pu in liver, spleen, and kidneys of the beagle. *Health Phys.* 14:2, 193-197 (1968).
4. Betsy J. Stover, D.R. Atherton, F.W. Bruenger, and Dawn S. Buster: $^{239}\text{Pu(IV)}$: Its distribution in the beagle. In Delayed Effects of Bone-Seeking Radionuclides. C.W. Mays et al. Eds., University of Utah Press, Salt Lake City, pp 109-123 (1969).
5. Betsy J. Stover, David R. Atherton, and Dawn S. Buster: Protracted hepatic splenic, and renal retention of ^{239}Pu in the beagle. *Health Phys.* 20:369-374 (1971).
6. David R. Atherton. EHPA-Scintillator method for the analysis of biological material for small quantities of ^{90}Sr , ^{228}Th , and ^{239}Pu . *Research in Radiobiology*, University of Utah, C00-119-240, March 1969, pp. 181-187.
7. D.R. Atherton, C.W. Mays, and Betsy J. Stover: Radionuclide distribution in adult beagle bones. *Radiobiology Laboratory Semi-Annual Progress Report*, C00-217, Sept. 1958, pp. 118-125.
8. Betsy J. Stover, D.R. Atherton, D.S. Buster, and F.W. Bruenger: Retention of $^{239}\text{Pu(IV)}$ by the young adult beagle. Abstracts of papers for the 19th Annual Meeting of the Radiation Research Society, 1971, p. 97.

9. W.S.S. Jee, H.Z. Park, and R. Burggraaf: Estimates of residence time of ^{239}Pu in trabecular bones of beagles. Research in Radiobiology, University of Utah, C00-119-240, March 1969, pp. 188-198.

THE INTERACTION OF SOME ACTINIDE ELEMENTS WITH BLOOD CONSTITUENTS*

D.R. Atherton, F.W. Bruenger and Betsy J. Stover

The distribution of a number of actinide elements in canine and human blood were studied and compared to extensive, already existing data on $^{239}\text{PuIV}$ and $^{241}\text{AmIII}$ in dogs. ^{227}Ac , ^{228}Th , ^{233}Pa , ^{233}UIV , ^{233}UVI , $^{137}\text{NpIV}$, $^{237}\text{NpIV}$, and $^{239}\text{PuIII}$ were used in these studies. Each nuclide in 0.08 M citrate, pH 3.5, was incubated in vitro with canine and/or human whole blood. Cells were removed and evaluated for nuclide retention. Except for $^{239}\text{PuIII}$, the concentration of the nuclide in the plasma was at least 10X that found with cells. This factor was about 5 for $^{239}\text{PuIII}$. Following gel-chromatography on G-25, varying percentages of the nuclides were associated with the peak eluting at the void volume. These are ^{227}Ac -42%, ^{228}Th -78%, ^{233}Pa -69%, ^{233}UIV -7%, ^{233}UVI -9%, $^{237}\text{NpIV}$ -20%, $^{237}\text{NpVI}$ -21% and $^{239}\text{PuIII}$ -65%. Nuclides forming strong complexes with inorganic entities (HCO_3^-) show limited binding to protein constituents. Further separation of protein-bound nuclides was achieved by gel-chromatography on gels of lower cross linkage and by anion-exchange procedures. Regions of protein-complex stability were determined in some cases by difference-spectrophotometry.

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INTERACTION BETWEEN BLOOD CONSTITUENTS AND SOME ACTINIDES

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Abstract: The interaction of a number of actinide elements with constituents of canine and human blood was studied and compared to extensive, already existing data on $^{239}\text{PuIV}$ and $^{241}\text{AmIII}$ in beagles. ^{228}Th , ^{233}Pa , ^{233}U , ^{237}Np and ^{249}Cf were used for these experiments. Each of these nuclides in 0.08 M citrate buffer was incubated in vitro with canine and/or human blood. None of the nuclides had a marked affinity for blood cells. High and low molecular weight components of plasma were separated by gel chromatography on Sephadex G-25 and amounts of nuclide associated with each fraction were evaluated. Binding to plasma proteins was further evaluated by gel chromatography on resins of low cross linkage and by anion exchange chromatography. Nuclides forming strong complexes with inorganic constituents of plasma showed only limited binding to protein constituents. The pH-ranges of protein complex stabilities were determined in some cases by difference spectrophotometry.

INTRODUCTION

Considerable differences are observed in the distribution of the actinide elements when these are injected intravenously in citrate buffer of low pH. The reaction at the site of deposition not only depends on the reactivity of certain specific biochemical entities which form stable bonds with the nuclides, but, also, the chemical form of the nuclide when it reaches the deposition site. Nuclides may bind to plasma proteins and form metalloproteins and also may be complexed by the various inorganic constituents of plasma or may simply hydrolyze and form polymers. The type of product formed in plasma, its stability, and the molecular size will have a strong influence on the subsequent reaction at the site of deposition. Extensive data on the reaction of plutonium and americium with plasma constituents are available (1-5). Some data are also available for curium(6).

No attempt has been made to study and to correlate the interaction of a larger number of these important actinide nuclides with blood constituents. This preliminary communication will provide some comparative information on this subject.

METHODS

The following nuclides were available for the experiment: ^{227}Ac , ^{228}Th , ^{233}Pa , ^{233}U , ^{237}Np , ^{239}Pu , ^{241}Am , and ^{249}Cf . Purification (when necessary) and attainment of specific oxidation states were done by standard techniques. Blood was obtained from healthy adult beagles or a healthy male human. The element under study was dissolved in 0.08 M citrate of pH ~ 3.5 , and added to the blood sample immediately after drawing. The mixture was then incubated for 30 min. at 40°C . After incubation, the blood was centrifuged and plasma and cells were separated. Distribution of the nuclides in the blood was determined by the following procedures: separation and purification of cells, gel filtration of plasma on Sephadex G-25 and G-100 - G-200, ion exchange chromatography, and difference spectrophotometry of isolated proteins.

Gel chromatography on Sephadex G-25 was performed on a 1 cm x 35 cm column. A known quantity of tagged plasma was applied and eluted with a buffer containing 0.1 M TRIS (Tris(hydroxymethyl)aminomethane) and 1% NaCl. Fractions were collected for protein determination and counting of radioactivity. Gel chromatography on Sephadex G-100 - G-200 was carried out as described earlier⁽¹⁾. Plasma was separated on G-100 - G-200 into three protein peaks designated I, II, and III in order of descending molecular weight. Fractions comprising the third protein peak were pooled, dialyzed against 0.1 M TRIS, pervaporated to a small volume, and

rechromatographed on DEAE-anion exchange resin. Anion exchange chromatography was carried out with Sephadex DEAE A-50, equilibrated with 0.05 M TRIS buffer of pH 8. Columns were eluted with a linear salt gradient consisting of 0.1 M TRIS $\rightarrow$ 0.1 M TRIS + 0.5 M NaCl.

Difference spectra were taken on samples prepared by the following method: An appropriate quantity of purified apo-conalbumin was dissolved in saline for spectrophotometry. Each sample was then divided into two and a sufficient quantity of nuclide was added to one with the other serving as a reference. Both tubes were then adjusted to equal pH-values and readings were taken over an appropriate range on either side of physiological pH. The difference in adsorption seen was due to the interaction of the metal and conalbumin.

RESULTS

The associations of nuclides with the cellular component of blood were expressed as the ratio of concentration of nuclide in cells to the concentration of nuclide in plasma, $\left(\frac{C_{\text{cells}}}{C_{\text{plasma}}} = \frac{C_c}{C_p} \right)$. These data are listed in Table 1. The affinity of these elements for blood cells was low. In the case of PuIII the reaction exceeded 10% but the cause for the relatively strong association is unknown.

The separation of plasma on Sephadex G-25 served two purposes.

- 1) To separate protein bound nuclides and other high molecular weight material from low molecular weight and largely ionic material. Should a nuclide in this system undergo hydrolysis, its products would also appear in the high M. W. region of the chromatogram.

- 2) To show the relative affinity of the element for protein as compared to the gel surface measured by resin sectioning.

Non-protein bound, ionic nuclide might be lost by surface adsorption on the resin and the estimate of the protein bound material might be too high.

In all but ^{227}Ac , ^{228}Th , and ^{233}Pa , the peak of protein and high molecular weight material was followed by a peak of high radioactivity about five or six fractions later and this material was not associated with protein. Uranium IV showed the greatest association with material of low molecular weight. The data are given in Table 2. (^{239}Pu and ^{249}Cf are not included in this listing.)

A more detailed study of the distribution of the nuclides in plasma was made by chromatography on gels of low cross linkage, i.e. G-100 - G-200. Reproducibility was generally good in this system and as long as plasma was obtained from the same source, little variation between individual samples was seen. A typical result of this method of separation is seen in Fig. 1. The upper part shows the elution spectrum of beagle plasma tagged with ^{59}Fe and $^{239}\text{PuIV}$ and subjected to gel chromatography on Sephadex G-100-G-200. Both nuclides in this case were administered intravenously in 0.08 M citrate buffer. The solid line shows the familiar three major protein peaks. The small peak to the right represents low molecular weight U. V. light absorbing material, and the dashed and dotted lines represent the plutonium and iron respectively. The peak of ^{59}Fe and ^{239}Pu are not precisely coincident with protein peak III, but are slightly displaced to the left, indicating that the protein-nuclide complex is associated with a minor, slower moving component of peak III.

Material from peak III was pooled and rechromatographed on the ion exchange resin DEAE-Sephadex and the results are seen, in the insert. During this operation, the protein of the pooled material was resolved into 2 peaks, transferrin and albumin; Iron appears only with the transferrin peak and, while plutonium is mostly associated with transferrin, a small fraction does appear with the albumin peak. Earlier papers have shown that the most important protein in the transport of PuIV is transferrin, the iron transport protein (2,3,4). The amount of plutonium found associated with the transferrin peak can be altered by changing the degree to which transferrin is saturated with iron. Plutonium can be displaced from the protein by the addition of iron to an already equilibrated system of Pu-transferrin. This indicates the relative strength of the two metallo-protein complexes.

In the lower portion of Fig. 1 the distribution of the ^{239}Pu in canine blood after in vivo administration is compared with the chromatogram obtained following the in vitro tagging of human blood with plutonium. Both elution spectra are very similar and in each case plutonium is associated with, but appears slightly to the left of protein peak III. The in vitro procedure using human blood from a single source was used in most cases for subsequent experiments with the other nuclides.

Table 3 is an appraisal of the spectra of the activities of the 8 nuclides tested as these activities relate to the three major protein peaks: high molecular weight, intermediate molecular weight, and that of low molecular weight. Actinium, protactinium, neptunium, and californium show a moderate tendency to associate with the first protein peak, while thorium, plutonium, and americium have a lesser tendency to do so.

Uranium is the only one in this group which shows even a slight tendency to associate with protein peak II. All chromatograms show a peak of activity associated with the third protein peak; actinium, thorium, and plutonium having the greatest affinity, followed by uranium, neptunium, and californium. Protactinium and americium have only a moderate tendency to associate with proteins of peak III. The peaks of radioactivity seen in protein peak III are all displaced slightly to the left of the protein peak (Fig. 1). This indicates different degrees of association of these elements with transferrin. Neptunium and americium had only slight affinity to bind to material of medium molecular weight (15,000 - 17,000), the others have a somewhat greater tendency to do so. All show a moderate association with low molecular weight components. Plutonium and americium have the lowest affinity for constituents found in this weight range. Carbonate, bicarbonate and phosphate complexes are necessary for the associations described above to occur.

The results of rechromatography of the pooled fractions of protein peak III on DEAE-Sephadex are tabulated in Table 4. As before, the protein was resolved into a transferrin and an albumin peak. Actinium, thorium, protactinium, plutonium, and californium exhibit a strong association with transferrin, while uranium, neptunium, and americium do so only to a moderate degree. All elements are found to some degree with the albumin peak. Only uranium is equally divided between albumin and transferrin and there is more americium in the albumin peak than in the transferrin peak.

The existence of metalloprotein complexes was verified spectrophotometrically. With all other conditions equal except for the presence

of the metal, the spectra show only the effect of the nuclide on the U. V. light absorption properties of the protein. Spectra as a function of the pH were obtained with nuclides that had a sufficiently long half-life to be used in quantities so that saturation of the protein could be achieved without excessive radiation hazard. Spectra of uranium, neptunium, plutonium, and iron (the naturally associated metal) are presented in Figs. 2, 3, and 4. Attempts to obtain a spectrum of the thorium-conalbumin complex failed in spite of the fact that its existence was demonstrated from chromatographic data. All complexes show two major peaks of U. V. light absorption at approximately 243 and 294 nm. The highest absorption is observed between pH 7 and pH 8. At a pH < 5 the peaks vanish, indicating that the complex dissociates. At pH > 9 the absorption peak at 243 nm diminishes and shifts towards higher wave length. In the case of iron and U VI this gives rise to the appearance of a new peak at ~ 270 nm.

DISCUSSION

The data presented here are used in an attempt to correlate the binding properties of the actinide elements with constituents of blood. They are still incomplete since curium, berkelium, and, of course, all other actinides of atomic number > 98 are still missing. It is not justified to make a direct extrapolation of these qualitative data which describe the binding of the various nuclides by plasma constituents to the distribution in the body. A few conclusions do, however, seem obvious. A rapid rate of removal from the body would appear to reflect a low stability of the metalloprotein complex, possibly with subsequent formation of polymeric species because of hydrolysis as in the case of americium. A relatively strong, specific complex may be partially

responsible for low excretion rates and greater interaction with constituents of bone rather than the liver as in californium and plutonium (i.e. transferrin). Formation of strong complexes with low molecular weight inorganic constituents of blood will probably favor a high excretion rate as seen in uranium VI. The fact that bone-seeking U VI is first bound on bone surfaces but soon migrates through the whole volume of bone, may well be due to a competitive reaction between the binding species of bone (glycoprotein) and the complex formed with carbonate and bicarbonate ions (8).

A more complex study of this subject may pave the way for a better understanding of the differences seen in the final tissue distribution of these elements. Stability constants of the metalloprotein complexes are needed to make better predictions of the initial fate of the nuclide after injection. Difference spectra may be helpful in obtaining these when sufficient quantities of pure and undenatured transferrin become available. The present spectra have only qualitative value since they are based on the assumption that conalbumin protein has properties identical with transferrin, including molecular structure. If, in addition, it becomes possible to make a reasonable estimate of the concentration of the ionic species of the nuclides in this system, then stability constants can be calculated very easily.

REFERENCES

1. Stevens, W., Bruenger, F. W., Stover, Betsy J.; In vitro studies of the interactions of PuIV with blood proteins. Radiation Res. 26, 114 (1965).

2. Boocock, G., Popplewell, D. S.; Distribution of plutonium in serum proteins following intravenous injection into rats. *Nature* 208, 282 (1965).
3. Stevens, W., Bruenger, F. W., Stover, Betsy J.; In vivo studies on the interactions of PuIV with blood constituents. *Radiation Res.* 33, 490 (1968).
4. Stover, Betsy J., Bruenger, F. W., Stevens, W.; The reaction of PuIV with the iron transport system in human blood serum. *Radiation Res* 33, 381 (1968).
5. Bruenger, F. W., Stevens, W., Stover, Betsy J.; Americium-241 in blood: In vivo and in vitro observations. *Radiation Res.* 37, 349 (1969).
6. Boocock, G., Popplewell, D. S.; In vitro distribution of americium in human blood serum proteins. *Nature* 210, 1283 (1966).
7. Stover, Betsy J., Atherton, D. R., Bruenger, F. W., Mays, C. W.; Distribution of ^{226}Ra between blood cells and plasma. *Health Phys.* 11, 617 (1965).
8. Rowland, R. E., Farnham, J. E.; The deposition of uranium in bone. Argonne Natl. Laboratory Radiological Physics Division, Report ANL-7489, pp. 33-36 (July 1967-June 1968).

Table 1. Association of Nuclides with Blood Cells

Element	$\frac{C_c^*}{C_p}$
^{227}Ac	0.039
^{228}Th	0.08
^{233}Pa	0.023
$^{233}\text{U VI}$	0.045
$^{237}\text{Np VI}$	0.093
$^{239}\text{Pu III}$	0.202
^{241}Am	0.005

$$\frac{C_c}{C_p} = \frac{\text{Concentration of nuclide in cells}}{\text{Concentration of nuclide in plasma}}$$

Table 2. G-25 Sephadex Separation*

Element	% of Applied Recovered	3 Peak Protein Tubes	5th Tube Post Peak	10th Tube Post Peak	15th Tube Post Peak	20th Tube Post Peak	Resin Section I	Resin Section II	Resin Section III	Resin Section IV	Resin Section V
^{227}Ac	95.4	42.1	0.6	0.3	0.4	0.1	19.0	8.4	7.2	3.4	5.7
^{228}Th	96.6	78.5	0.4	0.03	0.01	0.01	0.5	0.1	0.03	0.06	0.04
^{233}Pa	100.	68.8	1.1	0.01	0	0	5.6	4.5	2.1	2.	1.6
^{233}UIV	92.	6.8	22.0	3.1	0.16	0.07	0.2	0.05	0.07	0.08	0.16
^{233}UVI	95.4	9.0	8.8	0.02	0.02	0	9.4	34.9	5.6	0.02	0.02
$^{237}\text{NpIV}$	80.	19.8	12.7	0.3	0.3	0.05	4.2	5.3	2.8	0.5	0.05
$^{237}\text{NpVI}$	99.2	20.8	7.8	0.5	0.4	0.1	0.1	0.7	32.7	0.6	0.07
$^{239}\text{PuIII}$	100.	64.7	4.	0.1	0	0	3.	0.5	0.4	0.4	1.5
^{241}Am	83.	13.2	12.8	0.2	0.04	0.01	2.6	2.7	3.1	2.5	9.8

* Figures in % of recovered activity.

Table 3. Gel Chromatography of Tagged Human Plasma on Sephadex G100-G200

Atomic Number	Mass Number	Element	Valence	Protein Peak			Intermediate MW	Low MW
				I	II	III		
89	227	Ac	III	++	-	++++	+	+++
90	228	Th	IV	+	-	++++	+	++++
91	233	Pa	V	++	-	++	+	+++
92	233	U	VI	-	+	+++	+	++++
93	237	Np	V	++	-	+++	-	++++
94	239	Pu	IV	+	-	++++	+	++
95	241	Am	III	+	-	++	-	++
98	249	Cf	III	++	-	+++	+	++++

Table 4. Ion-Exchange Chromatography of Tagged Human Plasma on DEAE Sephadex

Atomic Number	Mass Number	Element	Valence	Transferrin	Albumin
89	227	Ac	III	++++	+
90	228	Th	IV	++++	+
91	233	Pa	V	++++	+
92	233	U	VI	++	++
93	237	Np	V	++	+
94	239	Pu	IV	++++	+
95	241	Am	III	++	+++
98	249	Cf	III	++++	+

Fig. 1 Elution spectra of tagged plasma on G-100 - G-200. Top: elution spectrum of a canine plasma tagged with ⁵⁹Fe and ²³⁹Pu. Most of the radioactivity was associated with protein peak III (A = absorbance). Radioactivity peaks are slightly displaced to the left shoulder. Insert shows the result of rechromatography of pooled peak III material on DEAE ion exchange resin. Lower graph: elution spectrum of plutonium tagged human plasma after gel chromatography on G-100 - G-200.

Fig. 2 Difference spectrum of Fe-conalbumin as a function of pH.

Fig. 3 Difference spectra of UVI-(top) and NpVI-(bottom) conalbumin as a function of pH.

Fig. 4 Difference spectra of PuIV-(top) and PuVI-(bottom) cpnalbumin as a function of pH.

ACCUMULATION OF MACROPHAGES LABELLED WITH PLUTONIUM-239
IN MARROW SPACES OF LUMBAR VERTEBRAL BODIES

Webster S. S. Jee

Abstract: Labeled macrophages are generated when osteoclasts progressively concentrate plutonium after resorption of bone surfaces labeled with plutonium, and when subsequently the macrophages engulf the dead osteoclasts which contain the plutonium or bind the plutonium freed by the disintegrating osteoclast. The efflux of labeled macrophages occur when live, dead or fragmented macrophages laden with plutonium are swept out of the marrow into the circulation. The accumulation of labeled macrophages is an indicator of altered kinetics of both macrophages and bone cells. Labeled macrophages are observed in beagles injected with 2.7, 0.9, 0.3, 0.09 and 0.05 $\mu\text{Ci } ^{239}\text{Pu/kg}$, but not in 0.015 $\mu\text{Ci } ^{239}\text{Pu/kg}$ dogs at death (4 to 6 years post-injection). Optimal accumulation of labeled macrophages appears in the 0.05 $\mu\text{Ci } ^{239}\text{Pu/kg}$ injected dogs. At the lowest dose level (0.015 $\mu\text{Ci/kg}$ injected), there is peak labeling at 1/4 to 2 years post-injection and no evidence of labeled cells at post-injection times greater than 4 years.

Despite the accumulation of labeled macrophages, there is a paucity of death due to hematopoietic changes and hematopoietic neoplasms in the dogs. These dogs died mostly of osteosarcoma death.

INTRODUCTION

It was not until 1957 that the definitive report describing the autoradiographic distribution of plutonium-239 in rats by Arnold and Jee was published in the American Journal of Anatomy.⁽¹⁾ Until then, perfecting techniques to produce suitable detailed autoradiographs had proved a tough

nut to crack. There had been three brief communications from Hamilton's laboratory⁽²⁻⁴⁾ and numerous short communications from our laboratory.⁽⁵⁻¹⁰⁾

The autoradiographic findings we described proved not only to be most artistic, but have survived the test of time. Briefly, we observed:

(1) plutonium was found to localize in high concentration at endosteal surfaces and to a lesser degree at periosteal surfaces and vascular channels of cortical bone, (2) the strata of initially-deposited plutonium was overlayed by new bone formed after injection, (3) all post-injection bone was readily identified by its diffuse labeling, (4) osteoclasts resorbing bone labeled with plutonium were progressively concentrating plutonium, (5) plutonium in the osteoclast was later transferred to macrophages, (6) the labeled macrophages were also loaded with hemosiderin, (7) formative and destructive phases of bone reconstruction were uniquely demonstrated by tracing the fate of plutonium, and (8) plutonium concentration in osteoclast was interpreted as evidence of the active role of that cell in bone resorption.

DISTRIBUTION IN ADULT DOG BONES

Next came our autoradiographic studies on the distribution of plutonium-239 (+4) citrate (monomeric Pu) in young adult beagles' bones.⁽¹¹⁻²⁸⁾ One such effort was the description of the percentage of total plutonium associated with the key histological structures in the lumbar vertebral bodies of dogs injected with approximately 2.7 μCi of $^{239}\text{Pu}/\text{kg}$ in 18 dogs serially sacrificed up to 2059 days post-injection by Twente and co-workers.⁽²⁹⁾ Briefly, these findings include (Table I): (1) Initially the largest percentage of plutonium is concentrated on the endosteal surface of trabeculae (as high as 97%). This concentration on the surface decreased

with increasing burden times, (2) the concentration associated with cortical bone and periosteal surfaces does not constitute more than 5% of the total plutonium, (3) the percentage of plutonium found in osteoclast was found to be on the order of 10%, was first detected at about two weeks after injection, and showed spurts of activity, (4) macrophages laden with plutonium appeared in the marrow at approximately the same time as osteoclasts laden with plutonium. The Pu percentage in macrophages generally ranged between 6 and 10% and did not increase with burden time, indicating that these macrophages do not accumulate in the marrow, and (5) new bone (diffusely labeled post-injection bone) began to form three months after injection and eventually amounted to at least 10% of the total plutonium in dogs surviving three years after injection.

ACCUMULATION IN MACROPHAGES

Of particular interest was the accumulation of ^{239}Pu labeled macrophages in the marrow as a function of increasing burden time and dose levels. At one time we thought the accumulation was an important concentration of alpha irradiation for osteosarcomas induction. Despite the accumulation of labeled macrophages, there is a paucity of death due to hematopoietic changes and hematopoietic neoplasms in our dogs. Nevertheless, there is a slim chance that these aggregates may contribute to the induction of osteosarcomas.

The accumulation of labeled macrophages in marrow spaces is dependent upon the biologic factors which control the rate of influx (production) and efflux (attrition) of labeled macrophages. Labeled macrophages were generated when osteoclasts progressively concentrated plutonium after

TABLE I - Percentage of ^{239}Pu Associated with Sites Within Lumbar Vertebral Bodies, as Studied Autoradiographically in Beagles Injected with $2.7 \mu\text{Ci/kg}$ (Mean $\pm$ S.D.)

<u>Days post injection</u>	<u>Trabecular surfaces</u>	<u>Osteoclasts</u>	<u>Macrophages</u>	<u>Post inj. bone</u>	<u>Haversian systems</u>	<u>Periosteal surface</u>
1	96.2 $\pm$ 1.8	0	0	0	1.0 $\pm$ 0.4	2.8 $\pm$ 1.4
3	94.5 $\pm$ 2.3	0	0	0	2.7 $\pm$ 1.5	2.8 $\pm$ 0.8
7	97.1 $\pm$ 1.7	0	0	0	1.1 $\pm$ 0.7	1.8 $\pm$ 1.1
15	92.5 $\pm$ 5.2	3.9 $\pm$ 5.1	2.8 $\pm$ 2.2	0	1.5 $\pm$ 0.8	2.7 $\pm$ 1.1
15	87.5 $\pm$ 10.7	6.0 $\pm$ 7.4	3.0 $\pm$ 3.1	0	1.5 $\pm$ 0.6	2.0 $\pm$ 0.7
28	71.3 $\pm$ 10.8	16.5 $\pm$ 6.0	9.5 $\pm$ 5.3	0	1.2 $\pm$ 0.4	1.5 $\pm$ 0.7
28	79.5 $\pm$ 5.3	8.1 $\pm$ 4.7	7.7 $\pm$ 3.3	0	1.6 $\pm$ 0.3	3.2 $\pm$ 1.4
92	76.2 $\pm$ 14.0	7.9 $\pm$ 8.0	8.2 $\pm$ 7.5	4.1 $\pm$ 3.8	1.4 $\pm$ 0.6	2.3 $\pm$ 0.9
210	72.2 $\pm$ 4.1	10.4 $\pm$ 2.4	11.2 $\pm$ 0.9	3.1 $\pm$ 1.5	1.0 $\pm$ 0.4	2.2 $\pm$ 0.7
217	61.3 $\pm$ 9.8	6.7 $\pm$ 0.4	28.0 $\pm$ 8.8	1.8 $\pm$ 1.4	0.7 $\pm$ 0.4	1.6 $\pm$ 0.8
400	74.3 $\pm$ 6.9	9.9 $\pm$ 4.5	5.7 $\pm$ 1.7	6.5 $\pm$ 2.2	1.2 $\pm$ 0.4	2.7 $\pm$ 1.2
777	71.8 $\pm$ 10.3	11.2 $\pm$ 12.5	7.2 $\pm$ 5.3	6.0 $\pm$ 6.3	1.7 $\pm$ 1.3	2.2 $\pm$ 0.5
1192	72.6 $\pm$ 2.3	8.1 $\pm$ 3.4	6.5 $\pm$ 0.9	8.6 $\pm$ 0.4	1.7 $\pm$ 0.3	2.5 $\pm$ 1.4
1194	68.3 $\pm$ 2.0	6.9 $\pm$ 0.6	2.2 $\pm$ 0.6	18.5 $\pm$ 2.7	1.8 $\pm$ 1.5	2.2 $\pm$ 0.2
1491	76.8 $\pm$ 0.9	7.0 $\pm$ 0.9	5.0 $\pm$ 0.1	8.2 $\pm$ 0.3	1.5 $\pm$ 0.4	2.1 $\pm$ 0.4
1562	63.4 $\pm$ 5.5	9.5 $\pm$ 3.9	4.0 $\pm$ 0.9	19.2 $\pm$ 3.6	1.5 $\pm$ 0.2	2.2 $\pm$ 0.6
1576	67.5 $\pm$ 4.9	13.4 $\pm$ 5.7	7.2 $\pm$ 0.8	8.2 $\pm$ 1.3	1.3 $\pm$ 0.2	2.4 $\pm$ 0.3
2059	73.7 $\pm$ 5.1	10.1 $\pm$ 1.0	1.7 $\pm$ 1.6	10.4 $\pm$ 2.1	2.0 $\pm$ 0.7	2.3 $\pm$ 0.3

Sample size = 4

resorbing bone surfaces labeled with plutonium and when subsequently the macrophages engulfed the dead osteoclasts with the plutonium and concentrated the plutonium or bound the plutonium freed by the disintegrating osteoclast. The efflux of labeled macrophages occurred when live, dead or fragmented macrophages laden with plutonium were swept out of the marrow into the circulation. Thus, the influx of labeled macrophages is related to osteoclastic resorption of bone surface labeled with Pu, and the production of non-labeled macrophages. At high dose levels (2.7 and 0.9 Ci/kg), bone resorption was depressed and the bone marrow was depleted; therefore, few labeled macrophages were observed (Table II).

