204
COO-119-246

Research in Radiobiology

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

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

**Respectfully Submitted by:
THOMAS F. DOUGHERTY, Director**

MARCH 31, 1972

CONTRACT No. AT (11-1)-119

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

Respectfully Submitted by:

Thomas F. Dougherty, Director

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GROUP LEADERS AND SECTIONS

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

Section

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

AVAILABILITY OF PREVIOUS REPORTS

Copies of our reports may be obtained from the 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
C00-119-246*	Mar 1972	Research in Radiobiology

* Also available on request from this laboratory.

CURRENT CENSUS OF THE BEAGLE COLONY

March 31, 1972

NUCLIDE	NUMBER OF DOGS
^{252}Cf (californium)	11
^{249}Cf (californium)	14
^{241}Am (americium)	83
^{239}Pu (plutonium)	149
^{228}Th (radiothorium)	25
^{228}Ra (mesothorium)	18
^{226}Ra (radium)	77
^{224}Ra (quickradium)	11
^{210}Pb (lead)	2
^{90}Sr (strontium)	31
X-ray	3
Ancillary (breeding and others)	43
Unassigned dogs	<u>126</u>
TOTAL	<u><u>593</u></u>

PREFACE TO THE 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" $\mu\text{Ci/kg}$ of ^{226}Ra , ^{228}Ra (Mesothorium), and ^{90}Sr , and 1.11 times the desired "retained" $\mu\text{Ci/kg}$ of ^{239}Pu 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 body retention is obviously meaningless unless the time after injection is specified.

Our present measurements indicate that:

average	^{226}Ra	retention = 0.25 at about 271 days
average	^{239}Pu	retention = 0.90 at about 6 days
average	^{228}Ra	retention = 0.25 at about 214 days
average	^{228}Th	retention = 0.90 at about 6 days
average	^{90}Sr	retention = 0.25 at about 134 days
average	^{241}Am	retention = 0.90 at about 6 days
average	^{249}Cf	retention = 0.90 at about 1 day
average	^{252}Cf	retention = 0.90 at about 1 day

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. 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 MIR0 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).

The standard beagle, referred to above, is assumed to have a skeleton which, in vivo, weights 1/10 of its weight at the time of injection. Measured values for this quantity are slowly accruing which will provide the basis for a revision of the estimates of radiation doses to the skeleton shown in the following tables. The revisions will result in an increase in estimated doses of less than 25%.

^{228}Ra doses deserve special comment. The dose from "pure" ^{228}Ra and

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

TABLE 1. TOXICITY ANIMALS (MAR. 31 1972)

A. RADIUM-226

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
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	2949	
F014R0.0	536	10.80		23 10 64	2716	
M015R0.0	564	12.80		4 2 65	2612	
F016R0.0	469	10.00		7 4 65	2550	
M017R0.0	469	12.50		27 4 66	2165	
F018R0.0	497	12.00		25 5 66	2137	
F019R0.0	533	8.42		13 10 66	1996	
M020R0.0	536	9.70		29 12 66	1919	
F021R0.0	549	9.90		26 1 67	1891	
M022R0.0	533	12.10		22 3 67	1836	
F031R0.0B	536	10.60		23 10 64	2716	
F031R0.0C	536	9.88		23 10 64	2716	
F031R0.0D	542	9.90		21 9 65	2383	
F032R0.0B	542	7.80		21 9 65	2383	
F032R0.0C	532	11.70		21 9 65	2383	
F032R0.0D	532	9.70		21 9 65	2383	
F033R0.0B	532	9.80		21 9 65	2383	
F033R0.0C	496	9.50		25 5 66	2137	
F033R0.0D	496	11.80		25 5 66	2137	
F034R0.0B	525	8.20		26 1 67	1891	
F034R0.0C	520	8.90		22 3 67	1836	
F034R0.0D	484	9.90		22 3 67	1836	
F035R0.0B	502	9.41		1 2 68	1520	
F035R0.0C	502	9.38		1 2 68	1520	
F035R0.0D	552	8.86		9 1 69	1177	
F036R0.0B	467	10.10		2 7 68	1368	
F036R0.0C	467	9.17		2 7 68	1368	
F036R0.0D	467	9.08		2 7 68	1368	
F037R0.0B	601	11.10		20 5 69	1046	
F037R0.0C	501	10.40		23 9 70	555	
F037R0.0D	501	10.90		23 9 70	555	

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 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F038R0.0B	501	11.50		23	9	70	555	
F042R0.0B	338	8.00		25	4	69		33
M013R0.2	529	9.77	0.00577	4	3	64	2949	
F014R0.2	460	8.10	0.00836	23	10	64	2716	
M015R0.2	504	10.80	0.00873	4	2	65	2612	
F016R0.2	486	8.90	0.00665	7	4	65	2550	
M017R0.2	494	11.80	0.00711	27	4	66	2165	
F018R0.2	497	9.30	0.00652	25	5	66	2137	
F019R0.2	533	10.60	0.00785	13	10	66	1996	
M020R0.2	546	11.40	0.00676	29	12	66	1919	
F021R0.2	549	11.50	0.00687	26	1	67	1891	
M022R0.2	533	12.90	0.00961	22	3	67	1836	
M013R0.5	529	11.00	0.0171	4	3	64	2949	
F014R0.5	510	9.75	0.022	23	10	64	2716	
M015R0.5	490	10.40	0.0263	4	2	65	2612	
F016R0.5	500	11.40	0.0205	7	4	65	2550	
M017R0.5	494	9.20	0.0215	27	4	66	2165	
F018R0.5	496	9.10	0.0197	25	5	66	2137	
F019R0.5	533	10.00	0.023	13	10	66	1996	
M020R0.5	536	13.20	0.0206	29	12	66	1919	
F021R0.5	538	8.80	0.0208	26	1	67	1891	
M022R0.5	520	12.30	0.029	22	3	67	1836	
M031R0.5B	508	11.40	0.021	27	4	66	2165	
F031R0.5C	537	9.40	0.0235	22	12	65	2291	
F031R0.5D	537	11.70	0.0238	22	12	65	2291	
M032R0.5B	496	13.40	0.0196	25	5	66	2137	
F032R0.5C	519	10.10	0.0239	22	12	65	2291	
F032R0.5D	509	10.10	0.024	22	12	65	2291	
M033R0.5B	497	12.90	0.0194	25	5	66	2137	
F033R0.5C	527	10.60	0.0212	27	4	66	2165	
F033R0.5D	527	8.70	0.0217	27	4	66	2165	
M034R0.5B	496	10.50	0.0196	25	5	66	2137	
F034R0.5C	524	9.90	0.0215	27	4	66	2165	
F034R0.5D	508	9.70	0.0212	27	4	66	2165	
M035R0.5B	536	10.40	0.0205	29	12	66	1919	
F035R0.5C	532	9.00	0.0201	29	12	66	1919	
F035R0.5D	532	10.20	0.0202	29	12	66	1919	

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 31/3/72 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	5462	150
F012R1.0	501	9.71	0.0444	17 1 56	3978	124
M013R1.0	529	11.70	0.0527	4 3 64	2949	
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	2550	
M017R1.0	494	11.40	0.0639	27 4 66	2165	
F018R1.0	496	10.00	0.0589	25 5 66	2137	
F019R1.0	526	11.60	0.0682	13 10 66	1996	
M020R1.0	536	10.00	0.061	29 12 66	1919	
F021R1.0	525	8.10	0.0633	26 1 67	1891	
M022R1.0	484	10.90	0.0861	22 3 67	1836	
F031R1.0B	509	10.40	0.0712	22 12 65	2291	
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	3254	730
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	4832	
F011R1.7	598	8.17	0.165	30 11 56	5324	300
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	MELANOMA (EYE); MAMMARY CARCINOMA
F012R1.0	MELANOMA, GINGIVA
M013R1.0	
F014R1.0	UNDETERMINED (NO NEOPLASIA)
M015R1.0	UNDETERMINED (NO NEOPLASIA)
F016R1.0	
M017R1.0	
F018R1.0	
F019R1.0	
M020R1.0	
F021R1.0	
M022R1.0	
F031R1.0B	
M001R1.7	OBTURATING ABDOMINAL AORTA AND PULMONARY EMBOLISM
M002R1.7	LYMPHOSARCOMA
M002R1.7A	FIBROSARCOMA (GINGIVA)
F003R1.7	MAMMARY GLAND CARCINOMA
M004R1.7	NEPHRITIS
M005R1.7	OSTEOSARCOMA
F006R1.7	PULMONARY CALCIFICATION (NO BONE TUMOR)
M007R1.7	BACTERIAL TOXEMIA, INTERSTITIAL CELL ADENOMA
F008R1.7	DRUG ALLERGY
F009R1.7	PYOMEIRA
M010R1.7	TRAUMA
M010R1.7A	
F011R1.7	MELANOMA (ORAL); THYROID CARCINOMA
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 31/3/72	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	525	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	PYOMEIRITIS + 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		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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 (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72	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		2949	
F014P0.0	452	9.89		12 5 64		2880	
M015P0.0	527	12.10		23 10 64		2716	
M016P0.0	486	13.90		7 4 65		2550	
M017P0.0	551	12.20		18 11 66		1970	
F018P0.0	536	11.40		29 11 66		1949	
M019P0.0	536	13.10		29 11 66		1949	
F020P0.0	546	8.50		29 12 66		1919	
M021P0.0	549	13.30		26 1 67		1891	
F022P0.0	489	10.60		25 5 67		1772	
M031P0.0B	452	11.80		12 5 64			1763
M031P0.0C	452	12.60		12 5 64		2880	
M032P0.0B	452	11.20		12 5 64		2880	
M032P0.0C	542	10.30		21 9 65		2383	
M033P0.0B	517	12.10		21 9 65		2383	
M033P0.0C	503	11.70		18 11 65		2325	
M034P0.0B	525	13.50		26 1 67		1891	
M034P0.0C	484	12.70		22 3 67		1836	
M035P0.0B	484	12.50		22 3 67		1836	
M035P0.0C	484	13.10		22 3 67		1836	
M036P0.0B	489	11.00		25 5 67		1772	
M036P0.0C	485	12.20		25 5 67		1772	
M037P0.0B	507	11.70		22 6 67		1744	
M037P0.0C	493	10.40		22 6 67		1744	
M038P0.0B	529	10.70		16 11 67		1597	
M038P0.0C	529	12.20		16 11 67		1597	
M039P0.0B	501	10.70		21 12 67		1562	
M039P0.0C	501	10.10		21 12 67		1562	
M040P0.0B	484	10.30		30 7 68		1340	
M040P0.0C	552	11.40		9 1 69		1177	
M041P0.0B	560	9.49		17 1 69		1169	

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		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M042P0.0B	538	11.60		25	4	69	32	
M081P0.0Y	105	7.10		9	3	72	22	
F082P0.0Y	105	7.30		9	3	72	22	
F013P0.1	515	9.46	0.00068	4	3	64	2949	
F014P0.1	452	10.30	0.00055	12	5	64	2880	
M015P0.1	527	9.67	0.00071	23	10	64	2716	
M016P0.1	501	12.00	0.00059	7	4	65	2550	
M017P0.1	551	12.20	0.00057	18	11	66	1970	
F018P0.1	536	9.28	0.0007	29	11	66	1949	
M019P0.1	536	11.60	0.00063	29	11	66	1949	
F020P0.1	536	9.80	0.00075	29	12	66	1919	
M021P0.1	538	11.30	0.00059	26	1	67	1891	
F022P0.1	489	9.80	0.00059	25	5	67	1772	
M031P0.1B	517	12.20	0.00068	4	3	64	2760	1
F032P0.1B	549	10.40	0.00059	18	11	65	2325	
M033P0.1B	549	10.80	0.00079	18	11	65	2325	
F034P0.1B	533	11.10	0.00058	18	11	66	1970	
M035P0.1B	489	10.30	0.00059	25	5	67	1772	
F036P0.1B	495	9.79	0.0006	22	6	67	1744	
M037P0.1B	493	11.30	0.00059	22	6	67	1744	
F038P0.1B	513	9.52	0.00057	21	12	67	1562	
M039P0.1B	490	10.50	0.00058	21	12	67	1562	
F044P0.1C	569	8.34	0.00072	2	12	70	485	
F013P0.2	517	9.44	0.00206	4	3	64	2949	
F014P0.2	516	7.44	0.00173	12	5	64	2880	
M015P0.2	505	10.90	0.00201	23	10	64	2716	
M016P0.2	500	11.40	0.00163	7	4	65	2550	
M017P0.2	533	11.80	0.00171	18	11	66	1970	
F018P0.2	530	9.46	0.002	29	11	66	1949	
M019P0.2	530	12.10	0.00198	29	11	66	1949	
F020P0.2	532	8.30	0.00224	29	12	66	1919	
M021P0.2	538	12.10	0.00181	26	1	67	1891	
F022P0.2	485	8.30	0.00176	25	5	67	1772	
M031P0.2B	515	10.70	0.00185	4	3	64	2640	1
F031P0.2C	452	11.90	0.00169	12	5	64	2880	
F031P0.2D	429	9.35	0.00186	12	5	64	2880	
M032P0.2B	549	13.60	0.00178	18	11	65	2325	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M042P0.0B SPECIAL STUDY
M081P0.0Y
F082P0.0Y

F013P0.1
F014P0.1
M015P0.1
M016P0.1
M017P0.1
F018P0.1
M019P0.1
F020P0.1
M021P0.1
F022P0.1
M031P0.1B STATUS EPILEPTICUS
F032P0.1B
M033P0.1B
F034P0.1B
M035P0.1B
F036P0.1B
M037P0.1B
F038P0.1B
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 MELANOMA (ORAL)
F031P0.2C
F031P0.2D
M032P0.2B

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F032P0.2C	494	10.10	0.00183	4	2	65	2612	
F032P0.2D	490	8.04	0.00193	4	2	65	2612	
M033P0.2B	513	14.50	0.00178	18	11	65	2325	
F033P0.2C	549	12.50	0.00176	18	11	65	2325	
F033P0.2D	513	12.70	0.00178	18	11	65	2325	
M034P0.2B	533	12.70	0.0017	18	11	66	1970	
F034P0.2C	533	11.50	0.00172	18	11	66	1970	
F034P0.2D	519	9.92	0.00167	18	11	66	1970	
M035P0.2B	489	11.20	0.00173	25	5	67	1772	
F035P0.2C	507	10.50	0.00175	22	6	67	1744	
F035P0.2D	507	9.10	0.00175	22	6	67	1744	
M036P0.2B	479	12.90	0.00177	25	5	67	1772	
F036P0.2C	493	10.40	0.00177	22	6	67	1744	
F036P0.2D	569	8.74	0.00146	16	11	67	1597	
M037P0.2B	529	10.60	0.00149	16	11	67	1597	
F037P0.2C	529	10.10	0.0015	16	11	67	1597	
F037P0.2D	529	7.14	0.00153	16	11	67	1597	
M038P0.2B	517	10.00	0.00152	16	11	67	1597	
F038P0.2C	501	7.95	0.00211	21	12	67	1562	
F038P0.2D	499	9.08	0.00176	21	12	67	1562	
M039P0.2B	542	11.60	0.00214	2	12	70	485	
F039P0.2C	499	9.46	0.00173	21	12	67	1562	
F039P0.2D	499	9.34	0.00176	21	12	67	1562	
F042P0.2C	589	9.55	0.00176	4	9	69	939	
F043P0.2C	542	9.50	0.00239	2	12	70	485	
M044P0.2B	540	11.60	0.00177	17	11	71	97	1
F013P0.5	517	9.93	0.0054	4	3	64	2388	19
F013P0.5A	501	11.20	0.00495	23	9	70	555	
F014P0.5	516	9.98	0.00493	12	5	64	2880	
M015P0.5	505	8.41	0.00627	23	10	64	2716	
M016P0.5	501	12.60	0.00521	7	4	65	2550	
M017P0.5	533	13.40	0.00506	18	11	66	1970	
F018P0.5	530	8.98	0.00594	29	11	66	1949	
M019P0.5	530	11.90	0.00645	29	11	66	1949	
F020P0.5	532	9.30	0.00553	29	12	66	1919	
M021P0.5	538	9.80	0.00526	26	1	67	1891	
F022P0.5	485	8.10	0.00525	25	5	67	1772	
M031P0.5B	515	10.50	0.00549	4	3	64	1648	14
F031P0.5C	494	8.44	0.00572	4	2	65	2546	14
M032P0.5B	549	13.60	0.00546	18	11	65	2275	18

DOG NUMBER	COMMENTS ON DEAD DOGS
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F032P0.2C
F032P0.2D
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
M044P0.2B

SPECIAL STUDY

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 SPECIAL STUDY
M032P0.5B SPECIAL STUDY

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F033P0.5B	503	10.10	0.00559	18	11	65	2325	
M034P0.5B	530	12.50	0.00642	29	11	66	1920	18
F035P0.5B	501	9.54	0.0052	22	6	67	1744	
M036P0.5B	479	11.50	0.00527	25	5	67	1772	
F037P0.5B	517	8.39	0.00454	16	11	67	1597	
M038P0.5B	517	10.50	0.00448	16	11	67	1597	
F039P0.5B	490	10.90	0.00528	21	12	67	1562	
M042P0.5B	542	13.10	0.00675	2	12	70	485	
M042P0.5C	542	12.20	0.00668	2	12	70	485	
F042P0.5D	542	9.71	0.00668	2	12	70	485	
F043P0.5B	545	11.60	0.00484	3	10	69	910	
F043P0.5C	535	10.70	0.0048	3	10	69	910	
M044P0.5B	445	11.50	0.0036	3	6	69	99	1
M045P0.5B	472	10.30	0.0035	3	6	69	42	1
F045P0.5C	540	10.00	0.00516	17	11	71	35	1
M046P0.5B	484	11.80	0.00336	3	6	69	7	1
F046P0.5C	540	9.15	0.00516	17	11	71	7	1
F014P0.7	533	8.98	0.00947	22	7	69	983	
M015P0.7	533	10.30	0.00941	22	7	69	983	
M016P0.7	516	11.90	0.0102	4	9	69	939	
M017P0.7	540	8.04	0.0103	3	10	69	910	
F018P0.7	521	9.66	0.00942	22	7	69	983	
M019P0.7	501	11.60	0.0104	23	9	70	555	
F020P0.7	521	9.18	0.00926	22	7	69	983	
M021P0.7	499	11.10	0.0104	23	9	70	555	
F022P0.7	538	9.69	0.0108	4	9	69	939	
F023P0.7	538	9.56	0.0108	4	9	69	939	
F024P0.7	516	8.90	0.011	4	9	69	939	
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

DOG
NUMBER

COMMENTS ON DEAD DOGS

F033P0.5B	
M034P0.5B	SPECIAL STUDY
F035P0.5B	
M036P0.5B	
F037P0.5B	
M038P0.5B	
F039P0.5B	
M042P0.5B	
M042P0.5C	
F042P0.5D	
F043P0.5B	
F043P0.5C	
M044P0.5B	SPECIAL STUDY
M045P0.5B	SPECIAL STUDY
F045P0.5C	SPECIAL STUDY
M046P0.5B	SPECIAL STUDY
F046P0.5C	SPECIAL STUDY

F014P0.7
M015P0.7
M016P0.7
M017P0.7
F018P0.7
M019P0.7
F020P0.7
M021P0.7
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 LYMPHADENOPHATHY
M008P1.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	4958	
F014P1.0	533	10.40	0.0141	22	7	69	983	
M015P1.0	516	12.80	0.0159	4	9	69	939	
M016P1.0	516	10.60	0.0165	4	9	69	939	
M017P1.0	537	10.90	0.0151	3	10	69	910	
F018P1.0	531	9.89	0.014	22	7	69	983	
M019P1.0	501	9.82	0.0159	23	9	70	555	
F020P1.0	521	10.40	0.0141	22	7	69	983	
M021P1.0	499	10.00	0.0156	23	9	70	555	
F022P1.0	531	9.04	0.0139	22	7	69	983	
F023P1.0	538	11.20	0.0163	4	9	69	939	
F024P1.0	516	10.40	0.0163	4	9	69	939	
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

DOG NUMBER	COMMENTS ON DEAD DOGS
F009P1.0	OSTEOSARCOMA
F010P1.0	MAMMARY CARCINOMA
M011P1.0	THYROID CARCINOMA
M012P1.0	CHRONIC PANCREATITIS
M013P1.0	
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)

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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
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

DOG
NUMBER

COMMENTS ON DEAD DOGS

M008P2.0	ASPIRATION PNEUMONIA
F009P2.0	OSTEOSARCOMA
F010P2.0	OSTEOSARCOMA
M011P2.0	OSTEOSARCOMA
M012P2.0	OSTEOSARCOMA

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

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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
M081P5.0Y	94	4.60	2.68	1	3	72	30	
F082P5.0Y	94	4.80	2.66	1	3	72	30	
F083P5.0Y	94	4.00	2.66	1	3	72	30	
F084P5.0Y	94	3.55	2.68	1	3	72	30	
F085P5.0Y	94	4.15	2.64	1	3	72	30	

DOG

NUMBER

COMMENTS ON DEAD DOGS

F006P5.0	OSTEOSARCOMA
F007P5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
M008P5.0	GINGIVITIS
F009P5.0	OSTEOSARCOMA, EPISTAXIS + CIRCULATORY COLLAPSE
M001P5.0Y	
F002P5.0Y	
F003P5.0Y	
F004P5.0Y	
F005P5.0Y	

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F001M0.0	732	7.33		4	1	54	3451	
F002M0.0	545	6.94		29	11	54	6155	
M003M0.0	579	13.00		13	3	56	5056	
M004M0.0	601	10.30		15	1	57	4816	
F005M0.0	671	11.20		5	3	57	4581	
M006M0.0	492	7.56		23	4	57	4934	
F007M0.0	395	8.71		4	6	57	1414	
F007M0.0A	594	10.90		15	1	63	3363	
F008M0.0	654	11.60		9	3	60	4405	
M009M0.0	575	12.40		13	4	60	4132	
M010M0.0	581	13.30		17	7	62	2991	
F011M0.0	475	9.31		18	9	62	3248	
M012M0.0	695	10.00		22	12	60	4117	
F001M0.5	492	9.47	0.0173	17	7	62	3545	
F002M0.5	492	9.15	0.0173	17	7	62	3545	
M003M0.5	493	10.80	0.0199	18	9	62	3482	
M004M0.5	475	12.80	0.0199	18	9	62	3482	
F005M0.5	534	7.83	0.0172	23	10	62	3447	
M006M0.5	510	10.30	0.0171	23	10	62	3019	71
F007M0.5	492	8.87	0.0172	17	7	62	3545	
F008M0.5	654	12.60	0.0159	9	3	60	4208	70
M009M0.5	485	11.90	0.017	13	4	60	4370	
M010M0.5	492	10.60	0.0174	17	7	62	3545	
F011M0.5	505	7.82	0.0202	18	9	62	3482	
M012M0.5	510	10.60	0.0165	23	10	62	3447	
F001M1.0	718	7.75	0.0463	4	1	54	2952	196
F001M1.0A	590	8.07	0.0512	23	10	62	3447	
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	4370	
M010M1.0	492	9.43	0.0501	17	7	62	2966	213
F011M1.0	505	8.91	0.0613	18	9	62	3482	

DOG
NUMBER

COMMENTS ON DEAD DOGS

FG01M0.0	PURULENT MENINGOENCEPHALITIS
FG02M0.0	UNDETERMINED; SENILITY
MG03M0.0	BRAIN INFARCTION
MG04M0.0	VALVULAR ENDOCARDITIS; MYOCARDIAL INFARCTION
FG05M0.0	MAMMARY CARCINOMA
MG06M0.0	NEPHRITIS
FG07M0.0	STATUS EPILEPTICUS
FG07M0.0A	
FG08M0.0	
MG09M0.0	VIRUS PNEUMONIA
MG10M0.0	MALIGNANT MELANOMA (ORAL)
FG11M0.0	ASPIRATION PNEUMONIA
MG12M0.0	

FG01M0.5	
FG02M0.5	
MG03M0.5	
MG04M0.5	
FG05M0.5	
MG06M0.5	MALIGNANT MELANOMA (EYE)
FG07M0.5	
FG08M0.5	BACTERIAL ENTERITIS
MG09M0.5	
MG10M0.5	
FG11M0.5	
MG12M0.5	

FG01M1.0	SARCOMA (SPLEEN)
FG01M1.0A	
FG02M1.0	OSTEOSARCOMA
MG03M1.0	OSTEOSARCOMA
MG04M1.0	PNEUMONIA; PANCREATITIS
FG05M1.0	MALIGNANT MELANOMA (EYE)
MG06M1.0	EPIDERMOID CARCINOMA (PENIS)
FG07M1.0	SARCOMA (HEART)
FG08M1.0	MALIGNANT MELANOMA (EYE)
MG09M1.0	
MG10M1.0	MALIGNANT MELANOMA (EYE)
FG11M1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
M012M1.0	528	9.27	0.0498	23 10 62	3447	
F001M1.7	510	7.52	0.151	23 10 62	3447	
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	3325	1010
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

MO12M1.0

FO01M1.7	
FO02M1.7	OSTEOSARCOMA
MO03M1.7	OSTEOSARCOMA
MO04M1.7	CARCINOMA SMALL INTESTINE
FO05M1.7	OSTEOSARCOMA
MO06M1.7	OSTEOSARCOMA
FO07M1.7	OSTEOSARCOMA
FO08M1.7	OSTEOSARCOMA
MO09M1.7	OSTEOSARCOMA
MO10M1.7	OSTEOSARCOMA
FO11M1.7	OSTEOSARCOMA
MO12M1.7	UNKNOWN

FO01M2.0	OSTEOSARCOMA
FO02M2.0	INTESTINAL HEMORRHAGE
MO03M2.0	PNEUMONIA
MO04M2.0	OSTEOSARCOMA
FO05M2.0	OSTEOSARCOMA
MO06M2.0	OSTEOSARCOMA
FO07M2.0	CHRONIC PANCREATITIS
FO08M2.0	OSTEOSARCOMA
MO09M2.0	OSTEOSARCOMA
MO10M2.0	OSTEOSARCOMA
FO11M2.0	OSTEOSARCOMA
MO12M2.0	OSTEOSARCOMA

FO01M3.0	OSTEOSARCOMA
FO02M3.0	OSTEOSARCOMA
MO03M3.0	OSTEOSARCOMA
MO04M3.0	OSTEOSARCOMA
FO05M3.0	OSTEOSARCOMA
MO06M3.0	OSTEOSARCOMA
FO07M3.0	OSTEOSARCOMA
FO08M3.0	OSTEOSARCOMA
MO09M3.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 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 Ci $^{228}\text{Th}/\mu$ Ci ^{228}Ra) injected = 0.15 for F1M1.0, 2.0, 3.0, 4.0, 5.0.
= 0.03 for F2M1.0, 1.7, 2.0, 3.0, 4.0, 5.0,
M3M1.0, 1.7, 2.0, 3.0, 4.0, 5.0.
= 0.006 for groups 4, 5, 6, 7, 8, 9, 10, 11,
12, and dogs F1M0.5, F2M0.5,
M3M0.5, F1M1A, F1M1.7 and M3M4.0A.

DOG
NUMBER

COMMENTS ON DEAD DOGS

M010M3.0 OSTEOSARCOMA
F011M3.0 OSTEOSARCOMA
M012M3.0 OSTEOSARCOMA

F001M4.0 OSTEOSARCOMA + CRIPPLING FRACTURE
F002M4.0 OSTEOSARCOMA
M003M4.0 STRANGULATED INGUINAL HERNIA
M003M4.0A OSTEOSARCOMA, NEPHRITIS, ULCERATIVE GINGIVITIS + PNEUMONIA
M004M4.0 FRACTURED MANDIBLE + ULCERATIVE GINGIVITIS
F005M4.0 OSTEOSARCOMA
M006M4.0 OSTEOSARCOMA
F007M4.0 OSTEOSARCOMA

F001M5.0 NEPHRITIS + SEVERE ANEMIA
F002M5.0 CRIPPLING FRACTURES
M003M5.0 ULCERATIVE GINGIVITIS
M004M5.0 CRIPPLING FRACTURE
F005M5.0 ULCERATIVE GINGIVITIS
M006M5.0 OSTEOSARCOMA + CRIPPLING FRACTURE
F007M5.0 ULCERATIVE GINGIVITIS, MYOCARDIAL INFARCTION + GLAUCOMA

D. THORIUM-228 (RADIOTHORIUM)

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	4917	
F006T0.0	489	9.64		10	1	61	171	
F006T0.0A	686	8.61		15	12	60	4124	
M007T0.0	517	10.50		7	2	61	1412	
M008T0.0	533	10.80		24	5	61	3964	
F009T0.0	569	8.28		29	6	61	3928	
F010T0.0	536	10.40		28	7	61	3899	
F011T0.0	530	9.45		4	6	63	3223	
F012T0.0	492	9.09		9	7	63	3188	
M001T0.2	682	11.40	0.00164	27	3	62	3657	
M002T0.2	682	10.40	0.00166	27	3	62	3657	
F003T0.2	478	9.86	0.00163	27	3	62	3657	
M004T0.2	478	10.00	0.00166	27	3	62	3657	
M005T0.2	625	13.80	0.00162	9	2	60	889	10
M005T0.2A	530	13.40	0.00173	4	6	63	3223	
F006T0.2	489	8.85	0.00176	10	1	61	4098	
M007T0.2	532	10.50	0.00159	7	2	61	3897	15
M008T0.2	494	13.90	0.00189	24	5	61	3964	
F009T0.2	569	7.82	0.00171	29	6	61	3897	16
F010T0.2	508	10.50	0.0017	28	7	61	3899	
F011T0.2	530	9.76	0.00171	4	6	63	3223	
F012T0.2	492	7.37	0.0019	9	7	63	3188	
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	4856	50
F006T0.5	489	9.66	0.0051	10	1	61	4098	
M007T0.5	532	9.11	0.00491	7	2	61	4070	
M008T0.5	533	9.53	0.00562	24	5	61	3964	
F009T0.5	569	8.62	0.00529	29	6	61	3928	
F010T0.5	508	10.20	0.0051	28	7	61	3899	
F011T0.5	530	7.78	0.00518	4	6	63	3223	

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	UNDIFFERENTIATED MALIGNANCY (INTESTINE)
M008T0.2	
F009T0.2	PULMONARY THROMBO EMBOLISM
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	PROSTATITIS
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 31/3/72 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	3964	
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	3223	
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	3110	280
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 OSTEOSARCOMA; (PNEUMONIA)
M008T1.5 OSTEOSARCOMA
F009T1.5 OSTEOSARCOMA
F010T1.5 OSTEOSARCOMA
F011T1.5 OSTEOSARCOMA
F012T1.5 OSTEOSARCOMA

M001T2.0 OSTEOSARCOMA
M002T2.0 OSTEOSARCOMA
F003T2.0 OSTEOSARCOMA
M004T2.0 TRAUMA
M004T2.0A OSTEOSARCOMA
M005T2.0 OSTEOSARCOMA
F006T2.0 OSTEOSARCOMA
M007T2.0 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	31/3/72	DEATH	
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 AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72	DEATH	DOSE TO SKELETON (RADS)
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	5281		
M005S0.0	542	10.60		19 11 57		4158	
M006S0.0	466	9.68		27 5 58		4726	
F007S0.0A	462	9.46		7 1 59		3303	
F008S0.0	483	9.29		19 5 59		4482	
F009S0.0	549	12.40		11 8 59		708	
F009S0.0A	535	11.20		4 6 63	3223		
M010S0.0	522	13.90		29 9 59	4567		
F011S0.0	541	9.60		3 11 59	4532		
M012S0.0	605	8.99		6 1 60	4468		
F001S1.0	1525	6.84	0.573	18 1 55		308	21
F001S1.0A	521	9.38	0.588	14 2 56	5890		
M002S1.0	567	8.81	0.606	14 2 56		5077	90
M003S1.0	493	10.90	0.572	11 9 57	5315		
F004S1.0	525	8.96	0.56	15 10 57	5281		
M005S1.0	555	10.20	0.532	19 11 57		2705	71
M006S1.0	466	9.56	0.581	27 5 58	5057		
F007S1.0	524	9.94	0.517	11 11 58	4889		
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	4567		
F011S1.0	543	10.30	0.55	3 11 59	4532		
M012S1.0	607	13.70	0.559	6 1 60		4184	100
F001S1.7	526	7.41	1.78	14 2 56		5624	470
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	3313		
M006S1.7	466	10.60	1.72	27 5 58	5057		
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	4616		
M010S1.7	519	13.60	1.66	29 9 59		2947	306

DOG NUMBER	COMMENTS ON DEAD DOGS
F001S0.0	CARCINOMA PANCREAS
M002S0.0	BRONCHOGENIC CARCINOMA
M003S0.0	OBTURATING AORTIC EMBOLISM, NEPHRITIS
F004S0.0	
M005S0.0	TRANSITIONAL CELL CARCINOMA
M006S0.0	MELANOMA (ORAL)
F007S0.0A	DIABETES MELLITUS
F008S0.0	DIABETES MELLITUS
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	BILIARY OBSTRUCTION
F001S1.7	HEMANGIOENDOTHELIAL SARCOMA (LIVER)
M002S1.7	HEMANGIOSARCOMA (SOFT TISSUE ORIGIN)
M003S1.7	OBTURATING THROMBOEMBOLISM, AORTA
F004S1.7	PANCREATIC CARCINOMA
M005S1.7	COMA OF UNKNOWN ETIOLOGY (NO BONE TUMOR)
M005S1.7A	
M006S1.7	
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 31/3/72 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	4468	
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	5057	
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	4532	
M012S2.0	607	11.60	3.49	6 1 60	4468	
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	5057	
F007S3.0	486	9.80	10.1	11 11 58	4018	1816
F008S3.0	465	12.50	12.9	19 5 59	4700	
F009S3.0	468	10.00	10.1	11 8 59	4599	2660
M010S3.0	519	12.50	10.3	29 9 59	2898	2099
F011S3.0	541	9.00	10.8	3 11 59	4532	
M012S3.0	605	8.43	10.2	6 1 60	4468	
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	4664	7920
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 CHROMAPHOBE ADENOMA
M010S3.0 FIBROSARCOMA (GINGIVA)
F011S3.0
M012S3.0

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

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
F009S4.0	468	9.56	30.6	11 8 59	4616	
M010S4.0	517	8.20	31.3	29 9 59	4242	6370
F011S4.0	542	8.86	32.7	3 11 59	2114	3324
M012S4.0	605	10.90	32.3	6 1 00	4226	5290
F001S4.5	530	9.00	64.2	16 3 06	2207	
M002S4.5	530	12.20	63.6	16 3 06	2207	
M003S4.5	530	11.90	63.8	16 3 66	1493	5949
F004S4.5	530	9.80	64.5	16 3 66	2197	9100
M005S4.5	496	13.30	61.3	16 3 66	993	5424
M006S4.5	496	12.00	63.8	16 3 66	2207	
F007S4.5	511	9.90	64.5	16 3 66	2207	
F008S4.5	511	9.90	64.5	16 3 06	2207	
F009S4.5	511	10.30	64.0	16 3 06	1028	5201
M010S4.5	496	14.00	60.9	16 3 66	2064	10680
F011S4.5	496	11.90	63.8	16 3 66	1758	6434
M012S4.5	485	11.40	63.7	16 3 66	2207	
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

MENINGIOMA; PERIANAL GLAND CARCINOMA
BLOOD DYSCRASIA, PYOMETRA
ADENOCARCINOMA NASAL CAVITY

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
HEMANGIOSARCOMA (SPLEEN)
OSTEOSARCOMA

OSTEOSARCOMA
SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
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	AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
F013W0.2	533	10.60	0.00179	13 10 66	1996	
M014W0.2	533	14.90	0.00178	13 10 66	1996	
F024W0.2	477	11.30	0.00181	21 3 68	1471	
F031W0.2	472	10.90	0.0018	8 5 68	1423	
M041W0.2	467	11.90	0.0018	2 7 68	1368	
M048W0.2	484	11.00	0.00174	30 7 68	1340	
M049W0.2	490	10.70	0.00175	25 11 69	857	
F058W0.2	496	10.40	0.00168	26 1 70	795	
M062W0.2	486	13.30	0.00175	24 2 70	766	
F069W0.2	542	10.80	0.0018	22 4 70	709	
M078W0.2	501	13.50	0.00178	16 7 70	624	
M079W0.2	501	10.00	0.00179	16 7 70	624	
F088W0.2	531	8.36	0.00177	25 8 70	584	
M095W0.2	526	13.10	0.00173	25 8 70	584	
F011W0.5	533	8.17	0.00532	13 10 66	1996	
M012W0.5	533	11.90	0.00539	13 10 66	1996	
M023W0.5	487	12.20	0.0053	21 3 68	1471	
M029W0.5	472	10.40	0.00548	8 5 68	1423	
F030W0.5	472	10.60	0.00538	8 5 68	1423	
F040W0.5	467	9.40	0.00528	2 7 68	1368	
M050W0.5	552	11.80	0.00526	25 11 69	857	
M059W0.5	496	11.50	0.00503	26 1 70	795	
M063W0.5	486	11.40	0.00524	24 2 70	766	
M070W0.5	497	12.80	0.00531	22 4 70	709	
F080W0.5	501	11.90	0.00545	16 7 70	624	
F081W0.5	501	12.10	0.00548	16 7 70	624	
F089W0.5	531	9.66	0.00527	25 8 70	584	
M096W0.5	490	12.00	0.00533	25 8 70	584	
F009W1.0	517	8.60	0.016	15 9 66	2024	
F010W1.0	517	9.90	0.0162	15 9 66	2024	
M020W1.0	513	10.80	0.0161	21 3 68	1471	
F021W1.0	513	9.36	0.0166	21 3 68		
F021W1.0A	552	11.40	0.0159	25 11 69	857	
M022W1.0	487	11.60	0.0164	21 3 68	1471	
M028W1.0	472	12.10	0.0158	8 5 68	1423	
F051W1.0	552	8.25	0.0163	25 11 69	857	
M060W1.0	496	10.00	0.0157	26 1 70	795	

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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 AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
M064W1.0	486	10.40	0.0158	24 2 70	766	
F071W1.0	485	12.10	0.0157	22 4 70	709	
M082W1.0	501	12.40	0.0163	16 7 70	624	
F090W1.0	526	10.90	0.016	25 8 70	584	
M097W1.0	490	10.40	0.016	25 8 70	584	
F042W1.7	495	9.26	0.0484	30 7 68	1340	
F043W1.7	492	10.40	0.0481	30 7 68	1340	
F044W1.7	492	7.46	0.0473	30 7 68	1340	
M045W1.7	492	11.90	0.0486	30 7 68	1340	
M046W1.7	484	8.42	0.0479	30 7 68	1340	
M047W1.7	484	11.10	0.0486	30 7 68	1340	
F052W1.7	552	9.57	0.0493	25 11 69	857	
M061W1.7	496	10.70	0.0458	26 1 70	795	
M065W1.7	486	11.20	0.0471	24 2 70	766	
F072W1.7	500	11.10	0.0479	22 4 70	709	
M083W1.7	501	12.60	0.0493	16 7 70	624	
F091W1.7	490	13.30	0.048	25 8 70	584	
M098W1.7	490	13.30	0.048	25 8 70	584	
F007W2.0	561	12.60	0.0952	15 9 66	1847	190
F008W2.0	561	11.70	0.0957	15 9 66	2024	
M019W2.0	513	13.40	0.097	21 3 68	1471	
M027W2.0	473	12.70	0.0961	8 5 68	1423	
M038W2.0	477	9.88	0.0945	2 7 68	1368	
F039W2.0	468	9.21	0.0948	2 7 68	1368	
M053W2.0	498	9.24	0.096	25 11 69	857	
F066W2.0	486	9.12	0.0935	24 2 70	766	
M073W2.0	552	14.30	0.0965	17 6 70	653	
F084W2.0	493	10.60	0.0984	16 7 70	624	
M085W2.0	493	10.80	0.0987	16 7 70	624	
F092W2.0	490	10.60	0.0962	25 8 70	584	
M005W3.0	561	15.00	0.305	15 9 66	1917	660
F006W3.0	561	11.90	0.31	15 9 66	1510	547
F018W3.0	523	8.60	0.307	21 3 68	1471	
M026W3.0	473	12.40	0.31	8 5 68	1423	

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

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

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

M005W3.0 OSTEOSARCOMA
F006W3.0 OSTEOSARCOMA
F018W3.0
M026W3.0

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M036W3.0	477	11.00	0.305	2	7	68	1368	
F037W3.0	468	8.44	0.294	2	7	68	1368	
M054W3.0	498	10.50	0.306	25	11	69	857	
M067W3.0	485	11.80	0.295	24	2	70	766	
F074W3.0	542	10.00	0.302	22	4	70	709	
F075W3.0	555	9.42	0.308	17	6	70	653	
M086W3.0	493	11.30	0.312	16	7	70	624	
F093W3.0	490	11.20	0.301	25	8	70	584	
M100W3.0	542	11.00	0.304	2	12	70	485	
M003W4.0	517	12.60	0.897	28	6	66	1779	2220
F004W4.0	517	9.40	0.911	28	6	66	1533	1976
M017W4.0	523	9.87	0.924	21	3	68	1132	1360
F025W4.0	473	10.50	0.927	8	5	68	1423	
M034W4.0	477	10.70	0.893	2	7	68	1368	
F035W4.0	477	8.87	0.902	2	7	68	1323	1660
F055W4.0	498	8.37	0.914	25	11	69	857	
M068W4.0	485	11.80	0.89	24	2	70	766	
F076W4.0	485	9.37	0.899	22	4	70	709	
M077W4.0	500	10.50	0.906	22	4	70	633	750
M087W4.0	501	13.10	0.916	16	7	70	624	
F094W4.0	490	11.30	0.912	25	8	70	584	
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.

