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Research in Radiobiology

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

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

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

MARCH 31, 1973

CONTRACT No. AT (11-1)-119

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RESEARCH IN RADIOBIOLOGY

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

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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 National Technical Information Service, Springfield, Virginia. Paper copies as indicated; microfiche copies \$0.95 each.

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

*Also available on request from this laboratory.

AVAILABILITY OF PREVIOUS REPORTS (Con't.)

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C00-119-230*	Jul 1964	(Superseded by C00-119-245)	4.00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

*Also available on request from this laboratory.

CURRENT CENSUS OF THE BEAGLE COLONY

March 31, 1973

NUCLIDE	NUMBER OF DOGS
^{252}Cf (californium)	36
^{249}Cf (californium)	24
$^{243/244}\text{Cm}$ (curium)	2
^{241}Am (americium)	82
^{239}Pu (plutonium)	168
^{228}Th (radiothorium)	21
^{228}Ra (mesothorium)	14
^{226}Ra (radium)	76
^{224}Ra (quickradium)	10
^{210}Pb (lead)	1
^{90}Sr (strontium)	16
X-ray	2
Ancillary (breeding and others)	36
Unassigned dogs	<u>120</u>
TOTAL	<u><u>608</u></u>

PREFACE TO THE INJECTION TABLES

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

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

The five injection levels designated by integers are those specified at the early meetings of the consultants, and those designated by nonintegers have been added by the laboratory staff. Since those injection levels were originally specified in "retained" activities, the actual injections are four times the desired "retained" $\mu\text{Ci/kg}$ of ^{226}Ra , ^{228}Ra (Mesothorium), and ^{90}Sr , and 1.11 times the desired "retained" $\mu\text{Ci/kg}$ of ^{239}Pu , ^{228}Th (Radiothorium), and ^{241}Am .^{*} 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
av. $^{243}/^{244}\text{Cm}$ 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 = $10 \times \frac{0.1 \text{ } \mu\text{Ci } ^{226}\text{Ra}}{70 \text{ kg man}} = 0.0143$ "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 , C = $^{243/244}\text{Cm}$, and A = Ancillary.

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

DOSIMETRY

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

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

(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)

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

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

(pure at $t = 0$)

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

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

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

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

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

(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, weighs 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 I. TOXICITY ANIMALS (MAR. 31 1973)

A. RADIUM-226

DOG NUMBER	AT INJECTION		INJECTED (MC /KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	3314	
F014R0.0	536	10.80		23	10	64	3081	
M015R0.0	564	12.80		4	2	65	2977	
F016R0.0	469	10.00		7	4	65	2915	
M017R0.0	469	12.50		27	4	66	2530	
F018R0.0	497	12.00		25	5	66	2502	
F019R0.0	533	8.42		13	10	66	2361	
M020R0.0	536	9.70		29	12	66	2284	
F021R0.0	549	9.90		26	1	67	2256	
M022R0.0	533	12.10		22	3	67	2201	
F031R0.0B	536	10.60		23	10	64	3081	
F031R0.0C	536	9.88		23	10	64	3081	
F031R0.0D	542	9.90		21	9	65	2748	
F032R0.0B	542	7.80		21	9	65	2748	
F032R0.0C	532	11.70		21	9	65	2748	
F032R0.0D	532	9.70		21	9	65	2748	
F033R0.0B	532	9.80		21	9	65	2748	
F033R0.0C	496	9.50		25	5	66	2502	
F033R0.0D	496	11.80		25	5	66	2502	
F034R0.0B	525	8.20		26	1	67	2256	
F034R0.0C	520	8.90		22	3	67	2201	
F034R0.0D	484	9.90		22	3	67	2201	
F035R0.0B	502	9.41		1	2	68	1885	
F035R0.0C	502	9.38		1	2	68	1885	
F035R0.0D	552	8.86		9	1	69	1542	
F036R0.0B	467	10.10		2	7	68	1733	
F036R0.0C	467	9.17		2	7	68	1733	
F036R0.0D	467	9.08		2	7	68	1733	
F037R0.0B	801	11.10		20	5	69	1411	
F037R0.0C	501	10.40		23	9	70	920	
F037R0.0D	501	10.90		23	9	70	920	

DOG
NUMBER

COMMENTS ON DEAD DOGS

MU01R0.0	SEMINOMA, LYMPHOSARCOMA
MU02R0.0	TRANSITIONAL CELL CARCINOMA
F003R0.0	STATUS EPILEPTICUS
MU04R0.0	CHRONIC INTERSTITIAL NEPHRITIS; THROMBOSIS
MU05R0.0	OBTURATING PULMONARY EMBOLISM
F006R0.0	STATUS EPILEPTICUS
MU07R0.0	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	
MU22R0.0	
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 AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
F038R0.0B	501	11.50		23 9 70	920	
F042R0.0B	338	8.00		25 4 69		33
M013R0.2	529	9.77	0.00577	4 3 64	3314	
F014R0.2	460	8.10	0.00836	23 10 64	3081	
M015R0.2	504	10.80	0.00873	4 2 65	2977	
F016R0.2	486	8.90	0.00665	7 4 65	2915	
M017R0.2	494	11.80	0.00711	27 4 66	2530	
F018R0.2	497	9.30	0.00652	25 5 66	2502	
F019R0.2	533	10.60	0.00785	13 10 66	2361	
M020R0.2	546	11.40	0.00676	29 12 66	2284	
F021R0.2	549	11.50	0.00687	26 1 67	2256	
M022R0.2	533	12.90	0.00961	22 3 67	2201	
M013R0.5	529	11.00	0.0171	4 3 64	3314	
F014R0.5	510	9.75	0.022	23 10 64	3081	
M015R0.5	490	10.40	0.0263	4 2 65	2977	
F016R0.5	500	11.40	0.0205	7 4 65	2915	
M017R0.5	494	9.20	0.0215	27 4 66	2530	
F018R0.5	496	9.10	0.0197	25 5 66	2502	
F019R0.5	533	10.00	0.023	13 10 66	2361	
M020R0.5	536	13.20	0.0206	29 12 66	2284	
F021R0.5	538	8.80	0.0208	26 1 67	2256	
M022R0.5	520	12.30	0.029	22 3 67	2201	
M031R0.5B	508	11.40	0.021	27 4 66	2530	
F031R0.5C	537	9.40	0.0235	22 12 65	2656	
F031R0.5D	537	11.70	0.0238	22 12 65	2656	
M032R0.5B	496	13.40	0.0196	25 5 66	2502	
F032R0.5C	519	10.10	0.0239	22 12 65	2656	
F032R0.5D	509	10.10	0.024	22 12 65	2656	
M033R0.5B	497	12.90	0.0194	25 5 66	2502	
F033R0.5C	527	10.60	0.0212	27 4 66	2530	
F033R0.5D	527	8.70	0.0217	27 4 66	2530	
M034R0.5B	496	10.50	0.0196	25 5 66	2502	
F034R0.5C	524	9.90	0.0215	27 4 66	2530	
F034R0.5D	508	9.70	0.0212	27 4 66	2530	
M035R0.5B	536	10.40	0.0205	29 12 66	2284	
F035R0.5C	532	9.00	0.0201	29 12 66	2284	
F035R0.5D	532	10.20	0.0202	29 12 66	2284	

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		INJECTED (μ ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	3314	
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	2915	
M017R1.0	494	11.40	0.0639	27	4	66	2530	
F018R1.0	496	10.00	0.0589	25	5	66	2500	
F019R1.0	526	11.60	0.0682	13	10	66	2361	
M020R1.0	536	10.00	0.061	29	12	66	2284	
F021R1.0	525	8.10	0.0633	26	1	67	2256	
M022R1.0	484	10.90	0.0861	22	3	67	2201	
F031R1.0B	509	10.40	0.0712	22	12	65	2656	
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	4903	511
F011R1.7	598	8.17	0.165	30	11	56	5320	300
F012R1.7	590	8.95	0.167	30	11	56	2399	210

DOG
NUMBER

COMMENTS ON DEAD DOGS

MU01R1.0	MELANOMA ORAL CAVITY
MU02R1.0	SEMINOMA
FU03R1.0	MAMMARY GLAND CARCINOMA
MU04R1.0	TRAUMA
MU05R1.0	TRANSITIONAL CELL CARCINOMA, HYDRONEPHROSIS
FU06R1.0	NEPHRITIS
MU07R1.0	STATUS EPILEPTICUS
FU08R1.0	LYMPHOSARCOMA
FU09R1.0	PNEUMONIA
MU10R1.0	FIBROSARCOMA (GINGIVA)
FU11R1.0	MELANOMA (EYE); MAMMARY CARCINOMA
FU12R1.0	MELANOMA, GINGIVA
MU13R1.0	
FU14R1.0	UNDETERMINED (NO NEOPLASIA)
MU15R1.0	UNDETERMINED (NO NEOPLASIA)
FU16R1.0	
MU17R1.0	
FU18R1.0	
FU19R1.0	
MU20R1.0	
FU21R1.0	
MU22R1.0	
FU31R1.0B	

MU01R1.7	OBTURATING ABDOMINAL AORTA AND PULMONARY EMBOLISM
MU02R1.7	LYMPHOSARCOMA
MU02R1.7A	FIBROSARCOMA (GINGIVA)
FU03R1.7	MAMMARY GLAND CARCINOMA
MU04R1.7	NEPHRITIS
MU05R1.7	OSTEOSARCOMA
FU06R1.7	PULMONARY CALCIFICATION (NO BONE TUMOR)
MU07R1.7	BACTERIAL TOXEMIA, INTERSTITIAL CELL ADENOMA
FU08R1.7	DRUG ALLERGY
FU09R1.7	PYOMETRA
MU10R1.7	TRAUMA
MU10R1.7A	MAST CELL SARCOMA; NEPHRITIS; MELANOMA (EYE)
FU11R1.7	MELANOMA (ORAL); THYROID CARCINOMA
FU12R1.7	UNDETERMINED (NO BONE TUMOR)

QUG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D.	MO	YR		
M001R2.0	471	8.74	0.382	20	4	53	3440	860
M002R2.0	592	8.21	0.387	16	11	53	3775	625
F003R2.0	541	8.53	0.347	10	3	54	4459	872
M004R2.0	414	10.50	0.361	7	4	54	325	189
M004R2.0A	420	10.60	0.306	11	4	55	4368	1142
M005R2.0	461	11.50	0.267	22	6	54	4703	994
F006R2.0	486	10.60	0.36	27	7	54	4615	1264
M007R2.0	514	11.10	0.413	24	8	54	3425	922
F008R2.0	572	6.95	0.331	21	12	54	4781	996
F009R2.0	592	9.38	0.317	11	4	55	3998	1016
M010R2.0	523	9.95	0.345	27	7	55	3569	1220
F011R2.0	495	9.30	0.31	20	12	55	3297	728
F012R2.0	497	10.30	0.281	17	1	56	2948	742
M001R3.0	473	8.91	1.2	20	4	53	2850	2395
M002R3.0	470	9.02	1.21	16	11	53	2226	1727
F003R3.0	386	7.74	1.11	10	3	54	2497	2323
M004R3.0	412	11.70	1.16	7	4	54	1917	2361
M005R3.0	461	13.00	0.846	22	6	54	2955	2317
F006R3.0	486	9.75	1.14	27	7	54	1932	2246
M007R3.0	514	12.30	1.29	24	8	54	2099	3029
F008R3.0	542	7.76	1.03	21	12	54	2612	1916
F009R3.0	551	8.02	0.987	11	4	55	2487	1839
M010R3.0	525	10.10	1.06	27	7	55	1737	2336
F011R3.0	495	12.90	0.938	20	12	55	1610	1333
F012R3.0	497	11.40	0.883	17	1	56	1897	1639
M001R4.0	471	9.08	3.51	20	4	53	1606	6575
M002R4.0	470	9.53	3.55	16	11	53	1884	6150
F003R4.0	384	8.65	3.33	10	3	54	490	2208
F003R4.0A	598	7.20	3.1	30	11	56	1614	3855
M004R4.0	408	8.83	3.47	7	4	54	1518	6063
M005R4.0	461	13.20	2.42	22	6	54	1659	4505
F006R4.0	486	8.55	3.44	27	7	54	1939	7133
M007R4.0	453	9.55	3.88	24	8	54	1647	5844
F008R4.0	474	8.94	3.14	21	12	54	1324	4615
F009R4.0	542	8.53	3.02	11	4	55	1471	4095
M010R4.0	527	10.80	3.28	27	7	55	1553	7582
F011R4.0	491	10.40	2.84	20	12	55	1469	5273

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001R2.0	HEMANGIOSARCOMA (SPLEEN)
M002R2.0	OSTEOSARCOMA
F003R2.0	RETICULUM CELL SARCOMA (NON-SKELETAL)
M004R2.0	PERFORATED ILEUM
M004R2.0A	VALVULAR ENDOCARDITIS
M005R2.0	OSTEOSARCOMA, ADRENAL CORTICAL CARCINOMA
F006R2.0	EPIDERMOID CARCINOMA (TYMPANIC BULLA)
M007R2.0	OSTEOSARCOMA, CUSHING SYNDROME
F008R2.0	UNDIFFERENTIATED MALIGNANCY, SMALL INTESTINE
F009R2.0	MAMMARY CARCINOMA
M010R2.0	OSTEOSARCOMA
F011R2.0	OSTEOSARCOMA
F012R2.0	MAMMARY ADENOCARCINOMA

M001R3.0	OSTEOSARCOMA
M002R3.0	OSTEOSARCOMA
F003R3.0	OSTEOSARCOMA
M004R3.0	OSTEOSARCOMA
M005R3.0	OSTEOSARCOMA
F006R3.0	OSTEOSARCOMA
M007R3.0	OSTEOSARCOMA
F008R3.0	OSTEOSARCOMA
F009R3.0	OSTEOSARCOMA
M010R3.0	OSTEOSARCOMA
F011R3.0	PYOMETRITIS + SECONDARY PERITONITIS
F012R3.0	OSTEOSARCOMA

M001R4.0	OSTEOSARCOMA
M002R4.0	OSTEOSARCOMA
F003R4.0	CANINE DISTEMPER
F003R4.0A	OSTEOSARCOMA
M004R4.0	OSTEOSARCOMA
M005R4.0	OSTEOSARCOMA
F006R4.0	OSTEOSARCOMA
M007R4.0	OSTEOSARCOMA
F008R4.0	OSTEOSARCOMA
F009R4.0	OSTEOSARCOMA
M010R4.0	OSTEOSARCOMA
F011R4.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
F012R4.0	496	9.61	2.81	17 1 56	1435	3877
M001R5.0	473	9.87	10.5	20 4 53	908	14943
M002R5.0	470	8.85	10.8	16 11 53	1380	18071
F003R5.0	380	7.82	10.1	10 3 54	481	7147
M004R5.0	408	8.90	10.6	7 4 54	1091	16417
M005R5.0	458	10.90	10.1	22 6 54	1220	15433
F006R5.0	486	9.66	10.2	27 7 54	1015	15414
M007R5.0	453	8.85	11.9	24 8 54	1288	16708
F008R5.0	474	7.76	9.68	21 12 54	968	11564
F009R5.0	420	9.16	9.48	11 4 55	1288	15941
M010R5.0	527	10.70	10.2	27 7 55	825	11179

DOG
NUMBER

COMMENTS ON DEAD DOGS

FU12R4.0 OSTEOSARCOMA

MU01R5.0 OSTEOSARCOMA

MU02R5.0 OSTEOSARCOMA

FU03R5.0 CANINE DISTEMPER

MU04R5.0 OSTEOSARCOMA

MU05R5.0 OSTEOSARCOMA

FU06R5.0 OSTEOSARCOMA

MU07R5.0 OSTEOSARCOMA

FU08R5.0 OSTEOSARCOMA

FU09R5.0 OSTEOSARCOMA + ANEMIA

MU10R5.0 OSTEOSARCOMA + FRACTURED MANDIBLE

R. PLUTONIUM-239

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
MU01P0.0	443	9.70		1 12 52		4003
F002P0.0	424	6.36		2 3 53		2755
MU03P0.0	515	10.80		1 6 53		5362
MU04P0.0	426	10.70		16 9 53		5138
F005P0.0	620	9.75		14 10 53		4088
F006P0.0	410	5.59		12 5 54		4490
FU07P0.0	515	6.90		25 10 54		5344
MU08P0.0	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
FU13P0.0	517	9.47		4 3 64	3314	
FU14P0.0	452	9.89		12 5 64	3245	
MU15P0.0	527	12.10		23 10 64	3081	
M016P0.0	486	13.90		7 4 65	2915	
MU17P0.0	551	12.20		18 11 66	2335	
FU18P0.0	536	11.40		29 11 66	2314	
MU19P0.0	536	13.10		29 11 66	2314	
F020P0.0	546	8.50		29 12 66	2284	
M021P0.0	549	13.30		26 1 67	2256	
FU22P0.0	489	10.60		25 5 67	2137	
MU31P0.0B	452	11.80		12 5 64		1763
MU31P0.0C	452	12.60		12 5 64	3245	
M032P0.0B	452	11.20		12 5 64	3245	
M032P0.0C	542	10.30		21 9 65	2748	
MU33P0.0B	517	12.10		21 9 65	2748	
MU33P0.0C	503	11.70		18 11 65	2690	
M034P0.0B	525	13.50		26 1 67	2256	
M034P0.0C	484	12.70		22 3 67	2201	
MU35P0.0B	484	12.50		22 3 67	2201	
M035P0.0C	484	13.10		22 3 67	2201	
MU36P0.0B	489	11.00		25 5 67	2137	
M036P0.0C	485	12.20		25 5 67	2137	
MU37P0.0B	507	11.70		22 6 67	2109	
MU37P0.0C	493	10.40		22 6 67	2109	
MU38P0.0B	529	10.70		16 11 67	1962	
M038P0.0C	529	12.20		16 11 67	1962	
MU39P0.0B	501	10.70		21 12 67	1927	
M039P0.0C	501	10.10		21 12 67	1927	
M040P0.0B	484	10.30		30 7 68	1705	
M040P0.0C	552	11.40		9 1 69	1542	
MU41P0.0B	560	9.49		17 1 69	1534	

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 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M042P0.0B	338	11.60		25	4	69	32	
M081P0.0Y	105	7.10		9	3	72	387	
F082P0.0Y	105	7.30		9	3	72	387	
F083P0.0Y	91	3.97		25	4	72	340	
F084P0.0Y	89	3.67		25	4	72	340	
M086P0.0Y	91	4.37		25	4	72	340	
F013P0.1	515	9.46	0.00068	4	3	64	3314	
F014P0.1	452	10.30	0.00055	12	5	64	3245	
M015P0.1	527	9.67	0.00071	23	10	64	3081	
M016P0.1	501	12.00	0.00059	7	4	65	2915	
M017P0.1	551	12.20	0.00057	18	11	66	2335	
F018P0.1	536	9.28	0.0007	29	11	66	2314	
M019P0.1	536	11.60	0.00063	29	11	66	2314	
F020P0.1	536	9.80	0.00075	29	12	66	2284	
M021P0.1	538	11.30	0.00059	26	1	67	2256	
F022P0.1	489	9.80	0.00059	25	5	67	2137	
M031P0.1B	517	12.20	0.00068	4	3	64	2760	1
F032P0.1B	549	10.40	0.00059	18	11	65	2690	
M033P0.1B	549	10.80	0.00079	18	11	65	2690	
F034P0.1B	533	11.10	0.00058	18	11	66	2335	
M035P0.1B	489	10.30	0.00059	25	5	67	2137	
F036P0.1B	493	9.79	0.0006	22	6	67	2109	
M037P0.1B	493	11.30	0.00059	22	6	67	2109	
F038P0.1B	513	9.52	0.00057	21	12	67	1927	
M039P0.1B	490	10.50	0.00058	21	12	67	1927	
F044P0.1C	569	8.34	0.00072	2	12	70	850	
F013P0.2	517	9.44	0.00206	4	3	64	3221	10
F014P0.2	516	7.44	0.00173	12	5	64	3245	
M015P0.2	505	10.90	0.00201	23	10	64	3081	
M016P0.2	500	11.40	0.00163	7	4	65	2841	7
M017P0.2	533	11.80	0.00171	18	11	66	2335	
F018P0.2	530	9.46	0.002	29	11	66	2314	
M019P0.2	530	12.10	0.00198	29	11	66	2314	
F020P0.2	532	8.30	0.00224	29	12	66	2284	
M021P0.2	538	12.10	0.00181	26	1	67	2256	
F022P0.2	485	8.30	0.00176	25	5	67	2137	
M031P0.2B	515	10.70	0.00185	4	3	64	2640	1

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

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

F013P0.2 INTESITINAL ILEUS
F014P0.2
M015P0.2
M016P0.2 ENCEPHALITIS
M017P0.2
F018P0.2
M019P0.2
F020P0.2
M021P0.2
F022P0.2
M031P0.2B MELANOMA (ORAL)

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F031P0.2C	452	11.90	0.00169	12	5	64	3245	
F031P0.2D	429	9.35	0.00186	12	5	64	3245	
M032P0.2B	549	13.60	0.00178	18	11	65	2690	
F032P0.2C	494	10.10	0.00183	4	2	65	2977	
F032P0.2D	490	8.04	0.00193	4	2	65	2977	
M033P0.2B	513	14.50	0.00178	18	11	65	2690	
F033P0.2C	549	12.50	0.00176	18	11	65	2690	
F033P0.2D	513	12.70	0.00178	18	11	65	2690	
M034P0.2B	533	12.70	0.0017	18	11	66	2335	
F034P0.2C	533	11.50	0.00172	18	11	66	2335	
F034P0.2D	519	9.92	0.00167	18	11	66	2335	
M035P0.2B	489	11.20	0.00173	25	5	67	2137	
F035P0.2C	507	10.50	0.00175	22	6	67	2109	
F035P0.2D	507	9.10	0.00175	22	6	67	2109	
M036P0.2B	479	12.90	0.00177	25	5	67	2137	
F036P0.2C	493	10.40	0.00177	22	6	67	2109	
F036P0.2D	569	8.74	0.00146	16	11	67	1962	
M037P0.2B	529	10.60	0.00149	16	11	67	1962	
F037P0.2C	529	10.10	0.0015	16	11	67	1962	
F037P0.2D	529	7.14	0.00153	16	11	67	1962	
M038P0.2B	517	10.00	0.00152	16	11	67	1962	
F038P0.2C	501	7.95	0.00211	21	12	67	1927	
F038P0.2D	499	9.08	0.00176	21	12	67	1927	
M039P0.2B	542	11.60	0.00214	2	12	70	850	
F039P0.2C	499	9.46	0.00173	21	12	67	1927	
F039P0.2D	499	9.34	0.00176	21	12	67	1927	
F042P0.2C	589	9.55	0.00176	4	9	69	1304	
F043P0.2C	542	9.50	0.00239	2	12	70	850	
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	920	
F014P0.5	516	9.98	0.00493	12	5	64	3245	
M015P0.5	505	8.41	0.00627	23	10	64	3081	
M016P0.5	501	12.60	0.00521	7	4	65	2915	
M017P0.5	533	13.40	0.00506	18	11	66	2335	
F018P0.5	530	8.98	0.00594	29	11	66	2314	
M019P0.5	530	11.90	0.00645	29	11	66	2314	
F020P0.5	532	9.30	0.00553	29	12	66	2284	
M021P0.5	538	9.80	0.00526	26	1	67	2256	
F022P0.5	485	8.10	0.00525	25	5	67	2137	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F031P0.2C
F031P0.2D
M032P0.2B
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
F013P0.5A
F014P0.5
M015P0.5
M016P0.5
M017P0.5
F018P0.5
M019P0.5
F020P0.5
M021P0.5
F022P0.5

MAMMARY CARCINOMA

DUG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
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	2270	18
F033P0.5B	503	10.10	0.00559	18 11 65	2690	
M034P0.5B	530	12.50	0.00642	29 11 66	1920	18
F035P0.5B	501	9.54	0.0052	22 6 67	2109	
M036P0.5B	479	11.50	0.00527	25 5 67	2137	
F037P0.5B	517	8.39	0.00454	16 11 67	1962	
M038P0.5B	517	10.50	0.00448	16 11 67	1962	
F039P0.5B	490	10.90	0.00528	21 12 67	1927	
M042P0.5B	542	13.10	0.00675	2 12 70	850	
M042P0.5C	542	12.20	0.00668	2 12 70	850	
F042P0.5D	542	9.71	0.00668	2 12 70	850	
F043P0.5B	545	11.60	0.00484	3 10 69	1275	
F043P0.5C	535	10.70	0.0048	3 10 69	1275	
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	1340	
M015P0.7	533	10.30	0.00941	22 7 69	1348	
M016P0.7	516	11.90	0.0102	4 9 69	1304	
M017P0.7	540	8.04	0.0103	3 10 69	1275	
F018P0.7	521	9.66	0.00942	22 7 69	1348	
M019P0.7	501	11.60	0.0104	23 9 70	920	
F020P0.7	521	9.18	0.00926	22 7 69	1348	
M021P0.7	499	11.10	0.0104	23 9 70	920	
F022P0.7	538	9.69	0.0108	4 9 69	1304	
F023P0.7	538	9.56	0.0108	4 9 69	1304	
F024P0.7	516	8.90	0.011	4 9 69	1304	
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

DOG
NUMBER

COMMENTS ON DEAD DOGS

M031P0.5B	STATUS EPILEPTICUS
F031P0.5C	SPECIAL STUDY
M032P0.5B	SPECIAL STUDY
F033P0.5B	
M034P0.5B	SPECIAL STUDY
F035P0.5B	
M036P0.5B	
F037P0.5B	
M038P0.5B	
F039P0.5B	
M042P0.5B	
M042P0.5C	
F042P0.5D	
F043P0.5B	
F043P0.5C	
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

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73	DEATH	DOSE TO SKELETON (RADS)
F006P1.0	410	7.38	0.014	12 5 54		4292	86
F007P1.0	510	6.36	0.0167	25 10 54		3981	96
M008P1.0	453	10.60	0.0172	15 3 55		3367	83
F009P1.0	556	7.87	0.0168	9 9 55		2257	60
F010P1.0	641	12.00	0.0152	22 11 55		3649	80
M011P1.0	602	8.90	0.0157	24 4 56		5160	116
M012P1.0	629	9.67	0.0167	29 5 56		2374	60
M013P1.0	504	12.70	0.0153	3 9 58		5277	115
F014P1.0	533	10.40	0.0141	22 7 69	1348		
M015P1.0	516	12.80	0.0159	4 9 69	1304		
M016P1.0	516	10.60	0.0165	4 9 69	1304		
M017P1.0	537	10.90	0.0151	3 10 69	1275		
F018P1.0	531	9.89	0.014	22 7 69	1348		
M019P1.0	501	9.82	0.0159	23 9 70	920		
F020P1.0	521	10.40	0.0141	22 7 69	1348		
M021P1.0	499	10.00	0.0156	23 9 70	920		
F022P1.0	531	9.04	0.0139	22 7 69	1348		
F023P1.0	538	11.20	0.0163	4 9 69	1304		
F024P1.0	516	10.40	0.0163	4 9 69	1304		
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

DOG
NUMBER

COMMENTS ON DEAD DOGS

F006P1.0	CARCINOMA OF COLON
F007P1.0	TRAUMA LYMPHADENOPHATHY
M008P1.0	OSTEOSARCOMA
F009P1.0	OSTEOSARCOMA
F010P1.0	MAMMARY CARCINOMA
M011P1.0	THYROID CARCINOMA
M012P1.0	CHRONIC PANCREATITIS
M013P1.0	SENILITY, HYDROCEPHALUS
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

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
FU05P2.0	594	8.12	0.0846	14	10	53	2423	310
FU06P2.0	417	7.54	0.0902	12	5	54	2947	370
FU07P2.0	485	8.40	0.0996	25	10	54	2093	310
MU08P2.0	406	9.73	0.0957	15	3	55	1761	250
FU09P2.0	552	9.72	0.101	9	9	55	2014	300
FU10P2.0	551	7.94	0.0968	22	11	55	2912	410
MU11P2.0	599	10.30	0.0961	24	4	56	1617	230
MU12P2.0	622	9.98	0.1	29	5	56	2284	370
MU01P3.0	443	8.00	0.261	1	12	52	1476	580
FU02P3.0	422	6.85	0.312	2	3	53	1947	900
MU03P3.0	485	8.74	0.291	1	6	53	1604	700
MU04P3.0	608	8.51	0.292	16	9	53	1950	840
FU05P3.0	650	8.22	0.288	14	10	53	1504	630
FU06P3.0	415	8.38	0.282	12	5	54	1617	670
FU07P3.0	485	9.00	0.314	25	10	54	1627	750
MU08P3.0	406	9.73	0.3	15	3	55	1770	780
FU09P3.0	552	7.67	0.3	9	9	55	1894	840
FU10P3.0	533	8.94	0.298	22	11	55	1547	700
MU11P3.0	599	10.50	0.309	24	4	56	1198	550
MU12P3.0	622	10.20	0.308	29	5	56	1659	760
MU81P3.0Y	91	4.03	0.32	25	4	72	340	
MU86P3.0Y	89	3.29	0.319	25	4	72	340	
MU89P3.0Y	89	4.23	0.312	25	4	72	340	
MU01P4.0	443	7.61	0.823	1	12	52	1724	2100
FU02P4.0	568	8.65	1.03	2	3	53	1556	2380
MU03P4.0	485	9.36	0.929	1	6	53	1198	1680
MU04P4.0	566	8.74	0.974	16	9	53	1066	1560
FU05P4.0	650	7.05	0.872	14	10	53	1245	1650
FU06P4.0	420	9.26	0.811	12	5	54	1357	1660
FU07P4.0	485	8.45	0.963	25	10	54	1198	1730
MU08P4.0	651	9.22	0.887	15	3	55	1157	1560
FU09P4.0	552	8.58	0.96	9	9	55	1343	1920
FU10P4.0	527	6.48	0.868	22	11	55	1241	1660
MU11P4.0	596	9.56	0.927	24	4	56	1288	1790
MU12P4.0	598	11.40	0.838	29	5	56	1463	1840

DOG
NUMBER

COMMENTS ON DEAD DOGS

F005P2.0	OSTEOSARCOMA
F006P2.0	OSTEOSARCOMA
F007P2.0	SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
M008P2.0	ASPIRATION PNEUMONIA
F009P2.0	OSTEOSARCOMA
F010P2.0	OSTEOSARCOMA
M011P2.0	OSTEOSARCOMA
M012P2.0	OSTEOSARCOMA

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

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

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
M001P5.0	413	8.86	2.67	1 12 52	1324	5370
F002P5.0	1151	8.75	3.3	2 3 53	1576	7830
M003P5.0	515	8.10	3.0	1 6 53	499	2340
M004P5.0	566	9.18	3.17	16 9 53	1562	7380
F005P5.0	691	8.77	2.77	14 10 53	2059	8690
F006P5.0	407	7.90	2.57	12 5 54	1194	4620
F007P5.0	482	8.33	2.99	25 10 54	1491	6630
M008P5.0	497	9.55	2.69	15 3 55	1192	4840
F009P5.0	552	9.45	2.73	9 9 55	1145	4750
M081P5.0Y	94	4.60	2.68	1 3 72	395	
F082P5.0Y	94	4.80	2.66	1 3 72	395	
F083P5.0Y	94	4.00	2.66	1 3 72	395	
F084P5.0Y	94	3.55	2.68	1 3 72	395	
F085P5.0Y	94	4.15	2.64	1 3 72	395	
M086P5.0Y	93	3.75	2.95	25 4 72	340	
F087P5.0Y	93	4.15	2.93	25 4 72	340	
F088P5.0Y	93	3.65	2.96	25 4 72	340	
M089P5.0Y	93	3.79	2.92	25 4 72	340	
M090P5.0Y	93	4.38	2.97	25 4 72	340	
M091P5.0Y	91	3.78	2.9	25 4 72	340	
M092P5.0Y	91	3.82	2.87	25 4 72	340	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001P5.0	OSTEOSARCOMA
F002P5.0	OSTEOSARCOMA + FRACTURED MANDIBLE
M003P5.0	LIVER DEGENERATION + ASCITES
M004P5.0	OSTEOSARCOMA
F005P5.0	OSTEOSARCOMA, LIVER DEGENERATION + HEPATIC HEMORRHAGE
F006P5.0	OSTEOSARCOMA
F007P5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
M008P5.0	GINGIVITIS
F009P5.0	OSTEOSARCOMA, EPISTAXIS + CIRCULATORY COLLAPSE
M081P5.0Y	
F082P5.0Y	
F083P5.0Y	
F084P5.0Y	
F085P5.0Y	
M086P5.0Y	
F087P5.0Y	
F088P5.0Y	
M089P5.0Y	
M090P5.0Y	
M091P5.0Y	
M092P5.0Y	

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 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	3624	
F008M0.0	654	11.60		9	3	60	4770	
M009M0.0	575	12.40		13	4	60	4130	
M010M0.0	581	13.30		17	7	62	2991	
F011M0.0	475	9.31		18	9	62	3248	
M012M0.0	695	10.00		22	12	60	4482	
F001M0.5	492	9.47	0.0173	17	7	62	3910	
F002M0.5	492	9.15	0.0173	17	7	62	3689	93
M003M0.5	493	10.80	0.0199	18	9	62	3847	
M004M0.5	475	12.80	0.0199	18	9	62	3847	
F005M0.5	534	7.83	0.0172	23	10	62	3812	
M006M0.5	510	10.30	0.0171	23	10	62	3019	71
F007M0.5	492	8.87	0.0172	17	7	62	3910	
F008M0.5	654	12.60	0.0159	9	3	60	4208	70
M009M0.5	485	11.90	0.017	13	4	60	4735	
M010M0.5	492	10.60	0.0174	17	7	62	3910	
F011M0.5	505	7.82	0.0202	18	9	62	3847	
M012M0.5	510	10.60	0.0165	23	10	62	3812	
F001M1.0	718	7.75	0.0463	4	1	54	2950	196
F001M1.0A	590	8.07	0.0512	23	10	62	3812	
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	4670	322
M010M1.0	492	9.43	0.0501	17	7	62	2966	213
F011M1.0	505	8.91	0.0613	18	9	62	3847	