TABLE II - Relationship of numbers of macrophages with star-producing aggregates in a longitudinal section of lumbar vertebral bodies marrow to injected doses of ^{239}Pu in young adult Beagles as studied by autoradiography. (Mean $\pm$ S.D.)

Injected dose ($\mu\text{Ci/kg}$)	No. dogs	Age at injection (days)	Burden time* (days)	No. of Macrophages with ^{239}Pu
2.7	6	632 $\pm$ 248	1518 $\pm$ 303	77 $\pm$ 34
0.9	9	522 $\pm$ 68	1316 $\pm$ 197	135 $\pm$ 98
0.3	3	503 $\pm$ 80	1724 $\pm$ 160	1388 $\pm$ 293
0.09	3	538 $\pm$ 114	2109 $\pm$ 730	9929 $\pm$ 5007
0.015	2	588 $\pm$ 45	1899 $\pm$ 509	0

* Time between injection and death

A direct relationship of numbers of labeled macrophages to burial of plutonium was observed in 0.3 μCi dose level dogs (Table III). Thus, incidence of plutonium burial (bone formation) was used as an index of bone remodeling assuming bone formation equals bone resorption.

TABLE III - Relationship of numbers of macrophages with star-producing aggregates to index of bone remodelling as measured by burial of bone surface plutonium in young adult Beagles injected with ~ 0.3 μCi of $^{239}\text{Pu}/\text{kg}$ as studied by autoradiography.

<u>Time after injection</u>	<u>Age at inj. (days)</u>	<u>Remodelling index* Buried/Surface</u>	<u>Number of macrophages with ^{239}Pu</u>
40 days	519	1.62	1955
187 days	489	2.57	2888
362 days	550	2.24	2300
1476 days	417	5.61	5780
1771 days	406	5.51	4910

* Assumes bone formation is coupled (equal) to bone resorption. Therefore burial (bone formation) represents bone resorption.

Increasing numbers of labeled macrophages occurred in dead dogs which had been injected with decreasing dose-levels to 0.05 μCi of $^{239}\text{Pu}/\text{kg}$ (Table II). Optimal accumulation of labeled macrophages appeared in dead dogs injected with 0.05 μCi of $^{239}\text{Pu}/\text{kg}$. The actual number of macrophages in lumbar vertebral bodies of these dogs could not be counted because of the marked clumping of labeled macrophages into aggregates, which made counting impossible. Surprisingly, the two 0.015 μCi injected dogs sacrificed at 1539 and 2257 days did not contain labeled cells.

The studies of numbers of labeled cells with increasing burden times showed peak labeling varied with dose-levels. Peak labeling occurred at 217 days in 2.7 μCi injected dogs, at 1771 days (death) in 0.3 μCi injected dogs and 560 days in 0.015 μCi injected dogs (Tables I, III, IV). At high dose-levels there was an early and transient spurt of bone resorption at 7 months post injection (Table I), but in general the level of osteoclastic resorption, as shown in the small numbers of labeled macrophages

per section, was depressed because of the intense irradiation. At the intermediate dose, peak labeling was observed at post mortem, indicating that there was a continual influx of labeled macrophages into the marrow throughout the life span of these dogs. This in turn means there was a depressed rate of resorption (remodeling) of labeled bone surfaces and labeled post-injection bone. On the contrary, at the lowest dose-levels (0.015 μ Ci injected), there was peak labeling at 2 years post-injection and no evidence of labeled cells at post-injection times greater than 1500 days. This indicates that at low doses all labeled bone can be resorbed by osteoclasts well within 2 years post-injection; thus, wiping out the source of ^{239}Pu on which macrophages concentrate. Furthermore, the post-injection bones were so lightly labeled that labeled osteoclasts and macrophages did not accumulate sufficient Pu to register star-producing aggregates by autoradiography.

One possible method by which macrophages accumulate plutonium is the binding of plutonium freed from osteoclasts with the hemosiderin residing in macrophages. Hemosiderin is an iron-containing substance (hydrated ferrous oxide in combination with protein), resulting from the splitting up of hematin during the phagocytic digestion of effete erythrocytes. Further electron microscopic autoradiography of labeled macrophages should clarify whether hemosiderin is essential for the binding of ^{239}Pu in macrophages.

Until now, we have neglected to study the impact of moderate doses of alpha irradiation upon the population of macrophages. We know from our histological studies that high dose-levels obviously depressed this population. It is not known what moderate and low doses will do. If

TABLE IV - Incidence of macrophages with star-producing aggregates in lumbar vertebral bodies from young adult Beagles given 0.015 μ Ci of ^{239}Pu /kg as studied by autoradiography.

<u>Time after injection</u>	<u>Age at injection (days)</u>	<u>Number of macrophages with ^{239}Pu</u>
5 days	554	0
35 days	548	525
96 days	1485	1968
97 days	559	3267
186 days	542	2173
375 days	550	1913
376 days	1534	1246
559 days	647	1807
647	556	1980
1539 days	620	0
2257	556	0

Exposure time was 2519 days

the source of alpha irradiation from labeled macrophages is important in radiation carcinogenesis, it is imperative that we do further study of the effect of graded doses of ^{239}Pu upon cells.

The efflux of labeled macrophages from marrow spaces was depressed in all but the 0.015 μ Ci injected dogs. The time necessary to clear these cells from the marrow of 0.015 μ Ci injected dogs was, at most, 2 years. No evidence of labeled macrophages was seen at about 4 years post-injection, when at 2 years post-injection the lumbar vertebral bodies contained half the peak load of these cells (Table IV). Previously, we reported that

in the 2.7 μCi injected dogs, labeled macrophages did not increase with burden times and concluded that this was evidence that the macrophages were cleared from the marrow. Subsequent studies at lower dose-levels (0.09 and 0.05 μCi injected) showed that in these dogs there was ample time for the macrophages to translocate to other sites. There is no doubt that the clearance of labeled macrophages has been slowed, and that the piling up of labeled cells is the consequence of depressed rate of removal of plutonium labeled bone and labeled macrophages from the marrow.

It must be emphasized that in acute cases of humans contaminated with ^{239}Pu , the accumulation of labeled macrophages will be similar to the 0.015 μCi injected dogs. Obviously, bones from individuals with extremely long burden times will not show many labeled macrophages.

REFERENCES

1. J. S. Arnold and W. S. S. Jee: Bone growth and osteoclastic as indicated by radioautographic distribution of Pu^{239} : Am. J. Anat.: 101: 367-417 (1957).
2. J. G. Hamilton: The metabolism of the fission products and the heaviest elements: Radiology, 49: 325-343 (1947).
3. J. G. Hamilton: The metabolic properties of the fission products and actinide elements: Rev. Modern Phys. 20: 718-728 (1948).
4. D. H. Copp, D. J. Axelrod and J. G. Hamilton: The deposition of radioactive metals in bone as a potential health hazard: Amer. J. Roent. Rad. Ther. 58: 10-16 (1947).
5. J. S. Arnold: Metabolism of bone as studied by radioautographic distribution of calcium, plutonium and radium: Am. J. Physiol. 167: 765 (1951).

6. J. S. Arnold: General features of bone deposition: Second Annual Conference on Plutonium, Radium and Mesothorium: Ed. by C. N. Stover, Jr., Radiobiology Laboratory, U. of Utah Report, TID-7639, 4-18 (1954).
7. J. S. Arnold: Some observations on plutonium and radium in bone: Therapy of Radioelements Poisoning, Ed. by M. W. Rosenthal, Argonne National Laboratory, ANL-5584, 131-143 (1955).
8. J. S. Arnold: Skeletal changes correlated with irradiation in radium and plutonium intoxicated dogs: Anat. Rec. 124: 451 (1956).
9. J. S. Arnold and W. S. S. Jee: Radioautographic and pathologic changes in bone of dogs receiving ^{239}Pu and ^{226}Ra : Rad. Res. 5: 466 (1956).
10. W. S. S. Jee: Osteoclastic activity in beagles and rats as shown by detailed radioautography distribution of plutonium-239: Anat. Rec. 124: 313 (1956).
11. W. S. S. Jee, J. S. Arnold and C. R. Mott: Distribution of plutonium-239 in skeleton of normal, rachitic and healing rachitic rats: Anat. Rec. 130: 420 (1958).
12. W. S. S. Jee, J. S. Arnold and T. H. Cochran: Skeletal changes induced by chronic doses of plutonium, radium, mesothorium, and radiothorium: Anat. Rec. 130: 421 (1958).
13. J. A. Twente, J. S. Arnold, C. W. Mays, D. H. Taysum and W. S. S. Jee: Localized dosimetry in bone. I. Instrumentation; Radiobiology Laboratory, U. of Utah Report C00-215, 98-101 (1958).
14. J. A. Twente and W. S. S. Jee: Localized dosimetry in bone: Radiobiology Laboratory, U. of Utah Report C00-217, 147-160 (1958).
15. J. A. Twente, E. G. Butler, O. Freudenberger and W. S. S. Jee: Localized plutonium dosimetry in bone: Radiobiology Lab., U. of

Utah Report C00-218, 190-206 (1959).

16. J. A. Twente and D. R. Atherton: Determination of Pu^{239} content of bone tissue: A comparison of radiochemical and photoelectric density techniques: Radiobiology Lab., U. of Utah Report C00-218, 207-209 (1959).
17. J. S. Arnold and W. S. S. Jee: Autoradiography in the localization of radiation dosage of Ra^{226} and Pu^{239} in bones of dog: Lab. Invest. 8: 194-204 (1959).
18. W. S. S. Jee: Microradiographic and autoradiographic studies of bones containing radioisotopes: Anat. Rec. 133: 395-396 (1959).
19. W. S. S. Jee and J. S. Arnold: The effect of internally deposited irradiation upon the vascular supply of cortical bone: Anat. Rec. 133: 293 (1959).
20. W. S. S. Jee and J. S. Arnold: Structural changes in dog skeleton containing plutonium: J. Bone and Joint Surg. 41A: 771 (1959).
21. W. S. S. Jee and J. S. Arnold: Structural changes in teeth and their supporting structures containing bone-seeking radioisotopes: J. Dent. Res. 38: 692 (1959).
22. W. S. S. Jee and J. S. Arnold: The indirect effect of internally deposited radioisotopes on bone: Rad. Res. 11: 447 (1959).
23. J. A. Twente, W. S. S. Jee and J. S. Arnold: A new technique for measuring localized tissue content of alpha emitting isotopes: Rad. Res. 11: 474 (1959).
24. W. S. S. Jee and J. S. Arnold: Remodelling of bones as visualized by autoradiography: Anat. Rec. 136: 217 (1960).
25. W. S. S. Jee and J. S. Arnold: Internally deposited radioisotopes in dog teeth: Rad. Res. 12: 445-446 (1960).

26. W. S. S. Jee, J. A. Twente and J. S. Arnold: The toxicity of plutonium deposited in skeletal tissues of beagles: in Radiation Damage in Bone - Conference on the Relation of Radiation Damage to Radiation Dose in Bone, International Atomic Energy Agency, Karntnerring, Vienna, 30-31 (1960).
27. W. S. S. Jee and J. S. Arnold: Radioisotopes in the teeth of dogs.
I - The distribution of plutonium, radium, radiothorium, mesothorium and strontium and the sequence of histopathologic changes in teeth containing plutonium: Arch. Oral Biol. 2: 215-238 (1960).
28. W. S. S. Jee and J. S. Arnold: Effects of internally deposited radioisotopes upon blood vessels of cortical bones: Proc. Soc. Biol. and Med. 105: 351-356 (1960).
29. J. A. Twente, E. G. Butler and W. S. S. Jee: The localized distribution of Pu^{239} in the lumbar vertebral centra of 5-level dogs: Radiobiology Laboratory, U. of Utah Report C00-220, 168-196 (1960).

HIGH RESOLUTION NEUTRON-INDUCED AUTORADIOGRAPHY OF BONE
CONTAINING PLUTONIUM-239

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Abstract: The detailed neutron-induced autoradiographic process for bones containing plutonium-239 produced autoradiographs with both fission fragment tracks and a corresponding bone image. Seven microns thick undecalcified bone sections were apposed on 300 microns thick Lexan (polycarbonate) film and exposed to neutrons in the thermal column of the Material Testing Reactor (MTR) at the National Reactor Testing Station in Idaho. The specimens were immersed in 28% KOH at 50°C for one hour to etch pinholes at the location of each fission track and pitting of the film at the location of the bone. The film, when inspected microscopically, exhibited both fission fragment tracks and a corresponding bone image. When the films were etched immediately after irradiation, no bone image was produced. When the bone sections were allowed to remain on the film for several weeks after irradiation, the bone caused a pitting of the film surface. Betas from phosphorus-32 seemed to be the main charged particles available to produce the pitting. This detailed neutron-induced autoradiographic process has advantages over the nuclear emulsion autoradiography of bones because it eliminated long exposure times and fading of latent image.

INTRODUCTION

Previous to 1967, conventional nuclear emulsion photographic autoradiography was used to determine the distribution of ^{239}Pu in bone.⁽¹⁻³⁾

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More recently, the technique of neutron-induced fission track autoradiography has been used to characterize the temporal and quantitative distribution of plutonium in bone.⁽⁴⁻⁷⁾ Thin bone sections are attached to thin polycarbonate films and irradiated in a high neutron flux. The neutron-irradiation of the very small quantities of plutonium produce fission tracks in the polycarbonate film. Using track etching techniques, the fission tracks become visible in the film. This neutron-induced autoradiography has advantages over the conventional nuclear emulsion because it eliminates long exposure times for bone containing only a small quantity of plutonium (in the order of pico pico curies per cm^2). It also eliminates the fading of latent image (loss of the photographic image) which plagues nuclear emulsion autoradiography.

In the studies of Bleaney,⁽⁴⁾ bone sections were sandwiched between polycarbonate films and exposed to thermal neutrons. The films were detached following irradiation and then etched to reveal the fission fragment tracks. The fission fragment tracks recorded by the "sandwich" technique are technically contact autoradiographs. This technique made it difficult to know, by superposition of foil and section, whether fission fragment tracks arose from the bone surface, bone or marrow cells. The work described in this paper demonstrates the feasibility of producing high resolution detailed neutron-induced autoradiographs that give superb resolution, equal to that of liquid emulsion coating of thin bone sections.⁽¹⁾ The detailed neutron-induced autoradiographic process produces autoradiographs with both fission fragment tracks and a corresponding bone image. A description of the process and an attempt to explain what causes the bone image is presented in the following sections.

PROCESS DESCRIPTION

Thin undecalcified bone sections are a prerequisite to high resolution autoradiography, whether the conventional nuclear or neutron-induced, autoradiographic techniques are employed. Blocks of undecalcified dog bones are fixed in absolute acetone, defatted in ether, embedded in a combination of ethyl/butyl methacrylate and sectioned on a mechanized heavy-duty Jung microtome.⁽⁸⁾ The 7 μ bone sections are floated off the knives with the aid of 80% alcohol, a brush and spatula, and apposed on a 300 microns thick Lexan (polycarbonate) film (Westlake Plastics, Lenni Mills, Pa.) which has been previously coated with 0.5% calf skin gelatin. The specimens (bone section apposed to Lexan foil) are allowed to air dry. They are then exposed to neutrons in the thermal column of the Material Testing Reactor (MTR) at the National Reactor Testing Station in Idaho. Neutron fluence in the range of 10^{13} to 10^{14} n/cm² is used. During the irradiation the temperature must be kept below 32⁰ C. Higher temperatures will anneal the tracks. Neutron irradiations were made in the MTR thermal column because of its high thermal neutron flux and low gamma heating. Subsequent to neutron irradiation, the specimens are allowed to age for a period greater than several weeks before immersing in 28% KOH at 60⁰ C for one hour to etch pinholes several microns in diameter at the location of each fission track. The bone section separates from the film during the etching process. The etched film is rinsed in tap water, air dried and inspected microscopically. The film exhibits both the fission fragment tracks and a corresponding bone image.

In a contact neutron-induced autoradiograph of a bone section with plutonium localized in both the bone and bone marrow, the exact position

Fig. 1. Detailed neutron-induced autoradiograph of portions of two bone spicules, showing fission fragment tracks distributed mostly upon bone surfaces in the lumbar vertebral body from a dog given $2.7 \mu\text{Ci}$ of $^{239}\text{Pu}/\text{kg}$ and killed 28 days later. Few isolated tracks are found in the bone marrow. B denotes bone, M denotes marrow (X 210).

of the fission tracks with respect to bone surface or bone marrow is uncertain because a bone image is not present to give the investigator the necessary orientation.⁽⁴⁻⁷⁾ If the polycarbonate film is etched immediately

after thermal neutron irradiation, we obtain the same result as with a contact neutron-induced autoradiograph. If the bone sections are allowed to remain on the polycarbonate film for several weeks after irradiation, the decay radiation from the bone causes a pitting of the film surface with results shown in Figures 1-3. In these figures, the bone was left on the polycarbonate film for 4 weeks after irradiation. As is quite evident, the radiation from the bone produced small pitting in the surface, which show as grey areas. The etched film exhibiting both fission fragment and bone images permits a more accurate characterization of the plutonium distribution.

ATTEMPTS TO EXPLAIN BONE IMAGE

Since the film pitting occurs after irradiation, reactions such as (n, α) , (n, p) , (n, d) or (n, n) by fast neutrons are ruled out. Radiation must be from the activation of bone constituents, i.e. calcium, phosphorus, etc. with a short half-life. The composition of bone indicates no alpha emitter would be produced and no alpha activity was found on control (plutonium-free) dog bone sections after irradiation.

Only beta activity was found in the sample. Absorption curves with aluminum absorbers indicated that most of the activity was from phosphorus-32, a 1.71 MeV beta emitter with a 14-day half-life. Some calcium activation should have occurred, but little activity other than phosphorus-32 was observed.

Human bone marrow produce greater amounts of film pitting than dog bone marrow. This clue so far has not helped to identify the decay radiation causing this pitting. Betas from phosphorus-32 seem to be the main charged particles available to produce the pitting. Pitting by betas has

Fig. 2. Detailed neutron-induced autoradiograph showing a portion of a bone spicule diffusely labeled with tracks (B) and star-producing aggregates of fission fragment tracks in the bone marrow in the lumbar vertebral body from a dog given $0.96 \mu\text{Ci}$ of ^{239}Pu /kg and killed 1617 days later (X 210).

never been reported in the literature. More work is needed before the source of pitting is known.

Previously, Kleeman and Lovering⁽⁹⁾ obtained prints of their ore sample with thick Lexan foils that were exposed to neutrons in contact with certain minerals. They too have observed a fine pitting of the foil surface

in contact with certain types of minerals. Although this is not caused by decay radiation from activation of the sample as we have observed, it is caused by small charged particles lighter than alphas. Nevertheless, the prints of the bone image allowed us to study the distribution of plutonium-239 in bone, with considerable saving in time and without fear of having the latent image fade.

APPLICATION OF TECHNIQUE

In order to express properly the radiation dose delivered to the bone lining cells in radiation carcinogenesis of bone seeking radionuclides, the residence time (reciprocal of "turnover rate") of the plutonium must be known. We are in the process of determining the resident time of plutonium on lumbar vertebral body bone surfaces in young adult beagles (15 months old) given a single intravenous injection of 0.015 μCi of ^{239}Pu citrate/kg using detailed neutron-induced autoradiography (Table I). The percent bone surface with plutonium (fission fragment tracks) and percent of bone with buried plutonium was determined by microscopic sampling. Details of the methods have been reported in a study using detailed autoradiographic methods.⁽¹⁰⁾ The ongoing neutron-induced autoradiographic study showed that 69.6% of the bone surface contains plutonium, 30.4% of the bone surface is free of plutonium and 13.2% of the bone surface contains buried plutonium at 5 days post injection. In other words, 43.4% of the bare bone surfaces exhibit buried plutonium. The buried plutonium was formerly located on bone surfaces at day 1. The bone surface deposits drop to 21.4% and 3.4% at 97 and 186 days post injection respectively.

Our current program calls for the use of neutron-induced autoradiography to determine the quantity of plutonium in both the marrow and bone,

Fig. 3. Detailed neutron-induced autoradiograph of a portion of two bone spicules showing diffusely labeled bone in upper spicules, and a band of tracks in the lower spicules in a lumbar vertebral body from a dog injected with $0.0142 \mu\text{Ci}$ of $^{239}\text{Pu}/\text{kg}$ and sacrificed 1539 days later. Very few tracks are located in the marrow.

B denotes bone and M denotes marrow (X 210).

using automatic track counting techniques. Previously, visual track counting proved to be time consuming and demoralizing. We will subject these bones to neutron irradiation and immediately etch them. Total plutonium value in the bone sections can be determined by spark counting¹¹ or by

track counting with the Quantitative Television Microscope (QTM¹²). Using the detailed neutron-induced autoradiography on the next serial section, the plutonium-in-bone value can be obtained by counting fission fragment tracks localized within bone with the QTM. Thus, the marrow Pu content can be derived by subtracting the bone count from the total Pu count.

TABLE I

Distribution of Plutonium-239 in Lumbar Vertebral Trabeculae of Beagles Injected with 0.015 $\mu\text{Ci/kg}$

<u>Time after injection</u>	<u>% Surface</u>	<u>% Buried</u>
5 days	69.6	13.2
97 days	21.4	53.1
186 days	3.4	43.5

REFERENCES

1. J. S. Arnold. "An Improved Technique for Liquid Emulsion Autoradiography." Proc. Soc. Exptl. Biol. and Med. 85: 113 (1954).
2. J. S. Arnold and W. S. S. Jee. "Bone Growth and Osteoclastic Activity as Indicated by Radioautographic Distribution of Pu^{239} ." Am. J. Anat. 101: 367 (1957).
3. J. S. Arnold and W. S. S. Jee. "The Toxicity of Plutonium Deposited in Skeletal Tissues of Beagles. I. The Relation of Distribution of Plutonium to the Sequence of Histopathologic Bone Changes." Lab. Invest. 7: 797 (1961).
4. B. Bleaney. "Radiation Dose Rates Near Bone Surfaces in Rabbits After an Injection of Plutonium." Phys. Med. Biol. 12: 145 (1967).

5. B. Bleaney. "The Radiation Dose-rates Near Bone Surface in Rabbits after I.V. or I.M. Injection of Plutonium-239." Brit. J. Radiol. 42: 51 (1969).
6. B. Bleaney. "Plutonium Deposition on Bone Surface and in Bone Marrow Following Intravenous and Intramuscular Injections." In Delayed Effects of Bone-Seeking Radionuclides, Ed. By C. W. Mays, R. D. Lloyd and W. S. S. Jee, p. 125, U. of Utah Press, Salt Lake City (1969).
7. K. Becker and D. R. Johnson. "Non-photographic Alpha Autoradiography and Neutron-Induced Autoradiography." Science, 167: 1370 (1970).
8. R. Dell, R. S. Mical and W. S. S. Jee. "Modified Techniques in Successful Thin Sectioning and Detailed Autoradiography of Undecalcified Bone" (in preparation).
9. J. D. Kleeman and J. F. Lovering. "Uranium Distribution Studies by Fission Track Registration in Lexan Plastic Prints." In Proceedings of the International Conference on Nuclear Track Registration in Solids, Sect. VI, p. 41, Univ. of Clermont-Ferrand, France (1969).
10. W. S. S. Jee, H. Z. Park and R. Burggraaf. "Estimates of Residence Time of ^{239}Pu in Trabecular Bone of Beagles." Research in Radiobiology, U. of Utah Report C00-119-246, p. 188 (1969).
11. D. R. Johnson, R. H. Boyett and K. Becker. "Sensitive Automatic Counting of Alpha Particle Tracks in Polymers and Its Applications in Dosimetry." Health Physics, 18: 424 (1970).
12. M. H. Bartley, S. Targonski, S. Seamans and W. S. S. Jee. "Comparison of Some Quantitative Studies of Trabecular Bone." In Stereology, Ed. by H. Elias, p. 68, Springer-Verlag, New York (1967).

ELECTRON MICROSCOPY OF RADIATION DAMAGE IN BONE

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Abstract: Some morphological effects of radionuclides on bone are described with the use of the electron microscope. Haversian canal and osteocytic lacunar fibrosis, perivascular cell reduction and osteolysis and subsequent osteostasis are present. Endothelial vacuolization increases with dosage level. Kidney failure leads to an increase in the number of osteoclasts and fusion between osteoclasts and adjacent cells are found.

INTRODUCTION

The purpose of this study with the electron microscope is to confirm and possibly add to observations made with the light microscope on changes in the bone morphology induced by alpha irradiation.

The numbers of radionuclides used and the variety of dosage levels given, together with the natural morphological changes in bone which occur with age, make comparison between the effects of different radionuclides difficult. However, some general findings on the effects of radiation on bone as a whole are possible and will be described briefly.

MATERIALS AND METHODS

At autopsy one metatarsal is removed from the dog and small pieces of spongy bone, cortical bone, and articular cartilage are fixed for electron microscopy in 2% cacodylate buffered glutaraldehyde (final buffer = .025 M). Sucrose is added to the fixative (.045 g/ml) to minimize inherent osmotic problems in bone. Part of the bones are decalcified in 4% EDTA and part remain undecalcified. All bone and cartilage is post-fixed in cacodylate buffered osmium tetroxide, dehydrated with graded ethyl alcohols and embedded in Epon 812. Sections for light and electron microscopy are obtained on a MT-1 Porter-Blum ultramicrotome. One micron

sections are stained with Paragon stain for light microscopy and thin sections, stained with uranyl acetate and lead citrate for electron microscopy. Electron micrographs are taken on an RCA electron microscope at 50 KV.

RESULTS

A summary of our preliminary findings follows:

A. Perivascular Observations:

1. There is a decrease in the number of perivascular cells in the Haversian canals of both ageing controls and irradiated dogs (Figs. 1,2,3).
2. At low injection levels of radionuclide normal bone formation can occur and tight junctions are prevalent between osteoblasts and periosteoblasts ⁽¹⁾ (Figs. 5,6,7).
3. Perivascular cells in irradiated animals have more lysosomal-like inclusions in their cytoplasm than controls (Figs. 7,8).
4. In perivascular fibrosis, which occurs subsequent to radiation damage, the collagen fibril present is of smaller caliber than the normal bone collagen fibril (Fig. 9).

B. Vascular Observations:

1. With increasing dosage levels the small pinocytotic vesicles normally present are replaced to varying degrees with larger vacuoles (Figs. 7,8).
2. The basement membrane appears to become somewhat thicker and more ragged in appearance (Fig. 8).
3. The endothelial cell acquires an increased number of inclusion particles (lysosomes) (Figs. 7,8).

4. Blistering of the endothelial cytoplasm can occur also (Fig. 4).

C. Osteocytic Changes:

1. The lacunar borders of some osteocytes show evidence of lacunar repair after apparent initial osteolysis (Figs. 10,11,12).
2. Empty lacunae can be found which are filled to various degrees with fine fibrillar material (Figs. 3, 13).