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

M036W3.0
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

OSTEOSARCOMA; NEPHRITIS
KIDNEY, THYROID AND LIVER DEGENERATION
OSTEOSARCOMA; THROMBOSIS

PANCREATIC ADENOCARCINOMA

LIVER DEGENERATION

M001W5.0
M002W5.0

LIVER DEGENERATION; KIDNEY DEGENERATION
LIVER DEGENERATION; KIDNEY DEGENERATION

6. CALIFORNIUM-252

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M001F0.0	562	12.00		1	2	72	59	
M005F0.0	562	10.40		1	2	72	59	
M001F1.0	586	9.69	0.0163	8	9	71	205	
F002F1.0	586	8.28	0.0167	8	9	71	205	
F003F1.0	539	8.89	0.0167	8	9	71	205	
F004F1.0	539	10.00	0.0165	8	9	71	205	
M005F1.0	539	12.90	0.0165	8	9	71	205	
M001F3.0	583	11.60	0.289	3	3	71	394	
F002F3.0	583	10.60	0.289	3	3	71	394	
F003F3.0	583	8.66	0.292	3	3	71	394	
F004F3.0	583	9.69	0.295	3	3	71	394	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001F0.0
M005F0.0

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

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

H. CALIFORNIUM-249

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M002G0.5	514	12.00	0.00514	29	2	72	31	
M003G0.5	514	12.60	0.00518	29	2	72	31	
M006G0.5	514	11.80	0.00511	29	2	72	31	
F001G1.0	555	8.58	0.0154	16	12	71	106	
F004G1.0	555	10.50	0.0154	16	12	71	106	
F001G2.0	558	9.32	0.0905	16	12	71	106	
M002G2.0	555	11.00	0.0916	16	12	71	106	
F004G2.0	558	10.30	0.0915	16	12	71	106	
F005G2.0	555	9.44	0.0913	16	12	71	106	
F001G3.0	584	11.60	0.29	24	2	71	401	
M002G3.0	580	13.20	0.282	24	2	71	401	
M003G3.0	580	13.70	0.284	24	2	71	401	
F004G3.0	580	8.79	0.283	24	2	71	401	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M002G0.5
M003G0.5
M006G0.5

F001G1.0
F004G1.0

F001G2.0
M002G2.0
F004G2.0
F005G2.0

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

TABLE II. TEST ANIMALS (MAR. 31 1972)

A. RADIUM-226*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 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 31/3/72 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	610	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 31/3/72 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 - T27R5H; and 2.16 μCi for T28R3H. Tabulated for each dog are his age at 1st injection, his average weight during the injection period, total μCi /average weight, the date of 1st injection, the time from 1st injection to death, and sum of the skeletal doses computed from each injection to death.

T35R3J also received 99 μCi ^{85}Sr .

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

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

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

T071R5.0	MELANOMA ORAL CAVITY
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B. PLUTONIUM-239*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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
T028P1.0	SPECIAL STUDY
T029P3.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
T030P1.0	546	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	606	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 31/3/72 DEATH	DOSE TO SKELETON (RADS)
T049P1.0	103	5.00	0.0162	5 7 66	2096	
T050P3.0	103	5.30	0.296	5 7 66	2096	
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	1116	
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	933	
T058P3.0E	573	52.30	0.291	10 9 69	917	408
T059P3.0E	591	44.50	0.29	5 11 69	877	
T060P3.0E	567	45.20	0.314	6 1 70	784	379
T061P2.0E	581	47.20	0.0983	6 1 70	815	
T062P2.0E	583	52.50	0.156	22 1 70	799	

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 OSTEOSARCOMA
T059P3.0E
T060P3.0E OSTEOSARCOMA

T061P2.0E
T062P2.0E

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T063P5.0	581	9.13	2.77	14	12	70	473	
T064P0.0	595	8.60		28	12	70	459	
T065P4.0P	542	11.50	0.904	30	11	71	14	2
T066P4.0P	542	10.60	0.913	30	11	71	135	
T067P4.0P	542	10.40	0.907	30	11	71	135	

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

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

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

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T063P5.0

T064P0.0

T065P4.0P SPECIAL STUDY

T066P4.0P

T067P4.0P

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 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 Ci $^{228}\text{Th}/\mu$ Ci ^{228}Ra) injected = 0.03.

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

D. THORIUM-228 (RADIOTHORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/72 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 mg ^{232}Th , 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 31/3/72 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		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	563	7.20	8.3	2	10	63	13	143
T022S5.0	545	9.01	99.0	1	4	69	1095	
T023S5.0	545	11.60	100.0	1	4	69	1095	

* 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 31/3/72 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	3040	
T012Q3.0	495	13.50	0.889	4 12 63	3040	
T013Q3.0	495	11.30	0.912	4 12 63	3040	
T014Q3.0	438	10.30	0.87	4 12 63	3040	
T015Q4.0	515	12.70	2.73	1 2 68	1520	
T016Q2.0	515	9.36	0.31	1 2 68	1520	
T017Q2.0	515	10.20	0.311	1 2 68	1520	
T018Q2.0	502	9.68	0.306	1 2 68	1520	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T00103.0J SPECIAL STUDY

T00204.00 OSTEOSARCOMA
T00304.0 HEMANGIOSARCOMA

T00405.0 OSTEOSARCOMA, EPIDERMOID CARCINOMA (FRONTAL SINUS)
T00505.0 OSTEOSARCOMA

T00606.0 PURPURA HEMORRHAGICA

T00705.0 OSTEOSARCOMA
T00805.0 PURPURA HEMORRHAGICA

T00904.0 STRANGULATION ON VOMITUS AND GRAND MAL
T01004.0 STATUS EPILEPTICUS

T01103.0
T01203.0
T01303.0
T01403.0

T01504.0

T01602.0
T01702.0
T01802.0

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED		DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO YR		
T019Q1.0	515	11.80	0.0475	1	2 68	1520	
T020Q1.0	515	10.40	0.0472	1	2 68	1520	
T021Q1.0	502	9.08	0.0447	1	2 68	1520	

* 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

T01901.0
T02001.0
T02101.0

G. AMERICIUM-241

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED		DAYS SINCE INJECTION 31/3/72 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	252	910

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

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 SPECIAL STUDY

H. LEAD-210*

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001L5.0	522	9.78	10.7	24	6	69	1011	
T002L5.0	522	9.16	10.7	24	6	69	28	180
T003L5.0	522	9.78	10.7	24	6	69	1011	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001L5.0

T002L5.0

T003L5.0

SPECIAL STUDY

I. CALIFORNIUM-252

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T001F5.0	586	11.40	2.81	8	9	71	36	340
T002F5.0	540	10.70	2.87	17	11	71	13	110

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001F5.0	SPECIAL STUDY
T002F5.0	SPECIAL STUDY

J. CALIFORNIUM-249

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/72 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T00165.0	597	12.20	2.84	24	2	71	401	
T00265.0	584	10.70	2.77	24	2	71		7
T00365.0	584	9.89	2.8	24	2	71		21

DOG
NUMBER

COMMENTS ON DEAD DOGS

T00165.0

T00265.0

T00365.0

SPECIAL STUDY

SPECIAL STUDY

K. ANCILLARY*

DOG NUMBER	AGE (DAYS) AS OF 31/3/72 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	AGE (DAYS) AS OF 31/3/72 DEATH	DOSE TO SKELETON (RADS)
F045A	6182	
F047A	1732	
F048A	5075	
F049A	5612	
M050A	2264	
F051A	1089	
F052A	509	
F053A	5376	
F054A	3190	
F055A	4563	
M056A	701	
F057A	4322	
M058A	767	
M059A	567	
M060A	4025	
M061A	5141	
F062A	5086	
F063A	4530	
F068A	4521	
F070A	4497	
M071A	1472	
M073A	5695	
F074A	5553	
M075A	5284	
F076A	4407	
F077A	4392	
F078A	4126	
F079A	4037	
F080A	3981	
F081A	3981	
M082A	3627	
F083A	3668	
M084A	3668	
M085A	3938	
M086A	499	
F087A	3176	
F088A	3176	
F089A	3332	
F090A	3116	
F091A	3106	
F092A	3106	
M093A	3051	
F094A	3051	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F045A	PNEUMONIA; SENILITY
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	BRONCHIOGENIC CARCINOMA
F083A	
M084A	
M085A	
M086A	SPECIAL STUDY
F087A	
F088A	
F089A	
F090A	
F091A	
F092A	
M093A	
F094A	

DOG NUMBER	AGE (DAYS) AS OF 31/3/72 DEATH	DOSE TO SKELETON (RADS)
F095A	3036	
F096A	3019	
F097A	3019	
F098A	2925	
F099A		479
M100A		407
M101A		290
F102A		243
M103A		217
M104A		188
F105A		157
F106A	2620	
F107A	2529	
F108A		1969
F109A		2253
F110A	2126	
F111A	2114	
F112A	1845	
F113A	981	
F114A	985	
F115A	1134	
F116A	621	
F117A	621	
F118A	621	
F119A	545	
F120A	545	
F121A	621	
F122A	377	
F123A		375
F124A	377	
F125A	327	
F126A	327	

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
F115A
F116A
F117A
F118A
F119A
F120A
F121A
F122A
F123A
F124A
F125A
F126A

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

ENCEPHALOMALACIA (BACTERIAL)
METRIIIS

ACCIDENTAL STRANGULATION

ANATOMIC DISTRIBUTION OF MONOMERIC AND POLYMERIC ^{239}Pu

G. N. Taylor, W. S. S. Jee, R. Dell, J. Williams
and L. Shabestari

ABSTRACT: Polymeric Pu-239 given intravenously to beagles was retained principally in the reticuloendothelial cells (RE) of the liver and the spleen. Retention in the skeleton was limited to the RE cells of the bone marrow with very little, if any, uptake in the bone proper. Except in the lung, where some mechanical filtration of larger particles may have occurred, the retention was principally governed by phagocytic factors.

INTRODUCTION

Marked dissimilarities in the distribution pattern of monomeric and polymeric plutonium were observed following intravenous injection even though retention in some instances occurred in the same organ and in some of the same cell types. The differences were especially apparent with respect to local concentration. One of the most significant contrasts occurred in the skeleton.

It is the purpose of this paper to intercompare the anatomical distribution of these two forms of ^{239}Pu following a single intravenous injection in dogs and after relatively short post-injection times.

METHODS

The polymeric plutonium used in this study was prepared by Dr. Arthur Lindenbaum of the Argonne National Laboratory by differential precipitation of plutonium nitrate with sodium hydroxide. Based on millipore filtration, 82.8% of the activity was contained in particles greater than 8 microns and 17% was included in particles less than 8 microns but greater than 0.025 microns, and 0.2% of the activity was ultrafilterable.⁽¹⁾ Figure 1 illustrates the appearance of the particles as seen with the electron

microscope. The colloidal plutonium was given via a single intravenous injection approximately two days following preparation.

The monomeric ^{239}Pu was administered in the tetravalent form in a citric acid-sodium citrate buffer (pH 3.5).⁽²⁾

The autoradiograms were prepared from acetone fixed and paraffin embedded tissues.

All of the dogs used in these studies were healthy purebred beagles approximately 16 months of age at the time of injection.⁽²⁾

RESULTS

The retention of polymeric ^{239}Pu was principally in those organs with a significant number of reticulendothelial (RE) cells monitoring the blood vascular system, thus, the major deposition was in the liver and spleen with lesser amounts in the lungs and bone marrow. Retention in the lymph nodes was very low.

Liver

Retention of polymeric ^{239}Pu in the liver was within the RE cells lining the sinusoids (Fig. 2). Selective deposition in specific regions of the lobule was not apparent. The amount of plutonium in individual cells varied widely, at least partly because of the heterogeneous particle size of the injected material, and, undoubtedly, because of the differences in the phagocytic activity of various RE cells. Significantly more than one-half of the RE cells did not contain plutonium.

This early retention pattern was much different than that of monomeric plutonium where the initial deposition was principally in the hepatic epithelium with little, if any, retention in the RE cells (Fig. 3).^(3,4)

Localization in the RE cells of monomeric plutonium occurred only at relatively late post-injection times during its translocation from the hepatic cells to the periphery of the acini and to some of the periportal tissues. The hepatic distribution of the polymeric plutonium most nearly resembled the pattern of intravenously injected thorotrast and other colloids where the uptake is also governed by phagocytic mechanisms. (5,6)

Kidney

Very little polymeric plutonium was retained in the kidney. A survey of several autoradiograms was necessary before finding a single star or alpha track. This, again, was different than the monomeric retention where approximately 0.5% of the injected dose was initially retained in the kidney. (7,8) Localization of the monomeric form was principally in the basement membranes, including those of the glomerular tuft and within tubular epithelial cells (Figs. 4, 5). Epithelial deposition was much higher at relatively short post-injection times and was very non-uniform, with selected cells forming hot spots. In many instances the tubular deposition was associated with iron staining pigment. Hot spots were also present at long post-injection times, but these were principally in the interstitial areas and were frequently associated with iron staining pigment (Fig. 6).

Spleen

Localization of polymeric plutonium in the spleen was almost entirely in the RE tissue. It was especially concentrated in the areas immediately peripheral to the follicles, becoming more sparse in the remainder of the red pulp (Fig. 7).

Retention of monomeric plutonium in the spleen was much more uniform,

being distributed throughout the red pulp and to a much lesser degree within the follicles. Like the polymeric form it appeared to be principally in the RE cells. At short post-injection times, the monomeric form was usually not associated with hemosiderin particles, however, the activity became much more focal at relatively long post-injection times, and a high percentage of the monomeric plutonium ultimately became localized in iron staining granules (Figs. 8, 9).

Lymph Node

Retention of polymeric plutonium in the lymph nodes was low. However, a few phagocytized particles were retained in the RE cells of the red pulp. The distribution of monomeric plutonium in the lymph nodes was similar to that of monomeric Pu within the spleen.

Bone

Within the skeletal tissues, retention of colloidal plutonium was principally in the RE cells of the bone marrow, with little, if any, uptake occurring in the bone proper (Fig. 10). This is in marked contrast to the extracellular distribution of the monomeric form which is retained principally on bone surfaces (Fig. 11).⁽⁹⁾ Toxicologically this was probably the most significant difference in the distribution pattern of these two forms of plutonium.

Lung

The distribution of the plutonium particles within the lung was sparse, and it frequently was not possible to unequivocally determine the precise site of retention (Fig. 12). However, some of the smaller particles were clearly within the RE cells. A few of the larger particles may have been mechanically

lodged within the capillary lumen. This would explain why the largest particles found in any organ were in the lung - this being the first capillary bed encountered by the particulates after intravenous injection.

DISCUSSION

The intercomparison of intravenously injected polymeric and monomeric ^{239}Pu contrasts the deposition pattern of a colloid with a form in which the uptake is governed by chemotactic factors. Thus, the early retention of polymeric plutonium in the RE cells is a function of particle size, the number of particles, colloid stabilization, etc.⁽¹⁰⁾ Such factors, especially particle size, preclude intercomparison of various data unless they are specified in terms of a given spectrum of colloidal characteristics. For example, the bone marrow tends to discriminate in favor of the medium sized particles, and it is possible to selectively vary the uptake in this organ over a relatively wide range by manipulating the particulate size.⁽¹⁰⁻¹¹⁾ Particle size is of further importance from the standpoint of biological effects since the radiation dose is a function of the particle size. A small particle might produce degeneration and malignant transformation, whereas, the effect of a large particle could be entirely necrotizing. It is obvious that this factor could produce extreme difficulty in attempting to establish a reproducible dose-response relationship unless the particle size were standardized within a narrow range.

Factors related to phagocytosis also present an additional variable in extrapolating radiotoxicological data from one species to another.⁽¹²⁾ This arises in part because of marked differences in phagocytic function which exist among the various animal types. For example, the uptake of an

injected dose of colloidal chromium phosphate in the relatively large dog spleen is over 20% but only about 5% in the small rabbit spleen and seldom reaches 10% in the mouse spleen. Also, the uptake in bone marrow varies significantly among various animal types.⁽¹¹⁾

The distribution of monomeric plutonium obviously is not governed by phagocytic factors even though in the spleen both monomeric and polymeric plutonium are initially retained in RE cells. The zero or near zero uptake in the RE cells of the liver and the relatively long post-injection blood levels of the monomeric form⁽⁷⁾ indicate that phagocytosis is not involved, and that significant intravascular clumping of the injected material does not occur.

The transport of monomeric plutonium within the blood stream via transferrin and other blood proteins and the ultimate binding to ferritin in the hepatic parenchyma is well established.⁽¹³⁻¹⁴⁾ Retention of monomeric plutonium in the tubular epithelium of the kidney and the RE cells of the spleen and lymph nodes is possibly via the same mechanism. This seems especially likely since each of these sites contains a significant amount of stainable iron and it has been shown that the amount of ferritin in various cells runs parallel to the amount of intracellular iron.⁽¹⁵⁾

ACKNOWLEDGEMENTS

The polymeric plutonium used in these experiments was prepared by Dr. Arthur Lindenbaum of the Argonne National Laboratory. The authors wish to extend their special thanks for his assistance in this study.

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Fig. 1. Electron micrograph showing some of the characteristics of the colloidal Pu-239 given intravenously to the beagle summarized in this study (X 5000). Dog T65P4.

Fig. 2. Autoradiogram of beagle liver 14 days after injection of approximately 1 $\mu\text{Ci/kg}$ of colloidal Pu-239. The retention is principally or entirely within the RE cells lining the sinusoids. Marked variation in the activity of individual cells is apparent. 14 day exposure. H & E stain (X 200). Dog T65P4.

Fig. 3. Autoradiogram of beagle liver 13 days post-injection of 2.4 $\mu\text{Ci/kg}$ of monomeric Pu-239. It shows a uniform distribution of the plutonium in the hepatic parenchyma and an absence of focal hotspots or aggregates in the RE cells. 50 day exposure. H & E stain (X 657). Dog T42P5.

Fig. 4. Autoradiogram of a beagle renal cortex 1400 days post-injection of 2.91 $\mu\text{Ci/kg}$ of monomeric Pu-239. The plutonium concentration is highest in the glomerular and capsular basement membranes. 50 day exposure. H & E stain (X 657). Dog T19P5.

FIG. 5. Autoradiogram of tubules in beagle kidney 13 days after injection of 2.4 Ci/kg of monomeric Pu-239. The hotspots are principally in tubular epithelial cells. 30 day exposure. H & E stain (X 657). Dog T42P5.

Fig. 6. Autoradiogram of beagle kidney 1400 days after injection of monomeric Pu-239. It shows hotspots in the interstitial region. Same dog as Fig. 4. 50 day exposure. H & E stain (X 657).

Fig. 7. Autoradiogram of beagle spleen 14 days after injection of colloidal plutonium. It shows minimal retention of plutonium in the white pulp and relatively high localization in the RE cells of the red pulp immediately peripheral to a follicle. Retention in other regions of the red pulp is more diffuse. Same dog as Fig. 2. Seven day exposure. H & E stain (X 200).

Fig. 8. Autoradiogram of beagle spleen 13 days after injection of monomeric Pu-239. The alpha tracks are distributed uniformly throughout the red pulp without any hotspots and without any association with hemosiderin. Same dog as Fig. 3. 47 day exposure. H & E stain (X 657).

Fig. 9. Autoradiogram of beagle spleen 474 days after injection of 2.68 $\mu\text{Ci/kg}$ of monomeric Pu-239. The plutonium is principally in RE cells, and, although not apparent in this photograph, the alpha activity is associated with hemosiderin. 47 day exposure. H & E stain (X 200). Dog T20P5.

Fig. 10. Autoradiogram of beagle bone marrow 14 days after injection of polymeric Pu-239. It shows a sparse distribution within the RE cells and no localization on the endosteum or within the bone proper. Same dog as Fig. 2. 5 week exposure. H & E stain (X 657).

Fig. 11. Autoradiogram of bone spicule and marrow in lumbar vertebra of beagle 474 days following injection of monomeric plutonium. The retention is principally on bony surfaces, with some hotspots in macrophages. Same dog as Fig. 9. H & E stain (X 200).

Fig. 12. Autoradiogram of lung from a beagle 14 days following injection of polymeric Pu-239 showing very intense hotspots but a relatively low incidence of phagocytosis. Same dog as Fig. 2. 5 week exposure. H & E stain (X 200).

SKELETAL RETENTION AND DISTRIBUTION OF POLYMERIC
AND MONOMERIC ^{239}Pu IN BEAGLES

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ABSTRACT: $^{239}\text{Pu}(\text{IV})$ was given intravenously to beagles in: 1) the Pu-transferrin complex (Pu-T_f), 2) the 0.08 M citrate buffer (Pu-M), and 3) as a suspension of near colloid size particles (Pu-P) at $\text{pH} \sim 6$.

As expected, the overall distribution of the polymeric form of plutonium does not resemble that observed when it is administered as the monomeric complex with citrate or transferrin. The skeletal retention of plutonium, when injected in a monomeric form, is 50% of the injected dose. The retention of Pu-P in the skeleton was less than 1/20 that seen in Pu-M or Pu-T_f at comparable times.

The uniformity of skeletal distribution of Pu-P from bone to bone is markedly less than that seen with the two monomeric forms. Reduced skeletal retention in animals injected with Pu-P indicates a different skeletal retention mechanism in animals injected with polymeric plutonium. Autoradiographs clarify this point.

For many years, one of the goals of our laboratory has been to obtain an accurate accounting of the retention and distribution of ^{239}Pu in the beagle. The nuclide has been given in the monomeric form, as a citrate complex of its + 4 state.⁽¹⁾ In addition to the ease with which injection solutions can be replicated, such a procedure presents plutonium to the beagle in a form which lends itself to chemical reaction - that is to say, the biochemical process, rather than to the cellular processes, such as phagocytosis, involved in the disposition of small particulate foreign bodies which one would expect to be formed in the blood, at the time of injection,

should plutonium be given in non-complex form.

MATERIALS AND METHODS

From time to time we have been questioned and have ourselves questioned whether the plutonium we were injecting was, in fact, in monomeric form. Over the years we have documented techniques^(1,2) with which our methodology has been scrutinized and no evidence has been found to cause any doubt about the chemical state of the plutonium in the injection solutions used in these studies. Recently, as a further check, plutonium was injected as the plutonium transferrin complex. The material was prepared by obtaining plasma from a beagle which was subsequently the recipient of the complex. Plutonium was added to the plasma as the citrate complex of PuIV at pH 3.5 in the ratio of about 1 volume citrate to 100 volumes of plasma. Incubation was carried out at pH 7.4. Subsequently the transferrin was recovered by anion exchange chromatography (DEAE A-50) and rendered acceptable for injection by dialysis against physiologic saline. This procedure assures that injected plutonium will be exclusively the transferrin complex.

The retention and distribution observed in this animal was compared with the calculated 14 day retentions derived from the average observed in 15 beagles⁽³⁾ which had been injected with plutonium as the citrate complex.

Polymeric plutonium was prepared for this study by Dr. A. Lindenbaum of Argonne National Laboratory. "A highly purified solution of $^{239}\text{Pu}(\text{NO}_3)_4$ in concentrated nitric acid was neutralized by dropwise addition of NaOH to the point of precipitation. The preparation is the near-colloidal supernate recovered about 2 hours after the precipitate had been allowed to settle out." Measurements showed the range of particle sizes tabulated on the next page.⁽⁴⁾

Size (μm)	Percent
Larger than 8	82.8
5 to 8	3.5
1.2 to 5	5.3
0.45 to 1.2	4.2
0.025 to 0.45	4.0
Less than 0.025 (ultrafilterable)	0.2
	100.0

Upon receipt of the material, energy spectra of the α -particles and γ -rays arising from the decay of plutonium in it were obtained and they failed to suggest that any radionuclide (detectable by these methods) other than ^{239}Pu was present. The pH of the injection medium was ~ 6 . It was given intravenously.

All of the animals in these studies were anesthetized and then euthanized by exsanguination. In general the analysis of skeletal retention was made by analysing 1/2 of the symmetrical skeleton and all or a known fraction of the asymmetrical skeleton of each beagle. Plutonium retention was measured by liquid scintillation counting as reported previously⁽⁵⁾ or by a slightly modified version of the method reported by Keough and Powers.⁽⁶⁾

RESULTS AND DISCUSSION

The skeletal retentions at 14 days following injection were:

Monomeric ^{239}Pu (Pu-M) Citrate	48.5%
Monomeric ^{239}Pu (Pu-T _f) Transferrin	49.5%
Polymeric ^{239}Pu (Pu-P)	2.2%

About 22 times as much plutonium is retained by the skeleton when it is given as either the citrate or transferrin complex as when it is given in polymeric form.

In order to appraise the uniformity of skeletal retention, analysis was done bone by bone, the results of which appear in Table 1. The similarity of the distribution of retained plutonium among the bones of those animals receiving the nuclide as a monomer and the contrast seen when the polymer was given is obvious from the first three columns. In the final 4 columns the relative concentrations are compared. The relative concentration compares the contribution to total skeletal retention made by a bone with that bone's contribution to total skeletal mass (based on wet weights). The last of these 4 columns compare the relative concentrations of the bones from the dog receiving the polymeric plutonium with the average of their counterparts from the 16 dogs which had been given plutonium as the monomer.

Retention patterns for the two forms of plutonium are not as divergent when relative concentrations are observed as when fraction of injected dose is considered. However, upon close examination, it is seen that there are differences here too. Bones having a relatively small amount of organic material (a high value for the ashed weight to wet weight ratio⁽⁷⁾) make a less than average contribution to skeletal retention and their counterparts from the animal receiving polymeric plutonium make an even smaller contribution to total skeletal retention. A reciprocal relationship is seen in the case of those skeletal parts having a relatively large amount of organic material in their composition (low ash to wet weight ratio).

In Figure 1, an autoradiogram is taken from the animal which had been given the plutonium-transferrin complex 14 days prior to obtaining the bone

specimen. It shows the typical early distribution of plutonium following its administration as the monomer. α -tracks can be seen outlining the bone spicule while very few tracks arise from the space occupied by marrow.

An autoradiogram typical of those made from bone of the animal receiving the polymeric plutonium is seen in Figure 2. Few tracks, if any, are ever found on bone surfaces. The tracks that are found are seen arising as clusters, the arrangement expected from particulate plutonium.

Figures 3 and 4 are included to demonstrate that the plutonium particles found in the bone marrow vary considerably in size, although the variation shown here is not intended to indicate the range of sizes in the material used.

To summarize then, it is seen that: 1) the intravenous administration of plutonium either in the form of a citrate complex or as the transferrin complex results in identical patterns of skeletal retention. This was an expected result, and gratifying to observe because it once again reinforced our belief of many years that the original objective of giving monomeric plutonium was accomplished.

2) There is a marked contrast between the skeletal retention observed when plutonium is given as a monomer with that seen when polymeric plutonium is used. The 14 day retention for the monomer being about 50% - a factor of more than 20 times that which was seen in the study of the retention of polymeric plutonium.

And, 3) it seems probable that skeletal retention of the polymer is not associated with bone per se but likely increases with the amount of red marrow a bone contains.

TABLE 1. SKELETAL RETENTION OF MONOMERIC AND POLYMERIC ^{239}Pu

	Retention: % of Injected Pu			Relative Concentration			
	15(1&3) level beagles (Pu-M)	4-level beagle (T52P4) (Pu-T _f)	4-level beagle (T65P4P) (Pu-P)	15 beagles (Pu-M)	1 beagle (Pu-T _f)	1 beagle (Pu-T _p)	$\frac{\text{Pu-P}}{\text{Pu-M}}$
Skull	3.40	2.87	0.0587	0.533	0.378	0.164	0.313
Mandibles	1.11	1.18	0.0181	0.430	0.397	0.117	0.273
Cervical Vert.	2.42	2.59	0.164	0.766	1.06	1.34	1.71
Thoracic Vert.	7.11	7.14	0.219	2.12	1.88	1.63	0.774
Lumbar Vert.	6.06	5.21	0.289	1.88	1.64	2.24	1.20
Sacrum	1.23	1.45	0.0142	2.34	2.18	0.533	0.229
Caudal Vert.	0.275	0.467	0.0035	0.282	0.758	0.116	0.372
Paws	1.59	1.36	0.0090	0.398	0.347	0.023	0.582
Radii	0.411	0.357	0.0041	0.453	0.323	0.079	0.178
Ulnae	0.449	0.408	0.0041	0.425	0.298	0.089	0.213
Humeri	5.02	5.23	0.212	1.66	1.54	1.49	0.902
Tibiae-Fibulae	1.94	1.63	0.0221	0.646	0.548	0.179	0.230
Femora	4.22	3.78	0.255	1.31	1.09	1.91	1.47
Pelvis	3.34	3.92	0.223	1.44	1.48	2.23	1.55
Scapulae	2.58	2.77	0.187	1.35	1.30	2.31	1.72
Ribs	6.17	6.95	0.483	1.14	1.18	5.11	4.47
Sternum	0.923	2.13	0.0070	1.66	1.86	0.119	0.071
Os Penis		0.068			0.246		
Total Skeleton	48.5	49.5	2.17	1.000	1.000	1.000	

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. A. Lindenbaum, Division of Biological and Medical Research, Argonne National Laboratory, for preparing the polymeric plutonium used in this and two companion studies now in progress.

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Fig. 1. Autoradiogram of a bone from the Pu-T_f injected animal showing early distribution of plutonium following its administration as a monomer.

Fig. 2. Autoradiogram typical of those made from bones of the animal receiving polymeric plutonium (Pu-P).

Figs. 3 & 4. Autoradiograms demonstrating the variance in size of plutonium particles found in bone marrow.

THE EFFECT OF THE PHYSICAL-CHEMICAL STATE OF ^{239}Pu ON ITS EARLY
RETENTION IN PLASMA AND SELECTED SOFT TISSUES OF BEAGLES

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ABSTRACT: The early distribution and retention of $^{239}\text{PuIV}$ in plasma, kidney, spleen, and other selected soft tissues was studied in beagles after injection of the strictly monomeric Pu-transferrin complex (Pu-T_f), PuIV in citrate buffer of pH 3.5 (Pu-M), and polymeric plutonium (Pu-P) prepared by differential precipitation from a purified solution of plutonium nitrate. It was given at pH ~ 6 . Pu-T_f was removed from the circulation at a rate slower than Pu-M , whereas, Pu-P initially left the blood at a very rapid rate. At 15' after injection 87%, 64%, and $< 2\%$ of the three forms of plutonium were circulating in plasma respectively; 100% of Pu-T_f and the larger fraction of Pu-M was protein bound. No protein binding of Pu-P was seen. At two weeks after injection, the concentration of plutonium in most soft tissues was quite similar for Pu-T_f and Pu-M . Compared to Pu-M , kidney retention was less by a factor of 20, whereas, spleen retention was elevated by a factor of 10 in the Pu-P injected animal. The concentration of Pu-P in the spleen was 1.6 times greater than the concentration in the liver. Following differential centrifugation of tissue homogenates from Pu-P injected animals, the percentage of plutonium in the soluble fraction was greatly reduced in spleen but not in similar fractions of kidney when compared to Pu-T_f and Pu-M injected dogs. Most of the nuclide in the kidney was found in the fraction rich in mitochondria. In the case of Pu-P , a large concentration of plutonium also was found in the nuclear fraction of spleen homogenates. The concentration of plutonium in thyroid, adrenal, pituitary, dura mater, subcutaneous loose connective tissue and other soft tissues are compared.

INTRODUCTION

In the original plutonium experiments performed in our laboratory, $^{239}\text{PuIV}$ was injected as citrate complex, pH 3.5, and was at least 95% in the monomeric form. Shortly after intravenous injection, the $^{239}\text{PuIV}$ reacts with transferrin to form a transferrin-Pu complex. A small fraction of the plutonium, however, reacts with other inorganic elements of the plasma to form low molecular weight products. Another small portion may undergo hydrolysis at the neutral pH of plasma and form polymers. In order to determine the effect of the physical-chemical form of plutonium on its ultimate distribution and metabolism, the deposition patterns of Pu- T_f and Pu-polymeric were compared with our extensive data on Pu-citrate (Pu-M).

MATERIALS AND METHODS

The monomeric plutonium (Pu-M) was prepared by our standard methods⁽¹⁾ and was injected as a citrate at pH 3.5. The preparation of Pu-transferrin is described elsewhere.⁽²⁾ The polymeric plutonium was prepared by A. Lindenbaum from the Argonne National Laboratories and was in saline, pH 6. The size distribution of this material is given elsewhere.⁽²⁾

Blood samples were counted by liquid scintillation methods or they were wet ashed and counted in a proportional counter. All soft tissue samples were taken 14 days after injection, immediately subjected to a low temperature ashing procedure, and counted by α -scintillation counting methods.

Spleen and kidney samples were homogenized in 0.25 M Sucrose, 0.1 N NaCl + 3 mM Ca^{++} and fractionated by standard differential centrifugation methods. Proteins were determined by the method of Lowry et al.⁽³⁾ Data were expressed as CPM/mg protein.

RESULTS

In Figure 1 the percent of each of the three forms of ^{239}Pu circulating in plasma is compared during the first two days after injection. The exponential⁽⁴⁾ was derived from a large number of dogs injected with PuIV citrate, pH 3.5. The middle curve represents the percent of ^{239}Pu still circulating after injection as the Pu-transferrin complex. The rate of removal of this form of plutonium was essentially the same as that calculated for our 15 Pu-M dogs. The lower curve shows the average ^{239}Pu still circulating in blood taken from two dogs injected with Pu-M and studied extensively for 48 hours after injection. The removal of Pu-P from blood was so rapid that the data could not be shown clearly in this graph and they, therefore, are included as a small table at the lower left of the figure. At 5 minutes, less polymeric-Pu was circulating than was seen in the other forms at 48 hours. By 2 hours after injection Pu-P had decreased to 0.1% of the injected dose circulating and essentially no further decrease was seen. These data indicate that different factors are involved in the removal of Pu-M and Pu-T_f from the circulation than are involved in removal of Pu-P from the blood.

Elution profiles of protein and plutonium radioactivity from G100-G200 Sephadex columns for plasma taken from dogs injected with three forms of plutonium are shown in Fig. 2. The upper graph is the elution profile of plasma from a Pu-T_f injected dog. Most of the plutonium was eluted in the third protein peak which contains transferrin. A small portion of the plutonium was eluted with low molecular weight material. The middle graph is the elution profile of Pu-M radioactivity when it is injected as the citrate complex (pH 3.5). A small amount of plutonium was eluted in the

void volume and a larger amount as low molecular weight material, $MW < 5000$. The majority, however, was in the third protein peak which is the transferrin bound material. In the blood of Pu-M injected dogs, the plutonium which is non-protein bound and of intermediate molecular weight leaves the plasma most rapidly followed by the low molecular weight materials. The bottom profiles are those of Pu-P in the absence of plasma and of Pu-P in the plasma of the dogs receiving polymeric plutonium. The elution profiles for each are very similar and are characterized by a large peak of activity in the gel-excluded volume followed by a long, gradually decreasing, tail of lower molecular weight material. No radioactivity was seen with the transferrin peak.

In spite of the large fractions of plutonium which deposit in skeleton and liver after injection of monomeric plutonium, significant concentrations are seen in other soft tissues. The relative concentrations of plutonium in selected soft tissues from animals injected with either Pu-M or Pu-P are shown in Table 1. Tissues were taken at 14 days except for the Pu-M lung which was removed at autopsy one day after injection. In animals injected with either Pu-M or Pu-P the lungs contain significant quantities of radioactivity. A greater concentration of plutonium was found in those animals which received Pu-P in all the tissues shown in this table. The greatest ratio of Pu-P/Pu-M, a value of 53 occurred in the subcutaneous LCT (loose connective tissue), followed by adrenal gland and dura mater which had a ratio of 10. In the dog injected with Pu-P the combined, miscellaneous soft tissues contained a large amount of plutonium. This amount was ten times larger than that contained in the same tissues taken from Pu-M injected animals. These values represent percent of total injected dose rather

TABLE 1. ^{239}Pu CONCENTRATIONS IN SELECTED SOFT TISSUES OF BEAGLES
INJECTED WITH Pu-M, Pu-T_f, or Pu-P

Tissue	Percent Dose/gram		
	Pu-M	Pu-P	Pu-P/Pu-M
Lung	$8.0 \times 10^{-3} *$	6.4×10^{-2}	8.0
Thyroid	8.2×10^{-5}	1.6×10^{-4}	2.0
Adrenal	1.4×10^{-4}	1.4×10^{-3}	10.0
Pituitary	4.1×10^{-5}	3.0×10^{-4}	7.3
Dura	1.3×10^{-5}	1.4×10^{-4}	10.8
Salivary	8.2×10^{-6}	4.3×10^{-5}	5.2
Muscle	2.0×10^{-6}	9.5×10^{-6}	4.8
LCT	7.6×10^{-6}	4.0×10^{-4}	52.6
Misc. Tissue**	9.0×10^{-1}	10.1	

* Measurement taken at 1 day post injection

** Muscle, fat, intestine, etc. (% of total injected dose)

than concentration. In the lung taken from the Pu-P injected dog, the amount of ^{239}Pu found represents 4% of the total retained plutonium.

Comparative data for kidney and spleen taken from dogs injected with the three different forms of plutonium are compared in Table 2. The percent of injected dose of plutonium in kidneys and spleens is about the same in Pu-T_f and Pu-M injected dogs, however, the spleen contains slightly more plutonium than the kidney in the animals injected with Pu-M. In the Pu-P dogs a drastic reduction is seen in the amount of plutonium in the kidney and a large increase in the spleen. The spleen represents 9.4% of the total

TABLE 2. THE PERCENT OF INJECTED DOSE IN KIDNEY AND SPLEEN OF DOGS INJECTED WITH Pu-M, Pu-T_f, or Pu-P

	Pu-T _f	Pu-M	Pu-P
	% Total	% Total	% Total
Kidney	0.362	0.547	0.0267
Spleen	0.321	0.812	9.4*

* Concentration in spleen 1.6 x that of liver.