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

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

F001M1.0	SARCOMA (SPLEEN)
F001M1.0A	
F002M1.0	OSTEOSARCOMA
M003M1.0	OSTEOSARCOMA
M004M1.0	PNEUMONIA; PANCREATITIS
F005M1.0	MALIGNANT MELANOMA (EYE)
M006M1.0	EPIDERMOID CARCINOMA (PENIS)
F007M1.0	SARCOMA (HEART)
F008M1.0	MALIGNANT MELANOMA (EYE)
M009M1.0	VALVULAR ENDOCARDITIS
M010M1.0	MALIGNANT MELANOMA (EYE)
F011M1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
M012M1.0	528	9.27	0.0498	23 10 62	3786	231
F001M1.7	510	7.52	0.151	23 10 62	3812	
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
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MU12M1.0	LYMPHATIC LEUKEMIA
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F001M1.7	
F002M1.7	OSTEOSARCOMA
M003M1.7	OSTEOSARCOMA
M004M1.7	CARCINOMA SMALL INTESTINE
F005M1.7	OSTEOSARCOMA
M006M1.7	OSTEOSARCOMA
F007M1.7	OSTEOSARCOMA
F008M1.7	OSTEOSARCOMA
M009M1.7	OSTEOSARCOMA
MU10M1.7	OSTEOSARCOMA
F011M1.7	OSTEOSARCOMA
MU12M1.7	UNKNOWN

F001M2.0	OSTEOSARCOMA
F002M2.0	INTESTINAL HEMORRHAGE
M003M2.0	PNEUMONIA
M004M2.0	OSTEOSARCOMA
F005M2.0	OSTEOSARCOMA
M006M2.0	OSTEOSARCOMA
F007M2.0	CHRONIC PANCREATITIS
F008M2.0	OSTEOSARCOMA
M009M2.0	OSTEOSARCOMA
MU10M2.0	OSTEOSARCOMA
F011M2.0	OSTEOSARCOMA
MU12M2.0	OSTEOSARCOMA

F001M3.0	OSTEOSARCOMA
F002M3.0	OSTEOSARCOMA
M003M3.0	OSTEOSARCOMA
M004M3.0	OSTEOSARCOMA
F005M3.0	OSTEOSARCOMA
M006M3.0	OSTEOSARCOMA
F007M3.0	OSTEOSARCOMA
F008M3.0	OSTEOSARCOMA
M009M3.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
MU10M3.0	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	770	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
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COMMENTS ON DEAD DOGS

MU10M3.0	OSTEOSARCOMA
FU11M3.0	OSTEOSARCOMA
MU12M3.0	OSTEOSARCOMA
F001M4.0	OSTEOSARCOMA + CRIPPLING FRACTURE
FU02M4.0	OSTEOSARCOMA
M003M4.0	STRANGULATED INGUINAL HERNIA
MU03M4.0A	OSTEOSARCOMA, NEPHRITIS, ULCERATIVE GINGIVITIS + PNEUMONIA
MU04M4.0	FRACTURED MANDIBLE + ULCERATIVE GINGIVITIS
FU05M4.0	OSTEOSARCOMA
MU06M4.0	OSTEOSARCOMA
FU07M4.0	OSTEOSARCOMA
F001M5.0	NEPHRITIS + SEVERE ANEMIA
FU02M5.0	CRIPPLING FRACTURES
M003M5.0	ULCERATIVE GINGIVITIS
MU04M5.0	CRIPPLING FRACTURE
F005M5.0	ULCERATIVE GINGIVITIS
MU06M5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
FU07M5.0	ULCERATIVE GINGIVITIS, MYOCARDIAL INFARCTION + GLAUCOMA

D. THORIUM-228 (RADIOTHORIUM)

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73	DEATH	DOSE TO SKELETON (RADS)
M001T0.0	493	8.24		8 2 54		4895	
M002T0.0	488	7.28		28 9 54		5510	
F003T0.0	797	11.60		6 6 55		2592	
M004T0.0	591	8.10		18 10 55		3072	
M005T0.0	458	10.40		14 10 58	5282		
F006T0.0	489	9.64		10 1 61		171	
F006T0.0A	688	8.61		15 12 60	4489		
M007T0.0	517	10.50		7 2 61		1412	
M008T0.0	533	10.80		24 5 61	4329		
F009T0.0	569	8.28		29 6 61	4293		
F010T0.0	536	10.40		28 7 61	4264		
F011T0.0	530	9.45		4 6 63	3588		
F012T0.0	492	9.09		9 7 63	3553		
M001T0.2	682	11.40	0.00164	27 3 62	4022		
M002T0.2	682	10.40	0.00166	27 3 62	4022		
F003T0.2	478	9.86	0.00163	27 3 62	4022		
M004T0.2	478	10.00	0.00166	27 3 62	4022		
M005T0.2	625	13.80	0.00162	9 2 60		8A9	10
M005T0.2A	530	13.40	0.00173	4 6 63	3588		
F006T0.2	489	8.85	0.00176	10 1 61	4463		
M007T0.2	532	10.50	0.00159	7 2 61		3897	15
M008T0.2	494	13.90	0.00189	24 5 61	4329		
F009T0.2	569	7.82	0.00171	29 6 61		3897	16
F010T0.2	508	10.50	0.0017	28 7 61		4217	16
F011T0.2	530	9.76	0.00171	4 6 63	3588		
F012T0.2	492	7.37	0.0019	9 7 63		3350	17
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	4463		
M007T0.5	532	9.11	0.00491	7 2 61	4435		
M008T0.5	533	9.53	0.00562	24 5 61	4329		
F009T0.5	569	8.62	0.00529	29 6 61		4149	49
F010T0.5	508	10.20	0.0051	28 7 61	4264		
F011T0.5	530	7.78	0.00518	4 6 63	3588		

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	BILE DUCT OBSTRUCTION; MAMMARY CARCINOMA
F011T0.2	
F012T0.2	HEPATIC CELL CARCINOMA

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

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
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	4329	
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	3588	
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	380	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 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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
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COMMENTS ON DEAD DOGS

MU08T2.0 OSTEOSARCOMA
F009T2.0 OSTEOSARCOMA
F010T2.0 OSTEOSARCOMA
F011T2.0 OSTEOSARCOMA
F012T2.0 OSTEOSARCOMA

MU01T3.0 OSTEOSARCOMA + SEVERE ANEMIA
MU02T3.0 OSTEOSARCOMA + TRAUMA
F003T3.0 OSTEOSARCOMA
MU04T3.0 OSTEOSARCOMA
MU05T3.0 OSTEOSARCOMA
F006T3.0 OSTEOSARCOMA
MU07T3.0 OSTEOSARCOMA
MU08T3.0 OSTEOSARCOMA
F009T3.0 OSTEOSARCOMA
F010T3.0 OSTEOSARCOMA
F011T3.0 OSTEOSARCOMA
F012T3.0 HEMANGIOSARCOMA (HUMERUS)

MU01T4.0 OSTEOSARCOMA + CRIPPLING FRACTURE
MU02T4.0 OSTEOSARCOMA, CRIPPLING FRACTURE + NEPHRITIS
F003T4.0 ULCERATIVE GINGIVITIS + NEPHRITIS
MU04T4.0 ULCERATIVE GINGIVITIS

MU01T5.0 NEPHRITIS
MU02T5.0 PANCYTOPENIA

E. STRONTIUM-90

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 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	5646	
M005S0.0	542	10.60		19 11 57	4158	
M006S0.0	466	9.68		27 5 58	4726	
F007S0.0A	462	9.46		7 1 59	3303	
F008S0.0	483	9.29		19 5 59	4482	
F009S0.0	549	12.40		11 8 59	708	
F009S0.0A	535	11.20		4 6 63	3425	
M010S0.0	522	13.90		29 9 59	4932	
F011S0.0	541	9.60		3 11 59	4831	
M012S0.0	605	8.99		6 1 60	4833	
F001S1.0	1525	6.84	0.573	18 1 55	308	21
F001S1.0A	6521	9.38	5.88	14 2 56	5219	90
M002S1.0	567	8.81	0.606	14 2 56	5077	90
M003S1.0	493	10.90	0.572	11 9 57	5363	142
F004S1.0	525	8.96	0.56	15 10 57	5646	
M005S1.0	555	10.20	0.532	19 11 57	2705	71
M006S1.0	466	9.56	0.581	27 5 58	5422	
F007S1.0	524	9.94	0.517	11 11 58	5254	
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	4932	
F011S1.0	543	10.30	0.55	3 11 59	4897	
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	3678	
M006S1.7	466	10.60	1.72	27 5 58	5422	
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	4803	414
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	MAMMARY CARCINOMA
M010S0.0	
F011S0.0	MAMMARY CARCINOMA
M012S0.0	
F001S1.0	SACRIFICED -IMPROPER INJECTION AGE-
F001S1.0A	PULMONARY THROMBO-EMBOLISM
M002S1.0	AORTIC BODY TUMOR
M003S1.0	SQUAMOUS CELL CARCINOMA
F004S1.0	
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	LYMPHOSARCOMA; NEPHRITIS
M010S1.7	OBTURATING PULMONARY EMBOLISM, NEPHRITIS

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F011S1.7	543	11.00	1.68	3	11	59	3180	239
M012S1.7	607	11.90	1.68	6	1	60	4717	355
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	5193	848
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	3430	593
F011S2.0	543	10.40	3.41	3	11	59	4880	659
M012S2.0	607	11.60	3.49	6	1	60	4584	707
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	5422	1816
F007S3.0	486	9.80	10.1	11	11	58		
F008S3.0	465	12.50	12.9	19	5	59	4018	2812
F009S3.0	468	10.00	10.1	11	8	59	4832	2660
M010S3.0	519	12.50	10.3	29	9	59	4599	2099
F011S3.0	541	9.00	10.8	3	11	59	2898	955
M012S3.0	605	8.43	10.2	6	1	60	4831	1632
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 ISLET CELL TUMOR

F001S2.0 BACTERIAL PNEUMONIA
M002S2.0 UNDETERMINED SOFT TISSUE SARCOMA +BRONCHIOGENIC CARCINOMA
M003S2.0 STATUS EPILEPTICUS; THYROID CARCINOMA
F004S2.0 MAMMARY CARCINOMA
M005S2.0 ULCERATIVE STOMATITIS
M006S2.0 UNDIFFERENTIATED SARCOMA (LIVER); NEPHRITIS
F007S2.0 PANCREATIC ISLET CELL ADENOMA
F008S2.0 PNEUMONIA
F009S2.0 UNDETERMINED (NO BONE TUMOR)
M010S2.0 BACTERIAL VALVULAR ENDOCARDITIS
F011S2.0 MAMMARY SARCOMA
M012S2.0 HEPATIC CELL CARCINOMA

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

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

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F009S4.0	468	9.56	30.6	11	8	59	4942	7599
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	60	4226	5290
F001S4.5	530	9.00	64.2	16	3	66	2572	
M002S4.5	530	12.20	63.6	16	3	66	2572	
M003S4.5	530	11.90	63.8	16	3	66		
F004S4.5	530	9.80	64.5	16	3	66	1493	5949
M005S4.5	496	13.30	61.3	16	3	66	2197	9100
M006S4.5	496	12.00	63.8	16	3	66	993	5424
F007S4.5	511	9.90	64.5	16	3	66	2572	
F008S4.5	511	9.90	64.5	16	3	66	2572	
F009S4.5	511	10.30	64.0	16	3	66	2325	9544
M010S4.5	496	14.00	60.9	16	3	66	1028	5201
F011S4.5	496	11.90	63.8	16	3	66	2064	10680
M012S4.5	485	11.40	63.7	16	3	66	1758	6434
							2253	9837
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	UNDETERMINED
M010S4.0	MENINGIOMA; PERIANAL GLAND CARCINOMA
F011S4.0	BLOOD DYSCRASIA, PYOMETRA
M012S4.0	ADENOCARCINOMA NASAL CAVITY
F001S4.5	
M002S4.5	
M003S4.5	SEVERE ANEMIA, INFARCTION, MYELOID METAPLASIA
F004S4.5	HEMANGIOSARCOMA (SPLEEN)
M005S4.5	OSTEOSARCOMA
M006S4.5	
F007S4.5	
F008S4.5	HEMANGIOSARCOMA (BONE)
F009S4.5	OSTEOSARCOMA
M010S4.5	SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
F011S4.5	OSTEOSARCOMA
M012S4.5	OSTEOSARCOMA; HEMANGIOSARCOMA (BONE)
F001S5.0	OSTEOSARCOMA
M002S5.0	STRANGULATED INGUINAL HERNIA
M002S5.0A	OSTEOSARCOMA
M003S5.0	OSTEOSARCOMA
F004S5.0	OSTEOSARCOMA
M005S5.0	SEVERE ANEMIA, AUTOAGGLUTINATION, INFARCTION, SPLENOMEGALY
M006S5.0	INTESTINAL HEMORRHAGE
M006S5.0A	OSTEOSARCOMA, INFARCTION + THROMBOCYTOPENIA
F007S5.0	STATUS EPILEPTICUS
F008S5.0	OSTEOSARCOMA
F009S5.0	SQUAMOUS CELL CARCINOMA ARISING FROM FRONTAL SINUS
M010S5.0	SEVERE ANEMIA + THROMBOCYTOPENIA
F011S5.0	HEMANGIOSARCOMA (LEFT MANDIBLE)
M012S5.0	HEMANGIOSARCOMA (RIB)

F. AMERICIUM-241*

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F013W0.2	533	10.60	0.00179	13	10	66	2361	1478 3
M014W0.2	533	14.90	0.00178	13	10	66	2361	
F024W0.2	477	11.30	0.00181	21	3	68	1836	
F031W0.2	472	10.90	0.0018	8	5	68		
M041W0.2	467	11.90	0.0018	2	7	68	1733	
M048W0.2	484	11.00	0.00174	30	7	68	1705	
M049W0.2	498	10.70	0.00175	25	11	69	1222	
F058W0.2	496	10.40	0.00168	26	1	70	1160	
M062W0.2	486	13.30	0.00175	24	2	70	1131	
F069W0.2	542	10.80	0.0018	22	4	70	1074	
M078W0.2	501	13.50	0.00178	16	7	70	989	
M079W0.2	501	10.00	0.00179	16	7	70	989	
F088W0.2	531	8.36	0.00177	25	8	70	949	
M095W0.2	526	13.10	0.00173	25	8	70	949	
F011W0.5	533	8.17	0.00532	13	10	66	2361	232 5
M012W0.5	533	11.90	0.00539	13	10	66	2361	
M023W0.5	487	12.20	0.0053	21	3	68	1836	
M029W0.5	472	10.40	0.00548	8	5	68	1788	
F030W0.5	472	10.60	0.00538	8	5	68	1788	
F040W0.5	467	9.40	0.00528	2	7	68	1733	
M050W0.5	552	11.80	0.00526	25	11	69	1222	
M059W0.5	496	11.50	0.00503	26	1	70	1160	
M063W0.5	486	11.40	0.00524	24	2	70	1131	
M070W0.5	497	12.80	0.00531	22	4	70	1074	
F080W0.5	501	11.90	0.00545	16	7	70	989	
F081W0.5	501	12.10	0.00548	16	7	70	989	
F089W0.5	531	9.66	0.00527	25	8	70	949	
M096W0.5	490	12.00	0.00533	25	8	70	949	
F009W1.0	517	8.60	0.016	15	9	66	2380	232 5
F010W1.0	517	9.90	0.0162	15	9	66	2389	
M020W1.0	513	10.80	0.0161	21	3	68	1836	
F021W1.0	513	9.36	0.0166	21	3	68		
F021W1.0A	552	11.40	0.0159	25	11	69	1222	
M022W1.0	487	11.60	0.0164	21	3	68	1836	
M028W1.0	472	12.10	0.0158	8	5	68	1780	
F051W1.0	552	8.25	0.0163	25	11	69	1222	
M060W1.0	496	10.00	0.0157	26	1	70	1160	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F013W0.2
M014W0.2
F024W0.2
F031W0.2 BRONCHOGENIC CARCINOMA
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 ACCIDENTAL STRANGULATION
F021W1.0A
M022W1.0
M028W1.0
F051W1.0
M060W1.0

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
M064W1.0	486	10.40	0.0158	24 2 70	1131	
F071W1.0	485	12.10	0.0157	22 4 70	1074	
M082W1.0	501	12.40	0.0163	16 7 70	989	
F090W1.0	526	10.90	0.016	25 8 70	949	
M097W1.0	490	10.40	0.016	25 8 70	949	
F042W1.7	495	9.26	0.0484	30 7 68	1705	
F043W1.7	492	10.40	0.0481	30 7 68	1705	
F044W1.7	492	7.46	0.0473	30 7 68	1705	
M045W1.7	492	11.90	0.0486	30 7 68	1705	
M046W1.7	484	8.42	0.0479	30 7 68	1705	
M047W1.7	484	11.10	0.0486	30 7 68	1705	
F052W1.7	552	9.57	0.0493	25 11 69	1222	
M061W1.7	496	10.70	0.0458	26 1 70	1160	
M065W1.7	486	11.20	0.0471	24 2 70	1131	
F072W1.7	500	11.10	0.0479	22 4 70	1074	
M083W1.7	501	12.60	0.0493	16 7 70	989	
F091W1.7	490	13.30	0.048	25 8 70	949	
M098W1.7	490	13.30	0.048	25 8 70	949	
F007W2.0	561	12.60	0.0952	15 9 66	1847	190
F008W2.0	561	11.70	0.0957	15 9 66	2389	
M019W2.0	513	13.40	0.097	21 3 68	1836	
M027W2.0	473	12.70	0.0961	8 5 68	1788	
M038W2.0	477	9.88	0.0945	2 7 68	1733	
F039W2.0	468	9.21	0.0948	2 7 68	1733	
M053W2.0	498	9.24	0.096	25 11 69	1222	
F066W2.0	486	9.12	0.0935	24 2 70	1131	
M073W2.0	552	14.30	0.0965	17 6 70	1018	
F084W2.0	493	10.60	0.0984	16 7 70	989	
M085W2.0	493	10.80	0.0987	16 7 70	989	
F092W2.0	490	10.60	0.0962	25 8 70	949	
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	1756	570
M026W3.0	473	12.40	0.31	8 5 68	1788	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M064W1.0
FU71W1.0
M082W1.0
FU90W1.0
M097W1.0

FU42W1.7
F043W1.7
FU44W1.7
M045W1.7
M046W1.7
M047W1.7
FU52W1.7
M061W1.7
M065W1.7
F072W1.7
M083W1.7
FU91W1.7
M098W1.7

FU07W2.0 OSTEOSARCOMA
FU08W2.0
M019W2.0
M027W2.0
M038W2.0
FU39W2.0
M053W2.0
FU66W2.0
M073W2.0
FU84W2.0
M085W2.0
FU92W2.0

M005W3.0 OSTEOSARCOMA
FU06W3.0 OSTEOSARCOMA
FU18W3.0 OSTEOSARCOMA
M026W3.0

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/73	DEATH	
M036W3.0	477	11.00	0.305	2	7	68		1696	490
F037W3.0	468	8.44	0.294	2	7	68		1733	
M054W3.0	498	10.50	0.306	25	11	69		1222	
M067W3.0	485	11.80	0.295	24	2	70		1131	
F074W3.0	542	10.00	0.302	22	4	70		1074	
F075W3.0	555	9.42	0.308	17	6	70		1018	
M086W3.0	493	11.30	0.312	16	7	70		989	
F093W3.0	490	11.20	0.301	25	8	70		949	
M100W3.0	542	11.00	0.304	2	12	70		850	
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		1527	1571
M034W4.0	477	10.70	0.893	2	7	68		1566	1212
F035W4.0	477	8.87	0.902	2	7	68		1323	1660
F055W4.0	498	8.37	0.914	25	11	69		1222	
M068W4.0	485	11.80	0.89	24	2	70		1131	
F076W4.0	485	9.37	0.899	22	4	70		1074	
M077W4.0	500	10.50	0.906	22	4	70		633	750
M087W4.0	501	13.10	0.916	16	7	70		989	
F094W4.0	490	11.30	0.912	25	8	70		949	
M001W5.0	517	10.40	2.78	28	6	66		401	1636
M002W5.0	517	12.70	2.83	28	6	66		448	1734

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

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

M036W3.0 OSTEOSARCOMA
F037W3.0
M054W3.0
M067W3.0
F074W3.0
F075W3.0
M086W3.0
F093W3.0
M100W3.0

M003W4.0 OSTEOSARCOMA; NEPHRITIS
F004W4.0 KIDNEY, THYROID AND LIVER DEGENERATION
M017W4.0 OSTEOSARCOMA; THROMBOSIS
F025W4.0 MESOTHELIOMA; KIDNEY DEGENERATION
M034W4.0 OSTEOSARCOMA
F035W4.0 PANCREATIC ADENOCARCINOMA
F055W4.0
M068W4.0
F076W4.0
M077W4.0 LIVER DEGENERATION
M087W4.0
F094W4.0

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

6. CALIFORNIUM-252

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
M001F0.0	562	12.00		1 2 72	424	
F002F0.0	545	10.60		3 1 73	87	
F003F0.0	545	9.43		3 1 73	87	
F004F0.0	492	10.60		27 2 73	32	
M005F0.0	562	10.40		1 2 72	424	
M006F0.0	509	10.90		28 11 72	123	
M001F0.1	498	13.00	0.0006	26 7 72	248	
F002F0.1	524	9.15	0.00064	2 11 72	149	
F003F0.1	545	10.10	0.00075	3 1 73	87	
F003F0.1	492	9.44	0.0006	27 2 73	32	
M005F0.1	498	10.40	0.0006	26 7 72	248	
M006F0.1	524	10.30	0.00062	2 11 72	149	
M001F0.5	498	12.20	0.00525	26 7 72	248	
F002F0.5	513	11.00	0.00529	2 11 72	149	
F003F0.5	511	8.89	0.00525	27 2 73	32	
F004F0.5	585	11.20	0.00518	27 2 73	32	
M005F0.5	494	9.44	0.0053	26 7 72	248	
M006F0.5	524	11.00	0.00529	2 11 72	149	
M001F1.0	586	9.69	0.0163	8 9 71	570	
F002F1.0	586	8.28	0.0167	8 9 71	570	
F003F1.0	539	8.89	0.0167	8 9 71	570	
F004F1.0	539	10.00	0.0165	8 9 71	570	
M005F1.0	539	12.90	0.0165	8 9 71	570	
M006F1.0	513	9.67	0.0165	2 11 72	149	
M001F2.0	498	11.50	0.0922	26 7 72	248	
F002F2.0	545	9.85	0.0905	3 1 73	87	
F003F2.0	511	9.16	0.0907	27 2 73	32	
F004F2.0	573	9.33	0.091	27 2 73	32	
M005F2.0	494	10.20	0.0905	26 7 72	248	
M006F2.0	513	11.40	0.0912	2 11 72	149	

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

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

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

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

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

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M001F3.0	583	11.60	0.289	3	3	71	759	
F002F3.0	583	10.60	0.289	3	3	71	759	
F003F3.0	583	8.66	0.292	3	3	71	759	
F004F3.0	583	9.69	0.295	3	3	71	750	
F005F3.0	524	11.10	0.284	28	11	72	123	
M006F3.0	513	10.20	0.293	2	11	72	149	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M001F3.0

F002F3.0

F003F3.0

F004F3.0

F005F3.0

M006F3.0

H. CALIFORNIUM-249

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
M002G0.0	509	10.50		28 11 72	123	
M003G0.0	509	10.10		28 11 72	123	
M002G0.1	486	13.10	0.00063	5 7 72	269	
M003G0.1	486	10.10	0.00063	5 7 72	269	
M006G0.1	486	11.60	0.00064	5 7 72	269	
M002G0.5	514	12.00	0.00514	29 2 72	396	
M003G0.5	514	12.60	0.00518	29 2 72	396	
M006G0.5	514	11.80	0.00511	29 2 72	396	
F001G1.0	555	8.58	0.0154	16 12 71	471	
M002G1.0	486	11.40	0.0152	5 7 72	269	
M003G1.0	486	11.50	0.0154	5 7 72	269	
F004G1.0	555	10.50	0.0154	16 12 71	471	
M006G1.0	524	10.60	0.016	28 11 72	123	
F001G2.0	558	9.32	0.0905	16 12 71	471	
M002G2.0	555	11.00	0.0916	16 12 71	471	
M003G2.0	486	10.80	0.0935	5 7 72	260	
F004G2.0	558	10.30	0.0915	16 12 71	471	
F005G2.0	555	9.44	0.0913	16 12 71	471	
M006G2.0	524	10.00	0.0963	28 11 72	123	
F001G3.0	584	11.60	0.29	24 2 71	766	
M002G3.0	580	13.20	0.282	24 2 71	766	
M003G3.0	580	13.70	0.284	24 2 71	766	
F004G3.0	580	8.79	0.283	24 2 71	766	
M006G3.0	524	10.10	0.293	28 11 72	123	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M002G0.0
M003G0.0

M002G0.1
M003G0.1
M006G0.1

M002G0.5
M003G0.5
M006G0.5

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

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

F001G3.0
M002G3.0
M003G3.0
F004G3.0
M006G3.0

TABLE II. TEST ANIMALS (MAR. 31 1973)

A. RADIUM-226*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	220	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	1140	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		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	471	15.70	1.12	3	3	59	1737	1603
T035R3.0J	570	9.44	0.951	5	5	59	8	14
T036R4.0	695	10.20	2.99	22	12	60	1154	3647
T037R4.0	695	9.53	3.0	22	12	60	1627	3234
T038R4.0	695	10.10	3.02	22	12	60	1503	3657
T040R1.0	899	13.00	0.0483	3	4	62	7	1
T041R1.0	899	12.70	0.0487	3	4	62	63	3
T042R1.7	967	14.00	0.146	4	4	62	7	2
T043R1.7	963	13.20	0.145	4	4	62	64	11
T044R3.0	938	11.10	0.937	4	4	62	68	71
T045R3.0	939	13.60	0.941	5	4	62	7	12
T046R3.0	810	12.50	0.928	5	4	62	69	103
T047R6.0	99	5.27	29.4	11	6	62		352
T048R6.0	2342	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
T035R3.0 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 AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
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	60	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
T072R5.0	4776	9.45	12.4	17 8 72	54	1195

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

T35R3J also received 99 μCi ^{85}Sr .

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

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

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

T071R5.0 MELANOMA ORAL CAVITY
T072R5.0 SPECIAL STUDY

B. PLUTONIUM-239*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 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

TU00P5.0	SPECIAL STUDY
TU01P5.0	SPECIAL STUDY
TU02P5.0	SPECIAL STUDY
TU03P5.0	SPECIAL STUDY
TU04P5.0	SPECIAL STUDY
TU05P5.0	SPECIAL STUDY
TU06P5.0	SPECIAL STUDY
TU07P5.0	SPECIAL STUDY
TU08P5.0	SPECIAL STUDY
TU09P5.0	SPECIAL STUDY
TU10P5.0	SPECIAL STUDY
TU11P5.0	SPECIAL STUDY
TU12P5.0	SPECIAL STUDY
TU13P5.0	SPECIAL STUDY
TU14P5.0	SPECIAL STUDY
TU15P5.0	SPECIAL STUDY
TU16P5.0	SPECIAL STUDY
TU17P5.0	SPECIAL STUDY
TU18P5.0	SPECIAL STUDY
TU19P5.0	OSTEOSARCOMA, BLOOD DYSCRASIA, LIVER DEGENERATION
TU20P5.0	LIVER DEGENERATION, ASCITES + THROMBOCYTOPENIA
TU21P5.0	TOXIC NEPHRITIS + LIVER DEGENERATION

TU23P1.0	SPECIAL STUDY
TU24P1.0	SPECIAL STUDY
TU25P1.0	SPECIAL STUDY
TU26P1.0	SPECIAL STUDY

TU27P3.0	SPECIAL STUDY
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TU28P1.0	SPECIAL STUDY
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TU29P3.0	SPECIAL STUDY
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DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
TU30P1.0	548	12.40	0.0148	9	8	61	30	1
TU31P3.0	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
TU35P3.0	550	11.90	0.303	15	9	61	362	180
T036P1.0	544	10.40	0.0158	15	9	61	5	
TU37P1.0	542	8.59	0.0148	15	9	61	186	4
T038P3.0	489	7.96	0.304	15	9	61	187	90
T039P1.0	1534	10.70	0.0151	15	9	61	376	10
T040P1.0	1534	9.92	0.0177	15	9	61	769	20
T041P5.0	543	8.50	3.01	30	11	64	1227	5586
T042P5.0	510	11.40	2.4	10	2	65	13	57
T043P5.0H	600	14.00	2.86	15	7	65	40	200
T044P5.0H	517	12.00	2.72	21	9	65	35	168
T045P5.0H	420	12.30	2.98	28	10	65	5/24	1
T046P5.0	420	11.90	3.01	28	10	65	732	3402
T047P5.0	806	12.40	3.02	30	11	65	69	353
T048P5.0	554	8.50	2.61	11	3	66	1327	5222

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

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ CI/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
T049P1.0	103	5.00	0.0162	5 7 66	2461	
T050P3.0	103	5.30	0.296	5 7 66	2461	
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	1481	
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	1298	
T058P3.0E	573	52.30	0.291	10 9 69	917	408
T059P3.0E	591	44.50	0.29	5 11 69	973	431
T060P3.0E	567	45.20	0.314	6 1 70	784	379
T061P2.0E	581	47.20	0.0983	6 1 70	1180	
T062P2.0E	583	52.50	0.156	22 1 70	1164	

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 OSTEOSARCOMA

T060P3.0E OSTEOSARCOMA

T061P2.0E

T062P2.0E

DUG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
T063P5.0	581	9.13	2.77	14 12 70	490	2129
T064P0.0	595	8.60		28 12 70	824	
T065P4.0P	542	11.50	0.904	30 11 71	14	2
T066P4.0P	542	10.60	0.913	30 11 71	500	
T067P4.0P	542	10.40	0.907	30 11 71	500	
T068P1.0E	569	44.20	0.0158	20 4 72	345	
T069P1.0E	569	32.00	0.016	20 4 72	345	
T070P1.0E	588	40.60	0.0152	9 5 72	326	
T071P0.5E	588	48.90	0.00521	9 5 72	326	
T072P0.5E	611	44.50	0.00512	1 6 72	303	
T073P0.5E	611	38.80	0.00507	1 6 72	303	
T074P4.0	3694	7.73	0.937	28 3 73	3	
T075P4.0	3478	8.47	0.897	28 3 73	3	
T076P4.0	3413	10.70	0.894	28 3 73	3	
T077P3.0	3413	9.95	0.31	28 3 73	3	
T078P3.0	2488	8.21	0.32	28 3 73	3	

* 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 SPECIAL STUDY

T064P0.0

T065P4.0P SPECIAL STUDY

T066P4.0P

T067P4.0P

T068P1.0E

T069P1.0E

T070P1.0E

T071P0.5E

T072P0.5E

T073P0.5E

T074P4.0

T075P4.0

T076P4.0

T077P3.0

T078P3.0

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 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

TU03M5.0 ULCERATIVE GINGIVITIS, SEVERE ANEMIA + CRIPPLING FRACTURE

D. THORIUM-228 (RADIOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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 31/3/73 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
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T001S0.0	SPECIAL STUDY
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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
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T008S2.0H	SPECIAL STUDY
T009S2.0H	SPECIAL STUDY
T010S2.0H	SPECIAL STUDY
T011S2.0H	SPECIAL STUDY

T012S3.0	BREMSSTRAHLUNG PHANTOM
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T013S4.0	BREMSSTRAHLUNG PHANTOM SAM MCGEE
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T014S5.0	SPECIAL STUDY
T015S5.0	SPECIAL STUDY

T016S2.0	SPECIAL STUDY
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T017S6.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
T018S6.0	HEMANGIOSARCOMA (ISCHIUM)

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 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	363	7.20	8.3	2	10	63	13	143
T022S5.0	545	9.01	99.0	1	4	69	1460	
T023S5.0	545	11.60	100.0	1	4	69	1379	10591

* 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 in-
jection 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 OSTEOSARCOMA

F. RADIUM-224*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ Ci/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/73 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	3405	
T012Q3.0	495	13.50	0.889	4 12 63	3405	
T013Q3.0	495	11.30	0.912	4 12 63	3405	
T014Q3.0	438	10.30	0.87	4 12 63	3405	
T015Q4.0	515	12.70	2.73	1 2 68	1692	100
T016Q2.0	515	9.36	0.31	1 2 68	1885	
T017Q2.0	515	10.20	0.311	1 2 68	1885	
T018Q2.0	502	9.68	0.306	1 2 68	1885	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001Q3.0J SPECIAL STUDY

T002Q4.00 OSTEOSARCOMA
T003Q4.0 HEMANGIOSARCOMA

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

T006Q6.0 PURPURA HEMORRHAGICA

T007Q5.0 OSTEOSARCOMA
T008Q5.0 PURPURA HEMORRHAGICA

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

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

T015Q4.0 OSTEOSARCOMA

T016Q2.0
T017Q2.0
T018Q2.0

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T019Q1.0	515	11.80	0.0475	1	2	68	1885	
T020Q1.0	515	10.40	0.0472	1	2	68	1885	
T021Q1.0	502	9.08	0.0447	1	2	68	1885	

* 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 31/3/73 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	20	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
T101W5.0	399	10.40	2.98	17	8	72	1	3
T102W3.0	515	11.60	0.28	10	10	72	17	5
T103W3.0	501	10.60	0.283	10	10	72	172	
T104W3.0	2658	7.67	0.305	28	11	72	123	
T105W3.0	2224	7.78	0.301	28	11	72	123	
T106W3.0	2224	13.80	0.308	28	11	72	123	

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

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

T101W5.0 SPECIAL STUDY

T102W3.0 SPECIAL STUDY

T103W3.0

T104W3.0

T105W3.0

T106W3.0

H. LEAD-210*

DOG NUMBER	AT INJECTION		INJECTED (μ CI/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/73	DEATH	
T001L5.0	522	9.78	10.7	24	6	69	1376		
T002L5.0	522	9.16	10.7	24	6	69		28	180
T003L5.0	522	9.78	10.7	24	6	69	1100		10992

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001L5.0

T002L5.0

T003L5.0

SPECIAL STUDY
OSTEOSARCOMA

I. CALIFORNIUM-252

DOG NUMBER	AT INJECTION		INJECTED (μ ci/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/73 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 31/3/73 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T00165.0	597	12.20	2.84	24	2	71	500	2348
T00265.0	584	10.70	2.77	24	2	71	7	26
T00365.0	584	9.89	2.8	24	2	71	21	95

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001G5.0	NEPHRITIS; MYOCARDIAL INFARCTION
T002G5.0	SPECIAL STUDY
T003G5.0	SPECIAL STUDY