D. Osteoclast Formation:

1. Several dogs have become terminal because of kidney failure. In each case of kidney failure there is an increase in the number of osteoclasts observed in compact bone, even though some of these dogs are quite old (Fig. 14,15,16).
2. Some osteoclasts have been found which appear to be fusing with another cell (Figs. 15,16).

DISCUSSION

The overall vascular findings in the bone vascular system of internally irradiated dogs are in general agreement with those described by Berdjis⁽²⁾ for externally irradiated animals. It is interesting to note that examples of each stage of vascular deterioration can be found in the bone vasculature of dogs injected with radionuclides many months before. The question naturally arises whether the vascular injury resulted from the latent effects of initial irradiation or whether, since much of the residual radioactivity resides in bone, the visible effects are the result of recent radiation damage.

The extent of initial osteolysis can be seen by the reversal lines around the perimeter of the osteocytic lacuna. Some osteocytes have

more than one line which suggests several periods of recovery and variable osteocytic activity. The osteocytes pictured (Figs. 10, 11, 12) all have new osteoid on the inside of the lacunar border indicating that the osteocytes have entered a synthesizing phase (osteostasis). Correlations between the current osteocytic activity and the appearance of vascular supply may be an important factor in evaluating this "life history."

Kidney failure in the dog brings about predictable morphological changes in bone of control or irradiated dogs. Osteoclast formation is increased and, depending on the time course of the disease, can be of value in studying osteoclast formation and osteoclasts. Fusion of osteoclasts is found and is less confusing to study because the numbers of perivascular cells are decreased.

REFERENCES

1. S. W. Whitson; Tight junction formation in the osteon; Accepted for publication, *Clinical Orthopaedics* (1971).
2. C. C. Berdjis; The cardiovascular system; In Pathology of Irradiation; Ed. by C. C. Berdjis, Williams and Wilkins, Baltimore, 377-407 (1970).

Fig. 1. Area of haversian canal of metatarsal shaft from a 5426 days old control beagle (M6M0). The endothelium (en) has many small pinocytotic vesicles and there are few cells in the perivascular space (ps). x 21,000

Fig. 2. Haversian canal showing absence of perivascular cells, with the exception of a small pericytic process (pc), in the metatarsal shaft of a dog (M4R1.7) given an i.v. injection of 0.163 μ Ci of ^{226}Ra /kg at 514 days and sacrificed 5495 days later. Endothelium (en), bone matrix (bm) X 15,300

Fig. 3. Haversian Canal (h) from the same dog as figure 2 (M4R1.7).
No blood capillary is present. If the limit of the original canal border is traced (arrows), it is evident that a new concentric layer of dense matrix (osteoid) has reduced its size. The resulting canal is filled with debris as is the empty osteocytic lacunae peripherally. x 12,800

Fig. 4. Capillary endothelium (en) exhibiting a blister (b) which protrudes into the capillary lumen in a haversian canal of the metatarsal shaft of an adult dog (T6W3) given an i.v. injection of 0.31 μ Ci of ^{241}Am and sacrificed 1510 days post injection at 2027 days of age. x 25,200

Fig. 5. Tight-junction formation (arrow) between osteoblasts in a haversian canal from the metatarsal of the same dog as in figure 2 (M4R1.7). Endothelium (en) x 21,000

Fig. 6. New osteoid (arrows) is being formed by the overlapped osteoblasts on the perimeter of the haversian canal in the metatarsal shaft of a dog (F10T1) given an i.v. injection of 0.015 μ Ci of ^{228}Th at 508 days old and killed 3420 days later. Endothelium (en) x 13,400

Fig. 7. Haversian canal from a 5297 days old dog (F4S2) injected with 3.41 μCi of $^{90}\text{Sr}/\text{kg}$ and sacrificed at 4775 days post injection (tangentially cut). Bone formation is occurring (arrows) and a large myelin figure is present partially surrounded by endothelial cytoplasm (en). $\times 10,300$

Fig. 8. An area of haversian canal (longitudinal section) from the metatarsal shaft of the same dog as figure 7 (F4S2). The basement membrane (ba) appears thickened and the preosteoblasts have large lysosomes (l). A loose network of collagen fibrils lie between the preosteoblast and the thin osteoblastic processes nearer the bone matrix. x 15,600

Fig. 9. Area of haversian canal from the metatarsal shaft of the same dog as in figure 6 (F10T1). The entire perivascular space surrounding endothelium (en) and the pericyte (pc) is completely surrounded by a dense, but small-fibered (arrow) matrix. x 11,400

Fig. 10. Osteocyte from the metatarsal shaft of the same dog as figure 7 (F4S2). The lacunar border presents a very irregular border (lb) and together with the small islands of collagen (ls, arrows) located in the lacunar space typically result from osteolytic activity. Fibrillar material is minimal. x 18,200

Fig. 11. Osteocyte, same treatment as animal in figure 7 (F4S2). The osteolysis evident along the lacunar border appears to be secondary to an attempt at lacunar repair as represented by the "reversal" line (arrows) of an earlier border limit. Some new collagen fibrils (f) are present. x 18,200

Fig. 12. Osteocyte, same animal as figure 7 (F4S2). The lacunar border is smooth in appearance but exhibits a new thin layer of collagen fibrils (f) along the entire extent of the lacunar border.

x 12,000

Fig. 13. Osteocytic lacunae from metatarsal midshaft of a dog (F11S4.5) injected i.v. with $63.8 \mu\text{Ci}$ of $^{90}\text{Sr/kg}$ at age 496 days and sacrificed 1758 days later. Three cytoplasmic processes (p) are evident but the rest of the lacunar space (ls) is filled with fine fibrillar material. $\times 15,400$

Fig. 14. Osteoclast from the metatarsal midshaft of the same dog as figure 2 (M4R1.7). Note the parts of two nuclei (n), the numerous mitochondria (m), and the modification of the plasma membrane into many irregular processes (p), some with collagen fibrils between them (arrows). Resorbing bone matrix (rbm) x 18,000

Fig. 15. Osteoclast from the epiphysial bone of the metatarsal of the same control as figure 1 (M6M0). In addition to the organelles previously mentioned note the prominent nucleoli (nu), within the nuclei, the occurrence of large vacuolated areas of cytoplasm (v), and the apparent fusion of a neighboring cell to the osteoclast (between arrows). x 10,400

Fig. 16. High magnification of figure 15 (M6M0) in the area of fusion (between arrows). Besides the fusion the ragged appearance of the bone matrix (bm) and lysosomal formation (l) is seen to better advantage. x 20,600

AUTORADIOGRAPHIC STUDY OF THE EARLY DISTRIBUTION OF ^{45}Ca IN ALVEOLAR BONE OF DOGS

W. W. S. Jee, Rebecca B. Dell and M. A. Davis*

Abstract: An autoradiographic study designed to yield information about the prompt uptake of ^{45}Ca in alveolar bones of adult mongrels at 2 and 30 hours after injection of ^{45}Ca found the greatest darkening at alveolar bone surfaces located at or near the alveolar crest and at forming bone surfaces of trabeculae and forming osteons. The next greatest darkening is seen at cemental and the remaining alveolar bone surfaces. Haversian canals of formed osteons of intermediate mineralization are poorly labeled with ^{45}Ca . Our findings suggest that the greatest exchange of calcium between bone and blood takes place at the bone surfaces with the richest blood supply (i.e. alveolar crest and areas of bone formation).

INTRODUCTION

The bone group in cooperation with Drs. M. A. Davis and J. James Adelstein of the Division of Nuclear Medicine, Peter Bent Brigham Hospital, Harvard Medical School, Dr. D. Garcia, Department of Radiology, Harvard Dental School and Dr. L. Blau of the Hospital for Special Surgery, New York City, are developing methods needed to apply radionuclide scanning techniques to the mineral metabolism of the jaws and alveolar bone of the beagles with and without periodontal diseases. The above individuals will develop the scanning methods and our group will carry out histological studies to correlate external nuclide counts with autoradiographic evidence of calcium-45 incorporation.

Scanning of the skeleton following administration of bone-seeking radionuclide has found an important role in many diagnostic procedures in the practice of orthopaedics. Detectable differences in the avidity for

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radioactivity have been found in bone surrounding tumors, fractures and infections. Early changes in bone pathology were detected by bone scanning weeks and months prior to detectable density differences in conventional radiographs. These scans were performed using ^{85}Sr . It is our plan to use strontium-87m. The clinical use of $^{87\text{m}}\text{Sr}$ will permit repetitive use and still be within acceptable radiation dose limits for humans. The calculated total bone dose of 0.14 rads for $^{87\text{m}}\text{Sr}$ (half-life: 2.8 hrs) per mCi injected in a 70 kg. man is less than 10% that received with 0.1 mCi of longer lived bone-seeking radionuclides (calcium-47 and strontium-85).

The research plan for this study will initially involve mongrel dogs given a dose ranging from 1 to 4 mCi of $^{87\text{m}}\text{Sr}$ per scan. With a physical half life of 2.8 hours, the radioactivity should disappear in 24 to 28 hours. Thus, it is imperative to determine whether there is a differential uptake of calcium-like radionuclides in areas of bone remodeling during this short time interval.

In 1935, Chiewitz and Hevesy⁽¹⁾ indicated that the rate of uptake of ^{32}P by bone was too rapid to be due to growth alone and there exists an exchange reaction between the bone mineral and the bone-seeking radioisotopes in the circulating fluids. More recently, Rowland⁽²⁾ identified the locations in compact bone of the femur which took up calcium-45 between 12 minutes to several days after intravenous administration of the bone-seeking radionuclide. He demonstrated that those bone structures with the most intimate contact with the circulating fluids were the primary sites of rapid exchange between blood and bone and that lowly mineralized structures are not the major sites of exchange between bone and blood.

The pilot study reported here was designed to yield qualitative

autoradiographic information about the prompt uptake of calcium-45 in alveolar bones of adult mongrels at 2 and 30 hours after intravenous injection of calcium-45.

METHODS AND MATERIALS

Two adult mongrel dogs (75% beagle) were sacrificed at 2 and 30 hours after intravenous injection of 2 mCi of ^{45}Ca by Dr. M. A. Davis. Immediately after sacrifice, the mandibles and tibiae were rapidly removed and fixed in 3 changes of absolute acetone and shipped to Utah. At Utah, the segments of the mandible with the first and second premolars and third and fourth premolars and one of the proximal tibiae were sawed, defatted in several changes of ether, embedded in bioplastic and ground into 100 micron-thick sections. These sections were microradiographed and then contact autoradiographed on Eastman No-Screen film and Eastman NTB 10 micron-thick plates. Subsequently, these same sections were dipped in Eastman NTB liquid emulsion.

RESULTS

Shown in Figures 1 through 3 are microradiographs, contact autoradiographs and detailed autoradiographs of the interradicular alveolar bone of the second premolar tooth section from a dog sacrificed 2 hours post ^{45}Ca administration. At this early time period, all the ^{45}Ca is deposited upon bone surfaces. In the autoradiographs, there is high uptake of ^{45}Ca at the cementum and alveolar bone surfaces. The deposit at the cemental surface was of uniform intensity, while the deposition on the alveolar bone surface was more irregular. Higher deposits of ^{45}Ca were located in a scalloped area (lower left) and on the coronal border (left portion of micrographs) of the alveolar bone. Two other hotspots are seen within the alveolar

bone. One corresponds to a forming Haversian system possessing a concentric layer of poorly mineralized bone (recently formed bone), while the other possesses very little lowly mineralized bone.

Figures 4 to 6 are microradiograph, contact autoradiograph and detailed autoradiograph of the interdental alveolar bone between the first and second premolars from a dog sacrificed 2 hours post injection. Again, there is a high uptake of ^{45}Ca at the cemental and alveolar bone surfaces. The heavier deposition areas occur at the alveolar crest (left) of and neighboring alveolar bone surfaces. Active bone resorption is restricted to the crest. Another region of the alveolar bone surface which has been resorbed (top right corner) shows a portion of its surface with a heavy ^{45}Ca deposition. It corresponds to an area of scalloped alveolar bone surface with a lowly mineralized osteon situated near the surface. The only other hotspot is located within the bone and near the crest. The hotspot corresponds to a forming osteon possessing poorly and highly mineralized bone surfaces.

Figures 7 through 10 are examples of active or recent bone resorption of alveolar bone surfaces, a consequence of periodontal diseases. Figure 7 shows the marked destruction of the interdental alveolar crest leaving behind trabeculae with poorly and highly mineralized bone surfaces. There is a high uptake of ^{45}Ca on these bone surfaces regardless of their state of mineralization (Fig. 8). The resulting trabeculation at this site made more surfaces available for ^{45}Ca deposition. In Figure 9, the coronal tip of the interradicular alveolar bone is well-scalloped by Howship's lacunae. The corresponding autoradiograph shows that the heavy deposition

on this surface is quite irregular (Fig. 10). Moreover, the hotspot corresponds to areas of lowly mineralized bone (newly formed or forming bone).

DISCUSSION

The autoradiographs from these two dogs clearly indicated that at 2 and 30 hours, the greatest darkening was seen over the alveolar bone surfaces located at or near the alveolar crest and bone surfaces of forming bone in trabeculae and osteons located within the alveolar bone. The next greatest darkening was seen at cemental and alveolar bone surfaces.

Our observations partially agree with Rowland's⁽²⁾ hypothesis "that the greatest exchange between bone and blood takes place at those bone surfaces with the most intimate contact with the circulating fluids, regardless of their degree of mineralization." Our tentative findings suggest at very short time intervals that the greatest exchange of calcium between bone and blood take place at those bone surfaces with the richest blood supply (i.e. the alveolar bone at the alveolar crest is probably better vascularized than alveolar bone surface adjacent to the periodontal ligament).

Heavy deposits of ^{45}Ca were located on bone surfaces of forming new bone at both 2 and 30 hours post-injection regardless of their location. Haversian canals of osteons of intermediate mineralization were poorly labeled. Most of the heavily labeled osteon possessed bits of poorly mineralized bone or smooth, highly mineralized bone surface. Even the cavities with highly mineralized bone surfaces may be actually laying down osteoid which microradiography could not detect. This is why we need a detailed autoradiographic method which will allow us to stain the undecalcified bone section to demonstrate vascularity, active bone formation and resorption.

In this study we were able to produce unstained, undecalcified liquid emulsion coated autoradiographs. We have not tried staining these specimens, but we expect some difficulty in finding a suitable stain to penetrate through the bioplastic, and stain the undecalcified bone with a low affinity for the liquid emulsion.

We were very fortunate that Dr. Davis chose to use two dogs with low grade periodontal disease. The destruction of the alveolar crest bone by periodontal disease increased the bone surfaces available to take up the heavy alveolar crest deposits of ^{45}Ca (Fig. 9 and 10). Whether these deposits are of sufficient quantity to allow detection by in vivo scanning of jaws must await development of suitable bone scanning instruments to view small tissue volumes.

REFERENCES

1. O. Chiewitz and G. Hevesy; Radioactive indicators in the study of phosphorus metabolism in rats; *Nature*, 136: 754 (1935).
2. R. E. Rowland, Exchangeable bone calcium; *Clinical Orthopaedics*, 49: 233-248 (1966).

Fig. 1. Microradiograph of the interradicular alveolar bone of the second premolar from a dog injected with 2 mCi of ^{45}Ca and sacrificed 2 hours later. (1) denotes cemental surface, (2) denotes alveolar bone surface (coronal portion), (3) denotes scalloped alveolar bone surface, (4) denotes forming osteon with lowly mineralized bone, (5) denotes cavity in alveolar bone with portion of bone surface lined with poorly mineralized bone, (6) denotes formed osteon with lowly mineralized bone and partial highly mineralized bone border and (7) denotes osteon with intermediate mineralized border to Haversian canal, X 56.

Fig. 2. Corresponding NTB contact autoradiograph to Fig. 1. Note the heavy darkening at structures (1) through (5). The much lower uptake of ^{45}Ca at structure (6) and (7).

Fig. 3. Detailed autoradiograph of same specimen as Fig. 1. Liquid emulsion coated 100 micron-thick section mounted in water.

Fig. 4. Microradiograph of the interdental alveolar bone between first and second premolars from a dog injected with 2 mCi of ^{45}Ca and sacrificed 2 hours later. (1) denotes cemental surfaces, (2) denotes alveolar bone surface (coronal portion), (3) denotes partially forming osteon and (4) a lowly mineralized osteon near a scalloped border of alveolar bone, X 56.

Fig. 5. Corresponding NTB contact autoradiograph to Fig. 4. Note the heavy deposits of ^{45}Ca at structures (2), (3) and (4). The cemental surface (1) possesses intermediate darkening.

Fig. 6. Detailed autoradiograph of same specimen as Fig. 4. Liquid emulsion coated 100 micron-thick section mounted in water.

Fig. 7. Microradiograph of the interdental alveolar bone of the third and fourth premolar from a dog injected with 2 mCi of ^{45}Ca and sacrificed 30 hours later. Note the spicules with lowly (grey) and highly (white) mineralized bone surfaces. (1) and (2) denote lowly mineralized bone surfaces, (3) a partially filled osteon and (4) a resorption cavity beginning to fill in, X 85.

Fig. 8. Corresponding NTB contact autoradiograph to Fig. 7. Note the heavy deposits upon the bone surfaces of the spicule in the area which was formerly the alveolar crest. Note the darker blackening at sites (1) and (2) which corresponds to lowly mineralized bone surfaces. A much lighter darkening is seen in filling osteon (3) as compared to forming bone at osteon (4).

Fig. 9. Microradiograph of the interradicular alveolar bone of the fourth premolar from a dog 30 hours after injection with 2 mCi of ^{45}Ca . Note the ragged alveolar bone surface. It is difficult to determine whether it is actively resorbing from a microradiograph. (1) denotes cemental surfaces, (2), (3) and (4) denote scalloped alveolar bone surfaces and (5) a partially forming osteon, X 85.

Fig. 10. Corresponding NTB contact autoradiograph to Fig. 9. Note the hotspots irregularly located at the alveolar bone surface. It is difficult to determine whether these hotspots correspond to resorbing or forming bone surfaces at sites (2), (3), and (4). Note right border of osteon (5) attracts a high concentration of ^{45}Ca .

EFFECT OF CORTISOL ON BONE ACCRETION, ^{45}Ca EFFLUX,
AND TRABECULAR BONE RESORPTION

H. Z. Park, K. W. Jee, and W. S. S. Jee

Abstract: In dose response studies using 0, 0.5, 1, 5, 20 and 75 mg of cortisol/kg/day for 7 days in 100 g rats, a significant reduction in tibial metaphyseal bone volume was measured in male rats injected with 1 and 5 mg of cortisol/kg/day. Female rats did not show such change. Endochondral growth of male rats measured by tetracycline labeling (25 mg/kg body weight) showed that there was significant inhibition of bone growth at dose levels of 20 and 75 mg of cortisol/kg/day. Decreased efflux of "deep store" ^{45}Ca was observed at day 3 of treatment with doses of 0.5, 5, and 20 mg of cortisol/kg/day. At day 6, the differences of efflux among these three dose levels became insignificant.

INTRODUCTION

Excessive dose of glucocorticoid is known to inhibit bone growth,⁽¹⁾ cause the loss of bone volume,⁽²⁾ and suppress the efflux of ^{45}Ca from the deep store pool of bone.⁽³⁾ In this study, the effects of various dose levels of cortisol on bone resorption, suppression of bone growth, and ^{45}Ca efflux were examined to determine the possible mechanism of osteoporosis induced by cortisol.

METHODS

Young male and female Sprague-Dawley rats weighing 100 to 200 g were used. Rats were injected with 0, 0.5, 1, 5, 20 and 75 mg of cortisol per kilogram body weight per day. At autopsy, the right tibia was fixed in acetone, defatted, dehydrated and embedded in Bioplastic and sawed. The sections were ground to 100 microns thickness for microradiographs and then reground to 50 microns and examined under a Leitz-Wetzlar U.V. microscope to determine the rate of endochondral bone formation. The microradiographs were examined with the Quantitative Television

Microscope (Metals Research, Ltd., Cambridge, England), which measures the percent bone volume in metaphysis of tibial bone.

The calcium-45 was allowed to enter the deep bone stores for two weeks after injection of 40 microcuries of ^{45}Ca (International Chemical and Nuclear Corporation, Irvine, California). One hundred lambda of blood serum samples were obtained from the tail vein and counted in a Packard refrigerated scintillation counter. Tetracycline (25 mg Achromycin/kg) was injected to label newly deposited bone 3 days prior to sacrifice of animal.

RESULTS

Effect of cortisol on bone volume in tibial spongiosa.

As shown in Fig. 1, control male rats show about 44% bone in the metaphyseal area of the proximal tibia. After 7 days of treatment with cortisol, low dose levels (1 and 5 mg) showed a decrease of bone to 33% and high doses (above 20 mg) increased slightly, but insignificantly, at day 7. Later studies show that this trend was definite and an increase of bone was observed.⁽⁴⁾ In females, a similar trend was observed, but it was insignificant statistically.

Effect of endochondral bone formation.

At dose levels of 1 and 5 mg, there were insignificant differences of endochondral bone growth rate. However, at 20 and 75 mg level, bone formation was completely suppressed (Fig. 2).

Efflux of deep store ^{45}Ca from bone to systemic fluid.

Mean value of ^{45}Ca in 100 lambda of serum was 2420 disintegrations per minute. Control rats responded to tail bleeding with fluctuation

between 94.7% to 97.9% of the day 0 value for ^{45}Ca concentration in serum. At a dose level of 0.5 mg/kg per day, calcium-45 efflux decreased to 90.6 (day 1), 76.9 (day 3) and 55.3 (day 6) percent of day 0 values. After day 6, the values leveled off. A dose response was observed at day 1 and 3 of treatment after 0.5, 5 and 20 mg/kg/day. At day 6, the differences in ^{45}Ca efflux among these three dose levels were insignificant.

DISCUSSION

The suppressed bone formation rate at higher doses of cortisol administration and the increase in metaphyseal bone volume could be accounted if there was less resorption at high doses as well, and less trabecular bone volume with low dose must be the consequence of stimulated bone resorption. Therefore, the bone change by cortisol administration can be attributed to super normal bone resorption with normal or subnormal bone formation at low dose, and super subnormal bone resorption with subnormal bone formation at high dose.

The efflux of calcium-45 pool, which is affected by various endocrine factors,⁽³⁾ shows the time-dose relationship in the effect of cortisol on mineral transport. This may account for the biphasic response observed in rat treated with low dose.⁽⁴⁾

REFERENCES

1. R. H. Follis, Jr.; Effect of cortisone on growing bone of the rat; Proc. Soc. Exp. Biol. Med., 76: 722 (1951).
2. F. Albright; Cushing's Syndrome: Its pathological physiology, its relationship to adreno-genital syndrome, and its connection with problems of reaction of body to injurious agents ("alarm reaction" of Selye); Harvey Lect., 1942-1943, 38, 123 (1943).

3. R. V. Talmage, H. Z. Park, and W. S. S. Jee; Parathyroid hormone and calcitonin function in cortisol treated rats; *Endocrinology*, 86: 1080 (1970).
4. G. H. Kenner, W. S. S. Jee and H. Z. Park; Cortisol and mineral transport -- effect of time and graded doses upon strontium-85 retention in young and adult rats; *Research in Radiobiology U.S.A.E.C. Report C00-119-242*, 282 (1970).

EFFECT OF CORTISOL ON % TRABECULAR BONE OF INTACT MALE AND FEMALE RATS

* SIGNIFICANT AT 0.05 LEVEL

Fig. 1. Bar graphs illustrating changes in quantity of proximal tibial metaphyseal bone after 7 days of cortisol treatment.

**EFFECT OF CORTISOL ON ENDOCHONDRAL
GROWTH OF INTACT MALE RATS
MEASURED BY TETRACYCLINE LABELING**

Fig. 2. Bar graphs showing change in endochondral bone formation rate measured by tetracycline labeling 3 days before sacrifice.

Fig. 3. Graph showing the efflux of calcium-45 given 14 days before the start of cortisol treatment. Serum concentration at the start of cortisol treatment (day 0) is expressed as 100%.

INTERRELATED EFFECTS OF GLUCOCORTICOID AND PARATHYROID
HORMONE UPON BONE REMODELLING

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H.Z. Park and M. Kramer

Abstract: A review of the animal and human data in the literature as well as the studies in the rat and rabbit in our laboratory shows that the possible mechanisms by which glucocorticoid acts in promoting bone resorption involves complex responses at the histological level as follows: (1) the effect of cortisol upon bone is dose dependent; (2) high doses suppress bone resorption; (3) cortisol acts upon bone cells by directly inhibiting precursor cell proliferation and by indirectly stimulating parathyroid hormone secretion; (4) inhibition of precursor cell proliferation is the overriding effect after high doses; and (5) the parathyroid hormone-mediated effect is the overriding effect after low doses.

INTRODUCTION

Excessive glucocorticoid is known to inhibit bone growth,⁽¹⁴⁾ suppress the synthesis of bone matrix proteins,^(1,2) and cause the loss of bone volume (osteoporosis)⁽³⁾. The cause of bone volume loss has been studied experimentally in animals and man, but there is disagreement about how the steroid effects bone remodeling to cause the osteoporosis. The osteoporosis has been attributed to (1) normal bone resorption with subnormal bone formation, (2) subnormal bone resorption with an even greater subnormal bone formation, and (3) supernormal bone resorption with subnormal bone formation.

The first purpose of this report is to develop a unified hypothesis of glucocorticoid action on bone remodeling by examining the recent pub-

lications and our studies on glucocorticosteroid actions on humans and experimental animals.

The second purpose is to examine whether or not this osteoporotic action is the direct effect of glucocorticoids, or is mediated through the parathyroid glands.

For clarity, this report will use a dose of 5.0 mg of cortisol (hydrocortisone) kg/day or less as low dose levels, and anything above as high dose levels. Most of the previous research has used cortisone and prednisone. It is not known on the organ level for bone whether a given amount of cortisone or prednisone is equivalent to cortisol. Unfortunately, we have not studied the potency relationships of cortisone and prednisone to cortisol like we have for other anti-inflammatory steroids (corticosterone, prednisolone, dexamethasone, etc.^(4,5)) The closest relative to cortisone and prednisone is corticosterone and prednisolone, possessing an 11 β -hydroxal instead of an 11-keto group. Corticosterone is one fourth as potent, while prednisolone is 2 times more potent than cortisol; therefore, tentatively at least, we assume that cortisone is one fourth and prednisone is twice as potent as cortisol.

From a review of the most recent literature (Table I), we can conclude that there is a consistent suppression in precursor cell division, bone formation, numbers of osteoblasts, bone mass and numbers of osteoclasts at higher dose levels. Moderated to massive dose levels were employed in the rat experiments while doses in the neighborhood of 2.5 mg cortisol/kg/day were used for rabbits. These investigators^(4,10) found dose levels exceeding 5 mg/kg/day for over 30 days were lethal.

Four reports in Table I showed a reduction in osteoclasts. Simmons

TABLE I MORPHOLOGICAL FEATURES OF BONES OF EXPERIMENTAL ANIMALS GIVEN GLUCOCORTICOIDS
(% OF CONTROL VALUE)

<u>Source</u>	<u>Species</u>	<u>Dose</u>	<u>Duration of treatment</u>	<u>Precursor Cell Division</u>	<u>Formation</u>	<u># Blasts</u>	<u>Bone Mass</u>	<u>Resorption</u>	<u># Clasts</u>
Simmons et al. (6)	rat	50mg/kg/d Cortisone	1-3d	+ 50%	--	+ 35%	--	--	+ 57%
Simmons et al. (6)	rat	50mg/kg/d Cortisone	19d	+ 50%	--	+ 50%	--	--	0
Roberts (7) (Stimulated PDL)	rat	5mg/kg/d Cortisol	8d	+ 40%	--	+ 40%	--	--	+ 45%
Storey (8)	rabbit	20mg/kg/d Cortisone	21d	--	↓	--	+ 57%	--	--
Thompson et al. (9)	rabbit (matrix implant)	4-15mg/kg/d Cortisone	28d	--	↓ 0%	--	+ 20%	--	+ 25%*
Blackwood (10)	rabbit (adult)	2.5mg/kg/d Cortisol	30d	--	↓ 25%	--	+ 50%	--	--
Blackwood (10)	rabbit	2.5mg/kg/d Cortisol	30d	--	↓ 33%	35%	+ 65%	--	--
Bartley (4)	rabbit	2.2mg/kg/d Cortisol	20d	--	↓ 5%	30%	+ 30%	--	+ 20%
Jett (11)	dog	1/mg/kg/d Dexamethasone	3 mo	--	↓ 0%	--	+ 65%	0	--

*Giant cells; -- no measurement; + - increase; ↓ - decrease; 0 - no change.

and Kunin⁽⁶⁾ reported a transient reduction in osteoclasts, and two other studies^(4,9) of slightly longer duration also showed reduction in numbers of osteoclasts.