TABLE 3. CONCENTRATION OF ²³⁹Pu IN SUBCELLULAR FRACTIONS OF KIDNEY*

	Pu-M	Pu-P
Homogenate	100	100
Cytosol	4	17
Mitochondria	320	273
Lysosomes	110	154

* Data expressed as CPM/mg protein, original homogenate arbitrarily = 100.

injected dose and has a tissue concentration of plutonium ~ 1.6 times that of the liver. In the Pu-M dog, the increase in plutonium in the spleen may represent the removal of the non-protein bound intermediate weight material that was seen in the G100 - G200 elution.

Separation of the kidney homogenates into subcellular fractions by centrifugation in both Pu-M and Pu-P injected dogs is shown in Table 3. The data are presented as CPM/mg protein with total homogenates normalized to 100. The concentration of plutonium in the cytosol was low for both

forms of plutonium, however, the Pu-P concentration was 4 times that of Pu-M. These data indicate that the highest concentration of plutonium in the kidney is associated with mitochondria or lysosomes or is in the form of particles which have densities equivalent to mitochondria and lysosomes.

The concentration of plutonium in subcellular fractions of the spleens is shown in Table 4. The greatest concentration of radioactivity was in the mitochondrial fraction, followed by that in the lysosomal fraction. In the animals injected with Pu-P, the concentration of plutonium in mitochondria of the spleen was 10 times that of the homogenate on the basis of CPM/mg protein. These data also indicate that the plutonium depositing in the spleen is either taken up by mitochondria or is in particulate form with a density equivalent to that of mitochondria. The total amount of polymeric plutonium in the spleen was 11 times that seen in spleens taken from dogs injected with Pu-M.

TABLE 4. CONCENTRATION OF ^{239}Pu IN SUBCELLULAR FRACTIONS OF SPLEEN*

	Pu-M	Pu-P
Homogenate	100	100
Cytosol	11	4
Mitochondria	786	1007
Lysosomes	417	341

* Data expressed as CPM/mg protein, original homogenate arbitrarily = 100.

DISCUSSION

The soft tissue deposition of Pu-M and Pu-T_f was nearly identical, although small differences occurred which were probably related to the presence of the low and intermediate weight material (non protein bound) which is seen in blood at early times after injection. Also, the removal of both of these injected forms of plutonium from the circulation occurred at about the same rate. The rate of removal of Pu-P, however, greatly exceed that of either of the monomeric forms. The amount of Pu-P remaining in the plasma at 5 minutes was equal to that seen with Pu-M and Pu-T_f at 48 hours.

The soft tissues examined for plutonium content showed that in every tissue the concentration of Pu-P exceeded that in respective tissues from Pu-M injected dogs. The greatest difference in concentration was seen in the loose connective tissue sample, a ratio of 53, followed by the dura mater and adrenal gland which each had a ratio of 10. The reasons for the greater concentration of Pu-P in soft tissue are obscure at this time and may be partially due to physical trapping of particulates.

Subcellular fractionation of the spleen and kidney indicate that the largest concentration of plutonium occurred in the fractions that contain mitochondria or lysosomes. In the Pu-P injected dogs the spleen contained 9.4% of the total injected dose which is more than 10 times that found in spleens taken from the Pu-M injected animals. Conversely, the amount in the kidney of the Pu-P dog was 1/20 of that seen in the Pu-M dogs.

The distribution of Pu-M and Pu-T_f seems to be determined by a series of reactions at the biochemical level. However, the distribution of Pu-P is determined by the size distribution of particles injected. These

particles are removed from the blood by the two major organs of the system of macrophages, i.e. liver and spleen. The spleen, which has the most active phagocytes, also has the highest concentration of Pu-P (1.6 x that of liver), whereas, the liver, which has the greatest number of reticuloendothelial cells, contains the greatest amount of the total injected Pu-P. The other factor is the physical removal of the larger Pu-P particles due to trapping in small vascular channels. This mechanism probably accounts for the rather large accumulation in the lung.

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Fig. 1. A semi-log plot of the percent of the injected dose circulating during the first two days after injection for the dogs injected with Pu-M and Pu-T_f. The data taken from the animal injected with Pu-P are inserted as a table in the lower left corner.

Fig. 2. Elution profiles of O.D. at 280 nm and ²³⁹Pu radioactivity from Sephadex G100-G200 columns. Upper graph: Plasma from the dog injected with Pu-T_f. Middle graph: Plasma from a dog injected with Pu-M. Lower graph: Plasma from the Pu-P injected dog and a sample of Pu-P (with no plasma) in saline solution.

THE EFFECT OF THE PHYSICAL-CHEMICAL STATE OF PLUTONIUM ON
ITS EARLY DISTRIBUTION IN THE LIVER

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

ABSTRACT: The early deposition and subcellular distribution of $^{239}\text{PuIV}$ in canine liver following administration of either strictly monomeric PuIV as a transferrin complex (Pu-T_f), PuIV in citrate buffer pH 3.5 (Pu-M), or largely polymeric PuIV in nitrate pH 6 (Pu-P) has been studied. Large differences in the quantities deposited and the intraorgan distribution pattern in the liver were observed. Liver depositions ranged from 25% of total dose for the strictly monomeric (Pu-T_f), an average of 32% for Pu-M , to ~ 70% for the largely polymeric material. Autoradiographs show that initially Pu-M was deposited uniformly and diffusely in hepatic cells whereas Pu-P was found largely in random clusters in R. E. cells. Cellular distribution patterns of Pu-T_f and Pu-M injected dogs were quite similar.

Liver homogenates from a dog injected with Pu-M and the animal injected with Pu-P also were studied by differential and isopycnic sucrose density gradient procedures. Monomeric plutonium was initially associated with soluble liver proteins, i. e. ferritin, and was found at later times with subcellular fractions rich in mitochondria and also with lysosomes. In these organelles, most of the nuclide was either membrane bound or possibly associated with heavy granular material. In liver homogenates obtained from dogs injected with Pu-P most of the nuclide sedimented with nuclei or mitochondria. No association of Pu-P with soluble proteins could be demonstrated at very short times after injection.

INTRODUCTION

The detailed distribution of plutonium in the liver has been of interest for a long time. Although the biological endpoint in most of our beagles

injected with plutonium is death due to osteogenic sarcomas, the liver carries an average higher concentration of the nuclide than the bone; however, in some areas of bone the concentration is much higher than in others. Death due to liver degeneration has occurred in seven beagles of our colony at the 2.8 $\mu\text{Ci/kg}$ dose level. One of these animals died as early as 474 days after injection. The latest incident occurred at 2059 days after injection. Early extensive liver degeneration is seen only in beagles receiving this dose level (2.8 $\mu\text{Ci/kg}$). At all lower dose levels there are extensive areas of liver regeneration. The physical and chemical state of the nuclide in the injection solution determines the different chemical reactions and interactions that will occur with cellular components of the liver. Also, reactions which lead to overall deposition and distribution in the liver will proceed at different rates and the distribution and deposition in the liver will change.

METHODS

Liver tissue obtained from dogs injected fourteen days before sacrifice with Pu-T_f (plutonium-transferrin), Pu-M (monomeric PuIV in citrate, pH $\sim$ 3.5) and Pu-P (polymeric plutonium) were wet ashed and analyzed for plutonium.⁽¹⁾ Liver tissue also was obtained from dogs injected with Pu-M and Pu-P by biopsy at various times after injection. These specimens were homogenized in 0.25 M sucrose containing 0.1 M NaCl and 3 mM Ca⁺⁺. Whole homogenates were subjected to differential centrifugation and part of the supernatant obtained by centrifugation at 6000 g-min was further separated by isopycnic density gradient centrifugation. Fractions were then characterized by biochemical methods and the plutonium concentrations were determined. A detailed description of the respective procedures has been given elsewhere.⁽²⁾ Polymeric Pu

was kindly supplied by Dr. A. Lindenbaum of Argonne National Laboratory. Preparation and sizing of the injection material is described elsewhere.⁽¹⁾

RESULTS

Two weeks after injection, the total liver deposition for the three different physical chemical forms of nuclide was 25% for Pu-T_f, an average of 32% ± 5.2% for Pu-M and 68% for the polymeric material. Using the above figures and the formula for the rejection of an aberrant individual, the difference between the amount of Pu-T_f and Pu-M deposited in the liver was insignificant.

$$\frac{|\bar{X} - X_{T_f}|}{\sigma_{\bar{X}}} = 1.4$$

In order for the difference to be statistically significant with a 95% confidence limit this expression has to assume a value of > 3.5.⁽³⁾

Deposition of polymeric plutonium in liver was about 2 1/2 times higher than for Pu-T_f and Pu-M and the rate of deposition also was much faster, a fact deduced from the rapid disappearance of polymeric plutonium from the circulation. Two completely different mechanisms for the deposition must be involved.

At 14 days after injection, as shown autoradiographically, the Pu-T_f and Pu-M distribution in the liver was very uniform, consisting almost entirely of single tracks originating from hepatic parenchymal cells. This supports previous findings that most of the plutonium injected in our long term toxicity study was initially deposited in liver as monomeric material. Pu-P, on the other hand, was strongly localized and gave rise to clusters of tracks.

These clusters appeared to originate from reticuloendothelial cells rather than parenchymal cells.⁽⁴⁾

Since liver deposition resulting from injection of Pu-T_f and Pu-M was similar and since all of our plutonium dogs have been injected with Pu-M, it seemed sufficient to compare only Pu-M with Pu-P injected livers in more detail. First, homogenates made from specimens obtained either at biopsy or at time of sacrifice were subjected to differential centrifugation. The various fractions sedimented with a considerable degree of overlap and therefore did not represent pure subcellular entities. Results of this procedure are given in Table 1 and 2. The original homogenate whose plutonium concentration was normalized to 100 CPM/mg protein is designated as (1 + 2). Nuclei, cellular debris, and unbroken cells are found in the (N + D) fraction. 3K is the supernatant free of mitochondria. The cytosol is the supernatant obtained following 600,000 g-min centrifugation. Microsomal fractions were contaminated with ferritin and not analyzed any further. Data on this fraction were, therefore, omitted. M and L are fractions containing mitochondria and lysosomes, respectively, but they cannot be regarded as "pure" fractions.

After injection of Pu-M (Table 1), the relative concentrations of the nuclide increased as a function of time in the N + D fraction. It decreased by a factor of > 5 in the supernatant free of mitochondria and by a factor of 4 in the cytosol. Consequently, the plutonium concentration in the fractions designated as mitochondria and lysosomes increased accordingly.

Table 2 depicts the corresponding data obtained from a Pu-P liver. An hour after injection the supernatant, from which fractions designated as mitochondria and lysosomes had been removed (3K), contained little or

no plutonium. The nuclide was found in the highest concentration in the mitochondrial fraction and in much lower concentration in the lysosomal fraction. With increasing time after injection, less nuclide sedimented with particles having the same density as mitochondria and more appeared in the supernatant fractions. Increased concentrations of plutonium were also found in the lysosomal fraction. Again the concentration in the N + D fraction was increased. Differential centrifugation of Pu-P injection solution in a medium containing 1.8% albumin (a protein concentration corresponding approximately to that of the cytosol) revealed that as much as 5% of the Pu-particles sedimented at 6000 g-min. Free Pu-P particles therefore might be sedimented in the N + D fraction.

Isopycnic density gradient centrifugation provides a more definitive means for separating mitochondria and lysosomes of liver homogenates. Separations were carried out after N + D fractions had been removed. The same general trends were apparent. Fractions derived from Pu-M (Table 3) had the highest plutonium concentration initially in the light, i.e. soluble, fractions. In fractions derived from Pu-P livers (Table 4), the plutonium concentration in heavy fractions was high from the very beginning and low in the light fractions, i.e. little or no plutonium was associated with soluble proteins.

Each fraction of the density gradient was also assayed for its protein concentration, acid phosphatase activity and, in some cases, cytochrome c oxidase activity. Data derived from these assays are presented in Figs. 1 and 2. The concentration of plutonium (CPM/mg protein) showed a peak occurring at intermediate densities, indicating an association with lysosomes as measured by its acid phosphatase activity (Sigma Units/mg protein).

However, the distribution of plutonium (% of total plutonium in each fraction) shifted from light to heavy fractions. The areas of greatest cytochrome c oxidase activity represent a heavy fraction containing intact mitochondria and a light fraction that is due to rupture and destruction of mitochondria with subsequent release of enzymes. In the case of Pu-P (Fig. 2), a correlation between acid phosphatase activity and plutonium concentration (CPM/mg protein) became higher only at longer times after injection. Initially almost all of the plutonium was associated with heavy fractions. In contrast to results with Pu-M, the amounts of Pu-P found in lighter fractions increased slightly at longer times after injection.

DISCUSSION

There are two different mechanisms involved in the deposition of the two different forms of plutonium, i.e. monomeric vs. polymeric. As long as the plutonium is present as a true monomer, or more explicitly, in the form of some anionic complex, the nuclide will undergo a series of chemical reactions on the molecular level, i.e. the initial complex ion reacts with transferrin or other transport proteins and after possibly going through some intermediate steps is finally found in the liver with proteins such as ferritin. The terminal point in this series of reactions is the form which is most stable under existing conditions. Of course, the molecules which finally bind plutonium will also ultimately be released due to death of the plutonium bearing cell and its fragments will be phagocytized. This leads to a rearrangement of the nuclide, and a change in its distribution pattern will occur.

Both Pu-T_f and Pu-M were in monomeric form and the results were similar retention and distribution patterns in the liver. Plutonium was uniformly

distributed and mostly associated with hepatic cells. At short times after injection, the nuclide was bound to soluble proteins, i.e. mostly ferritin. With increasing time, the Pu-ferritin pool was depleted and increasing concentrations of plutonium were found in the mitochondrial and lysosomal fractions and also in the N + D fraction. At the present time it is almost impossible to verify the exact locations in the cell once the nuclide has associated with subcellular components but more than one subcellular particle is probably involved.

If plutonium is presented to the animal as a polymer, the molecular reactions just described cannot occur. Instead deposition will be controlled by the filtering properties of certain organs. Filtration may be either of a physical nature, such as trapping of large particles in constrictions of the circulatory system, or it may be achieved metabolically. In the later case, macrophages recognize Pu-P as foreign material and phagocytize it. Macrophages of the spleen are the most active individually, but because of its size the liver is the most important from the standpoint of total phagocytic activity.⁽⁵⁾ The result is that we find a very high plutonium concentration in the spleen (1.6 x liver, ~ 9.4% dose) but a greater total quantity (70%) is in the liver.

After injection of Pu-P, hepatocytes seem unaffected, since most of the nuclide is taken up by reticuloendothelial cells. No association with ferritin was observed. At 1 hour, less than 1% of the total plutonium in the liver can be found in the cytosol. Immediately, the nuclide is found in fractions rich in mitochondria, lysosomes and the N + D fraction. This does not necessarily mean that Pu-P is initially associated with these subcellular particles. It may simply mean it has the same density as these

cell components and, therefore, sediments in the same fractions. The increase of plutonium concentration in the 3000 xg supernatant, the cytosol and light fractions after differential centrifugation as a function of time after injection can be interpreted as a result of slight depolymerization and solubilization of Pu-P by biochemical ligands which will lead to translocation within the cell.

SUMMARY

Pu-T_f and Pu-M when injected intravenously into beagles have very similar retention and distribution patterns in the liver. Plutonium is uniformly distributed and mostly associated with hepatocytes. With increasing time, a correlation between plutonium and lysosomes (as determined by acid phosphatase assay) becomes discernable.

Pu-P is deposited in the liver by a different mechanism. About 2 1/2 times as much nuclide is deposited in the liver. Most of it is phagocytized and is located in random clusters in reticuloendothelial cells. Evidence exists that at later times a little plutonium is depolymerized and, therefore, shifted to soluble protein fractions.

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TABLE 1. DIFFERENTIAL CENTRIFUGATION OF LIVER HOMOGENATES
AFTER INJECTION OF Pu-M

	1 D	8 D	71 D
1 + 2	100	100	100
N + D	36	54	66
3K (Supernatant)	157	75	29
Cytosol	105	30	25
Mitochondria	148	219	888
Lysosomes	---	91	183

* Data expressed as CPM/mg protein, original homogenate
arbitrarily = 100.

TABLE 2. DIFFERENTIAL CENTRIFUGATION OF LIVER HOMOGENATES
AFTER INJECTION OF Pu-P

	1 H	2 D	14 D	106D
1 + 2	100	100	100	100
N + D	187	192	145	260
3K (Supernatant)	---	11	15	26
Cytosol	1	2	3	8
Mitochondria	307	367	285	287
Lysosomes	31	55	100	109

* Data expressed as CPM/mg protein, original homogenate
arbitrarily = 100.

TABLE 3. ISOPYCNIC DENSITY GRADIENT CENTRIFUGATION OF
HOMOGENATES FREE OF NUCLEI AND DEBRIS (Pu-M)*

(Total of 16 Fractions)

ΔT	Pu in 1st Three Fractions (heavy)	Pu in Last Three Fractions (light)
1 D	8	66
4 D	4	34
8 D	23	35
30 D	32	13
204 D	58	8

*Percent of total radioactivity in gradient.

TABLE 4. ISOPYCNIC DENSITY GRADIENT CENTRIFUGATION OF
HOMOGENATES FREE OF NUCLEI AND DEBRIS (Pu-P)*

ΔT	Pu in 1st Three Fractions (heavy)	Pu in Last Three Fractions (light)
1 H	66	2
2 D	72	7
14 D	71	9
106 D	47	14

*Percent of total radioactivity in gradient.

Fig. 1. Density distribution of acid phosphatase, cytochrome c oxidase and plutonium in 600 xg supernatants at various times after injection of Pu-M. Heavy fractions are on the left side, low density material is in fractions on the right side.

Fig. 2. Density distribution of acid phosphatase and plutonium found in 600 xg supernatants at various times after injection of Pu-P.

CONCENTRATION OF $^{239}\text{PuIV}$ IN THE BLOOD PLASMA OF THE BEAGLE

Betsy J. Stover and David R. Atherton

ABSTRACT: The concentration of plutonium in plasma has been measured from 1 minute to 12.5 years following injection of the young adult beagle with $^{239}\text{PuIV}$ in 0.08 M citrate buffer, pH 3.5. The following sum of exponentials has been fit to the data:

$$P = 0.116 e^{-1.05t} + 0.017 e^{-0.47t} + 0.0015 e^{-0.080t} + 0.00016 e^{-0.00089t}$$

where P is the percent of injected $^{239}\text{Pu/g}$ of plasma and t is days. Samples were obtained from dogs at 6 dose levels, and from well, ill, and terminal dogs. Thus, because of the known effect of dose level on hepatic and skeletal retention and the unknown effect of the disease state of the animal, metabolic interpretation of the equation must be limited to the observation that, on the average, there is a continuous decrease in the concentration of ^{239}Pu in plasma with time. An effect on the fourth term of P was observed at the highest dose level only.

INTRODUCTION

In our first analysis of the concentration of plutonium in plasma as a function of time the data were divided into three groups (1 min-10 hrs., 1-22 days, 22 days-4 yrs), and a power function was fit to each group.⁽¹⁾ Subsequent samples obtained from terminal dogs at 3 to 8 years after injection yielded concentrations that were less than the values calculated from the power function for 22 days-4 yrs.⁽²⁾ For this reason, the fact that more interim and terminal data had been obtained, and the desirability of having a continuous function as opposed to a set of functions for several intervals, a re-analysis of all the data seemed appropriate.

METHODS

The analytical methods for the earlier data have been reported previously.^(1,2) Some of the more recent samples have been analyzed by extracting the plutonium

contained in a plasma sample into EHPA (di-2 ethyl hexyl phosphoric acid) in toluene liquid scintillator solution. The procedure has been described previously.⁽³⁾

RESULTS AND DISCUSSION

A sum of four exponentials was fit to the set of 224 points. The calculated equation and individual points are shown in Figs. 1 and 2, and the various parameters are given in Table 1. The equation is as follows:

$$P = 0.116 e^{-1.05t} + 0.017 e^{-0.47t} + 0.0015 e^{-0.080t} + 0.00016 e^{-0.00089t} \quad (1)$$

TABLE 1. THE EQUATION FOR CONCENTRATION OF
PLUTONIUM IN PLASMA, $P = \sum_{i=1}^4 a_i e^{-b_i t}$

Term	n	$a \pm \sigma_a$	$b \pm \sigma_b$	$t_{1/2}$	p^*
		% inj. $^{239}\text{Pu/g. plasma}$	d^{-1}	d	
1	63	0.116 ± 0.004	1.05 ± 0.04	0.660	<0.01
2	10	0.0170 ± 0.0256	0.471 ± 0.306	1.47	0.2-0.1
3	10	0.00153 ± 0.00065	0.0797 ± 0.0192	8.70	<0.01
4	141	0.000158 ± 0.000017	0.000894 ± 0.000076	775	<0.01

*Probability that b does not differ from zero.

The long (4 th) term includes data from 62 to 4572 days and approximately 35% of the samples were obtained from terminal dogs at sacrifice. Many of these dogs represent the very low and some of the very high points in

Fig. 2. It is of interest that when an exponential is fit to the 70 points obtained from sampling non-terminal dogs at the P5 and P4 level from 62 days to 4 years, the intercept is about the same as that in the 4th term of Eq(1), but the half-period is about 40% longer. The three possible factors in this longer half-period are dose level, disease state, and the longer interval over which the sampling was done.

The data from which the fourth term in Eq(1) was calculated were further analyzed by dose level and groupings of dose levels. The results are summarized in Table 2. Each value of b , the coefficient of t in the exponential, differs significantly from zero except that for the P3 level. However, there are the fewest points in this subgroup, they were obtained over the shortest sampling interval, and all but 3 samples came from terminal animals.

TABLE 2. ANALYSIS OF LAST TERM OF P BY DOSE LEVEL

Dose Level (s)	n	Interval days	$a \pm \sigma_a$ (% inj. Pu/g) $\times 10^5$	$-b \pm \sigma_b$ $d^{-1} \times 10^4$	p*
All	141	62-4572	15.8 ± 1.66	8.94 ± 0.763	<0.01
P5	54	62-2059	23.3 ± 3.44	13.4 ± 1.93	<0.01
P4	44	62-1724	14.2 ± 2.90	10.2 ± 2.13	<0.01
P3	13	362-1947	13.4 ± 12.2	9.09 ± 6.24	0.2-0.1
P1	15	96-4572	16.1 ± 4.66	6.70 ± 1.66	<0.01
P1 + P1.7	20	96-4572	16.6 ± 6.17	8.98 ± 1.69	<0.01
P1 + P1.7 + P2	30	96-4572	16.2 ± 5.40	8.18 ± 1.41	<0.01
All but P5	87	62-4572	12.5 ± 1.90	7.75 ± 0.926	<0.01

*Chance that $b = 0$

Using the "t" test, the significances of the differences between pairs of "b"'s in Table 2 were evaluated. The significant differences all involved the P5 group of data. It differed from the P1 and all but P5 groups ($p < 0.01$), and the P1 + P1.7 + P2 and the all groups ($0.05 > p > 0.02$). Thus, the dose level effect is not as well demonstrated for concentration of ^{239}Pu in plasma as it is in hepatic and skeletal retention.^(4,5)

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Fig. 1. Concentration of ^{239}Pu in plasma through 45 days after intravenous injection.

Fig. 2. Concentration of ^{239}Pu in plasma 62 to 4572 days. Many samples were obtained from terminal dogs.

COMPARISON OF SKELETAL AND HEPATIC DOSE RATES
FROM ^{239}Pu IN THE BEAGLE AS A FUNCTION OF DOSE LEVEL

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

ABSTRACT: The total skeletal retention has been measured in four beagles that were injected intravenously with $0.0059\mu\text{Ci } ^{239}\text{PuIV/kg}$ in 0.08 M citrate buffer, pH 3.5. The dogs were young adults at the time of injection and they were sacrificed at 1648 to 2546 days after injection at which times the removal of plutonium from trabecular surfaces by remodeling had decreased significantly. Retention of plutonium in 8 humeri and 8 third lumbar vertebrae was measured in dogs at low dose levels which were sacrificed at 35 to 4375 days after injection. These results are combined with previous data on early deposition and retention in the skeleton, and early and long term data on retention in the humerus and third lumbar vertebra, to obtain a set of four skeletal retention equations applicable to at least seven dose levels. From measurements of the defleshed skeletons of 18 beagles, the indicated wet skeletal weight was $7.5 \pm 0.9\%$ of the weight of the dog at injection, a value lower than the previously assumed 10%. Using the set of skeletal equations, and the measured skeletal weight, the cumulative rads doses to the skeleton and the liver are compared. The average dose to the liver exceeds that to the skeleton except at the $2.8 \mu\text{Ci/kg}$ dose level after 1300 days.

INTRODUCTION

Our progress in measuring the excretion, retention, and distribution has been summarized recently. ⁽¹⁾ In brief review, a skeletal retention equation

$$B (\% \text{ inj. } ^{239}\text{Pu}) = 72 t^{-0.040} \quad (1)$$

where t is days after injection, was derived from excretion equation, an hepatic retention equation based mainly on results from dogs at the P5-level, and limited early distribution data. ⁽²⁾ Equation (1) and the assumption that skeletal weight = 0.1 body weight at injection has served as the basis of the average skeletal radiation doses for dogs given ^{239}Pu since data of this kind were first published in the Semi-Annual Progress Report, September 1959, C00-219. As tissues from lower level dogs at longer time intervals became available, the effect of dose level on hepatic retention was quantitated, and it was

shown that the rate of decrease increased with increasing dose level.^(3,4,5) The above results plus measurements of retention of ^{239}Pu in the humerus and third lumbar vertebra of terminal dogs at the three highest dose levels suggested the opposite effect of dose level on skeletal retention.⁽⁶⁾ Next measurements of skeletal distribution at the Pl-level (0.016 $\mu\text{Ci/kg}$) showed that the early deposition was less and the decrease during the first two years was greater than would be anticipated by Eq. (1).^(7,8) However, estimated retention at 4375 days after injection of 0.048 $\mu\text{Ci/kg}$ (Pl.7-level) was found to be about 4 times greater than anticipated by extrapolation of the results obtained during the first two years for the Pl-level, and, similarly, long term retentions in the humerus and third lumbar vertebra were higher.⁽¹⁾ In addition, Dr. Jee has demonstrated the rapid removal of ^{239}Pu from trabecular surfaces of these Pl-level dogs during the first two years.⁽⁹⁾ In this paper we report additional long term retention at low dose levels which, in conjunction with prior data, are consistent with a two component equation which can be correlated with resorption, burial, and redeposition in bone and elimination (via macrophages) from bone.

MATERIALS AND METHODS

The beagles were given $^{239}\text{Pu IV}$ as the monomer in citrate buffer solution. Injection was by vein, and the procedure has been reported previously.⁽²⁾

Following sacrifice, the skeletons were dissected out and gross defleshing was done. Careful defleshing was done as soon as possible thereafter and prior to determining the wet weights of the various skeletal parts. Care was taken to minimize dehydration during the defleshing procedure. Bones were then reduced to ash in a muffle furnace ($\sim 500^\circ\text{C}$), weighed, put into solution with nitric acid, dried and resolubilized in 2 N HNO_3 . Appropriate aliquots

were taken for plutonium analysis which was done by liquid scintillation counting, according to the method of Keough and Powers.⁽¹⁰⁾

Fragments of the skeleton of one dog receiving $0.00549 \mu\text{Ci } ^{239}\text{Pu IV /kg}$ 1648 days prior to its death and another receiving $0.0473 \mu\text{Ci } ^{239}\text{Pu/kg}$ 4375 days before death were also analyzed for plutonium content. Unfortunately a considerable portion of each skeleton was missing (see Tables 5 and 6) and it was not possible to obtain wet weights because the bones had been dehydrated by fixation in acetone prior to their being stored by freezing. Since rehydration to a reasonable approximation of the in vivo condition was not possible, these partial skeletons were analyzed only for the weight of skeletal ash and percent of injected ^{239}Pu each part contained. Wet weights were calculated for the parts on which ashed weights were obtained by assuming that the average ash/wet weight ratios for those parts, measured in the 3 dog group, were applicable. The weight of each part was converted to the total wet skeletal weight by dividing it by the 3-dog average of the fractional contribution the part makes to total skeletal weight. Total skeletal retention (% of injected dose) was calculated by dividing the measured retention of a part by the average contribution to total skeletal retention made by that part. These calculations result in a series of values for the wet weight of total skeleton and its retention.

RESULTS AND DISCUSSION

Retention of ^{239}Pu in one humerus and the third lumbar vertebra of 8 dogs at the P0.5 dose level ($0.0055 \mu\text{Ci/kg}$) and of one at the P1.7 dose level has been measured at 7 to 4375 days after injection (Table 1). The 8 new points for 35 to 4375 days are shown in Figure 1 with the 44 points previously reported for 35 to 5200 days for beagles at the P2-level, ($0.095 \mu\text{Ci/kg}$),

Pl.7-level ($0.048 \mu\text{Ci/kg}$), and the Pl-level ($0.016 \mu\text{Ci/kg}$).⁽¹⁾ The data clearly indicate that the rate of decrease of retention in these two bones is greater during the first few years than at later times, and, thus, an equation of two terms is appropriate for description of the data. Although plutonium is most probably lost continuously from the bone of dogs at low dose levels through the normal processes of skeletal remodeling, the variation between dogs at long times masks the slow rate of decrease. Thus, the second rate constant cannot be distinguished from zero which leads to an equation of the form.

$$\text{Retention} = ae^{-bt} + c \quad (2)$$

To calculate an equation of this form, the average value of $H + V$ over 1600 to 5200 days was used as a first approximation for the constant, c . Then a single exponential was fit to the values of $H + V - c$ for $t < 1600$ days. The value of c was then decreased by successive increments and corresponding exponentials were calculated. The iterative process was continued until a value of c was determined which yielded an exponential of better fit than did either higher or lower values of the constant. The resulting equation is

$$H + V = (2.53 \pm 0.39) e^{-(0.00150 \pm 0.00040)t} + 1.20 \quad (3)$$

where $H + V$ is in % injected plutonium and t is in days. The criteria for goodness of fit were maximum value of the correlation coefficient for the exponential given by the computer and minimum value of the sum of the squares of the differences between observed and calculated values of the complete set of data ($n = 52$).

Our most complete sets of skeletal concentration of ^{239}Pu have been obtained for three dogs at the P0.5 level, which were recently sacrificed at 1920, 2275, and 2546 days after injection. At these times skeletal retention

is decreasing very slowly as shown by Figure 1. All of the asymmetrical and one half of the symmetrical skeletons of these dogs was measured. Thus, the only assumption required to obtain a value for total retention is that the distribution is symmetrical in the symmetrical skeleton. The total measured retentions in order of increasing time are 24.2, 22.8, and 20.8%. Detailed data on retention, concentration, and skeletal weights are given in Tables 2, 3, and 4.

Significant portions of the skeletons of a dog at the P0.5 level at 1648 days and one at the P1.7-level at 4375 days were also measured. Since these are also times at which the skeletal retention is decreasing very slowly, estimates of the amounts of ^{239}Pu in the missing bones can be made using the extensive data of Tables 2, 3, and 4. The bases for these estimates are summarized in Table 5. The measured and estimated data are given in detail in Tables 6 and 7. The estimated total skeletal retentions are 35.3 and 21.4%.

We now have a set of 16 values of skeletal retention available for calculation of an equation of the form of Eq. (2) for dogs at low dose levels. They are the five long term values (4.5 to 12 years after injection) presented above, and the early values for the 11 dogs at the P1-level which were sacrificed at 35 to 769 days after injection. (1,7) The iterative method and criteria described above were employed to obtain the following equation for skeletal retention at dose levels P2, P1.7, P1, and lower:

$$B = (28.6 \pm 4.7) e^{-(0.00110 \pm 0.00034) t} + 21.0 \quad (4)$$

where B is in % injected plutonium and t is in days. The data and the calculated curve are shown in Figure 2.

To estimate skeletal retention curves for beagles at the P5, P4, and P3

levels utilize the average retention in the humerus and third lumbar vertebra, $H + V$, in the toxicity dogs.⁽⁶⁾ (Data have also been obtained for serially sacrificed dogs at the P5-level, and these plus the terminal data have been analyzed by a variety of approaches but no significant time dependence can be shown, which is in marked contrast with the data in Figure 1.) The average values of $H + V$ and the average survival times for these dogs are given in Table 8. We assume that removal of plutonium from these bones of these terminal dogs has virtually ceased at these times as a consequence mainly of the radiation damage, i.e., death of osteoclasts, peritrabecular fibrosis, etc.^(9, 11) The value of $H + V$ for each dose level is then substituted into Eq. (3) which is then solved for t . This value of t is called $t_{\text{equivalent}}$ for it is the equivalent time at which $H + V$ for low level dogs would have decreased to the particular value, e.g., 2.94% for the P5-level (Table 8).

Comparison of Eqs. (3 and 4) shows that about 32% of the ^{239}Pu in the humerus and vertebra is retained throughout the dog's life and the remainder is removed with a half-period of about 460 days, while the corresponding values for the whole skeleton are 42% and 630 days. However, $t_{\text{equivalent}}$, provides a bridge to obtain equations analogous to Eq.(4) for the three higher dose levels. At $t_{\text{equivalent}}$ skeletal retention has become constant, and the value obtained by substituting $t_{\text{equivalent}}$ into Eq.(4) is the constant term, c (Eq.2). Since the initial deposition is independent of dose level, the coefficients of the exponential are obtained by difference, i.e., at $t = 0$, $B = 49.6$ for all dose levels. The deficit of this approach is that the same rate constant must be used for all dose levels since data are not available for all dose levels. (Autoradiographic evidence suggests that the rate constants could be expected to decrease with increasing dose level, and, thus,

use of the low level rate constant at higher dose levels would lead to a slight under-estimation of cumulative radiation doses at higher levels.) The resulting skeletal retention equations for higher dose levels are as follows.

$$B (P5) = 6.9 e^{-0.0011t} + 42.8 \quad (5)$$

$$B (P4) = 11.0 e^{-0.0011t} + 38.6 \quad (6)$$

$$B (P3) = 15.2 e^{-0.0011t} + 34.4 \quad (7)$$

Eqs. (5,6,7) are compared with eq. (4) in Figure 3, which also included four measured values at both the P5 and P3 levels. (6,7)

The primary purpose in obtaining the set of skeletal retention equations is to use them in calculating average radiation dose rates and cumulative radiation doses to the skeleton. Also of primary importance is determination of skeletal weight in relation to the weight of the dog at the time it is injected, especially since all of our average dose calculations to date have been based on the assumption that skeletal weight = 0.1 body weight. Data now have been accumulated for 18 dogs on wet and ashed skeletal weights obtained following meticulous defleshing of the bones and precautions to minimize dehydration. (12) These results are given in Tables 9, 10, and 11. The importance of these present measurements is that the skeletal weight is $7.5 \pm 0.9\%$ of the body weight at injection.*

This value, Eqs. (4,5,6,7), the average weights and injected doses (summarized in table 12 and found detailed in the tabular material on pages 30,32,34,36) have been used to calculate cumulative rad doses to the skeleton at 6 dose levels over the survival period for each dose level shown in Figures 4 and 5. Also shown are the cumulative rad doses to the liver calculated from equations reported previously. (5) Except at the

*This number represents the best presently available information for beagles in this colony. These studies will be continued and this value (7.5%) may be subsequently refined.

P5-level at times exceeding 1300 days, hepatic doses exceed skeletal doses.

In summary, skeletal retention of ^{239}Pu has been shown to be biphasic with respect to time dependence, and the relationship of skeletal weight to weight at injection has been determined experimentally. These findings permit a more accurate calculation of average radiation dose of the skeleton.

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Table 1
Retention of Injected ^{239}Pu by Humerus (H) and 3rd Lumbar Vertebra (V)

Dog	Injection to Death (days)	H		V		H + V	
		% of Injected	%/g (wet)	%of Injected	%/g (wet)	%of Injected	%/g (wet)
F46P0.5C	7	1.91	0.0642	0.658	0.0805	2.57	0.0677
F45P0.5C	35	2.28	0.0731	0.791	0.0893	3.07	0.0767
M45P0.5B	42	2.44	0.0832	1.30	0.161	3.74	0.100
M44P0.5B	99	1.92	0.0583	0.646	0.0654	2.57	0.0599
M31P0.5B	1648	1.46	0.0384	0.546	0.0484	2.00	0.0407
M34P0.5B	1920	0.826	0.0200	0.380	0.0326	1.21	0.0278
M32P0.5B	2275	0.850	0.0227	0.319	0.0294	1.17	0.0242
F31P0.5C	2546	0.843	0.0428	0.253	0.0453	1.10	0.0434
M13P1.7	4375	1.06	0.0324	0.258	0.0361	1.32	0.0331

Table 2

M34P0.5B

Weight at Injection 12,500 (g)
 Injection to Death: 1920 (days)

Weight of Wet Skeleton 1174 g
 Weight of Ashed Skeleton: 472 g

Part	Fraction Analyzed	% dose Part	% dose g wet bone	Ashed wt wet wt	% of Skeletal Retention	Relative Conc.
Skull	1/1	3.48	0.0198	0.412	14.8	0.965
Mandibles	2/2	1.29	0.0177	0.510	5.35	0.860
Cervical Vert.	7/7	1.70	0.0243	0.452	7.04	1.18
Thoracic Vert.	13/13	3.15	0.0360	0.352	13.0	1.75
Lumbar Vert.	7/7	2.67	0.0331	0.384	11.0	1.61
Sacrum	1/1	0.569	0.0367	0.328	2.35	1.78
Caudal Vert. ^a	17/17	0.290	0.0198	0.396	1.20	0.962
Paws ^a	2/4	1.30	0.0116	0.396	5.38	0.561
Radii	1/2	0.404	0.0145	0.462	1.67	0.705
Ulnae	1/2	0.435	0.0144	0.472	1.80	0.700
Humeri	2/2	1.65	0.0200	0.418	6.84	0.970
Tibiae-Fibulae	1/2	0.559	0.00775	0.448	2.31	0.377
Femora	1/2	1.33	0.0158	0.396	5.50	0.769
Pelvis	1/2	1.92	0.0307	0.419	7.93	1.49
Scapulae	1/2	1.30	0.0266	0.387	5.38	1.29
Ribs	13/26	1.35	0.0145	0.384	5.60	0.705
Sternum	1/1	0.768	0.0176	0.144	3.18	0.853
Total		24.16	0.0206	0.396	100.	1.00

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wet weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton.

Table 3

M32P0.5B

Weight at Injection: 13,600(g)
 Injection to Death: 2275(days)

Weight of Wet Skeleton: 1117 g
 Weight of Ashed Skeleton: 440 g

Part	Fraction Analyzed	%dose Part	% dose g wet bone	Ashed wt. Wet Wt.	% of Skeletal Retention	Relative Conc.
Skull	1/1	2.97	0.0183	0.431	13.1	0.897
Mandibles	2/2	1.15	0.0164	0.545	5.36	0.805
Cervical Vert.	7/7	1.38	0.0206	0.377	6.75	1.01
Thoracic Vert.	13/13	2.38	0.0289	0.311	10.4	1.42
Lumbar Vert.	7/7	2.14	0.0291	0.365	9.42	1.43
Sacrum	1/1	0.224	0.0194	0.302	0.985	0.950
Caudal Vert. ^a	17/17	0.250	0.0199	0.394	1.10	0.977
Paws ^a	2/4	1.32	0.0118	0.394	5.79	0.581
Radii	1/2	0.282	0.0114	0.473	1.24	0.561
Ulnae	1/2	0.358	0.0129	0.473	1.57	0.632
Humeri	2/2	1.70	0.0227	0.395	7.47	1.12
Tibiae-Fibulae	1/2	0.837	0.0126	0.447	3.68	0.617
Femora	1/2	1.35	0.0178	0.416	5.91	0.872
Pelvis	1/2	1.81	0.0290	0.424	7.94	1.42
Scapulae	1/2	1.22	0.0263	0.399	5.36	1.29
Ribs	13/26	2.29	0.0230	0.340	10.0	1.13
Sternum	1/1	1.11	0.0231	0.142	4.88	1.13
Total		22.76	0.0204	0.394	100.	1.00

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wet weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton.