K. CURIUM-243/244

DOG NUMBER	AT INJECTION		INJECTED (μ ci/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/73	DEATH	
T001C5.0	511	10.40	2.6	27	2	73	32		
T002C5.0	485	12.20	2.64	27	2	73		6	24
T003C5.0	485	11.40	2.64	27	2	73		13	52
T004C5.0	485	12.50	2.64	27	2	73		20	80
T005C5.0	485	12.80	2.63	27	2	73	32		

DOG
NUMBER

COMMENTS ON DEAD DOGS

T001C5.0

T002C5.0

T003C5.0

T004C5.0

T005C5.0

SPECIAL STUDY

SPECIAL STUDY

SPECIAL STUDY

L. ANCILLARY

[illegible]

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	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED		AGE (DAYS)	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	
F045A						6182	
F047A						1732	
F048A						5075	
F049A						4773	
M050A						2264	
F051A						1089	
F052A						509	
F053A						5520	
F054A						3190	
F055A						4563	
M056A						701	
F057A						4322	
M058A						767	
M059A						567	
M061A						5506	
F062A						5348	
F063A						4530	
F068A						4521	
F070A						4862	
M071A						1472	
M073A						5695	
F074A						5553	
M075A						5284	
F076A						4772	
F077A						4757	
F078A						4491	
F079A						4359	
F080A						4346	
F081A						4346	
M082A						3627	
F083A						4033	
M084A						4033	
M085A						4303	
M086A						499	
F087A						3541	
F088A						3541	
F089A							
F090A							
F091A						3471	
F092A						3471	
M093A							
F094A							
F095A						3401	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F045A	PNEUMONIA; SENILITY
F047A	SPECIAL STUDY
F048A	CHRONIC PANCREATITIS
F049A	ISLET CELL TUMOR; BRAIN HEMORRHAGE
M050A	SPECIAL STUDY
F051A	SPECIAL STUDY
F052A	SPECIAL STUDY
F053A	THYROID CARCINOMA
F054A	SPECIAL STUDY
F055A	NEPHRITIS; METASTATIC CALCIFICATION
M056A	VOLVULUS + PERITONITIS
F057A	UNDIFFERENTIATED MALIGNANCY
M058A	SPECIAL STUDY
M059A	SPECIAL STUDY
M061A	
F062A	AORTA THROMBO-EMBOLISM
F063A	MAMMARY CARCINOMA; TRANSITIONAL CELL CARCINOMA
F068A	UNDIFFERENTIATED CARCINOMA
F070A	
M071A	SPECIAL STUDY
M073A	DEGENERATION OF ADRENAL GLAND + DIABETES MELLITUS
F074A	LYMPHO SARCOMA
M075A	AORTIC THROMBUS
F076A	
F077A	
F078A	
F079A	PNEUMONIA
F080A	
F081A	
M082A	BRONCHIOGENIC CARCINOMA
F083A	
M084A	
M085A	
M086A	SPECIAL STUDY
F087A	
F088A	
F089A	REASSIGNED, SEE T074P40
F090A	REASSIGNED, SEE T075P40
F091A	
F092A	
M093A	REASSIGNED, SEE T076P40
F094A	REASSIGNED, SEE T077P30
F095A	

DOG NUMBER	AT INJECTION		INJECTED (μ Ci/KG)	DATE INJECTED		AGE (DAYS) AS OF 31/3/73	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO		
F096A						3384	
F097A						3384	
F098A						3290	
F099A							479
M100A							407
M101A							290
F102A							243
M103A							217
M104A							188
F105A							157
F106A						2985	
F107A						2894	
F108A							1969
F109A							2253
F110A							
F111A						2479	
F112A						2210	
F113A						1346	
F114A						1350	
F115A						1499	
F116A						986	
F117A						986	
F118A						986	
F119A						910	
F120A						910	
F121A						986	
F122A						742	
F123A							375
F124A						742	
F125A						692	
F126A						692	
F128A						662	
F129A						659	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F096A

F097A

F098A

F099A

SPECIAL STUDY

M100A

SPECIAL STUDY

M101A

SPECIAL STUDY

F102A

SPECIAL STUDY

M103A

SPECIAL STUDY

M104A

SPECIAL STUDY

F105A

SPECIAL STUDY

F106A

F107A

F108A

ENCEPHALOMALACIA (BACTERIAL)

F109A

METRITIS

F110A

REASSIGNED, SEE T078P30

F111A

F112A

F113A

F114A

F115A

F116A

F117A

F118A

F119A

F120A

F121A

F122A

F123A

ACCIDENTAL STRANGULATION

F124A

F125A

F126A

F128A

F129A

SOME SCIENTIFIC ADVANTAGES IN THE USE OF THE BEAGLE
IN LONG TERM RADIOBIOLOGICAL STUDIES*

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ABSTRACT: The beagle was originally chosen as an experimental animal in radiobiology primarily because of its relatively long lifespan and metabolic similarity to man. Some obvious scientific advantages in using the beagle are:

1. Skeletal similarity to man.
2. Important jaw syndrome induced in man and beagles, but not rodents.
3. Relevant radiographic information.
4. Osteosarcoma location.
5. Possible difference in shape of dose-response: Rodents vs. large mammals.
6. Unique sex difference in bone sarcoma induction: Mice vs. humans and beagles.
7. Growth dynamics of osteosarcomas.
8. Liver retention of ^{239}Pu .
9. Advantage in obtaining sequential blood samples in beagles.
10. Corticosteroid production similarity between man and beagle.
11. Transaminase and alkaline phosphatase similarities between man and beagle.
12. Advantage of larger organ size in radionuclide distribution studies.

INTRODUCTION

In 1950 the world's first large-scale radiation experiment with dogs was started at the University of Utah.⁽¹⁻³⁾ The primary objective was to predict the toxicity of ^{239}Pu in man from effects observed in beagles. The beagle was chosen as the experimental animal primarily because of its skeletal similarity to man and because the effects of internally-deposited radionuclides could be observed throughout its relatively long lifespan. The

beagle proved to be an excellent choice, and it has now become the standard dog in animal research.⁽⁴⁻⁶⁾

Beagle studies have provided a wealth of information on the toxicity of radionuclides.⁽⁷⁻¹⁴⁾ But the question is sometimes raised, despite the fact that humans are generally much more similar to beagles than to mice and rats, as to whether the beagle results could have been derived from rodents. Therefore, it is appropriate to review some radiobiological findings of importance to mankind that were derived from beagles, but were unlikely to have been discovered in mice and rats. Ancillary studies in mice, rats, rabbits, and chinchillas are very important and provide inexpensive insight into long term studies. It is obvious that more is likely to be learned from a variety of species, than by confining studies to only one type of experimental animal.

SOME SCIENTIFIC ADVANTAGES OF THE BEAGLE

The following topics are restricted to some of the more interesting findings in beagles from our own laboratory. The reader is also referred to important results on radionuclide toxicity from other beagle laboratories at Richland,⁽¹⁵⁾ Ft. Collins,⁽¹⁶⁾ Albuquerque,⁽¹⁷⁾ Davis,⁽¹⁸⁾ and Argonne.⁽¹⁹⁻²⁰⁾

1. SKELETAL SIMILARITY TO MAN.

The adult beagle skeleton is an excellent experimental model for studying the physiology, pharmacology, and disease processes of the human skeleton. Evidence is summarized concerning adequacy of various animal skeletons for research in the following two tables from a volume written by Dr. Harold M. Frost, Professor of Orthopedic Surgery, Henry Ford Hospital, Detroit, Michigan.⁽²¹⁾

The various bone activities listed in Table 1 are:

1. MODELING - The sculpturing, or architecturally oriented cellular activity which determines the gross architectural features of the skeleton and maintains them in a proportional sense throughout life.
2. SKELETAL MATURATION - Involves the removal of transient mineralized tissues (calcified cartilage cores and fibrous bone) with the subsequent replacement by lamellar bone.
3. REMODELING - The removal of older material and replacement with nearly equal amounts of newly produced lamellar bone, i.e., "turnover".

The two tables state clearly that only larger animals, such as dogs, cows, horses, sheep, goats, monkeys, apes, and man possess bone remodeling activity; small ones do not. This means that experimental animals such as the rat, mouse, shrew, and guinea pig do not exhibit useful amounts of secondary haversian (osteonal) bone remodeling, and, therefore, do not apply to the study of human bone remodeling.

Students of bone morphology agree that the bone histology of the dog and human skeleton are quite similar. The characteristics of dog bone are being studied in the Bone group headed by Dr. W. S. S. Jee at the Radiobiology Division, Salt Lake City, Utah; by Dr. Z. F. G. Jaworski at the Department of Medicine, Ottawa General Hospital, Canada; by Dr. G. Marotti at the Institute of Normal Human Anatomy, Faculty of Medicine and Surgery, Sassari, Italy.

The two types of bone to study are the trabecular (spongy) bone and cortical (compact) bone. The trabecular bone has large amounts of surface area which function actively in body physiology, while cortical bone serves more as support. In both beagles and humans, there is a loss of the quantity of both types of bone with age.

TABLE 1. BONE MODELING, MATURATION, AND REMODELING IN VARIOUS MAMMALS†

Mammal	Possesses Bone Modeling Activity	Exhibits Skeletal Maturation	Possesses Bone Remodeling Activity
Child	+	+	+
Adult human	0	n.a.*	+
Mouse	+	0	0
Shrew	+	0	0
Young rat	+	0	0
Very old rat	+	0	+
Young rabbit	+	+	0
Old rabbit	0	n.a.*	+
Dog (young)	+	+	+
Dog (old)	0	n.a.*	+
Monkey (young)	+	+	+
Monkey (old)	0	n.a.*	+
Shark, dogfish	0	0	0
Lamprey	0	0	0

*Not applicable.

† As an aid to interpreting research reports (and to designing better and more relevant experiments), this table lists common animals which include modeling and remodeling activities in their manifest of skeletal behavior. Note that some follow the human pattern of exhibiting both modeling and remodeling activities early in life, but the latter only after skeletal maturity; such animals provide the best experimental subjects to use in research oriented towards human skeletal disease. Those animals that do not attain a clear-cut skeletal maturity continue to grow throughout life, thus displaying modeling activities throughout life; they provide very poor model systems of skeletal disease acquired by man in adult life, a fact which inspection of current periodicals reveals needs for wider recognition. The functional unit of bone remodeling activity is the Basic Metabolic Unit (BMU). Each BMU begins in previously-formed bone when some stimulus causes mesenchymal cells near bone surfaces to proliferate and produce new daughter cells. This is followed by a batch of osteoclasts which resorb a "packet" of bone. The resorptive activity then subsides, the osteoclasts disappear, and osteoblasts appear at the same site, whereupon new bone formation begins. An amount nearly equal to that which was resorbed is formed in this position. Most small animals do not possess sufficient BMU-based remodeling activity to serve as valid models of diseases of human, a fact not previously published. The shark and lamprey, lacking any capacity for skeletal resorption, also lack totally any skeletal remodeling capacity, for which resorption forms an essential phase.⁽²¹⁾

TABLE II. SKELETAL TISSUE TURNOVER MODALITIES IN ANIMALS*

Morphological Behavioral Factor	Normally present during		Animal Groups Involved	Simple Tissues Involved
	Growth	Adult Life		
Lamellar bone remodeling (compacta and secondary spongiosa)	Yes	Yes	2,3	Lamellar bone
Bone modeling (compacta)	Yes	No	1,2,3	Lamellar bone
Replacement: Primary spongiosa by secondary spongiosa	Yes	No	1,2,3	Lamellar bone fibrous bone hyaline cartilage
Replacement: Calcified cartilage by primary spongiosa	Yes	No	1,2,3	Fibrous bone hyaline cartilage
Group (1) Animals: No significant amount of true lamellar bone remodeling activity: shrew, mouse, gerbil, hamster, small birds, small teleost fishes, immature rats, immature rabbits, chipmunk.				
Group (2) Animals: Significant amounts of true lamellar bone remodeling occur only in adult life: Rabbits, squirrels, rats, chickens, marmosets.				
Group (3) Animals: True bone remodeling occurs throughout postnatal life: dogs, cows, horses, sheep, goats, monkeys, apes, man.				

*A listing of the cellularly driven tissue modalities which, alone or when summed up over the skeleton, cause turnover of skeletal tissues to occur at the organ and higher levels of organization, and which studies of bone tissue turnover in intact animals by any appropriate means will reflect. One meaning of this table is as follows: If one's grant permits one to study a human disease that arises by virtue of an abnormality in true bone remodeling (postmenopausal osteoporosis, spontaneous fractures), then choosing mice or rats as one's experimental animal practically guarantees that the study will produce irrelevant information.(21)

Trabecular (spongy) bone of the beagle behaves like that of human. There is a comparable net loss of trabecular bone morphologically with age at various sites in both adult beagle and adult human. Trabecular bone of the human iliac crest shows a rate of loss of 0.1%/yr.⁽²²⁾ Further human studies in lumbar vertebral body trabecular bone showed a similar loss of 0.1%/yr. A sex difference was detected, females starting and ending with less trabecular bone, though rates of loss were the same.⁽²³⁾ A third study demonstrated a rate of loss of human vertebral body trabecular bone of 0.13%/yr.⁽²⁴⁾ Jee et al. showed a rate of loss of beagle vertebral body trabecular bone of 0.6%/yr.⁽²⁵⁾ In more recent work, Jee states that the loss of beagle vertebral body trabecular bone moves at a rate of 0.3%/yr for male animals and 0.6%/yr for female animals, with female animals ending far lower.⁽²⁶⁾ In summary, beagle and human studies show similar patterns of loss of trabecular bone and the loss occurs approximately 5 times faster in the dog than in the human.

The amount of trabecular bone surface is of special interest to the radiation biologist because it represents surface available for deposition of bone surface seeking radionuclides. Again, as in the bone volume studies, the amount of bone surface loss with age in both human and beagle studies are quite similar. The study of the human iliac crest cites a rate of loss of about 0.4%/yr.⁽²²⁾ Studies on the human vertebral body have shown respectively rates of loss of about 0.6% and 0.7%/yr.^(23,24) The studies of beagle lumbar vertebra for this feature show a loss rate of about 1.6%/yr.⁽²⁶⁾ In summation of this particularly important feature of morphology of bone, it is seen that the beagle again resembles the human in loss of bone surface with age, though the rate here is only about 3 times faster.

A study of trabecular thickness changes in aging is important in determining

the exact nature of bone volume loss noted earlier. One study on the human vertebra concludes that the trabecula become progressively thinner in older material.⁽²⁴⁾ The beagles studies show a similar loss of thickness of trabeculae with age.⁽²⁶⁾

The loss of cortical (compact) bone of humans and beagles is also similar. In a study on the human rib, the cortical cross-sectional rate of loss was 0.15%/yr from young to aged material.⁽²¹⁾ In work conducted on the beagle metatarsal, a rate of loss of cortical cross-sectional area of 0.4%/yr was shown.⁽²⁷⁾ This factor falls in the area of others of about 3 times as fast in the beagle as the human.

The rate of marrow cavity expansion in relation to total area of the bone shows a similar pattern in humans and beagles. Frost shows a rate of 0.6%/yr in human rib,⁽²¹⁾ while the beagle metatarsal studies show a more rapid, but still characteristic change in this feature, at a rate of 4.3%/yr.⁽²⁷⁾ This is a beagle/human ratio of seven.

The years of a beagle's life have been characterized as follows: The first two years are equal to 24 human years, then each ensuing year of beagle life is the same as 4 years of human life. Bone volume, bone surface, trabecular thickness, and cortical cross-sectional area disappear 3-5 times as fast in beagles as in humans. Biologically-speaking, then, the finding that most of the adult bone loss parameters proceed at a rate 3-5 times that of humans would lead us to a conclusion that the skeletons are similar (Table III). The advantage lies with the researcher who studies the beagle skeleton because the life story of the beagle is completed to the same degree as the human, but in a more rapid style.

TABLE III. COMPARATIVE YEARLY CHANGES IN BONES OF MAN AND BEAGLES

Worker	Adult Animal	Sites*	% Bone Change	Bone surface change	Reduction in thickness trabecular	Rate X-Sect. Enlarge	$\frac{M_a^{**}}{P_a}$
Schenk	Human	IC	-0.1%	-0.4%	n.a.	n.a.	n.a.
Bromley	Human	LVB	-0.1%	-0.7%	n.a.	n.a.	n.a.
Arnold	Human	LVB	-0.13%	-0.6%	yes	n.a.	n.a.
Frost	Human	Rib	n.a.	n.a.	n.a.	-0.15%	+0.6%
Jee	Beagle	LVB	-0.6%	n.a.	n.a.	n.a.	n.a.
Jee	Male beagle	LVB	-0.3%				
Jee	Female beagle	LVB	-0.6%	-1.6%	yes	-0.40%	+4.3%
Ratio (Beagle/human)			5x	3x	+	3x	7x

* IC - Iliac crest; LVB-Lumbar vertebral body

**M_a = Area of marrow cavity of bone

P_a = Total area of bone including cortex and marrow cavity

2. IMPORTANT JAW SYNDROME INDUCED IN MAN AND BEAGLES, BUT NOT RODENTS.

Necrosis of the mandible and maxilla, including infection, bone destruction, and loss of teeth, was the first evidence of damage to be recognized in the radium dial painters.⁽²⁸⁾ This condition, descriptively called "radium jaw", was initially described by Harrison Martland as follows: "We think that the bone necrosis in these cases has nothing to do with the actual deposition of radioactive element in the bone itself, but it leaves on the gum, on the roof of the mouth, and the teeth minute particles [of radioactive dial paint] which stimulate the normal bacteria of the mouth to increased activity."⁽²⁹⁾ Later, Martland changed his opinion and wrote, "...it was more logical to interpret the jaw necrosis as being primarily

due to a radiation osteitis similar to that occurring in the bones."⁽³⁰⁾ But it was not until over a quarter century later that the etiology of radium jaw was finally resolved as being mainly due to radium reaching the bones and teeth through the blood stream, rather than by direct deposition of the ingested dial paint on the inside of the mouth. The similar dental changes seen in the dial painters were also seen in beagles injected intravenously with radium, plutonium, and other α -emitters.⁽³¹⁾ This proved that radionuclide-induced dental changes do not require an oral route of entry but can be caused by high doses of bone-seeking α -emitters entering the bloodstream by a variety of routes. It seems unlikely that this question could have been resolved using rodents whose teeth grow continually throughout the animal's life, and whose tooth and bone structure is quite dissimilar to that in dog and man. This very important aspect of the "radium jaw" syndrome in man and dog has not been described in rodents.

3. RELEVANT RADIOGRAPHIC INFORMATION.

Because of much larger bone size, the beagle is decidedly superior to the smaller rodents for detailed radiography of bone conditions relevant to those in man. The following is a good example of this fact.

A sizable number of employees in the nuclear industry have accidentally acquired measurable body burdens of ^{239}Pu as a result of occupational exposure,⁽³²⁻³⁶⁾ and as the nuclear industry expands, it is inevitable that additional persons will become exposed to plutonium. Of particular interest are the detailed follow-up studies by Wright Langham and colleagues of Los Alamos plutonium workers whose estimated body burdens nearly all lie below $0.4 \mu\text{Ci}$ ^{239}Pu (presently permissible body burden of ^{239}Pu in radiation workers is 10 times lower, or $0.04 \mu\text{Ci}$).

Based on the experience of our radiologist, W. R. Christensen, on the ^{239}Pu -induced changes he had observed radiographically in the Utah beagles, the number of x-ray pictures taken during periodic medical examination of selected higher-burden plutonium workers was reduced to those radiographs that were best for the detection of Pu-induced injury. These were radiographs of the chest, pelvis, single projection film of the right femur, distal femur showing metaphyseal bone, and dental films.⁽³⁵⁾

Some concern was expressed about what appeared to be an excess of "bone islands" (patches of excess calcification in the skeleton) in persons with plutonium burdens. However, after finding this condition can occur in men without plutonium exposure, and with the evidence that plutonium does not induce an excess of bone islands in beagles, it was concluded that this observation in the plutonium workers was within normal limits.⁽³⁶⁾ This is an example of how careful observations in a long-lived mammal with a human-like skeletal structure and a much larger bone size than rodents can be applied directly to questions of radionuclide toxicity in man. It further indicates the importance of using an animal model in which precise radiography can be done.

4. OSTEOSARCOMA LOCATION.

An extremely important endpoint in bone-seeking radionuclide toxicity is the induction of osteosarcomas. Table IV lists the distribution of spontaneous and ^{226}Ra and ^{239}Pu induced osteosarcomas in man, dog, and mouse, and emphasizes the distribution in man is more similar to that in the dog than to that in the mouse.⁽³⁷⁻⁴²⁾

TABLE IV. DISTRIBUTION OF SPONTANEOUS AND RADIATION INDUCED OSTEOSARCOMAS ⁽⁴²⁾

Species	Treatment	% Distribution		Reference
		Axial	Appendicular	
Man	spontaneous	20	80	(37,38)
Man	Ra-226	10	90	(39)
Dog	spontaneous	22	77	(40)
Beagle	Ra-226	33	65	(42)
Beagle	Pu-239	44	55	(42)
Mice	Ra-226	73	27	(41)
Mice	Pu-239	78	22	(41)

5. POSSIBLE DIFFERENCE IN SHAPE OF DOSE-RESPONSE: RODENTS VS. LARGE MAMMALS.

In only 1 of 5 rodent experiments with α -emitters could a linear dose-response relationship be rejected, ⁽⁴³⁾ and in this one experiment ⁽⁴⁴⁾ the animals were plagued with intestinal infection and amyloid disease that may have shortened the average lifespan sufficiently to have prevented the appearance of bone sarcomas at the two lowest dose-levels. At present, the weight of evidence supports a linear relationship for incidence vs. dose in the induction of bone sarcomas by low doses of α -irradiation in rodents.

In contrast, when α -particles have induced bone sarcomas in humans, the dose-response relationship for the present data is best fit by a sigmoid (S-shaped), or practical threshold relationship in which very few cancers are induced at low doses, both for long-lived radium ^(43,45-48) and for short-lived ^{224}Ra . ^(43,49) Could it be that the dose-response relationship for α -particles is different in the higher mammals than in rodents? Because of this possible difference, results in beagles are of special interest. In all

4 beagle experiments with ^{239}Pu , ^{228}Th , ^{228}Ra , and ^{226}Ra , our present results support a sigmoid, or practical threshold relationship in accord with present results in humans. It must be pointed out that both the human and beagle experiments are still in progress. Future results could weaken (or strengthen) present trends. Additional follow-up is especially needed at dose-levels just below those at which cancers have already been induced. The question as to whether or not a fundamental difference exists between the shapes of the dose-response curves for higher mammals and rodents seems unlikely to be resolved if future studies were confined to rodents alone.

6. UNIQUE SEX DIFFERENCE IN BONE SARCOMA INDUCTION: MICE VS. HUMANS AND BEAGLES.

Effects seen in the radium dial painters are used to predict risks to plutonium workers. ^(43,45-48,50-52) But it must be remembered that the vast majority of the human radium cases are women, whereas nearly all plutonium workers are men. Therefore, it is not surprising that among the 54 cases of bone sarcoma that have occurred among the studied group of radium cases only 3 were in men. ⁽⁵³⁾ The radium human data alone are insufficient to make an accurate assessment of the risk to men vs. women. Is it reasonable to assume that males and females are equally sensitive to bone sarcoma induction?

The most extensive investigation on the induction of bone sarcomas in males vs. females has been done in NMRI mice injected with ^{224}Ra at Neuherberg, Germany. ^(54,55) An analysis indicated that at the doses below those producing peak incidence, the female mice were over 10 times more sensitive per rad than the male mice. ⁽⁴³⁾ A greater incidence of bone sarcomas in female mice, relative to males, has also been found in CFI mice by Miriam Finkel, ^(56,57) in C3H mice by John Loutit, ⁽⁵⁸⁾ and in C57 Bl mice at our own laboratory. ⁽⁵⁹⁾

If the sex differences observed in mice had been used in converting ^{226}Ra effects in women into ^{239}Pu predictions for men, the risk to plutonium workers would have been grossly underestimated. However, no sex differences were applied^(51,52) because prior results in our colony indicated an approximately equal sensitivity to bone sarcoma induction in male and female beagles,⁽⁶⁰⁾ and beagles were assumed to be more similar to man than were mice. Subsequently, the correctness of this assumption was confirmed by the equal sensitivity of German male and female patients to ^{224}Ra -induced bone sarcomas.⁽⁴⁹⁾

The cause of the very marked sex difference in osteogenic sarcoma incidence in mice is not known, but many rodents differ significantly from man and beagle in their sexual cycle in that they do not ovulate spontaneously.⁽⁶¹⁾

7. GROWTH DYNAMICS OF OSTEOSARCOMAS.

The best information on the growth rates for human bone sarcomas has come from sequential radiographs of pulmonary metastases. In a limited series of 6 patients with bone sarcomas (4 osteosarcomas and 2 Ewing's sarcomas) the pulmonary metastases appeared to grow exponentially with volume doubling times ranging from 19 to 72 days, with a log-normal mean of 32 days.⁽⁶²⁾ Excluding the 2 cases of Ewing's sarcoma, the corresponding log-normal mean for the 4 osteosarcoma cases is 33 days, which is almost identical to the combined mean of 32 days.

It requires 40 volume doubling times for a tumor to enlarge from a diameter of 0.001 cm (size of a typical tumor cell) to a diameter of 10 cm (the size of a typical human tumor at death).⁽⁶³⁾ Thus, assuming an origin from a single cell and a constancy of doubling time, the calculated time before death at which these human bone sarcomas typically began to enlarge would be,⁽⁶²⁾

$$\frac{(40 \text{ doublings}) (32 \text{ days/doubling})}{365 \text{ days/year}} = 3.5 \text{ years}$$

with individual growth periods ranging between 2 and 8 years. A typical growth period of 3.5 years is consistent with the shortest reported time of 4 years from the start of irradiation to death from an induced bone sarcoma in a human.⁽⁴⁹⁾

These preliminary evaluations of tumor growth dynamics, if confirmed, could have important application to post-operative follow-up of cancer patients.⁽⁶³⁾ It suggests that for cancers which grow exponentially, recurrence from incomplete ablation of tumor tissue should be detectable within 30 volume doubling times, the time required for a single tumor cell to grow into a detectable nodule of 1 cm.⁽⁶³⁾ If recurrence fails to appear within this time, the patient would probably remain free from his original malignancy. But not all tumors grow exponentially, i.e., not all tumors grow with constant doubling times. Do bone sarcomas grow exponentially?

Because we radiograph our beagles at frequent intervals it was possible to calculate volume doubling times for 249 osteosarcomas in beagles.⁽⁶⁴⁾ Growth appeared to remain exponential (constant doubling time) during the times the tumors were visible radiographically in beagles,⁽⁶⁴⁾ in contrast to a lengthening of the doubling time of radiation-induced osteosarcomas in mice⁽⁶⁵⁾ as the tumor volume expanded. Perhaps this was due to the inability of the small mouse to sustain the rapid growth of a large tumor mass. In beagle osteosarcomas, the volume doubling times ranged from 5 to 60 days, and the mean tumor growth rate corresponded to a doubling time of 12 days. As might be expected due to the greater metabolic rate of dogs, beagle osteosarcomas tended to enlarge more rapidly than did the limited sample of 6

human bone sarcomas⁽⁶²⁾ although a considerable overlap in doubling times occurred. Assuming exponential growth from a single tumor cell, the average estimated start of growth was 1.3 years before dog death in good agreement with the shortest time from start of irradiation to death with an osteosarcoma of 1.5 years for those beagles.⁽⁶⁴⁾

The extensive studies of growth rates in beagle osteosarcomas reinforces the limited observations that have been possible to obtain directly in man, and may make possible better follow-up of treated cancer patients.

8. LIVER RETENTION OF ^{239}Pu .

The induction of liver tumors by low doses of ^{239}Pu has been infrequent or absent in mice^(44,66) and rats.^(67,68) This occurred regardless of whether the quickly-excreted monomeric^(66,69) or the more slowly excreted polymeric^(66,69) form of ^{239}Pu was used. It is possible that the short lifespan of mice and rats (median lifespan, about 2 years) is insufficient to allow complete expression of low-dose damage.

In our beagles injected intravenously with monomeric ^{239}Pu in citrate solution, ^{239}Pu is retained tenaciously in both the liver and skeleton,⁽⁷⁰⁾ similarly to that in man.^(71,72) For the first 7.6 years after injection no liver tumors were observed, but as of 1 March 1971, ten cases of liver tumors had appeared, 6 in males, and 4 in females, among the 96 plutonium-injected beagles that had died at least 1000 days after injection.⁽⁷³⁾ These 10 liver tumors were all of the bile duct type. All of these tumors occurred in the lower-dose dogs injected with 0.014 to 0.097 $\mu\text{Ci } ^{239}\text{Pu/kg}$, with liver doses ranging from roughly 100 to 400 rads. Additional liver tumors would undoubtedly have occurred at higher dose levels except that all of the higher-level dogs died from other effects, mostly bone sarcomas, before reaching

7.6 years after injection. Because ^{241}Am deposits more in the liver and less in the skeleton than ^{239}Pu , we expect to obtain more liver tumors in our ^{241}Am -injected dogs (introduced in 1966 through 1970) than in our original ^{239}Pu -injected dogs (introduced in 1952 through 1958). This will be a welcome supplement to our liver tumor results for ^{239}Pu . Furthermore, starting in 1964, and still continuing, we are injecting additional beagles at very low levels (0.0006 to 0.015 $\mu\text{Ci } ^{239}\text{Pu/kg}$), but none of these dogs have yet developed liver tumors or bone sarcomas.

For ^{239}Pu reaching the blood via absorption from lung or from puncture wounds, roughly half deposits in the liver and half in the skeleton of humans.^(52,72) Using results from our lowest-level plutonium dogs at which radiation-induced tumors have definitely appeared, we estimated that the risk to humans from liver tumors could be twice the risk from bone sarcomas for equal amounts of ^{239}Pu in liver and skeleton.⁽⁵²⁾ Except for the long-term toxicity studies in beagles, the risk from ^{239}Pu -induced liver tumors could have remained unsuspected until perhaps tragically discovered in humans.

9. ADVANTAGE IN OBTAINING SEQUENTIAL BLOOD SAMPLES IN BEAGLES.

It has been very valuable to follow sequentially the blood cell changes produced by internal irradiation⁽⁷⁴⁾ and age⁽⁷⁵⁾ in a long-lived species whose body, and hence blood supply is sufficiently large that the amount of blood removed in prior samples has negligible distortion on subsequent results. Perhaps this is partially why few sequential hematological evaluations on the same mouse have been made in radiobiological studies.

Another factor of considerable importance is the excellent adaptability of the beagle to laboratory life such that blood samples, etc. can be obtained without excitement and stress. The beagle is much more adaptable in this respect than many laboratory animals, especially rodents.

In beagles the hematological RBE of α -emitters that deposit on bone surfaces (^{239}Pu and ^{228}Th) is greater than that of α -irradiation resulting from bone volume seekers (^{226}Ra and ^{228}Ra), while that from the β -emitting bone volume seeker ^{90}Sr is less.⁽⁷⁴⁾ These results are in good biological agreement with evaluation of bone sarcoma RBE in the same beagles:^(50,51) Both show that radiation emitted from bone surfaces is more damaging than radiation emitted from within bone mineral, and that α -particle radiation is more damaging per rad than β -radiation.

It is interesting that bone sarcomas have occurred at or below dose levels producing significant hematological changes for the α -emitters tested, but the reverse occurs for β -emitting ^{90}Sr .^(51,74) Perhaps this difference is due to the greater penetration into the marrow of long-range β -particles from the bone-deposited radionuclides.

10. CORTICOSTEROID PRODUCTION SIMILARITY BETWEEN MAN AND BEAGLE.

The secretion of glucocorticoids in beagles^(76,77) is the same as in man.⁽⁷⁸⁾ Both species produce greater amounts of cortisol than corticosterone. This secretory pattern is reversed in rodents, which produce corticosterone as their major adrenal corticosteroid.^(78,79) Studies in our laboratory have shown that internal emitters cause a decreased level of in vitro adrenal steroid biosynthesis.⁽⁸⁰⁾ This finding was made in adrenal tissue from beagles bearing burdens of ^{239}Pu , ^{90}Sr , ^{228}Th , ^{228}Ra , and ^{226}Ra . Reversal of decreased in vitro steroid synthesis was obtained on the addition of coenzymes necessary for steroid biosynthesis to incubations of irradiated tissue.⁽⁸¹⁾ Internal emitters have also been shown to decrease circulating plasma cortisol and corticosterone levels in beagles bearing burdens of ^{239}Pu

and ^{241}Am .⁽⁷⁶⁾ Because of the similarity of corticoid secretion, findings in the beagle will be more applicable to man.

11. TRANSAMINASE AND ALKALINE PHOSPHATASE SIMILARITIES BETWEEN MAN AND BEAGLE.

Both serum transaminase⁽⁸²⁾ and alkaline phosphatase⁽⁸³⁾ levels are utilized diagnostically in man. Serum transaminase levels indicate soft tissue damage, particularly in liver of man.⁽⁸²⁾ Serum glutamic pyruvic transaminase levels have been shown to be elevated specifically in response to hepatocellular necrosis in dogs.⁽⁸⁴⁾ Studies in our laboratory have shown that SGPT levels respond with an increase in beagles bearing burdens of ^{239}Pu , ^{228}Th , and ^{226}Ra .⁽⁸⁵⁾ In dogs bearing burdens of ^{239}Pu , the increase in transaminase is dose dependent⁽⁸⁶⁾ and precedes the appearance of otherwise clinically obvious illness.

Serum alkaline phosphatase levels are elevated in response to bone, liver or kidney damage in man⁽⁸³⁾ and in beagles.⁽⁸⁵⁾ Elevations of alkaline phosphatase precede clinical demonstration of bone tumors in beagles. This increase is associated with osteoblastic activity and tumor growth. There is a correlation between the mean serum alkaline phosphatase level and radionuclide dose.⁽⁸⁵⁾ Alkaline phosphatase levels also increase in response to bony metastases in man. Therefore, observations in the beagle on diagnostic applications of this enzyme should correlate well with radiation-induced changes in man.

12. ADVANTAGE OF LARGER ORGAN SIZE IN RADIONUCLIDE DISTRIBUTION STUDIES.

Because the size of the beagle facilitates detailed sampling of organs at autopsy, an unsuspected high uptake of ^{241}Am was discovered in the beagle thyroid gland.^(87,88) The concentration of ^{241}Am had not been reported in any of the previous studies of this nuclide in rodents, presumably, because

of the greater difficulty in dissecting out their tiny thyroids, and because the possibility of thyroid uptake of ^{241}Am had not been considered. While the high uptake of ^{241}Am in the beagle thyroid does not prove that a similar uptake will occur in man, it has alerted the scientific community to this possibility which we suspect will soon be resolved by analysis of human autopsy samples.