At this relatively high dose, (5mg/kg/day or more) loss of bone mass (osteoporosis) results from a subnormal bone resorption with an even greater subnormal bone formation. These high doses also depress the division of precursor cells, which may be an important reason why bone formation was more depressed than bone resorption. This will be discussed later in the report.

Reports dealing with experimental animals treated with low dose levels of cortisol are sparse. It is puzzling that investigators have avoided experimenting with doses below 2 mg of cortisol/kg/day when almost all of the clinical reports are below this dose level. Extensive dose response studies on the relationship of cortisol and bone remodeling have been and are being carried out in our laboratory. The most informative one is the study by Roberts⁽⁷⁾ using the unstimulated periodontal ligament (PDL) model in rats given 0.5, 2.5, 5, 25, and 50 mg of cortisol/kg/day for 8 days. He quantified the numbers of tritiated thymidine labeled PDL fibroblasts (precursor cells), the number of osteoblasts and osteoclasts, and the loss of alveolar bone. His studies showed that 0.5 and 2.5 mg/kg/day for 8 days stimulated PDL fibroblast labeling, decreased the number of osteoblasts, reduced bone mass, and dramatically increased the number of osteoclasts. Furthermore, despite the finding that osteoblasts were not made, there was an increase in the number of PDL fibroblasts. This is the best evidence of 'decoupling' or 'block differentiation' of precursor cells to osteoblasts. Moreover, the stimulated labeling of PDL fibroblast is not a transient

event. Elevated labeling index of PDL fibroblasts was observed in rats treated with 0.6 mg of cortisol/kg/day for 150 days. (12)

In summary, low doses of cortisol cause osteoporosis by supernormal bone resorption with subnormal bone formation. These events are accompanied by an increase in precursor cell proliferation.

Roberts' (7) dose response study needs to be discussed further. It is best to begin by characterizing the model, and follow with a description of the events occurring at all dose levels.

The unstimulated PDL of the mesial root of the first maxillary molar in 200 g rats is remodeling the alveolar bone of the tooth socket at a very low rate. Both bone formation and resorption are occurring. (The typical characteristics of the PDL for those cells involved with the remodeling of alveolar bone are 143.5 ± 1.2 (S.D.) fibroblasts, 0.83 ± 0.13 osteoblasts and 0.14 ± 0.08 osteoclasts per field ($4 \times 10^4 \mu^2$). Dose levels of 0.5 and 2.5 mg of cortisol/kg/day increase fibroblast proliferation, decrease number of osteoblasts, decrease bone mass, and increase the number of osteoblasts (Table II). At 5 mg of cortisol/kg/day, osteoblast numbers and bone mass were decreased, but osteoclast numbers were still dramatically elevated. Massive doses of 25 and 50 mg of cortisol/kg/day suppressed fibroblast proliferation and the number of osteoblasts, while bone mass and number of osteoclasts were unchanged.

We interpreted this action of cortisol to be (1) an increased morphogenesis of bone precursor cells to osteoclasts, (2) a decrease of morphogenesis of bone precursor cell to osteoblasts or 'decoupling' of bone formation, (3) decreased proliferation of precursor cell division by high doses, and (4) increased proliferation of precursor cell division at low doses. (7,12)

TABLE II

DOSE RESPONSE OF MORPHOLOGICAL FEATURES OF UNSTIMULATED PERIODONTAL
LIGAMENT (PDL) OF RATS TO CORTISOL (% OF CONTROL VALUE)
Adapted from Roberts (7)

<u>Dose</u>	<u>Duration</u>	<u>Precursor Cell Division</u>	<u>Blasts</u>	<u>Bone Mass*</u>	<u>Clasts</u>
0.5mg/kg/d	8 d	↑ 150%	↑ 0%	↑ 91%	↑ 1200%
2.5mg/kg/d	8 d	↑ 130%	↑ 0%	↑ 90%	↑ 1400%
5.0mg/kg/d	8 d	100%	↑ 0%	↑ 89%	↑ 1400%
25mg/kg/d	8 d	↓ 25%	↑ 0%	0	↑ 190%
50mg/kg/d	8 d	↓ 5%	↑ 0%	0	0

* ↓ in width of PDL = loss of alveolar bone; ↑-increase, ↓-decrease; 0-no change.

There is no way to escape the conclusion that cortisol favors morphogenesis of precursor cells to osteoclast. At 25 mg of cortisol/kg/day when the labeled PDL Fibroblast was 25% of normal, the number of osteoclasts was still elevated 200% and the number of osteoblasts was zero. No reduction of bone mass was observed, probably because the measured width of PDL is an insensitive index of alveolar bone resorption. Furthermore, at 50 mg of cortisol/kg/day, we found the labeling index of PDL fibroblasts to be 5% of normal, which apparently produces sufficient precursor cells for morphogenesis to osteoclasts only to achieve an osteoclast population comparable to control value. (Table II)

Besides the above experiment, which produced alveolar bone loss in rats given 0.5, 2.5, and 5 mg/kg/day for 8 days, we have been able to produce significant reduction in tibial metaphyseal bone mass in rats and rabbits with 'relative' low doses of cortisol.

Heretofore rats have been shown to resist developing osteoporosis

when glucocorticoids were administered.⁽¹⁴⁾ Unfortunately, these studies involve large doses of cortisone. In one of our dose response studies using 0, 1, 5, 20 and 75 mg of cortisol/kg/day for 8 days in 100 g rats, a significant reduction in tibial metaphyseal bone mass was measured in the 1 and 5 mg of cortisol/kg/day injected male rats⁽¹⁵⁾. Female rats did not show such changes.

Figure 1. Bar graphs illustrating significant reduction in quantity of proximal tibial metaphyseal bone in intact male rats given 1 or 5 mg of cortisol/kg/day for 8 days. The female rats did not show this change.

Suprisingly enough, we were able to produce a reduction in tibial metaphyseal bone mass in the rabbit with 1.0, 2.2 and 5 mg of cortisol/kg/

day. (Fig. 2,⁽⁴⁾) These dose levels are considerably lower than what has been reported thus far. Previously, doses of 10 mg of cortisone/kg/day or more, in combination with a calcium deficient diet and mechanical stress, have been used to demonstrate the osteoporotic effect of cortisone.⁽⁸⁾

Figure 2. Bar graphs showing significant reduction in quantity of proximal tibial metaphyseal bone in New Zealand female rabbits given 1.0, 2.2, or 5.0 mg of cortisol/kg/day for 20 days.

The cause of bone loss with excessive glucocorticoid has been the subject of both experimental studies in man and investigations of patients afflicted with Cushing's syndrome (Table III). All available information indicated a decrease in bone formation^(11,16,18). However, normal^(11,14)

increased^(16, 18), and decreased⁽¹¹⁾ bone resorption have been reported.

TABLE III

MORPHOLOGICAL FEATURES OF BONES IN PATIENTS WITH EXCESSIVE
GLUCOCORTICOID (% OF CONTROL VALUE)

<u>Source</u>	<u>#</u>	<u>Dose</u>	<u>Duration of Burden</u>	<u>Formation</u>	<u>Bone Mass</u>	<u>Resorption</u>
Jett, et al. ⁽¹¹⁾	1	Cushing	Chronic	+ 0%	+65%	0**
Jowsey, et al. ⁽¹⁶⁾	4*	Cushing	Chronic	+ 0%	--	+ 200%***
Riggs, et. al. ⁽¹⁷⁾	11	Cushing	Chronic	+ 4%	--	+ 240%***
	1	Exogenous				
Jowsey, et al. ⁽¹⁸⁾	2	Cushing	Chronic	+ 5%	--	+ 400%
	3	Exogenous				

*1 case with normal resorption; **0-no change; ***average of all cases reported;
↑-increase; ↓-decrease.

It is best to restrict most of our discussion on humans to experimentally treated man. Jowsey and co-workers^(17,18) have reported studies on one adrenalectomized patients given 30 mg of cortisone acetate and 3 patients given 10 to 60 mg of prednisone daily. These dose levels are within the range we have previously designated as low doses for experimental animals. If we assume that the potency for osteoporosis of cortisone and prednisone is about 1/4 and 2 times cortisol, then these patients would be receiving 15 to 120 mg or 0.2 to 1.7 mg/kg/day of cortisol for a 70 mg person. These 4 patients responded with subnormal bone formation and super-normal bone resorption (identical to the behavior of animals injected with low doses of cortisol).

All but two of the persons afflicted with Cushing's syndrome have increased bone resorption. In both cases, bone resorption was normal.^(11,17) It is conceivable that these individuals possessed extremely high blood glucocorticosteroid level, and can be classified under our category of

high dose level effects.

For the sake of simplicity, let us start with a four compartment model for bone remodeling (Fig. 3). These compartments are as follows: the proliferating precursors I (pool 1), proliferating precursor daughters II (pool 2), the fully differentiated osteoblasts (pool 3), and osteoclasts (pool 4). In order to transit out of pool 1 (proliferating precursors), there must be cell division. Transit out of pool 2 (proliferating precursor daughters II) involves differentiation. Furthermore, the model assumes that the proliferating precursor daughter cells differentiate first into osteoclasts (pool 3) and later into osteoblasts (pool 4). Thus, the normal sequence in bone remodeling involves 3 transits which sequentially connect 4 cellular pools. This is Frost's A R F⁽²⁰⁾. A remodeling unit is activated (A) by some stimulus to produce new, proliferating precursor daughter cells. These daughter cells are initially osteoclasts which resorb (R). The osteoclasts then disappear and new osteoblasts appear to lay down bone (F). We have experimental proof of such a sequence in the Roberts' stimulated PDL model.⁽⁷⁾ Briefly, it is a model in which after 5 days of cortisol injections, an orthodontic elastic band is placed between the first and second maxillary molars to initiate a sequence of 40 hours of bone resorption followed by 68 hours of bone formation in the tooth socket.

Low doses of cortisol favor transit from pool 1 (proliferating precursors I) to pool 2 (proliferating precursor daughters II) and to pool 3 (osteoclasts) by increasing precursor cell division and differentiation to osteoclasts, but block the transit into pool 4 (osteoblasts; Fig. 3).

High doses of cortisol depress transit from pool 1 (proliferating

Figure 3. A four compartment model for bone remodeling. The normal sequence in bone remodeling involves transits from the proliferating precursors (pool 1) to proliferating precursor daughters (pool 2) to osteoclasts (pool 3), and proliferating precursor daughters (pool 2) to osteoblasts (pool 4). Low dose levels of cortisol stimulated precursor cell division, stimulated differentiation to osteoclasts and blocked morphogenesis of osteoblasts. High dose levels of cortisol depressed precursor cell division, depressed differentiation to osteoclasts and blocked morphogenesis to osteoblasts.

precursors 1) to pools 2 (proliferating precursor daughters 11) and 3 (osteoclasts) by suppressing precursor cell proliferation, and block the transit into pool 4 (osteoblasts; Fig. 3.).

Our knowledge of the mechanism by which glucocorticoids effect changes in bone remodeling is not yet clear. It has been postulated that the cellular effect of cortisol on bone is the result of (1) direct inhibition of protein synthesis; (2) direct inhibition of precursor cell proliferation and/or (3) parathyroid hormone mobilization of skeletal calcium.

The glucocorticoids have been shown directly to depress protein synthesis.^(1,2) Most investigators also agree that these corticoids directly inhibit bone formation. Glucocorticoids are known to possess anti-proliferative potential.^(21,22) However, we reported a significant increase in thymidine incorporation into unstimulated PDL fibroblasts with 0.5 and 2.5 mg of cortisol/kg/day. Since this observation conflicted with the proven antiproliferative action of glucocorticoids, we attempted to explain it by (1) the possible conversion of cortisol to cortisone by the 11 β -hydroxydehydrogenase system in fibroblasts;⁽²²⁾ cortisone is a compound shown to stimulate fibroblastic proliferation in tissue culture; and (2) secondary hyperparathyroidism subsequent to cortisol administration.^(8,24-26)

There are many reports which support the hypothesis that the cellular effect of glucocorticoid actions is mediated through the parathyroids. Numerous investigators believe that the calcium-lowering effect of the corticosteroid stimulated the parathyroid glands to maintain serum calcium levels.^(27,28)

Cortisol is believed to lower serum calcium by depressing intestinal calcium absorption,^(24,30) promoting hypercalciuria,⁽³¹⁾ and depressing efflux of ⁴⁵Ca in previously labeled bone.^(12,32-35) Nevertheless, it is most

difficult to demonstrate depression of serum calcium level unless you deprive the animal of its parathyroid.^(28,36) Stoerk et al.⁽²⁸⁾ reported that parathyroidectomized (PTX) rats injected with 3 mg of cortisol/kg/day exhibited more pronounced hypocalcemia than PTX controls. The serum calcium in non-PTX rats was not lowered after injection of the steroid. Apparently the parathyroids compensate so rapidly that the effect of cortisol upon serum calcium level escapes detection unless the parathyroids are eliminated.

Our tritiated thymidine labeling of precursor cells of alveolar bone in PTX and endogenous parathyroid stimulated rats and rats given Lilly's parathyroid extract PTE, further support the hypothesis that the parathyroid hormone is involved in stimulating precursor cell division in rats injected with low doses of cortisol. We found that the addition of PTE or the stimulation of endogenous parathyroid hormone secretion increased precursor cell proliferation, and the removal of the parathyroids or the addition of low doses of cortisol to PTX rats diminished precursor cell division. These findings can be summarized as follows:

Table IV

RESPONSE OF RAT PDL FIBROBLASTS
(% OF CONTROL VALUE)

<u>Treatments</u>	<u>Unstimulated PDL Fibroblasts</u>	<u>Stimulated PDL Fibroblasts</u>	<u>Reference</u>
Intact + 125 u. PTE	↑ 185%	↑ 150%	(37)
Intact + 250 u. PTE	↑ 360%	↑ 200%	(37)
Intact + Ca free diet	---	↑ 150%	(38)
PTX	↓ 75%	↓ 75%	(39)
PTX + 125 u. PTE	---	↓ 75%	(39)
PTX + 250 u. PTE	---	↑ 125%	(39)
PTX + 0.5mg/kg/d	---	↓ 50%	(39)

(1) In intact rats the injection of 125 or 250 units of PTE/rat/12 hour period, tritiated thymidine labeled stimulated PDL fibroblasts were increased to 150 and 200 % of control value respectively. The labeled unstimulated PDL fibroblasts were increased to 185 and 360% of control value. (37)

(2) In intact rats fed a calcium-free diet supplemented with 5 mg % of CaCl_2 in their drinking water to stimulate endogenous parathyroid hormone secretion, labeled stimulated PDL fibroblasts were increased to 150% of control values. (38)

(3) In PTX rats without any supplemental treatment, the labeled stimulated and unstimulated PDL fibroblasts were decreased to 75% or less of control values. (39)

(4) In PTX rats given 125 or 250 units of PTE/rat/12 hour intervals, the labeled stimulated PDL fibroblasts were diminished to 75% and increased to 125% of control values respectively. (39)

(5) In PTX rats given 0.5 mg of cortisol/kg/day for 7 days, the labeled stimulated PDL fibroblasts were only 50% of control values. (39)

These findings best support the hypothesis that the cellular effects of cortisol upon bone tissue are a complex interaction of two antagonistic mechanisms: (a) direct inhibition of precursor cell proliferation by cortisol; and (b) indirect stimulation of parathyroid hormone secretion by cortisol. (17,13) Low dose levels of cortisol stimulated precursor cells proliferation, decreased bone formation, increased the number of osteoclasts and increased bone resorption. At low dose levels, the importance of the parathyroid's mediating effect is most apparent. Indeed, the cellular effect of low doses of cortisol on bone remodeling are identical to those for parathyroid hormone. Therefore, we believe that at low doses the parathyroid's

mediating effect overrides the anti-proliferative effect of the cortisol.

(Fig. 4)

Figure 4. Schema for cellular hypothesis of low dose level glucocorticoid induced osteoporosis. Cortisol acts by inhibiting precursor cell proliferation directly and stimulating parathyroid hormone secretion indirectly (via bone, kidney and intestine). At low dose levels, the parathyroid's mediating effect overrides the antiproliferative effect, while at high dose levels, the anti-proliferative action of cortisol prevails.

High dose levels of cortisol depressed precursor cell proliferation and decreased bone formation without any evidence of bone resorption. At

higher dose levels, the importance of the anti-proliferative action of cortisol is most apparent. Furthermore, cortisol can block the parathyroid's mediating effect by inhibiting parathyroid secretion directly and opposing the hormone action at its target sites in bone and kidney. (40)

Depression of precursor cells by cortisol would not allow the secondary hyperparathyroidism to express itself because of insufficient precursor cells. Moreover, severe depression of precursor cells would result in subnormal osteoclastic activity.

REFERENCES

1. Clark, I.; The effect of cortisone upon protein synthesis. *Journal of Biological Chemistry*, 200: 69-76, (1953).
2. Peck, W.A., Brandt, J. and Miller, I.; Hydrocortisone-induced inhibitors of protein synthesis and uridine incorporation in isolated bone cells in vitro. *Proceedings of National Academy of Science, U.S.A.*, 57, 1599-1606, (1967).
3. Albright, F.; Cushing's syndrome: its pathological physiology, its relationship to adreno-genital syndrome, and its connection with problem of reaction of body to injurious agents ("alarm reaction" of Selye). *Harvey Lect.* (1942-1943) 38: 123-186, (1943).
4. Bartley, M.H., Jr.; Structural Activities of the Anti-inflammatory Steroids and their Relationship to Osseous Tissue. Ph.D. Thesis, Department of Anatomy, University of Utah Salt Lake City, Utah, (1968).
5. Berliner, D.L., Bartley, M.H., Kenner, G.H. and Jee, W.S.S.; Activity of anti-inflammatory steroids upon fibroblasts and bones. *British Journal of Dermatology*, 82:Suppl. 6: 53-61, (1970).
6. Simmons, D.J. and Kunin, A.S.; Autoradiographic and biochemical investigations of the effect of cortisone on the bones of the rat. *Clinical Orthopaedics*, 55: 201-215, (1967).
7. Roberts, W.E.; The Effects of Cortisol on the Cellular Kinetics and Cell Population Dynamics of Periodontal Ligament Bone Cells. Ph.D. Thesis Department of Anatomy, University of Utah, Salt Lake City, Utah, (1969).
8. Storey, E.; The influence of adrenal cortical hormones on bone formation and resorption, *Clinical Orthopaedics*, 30: 197-217 (1963).
9. Thompson, J. and Urist, M.R.; Influence of cortisone and calcitonin on bone morphogenesis. *Clinical Orthopaedics*, 71: 253-270, (1970).
10. Blackwood, E.L.; The Effects of Cortisol on the Bones and Teeth of Young and Adult Rabbits. Ph.D. Thesis, Department of Anatomy, University of Utah, Salt Lake City, Utah, (1969).
11. Jett, S., Wu, K., Duncan, H., and Frost, H.M.; Adrenal-corticosteroid and salicylate actions on human and canine haversian bone formation and resorption. *Clinical Orthopaedics*, 68: 301-315, (1970).
12. Kenner, G.H.; Adrenal Cortical Hormone and Mineral Kinetics in Young and Adult Rats. Ph.D. Thesis, Department of Anatomy, University of Utah, Salt Lake City, Utah, (1970).

13. Jee, W.S.S., Park, H.Z., Roberts, W.E. and Kenner, G.H.; Corticosteroid and bone. *American Journal of Anatomy*, 129: 477-480, (1970).
14. Follis, R.H., Jr.; Effect of cortisone on growing bones of the rat. *Proceedings of the Society of Experimental Biology and Medicine*, 76: 722-724, (1951).
15. Park, H.Z., Jee, K.W., Burggraaf, R., and Jee, W.S.S.; Dichotomy of effects of cortisol upon metaphyseal bone. *Journal of Dental Research*, 49, abstract number 113, page 77, (1970).
16. Jowsey, J., Kelly, P.J., Riggs, L.B., Bianco, A.J., Scholz, D.A. and Gershon, Cohen, J.; Quantitative microradiographic studies of normal and osteoporotic bone. *Journal of Bone and joint Surgery*, 47A: 785-806, (1965).
17. Riggs, B.L., Jowsey, J. and Kelly, P.; Quantitative microradiographic studies of bone remodeling in Cushing's syndrome. *Metabolism*, 15: 733-780, (1966).
18. Jowsey, J. and Riggs, B.L.; Bone formation in hypercortisonism. *Acta Endocrinologica*, 63: 21-28, (1970).
19. Sissons, H.A.; The osteoporosis of Cushing's syndrome. *Journal of Bone and Joint Surgery*, 38B: 418-433, (1956).
20. Frost, H.M.; Mathematical Elements of Lamellar Bone Remodeling. Charles C. Thomas, Springfield, (1964).
21. Dougherty, T.F. and White, A.; Functional alterations in lymphoid tissue induced by adrenal cortical secretion. *American Journal of Anatomy*, 77: 81-116, (1945).
22. Dougherty, T.F., Berliner, D. L. and Berliner, M.L.; Corticosteroid-Tissue Interactions, *Metabolism*, 10: 966-989, (1961).
23. Berliner, D.L., and Ruhmann, A.G.; Comparison of the growth of fibroblasts under the influence of 11-hydroxy and 11-keto corticosteoids. *Endocrinology*, 78: 373-382, (1966).
24. Collins, E.J., Garrett, E.R and Johnston, R.L.; Effect of adrenal steroids on radio-calcium metabolism in dogs. *Metabolism* 11: 716-726, (1962).
25. Gordan, G.W., Hansen, J. and Lubick, W.; Effects of hormonal steroids on osteolysis. In: Proceedings of Second International Congress of Hormonal Steroids. Excerpta Medica International Congress Series No. 132: 786-793, (1967).
26. Rasmussen, H.; The parathyroids. In: Textbook of Endocrinology, R.H. Williams, editor, p. 895, W.B. Saunders Co., Philadelphia, (1968).

27. Pincus, J.B., Natelson, S. and Lugovoy, J.K.; V. Effect of epinephrine, ACTH and cortisone on citrate, calcium, glucose and phosphate levels in rabbits. *Proceedings of Society for Experimental Biology and Medicine*, 78: 25-27, (1951).
28. Stoerk, H.C., Peterson, A.C. and Jelinck, V.C.; The blood calcium lowering effect of hydrocortisone in parathyroidectomized rats. *Proceedings of Society for Experimental Biology and Medicine*, 114: 690-695, (1963).
29. Talmage, R.V.; Morphological and physiological considerations in a new concept of calcium transport in bone. *American Journal of Anatomy*, 129: 467-476, (1970).
30. Harrison, H.E. and Harrison, H.C.; Transfer of Ca^{47} across the intestinal wall in vitro in relation to action of vitamin D and cortisol. *American Journal of Physiology*, 199: 265-271, (1960).
31. Laake, H.; The action of corticosteroids on the renal reabsorption of calcium. *Acta Endocrinologica*, 34: 60-64, (1960).
32. Raisz, L.G.; Bone resorption in tissue culture: Factors influencing the response to parathyroid hormone. *Journal of Clinical Investigation*, 44: 103-116, (1965).
33. Schafer, S.A., Stevens, W., Jr. and Jee, W.S.S.; Calcium transport in bone organ culture by cortisol. *Journal of Dental Research*, 48, abstract number 679, p. 210, (1969).
34. Stern, P.H.; Inhibition by steroids of parathyroid hormone-induced Ca^{45} release from embryonic rat bone in vitro. *The Journal of Pharmacology and Experimental Therapeutics*, 168: 211-217, (1969).
35. Talmage, R.V., Park, H.Z. and Jee, W.S.S.; Parathyroid hormone and thyrocalcitonin function in cortisol-treated rats. *Endocrinology*, 86: 1080-1084, (1970).
36. Canas, F.M., Bergstrom, W.H. and Churgin, S.J.; Effects of the adrenal on calcium homeostasis in the rat. *Metabolism*, 16: 670-675, (1967).
37. Chase, D.C., Roberts, W.E. and Jee, W.S.S.; ^3H -thymidine evaluation of the effect of parathyroid extract on the cell kinetics of orthodontic tooth movements in the rat. *Journal of Dental Research*, 48, abstract number 524, page 171, (1969).
38. Kramer, M., Roberts, W.E., Park, H.A. and Jee, W.S.S.; Endogenous parathyroid hormone and the proliferation of periodontal ligament fibroblasts. *Journal of Dental Research*, 50, abstract number 827, p. 259, (1971).
39. Julian, G., Park, H.Z., Roberts, W.E. and Jee, W.S.S.; Parathyroid-cortisol relationships measured by periodontal ligament labeling indices. *Journal of Dental Research*, 50, abstract number 825, p. 259, (1971).

40. Eliel, L.P., Thomsen, C. and Chanes, R.; Antagonism between parathroid extract and adrenal cortical steroids in man. *Journal of Clinical Endocrinology*, 25: 457-464, (1965).

EFFECT OF CORTISOL ON INCORPORATION OF TRITIATED-TETRACYCLINE INTO BONE

S. A. Schafer and W. S. S. Jee

Abstract: Young-adult male mice were treated daily for eleven days with 25 micrograms cortisol per gram body weight. Three days before sacrifice, all mice were given a single injection of tritiated-tetracycline. Data indicate the following: 1) While control animals gained in total body weight throughout the experimental period, cortisol caused a rapid loss of weight, with a trend toward recovery after the first four days. The tritiated-tetracycline itself caused weight-loss in both groups of animals. 2) Blood radioactivity was the same in treated and control animals at five minutes after injection. The cortisol-treated animals showed less circulating tetracycline thereafter. 3) Bone weights at the time of sacrifice were less in cortisol-treated mice than in controls. 4) At sacrifice, total bone radioactivity was less in cortisol-treated mice than in controls. 5) Extraction of the tibiae with hydrochloric acid caused the release of the same percentage of tetracycline (diffuse component) in both groups.

INTRODUCTION

Previous studies in this laboratory have indicated that quantitative measurements of radioactive tetracycline could be used as an index of new bone formation if the diffuse component were first eluted from the bone (1). This experiment was designed to study the effect of cortisol on bone formation in young-adult mice, as determined by tritiated-tetracycline incorporation into the tibiae.

MATERIALS AND METHODS

Eight CBA male mice, 65 days of age with an average weight of 21.4 grams, were divided into two groups. The treated animals were given subcutaneous injections of cortisol (25 micrograms per gram body weight) at the same hour every morning for eleven days. The steroid was dissolved in an injection solution of saline with 16% propylene glycol and 8% ethyl alcohol. The control group received the carrier solution alone

at the same time periods. Three days before sacrifice (day 8 of the experiment) all mice were given 2 microcuries tritiated-tetracycline (specific activity 1.2 mCi/mg) per gram body weight intraperitoneally. Blood samples were taken by orbital sinus puncture at 5 minutes and 1, 2, and 3 days after injection of the radioactivity. On day 11, the animals were sacrificed and the tibiae were cleaned of connective tissue and muscle, and weighed. One tibia from each animal was extracted for one hour at room temperature with 3×10^{-3} N HCl. The bones were then pulverized, dissolved overnight in Soluene (Packard Sample Solubilizer), and prepared for liquid scintillation counting. The second tibia from half of the animals in each group was extracted and fixed in acetone. The remaining tibiae were fixed in acetone immediately after sacrifice. Blood samples, HCl eluants and dissolved bones were counted in a Packard Tricarb Liquid Scintillation Counter with an efficiency for tritium of 20%. The tibiae prepared for autoradiography were embedded undecalcified in ethyl-butyl-methacrylate, cut at 5 microns on a Jung Model K microtome, and dipped in Kodak NTB photographic emulsion. The autoradiographic results will not be discussed in this paper.