Table 4

F31P0.5C

Weight at Injection: 8,440 (g)
 Injection to Death: 2546(days)

Weight of Wet Skeleton: 641 g
 Weight of Ashed Skeleton: 226 g

Part	Fraction Analyzed	% dose Part	% dose g wet bone	Ashed wt Wet wt	% of Skeletal Retention	Relative Conc.
Skull	1/2	3.04	0.0266	0.347	14.6	0.818
Mandibles	2/2	1.12	0.0262	0.483	5.40	0.805
Cervical Vert.	7/7	1.19	0.0364	0.398	5.70	1.12
Thoracic Vert.	13/13	2.20	0.0492	0.277	10.6	1.51
Lumbar Vert.	7/7	1.84	0.0465	0.299	8.85	1.43
Sacrum	1/1	0.380	0.0371	0.192	1.82	1.14
Caudal Vert. ^a	17/17	0.290	0.0394	0.353	1.39	1.21
Paws ^a	2/4	1.25	0.0208	0.353	5.98	0.638
Radii	1/2	0.362	0.0245	0.435	1.74	0.755
Ulnae	1/2	0.358	0.0224	0.445	1.72	0.688
Humeri	2/2	1.68	0.0428	0.376	8.09	1.32
Tibiae-Fibulae	1/2	0.967	0.0250	0.385	4.64	0.770
Femora	1/2	1.50	0.0363	0.392	7.21	1.12
Pelvis	1/2	1.26	0.0394	0.366	6.03	1.21
Scapulae	1/2	0.933	0.0280	0.344	4.48	0.860
Ribs	13/26	2.01	0.0370	0.326	9.66	1.14
Sternum	1/1	0.451	0.0235	0.104	2.16	0.723
Total		20.83	0.0325	0.353	100.	1.00

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wt weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton.

Table 5

Average Values of Some Measurements of Weight and ^{239}Pu Retention on Three
P0.5 Level Beagle Skeletons

Part	Ashed Weight		Wet Wt. of Part		Retention of Part	
	Wet weight Av.	SD ^b	Wet Wt. of Total Skeleton Av.	SD ^b	Total Skeletal Retention Av.	SD ^b
Skull	0.396	0.0436	0.158	0.0179	0.142	0.0956
Mandibles	0.512	0.0311	0.0639	0.0027	0.0537	0.0003
Cervical Vert.	0.409	0.0390	0.0569	0.0052	0.0650	0.0070
Thoracic Vert.	0.313	0.0380	0.0726	0.0024	0.113	0.0146
Lumbar Vert.	0.349	0.0442	0.0655	0.0034	0.0977	0.0114
Sacrum	0.274	0.0719	0.0124	0.0041	0.0172	0.0069
Caudal Vert. ^a	0.381		0.0118		0.0123	0.0015
Paws ^a	0.381		0.0968		0.0572	0.0031
Radii	0.457	0.0198	0.0230	0.0008	0.0155	0.0027
Ulnae	0.463	0.0160	0.0252	0.0004	0.0170	0.0012
Humeri	0.396	0.0208	0.0663	0.0045	0.0747	0.0063
Tibiae-Fibulae	0.427	0.0361	0.0604	0.0009	0.0354	0.0117
Femora	0.401	0.0130	0.0680	0.0034	0.0621	0.0089
Pelvis	0.403	0.0322	0.0530	0.0030	0.0730	0.0110
Scapulae	0.377	0.0286	0.0450	0.0060	0.0507	0.0052
Ribs	0.350	0.0302	0.0845	0.0048	0.0844	0.0246
Sternum	0.130	0.0224	0.0367	0.0066	0.0341	0.0137
Total	0.381	0.0243	1.00		1.00	

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wet weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton.

^bStandard deviation of the average

Table 6

M31P0.5B

Weight at Injection 10,500 (g)

Injection to Death: 1648 (days)

Part	Fraction Available & Analyzed	Measured		Calculated	
		Ashed Weight (g) Part	Retention % of Inj. Dose	Wet weight (g) of Total Skeleton	Retention (%) by Total Skeleton
Skull	---	----	----	----	----
Mandibles	---	----	----	----	----
Cervical Vert.	4/7	32.27	1.86	1417	28.71
Thoracic Vert.	8/13	27.25	4.52	1199	39.89
Lumbar Vert.	3/7	31.55	3.81	1380	38.99
Sacrum	---	----	----	----	----
Caudal Vert.	17/17	3.57	0.187	794	15.20
Paws	2/4	43.22	1.53	1172	26.80
Radii	---	----	----	----	----
Ulnae	1/2	13.93	0.896	1194	52.70
Humeri	1/2	32.12	2.91	1146	38.96
Tibiae-Fibulae	---	----	----	----	----
Femora	---	----	----	----	----
Pelvis	1/2	27.16	2.65	1272	36.27
Scapulae	1/2	19.71	1.75	1160	34.54
Ribs	22/26	36.21	3.44	1224	40.77
Sternum	---	----	----	----	----
		Average		1196	35.28
		SD		168	10.0
		SD/Av.		0.141	0.284

Table 7

M31P1.7

Weight at Injection: 10,600(g)

Injection to Death: 4375(days)

Part	Fraction Available & Analyzed	Measured		Calculated	
		Ashed Weight(g) Part	Retention % of Inj. Dose	Wet Weight(g) of Total Skeleton	Retention(%) by Total Skeleton
Skull	<1/4	34.94	1.50	558	10.6
Mandibles	----	-----	-----	-----	-----
Cervical Vert.	4/7	24.13	1.42	1037	21.8
Thoracic Vert.	5/13	18.17	2.61	800	23.0
Lumbar Vert.	1/7	20.73	1.80	907	18.5
Sacrum	---	-----	-----	---	-----
Caudal Vert.	14/17	3.54	0.170	787	13.8
Paws	11/24	35.52	1.34	963	23.4
Radii	1/2	9.21	0.337	876	21.8
Ulnae	1/2	10.82	0.342	927	20.1
Humeri	1/2	25.00	2.12	952	28.4
Tibiae-Fibulae	---	-----	-----	---	-----
Femora	---	-----	-----	---	-----
Pelvis	1/2	19.19	1.82	932	24.9
Scapulae	---	-----	-----	---	-----
Ribs	13/26	29.40	2.41	994	28.5
Sternum	-----	-----	-----	---	-----
		Average		885	21.3
		SD		132	5.50
		SD/Av.		0.149	0.258

Table 8

Retention: H + V at Higher Dose Levels

Injected $\mu\text{Ci } ^{239}\text{Pu/kg}$	Average % Retention by H + V		Interval ($\bar{t}$) Injection to Death		t equivalent (Lower Dose Levels)
	% of Inj.	SD	Days	SD	
2.9	2.94	0.31	1338	424	250
0.91	2.51	0.69	1320	185	440
0.30	2.10	0.45	1650	218	690

Table 9

Measured Values of the Ratio of Ashed Weights to Wet Weights of Bones or Groups of Bones

	P1			P3			P0.5			All		
	n	Av.	SD	n	Av.	SD	n	Av.	SD	n	Av.	SD
Skull	10	0.444	0.030	4	0.468	0.031	3	0.396	0.044	17	0.441	0.038
Mandibles	11	0.538	0.015	4	0.554	0.014	3	0.512	0.031	18	0.538	0.021
Cervical Vert.	10	0.493	0.056	4	0.490	0.037	3	0.409	0.039	17	0.478	0.057
Thoracic Vert.	10	0.374	0.041	4	0.404	0.042	3	0.313	0.038	17	0.370	0.048
Lumbar Vert.	10	0.414	0.044	4	0.400	0.042	3	0.349	0.044	17	0.399	0.048
Sacrum							3	0.274	0.072			
Caudal Vert. ^a		0.418			0.432			0.377			0.414	
Paws ^a		0.418			0.432			0.377			0.414	
Radii	11	0.477	0.017	4	0.498	0.040	3	0.457	0.020	18	0.478	0.026
Ulnae	11	0.477	0.032	4	0.500	0.016	3	0.463	0.016	18	0.480	0.029
Humeri	10	0.440	0.019	3	0.370	0.023	3	0.396	0.021	16	0.419	0.035
Tibiae-Fibulae	11	0.462	0.023	4	0.475	0.036	3	0.427	0.036	18	0.459	0.031
Femora	11	0.439	0.019	4	0.451	0.040	3	0.401	0.013	18	0.435	0.028
Pelvis	10	0.442	0.031	4	0.473	0.033	3	0.403	0.032	17	0.442	0.038
Scapulae	11	0.416	0.037	4	0.448	0.023	3	0.377	0.029	18	0.420	0.042
Ribs	11	0.338	0.025	4	0.360	0.032	3	0.350	0.030	18	0.345	0.027
Sternum	9	0.172	0.032	4	0.184	0.019	3	0.130	0.022	16	0.167	0.033
Total	11	0.418	0.026	4	0.432	0.026	3	0.377	0.022	18	0.414	0.030

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wet weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton

Table 10

Measured Values of the Ratio of the Wet Weight of Bones or Groups of Bones to the Wet Weight of Total Skeleton

Part	P1			P3			P0.5			All		
	n	Av.	SD	n	Av.	SD	n	Av.	SD	n	Av.	SD
Skull	11	0.168	0.014	4	0.159	0.008	3	0.158	0.018	18	0.164	0.014
Mandibles	11	0.070	0.006	4	0.084	0.014	3	0.064	0.003	18	0.072	0.010
Cervical Vert.	11	0.067	0.005	4	0.069	0.006	3	0.057	0.005	18	0.067	0.008
Thoracic Vert.	11	0.068	0.009	4	0.065	0.005	3	0.073	0.002	18	0.069	0.008
Lumbar Vert.	11	0.061	0.004	4	0.059	0.003	3	0.066	0.003	18	0.061	0.004
Sacrum							3	0.012	0.004	3	0.012	0.004
Caudal Vert. ^a	12	0.022	0.007	4	0.030	0.002	3	0.012	0.001	19	0.022	0.079
Paws ^a	12	0.101	0.006	4	0.100	0.012	3	0.097	0.004	19	0.100	0.009
Radii	11	0.024	0.002	4	0.026	0.001	3	0.023	0.001	18	0.024	0.002
Ulnae	11	0.025	0.002	4	0.028	0.001	3	0.025	0.0004	18	0.026	0.002
Humeri	11	0.059	0.005	3	0.062	0.001	3	0.066	0.004	17	0.061	0.005
Tibiae-Fibulae	11	0.058	0.005	4	0.063	0.002	3	0.060	0.001	18	0.060	0.005
Femora	11	0.063	0.005	4	0.067	0.003	3	0.068	0.003	18	0.065	0.005
Pelvis	10	0.050	0.005	4	0.052	0.002	3	0.053	0.003	17	0.051	0.004
Scapulae	11	0.039	0.002	4	0.038	0.002	3	0.045	0.006	18	0.040	0.004
Ribs	11	0.104	0.0005	4	0.083	0.001	3	0.084	0.0005	18	0.096	0.001
Sternum	9	0.010	0.003	4	0.010	0.007	3	0.037	0.007	16	0.015	0.012

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wet weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton

Table 11

Measured Values of the Ratio of the Wet Weight of a Bone or Group of Bones (X 100) to the Weight of the Beagle at Time of Injection.

Part	P1			P3			P0.5			All		
	n	Av.	SD	n	Av.	SD	n	Av.	SD	n	Av.	SD
Skull	11	1.23	0.193	4	1.09	0.087	3	1.32	0.104	18	1.22	0.174
Mandibles	11	0.516	0.080	4	0.539	0.077	3	0.536	0.042	18	0.514	0.068
Cervical Vert.	10	0.492	0.070	4	0.506	0.101	3	0.480	0.088	17	0.493	0.076
Thoracic Vert.	11	0.505	0.110	4	0.504	0.048	3	0.611	0.085	18	0.523	0.100
Lumbar Vert.	11	0.449	0.071	4	0.463	0.065	3	0.552	0.087	18	0.469	0.087
Sacrum							3	0.110	0.022	3	0.110	0.022
Caudal Vert. ^a	12	0.158	0.069	4	0.216	0.020	3	0.099	0.016	19	0.161	0.066
Paws ^a	12	0.739	0.118	4	0.716	0.104	3	0.810	0.095	19	0.746	0.110
Radii	11	0.173	0.023	4	0.167	0.020	3	0.193	0.026	18	0.175	0.023
Ulnae	11	0.185	0.025	4	0.183	0.026	3	0.212	0.026	18	0.189	0.026
Humeri	11	0.431	0.063	3	0.506	0.031	3	0.559	0.098	17	0.467	0.081
Tibiae-Fibulae	11	0.429	0.064	4	0.422	0.056	3	0.508	0.061	18	0.441	0.066
Femora	11	0.462	0.056	4	0.474	0.074	3	0.572	0.092	18	0.483	0.074
Pelvis	11	0.367	0.057	4	0.361	0.058	3	0.446	0.062	18	0.379	0.062
Scapulae	11	0.288	0.037	4	0.268	0.036	3	0.376	0.030	18	0.298	0.050
Ribs	11	0.762	0.103	4	0.702	0.075	3	0.708	0.056	18	0.739	0.091
Sternum	9	0.071	0.022	4	0.127	0.038	3	0.310	0.072	16	0.130	0.099
Total	11	7.316 ^b	0.997	4	7.282 ^b	0.626	3	8.399	0.912	18	7.49	0.963

^aCaudal vertebra and paws were not defleshed. Ashed weights were obtained and wet weights calculated by assuming the ashed weight/wet weight ratio for these parts to be the same as the average such ratio for the rest of the skeleton.

^bIncludes a value for the sacrum derived from related data.(13)

Table 12
Average Weights and Doses

Dose Level	n	Average Dog Weight at Injection		Average Dose	
		kg	SD.	μCi/kg	SD.
P1	25	9.80	1.64	0.0155	0.00110
P1.7	14	9.97	1.55	0.0477	0.00179
P2	12	8.95	1.15	0.0951	0.00786
P3	12	8.72	1.04	0.296	0.0149
P4	12	8.70	1.28	0.907	0.0678
P5	9	8.76	0.56	2.88	0.294

Figure 1. Percent of injected ²³⁹Pu found in one Humerus plus 3rd Lumbar Vertebral body at times ranging from 35 to 5200 days following injection.

Figure 2. Percent of injected ^{239}Pu found in the beagle skeleton at time ranging from 35 to 4375 days following injection. Solid circles are the recently acquired values for the 4-P0.5 and the P1.7 level animals.

Figure 3. Skeletal Retention of ^{239}Pu following injection at the 6 levels shown in Table 12 and the P0.5 ($0.006 \mu\text{Ci/kg}$) level. B (low level) includes P0.5, P1, P1.7, and P2 levels.

Figure 4. Cumulative doses (rads) to the Skeleton and Liver following administration of ^{239}Pu at the P1, P1.7, and P2 level. (See Table 12).

Figure 5. Cumulative doses (rads) to the Skeleton and Liver following administration of ^{239}Pu at the P3, P4, and P5 level (See Table 12).

QUANTITATIVE MORPHOLOGY OF VERTEBRAL TRABECULAR BONE IN BEAGLES INJECTED WITH PLUTONIUM

W. S. S. Jee, R. B. Dell, and E. Hashimoto

ABSTRACT: The percentage of trabecular bone within the cortical bone envelope of lumbar vertebral bodies of beagles diminishes with age from a value of 25% at 500 days to a value of 16% at about 6000 days of age. Beagles injected with 2.7, 0.909, 0.301, 0.096, and 0.0485 μCi of $^{239}\text{Pu}/\text{kg}$ exhibit elevated percent trabecular bone values, but were within normal range for their respective age groups in the beagles injected with 0.0157 $\mu\text{Ci}/\text{kg}$. The dogs injected with 0.0157 to 2.7 μCi $^{239}\text{Pu}/\text{kg}$ show increased trabecular widths. In general, the effect of a single intravenous dose of plutonium results in the inhibition of bone resorption leading to thicker or mosaic trabeculae.

INTRODUCTION

Histopathological lesions in bones, induced by a single intravenous injection of plutonium ($+4$) citrate ranging between 2.7 to 0.0158 $\mu\text{Ci}/\text{kg}$, are listed in Table I.⁽¹⁾ These changes have also been observed for other transuranic elements. They have been reviewed recently⁽¹⁾ and only those changes contributing to the alterations in percentage of the lumbar vertebral body occupied by trabecular bone and frequency distribution of trabecular widths in lumbar vertebral bodies will be discussed in this progress report.

MATERIALS AND METHODS

Preparation of samples: Adult beagle lumbar vertebral bodies were collected from 109 autopsies of plutonium toxicity and aging control dogs (Table II). The second lumbar vertebral body was removed, cut longitudinally into 2 halves, fixed in acetone, defatted, dehydrated and embedded in a mixture of 45% ethyl and 55% butyl methacrylate. The embedded vertebral bodies were sectioned at 8 microns as undecalcified sections by a motor-driven

heavy duty "K" Jung microtome. The undecalcified sections were stained by the Von Kossa mineralized bone method.⁽²⁾

Method of quantitation: Percentage of trabecular bone in the Von Kossa stained section, which is the percentage of the area within the cortical bone envelope of a vertebral body was measured directly on the Quantitative Television Microscope (QTM).^{*} Also the percentage of the total numbers of trabeculae counted which are wider than a certain chord length was measured directly by the QTM. The chord length was later converted to micron lengths and plotted as the frequency distribution of trabecular widths.

RESULTS

The percentage of the lumbar vertebral body occupied by trabecular bone diminished with age. A maximum value of 25% was present at about 500 days of age with a minimum value of 16% at about 6000 days of age. There was a great deal of variation in this value for the 41 aging controls. They varied from 10 to 33% (Figs. 1-4).

The percent of trabecular bone in six, 5-level plutonium dogs (2.7 $\mu\text{Ci/kg}$ injected) between the ages of 1014 and 2130 days showed 2 dogs with increased and one dog with decreased percent bone values (Figs. 5-7).^{**}

The most consistent percent bone values for a group of dogs were measurements from the 4-level dogs (0.909 $\mu\text{Ci/kg}$ injected) dying between 1632 and 2167 days of age. They all exhibited increased percent bone values ranging from 24.5 to 48.5 percent (Figs. 8-9).

The percent bone values for the 3 level dogs (0.301 $\mu\text{Ci/kg}$ injected)

^{*}Metal Research Limited, Cambridge, England.

^{**}The value for percent bone was plotted against total age in days. The radio-nuclide burdened dogs were injected at about 500 days of age. See injection tables for exact age at injection.

were most surprising. There possibly may have been a transient increase in percent bone between ages 1000 to 1300; however, many of the remaining dogs' percent bone value fell within control values (Figs. 10-11).

Again the 2-level dogs (0.096 $\mu\text{Ci/kg}$ injected) exhibited elevated percent bone values in 11 out of 12 dogs ranging between 2167 and 3670 days of age. Their percent bone measurements ranged between 22 and 35% (Figs. 12-13).

At the 1.7-injection level (0.0485 $\mu\text{Ci/kg}$ injected), the trend was still toward elevated percent bone values. Four out of 8 animals possessed elevated values and only 2 dogs exhibited depressed values (Figs. 14-15).

When the percent trabecular bone for 1-level dogs (0.0157 $\mu\text{Ci/kg}$ injected) was considered collectively, the values were within the normal range for their respective age groups. However, when individual dog values were plotted, the values dipped as low as 12% at 3,000 and 5,400 days of age and a high of 24% at 4,500 days of age (Figs. 1 and 16).

Increased trabecular widths were observed in dogs injected with high dose levels of ^{239}Pu . For example, the median value for the frequency distribution of trabecular widths is 123 μm for dogs injected with 0.909 μCi of $^{239}\text{Pu/kg}$, 109 μm for dogs injected with 0.0157 $\mu\text{Ci/kg}$ and 93 μm for control dogs of comparable age (Fig. 17).

DISCUSSION

There is no doubt that control dogs tend to lose trabecular bone from the lumbar vertebral bodies as a function of age. However, it is much more difficult to characterize that a dog of a certain age possesses lumbar vertebral bodies with a given percentage of trabecular bone. It may be possible to do this when a larger population of control dogs is available for analysis.

The tremendous variation of percent bone at young and old ages suggests the possibility that there are two populations of dogs skeletally. One population of dogs has about 35% trabecular bone in vertebral bodies at 500 days, which diminishes to 21% at age 6,000 days. Another population of dogs has about 25% trabecular bone at young adulthood which involutes to 10-14% at old age. Further and more careful analyses may provide the answer.

In general, the effect of a single intravenous dose of plutonium results in increased percent trabecular bone in lumbar vertebral bodies. This was a result of the inhibition of bone resorption leading to thicker or mosaic trabeculae. When the injected doses are unusually high (greater than $0.909 \mu\text{Ci/kg}$), the entire bone remodeling process (both bone resorption and formation) was inhibited and the trabeculae are coated with a dense fibrotic layer which we have previously called peritrabecular fibrosis (Fig. 6). Often late excessive bone resorption can occur beneath the dense fibrotic layer (Fig. 7).

Elevated percent trabecular bone values were observed down to the $0.0485 \mu\text{Ci/kg}$ injected dose level, where autoradiographically there are few scattered foci of heavy buried deposits of plutonium in islands of mosaic trabeculae (Figs. 16 and 17). It should be remembered that ^{239}Pu is initially a bone surface seeker and labels all existing surface intensively in these young adult beagles. The labeled bone surfaces serve as an excellent bone marker. Thus, when any heavy plutonium deposits are still found in trabeculae at many days post injection, it will mean that particular piece of bone is the same bone found at the day of injection. In the P2 and P3 dose level vertebral bodies, large numbers of trabeculae contain the buried plutonium marker. In the P4 level, almost all trabeculae are labeled with the buried plutonium, while in the P5 level some of the Pu is found unremodeled on the bone surfaces.

It is difficult to tell whether the remodeling of the P1 level vertebral bodies ~~has~~ been altered. There are a few sites with buried plutonium in trabeculae indicating that some trabeculae of the lumbar vertebral body of these dogs had not yet turned over after an interval of 2394 days.

Non-radioactive trabecular labeling studies using tetracycline and fluorescent microscopy are in progress. In the future, more trabecular remodeling rate data will be available from old control dogs using the tetracycline procedure.

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Fig. 1. Percent trabecular bone inside lumbar vertebral bodies as a function of age for beagles injected with 0 or 0.016 μCi $^{239}\text{Pu}/\text{kg}$. The radionuclide was injected at about 500 days of age.

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Fig. 2. Appearance of trabecular bones in a portion of the lumbar vertebral body of a 767 day old, male beagle (M58A). Note the thicker plates of bone (arrows) interspersed between the thinner longitudinally oriented trabeculae and the frequent transverse trabeculae (vertically oriented). X 84.

Fig. 3. Appearance of trabecular bones in a portion of a lumbar vertebral body of 2755 day old, female beagle (F2P0). Trabecular surfaces show evidence of bone resorption (arrows). X 84.

Fig. 4. Appearance of trabecular bones in a portion of a lumbar vertebral body of a 5246 day old, female beagle (F49A). Fewer transverse trabeculae are apparent. X 84.

Fig. 5. Comparison of the distribution of percent trabecular bone in lumbar vertebral bodies of beagles injected with $2.7 \mu\text{Ci}$ of $^{239}\text{Pu}/\text{kg}$ against the corresponding curve for ageing controls.

Fig. 6. Appearance of necrotic trabeculae (n) generalized peritrabecular fibrosis, (f), fibrotic marrow and hypermineralized tissue (h) in lumbar vertebral body of a beagle (F9P5) injected with 2.73 μ Ci of ^{239}Pu /kg and surviving 1145 days. Note the relatively smooth trabecular bone surfaces. X 84.

Fig. 7. Appearance of a portion of the same vertebral body as Figure 6. Note the island of hyperplastic marrow (m), extensive peritrabecular fibrosis (f) and scalloped trabecular borders (arrows). The trabeculae per se were about the same width of those of comparable aged controls. X 84.

Fig. 8. Comparison of the distribution of percent trabecular bone in lumbar vertebral bodies of beagles injected with 0.909 μCi of $^{239}\text{Pu}/\text{kg}$ against the corresponding curve for aging controls.

Fig. 9. Typical appearance of a portion of a lumbar vertebral body from a beagle (M4P4) injected with 0.974 μCi of $^{239}\text{Pu}/\text{kg}$ and sacrificed at 1066 days later. Note the massive mosaic trabeculae (t), the spotty peritrabecular fibrosis (f), and the hyperplastic marrow (m). X 84.

Fig. 10. Comparison of the distribution of percent trabecular bone in the lumbar vertebral bodies of beagles injected with 0.303 μCi of ^{239}Pu /kg against the corresponding curve for ageing controls.

Fig. 11. Appearance of a portion of a lumbar vertebral body from a beagle (F10P3) injected with 0.298 μCi of $^{239}\text{Pu}/\text{kg}$ and sacrificed 1547 days later. Occasional thick transverse trabecula (arrow) occurs in this group of beagles. X 84.

Fig. 12. Comparison of the distribution of percent trabecular bone in lumbar vertebral bodies of beagles injected with 0.096 μCi of $^{239}\text{Pu}/\text{kg}$ against the corresponding curve for ageing controls.

Fig. 13. Appearance of a portion of the lumbar vertebral body from a beagle (F7P2) injected with 0.0996 μCi of $^{239}\text{Pu}/\text{kg}$ and sacrificed 2093 days later. The trabeculae were uniformly thicker.

Fig. 14. Comparison of the distribution of percent trabecular bone in lumbar vertebral bodies of beagles injected with 0.0485 μCi of $^{239}\text{Pu}/\text{kg}$ against the corresponding curve for ageing controls.

Fig. 15. Appearance of a portion of the lumbar vertebral body from a beag.
(M12P1.7) injected with 0.0491 μCi of ^{239}Pu /kg and sacrificed
2093 days later. Some of the trabeculae are much thicker than
others. X 84.

Fig. 16. Appearance of a portion of the lumbar vertebral body from a beagle (F9P1) injected with 0.0167 μCi of $^{239}\text{Pu}/\text{kg}$ and surviving 2257 days. Note the thinner than normal and scalloped trabeculae (arrows). X 84.

Fig. 17. Comparison of the frequency distribution of trabecular width in lumbar vertebral bodies of beagles injected with 0.909, 0.0157, and 0 μCi of $^{239}\text{Pu/kg}$.

Fig. 18. Moderate concentration of buried alpha tracks (arrows) in lumbar vertebral body trabeculae from a beagle (F6Pl.7) injected with $0.0459 \mu\text{Ci}$ of $^{239}\text{Pu}/\text{kg}$ and sacrificed 2221 days later. Buried alpha tracks were initially on trabecular surfaces. X 130.

Fig. 19. Heavy planar deposits (arrows) of ^{239}Pu within mosaic trabecula in a beagle (M11P2) injected with $0.0961 \mu\text{Ci}$ of $^{239}\text{Pu}/\text{kg}$ and sacrificed 1617 days later. X 130.

TABLE 1. SOME "HISTOLOGICALLY" DETECTED LESIONS IN BONE

Trabecular Bone	Cortical Bone
Extravasation of blood	Haversian canal plugs
Bone cell death	Inhibited canalicular transport
Depressed DNA synthesis (osteoprogenitor cells)	Osteocyte death
Diminished vascularity	Altered vascularity
Suppression of bone resorption	Excessive resorption
Acellular marrow	Atypical bone formation
Petrirabecular fibrosis	Endosteal bone formation
Atypical bone formation	Altered remodeling rate
Endosteal bone growth	Death to cells residing in Haversian canals
Mosaic trabeculae	Injury to cells residing in Haversian canals
Growth arrest plate	Damage to blood vessels
Devitalized bone	Spontaneous fracture
Spontaneous micro-fractures	Osteogenic sarcoma (rare)
Damage to progenitor cells	
Altered bone remodeling rate	
Pathologic bone resorption	
Regeneration of marrow	
Formation of osteogenic sarcoma	

CELLULAR KINETICS OF THE PERIODONTAL LIGAMENT SUBSEQUENT TO OSTEOGENIC STIMULUS

W. E. Roberts and W. S. S. Jee

ABSTRACT: Orthodontically stimulated rat periodontal ligament (PDL) has been used as an experimental model for studying osseous precursor cell proliferation associated with stimulated osteogenesis. Osteogenic stimulus by orthodontic elastics resulted in a progressive increase in tritiated thymidine labeling indices of PDL fibroblasts up to a peak of 25.2% at 27 hours. Percent labeled cells then dropped rapidly to a slowly descending plateau of approximately 10% from 30-72 hours. Percent labeled mitoses curves revealed average generation time for stimulated fibroblasts of 37, 36 and 32 hours respectively for samples of cells labeled at 8, 27 and 36 hours following initiation of osteogenic stimulus. Variation of individual cell cycle times was found to be $\pm 5\%$. Instead of approximately doubling after 16 hours (length of first mitotic peak of the labeled mitoses curves), the number of labeled PDL fibroblasts only increased about 57%, indicating DNA synthesis not immediately associated with mitosis. From interpretation of percent labeled mitoses curves, a multiple compartment model was proposed to explain the kinetics of the proliferating population of the stimulated PDL.

INTRODUCTION

The bone group is always in search of a useful experimental model to study the toxic effects of plutonium-239 upon hard tissues. Recently, one of the authors (W. E. Roberts) developed the rat periodontal ligament (PDL) model for studying the cellular kinetics of bone cells in response to an osteogenic stimulus. This has led to studies now in progress to learn more about the effects of plutonium-239, parathyroid hormone and hydrocortisone upon the cellular kinetics of bone cells.

The current report will deal with characterizing the labeling indices of unstimulated and stimulated PDL fibroblasts (precursors of bone cells), the percent labeled mitoses curves for 8, 27 and 36 hours post stimulus, total cell (generation) cycle experiments, the values for generation cell cycle time (average, minimum and maximum generation cycle time - t_G^{ave} , t_G^{min}

and $t_G^{\max}$) and average DNA synthesis time (t_S^{ave}) for the stimulated PDL fibroblasts and a proposed cytodifferentiation sequence to osteoblasts.

Most studies of the cellular kinetics of osteogenesis have been done in young growing animals.⁽¹⁻⁴⁾ The relationship of these systems to stimulated bone formation in adult animals such as in fracture repair and orthodontic tooth movement is uncertain. Tritiated thymidine ($^3\text{HTDR}$) labeling indices of fractured long bones has provided information on the proliferative response of resting osteogenic cells.⁽⁵⁻⁷⁾ Since fracture repair involves both appositional and endochondral bone formation,⁽⁸⁾ cell population studies are greatly complicated. Consequently, the nature of osteogenic proliferation and the kinetics of osteoblast formation remain to be illucidated.

It is well established that proliferating periodontal ligament (PDL) fibroblasts differentiate into osteoblasts and form bone in response to orthodontic stimulus,^(9,10) but detailed kinetics of cellular proliferation and specialization have not been studied. The objective of this study is not only to characterise the cellular kinetics of orthodontically stimulated PDL fibroblasts, but to suggest that this PDL reaction is characteristic of the general response of resting periosteum to an osteogenic stimulus.

MATERIALS AND METHODS

The cellular kinetics studies were divided into two parts: 1) the proliferative activities of both unstimulated (control) and stimulated PDL fibroblasts were studied between 8 and 72 hours after activation using the labeling index method⁽¹¹⁾ and 2) the generation cycle curves of the stimulated PDL fibroblasts at 8, 27 and 36 hours following the stimulus were determined using the percent labeled mitoses method.⁽¹²⁾

In both studies, male, 200 g Sprague-Dawley rats, approximately six weeks of age, were allowed to acclimate to their cages for four days. Between 9 and 10 a.m. on the fifth day, a 0.5 mm latex elastic (#J-104, Rocky Mountain Co., Denver, Colorado) was placed in the contact area between the maxillary right first and second molars of each animal (Fig. 1). The maxillary left first and second molars served as the unstimulated control side. In the labeling index experiment, two animals per group received a subcutaneous injection of 1.0 μCi $^3\text{HTDR}$ (New England Nuclear Corp., specific activity 16.7 Ci/mM)/g at each of the following time intervals: 8, 16, 20, 24, 27, 30, 32, 36, 48, and 72 hours following application of elastics. The animals were sacrificed by decapitation one hour later. The maxilla was rapidly removed from each animal, separated at the mid-palatal suture, and fixed separately in 2.5% buffered glutaraldehyde of pH 7.4. The fixative was prepared by mixing five parts 0.2M sodium phosphate buffer (92% dibasic and 8% monobasic sodium phosphate), four parts distilled water and one part 25% glutaraldehyde. Following a 24 hour fixation, tissues were rinsed with running tap water and decalcified for two weeks in repeated changes of 10% Versene adjusted to pH 7 with glacial acetic acid. To standardize the area of the PDL sampled, decalcified maxillae were leveled with a razor blade under a dissecting microscope to just short of the desired plane of sectioning, which was the sagittal plane along the long axis of the mesial (anterior) root of the maxillary first molar (Fig. 1). Specimens were oriented flat against the bottom of one ounce vial and embedded for thin sectioning according to the modified methyl methacrylate technique of Cathey.⁽¹³⁾ Following careful leveling to the desired plane, blocks were sectioned at $3 \pm 0.3 \mu\text{m}$ on a mechanized, heavy

sledge-type microtome (Jung, Heidelberg, Germany) and mounted on 0.5% gelatinized slides. As a quality control, random sections were measured between glass slides on an electronic caliper to a tolerance of 0.00001 in. (approximately $0.25\mu\text{m}$). With strict adherence to standardized procedures, section thickness was relatively uniform ($\pm 0.25\mu\text{m}$). Due to the low energy of tritium (average range $1\mu\text{m}$) and the rigid quantitation necessary for relatively precise labeled cell counts, it has been our experience that uniformly thin sections, $3\mu\text{m}$ or less, are essential.

Sections were run through three ten-minute changes of acetone to remove the embedding medium, then down through the alcohols to distilled water. Autoradiographs were prepared with Kodak NTB liquid emulsion according to Arnold⁽¹⁴⁾ and Fabrikant.⁽¹⁵⁾ Following three weeks' exposure in a light tight can, slides were developed for five minutes in Kodak D-19 at 65°F , fixed for ten minutes and stained with Mayer's hematoxylin and eosin. This produced autoradiographs with negligible background (less than 5 grains/ $1000\mu\text{m}^2$) and any cell with three or more grains over the nucleus was considered labeled.

As shown in Figure 1, the histologic area under study was the PDL along the mesial root of the maxillary first molar. The area is bound by the mesial surface of the maxillary first molar posteriorly, the alveolar bone anteriorly, a perpendicular to the alveolar bone from the apex of the root superiorly, and a perpendicular to the tooth surface from the alveolar crest inferiorly. Only labeled and unlabeled PDL fibroblasts were counted. All other cell types, including endothelial, epithelial, intravascular, and myelogenous cells were excluded. For each animal a sample (greater than 2000 cells) consisted of a count of all PDL fibroblasts in the area studied

from three serial sections. Figure 1 is representative of the histological appearance of sections counted.

The percent labeling index of PDL fibroblasts in the defined area was determined by dividing the number of labeled cells by the total number of cells present and multiplying by 100. Means and standard deviations were computed according to Wolff.⁽¹⁶⁾

For the 8 hour post stimulus generation cycle, 38 male, 200 g Sprague-Dawley rats were divided into nineteen groups. Elastics were inserted 8 hours prior to injection of 0.5 μ Ci of 3 HTDR/g and the animals were sacrificed in groups of two at each of the following time intervals: 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 16, 32, 36, 40, 42, 45, 50, and 72 hours after injection of 3 HTDR. The same protocol was used for the 27 and 36 hour post stimulus samples, differing only in the initial time period between insertion of elastics and injection of 3 HTDR.

Utilizing the same histologic area and autoradiographic procedures previously described for the labeling index counts, generation cycle curves were plotted by the percent labeled mitoses technique of Quastler and Sherman.⁽¹²⁾ A mitotic figure with three or more grains over the nucleus was considered labeled. Only the PDL of the stimulated side was studied because the unstimulated side with its extremely low number of mitoses did not lend itself to such studies.

The procedure for analyzing generation cycle curves is a modification of the Quastler and Sherman methods adapted to our system (Fig. 4). The average time a PDL fibroblast spends in S phase (t_s) is estimated by measuring between the 50% level on the ascending portion to the 50% level on the descending portion of the first peak of the generation cycle curve. The

minimum generation time ($t_G^{\min}$) is determined by measuring between the initial points of inflection of the first and second peaks. The average generation time (t_G^{ave}) is estimated by the distance between the apices of the two peaks. The apex represents the mode⁽¹⁶⁾ of the population of labeled cells passing through mitosis. Maximum generation time ($t_G^{\max}$) is determined by measuring between the points where the descending portions of the peaks return to zero.

Generation cycle curves were plotted on 20 x 50 cm. of linear graph paper graduated in mm. and the number of mm² under each peak was counted. Height of each peak was measured from the apex perpendicular to the time axis, and the base length was measured directly on the time axis. On the horizontal axis 1 cm = 5% labeled mitotic figures. The area under the first peak (A) is related to that of the second peak (A') by the ratio A'/A. Similar ratios for height (H) and base length (b) were also calculated (Table 3). Since the second peak of the 8 hour curve was incomplete (Figure 3), its area and base length were estimated by extrapolating the descending curve to zero percent labeled mitoses.

Cells which initially incorporated low levels of tritiated thymidine (fewer than 6 grains/nucleus) may be undetectable at the 3+ grains/nucleus level after one mitosis. To test the magnitude of this potential variable, grain counts were made of all labeled nuclei of two sections from each of four animals of the 36 hour generation cycle experiment. The 1.5 hour animals were selected as samples of the initially labeled population of proliferating fibroblasts and the 16 hour animals were chosen as a post mitotic sample. Scored grain counts were arranged in groups, tabulated in Table 4, and plotted in Figure 5.

RESULTS

A detailed histologic analysis of the unstimulated and stimulated osteogenic PDL will be published elsewhere. Suffice to say for this study, bone formation was first observed along the alveolar bone surface of the area studied approximately 40 hours after activation (placing of elastics). Rapid and continuous fibrous bone formation continued up through 108 hours post elastic, which is the longest time period under consideration in this study.

The autoradiographic results of labeling index counts are tabulated in Table 1 and plotted in Figure 2. The percent labeling index and its standard deviation of fibroblasts in the unstimulated PDL was $1.41 \pm 0.10\%$ averaged over all sacrifice times. The means and standard deviation of the percent labeling of fibroblasts in the stimulated PDL between 8 and 72 hours post stimulus rose from $2.90 \pm 0.71\%$ at 8 hours to a peak of $25.20 \pm 0.42\%$ at 27 hours, then dropped abruptly to $10.60 \pm 0.57\%$ at 30 hours and plateaued for a value of $9.00 \pm 0.28\%$ at 72 hours (Fig. 2).

The percent labeled mitoses counts of the 8, 27 and 36 hour generation cycle experiments are tabulated in Table 2 and plotted in Figure 3. In the 8 hour (pre-peak DNA synthesis) generation cycle experiment, the percent labeled mitoses curve began to rise by 2.5 hours, ascended to a peak of $97.90 \pm 0.49\%$ at 8 hours, dropped to $90.30 \pm 0.99\%$ at 10 hours and to 0% at 16 hours. The curve began to rise again at 36 hours, ascended to a second peak of $15.50 \pm 0.42\%$ at 45 hours, and dropped to $4.60 \pm 0.57\%$ at 50 hours (Table 2; Fig. 3).

The plot for the 27 hour (peak DNA synthesis) generation cycle was similar to that at 8 hours, except that the first peak occurred one hour

sooner while the second peak was two hours advanced.

The 36 hour (post-peak DNA synthesis) percent labeled mitoses curve was similar to those at 8 and 27 hours except the second peak was significantly higher (39.35 ± 0.35 opposed to 15.50 ± 0.42 and 16.00 ± 0.14 respectively).