DISCUSSION

Some of the most useful predictions for humans have been derived from interspecies correlations.^(89,100) More is likely to be learned from a variety of species than from only one type of experimental animal, and if the predictions are crucial to the protection of mankind in the present world of expanding nuclear technology, large and long-lived species should be included despite the costs in money and time. Although it may seem expensive to do these studies properly, what is the alternative cost to humanity of incorrect assessments of risk?

In summary, similarities in skeleton, dentition, growth dynamics of osteosarcomas, liver retention of ^{239}Pu , hematology, biochemistry, and the advantage of large-sized organs make the beagle an excellent experimental model for radiobiological and toxicological studies relevant to man.

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INCREASED TOXICITY OF Na_3CaDTP WHEN GIVEN BY PROTRACTED ADMINISTRATION

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ABSTRACT: Protracted administration greatly enhanced the toxicity of Na_3CaDTP in beagles. Injections equivalent to 0.0266 g DTPA given every 5 hours, were fatal as early as 4 days after the onset of treatment. The most serious lesions were induced in the epithelial lining and related structures of the intestinal tract. Protracted administration of equal molar amounts of Na_3ZnDTP was found to be much less toxic.

INTRODUCTION

During decorporation studies in beagles, the toxicity of the trisodium, calcium or zinc salt of diethylene-triamine pentacetic acid (Na_3CaDTP) was found to be markedly increased when a given amount was administered in divided doses rather than as a single daily injection. It is the purpose of this study to present some of the clinical and postmortem findings, thus observed, and, also, to present brief observations intercomparing the toxicity of Na_3ZnDTP and Na_3CaDTP .

METHODS

All of the dogs used in these studies were adult purebred beagles, born and reared within this laboratory, and averaging approximately 10 kg body weight.⁽¹⁾

The Na_3CaDTP was obtained, in part, from the Giegy Pharmaceutical Company, and part was prepared at this laboratory. All of the Na_3ZnDTP was prepared at this laboratory. Intravenous administration of the chelate was via the cephalic vein.

The weights given in Table 1 and Figure 1 indicate the grams of DTPA and do not include the sodium, calcium or zinc moieties.

Occult blood in the urine and feces was determined by "Hematest Reagent Tablets".* The urinary proteins were measured using the method described by Savory, et al.⁽²⁾

RESULTS

The toxicity of a given daily dose of CaDTP was markedly enhanced when the administration was protracted by giving one-fifth of the dose at approximately 5 hour intervals rather than via a single injection. Daily dose levels which produced only very mild symptoms when given as a single dose were fatal when the same amount was given in five divided doses. For example, 0.09 to 0.10 g given subcutaneously as a single daily injection for 8 days produced relatively mild symptoms consisting of slight anorexia, intermittent diarrhea, and occasional vomiting. Rapid recovery occurred following cessation of treatment (Table 1), whereas one-fifth of the above dose given subcutaneously at 5 hour intervals for 8 days or until death, was fatal in 3 out of 3 dogs within 3 to 9 days. A slightly increased toxicity was also noted when administered subcutaneously as compared to intravenously. The dose response relationship is presented in Fig. 1; however, this is very tentative in view of the small number of dogs studied. The curve suggests that the maximum Na_3CaDTP toxicity, when the injections are made every five hours, is reached at a dose level near 0.025 g, and shows the relatively narrow interval between the fatal and non-fatal dose levels.

The most prominent Na_3CaDTP induced symptoms were principally related to the intestinal tract and, to a lesser extent, the kidneys. The intestinal disturbance was characterized by loss of appetite, diarrhea, vomiting, melena

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

and abdominal tenderness (Table 1). Hematemesis occurred in some instances. In the advanced stages, the oral mucous membranes were usually extremely congested. The urinary symptoms included proteinuria, hematuria, and lowered specific gravity; however, the latter was always associated with polydipsia and did not go below 1.010. Hematuria invariably occurred in the fatal cases. Casts were present in the urinary sediment in less than half of the animals, but the total urine protein increased significantly in all of the dogs which developed marked symptoms and remained elevated for a variable period during the recovery phase. Up to 300 mg% or approximately 1 g. of protein was voided in the urine over a 24 hour period in some instances. Slight to moderate elevation of the blood urea nitrogen also occurred in most of the dogs which developed pronounced symptoms, but this was not a constant change.

Based on bromsulphalein (BSP) retention, the Van Den Bergh Test, and prothrombin time, Na_3CaDTP induced liver injury was minimal or absent. The BSP clearance time was increased in part of the dogs but only slightly. The serum glutamic pyruvic transaminase values generally remained within normal limits further indicating little if any hepatic cell injury or necrosis. The relatively slight changes may have been secondary to the severe enteritis.

On gross post mortem examination, the most obvious Na_3CaDTP induced lesions were seen in the small and large intestines, being most extreme in the duodenum and proximal jejunum. The mucous membranes were diffusely hemorrhagic and lacked the normal velvety appearance. Significant amounts of blood were usually present in the lumen, and prominent brush-like hemorrhages were evident beneath the serosal surface. Excess fluid was generally present in the peritoneal cavity. The kidneys, on gross examination, were frequently

hyperemic, especially in the medullary region; however, this was not a constant finding and they were, otherwise, normal on gross inspection. Additional grossly apparent hemorrhages were frequently seen in the lungs, adrenals, and the thymus. These ranged in size from small petechiae to frank effusions. Severe interstitial bleeding occurred in the lungs of one of the dogs. Varying degrees of congestion were invariably present in the liver.

Microscopically, the intestinal lesions were characterized by hemorrhages, loss of a high percentage of the villi, extreme degeneration and/or loss of the epithelial lining of the crypts and villi, and plasma cell infiltration in the interstitial regions between the crypts (Figs. 2-4). The remaining epithelium was low or squamous-like, and the number of mitotic figures and goblet cells was reduced. Many of the crypts were partially or completely denuded and frequently had significant amounts of cellular debris in the lumen. Similar, but less extreme, changes were seen in the large intestine; however, the ulcerations and hemorrhages were more focal and principally involved the crests of the longitudinal folds. Evidence of regeneration was minimal.

Microscopic changes in the kidney were variable and consisted of slight swelling of the proximal tubular epithelium in a few instances, cytoplasmic vacuole formation within the proximal tubules in one instance, and hyaline cast formation in less than half of the dogs. Amorphous eosinophilic material was frequently seen in Bowman's space and the endothelium of the glomerular capillaries was occasionally swollen. Vascular congestion and occasional interstitial hemorrhages were the most constant lesions. Based on the extent and the type of changes, renal injury tentatively did not appear to be a major factor in the cause of death, and most of the kidney changes may have been secondary, rather than primary Na_3CaDTP induced changes.

In all instances, very obvious symptoms were seen several days before death, and recovery patterns tentatively indicated that withdrawal of Na_3CaDTP treatment at the first indication of marked loss of appetite and/or emesis would prevent irreversible injury and death. For example, in three dogs the Na_3CaDTP injections were continued at a toxic dose level, 0.024 g DTP intravenously, every five hours, for four days, and were stopped when symptoms became severe. Based on clinical evaluations, each of these animals would likely have died if treatment were continued for one or two additional days. However, complete recovery occurred within approximately one week after the last treatment. Thus, the symptoms provide a significant safety factor in the use of this compound.

The protraction effect was also evaluated for Na_3ZnDTP , which has been shown in other studies to be less toxic than the calcium form.⁽³⁻⁶⁾ Two dogs were given subcutaneous injections every 5 hours, of Na_3ZnDTP equivalent to 0.0266 g DTPA for 19 days. In one dog, this treatment produced very mild symptoms consisting of intermittent diarrhea, slight melena, and hematuria, but only slight melena in the second dog (Table 1). The melena and hematuria were evident only on chemical analysis of the excreta and could not be seen grossly. On casual examination the dogs were asymptomatic, except for slight diarrhea which occurred on two different days in one of the dogs. Since an equal molar amount of Na_3CaDTP produced death in four days, these data further substantiated the very significant difference in the toxicity of trisodium calcium and zinc DTP.

DISCUSSION

The reason for the increased toxicity of Na_3CaDTP with protraction of administration is still speculative, but the relatively rapid excretion, up

to 65% by one hour and 95-98% by 6 hours,^(7,8) suggests a possible relationship to a more sustained inhibition of an essential factor or physiological process, resulting from insufficient recovery time between treatments. Weber, et al. have shown a significant decrease in ³H thymidine labeling in the duodenal epithelium at approximately 10 hours following Na₃CaDTP administration in rats and suggest that the effect is resultant to chelation of metals required by enzymes involved in DNA synthesis.⁽⁹⁾ This is supported by Lieberman's observations which indicate diminished DNA polymerase and thymidine kinase activity in vitro when zinc is chelated with the disodium, calcium salt of ethylene diamine tetraacetic acid (Na₃CaEDT).⁽¹⁰⁾ However, Cremona has presented the possibility of direct enzyme binding.⁽¹¹⁾ The DNA inhibition thesis is further substantiated by the fact that the site of the most significant injury observed when the administration was protracted, involved the intestinal epithelium, a tissue of intense mitotic activity where complete renewal occurs every 2 to 3 days.⁽¹²⁻¹⁴⁾ The marked differences in the intestinal toxicity of the calcium and zinc forms of EDTA and DTPA may be explained by the studies of Bohne which indicated transient inhibition of DNA synthesis in intestinal crypt cells by calcium chelates but not by Na₃ZnDTP.⁽¹⁵⁾

Although significant kidney lesions and functional changes were seen in some of the dogs presented in this study, the nephritic changes were considerably less extreme and less life threatening than those induced in the small and large intestines. In no instance did death appear to result from the kidney syndrome, and under the conditions of this study Na₃CaDTP would be more accurately classified as an enterotoxin rather than a nephrotoxin. In fact, many of the non-intestinal lesions, including the kidney changes, may have been secondary to the enteritis and the resultant toxemia.

The intestinal syndrome is not unique for Na_3CaDTP and Aronson, et al. have demonstrated similar lesions in cattle following continuous intravenous administration of Na_2CaEDT .⁽¹⁶⁾ Thus, a similar intestinal syndrome is induced by both Na_2CaEDT and Na_3CaDTP , although Na_3CaDTP reportedly produces greater DNA inhibition.⁽¹⁵⁾ It also appears that the intestinal lesions are produced principally when the administration is continuous or protracted by giving relatively frequent injections. This is possibly related to the relatively transient inhibition of DNA synthesis by the calcium chelates.⁽¹⁵⁾ Injury to the intestine has probably been less frequently reported than the renal syndrome, since the administration of the Na_2CaEDT or Na_3CaDTP has not been protracted in a high percentage of the experiments.⁽¹⁷⁻²³⁾

The effectiveness of Na_3CaDTP and Na_3ZnDTP in the removal of some of the transuranium elements has been shown, at least in some instances, to be comparable.⁽³⁾ In view of the much greater toxicity of the calcium form, its use appears to involve unnecessary risks, unless it is known to be the only effective chelating agent for the given circumstances. Also, the relatively low degree of toxicity involved in the protracted administration of Na_3ZnDTP provides the possibility of much more intensive and frequent treatments, which would be advantageous in some instances.

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Table 1. Summary of symptoms induced: anorexia (A), emesis (E), diarrhea (D), melena (M), and hematuria (H). The number of injections per day is shown in parentheses. The g/injection is the grams of DTPA in the given salt.

	Route Admin.	g/Inj.	Hrs. between Inj.	Dog No.	Days on Treatment																				
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Na ₂ CaDTP							AE	AE	AE	AD	AD	A	A	A	AD	A									
	S.C.	0.10	24	1227	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)													
							AE	AE	AE	A	AD														
	S.C.	0.09	24	1218	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)													
	S.C.	0.005	5	1496	(3)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	
	S.C.	0.018	5	1492	(3)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	
							AE	AE	AE		AE														
	S.C.	0.018	5	790	(3)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	
							AE	AD	AE																
	S.C.	0.0266	5	1463	(4)	(5)	(4)	(4)	(died)																
	I.V.	0.09	24	1216	(1)	(1)	(1)	(1)	(1)	(1)	AD	(1)	(1)												
	I.V.	0.13	24	1239	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)											
I.V.	0.024	5	1216*	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)		
I.V.	0.024	5	1217	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)		
I.V.	0.024	5	1218*	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)		
I.V.	0.072	5	1239*	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)		
I.V.	0.072	5	1227*	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)		
Na ₂ ZnDTP	S.C.	0.0266	5	1495	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(2)	
	S.C.	0.0266	5	1497	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(2)	

* Second sequence of treatment following recovery from earlier chelate therapy.

Fig. 1. Dose-survival relationship of beagles receiving protracted Co DTPA treatment

Figure 2. Microphotograph of the mucosa of the duodenum near the muscularis mucosae (M) showing degeneration and exfoliation of the disintegrating epithelial cells lining the crypts (arrow), which was associated with CaDTPA toxicity. Some of the crypts are cystic and variably denuded, and the remaining epithelium is of cuboidal or squamous type. Moderate leukocytosis is apparent in the interstitial regions. Dog number 1239. H & E. X 180.

Figure 3. Microphotograph of the inner aspect of the duodenal tunica mucosa, showing marked loss of villi (arrow) plus severe degenerative changes involving the deeper regions. Same dog as Fig. 2. H & E. X 180.

Figure 4. Microphotograph of basal aspect of duodenal tunica mucosa taken from dog number 1463 which died after three days of CaDTPA treatment. It shows partial denuding of the crypts, cuboidal and squamous dysplasia of the remaining epithelium, many necrotic cells within some of the crypts (arrow), and a moderate excess of plasma cells in the interstitial areas. The incidence of mitotic figures is abnormally low. H & E. X 500.

CALIFORNIUM RETENTION IN BEAGLES DURING THE FIRST
TWO YEARS AFTER INJECTION

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ABSTRACT: Retention of californium in the total-body and in the liver of 21 beagles injected with Cf-249 and 19 beagles injected with Cf-252 has been determined by total-body and partial-body counting techniques for burden times of more than 2 years. Total-body retention was as if 23.8% of the injected Cf were retained with a biological half-time ($T_{1/2}$) of about 1.6 days and 76.2% with a $T_{1/2}$ of about 3129 days = 8.6 years. Liver retention was as if 9.6% of the injected Cf were retained with a $T_{1/2}$ of 2.5 days and 18.5% with a $T_{1/2}$ of 1191 days = 3.3 years. It appeared that there were no important differences between these two californium isotopes in either total-body or liver retention.

INTRODUCTION

The biological retention of Cf-249 or Cf-252 in beagles has been reported for the first 82 days,⁽¹⁾ 160 days,⁽²⁾ and 368 days⁽³⁾ post-injection. Measurements have now been extended to 760 days after californium administration. The experimental design and analytical methods were described previously.⁽¹⁻³⁾ This report is based on 102 measurements of 21 dogs injected with 0.005 to 2.8 μ Ci Cf-249/kg and 81 measurements of 19 dogs injected with 0.016 to 2.9 μ Ci Cf-252/kg.

RESULTS AND DISCUSSION

Retention values for both Cf-249 and Cf-252 in the total-body and in the liver are shown in Figure 1. Equations which were fit to the data by the method of least squares resulted in the following descriptive expressions for the biological retention of Cf-249 and Cf-252 in our beagles at

t days after injection:

For both Cf-249 and Cf-252:

$$\% \text{ in total-body} = 23.8e^{-0.445t} + 76.2e^{-0.000222t}$$

$$\% \text{ in liver} = 9.6e^{-0.282t} + 18.5e^{-0.000582t}$$

For Cf-249 only:

$$\% \text{ in total-body} = 22.2e^{-0.681t} + 77.8e^{-0.000190t}$$

$$\% \text{ in liver} = 9.3e^{-0.243t} + 19.4e^{-0.000767t}$$

For Cf-252 only:

$$\% \text{ in total-body} = 26.4e^{-0.289t} + 73.6e^{-0.000227t}$$

$$\% \text{ in liver} = 9.2e^{-0.293t} + 17.3e^{-0.000375t}$$

These preliminary results indicate that the total-body biological half-times ($T_{1/2}$) of californium in beagles at more than about 20 days post-injection approximated 8.6 years for Cf-249 and Cf-252 combined, 10.0 years for Cf-249 only and 8.4 years for Cf-252 only, as shown in Table 1. It should be borne in mind that these studies are still in progress, so that the retention equations reported herein are only provisional.

Statistical analysis indicated that the total-body retention equation of the Cf-249 dogs was not significantly different from that of the Cf-252 dogs ($P > 0.2$) but that the corresponding liver retention curves were significantly different ($P < 0.05$). The contrast in slopes of the longest-lived components of the 2 liver equations (-0.000767 ± 0.000116 and -0.000375 ± 0.000106) rather than a dissimilarity in intercepts ($19.40 \pm 1.04\%$ and $17.35 \pm 1.04\%$) was responsible, since there were no important differences for the respective short-term components in either the slopes (-0.243 and

Table 1. Comparison of retention equations for Cf-249, Cf-252, and combined Cf-249 and Cf-252 in beagles for the first 2 years after injection.

Equation	Nuclide	1st Component		2nd Component	
		%	$T_{1/2}, d$	%	$T_{1/2}, d$
Total-body	Cf-249 and Cf-252	23.8	1.56	76.2	3129
	Cf-249	22.2	1.02	77.8	3648
	Cf-252	26.4	2.40	73.6	3054
Liver	Cf-249 and Cf-252	9.6	2.46	18.4	1191
	Cf-249	9.3	2.85	19.4	904
	Cf-252	9.2	2.37	17.1	1848

-0.293) or the intercepts (9.3% and 9.2%). If Cf-249 is removed more rapidly from the beagle liver than is Cf-252, Cf-249 retention in non-liver tissue must be correspondingly greater than Cf-252, since total-body retention of the 2 isotopes appears to be similar.

Inspection of data from which Figure 1 was constructed revealed that one Cf-249-injected dog (M3G3) showed a liver retention which seemed consistently much lower than the Cf-249 average, and that one Cf-252-injected dog (F4F3) showed a liver retention which seemed consistently much higher than the Cf-252 average. The data points representing these 2 dogs from about 160 to 760 days post-injection are identified by arrows in Figure 1 (open circles = F4F3; solid circles = M3G3). To what extent these 2 animals affected the significance of the difference between the Cf-249 and Cf-252 liver retention curves was tested by omitting them from the respective least squares fits. Without M3G3, the expression describing the liver retention of Cf-249 was:

Figure 1. Total body retention and liver retention of injected californium in beagles. Open circles represent data of Cf-252-injected dogs, while solid circles represent data of Cf-249-injected dogs. Percent retention of injected Cf in the total-body = $23.8e^{-0.445t} + 76.2e^{-0.000222t}$ and percent retention in the liver = $9.6e^{-0.282t} + 18.5e^{-0.000582t}$. Solid circles identified with arrows indicate the liver retention of M3G3, and open circles identified with arrows indicate the liver retention of F4F3, the 2 dogs omitted from consideration in one of the analyses (see text).

$$\% \text{ in liver} = 8.8e^{-0.285t} + 20.1e^{-0.000678t}$$

and without F4F3, the expression describing the liver retention of Cf-252 was:

$$\% \text{ in liver} = 9.6e^{-0.239t} + 16.9e^{-0.000434t}$$

Statistical analysis revealed that there was no significant difference between these 2 equations ($P > 0.10$). Therefore, it appears that although there is a significant difference between the liver retention curves for Cf-249 and Cf-252, the data for only 2 dogs was responsible for the difference so that the liver retention of these 2 californium isotopes might be considered to be about the same.

Our calibration⁽³⁾ for Cf-252 liver counting was based on only 2 dogs at 13 and 36 days after injection, and the calibration^(1,2) for Cf-249 liver counting was based on only 2 dogs at 7 and 21 days after injection. Since the total-body retention of both M3G3 and F4F3 appeared similar to the other dogs, it is possible that the average calibration factors determined for Cf-249 and Cf-252 liver counting are not applicable to these 2 dogs. Perhaps this question can be resolved when they come to autopsy.

It has been pointed out^(3,4) that the continuous gamma-ray spectrum^(2,3) of Cf-252 contains some contribution from the gamma-emitting fission products which result from the spontaneous fission of this isotope. Parker and Wright⁽⁵⁾ found that total-body retention curves of Cf-252-injected mice were essentially identical whether the retention measurements were based on gamma-gamma coincidence counting, which is specific for fission gamma-rays of Cf-252, or on what they termed "gross gamma-counting" between 100 and 800 keV, which includes much of the photon radiation of the fission products

as well as of the continuous fission gamma-ray spectrum. The early excretion of Cf-252 by our beagles⁽¹⁾ was reported to be not significantly different from that of Cf-249 based on counting of Cf-252 excreta samples and injection duplicates in suitable phantoms between about 50 and 750 keV. Both of these experiments, as well as the similarity of measured total-body retention of Cf-249 and Cf-252 in beagles, suggest that satisfactory results can be obtained by utilizing the photon spectrum of Cf-252 samples or standards below about 800 keV, even though the fission products contribute to the counting rates. Except for iodine isotopes in the thyroids,⁽³⁾ Ru-103 in urine samples,⁽³⁾ and the 2.23 MeV gamma-ray resulting from neutron capture by hydrogen in urine samples and distilled water phantoms, we have never observed detectable photopeaks superimposed upon the continuous photon spectrum of dogs, excreta samples, or injection standards containing Cf-252. If, however, a standard solution and, for example, a dog contained Cf-252 which was either enriched or depleted of fission products relative to that in the contrasting sample, the consequent counting rate per μCi Cf-252 would be different. This situation might also obtain among the various organs of a dog so that the counting rate per μCi of the total dog could be different from that of the dog with his liver shielded by 2 inches of lead. Because of these potential uncertainties in Cf-252 counting, metabolic data obtained with Cf-249 may be more reliable than that obtained with Cf-252.

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CURIUM EXCRETION, RETENTION, AND DISTRIBUTION STUDIES IN BEAGLES

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ABSTRACT: The metabolism of curium has been studied in 5 young adult beagles from 0 to 57 days after the intravenous injection of $^{244}\text{Cm} + ^{243}\text{Cm}$ in citrate buffer. Total excretion during the first 3 weeks after injection was mainly urinary. Of the entire Cm eliminated in this 3 week collection period, nearly 3/5 appeared in the first day's urine. About 5 times more Cm was excreted in the urine than in the feces on the first day. Following this, however, partitioning of Cm between urine and feces in the daily excretion was more nearly equal. A combination of total-body and partial-body counting of the living dogs utilizing the 209, 228, and 278 keV gamma-rays of ^{243}Cm indicated that at a week after injection about 35% of the injected Cm was deposited in the liver and about 53% was in non-liver tissue (mainly skeleton). Three dogs were sacrificed at 6, 13, and 20 days after injection. Although the liver and skeleton accounted for the bulk of the retained curium, some other tissues exhibited significant Cm concentrations, particularly the thyroid. Urinary excretion, retention, and tissue distribution of injected curium were similar to that of inhaled curium in 24 beagles reported by McClellan et al. (Health Physics 22: 877, 1972).

INTRODUCTION

Although discovered almost 3 decades ago,⁽¹⁾ curium has been extensively studied only recently because of increased availability and because the properties of its various isotopes make it potentially applicable to such uses as power production,⁽²⁻⁹⁾ as an energy source for neutron radiography,⁽¹⁰⁾ in x-ray excitation for element analysis,^(11,12) for spectrometer calibration,^(11,13) for producing higher number elements,⁽¹⁴⁾ and for the potential reduction in the costs of reactor-generated electric power through the sale of Cm isotopes as a valuable by-product.⁽¹⁵⁾ The yield (atoms of product ÷ atoms of primary target) is greatest when curium is purposefully made, for example, from neutron-irradiated americium, but both the total amount produced, and the production costs are most favorable for reactor-generated

curium.⁽¹⁵⁻¹⁷⁾ This element has also been produced in nuclear explosions.^(14,18) As the utilization and availability of curium increases, it is inevitable that human exposure to this material from processing, fabrication, and accidents will also increase. A number of people have already been exposed to curium contamination,⁽¹⁹⁻²¹⁾ and some of them have retained detectable body burdens as a consequence.

Previous studies of curium metabolism in animals⁽²²⁻³²⁾ indicated that curium resembles americium more than plutonium in its physiological behavior. No reports of the long-term biological effects in large mammals from internally-deposited curium have been published, but they will be of great importance in evaluating human exposures as well as in any future revision of maximum permissible levels when they finally appear.

METHODS

Five male beagles ranging in age from 485 to 511 days, and in body weight from 10.4 to 12.8 kg were injected intravenously with about 2.6 μCi Cm-citrate/kg. The curium activity of the curium stock, as of 21 December 1972, was about 58.7% ^{244}Cm , 41.2% ^{243}Cm and perhaps 0.1% of other curium isotopes. Dogs were housed in stainless steel metabolism cages for one week before and for three weeks after injection. All excreta were collected and assayed for Cm content by gamma-ray counting using the 209, 228, and 278 keV photons resulting from the radioactive decay of ^{243}Cm which occur, respectively, in 4%, 12%, and 14% of its disintegrations.⁽³³⁾ Retention in the total-body and in the liver was determined in the living dogs by a combination of total-body and partial-body counting using techniques similar to those reported previously for beagles injected with ^{241}Am ⁽³⁴⁾ and ^{249}Cf .⁽³⁵⁾ Since the same Pb shield 20 cm high x 18 cm wide x 2.54 cm thick was used to cover the liver for the partial-body

counting of these curium-injected dogs as was used with ^{249}Cf dogs, the calibration factor⁽³⁵⁾ determined for ^{249}Cf dogs was assumed to apply (i.e., counting rate of total non-liver Cm = counting rate of dog with Pb shield over liver $\div 0.62$). Distribution of Cm in the various organs and tissues of 3 dogs sacrificed at 6, 13, and 20 days after injection was also determined by gamma-ray counting using methods described in reference 34.

RESULTS

Excretion.

Table 1 summarizes the measured curium excretion during the first 3 weeks after injection. Curium output was mainly urinary, with almost 3/5 of the total 3 week excretion appearing in the first day's urine. On day 0-1, about 5 times more Cm was excreted in urine than in feces. Afterwards, partitioning of the daily excretion between urine and feces was more nearly equal.*

A comparison of curium excretion by beagles with the other transuranium elements plutonium, americium, and californium is made in Table 2. It appears that the partitioning of excreted curium between urine and feces is more like americium than either plutonium or californium. During the first 3 weeks after injection, Am, Cf, and Cm were excreted mainly in the urine, while Pu excretion was predominately fecal.

*The Cm activity found in cage washings was assigned to the urine and typically amounted to a small fraction of the total excretion.

Table 1. Curium excretion by 5 beagles during the first 3 weeks after injection. Values given in the table are in terms of percent of injected Cm.

<u>Dog</u>	<u>Sample</u>	<u>day</u> <u>0-1</u>	<u>day</u> <u>1-2</u>	<u>day</u> <u>2-3</u>	<u>day</u> <u>3-7</u>	<u>day</u> <u>7-14</u>	<u>day</u> <u>14-21</u>	<u>day</u> <u>0-21</u>
T1C5	Urine	3.253	0.413	0.395	0.635	0.398	0.145	5.239
	Feces	1.668	0.112	0.837	0.388	0.459	0.179	3.643
	Total	4.921	0.525	1.232	1.023	0.857	0.324	8.882
T2C5	Urine	7.404	0.316	0.334	0.543*			
	Feces	0.877	0.353	0.334	0.552*			
	Total	8.281	0.669	0.668	1.095*			
T3C5	Urine	5.065	0.660	0.167	0.475	0.230**		
	Feces	1.776	0.477	0.169	0.308	0.167**		
	Total	6.841	1.137	0.336	0.783	0.397**		
T4C5	Urine	9.142	0.469	0.265	0.619	0.383	0.121***	
	Feces	0.787	0.226	0.274	0.381	0.215	0.099***	
	Total	9.929	0.695	0.539	1.000	0.598	0.220***	
T5C5	Urine	7.713	0.499	0.563	0.980	0.321	0.142	10.218
	Feces	1.287	0.642	0.275	0.389	0.290	0.148	3.031
	Total	9.000	1.141	0.838	1.369	0.611	0.290	13.249
All dogs (Average)	Urine	6.515	0.471	0.345	0.677	0.367	0.144	7.728 ⁺
	Feces	1.279	0.362	0.378	0.366	0.321	0.164	3.337 ⁺
	Total	7.794	0.833	0.723	1.043	0.688	0.308	11.065 ⁺

* day 3-6

** day 7-13

*** day 14-20

+ 2 dogs only, T1C5 and T5C5

Table 2. Excretion of Pu, Am, Cm, and Cf by beagles during the first 3 weeks after intravenous injection.

<u>Number of dogs</u>	<u>Element Name & No.</u>	<u>Reference</u>	<u>% in Urine</u>	<u>% in Feces</u>	<u>Total % Excreted</u>
19	Pu (94)	36	4	8	12
4	Am (95)	34	10	1	11
5	Cm (96)	Table 1	8	3	11
11	Cf (98)	35	16	8	24

Retention.

The total-body retention and partitioning of retained curium activity between liver and non-liver tissue as determined by total-body and partial-body counting are shown in Table 3. For comparison, the total-body retention calculated by excreta subtraction at corresponding times is also given. The ratio of retention by in vivo counting to the retention by excreta subtraction averaged 0.98 for all measurements. At a week after injection, about 1/3 of the injected curium was found in the liver, and about 1/2 of the administered activity remained in the non-liver tissue (mainly skeleton). Retention values for plutonium, americium, and californium at corresponding times are compared with those for curium in Table 4. Partitioning of curium between liver and non-liver tissue appears to be intermediate between americium and californium.

Distribution.

The curium content and concentration in selected tissues of the 3 sacrifice dogs are exhibited in Table 5. A comparison of Tables 3 and 5 reveals that liver retention as measured by in vivo counting was similar to that

Table 3. Biological retention of injected curium in beagles.

Dog	Days after Injection	% of injected curium in			% Retention by excreta subtraction
		Liver	Non-liver	Total-body	
T1C5*	6	36.0	55.0	91.0	92.3
	13	31.2	58.7	89.9	91.4
	20	33.9	57.3	91.2	91.2
	27	31.5	58.9	90.4	
	57	30.3	57.7	88.0	
T2C5	1	42.9	45.8	88.7	91.7
	3	38.6	48.8	87.4	90.4
	6	35.7	49.2	84.9	89.3
T3C5	1	38.1	54.2	92.3	93.4
	3	34.0	56.5	90.5	91.9
	6	33.0	56.0	89.0	91.3
	13	35.6	55.1	90.7	90.5
T4C5	1	40.1	50.2	90.3	90.1
	3	37.1	51.1	88.2	88.8
	6	35.7	51.9	87.6	87.9
	13	31.9	54.5	86.4	87.3
	20	36.0	51.6	87.6	87.0
T5C5	1	42.6	48.3	90.9	97.1
	3	38.2	51.8	90.0	95.2
	6	37.4	52.1	89.5	94.1
	13	35.7	52.2	87.9	93.4
	20	36.2	52.1	88.3	91.4
	27	35.4	52.5	87.9	
	57	35.8	50.7	86.5	

*Liver biopsies taken on day 1 and day 47, about 5 g each.

Table 4. Retention of Pu, Am, Cm, and Cf by beagles one week after intravenous injection in citrate solution.

Reference	Element Name & No.		Percent of Injected Radioactivity		
			Liver	Non-liver	Total
36	Pu	(94)	30	60	90
34	Am	(95)	50	40	90
Table 3	Cm	(96)	35	53	88
35	Cf	(98)	20	60	80

Table 5. Distribution and concentration of injected curium in selected tissues of 3 male beagles.

	T2C5 (6 days)		T3C5 (13 days)		T4C5 (20 days)	
	% of injected Cm	%/kg	% of injected Cm	%/kg	% of injected Cm	%/kg
Liver	39.4	146.9	34.5	124.6	34.4	123.3
Skeleton	36.5	29.9	44.7	39.1	42.7	34.1
Thyroid	0.020	21.4	0.028	49.0	0.022	32.0
Kidneys	0.780	18.8	0.615	15.6	0.465	9.6
Lungs & Trachea	0.420	5.0	0.580	6.9	0.441	5.2
Spleen	0.085	3.5	0.079	3.5	0.091	3.3
Testes	0.013	0.5	0.017	0.7	0.010	0.4

determined in the excised livers. Like americium and californium, curium appears to concentrate in the thyroid in addition to the liver and skeleton.

DISCUSSION

It is of some interest to compare these results with those obtained by McClellan et al.⁽³²⁾ In their experiment, 24 beagles aged 11 to 47 months

were exposed by inhalation to ^{244}Cm either as the chloride or the oxide. They reported that the retention by dogs was in good agreement with the limited data from human accidental exposures to curium but differed markedly from the measured Cm retention in rats. There appeared to be no important differences between dogs exposed to the relatively soluble chloride or the relatively insoluble oxide in fraction of inhaled activity deposited in the lung, urinary excretion pattern, translocation from the lung, or distribution in tissues.

Figure 1 compares the daily urinary excretion of the five Cm-injected beagles with corresponding data for the 24 beagles of McClellan et al. that were exposed by inhalation.⁽³²⁾ The average content of the first day's urine for the animals exposed by inhalation was about 4% of what they termed the initial lung burden, ranging between about 0.4% and 9.4%, while the Cm-injected dogs averaged 6.5% of the injected activity, ranging between 3.2% and 9.1%. These results seem strikingly similar, considering the contrasting routes of administration, and may indicate that much of the inhaled ^{244}Cm was rapidly absorbed into the blood. After the first day, the average urinary excretion by the dogs exposed to the Cm aerosol appeared to remain consistently higher than that of the Cm-injected dogs, although some overlap in the ranges occurs throughout virtually all of the 20 day period which is depicted in Figure 1. This difference could reflect the continuing translocation of activity from lung to blood which is suggested during this time by Figure 3 of reference 32 for the beagles exposed by inhalation. The greater daily range of values for the dogs exposed to Cm aerosol is at least partly due to their greater range in age and the larger number of animals.