RESULTS

Figure 1 shows the average change in weight in the cortisol-treated and control mice for the eleven days of the experiment. Control animals showed a 2% increase in their original weights until the time of tetracycline injection (indicated by arrows). The radioactive antibiotic resulted in a 4% total loss in body weight, which appeared to have stabilized by the third day after the injection. The cortisol-treated animals showed a rapid loss of weight which reached a maximum loss of 14% of the

original weight by the fourth day of treatment. Thereafter the animals recovered 9% of their original weight by the sixth day. After the sixth day, the loss in weight began to occur again, compounded by the injection of tetracycline on the eighth day.

Figure 2 shows the distribution of the tetracycline in the blood for a period of three days after injection. The five minute values of the cortisol-treated and control animals are equivalent. The cortisol-treated animals show a faster than control loss of radioactivity from the blood. The radioactivity found in the tibiae upon sacrifice is indicated on the graph by X's on the eleventh day of the experiment. The cortisol-treated animals showed a decrease in total tetracycline associated with the bone. The bone in both groups however, has a higher tetracycline concentration than the corresponding blood.

Tables I and II show the results of the extraction of the tibiae with hydrochloric acid. Although the tibiae of the control animals contained 1.75 times more tetracycline than those of the treated animals, the partial demineralization released 77% of the tetracycline in both cases (Table I). Because the tibiae of the cortisol-treated animals weighed less than those of controls, the radioactivity values are also expressed as a function of unit weight (Table II).

DISCUSSION

Loss of total body weight by the cortisol-treated mice in this experiment is an indication of the anti-anabolic and/or catabolic effectiveness of this steroid in this species. Pharmacologic concentrations of cortisone have previously been reported to have no effect on mice (2). Reports of the general protein-inhibitory side effect of

Table I

³H-Tetracycline Incorporation Into Tibiae

Treatment	Activity (DPM x 10 ³)			Hot-Spots
	Total	HCl Wash	Bone	% Left In Bone
Cortisol	21.3±3.2	16.4±2.4	4.8±1.1	22.6±3.1
Control	37.3±5.9	28.6±4.1	8.6±2.8	22.9±5.8

Table II

³H-Tetracycline Incorporation Into Tibiae

Treatment	Tibial Weights	DPM/GM x 10 ⁵		
		Total	HCl Wash	Bone
Cortisol	39.1±3.7	5.47±0.88	4.23±0.74	1.23±0.23
Control	44.0±6.3	8.52±1.13	6.58±1.12	1.93±0.46

tetracycline are also substantiated by the loss in total body weights of both groups of mice after injection of the tetracycline (3). The recovery of some weight between days 4 and 6 in the cortisol-treated animals is an indication of compensatory homeostatic mechanisms. Among these would certainly be that of secondary hyperparathyroidism. However, the animals never regain their original weight and, in fact, appear to be failing again after the sixth day. This failure might indicate an exhaustion of the homeostatic mechanisms just mentioned.

Eight days of high-level cortisol treatment had no effect on the uptake of tetracycline from the peritoneal cavity. It did however cause an increased removal of the tetracycline from the blood. This could be accounted for in two ways: 1) increased pooling and/or binding of the

tetracycline in body tissues, particularly the skeleton and 2) increased excretion of tetracycline via the feces and urine (4). The values of bone radioactivity three days after injection show there is in fact decreased quantities of tetracycline associated with the calcified tissues. The second explanation of increased excretion seems to be the correct one.

The data obtained from the extraction of the bone with hydrochloric acid indicate that both components of tetracycline in the bone are decreased: that associated diffusely throughout all bone matrix and that actively incorporated into areas of newly-mineralizing osteoid (hot-spot). In addition, the decrease in diffuse tetracycline is equivalent to the decrease in hot-spot tetracycline. It seems likely that not only is bone formation depressed by cortisol, but the very transport or diffusion of tetracycline through the bone is also depressed. The anti-anabolic effect of the steroid may in fact be the result of a failure of metabolites in general to reach the bone cells.

REFERENCES

1. S. A. Schafer, D. Higgenbottom, H. Z. Park, and W. S. S. Jee; The distribution of tritiated-tetracycline in young-adult mouse bone - A rapid method for the quantitation of new bone formation. This publication.
2. R. H. Follis, Jr.; Non-effect of cortisone on growing bones of mice, guinea pigs, and rabbits. Proc. Soc. Exp. Biol. Med. 78: 723-724 (1951).
3. P. Demers, D. Fraser, R. B. Goldbloom, J. C. Haworth, J. LaRoche, R. MacLean, and T. K. Murray; Effects of tetracyclines on skeletal

growth and dentition. Canad. Med. Ass. J. 99: 849-854 (1968).

4. R. G. Kelly, and D. A. Buyske; Metabolism of tetracycline in the rat and the dog; J. Pharmacol. Exp. Ther. 130: 144-149 (1960).

Fig. 1. The effect of cortisol on percent change in total body weights. Solid circles represent the arithmetic means of control animals; open circles, of cortisol-treated animals. Arrows indicate the times of tetracycline injection.

Fig. 2. The effect of cortisol on tritiated-tetracycline incorporation into blood and bone. Solid circles represent the arithmetic mean of blood radioactivity in control animals; open circles, of cortisol-treated animals. The X indicates the radioactivity in control tibiae, the circled X, in cortisol-treated tibiae, three days after tetracycline injection.

THE DISTRIBUTION OF ^3H -TETRACYCLINE IN YOUNG-ADULT MOUSE BONE -
A RAPID METHOD FOR THE QUANTITATION OF NEW BONE FORMATION

S. A. Schafer, D. Higgenbottom, H. Z. Park,
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Abstract: Three-month-old female mice were injected with 2 microcuries tritiated-tetracycline per gram body weight. Blood samples were taken by orbital sinus puncture at 0, 5 minutes, 1, 2, 3, 4, and 5 days. Bone samples were taken at 1, 3, and 5 days. The association of the tetracycline with the lipid and mineral phases of the bone was then investigated by sequential extraction with chloroform, chloroform/methanol, and chloroform/methanol/HCl. The data indicate the following: 1) The bone loses radioactivity as rapidly as the blood, with a biological half-life of 3.65 days. 2) Neither diffuse nor hot-spot tetracycline shows any correlation with the neutral lipid compartment of the bone. 3) The diffuse tetracycline comprises the majority of the compound deposited in bone, possibly amounting to 70%. This fact makes it meaningless to use total radioactive tetracycline measurements as an index of new bone formation, at least in short time periods up to five days after injection. Since the only relevant fraction is that bound in newly-mineralizing areas, so called hot-spots, the two components must be dissociated. 4) We would like to suggest that bone formation can be quantitated by measurements of tetracycline radioactivity remaining in bone after partial, controlled dissolution of the hydroxyapatite. Since the diffuse tetracycline is loosely associated with the bone, possibly by electrostatic bonds, there is a point during demineralization where the diffuse component is virtually eliminated leaving a preparation of hot-spot tetracycline. We therefore have a technique which replaces the time-consuming classical techniques now employed to study bone formation.

INTRODUCTION

Since the initial isolation of chlortetracycline in 1948,⁽¹⁾ tetracycline has become the third major antibiotic after penicillin and streptomycin.⁽²⁾ Two decades of usage have brought to light certain important skeletal and dental side effects caused by its administration. One of these side effects is a specific yellowish fluorescence in the bones and in enamel and dentine of the teeth. Studies by Milch,⁽³⁾ by Frost,⁽⁴⁾ and by Harris⁽⁵⁾ all showed that the affinity of tetracycline for calcified

tissues was of two varieties. The major component was said to be actively deposited in all sites of newly-mineralizing bone and to be relatively permanently fixed in bone until resorption occurred. A so-called lesser component was said to be diffuse and not as tightly bound. Tetracycline bone labeling, followed by ultraviolet microscopic measurements of the width, area, or volume of hot-spot fluorescent zones is now commonly used as an index of skeletal metabolic activity.⁽⁶⁾ Such activity is expressed as appositional growth rate, radial rate of osteon closure, and osteon maturation rate.

This paper concerns our attempts to separate by chemical extraction the diffuse and hot-spot components of the tetracycline deposited in bone. The purpose of this research was two-fold. In the first place, we wanted to be able to make direct measurements of the total quantity of tetracycline bound to bone-forming sites, i.e. the hot-spot component. This would be a new tool for measuring bone formation, eliminating the tedious sectioning and microscopy currently employed. Secondly, the chemical nature of any substance which would bring about a successful separation of the two components would give us clues to the nature of the tetracycline affinities to the different skeletal areas.

MATERIALS AND METHODS

Ten C3Hf female mice, prior to closure of their growth plates, were used in this study. They were three months of age, with an average weight of 23 grams. At time 0, the mice were injected intraperitoneally with 2 microcuries tritiated-tetracycline (specific activity 1.2 mCi/mg) per gram body weight. Blood samples were taken by orbital sinus puncture at 0, 5 minutes, 12 hours, and each day for 5 days after injection.

Groups of animals were sacrificed and bone samples were taken at 1, 3, and 5 days after injection. After the connective tissue and muscle had been removed, the tibiae were sequentially extracted for one hour at room temperature in 1 ml of each of the following solvents:

chloroform

chloroform/methanol, 2/1

chloroform/methanol/HCl, 200/100/1.

Samples of bone at each step of the extraction were prepared for autoradiography. They were fixed in acetone, embedded undecalcified in ethyl-butyl-methacrylate and cut at 5 microns on a Jung Model K microtome. They were dipped in Kodak NTB emulsion and exposed for 28 to 56 days. After development and fixation, the sections were stained with Mayer's hematoxylin and eosin Y.

RESULTS

The results of this experiment are tabulated to show:

- 1) the distribution of radioactivity in the blood and bone at the time periods mentioned,
- 2) the amount of radioactivity eluted with the three solvents and that remaining in the bone after each extraction,
- 3) autoradiographic representations of the location of the tetracycline remaining in the bone after each extraction.

As seen in Figure 1, the bone of these animals had reached its maximum radioactivity within the first day. Thereafter blood and bone lost radioactivity at the same rate; the biological half-time of the tetracycline computed to be 3.65 days.

The results of the sequential extraction of the tibiae are shown in Figure 2. By statistical analysis of parallelism, the rates of loss of tetracycline from the bone as a whole and from the hydroxyapatite-lipid compartment in particular, i.e. the diffuse component, are not significantly different from each other. The biological half-time, as mentioned, is 3.65 days. Only that tetracycline eluting with the chloroform-neutral lipid fraction is lost at a different, faster rate, with a biological half-time of 1.86 days.

Expressing the value for radioactivity of each extraction as percentages of total radioactivity (Figure 3) makes evident the fact that most of the tetracycline, more than 90%, remains in the bone after the lipids have been removed. Seventy percent is eluted out with the partial dissolution of the hydroxyapatite. After one hour of extraction, an average of 23% of the original tetracycline remains tightly bound to the bone.

Autoradiographs of the bone before extraction and after passage through chloroform and chloroform/methanol are virtually identical. These show the diffuse component to be more outstanding than as seen under the ultraviolet microscope. The diffuse component completely overshadows the hot-spots (Figure 4).

In contrast, elution with acidic chloroform/methanol shows a loss of the majority of the label. Under high power, that tetracycline remaining is shown to be in areas deep to osteoid, i.e. those areas which were the calcifying front of newly-mineralizing bone at the time of injection (Figure 5).

DISCUSSION

Tetracycline is sequestered by bone almost immediately. After the first day, the bone loses radioactivity as rapidly as the blood. Of interest to this finding is the fact that the fluorescent picture of bone changes within five days after injection, hot-spots becoming more brilliant. Because it has been shown that tetracycline fluoresces in vitro only after chelation with calcium, the increase in fluorescence with time in vivo has been assumed to be due to an increase in the amount of the compound actively bound to hydroxyapatite and not to an increase in the total amount present. This assumption is supported by the fact that the total bone radioactivity in itself is not increasing after the first day.

The rationale for the bone extractions centered around two assumed properties of tetracycline:

- 1) Its solubility in or actual binding to lipids. This was assumed from reports that tetracycline fluorescence was used to identify lipids on chromatograms.⁽⁷⁾ This information appears to have been false as there is no reference to such a technique in the literature and further there is in fact no correlation between the lipid compartment and tetracycline.

- 2) The second property is well-substantiated, namely its chelation with divalent cations, specifically magnesium, manganese and calcium.^(8,9) This property could account for both components of tetracycline labeling. In any event, that tetracycline entering with calcium into newly-mineralizing bone might be more tightly bound or as Frost has suggested "fixed".⁽⁶⁾

Urist indicated that there were two types of hydroxyapatite.⁽¹⁰⁾ That first laid down on osteoid is loose, composed of larger lattices and less packed with collagen than the second variety. This structure leaves more positions in the crystal open for exchange with tetracycline. As the crystal ages (as rapidly as 24 hours) some positions in the crystal are buried by polysaccharides, by collagen and by packing. If these newly-buried positions were occupied by tetracycline, the antibiotic would indeed now be cemented in.

Shapiro (Table I) showed that the majority of lipids in anorganic bone is eluted out with chloroform and chloroform/methanol.⁽¹¹⁾ Less than 5% of the mean total eluted phosphatides are associated with the bone after treatment with acidified chloroform/methanol. The effect of the acidic chloroform/methanol solvent system is also to cause almost complete solubilization of the hydroxyapatite.

Table I
The Percentage Distribution of Phospholipids Removed
By Solvent Elution From Anorganic Bone⁽¹¹⁾

Expt. No.	% of Lipid Removed at Each Extraction Stage		
	Chloroform	Chloroform/ Methanol	Acidified Chloroform/ Methanol
1	82.4	11.7	5.9
2	79.6	16.5	3.9

The radioactivity eluted out with the lipid solvents shows that neither the diffuse nor the hot-spot tetracycline correlates with the neutral lipid compartment of the bone. The diffuse tetracycline comprises the majority of the compound deposited in bone, possibly amounting to

70%. This fact makes it meaningless to use total radioactive tetracycline measurements as an index of new bone formation, at least in short time periods up to five days after injection. Since the only relevant fraction is that bound in newly-mineralizing areas, so called hot-spots, the two components must be dissociated. We would like to suggest that bone formation can be quantitated by measurements of tetracycline radioactivity remaining in bone after partial, controlled dissolution of the hydroxapatite. Since the diffuse tetracycline is loosely associated with the bone, possibly by electrostatic bonds, there is a point during demineralization where the diffuse component is virtually eliminated leaving a preparation of only hot-spot tetracycline. We therefore have a technique which replaces the time-consuming classical techniques now employed to study bone formation.

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REFERENCES

1. B. M. Duggar; Aureomycin; A product of the continuing search for new antibiotics; Ann. N. Y. Acad. Sci. 51: 177-181 (1948).
2. E. M. Ory; The tetracyclines; Med. Clinics of No. Am. 54: 1173-1186 (1970).
3. R. A. Milch, D. P. Rall, and J. E. Tobie; Bone localization of the tetracyclines; J. Nat. Cancer Inst. 19: 87-93 (1957).
4. H. M. Frost, A. R. Villanueva, and H. Roth; Tetracycline staining of newly forming bone and mineralizing cartilage in vivo; Stain Technol. 35: 135-138 (1960).

5. W. H. Harris, R. H. Jackson, and J. Jowsey; The in vivo distribution of tetracyclines in canine bone; J. Bone Jt. Surg. 44A: 1308-1320 (1962).
6. H. M. Frost, A. R. Villanueva, H. Roth, and S. Stanisavljevic; Tetracycline bone labeling; J. New Drugs 1: 206-216 (1961).
7. H. Z. Park; personal communication.
8. K. W. Kohn; Mediation of divalent metal ions in the binding of tetracycline to macromolecules; Nature 191: 1156 (1961).
9. K. H. Ibsen, and M. R. Urist; Complexes of calcium and magnesium with oxytetracycline; Proc. Soc. Exp. Biol. Med. 109: 797-801 (1962).
10. M. R. Urist, and K. H. Ibsen; Chemical reactivity of mineralized tissue with oxytetracycline; Arch. Path. 76: 28-40 (1963).
11. I. M. Shapiro; The association of phospholipids with anorganic bone; Calc. Tiss. Res. 5: 13-20 (1970).

Fig. 1. Distribution of tritiated-tetracycline in blood cells, plasma and bone. Each point represents the arithmetic mean $\pm$ standard deviation.

Fig. 2. Tritiated-tetracycline removed by three solvents from tibiae.

Each point represents the arithmetic mean $\pm$ standard deviation.

Fig. 3. Percentage distribution tritiated-tetracycline in three elutions of tibiae. Each point represents the arithmetic mean $\pm$ standard deviation.

Fig. 4. Mouse tibia labeled with tritiated-tetracycline. Autoradiograph made before extraction procedure. Note size of diffuse component.

Fig. 5. Autoradiograph of labeled tibia after extraction with chloroform/methanol/HCl. Seventy percent of tetracycline has been eluted out; that remaining is predominantly in hot-spots.

STRUCTURAL ACTIVITY RELATIONSHIP OF CORTICOSTEROIDS TO GROWING
BONES: COMPARISON OF ACTIVITIES OF CORTISOL, PREDNISOLONE,
BETAMETHASONE AND DEXAMETHASONE

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Abstract: The relative potencies of betamethasone (9 α -fluoro-11 β , 17 α , 21-triol-16 β -methyl-pregnan-1, 4-diene-3, 20-dione) and dexamethasone (9 α -fluoro-11 β , 17 α , 21-triol-16 α -methyl-pregnan-1, 4-diene-3, 20-dione) and prednisolone (1, 4-pregnan-diene-11 β , 17 α , 21-triol-3, 20-dione) to cortisol (4-pregnen-11 β , 17 α , 21-triol-3, 20-dione) were assayed using morphometric techniques. The suppression of endochondral ossification (longitudinal growth), periosteal accretion and endosteal accretion were obtained by measuring tetracycline labels using fluorescence microscopy and morphometric techniques. Reduction in metaphyseal bone and cell counts were determined using the point-hit and the Quantimet Television Microscopic method. Prednisolone was found to be 2 to 3 times, betamethasone was 10 to 26 times and dexamethasone 9 to 44 times more potent than cortisol. Dexamethasone was consistently more effective in suppressing bone accretion and endochondral ossification than betamethasone. The addition of the fluorine atom at the C-9 position conferred an increased degree of bone growth suppression activity to the steroid molecule.

INTRODUCTION

The clinical use of anti-inflammatory hormones has been greatly inhibited by side effects produced by these corticosteroids (i.e., reduction in bone volume or osteoporosis). Since pharmaceutical companies have been synthesizing many corticosteroids in an attempt to find one which exhibits maximal anti-inflammatory activity with minimal osteoporotic side effects, it is important that the relative potencies of these hormones be determined. As a continuation of many experiments done in our laboratory on both naturally occurring and synthetically produced corticosteroids, this experiment was designed to determine structural activity relationships between betamethasone, dexamethasone, prednisolone and cortisol. The relative potencies, indices of precision and fiducial limits for these four corticosteroids were determined by analysis of dose response curves plotted for a number of bone

growth measurements.

MATERIALS AND METHODS

Eighty, 8 week old, male New Zealand White Rabbits were given daily subcutaneous injections of the corticosteroids for twenty days. The dose levels of the corticosteroids are listed in Table I while the molecular configurations of each corticosteroid are shown in figure 1. Estimated potency ratios were derived from fibroblastic assays⁽¹⁾ and then multiplied by the dose levels of cortisol being used. Control animals were given daily injections of vehicle only. Intramuscular injections of tetracycline (Achromycin, Lederle) were given on days 2 and 16 of the experiment to label new sites of bone formation. Animals were sacrificed and the bones were defleshed. The right side of the skeleton was fixed in acetone and the left side in 10% formalin. The left proximal tibia were embedded in plexiglass and 6 micron sections were cut on the Jung microtome. These were stained with H. and E. for bone cell counts. The number of osteoblasts and osteoclasts per unit area were counted in 10 random fields in a standardized area in the metaphysis. The right proximal tibia were embedded in bioplastic, sectioned on a bone saw and the sections ground between glass plates. The longitudinal frontal sections of the proximal tibia were first ground to 100 microns for microradiography and measurement of per cent trabecular metaphyseal bone. The sections were then reground to 50 microns for measurement of endochondral growth using ultraviolet microscopy. Fifty micron sections of the tibial midshaft were used for periosteal accretion measurements on the ultraviolet microscope. Table II summarizes the processing, method of analysis and data obtained from

various skeletal sites. The relative potencies, indices of precision, and fiducial limits for these four corticosteroids were determined by analysis of dose response curves plotted for endochondral bone growth, periosteal accretion, numbers of metaphyseal osteoblasts and per cent metaphyseal bone (Figs. 2-5). The methods used to analyze these values have been reported previously.⁽²⁾

Table I

<u>Injected dose levels (mg/kg/day)</u>	
Cortisol	2.2, 1.0, 0.44, 0.25
Prednisolone	2.2, 1.0, 0.3, 0.1, 0.03
Betamethasone	0.25, 0.09, 0.014, 0.006
Dexamethasone	0.25, 0.09, 0.014, 0.006

RESULTS AND DISCUSSION

Dose response curves were used to determine the relative potencies, indices of precision and fiducial limits of prednisolone, betamethasone and dexamethasone as compared to cortisol, the standard which was assigned a potency of 1.

The minimal dose at which significant changes from the control group were noted is found in Table III. Betamethasone and dexamethasone were similar in their effectiveness in causing significant changes in the parameters measured in both the proximal tibia and tibial midshaft. Prednisolone was shown to be just slightly more effective in causing significant differences than cortisol which was the least effective.

The potency ratios, fiducial limits, and indices of precision (λ values) are found in Table IV. Dexamethasone (9 α -fluoro, 16 β -methyl) was shown to be the most potent with potency ratios ranging from 9-44 times that of

Table II

Summary of Processings and Analyses

Skeletal Site	Tissue Plane	Product for Analysis	Method of Analysis	Data determined
tibia, diaphysis	x-section	50 μ undecal. (tet.)	u.v. microscopy linear-reticule	periosteal accretion (transverse growth)
tibia, proximal (reground section)	frontal	100 μ undecal. microradiograph	projection upon screen/pt.-hit	%metaphyseal bone
tibia, proximal	frontal	6 μ decal.	light microscopy	# of osteoblasts

cortisol. Betamethasone (9 α -fluoro, 16 β -methyl) was slightly less potent than dexamethasone, its potency being 10-26 times cortisol. Prednisolone was only 2-3 times more potent than the standard.

Table III

Minimal Dose of Steroids Producing Skeletal Changes

Anti-inflammatory steroids	Endochondral bone growth	Parameters periosteal accretion	% bone	Osteoblast depression
Cortisol	0.44	0.44	2.0	0.44
Prednisolone	0.10	0.10	*	*
Betamethasone	0.006	0.006	0.09	0.006
Dexamethasone	0.006	0.006	0.09	0.006

*data not compiled for these parameters as of yet

The best indices of precision were found for the endochondral bone growth data, ranging from 0.08 to 0.19, implying that the data for this parameter are consistently precise. These anti-inflammatory corticosteroids may be ranked as to their potency in the following order (from most to least potent): Dexamethasone, betamethasone, prednisolone and cortisol.

The differences in potency of these steroids in causing changes in the bone growth parameters measured may be said to arise from the substitutions made to the steroid molecule. Prednisolone which is only slightly more potent than cortisol, differs from the standard only by the addition of a double bond between C-1 and C-2. Dexamethasone and betamethasone which are 9-44 and 10-26 times more potent than cortisol respectively, differ quite markedly in that in addition to the Δ^2 double bond as in prednisolone, both molecules are halogenated at C-9 with fluorine in the alpha position, and both are methylated at C-16. The difference in potency existing between dexamethasone and betamethasone is probably due to the fact that the methyl

Table

Summary of Relative Potencies, Fiducial Limits and Indices of Precision Values
for Various Bone Bioassays

Parameter- Steroid	Potency Ratio	Fiducial Limits	Index of Precision -Values
periosteal accretion - tibial shaft			
cortisol	1	---	0.21
prednisolone	2.10	3.93-0.05	0.30
betamethasone	13.49	36.07-4.62	0.52
dexamethasone	16.92	44.88-6.14	0.56
% metaphyseal bone - prox. tibia			
cortisol	1	---	0.27
betamethasone	16.92	49.01-4.77	0.41
dexamethasone	22.07	55.69-9.33	0.38
endochondral bone growth - prox. tibia			
cortisol	1	---	0.08
prednisolone	3.03	3.96-0.37	0.15
betamethasone	21.62	29.28-15.87	0.19
dexamethasone	44.09	60.55-32.08	0.15
osteoblast depression - prox. tibia			
cortisol	1	---	0.23
betamethasone	12.53	19.23-8.05	0.23
dexamethasone	10.16	15.70-6.50	0.22

group is in the alpha position in dexamethasone and the beta position in betamethasone. The 16 α -methyl imparts slightly more potency to the molecule than the 16 β -methyl.

REFERENCES

1. A.G. Ruhmann and D.L. Berliner; Influence of steroids on fibroblasts.
II. The fibroblast as an assay system for topical anti-inflammatory potency of corticosteroids; J. Invest. Derm. 49: 123-130 (1967).
2. M.H. Bartley and W.S.S. Jee; Structural Activity Relationships of Anti-inflammatory Steroid Hormones to Growing Bone. I. Growth Parameters and Resorption of Proximal Tibia; Research in Radiobiology; Radiobiology Division, Department of Anatomy, University of Utah, College of Medicine, U.S.A.E.C. Report C00-119-240, 199-213 (1969).

Fig. 1. Molecular configuration of corticosteroids.

Fig. 2. Dose response curves for effects upon endochondral growth by 4 different corticosteroids.

Fig. 3. Dose response curves for effects upon periosteal accretion by 4 different corticosteroids.

Fig. 4. Dose response curves for effects upon total osteoblast numbers by 4 different corticosteroids.

Fig. 5. Dose response curves for effects upon per cent metaphyseal bone by 4 different corticosteroids.

KIDNEY FUNCTION IN AGING CONTROL BEAGLE DOGS

Charles J. Nabors, Jr. and Walter Stevens

Abstract: Normal blood levels of blood urea nitrogen and creatinine were determined in a group of control beagles for a period of 1 year. The age of the animals ranged from 1-203 months. No changes with age were noted and male dogs had slightly higher serum levels than females.

INTRODUCTION

In our last progress report⁽¹⁾ preliminary values for serum blood urea nitrogen (BUN) and creatinine determinations in aging control animals were reported. We now have collected data on this group of animals for a 12-month period. The animals range in age from 1 to 203 months. The purpose of this study is to establish normal values for BUN and creatinine of aging control animals in our colony. It has been shown that ²⁴¹Am and ²³⁹Pu deposit in the kidneys of beagles.⁽²⁾ Both of these radionuclides are also deposited in significant concentrations in other soft tissues. These blood biochemical measurements may be used to evaluate kidney function in irradiated dogs. This report constitutes the final normal serum levels for male and female Beagles.

METHODS

The animals were purebred beagles raised in our laboratory. All of the animals were either zero level animals or aging control dogs that showed no pathology, and were judged to be in good health. The determinations were made using routine clinical laboratory procedures.^(3,4) Blood urea nitrogen was measured using urease.⁽³⁾ Creatinine was determined by the method of Folin and Wu.⁽⁴⁾ Means and standard deviations for the various ages of animals were calculated by the use of the Wang

Series 700 computer.