For the 8 hour post stimulus study the average time a PDL fibroblast spends in DNA synthesis (t_S^{ave}) was 9 hours, the minimum generation cycle time (t_G^{min}) was 34 hours, t_G^{ave} was 37 hours, and t_G^{max} was estimated to be about 38 hours (Table 3). These same values for the 27 hour generation cycle were shorter by 1/2 to 2 hours. Similar values for the 36 hour group were likewise less by one to six hours, except for t_S^{ave} which was prolonged 1/2 hour.

The ratio of the area under the second peak divided by the area under the first peak (A'/A) was 0.160, 0.165, and 0.331 for the 8, 27, and 36 hour generation cycles respectively. The ratio of second to first peak heights (H'/H) was quite similar to area ratios and measured as 0.151, 0.160 and 0.394 respectively for the 8, 27, and 36 hour labeled mitoses curves. Base length ratios of the second to the first peaks (b'/b) were 1.07 for all three generation cycles. Since base length only increases by 7% from the first to the second peaks (Table 3), H'/H is a good estimate of A'/A and is easier to measure.

Grain counts from the 36 hour experiment show that less than 5% of the initially labeled sample of fibroblasts have fewer than six grains/nucleus. Total number of labeled cells per section increased from 55.8 ± 13.4 at 1.5 hours to 87.8 ± 10.0 cells at 16 hours following injection of $^3\text{HTDR}$. This difference was significant at the $P < .05$ level (Table 4 and Fig. 5).

DISCUSSION

The osteogenic model developed for these studies is a modification of the techniques of Waldo and Rothblatt⁽¹⁷⁾ who used a 0.8mm "rubber band" between the maxillary molars of rats to elicit the histologic reactions characteristic of orthodontic tooth movement. In the present experiments, reducing the size of the elastic to 0.5 mm, has resulted in a more uniform deposition of fibrous bone along essentially the entire alveolar surface of the PDL on the mesial aspect of the maxillary first molar (Fig. 1). Our total experience with this model^(10,28-25), which is in excess of 400 adult rats sacrificed from five minutes to fifteen days after placing elastics, has confirmed the observation of others^(17,26,27) that this is a relatively precise, reproducible osteogenic reaction resulting in rapid and continuous bone formation.

As shown in Figure 1, an elastic separating the contact areas of maxillary first and second molars consistently resulted in generalized bone apposition along the alveolar margin of the mesial root of the maxillary first molar. The only bone resorption noted in the area studied was occasional, localized osteoclasts at the alveolar crest. This histological observation is in apparent conflict with Macapanpan et al.,⁽²⁶⁾ who reported bone resorption on the "mesial of maxillary first molars." This discrepancy may be due to the thinner elastic which they used (a strip of rubber band) and uncertainty as to the precise root surface studied. The rat maxillary first molar has five roots and detailed information on the plane of sectioning was not given.

In the present study the unstimulated PDL fibroblast labeling index

($1.41 \pm 0.10\%$) was similar to that observed by Weiss, Stahl and Tonna,⁽²⁸⁾ while Toto and Borg⁽²⁹⁾ noted a labeling index of only 0.38% in PDL fibroblasts of 60 day-old rats. The discrepancy between their data and the current study may be explained by the following differences in experimental design: 1) lower dose of $^3\text{HTDR}$ ($0.7 \mu\text{Ci/gm}$), 2) thicker sections ($4-6 \mu\text{m}$), and 3) older experimental animals. These factors are known to reduce the number of labeled cells. Manspeizer and Tonna⁽¹¹⁾ have shown that a dose of less than $1.0 \mu\text{Ci/gm}$ $^3\text{HTDR}$ results in significantly fewer osteogenic cells. Owen and MacPherson⁽⁴⁾ pointed out that due to the low energy of ^3H beta particles (average range $1 \mu\text{m}$ in soft tissue), thicker sections restrict the percentage of radioactive nuclei recording disintegrations in the autoradiographic emulsion layer. Furthermore, Jensen and Toto,⁽³⁰⁾ and Toto and Borg⁽²⁹⁾ have observed a progressive decrease in the labeling index of PDL fibroblasts as a function of age.

Although increased uptake of $^3\text{HTDR}$ in orthodontically stimulated PDL fibroblasts has been observed,^(31,32) labeling index and generation cycle curves have not been previously reported.

Prior to designing the generation cycle experiments, peak DNA synthesis (maximum percent labeled cells) was found to occur at 27 hours following activation. As shown in Figure 2, the 8, 27, and 36 hour post elastic time periods, prior to injection of $^3\text{HTDR}$, allowed the study of generation cycle characteristics of three separate samples of cells from the proliferating population. The fraction of the population of proliferating fibroblasts in DNA synthesis (S) phase at 8, 27, and 36 hours following activation is 2.9%, 25.2%, and 10.6% respectively (Fig. 2). Referring to the portion of PDL fibroblasts in S phase at the time of $^3\text{HTDR}$ injection, the 8, 27,

and 36 hour groups are the pre-peak, and post-peak DNA synthesis samples of proliferating fibroblasts. The relationship of the generation cycle samples to the labeling index characteristics of the model system are graphically presented in Figure 2.

Variability of individual cell cycle times can be estimated by comparing the range from $t_G^{\min}$ to $t_G^{\max}$:

$$\text{Variation Index (VI)} = \frac{t_G^{\max} - t_G^{\min}}{t_G^{\text{ave}}}$$

From data in Table 3, VI for the 8, 27, and 36 hour generation cycles is 10.6%, 11.1%, and 6.3% respectively. Another method for measuring variability is comparison of base lengths of the first and second peaks of labeled curves. The base length of the peak represents the total time for all proliferating labeled cells to pass through mitosis. For all three generation cycles the base of the second peak was approximately 7% longer than that for the first peak (Table 3). Comparison of these six estimates indicates that variability of cell cycle times is on the order of 10%. Assuming that the mean t_G is approximately midway between the two extremes, the variation of cell cycle times of the samples studied is about $\pm 5\%$.

Studying labeling indices of fracture stimulated periosteum of long bones in mice, Tonna and Cronkite⁽⁶⁾ observed a rise in $^3\text{HTDR}$ labeling from 2% to 25.4% at 32 hours, followed by a slow descent to about 10% by four days. In a similar experiment in young rats, Nichols, Toto and Choukas⁽⁷⁾ reported peak labeling of 30% at 24 hours which then fell to about 10% by 56 hours. Comparing these data to the 25.2% at 27 hours with a drop to 10.6% at 30-36 hours observed in the present study, the initial osteogenic

response of the orthodontically stimulated PDL is similar to that for osseous precursor cells of long bone periosteum. The persistence of elevated labeling index (LI) values for up to four days in fracture stimulated periosteum may indicate that a larger fraction of the resting osteogenic population is being stimulated to proliferate than in the stimulated PDL, or longer duration of maximal proliferation. This correlates with one of the interpretations of our findings that only about 35% of PDL fibroblasts in the area studied respond to osteogenic stimulus by synthesizing DNA. This point will be more fully developed later in discussion. It appears the initial osteogenic response of stimulated PDL and fractured bone periosteum are similar phenomena. The major difference of fracture being the magnitude of proliferation of osteogenic cells leading to relative ischemia in more superficial regions of the callus, which encourages formation of cartilage rather than bone.^(8,33) Therefore, osteogenesis of stimulated PDL and fractured bone periosteum may not be so different as they at first appear. Studying cellular kinetics of proliferation and specialization associated with osteogenesis, the PDL offers several experimental advantages over fractured bone periosteum: 1) relatively atraumatic osteogenic stimulus, 2) free of inflammation, 3) cell specialization exclusively to osteoblasts, simplifying cell population studies, and 4) reaction is of shorter duration with significant amounts of bone formation by 72 hours.

Correcting for the less than 5% of the labeled population with fewer than six grains/nucleus (Table 4 and Figure 5), a 90% increase in labeled cells would be expected if all initially labeled cells mitose within 16 hours (the length of the first peak of the labeled mitoses curves). Mean number of labeled cells increased from 55.8 to 87.8, which is only about

a 57% increase. From this evidence it appears that $^3\text{HTDR}$ may not be a true premitotic label in this system. These results are similar to those of Owen and MacPherson,⁽⁴⁾ who identified DNA synthesis, which was not associated with cell division, in osseous precursor cells of rapidly growing rabbit femoral periosteum. Since it is widely accepted that $^3\text{HTDR}$ is incorporated only by osseous precursor cells prior to an imminent mitosis,⁽³⁴⁾ these findings in two independent bone forming systems require further study to identify this non-dividing population, before any conclusions can be drawn from labeling index data concerning the mechanism of osteogenic proliferation and cell specialization. For the present analysis of the proliferating pool of the stimulated PDL, we will depend primarily on generation cycle data which is based on mitotic figure counts.

From the data presented in this study two basic interpretations have been considered. Each possibility, as outlined below, will be discussed separately in an effort to establish by process of elimination a model which best explains available data.

PROLIFERATIVE POOL

A. Single Compartment.

1. Maximal Response.
2. Sub-maximal Response.

B. Multiple Compartment.

1. Primary Group -- enter mitosis first.
2. Secondary Group -- enter mitosis later.

A. Single Compartment.

The simplest view to be tested is that the proliferative pool is a

single population of cells mitosing every t_G .

1. Maximal Response - From 30-72 hours, the LI curve is relatively constant at about 10% (Fig. 2), which is consistent with a non-synchronous proliferating population. Assuming at this time period the proliferative pool is a single, non-synchronous dividing population, the percentage of PDL fibroblasts proliferating can be calculated using t_G^{ave} and t_S^{ave} for the 36 hour generation cycle (Table 3).

$$\% \text{ proliferating} = \frac{t_G^{ave} (LI)}{t_S} = \frac{32 \text{ hours } (10)}{9.5} = 34.7\%$$

Assuming this system is a maximal response, only about 35% of PDL fibroblasts entered the proliferative pool. This suggests that PDL fibroblasts are functionally compartmentalized so that only a portion of the resting population is osteogenic.

2. Submaximal Response - An average LI of 10% after 30 hours (Fig. 2) is also consistent with a sub-maximal response. As previously mentioned, the longer duration of peak LI values in fractured bone periosteum could be interpreted as suggesting that the stimulated PDL is a submaximal response.

Assuming that a block of cells in S phase at time of $^3\text{HTDR}$ injection progresses through mitosis in a reasonably uniform manner, the comparison of first and second peaks of generation cycle curves yields information on the kinetics of unlabeled cells entering the proliferative pool. The first peak of all generation cycle curves (Table 2 and Fig. 3) reached about 100% labeled mitotic figures, indicating that the entire sample of proliferating fibroblasts mitosing at that time, were in S phase when $^3\text{HTDR}$ was

injected. If the proliferative pool is a single population of cells mitosing every t_G , then the area under the second peak divided by the area under the first peak A'/A should approximate 1. It would never reach 1 because some cells, less than 5% of the initially labeled population, incorporated less than six grains per nucleus (Table 4 and Fig. 5). After dilution of the label by one cell division, this small fraction would be lost from the autoradiographically identifiable population. The ratio A'/A never exceeded 0.331 (Table 3), indicating an influx of unlabeled dividing cells diluting the labeled population which is undergoing its second mitosis. This can be explained by two possibilities: 1) either a secondary group of unlabeled cells has begun proliferation by this time, or 2) the entire primary compartment of cells has not completed its first cell division by the time of the second peaks of labeled mitoses (approximately 40-86 hours post stimulus). Mitotic figure counts in stimulated PDL argue against this latter possibility. Macapanpan et al.,⁽²⁶⁾ and Roberts and Jee (unpublished), have noted that numbers of mitoses are elevated at 24 and 36 hours followed by a drop to near control levels by 48 hours, suggesting that the group of cells initially entering the proliferative pool have completed their first mitosis by 48 hours. Therefore, from percent labeled mitoses curves and mitotic indices it appears that the proliferating pool is not a single compartment of cells.

B. Multiple Compartment.

In addition to osteogenic potential, PDL fibroblasts synthesize collagen and mucopolysaccharides necessary for maintenance of the PDL, and are probably the source of cementoblasts and osteoclasts.⁽³⁵⁾ From ultrastructure autoradiography of embryonic rat tibia, Scott⁽³⁶⁾ identified $^3\text{HTDR}$

incorporation in two general types of osseous precursor cells: 1) spindle shaped cells with morphology suggesting partial differentiation to osteoblasts, and 2) rounded cells with characteristics consistent with developing osteoclasts. Recognizing various stages of development of each cell type, with some forms retaining DNA synthesis capability, she concluded that cell specialization to osteoblasts and osteoclasts may involve separate pathways of cytodifferentiation. Extending these observations to the mitotically active stimulated PDL, a functionally compartmentalized population of PDL fibroblasts is considered most consistent with the present kinetic data. As shown in Fig. 6, the morphological entity "PDL fibroblast" may actually be a spectrum of various cell types representing all stages of cytodifferentiation from undifferentiated, multipotential cells, referred to as mesenchymal cells by Kember⁽³⁷⁾ to osteoblasts, osteoclasts, cementoblasts, and functional fibroblasts (cells primarily concerned with synthesis of collagen and mucopolysaccharides for maintenance of the PDL).

Utilizing the concepts of separate pathways of cytodifferentiation and DNA synthesis independent of mitosis, we propose the following sequence for cytodifferentiation to osteoblasts: mesenchymal cells → osteoblastic cells (maintain mitotic potential) → preosteoblasts (synthesize DNA but do not divide) → osteoblasts (functionally mature cells located immediately adjacent to bone forming surfaces).

Based on a heterogeneous PDL population of mesenchymal cells and partially differentiated cells along various cytodifferentiation pathways, a two compartment model with primary and secondary influxes of proliferating cells is suggested to explain the observed kinetics of the proliferating population of the stimulated PDL.

1. Primary Group - The first group of cells to enter the proliferative pool is probably composed primarily of mesenchymal and osteoblastic cells (Fig. 6). Following completion of an initial cell division by 48 hours post stimulus, at least part of these cells go on to a second mitosis as shown by the second peaks in labeled mitoses curves (Table 2 and Fig. 3).

2. Secondary Group - In an extensive review of in vivo and in vitro studies in various skeletal systems, Hall⁽³³⁾ concluded that, probably by reverting to a less differentiated intermediary, fibroblasts, osteoblasts, and osteoclasts are capable of interconversion depending on the dictates of the microenvironment. In conjunction with this evidence, we suggest that a secondary group of cells enters the proliferative population following an initial period of dedifferentiation from other cell types such as functional fibroblasts, osteoclastic cells, osteoclasts, and cementoblasts (Fig. 6). These cells, since they are entering mitosis at a later time interval than the primary group, would explain the large number of unlabeled mitotic figures observed during the second peak of generation cycle curves at 40-86 hours post stimulus (Fig. 3). Another interesting piece of data is that A'/A (Table 3) is greater for the 36 hour generation cycle than for those at 8 and 27 hours after stimulus, suggesting that at the time interval of the second peak of the 36 hour generation cycle (70-86 hours post stimulus) the secondary group of cells is entering mitosis at a decreasing rate.

In conclusion, the rat PDL has proven to be a useful experimental model for studying the cellular kinetics of stimulated osteogenesis. Further studies, with emphasis on differential cell and mitoses counts at hourly intervals are currently in progress in an attempt to elucidate the mechanism of osteoblast formation in response to an osteogenic stimulus.

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Table 1. Labeling Indices of PDL Fibroblasts

			n	Mean $\pm$ S.D.
Unstimulated Side			22	1.41 $\pm$ 0.10
Stimulated Side				
Hours Post Elastic	% Labeled PDL Fibroblasts			
	A	B		
8	2.4	3.4	2	2.9 $\pm$.71
16	8.1	7.5	2	7.8 $\pm$.42
20	11.8	12.4	2	12.1 $\pm$.42
24	15.8	16.3	2	16.1 $\pm$.36
27	25.5	24.9	2	25.2 $\pm$.42
30	10.2	11.0	2	10.6 $\pm$.57
36	10.1	11.1	2	10.6 $\pm$.50
40	10.0	9.3	2	9.65 $\pm$.49
48	9.5	8.6	2	9.05 $\pm$.64
60	9.0	8.8	2	8.90 $\pm$.14
72	9.2	8.8	2	9.00 $\pm$.28

Table 2. Generation Cycles of PDL Fibroblasts

Hours post injection of ³ H-Thymidine	Percent Labeled Mitotic Figures								
	8 Hour			27 Hour			36 Hour		
	A	B	Mean±S.D.	A	B	Mean±S.D.	A	B	Mean±S.D.
1	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0
1.5	0.0	1.0	0	1.6	3.1	2.35±1.06	0.8	2.7	1.75±1.34
2	0.9	2.5	1.70±1.13	40.5	40.1	40.30±.28	37.5	28.6	33.05±6.29
2.5	33.3	37.5	35.4±2.97	50.0	67.5	58.75±8.75	65.7	53.1	59.4±8.90
3	67.5	60.1	63.8±5.23	91.6	85.8	88.70±4.10	85.7	93.1	89.4±5.23
4	90.5	87.5	89.0±2.12	100.0	99.4	99.70±.42	97.5	100.0	98.75±1.77
6	94.2	94.8	94.5±0.42	99.5	96.4	97.95±2.19	100.0	99.4	99.70±.42
8	97.5	98.2	97.9±0.49	94.1	94.8	94.45±.49	97.5	98.4	97.95±.64
10	89.6	91.0	90.3±0.99	61.3	60.0	60.65±.92	75.8	85.7	80.75±7.00
12	50.0	56.3	53.2±4.45	35.0	34.5	34.75±.35	41.1	53.2	47.15±8.56
14	12.5	20.0	16.3±5.30	0.0	1.4	0.70±.99	11.0	22.6	16.80±8.20
16	0.0	0.0	0	0.0	1.3	.65±.92	0.0	0.0	0
32	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0
34	0.0	0.0	0	2.4	2.4	2.4±0.0	11.5	19.8	15.65±5.87
36	2.0	2.5	2.25±0.35	6.3	4.6	5.45±1.2	31.2	34.2	32.70±2.12
38	7.5	6.8	7.15±0.49	10.3	12.5	11.4±1.56	39.6	39.1	39.35±.35
40	10.0	8.6	9.30±0.99	13.6	16.7	15.15±2.19	25.4	22.6	24.00±1.98
42	13.1	12.5	12.80±0.42	16.1	15.9	16.00±.14	17.8	22.3	20.05±3.18
45	15.8	15.2	15.5±0.42	11.6	4.9	8.25±4.74	8.0	11.3	9.65±2.33
50	5.0	4.2	4.60±0.57	0.0	0.0	0	0.0	0.0	0
72	0.0	0.0		5.3	9.8	7.55±3.18	11.6	19.2	15.40±5.37

Table 3. Kinetic Data from Generation Cycle Curves

	Pre-Peak DNA Synthesis	Peak DNA Synthesis	Post Peak DNA Synthesis
Time post elastic prior to injecting ^3H -Thymidine	8 Hour	27 Hour	36 Hour
t_s^{ave}	9 hours	8.5 hours	9.5 hours
t_G^{min}	34 hours	33 hours	32 hours
t_G^{ave}	37 hours	36 hours	32 hours
t_G^{max}	38 hours	37 hours	34 hours
A-area under first peak	178.55 cm^2	166.00 cm^2	185.39 cm^2
A'-area under second peak	28.50 cm^2 *	27.41 cm^2	61.39 cm^2
A'/A	0.160	0.165	0.331
A'/A expressed as percent	16.0%	16.5%	33.1%
H-height of first peak on % labeled mitoses axis	97.90	99.70	99.70
H'-height of second peak on % labeled mitoses axis	15.50	16.00	39.35
H'/H	0.157	0.160	0.394
H'/H expressed as percent	15.7%	16.0%	39.4%
b-base length of first peak in hours	14	14	15
b'-base length of second peak	15*	15	16
b'/b	1.07	1.07	1.07
b'/b expressed as percent	107%	107%	107%

*Estimated by extrapolating the descending curve of the second peak to zero percent labeled mitotic figures.

Table 4. Grain counts from 36-hour generation cycle cells initially labeled (1.5 hours) and following the first mitotic peak (16 hours). Two random sections per animal were scored. Note that the number of cells with under six grains is less than 5% of the total initially labeled samples.

	Animal No.	Number Grains Per Nucleus						Total # Labeled Cells
		3-5	6-15	16-25	26-35	36-45	46+	
Initially Labeled Cells	1.5AL	3	28	23	6	2	1	63
		2	29	27	8	3	1	70
	1.5BL	1	15	15	9	0	0	40
		3	16	13	14	4	0	50
After First Mitotic Peak	Mean±S.D.	2.3±1.0	22.0±7.5	19.5±6.6	9.3±3.4	2.3±1.7	0.5±0.6	55.8±13.4
	16AL	3	39	27	7	0	0	76
		5	37	30	12	1	0	85
	16BL	2	45	30	12	1	0	90
		6	44	36	14	0	0	100
	Mean±S.D.	4.0±1.8	41.3±3.9	30.8±3.8	11.3±3.0	0.5±0.6	0	87.8±10.0*

*Significantly different at 0.05 level from total number cells at 1.5 hours.

Fig. 1. A diagram of the area of periodontal ligament (PDL) studied. An elastic (A) in the contact area between maxillary left first and second molars of an adult rat produces an osteogenic reaction in the PDL along the mesial surface of the first molar with subsequent formation of new bone (B). The PDL unit (outlined in black) is bordered by the tooth, the original alveolar bone surface, a line perpendicular to the tooth surface from the alveolar crest, and a line perpendicular to the bone surface from the tooth apex. (C, cementum; D, dentin; E, enamel; P, periodontal ligament; X, alveolar crest; O, alveolar bone).

Fig. 2. The percent labeling index of the orthodontically stimulated (open circles and solid line) and unstimulated (closed circles and dotted line) PDL fibroblasts at different time periods. Arrows on the horizontal scale mark the times post stimulus of the proliferating fibroblast population which were selected for generation cycle determinations.

Fig. 3. Comparison of generation cycle curves from animal labeled at pre-peak (8 hours), peak (27 hours) and post-peak (36 hours) DNA synthesis times. Osteogenic stimulus was initiated at 8, 27 or 36 hours prior to injection of $^3\text{HTDR}$ at time zero.

Fig. 4. Methods used to determine average time of DNA synthesis (t_s), minimum time of generation cycle ($t_G^{\min}$) and average time of generation cycle (t_G^{ave}). See text for explanation. Area under first peak (A) and area under second peak (A') determined by plotting on graph paper graduated in mm² with 20 cm = 100% on vertical axis and 1 cm = 1 hour on horizontal axis. Note that the base of the first peak (b) is approximately equal to the base of the second peak (b'), but the height of the first peak (H) is higher than that of the second peak (H').

Fig. 5. Distribution of ^3H grains (tracks) per fibroblast nucleus at 1.5 and 16 hours after $^3\text{HTDR}$ injection. Note that less than 5% of the labeled nuclei population at 1.5 hours contained 3 to 5 grains (see Table 4).

Fig. 6. Proposed cytodifferentiation sequence for PDL cells. Since cementoblasts are assumed to be derived from the PDL,⁽³⁵⁾ they are included in the schema for completeness. The kinetics of the mitotic population (proliferative pool) have been explained on the basis of a two compartment model with a primary group of cells entering mitosis by 24 hours post stimulus and a secondary group beginning mitosis sometime prior to the second peaks in percent labeled mitoses (Fig. 3). Dashes in boxes indicate intermediaries for which there is no evidence for or against.

SKELETAL DISTRIBUTION OF ^{241}Am AND ^{239}Pu IN BEAGLES:
CAN THEY BE COMPARED?

Ray D. Lloyd, Charles W. Mays, Webster S. S. Jee,
and Glenn N. Taylor

ABSTRACT: No statistical justification was found for rejecting data of a dog sacrificed 1 day after the injection of ^{239}Pu from the mean percent skeletal Pu per bone values which included data from 19 other dogs sacrificed 5 to 769 days after ^{239}Pu injection. An equivalent conclusion was drawn from corresponding data for dogs injected with ^{241}Am and sacrificed 1 and 7 to 1533 days later. Comparison of the mean skeletal distribution of retained Pu with retained Am emphasizes similarities more than differences. Whereas the relative distribution of Pu in the skeleton was not generally dependent upon time after injection, a highly significant relationship between relative skeletal distribution and burden time was revealed for Am, with vertebrae, tail, and sternum, which have much trabecular bone, exhibiting a decreasing fraction of retained Am with increasing time.

INTRODUCTION

A recent publication⁽¹⁾ by our laboratory compared the skeletal distribution of injected ^{241}Am with that of ^{239}Pu . The animals included in the comparison consisted of 10 dogs sacrificed 1 to 1533 days after the injection of 0.3 to 4.53 $\mu\text{Ci } ^{241}\text{Am/kg}$ and 20 dogs sacrificed 1 to 769 days after the injection of 0.015 to 3.2 $\mu\text{Ci } ^{239}\text{Pu/kg}$. A question has been raised as to whether the inclusion of a dog sacrificed at 1 day after injection of ^{239}Pu might distort the data, since a significant amount of ^{239}Pu is still circulating at that time.⁽²⁾ Because the nuclide distribution data tabulated in our publication⁽¹⁾ were given in terms of percent of retained skeletal Am or Pu in each bone or bone group, circulating Pu (or Am) should have had little effect upon the values listed. The same is true of any soft-tissue deposition or of excretion. An analysis of our original data has been made to determine whether the values for the 1-day sacrifice dog seriously

affected our conclusions.

DISTRIBUTION OF ^{239}Pu

Table 1 lists the distribution data for the 20 dogs containing ^{239}Pu . Percent of injected ^{239}Pu data from which all percent of skeletal ^{239}Pu values for the 1-level and the 3-level dogs had been calculated were found in Reference 3. Data for the 5-level animals were originally taken from Reference 4, a 1958 report, where only the means and standard deviations for the total of these 4 animals were shown. In order to obtain the data for the individual dogs, a search of our archival files was undertaken. It was found that a few of the means reported in Reference 4 did not include bones of TOP5, the 1 day sacrifice dog, although some of these samples had been analyzed. All available data for TOP5 were, therefore, included in this report. Because the cervical vertebrae, most of the ribs and in all probability the sternum of TOP5 had been pooled for analysis, it was not possible to determine individual values for these bone groups. A single rib had been analyzed separately, however, and an estimate of the percent skeletal ^{239}Pu in all ribs was made therefrom. Data used to make the estimate included:

$$\text{for dog TOP5, } \frac{\% \text{ injected } ^{239}\text{Pu in 1 rib}}{\% \text{ injected } ^{239}\text{Pu in skeleton}} = 0.570\%$$

$$\text{ashed weight of 1 rib} = 1.23 \text{ g}$$

$$\text{for dog M3P5, ashed weight of all ribs} = 21.34 \text{ g}$$

ratio of ashed weights of skull, forelimbs, and hindlimbs of TOP5 to those of M3P5,

$$\frac{\text{g ash TOP5}}{\text{g ash M3P5}} \approx \frac{50.3 \text{ g} + 31.5 \text{ g} + 36.1 \text{ g}}{47.3 \text{ g} + 28.5 \text{ g} + 34.1 \text{ g}} = \frac{1.10 \text{ g ash TOP5}}{\text{g ash M3P5}}$$

so TOP5 ash weight all ribs $\approx$

$$\frac{1.10 \text{ g ash TOP5}}{\text{g ash M3P5}} \times 21.34 \text{ g ash M3P5 all ribs} =$$

23.5 g ash all ribs TOP5, and

$$\text{TOP5 \% skeletal } ^{239}\text{Pu all ribs} \approx 0.570\% \text{ 1 rib} \times \frac{23.5 \text{ g ash all ribs}}{1.23 \text{ g ash 1 rib}}$$

$$= 10.89\% \text{ skel. } ^{239}\text{Pu all ribs.}$$

Examination of the original data sheets for the 3 other 5-level dogs (T4P5, M3P5, and T3P5) revealed that the percent of skeletal ^{239}Pu in all bones did not add exactly to 100% in each case, so appropriate adjustments were made. Although the means for the various bones and bone groups calculated from the corrected data for these four 5-level dogs were somewhat different from those reported in Reference 4, none of the differences was significant. Means given in Ref. 1 were similarly but even less significantly affected.

Table 2 gives the corrected means and standard deviations for all 20 animals and compares them with corresponding means and standard deviations of the 19 dogs, excluding TOP5, the 1 day sacrifice dog. No obvious distortion of the means for the 19 dogs by inclusion of data for TOP5 is evident. Group comparison tests⁽⁵⁾ ("t" test) were done on corresponding means for 19 vs. 20 dogs. None of the means of 20 dogs was significantly different from the corresponding means of 19 dogs, excluding TOP5.

The data for TOP5 were also analyzed using the test⁽⁶⁾ for an aberrant individual to determine whether any value could be rejected statistically as an extreme observation. It was found that no value of TOP5 was significantly different from the corresponding mean of the other 19 dogs.

Results of linear regression analyses ("F" test) indicated that for no bone or bone group but the skull and ulna was there a significant dependence⁽⁵⁾ of percent retained skeletal ^{239}Pu on time after injection using data for all 20 dogs. Similar analyses⁽⁵⁾ of data for 19 dogs, excluding TOP5, indicated that for no bone or bone group but the skull and ribs was there a significant dependence of percent skeletal ^{239}Pu on time after injection. Analyses⁽⁵⁾ of the data for only the twelve 1-level dogs showed a significant relationship between percent skeletal ^{239}Pu and time after injection for skull, lumbar vertebrae, paws,* ulnae, and ribs, while combined data for the 1-level and 3-level animals (16 dogs total) indicated that the relationship was significant⁽⁵⁾ for skull, mandible, lumbar vertebrae, paws, radii, ulnae, and ribs. It appears that the inclusion of TOP5 did not intensify any time dependence of ^{239}Pu skeletal distribution, but, instead, had the opposite effect. Therefore, it appears that the values for the dog sacrificed at 1 day after injection had a negligible effect on the averages for ^{239}Pu .

The ^{239}Pu found in a bone soon after injection may have included some activity contributed by ^{239}Pu in trapped blood or in other fluids. Because there was no significant difference in relative distribution of ^{239}Pu in the skeleton at 1 day as compared to greater than 1 day after injection,

*Carpals, tarsals, metacarpals, metatarsals, and digits.

either the fraction of the total Pu in each bone contributed by this source was unimportant, or the relative distribution of Pu in trapped fluids within the skeleton was proportional to the ultimate distribution. In either case, the inclusion of the 1 day sacrifice dog had no significant effect on the means for ^{239}Pu distribution as reported in Reference 1.

DISTRIBUTION OF ^{241}Am

Skeletal distribution data for ^{241}Am in beagles appear in tabular form in Reference 7 for the 10 animals reported in our original publication⁽¹⁾ and in Reference 8 for 6 additional dogs. Table 3 compares the means and standard deviations for all 10 of the original⁽¹⁾ ^{241}Am dogs with those of 9 of the dogs, excluding the 1 day sacrifice dog T15W5.5. Results of group comparison tests⁽⁵⁾ and tests for an aberrant individual⁽⁶⁾ revealed no significant differences in corresponding means.

Linear regression analyses⁽⁵⁾ indicated that, for the original 10 dogs, there was a significant dependence of percent skeletal ^{241}Am on time after injection for the skull, thoracic vertebrae, lumbar vertebrae, paws, and pelves. When data for all 16 dogs^(7,8) were considered, however, results were significant⁽⁵⁾ for every bone or bone group except the ribs, with highly significant results for 12 of the 16 analyses (Table 4).

Results shown in Table 4 suggest that the relative fraction of retained skeletal ^{241}Am decreased as a function of time (negative slope) in bones containing a high proportion of trabecular bone (vertebrae, tail, sternum) while there was a corresponding increase (positive slope) in the relative fraction of retained skeletal ^{241}Am in bones containing a higher proportion of cortical bone (paws, leg bones, pelves and scapulae). This may reflect the more rapid remodelling in trabecular as compared with cortical bone.

COMPARISON OF ^{241}Am WITH ^{239}Pu

Group comparison tests⁽⁵⁾ of means for the 20 ^{239}Pu dogs and means for the ^{241}Am dogs revealed significant differences for many bones and bone groups (Table 5). For only the skull and mandible were there no significant differences between Pu and Am in any comparison. Means for paws, radii, and tibiae + fibulae were not significantly different from the ^{239}Pu means for the 10 original ^{241}Am dogs while those for cervical vertebrae, humeri, femora, pelvis, scapulae, and sternum lacked significance in the comparison with the 6 additional ^{241}Am dogs. Only means for the ulnae, ribs, and tail were significantly different in all the groupings of ^{241}Am dogs as compared with the corresponding means for ^{239}Pu . This analysis reaffirms the observation made in our original publication⁽¹⁾ that "... similarities in gross distribution appear to be more pronounced than the differences."

ACKNOWLEDGEMENTS

We appreciate the help of David R. Atherton for supplying the data on which Table 1 is based, and Betsy J. Stover for suggesting the need for this detailed analysis.

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Table 1. Distribution of injected ^{239}Pu in the beagle skeleton.

A. 5-level* and 3-level dogs

Days after injection	1	365	499	610	40	187	362	560
Injected $\mu\text{Ci/kg}$	3.05	3.02	3.00	3.22	0.305	0.304	0.303	0.296
Sex	M	F	M	F	M	F	M	M
Dog	TOP5	T4P5	M3P5	T3P5	T31P3	T38P3	T35P3	T29P3
PERCENT OF <u>RETAINED</u> SKELETAL ^{239}Pu								
Skull	5.81	4.32	10.86	8.61	6.61	8.11	7.43	9.00
Mandible	1.59	1.44	3.51	0.97	2.12	2.77	2.65	3.37
Cerv. Vert.	----	7.42	4.71	7.48	4.85	5.25	5.03	4.68
Thor. Vert.	11.26	18.01	13.26	13.79	14.31	14.65	18.26	14.26
Lumb. Vert.**	12.03	13.93	11.15	13.69	17.25	13.59	12.26	14.99
Tail	0.94	0.62	0.76	0.74	0.69	0.54	0.70	0.68
Paws	2.54	1.82	5.78	1.59	2.60	3.20	3.92	3.07
Radii	0.77	0.46	1.21	0.52	1.03	0.90	0.95	1.13
Ulnae	0.59	0.47	1.42	0.41	1.01	0.86	0.93	1.38
Humeri	6.92	8.49	8.89	11.44	8.93	7.82	7.14	9.20
Tib. + Fib.†	1.66	1.53	3.75	3.23	4.68	3.52	3.74	3.67
Femora	4.82	4.92	8.59	6.39	8.71	8.30	9.47	7.34
Pelves	6.78	8.53	7.34	6.66	7.81	7.17	7.93	7.77
Scapulae	5.34	4.62	4.89	5.81	5.49	6.40	5.08	4.90
Ribs	10.89 [‡]	12.81	10.40	12.11	11.42	14.31	10.34	12.12
Sternum	-----	10.61	3.48	6.56	2.49	2.61	4.17	2.44
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
% of inj. ^{239}Pu	30.9	53.0	55.0	55.0	46.6	44.4	44.1	39.8

* See reference 9 for skeletal distribution of ^{239}Pu in dog MIP5 which was not among the animals included in Reference 1.

** including sacrum

+ including patellae

+ see text

----- no data

Table 1 (continued)

B. 1-level dogs, 5 to 375 days after injection

Days after injection	5	35	97	186	274	375
Injected $\mu\text{Ci/kg}$	0.0158	0.0148	0.0172	0.0148	0.0162	0.0153
Sex	M	M	M	M	M	M
Dog	T36P1	T30P1	T24P1	T37P1	T32P1	T33P1
PERCENT OF <u>RETAINED</u> SKELETAL ^{239}Pu						
Skull	5.24	5.66	7.72	7.82	11.94	10.09
Mandible	2.49	1.78	2.48	2.27	5.31	2.97
Cerv. Vert.	4.83	5.24	4.82	4.35	4.46	4.79
Thor. Vert.	16.60	17.79	11.49	14.23	11.92	14.24
Lumb. Vert.*	16.10	15.22	13.59	14.55	14.18	13.98
Tail	0.35	0.40	0.60	0.53	0.51	0.52
Paws	2.38	3.41	4.21	4.31	3.85	3.42
Radii	0.71	0.78	0.79	1.11	1.04	0.93
Ulnae	0.65	0.89	0.99	1.04	1.15	0.97
Humeri	10.61	11.52	10.77	10.06	15.83	12.01
Tib. + Fib.†	3.41	4.03	4.60	4.75	2.97	2.87
Femora	9.36	7.36	9.52	8.44	7.27	7.83
Pelves	7.45	7.60	7.04	7.20	3.99	6.96
Scapulae	6.29	5.17	6.23	4.86	4.21	4.77
Ribs	11.68	11.72	13.07	13.04	10.54	12.20
Sternum	1.85	1.43	2.08	1.44	0.83	1.45
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00
% of inj. ^{239}Pu	46.6	44.9	49.6	45.0	47.1	42.1

*including sacrum

†including patellae

Table 1 (continued)

C. 1-level dogs, 376 to 769 days after injection

Days after injection	376	467	559	647	746	769
Injected $\mu\text{Ci/kg}$	0.0151	0.0167	0.0150	0.0160	0.0154	0.0177
Sex	M	M	M	M	M	M
Dog	T39P1	T25P1	T28P1	T26P1	T34P1	T40P1
PERCENT OF <u>RETAINED</u> SKELETAL ^{239}Pu						
Skull	8.07	9.28	8.97	11.80	11.20	9.39
Mandible	1.91	2.83	3.34	4.12	3.88	3.21
Cerv. Vert.	8.02	4.62	5.38	5.37	4.99	5.25
Thor. Vert.	15.66	14.07	14.12	11.83	12.15	17.26
Lumb. Vert.*	14.52	13.00	12.44	12.17	11.39	15.35
Tail	1.03	1.45	0.67	0.49	0.71	0.65
Paws	3.48	5.95	5.38	4.54	5.15	4.66
Radii	0.60	2.05	1.19	1.04	1.41	1.23
Ulnae	0.77	1.28	1.31	1.34	1.63	1.13
Humeri	9.76	9.14	9.49	7.90	11.85	9.10
Tib. + Fib.†	2.60	3.90	3.74	3.81	4.31	3.40
Femora	8.67	7.85	8.25	10.95	7.32	5.83
Pelves	7.19	6.71	7.14	7.44	6.65	8.42
Scapulae	4.45	4.21	5.40	4.85	5.21	5.60
Ribs	11.44	12.14	11.84	10.79	11.12	7.09
Sternum	1.83	1.52	1.34	1.56	1.03	2.43
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0
% of inj. ^{239}Pu	35.0	42.0	40.4	32.8	36.9	30.9

*including sacrum

†including patellae

Table 2. Comparison of the means for all 20 ^{239}Pu dogs with those of 19 dogs, excluding the 1 day sacrifice animal, TOP5.

<u>Bone or Bone Group</u>	<u>Mean $\pm$ S.D. All 20 dogs</u>	<u>Mean $\pm$ S.D. 19 dogs</u>
Skull	8.40 $\pm$ 2.16	8.53 $\pm$ 2.14
Mandible	2.75 $\pm$ 1.02	2.81 $\pm$ 1.01
Cerv. Vert.	†	5.34 $\pm$ 1.07
Thor. Vert.	14.46 $\pm$ 2.20	14.63 $\pm$ 2.13
Lumb. Vert.*	13.77 $\pm$ 1.59	13.86 $\pm$ 1.58
Tail	0.68 $\pm$ 0.24	0.67 $\pm$ 0.24
Paws	3.76 $\pm$ 1.25	3.83 $\pm$ 1.25
Radii	0.99 $\pm$ 0.35	1.00 $\pm$ 0.36
Ulnae	1.01 $\pm$ 0.33	1.03 $\pm$ 0.32
Humeri	9.84 $\pm$ 2.06	10.00 $\pm$ 1.99
Tib. + Fib.†	3.51 $\pm$ 0.87	3.61 $\pm$ 0.78
Femora	7.86 $\pm$ 1.54	8.02 $\pm$ 1.40
Pelves	7.19 $\pm$ 0.92	7.21 $\pm$ 0.94
Scapulae	5.19 $\pm$ 0.64	5.18 $\pm$ 0.66
Ribs	11.55 $\pm$ 1.45	11.59 $\pm$ 1.49
Sternum	‡	2.69 $\pm$ 2.33

*including sacrum

†including patellae

‡not determined for TOP5

Table 3. Comparison of the means for all 10 ^{241}Am dogs with those of 9 dogs, excluding the 1 day sacrifice animal T15W5.5.