Figure 3 of reference 32 also indicates that the deposition of inhaled ^{244}Cm in the liver and skeleton approximated 30% and 40%, respectively, of

Figure 1. Daily urinary excretion of inhaled ^{244}Cm in 24 beagles during the first 20 days after exposure (left) compared with the daily urinary excretion during a corresponding period of 5 beagles injected with $^{243}\text{Cm} + ^{244}\text{Cm}$ (right). Plotted points represent daily averages, and vertical bars indicate the range of measured values. Data for the inhalation-exposure animals were taken from Figure 2 of Reference 32.

the initial lung burden beginning about 2 weeks following administration. This is in remarkable agreement with the corresponding values for the Cm-injected beagles shown in Table 5 of 34.5% (liver), and 44.7% (skeleton) of the injected activity for T3C5 at 13 days, and 34.4% (liver), and 42.7% (skeleton) for T4C5 at 20 days after injection.

Table 2 of reference 32 shows that the relative concentrations of inhaled ^{244}Cm among the liver, skeleton, kidneys, spleen, thyroid, and testes resembled that of injected curium at comparable times (Table 5). It might be expected that the relative concentration in such tissues as lung, tracheo-bronchial lymph nodes, and turbinate would be higher in the animals exposed by inhalation than in those injected with curium.

Because of similarities in urinary excretion, retention, and tissue deposition of inhaled and injected curium, it appears that most of the curium which is deposited in the lung by inhalation may be translocated quite rapidly to the blood. Furthermore, the chemical forms in which injected Cm and inhaled Cm travel in the blood must be alike, or at least they must behave in an analagous fashion metabolically.

McClellan et al.⁽³²⁾ recognized that there may be important differences in the biological consequences of inhaled and injected curium of equal activities and identical isotopic composition, since nasopharyngeal and respiratory tissues receive significantly greater radiation doses from an inhalation exposure. Their Table 3 gives the estimated integral doses to liver, skeleton, and lung as a function of time after the inhalation of $1 \mu\text{Ci } ^{244}\text{Cm}$. By 256 days after exposure, the accumulated dose indicated for the liver is greater than that tabulated for the lung, and by 1000 days, the indicated skeletal dose has also exceeded the accumulated lung dose. Because

the dose-rates at which the resulting doses would be delivered differ for these 3 organs during successive periods of time, expression of damage might not be simply a function of accumulated rads,⁽³²⁾ particularly since the induction of bone sarcomas by alpha emitters can be enhanced by protraction of the radiation dose.^(37,38) Comparison of final results from inhalation and injection experiments will undoubtedly answer some of the important questions of curium toxicity as well as provide information of basic radiobiological significance.

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EARLY RETENTION AND DISTRIBUTION OF CURIUM IN SOFT TISSUES AND BLOOD OF THE BEAGLE

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ABSTRACT: Following intravenous administration of curium to the beagle, the concentration of the nuclide in circulating blood diminishes very rapidly, and after 2 days the concentration is of the order of 0.4% per kg of blood. Soft tissue concentration is highest in the liver, followed by thyroid, kidney, dura mater, and lymph nodes. The concentration in other soft tissues are less by substantial factors.

INTRODUCTION

On January 16, 1973, it became possible to expand this laboratory's investigations of the chronic biologic effects of actinide elements when a modest supply of ^{244}Cm (enriched with ^{243}Cm) arrived.

The chemistry and physics groups cooperated in the qualitative analysis of the material which was supplied by O.R.N.L. No evidence was obtained by γ -ray and α -particle spectroscopy to indicate the presence of radionuclides other than the 7 isotopes of curium found by the mass analysis done by the supplier. On an activity basis 41.2% is ^{243}Cm , 58.7% is ^{244}Cm , and the remainder ($\sim 0.1\%$) is made up of traces of curium isotopes of mass 242, 245, 246, 247, and 248, as of 21 December 1972.

MATERIALS AND METHODS

A pilot study was begun on February 27, 1973, which involved the intravenous administration of $2.6 \mu\text{Ci Cm/kg}$ to each of 5 beagles. One of the animals (T1C5) was included for the purpose of providing a series of liver biopsies so that the subcellular distribution of curium could be observed as a function of time. Three animals (T2C5, T3C5, and T4C5) were included for the purpose of obtaining data on the early retention and distribution

of curium. They were sacrificed at 6, 13, and 20 days following injection. The remaining dog (T5C5) will be maintained for its lifetime.

Retention studies by the physics group were carried out by whole body counting. Complementary studies of excretion were also done by them. Results of these studies appear elsewhere in this report.⁽¹⁾

The chemistry group measured the concentration of curium in the circulating blood at intervals during the first few days following injection, and following autopsy, measured the curium retained in a variety of soft tissues.

RESULTS AND DISCUSSION

Data accumulated to date on the subcellular distribution of curium in liver, kidney, and spleen are presented elsewhere in this report.⁽²⁾

Results of the measurements obtained by sampling blood from these animals are presented graphically in Figure 1. The vertical bars indicate the range, and the points are the averages of the values obtained at the indicated times after injection.

The percent of the injected nuclide recovered from a variety of soft tissues obtained at autopsy are listed in Table 1, while the concentration of the retained curium is shown for these and several other tissues in Table 2.

Curium leaves the circulating blood at a rate reminiscent of the very rapid removal of ^{241}Am .⁽³⁾ This similarity and the contrast these nuclides exhibit with some other actinides is shown in Figure 2. In this figure the percent of nuclide per kg of blood is plotted vs. time for the first 6 hours post-injection. The amount of nuclide in 1 kg of blood is almost exactly the total nuclide circulating in a 10 kg dog according to Parkinson⁽⁴⁾ who reports the beagles of our colony have 99.7 g of blood per kg of body weight. Figure 2 also uses

Table 1. Retention (% dose per part).

Part	T2C5 (6d)	T3C5 (13d)	T4C5 (20d)
Liver	39.7	35.0	35.6
Kidney	0.917	0.712	0.495
Spleen	0.0916	0.0748	0.0913
Thyroid	0.0196	0.0277	0.0218
Adrenals	0.00299	0.00320	0.00363
Pituitary	0.00026	0.00028	0.00029

Table 2. Concentration (% dose per kg of tissue).

Part	T2C5 (6d)	T3C5 (13d)	T4C5 (20d)
Liver	148	126	128
Kidney	20.2	18.0	12.2
Spleen	3.73	3.31	3.32
Thyroid	21.4	49.0	32.0
Adrenals	3.05	3.21	2.99
Pituitary	4.72	4.57	4.80
Loose Connective Tissue	0.62	0.60	1.26
Skeletal Muscle	0.38	0.40	0.30
Salivary Glands	3.82	4.59	2.81
Thymus	1.04	1.09	0.79
Dura	12.0	14.7	10.3
Lymph Nodes	9.38	6.79	9.23
Eyes	0.9	1.5	1.0
Pancreas	0.6	0.7	0.6
Brain	0.1	0.2	0.1
Heart	1.4	1.1	0.8
Prostate	2.2	2.6	1.2
Testes	0.5	0.7	0.4
Spinal Nerve Cord	0.7	---	0.9
Aorta	3.1	4.8	2.9
Bladder	2.6	2.1	1.2
G. I. Tract	1.5	1.2	0.9
Pelt	1.3	1.6	0.9
Lungs and Trachea	5.0	6.9	5.2

Parkinson's value for red cell and plasma ratios of 47.0 g and 52.6 g per kg of body weight. A further assumption that all of each nuclide shown is associated in the circulating blood exclusively with plasma introduces a very small error, if any. Values of ^{239}Pu , ^{228}Th , ^{249}Cf , and ^{252}Cf are taken from previous reports.^(5,6,7)

As with other actinide nuclides studied in this laboratory, the major site of soft tissue deposition is the liver. The liver also has the highest concentration of the nuclide followed by thyroid, kidney, dura mater, and lymph nodes, in that order. The skeletal concentration of retained curium⁽¹⁾ is similar to that of the thyroid.

Tables 3, 4, and 5 compare the retention concentrations of ^{228}Th , ^{239}Pu , ^{241}Am , ^{249}Cf , ^{252}Cf , and $^{243/244}\text{Cm}$ at 1, 2, and 3 weeks in a variety of soft tissues.

Table 3. Concentration at 1 week (% dose/kg tissue).

	^{228}Th	^{239}Pu	^{241}Am	$^{249} \text{ \& } ^{252}\text{Cf}$	$^{243/244}\text{Cm}$
Liver	13.4	87.4	174.	77.0	148.
Kidney	82.3	9.4	14.8	23.1	20.2
Spleen	15.8	3.8	2.2	7.7	3.7
Thyroid			45.5	57.5	21.4
Adrenals			2.7	6.3	3.0
Pituitary			3.5	3.8	4.7
Loose Con. Tissue			0.4	0.9	0.6
Skeletal Muscle			0.3	0.4	0.4
Salivary Glands			4.2		3.8
Thymus			1.1	1.7	1.0
Dura			11.8	18.6	12.0
Lymph Nodes			5.6		9.4

Table 4. Concentration at 2 weeks (% dose/kg tissue).*

	^{239}Pu	^{241}Am	$^{249} \text{ \& } ^{252}\text{Cf}$	$^{243}/^{244}\text{Cm}$
Liver	84.7	216.		126.
Kidney	8.8	20.2		18.0
Spleen	15.2	5.7		3.3
Thyroid	5.3		33.9	49.0
Adrenals			5.7	3.2
Pituitary		4.7		4.6
Loose Con. Tissue				0.6
Skeletal Muscle		0.3		0.4
Salivary Glands				4.6
Thymus				1.1
Dura				14.7
Lymph Nodes				6.8

* No data available for ^{228}Th at 2 weeks post-injection.

Table 5. Concentration at 3 weeks (% dose/kg tissue).

	^{228}Th	^{239}Pu	^{241}Am	$^{249} \text{ \& } ^{252}\text{Cf}$	$^{243}/^{244}\text{Cm}$
Liver	11.4		204.	85.2	128.
Kidney			9.7	22.1	12.2
Spleen	21.7		3.0	6.4	3.3
Thyroid			20.4	16.2	32.0
Adrenals				5.9	3.0
Pituitary				3.9	4.8
Loose Con. Tissue				0.7	1.3
Skeletal Muscle				0.5	0.3
Salivary Glands					2.8
Thymus				1.4	0.8
Dura				20.5	10.3
Lymph Nodes					9.2

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Figure 1. Concentration of curium in the circulating blood during first 6 post-injection days.

Figure 2. Comparison of the concentrations of several actinides in the circulating blood during the first 6 post-injection hours.

THE EARLY SUBCELLULAR DISTRIBUTION OF CURIUM IN CANINE LIVERS

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

Abstract: Previous studies of the subcellular distribution of ^{239}Pu , ^{241}Am and $^{249\&252}\text{Cf}$ in canine livers were extended to include $^{243/244}\text{Cm}$. Beagles were injected with 3 μCi of the nuclide in citrate solution of pH 3.5. Following injection liver specimen were obtained at 0.092 and 47 days by surgical liver biopsy and at 6, 13 and 30 days at autopsy. As observed with other nuclides, curium in the liver was first associated with soluble liver proteins, mainly ferritin, and was found later in increasing concentrations in subcellular organelles. The highest concentration was found in the crude mitochondrial fraction and somewhat less in the crude lysosomal fraction. A considerable part of the nuclide remained in the cytosol. Sucrose density gradients showed a variable relation between the lysosomal enzyme marker activity and the nuclide concentration. Heavy subcellular organelles other than lysosomes (probably mitochondria) must, therefore, be involved in the binding of the nuclide. With respect to liver retention and subcellular distribution, curium occupies a position between AmIII and CfIII . However, it resembles AmIII more closely than CfIII .

Introduction

The liver is the principal target organ for a number of actinide elements when injected intravenously in solution. The subcellular distribution of ^{239}Pu , ^{241}Am and $^{249\&252}\text{Cf}$ in the liver has been studied by a number of workers in this and other laboratories. The association of ^{239}Pu with the lysosomal particles was reported by Rahman and Lindenbaum as early as 1964 (1). Later a mechanism for the transfer of plutonium from soluble liver proteins into subcellular organelles was proposed by Boocock (2) and by Taylor (3) in rat livers. A similar transfer mechanism for ^{239}Pu , ^{241}Am ,

^{249}Cf and ^{252}Cf from soluble liver proteins to subcellular organelles in beagles has been presented by this laboratory (4). An extension of these studies to the liver distribution of other actinide elements is necessary for two reasons. First, the expected proliferation of nuclear reactors will increase the availability and therefore the potential hazards from accidental exposure to other transuranium elements up to ^{258}Fm (5). Second, a clearer understanding of the basic behavior of these elements in the body can be gained by comparing and correlating the biological associations of all of the 5f elements with their known chemical and physico-chemical properties. Thus, it may become possible to replace purely empirical data concerning the deposition, retention and distribution in the body by more accurate, calculated predictions which hopefully may provide a basis for their decorporation.

A study of the biological properties of curium has been initiated in this laboratory to elucidate the mechanism of deposition, retention and microdistribution of this actinide. It has been projected that in the U. S. 45 kg of ^{242}Cm and ~ 5 kg of ^{244}Cm will be produced yearly by 1980 (6). The increasing availability and the resulting biological hazards make it prudent and highly desirable to conduct these studies.

The preliminary work presented here describes the distribution of curium in the beagle liver up to 47 days after injection.

Methods

Four beagles were injected with ~ 3 $\mu\text{Ci/kg}$ of curium in 0.08 M

citrate buffer of pH 3.5. The isotopic composition of the nuclide is described elsewhere in this report (7). Liver specimen were obtained either by serial liver biopsy from one animal at 0.092 days and at 47 days (T1C5) or at time of sacrifice, 6 days (T2C5), 13 days (T3C5), and at 20 days (T4C5). Liver tissue was immediately chilled in ice, weighed, finely minced and then homogenized twice each time in two volumes of 0.25 M sucrose in 0.1 M NaCl + 0.03 M Ca^{++} . Fractionation of the homogenate by differential centrifugation was then carried out according to previously described methods (8). A fraction containing nuclei and debris (6000 g-min), a crude mitochondrial fraction (60,000 g-min), a crude lysosomal fraction (800,000 g-min), a microsomal fraction (6×10^6 g-min) and a cytosol fraction were collected. Fractionation by differential centrifugation results in considerable overlap. Fractions designated as mitochondria, lysosomes, etc. are enriched in these organelles but are not pure fractions.

A further separation of the 6000 g-min supernatant and - in some cases - of the rehomogenized mitochondrial and/or lysosomal pellet was accomplished by isopycnic sucrose density gradient centrifugation using a linear sucrose gradient of 10 - 52%. Twenty or twenty-one samples were collected from each of these gradients.

All samples were analyzed for protein, Cm-radioactivity and acid phosphatase. When possible, samples were analyzed for cytochrome c oxidase activity.

Results

The concentration of curium in some of the liver fractions

obtained by differential centrifugation is listed in Table 1. In order to facilitate comparison between the samples obtained from different dogs and at different times, data are expressed as Cm-activity/mg protein with the Cm-activity of the original homogenate in each case arbitrarily normalized to 100. Limited data, presently available, indicate a noticeable drop in total liver retention during the first two weeks after injection. After this time the liver retention seems to stabilize. Initially, the concentration of nuclide in the cytosol, mitochondrial and lysosomal fractions is approximately the same. The cytosol is the largest of these three components of the liver cell and, therefore, contains the greatest quantity of curium. At least 89.5% of the nuclide in the cytosol fraction was bound to ferritin. The concentration of nuclide in the cytosol decreases significantly as a function of time with a concomitant increase in the lysosomal and mitochondrial fractions. The rate of increase is similar in both the lysosomes and mitochondria up to 20 days, followed by a continuing increase in the mitochondria and a decline in the lysosomes to day 47. It must be emphasized, however, that not all of the nuclide leaves the cytosol and undergoes binding with subcellular organelles. A substantial quantity of the curium remains associated with soluble proteins.

Since the results from differential centrifugation indicate an increasingly strong association of curium with lysosomes and mitochondria with respect to time, the 6000 g-min homogenate, and the mitochondrial and lysosomal fractions were analyzed for nuclide and lysosomal marker activity. For these three fractions, the

concentration of Cm/unit of acid phosphatase was calculated after setting the enzyme marker activity of the 6000 g-min homogenate arbitrarily equal to 10. Results are presented in Table 2. This ratio increases moderately in the 6000 g-min fraction, increases by a factor of 5 in the mitochondrial fraction and by a factor of 4 in the lysosomal fraction at 20 days after injection. The value of this ratio is considerably greater in the mitochondrial than in the lysosomal fraction except at very early times after injection (2 hours) when concentrations were almost equal.

The 6000 g-min, mitochondrial and lysosomal fractions were further separated by sucrose density gradients. Table 3 shows the distribution of curium between the light and heavy fractions after the sucrose density gradient centrifugation of the 6000 g-min homogenate. The first three and the last three fractions were pooled from a total of 20-21 fractions and the curium content in these fractions was determined. The numbers given represent the percent of the total curium in the gradient found in each of these pooled fractions. As expected, initially most of the nuclide is in the light fractions which represent mostly soluble proteins. At later times, the nuclide migrates from the soluble proteins (low density fractions) into subcellular particles and is, therefore, found in the heavy fractions. The result of the gradient centrifugation of the 6000 g-min homogenate is shown in Fig. 1. The percent of nuclide in the whole gradient which is found in each fraction is presented in the lower curve. With increasing time, the distribution of nuclide shifts from light to heavy fractions. Within

the gradient, a correlation between acid phosphatase activity and radioactivity exists, indicating an association of nuclide with lysosomes. It must, however, be emphasized that this correlation between acid phosphatase and radioactivity exists primarily in an area which contains a relatively small quantity of nuclide when compared to the large amounts of curium found in the light fractions initially or in the heavy fractions at later times. Lysosomes, therefore, bind some of the subcellularly bound nuclide but not all of it. Fig. 2 presents the result of sucrose gradient centrifugation of an homogenate prepared from the 60,000 g-min pellet (mitochondrial fraction). Again a correlation between acid phosphatase and nuclide activity exists, but, once again, this correlation exists in those fractions which contain a minimal amount of nuclide.

The constancy of the ratio of radioactivity/phosphatase was tested in the lysosomal and mitochondrial fractions. In Fig. 3 and Fig. 4, the ratio of nuclide activity/phosphatase activity is plotted against the fraction number and density of each fraction. The last three or four fractions contain soluble Cm-protein and, therefore, cannot be considered. In Fig. 3, the respective data obtained from the lysosomal gradient are shown. Disregarding fractions containing soluble Cm-proteins, curves are reasonably flat, i.e. within reasonable limits the ratio is approximately constant. However, in the mitochondrial gradient the relationship between Cm/phosphatase deviates sharply from the expected constant ratio seen in the lysosomal gradient with a distinctly steep increase with decrease of density (Fig. 4).

Discussion

After intravenous injection of curium in citrate buffer of pH 3.5, the reaction of the nuclide with the various constituents of the liver parenchyma (9) follows a pattern similar to that observed with ^{241}Am and $^{249\&252}\text{Cf}$. Initially all of the nuclide binds to soluble protein (mostly ferritin) and is found in the cytosol. With increasing time after injection, increasing amounts of the curium are found with subcellular organelles but substantial amounts also remain bound to proteins in the cytosol. The correlation between lysosomal enzyme activity and curium activity in fractions of medium density is an indication that some of the nuclide is associated with lysosomes. It must, however, be considered that the correlation exists mainly in an area of the gradient which contains only a small fraction of the total nuclide present. Lysosomes are organelles which contribute only about 1% or less of the total liver protein and in canines have a strongly heterogeneous density distribution. They can, therefore, be found in practically all fractions of the gradient except in the very light ones which represent soluble proteins and the microsomes. This makes it difficult to determine whether all of the nuclide is associated with lysosomes. Assuming that within limits, the marker enzyme - in this case acid phosphatase - is a quantitative marker, i.e., every unit of enzyme represents a certain amount of lysosomes, then a constant amount of nuclide should be associated with each unit of enzyme marker. The curium to enzyme ratio then should be nearly constant throughout the gradient. It is nearly constant - within a factor of about two -

in the gradient prepared from the lysosomal fraction. In the gradient prepared from the mitochondrial fraction a rather large deviation from constancy is observed. This indicates that some other organelle, different from the lysosome, also binds the nuclide. Since the gradient yields a quite pure mitochondrial preparation, we must believe that curium is also associated with mitochondria. More accurate and more quantitative analyses of either the whole spectrum of marker enzymes in the liver are necessary to determine all the binding sites or means must be found to prepare pure preparations of all major organelles in this organ.

Comparing the distribution and retention of this nuclide to that of the other trivalent actinides which have been tested in this laboratory, curium occupies a position between AmIII and CfIII, but retention and distribution are closer to AmIII than to CfIII. The initial liver deposition (35-40%) is lower than that observed with AmIII (50%), but higher than that seen with CfIII (28-30%). As long as there is no liver damage, AmIII leaves this organ very slowly and initially CfIII is eliminated from the liver at a rapid rate. After about one week elimination of CfIII slows down, but retention in the liver never stabilizes. CmIII leaves the liver initially quite rapidly but after a few days the retention seems to stabilize. Likewise, the amount of CmIII which is bound to soluble liver proteins at a given time is much greater than observed with PuIV, and is between AmIII and CfIII, being somewhat closer to AmIII.

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Table 1
Concentration of Curium in Liver Fractions Obtained by Differential
Centrifugation*

	T1C5	T2C5	T3C5	T4C5	T1C5
ΔT (Days)	0.092d	6d	13d	20d	47d
Original homogenate	100.	100.	100.	100.	100.
Homogenate free of debris and nuclei	120.2	101.6	116.6	124.2	94.7
Homogenate free of mitochondria	135.2	49.6	66.9	63.8	29.3
Cytosol	85.3	25.1	17.8	22.6	16.
Mitochondrial fraction	85.9	220.	262.7	266.2	487.4
Lysosomal fraction	83.7	166.3	242.2	259.9	117.7
% of inj. dose/liver (Measured by direct counting of known liver aliquot)		39.7%	35. %	35.6%	

*Data expressed as Cm-activity/mg protein with the original homogenate arbitrarily set = 100.

Table 2

Concentration of Curium/Unit of Lysosomal Enzyme Marker*

ΔT (Days)	0.092d	6d	13d	20d	47d
Homogenate free of Nuclei and Debris	9.16	10.46	11.84	14.01	8.92
Mitochondrial Fraction	4.69	14.96	21.31	24.16	20.84
Lysosomal Fraction	4.01	8.91	16.62	15.33	4.99

* Acid Phosphatase Activity of homogenate free of nuclei and debris arbitrarily set = 10.

Table 3

Distribution of Curium between Light and Heavy Fractions
Obtained after Sucrose Density Gradient Separation

ΔT	Light	Heavy	H/L
0.092 d	72.8	4.2	0.06
6 d	33.1	33.4	1.
13 d	18.4	31.6	1.7
20 d	14.2	35.2	2.5
47 d	16.8	20.1	1.2

Fig. 1 Result of density gradient centrifugation of 6000 g-min liver homogenate. Data presented show the distribution of CmIII (CPM/mg protein) and of acid phosphatase (Σ -units/mg protein) at times between 6 days and 47 days after injection. A correlation between radioactivity and the lysosomal enzyme marker activity in areas containing a small amount of radioactivity is indicated.

Fig. 2 Result of density gradient centrifugation of rehomogenized mitochondrial fraction. The distribution of CmIII (CPM/mg protein) and of acid phosphatase (Σ -units/mg protein) at times between 6 days and 20 days is shown. The correlation between radioactivity and lysosomal enzyme marker activity is again pronounced in an area containing a small amount of radioactivity and not in the area in which the bulk of radioactivity is found.

X

Fig. 3 The ratio of nuclide concentration to lysosomal enzyme concentration in the rehomogenized lysosomal pellet is plotted against the density of the gradient medium. Shown are the respective curves for 6 days, 13 days and 20 days. Slopes in the area in which lysosomes are found show a more or less constant relationship, indicating that little curium in this subcellular fraction is bound to organelles other than lysosomes.

Fig. 4 The ratio of nuclide concentration to acid phosphatase concentration in the rehomogenized mitochondrial fraction is plotted against the density of the sucrose gradient medium. Slopes in the area in which subcellular organelles (other than microsomes) are found are steep, indicating a strong deviation from constancy and therefore involvement of organelles other than those showing acid phosphatase activity, probably mitochondria.

COMPARISON OF THE INTRACELLULAR DISTRIBUTION OF ^{239}Pu , ^{241}Am
AND ^{249}Cf IN LIVERS AFTER INTRAVENOUS ADMINISTRATION

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

ABSTRACT: The intracellular distribution in livers of beagles injected intravenously with either $^{239}\text{PuIV}$, $^{241}\text{AmIII}$, $^{249}\text{CfIII}$ or $^{252}\text{CfIII}$ in 0.08 M citrate, pH 3.5 was studied serially. Liver homogenates were fractionated by differential centrifugation. A mitochondrial fraction was obtained by centrifugation at 60,000 g-min, a lysosomal fraction at 8×10^5 g-min and a cytosol at 6×10^5 g-min. Initially all three nuclides were found in the cytosol and mostly associated with ferritin. Considerably higher concentrations of americium and californium than plutonium remained in the cytosol at progressively longer times after administration. The order of the rate of removal of nuclide from the cytosol was $\text{Pu} \gg \text{Cf} > \text{Am}$. As the nuclide was removed from cytosol it became associated with subcellular organelles. No constant relationship was observed between concentration of nuclide and mitochondrial or lysosomal marker. The ratio of nuclide to lysosomal marker was consistently higher in the mitochondrial fraction than in the lysosomal fraction. However, we have not been able to demonstrate significant contamination of the mitochondrial fraction with lysosomes using electron microscopy. It is concluded from this study that mitochondria and lysosomes and possibly other organelles are involved with nuclide binding in canine liver cells.

INTRODUCTION

The subcellular distribution of actinide elements in liver has been of interest to this laboratory for a number of years. Two laboratories have reported the association of polymeric (1) and monomeric ^{239}Pu (2) with lysosomal fractions of rat liver. Previously we reported on the subcellular distribution of plutonium, americium and californium in the liver of canines (3,4). In these studies an initial deposition of nuclide was seen in the soluble fraction, but there was a gradual movement of americium, californium and plutonium into a heavy particulate fraction composed primarily

of mitochondria during the first 70 days after injection. The results reported here extend these studies in time (250 days for AmIII, 500 days for PuIV and 500 days for CfIII) and include similar and more recent observations with CmIII (15 days).

MATERIALS AND METHODS

The beagles used in this study were injected intravenously with either ^{241}Am , ^{239}Pu , ^{249}Cf , ^{252}Cf or $^{243/244}\text{Cm}$ at a dose level of $3.0 \mu\text{Ci/kg}$. All nuclides were injected in 0.08 M citrate buffer pH 3.5. The liver specimens were obtained by serial surgical biopsy or at time of autopsy. Immediately after obtaining the liver samples, they were chilled, minced, and homogenized in 2 volumes of a 0.25 M sucrose, 0.1 M NaCl, 0.0003 M CaCl_2 solution at 4°C . The resulting homogenate was then subjected to a differential centrifugation procedure as shown in Fig. 1.

Whole cells, nuclei, debris and connective tissue fragments were removed by centrifuging the homogenate for 6000 g-min. This supernatant was further centrifuged for 60,000 g-min to sediment mitochondria and other heavy particles. The lysosomes, peroxisomes and other light particles were removed from this supernatant by centrifugation 6×10^5 g-min leaving the cytosol free of cellular organelles. The 6000 x g-min supernatant was further analyzed by isopycnic density centrifugation layering a sample of the supernatant on a continuous sucrose gradient of 10-50%. This sample was centrifuged at $1850 \times \text{g}$ for 120 min (2.2×10^5 g-min). At the end of centrifugation, fractions were collected by bottom unloading of the tube. All resulting fractions were analyzed for

1) protein content by the method of Lowry et al. (5), 2) acid phosphatase (Sigma) (6), and, 3) cytochrome c oxidase activity (7).

RESULTS

The relative volumes of the subcellular components in a typical hepatocyte from a rodent liver are shown in Fig. 2. We have no reason to believe that the gross distribution of subcellular particles in the liver of dogs (or man) differs significantly from this model. Metabolic differences, however, do exist. This figure provides the reader with an idea of the relative volume which each particle compartment contributes to the total cell volume. Nuclei are 6%, membranes-golgi, smooth and rough endoplasmic-reticulum minus microsomes 15%, mitochondria 22% and lysosomes + peroxisomes 2% and the remaining cytoplasm which contains granules, lipid droplets and glycogen, 54%. This would indicate that the lysosome-peroxisome compartment comprises only 1/10 the volume occupied by the mitochondrial compartment. These relative proportions should be considered when analyzing the following data.

Fig. 3 illustrates the loss of nuclide from the supernatant (60,000 g-min) which remains after the removal of mitochondria but contains the lysosomes, peroxisomes and the cytosol (6×10^6 g-min). All data have been normalized by arbitrarily setting the nuclide concentration (quantity of nuclide/mg protein) in the homogenate equal to 100. All other data given are relative to this number. Previous studies in this and other laboratories have shown that initially plutonium and americium in the cytosol are primarily associated with ferritin (8). Thus, at very short times after injection the concentration of plutonium and americium in

cytosol both exceed that of the homogenate, whereas in the supernatant free of mitochondria the concentrations of plutonium and curium were greater than the concentration in the homogenate at very short times after injection. The concentration of americium in this fraction is less than that in the homogenate. At longer times after injection a rapid decrease in the concentration of all 4 nuclides occurs in both of these fractions. After the early rapid decline in these supernatants, the concentration of all the nuclides studied showed a slow but steady decrease in concentration over the experimental period.

The relative concentration of plutonium, americium, curium and californium in the fraction containing mitochondria and lysosomes are shown in Fig. 4. The concentration of plutonium, curium and californium increased in the mitochondrial fraction for 3 weeks. At this time the concentration of californium began a gradual decrease but was still greater than the concentration in the homogenate at 450 days. The concentration of plutonium continued to increase with the highest value occurring at 500 days. In contrast, ^{241}Am concentration began at relatively high values and underwent a slow but steady decrease for up to 250 days. The concentration of all 4 nuclides in the mitochondrial fraction was greater than that in the original homogenate during the period studied.

Except for plutonium, concentrations of these nuclides in lysosomal fractions showed similar changes. An early steady increase in concentration of californium, curium and plutonium was followed by a rapid decrease which begins at 3 weeks and levels

out at about 200 days into a slow steady decline to levels less than those of the original homogenate. The concentration of americium in lysosomes is high immediately after injection, but rapidly declines over the 250 day period studied.

TABLE 1
DISTRIBUTION OF NUCLIDES BETWEEN CYTOSOL AND
HEAVY PARTICLE FRACTIONS OF LIVER HOMOGENATES*

Time After Injection	Nuclide			
	Pu	Am	Cf	Cm
1 Day	0.1	0.05	0.2	0.06
1 Week	0.7	0.3	1.4	1.
1 Month	2.5	0.35		2.5
6 Month	6.7	0.36	1.1	

* Data expressed as the ratio of % of total activity in three heaviest fractions and the % of total activity in three lightest fractions. Total gradient collected in 17 tubes.

These results obtained by differential centrifugation were confirmed by layering an aliquot of the 6000 g-min supernatant - without nuclei and debris - on a 10-50% continuous sucrose gradient and centrifuging until equilibrium was reached. The data were computed as the ratio of the percent of the total radioactivity in the gradient in the three heaviest fractions to that of the three lightest fractions (Table 1). The heaviest fractions contain heavy particles which include mitochondria and the light fraction is composed of soluble material and also contains some microsomes. In the liver of dogs injected with plutonium, this ratio increased until

the amount of nuclide in the mitochondria was 7 times that of the cytosol at 6 months after injection. Curium seems to behave in a similar manner during the first month after injection. The americium ratio appears to be constant after about one week. The amount of californium in these same fractions is intermediate to that of americium and plutonium.

A graphical presentation of the distribution of plutonium (70 days after injection) and the marker enzyme for lysosomes in the 6000 g-min supernatant of canine liver is seen in Fig. 5. The peak of acid phosphatase activity indicates the principal density at which relatively pure lysosomes are found. However, the concentration of nuclide in this area is not as great as it is in the heavier fractions. If we consider the total activity in the gradient, about 50% is with the heavy - mitochondrial - fractions while only 12% is with lysosomes and 30% associated with the cytosol.

DISCUSSION

Using current methods it is nearly impossible to obtain pure fractions of organelles whether it be mitochondria or lysosomes. Any particular fraction can be enriched with particles of similar or identical densities, but fractions remain heterogeneous. Also we should caution the reader to be aware that a high concentration of nuclide in a particular fraction does not necessarily indicate that the nuclide is associated with the predominant particle of that fraction. Further studies need to be performed to isolate and identify specific sites with which actinide elements are associated. We have done this to a limited extent with mitochondria and lysosomes

and found that 60-70% of the plutonium and americium was bound to membranes.

Previous reports have indicated that polymeric (1) and monomeric (2) plutonium was predominantly associated with lysosomes in rat liver, whereas we have found it to be associated with the mitochondrial fraction in canine liver. Perhaps the difference in experimental finding is due to metabolic differences between canine and rodent livers.

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FLOW SHEET FOR ANALYSIS OF LIVER HOMOGENATES

Fig. 1 Flow sheet for analysis of liver homogenates.

VOLUMETRIC SUBDIVISION OF LIVER TISSUE, HEPATOCYTE AND HEPATOCYTIC CYTOPLASM

"Correlated Morphometric and Biochemical Studies on the Liver Cell,"
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Fig. 2 Relative volumes of the subcellular components in a typical hepatocyte from rodent liver.

Fig. 3 Nuclide concentration in liver cytosol and supernatant free of mitochondria but containing lysosomes and peroxisomes.

Fig. 4 The relative concentration of plutonium, americium, curium and californium in the fractions containing mitochondria and lysosomes.

Fig. 5 Distribution of plutonium (70 days after injection) and the marker enzyme for lysosomes in the 6000 g-min supernatant of canine liver.