RESULTS

Table I gives the means and standard deviations obtained in male and female dogs at 4 time intervals of measurement in this study. There are no differences greater than 2 standard deviations from the mean between the sexes or among the various time periods that we have measured. Note that the total mean values for BUN for male animals is slightly higher (20.68) than those for female dogs (18.138). The same sex difference exists in the creatinine values.

Table II shows the blood urea nitrogen data obtained when animals were grouped by 2-year age periods. These are all of the values obtained for animals of a particular age measured over the same time period reported in Table I. There is a slight tendency for serum creatinine determinations in females to decrease with age. This tendency is less marked in male dogs. However, no statistically significant differences exist among the values in this table.

Data on serum creatinine values obtained from female and male control dogs are presented in Table III. As was the case with BUN, there is a tendency for creatinine values to decrease with age in female beagles. However, no differences could be found greater than two standard deviations from the mean. The male animals again demonstrate slightly higher values.

DISCUSSION

Measurements for BUN and creatinine reported here indicate slight decreases with increasing age. This is most clearly evident in female animals. No statistically significant differences could be discovered.

The slight elevation of both values in males as compared to females may be due to greater muscle mass. These data will provide a baseline for future determinations on radionuclide bearing animals or on animals with suspected kidney pathology. The data reported here are done on the basis of age from birth. Elsewhere in this progress report, we are reporting differences measured in terms of radionuclide burden time. This is not possible with dogs that have not been injected, although it appears that radionuclide burden time is a very significant factor with actual age being of less significance in radionuclide bearing dogs in our colony.

REFERENCES

1. Nabors, Charles J., Jr. and W. Stevens; Preliminary study of kidney function in beagle dogs: Aging control dogs; Res. in Radiobiology; U. of Utah Research Report C00-119-242; pp. 205-208 (1970).
2. Atherton, D. R., R. D. Lloyd, G. N. Taylor, Betsy J. Stover, and C. W. Mays; Distribution of ^{241}Am in the beagle; Res. in Radiobiology; U. of Utah Research Report C00-119-237; pp. 117-123 (1968).
3. Technical bulletin #14; Sigma Chemical Co., St. Louis, Mo.
4. Folin, O. and H. Wu; A system of blood analysis; J. Biol. Chem. 38: 81-88 (1919).

BLOOD UREA NITROGEN AND CREATININE AVERAGES

Female Dogs

	<u>n</u>	<u>Feb. '70</u> <u>$\bar{x} \pm S.D.$</u>	<u>n</u>	<u>May '70</u> <u>$\bar{x} \pm S.D.$</u>	<u>n</u>	<u>Oct. '70</u> <u>$\bar{x} \pm S.D.$</u>	<u>n</u>	<u>Feb. '71</u> <u>$\bar{x} \pm S.D.$</u>	<u>n</u>	<u>Total</u> <u>$\bar{x} \pm S.D.$</u>
BUN	55	16.18 $\pm$ 2.50			50	20.05 $\pm$ 3.019	46	18.41 $\pm$ 2.99	151	18.14 $\pm$ 8.06
CREATININE			52	1.23 $\pm$.19	51	1.03 $\pm$.16	46	1.06 $\pm$.16	149	1.11 $\pm$.19

Male Dogs

BUN	45	18.91 $\pm$ 3.02			45	23.1 $\pm$ 4.82	42	19.98 $\pm$ 3.09	132	20.68 $\pm$ 4.14
CREATININE			44	1.30 $\pm$.12	45	1.14 $\pm$.14	42	1.16 $\pm$.13	131	1.20 $\pm$.15

TABLE 1. Mean values and number of measurements for serum blood urea nitrogen and creatinine determinations in beagle dogs 1-203 months of age.

AGING SERUM CREATININE DATA

Age (Months)	FEMALE		MALE	
	<u>n</u>	<u>$\bar{x} \pm S.D.$</u>	<u>n</u>	<u>$\bar{x} \pm S.D.$</u>
1-24	13	1.2 $\pm$.14	36	1.24 $\pm$.15
25-48	32	1.18 $\pm$.15	29	1.23 $\pm$.13
49-72	23	1.24 $\pm$.15	21	1.25 $\pm$.12
73-96	32	1.13 $\pm$.21	18	1.19 $\pm$.09
97-120	14	1.02 $\pm$.14	4	1.15 $\pm$.12
121-144	18	.98 $\pm$.10	12	1.07 $\pm$.12
145-168	11	.90 $\pm$.17	9	1.02 $\pm$.17
169-203	6	.91 $\pm$.14	2	1.3 $\pm$.00

TABLE III. Serum creatinine data for dogs grouped in two-year age periods. The number of measurements, mean and standard deviation are shown for each group.

AGING BLOOD UREA NITROGEN DATA

Age (Months)	FEMALE		MALE	
	<u>n</u>	<u>$\bar{x} \pm \text{S.D.}$</u>	<u>n</u>	<u>$\bar{x} \pm \text{S.D.}$</u>
1-24	13	19.26 $\pm$ 2.42	38	20.09 $\pm$ 2.50
25-48	33	18.43 $\pm$ 3.41	30	22.08 $\pm$ 5.41
49-72	28	18.85 $\pm$ 2.67	19	19.39 $\pm$ 2.50
73-96	28	18.38 $\pm$ 3.61	17	20.67 $\pm$ 2.29
97-120	14	17.75 $\pm$ 3.26	4	22.17 $\pm$ 2.38
121-144	18	17.29 $\pm$ 2.90	14	19.25 $\pm$ 4.13
145-168	11	16.15 $\pm$ 3.93	7	20.94 $\pm$ 5.50
169-203	6	16.76 $\pm$ 3.29	3	26.63 $\pm$ 12.20

TABLE II. Blood urea nitrogen data for dogs grouped by age in two-year periods. The number of measurements, mean and standard deviation are given for each age grouping.

GENETICS OF CANINE BLOOD GROUP "A" AND HYPOTHETICAL GROUPING OF THE BLOOD ANTIGENS ACCORDING TO THEIR SEROLOGICAL PROPERTIES

Stephen G. Jolley and Glenn N. Taylor

Abstract: The observed incidence of the canine blood group A in our Beagle colony and another study involving mixed breeds differed significantly. Deviations were possibly due to geographical selection or differences among various canine breeds as to the incidence of group A cells. Our pedigrees showed that the A factor could be explained in a system of multiple alleles or non-Mendelian, random traits. Canine blood group A could be a simple Mendelian dominant if blood group chimerism occurred in our Beagles, but our studies are presently insufficient to confirm this uncommon possibility. Certain serological characteristics of the canine A system, such as the ability to fix complement, were accepted as criteria for differentiating canine anti-A sera from canine anti-B, anti-C, anti-D, anti-E, anti-F, and anti-G sera. Antiglobulin reactions were proposed as a means of segregating anti-E and anti-F from anti-B, anti-C, anti-D, and anti-G. These serological properties of the various isohemagglutinins were used to theoretically group antigens A₁ and A₂ together; antigens B, C, D, and G into one system; and antigens E and F as a separate system.

INTRODUCTION

For many years, scientists have paid little attention to compatibility factors when transfusing dogs with blood from randomly chosen canine donors. These transfusions seldom produced visible reactions, since it was uncommon for a donor to be used more than once with the recipient. However, multiple transfusions with a common donor often produced violent hemolytic reactions in the recipient.^(14,15,16) Ottenberg, et al.⁽¹¹⁾ were one of the first groups to study this hemolytic phenomenon. Many of those who followed began to recognize several antigenic differences among canine red cells.^(1,4,9,10) Swisher, et al.⁽¹³⁾ were the first to fully characterize eight antigenic differences on the canine erythrocyte distinct from those on human red cells. Seven (A₁, B, C, D, E, F, and G) were believed to give rise to typical divalent isohemagglutinins.

The reactivity of A₂, particularly in the Coombs' or anti-canine globulin (ACG) reaction, lent itself towards the formation of a possible "incomplete" or blocking isohemagglutinin; similar, in some respects, to the human Rh₀ antibody.

The relationship of canine blood groups to the ABO system of man is not completely understood. Difficulties arose because of the apparent inconsistent genetic patterns of canine blood groups. Cohen, et al.⁽³⁾ attempted to explain the canine A, B, C, and D systems in terms of autosomal dominant mechanisms. Swisher, et al.⁽¹³⁾ also proposed a genetic pattern for canine A, but encountered problems in using data from random street dogs. Of the two, autosomal dominant mechanisms appeared to play the most important role in the acquisition of blood types.

METHODS

In 1964, our laboratory obtained some canine anti-A serum from Dr. S. N. Swisher, Department of Medicine, University of Rochester School of Medicine, for the purpose of typing our Beagle colony. We felt this colony would be excellent for studying the genetic distribution or incidence of any red cell antigens because of its well kept pedigree records. The antiserum was initially used in selecting A and non-A dogs for the further preparation of anti-A serum. Dog no. 613 (non-A) was immunized with type A erythrocytes from dog no. 035. Washed, packed 035 red cells were given daily in doses of 3.0 ml intravenously and 3.0 ml intraperitoneally. Aliquots of blood were collected at intervals throughout the immunization period, and titers determined in an agglutination test with diluted sera. Anti-035 RBC serum was harvested when the titer reached a second plateau (Figure 1).

The anti-A serum used in this study was collected from dog no. 613, inactivated at 56°C for thirty minutes, and the non-A isohemagglutinins adsorbed by a composite of red cells from eight non-A dogs. Adsorption was carried out by rotation of 1.0 ml packed RBC and 4.0 ml serum for an interval of fifteen minutes. Three to five adsorptions were carried out on each sample, depending on how much agglutination was still present. Red cells were removed from the suspension by centrifugation at 850 xg for fifteen to twenty minutes. Desired antisera did not produce agglutination with non-A erythrocytes after adsorption. Isohemagglutinin A in each case was characterized by complement (C') fixation.^(2,7,13)

Contrary to Stohlman,⁽¹²⁾ we found that the hemolytic properties of canine blood group A could be enhanced by non-canine C', namely guinea pig C'. An important point with respect to anti-A's complement-fixing ability is also expressed in Tables 1 and 2. Our experience with C' fixation tests showed that canine anti-A doesn't perform well in the full-scale Kolmer⁽⁷⁾ test with sheep red blood cells (SRBC) and amboceptor. Limitations occurred in reading tubes when two hemolytic systems were involved, where hemolysis at high anti-A antibody concentration (test system) and at low anti-A antibody concentration (indicator system) resulted. One way of getting around this was to test the supernatant fluid from a mixture of anti-A serum, A cells, and C' for unbound C' using the SRBC-amboceptor indicator system. But a more direct method, as used in this study, was a hemolysin titration technique; substituting canine anti-A for amboceptor and 2% group A cells for SRBC. This allowed the observance of hemolysis only when C' was fixed in the test system.

Antisera shown to possess specific anti-A properties were used to type our Beagle colony. The method employed here was a modification

of the tube agglutination technique.⁽¹⁾ Fresh autologous serum from our Beagles was omitted as a red cell diluent because it produced non-specific hemolysis on several occasions. We also avoided using detergents to wash glassware because of their hemolytic tendencies.⁽⁴⁾

In lieu of autologous serum we mixed one drop (9" Pasteur pipette) of thrice-washed and packed red cells with 0.2 ml antiserum in a 9 x 75 mm Kahn tube. Such a system allowed us to detect both A₁ and A₂ cells as group A without the use of ACG. Even though A₂ cells react weakly with anti-A₁ serum they react better with anti-A serum because of the possible A₂ isohemagglutinins in addition or in lieu of A₁ antibodies.

The absence of saline in these tests also enhanced the reactivity of A cells.⁽¹⁾ Controls consisted of homologous serum from non-immunized dogs in place of antiserum. Incubation of the tubes was carried out in a 37°C water bath for fifteen minutes and at room temperature for an additional fifteen minutes. Each tube was read by pouring the contents onto a micro-slide and examining for gross agglutination. Anti-A titers determined by this method averaged from 128 to 256.

RESULTS AND DISCUSSION

The incidence of group A dogs in our colony differed significantly from other studies shown in Table 3. Since the Rochester group utilized the largest number of animals it seemed reasonable to assume that it would be the most significant of the three. But, because the Rochester data includes only observed incidences of canine blood groups, it may or may not represent the true blood group distribution in dogs. The validity of any such study cannot be checked due to the lack of a known genetic mechanism defining canine blood group inheritance.

With this in mind we used chi-square (χ^2) to inter compare the Salt Lake data, the Jackson results, and the Rochester study. The χ^2 value in Table 3 indicated that all group A incidences differed significantly from each other. The significant differences observed raised two important possibilities: (1) Do the large deviations from the Rochester group indicate a geographical localization of canine blood group A?

(2) Is canine group A a genetic trait according to the Mendelian theory?

Of the three studies compared the Jackson study supported the first possibility. Here we saw an extremely high incidence of blood group A in an in-bred colony of Basenjis and Cocker Spaniels. Such a high degree of in-breeding could ultimately result in the selection and high incidence of one or two blood groups. On the other hand, we expected that the Salt Lake colony would represent a true Beagle population, due to a moderate degree of in-breeding (C. E. Rehfeld, unpublished observations). The degree of in-breeding in the Salt Lake colony is somewhat less than a cross between two siblings. Therefore, the incidence of A should have been similar to the Rochester group, however it was not. This might suggest that the group A incidence varies according to breed. This would certainly produce significant differences between the occurrence of canine group A in pure-bred colonies and mongrels. At the present, though, there aren't any known breeds lacking type A completely.

Our second question deals with the inheritance properties of canine blood group A. Thus far, there hasn't been any correlations between canine blood groups and the human blood groups. Referring to Figure 2 we see a simple cross of two type A Beagles. Testing the application of simple Mendelian genetics we postulated that both animals were heterozygous A, or $AO \times AO$. As a result, such a cross was expected to give

both type A and non-A offspring. Thus, there appeared to be a non-detectable or recessive allele participating. Likewise, in Figure 3, an A0 x 00 cross gave both types of offspring. Figure 4, however, showed a high incidence of non-A offspring and one A animal. Such was not expected in any of the crosses AA x 00, A0 x 00, and 00 x 00. However, segregation of alleles in A0 x 00 crosses can produce this effect.

Referring to Figure 6 it appeared that a simple Mendelian theory didn't fit. We had an 00 x 00 cross giving both type A and non-A offspring. Similarly, Figure 7 showed all A offspring from another 00 x 00 cross. Even if the 0 gene was dominant we would not expect to see all type A offspring in a A0 x A0 cross, nor any non-A pups in an AA x AA cross. Hence, the inheritance of type A did not follow a simple Mendelian pattern. The phenomenon of blood group chimerism⁽⁸⁾ might have been a factor in these crosses. We noted that both parents in each of the 00 x 00 crosses had siblings of blood type A (Figures 4 and 5). If blood group chimerism occurred then simple Mendelian genetics could have been operating and still have given the appearance of non-Mendelian incidences. However, homografts have not yet been done in each case to determine the possibility of chimerism. Figures 8 and 9 show more offspring distributions with various forms of blood type segregation.

Cohen, et al.⁽³⁾ postulated that the A factor was an autosomal dominant. They felt that if an animal had the A antigen it would have the X^a gene. Similarly, those lacking the A antigen would have an X^0 gene. Thus, the possible genotypes for a type A dog would be X^aX^a and X^aX^0 . A non-A dog would be X^0X^0 . Again, using Figure 6 and the Jackson group's genotype for non-A animals, we see that this theory doesn't fit, since it cannot allow for type A offspring in non-A x non-A

pedigrees. With this in mind, we felt that the inheritance of type A wasn't occurring as a simple autosomal dominant.

One explanation of this may be that the blood groups are non-Mendelian random traits selected during fertilization by some random process as determinants of the red cell antigens. Another possibility may lie in the presence of numerous alleles. The process of allelic pairing may involve so many combinations that the traits appear to be randomly distributed. One such theoretical situation is given in Figure 10. The hypothetical mechanism is outlined by tracing the distribution of alleles in two parents via meiotic division and gamete formation. Here there are five alleles with a dominance gradient of $z_1 > a > z_2 > z_3 > z_4$. The randomness with which the alleles can pair and the random position of the three determinants on the genome result in 2,080 possible genotypes. Table 4 gives the genotypes for possible determinants. Such a system may also account for A offspring in a non-A x non-A cross. For this to occur the non-A parents would need a Y determinant of genotype $z_1 a$. Hence, the inheritance of A could involve a multiple allele or non-allele random mechanism.

The third possibility may involve the presence of secretory substances or abnormalities which alter the red cell surface. If this occurred then a type A dog could be transformed from a non-A dog by the secretion of A-like substances (not related to man) which adsorb to the red cell walls. However, Kolmer⁽⁷⁾ C' fixation tests done in our laboratory with anti-A serum and sera from type A dogs failed to give positive results (unpublished observations). Therefore, we concluded that secretory substances in the sera of group A dogs probably don't contribute significantly to the red cell agglutinogenicity.

Another interesting aspect of canine blood groups which demands some attention is the arrangement of blood antigens. The main question to be answered is whether the eight antigens mentioned by Swisher, et al.⁽¹³⁾ are components of one or many systems. For our purpose in this discussion we used serological data in an attempt to resolve this point. To begin with, we reviewed some of the factors influencing the formation of antibodies. It has been firmly established that the structure of antigens explicitly determines the chain conformation of the specific antibody,⁽⁵⁾ Hence, antibodies produced by stereochemically similar antigens usually have similar serological properties in the presence of their respective antigen. In some cases cross-reactions occur. Antigens do not have to cross-react in order to be in the same genetically-determined group, however. The best examples of this are the ABO and Rh systems in man where antigens within a system do not cross-react. Here the reactivity of antibodies from each group in saline might be a means for segregating the two in genetic studies.

The serological characteristics we used to group the blood antigens are C' fixation and the ACG reaction. All canine blood groups have moderate to strong agglutinative power in vitro.⁽¹³⁾ Of the eight, however, only four (A₁, A₂, E, and F) have the ability to react with ACG. Two of these (A₁ and A₂) can fix C'. From this one might be able to group B, C, D, and G into one system because of their inability to fix C' or react with ACG. Usually the structure of the crystallizable fragment (fc) in an antibody, determines whether it can undergo C' fixation or reaction with ACG. Since it doesn't do either, one could theorize that a bulky fc sterically hinders attachment. E and F could be grouped together because of their reactivity with ACG and not C'. In this case,

the fc may not be as sterically hindered at the site of anti-canine antibody attachment as it is at the C'-binding portion. A third group may contain A₁ and A₂ because they possess both of the aforementioned characteristics of C' fixation and ACG reactivity. Johnson, et al.⁽⁶⁾ has shown that anti-A is predominately 7S globulin. The low molecular weight of anti-A may account for the loss of theoretical steric problems in the fc.

Nevertheless, we feel that serological factors such as C' fixation and reaction of sensitized RBC's in the ACG reaction may be important criteria in the grouping of canine blood antigens into systems. If the blood group antigens could be grouped into systems it would simplify genetic studies.

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REFERENCES

1. R. M. Christian, D. M. Ervin, and L. E. Young; Observations on the in vitro behavior of dog isoantibodies; Jour. Immunol. 66: 37 (1951).
2. R. M. Christian, W. B. Stewart, C. L. Yuile, D. M. Ervin, and L. E. Young; Limitation of hemolysis in experimental transfusion reactions related to the depletion of complement and isoantibody in the recipient; Blood 6: 142 (1951).
3. C. Cohen and J. L. Fuller; The inheritance of blood types in the dog; Jour. Heredity 44: 225 (1953).
4. A. S. Hamilton; Study of in vitro methods for the demonstration of isoagglutination with the bloods of normal and ill dogs; Am. Jour.

Physiol. 154: 525 (1949).

5. F. Haurowitz; The formation of antibodies; Molecular Structure and Biological Specificity, Ed. by L. Pauling and H. A. Itano; Waverly Press, Baltimore, pp. 18-27 (1957).
6. J. S. Johnson, J. H. Vaughan, and S. N. Swisher; Canine immunoglobulins. II. Antibody activities in six immunoglobulin classes; Jour. Immunol. 98: 935 (1967).
7. J. A. Kolmer, E. H. Spaulding, and H. W. Robinson, Approved Laboratory Technic, Appleton-Century-Crofts, Inc., 5th Ed., pp. 797-939 (1951).
8. J. W. Nicholas, W. J. Jenkins, and W. L. Marsh; Human blood chimeras. A study of surviving twins; Brit. Med. Jour., 1: 1458 (1957).
9. W. A. O'Brien, S. N. Swisher, Jr., and L. E. Young; Observations on the wide spectrum of in vitro and in vivo behavior of immune iso-antibodies in dogs; Clin. Res. Proc. 1: 67 (1953).
10. W. H. Olson; Natural isohemagglutination in dogs; Am. Jour. Physiol. 123: 203 (1940).
11. R. Ottenberg, D. J. Kaliski, and S. S. Friedman; Experimental agglutinative and hemolytic transfusions; Jour. Med. Res. 28: 141 (1913).
12. F. Stohlman; The nature of serum enhancement of the canine isohemagglutinin A: Role of conglutinin; Clin. Res. Proc. 1: 67 (1953).
13. S. N. Swisher and L. E. Young; The blood grouping systems of dogs; Physiol. Rev. 41: 495 (1961).
14. L. E. Young, D. M. Ervin, and C. L. Yuile; Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. I. Serologic and hematologic aspects; Blood, 4: 1218 (1949).
15. L. E. Young, R. M. Christian, D. M. Ervin, R. W. Davis, W. A.

O'Brien, S. N. Swisher, and C. L. Yuile; Hemolytic disease in newborn dogs; Blood, 6: 291 (1951).

16. C. L. Yuile, T. F. Van Zandt, D. M. Ervin, and L. E. Young; Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. II. Renal aspects following whole blood transfusions; Blood, 4: 1232 (1949).

Table 1. Kolmer Complement Fixation Test with Canine Anti-A, 893 RBC (A-negative), 035 RBC (A-positive), SRBC, and Amboceptor (one-fifth method)

<u>Serum Dilution</u>	<u>Anti-A Serum and 035 RBC</u>	<u>Anti-A Serum and 893 RBC</u>
1:1	2*	2
1:2	3	2
1:4	3	2
1:8	3	2
1:16	2	2
1:32	2	2
1:64	2	2
1:128	2	2
1:256	2	2
1:512	2	2
Hemolytic Control	4	4
Serum Control	4	4
Antigen Control	2	2
SRBC Control	0	0

* 0 - 4 = Degree of Hemolysis.

Table 2. Comparison of Anti-A Titers Obtained in a Complement Fixation Test without SRBC and Amboceptor to Those Obtained in Tube Agglutination (035 RBC source of cells).

Serum Dilution	Anti-A Serum in CF Test*	Anti-A Serum in Aggl. Test**
1:1	4	4+
1:2	4	3+
1:4	4	3+
1:8	4	2+
1:16	4	2+
1:32	4	2+
1:64	3	2+
1:128	3	0+
1:256	2	0+
1:512	0	0+
Homologous Control	0	0+
Complement Control	0	-
035 RBC Control	0	0+

* 0 - 4 = Degree of Hemolysis

** 0+ - 4+ = Degree of Hemagglutination

Table 3. Comparison of Observed Incidences from Various Studies on Canine Blood Group A.

<u>Group</u>	<u>Salt Lake Beagles</u>	<u>Jackson (3) Basenjis and Cocker Spaniels</u>	<u>Rochester (13) Mongrels</u>	<u>Total dogs</u>
A	284	101	597	982
non A	214	23	357	594
Total	498	124	954	1,576
$\chi^2 = 25.25$ (d.f. = 2)				

Table 4. Possible Genotypes of the Antigenic Determinants using Five Alleles.

<u>Determinant</u>	<u>Genotypes</u>
γ	$z_1 z_1, z_1 a, z_1 z_2, z_1 z_3, z_1 z_4$
A	aa, az_2, az_3, az_4
δ	$z_2 z_2, z_2 z_3, z_2 z_4$
Σ	$z_3 z_3, z_3 z_4$
II	$z_4 z_4$

Fig. 1. Curve representing the titer of anti-035 RBC isohemagglutinin in dog no. 613's serum during repeated immunization with dog no. 035 erythrocytes (Sensitivity of test = $\pm$ one two-fold dilution).

Fig. 2. Possible heterozygosity of two group A Beagles expressed by the ratios of group A and non-A offspring in their litters.

Fig. 3. Typical cross showing preference of a single blood type in each litter, but differences among the prevalent blood types of two litters.

Fig. 4. Lineage of blood group A in dog no. 879 showing a type A sibling (dog no. 878) which could have participated in an in utero exchange of erythrocyte stem cells with dog no. 879.

Fig. 5. History of group A in dog nos. 893 and 894. Blood group chimerism may have occurred here with dog no. 895 acting as the donor of group A erythrocyte stem cells.

Fig. 6. A cross giving ratios of group A and non A pups not compatible with an autosomal dominant theory.

Fig. 7. Pedigree showing that segregation and prevalence of a single blood type can occur in crosses which possibly reject the notion that the A factor is a simple Mendelian dominant.

Fig. 8. A typical cross involving segregation of group A pups from non-A pups in contrast to the form of segregation in Fig. 4.

Fig. 9. An example of matings within our colony that do not show any dominance nor preference of a single blood type within their litters.

*Orientation of genome

Fig. 10. Hypothetical genetic mechanism for the inheritance of A, γ, δ, Σ, and Π blood antigens.

GROWTH DYNAMICS OF OSTEOSARCOMAS INDUCED IN BEAGLES BY
BONE-SEEKING RADIONUCLIDES*

Gary B. Thurman, Charles W. Mays, Glenn N. Taylor,
and Thomas F. Dougherty

Abstract: This study reports an analysis of the growth dynamics of 256 radiation-induced osteosarcomas in adult Beagles. The osteosarcomas were induced by intravenous injection of ^{226}Ra (80 tumors), ^{239}Pu (64 tumors), ^{228}Ra (43 tumors), ^{228}Th (56 tumors), ^{90}Sr (8 tumors) or ^{224}Ra (5 tumors). Radiographs of each osteosarcoma were taken during the period of the tumor's growth. The growth dynamics of these osteosarcomas were estimated by measuring tumor dimensions on as many radiographs as possible. During the period of measurements, the tumor volumes appeared to enlarge exponentially with time. Exponential growth constants were calculated for 249 tumors, and the corresponding volume doubling times ranged from 4.7 to 60.3 days and averaged 12.4 days. The exponential growth constants (rates of tumor growth) were not significantly dependent upon sex, administered radionuclide, injected $\mu\text{Ci/kg}$, tumor location, tumor calcification, tumor volume at autopsy, or age of the animal at death.

* Abstract, Presented at the 19th Annual Meeting of the Radiation Research Society at Boston, Uncorrect Preprints, p. 22 (9-13 May 1971).

RETENTION AND DISTRIBUTION OF INJECTED ^{210}Pb IN BEAGLES*

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

Abstract: ^{210}Pb was administered to 10 young adult Beagles as a single intravenous injection in a citric acid-sodium citrate buffer 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 by γ -ray counting. About 3/4 of the excreted ^{210}Pb appeared in the feces. The fraction of the injected activity still in the circulating blood was about 0.40 at 1 day, 0.14 at 1 week, and 0.03 at 1 month. After 1 hour following injection, red cells had more than 99% of the ^{210}Pb in the blood. Preliminary results indicate that, during the first 700 days, 36% of the injected ^{210}Pb is retained in the dog with a biological half-life of 4 days, 43% with 33 days, and 21% with 990 days. One dog sacrificed 28 days after injection had about 63% of the retained ^{210}Pb in the skeleton, 20% in the liver, 8% in the blood and 9% in other soft tissues. Liver had the highest ^{210}Pb concentration of any tissue measured, 1.6 times that in the skeleton.