<u>Bone or Bone Group</u>	<u>Mean $\pm$ S.D. All 10 dogs</u>	<u>Mean $\pm$ S.D. 9 dogs</u>
Skull	8.44 $\pm$ 1.16	8.58 $\pm$ 1.13
Mandible	2.72 $\pm$ 0.62	2.80 $\pm$ 0.60
Cerv. Vert.	7.94 $\pm$ 1.11	7.88 $\pm$ 1.17
Thor. Vert.	16.95 $\pm$ 2.86	16.80 $\pm$ 2.99
Lumb. Vert.*	17.53 $\pm$ 3.26	17.21 $\pm$ 3.28
Tail	1.77 $\pm$ 0.13	1.80 $\pm$ 0.11
Paws	2.77 $\pm$ 1.98	2.90 $\pm$ 2.06
Radii	1.19 $\pm$ 0.27	1.22 $\pm$ 0.26
Ulnae	1.45 $\pm$ 0.33	1.49 $\pm$ 0.32
Humeri	6.34 $\pm$ 0.48	6.34 $\pm$ 0.51
Tib. + Fib.†	3.65 $\pm$ 0.82	3.74 $\pm$ 0.82
Femora	6.06 $\pm$ 0.75	6.14 $\pm$ 0.74
Pelves	5.26 $\pm$ 0.80	5.28 $\pm$ 0.85
Scapulae	3.71 $\pm$ 0.55	3.80 $\pm$ 0.48
Ribs	8.60 $\pm$ 0.83	8.59 $\pm$ 0.88
Sternum	5.53 $\pm$ 0.99	5.34 $\pm$ 0.84

*including sacrum

†including patellae

Table 4. Relationship of percent skeletal ^{241}Am and time after injection in 16 beagles.

Bone or Bone Group	Calculated Value of		Level of Significance	Correlation Coefficient
	a ⁺	b ⁺⁺		
Skull	8.18	+0.0009061	0.05	0.56
Mandible	2.63	+0.0005507	0.05	0.60
Cerv. Vert.	7.97	-0.0009901	0.05	-0.57
Thor. Vert.	17.92	-0.0041888	0.01	-0.83
Lumb. Vert.*	18.63	-0.0043519	0.01	-0.83
Tail	1.77	-0.0002148	0.01	-0.63
Paws	2.03	+0.0025986	0.01	0.84
Radii	1.15	+0.0003806	0.01	0.71
Ulnae	1.33	+0.0004756	0.01	0.78
Humeri	6.24	+0.0011486	0.01	0.73
Tib. + Fib.**	3.39	+0.0011159	0.01	0.78
Femora	5.81	+0.0011271	0.01	0.81
Pelves	4.97	+0.0013105	0.01	0.84
Scapulae	3.55	+0.0006582	0.01	0.74
Ribs	8.64	+0.0006845	0.10	0.47
Sternum	5.64	-0.0011885	0.01	-0.65

* including sacrum

**including patellae

+ percent skeletal ^{241}Am at 0 days after injection

++ change in percent skeletal ^{241}Am per day following injection such that
 $\% \text{ skel. Am} = a + bt$, where t = days after injection

Table 5. Statistical comparison of means for ^{239}Pu dogs^(3,4)
and ^{241}Am dogs.^(7,8)

Bone or Bone Group	Level of Significance for Difference from ^{239}Pu means		
	All 16 Am dogs	10 Am dogs ⁽⁷⁾	6 Am dogs ⁽⁸⁾
Skull	_____	_____	_____
Mandible	_____	_____	_____
Cerv. Vert.	0.01	0.01	_____
Thor. Vert.	_____	0.02	0.01
Lumb. Vert.*	_____	0.01	0.01
Tail	0.01	0.01	0.01
Paws	_____	_____	0.01
Radii	0.01	_____	0.01
Ulnae	0.01	0.01	0.01
Humeri	0.01	0.01	_____
Tib. + Fib. ⁺	0.05	_____	0.01
Femora	0.02	0.01	_____
Pelves	0.01	0.01	_____
Scapulae	0.01	0.01	_____
Ribs	0.01	0.01	0.05
Sternum	0.01	0.01	_____

*including sacrum

⁺including patellae

_____ not significant

Am-241 SKELETAL DISTRIBUTION IN BEAGLES

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

ABSTRACT: This paper corrects a transcribing error in an earlier report of similar title (C00-119-244) and provides additional data on the skeletal distribution of ^{241}Am .

INTRODUCTION

Studies of the metabolism and radiotoxicity of ^{241}Am in beagles have been in progress at this laboratory since 1966. Preliminary results and the experimental design are summarized in Ref. 1 and in the first 15 references to Ref. 2.

The distribution of injected Am-241 citrate within the skeletons of 10 beagles was reported previously.⁽²⁾ Patricia Durbin⁽³⁾ has pointed out a transcribing error in that summary consisting of the reported values for "percent of injected ^{241}Am in the total skeleton", the last line in Table 1 of Ref. 2. These values should be replaced by those listed in Table 1 of this report. The correction does not affect the percent skeletal ^{241}Am data or the comparison of percent skeletal ^{241}Am and percent skeletal ash reported in Ref. 2, however.

Since Ref. 2 appeared, 6 additional beagles containing ^{241}Am have come to autopsy. Table 2 summarizes these data in the same form as Table 1 of Ref. 2, except that the values exhibited in the last line are not in error.

Currently there are far too few data in hand to make meaningful analyses of the time function relative to tissue retention and/or concentration. At the present time, however, it is obvious that retention patterns are determined by dose level.

Table 1. Percent of injected ^{241}Am in the total skeletons of 10 beagles (see Table 1 of Ref. 2).

<u>Dog</u>	<u>Days after Injection</u>	<u>% injected ^{241}Am in the total skeleton</u>
T15W5.5	1	28.3%
T32W5.5	7	28.6
T33W5.5	8	28.8
T56W5	15	36.5
T57W5	15	34.8
T16W5	22	29.3
M1W5	401	45.4
M2W5	448	50.5
F6W3	1510	33.4
F4W4	1533	46.7

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

Days after injection	633	1132	1323	1779	1847	1917
inj. $\mu\text{Ci/kg}$	0.906	0.924	0.902	0.897	0.0952	0.305
Sex	M	M	F	M	F	M
Dog	M77W4	M17W4	F35W4	M3W4	F7W2	M5W3
	PERCENT OF <u>RETAINED</u> SKELETAL ^{241}Am					
Calvarium	3.13	2.96	2.87	2.14	2.06	2.28
Maxillae	7.78	6.75	7.01	5.70	7.65	7.57
Mandibles	3.81	3.48	3.67	3.05	3.55	4.24
Cerv. Vert.	6.20	5.30	6.17	5.60	6.19	6.23
Thor. Vert.	11.80	11.03	12.06	10.63	9.33	9.57
Lumb. Vert.*	11.82	12.27	11.93	11.41	10.39	10.22
Caud. Vert.	1.20	1.30	1.22	1.29	1.49	1.39
Paws	5.43	6.21	5.29	7.16	6.18	7.20
Radii	1.94	2.04	1.61	1.81	1.89	1.96
Ulnae	1.94	2.21	1.85	1.89	2.32	2.63
Humeri	7.63	8.95	8.41	9.51	8.23	8.18
Tib.+ Fib.+	5.12	5.01	4.60	5.18	5.78	6.19
Femora	7.37	7.55	7.52	8.50	7.99	7.72
Pelves	7.00	6.63	6.90	7.38	7.97	7.53
Scapulae	4.19	4.80	4.84	5.17	4.82	4.12
Ribs	10.10	10.27	11.45	8.88	10.44	9.72
Sternum	3.25	2.96	2.60	4.53	3.72	2.96
Os Penis	0.29	0.28		0.17		0.29
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0
% of Inj. ^{241}Am in Skeleton	46.80	42.75	45.07	49.82	29.88	34.88

*Including sacrum

+ Including patellae

TISSUE CONCENTRATION OF INJECTED ^{241}Am IN BEAGLES

Ray D. Lloyd, David R. Atherton, and Glenn N. Taylor

ABSTRACT: The concentration (% injected activity/kg) of ^{241}Am in 16 beagles sacrificed 1 to 1917 days after injection is presented in tabular form. Tissues of highest concentration typically were found to be the liver, thyroid, skeleton, and kidney. In only 2 dogs was the skeletal concentration higher than that in the thyroid. Liver exhibited the highest concentration in all 16 animals. In a few individual dogs, some other tissues such as the dura, spleen, aorta, mesentery or lymphatics showed significant concentrations.

INTRODUCTION

The ^{241}Am concentration or content of some tissues of several of our beagles has been reported previously.^(1,3-8) This report tabulates all of the tissue concentrations which have been measured so far (Table 1). Experimental techniques are reported in references 1 and 2.

Our earlier statement⁽¹⁾ that the liver, thyroid, skeleton, and kidney appeared to be the 4 tissues of highest concentration in the first 6 dogs studied is generally confirmed by the inclusion of data for 10 additional animals. The liver was the tissue of highest concentration in all cases; but for 2 dogs (T16W5 and F7W2) the value for the skeleton was greater than that for the thyroid. Except for T15W5.5, sacrificed at 1 day after injection, at which time urinary excretion is most pronounced, the kidney had a lower concentration than liver, thyroid, or skeleton. In a few cases, other tissues (dura, spleen, aorta, mesentery, lymphatics) exhibited values approaching or similar to those of the kidney, skeleton, or thyroid.

At least in the higher level dogs, radiation damage produces a loss of liver mass accompanied by a loss of liver ^{241}Am , which is to some extent redeposited in other tissues, principally the skeleton.⁽¹⁾ This effect becomes progressively less obvious as dose levels decrease. Comparison

of tissue content or concentration among dogs at various times after injection is complicated by this redistribution effect which depends strongly on the injected activity and resulting radiation dose. When a number of additional animals at lower levels (W3, W2, W1.7, W1) have come to autopsy, we plan to report an analysis of the effects of time after injection on relative tissue concentration for individual dose levels.

ACKNOWLEDGEMENTS

Values in Table I were obtained with the assistance of C. W. Mays, D. H. Taysum, W. S. S. Jee, F. W. Bruenger, W. W. Wagner, Susan S. Gaufin, R. W. Holmgren, P. J. Diehl, G. B. Thurman, G. B. Goates, T. R. Lloyd, C. McRoberts, W. Angus, and Darcie Smith.

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Table 1. Concentration of injected ^{241}Am in beagle tissue at autopsy (percent of injected ^{241}Am per kg of tissue).

Days after Injection →	1	7	8	15	15	22
Injected						
μCi/kg →	4.53	4.46	4.47	2.90	2.77	2.78
Sex →	M	M	M	M	F	M
Dog →	T15W5.5	T32W5.5	T33W5.5	T56W5	T57W5	T16W5
Liver	175.3	195.1	153.2	153.6	267.2	203.8
Thyroid	114.6	46.8	42.0	45.6	56.2	20.4
Skeleton	24.6	27.5	28.6	32.3	49.6	27.5
Kidneys	27.4	13.6	11.7	14.8	25.6	9.7
Dura	---	8.1	8.9	---	---	---
Lymphatics	---	6.9	4.5	4.6	6.2	7.4
Lungs + Trachea	7.5	3.4	3.0	5.2	6.3	5.1
Aorta	11.2	1.6	4.6	4.2	1.6	2.0
Larynx	---	2.5	4.4	2.8	---	2.8
Salivaries	---	2.6	3.8	3.7	3.8	---
Adrenals	4.8	2.4	2.2	9.8	7.8	---
Pituitary	---	2.7	2.5	---	---	2.7
Prostate	3.0	0.85	2.3	1.7	---	1.2
Spleen	---	0.56	1.2	6.2	5.4	2.8
Eyes	2.3	0.81	0.62	1.2	1.6	---
G.I. tract	2.1	0.77	1.4	1.9	1.6	0.88
Heart	1.3	0.51	0.70	0.94	1.2	0.74
Ur. bladder	---	0.50	1.1	---	1.7	---
Testes	1.9	0.17	0.30	0.93	---	0.82
Pelt	1.4	0.65	0.62	0.99	1.2	0.45
Pancreas	0.77	---	0.26	0.66	0.62	---
Muscle	0.56	0.17	0.17	0.34	0.44	0.25
Fat	---	0.13	0.05	---	---	---
Thymus	---	<0.41	---	---	1.3	---
Brain	0.03	<0.02	<0.03	0.09	0.10	0.04
Parathyroid	---	3.5	---	---	---	---
Ovaries	---	---	---	---	2.3	---
Diaphragm	---	---	---	---	---	---
Mesentery	---	---	---	0.30	---	21.3
Conn. tiss.	---	0.30	0.24	---	---	---
Uterus	---	---	---	---	0.97	---

--- Not measured.

Table 1 (continued)

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Days after Injection →	401	448	633	1132	1323
Injected					
μCi/kg →	2.78	2.83	0.906	0.924	0.902
Sex →	M	M	M	M	F
Dog →	M1W5	M2W5	M77W4	M17W4	F35W4
Liver	158.5	91.6	180.6	102.0	95.2
Thyroid	133.8	80.1	144.3	275.8	157.0
Skeleton	43.7	39.8	44.6	43.3	50.8
Kidneys	18.7	17.4	13.6	13.0	29.1
Dura	---	---	21.3	---	25.7
Lymphatics	16.3	16.8	9.3	8.8	4.8
Lungs + Trachea	---	7.5	6.5	8.5	8.3
Aorta	4.3	6.0	6.2	6.5	3.7
Larynx	---	---	---	4.8	---
Salivaries	---	---	5.1	8.1	4.3
Adrenals	---	3.4	3.0	1.6	1.8
Pituitary	<13.3	14.4	3.8	2.3	---
Prostate	2.4	7.8	3.9	2.9	
Spleen	10.7	22.3	18.0	7.2	9.9
Eyes	1.5	1.3	0.94	2.7	---
G.I. Tract	0.53	1.1	0.43	1.2	0.46
Heart	1.42	2.0	6.7	1.2	1.6
Ur. Bladder	---	---	---	1.2	---
Testes	0.78	1.2	0.88	1.1	
Pelt	0.75	1.2	0.72	1.2	0.75
Pancreas	0.64	0.62	0.83	1.3	0.93
Muscle	0.44	0.83	0.26	0.62	0.55
Fat	---	---	---	---	---
Thymus	---	---	---	---	2.2
Brain	0.11	0.08	0.09	0.15	0.18
Parathyroid	---	---	0.39	12.0*	---
Ovaries					<7.4
Diaphragm	0.78	---	---	---	---
Mesentery	---	---	---	---	---
Conn. Tiss.	---	---	---	---	0.74
Uterus					---

--- Not measured.

* May have included some thyroid tissue.

Table 1 (continued).

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Days after Injection →	1510	1533	1779	1847	1917
Injected					
μCi/kg →	0.310	0.910	0.897	0.095	0.305
Sex →	F	F	M	F	M
Dog →	F6W3	F4W4	M3W4	F7W2	M5W3
Liver	147.9	96.6	55.6	132.6	67.4
Thyroid	69.4	88.9	67.2	9.8	30.8
Skeleton	28.1	49.7	39.5	29.9	23.2
Kidneys	---	37.3	25.9	8.3	14.2
Dura	---	---	---	9.0	1.3
Lymphatics	1.7	2.0	<1.8	0.70	1.5
Lungs + Trachea	2.5	9.3	5.2	3.2	3.2
Aorta	---	---	4.0	2.6	1.3
Larynx	---	---	---	---	2.1
Salivaries	---	---	2.3	0.84	1.6
Adrenals	1.1	2.2	<3.0	---	1.0
Pituitary	1.8	4.6	1.8	---	0.64
Prostate	---	---	1.1	---	0.68
Spleen	3.3	6.5	5.2	2.4	3.8
Eyes	0.85	---	1.9	---	<0.90
G.I. Tract	0.32	0.91	2.2	0.19	0.24
Heart	0.48	0.63	1.0	5.9	0.60
Ur. Bladder	---	---	0.4	---	0.68
Testes	---	---	0.6	---	0.45
Pelt	0.30	1.0	1.2	0.38	0.28
Pancreas	0.31	---	1.0	5.5	0.41
Muscle	0.13	0.64	0.77	0.19	0.18
Fat	---	0.34	---	---	---
Thymus	---	---	---	---	<1.3
Brain	0.12	---	0.10	0.48	0.17
Parathyroid	6.0	33.9*	4.0	---	2.6
Ovaries	1.1	---	---	1.0	---
Diaphragm	---	---	---	---	---
Mesentery	---	---	---	---	---
Conn. Tiss.	---	---	---	0.23	---
Uterus	---	---	---	---	---

--- Not measured.

* May have included some thyroid tissue.

TOXICITY TO BLOOD CELLS OF ^{241}Am COMPARED TO OTHER TRANSURANIUM NUCLIDES.

Jean H. Dougherty

Abstract: A comparison of the effects of the bone surface seekers ^{239}Pu , ^{241}Am and ^{228}Th and the bone volume seeker ^{226}Ra on leukocytes has been made for the first year following injection using the technic of probit analysis. Hematological responses for total white blood cells, polymorphonuclear leukocytes, lymphocytes and monocytes were evaluated at six time periods from 30-360 days post-injection. The probit of the percentage depression of the various cells when plotted against the log of the injected activity yielded approximately straight lines from which ED 50's (50% depression of leukocytes) were calculated. Using ^{226}Ra as a standard nuclide, toxicity indices (^{226}Ra μCi per kg/nuclide μCi per kg for 50% depression) were determined. From these data the hematological RBE's (^{226}Ra rads per day/nuclide rads per day for 50% depression) were computed for the various leukocytes over the first year. The surface seekers, ^{239}Pu , ^{241}Am and ^{228}Th showed considerably greater toxicity than ^{226}Ra . The pattern of depression and recovery of leukocytes is most similar for ^{241}Am and ^{239}Pu as shown by the probit method.

INTRODUCTION

A preliminary report on the hematological effects of ^{241}Am in the beagle was made two years ago.⁽¹⁾ At this time only one-half of the proposed number of dogs had been injected. At the present time all of the dogs have been injected (usually 12 dogs at each dose level) for at least a year so that the studies have been extended and the results will be compared to those of dogs injected with other alpha emitters, namely ^{239}Pu and $^{228}\text{Th}(\text{Rdth})$, as well as to our standard nuclide ^{226}Ra .

METHODS

Several methods have been used to analyze the hematological responses to the various internal emitters under study in our laboratory. The analyses of long-term effects were made according to the basic experimental

design, i.e., randomized complete blocks.⁽²⁾ The method that has proven most sensitive particularly during the first year following injection is the use of probits.⁽³⁾ The probit technic is applicable when the post-radiation response is not linear, but follows a sigmoid curve as is the case with the hematological data on the beagles. It has the advantage of relating post-injection values to pre-injection controls.

For purposes of this report the hematological responses for ^{226}Ra , ^{241}Am , ^{239}Pu and ^{228}Th have been evaluated at six time periods post-injection, i.e. at 30, 60, 90, 180, 270 and 360 days. The most sensitive cells to reflect hematological damage to internal irradiation are the leukocytes. Thus, major attention will be given to the responses of these cells; namely total leukocyte count (WBC) and absolute numbers of segmented polymorphonuclear cells (pmns), lymphocytes (lymph) and monocytes.

The leukocyte depressions were analyzed by a modification⁽⁴⁾ of the standard probit technic which could not be used here as the size of the population of cells at risk was not known. The percentage of depressions of the various leukocytes relative to pre-injection means was transferred on the probit scale and plotted against the logarithmic transforms of injected activities expressed as nanocuries per kilogram. Linear regression analyses weighed for the number of samples at each point were performed using the method of least squares. The dose levels which did not show a depression in leukocytes were not included in the plots of the data. By using the probit transform, the leukocyte responses become linear when plotted against dose enabling a more accurate comparison of the effects

of the different nuclides.

From the fitted probit line, ED_{50} values were estimated. The ED_{50} (median effective dose) is the injected activity ($\mu\text{Ci/kg}$) which will produce 50% reduction in leukocytes relative to pre-injection values. Thus a probit of 5 is equal to a 50% depression of the cell type in question. Toxicity indices were computed from these values as follows using ^{226}Ra as a standard:

$$\text{Toxicity index} = \frac{(^{226}\text{Ra } \mu\text{Ci/kg at } ED_{50})}{(\text{nuclide } \mu\text{Ci/kg at } ED_{50})}$$

Toxicity indices while they permit inter-radionuclide comparison do not account for differences in retention and radiation energies. By using a conversion factor based on ^{226}Ra rads per day/nuclide rads per day, the hematological relative biological effectiveness (RBE) was computed for each cell type and for total WBC at each time period. Conversion to RBE allows comparisons in terms of average dose rate to the skeleton at the time of blood sampling.

RESULTS AND DISCUSSION

The results of the probit analyses for ^{226}Ra , ^{239}Pu , ^{228}Th and ^{241}Am are shown in Figs. 1-4 for WBC, pmns, lymphocytes and monocytes, respectively. For each time period the fitted regression lines are depicted along with the observed data points. Lower dose levels which did not show a depression in leukocytes were not included.

The regression lines for total leukocytes (WBC), Fig. 1 for ^{239}Pu , ^{228}Th and ^{241}Am are displaced to the left of those of the ^{226}Ra dogs indicating the greater toxicity of these nuclides. The dose level points for the most part fit well to the regression line. The earlier time periods show steeper slopes indicating a greater depression at these periods.

Also the regression lines are quite parallel at most intervals and in the case of ^{241}Am and ^{239}Pu are very close together and in some cases overlying each other.

The segmented pmns at 30 days (Fig. 2) show the steepest slope as this is the period of greatest depression of pmns. These cells constitute the majority of the circulating leukocytes (50-65%) so that the regression lines at most time intervals are similar to those for WBC. The slope of the regression lines for ^{226}Ra at 180, 270 and 360 days is less steep again indicating less toxicity of this nuclide to leukocytes.

The non-granular leukocytes, lymphocytes (Fig. 3) and monocytes (Fig. 4) are present in fewer numbers in the blood (lymphocytes $\approx$ 25-35%, monocytes $\approx$ 5-10%). At most time periods they do not show as good parallelism as the pmns but in most all cases the regression line for ^{241}Am and ^{239}Pu are extremely close together or superimposed. Again ^{226}Ra has a shallower slope for 180-360 days indicating less toxicity at the lower dose levels.

The lymphocyte regression lines are steeper at 180-360 days indicating less recovery of these cells during the first year post-injection. Note that the probits for lymphocytes range from 2 to 6 in contrast to the other cells from 3 to 7 which means there is a lesser toxicity of these nuclides to the lymphocytes.

From the regression equation the ED 50's (median effective doses) were estimated for the various cell types at each time period. These are converted to toxicity indices relative to ^{226}Ra (see Methods). From the toxicity indices the hematological RBE is computed for each cell type and time interval. Table 1 summarizes these data. The median toxicity index and RBE for each cell type is determined over the six time periods

Based on toxicity indices alone, ^{228}Th appears to be five times as toxic as ^{239}Pu and ^{241}Am . However, when dose rates to the skeleton are taken into account, its effect on blood cells is similar to ^{239}Pu and ^{241}Am rad for rad. All three are more toxic than the volume seeker, ^{226}Ra .

ACKNOWLEDGEMENTS

The probit analyses of ^{226}Ra , ^{239}Pu and ^{228}Th were performed by Leon S. Rosenblatt. Lowell S. Woodbury added the analyses of ^{241}Am . The author also wishes to thank Lois Russell and Peggy Soren for their technical assistance.

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Table 1
HEMATOLOGICAL TOXICITY INDICES AND RBE VALUES RELATIVE TO $^{226}\text{Ra} = 1^*$

Cell Type	^{228}Th		^{239}Pu		^{241}Am	
	Median	Range	Median	Range	Median	Range
Toxicity indices WBC Pmn Lymph Mono Eos	27 26 21 16 10	11-50 9-55 6-28 7-35 6-20	5 5 3 3 4	2.8-7.1 2.5-5.5 1.7-6.5 2.2-7.8 2.9-8.5	3 3 5 3 2	1.6-5.9 1.7-6.5 2.4-8.4 1.2-3.3 0.8-5.1
Hematological RBE WBC Pmn Lymph Mono Eos	4 4 4 2 2	2.0-7.4 1.7-7.9 1.1-4.4 1.3-5.5 1.1-2.8	4 4 3 3 3	2.9-6.5 2.4-5.0 1.8-5.0 1.5-7.4 2.3-6.5	5 3 6 3 2	2.5-5.8 1.7-6.5 3.0-8.8 1.3-4.4 0.7-7.6

* During 30-360 days post-injection.

Figure 1. WBC: Relation of log of injected activity to probit transform of percent depression.

Figure 2. Segmented pmns: Relation of log of injected activity to probit transform of percent depression.

Fig. 3. Lymphocytes: Relation of log of injected activity to probit transform of percent depression.

Figure 4. Monocytes: Relation of log of injected activity to probit transform of percent depression.

BONE SARCOMA INDUCTION IN THE ST. BERNARD,
A PILOT STUDY IN DOGS OF HIGH NATURAL INCIDENCE

G.N. Taylor and C.W. Mays

Abstract: Because of their high spontaneous incidence of bone cancer, young adult St. Bernards were given a single intravenous injection of ^{239}Pu . The appearance time of radiation induced osteosarcomas in this giant breed was approximately 1/2 of that observed in beagles when both were injected with $0.3 \mu\text{Ci } ^{239}\text{Pu/kg}$ of body weight.

In dogs the spontaneous incidence of bone cancer is a function of body size, being rare in the smaller types and high in the giant breeds. (1-3) Osteosarcoma is the most frequent form of malignancy in very large dogs. Utilizing this marked difference in the spontaneous incidence of bone cancer within the canine species, the relationship between bone tumor induction by ^{239}Pu and natural susceptibility to bone neoplasia is being evaluated. The experiment includes 18 St. Bernards, weighing at least 100 lbs. and ranging from 16 to 19 months of age at the time of administration of the radionuclide via a single intravenous injection. Several dose levels are being studied on a comparable $\mu\text{Ci/kg}$ basis to some of those used in our beagle experiments. Ultimately these data will enable a comparison of some factors related to plutonium induction of bone cancer in two different breeds of the same species, which represent opposite extremes in natural susceptibility to skeletal neoplasia. It is anticipated that the study will provide information concerning some of the fundamental aspects of carcinogenesis.

The study was begun in 1969 and 12 of the dogs have presently been injected with various dose levels of ^{239}Pu .

Thus far, it has been determined that the appearance time of ^{239}Pu induced osteosarcomas in St. Bernards is approximately 1/2 of that observed in beagles when both were injected with $0.3 \mu\text{Ci } ^{239}\text{Pu/kg}$ (our 3-level).

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^{252}Cf , ^{249}Cf , ^{239}Pu , AND ^{226}Ra TOXICITY STUDIES IN MICE

D.H. Taysum and G.N. Taylor

Objectives:

1. Establish the toxicity of fission fragment emitting ^{252}Cf relative to α -emitting ^{249}Cf , ^{239}Pu , and ^{226}Ra .
2. Examine the shape of the dose-response curves for each of these nuclides.

Plan:

1. Replicate studies are being made in the Clinical Group mouse room (G. N. Taylor) and in the Physics Group mouse room (D. H. Taysum). Thus, if 1 colony is wiped out, as by infection, the other can still provide results. Hopefully, both studies will be successful and each will provide an independent check of the other.
2. The C57BL mouse was selected because:
 - (a) It has a very low natural incidence of bone tumors, similar to man. (The "linear" dose-response results in Miriam Finkel's⁽¹⁾ ^{226}Ra mice may have been due to the high natural incidence in her CF-1 females).
 - (b) The C57BL mouse is already established and stabilized in both mouse rooms.
3. Based on the difference in response observed in male and female mice of the NMRI strain injected with ^{224}Ra at Neuherberg, Germany,⁽²⁾ both male and female mice are being studied. Six mice of the same sex are in each cage.
4. To compare directly with our dog studies and with Miriam Finkel's

mice, the radionuclides are being injected in a citrate solution into young adults at about 10 ± 1 weeks of age. Intraperitoneal injections were chosen after establishing that there was no significant difference in retention or distribution as compared to the more difficult intravenous route of administration. The skeleton is the chief retention site, and bone sarcomas are expected to be the chief induced effect.

5. Animals will be examined visually each day. Dead and moribund animals will be autopsied by G. N. Taylor, or others designated by him. Due to the pathology work load, histological sections will be taken only from those tumors which are evident at gross autopsy and from radiographic examination of the eviscerated carcass.
6. Injection levels were selected so that the $\mu\text{Ci/kg}$ values correspond to the intended values for the Utah beagles.
7. Dose levels for ^{226}Ra are intended to extend from those with a resulting bone tumor incidence near that of the control animals up to the region of peak incidence for Miriam Finkel's mice.
8. Due to the more rapid excretion of ^{226}Ra , the injected $\mu\text{Ci } ^{226}\text{Ra/kg}$ for each level is about 3 times greater than for ^{252}Cf , ^{249}Cf or ^{239}Pu so that retained activities will be approximately equal at each injection level.
9. This pilot study should require about 5 years to complete, but even so, it will provide the first experimental evidence for cancer induction by ^{252}Cf . It will be completed before our Cf studies in beagles. The experiment may require modification if unexpected toxicity results appear. More animals could be added to existing

injection levels to refine statistics. Serial sacrifice animals will be included in addition to the toxicity mice to establish tissue distribution and concentration as necessary.

10. No additional personnel are anticipated for this experiment, and minimal additional costs are expected.

INJECTION LEVEL SCHEME FOR MOUSE STUDY

Injection level designation	Expected bone sar. incidence (Ra mice)	Injected $\mu\text{Ci/kg}$			
		^{226}Ra	^{239}Pu	^{249}Cf	^{252}Cf
5	75%	9.26	2.57	2.57	2.57
4	40%	3.09	0.857	0.857	0.857
3	23%	1.03	0.286	0.286	0.286
2	8%	0.343	0.0952	0.0952	0.0952
1	2%	0.0571	0.0159	0.0159	0.0159
0	1%	0.0	0.0	0.0	0.0

The experimental design calls for about 24 toxicity mice (12 males and 12 females) at each dose level for each nuclide to be replicated in the two separate mouse rooms. Each part of the experiment will have about (24 animals/dose level) $\times$ (6 dose levels/nuclide) $\times$ (4 nuclides) = 576 animals or 96 cages of mice per experiment, 6 per cage, for a total of about 1200 mice, including serial sacrifice animals. Injections began in August 1971, and a total of about 700 mice have been injected as of May 1972.

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²⁵²Cf PILOT STUDY IN BEAGLES

at the Radiobiology Division, University of Utah,

Radiobiology Staff

Because of increasing use of spontaneously fissioning ²⁵²Cf and the need to establish the delayed biological effects from its radiations, the following pilot study was agreed upon between the AEC Division of Biology and Medicine and this laboratory. The study is now in progress.

Dose Level Designation	Injected μCi/kg	Cf-252 Toxicity Dogs	Cf-249 Toxicity Dogs
3	0.296	6	6
2	0.0951	6	6
1	0.0154	6	6
0.5	0.00553	6	6
0.1	0.00064	6	6
0	0.0	6	6
		<u>36</u>	<u>36</u>

In addition, a few serial sacrifice dogs are to be included for tissue distribution and concentration studies as needed. Based on results to be forthcoming, it might be desirable to expand the study in the future.

²⁵²Cf decays by α-emission in 97% of its disintegrations and by spontaneous fission in the remaining 3%, but the kinetic energy of the fission fragments accounts for about half of the total energy absorbed locally. Fission fragments have an extremely high linear energy transfer (LET) of about 4000 keV/micron of tissue (about 30 times greater than the LET of α-particles), but the cancer inducing effects of fission fragments are completely unknown.

Also included in the experimental design was ²⁴⁹Cf. Because its radioactive decay is virtually all by alpha emission, comparison of the biological

effects of ^{249}Cf with those of ^{252}Cf can provide a method of identifying the separate effects of alpha and fission fragment emission by ^{252}Cf . In addition, the discrete 333 and 388 keV gamma-rays of ^{249}Cf make this isotope much more convenient to use in tracer and metabolic studies than does the continuous spectrum of fission gamma-rays from ^{252}Cf .

Most of the dogs at the 3 and 2 levels are expected to die with osteosarcomas. Survival times for ^{252}Cf -injected dogs should be shorter than for corresponding ^{239}Pu levels if the effectiveness of fission fragments for producing pathology is high. About half of the 1-level animals are expected to die with osteosarcomas or liver tumors. Some radiation-induced tumors may occur at the 0.5-level. Probably, no effects will appear at the 0.1-level. The retention of ^{252}Cf and ^{249}Cf in the liver and total body is being followed by γ -ray counting of living dogs.

Dogs are being introduced as kennel space becomes available. Injections of toxicity (lifespan) and test (sacrifice) dogs began in Feb. 1971, and as of May 1972, 1/3 of the presently planned toxicity dogs have been injected. The remaining injections should be completed in another 1-2 years. The yield of new information per dog should be very high in this experiment.

The ^{252}Cf and ^{249}Cf being used was produced by the Chemical Technology Division of the Oak Ridge National Laboratory.

CALIFORNIUM RETENTION IN BEAGLES DURING THE FIRST YEAR AFTER INJECTION

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

ABSTRACT: Measurements of the retention of citrate complexed, intravenously injected Cf-249 and Cf-252 in the total-body and liver of beagles have been extended to one year after injection. The biological half-time of Cf in the total body appears to be in the order of 6 years. Sacrifice of 2 additional dogs for distribution studies confirmed our earlier conclusion that liver, skeleton, thyroid, and kidney were the tissues with highest Cf concentration. Fission product iodine isotopes were found in the thyroid glands of Cf-252 injected dogs.

INTRODUCTION

Studies of the metabolism and biological effects of injected Cf-249 and Cf-252 in beagles were initiated at this laboratory in 1971. Preliminary results, investigative techniques, and the experimental design have been reported.⁽¹⁻⁵⁾ Observations have now been extended to about a year after injection. These and other findings are reported herein.

Cf DISTRIBUTION IN TISSUES

Two Cf-249 injected dogs were previously sacrificed for distribution studies at 7 and 21 days after injection.⁽¹⁾ Since reference 1 appeared, 2 additional dogs were sacrificed 13 and 36 days after the injection of Cf-252. Results for all 4 dogs are compared in Table 1. Californium was found in virtually every tissue which was measured. The highest concentration was observed in the liver, while the skeleton, thyroid, and kidney exhibited smaller but significant values. Total californium content of the tissues agreed within a few percent with the Cf retention measured by total-body counting and by excreta subtraction.

Table 1. Distribution and concentration of injected Cf-249 or Cf-252 in the tissues of 4 beagles.

	<u>♂ T2G5</u> <u>(7 days)</u>		<u>♀ T2F5</u> <u>(13 days)</u>		<u>♀ T3G5</u> <u>(21 days)</u>		<u>♂ T1F5</u> <u>(36 days)</u>	
	% of injected Cf	%/kg	% of injected Cf	%/kg	% of injected Cf	%/kg	% of injected Cf	%/kg
Liver	19.2	63.7	15.6	61.8	18.3	77.7	16.5	45.8
Skeleton	44.13	41.2	49.15	46.0	53.13	53.7	56.33	49.3
Thyroid	0.0491	57.6	0.0203	33.9	0.00908	16.2	0.0208	28.3
Kidneys	0.927	21.8	1.245	30.5	0.886	23.7	0.630	13.6
Dura	0.020	19.6	---	---	0.016	20.0	0.007	8.0
Connective Tissue*	0.185	18.9	---	---	---	---	0.002	1.1
Lymphatic Tissue*	0.123	13.9	0.0843	8.1	0.075	11.8	0.056	8.7
Lungs and Trachea	1.21	14.5	0.653	8.8	0.559	8.3	0.611	6.8
Spleen	0.224	9.3	0.242	9.7	0.117	6.4	0.158	6.4
Aorta	0.037	9.7	<0.009	<1.2	0.025	5.1	0.029	1.9
Salivary Glands	---	---	<0.009	<1.8	0.074	6.3	0.039	5.1
Adrenals	0.008	6.1	0.007	5.7	0.006	6.0	0.004	3.4
Prostate	0.030	4.2					0.050	5.4
Pituitary	0.00027	3.9	0.0002	2.5	0.00023	3.8	0.00092	12.1
Ovaries			0.006	4.9	0.002	3.4		
G.I. Tract	1.685	3.8	1.428	3.9	0.525	1.4	0.605	1.9
Pelt	4.132	2.6	4.511	3.1	2.095	1.3	1.640	1.3
Heart	0.207	2.0	0.128	1.8	0.118	1.5	0.097	1.0
Eyes	0.026	2.0	<0.009	<0.8	0.017	1.5	<0.007	<1.3
Thymus	0.0095	1.7	<0.009	<1.2	0.030	1.6	0.0074	3.3
Pancreas	0.044	2.3	<0.009	<0.6	0.015	0.9	0.032	1.8
Testes	0.033	1.4					0.017	0.8
Muscle	3.400	0.8	5.133	1.1	2.657	0.5	2.549	0.5
Brain	0.013	0.2	<0.011	<0.2	0.017	0.2	0.014	0.2
Omentum	---	---	---	---	0.027	0.2	---	---
Uterus			<0.009	<0.6	---	---		
Total Of Above	75.7		78.2		78.7		79.4	
By Total Body Count	78.3		73.7		72.2		74.9	
By Excreta Subtraction	81.6		82.5		75.1		79.0	

* Incomplete sample

---Not measured

LIVER RETENTION OF Cf-252

We were able to report in reference 1 the results of total-body and partial-body counting as retention of Cf-249 in the liver and in non-liver tissue by the living dog. Inadequate calibration of partial-body counting for Cf-252 precluded the reporting at that time of similar data for dogs injected with Cf-252. The sacrifice of 2 animals containing Cf-252 (Table 1) furnished the opportunity to complete the required calibration.

Immediately before sacrifice, each dog was total-body counted alive and intact. The animal was also counted with a 2 inch thick Pb shield, 20 cm high and 18 cm wide, covering the liver (see Figure 1 of reference 6). After sacrifice by exsanguination, the dog was again counted with and without the Pb shield, but with the liver removed and replaced by an equal mass of non-Cf-containing beef liver. The Cf-252 content of each excised liver was also determined (Table 1).