BINDING OF ^{241}Am BY BONE PROTEIN

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Abstract: A study to characterize some details involved in the binding of actinide elements to skeletal tissue has been initiated. The present studies have been limited to ^{241}Am as a model representative of the actinide series. Experiments were carried out first with mature skeletal tissue obtained from a beagle which had been injected with ^{241}Am . A protein fraction of high but as yet undetermined M. W. which was associated with ^{241}Am was extracted from the whole bone homogenate. Extracts were also prepared from embryonic or neonatal skeletal tissue of rats and beagles. These extracts were incubated in vitro with ^{241}Am and ^{90}Sr . These experiments showed that beginning with the onset of ossification a protein of M. W. $\approx 500,000$ emerged which had a high affinity for ^{241}Am . With increasing time after conception another protein of very high M. W. emerged to which the nuclide had an affinity which was even higher than the affinity for the protein of M. W. $\approx 5 \times 10^5$. ^{90}Sr exhibited a much lower affinity for both of these proteins, but qualitative similarities existed between the uptake of ^{241}Am and ^{90}Sr by both of these proteins. The protein of M. W. $\approx 5 \times 10^5$ and the very high M. W. protein both gave positive tests for glycoprotein and sialic acid.

Introduction

All actinide elements deposit to a significant but variable degree in the skeleton and the damage to the skeleton (cells) produced by these nuclides represents a substantial biological hazard. Very little knowledge of the biochemical events involved in the deposition of these nuclides in bone is presently available. Herring and coworkers have isolated from decalcified bone a number of proteins of relatively low M. W. ($< 50,000$) and characterized these in some detail (1-3). The capacity for binding of some of the actinides by these proteins was studied at the Institute of Cancer

Research in Sutton, England (4-7). Both of these groups have provided valuable basic information on substances derived from bone which can bind these elements. They have implicated the involvement of glycoproteins and sialoglycoproteins in this binding by extracting and isolating proteins with these characteristic properties. A study to extend these earlier observations was initiated in this laboratory and some preliminary results are presented in this report.

Methods

The isolation of specific but yet unknown proteins which are capable of binding actinides such as ^{239}Pu or ^{241}Am from bone differed from that of previous workers in that the initial step of bone decalcification with EDTA was omitted. A flow sheet for the procedure used in this laboratory is shown in Fig. 1. Fresh skeletal tissue from a mature beagle which had been injected with 3 μCi of ^{241}Am in citrate of pH 3.5 intravenously one day prior to sacrifice was frozen at -70°C , ground to a fine powder, sifted, homogenized in distilled H_2O and separated into a soluble and insoluble phase by centrifugation. The resulting supernatant was free of ^{241}Am and did not contain protein in a concentration sufficient for precipitation with either alcohol or cetylpyridinium (C.P.C.) The nuclide was in the pellet which was extracted with a buffer of 0.067 M PO_4^{3-} and 10% KCl, pH 7.5. After extraction the supernatant and pellet were separated by centrifugation at 15,000 $\times g$ for 45 minutes. This cycle of extraction can be carried out 3 or 4 times

with sustained yields of protein and ^{241}Am . The large volume of supernatant containing ^{241}Am was reduced by molecular filtration using a Pellicon filter of 10,000 M. W. cutoff. The soluble ^{241}Am protein was retained by the filter (Fraction A). Precipitation of protein from the filtrate with C.P.C. was unsuccessful. The material retained by the filter was dissolved either in H_2O or in KCl-PO_4 buffer. Fresh skeletal tissue taken from rat or beagle fetuses or from a neonatal dog also was extracted according to this procedure. After molecular filtration Fraction A was incubated with ^{241}Am in citrate of pH 3.5 for 20 minutes at $\sim 40^\circ\text{C}$. Separation of the incubation mixture was carried out by high speed sucrose density gradient centrifugation ($2.2 \times 10^5 \text{ xg}$ for 20 hours) in a gradient of 6.5 to 26% sucrose and by gel chromatography on Sephadex G-100 or by chromatography on Sepharose 4B. The presence of glycoproteins was determined qualitatively by the Periodic Acid Schiff's Reaction. Sialic acid was determined by the Warren Reaction with thiobarbituric acid.

Results

The result of chromatography on G-100 of the 15,000 xg supernatant from bone tagged in vivo with ^{241}Am is presented in Fig. 2. All of the ^{241}Am eluted with the void volume, indicating that ^{241}Am was associated with a high molecular weight protein. There were no other areas of ^{241}Am activity throughout the elution volume.

The skeletons from rat fetuses taken at 14, 17, and 21 days of gestation were extracted. Fraction A, i.e. the sample corresponding

to the one on which chromatography on Sephadex G-100 was carried out, was incubated with ^{241}Am and subjected to a high speed sucrose density gradient separation. After centrifugation, the gradients were collected into 14 fractions of decreasing density with approximately equal volumes and the ^{241}Am content of each fraction including that which was retained in the gradient tube, was determined (Fig. 3). Fraction numbers are plotted in the order of decreasing densities vs. the ^{241}Am content of each fraction. At day 14, before the onset of ossification, almost all of the nuclide remained in the light gradient fractions. At day 17, the beginning of ossification, the nuclide was found in light, medium and a small quantity in the very dense region of the gradient and at 21 days of gestation, birth, practically all of the americium was associated with a protein which sedimented with the applied g-force.

A parallel study was then done on the fetus of the beagle. In Fig. 4, results of the same gradient procedures are shown for extracts of canine fetal cartilage at 39, 49, 54 days of gestation and at time of birth. The radioactivity of the total ^{241}Am applied to the gradient was normalized to 10^5 CPM in order to make a direct comparison between the various times of gestation possible. The solid line on this graph is that part of the ^{241}Am which was found in the soluble fractions of the gradient. The amount of ^{241}Am associated with protein heavy enough to sediment in the gradient tube is not shown in this figure. Ossification in the beagle commences between the 37th and 39th day as determined using the Von Kossa staining procedure (8). Calcification is quite slow initially.

The appearance of an ^{241}Am -binding protein is represented at 39 days after conception by the shoulder at fraction #4. By 49 days and 56 days, this shoulder has changed into a peak of ^{241}Am activity. The quantities of ^{241}Am found in these soluble fractions of the gradient decreased with time and at birth (64 days) most of the nuclide sedimented in the high density fractions, indicating the presence of a very high M. W. constituent. This shift of ^{241}Am activity from fractions of medium density into those of very high density is most clearly seen in Fig. 5. In this figure, the time after conception is plotted against the % of total nuclide applied to the gradient tube which is sedimentable. This percentage increased from about 4% at day 39 to 97% at time of birth.

Identical extracts (Fraction A) from dog fetuses were then incubated with ^{90}Sr instead of ^{241}Am and subjected to the procedure as outlined before for americium (Fig. 6). Fig. 6 shows that there are major quantitative differences but qualitatively the results obtained after incubation with ^{241}Am and ^{90}Sr are comparable.

In addition to the sucrose density separation, the fetal extracts of canine skeletons also were separated by gel chromatography. Since during chromatography of the ^{241}Am bone extracts on Sephadex G-100 the protein-bound ^{241}Am was eluted at the void volume, gels with separating capabilities for larger molecules were applied and final chromatography was done using Sepharose-4B, an agarose gel with an effective range of separation between molecular weights of 3×10^6 and 3×10^5 . Three of these chromatograms are shown in Fig. 7. For a better comparison a correction was applied to normalize

the total activity for the three given spectra; no correction however was applied to the corresponding optical density data. The change in the elution pattern of ^{241}Am following gel chromatography as a function of time was similar to the density distribution observed following sucrose gradient analysis. At the beginning of ossification, most of the ^{241}Am elutes in the low M. W. region. Some of the nuclide found in this area is dialysable in Tris-buffer, but part of it may be protein bound. In this low M. W. region, tests of the eluate for glycoprotein and sialic acid were negative. As the skeleton developed with increasing time after conception the intermediate ^{241}Am peak representing protein of a M. W. of approximately 5×10^5 increased and a test for both glycoprotein and sialic acid was positive. In the third peak, nuclide associated with strongly glycoprotein and sialoprotein-positive protein of high molecular weight which eluted with the void volume.

Discussion

The studies by Herring and Taylor and their coworkers were carried out with mucosubstances isolated from supernatants obtained during decalcification of bovine cortical bone with EDTA. All of these proteins had a relatively low molecular weight. In our laboratory proteins were extracted from whole bone homogenate, leaving the mineral phase of the bone more or less unchanged.

Knowing that the highest concentration of the nuclide is found in those areas in which new bone is being laid down, we assumed that the active process of bone mineralization and that of deposition

of the nuclide were related chemically. We expected that at the onset of calcification in the fetus, the presence of such a substance could be most easily demonstrated.

Using bone from a beagle injected with Am-citrate, direct recovery of an actinide-protein complex should be possible. This procedure involves a slow and gentle process of solubilization of glycoproteins from bone. Only small quantities of protein and nuclide can be extracted during each cycle. The fraction of total nuclide in bone which can be recovered by these repeated extractions is likely to be dependent on such factors as age of the animal and time after injection. Most old deposits of the nuclide seem to be associated with the bone mineral and are thus not available through extraction of proteins.

Chromatography of the protein extract obtained from bone of a beagle injected with ^{241}Am produced an ^{241}Am -binding protein of M. W. > 100,000.

Results of both the sucrose gradient separation and gel chromatography of ^{241}Am tagged extracts of fetal and neonatal skeletons showed the emergence of a protein with a high affinity for the nuclide coincident with onset of ossification. The M. W. of this protein was estimated as being close to 500,000. This is the area coinciding with the peak of ^{241}Am at fraction #4-5 and the middle peak of ^{241}Am activity obtained after gel chromatography. As the skeleton becomes progressively calcified, more and more of the nuclide becomes associated with a protein of very high M. W..

When ^{241}Am was replaced by ^{90}Sr , results from sucrose density

gradient separation of fetal extracts showed that there was an appreciable difference in the quantitative distribution of the alkaline earth metal and therefore, in the affinity of these two elements for the binding proteins. The fraction of ^{90}Sr bound to both of the high M. W. proteins was considerably smaller than with ^{241}Am . Nevertheless the general pattern of ^{90}Sr -protein associations resembles those observed with the actinide. Results similar to this were observed earlier by Williams (9,10). A more detailed study is necessary to elucidate the interaction of the alkaline earth elements with the ^{241}Am -binding proteins and the position which these proteins occupy during bone mineralization.

It is presently unknown whether this very high M. W. material is related to the protein of medium M. W. in the sense that it consists of subunits of such a protein or if it is a completely different protein. This very high M. W. material seems to have a high affinity for the nuclide as indicated by the fraction of the total nuclide that is associated with it. Both of the high M. W. components, which do not yet represent single protein fractions and therefore have to be purified further, contain glycoprotein and sialoprotein. A connection between the proteins described here and those isolated by Herring is likely to exist. Work presently in progress in this laboratory may answer these questions.

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Fig. 1 Flowsheet for separation of bone protein.

Fig. 2 Elution pattern after chromatography on Sephadex G-100 of supernatant of bone extract obtained from dog injected with ^{241}Am one day before sacrifice. This supernatant was not subjected to Diaflow separation. ^{241}Am was quantitatively eluted with protein at the void volume, indicating the presence of an high M.W. Am-protein.

Fig. 3 Distribution of ^{241}Am after high speed sucrose density centrifugation (SW 50, 50,000 RPM, 22 hrs) of in vitro tagged supernatant obtained from rat fetal skeletons at various times of gestation. Ossification commences between day 16 and 17 after conception.

Fig. 4. Distribution of ^{241}Am after high speed sucrose density centrifugation of in vitro tagged supernatant obtained from canine fetal skeletons at various times of gestation (Fraction A). Ossification in the beagle begins between the 37th and 39th day after conception. ^{241}Am which sedimented with high M.W. material in gradient tubes was omitted from this graph and is presented in Fig. 5.

Fig. 5 Increase in the percentage of total ^{241}Am sedimenting with high M.W. material after high speed sucrose density centrifugation. The total ^{241}Am applied to each gradient was normalized to 10^5 CPM before calculating the fractions.

Fig. 6 Distribution of ^{90}Sr after high speed sucrose density centrifugation. Protein samples in this gradient are identical to those taken for the ^{241}Am gradient seen in Fig. 4. ^{90}Sr which sedimented with high M. W. material in gradient tubes is given as shaded bars.

COLUMN CHROMATOGRAPHY OF CARTILAGE EXTRACT ON SEPHAROSE - 4B

Fig. 7 Elution pattern of ^{241}Am tagged protein obtained from canine fetal skeletons (Fraction A) after chromatography on Sepharose 4B. High M.W. material is eluted in fractions of low number (left side of spectrum); low M.W. material is eluted in fractions having a high number (right side of spectrum). Total ^{241}Am eluted was normalized to the same value for each of the three chromatograms. At the onset of ossification, most of the ^{241}Am is found in low M.W. fractions. With increasing time after conception, most of the nuclide becomes associated with high M.W. material. The middle peak of ^{241}Am activity corresponds to a M.W. of about 500,000. The optical density curve was not normalized.

RETENTION OF ^{239}Pu IN THE ULNA OF THE BEAGLE COMPARED WITH RETENTION IN THE HUMERUS AND THE THIRD LUMBAR VERTEBRA

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ABSTRACT: The initial deposition of ^{239}Pu and the rate of decrease in retention are both higher in bones which have a relatively greater amount of trabecular bone. For this reason the ulna, which has relatively less trabecular bone, was chosen for an extensive comparison with the humerus and third lumbar vertebra. These three bones were obtained from 40 beagles at dose levels from 0.00064 to 0.095 $\mu\text{Ci } ^{239}\text{Pu/kg}$ (P0.1 to P2) and at times from 35 to 4549 days after injection. Analyses showed that the initial concentration of ^{239}Pu in the ulna was about 25% that in the other two bones. Retention of ^{239}Pu in the ulna was approximately constant over the 12.5 year period. In contrast, retention in both the humerus and third lumbar vertebra decreased during the first several years and then approached approximately constant values. A kinetic model for the observed retention has been formulated.

INTRODUCTION

In the continuing extension of measurements of skeletal retention of ^{239}Pu to longer times and lower dose levels, it has been shown that, as a result of the effect of α -irradiation on bone remodeling processes, separate retention equations are required for the high dose levels, P5, P4, and P3. For dose levels P2 and lower a single equation is applicable on the basis of existing data. From skeletal retention data for 16 dogs at the P1.7, P1, and P0.5 dose levels it is estimated that 58% of the ^{239}Pu initially deposited in the skeleton leaves with a half-period of about 630 days and 42% remains throughout the life-time of the beagle.⁽¹⁾ Analysis of the skeletal retention of 11 dogs at the P1 dose level over the period 35 to 769 days showed significant ($p < 0.05$) decrease in total skeletal retention, and in retention in lumbar and thoracic vertebrae, ribs, humeri, and femora. No significant decreases (or increases) were found for other bones or groups of bones.⁽²⁾ The most extensive set of measurements of skeletal retention at low dose

levels was made on 44 humeri and 46 third lumbar vertebrae from dogs at the P2, P1.7, and P1 dose levels over the period 35 to 5200 days after injection.⁽²⁾ In the humerus 71% of the ^{239}Pu left with a half-period of about 320 days and 29% remained throughout the beagle's life-time. Corresponding data for the lumbar vertebra were essentially the same, for 69% left with a half-period of about 380 days, and 31% remained. Thus, retention in these two bones decreases more rapidly and to a lower constant value than does total skeletal retention. In addition, the initial concentration of ^{239}Pu is higher in these two bones than the average initial skeletal concentration. A similar study of the retention of ^{239}Pu at low dose levels in the ulna has since been done. The ulna was chosen since it has a relatively higher proportion of cortical bone, its initial concentration of ^{239}Pu is lower than the average initial skeletal concentration, and a sufficient number of ulnae were available for a meaningful comparison with the humeri and lumbar vertebrae.

MATERIALS AND METHODS

The skeletal remnants of beagles which had been injected as young adults with low levels (0.1, 0.2, 0.5, 1, 1.7 and 2 level) of $^{239}\text{PuIV}$ in 0.08 M citric acid-sodium citrate buffer were surveyed and it was found that, of the bones which might be expected to show a contrasting retention to the humerus and vertebrae, only the ulna could be collected with any regularity. Thirty three were analyzed, 29 of which came from animals on which retention by the humerus and 3rd lumbar vertebra had already been measured. Data on these bones were added to that previously obtained from 11 test run dogs on which complete skeletal analysis had been done.⁽³⁾

As the exhumed ulnae had been preserved for variable times and by several methods, it was not possible to obtain values for their 'wet weights'. Wet weights were, however, calculated using an ashed weight/wet weight ratio of 0.480, the average value for 18 previously reported measurements.⁽¹⁾

The ulnae were reduced to ash, put into solution, sampled and plutonium content determined as described previously.⁽¹⁾

RESULTS

The results of the measurement of both amount and concentration of ^{239}Pu in the ulna are presented in detail in Table 1. Ulnae were obtained from 44 dogs at dose levels P2, P1.7, P1, P0.5, P0.2, and P0.1 and at times ranging from 35 to 4549 days after injection. An attempt to fit a single exponential to these retention data showed no significant change with time. The mean retention with standard deviation is $(0.219 \pm 0.045)\%$ dose for these 44 ulnae. The humerus and third lumbar vertebra from 40 of these 44 dogs were also measured, and the retention in each of the three bones is given in Table 2. The data fall into two groups with respect to time after injection. Twelve sets were obtained during the first two years and 28 during the period 4.8 to 12.5 years. The average retention for the humerus, H, third lumbar vertebra, V3, and the ulna, U1, for the entire group and the two subgroups is given in Table 3, which also includes the average of the ratios, H/U1 and V3/U1. The average values for U1 for the two time periods are the same while those for H and V3 differ significantly and have greater fractional standard deviations.

The following three equations were fit to the sets of data for the three bones:

$$\text{Retention} = ae^{-bt} \quad (1)$$

$$\text{Retention} = a_1e^{-b_1t} + a_2 \quad (2)$$

$$\text{Retention} = a_1e^{-b_1t} + a_2e^{-b_2t} \quad (3)$$

The parameters appear in Tables 4, 5, and 6. None of the equations fit the data for the ulna, and Eq. (2) gave the best fit for the humerus and the third lumbar vertebra. Thus retention of ^{239}Pu in these bones is given by

$$U1 = 0.214 \quad (4)$$

$$H = 1.95 e^{-0.0018t} + 0.97 \quad (5)$$

$$V3 = 0.72e^{-0.0018t} + 0.33 \quad (6)$$

where retention is expressed as percent dose and t is in days. The initial (and approximately time independent) concentration of ^{239}Pu in the ulna is only about 1/4 that in the humerus and third lumbar vertebra.

DISCUSSION

The autoradiographic determination of the microdistribution of ^{239}Pu in bone by Dr. W.S.S. Jee and associates provides a basis for developing a kinetic model for skeletal retention.⁽⁴⁾ Initially ^{239}Pu is deposited non-uniformly on endosteal, periosteal and haversian system surfaces. The major deposition is on trabecular surfaces which correlates with the macro observation that initial deposition is highest in bones with a high proportion of trabecular bone. The continuous remodeling of bone removes ^{239}Pu from the surface by new bone formation and by resorption of the surface. The new bone which covers the initial high surface concentration of ^{239}Pu has a low concentration of ^{239}Pu . When ^{239}Pu on the surface is resorbed it is temporarily contained in the osteoclasts and then transferred to macrophages in the marrow. The macrophages are swept out of the marrow into the circulation.

The following kinetic model is proposed to relate the measured retention in whole bones to the autoradiographic observations of the microdistribution of ^{239}Pu . Assume that the ^{239}Pu that deposits is distributed in four compartments on a time dependent basis. Let $A = ^{239}\text{Pu}$ on bone surfaces, $B = ^{239}\text{Pu}$ that has been buried by new bone formation and also that deposited diffusely in new bone, $C = ^{239}\text{Pu}$ that has been resorbed and is present in the whole bone in osteoclasts and macrophages (and perhaps bound to transferrin), and $D = ^{239}\text{Pu}$ that has been transported out of the bone by the macrophages (and transferrin). Assume that all ^{239}Pu is initially in A, i.e., at $t = 0$, $A = A_0$ and $B = C = D = 0$ and at $t > 0$, $A_0 = A + B + C + D$. If bone remodeling occurs at an approximately constant rate, the rate of removal of ^{239}Pu will be proportional to the chance that there is ^{239}Pu on the increment of surface undergoing remodeling. In this model the A decreases by two competitive first order processes, burial by new bone formation with rate constant, k_1 , and resorption with rate constant, k_2 . The resorbed ^{239}Pu in cells then exits from the bone with the rate constant, k_3 . The rate equations for these processes and their solutions are as follows:

$$-dA/dt = (k_1 + k_2)A \quad (1)$$

$$A = A_0 e^{-(k_1 + k_2)t} \quad (2)$$

$$dB/dt = k_1 A_0 e^{-(k_1 + k_2)t} \quad (3)$$

$$B = \frac{k_1 A_0}{k_1 + k_2} \{1 - e^{-(k_1 + k_2)t}\} \quad (4)$$

$$dC/dt = k_2 A_0 e^{-(k_1 + k_2)t} - k_3 C \quad (5)$$

$$C = \frac{k_2 A_0}{k_3 - k_2 - k_1} \left\{ e^{-(k_1 + k_2)t} - e^{-k_3 t} \right\} \quad (6)$$

$$dD/dt = k_3 C \quad (7)$$

$$D = \frac{k_3 k_2 A_0}{k_3 - k_1 - k_2} \left[\frac{\left\{ 1 - e^{-(k_1 + k_2)t} \right\}}{(k_1 + k_2)} - \frac{(1 - e^{-k_3 t})}{k_3} \right] \quad (8)$$

Thus A decreases exponentially with time, B increases from 0 to $(k_1 A_0)/(k_1 + k_2)$, C increases from 0 to a maximum value then decreases to 0, and D increases from 0 to $(k_2 A_0)/(k_1 + k_2)$. If the ^{239}Pu were deposited uniformly, and if k_1 and k_2 were the rate constants for new bone formation and resorption of bone and the two processes balanced so that there was no net change in bone mineral, then k_1 would equal k_2 . However, even though ^{239}Pu is deposited nonuniformly and the presence of ^{239}Pu may have some effect on rates of remodeling, k_1 and k_2 should not differ greatly at low dose levels during the first few years after injection of the young adult beagles.

Measurement of retention of ^{239}Pu in a whole bone including marrow corresponds to measuring the sum, $A + B + C$:

$$\begin{aligned} A + B + C &= A_0 \left[e^{-(k_1 + k_2)t} + \frac{k_1}{k_1 + k_2} \left\{ 1 - e^{-(k_1 + k_2)t} \right\} \right. \\ &\quad \left. + \frac{k_2}{k_3 - k_1 - k_2} \left\{ e^{-(k_1 + k_2)t} - e^{-k_3 t} \right\} \right] = \quad (9) \\ &A_0 \left[\frac{k_1}{k_1 + k_2} + \left\{ \frac{k_2}{k_1 + k_2} + \frac{k_2}{k_3 - k_1 - k_2} \right\} e^{-(k_1 + k_2)t} - \frac{k_2}{k_3 - k_1 - k_2} e^{-k_3 t} \right] \end{aligned}$$

To apply Eq. (9) to the observed data the rate limiting step must be identified. If $k_3 \gg k_1 \approx k_2$, which would be the case if the remodeling processes are rate limiting, then

$$\frac{k_2}{k_3 - k_1 - k_2} \approx \frac{k_2}{k_3} \ll \frac{k_2}{k_1 + k_2} \quad (10)$$

and

$$A + B + C \approx A_0 \left[\frac{k_1}{k_1 + k_2} + \frac{k_2}{k_1 + k_2} e^{-(k_1 + k_2)t} \right] \quad (11)$$

This condition also requires that

$$dC/dt \approx 0, \quad C = \frac{k_2 A_0}{k_3} e^{-(k_1 + k_2)t} \quad (12)$$

which is contradicted by the autoradiographic evidence. The number of macrophages containing ^{239}Pu in the lumbar vertebral bodies from beagles at the P1 dose level were counted. These were some of the same dogs from which the humerus, third lumbar vertebra, and ulna were obtained, and total skeletal retention was determined. Labeled macrophages were absent at 5 days, present at 35 days, present in large numbers at 96 through 647 days, and absent in vertebral bodies from dogs that survived 1539 and 2257 days. The deposition of ^{239}Pu on trabecular surfaces was high at 5 days but low at 97, 186, 376, and 467 days.

Thus, efflux of labeled macrophages from the marrow is the rate limiting step, and $k_3 \ll k_1 \approx k_2$ so that

$$\frac{k_2}{k_3 - k_1 - k_2} \approx - \frac{k_2}{k_1 + k_2} \quad (13)$$

and

$$A + B + C \approx A_0 \left[\frac{k_1}{k_1 + k_2} + \left\{ \frac{k_2}{k_1 + k_2} - \frac{k_2}{k_1 + k_2} \right\} e^{-(k_1 + k_2)t} + \frac{k_2}{k_1 + k_2} e^{-k_3 t} \right] = A_0 \left[\frac{k_2}{k_1 + k_2} e^{-k_3 t} + \frac{k_1}{k_1 + k_2} \right] \quad (14)$$

which is of the form

$$\text{Retention} = a_1 e^{-b_1 t} + a_2$$

which was observed for the humerus and third lumbar vertebra.

From Eqs. (5) and (6) (and Table 5) it is seen that in both of these bones 1/3 of the initial ^{239}Pu is buried or deposited in new bone, 2/3 is resorbed and transported out of H and V3 with a half-period of about one year, and $k_1 \approx 1/2 k_2 > k_3 = 0.0018\text{d}^{-1}$.

The constant retention observed for the ulna is more difficult to interpret. Two factors undoubtedly are involved. First is the higher proportion of cortical bone in the ulna, with trabecular bone limited mainly to the proximal end. Second is the high variation between dogs (fractional standard deviation = 0.20), which is characteristic of the retention, distribution, and excretion of ^{239}Pu . It can also be speculated that, since there is less red marrow in the ulna, there is a smaller population of macrophages to transport ^{239}Pu from the ulna. Mathematically, constant retention would result if $k_2 = 0$, but this is not biologically meaningful. A more realistic possibility is that the values of k_1 , k_2 , and k_3 are all less than those for the humerus and third lumbar vertebra, and that $k_1 > k_2$. If this is the case, decrease in retention could be masked by variation between dogs. The values of k_1 and k_2 for a whole bone very probably reflect the relative amounts of trabecular and cortical bone since both surface area and the rate of surface renewal are

greater in a unit amount of trabecular bone. The higher initial concentrations in the humerus and vertebra also reflect the greater proportion of trabecular bone. A more definitive interpretation of retention of ^{239}Pu in the ulna will be possible when correlative autoradiographic results are complete.

Some important questions which remain unanswered are as follows: Is transport by macrophages the only mechanism for removal of ^{239}Pu from bone? Do all the macrophages which contain ^{239}Pu remain intact while in bone or do some rupture and release ^{239}Pu to be redeposited? When the macrophages leave the bone, where do they go? (Is the ^{239}Pu simply transported to lymph nodes or other sites or does some or all become available for excretion?) Can the rate of efflux of macrophages be increased? Answers to these questions would be useful in efforts to devise new or improved methods of decorporation therapy.

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TABLE 1

Weight and ^{239}Pu Retention by the Ulna of the Beagle

Dog	Interval Inj. to Death (Days)	Ashed Weight	Estimated Wet Weight	% Injected Dose per Ulna	% Injected Dose per gm Wet Ulna	% Injected Dose per gm Ashed Ulna
M31P0.1B	2760	6.38	13.3	0.278	0.0209	0.0436
M44P0.2B	97	5.96	12.4	0.186	0.0150	0.0312
M31P0.2B	2640	6.40	13.3	0.273	0.0205	0.0426
F13P0.5	2388	5.18	10.8	0.183	0.0170	0.0353
F45P0.5C	35	5.07	10.6	0.146	0.0138	0.0288
M45P0.5B	42	5.36	11.2	0.200	0.0179	0.0373
M3P1	4292	6.44	13.4	0.216	0.0161	0.0335
M4P1	4549	5.96	12.4	0.209	0.0168	0.0351
F6P1	4292	3.93	8.18	0.287	0.0351	0.0730
F7P1	3981	3.11	6.48	0.240	0.0370	0.0771
M8P1	3367	5.55	11.6	0.189	0.0164	0.0341
F9P1	2257	4.30	8.96	0.231	0.0258	0.0537
F10P1	3649	5.11	10.6	0.227	0.0213	0.0444
M12P1	2374	4.72	9.83	0.136	0.0138	0.0288
T24P1 ^a	97	5.47	11.2	0.245	0.0215	0.0448
T25P1	467	6.16	12.9	0.273	0.0213	0.0443
T26P1	647	4.89	10.4	0.219	0.0215	0.0448
T28P1	559	4.40	9.24	0.265	0.0289	0.0602
T30P1	35	4.92	9.71	0.203	0.0198	0.0413

^a Wet weight for T24 . . .40P1 are measured values.

TABLE 1 (continued)

T32P1	274	2.32	5.81	0.271	0.0561	0.117
T33P1	375	5.39	11.2	0.205	0.0183	0.0380
T34P1	746	4.57	9.46	0.300	0.0315	0.0656
T37P1	186	4.18	8.99	0.235	0.0270	0.0562
T39P1	376	5.33	10.1	0.134	0.0121	0.0252
T40P1	769	5.42	11.0	0.175	0.0154	0.0323
M1P1.7	3025	4.26	8.88	0.151	0.0170	0.0354
F2P1.7	3430	3.56	7.42	0.194	0.0262	0.0545
M3P1.7	3430	5.06	10.6	0.198	0.0188	0.0391
M4P1.7	3312	5.20	10.8	0.241	0.0222	0.0463
F5P1.7	2659	4.03	8.39	0.144	0.0172	0.0357
F6P1.7	2221	3.67	7.64	0.224	0.0293	0.0611
F7P1.7	3353	4.81	10.0	0.174	0.0174	0.0362
M8P1.7	3282	6.71	14.0	0.198	0.0142	0.0295
F9P1.7	2500	4.19	8.73	0.197	0.0226	0.0470
M12P1.7	2973	5.64	11.7	0.222	0.0189	0.0394
M1P2	2985	5.76	12.0	0.324	0.0270	0.0563
M3P2	3185	6.47	13.5	0.247	0.0183	0.0382
F6P2	2947	3.82	7.95	0.280	0.0352	0.0734
F7P2	2093	4.38	9.13	0.216	0.0237	0.0493
M8P2	1761	5.54	11.5	0.230	0.0199	0.0415
F9P2	2014	3.94	8.20	0.201	0.0245	0.0510
F10P2	2912	3.77	7.86	0.172	0.0219	0.0456
M11P2	1617	5.25	10.9	0.228	0.0209	0.0434
M12P2	2284	5.92	12.3	0.261	0.0212	0.0441

TABLE 2

^{239}Pu Retention by ulna, humerus and 3rd lumbar vertebra
of the beagle

Dog	Interval: Injection to Death (Days)	Retention: % of Injected ^{239}Pu				
		Humerus (H)	3rd Lumbar Vertebra (V3)	Ulna (U1)	$\frac{H}{U1}$	$\frac{V3}{U1}$
F45P0.5C	35	2.28	0.971	0.146	15.6	5.42
T30P1	35	2.59	0.889	0.203	12.8	4.38
M45P0.5B	42	2.44	1.30	0.200	12.2	6.50
T24P1	97	2.67	0.859	0.245	10.9	3.51
T37P1	186	2.26	0.928	0.235	9.62	3.95
T32P1	274	3.72	0.946	0.271	13.7	3.49
T33P1	375	2.53	0.745	0.205	12.3	3.63
T39P1	376	1.71	0.703	0.134	12.8	5.28
T25P1	467	1.92	0.689	0.273	7.03	2.52
T28P1	559	1.92	0.477	0.265	7.24	1.80
T26P1	647	1.29	0.461	0.219	5.89	1.90
T40P1	769	1.41	0.618	0.175	8.06	7.69
M8P2	1761	0.758	0.254	0.230	3.30	1.10
M34P0.5B	1920	0.826	0.380	0.217	3.81	1.75
F9P2	2014	1.28	0.562	0.201	6.37	2.80
F7P2	2093	0.877	0.282	0.216	4.06	1.31
F6P1.7	2221	1.00	0.313	0.224	4.46	1.40
F9P1	2257	0.820	0.226	0.231	3.55	0.978
M32P0.5B	2275	0.850	0.253	0.179	4.75	1.41
M12P2	2284	1.08	0.347	0.261	4.14	1.33
M12P1	2374	1.42	0.492	0.136	10.4	3.62
F9P1.7	2500	0.982	0.322	0.197	4.98	1.63
F31P0.5C	2546	0.843	0.253	0.179	4.28	1.41
F5P1.7	2659	0.790	0.271	0.144	5.49	1.88
F10P2	2912	1.81	0.672	0.172	10.5	3.91
F6P2	2947	0.920	0.277	0.280	3.28	0.989
M12P1.7	2973	0.925	0.368	0.222	4.17	1.66
M1P2	2985	1.23	0.272	0.324	3.80	0.840
M1P1.7	3025	0.575	0.207	0.151	3.81	1.37
M3P2	3185	1.22	0.425	0.247	4.94	1.72
M8P1.7	3282	0.773	0.326	0.198	3.90	1.65
M4P1.7	3312	0.893	0.275	0.241	3.70	1.14
M8P1	3367	1.40	0.428	0.189	7.41	2.26
F2P1.7	3430	0.867	0.279	0.194	4.47	1.44
M3P1.7	3430	1.01	0.371	0.198	5.10	1.87
F10P1	3649	0.841	0.269	0.227	3.70	1.18
F7P1	3981	0.973	0.371	0.240	4.05	1.55
M3P1	4292	0.829	0.187	0.216	3.84	0.866
F6P1	4292	0.904	0.270	0.287	3.15	0.941
M4P1	4549	0.714	0.164	0.209	3.42	0.785

TABLE 3

Average retention of ^{239}Pu in the ulna, humerus,
and third lumbar vertebra

Bone	n = 40 35 to 4549 days			n = 12 35 to 769 days			n = 28 1761 to 4549 days		
	$\bar{x}$	σ	$\sigma/\bar{x}$	$\bar{x}$	σ	$\sigma/\bar{x}$	$\bar{x}$	σ	$\sigma/\bar{x}$
U1	0.214	0.043	0.20	0.214	0.046	0.22	0.215	0.042	0.20
H	1.35	0.71	0.53	2.23	0.66	0.29	0.973	0.264	0.27
V3	0.465	0.261	0.56	0.784	0.228	0.29	0.328	0.111	0.34
H/U1	6.52	3.57	0.55	10.7	3.1	0.29	4.73	1.89	0.40
V3/U1	2.28	1.42	0.62	3.83	1.42	0.37	1.61	0.76	0.47

TABLE 4

Parameters for retention = ae^{-bt}

35-4549 days, n = 40

Bone	a	b x 10 ³	b = 0 ?
U1	0.204 ± 0.013	+0.020 ± 0.025	0.4 < p < 0.5
H	1.98 ± 0.18	-0.236 ± 0.036	p < 0.01
V3	0.778 ± 0.0695	-0.300 ± 0.035	p < 0.01

TABLE 5

Parameters for retention = $a_1 e^{-b_1 t} + a_2$
 35-769 days, n = 12 and 1761-4549 days, n = 28

Bone	a_1	$b \times 10^3$	$b = 0 ?$	a_2
U1	-----	-----	-----	0.215 ± 0.042
H	1.95 ± 0.38	1.82 ± 0.49	$p < 0.01$	0.973 ± 0.264
V3	0.717 ± 0.126	1.82 ± 0.44	$p < 0.01$	0.328 ± 0.111

TABLE 6

Parameters for retention = $a_1 e^{-b_1 t} + a_2 e^{-b_2 t}$
 35-769 days, n = 12 and 1761-4549 days, n = 28

Bone	a_1	b_1	$b_1 = 0 ?$	a_2	b_2	$b_2 = 0 ?$
U1	-----	-----	-----	0.214 ± 0.029	0.059 ± 0.053	$0.2 < p < 0.3$
H	1.93 ± 0.38	1.83 ± 0.49	$p < 0.01$	0.989 ± 0.153	0.017 ± 0.052	$0.7 < p < 0.8$
V3	$0.594 \pm 0.259^*$	$2.92 \pm 1.17^*$	$0.02 < p < 0.05$	0.469 ± 0.109	0.138 ± 0.077	$0.05 < p < 0.01$

*n = 11, computer rejected number for T26P1

JEE-MILLER DETAILED NEUTRON-INDUCED AUTORADIOGRAPHY
OF ^{239}Pu BONES

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ABSTRACT: Neutron-induced autoradiography (NIAR) of bones from beagle dogs injected with as little as $0.0006 \mu\text{Ci}$ of ^{239}Pu /kg body weight is now possible. Undecalcified 7μ thick sections are affixed to Lexan film and irradiated with thermal neutrons at the Ames Laboratory Research Reactor, Iowa State University, Ames, Iowa, or the Massachusetts Institute of Technology Research Reactor, Massachusetts Institute of Technology, Cambridge, Massachusetts, for about one month. Recent improvements in developing techniques have given reproducible films showing fission fragment tracks upon a bone image. This method allows rapid, accurate localization of the ^{239}Pu in bones from animals receiving very small amounts of ^{239}Pu .