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THE ASSOCIATION OF ^{210}Pb WITH CONSTITUENTS OF ERYTHROCYTES

F. W. Bruenger, W. Stevens, and Betsy J. Stover

Abstract: The interaction of trace amounts of lead with constituents of blood was investigated. In vivo experiments using ^{210}Pb were performed in beagles and in vitro studies were done with human and canine whole blood or isolated blood cells. At low concentrations of lead, about 90% of the nuclide found with red cells was associated with red cell cytoplasm and less than 10% was bound to stroma. Transfer of Pb across the red cell membranes did not require binding of the nuclide to plasma proteins. Red cell cytoplasm analyzed by electrophoresis showed that most of the lead was found in the hemoglobin containing fractions. In the dog varying amounts were also found in fractions with an electrophoretic mobility greater than hemoglobin. The HbA_2 ($\alpha_2\delta_2$) component of human erythrocytes had a much higher affinity for ^{210}Pb than the main HbA ($\alpha_2\beta_2$) fraction. Only after saturation of the HbA_2 fraction were greater quantities of lead found in the main hemoglobin band. Lead also was bound to erythrocuprein.

INTRODUCTION

Lead has received considerable attention lately as an environmental pollutant. Effects of long term exposure to small concentrations of lead may exist but have not been explored to any extent. Also exposure to consistently high levels of radioactive lead is a major occupational hazard among uranium miners. In blood, the majority of the lead is associated with erythrocytes and only a very small amount is found in plasma. It was accepted that the metal would bind mainly to the erythrocyte membrane.⁽¹⁾ In a preliminary report it was suggested that lead would bind to cytoplasmic components, i.e. hemoglobin (2,3). Recently Barltrop and Smith⁽⁴⁾ described the binding of ^{203}Pb by "the cell contents rather than to stromal material". They concluded that lead in erythrocytes was bound primarily to hemoglobin. The reaction kinetics has been studied by Schubert and White,⁽⁵⁾ by Stover,⁽⁶⁾ and a number of other workers using stable lead, ^{210}Pb and ^{212}Pb . In this paper the

interaction of lead with constituents of canine and human blood in vivo and in vitro is described.

METHODS

For the in vivo experiments, ^{210}Pb in citrate buffer of pH 3.5 was injected intravenously into beagles. In the in vitro studies ^{210}Pb in physiological saline solution was incubated with either whole blood or washed red cells.

Blood was obtained from healthy, young adult beagles or from humans free from any hematological disorders. Incubations were performed for 20 minutes at 40°C with quantities of ^{210}Pb from 0.1 - 0.3 $\mu\text{Ci/ml}$ of blood (0.014 - 0.042 $\mu\text{g/ml}$). Blood cells were then separated by centrifugation, washed in physiological saline solution and after removal of the buffy coat they were laked and stroma and soluble material were separated by standard techniques. In some incubations ^{210}Pb was either replaced by other metals as ^{203}Hg , ^{65}Zn , ^{64}Cu , etc. or these metals were used in addition to lead.

The supernatant red cell cytoplasm (cytosol) was analyzed by gel filtration and/or disc electrophoresis. Gel filtration was done on Sephadex G-100 with an eluant containing 0.1 M Tris-(hydroxymethyl)-aminomethane (TRIS) and 0.68 M NaCl. Analytical disc electrophoresis was carried out in duplicate for 70 minutes at 200 V using a separating gel with 7% acrylamide containing 0.25% N,N'-methylenebisacrylamide. One gel cylinder was stained for detection of protein bands, the other was cut into slices of equal thickness (~1.3mm) and counted. High and low molecular weight components of the cytosol were separated by dialysis through Visking Nojax casing using a 0.05 M TRIS-HCl buffer of pH 7.5 at 4°C .

All counting was done with a NaI (Tl) detector using the 47 keV γ emission of ^{210}Pb .

RESULTS

In tracer quantities, about 10% of ^{210}Pb was associated with the stroma and 90% with cytoplasmic components. Transfer across the membrane was time dependent (being complete in about 30 minutes) and did not require the presence of plasma constituents although lead can form complexes with plasma proteins. Raising the lead concentration to mg levels by addition of stable metal increased the fraction of lead associated with the red cell stroma as observed before.⁽⁵⁾

Lead in the ghost-free cytosol was not dialysable but it became ultrafiltrable in the presence of high concentrations of inorganic salts such as phosphate or 6 M urea. After precipitation of the hemoglobin with 0.88 volumes of EtOH-CHCl_3 , 80 - 90% of the lead was found in the precipitate and 10 - 20% was rendered ultrafiltrable.

After gel chromatography of the cytosol on Sephadex G-100, a large fraction of the nuclide eluted with the hemoglobin peak (Fig. 1). An attempt to separate the cytosol on DEAE-ion exchange resin under conditions which separate the various fractions of hemoglobin, was unsuccessful since practically all the lead remained on the column. Separation then was performed by gel disc-electrophoresis. The results of this procedure when applied to a human cytosol are presented in Fig. 2. Hemoglobin was resolved into two bands, a main band ($\text{HbA}(\alpha_2\beta_2)$) and a minor band ($\text{HbA}_2(\alpha_2\delta_2)$). The A_2 -band is typical for humans and was not found in cytosols derived from canine RBC. Since in RBC-cytosol approximately 95% of the protein is hemoglobin, it is quite difficult to

separate nondestructively any minor protein components having the same range of mobility as Hb. The position of some nonhemoglobin proteins, however, was determined as outlined by Haut et al. (7). These were catalase, carbonic anhydrase and erythrocyte. Catalase and proteins with lower mobilities did not carry any ^{210}Pb . The question whether carbonic anhydrase or erythrocyte, whose mobilities were close to that of Hb, carried lead was later solved by a double tracer technique since both are metalloproteins. The major fraction of ^{210}Pb associated with the minor (A_2) band comprising only 2% of the total hemoglobin. Only after saturation of this band was a greater fraction of ^{210}Pb found in the main band.

In Fig. 3 electrophoretic patterns of human RBC-cytosol are compared to canine RBC-cytosol and to the mobility of ^{210}Pb subjected to the same experimental conditions but without any protein. The A_2 -band was missing in the dog and so was the corresponding peak of ^{210}Pb activity. In the beagle no difference was observed between cytosols tagged in vivo and in vitro, and results did not differ greatly when breeds other than the beagle were used. ^{210}Pb had always a slightly higher mobility than the corresponding hemoglobin but any modification in the mobility of the Hb also resulted in a corresponding shift in the appearance of ^{210}Pb . Also, in the dog some lead remained unbound and was therefore found in the front band.

Since hemoglobin has free -SH groups, it seemed likely that these were involved in lead binding. Consequently, the binding of lead was compared to that of mercury, which is known to interact with the thiol-groups of hemoglobin (Fig. 4, left side). Two things became evident: a) two of the ^{203}Hg peaks were positioned as ^{210}Pb ; b) ^{203}Hg reacting

with Hb was leading the hemoglobin peak just as ^{210}Pb , indicating that both nuclides impart a higher mobility when combined to Hb. Also there was more ^{203}Hg in the HbA_2 area than in the main band.

Since mercury forms a strong bond with sulfhydryl groups, prior incubation of hemoglobin with Hg should block the uptake of ^{210}Pb if -SH groups are involved. As seen in the right half of Fig. 4, sulfhydryl inhibition with mercury blocked the uptake of ^{210}Pb completely. No difference was observed between cytosols whose thiol-groups were blocked by Hb^{++} and subsequently incubated with ^{210}Pb while the red cells were intact as compared to those which were lysed before incubation. ^{210}Pb in both cases moved as unbound lead.

Purification of hemoglobin by crystallization in phosphate buffer⁽⁸⁾ and subsequent activation of thiol-groups with dithioerythrol did not greatly alter the pattern of lead uptake, except that more lead was found in the main Hg band. Thus, the availability of thiol-groups seems to be a necessary but not in itself a sufficient condition for the association of the metal with hemoglobin.

Carbonic anhydrase and erythrocyte have electrophoretic mobilities similar to hemoglobin. Since both are metalloproteins, (Zn and Cu, respectively), a double tracer study using ^{210}Pb and the metal associated with the respective protein was performed and the electrophoretic mobilities of Zn, Cu and ^{210}Pb were compared. As seen on the left side of Fig. 5, the HbA_2 compound was clearly separated from the carbonic anhydrase. ^{210}Pb appeared only in the A_2 area while ^{65}Zn carbonic anhydrase had a lower mobility. Incubation of a semipurified preparation of human carbonic anhydrase with ^{210}Pb showed no association of the protein with lead.

A similar experiment using ^{64}Cu and ^{210}Pb was carried out to determine the position of erythrocyte. As seen on the right side of Fig. 5, ^{64}Cu after electrophoresis yielded a rather broad spectrum and the position of erythrocyte could not be determined by this method. However, erythrocyte separated from swine RBC and later from human RBC, when incubated with ^{210}Pb and separated, indicated a strong association of lead and protein, as was expected since lead is used as a precipitant in the preparation of erythrocyte.⁽⁹⁾

DISCUSSION

In the red cell lead was associated with both stroma (10%) and cytosol (90%). The association of lead with the membranes causes basophilic stippling which was described years ago. This phenomenon occurs mainly when larger quantities of lead are available. The other is the interaction of lead with hemoglobin which is the main topic of this communication. This interaction may be the most important because when exposed to small quantities of the metal, as occurs when radon is inhaled by uranium workers, 9/10 of the lead would be bound to Hb. In cases of pathological lead poisoning, the metal will be associated with the red cell membrane and the red cell cytosol.

Because of the high hemoglobin content of the cytosol, it would be very difficult to resolve any minor protein fractions having the same mobility as hemoglobin and a possible association of any such fractions with lead cannot be excluded at present. The high affinity of ^{210}Pb with the HbA₂ component was surprising. It showed that small changes in the hemoglobin molecule (β and δ chains differ in six amino acid positions) may greatly alter the metal binding properties of Hb. The small difference

between the peaks of ^{210}Pb and hemoglobin is not inconsistent with a binding of the nuclide as has been observed by Charache et al.⁽¹⁰⁾ as demonstrated by the identical behaviour of mercury tagged Hb. Mercury however, is known to form a strong bond with hemoglobin. The stoichiometry of the reaction is difficult to establish. The fact that protein bound lead became dialysable in solutions of high salt or urea concentration indicates that the metal-protein bond is dependent on the steric configurations of the protein molecule. Inhibition by mercury ions proves that thiol-groups are involved in this binding. On the other hand, chemical activation of SH-groups did not change the pattern of lead binding drastically, which is not surprising since lead, despite its high activity for SH-groups is known to be an ineffective inhibitor of sulfhydryl proteins (those with only one thiol group). Chelate chemistry tells us that lead binding is much stronger when the metal interacts with with a second ligand such as another sterically available thiol-group or neighboring histidine. This would explain the sensitivity of the reaction of lead with proteins of red cell cytoplasm.

No attempt was made to assess or evaluate any of the consequences arising from such a bond between lead and hemoglobin.

The presence of lead in erythrocyte may contribute to the diminished hemoglobin synthesis seen in lead poisoning.

REFERENCES

1. J. C. Aub, L. T. Fairhall, A. S. Minot, and P. Reznikoff; Lead Poisoning; Baltimore; Williams and Wilkins (1962).
2. F. W. Bruenger, W. Stevens, and Betsy J. Stover; The distribution of ^{210}Pb in canine blood after intravenous injection and the

- association of the nuclide with blood constituents in dogs and humans; Res. in Radiob., P. R. C00-119-242, 152, Univ. of Utah (1970).
3. C. W. Mays, R. D. Lloyd, F. W. Bruenger, D. R. Atherton, and J. L. Williams; ^{210}Pb metabolism in beagles; Meeting on biol. and ecol. of polonium and radiolead; Sutton, England (1970).
 4. D. Barltrop and A. Smith; Interaction of lead with erythrocytes; *Experientia* 27: 92 (1971).
 5. J. Schubert and M. R. White; Effect of sodium and zirconium citrate on distribution and excretion of radiolead; *Arch. Biochem.* 21: 224 (1949).
 6. Betsy J. Stover; ^{212}Pb (ThB) tracer studies in adult beagle dogs; *Proc. Soc. Exp. Biol. Med.* 100: 269 (1959).
 7. A. Haut, G. R. Tudhope, G. E. Cartwright, and M. M. Wintrobe; The nonhemoglobin erythrocytic proteins, studied by electrophoresis on starch gel; *J. Clin. Invest.* 41: 579 (1962).
 8. D. L. Drabkin; A simplified technique for a large scale crystallization of human oxyhemoglobin; *Arch. Biochem.* 21: 224 (1949).
 9. J. R. Kimmel, H. Markowitz, and D. M. Brown; Some chemical and physical properties of erythrocytorein; *J. Biol. Chem.* 237: 47 (1959).
 10. S. Charache and D. J. Weatherall; Fast hemoglobin in lead poisoning; *Blood*, Vol. 28, 3: 337 (1966).

Fig. 1. Elution spectrum of dog red cell cytoplasm tagged with ^{210}Pb on Sephadex G-100. ^{210}Pb is associated with the Hb-peak and also with low molecular weight components. The lead content is presented on a logarithmic scale. The optical density is shown on the left side.

Fig. 2. The results of disc gel-electrophoresis of red cell cytosol.

The top diagram represents the picture of a developed gel cylinder to which a human cytosol was applied. The solid curve on the bottom is the densitometric scan of the above gel and is a measure of the approximate protein content. The dashed line is the ²¹⁰Pb content.

Fig. 3. Comparison of canine and human RBC-cytosol after separation by disc gel-electrophoresis. Canine Hb has a slightly higher mobility than human Hb and the A₂-band is missing. The dotted line represents the position of protein-free ²¹⁰Pb subjected to electrophoresis under the same conditions.

Fig. 4. Left: Spectrum of ^{203}Hg and ^{210}Pb activity after electrophoresis of two tagged human RBC-cytosols obtained from the same source. The position of the main Hb-band is indicated by x's.

Right: Comparison of electrophoretic mobilities of two human cytosols; one saturated with Hg and one that has no mercury (control).

Fig. 5. Left: Result of a double tracer study using ^{65}Zn and ^{210}Pb tagged human RBC-cytosol. ^{65}Zn associated with carbonic anhydrase has a lower mobility than HbA_2 .

Right: A similar double tracer study using ^{64}Cu and ^{210}Pb to determine the position of erythrocyte. The spectrum of ^{64}Cu is rather broad and the position of erythrocyte could not be determined this way.

Peaks of HbA and HbA_2 are marked by x- and #-marks, respectively.

A NON-LINEAR INTERPRETATION
TO THE "DOUBLING DOSE" CONCEPT*

CHARLES W. MAYS

ABSTRACT

Radiation is known to induce cancer. If the increased incidence per additional rad is proportional to the total incidence existing before each additional dose is received:

$$I_r = I_o [e^{kx} - 1]$$

where I_r is the incidence of a radiation-induced cancer, I_o is its natural incidence, x is the cumulative dose of radiation, and k is a constant. The assumptions, derivation, and properties of this exponential, non-linear, equation are presented and compared with those of the traditional linear dose-response equation.

INTRODUCTION

As applied to the radiation induction of a particular type of cancer, the doubling dose is defined as the quantity of radiation which will cause its total incidence to double. For example, if the natural incidence is 1%, and 100 rads induces an additional 1% so that the total incidence is 2%, then 100 rads is the first doubling dose. But what might be induced by a second doubling dose? Might it induce an additional 1% (equal to the original natural incidence) or might it induce an additional 2% (equal to the total incidence following the first doubling dose)? Obviously, two different interpretations are possible, as has been pointed out by Brues (1970), and the purpose of this analysis is to explore them both. Much hinges on the factors which determine the so-called "natural" incidence, as will be examined

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later in the discussion section.

DERIVATION OF EQUATIONS

let I = total incidence = $I_r + I_o$

I_r = radiation induced incidence

I_o = "natural" incidence, assumed constant for fixed conditions

x = radiation dose

b and k are constants

(a) Linear Interpretation ("simple interest")

Assume that the additional incidence (dI) from additional dose (dx) is proportional to the natural incidence (I_o).

$$\frac{dI}{dx} = b I_o x \quad (1)$$

The solution to Eq. 1 is:

$$I = b I_o x + I_o \quad (2)$$

And since $[I = I_r + I_o]$ then:

$$I_r = b I_o x \quad (3)$$

Thus, for the linear interpretation, the incidence of radiation induced cancer should be directly proportional to the amount of radiation. The doubling dose equals $1/b$. Therefore, the induced incidence should be I_o for 1 doubling dose, $2I_o$ for 2 times the doubling dose, $3I_o$ for 3 times the doubling dose, etc., for this model.

(b) Non-Linear Interpretation ("compound interest")

Assume that the additional incidence (dI) from additional dose (dx) is proportional to the total incidence (I) existing just before the additional dose (dx) is received:

$$\frac{dI}{dx} = k I \quad (4)$$

The solution to Eq. 4 is:

$$I = I_0 e^{kx} \quad (5)$$

And since $[I = I_r + I_0]$ then:

$$I_r = I_0 [e^{kx} - 1] \quad (6)$$

Thus, under the non-linear interpretation the incidence of radiation induced cancer should increase as an exponential function of dose.

DISCUSSION

Equations 3 and 6 are compared in Fig. 1 where, as an illustrative example, both curves are fit through $I_r = 10\%$ at 1000 rads.

Non-linear Eq. 6 has interesting properties which will now be explored. As shown in Fig. 2, the higher the natural incidence, the more "linear" the "non-linear" model becomes. This prediction supports the impression stated by Albert (1970) that dose-incidence curves often tend to be non-linear for types of cancer having a low natural occurrence. Similarly, during the recorded discussion of a paper by Finkel (1969), I suggested that the different shapes of the observed dose-response relationships for radium in CF1 mice (linear) and in humans (non-linear) might be related to their different natural incidences (~ 1 osteosarcoma/70 deaths in control mice vs. ~ 1 osteosarcoma/10,000 deaths in humans).

The slope of non-linear Eq. 6 is of interest, and can be obtained by differentiation:

$$\frac{dI_r}{dx} = k I_0 e^{kx} \quad (7)$$

For small values of x , $[e^{kx} \sim 1]$ and $[\frac{dI_r}{dx} \sim k I_0]$, so that:

$$I_r \sim k I_0 x \quad [\text{for small doses}] \quad (8)$$

Thus, at small doses, "non-linear" Eq. 6 approaches a "linear" rela-

tionship, but one whose slope is more shallow than that of the conventional linear relationship (see Figs, 1 & 2 and their captions).

Traditionally, the conventional linear relationship (Eq. 3) has been used to express the "doubling dose" concept, but with perhaps insufficient thought given to the make-up of the so-called "natural" incidence. Recently Gofman and Tamplin (1969 & 1970) have indicated that the natural incidence is not a fixed quantity, but can be influenced by various factors such as country of residence, cigarette smoking, exposure to asbestos, and background radiation. They claim that the average background of about 0.1 rad per year causes about 3% of the naturally-occurring cancers. If this were true and if the dose-response were linear, then in some regions of high background, well over half of the "natural" cancers should be radiation-induced, since the background in some inhabited parts of the world ranges up to about 10 rads per year, which is 100-fold higher than the average (Penna Franca et. al., 1965).

This raises an interesting point. Gofman and Tamplin (1969 & 1970) claim that (a) the doubling dose for each type of cancer is similar and is about 100 rads for adults, (b) each type of cancer is induced in direct proportion to its natural incidence, and (c) the natural incidence is not a fixed quantity but is determined by a number of factors including background radiation. Suppose a prior dose of artificial radiation had been received. Then it would seem consistent to add its resulting incidence to the natural incidence, and to use this new total incidence in predicting the effect of a subsequent dose, as has been done in the derivation of Eq. 6. Obviously, cells cannot distinguish between radiations which differ only in their natural vs. artificial origin.

This suggests that non-linear Eq. 6 forms a more consistent model for cancer induction than does linear Eq. 3. In financial analogy, continually compounded interest (based on the accrued interest plus the original principal) is more fair than simple interest (based only on the principal). In fact, Eqs. 3 & 6 apply to banking when I_t is the accrued interest, I_0 is the invested principal, x is the time since the investment was made, and b & k are the simple and compound interest rates, respectively.

But one must realize that the fundamental mechanisms by which cancer induces radiation may be quite complex, and any relation as simple as Eq. 6 should not be expected to describe accurately the actual incidence over a wide dose range. For example, it must break down at very high doses due to the radiation-killing of potentially malignant cells. It may, however, prove useful in testing hypotheses, such as those by Gofman and Tamplin, against experimental data, and it may give insight into alternative models which hopefully may more closely approximate that elusive goal called reality.

REFERENCES

- Albert, R. (1970). Unrecorded discussion, Conference on the Estimation of Low-Level Radiation Effects, Argonne National Laboratory, U.S.A. 7-9 Dec. 1970.
- Brues, A. M. (1970). Ibid.
- Finkel, M. P. Biskis, B. O., and Jinkins, P. B. (1969). Toxicity of radium-226 in mice, in "Radiation-Induced Cancer," pp. 369-391. (A. Ericson, ed.) Vienna: International Atomic Energy Agency.
- Gofman, J. W. and Tamplin, A. H. (1969), Low dose radiation, chromosomes and cancer, in "Environmental Effects of Producing Electric Power," Hearings before the U. S. Congressional Joint Committee on Atomic Energy, Oct. 28-31 and Nov. 4-7, 1969, pp. 641-652.
- Gofman, J. W., and Tamplin, A. H. (1970). Major fallacies in the AEC staff comments on the Gofman-Tamplin papers and congressional testimony, in "Environmental Effects of Producing Electric Power," Hearings of the U. S. Congressional Joint Committee on Atomic Energy, Part 2 (Vol II), Jan. 27-30 and Feb. 24-26, 1970, pp. 2113-2127.
- Penna Franca, E., and 10 others (1965). Status of investigations in the Brazilian areas of high natural radioactivity, Health Physics 11:8 pp. 699-712.

Fig. 1. Linear and exponential models for radiation induced cancer. Both of these illustrative models were fit through 0% induced cancer at zero dose and 10% induced cancer at 1000 rads. For a 1% natural incidence the initial slope of this particular exponential model (2.42% per 1000 rads) is about 1/4 of that for the linear model (10% per 1000 rads).

Fig. 2. Exponential models for 10%, 1% or 0.1% natural incidence. Each of these illustrative models was fit through 0% induced cancer at zero dose and 10% induced cancer at 1000 rads. The initial slopes of the 3 curves are 6.93% per 1000 rads (for 10% natural incidence), 2.40% per 1000 rads (for 1% natural incidence) and 0.462% per 1000 rads (for 0.1% natural incidence). The higher the natural incidence, the more closely the exponential model approaches the linear model.

ADDITIONS TO BIBLIOGRAPHY

(1 April 1970 through 31 March 1971)

PUBLISHED ARTICLES

1. Bartley, M. H. and W. S. S. Jee; Skeletal system/teeth and mandible; in The Beagle; edited by A. C. Andersen, Iowa State U. Press, Ames, Iowa; pp. 162-188 (1970).
2. Dewey, J. R., M. H. Bartley, Jr., and G. J. Armelagos; Rates of femoral cortical bone loss in two nubian populations; Clinical Orthopaedics 65: 61-66 (1969).
3. Dougherty, J. H. and L. S. Rosenblatt; The comparative toxicity of ^{226}Ra , ^{239}Pu , ^{228}Th , ^{228}Ra and ^{90}Sr to leukocytes of beagles; Radiation Res. 43: 56-70 (1970).
4. Dougherty, T. F. and D. A. Dolowitz; Physiologic actions of heparin not related to blood clotting; in Chemical Dynamics; edited by Joseph O. Hirschfelder and Douglas Henderson; Wiley-Interscience, a Division of John Wiley & Sons, Inc., pp. 633-646 (1971).
5. Eyring, Henry and Betsy J. Stover; The Dynamics of Life, II. The steady-state theory of mutation rates; Proc. Natl. Acad. Sciences 66: 441-444 (1970).
6. Jee, W. S. S.; Bone seeking radionuclides and bones; in Pathology of Irradiation; edited by C. C. Berdjis; Williams and Wilkins, Baltimore; pp. 186-212 (1970).
7. Jee, W. S. S., M. H. Bartley, R. D. Cooper and N. L. Dockum; Skeletal system/bone structure; in The Beagle; edited by A. C. Andersen; Iowa State U. Press, Ames, Iowa, p. 162-188 (1970).
8. Jee, W. S. S., H. Z. Park, W. E. Roberts, E. L. Blackwood, and

- G. H. Kenner; Corticosteroid and bone; Amer. J. Anat., 129: 477-480 (1970).
9. Lloyd, R. D., C. W. Mays, B. W. Church, R. C. Pendleton, and Susan F. Mays; Does the elimination rate of cesium in humans change with the seasons; Health Physics 18: 623-629 (1970).
 10. Mays, C. W., T. F. Dougherty, B. J. Stover, W. S. S. Jee, W. R. Christensen, J. H. Dougherty, W. Stevens, and C. J. Nabors; Bone cancer induction by radionuclides: incidence vs. dose; in Environmental Effects of Producing Electric Power; Hearings before the Joint Committee on Atomic Energy, U. S. Congress Vol. II, Part 2; pp. 2192-2210 (Jan. 27-30 and Feb. 24-26, 1970).
 11. Mays, C. W., G. N. Taylor, W. S. S. Jee, and T. F. Dougherty; Speculated risk to bone and liver from ^{239}Pu ; Health Physics 19: 601-610 (1970).
 12. Pendleton, R. C. and R. D. Lloyd; Zirconium-95 in Utah vegetation produced during the 1966 growing season; Radiological Health Data 11: 227-281 (1970).
 13. Spiess, H. and C. W. Mays; Bone cancers induced by ^{224}Ra (ThX) in children and adults; Health Physics 19: 713-729 (1970).
 14. Stover, Betsy J., David R. Atherton and Dawn S. Buster; Protracted hepatic, splenic and renal retention of ^{239}Pu in the beagle; Health Phys. 20: 369-374 (1971).
 15. Stover, Betsy J., F. W. Bruenger and W. Stevens; Association of americium with ferritin in the canine liver; Radiation Res. 43: 173-186 (1970).
 16. Stover, Betsy J.; The Dynamics of Life, IVa. Lenticular Cataracts induced by aging and irradiation; Chemical Dynamics; (Ed.

by Hirschfelder); pp. 661-667, J. Wiley and Sons, New York (1971).

17. Stover, Betsy J. and Henry Eyring; The Dynamics of Life, I. Death from internal irradiation by ^{239}Pu and ^{226}Ra , aging, cancer and other diseases; Proc. Natl. Acad. Sciences 66: 132-139 (1970).
18. Stover, Betsy J. and Henry Eyring; The Dynamics of Life, III. Mechanisms of nonsurvival and the relation of dose size; Proc. Natl. Acad. Sciences 66: 672-676 (1970).