Since about 20% of the fission gamma-rays of Cf-252 (Figure 1) penetrate a 2 inch thickness of Pb and appear in the emergent spectrum, correction for this effect had to be made. (For gamma-rays of Cf-249, the penetration of the 333 and 388 keV photons through the 1 inch thick Pb shield used for the Cf-249 dogs was negligible.) To determine the counting rate of only that portion of the dog not covered by the shield in the presence of the radiation transmitted through the shield,

let

- C = counting rate of tissue to be Covered by 2" Pb shield, unshielded dog.
- N = counting rate of tissue Not to be covered by 2" Pb shield, unshielded dog.
- US = counting rate of Unshielded Cf-252 Standard in dog phantom.⁽⁷⁾
- SS = counting rate of Shielded Cf-252 Standard in dog phantom (completely covered by 2" Pb shield.)
- UD = counting rate of Unshielded Dog.
- SD = counting rate of Shielded Dog (with 2" Pb shield covering the liver.)

then

$$(a) \quad UD = N + C$$

$$(b) \quad SD = N + C \left(\frac{SS}{US} \right), \text{ but from (a)}$$

$$(c) \quad C = UD - N \quad \text{so}$$

$$(d) \quad SD = N + (UD - N) \left(\frac{SS}{US} \right)$$

$$(e) \quad SD = N + UD \left(\frac{SS}{US} \right) - N \left(\frac{SS}{US} \right)$$

$$(f) \quad SD - UD \left(\frac{SS}{US} \right) = N \left(1 - \frac{SS}{US} \right)$$

$$(g) \quad N = \frac{SD - UD \left(\frac{SS}{US} \right)}{1 - \left(\frac{SS}{US} \right)}$$

Using equations (g) and (c), above, it was determined that in the carcass of T1F5, with the liver removed, the fraction of non-liver Cf-252 which was covered by the Pb shield was 0.401. The corresponding value for T2F5 was 0.456, averaging about 0.43 for both animals. Therefore, the calculated counting rate of the tissue not covered by the shield (N in eq (c)) could be divided by $(1 - 0.43) = 0.57$ to obtain an estimate of the total counting rate of non-liver Cf-252 in the living dog. The corresponding value found for Cf-249 dogs⁽¹⁾ was $(1 - 0.38) = 0.62$, in good agreement, considering that the 2 inch thick Pb shield used with Cf-252 dogs actually shields a larger fraction of the dog from the detector than does the 1 inch thick Pb shield of the same height and width used with Cf-249 dogs because of the greater solid angle it subtends.

The results of this calibration study were used to calculate the

retention of Cf-252 in the livers of Cf-252 injected dogs previously counted alive and intact. Our speculation⁽¹⁾ that the partitioning of Cf-252 between liver and non-liver tissue was similar to that of Cf-249 was thereby confirmed (Figure 2).

CALIFORNIUM EXCRETION

Table 2 of reference 1 indicated that a significant fraction of excreted Cf appeared in cage washings. We tentatively assigned the activity found in cage washings to the urine, pending further evidence.

The first day's urine collection of both T1F5 and T2F5 exhibited a fairly prominent gamma-ray peak in their photon spectra at about 0.5 MeV superimposed upon the continuous fission gamma-ray spectrum. This gamma-ray peak was also observed in the photon spectra of cage washings but never in that of fecal samples. These observations tend to verify our earlier speculation concerning the origin of Cf in the cage washings.

The nuclide found in the urine samples has not yet been positively identified, but results of serial counting suggest it to be a fission product of Cf-252 with a physical half-period of just under 46 days. Ru-103 (half-period = 39.8 days, gamma-ray energy = 0.498 MeV) appeared to be the most likely emitter of suitable half-period, photon energy, mass number and resulting abundance in fission that may have accounted for at least part of this activity.

FISSION PRODUCTS IN Cf-252 INJECTED DOGS

Gamma-radiation from fission products undoubtedly contributes to the Cf-252 photon spectrum of the injection solution, Cf-252 injected dogs, excreta, and excised tissues, but we have not been able to detect their

photopeaks in the presence of the continuous fission gamma-ray spectrum (Figure 1) except for few urine and cage washings samples and the thyroid glands of T1F5 and T3F5. The 0.7363 gram thyroid of T1F5 was found to contain 1479 pCi I-133/g and 1483 pCi I-131/g, at 36 days after injection, corrected to time of death, while the corresponding values for the 0.6004 gram thyroid of T2F5 were 3051 pCi I-133/g and 3043 pCi I-131/g at 13 days after injection. The iodine isotopes were identified by their respective 20.8 hour and 8.0 day half-periods and by their 0.53 and 0.36 + 0.64 MeV photon energies. I-134 ($T_{1/2}$ = 52 min), I-132 ($T_{1/2}$ = 2.3 hr), and I-135 ($T_{1/2}$ = 6.7 hr) could not be detected with any certainty in either of these thyroids, although there was a suggestion of some photopeaks of I-132 and I-135 in the thyroid of T2F5 which was counted about 1 hour after death.

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Table 2. Concentration of Cf, Am, and Pu in skeleton, liver, kidney, and thyroid of beagles soon after injection

<u>% of injected activity per kg</u>					
<u>Days After Injection</u>	<u>Dog</u>	<u>Skeleton</u>	<u>Liver</u>	<u>Kidney</u>	<u>Thyroid</u>
7	T2G5	41.2	63.7	21.8	57.6
13	T2F5	46.0	61.8	30.5	33.9
21	T3G5	53.7	77.7	23.7	16.2
36	T1F5	49.3	45.8	13.6	28.3
	Average Cf	47.6	62.2	22.4	34.0
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	T16W5	27.5	194.2	9.7	20.4
	Average Am	27.0	178.5	15.6	55.1
5	T36P1	44.8	95.9	18.3	
29	T1P5	49.2	73.0		
35	T30P1	36.2	159.7	18.7	
40	T31P3	35.8	102.9	7.4	
44	T2P5	55.8	76.6		
7	T56P5		106.1		
7	T14P5		68.7	18.8	
14	T55P5		110.6	13.4	
13	T42P5				5.3
35	T44P5				6.8
(404)	(T54P5)*				6.6*
	Average Pu	44.4	99.2	15.3	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.

Figure 1. Comparison of the gamma-ray spectra of Cf-249 and Cf-252. Note that the activities of the two sources used to obtain these spectra differed by a factor of 10. The detector in each case was an 8 x 4 inch Na I (Tl) crystal in a 0.02 inch thick stainless steel housing.

Figure 2. Retention of injected californium in the total-body and liver of beagles. Note that the retention of Cf-252 (closed circles) was similar to that of Cf-249 (open circles). Each point represents the mean of 4 dogs injected with 0.28 μCi Cf-249/kg or the mean of 4 dogs injected with 0.29 μCi Cf-252/kg. Vertical bars represent ± 1 standard deviation of the individual values for the 4-dog group (livers). The standard deviation bars for total-body retention are in all cases enclosed within the circles. Provisional indicated biological half-times of Cf are about 6 years in both the total body and liver for the combined data, but about 7.9 years in the total body and about 3.3 years in the liver for Cf-249 alone.

THE DISTRIBUTION AND RETENTION OF ^{249}Cf IN BEAGLE SOFT TISSUE

David R. Atherton and Ray D. Lloyd

ABSTRACT: Beagles injected intravenously with ^{249}Cf in citrate buffer of pH 3.5 were sacrificed 7 days and 3 weeks after injection. As with americium, the concentration of californium in the liver was highest of all soft tissue but the total was about 18 to 20%, whereas the beagle liver retains about 50% of injected americium at comparable times. Concentrations in other soft tissue ranged from 70% of that in the liver downward. The thyroid, kidney, spleen, lymphatic tissue and dura mater showed significant concentrations. The presence of californium was ubiquitous but in very low concentrations throughout all other soft parts; the brain, lungs, heart, gastrointestinal tract, pancreas, gonads, thymus, pituitary, and adrenals being measured specifically, as were large samples of fat, muscle and pelt. From these initial studies it is seen that of the soft tissues, liver, thyroid, and kidneys will be at greatest risk following administration of ^{249}Cf .

INTRODUCTION

Recently the Radiobiology Laboratory at the University of Utah initiated studies designed to elucidate details of the chronic effects of californium in the beagle. The practical reasons for the study of californium along with details of the physical characteristics of the two isotopes of the nuclide being used are presented elsewhere (see reference 1); included are details of preparing the nuclide for injection as well as pertinent information about the beagles serving as the subjects for this report.

From experience gained in studies of the chronic effects of ^{239}Pu and ^{241}Am , it was expected that the sites of major deposition would be skeleton and liver. Experience had also shown that on occasion a tissue may be found which retains relatively high concentrations of a radionuclide even though the retention in that tissue would make no significant contribution to the total body retention. This was the case in studies of ^{241}Am which have shown that the thyroid exhibits relatively high concentrations of that nuclide

for prolonged periods of time.⁽²⁾ It was therefore decided that the analysis of the distribution of retained californium in beagle soft tissues would be carried out in considerable detail.

MATERIALS AND METHODS

The two beagles selected for study were given 2.8 μCi of ^{249}Cf per kg and were designated as T2G5 and T3G5.⁽¹⁾ They were sacrificed for detailed studies of early retention and distribution at 7 and 21 days, respectively.

In an effort to make the study as meaningful as possible, these results were compared to results obtained on 2 dogs which had been given 4.5 $\mu\text{Ci/kg}$ of ^{241}Am ,⁽²⁾ designated as T32W5.5 and T33W5.5 and which were sacrificed for studies of the early retention and distribution of americium at 7 and 8 days, respectively, following their injection.

At autopsy, skeletal parts and the larger soft tissues such as liver and collections of muscle were examined for californium or americium content by γ -ray spectroscopy as described by Lloyd et al.^(2,3) Smaller parts were evaluated by γ -ray spectroscopy using a small sodium iodide crystal-well,⁽⁴⁾ (Knothole 1), which with small volumes (≤ 10 ml) is quite insensitive to changes in geometry and in which the smaller samples were compared to standard sources whose volumes were similar to those of the samples.

RESULTS

The age and body weights at injection along with the intervals from injection to death (days) and the injected dose ($\mu\text{Ci/kg}$) for each of the beagles used for this report are shown in Table 1. At the ages shown the animals are young adults having achieved skeletal maturity as demonstrated radiographically by closure of all epiphyses except a few at the costo-chondryl

junction. The body weights are in the average range for our colony.

Gross distribution and concentrations of the retained nuclide are shown in Table 2. The skeleton and liver combine to account for essentially all of the retention of each nuclide. The total retention amounts to about 80% for both nuclides. In the case of californium, about 48% was found in the skeleton and about 22% was in the liver; while 29% of the americium was in the skeleton and about 47% in the liver.

Although, as can be seen in Table 3, the percentages of the injected dose of either nuclide retained by soft parts other than livers are very small, the concentrations in some of them, as seen in Table 4, indicate that certain organs are exposed to radiation dose rates approaching those of the skeletal and hepatic systems.

A final tabulation (Table 5) is included to provide another basis for judging the radiation hazard to each of the tissues relative to the average for the whole body. This is done simply by comparing the concentration (% dose/g) of each tissue to the total retention divided by the weight of the animal.

DISCUSSION

It should not be assumed that the relative concentrations shown in Table 5 can be directly related to actual radiation doses to critical cells or portions of cells. Indeed, it is well known that americium shows mainly a surface deposition in bone⁽⁵⁾ and, therefore, the relative dose-rate in critical bone volumes will be very much higher than indicated. The whole body concentration of retained californium is virtually the same as that for americium, which permits easy comparison of the deposition patterns of the two nuclides.

Although data on the chronic effects of metabolized americium is in the very early stages of accumulation in this laboratory, the effects at dose levels of 2.8 and 0.9 $\mu\text{Ci/kg}$ have been severe damage to liver, kidney, and thyroid, with death occurring at about 400 and 1500 days following administration, respectively. Direct extrapolations from these, admittedly very sparse, data on the effects of americium should not be made because of the uncertainty as to how the local distribution of americium and californium compare within a given tissue. However, the implications of this study are that the skeleton, liver, thyroid, kidney, and, perhaps, lymphatic tissue should be closely observed as possibly important targets in californium intoxication.

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TABLE 1. AGES, WEIGHTS AND DOSES OF EXPERIMENTAL ANIMALS

	Nuclide	Age At Injection (Days)	Weight At Injection (kg)	Interval: Injection-Death (Days)	Dose (μ Ci/kg)
T2G5	^{249}Cf	584	10.7	7	2.77
T3G5	^{249}Cf	584	9.89	21	2.80
T32W5.5	^{241}Am	553	10.4	7	4.46
T33W5.5	^{241}Am	393	10.5	8	4.47

TABLE 2. GROSS DISTRIBUTION OF RETAINED NUCLIDES

	Skeleton		Liver		Other		Whole Body	
	% Dose	% Dose/g	% D	% D/g	% D	% D/g	% D	% d/g
T2G5	44.1	0.0565	24.6	0.0815	10.7	0.0011	80.8	0.00755
T3G5	53.1	0.0736	20.1	0.0852	6.0	0.0007	80.3	0.00812
T32W5.5	28.6	0.0376	47.7	0.195	3.2	0.0003	79.5	0.00764
T33W5.5	30.0	0.0392	46.2	0.148	5.3	0.0006	81.5	0.00776

TABLE 3. RETENTION: % OF INJECTED DOSE

	T2G5	T3G5	T32W5.5	T33W5.5
Liver	24.6	20.1	47.7	48.0
Skeleton	44.1	53.1	28.6	30.0
Thyroid	0.0491	0.0091	0.0247	0.0574
Kidneys	0.895	0.827	0.475	0.453
Dura mater	0.0190	0.0205	0.0105	0.0131
Spleen	0.236	0.117	0.014	0.024
Lymph Nodes	0.123	0.075	0.026	0.044
Adrenals	0.0082	0.0058	0.0013	0.0015
Pituitary	0.0003	0.0002	0.0002	0.0002
Prostate	0.030	-----	0.006	0.009
Testes	0.033	-----	0.002	0.006
Ovaries	-----	0.002	-----	-----
Thymus	0.0095	0.028	0.002	-----
Eyes	0.026	0.017	0.009	0.007
Loose Connective Tissue	0.0032	0.0012	0.0007	0.0002
Brain	0.013	0.017	0.002	0.002
Other	10.7	5.99	1.60	2.97
Total	80.8	80.3	78.5	81.6

TABLE 4. CONCENTRATION OF RETAINED NUCLIDE ((%/gram) $\times 10^3$)

	T2G5	T3G5	T32W5.5	T33W5.5
Liver	81.5	85.2	195.	148.
Skeleton	56.5	73.6	37.6	39.2
Thyroid	57.5	16.2	50.0	42.0
Kidneys	21.1	22.1	13.5	11.7
Dura mater	18.6	20.0	8.1	8.9
Spleen	9.8	6.4	0.56	1.2
Lymph Nodes	14.0	12.0	6.86	4.49
Adrenals	6.3	5.9	2.3	2.2
Pituitary	3.8	3.9	2.7	2.5
Prostate	4.0	---	0.85	2.3
Testes	1.0	---	0.17	0.30
Ovaries	---	3.0	----	----
Thymus	1.7	1.4	0.4	----
Eyes	2.0	2.0	0.81	0.62
Loose Connective Tissue	0.94	---	0.30	0.24
Brain	0.2	0.2	0.02	0.03
Other	1.14	0.69	0.17	0.32
Whole Body	7.55	8.12	7.54	7.77

TABLE 5. RELATIVE NUCLIDE CONCENTRATION BY VARIOUS TISSUES

	T2G5	T3G5	T32W5.5	T33W5.5
Liver	10.8	10.5	25.9	19.0
Skeleton	7.5	9.1	5.0	5.0
Thyroid	7.6	2.0	6.6	5.4
Kidneys	2.8	2.7	1.8	1.5
Dura mater	2.5	2.5	1.1	1.1
Spleen	1.3	0.8	0.07	0.2
Lymph Nodes	1.8	1.5	0.9	0.6
Adrenals	0.8	0.7	0.3	0.3
Pituitary	0.5	0.5	0.4	0.3
Prostate	0.5	---	0.1	0.3
Testes	0.1	---	0.02	0.04
Ovaries	---	0.4	---	----
Thymus	0.2	0.2	0.05	----
Eyes	0.3	0.2	0.1	0.08
Loose Connective Tissue	0.1	---	0.04	0.03
Brain	0.03	0.02	0.003	0.004
Other	0.2	0.08	0.02	0.04
Average Whole Body	1.0	1.0	1.0	1.0

INTRACELLULAR DISTRIBUTION OF ^{249}Cf IN CANINE LIVER

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

ABSTRACT: Three beagles were injected intravenously with ^{249}Cf in citrate buffer. One of these dogs was sacrificed at 7 days, another at 21 days; 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. Most of the ^{249}Cf was initially bound by soluble proteins, probably ferritin. At later times increasing quantities were associated with intracellular organelles. 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 12-52%. 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. This pattern 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 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.

INTRODUCTION

The liver is a principal target organ for a number of transuranium elements when they are delivered in solution by intravenous injection. Subcellular distributions of plutonium and americium have been studied by a number of workers in this and other laboratories. ^(1,2,3) The lysosomal uptake of polymeric plutonium in rats was first reported by Rahman and Lindenbaum ⁽⁴⁾ and the transfer of soluble plutonium-protein constituents of the liver into the lysosomal fraction of rodent livers was studied as a function of time by Boocock et al. ⁽¹⁾ A similar mechanism of plutonium transfer from soluble components into subcellular particles in canines has

been presented.⁽⁵⁾ With the increasing use of isotopes of californium, especially ^{252}Cf , distribution and retention data for this transuranium element in animals are needed. Although some predictions regarding its distribution were made earlier by Durbin⁽⁶⁾ and some general data derived from rats became available more recently,⁽⁷⁾ no detailed studies regarding its subcellular distribution in the liver are as yet available. This presentation is a preliminary attempt to elucidate the distribution of this element in the liver as a function of time after the injection of californium in canines.

METHODS

Three beagles were injected intravenously with $2.8 \mu\text{Ci/kg}$ body weight of ^{249}Cf in citrate buffer, pH 3.5. One of the animals (T1) was set aside for serial liver biopsies; T2 was sacrificed at 7 days and T3 at 21 days after injection. Fresh liver specimens were immediately cooled on ice, finely minced with razor blades, and homogenized 2X in two volumes of 0.25 M sucrose containing 0.1 M NaCl and 3mM of Ca^{++} . The homogenates were then subjected to differential centrifugation according to a scheme which is essentially the same as that used by Boocock⁽¹⁾ (Fig. 1). The homogenates, free of nuclei and debris, obtained after centrifugation for 6000 $\text{g}\cdot\text{min}$ were layered on a continuous sucrose density gradient of 12-52% and centrifuged for 220,000 $\text{g}\cdot\text{min}$ ($1850 \times \text{g}$ for 120 min). After centrifugation 16 to 18 fractions were collected. All samples were analyzed for protein,⁽⁸⁾ acid phosphatase,⁽⁹⁾ cytochrome oxidase,⁽¹⁰⁾ and ^{249}Cf radioactivity. Some data were also obtained by differential centrifugation of kidney and spleen homogenates.

RESULTS

The retention of ^{249}Cf in the liver, as extrapolated from whole and partial body counting data,⁽¹¹⁾ was 28.4% of injected dose at one day after injection for T1, and 26.0 and 23.5% of injected dose at two days after injection for dogs T2 and T3, respectively. These data are comparable to retention data obtained for $^{239}\text{Pu IV}$.

The results of differential centrifugation of liver homogenates are listed in Table 1. Data are expressed as the concentration of ^{249}Cf (% of injected dose/mg protein). The concentration in fraction 1 (N + D), varied a great deal due to the presence of whole cells. It did, however, show no change in ^{249}Cf concentration as a function of time. At one day after injection the concentration as a function of protein content in each fraction did not vary. This indicates that, to a large extent, the ^{249}Cf was not associated with subcellular organelles but was present as a soluble californium-ferritin complex. At later times after injection decreasing concentrations of the nuclide were found in cytosol (fraction 5). The 60,000 g-min centrifugation pelleted most of the nuclide and consequently the concentration in this fraction (fraction 6) increased as a function of time. The fact that fraction 6 values for T1 at 110 and 174 days (9.4 and 10.5) were lower than the values at 7 and 21 days for T2 and T3 must be considered in the light of the decrease in total liver retention. Thus, the values of 9.4 and 10.5 should be doubled to compensate for this loss if comparisons are to be made with T2 and T3. The concentration in fraction 7 (designated as lysosomal fraction) varied but was generally lower than that of the mitochondrial fraction. Acid phosphatase and cytochrome oxidase was measured in the homogenate free of nuclei and debris, and in the two fractions

designated as mitochondria and lysosomes. There was no linear relationship between either of these enzyme activities and the amount of ^{249}Cf in similar fractions. Organelles contained in fraction 6 were ruptured by sonic shock or treatment with Na-deoxycholate. Subsequent centrifugation at 60,000 $\times g$ for 20 minutes showed that approximately 70% of the nuclide in this fraction was found in the sediment and was either membrane bound or otherwise particulate. Most of the ^{249}Cf remaining in the supernatant was bound by ferritin.

Homogenates free of nuclei and debris were subjected to isopycnic density gradient centrifugation and the protein content and enzyme activities of different fractions were determined. As expected from results of density gradient centrifugation, most of the ^{249}Cf , at early times after injection, was found in the light fractions containing mostly soluble proteins and some very light subcellular organelles. As the concentration of ^{249}Cf in these fractions decreased, the amount of nuclide found in the heavy fractions increased. This is shown in Table 2. At later times after injection, increasing quantities of ^{249}Cf were found in fractions of intermediate densities. Thus, a second redistribution occurred at long times after injection. It is too early to make definite quantitative predictions about the redistribution of californium at this time. Gradient fractions which contained high levels of both acid phosphatase and cytochrome oxidase also had high californium levels, but no direct linear relationship between either enzyme and radioactivity was observed. Enzyme activities (units/volume) are shown in Fig. 2 together with cpm of ^{249}Cf found in the same volume of each fraction. The shift in ^{249}Cf from light to heavy fractions is again clearly evident.

Data on the distribution of ^{249}Cf in kidney and spleen are available only from the two sacrificed dogs. Results of separation by differential

centrifugation are shown in Table 3. In the kidney, the highest concentration was found in the N + D fraction, the remaining radioactivity was more or less ubiquitously distributed over all other fractions. The concentration in the mitochondrial and lysosomal fractions was not much higher than that in other fractions, indicating these two organelles in the kidney are not the predominant carriers of the nuclide. In the spleen the highest concentration of the ^{249}Cf was found in the two fractions designated as mitochondria and lysosomes. Almost all of the ^{249}Cf remaining after 800,000 $\text{g}\cdot\text{min}$ was sedimentable at $6 \times 10^6 \text{ g}\cdot\text{min}$ and the concentration in the cytosol was correspondingly low.

DISCUSSION

Our data on liver retention are slightly higher than those reported by Durbin⁽⁶⁾ and considerably higher than those of Mewhinney.⁽⁷⁾ Because of the short half life of californium isotopes, there are fewer atoms per μCi and this reduces the probability of formation of hydrolysis products. The presence of definite californium-protein complexes of intermediate stability (less stable than the similar plutonium-protein complexes) may also inhibit the formation of hydrolysis products and polymeric material. Californium-protein complexes present in the blood stream are sufficiently unstable so that californium may interact with other biochemical substances in the body which can form more stable associations. In the liver, the main association of californium was with ferritin, as has been described for Pu IV and Am III. Lack of hydrolysis was demonstrated by the large fraction of ^{249}Cf found at early times after injection in the cytosol, and after the sucrose density separation in the light fractions.

At later times after injection (7 days), as was observed with Pu IV

and Am III, increasing quantities of the nuclide were found in subcellular organelles. It is unfortunate that a clear separation of these organelles has not yet been accomplished in any species.⁽¹²⁾ In canines, density distributions, especially of lysosomes, have an extremely wide range and specific fractions cannot be readily prepared in purified form. Estimation of californium distribution is further complicated by the fact that mitochondria account for about 20% of the total liver protein whereas the lysosomes account for less than 1%. Even in so-called pure preparations of lysosomes, the majority of the protein is contained in peroxisomes. High concentrations of ^{249}Cf at later times were associated with high concentrations of both acid phosphatase and cytochrome oxidase, i.e. lysosomes and mitochondria; but a direct linear relationship between enzyme concentration and ^{249}Cf concentration was not seen. A high concentration of ^{249}Cf was always found in heavy particles (rich in mitochondria) and not so much within the lighter fractions usually designated as lysosomes.

There are indications that at least partially association with mitochondria is an intermediate step in the metabolism and the hypothetical scheme outlined in Fig. 3 may exist. Mitochondria contain ferritin, thus providing a link between cellular Cf-ferritin and Cf in subcellular organelles. Catabolic products could then be engulfed by lysosomes and a direct route from Cf-ferritin into lysosomes appears also possible. A similar scheme for Pu and Am was proposed earlier by this laboratory. Association of plutonium with lysosomal particles was described earlier by Lindenbaum,⁽⁴⁾ Boocock,⁽¹⁾ and Taylor.⁽³⁾

Because of the complications presented above, more data must be collected and separation methods improved before definite quantitative statements

can be made about the distribution of californium in these organelles.

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TABLE 1. CONCENTRATION OF ^{249}Cf IN VARIOUS LIVER FRACTIONS AFTER DIFFERENTIAL CENTRIFUGATION ($10^4 \times \% \text{ D/mg PROTEIN}$)

		Dog T1			Dog T2	Dog T3
		1 day Sample	110 day Sample	174 day Sample	7 day Sample	21 day Sample
Fract. 1	N + D	3.3	4.	4.5	5.9	3.6
Fract. 2	600 $\underline{\text{g}}$	5.2	4.	3.2	6.2	6.3
Fract. 3	3000 $\underline{\text{g}}$	5.0	2.4	1.5	3.7	2.
Fract. 4	40,000 $\underline{\text{g}}$	5.0	1.9	1.4	4.	1.
Fract. 5	cytosol	4.2	1.4	1.1	3.2	1.3
Fract. 6	Mitoch.	6.7	9.4	10.5	15.4	20.
Fract. 7	Lysos.	9.4	3.5	3.1	2.6	7.1
Total Liver Retention		28.4	11.9	9.6*	19.2	18.3

* Obtained by interpolation between 110 and 204 days.

TABLE 2. RESULT OF DENSITY GRADIENT CENTRIFUGATION OF LIVER HOMOGENATE

Time After Injection	1 Day	7 Days	21 Days
% of total ^{249}Cf found in three heaviest fractions	10.9	36.5	43.1
% of total ^{249}Cf found in three lightest fractions	52.5	27.2	9.5

TABLE 3. CONCENTRATION OF ^{249}Cf IN VARIOUS FRACTIONS OF KIDNEY AND SPLEEN AFTER DIFFERENTIAL CENTRIFUGATION ($10^4 \times \% \text{ D/mg protein}$)

	Kidney		Spleen	
	<u>Dog T2</u> <u>7 Days</u>	<u>Dog T3</u> <u>21 Days</u>	<u>Dog T2</u> <u>7 Days</u>	<u>Dog T3</u> <u>21 Days</u>
Fract. 1 (N + D)	3.2	1.7	0.7	0.3
Fract. 2 (600 g)	0.9	0.9	1.1	0.5
Fract. 3 (3000 g)	0.7	0.9	0.7	0.4
Fract. 4 (40,000 g)	1.0	1.1	0.3	0.3
Fract. 5 (Cytosol)	0.9	0.8	0.04	---
Fract. 6 (Mitoch.)	1.6	1.0	3.9	1.4
Fract. 7 (Lysos.)	0.6	0.9	1.4	1.1
% D/g whole tissue	0.0211	0.0221	0.0098	0.0064
Total retention (% of injected dose)	0.895	0.627	0.236	0.117

Fig. 1.

Fig. 2. Result of density gradient distribution. At day 1 most of the ^{249}Cf is found in the light fractions, at later times it is associated with subcellular organelles with high acid phosphatase and high cytochrome oxidase activity.

Fig. 3. Hypothetical scheme of the possible pathway of ^{249}Cf in liver tissue.

INTERACTION OF ^{249}Cf AND ^{252}Cf WITH CONSTITUENTS
OF DOG AND HUMAN BLOOD

Walter Stevens and F. W. Bruenger

ABSTRACT: Beagles were injected with ^{249}Cf or ^{252}Cf in citrate buffer. The concentration of nuclide in plasma and whole blood was measured from 5 minutes to 48 hours post-injection. Small amounts of californium were found associated with the cellular elements. The concentration of californium in plasma decreased rapidly during the first few hours after injection. At twenty four hours post injection less than 1% of the injected dose was circulating. Californium-249 and -252 disappeared from the circulation at the same rate. Separation of plasma constituents by gel filtration demonstrated that californium in plasma was associated with compounds with a molecular weight of approximately 70,000. Additional information obtained by ion exchange chromatography indicated that this protein was transferrin. Data obtained in vivo from canines were extended by in vitro experiments with human blood. Gel chromatography of a human plasma protein incubated in vitro with californium indicated that a Cf-protein complex was formed and appeared to be nearly identical to that seen in dogs. Re-chromatography of these fractions on DEAC-Sephadex showed that transferrin and californium did not coincide exactly. Thus californium-transferrin is either held more tenaciously by the ion exchange resin than iron-transferrin or apo-transferrin, or the shift was caused by the presence of another as yet unidentified minor protein component. The stability of the protein complex(s) formed is less than that of the plutonium-transferrin complex and this decreased stability is responsible for the high rate of its disappearance from the bloodstream.

INTRODUCTION

The increasing use of ^{252}Cf for a variety of medical and industrial applications has necessitated an evaluation of its potential radiation hazard when introduced internally. Extensive studies of the distribution and retention of plutonium and americium have been carried out by this laboratory for many years and as part of this work the rate of loss of these nuclides from the circulation after intravenous injection into beagles has been evaluated. In addition the chemical interactions and

associations of these nuclides with constituents of canine and human blood were studied by this and other laboratories,^(1,2,3,4,5) and specific proteins that reacted with plutonium and/or americium were isolated and identified. Recently, a study to determine the retention, distribution, biochemical associations and biological end-points of californium in beagles injected with ^{249}Cf and ^{252}Cf in citrate buffer of pH 3.5 was initiated. In this paper we will examine the distribution and the removal of the two nuclides from canine blood and their interaction with blood proteins. Associated studies were performed in vitro with human blood.

METHODS

Californium-252 and -249 used in these studies were obtained from the Oakridge National Laboratory. Injection solutions of tri-valent ^{249}Cf and ^{252}Cf were prepared in a sodium citrate-citric acid buffer solution of pH 3.5. Young adult beagles were injected intravenously with either ^{249}Cf or ^{252}Cf . In the case of ^{249}Cf blood samples were taken at 5 and 30 minutes and 1, 2, 4, 7, 24, and 48 hours after injection. Similar time intervals were chosen for ^{252}Cf . Blood was centrifuged and the cells were removed from the plasma and washed in saline solution. The amount of activity associated with the washed cells and the plasma was determined. Californium-249 activity was determined in a gamma-ray spectrometer and the activity of the ^{252}Cf was determined in a liquid scintillation spectrometer.

Plasma constituents were separated by gel filtration and ion-exchange chromatography. Serum proteins were separated using a double

column of Sephadex G-100-G-200 which was eluted with 0.1 M TRIS + 0.7 M sodium chloride with 0.0015 M sodium azide, pH 7.5. Under these conditions the proteins were separated into three peaks. Peak 1 contained macroglobulins and high molecular weight lipoproteins. The bulk of the globulins comprised peak 2, and peak 3 includes albumin, transferrin and some alpha globulins. Low molecular weight materials were eluted after the proteins. Ion exchange chromatography of serum proteins on DEAE-Sephadex produced three distinct protein peaks. The serum was placed on a column containing 7.5 g of resin at pH 8, and eluted with a linear gradient formed with 0.1 M TRIS $\rightarrow$ 0.1 M TRIS + 0.5 M NaCl. The first eluting peak (left) contained beta and gamma globulins including the beta globulin transferrin. The second peak was mainly alpha globulins plus some beta globulins, and the third peak contained albumin and traces of the alpha and beta globulins. Protein concentration was determined by measuring the optical density at 280 nm.

RESULTS

The concentration of ^{249}Cf associated with the cellular elements during the first day was one eighth that found in plasma and decreased further by 48 hours. The rate of disappearance of ^{249}Cf and ^{252}Cf from the blood of beagles was studied from 5 minutes to 48 hours post-injection. The results of these studies are found in Table 1 and are shown graphically in Fig. 1. In both Table 1 and Fig. 1 similar data for ^{241}Am , ^{239}Pu , and ^{228}Th are also shown so that a direct comparison can be made.

The initial loss of californium from the bloodstream was rapid. At 5 minutes, approximately 50% of the injected ^{249}Cf was circulating.

By 10 minutes this figure had dropped to 33%. After 1 hour, the rate of elimination began to decrease gradually. At 7 hours after injection 2.5% was circulating and by one and two days the value decreased to 0.5 and 0.4%, respectively. Data obtained for dogs injected with ^{252}Cf are very similar although they are slightly higher. Both sets of data are average plasma retention data for two dogs (^{252}Cf) or three dogs (^{249}Cf). Compared to data on americium, the initial decrease of nuclide concentration in plasma is similar, but by 2 to 7 hours, values for americium are approximately one half of those seen for californium. At later times, americium continues to leave the blood at a faster rate than do either of the californium isotopes. Plutonium, on the other hand, is leaving the circulation at a very slow rate. At 5 minutes, about 85% of the injected dose is circulating. At 7 and 24 hours, the respective values are 53% and 28%. Eighty five percent of the injected $^{228}\text{ThIV}$ nuclide is circulating at 5 minutes after injection. The rate of elimination is intermediate between plutonium and californium and the values for 7 and 24 hours are 19% and 2.3%, respectively.

In previous papers the association of plutonium and americium with the transferrin component of beagle and human blood has been described.^(3,5) Plutonium is bound tenaciously by the transferrin and this may account for its slower removal from the blood of the injected animals. Americium on the other hand forms only loose bonds with plasma proteins and reacts with the inorganic plasma constituents very rapidly. In order to understand the distribution and deposition of californium in the body it was necessary to establish the degree of interaction of these nuclides with the constituents of blood. According to earlier procedures blood samples

from beagles injected with californium were submitted to gel chromatography on Sephadex G 100-G-200 columns. Samples were also subjected to ion exchange chromatography on DEAE Sephadex before G-100-G-200 chromatography and also after. The result of these studies carried out with canine plasma are shown in Fig. 2.

A sample of canine plasma was obtained at 5 minutes after injection. Gel chromatography separated the plasma proteins into three peaks according to molecular size and weight. With these three protein peaks two peaks of californium activity were seen, the largest occurring with the third protein peak and a smaller but significant peak in the area of very high molecular weight material. The californium peak associated with protein peak III is displaced slightly to the left indicating, as with PuIV, binding to transferrin. The concentration of ^{249}Cf activity found with proteins of peak III after chromatography of this sample was low, making rechromatography on DEAE ion exchange resin quite difficult. Rechromatography was therefore omitted. Results of direct ion exchange chromatography of a sample obtained 10 minutes after injection are presented in the lower half of Fig. 2. Here the protein was resolved into three peaks, the one on the right representing mostly albumin, the one in the middle containing the bulk of the globulins and the one on the left, globulins including transferrin. Two peaks of californium activity are seen, the largest one occurring just on the left of the transferrin-containing peak. This represents the greatest amount of activity. A second much smaller peak was found on the right shoulder of the middle peak and probably represents material associated with albumin and perhaps a third unknown protein. The picture represented in Fig. 2 is similar to

that we have seen previously with americium and plutonium in canine blood.

In Fig. 3 the results of gel chromatography and ion exchange chromatography of human plasma proteins incubated in vitro with ^{249}Cf are shown. Chromatography of human plasma proteins on Sephadex G-100-G-200 yielded three major peaks. Two rather sharp peaks of californium are seen, one occurring with a high molecular weight material which would be near the exclusion limit of the column. A second californium peak occurs just slightly to the left of the third protein peak. This elution pattern is similar to previous observations with plutonium or americium transferrin complexes^(3,5) and with data described in Fig. 2 for canines.

The chromatogram seen on the lower left side of Fig. 3 is the result of re-chromatography of the pooled proteins of peak III (G-100-G-200) on DEAE Sephadex. The small peak on the left is probably a minor protein component that passed through the DEAE unabsorbed. The middle peak is primarily transferrin and the large peak on the right mostly albumin. A small peak of ^{249}Cf appeared at the left shoulder of the transferrin peak, a large ^{249}Cf peak is on the right shoulder and in the valley between transferrin and albumin, and a third small californium peak coincides with the albumin. Essentially the same results are seen on the right side of Fig. 3. Here a small sample of diluted plasma was subjected directly to DEAE ion exchange chromatography. The ^{249}Cf distribution was nearly the same as before but it must be kept in mind that the protein peaks now represent a great number of individual proteins which were previously eliminated by gel chromatography on G-100-G-200.

DISCUSSION

Knowledge of the interaction of actinide elements with constituents of the blood is essential for the interpretation of deposition patterns. From results obtained with ^{241}Am whose ionic radius is $0.99 \text{ \AA}$, it was expected that Cf(III) would be removed from plasma slower than americium and that complex of chelate formation with plasma constituents would be more pronounced since the ionic radius was appreciably smaller ($0.94 \text{ \AA}$).⁽⁶⁾ An atom with a smaller ionic radius should form more stable complexes with a higher solubility than those with larger ionic radii. Our results established that these predictions were true. ^{249}Cf cleared from plasma somewhat slower than americium, definite protein complexes were formed, and there was little or no formation of hydrolysis products as inferred from tissue distribution patterns. The formation of a ^{249}Cf -transferrin complex is indicated. However the shift in the respective protein and ^{249}Cf peaks cannot be explained at this time. It may be that ^{249}Cf -transferrin is held more tenaciously by the resin or that ^{249}Cf also reacts with another very minor protein component whose molecular size is similar to transferrin and whose effective charge is only slightly greater. Further studies under carefully controlled conditions are needed to establish the presence of a Cf-transferrin complex in canine and human plasma.

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Table 1. Percent of Injected Dose Circulating in Blood at Times Post-Injection.

Time Post-Injection	$^{249}\text{Cf}^*$	$^{252}\text{Cf}^*$	$^{241}\text{Am}^*$	$^{239}\text{Pu}^{**}(1)$	$^{228}\text{Th}^{**}(2)$
5 min	49.18	-----	39.4	85.3	85.07
10 "	33.88	39.87	-----	79.1	81.87
30 "	20.71	23.2	15.80	70.2	70.89
1 hr	13.76	16.25	9.82	65.1	58.65
2 "	8.89	10.08	5.08	60.36	43.53
4 "	4.52	5.10	2.35	55.97	29.21
7 "	2.51	2.85	1.29	52.65	18.97
24 "	0.55	0.8	0.28	27.7	2.27
48 "	0.36	0.54	0.033	9.47	0.352

* Actual experimental measurements.

** Calculated from equations derived from experimental data.

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Fig. 1 The disappearance of ^{249}Cf and ^{252}Cf from the blood of beagles. Data on ^{239}Pu , ^{241}Am and ^{228}Th are included for comparison.

Fig. 2 Elution profiles of canine plasma proteins and ²⁴⁹Cf from Sephadex G-100-G-200 (upper) and from DEAE Sephadex ion exchange columns (lower) following I. V. injection.

Fig. 3 Elution profiles of human plasma proteins and ^{249}Cf from Sephadex G-100-G-200 (upper) and from DEAE Sephadex ion exchange resins (lower).

ON THE GENETICS OF CANINE BLOOD GROUPS

Stephen G. Jolley, Glenn N. Taylor, and Walter Angus

Abstract: The behavior of canine blood groups A and D was studied in a colony of 498 beagles. Blood groups A's distribution in four pedigrees deviated from that expected for an autosomal dominant factor. Blood group D appeared to be a typical autosomal dominant allele. No conclusions were drawn on the genetic segregation of blood groups A and D because of group A's unexpected behavior.

Human ABO analysis and a mathematical method devised by the authors were used to project the likelihood of various genetic mechanisms and models in 4 different canine populations. No Human ABO analogs were found in either of these canine groups. A probable genetic model for the basenjis and cocker spaniels was the location of canine blood groups A and D at a common position on the chromosome. For mongrels, the likely genetic model was variable.

INTRODUCTION

At the present, canine blood group genetics does not have all of the practical applications like those found with human blood groups. The primary concern is the potential incompatibility of blood group factors (primarily group A) between the bitch and her suckling offspring.⁽⁸⁾ Erythroblastosis fetalis can occur in these pups if the mother becomes sensitized and produces antibody against the offspring's red blood cells. Practicing veterinarians encounter this hemolytic disease in dogs that have received multiple transfusions of incompatible canine blood.^(9,10) The only requirement for preventing these mis-transfusions is the proper cross-matching of recipient and donor dogs.