INTRODUCTION

Recently, the technique of fission fragment track production has been used to characterize the distribution of plutonium (^{239}Pu) in bone.⁽¹⁻³⁾ The neutron irradiation of very small quantities of plutonium produces fission fragments which bombard a polycarbonate film. Using track etching techniques the fission tracks become visible on the film. The above method has advantages over conventional nuclear emulsion autoradiography of ^{239}Pu because it eliminates long exposure times for bone containing minute quantities of plutonium and it eliminates the fading of the latent image which plagues nuclear emulsion autoradiographers. In earlier studies, bone sections were sandwiched between polycarbonate films and exposed to thermal neutrons. The films were detached following irradiation, then etched to reveal the fission fragment tracks; later the bone specimen was superpositioned upon the etched

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film. The necessity for removal and replacement of the bone section makes it difficult to specify whether fission fragment tracks arise from the bone surface, from buried sites in bone, or from bone marrow cells.⁽¹⁻³⁾

More recently, the Jee-Miller detailed neutron-induced autoradiographic process for bone containing ^{239}Pu was described.⁽⁴⁾ This method produced autoradiographs with both fission fragment tracks and a corresponding bone image, with no requirement for section repositioning. Seven micron thick undecalcified bone sections were affixed to 300 microns thick Lexan (polycarbonate) film coated with 0.5% calfskin gelatin and exposed to thermal neutrons in the thermal column of the Material Testing Reactor (MTR) at the National Reactor Testing Station in Idaho. The irradiated specimens were immersed in 6N KOH at 50°C for 1.5 hours to etch pinholes at the location of each fission track and to pit the film at the location of the bone.

Description of Current Process.

Section preparation and irradiation. Thin undecalcified bone sections are a prerequisite to high resolution neutron-induced autoradiography. Blocks of undecalcified dog bones are fixed in absolute acetone, defatted in ether, embedded in a combination of ethyl-butyl methacrylate, and sectioned on a Jung microtome.^(5,6) The 7 μ bone sections are floated off the knife with aid of 70-80% alcohol, a brush, and a sharpened section lifter, then affixed to a 300 μ thick Lexan (polycarbonate) film (Westlake Plastics, Lenni Mills, Pa.) which has been previously coated with 0.5% calfskin gelatin. The specimens (bone section affixed to Lexan) are allowed to air dry. They are then exposed to neutrons in the thermal column of the Ames Laboratory Research Reactor TV-1 Facility (ALRR), Iowa State University, Ames, Iowa, or the Massachusetts Institute of Technology Research Reactor (MITRR), Cambridge,

Massachusetts. Neutron fluence in the range of 10^{15} to 10^{16} n/cm² is used. During the irradiation, the temperature must be kept below 32°C, because higher temperatures will anneal the tracks. Subsequent to neutron irradiation, the specimens are allowed to cool before the developing procedure is initiated.

Developing procedure. The specimens are immersed in 6N KOH (low chloride) at 50°C for 1.5 hours to etch pinholes at the location of each fission track. The bone section separates from the film during this process. The etched film is rinsed in tap water, air dried, mounted between two microscope slides. The film exhibits both the fission fragment tracks and a bone image (see Figures 1-4).

Characteristics of the Thermal Column.

Currently, we are obtaining satisfactory neutron-induced autoradiographs of bones containing ²³⁹Pu from the ALRR and the MITRR.

The ALRR is a large scale enriched uranium-heavy water reactor. The TV-1 facility has a five foot square opening in one of the shielding faces of the reactor which penetrates the concrete and terminates near the reactor core tank. This large opening is filled with graphite blocks beyond an outer removable shield which is four feet thick. This horizontal facility is called a thermal column. In the thermal column, neutrons diffuse through the graphite without being captured, but are gradually reduced in speed until they are thermalized. That is, they are brought down to speeds that are in equilibrium with those of hydrogen atoms in the air at the temperature of the graphite which is approximately room temperature. The thermal column thus supplies thermal neutrons to irradiate the bone specimens. The critical point about the ALRR is that it produces room-temperature thermal neutrons at a low enough

level of radiation (to limit heat production) such that the Lexan is not melted or fogged irreparably. More specifically, the TV-1 facility possesses a vertical penetration, terminating in the top of the large graphite block comprising the main body of the thermal column. It utilizes a rotary shutter on top of the reactor, permitting sample manipulation without requiring the reactor to be shut down. A low-velocity air circulation system moves air through the facility and out the building ventilation stack. Radiation levels are sufficiently low to eliminate sample heating problems. Its thermal neutron flux is 6.0×10^7 n/cm²/sec with a cadmium ratio of 2600 and a gamma dose of 5.5×10^4 R/hour.

The MITRR possesses a good thermal to fast neutron ratio to avoid excessive fogging of the Lexan. It has a cadmium ratio using gold of around 700 in the thermal column.

Table 1 gives the approximate required fluence to produce a suitable number of fission tracks in our low dose samples.

Table 1.

<u>Sample Type</u>	<u>Injection Dose (μCi $^{239}\text{Pu/kg}$)</u>	<u>Required Fluence (Neutrons/cm²)</u>
P1	0.015	10×10^{15}
P0.5	0.005	30×10^{15}
P0.2	0.002	90×10^{15}
P0.1	0.0006	270×10^{15}

These fluences have been achieved at the ALRR and successful neutron induced autoradiography of a lumbar vertebra section from M31P0.1B injected with 0.00068 μ Ci of $^{239}\text{Pu/kg}$ and surviving 2760 days has been obtained.

Attempts to Explain Bone Image.

Previously, we thought that the beta emissions from ^{32}P , activated from ^{31}P , which is a major component of hard tissue, were the cause of the pitting of the film resulting in the bone image.⁽⁴⁾ Further studies will be performed by Mr. Mike Fellows as a basis for a Master's thesis at the Department of Nuclear Engineering, MIT, under Lincoln Clark, Jr., Director.⁽⁷⁾

Application of Technique.

The neutron-induced autoradiographic technique plays an integral role in delivery of the local ^{239}Pu data. Currently, we are concentrating our entire efforts to characterize: (1) the local variation of initial uptake of low levels of ^{239}Pu on bone surfaces, (2) the variation of plutonium deposits with time as bone remodeling proceeds, and (3) the variation in the volume of cells at risk with time in beagles injected with 0.015 μCi of ^{239}Pu or less/kg.

The program calls for the use of neutron-induced autoradiography to determine the residence time of ^{239}Pu on various bone surfaces using visual counting techniques and to measure the quantity of plutonium in both bone and marrow as a function of time using automatic track counting techniques.⁽⁸⁾ Currently, we are experimenting with ^{239}Pu standards of various concentrations affixed to the Lexan adjacent to the bone sample in hopes of solving the problem of varying neutron doses, resulting in varying track density between different sections of the same bone. Once this is resolved, we should be able to make better progress in the area of dosimetry.⁽⁹⁾

Broad-Spectrum.

The overall aim of the local distribution without losing sight of events at the cellular level, is in relation to carcinogenesis. In spite of the

magnitude of the problem involved, it must be accepted that studies of radiation dosage distribution in bone offer one of the few ways of gaining information of bone tumor production by radiation. Local dose distribution studies are most valuable in the investigation of comparative carcinogenic effectiveness in different species and different bones in the same species. There is no doubt that without some knowledge of the distribution of radiation doses in any given case, it is only too easy to make false deductions or to miss an important conclusion.

Pilot Studies With Human Transuranium Tissue Analysis Program.

Currently, we have a pilot project in cooperation with the various installations involved in the Human Transuranium Tissue Analysis Program (Los Alamos Scientific Laboratory, Battelle-Northwest, Dow Chemical, and Hanford Human Transuranic Registry) to determine the feasibility of using neutron-induced autoradiography to characterize the local distribution of ^{239}Pu in soft and hard tissues. Bone samples are being and will be irradiated at the ALRR for 30 or more days.

If this pilot study proves successful, a full scale joint program involving the above installations will be proposed to the Division of Biomedical and Environmental Sciences, USAEC.

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Figure 1. Neutron-induced autoradiograph of trabecular bone of the lumbar vertebral body of beagle T36P1 given 0.0158 μCi of $^{239}\text{Pu}/\text{kg}$ and killed five days later. Note the bone image which is dark and the marrow which is light. Surface deposition of ^{239}Pu is clearly non-uniform; there are only a few isolated tracks in the bone marrow. B denotes bone; M denotes marrow. (X88).

Figure 2. Neutron-induced autoradiograph of trabecular bone from the distal femoral metaphysis of beagle T36P1 given 0.0158 μCi of $^{239}\text{Pu}/\text{kg}$ and killed five days later. Note the bone image which is much less distinct than that in Figure 1, but remains easily interpretable. Surface deposition is non-uniform in this region as well. B denotes bone; M denotes marrow. (X88).

Figure 3. Neutron-induced autoradiograph of trabecular bone from the distal femoral metaphysis of beagle M12P1 given $0.0167 \mu\text{Ci}$ of $^{239}\text{Pu}/\text{kg}$ and killed 2374 days later. Note the distinct bone image. After this amount of time, most of the tracks are buried within the bone. There are a few star-producing aggregates within the bone marrow. B denotes bone; M denotes marrow. (X88).

Figure 4. Neutron-induced autoradiograph in this case has produced a result in which the bone appears lighter than the marrow, but remains easily discernible. (T33P1 given 0.0153 μCi of $^{239}\text{Pu}/\text{kg}$ and sacrificed 375 days later.) B denotes bone; M denotes marrow (X88).

QUANTITATIVE STUDIES OF BEAGLE LUMBAR VERTEBRAL BODIES

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ABSTRACT: The QUANTIMET image analyzing computer was used to evaluate lumbar vertebral body samples from a colony of aging beagle dogs. The following three features were measured: (1) Percent Bone; (2) Perimeter; and (3) Mean Dorsal-Ventral Trabecular Width. A fourth, Perimeter/Area, was calculated.

Percent Bone decreased with age in both females and males, though faster in females. Perimeter decreased only in selected areas of the female lumbar vertebral body. Mean Dorsal-Ventral Trabecular Width decreased significantly in both females and males. Perimeter/Area increased with age in both females and males. In general, the dorsal region was the most sensitive to these changes which were studied.

When compared to human bone quantitation reports, percent bone in the beagle decreases more rapidly. However, the perimeter does not decrease with age in beagles, as it does in humans. Thus, perimeter/area in the beagle rises, while in the humans it remains constant.

INTRODUCTION

The successful processing of large sections of undecalcified bone in exactly the spatial arrangement they are in life has allowed the measurement of bone to progress. The name which has been applied to the process of evaluating various features of bone, such as amount, shape, perimeter, number of individual spicules, and distribution of spicules, is bone morphometry. The methods of quantitation which have been applied are those used in the newly-expanded field of stereology, a science designed to evaluate three-dimensional structures on the basis of two-dimensional sampling techniques. To evaluate the area of a feature, a random point-counting technique is used. To evaluate the perimeter (boundary), a random linear intercept method is used. And to evaluate the actual number of features, a manual count is used.

Since the field of bone morphometry is very new, the investigators have yet to reach a common terminology. One of the aims of this report is to equate our terms to those of a paper written by Merz and Schenk.⁽¹⁾

Percent Bone is the same as volumetric density (V_v). Though it is actually a dimensionless quantity, it can be thought of as being mm^2 "bone"/ mm^2 "bone tissue" examined, where the term "bone" refers only to the hard tissue, while the term "bone tissue" refers to both the hard tissue and bone marrow areas of the sample examined.

Perimeter is the same as surface density (S_v). It is expressed as mm "bone" surface/ mm^2 "bone tissue" examined.

Perimeter/Area is the same as specific surface (S_s), a factor used to describe in indirect terms the diameter of individual pieces of bone. It is expressed as mm "bone" surface/ mm^2 "bone".

The remaining difference in terms used is merely that of this paper's units of two dimensions while that of Merz and Schenk uses three dimensions.

In addition to these features, it is desirable to measure the Mean Dorsal-Ventral Width of Trabecula in the beagle lumbar vertebra.

It is now possible to do these procedures on a machine very quickly and with few errors on thin section prepared to meet simple requirements of contrast. The QUANTIMET (Metals Research, Ltd., Cambridge, England) has the ability to determine the exact amount in percent which each well-contrasted feature occupies in a selected field.⁽²⁾ It also has the ability to determine the exact distance occupied by the boundary between two well-contrasted features within a selected field. As well as these abilities, the QUANTIMET has the power to rule out features of a given thickness over regular intervals along a standardized scale.

This machine thus accomplishes two of the time-honored methods of feature evaluation mechanically---horizontal intersection counts; and point counts. Its screen divides a distance of 1 mm into 55 equal parts, both

horizontally and vertically. This allows a large number of horizontal intersections to be evaluated per section, limiting randomness errors, while mechanizing and speeding the process. It also allows the analysis of a grid of 55X55 points in each mm² in the evaluation of percent area. In addition, it does a job which the eye is hard-pressed to do, in that, along a horizontal axis, it can judge the length of each of its own 55 lines in each mm which occupy a certain feature, then successively exclude features of shorter lengths, and end by detecting only those of longest size.

The purpose of this study is to use the QUANTIMET to evaluate the bone of lumbar vertebral bodies selected from a controlled population of aging beagle dogs for changes in the following features: (1) Percent Bone, (2) Perimeter of trabecular bone, (3) Mean Dorsal-Ventral Trabecular Width, and (4) Perimeter/Area. The reason for examining the percent bone is to compare the aging beagle skeleton to reported values of human data for this feature. In this way, we can draw conclusions about the validity of bone amount changes in the beagle in its application to human bone research. The purpose for perimeter evaluation is to find the change in amount of perimeter with age upon which surface-seeking elements of the transuranium series may be deposited. In this way, one can begin to discover the number of cells potentially at risk at different ages soon after an acute exposure. An additional reason is to measure bone surface available for metabolism by cells as a function of age. Measurement of mean trabecular width allows one to attribute the change in percent bone to generalized bone disappearance or loss of certain whole spicules of bone. The more general purpose of the four parameters' measurement is to develop the figures for a control population, so that changes induced by the transuranium elements which reside in the bone for extended periods of time can be measured.

MATERIALS AND METHODS

Lumbar vertebral bodies from representative members of a colony of aging beagles were collected. The samples were opened, fixed in acetone, defatted in ether, and embedded undecalcified in 45/55 Ethyl-Butyl Methacrylate.⁽³⁾ Sections were prepared at 7 microns thickness on a motorized Jung sliding microtome and affixed to glass slides coated previously with 0.5% calfskin gelatin. The embedding medium was removed and the sections were stained by the Von Kossa method.⁽⁴⁾

The samples were blind-coded and analyzed on the QUANTIMET in twelve separate 3mmX3mm squares arranged as shown in Figure 1. For each region, the following features were examined: (1) Percent Bone, (2) Perimeter of trabecular bone surface, and (3) Mean Dorsal-Ventral Trabecular Width. Thus, a total area of 108 mm² was examined for each sample.

For the purposes of analysis, the dogs were grouped according to sex. Two particular regions of the lumbar vertebral body from each dog were evaluated separately by a computer program, the dorsal area (numbers 8-11 on Figure 1) and the ventral area (numbers 2-5 on Figure 1). As well, a value for the whole bone (numbers 1-12 on Figure 1) was obtained.

The values obtained were plotted with the age on the abscissa and the log of the feature on the ordinate. Regression analysis was performed and significance levels were calculated for each group. Graphs for all dorsal bone features are presented; other values appear in the tables.

RESULTS

The beagle lumbar vertebral body when sectioned parasagittally at seven microns thickness shows a series of antero-posteriorly oriented pieces of bone. There are usually a few thickened pieces in each section which may be the

result of tangentially-sectioned areas of union of adjacent pieces of antero-posteriorly oriented bone, or simply thickened areas within each piece (Figure 2a and 2b).

Percent Bone. In the whole bone analysis, the females showed a significant ($p < 0.001$) loss of bone area with age (Table 1). The loss in the males was much less significant ($p < 0.05$). The difference between the sexes in the amount of loss was significant ($p < 0.05$).

In the dorsal portion of the bone, the females ($p < 0.001$) and males ($p < 0.01$) showed very significant losses in the amount of bone with age. However, there was only a mild difference between the amount of loss in the two groups (Figures 3 and 4).

In the ventral portion of the bone, the females again showed a significant amount of loss ($p < 0.01$) while the males showed no significant change. The rate of female bone loss was significantly greater ($p < 0.01$). Comparison of the dorsal and ventral portion of the bone showed the dorsal bone disappeared significantly faster in both males and females ($p < 0.05$).

Table 1. Changes in Percent Bone with age of beagle lumbar vertebral body.

AREA	SEX	% - BONE AT AGE:		SIGNIFICANCE OF RATE OF LOSS	SEX DIFFERENCE IN RATE OF LOSS
		500 DAYS	6000 DAYS		
WB *	F	29.7	18.9	$p < 0.001$	$p < 0.05$
WB	M	28.7	23.7	$p < 0.05$	
DB **	F	25.2	10.7	$p < 0.001$	Mild
DB	M	22.0	13.6	$p < 0.01$	
VB ***	F	28.9	16.1	$p < 0.01$	$p < 0.05$
VB	M	26.8	22.4	none	

* Whole Bone; ** Dorsal Bone; *** Ventral Bone.

Perimeter. The female beagles showed a significant rate of loss of perimeter in the whole bone ($p < 0.01$), while the males did not (Table 2).

In the dorsal area, the females again showed a very significant loss of perimeter with age ($p < 0.001$), while the males showed no change (Figures 5 and 6).

Neither group showed a significant loss of perimeter in the ventral region. Although the females showed a significant rate of loss in the dorsal region, when the rates of the perimeter loss with time in the dorsal and ventral regions were compared, there was no significant difference.

Table 2. Changes in Perimeter with age in beagle lumbar vertebral body (mm "bone" surface/mm² "bone tissue" examined)

AREA	SEX	% - BONE AT AGE:		SIGNIFICANCE OF RATE OF LOSS	SEX DIFFERENCE IN RATE OF LOSS
		500 DAYS	6000 DAYS		
WB*	F	5.89	5.08	$p < 0.01$	$p < 0.01$
WB	M	5.17	5.72	none	
DB**	F	5.17	3.68	$p < 0.001$	$p < 0.01$
DB	M	4.39	4.52	none	
VB***	F	5.58	4.86	none	none
VB	M	5.02	5.68	none	

* Whole Bone; ** Dorsal Bone; *** Ventral Bone.

Mean Dorsal-Ventral Trabecular Width. The mean width was found to decrease ($p < 0.001$) in all instances, except for the ventral area of the male lumbar vertebral body. The changes in the dorsal region were particularly marked (Figures 7 and 8). There was, however, no detectable sex difference in the patterns or amounts of loss (Table 3).

Table 3. Changes in Mean Dorsal-Ventral Trabecular Width with age in beagle lumbar vertebral body (microns)

AREA	SEX	MEAN DORSAL-VENTRAL TRABECULAR WIDTH AT AGE:		SIGNIFICANCE OF RATE OF THINNING	SEX DIFFERENCE IN RATE OF THINNING
		500 DAYS	6000 DAYS		
WB [*]	F	96.3	54.5	p<0.001	none
WB	M	94.2	66.7	p<0.001	
DB ^{**}	F	98.8	41.4	p<0.001	none
DB	M	99.5	48.7	p<0.001	
VB ^{***}	F	97.7	55.1	p<0.001	p<0.01
VB	M	83.0	72.0	none	

* Whole Bone; ** Dorsal Bone; *** Ventral Bone.

DISCUSSION

Evaluation of the beagle lumbar vertebral body by the QUANTIMET image-analyzing computer showed us the characteristics of percent bone (volumetric density (V_v)), perimeter (surface density (S_v)), and mean dorsal-ventral trabecular width, as well as allowing us to calculate the perimeter/area (specific surface (S_s)) for several regions and the whole bone.

Table 4. Rate of loss of Percent Bone in humans

<u>INVESTIGATORS</u>	<u>SITE</u>	<u>RATE/YEAR</u>	<u>REFERENCE</u>
Arnold and Wei	LVB [*]	0.16%	(5)
Bromley et al.	LVB	0.12%	(6)
Merz and Schenk	IC ^{**}	0.11%	(1)

* Lumbar Vertebral Body; ** Iliac Crest.

A combined percent bone figure for male and female dogs suggests a loss of about 8% bone in 16 years, a rate of 0.5%/year. Values found in three human studies are listed in Table 4.

The rate of loss of percent bone in the beagle lumbar vertebral body is about 3-4 times that reported for human vertebral body, and near five times that for iliac crest.

A combined perimeter figure for male and female dogs does not indicate a significant change in the amount of perimeter with age. The human studies indicate that human trabecular bone shows a decrease in this feature (Table 5). It does not appear, then, that the beagle vertebral body is exactly like the human bone in this particular feature.

Table 5. Rate of loss of Perimeter in humans

<u>INVESTIGATORS</u>	<u>SITE</u>	<u>RATE/YEAR</u>	<u>REFERENCE</u>
Arnold and Wei	LVB*	0.71%	(5)
Bromley et al.	LVB	0.54%	(6)
Merz and Schenk	IC**	0.48%	(1)

* Lumbar Vertebral Body; ** Iliac Crest.

From the radiobiologist's viewpoint, it is significant to see that the beagle does not lose bone surface with age; this means that the initial uptake of surface-seeking radionuclides in the beagle lumbar vertebral body could be predicted to be non-age dependent. While this static figure is important, it is also necessary to determine bone remodelling rates as well as cells at risk before one can intelligently determine the actual toxicities of various radionuclides.

There are two methods of evaluating diameter changes of trabecular bone used in this study. The first approach is the mean dorsal-ventral trabecular width measurement which has been seen to decrease. This suggests a generalized reduction in the amount of bone, rather than loss of individual trabeculae. The second is the Perimeter/Area (specific surface (S_s)), which is calculated from the measured values for percent bone and perimeter (Table 6).

Table 6. Changes in Perimeter/Area with age in beagle lumbar vertebral body (mm "bone" surface/mm² "bone")

<u>AREA</u>	<u>SEX</u>	<u>PERIMETER/AREA AT AGE:</u>	
		<u>500 DAYS</u>	<u>6000 DAYS</u>
WB [*]	F	20.04	26.88
WB	M	18.5	24.14
DB ^{**}	F	21.20	34.40
DB	M	20.46	33.24
VB ^{***}	F	19.69	30.18
VB	M	18.83	25.36

* Whole Bone; ** Dorsal Bone; *** Ventral Bone.

It is quite clear that the general trend of the beagle lumbar vertebral body is to have increased Perimeter/Area, indicating the presence of more and thinner spicules. The three human studies show that there is little or no change in Perimeter/Area with age (Table 7), which would seem to say that there is a loss of whole spicules of bone, a fact pointed out by Arnold and Wei.⁽⁵⁾

The female beagles show a greater decrease in all corresponding features when compared to the males. It is also demonstrated that there are regions

of varying sensitivity within the lumbar vertebral body, the portion nearer to the dorsal cortex appearing to decrease in bone mass features at a more rapid rate than the portion near the ventral cortex.

Further measurements which are possible with the QUANTIMET include evaluation of the number of bone spicules within an area and measurement of shapes of bone spicules by a series of form factors. These readings are now being made in a continued attempt to adequately quantitate the trabecular bone of the lumbar vertebral body of the beagle.

Table 7. Changes in Perimeter/Area in humans

<u>INVESTIGATORS</u>	<u>SITE</u>	<u>PERIMETER/AREA AT AGE:</u>		<u>REFERENCE</u>
		<u>20 YEARS</u>	<u>80 YEARS</u>	
Arnold and Wei	LVB [*]	19.4	20.0	(5)
Bromley et al.	LVB	18	15	(6)
Merz and Schenk	IC ^{**}	17.3	17.1	(1)

* Lumbar Vertebral Body; ** Iliac Crest.

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Figure 1. The beagle lumbar vertebra was divided as arranged here into 12 equal sections for analysis on the QUANTIMET. The "dorsal area" refers to areas 8-11, while the "ventral area" refers to areas 2-5. (x8).

Figure 2a. This parasagittal section of lumbar vertebral body from a beagle 843 days old at sacrifice is stained by the Von Kossa method. It shows a number of longitudinally-oriented trabeculae with connecting areas between trabeculae. (X8).

Figure 2b. This identically-prepared section from a beagle 5075 days old at sacrifice shows relatively thinner trabeculae, mainly in the dorsal region. (X8).

Figure 3. The log Percent Bone is plotted against age for the dorsal area of the female beagles. There is a very significant rate of loss with increasing age.

Figure 4. The log Percent Bone is plotted against age for the dorsal area of the male beagles. There is a significant rate of loss with increasing age.

Figure 5. The log Perimeter is plotted against age for the dorsal region of the female beagles. There is a significant rate of loss with increasing age.

Figure 6. The log Perimeter is plotted against age for the dorsal area of the male beagles. There is no significant loss with increasing age.

Figure 7. The log Mean Dorsal-Ventral Trabecular Width is plotted against age for the dorsal area of the female beagles. There is a significant rate of decrease with increasing age.

Figure 8. The log Mean Dorsal-Ventral Trabecular Width is plotted against age for the dorsal area of the male beagles. There is a significant rate of decrease with increasing age.

THE EFFECTS OF DISODIUM ETHANE-1-HYDROXY-1, 1-DIPHOSPHONATE (EHDP)
AND DISODIUM DICHLOROMETHANEDIPHOSPHONATE (Cl₂MDP) ON BONE
OF THE PROXIMAL TIBIA OF THE GROWING RAT

S. C. Miller and W. S. S. Jee

ABSTRACT: In rats treated for 10 days with EHDP and Cl₂MDP, many past observations, such as endochondral growth inhibition, bone formation decreases, mineralization defects, and abnormal metaphyseal modeling were confirmed. The current studies also found the labeling index of osteoprogenitor cells was depressed by higher doses (1.0 and 5.0 mg P/kg/day) of both compounds. As well, the number of osteoblasts was lower for all doses and further evidence of decreased bone formation. Only 2.5 mg P/kg and 5.0 mg P/kg lowered appositional bone formation rates. The number of osteoclasts along with the number of nuclei/osteoclast, was higher at all doses.

INTRODUCTION

The diphosphonates are of special interest to us because of the possibility that they may effect the skeletal binding and the metabolism of bone-seeking radionuclides such as ²³⁹Pu. They are synthetic compounds which structurally resemble naturally occurring pyrophosphate. However, unlike the P-O-P bond of pyrophosphate, the diphosphonates contain a P-C-P bond which is resistant to enzymatic hydrolysis.^(1,2) The diphosphonates stabilize hydroxyapatite mineral by surface chemisorption in vitro,^(3,4) and affect mineral metabolism, and prevent certain types of bone loss in vivo.^(1,5-11)

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

MATERIALS AND METHODS

Male Sprague-Dawley rats initially weighing 100 grams were used (Hormone Assay, Chicago, Illinois) and maintained on a normal diet of lab chow (Wayne Lab Blox, Allied Mills Inc., Chicago, Ill.)

Two experiments were performed. In both, two diphosphonates, disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP) and disodium dichloromethane-diphosphonate (Cl_2MDP), were studied. The diphosphonates^{*} were supplied in the powdered form, mixed in water, and adjusted to pH 7.4 with NaOH. The compounds were administered on the basis of their phosphorus content so that the effects could be compared on a %P basis.^{**}

The purposes and protocol of Experiments 1 and 2 were as follows:

Experiment 1.

This experiment was designed to measure the effects of the two diphosphonates upon the quantity of hard tissue (calcified cartilage cores and bone), as well as the amount of bone matrix formation in the proximal tibia.

Fifty rats were given daily subcutaneous injections of 0.0, 0.1, 0.5, 2.5, and 5.0 mg P/kg/day for 10 days of EHDP or Cl_2MDP . The animals were weighed daily and the dosage, given in a volume of 0.1 ml/100 g animal weight, was adjusted accordingly. Three days prior to sacrifice the animals were given an intraperitoneal injection of $2\mu\text{Ci } ^3\text{H-proline}$ (Schwarz/Mann, specific activity = 31 Ci/mM) per gram body weight.

At autopsy proximal tibias and distal femurs were collected. One tibia from each animal was fixed in 10% phosphate-buffered formalin, decalcified in 10% EDTA, and embedded in a modified methyl methacrylate.⁽¹²⁾ Consistent 3.5 micron thick sections of both frontal sections and cross-sections of the proximal tibia were cut on the Jung microtome. Some were stained in hematoxylin and eosin (H & E) while others were used for detailed autoradiography. These slides were coated with NTB liquid emulsion (Eastman Kodak, Rochester, New York) and allowed to expose for 3 weeks in a dry, cold

*Obtained from Procter and Gamble, Cincinnati, Ohio.

**The disodium of EHDP contains 25% elemental phosphorus.

atmosphere. The autoradiographs were developed in Kodak D-19 for 3.5 minutes, fixed in Kodak fixer for 10 minutes, and rinsed in tap water (27°C) for 2 hours. They were then stained with H & E.⁽¹³⁾ The femur from each animal was embedded undecalcified in ethyl/butyl (45/55) methacrylate,⁽¹⁴⁾ sectioned at 5 microns on the Jung microtome, stained by the von Kossa method,⁽¹⁵⁾ and counter-stained with safranine red.

The other tibia was defatted and dehydrated in a series of acetone and ether changes. It was embedded in bioplastic and sawed in 250 μ thick sections which were then ground to 100 μ . These 100 μ sections were micro-radiographed at 12 kV and 30 mA for 2 minutes.

Bone Matrix Formation. The depth of ³H-proline burial was determined in a 1.55 mm² area of the proximal tibial epiphysis. In areas where the ³H-proline was buried by new bone, the distance from surface-to-label (depth) along a horizontal line was measured. Endosteal ³H-proline burial was measured at 88 micron intervals in a cross-section of the proximal shaft of the tibia.

Quantity of Hard Tissue (calcified cartilage cores and bone). The percent calcified cartilage cores in a 2 mm² area of the proximal tibial metaphysis was sampled by the point counting method in the H & E stained thin sections. The percent bone in the microradiographs in a 6 mm² area of the proximal tibial metaphysis was determined by the Quantitative Television Microscope (QTM, Metals Research, Cambridge, England) image-analyzing computer.

Presence of Osteoid. The presence of osteoid was qualitatively observed in the von Kossa and safranine red stained thin sections of the femurs.

Experiment 2.

This experiment was designed to determine the effects of the diphosphonates

on endochondral growth, osteoprogenitor cell DNA synthetic activity, and numbers of functional bone cells.

The rats received daily subcutaneous injections of 0.0, 0.1, 1.0, and 5.0 mg $P/kg/day$ EHDP or Cl_2MDP for ten days. Three days prior to sacrifice the rats were given 15 mg tetracycline (Acromycin, Lederle) per kilogram body weight intravenously. One hour prior to sacrifice, the animals were given 1 μCi tritiated thymidine (3HTdR) (methyl- 3H , ICN, SA = 14.1 Ci/mM) per gram body weight intraperitoneally. Autopsy procedures, tissue preparation, and microradiographic and autoradiographic techniques were the same as those employed in Experiment 1. However, following microradiography of the 100 micron ground sections of the proximal tibial metaphysis they were further ground to 50 microns for ultraviolet microscopy (tetracycline analysis).

Endochondral Bone Growth. The distance from the zone of cell hypertrophy of the growth cartilage to the center of the tetracycline label was measured on the ultraviolet microscope from the 50 micron ground sections.

Labeling Index of Osteoprogenitor Cells. The percentage of 3HTdR -labeled nuclei was determined collectively for the mesenchymal and the endothelial cells, in a 0.29 mm^2 area of the proximal tibial metaphyseal primary spongiosa within 300μ of the zone of cell hypertrophy of the growth cartilage. These two types of cells comprise the osteoprogenitor cell pool in this area.

Endothelial and mesenchymal cells have inconspicuous cytoplasm and generally dark-staining, oval or fusiform nuclei. A labeled nucleus was one having five or more silver grains directly over it.