PUBLISHED ABSTRACTS

1. Atherton, D. R., F. W. Bruenger, and Betsy J. Stover; The interaction of some actinide elements with blood constituents; Abstracts of Papers for Nineteenth Annual Meeting of the Radiation Research Society, p. 97 (1971).
2. Bruenger, F. W., W. Stevens and Betsy J. Stover; The association of ^{210}Pb with constituents of erythrocytes; Abstracts of Papers for the Nineteenth Annual Meeting of the Radiation Research Society, p. 50 (1971).
3. Eyring, Henry and Betsy J. Stover; The Dynamics of Life, IV. The steady state theory of mutations causing cancer and other degenerative diseases; Abstracts of Papers for the Division of Physical Chemistry, American Chemical Society, 160th National Meeting, Chicago (1970).
4. Julian, G., H. Z. Park, W. E. Roberts, and W. S. S. Jee; Parathyroid-cortisol relationship measured by periodontal ligament labeling indices; 49th Annual Meeting Int. Assoc. for Dental Res. (I.A.D.R.); Abstracts of Papers, p. 259 (1971).
5. Kimmel, D., H. Z. Park, R. B. Dell, and W. S. S. Jee; Effects of calcitonin, cortisol and hypophysectomy upon ^{91}Y uptake; 49th Annual Meeting IADR; Abstracts of Papers, p. 258 (1971).
6. Kramer, M., W. E. Roberts, H. Z. Park, and W. S. S. Jee; Endogenous parathyroid hormone and the proliferation of periodontal ligament fibroblasts; 49th Annual Meeting IADR; Abstracts of Papers, p. 259 (1971).
7. Lloyd, R. D., C. W. Mays, D. R. Atherton, F. W. Bruenger, and J. L. Williams, Retention and distribution of injected ^{210}Pb

- in beagles; Health Physics 19: 337 (1970).
8. Mays, C. W., R. D. Lloyd, F. W. Bruenger, D. R. Atherton, and J. L. Williams; ^{210}Pb metabolism in beagles; Published Abstracts of the Meeting on the Biology and Ecology of Polonium and Radiolead; Belmont, England, p. 24 (30 April-1 May, 1970).
 9. Mays, C. W., G. N. Taylor, W. S. S. Jee, and T. F. Dougherty; Speculation on the risk to adult man from a permissible body burden of ^{239}Pu (0.04 μCi) equally divided between bone and liver; Book of Abstracts, 2nd International Congress of Radiation Protection, Brighton, England, p. 56 (3-8 May 1970) and also in Rad. Res. 43:1, p. 264 (July, 1970).
 10. Nabors, C. J., Jr.; Altered corticosteroid biosynthesis in adrenals from cystic fibrosis patients; 12th Annual Meeting of Cystic Fibrosis Club, Atlantic City, N. J., (April 1971).
 11. Park, H. Z., J. M. Jurancich and W. S. S. Jee; Efflux of deep mineral store by parathyroid hormone and bone cells; 49th Annual Meeting IADR; Abstracts of Papers, pp. 107 (1971).
 12. Roberts, W. E. and W. S. S. Jee; Cortisol and the cellular kinetics of stimulated rat periodontal ligament; 49th Annual Meeting IADR; Abstracts of Paper, p. 257 (1971).
 13. Schafer, S., D. Higgenbottam, H. Z. Park, and E. I. Hashimoto; The distribution of ^3H -tetracycline in young mouse bone; Anat. Rec. 169: 418 (1971).
 14. Schafer, S., W. S. S. Jee, and R. B. Dell; The effect of cortisol on bone cell differentiation in vitro; 49th Annual Meeting IADR; Abstracts of Papers, p. 257 (1971).

15. Stevens, W., F. W. Bruenger, D. R. Atherton and Betsy J. Stover; Subcellular distribution of ^{241}Am (III) and ^{239}Pu (IV) in livers studied serially; Abstracts of Papers for the Nineteenth Annual Meeting of the Radiation Research Society, p. 97 (1971).
16. Stover, Betsy J., D. R. Atherton, D. S. Buster and F. W. Bruenger; Retention of ^{239}Pu (IV) by the young adult beagle; Abstracts of Papers for the Nineteenth Annual Meeting of the Radiation Research Society, p. 99 (1971).
17. Taylor, G. N., R. D. Lloyd, D. R. Atherton, and C. W. Mays; Radium induced eye tumors; 4th International Congress of Radiation Research, Evian, France; Livre des Resumes; p. 216 (1970).
18. Thurman, G. B., W. C. Mays, G. N. Taylor, and T. F. Dougherty; Growth dynamics of osteosarcomas induced in beagles by bone-seeking radionuclides; Book of Abstract Preprints for 19th Annual Meeting of the Radiation Research Society, Boston; Abstract Ce-11, pg. 22 (1971).
19. Whitson, S. W.; Microtubules and tight junctions in bone; Anat. Rec. 169: 454 (1971).
20. Whitson, S. W., J. Yee, H. Z. Park, and W. S. S. Jee; Dose response studies of estradiol on growing rat bones; 49th Annual Meeting IADR; Abstracts of Papers, pp. 106 (1971).
21. Yee, J., D. W. Young, G. H. Kenner, H. Z. Park and W. S. S. Jee; Structural activity relationship of corticosteroids to growing rabbit bones; 47th Annual Meeting IADR; Abstracts of papers, pp. 106 (1971).

COMPARATIVE EFFECTS OF ^{239}Pu AND ^{241}Am on BIOCHEMICAL PARAMETERS
EFFECTS OF DOSE AND RADIONUCLIDE BURDEN TIME

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Abstract: A comparison of the effects of ^{239}Pu and ^{241}Am on serum biochemical parameters has been made using the technique of multiple regression analysis. A separate analysis was made of data from male and female animals with plutonium animals also being separated into tumor and non-tumor groups. Two major differences were evident: ^{241}Am appears to produce significant changes in SGPT and serum alkaline phosphatase at earlier times post-injection than ^{239}Pu ; male beagle dogs appear to be more susceptible to radiation induced changes in SGPT, SGOT and serum alkaline phosphatase than females. The significance of these findings and their possible relationship to carcinogenesis and hormonal effects is discussed.

INTRODUCTION

Changes in serum biochemistry due to injected radionuclides have been studied in this laboratory since its inception 20 years ago. Thus far, the most sensitive responses have been changes in serum transaminases and alkaline phosphatase. Of the nuclides in our long-term study group, ^{239}Pu has produced the most marked alterations in serum levels of these enzymes. More recently, ^{241}Am has been added to the nuclides under investigation in our laboratory. Preliminary observation showed that these nuclides have two common features: (1) both deposit large amounts in the liver⁽¹⁾ and (2) both elicit large increases in the same serum enzymes⁽²⁾.

Increases in serum alkaline phosphatase correlate with both osteoblastic activity and liver function⁽³⁾. Increased alkaline phosphatase activity precedes the onset of clinically detectable osteogenic sarcomas and correlates with the incidence of this tumor, which is also produced by a number of other

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radionuclides^(4,5). Bone-seeking radionuclides produce osteogenic sarcomas as their major biologic end-point⁽⁶⁾.

Elevations in serum glutamic-pyruvic transaminase levels are specific indicators of hepatocellular necrosis in dogs^(7,8). Our laboratory has previously shown that changes in serum levels of this enzyme correlate with ^{239}Pu dosage⁽⁸⁾. In a preliminary report, we have also shown that ^{241}Am produces earlier post-injection rises in SGPT than ^{239}Pu . Serum glutamic oxaloacetic transaminase activity is a less specific indicator of hepatic damage, but dogs bearing ^{239}Pu also have significant increases in SGOT⁽²⁾.

Since ^{241}Am and ^{239}Pu appear to be potentially harmful to our ecosystem, a comparative evaluation of the effects of the two nuclides was undertaken. This study includes an evaluation of the effects of these two nuclides with respect to the sex of the animal and also with respect to radionuclide burden time and dose.

MATERIALS AND METHODS

The dogs used were purebred Beagles born and reared in our laboratory. The animals were injected with either ^{239}Pu or ^{241}Am in young adulthood. Twelve or more ^{239}Pu dogs were injected at each dose level in our regular program⁽⁹⁾. Five or more animals were injected as ^{241}Am test animals in a special study. While plutonium injected animals have been studied in this laboratory for 20 years, the more recent americium group has been studied for the last five years. Each animal received a single intravenous injection of the soluble nuclide in sodium citrate-citric acid buffer, pH3.5. Blood samples were taken for biochemical analysis at 1 to 3 month intervals. All biochemical determinations

were made using standard laboratory procedures. The control mean and 95% confidence limits used in this study were computed from data on zero level animals in our colony. The statistical technique used for the analysis of the data was multiple regression analysis. A similar technique has been reported previously⁽²⁾. Calculations for the statistical analysis were done on a Univac 1108 computer. The mathematical model for this analysis is: $Z = a + b(\mu) + c(t) + d(\mu)(t)$, where μ is dose in $\mu\text{C/kg}$, t is radionuclide burden time in months post-injection (MPI), and Z is the parameter being approximated. This model constitutes a truncated biquadratic, as the squared terms $e(\mu^2)$ and $f(t^2)$ have been deleted. It was found that addition or deletion of these squared terms did not significantly ($p < .05$) change the interpolatory character of this model for the data analyzed. Hence, this model was chosen for its simplicity and statistical significance. The equation can be rearranged to: $Z = (a + b\mu) + (c + d\mu) t$. For fixed values of μ , the dose level, this equation is a linear function of t . The intercept slope of this line can be interpreted as follows:

The intercept $(a + b\mu)$ can be thought of as the immediate effect produced by an injected burden and the sign of the constant b determines whether an increase in dose will cause an increase $(+b)$ or a decrease $(-b)$ in a particular parameter. If the constant b does not differ significantly from zero, then the isotope in question does not have a significant immediate effect upon the parameter.

The slope $(c + d\mu)$ of the line is the time rate of change effect produced by the isotope burden (μ) . Thus the effect measured by the slope becomes more

pronounced as time increases, for the magnitude of the slope measures the effect per unit of time. As for the constant d , the sign and magnitude of d is important in determining the burden's effect upon the parameter as time after injection increases.

Since the multiple regression technique was used for this statistical analysis, the slope and intercept determined are the maximum likelihood estimators. The maximum likelihood estimator of slope is used to compare dose effects in Table 1.

RESULTS

Figure 1 depicts the results obtained for SGPT measurements in male and female animals injected with ^{239}Pu and ^{241}Am . The plutonium animals were further separated into tumor and non-tumor groups. Since only one osteosarcoma has been identified in americium animals, this distinction was not applicable. The regression lines on the graphs are solid where the data is actual and dashed where extrapolations have been made. Note that as the dose increases from the 1 to the 3 to the 5 level, the effect of plutonium in female tumor animals appears to increase in terms of the slope of the regression line, while in males it is marked even at the 1 level. Some cross-over of the data appears between plutonium tumor and non-tumor animals in the females at 1 level, and in both sexes at the 3 and 5 levels. A rather interesting change occurs in americium male animals with the regression line changing from a negative slope at the 1 level to a positive slope in 5-level animals. A similar although somewhat less marked change occurs in female animals. The 1 level slope for Am male dogs is more negative than for females. Plutonium non-tumor

animals at the 1 and 3 levels show steeper slopes than the tumor animals. The negative slope obtained in 1 level americium animals is most prominent in male animals.

The results obtained from analysis of the data obtained by measuring serum glutamic oxaloacetic transaminase is presented in Figure 2. A tendency of the non-tumor plutonium injected animal to exhibit higher values than the tumor bearing plutonium animal is evident. This is true in all cases except for the 5 level male animal. The americium data shows some interesting results with the slope of the americium male regression line proceeding from a negative to a positive slope with increasing dose level. At level 5, the male values are higher than female values. Also, 1 level values for female americium non-tumor animals are higher throughout than for the plutonium non-tumor animals with lower values obtained at the 3 and 5 level. This may indicate a more drastic effect of americium. In terms of overall effects, the male animal again appears to evidence greater radiation-induced changes at the 3 and 5 dose levels. Note that the values for SGOT units are generally higher than those obtained in females.

Figure 3 contains the data obtained for alkaline phosphatase measurements in plutonium tumor and non-tumor animals and in americium animals without tumors. Generally, the slopes of the regression lines for plutonium tumor animals are in all cases steeper than those for plutonium non-tumor animals in both sexes. It should be noted that in the 5 level group, many animals die due to other effects of the radiation prior to the onset of osteogenetic sarcoma, a pathology known to cause elevated alkaline phosphatase measurements. In male plutonium

1 to 3 level animals, there is a cross-over of the tumor and non-tumor regression lines, while this is evident only in the 1 level female plutonium animals. The absolute values of the measurements at 120 days again increases in male animals as a function of increased dose. The same change occurs in female animals although the 120-day values are lower than in males. These findings taken along with those for SGPT and SGOT may mean a greater sensitivity to radionuclide induced changes in male animals than similar changes in females. The pattern observed in the americium-injected animals is that of a regression line that shows a negative slope which becomes increasingly positive in magnitude with increasing dose and actually positive in the 5 level male animals. Again, the change occurring in male americium-injected animals is much more drastic than the change in female animals receiving this radionuclide.

Table 1 shows the results obtained when the maximum likelihood estimator of slope of the line at various dose levels is calculated. Analysis of a maximum likelihood estimator in this fashion provides a comparison of the effect of dose. Two facts are most obvious from this table. First of all, the slope of the line increases with increasing dose level as we proceed from the 1 level figures on the left to the 5 level figures on the right. Secondly, the slope of the line is higher in male than in female animals within each test group compared (0.48 for plutonium male tumor at the 1 level vs. 0.031 for plutonium female tumor). Generally the absolute values of the slopes for SGPT are higher than those for SGOT. One exception for this obtains in americium non-tumor animals at the 1 and 3 levels. This may be due to the fact that the slopes are generally negative in these groups and that this parameter is being measured at

very early times in terms of months post-injection. This difference reverses for SGPT and SGOT values in the 5 level animals.

A difference obtains in alkaline phosphatase measurements where the plutonium injected female 1 level tumor animals show lower alkaline phosphatase values than female 1 level non-tumor animals. This difference reverses with the tumor animal's values being higher at the 3 and 5 levels where tumor incidence is higher. Again, the values for male animals are higher than for female with the exception of the 1 level non-tumor plutonium animals. The same sex difference obtains in americium-injected dogs for alkaline phosphatase. Also, these measurements in americium-injected dogs tend to become more positive with increasing dose level. These results show that dose level has a distinct effect on alkaline phosphatase values.

Table 2 shows the results obtained when the standard partial regression coefficients for the effects of $\mu\text{Ci/kg}$, months post-injection, and interaction are put into standard measure. The measurement with the smallest standard partial regression coefficient becomes the least common denominator, with a value of unity. The results show that the least effective variable in tumor animals tends to be months post-injection while $\mu\text{Ci/kg}$ and interaction [$\mu\text{C/kg (MPI)}$] are generally much more effective in all three of the tests compared in this study. In both non-tumor and tumor animals, interaction tends to be the most potent variable of the three, indicating that both dose and radionuclide burden time interact to cause changes in SGPT, SGOT and alkaline phosphatase.

Table 3 shows the cumulative data on the incidence of osteosarcomas in beagle dogs injected with plutonium-239. With exception of the 1 and 5 levels,

distribution of osteosarcomas is approximately equal between male and female animals. The incidence of osteosarcomas is slightly lower in males at the 5 level and higher in males at the 1 level as compared to females. Insufficient data are available to draw any significance from these differences at this time. The 3 and 5 level animals have all expired, while some of the 1 level animals are still living. The over-all incidence of osteosarcomas is approximately equal between the two sexes.

Figure 4 shows the results for individual measurements of SGPT in two plutonium 5 level and two americium 5 level animals. The time scale on the abscissa is the same for both graphs. However, the ordinate scale differs for the two nuclides. The horizontal line across the graph indicates a 95% confidence level for control (0 level) dogs in our colony. The SGPT measurement in americium treated animals rises above the 95% confidence level at 5 months post-injection while the first detectable increase in plutonium animals does not occur until the eighth month post-injection. Also, the elevation of this value in americium dogs is from two to four times the highest elevation in animals injected with plutonium-239.

Figure 5 shows a similar comparison for animals injected with plutonium and americium for the measurement of alkaline phosphatase. Again, the horizontal line of the graph indicates a 95% confidence level obtained from measurements in zero level dogs. The earliest increase in americium treated animals is at 5 months post-injection while the earliest increase in plutonium injected animals occurs 14 months post-injection. Again, increases in serum alkaline phosphatase in americium injected animals are far greater than those obtained

in plutonium injected animals. Thus an earlier and more marked elevation in this serum biochemical perimeter appears to occur in americium injected beagle dogs.

DISCUSSION

The regression lines for transaminase measurements in animals bearing burdens of plutonium and americium show several distinct effects. The alterations in these serum enzymes are frequently the greatest in male animals. This is indicated by the slopes of the regression lines, where the curves for males tend to be more positive in ^{239}Pu animals and more negative in ^{241}Am males than females. The transaminases measure hepatocellular necrosis and there may be a tendency in female animals to resist this type of damage more than in males. The negative slope of the line describing the response to americium is quite surprising. Since this measurement indicates hepatocellular necrosis, this slope may be a reflection of the shorter length of time that ^{241}Am has been studied. Only one osteogenic sarcoma tumor animal has been discovered in the americium study this far. Therefore, it was not possible to plot a line for americium tumor animals. Also, the 5-level americium animals died quite rapidly, precluding tumor development.

It is also interesting to note that in some instances the plutonium non-tumor animals exhibited higher transaminase levels than in the tumor animals. This may be indicative of future tumor incidence or a precancerous condition. The individual dogs will have to be studied further to establish whether or not this observation is of biologic significance. Prior studies on alkaline phosphatase have shown that this parameter is elevated before the appearance of

radiologically detectable disease⁽⁵⁾.

Changes observed in alkaline phosphatase measurements follow a pattern similar to that observed for transaminases. Again, the male animal exhibits a greater susceptibility to elevations of serum alkaline phosphatase activity. In this test, however, the slopes of the regression lines for plutonium tumor animals were higher than those for plutonium non-tumor animals. The slope of the regression line does not, however, reflect the elevation in serum alkaline phosphatase that occurs prior to the onset of clinically detectable osteosarcoma^(4,5). The slope of the regression line for americium injected animals is again negative at lower dose levels and becomes positive with a 5 level dose.

As another parameter of effect of dose level, we have calculated a maximum likelihood estimator for the slope of the regression line. These values, shown in Table 1 again indicate a more drastic response in male animals than in females and an increase in the response with increasing dose. Also, the response in the plutonium tumor animals is generally higher than that in non-tumor animals with some exceptions in the measurements made in female animals. Since dose-time interaction is a very significant factor (Table 2), this may account for the fact that our maximum likelihood estimators for americium are lower than those for plutonium. Americium treated animals have been injected for only a short period of time and although drastic changes occurred as reflected in the slope of the regression line, the maximum likelihood estimator of dose effect is not higher than that obtained with plutonium. The negative figures obtained with americium are from groups of animals that showed a negative regression at

lower dose levels.

When the standard partial regression coefficients from various groups of animals are put into standard measure, it appears that dose and dose-time interaction are the most significant factors for most of the plutonium tumor animals that we have studied. Radionuclide burden time tends to be more of a significant factor for changes in serum biochemical measurements in plutonium non-tumor animals than in tumor animals. Again, this may be due to the fact that we are dealing with a precancerous condition. Once pathology becomes clinically evident, dose becomes more statistically relevant. This is less true of americium treated animals, where the standard partial regression coefficient for dose is more prominent, particularly in the SGPT and alkaline phosphatase tests.

The comparison of tumor incidence in male and female plutonium injected animals shows no remarkable sex difference over the dose levels and time period that we have studied. This contrasts, of course, with the different responses in transaminases and alkaline phosphatase which we have observed in our study. Therefore, it appears that biochemical studies may reveal more subtle differences than clinically detectable carcinoma. Plutonium animals at level 1 and below are still surviving, and more obvious sex differences in the occurrence of osteosarcoma may appear in these animals.

One other indication of the differences between the effects of americium and plutonium on SGPT and alkaline phosphatase may be seen in Figures 4 and 5 where in both instances americium produced an earlier rise above the 95% confidence limit in the particular enzyme and also increased enzymatic activity to a

far higher level than plutonium injection. These results are quite comparable since both plutonium and americium animals have been studied over these shorter periods of time. A possible explanation for the difference is the amount of injected nuclide initially deposited in the liver. Plutonium injection deposits 32.5% of an injected dose⁽¹⁰⁾, while 48.7% of an injected dose of americium deposits⁽¹⁾ at the 3 dose level. The larger initial deposition of ^{241}Am could be responsible for the early onset of enzymatic changes.

The sex difference in the degree of change induced by these two nuclides is perhaps the most interesting finding in this study. Previously, we have demonstrated radiation-induced impairment of adrenal cortical steroid production^(11,12), hepatic steroid conjugation⁽¹³⁾, and formation of androgens in the testes^(14,15). All of these activities are intimately involved with the function of the reticuloendothelial system⁽¹⁶⁾. Fisher and Fisher have reported that substances that blockade the RES will produce an increase in serum transaminase⁽¹⁷⁾. Estrogens have been shown to stimulate RES activity⁽¹⁸⁾. It is possible that the presence of estrogen in the liver of female beagles contributes to the relative resistance to radiation reported here. Further studies on endocrine relationships will be the subject of future investigations.

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REFERENCES

1. Lloyd, R. D., C. W. Mays, G. N. Taylor and D. R. Atherton; Americium-241 retention in beagles; Research in Radiobiology, Univ. of Utah Report C00-119-241, 123-134 (1970).
2. Berliner, D. L., W. Stevens, C. J. Nabors, Jr. and R. E. Maxwell; Biochemical changes induced by internally deposited radionuclides in beagle dog blood. A statistical study. In: Delayed Effects of Bone-Seeking Radionuclides, C. W. Mays et al., Eds., Univ. of Utah Press, Salt Lake City, Utah, 471-488 (1969).
3. Dalgaard, J. B.; Serum and bile phosphatase in biliary fistula dogs; ACTA Physiol. Scandia. 16: 293-311 (1949).
4. Stevens, W., C. J. Nabors, Jr. and D. L. Berliner; A comparison of serum transaminase levels and other serum constituents in dogs burdened with ^{239}Pu , ^{228}Th , ^{228}Ra and ^{226}Ra ; Ann. N. Y. Acad. Sci. 145: 817-829, (1967).
5. Berliner, D. L., C. J. Nabors, Jr. and W. Stevens; Analysis of serum in beagles and possibilities of its use as predictors of radiation induced pathology. Bio-medical directors meeting of the A.E.C., Park City, Utah (1969).
6. Dougherty, T. F.; Incidence of bone cancer in internally irradiated dogs. In: Some Aspects of Internal Irradiation, T. F. Dougherty et al., Eds, Pergamon Press, New York, 47-57 (1962).
7. Zontine, W. J.; The transaminase test for liver necrosis in dogs; Can. Vet. J., 2: 9-19 (1961).
8. Stevens, W. and D. L. Berliner; Serum transaminase levels in beagle dogs burdened with plutonium-239; Radiation Res. 23: 420-429 (1964).

9. Dougherty, T. F., B. J. Stover, J. H. Dougherty, W. S. S. Jee, C. W. Mays, C. E. Rehfeld, W. R. Christensen and H. C. Goldthorpe; Studies of the biological effects of ^{226}Ra , ^{239}Pu , ^{228}Ra (MsTh.), ^{228}Th (RdTh) and ^{90}Sr in adult beagles; *Radiation Res.* 17: 625-681 (1962).
10. Stover, B. J., D. R. Atherton and D. S. Buster; $^{239}\text{Pu}(\text{IV})$: Its retention in the skeleton of the beagle (in press, 1971).
11. Nabors, C. J., Jr., D. M. Cazes and D. L. Berliner; A model system for the study of radiation effects on adrenal steroidogenesis; *Radiation Res.* 16: 557 (1962).
12. Berliner, D. L., W. Stevens and T. F. Dougherty; The effects of ionizing radiation on the biosynthesis and biotransformation of corticosteroids. In: Effects of Ionizing Radiation on the Reproductive System, W. D. Carlson and F. X. Gassner, Eds., Pergamon Press, New York, 433-449 (1964).
13. Stevens, W. and D. L. Berliner; The effect of X-irradiation on the conjugation of steroids by mouse liver and kidney; *Radiation Res.* 20: 510-518 (1963).
14. Ellis, L. C. and D. L. Berliner; The effects of ionizing radiation on endocrine cells. I. Steroid biotransformations and androgen production by testes from irradiated mice; *Radiation Res.* 20: 549-563 (1963).
15. Ellis, L. C. and D. L. Berliner; The effects of ionizing radiations on endocrine cells. III. Restoration of testosterone production with a reduced nicotinamide adenine dinucleotide phosphate-generating system after localized irradiation to heads of rats; *Radiation Res.* 25: 156-194 (1964).
16. Berliner, D. L., C. J. Nabors, Jr. and T. F. Dougherty; The role of hepatic and adrenal reticuloendothelial cells in steroid biotransformation; *J. Reticuloendothelial Soc.* 1: 1-17 (1964).

17. Fisher, E. R. and B. Fisher; Hepatic damage by reticuloendothelial interference; Arch. Pathol. 75: 191-195 (1963).
18. Nicol, T. and D. L. J. Bilbey; The effect of various steroids on the phagocytic activity of the reticuloendothelial system. In: Reticuloendothelial Structure and Function, J. H. Heller, Ed., Ronald Press, New York, 301-320 (1960).

Figure 1. Regression lines for serum glutamic pyruvic transaminase levels in ^{239}Pu injected osteosarcoma tumor animals (Δ), non-tumor animals ($\circ$) and ^{241}Am injected non-tumor animals ($\square$). The dashed portion of the lines represents an extrapolation of the data. Female animals injected with dose levels 1, 3 and 5 are on the left and males are on the right side of the page. The 95% confidence limit for elevated SGPT is 52 units.

Figure 2. Serum glutamic oxaloacetic transaminase regression lines for ^{239}Pu osteosarcoma animals (Δ), non-tumor animals ($\circ$) and ^{241}Am -injected non-tumor animals ($\square$). The dashed portion of the line represents extrapolated data. Female animals injected at dose levels 1, 3 and 5 are on the left, with comparable doses for males in the right hand column. The 95% confidence limit for elevated SGOT is 38 units.

Figure 3. Regression lines for serum alkaline phosphatase measurements in beagles bearing ^{239}Pu or ^{241}Am burdens. Data for ^{239}Pu osteosarcoma dogs (Δ), non-tumor dogs ($\circ$) and ^{241}Am non-tumor ($\square$) animals were analyzed by multivariable regression analysis. The dashed portion of the line represents extrapolated data. Female animals bearing 1, 3 and 5 level doses are graphed on the left and comparable males on the right.

Figure 4. Individual SGPT measurements for four 5 level dogs. The time scale on the abscissa is the same for both graphs, with the exception of the interruptions in the plutonium dogs' graph. Note the difference in the values for SGPT units on the ordinates of the two graphs. The horizontal lines represent the 95% confidence level for elevated measurements. The dogs, sexes and graph symbols are as follows: T41P5 (♀, ●); T53P5 (♂, ○); T1W5 (♂, △); T2W5 (♂, ▲).

Figure 5. Serum alkaline phosphatase values in four 5 level animals measured for the first 15 months post-injection. Note the different values for alkaline phosphatase units on the ordinates of the two graphs. The horizontal lines represent 95% confidence levels for elevated measurements. The dogs, sexes and graph symbols are as follows: T41P5(♀, ●); T53P5(♂, ○); T1W5(♂, △); T2W5(♂, ▲).

Maximum likelihood estimators for slopes of regression lines

$$\text{SLOPE} = c + d (\mu\text{c/kg})$$

1 LEVEL .016 $\mu\text{c/kg}$				3 LEVEL .30 $\mu\text{c/kg}$				5 LEVEL 2.8 $\mu\text{c/kg}$			
TEST	TUMOR Pu	NON TUMOR Pu	W	TEST	TUMOR Pu	NON TUMOR Pu	W	TEST	TUMOR Pu	NON TUMOR Pu	W
<u>SGPT</u>				<u>SGPT</u>				<u>SGPT</u>			
Female	.031	.722	-.037	Female	.074	1.269	.077	Female	8.4274	6.0782	1.0731
Male	.480	.469	-.285	Male	3.396	1.50	.080	Male	29.063	10.571	3.2902
<u>SGOT</u>				<u>SGOT</u>				<u>SGOT</u>			
Female	.051	.045	.121	Female	.177	.290	.156	Female	1.2839	2.4499	.4647
Male	.045	.205	-.308	Male	.549	.574	-.076	Male	4.9817	3.82	1.967
<u>Alk. Phos.</u>				<u>Alk. Phos.</u>				<u>Alk. Phos.</u>			
Female	.036	.071	-.018	Female	.120	.096	-.040	Female	.8623	.314	.23
Male	.107	.046	-.111	Male	.189	.122	-.049	Male	.9108	.7885	.499

Table 1. The slopes of the regression lines for SGPT, SGOT and serum alkaline phosphatase, calculated for three dose levels.