The genetic transmission of the canine blood antigens A, B, C and D is thought by some investigators to occur by the autosomal dominant mechanism.^(2,4,6,7,8) Canine blood antigen pairs A and B; A and C; B and D;

and C and D have no interactions comparable to those of the human ABO or MN system. Pedigrees have not been available to determine the segregation of A and D or B and C. The human blood grouping system whose genetic mechanism most resembles the transmission of canine blood groups is the P system.

The purpose of the present study is to present the genetic behavior of canine blood factors A and D, and two mathematical methods for projecting the likelihood of various genetic mechanisms and models within the canine blood groups.

MATERIALS AND METHODS

Typing -- Four-hundred and ninety-eight purebred beagles from the University of Utah A.E.C. Colony were typed for canine blood groups A and D. The anti-A serum was prepared by our laboratory.⁽³⁾ Dr. R. W. Bull, Department of Medicine, Michigan State University, supplied us with the anti-D serum. Typing was accomplished by the tube hemagglutination technique.⁽¹⁾ Complement fixation and antiglobulin reactivity were also utilized in the detection of blood groups A.^(3,6) Pedigrees were available to us for the study of A and D segregation.

Reported data on the distribution of canine blood factors, A, B, C and D were used in the mathematical analyses. One study included 124 purebred basenjis and cocker spaniels from Bar Harbor, Maine.⁽²⁾ The data we used from another study had blood type distributions on a combined total of 783 mongrels in the Rochester, N.Y. area.^(1,6,7) In our analyses, each population was treated separately (Table 1). Both of them were used for comparison with populations that had been derived from

hypothetical mechanisms or models.

Mathematical Method 1. -- The form of mathematical analysis used in the human ABO system⁽⁵⁾ was applied to the canine blood groups. Six hypothetical systems (ABO, ACO, ADO, BCO, BDO and CDO) were constructed from 4 canine blood groups. The likelihood of each system was determined by comparing the observed and expected numbers of dogs possessing both of the system's detectable blood groups using the Chi-square (χ^2) test. Expected numbers were derived from:

f_X = observed incidence^a of blood type X

f'_X = expected incidence of blood type X

$$f'_{XY} = 2(\sqrt{f_0 + f_X} - \sqrt{f_0}) \times (\sqrt{f_0 + f_Y} - \sqrt{f_0})$$

Mathematical Method 11. -- This method was employed to project the probability of any 2 blood group determinants at a common genetic locus on the chromosome. Since we dealt with 4 blood factors, the smallest number of blood group loci in our hypothetical models was 2 (i.e., 2 determinants at each position), and the largest was 4 (i.e., no 2 factors at the same genetic locus).

Expected blood type distributions for each canine population were based on ten hypothetical models (Fig. 1). Expected incidences of the 16 blood phenotypes (A,B,C,D,O, AB,AC,AD,BC,BD,CD,ABC,ABD,ACD,BCD and ABCD) for each hypothetical model were obtained directly from the products of multiplication:

Sample Calculation Based on Model A (Autosomal)

$$f_a = \text{incidence of blood antigen A} = f_A + f_{AB} +$$

^aAll incidences used throughout this study were phenotypic

$$f_{AC} + f_{AD} + f_{ABC} + f_{ABD} + f_{ACD} + f_{ABCD}$$

$$f_{\text{non-a}} = 1 - f_a$$

$$f_b = f_B + f_{AB} + f_{BC} + f_{BD} + f_{ABC} + f_{ABD} + f_{BCD} + f_{ABCD}$$

$$f_{\text{non-b}} = 1 - f_b$$

$$f_c = f_C + f_{AC} + f_{BC} + f_{CD} + f_{ABC} + f_{ACD} + f_{BCD} + f_{ABCD}$$

$$f_{\text{non-c}} = 1 - f_c$$

$$f_d = f_D + f_{AD} + f_{BD} + f_{CD} + f_{ABD} + f_{ACD} + f_{BCD} + f_{ABCD}$$

$$f_{\text{non-d}} = 1 - f_d$$

Expected blood type incidences derived from the expansion of

$$(f_a + f_{\text{non-a}}) \times (f_b + f_{\text{non-b}}) \times (f_c + f_{\text{non-c}}) \times (f_d + f_{\text{non-d}}) = 1,$$

$$f'_A = f_a \times f_{\text{non-b}} \times f_{\text{non-c}} \times f_{\text{non-d}}$$

$$f'_B = f_{\text{non-a}} \times f_b \times f_{\text{non-c}} \times f_{\text{non-d}}$$

$$f'_C = f_{\text{non-a}} \times f_{\text{non-b}} \times f_c \times f_{\text{non-d}}$$

$$f'_D = f_{\text{non-a}} \times f_{\text{non-b}} \times f_{\text{non-c}} \times f_d$$

$$f'_O = f_{\text{non-a}} \times f_{\text{non-b}} \times f_{\text{non-c}} \times f_{\text{non-d}}$$

$$f'_{AB} = f_a \times f_b \times f_{\text{non-c}} \times f_{\text{non-d}}$$

$$f'_{AC} = f_a \times f_{\text{non-b}} \times f_c \times f_{\text{non-d}}$$

$$f'_{AD} = f_a \times f_{\text{non-b}} \times f_{\text{non-c}} \times f_d$$

$$f'_{BC} = f_{\text{non-a}} \times f_b \times f_c \times f_{\text{non-d}}$$

$$f'_{BD} = f_{\text{non-a}} \times f_b \times f_{\text{non-c}} \times f_d$$

$$f'_{CD} = f_{\text{non-a}} \times f_{\text{non-b}} \times f_c \times f_d$$

$$f'_{ABC} = f_a \times f_b \times f_c \times f_{\text{non-d}}$$

$$f'_{ABD} = f_a \times f_b \times f_{\text{non-c}} \times f_d$$

$$f'_{ACD} = f_a \times f_{\text{non-b}} \times f_c \times f_d$$

$$f'_{BCD} = f_{\text{non-a}} \times f_b \times f_c \times f_d$$

$$f'_{ABCD} = f_a \times f_b \times f_c \times f_d$$

Expected numbers of dogs ($f'_X \times \text{sample size}$) less than 2.5 were combined to give a single expected value. The expected and observed numbers of dogs were compared in a (d.f. + 1) - x-2 contingency table by χ^2 analysis. The degrees of freedom (d.f.) were given by:

$$\text{d.f.} = \begin{cases} S - 1 & n = 0 \\ S - n & n > 0 \end{cases} \quad \text{where } S = \text{number of possible}$$

blood types, and n = number of expected values < 2.5

Mathematical Method II was programed in Fortran V for use on a UNIVAC 1108 Computer.

RESULTS

We had no reason to believe that blood factor A was a typical autosomal dominant factor in our beagle colony. Four matings (Fig. 2 and Table 2) indicated that group A had recessive properties. Not every non-A x non-A cross yielded group A pups though. Litters from 6 of these matings were composed entirely of non-A pups. Several crosses revealed some dominant behavior associated with group A. The unusual distribution of blood group A implied that possibly 2 or more classes of group A determinants were present in the beagles.

Despite the occurrence of group A pups in non-A x non-A crosses,

no clinical signs of erythroblastosis fetalis were apparent in any of these pups (Table 2). However, these group A feti stimulated their mothers to produce anti-A antibody, with titers reaching 1:8 (Table 2).

Blood group D appeared to be a dominant factor, with no unusual pedigrees in its distribution. Both homozygous and heterozygous group D dogs were seen. When matings between group D and non-D dogs occurred, the litters always had at least 1 group D pup. The segregation of D from A appeared to be independent of the A factor (Fig. 3), since some AD x non-AD crosses resulted in progeny with both A and D.

Chi-square probabilities obtained by applying the human ABO analysis to canine blood groups projected that a human-ABO analog in dogs was unlikely. The f'_{XY} for each of 5 hypothetical systems (ACO, ADO, BCO, BDO, and BDO) in the basenjis and cocker spaniels was zero whereas the f'_{XY} was non-zero. The hypothetical canine ABO system for this population was significant below the 0.002 probability level. In the mongrels, all 6 systems were significant at ≤ 0.000 probability level.

However, there was a difference between the blood groups chromosomal positioning of purebreds and mongrels, as projected by Mathematical Method II (Table 3). In cocker spaniels and basenjis, the positioning of A and D at a common point on the chromosome seemed the most likely. The likelihood of B and D at a single genetic locus was also within an acceptable range. All other models were unlikely. For mongrels, the location of B and D at a common locus on the chromosome was the most probable. The other models were also likely for this population though. We found it difficult to discern between models with low p values ($p < 0.300$) because of variations within both populations (i.e., neither

population was ideal).

DISCUSSION

We were unable to draw conclusions on the allelic association of blood antigens A and D. This was primarily due to the unusual behavior of group A in our beagles. We considered blood group chimerism as a means of explaining the genetic properties of group A, but rejected it because of its uncommon occurrence in dogs.⁽³⁾ If a non-serologically-detectable factor was dominant over group A in crosses, I, II, V and VI (Table 2) it could have also been dominant over group D; providing that A and D were at a common chromosomal position. Hence, our pedigrees that showed the independent segregation of A and D may have resulted from the possession of this non-detectable, dominant factor by the non-AD parents.

The mating of dog no. 879 and dog no. 762 with non-A females other than dog nos. 890, 858, 893, and 894 introduced another peculiarity of group A (Table 2). The non-A males were unable to produce offspring with group A in these crosses. Only dogs nos. 890, 858, 893, and 894 possessed what appeared to be a recessive A trait. The absence of high anti-A titers in these females, and erythroblastosis fetalis in their pups, lead us to believe that the recessive A factor prevented them from becoming highly sensitized to group A red cells.

Some of the likelihoods projected in our mathematical analyses paralleled pedigree data reported in other studies. Kamel and Ezzat⁽⁴⁾ found that A and B segregated independently of each other. Cohen and Fuller⁽²⁾ discovered that B and D, in addition to A and B, were not alleles

of a common locus on the purebred chromosome. Antigens A and C have also been shown to be independently segregated.⁽⁷⁾

Differences between the purebred and mongrel populations shown by Mathematical Method II may have resulted from dissimilar positioning of their blood antigen determinants on the chromosome pair. In other words, if the chromosomal arrangement of blood antigen determinants in the purebreds were fixed, the same arrangement in the mongrels could have been variable. We theorized that model D or J (Fig. 1) actually existed in purebred basenjis and cocker spaniels; since there was little or no evidence to disprove either.⁽²⁾ The variability of blood group positions on a mongrel's chromosome was also compatible with blood type distributions in reported pedigrees. All of this inferred that purebred strains differed as to the positioning of their blood group determinants on the chromosome.

This study introduced some unique concepts into canine blood group genetics. Group A appeared to be a recessive trait on several occasions. Only non-A female beagles carried the recessive A factor. The likely chromosomal positioning of canine blood group determinants in purebreds and mongrels deviated from that expected for the autosomal genetic theory. All of these indicate that the genetics of canine blood groups is unsettled.

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TABLE 1 -- The Distribution of Blood Types in 2 Reported
Populations of Dogs.

	124 Purebred Basenjis and Cocker Spaniels from Maine ⁽²⁾	783 Mongrels from New York ^(1,6,7)
Blood Type	Incidence	Incidence
A	0.0000	0.0157
B	0.0000	0.0000
C	0.1530	0.2640
D	0.0000	0.0024
O	0.0000	0.0038
AB	0.0000	0.0013
AC	0.4660	0.4458
AD	0.0000	0.0024
BC	0.0320	0.0114
BD	0.0000	0.0000
CD	0.0000	0.0831
ABC	0.0400	0.0280
ABD	0.0000	0.0028
ACD	0.2500	0.1163
BCD	0.0000	0.0026
ABCD	0.0560	0.0204

TABLE 2 -- Blood Type Data Resulting from Nine Non-A x Non-A Crosses within the University of Utah - AEC Beagle Colony.

Cross	Sire's No.	Dam's No.	No. of Litters	Number of Off-spring having Given Blood Type		Anti-A Antibody Present in Dam's Serum (Titer)	Hemolytic Disease in Pups
				A	Non-A		
I	762	858	1	1	1	No	No
II	"	890	1	1	3	Yes(1:2)	No
III	"	869	1	0	2	No	No
IV	"	1012	1	0	2	No	No
V	879	893	6	16	12	Yes(1:4)	No
VI	"	894	3	10	0	Yes(1:8)	No
VII	"	704	1	0	4	No	No
VIII	"	760	2	0	5	No	No
IX	"	865	1	0	3	No	No

TABLE 3 -- The Projection of Various Genetic Models Concerned with the Positioning of Canine Blood Group Determinants on the Chromosome Pair.

Model	124 Basenjis and Cocker Spaniels			783 Mongrels		
	d.f.	χ^2	p-level	d.f.	χ^2	p-level
A	6	13.98	0.029	11	19.50	0.055
B	7	16.63	0.020	10	12.42	0.258
C	6	14.08	0.028	11	19.12	0.061
D	6	3.51	0.743	11	19.32	0.058
E	6	13.98	0.029	11	19.10	0.062
F	6	11.33	0.078	11	10.81	0.459
G	6	13.94	0.030	11	19.54	0.053
H	7	16.59	0.021	10	12.55	0.250
I	6	11.43	0.076	11	10.35	0.498
J	6	3.50	0.744	11	19.01	0.063

Figure 1. Ten hypothetical models used to depict the relative positions of canine blood group determinants A, B, C, and D on the chromosome pair for Mathematical Method II.

Figure 2. The distribution of blood groups A and D in four beagle pedigrees. Group A exhibited both dominant (A) and recessive (C and D) genetic properties. The distribution of group D appeared to be that of autosomal dominant factor (A,B,C, and D). Selection of a particular blood type within litters also occurred (B and C).

Figure 3. A beagle pedigree showing what appeared to be the independent segregation of blood group D from blood group A.

A NOTE ON THE HALF-PERIOD OF Ba-133

Ray D. Lloyd and Charles W. Mays

ABSTRACT: The Ba-133 in a sealed mock I-131 source was found to have decayed with a half-period of about 11 years over a 4 1/2 year span of time, based on a comparison with the decay of the Cs-137 which it also contained. This observation tends to confirm the values for the Ba-133 half-period of about 10 to 11 years reported by several authors but disagrees with values of about 7 to 8 years reported by other investigators. An intensive search of the literature revealed a phantom reference for the previously "accepted" 7.2 year value of the Ba-133 half-period. From recent work of other investigators, we regard 10.5 ± 0.5 years as our best estimate of the true half-period of Ba-133.

INTRODUCTION

Since the existence and radioactive properties of Ba-133 were first published by Katcoff⁽¹⁾ in 1947, various estimates of its half-period have been reported.⁽²⁻⁹⁾ These values tended to fall into 2 groups, one of about 7 to 8 years,^(3,6,8,9) and the other of about 10 to 11 years.^(2,4,5,7) Recent measurements made in our laboratory seem to corroborate the longer half-period, and a search of the literature has revealed some interesting information about the historical evolution of "accepted" values, including the discovery of a phantom reference.

METHODS

Our Ba-133 sample was a sealed rod 1.2 cm in diameter and 6.2 cm long containing a combination of Ba-133 and Cs-137 in a resin filler, shielded by 1 mm of Babbitt metal and 1 mm of rigid polyvinyl chloride (Trovidur). It was prepared in 1963 by the International Atomic Energy Agency and was labelled "Mock I-131 (Ba-133 + Cs-137) 0.05 μ Ci July 1963 I.A.E.A. #64." Its photon spectrum had been recorded on 5 August 1967 and on 21 September 1967 inside of a cylindrical lucite neck phantom 12.2 cm in diameter and 12.6 cm long. The phantom contained a tunnel parallel to the axis of the

cylinder but centered 2 cm from its front surface into which the mock I-131 source could be placed.

On 12 January 1972 and again on 11 February 1972, the neck phantom containing the mock I-131 source was positioned in our human total-body counter with its front surface 33.5 cm directly below the face of the nearest of the two 8 x 4 inch Na I(Tl) crystals,⁽¹⁰⁾ exactly as was done in 1967. The spectrum was again recorded on each of these days, as were the spectra of a Cs-137 standard and a Ba-133 standard. Each of the recorded spectra, corrected for background, was then integrated over two pulse-height bands, one corresponding to the Ba-133 photon cluster at about 0.36 MeV (0.25-0.4 MeV) and the other corresponding to the 0.66 MeV gamma-ray associated with the decay of Cs-137 (0.62-0.70 MeV). Counting times were sufficiently long that expected statistical uncertainties were reduced to $\pm 1\%$ or less.

After adjusting the various mock I-131 spectra for the contribution of the Cs-137 to the counting rate in the Ba-133 band, the net corrected integral counting rates of the Ba-133 and Cs-137 in 1972 were compared with their 1967 counterparts. In none of the mock I-131 spectra which were recorded was there any evidence of photon emitters other than Ba-133 and Cs-137.

RESULTS AND DISCUSSION

It was found that the observed change in the Cs-137 counting rate of the mock I-131 source during the intervening 4 1/2 years could be exactly accounted for by the radioactive decay of Cs-137 ($T_{1/2} = 30$ years.⁽¹¹⁾) This we interpreted as evidence that the experimental conditions in 1972 accurately duplicated those existing in 1967. The diminution of the corrected

Ba-133 counting rate corresponded to a half-period of 11.3 ± 0.7 years, assuming that radioactive decay was the only effect contributing to the observed change. Our value tends to support those reported half-periods for Ba-133 of about 10 to 11 years, but differs decidedly (> 4 S.D.) from those of about 7 to 8 years.

The accepted half-period for Ba-133 has been the 7.2 year value since about 1958.^(8,9) An intensive search of the literature on this subject,^(1-9,12-16) however, failed to locate the published source of this accepted half-period. The compilation of reference 8 (1958) lists 3 references for the single value given of 7.2 years: our reference 2 ($T_{1/2} = 9.5 \pm 2$ years, unpublished), our reference 16 (which contains no mention whatsoever of Ba-133) and "S. Katcoff-private communication from G. Friedlander, Nov. 1957." The 1967 compilation⁽⁹⁾ preserves the 7.2 year half-period as the preferred value but omits the 2 previous unpublished references, leaving only our reference 16 as substantiation, which contains nothing at all about Ba-133. Also listed as second choice is the 10.7 year value as given in our reference 4.

Katcoff's 1952 value of 9.5 ± 2 years was obtained from the serial measurement of two Ba-133 samples over a 6 year period using a series of end-window Geiger counters.^(2,17) Counting rates were compared to a uranium standard. In 1954, he followed the decay of still another Ba-133 source over a period of only about 200 days using a balanced ionization chamber, comparing the photon emission rate of the Ba-133 to that of a Ra-226 standard. Although the individual measurements in the second experiment clustered more tightly around a 7.2 year decay line than the measurements in the first experiment clustered around their best-fit decay lines ranging between

half-periods of about 8 and 12 years, he was " ... never completely happy with ... " the second experiment and its resulting 7.2 year half-period, principally because of continuing instrument difficulties.⁽¹⁷⁾ Consequently, he never reported the 7.2 year value, although it was informally communicated to the authors of reference 8 by an associate.⁽¹⁷⁾

A probable half-period for Ba-133 appears to occur in the neighborhood of 10 to 11 years.^(2,4,5,7) Based on more recent data of his laboratory which yielded a value of 10.66 ± 0.12 years,⁽⁵⁾ the author of reference 4, cited in reference 9 as the source of the 10.7 ± 0.2 year half-period, has more confidence than ever that his 1961 value was accurate.⁽¹⁸⁾ Estimates of or attributed directly or indirectly to Katcoff^(2,12,13,14,15) as well as reported values of Wyatt and his associates^(4,5,18) seem to be confirmed by preliminary measurements made in our laboratory. From recent work of other investigators,^(5,7) we regard 10.5 ± 0.5 years as our best estimate of the true half-period.

ACKNOWLEDGEMENTS

Our interest in the Ba-133 half-period was stimulated by an inquiry of John H. Marshall (Argonne National Laboratory). We are grateful to Seymour Katcoff (Brookhaven National Laboratory) for details of his early experiments and for confirming our suspicions that a published reference to the 7.2 year value did not exist. Communications from E. I. Wyatt (Oak Ridge National Laboratory), C. M. Lederer (Lawrence Radiation Laboratory) and Charles Rohrman (Battelle Northwest Laboratory) were also most valuable.

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RUBIDIUM-83 HALF-PERIOD DETERMINATION

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ABSTRACT: Serial measurement of the gamma-ray emission rate from 2 Rb-83 sources over periods of 312 and 366 days yielded calculated half-periods of 85.0 ± 2.1 and 86.3 ± 1.8 days, respectively. These values are in substantial agreement with, but are not as mnemonically appealing as, the accepted half-period for Rb-83 of 83 days.

INTRODUCTION

Several shipments of Rb-83 solution were received by our laboratory from the Isotopes Development Center, Oak Ridge National Laboratory, from 1966 through 1970. This material had been produced by proton bombardment of isotopically enriched Sr-84 and the subsequent chemical separation of Rb. Some of the Rb-83 was used in animals and in human tracer studies.^(1,2) All stocks were investigated for their radiochemical purity. No alpha emitters could be found in any Rb-83 which we received. Gamma-ray analysis revealed traces of contamination by Na-22, Y-88, or Co-56 in some batches. In others, however, we could detect photon radiation only from Rb-83, principally the primary cluster of photon energies at about 0.53 MeV (0.52 - 0.55 MeV) and a secondary photon energy of about 0.79 MeV. The intensity at 0.79 MeV appeared to diminish with time at the same rate as the intensity at about 0.53 MeV. Rb-83 from two of the uncontaminated batches was administered to a number of human volunteers, who also were given Cs-137, and their total-body retention was measured periodically for about one year thereafter (see Fig. 1. of Ref. 2). The photon spectra of a duplicate of the administered Rb-83 sealed in a 10 ml ampoule and a duplicate of the administered Cs-137 similarly sealed were recorded on each day human

measurements had been made. Therefore, the radioactive half-period of Rb-83 could be determined readily from these serial measurements of the Rb-83 sources.

METHODS

Every day the retention of Rb-83 and Cs-137 was determined in our human subjects, the photon spectra of the Rb-83 ampoule, and the Cs-137 ampoule were measured in our human phantom⁽³⁾ so that geometry, self-absorption, scattering, etc. remained constant throughout the experiment. The integral counting rate of the Rb-83 ampoule from 430 to 600 keV and the integral counting rate of the Cs-137 ampoule from 560 to 730 keV were determined for each day that the two sources were both counted. The integral counting rates of the Cs-137 source were corrected for radioactive decay back to the first day of the study, using the 30 year half-period of Cs-137.⁽⁴⁾ A ratio was then calculated of the integral counting rate of the Rb-83 sample to the corrected integral counting rate of the Cs-137 sample. Thus, the resulting array of ratios represented the decrease of Rb-83 activity as a function of time, adjusted for geometry and self absorption, referred to a "constant activity" Cs-137 standard. Least squares fits were then calculated for the array of ratios generated for each of the two Rb-83 sources.

RESULTS AND DISCUSSION

Figures 1 and 2 display the lines of best fit for each of the Rb-83 sources and the data points from which they were calculated. The half-period of the source measured over 312 days from 29 December 1969 to 6 November 1970 was 85.0 ± 2.1 days, and the half-period of the source measured

over 366 days from 9 August 1970 to 10 August 1971 was 86.3 ± 1.8 days. These values are in excellent agreement, and this indicates the degree of radioactive purity of the two different batches of Rb-83, produced almost 9 months apart.

If there had been a short-lived contaminant in the Rb-83, the calculated half-period would have been erroneously short; conversely, if there had been a long-lived contaminant in the Rb-83, the calculated half-period would have been erroneously long. The effects of a short-lived contaminant would have been most pronounced at early times and the effects of a long-lived contaminant would have been most pronounced much later on. As a test for both long- and short-lived contaminants, the first and second halves of the observed decay curves (Figures 1 and 2) were analysed separately. The resulting half-periods calculated for the first and last halves of each of the 2 decay lines were not significantly different from the respective half-periods calculated for the entire line. Therefore, this analysis did not reveal any contamination that seriously affected the results.

The 2 Rb-83 sources were recounted 828 and 605 days after the beginning of the respective experiments at which time the Rb-83 activities had decayed to less than 0.002 and less than 0.008 of their initial values. No evidence of any long-lived contamination could be seen in the photon spectrum of either source. Moreover, the relative counting-rate ratios of (Rb-83/corrected Cs-137) closely matched those predicted by the decay constants derived from the half-period determinations.

The uncertainties associated with our estimates of the Rb-83 half-period are the root-mean-square errors of the two major sources of uncertainty in the determination, the errors in the slope of the least-squares lines

(Figures 1 and 2) and the reported uncertainty⁽⁵⁾ in the accepted half-period of Cs-137. The combined half-period and standard deviation of 85.7 ± 1.3 days derived from the two Rb-83 sources in our studies are not in serious disagreement with the accepted value of 83 days.⁽⁶⁾

ACKNOWLEDGEMENTS

The Rb-83 used in these studies was produced, processed, and furnished by the Isotopes Development Center, Oak Ridge National Laboratory. The valuable cooperation of their staff, particularly Homer B. Hupf and Larry C. Brown, is acknowledged with thanks.

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Fig. 1. Decay of a Rb-83 source, 29 December 1969 to 6 November 1970.

Fig. 2. Decay of a record Rb-83 source, 9 August 1970 to 10 August 1971.

BIOCHEMICAL CHANGES PRODUCED BY LOW DOSE OF RADIATION

Charles J. Nabors, Jr.*

ABSTRACT: Long term studies on low dose level nuclide bearing animals are reported. Beagles bearing ^{239}Pu burdens show the most marked alterations in serum alkaline phosphatase and serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminases. Low dose level animals appear to have a much longer latent period prior to the appearance of altered serum biochemistry than do animals with higher dose levels.

INTRODUCTION

Prior to this time, insufficient data have been available to demonstrate that the effect of the lower doses of radionuclides administered to beagles in our project can produce biochemical changes over longer periods after injection. The length of exposure time to radionuclide appears to be the controlling factor in determining the onset of biochemical changes.⁽¹⁾ Statistical analysis of the data gathered over a twenty year period in this laboratory shows that radionuclide burden time is the controlling factor in the respect to biochemical alterations, without regard to age.⁽²⁾ At higher doses of radiation, pathology is both more immediate and in some instances of a different nature. The present study deals with biochemical changes in animals bearing low level radionuclide burdens (i.e. < 1.0). Previous studies have shown that serum alkaline phosphatase and serum transaminase measurements are the most sensitive indicators of radiation-induced damage currently available in the laboratory.⁽²⁾ Elevated serum glutamic pyruvic transaminase is indicative of hepatocellular necrosis.^(3,4) A previous report from this laboratory gave data on earlier measurements, primarily in higher dose level animals (greater than $0.02 \mu\text{Ci/kg}$ for ^{239}Pu). Alkaline phosphatase levels in serum are indicators of osteoblastic

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activity and/or hepatocellular disfunction.^(4,5) Studies in our laboratory have shown that serum alkaline phosphatase is elevated prior to radiologically detectable osteosarcoma.^(2,4) The purpose of this report is to describe the first observable alterations in these serum enzymes in animals receiving less than 0.002 - 0.005 $\mu\text{Ci/kg}$ ⁽²⁾ for at least 50 months after injection.

METHODS

The dogs were purebred beagles born and reared in our laboratory. The animals were injected in young adulthood. Twelve or more dogs were injected at each dose level in our regular program. Five or more animals were injected as ^{241}Am test animals in a special study. Details of the injected dose and number of animals are given in the injection table section in this progress report. Each animal received a single intravenous injection of the soluble nuclide in sodium citrate-citric acid buffer, pH 3.5. Blood samples were taken for biochemical analysis at 1 to 3 month intervals prior to and after injection. 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.

RESULTS

Table 1 contains the data on long term ^{239}Pu animals which currently exhibit elevated serum transaminase or serum alkaline phosphatase measurements. The criterion for an elevated measurement is that it exceeds the 95% confidence level established for these serum enzymes in control animals. These limits are 52.0 units for serum glutamic pyruvic transaminase; 38.0 units for serum glutamic oxaloacetic transaminase; and 8.3 units for serum alkaline phosphatase.

Only one control plutonium dog has exhibited an SGPT value equal to the 95% confidence limit. In contrast, eleven dogs bearing ^{239}Pu burdens have exhibited elevated transaminase or phosphatase measurements. Seven of these animals have elevated transaminase measurements, two had elevated alkaline phosphatase levels, and in two other dogs, both types of serum enzymes were increased. There does not as yet appear to be a strict correlation between dose level and the onset of altered enzyme level although the two dogs with the shortest burden times are in the 1.0 and 0.7 level groups, the highest doses compared here. Only one of these dogs (M34P0.5B) was sacrificed as part of a special study and histology findings are not yet available. Therefore, it will be possible to follow changes in serum enzymes in the remainder of these animals in the future.

Table II contains the results to date for all other radionuclide groups. One low level ^{241}Am injected dog has evidenced an increased serum transaminase level. The SGPT rise that occurred in this animal 42 months post-injection as compared with a 57 month average latent period for a similar increase in one-level plutonium animals and a 63.8 month average latent period for all ^{239}Pu animals included in this report (Table I). Some elevated measurements appear in low level animals injected with ^{90}Sr , ^{226}Ra and ^{228}Ra . These animals are of advanced age and control dogs in these groups also have elevated serum enzyme levels. Two animals injected with ^{228}Th also exhibit elevated serum enzymes.

DISCUSSION

The data reported here indicate that the increases in serum enzymes previously reported in dogs with high levels of radiation appear in lower

level animals. It appears that a much longer latent period prior to the onset of these changes will be necessary. Of the radionuclides studied to date, ^{239}Pu appears to be the most toxic at low dose levels. This finding is consistent with an earlier report from our laboratory.⁽⁴⁾ Elevated SGPT and alkaline phosphatase measurements were found in 1.0 dose level animals in a previously injected group.⁽⁴⁾ Of these fourteen earlier animals, 9 died of carcinoma, four with osteosarcoma, and two with hepatic pathology. One animal, M13P1.0, is still surviving. The other dose levels of ^{239}Pu reported here have elapsed burden times more similar to the second group of 1.0 level ^{239}Pu dogs. However, ^{241}Am , a nuclide more recently studied in our laboratory, may produce changes with a shorter latent time. This is the case with high doses of ^{241}Am (3.0 - 5.0 dose levels).⁽¹⁾ The other radionuclides reported are judged to be less toxic than plutonium for two reasons: a longer latent period before the appearance of biochemical changes; and the simultaneous occurrence of similar changes in zero level animals.

There is another interesting aspect to the study in plutonium animals. Seven of the animals exhibited elevated transaminase levels only. SGPT has been shown to be specific for hepato-cellular necrosis in beagles.⁽³⁾ Therefore, these data demonstrate that the site of the biochemical lesion is in the liver. Two of the ^{239}Pu injected animals have elevated serum alkaline phosphatase levels only. Alkaline phosphatase can indicate osteoblastic activity and/or liver damage.^(4,5) Since transaminase levels are not elevated in these dogs, the data suggest the absence of hepatic lesions and perhaps the presence of early bone tumors. These animals probably have abnormal osteogenic activity. Overall, it appears that low level

plutonium doses produce liver damage as their main consequence at long times post-injection.

ACKNOWLEDGEMENT

I wish to thank Ms. Ann Bailly for her excellent assistance.

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Table I
Abnormal Test Results in Beagles injected with ^{239}Pu at
the 1.0 Dose Level and Below

Dog Number	Months Post Injection	SGPT units	SGOT units	Alkaline Phosphatase units
F20P0	60	52.0		
M15P0.1	85	140.5	61.5	28.27
F34P0.1B	60	56.0	83.0	
M15P0.2	84	79.0		
F20P0.2	60	999.9*	65.0	32.31
F33P0.2D	72			9.05
F34P0.2D	60	62.0		
M15P0.5	84	55.0		
M34P0.5B	59	122.0		
F20P0.7	24			12.40
M13P1	90	62.0		
F18P1	24	200.0		

*This enzyme measurement was above the range of sensitivity of the test.

Table II

Abnormal Test Results in Beagles injected with Radionuclides
at the 1.0 dose level and below.

Dog Number	Months Post Injection	SGPT units	SGOT units	Alkaline Phosphatase units
M22W1	42	69.0		
F4S0	168	54.0		8.26
F8S0	146			29.47
F8S0	147	72.0		35.23
M10S0	143			8.38
F11S1	144			10.70
M10S1	143	99.5		
F31ROC	84	195.0		10.02
F32R0.5C	72	64.0		
M4M0	108	685.0	69.5	35.53
F11M1	108	74.5		
M12M1			12.42	
F12T0.2	96	112.5		11.29
M7T0.2	127		51.0	

PLASMA CORTISOL AND CORTICOSTERONE LEVELS IN BEAGLES. I. PRELIMINARY
VALUES FOR CONTROL AND RADIONUCLIDE BEARING ANIMALS

Charles J. Nabors, Jr.*

ABSTRACT: Beagle plasma cortisol was measured by a competitive protein binding technique and plasma corticosterone measured by radioimmunoassay. A group of nineteen control and nine radionuclide bearing dogs studied showed higher steroid levels in female beagles than in males. It also appears that both cortisol and corticosterone blood levels are diminished in radionuclide bearing dogs.

Radiation has long been known to elicit a stress response with respect to corticosteroid levels in blood,^(1,2) and blood lymphocytes, and eosinophils.⁽¹⁾ Blood steroid levels respond with an initial increase and subsequent decrease. Previous studies from this laboratory and others have shown that radiation produces decreases in hydroxylation of the steroid molecule.^(3,4) This alteration in steroid biosynthesis can be reversed in vitro by addition of coenzymes to incubations.^(3,5) Newer methods for measurement of small quantities of corticosteroids in peripheral blood are now available in our laboratory. A study on the effects of long term radionuclide burdens on blood steroid levels has been initiated to determine what physiological effects occur. This is the first report on peripheral blood steroids in our colony.

METHODS

Peripheral blood samples were taken from control and radionuclide bearing dogs in our colony. Serum for corticosteroid determinations was frozen and stored for analysis. Only female dogs in diestrus were selected in an attempt

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to determine base line values. Cortisol was measured by the competitive protein binding method of Murphy.⁽⁶⁾ Corticosterone was measured by a radioimmunoassay developed in collaboration with Dr. C. D. West of the Clinical Research Center, University of Utah.⁽⁷⁾ Three ml of serum is extracted with benzene and chromatographed in the Bush B₃ system. The corticosterone area of the chromatogram is eluted with methanol, concentrated by evaporation and assayed.⁽⁶⁾ In this system, no other steroid of similar chromatographic mobility cross-reacts more than 3% with the antibody. The amount of corticosterone bound is determined by measuring its displacement of known radioactive ligand.^(6,7)

RESULTS

The results obtained for male animals to date are given in Table I. The average amount of cortisol in control animals serum is 2300 nanograms (ng) per hundred ml (8:30 a.m.). The average corticosterone value (8:30 a.m.) is 158 nanograms percent. The average cortisol: corticosterone ratio is 15.5 in control beagles. In contrast there appears to be a decreased cortisol: corticosterone ratio in the radionuclide bearing animals measured thus far. This decrease is largely at the expense of cortisol. The one exception observed is T67P4P, a dog bearing polymeric ²³⁹Pu. Also, corticosterone levels appear to be somewhat more variable in radionuclide injected animals which exhibit a range extending from 116 to 390 ng%.

Cortisol and corticosterone levels measured in female animals are presented in Table II. The levels of both steroids appear to be higher, with corticosterone being the most elevated. The average control plasma cortisol level is 3450 ng percent (8:30 a.m.) and the average corticosterone level is 361 ng percent. Both of these values are higher than those

observed in males, and the increase in corticosterone is greatest. The average cortisol: corticosterone ratio is 10.4, lower than the ratio in males. This is probably due to the disproportionate increase in corticosterone.

The data for these same steroids in the group of female radionuclide bearing animals measured, thus far, suggest absolute decreases for both cortisol and corticosterone. However, two of the three cortisol: corticosterone ratios are higher (F18W3 and F44W4). This is apparently due to the fact that the decrease in the amount of corticosterone in blood is greater than the decrease in cortisol.

DISCUSSION

The data obtained for corticosteroids in peripheral blood of beagles suggests that this may be a significant parameter of radiation-induced damage. Altered levels of both cortisol and corticosterone have been observed in both male and female animals. Previous results, indicating that there is direct damage to the adrenal in radionuclide bearing dogs now have new significance. In these earlier studies, hydroxylations of the steroid molecule in the process of both corticosteroid and androgen formation were shown to be inhibited.^(3,8,9) The evidence available for decreased in vivo levels in a small group of injected dogs suggests a similar depression in long term animals.

The differences in corticosteroid levels in peripheral blood of male and female animals are similar to those observed in humans. Higher levels of corticosteroid binding globulin in females may be the cause of this discrepancy. More animals and additional measurements will be needed. The reproducibility of replicate measurements in the same animal gives further

validity to these findings. It is possible that corticosteroid levels in peripheral blood will provide a sensitive measure of radionuclide induced damage not detectable by other clinical means.

ACKNOWLEDGEMENTS

The author wishes to thank Ms. R. Imelda Zizumbo, Ms. Virginia Schmidt, and Mr. Kenneth Fowkes for their assistance.

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TABLE 1. PERIPHERAL BLOOD CORTICOSTEROID LEVELS IN MALE BEAGLE DOGS

Dog Number	Cortisol	Corticosterone	Cortisol: Corticosterone
	 nanograms per 100 ml		
M15P0	2000	90	22.2
M16P0	4500	335	13.4
M17P0	2700	241	11.2
M19P0*	2200	148	14.8
M19P0*	1800	146	12.3
M21P0C	2800	206	13.6
M31P0C	2500	173	14.5
M36P0C	2500	114	21.9
M38P0B	1200	101	11.9
M40P0B*	1400	67	20.9
M40P0B*	1700	118	14.4
.....			
mean (n = 11)	2300	158	15.5
T30P3	2000	281	7.1
T66P4P	1900	116	16.4
T67P4P	4300	390	11.6
M36W3	1700	174	9.7
M100W3	2000	153	13.1
M34W4	1100	138	7.9

* These are replicate measurements made at least four months apart.

TABLE II. PERIPHERAL BLOOD CORTICOSTEROID LEVELS IN FEMALE BEAGLE DOGS

Dog Number	Cortisol nanograms per 100 ml	Corticosterone	Cortisol: Corticosterone
F13P0*	2900	263	11.0
F13P0*	3300	271	12.1
F14P0*	5300	439	12.1
F14P0*	4600	402	11.4
F22P0*	4100	349	11.7
F22P0*	4100	440	9.3
F18P0	3300	497	6.6
F31R0C	2100	233	9.0
.....			
mean (n = 8)	3450	361	10.4
T63P5	1500	261	5.7
F18W3	2600	109	23.9
F55W4	1500	66	22.7

* These are replicate measurements made at least four months apart.

ADDITIONS TO BIBLIOGRAPHY

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1. Radiobiology of Plutonium; edited by Betsy J. Stover and W. S. S. Jee; J. W. Press, Salt Lake City; 552 pages (1972).

Recognizing the increasing use of plutonium and the importance of protecting people from overexposure, our laboratory, in commemoration of its 20 years of plutonium research, has recently published the 552-page book indicated above. The book's 32 chapters, written by world-recognized authorities throughout in the U.S.A. and abroad, provide an extensive summary on the biological effects of plutonium and other radionuclides when deposited in tissue.

Research includes (a) the transport of plutonium from its entry into the body to its deposition in tissue, (b) the resulting radiation dose patterns, (c) the induction of various types of cancers, and (d) methods for removal of plutonium from the body. The objective of this research is to predict the potential risks to man, and how they can be minimized, so that society may safely enjoy the benefits of the nuclear age.

The book is available at \$17.50 per copy from the J. W. Press, Anatomy Department, University of Utah, Salt Lake City, Utah 84112.