Number of Functional Bone Cells. The number of osteoblasts, osteoclasts, average nuclei per osteoclast, and bone surface intersections on a horizontal grid were counted in five 0.94 mm^2 areas of the metaphysis. Bone surface perimeter was estimated by applying the following formula:

$$\text{Perimeter} = \pi/2 \times H \times D$$

(1)

where H = number of intersections with bone, D = distance between the lines on the grid, and $\pi/2$ is a shape correction factor.

RESULTS

Depth of ^3H -proline burial in the epiphysis decreased at doses of 2.5 and 5.0 mg P/kg/day EHDP and 0.5, 2.5, and 5.0 mg P/kg/day Cl_2MDP . On the endosteal surface of the proximal shaft, doses of 2.5 and 5.0 mg P/kg/day EHDP decreased the depth of burial, while Cl_2MDP had no significant effect at this site (Table 1).

Table 1. ^3H -proline burial in three days

DOSE mg P/kg/day	^3H -Proline Burial	
	Proximal shaft ⁺ (microns)	Epiphyseal trabeculae (microns)
	$\pm$ S.E.	$\pm$ S.E.
CONTROL	25.1 ± 0.8	10.9 ± 0.7
0.1 EHDP	25.5 ± 1.1	10.4 ± 1.1
0.5 EHDP	22.8 ± 1.4	10.1 ± 0.8
2.5 EHDP	21.2 ^{**} ± 1.2	8.7 [*] ± 0.8
5.0 EHDP	6.9 ^{***} ± 1.3	5.7 ^{***} ± 0.4
0.1 Cl_2MDP	24.9 ± 1.0	10.7 ± 0.9
0.5 Cl_2MDP	22.8 ± 1.2	8.7 [*] ± 0.4
2.5 Cl_2MDP	21.5 ± 0.7	7.6 ^{**} ± 0.5
5.0 Cl_2MDP	23.6 ± 1.6	6.4 ± 0.4

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; + one cm distal to growth cartilage.

The percent metaphyseal area occupied by hard tissue increased with increasing doses of both EHDP and Cl_2MDP , Cl_2MDP having a greater effect than equivalent doses of EHDP. The one exception was at 5 mg P/kg/day EHDP, where there was less percent hard tissue (Table 2).

Table 2. Endochondral growth in the 3 days.

	CONTROL	EHDP mg P/kg/day			Cl_2MDP mg P/kg/day		
		0.1	1.0	5.0	0.1	1.0	5.0
MEAN	701	670	649*	590**	709	626**	505**
GROWTH (MICRONS)	± 10	± 22	± 15	± 17	± 32	± 14	± 18
S.E.							

* $p < 0.01$

** $p < 0.001$

Examination of the von Kossa and safranine red stained sections showed large amounts of osteoid only at 5.0 mg P/kg/day EHDP.

Longitudinal growth was slightly decreased in the rats receiving 1.0 and 5.0 mg P/kg/day of Cl_2MDP and EHDP (Table 3). The highest dose of EHDP inhibited the incorporation of tetracycline into bone; growth was determined by using growth arrest lines on the microradiographs.

The labeling index of the osteoprogenitor cells in the tibial primary spongiosa decreased in the 1.0 and 5.0 mg P/kg/day doses of both EHDP and Cl_2MDP . EHDP depressed the labeling index more than equivalent doses of Cl_2MDP (Table 4).

There were fewer osteoblasts present in the 5.0 mg P/kg/day dose of EHDP and both 1.0 and 5.0 mg P/kg/day doses of Cl_2MDP . There were more

osteoclasts in the 1.0 and 5.0 mg P/kg/day EHDP groups and in all dose groups of Cl_2MDP . There were also increases in the average number of nuclei per osteoclast in all doses of both EHDP and Cl_2MDP . Osteoblasts/mm bone surface was greatly reduced with increasing doses of diphosphonates, while osteoclasts/mm bone surface was increased at both 1.0 and 5.0 mg P/kg/day compound (Table 4).

DISCUSSION

Past work in growing rats has shown that the diphosphonates, EHDP, and Cl_2MDP , inhibit certain types of bone loss.^(1,8,9,16-19) Both EHDP and Cl_2MDP at high doses inhibit the endochondral growth rate.^(18,20) Both compounds inhibit bone formation rates in endosteal and periosteal surfaces of the tibial shaft, but dose-response relationships are not clearly defined.⁽²⁰⁾ EHDP at higher doses impairs mineralization in cartilage and osteoid.⁽¹⁸⁾ EHDP produces "wide osteoid seams" in adult cats,⁽²¹⁾ growing rats,⁽²²⁾ and young dogs.⁽²³⁾ Both compounds produce abnormal modeling of the metaphysis of the growing rat with the net result of an increased amount of calcified material in it. There was also a "clubbing" of the proximal tibial end. In addition, it has been shown that there are greater numbers of osteoclasts with abnormal appearance and increased numbers of nuclei.⁽²⁰⁾

The current study confirms the abnormal modeling of the metaphysis of a growing rat. First, higher doses of both compounds reduce endochondral growth rate. Second, the quantitation of the increased hard tissue density in the metaphysis shows an increased amount of bone with increasing dose of diphosphonates. Third, higher doses of both compounds decreased appositional bone formation rates at some sites.

The new findings in this report are:

(1) Doses of 1.0 and 5.0 mg P/kg/day of both compounds decreased the labeling index of the osteoprogenitor cells of the primary spongiosa of the proximal tibia.

(2) Cl_2MDP was the less potent of the two in depressing the labeling index.

(3) The number of osteoblasts/mm bone surface was lower for both compounds at all doses used in this experiment.

(4) The number of osteoclasts/mm bone surface increased at 1.0 and 5.0 mg P/kg/day for both compounds.

(5) The number of nuclei/osteoclast increased at all dose levels for both EHDP and Cl_2MDP .

(6) The epiphyseal trabeculae are a more sensitive site at which to sample appositional bone formation, when compared to the proximal tibial shaft.

It is tempting to tie the lack of osteoblasts partly to the decreased labeling index, but this, in any case, does not account for the whole decrease. There is most likely also a decrease in the rate of differentiation from osteoprogenitor cells to osteoblasts in this site. That the major effect is at the differentiation level is supported by the fact that EHDP and Cl_2MDP have similarly reduced numbers of osteoblasts/mm bone surface at identical doses, even though the labeling index of EHDP-treated animals is much lower at corresponding doses.

The increase in the number of osteoclasts/mm bone surface and osteoclasts' nuclei is more difficult to explain. It certainly cannot be explained on the basis of labeling index, which was decreased at doses where the number of osteoclasts was increased. The three explanations which seem possible are:

(1) an effect promoting the differentiation of cells from osteoprogenitor cells

to osteoclasts, or (2) a prolonged lifespan of the nuclei inside osteoclasts, or (3) a combination of both.

Past work has confirmed many times the effect of diphosphonates on inhibiting mineral dissolution. The dichotomy of more and larger osteoclasts with larger amounts of bone would imply that the osteoclasts are unable to digest bone with diphosphonate on its surface.

The importance of defining dose-response relationships lies in locating a dose with optimal beneficial effects with minimal undesirable side effects during treatment. The specific goal for bone is to create a mature skeleton with more trabecular bone for metabolism and support, while not damaging the subject's cellular populations and bone formation capacities. While the 0.1 mg P/kg/day dose of either compound may not be an optimum, it does represent one whose effects of more bone present are not offset by undesirable effects (i.e., lower labeling index and dramatic osteoblast and osteoclast population changes).

Thus, the search for an optimal dose of either or both of the two diphosphonates, EHDP and Cl_2MDP , must continue. It does appear from this study that such a dose does exist, which will diminish bone loss, while not harming functional cell populations in bone. A further object in attempting to understand the diphosphonates is to characterize their effects upon the metabolism of ^{239}Pu and other bone-seeking radionuclides.

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Table 3. Percent area of hard tissue

Dose mg $\bar{P}$ /kg/day	% Metaphyseal bone	% Metaphyseal calcified cartilage cores
	$\pm$ S.E.	$\pm$ S.E.
CONTROL	23.7 ± 0.5	8.1 ± 0.3
0.1 EHDP	25.8* ± 0.6	9.0 ± 0.3
0.5 EHDP	28.4*** ± 1.5	9.6*** ± 0.3
1.0 EHDP	30.9*** ± 0.7	11.0*** ± 0.3
2.5 EHDP	35.6*** ± 1.1	12.5*** ± 0.3
5.0 EHDP	29.3*** ± 0.9	14.9*** ± 0.6
0.1 Cl_2 MDP	29.7*** ± 2.6	10.2*** ± 0.2
0.5 Cl_2 MDP	37.1*** ± 1.1	11.7*** ± 0.4
1.0 Cl_2 MDP	40.1*** ± 1.0	13.3*** ± 0.5
2.5 Cl_2 MDP	44.1*** ± 3.0	14.1*** ± 0.2
5.0 Cl_2 MDP	51.5*** ± 0.5	16.0*** ± 0.4

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 4. Labeling index and cell population analysis

Dose mg $\overline{P}$ /kg/day	Labeling Index osteoprogenitor cells $\pm$ S.E.	Total osteoblasts $\pm$ S.E.	Total osteoclasts $\pm$ S.E.	Osteoblasts per/mm surface $\pm$ S.E.	Osteoclasts per/mm surface $\pm$ S.E.	Ave number nuclei per osteoclast $\pm$ S.E.
CONTROL	15.7 $\pm$ 0.7	326 $\pm$ 13	34 $\pm$ 2	10.2 $\pm$ 0.3	1.1 $\pm$ 0.1	3.4 $\pm$ 0.1
0.1 DHDP	13.6 $\pm$ 0.6	311 $\pm$ 21	38 $\pm$ 2	9.0* $\pm$ 1.3	1.1 $\pm$ 0.1	4.2*** $\pm$ 0.1
1.0 EHDP	11.5*** $\pm$ 0.5	321 $\pm$ 27	55*** $\pm$ 4	8.1*** $\pm$ 1.6	1.5*** $\pm$ 0.1	5.6*** $\pm$ 0.3
5.0 EHDP	6.1*** $\pm$ 0.4	116*** $\pm$ 15	48*** $\pm$ 3	3.4*** $\pm$ 0.4	1.5*** $\pm$ 0.1	6.2*** $\pm$ 0.2
0.1 Cl_2 MDP	15.0 $\pm$ 1.3	321 $\pm$ 43	43*** $\pm$ 2	8.5 $\pm$ 2.2	1.2 $\pm$ 0.1	4.5*** $\pm$ 0.2
1.0 Cl_2 MDP	13.4** $\pm$ 0.6	229*** $\pm$ 22	66*** $\pm$ 2	5.2*** $\pm$ 1.4	1.5*** $\pm$ 0.1	6.0*** $\pm$ 0.4
5.0 Cl_2 MDP	12.5*** $\pm$ 0.3	175*** $\pm$ 14	65*** $\pm$ 6	3.5*** $\pm$ 0.2	1.3* $\pm$ 0.1	5.8*** $\pm$ 0.3

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

EVALUATION OF PROGRESSIVE PERIODONTAL DISEASE IN BEAGLES

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and W. S. S. Jee

ABSTRACT: This preliminary study deals with quantitative histological aspects and distribution of ^{45}Ca in the interradicular and interdental hard tissue areas of the jaw from four beagles. These values will be part of the baseline data for the study of beagles placed on periodontal disease inducing diet.

The percent bone in the various areas as follows:

- (1) The interradicular area of the mandibular first molar - 31 to 39%.
- (2) The interdental area between the mandibular first molar and the fourth premolar - 52 to 72%.
- (3) The interradicular area of the mandibular third premolar - 73 to 77%.
- (4) The interdental area between the mandibular second and third premolars - 72 to 87%.
- (5) The interradicular area of the maxillary first molar - 33 to 35%.
- (6) The interdental area between the maxillary first molar and the third premolar - 53 to 60%.

Counts of "hot spot" distribution indicate that 20 to 35% of the interdental and interradicular bones were occupied by "hot spots". The ratios of "hot spots/diffuse" ranged from 5 to 7.

INTRODUCTION

Periodontal disease is a destructive process involving the supporting structures of the teeth. Presently, it is felt that its first sign is inflammation of the gingiva (gingivitis) around the cervical region of the teeth. The next stage involves loosening of the gingival attachment to the teeth with the formation of periodontal pockets. Later, there appears radiographic loss or rarefaction of the interdental portion of alveolar bone (periodontitis is confirmed by this appearance). Finally, clinical mobility with nearly total loss of the alveolar process results in the loss of the teeth.

Since the realistic clinical finding is that the disease can be cured if the soft tissues are involved, but only palliatively-treated if a significant amount of the alveolar process bone is involved in any given area,

It is desirable to know the exact involvement of the bone in the face of certain clinical appearances. Garcia, Davis, and Kapur (IADR Abstracts #13, 51st General Session, Washington, D. C., p. 62, 1973) have reported that Technetium-99m-labelled polyphosphate (Tc-PP) can be used to detect increases in "mineral metabolism" in the jaws. A part of this study involves the in vivo scanning of the jaw after injection with Tc-PP, in an attempt to characterize the pattern of mineral metabolism in states of disease and health of the periodontium.

It is desirable as well to know the distribution of ^{45}Ca , a radionuclide which localizes as "hot spots" in areas of mineralization and as a "diffuse component" in existing bone. We will attempt by contact autoradiography of 100μ thick sections to determine this distribution in the jaws.

The goals of our portion of this study are:

- (1) To describe in bone quantity the state of specific areas of the hard tissue of the jaw from the microradiographs of specific areas.
- (2) To characterize sites of new bone formation from studies of local distribution of tetracycline in these same areas.
- (3) To evaluate the distribution of ^{45}Ca in specific areas of the jaw.
- (4) To correlate the combined tetracycline and ^{45}Ca pictures with the in vivo studies of the same region.

MATERIALS AND METHODS

A group of beagle dogs has been placed on a periodontal disease-inducing diet. The plan is for a series of timed sacrifices over a period of two to three years. At this time, we have in hand jaws from Dogs BT and BR, sacrificed on September 21, 1972, from dogs OS and ON, sacrificed on November 9, 1972, and from dogs MF and MC, sacrificed on March 15, 1972. All dogs received

1 mCi ^{45}Ca 2 days prior to sacrifice. All dogs received some form of tetracycline about three weeks prior to sacrifice. They received an additional dose of a different tetracycline several hours prior to sacrifice (this represents a mistake in the protocol which has since been corrected).

Upon receipt of the jaws in our laboratory, they were sawed on a band saw into three sections. The sections in the mandible contained (1) all teeth anterior to/and including the cuspid, (2) the first through third premolars, and (3) the fourth premolar and first and second molars. The sections of the maxilla contained (1) all teeth anterior to/and including the cuspid, (2) the first and second premolars, and (3) the third premolar, and first and second molars (this particular method has been modified as agreed upon in a meeting in April).

These portions of the jaw were then thoroughly defatted and dehydrated in successive changes of acetone and embedded in bioplastic. Following hardening, they were sawed mesio-distally into 350μ sections. The sections which contained teeth were ground to 100μ between glass plates. The 100μ sections were then sandwiched between Kodak's NTB autoradiographic plates for 20 days. The autoradiographs were developed at 68°C in Kodak's D-19 for three minutes, placed in a stop bath, then fixed for six minutes (Figure 1).

These 100μ sections were microradiographed on Kodak's large spectroscopic plates for 30 minutes. The sections were placed between two glass slides without mounting medium in preparation for future ultraviolet examination (Figure 2).

The following areas of the jaw were chosen for analysis on the microradiographs and autoradiographs:

- (A) The interradicular area of the mandibular first molar.

- (B) The interdental area between the mandibular first molar and the fourth premolar.
- (C) The interradicular area of the mandibular third premolar.
- (D) The interdental area between the mandibular second and third premolars.
- (E) The interradicular area of the maxillary first molar.
- (F) The interdental area between the maxillary first molar and the third premolar.

(1) Point counts were performed on a 10 X 10 grid to determine the %-bone in each area. The apex of the shortest root adjacent to a given interdental area was used as the apical boundary of the alveolar process. A line connecting the dento-enamel junctions of the two teeth was used as the coronal boundary. An imaginary line connecting the root apices was used as the apical boundary of the interradicular bone.

(2) These same areas were analyzed by point-counting on the autoradiographs to determine the distribution of ^{45}Ca into concentrated ("hot spot") and diffuse components.

(3) A microdensitometer was designed which would read density in a circular area with a diameter of 34 microns. The machine was calibrated from a ^{90}Sr standard of 10 rads/hr intensity, exposed on a Kodak NTB plate for 1 to 10 hours. A standard curve was prepared relating the density of the standard to the output of the microdensitometer. Unknown values from the autoradiographs were compared to this curve. A 4-hour exposure of the ^{90}Sr standard was made on each autoradiograph, which was used to adjust the unknown values to correct for differences in film and developing procedures. A Polaroid photograph of each area to be examined was prepared. Selected

area of varying densities were read on the autoradiograph and recorded on the photograph. The density of a series of "diffuse component" readings was averaged against which all of the more concentrated areas could be compared. Final results were expressed as a ratio of "hot spot" density to "diffuse" density.

RESULTS

(1) In dogs from Group 1 (BR and BT), the percent bone in Area A was 39%. In dogs from Group 2 (OS and ON) the corresponding area was about 34% (see Table 1).

Table 1. Percent bone.

Animals #	Area of Analysis					
	A	B	C	D	E	F
Group 1						
BR	39% (6) [*]	53% (6)	73% (2)	72% (2)	35% (3)	53% (2)
BT	39% (3)	72% (2)	77% (1)	87% (1)	-	-
Group 2						
ON	31% (3)	52% (3)	75% (1)	73% (1)	-	63% (2)
OS	36% (3)	61% (2)	-	-	33% (2)	60% (1)

^{*}(6) - number of samples examined.

In dogs from Group 1, the percent bone in Area B was 53%, while dogs from Group 2 showed the same amount.

In dogs from Group 1, the percent bone in Area C was about 75%, a level which persisted in Group 2.

In dogs from Group 1, the percent bone in Area D was also about 75%. Group 2 animals showed the same.

In dogs from Group 1, the percent bone in Area E was about 35%, while the amount was 33% in dogs of Group 2.

In dogs from Group 1, the percent bone in Area F was about 53%; this number persisted in Group 2.

(2) Preliminary counts of "hot spot" distribution indicated that 32% of the bone in the region of the mandibular first molar, both mesial interdental and interradicular was occupied by "hot spots". In the area of the mandibular third premolar, this number was 20% of the bone in "hot spots", both interdentally and interradicularly. In the area of the maxillary first molar, about 35% of the bone was occupied by "hot spots".

(3) The ratio of the densest areas of radioactivity to the light regions show the relative degree of activity of the "hot spots and diffuse" regions in the interdental regions. These approximate values show that the ratios ranged from 5 to 7 with reasonable uniformity from area to area in the same dog (see Table 2). There was most likely no difference between the two groups.

Table 2. Hot spot/diffuse ratios.

Animal #	Hot Spot/Diffuse		
	Lower Molar	Lower Premolar	Upper Molar
<u>Group 1</u>			
BR*	9	10	9
BT	7	7	6
<u>Group 2</u>			
OS	6	5	5
ON	6	7	6

*"diffuse component" levels in Animal BR were 30% lower than in the three other animals, resulting in the higher ratios.

DISCUSSION

The only conclusion which one can reach in this part of the study thus far is that there is no morphologic evidence of what one would expect to be the picture of periodontitis in the two groups of dogs which have been examined. Neither is there evidence of change in autoradiographic distribution of ^{45}Ca by these methods of analysis.

However, at this point, it is important to discuss the progress in overcoming the procedural difficulties which we have encountered:

(1) Since it is desirable to examine specifically the areas around the third and fourth premolars, and the first molar of the mandible, we have revised the manner in which we section the bone, so that we will attempt to obtain longer sections. This plan will take effect with the fourth group of dogs. However, we may encounter problems in the sawing of a reasonable number of appropriate sections, due to the curvature of the dental arch, as well as contacting this large a section to an NTB plate. We have experienced difficulties in obtaining appropriate sections of the maxilla, due to the curvature of the arch, and the three smaller roots of the teeth in the maxilla. We would expect that with the attempt at sectioning of larger portions of the maxilla, that these difficulties would be greatly increased.

(2) The protocol for the tetracycline injections now calls for five daily injections and beginning at three weeks pre-autopsy. We will evaluate longitudinally sectioned material in the sites we have been examining.

(3) The sectioning of the embedded jaw provides a challenge as to orientation of the specimens. The most reproducible sections came from the lower first molar region, presumably due to the more straight alignment of the teeth in the jaw and the large mesial and distal roots of the lower teeth. The sections

prepared of the maxillary molar region were quite variable, due to the root configuration.

(4) The distribution of ^{45}Ca from one side of a 100μ section to the other does not vary enough to warrant study.

(5) It apparently may be possible to construct a three-dimensional view of the jaw, both from the microradiographs and autoradiographs, though we have not tackled this problem yet. We will attempt this on the third group of animals.

Figure 1. Contact autoradiograph (exposed 24 days) of the same 100 micron ground section seen in Figure 2. Note the distribution of ^{45}Ca into "hot spot" (HS) and "diffuse" (D) fractions. It is possible to relate the location of ^{45}Ca "hot spots" to some, but not all bone surfaces in the region (X8.5).

Figure 2. Microradiograph of 100 micron ground section of lower first molar region of Dog ON. Note the character of the bone of the alveolar process, both interradicularly and interdentally, a dense "shell" of alveolar bone proper with randomly-oriented trabeculae (X8.5).

A RAPID PLASTIC EMBEDDING TECHNIQUE FOR PREPARATION OF THREE-MICRON SECTIONS

D. Kimmel and W. S. S. Jee

ABSTRACT: A 24 hour start-to-finish method is now available for the preparation of three micron thick sections of decalcified bone tissue. Following acetone dehydration, the tissue to be embedded is placed in a vacuum, in a series of graded clearing solutions approaching the content of the final methyl methacrylate mixture. Polymerization takes place overnight in a 35°C oven. Three micron thick sections are easily prepared the next day on a Jung microtome for use in high quality histologic or detailed autoradiographic preparations.

INTRODUCTION

A recent paper by Walker and Kember⁽¹⁾ emphasizes the need for thin sectioning (three microns or less) prior to autoradiographic procedures involving the use of tritiated thymidine (³HTdR). The reason is to eliminate "false negatives", that is, cells which incorporate ³HTdR, but are not detected due to the fact that they are too far from the autoradiographic liquid emulsion. Caviness and Barkley,⁽²⁾ conclude that because the tritium beta particle has such a short range, three microns is the ideal thickness for sections to be used in tritium grain-counting procedures.

The most reliable method of obtaining three micron thick sections is from tissue embedded in one of several types of plastics. Some problems with plastic embedding have been cited, but solved in recent years:

- 1) Improper tissue infiltration has been remedied by using serial changes of graded clearing solutions approaching the casting syrup composition.^(4,5,6,7) Some authors use a series of daily changes, while others choose to vacuum the clearing solutions into the tissue to assure infiltration.^(4,5,6)
- 2) Bubbling of plastic during polymerization is eliminated by using

high pressure vessels.^(8,9) However, recent workers have found that very careful attention to temperature conditions during polymerization is the most vital step in control of bubbling.

- 3) Variability in block hardness has been overcome by paying careful attention to proportions of ingredients and conditions during polymerization.^(5,10,11)
- 4) Rapid expansion of blocks during sectioning was eliminated by the introduction of a polyethylene glycol distearate.⁽³⁾ This improvement permitted the preparation of serial sections.
- 5) Difficulties in cutting and handling sections in plastic were eliminated by employing a Jung microtome with carbide-tipped knives, removing sections from the blade in 80% ethanol onto a sharpened section-lifter, and flattening sections onto a slide with a roller.

Other often mentioned pitfalls which have not been solved are:

- 1) Tearing of tissue which makes it difficult to prepare areas of tissue larger than 1 cm² at three microns thickness. Indeed, all techniques of plastic embedding recommend that the smallest possible informative piece of tissue be selected for sectioning, due to inadequate support for large areas of embedded material.
- 2) Differential staining of plastic sections which is difficult when the stain must penetrate the plastic, as well as the tissue.
- 3) Speedy processing of plastic-embedded tissue which is prolonged due to a need for a 14-21 day "seasoning" or post-hardening period before cutting is possible.

Our main goal was to find an embedding procedure which eliminates the above pitfalls, and allows us to prepare relatively large pieces of decalcified

cortical and cancellous bone in one section at three microns thickness; this would enable us to do accurate $^3\text{HTdR}$ autoradiography. We were also seeking improved one cell layer sections for histologic preparations. The final purpose was to find a technique which was reliable if a reasonable amount of care was taken, and was relatively rapid to the ready-to-cut stage.

MATERIALS AND METHODS

Recipes for solutions.

Solution 1: To 1260 ml of methyl methacrylate monomer (Matheson, Coleman, and Bell Co., Norwood, Ohio) dried previously with anhydrous Na_2SO_4 (which will not dissolve in the monomer), add 5.3 g of benzoyl peroxide. Shake until all particles are dissolved.

Solution 2: To 891 ml Solution 1 (the rest is for the first clearing solution change), add 130 ml dibutyl phthalate (Mallinkrodt Co., St. Louis, Missouri) and 200 g finely-chopped polyethylene glycol 1540 distearate (Kessler Co., Philadelphia, Pennsylvania). Shake or heat lightly until all particles are dissolved.

Solution 3: Do not mix this solution more than 15 minutes before using. To 867 ml Solution 2 (the rest is for the second clearing solution change), add a total of 231 g methacrylate polymer fine-grained beads (Polysciences Co., Warrington, Pennsylvania) in 50 g increments, being sure that all the particles dissolve and remain in solution for the duration of the procedure. Heat lightly until this solution becomes a thin syrup, then pour over the tissues as outlined below.

Embedding Procedure.

- 1) Tissue is fixed in phosphate-buffered formalin (pH7.4), then decalcified

- in 14% EDTA buffered to pH7.4 with glacial acetic acid. Tissue is trimmed to a size convenient to embed.
- 2) Processing and serial solution changes are easily accomplished in 4-dram Opticlear vials (Intermountain Scientific Co.). The amounts in the above recipe will fill 100 of these vials half-way, making adequate size blocks for clamping in the Jung microtome.
 - 3) Tissues are placed in 70% alcohol for at least one overnight period.
 - 4) Three one-hour acetone changes are done during the morning of the embedding procedure, while the clearing solutions are being prepared.
 - 5) After the third acetone change, Solution 1 is poured over the tissue and vacuumed for sixty minutes.
 - 6) Solution 1 is decanted and Solution 2 is poured on the tissue, then vacuuming is continued for another ninety minutes.
 - 7) Solution 2 is discarded and Solution 3 (the casting syrup) is placed on the tissue; thirty minutes of vacuuming and appropriate orientation of the tissue completes the procedure.
 - 8) Mixture is then placed in an oven set at 35°C overnight.
 - 9) The next morning, the partially-polymerized blocks are heated to 42°C gradually over a period of several hours. This will complete polymerization.
 - 10) The blocks are cooled in a refrigerator, then cracked from the vials.

Sectioning Procedure.

Sections are prepared at three microns thickness on a Jung microtome equipped with a heavy-duty carbide-tipped knife, using a sharpened section lifter and 80% ethanol. The sections are affixed to slides coated with 0.5%

calfskin gelatin. Slides may be dried at 60°C for one hour (for histology only) or left in folders a minimum of three days (prior to autoradiography).

Staining or autoradiographic procedures.

The plastic is removed by three ten minute changes of acetone, prior to either histologic or autoradiographic preparation. Mayer's hematoxylin and eosin staining is then completed. For autoradiography, unstained sections are dipped in Kodak's NTB liquid emulsion (Rochester, New York), then allowed to dry, stored in light-tight cans for an appropriate period of time, then developed and stained.

RESULTS

Tissue of up to 2.5 cm² has been sectioned at three microns thickness within 24 hours of completion of vacuuming. This includes large samples of cortical bone combined with a mixture of hard and soft tissue (bone marrow, cartilage, loose and dense connective tissues, muscle, etc.). The quality of a histologic stained decalcified section of bone is shown in Figures 1 and 2. Of particular note is the presence of only one layer of cells.

A serial thickness study of ³HTdR autoradiographs indicated there was a 20% decrease in labeling index from the 2-4 microns sections, to the ones cut at 4-6 microns (see Table 1).

DISCUSSION

The application of this technique allows the examination of sections of one cell layer, making for clearer staining. These single cell layers are relatively easy to quantitate. The preparation of large pieces of tissue allows examination of many different areas in one section, eliminating such problems as variable staining between different slides. This procedure is

Table 1. Effect of section thickness on labelling index

<u>Section Thickness</u>	<u>Number of Cells</u>	<u>Number of Labelled Cells</u>	<u>Labeling Index</u>	<u>% Change from Ideal (2u)</u>
2	1574	849	54.0	-
2.5	1724	893	52.4	-3.0
3	1766	910	51.6	-4.4
3.5	1701	935	55.0	+1.9
4	1841	1037	56.3	+4.3
4.5	1972	999	45.6	-15.5*
5	1937	814	42.0	-22.2*
5.5	2235	957	42.8	-20.8*
6	2140	1015	47.5	-12.0*

*Significantly different ($P < .05$) from 2u section.

also less time-consuming because one block may be processed instead of several. Furthermore, other methacrylate techniques are more time-consuming due to the complexity of the embedding procedures and the prolonged seasoning (post-hardening) period.

Another advantage of this procedure is that the plastic is easily removed following section preparation, allowing good infiltration of stains into the tissue.

Autoradiography of $^3\text{HTdR}$ -labeled cells demands sections of four microns or less, according to a serial thickness experiment; many other authors indicate that on a theoretical basis, three microns is a desirable thickness. This procedure meets the demands of three micron thickness sectioning routinely. A sample of $^3\text{HTdR}$ autoradiography on these sections is seen in Figure 3.

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Figure 1. This low power photomicrograph of a three micron section of an 8-week old rat tibia shows a broad area of cells, cartilage, and bone. H & E Stain. (X88).

Figure 2. This high power photomicrograph of the primary spongiosa of an 8-week old rat tibia demonstrates the single layer of cells. Osteoblasts (Ob), osteoclasts (Oc), and bone lining cells (LC) are clearly demonstrated. H & E Stain. (X560).

Figure 3. This detailed autoradiograph of cells in the tibial primary spon-
giosa shows the tracks in the emulsion clearly localized over
the ³HTdR-containing bone lining cells. Kodak NTB emulsion. H &
E Stain. (X1400).

PLUTONIUM-239 AND THE STIMULATED PERIODONTAL LIGAMENT CELLS

John Hess and W. S. S. Jee

ABSTRACT: Following a single i.p. injection of 3.0 μCi $^{239}\text{Pu}/\text{kg}$ body wt., the quantity of ^{239}Pu on the alveolar bone surface mesial to the periodontal ligament (PDL) of the maxillary first molar of male Sprague-Dawley rats increased from 1 to 9 days, then decreased by 15 days. At 15 days post-injection, the amount continued to decrease for 22 hours in both an orthodontically-stimulated area and a non-stimulated area in the opposite jaw of the same animal. However, the amount continued to fall in the stimulated PDL until 48 hours post-stimulation while the non-stimulated side remained constant from 22 to 48 hours.

The labeling index of the stimulated PDL fibroblasts of control and ^{239}Pu -injected female Sprague-Dawley rats rose to a peak of 12% at both 22 and 27 hours post-stimulation. Regional analysis of labeling index divided the PDL fibroblasts into "at risk" and "safe" groups. The "at risk" cells showed a labeling index of 10% at 22 hours compared to the cells of the same area in a control animal whose labeling index was 22%. The "safe" cells showed a labeling index of 15% vs. a labeling index of 10% in corresponding controls.

In stimulated controls, the number of osteoblasts increased by three-fold after 48 hours, while the number of osteoclasts decreased to zero. In stimulated ^{239}Pu -treated animals, the number of osteoblasts doubled in 48 hours, while the number of osteoclasts doubled also.

INTRODUCTION

The eventual goal of this study is to measure the effect of graded doses of ^{239}Pu on the cells of the stimulated rat periodontal ligament (PDL).⁽¹⁾

In this preliminary report, an intraperitoneal injection of 3.0 μCi $^{239}\text{Pu}/\text{kg}$ body weight was used. The quantitative distribution of plutonium along the alveolar bone adjacent to the PDL is presented, along with a regional analysis of the ability of the fibroblasts to respond to a stimulus of the PDL.

MATERIALS AND METHODS

Experiment 1 - The purpose of this experiment was to evaluate the qualitative and quantitative distribution of ^{239}Pu in the alveolar bone bordering

the PDL, as a function of time after injection and time after stimulation.

Twenty-eight 100-gram male Sprague-Dawley rats were given intraperitoneal injections of 3.0 μ Ci plutonium-239 citrate/kg body weight. Sacrifices were conducted at 1, 3, 5, 9, and 15 days post-injection. On day 15, stimulation of the left maxillary first molar was begun. The histologic area under study was the PDL along the mesial aspect of the mesial root of the maxillary first molar. A 0.5 mm thick latex elastic (#J-104, Rocky Mountain Co., Denver, Colorado) was secured in the interproximal space between the maxillary left first and second molars of the rats. The corresponding area of the maxillary right first molar served as an unstimulated control. Animals were sacrificed at 8, 16, 22, 27, 36, and 48 hours post-stimulation. Following dehydration and defatting procedures, the maxillas were embedded in bioplastic and sawed into 250 μ thick sections. The section best approximating the apical foramen of the mesial root of the first molar was ground to 50 μ thickness and dipped in Kodak's NTB liquid emulsion (Eastman Kodak, Rochester, New York). Sections were exposed for 10 days in a light-tight can in a cold, dry atmosphere. They were then developed in Kodak's D-19. Alpha tracks were counted along the alveolar bone surface in the area studied. The mean local dose rate delivered to the 35 μ soft tissue layer from the bone surface was calculated from the following formula:

$$\text{Dose rate (in rads/day)} = 290 \frac{T}{L}$$

where T is the number of tracks per day, L is the length of alveolar bone surface scanned and 290 is a constant which considers the energy of the ^{239}Pu alpha particle, its soft tissue range, and the density of soft tissue.⁽²⁾

Experiment 2 - The purpose of this experiment was to examine the regional effects of ^{239}Pu on labeling index of the PDL fibroblasts, as well as the numbers of osteoblasts and osteoclasts at various times post-stimulation.

Twenty 200 gram female Sprague-Dawley rats were injected with $3.0 \mu\text{Ci}$ plutonium citrate/kg body weight; twenty other female Sprague-Dawley rats were used as untreated controls. After 15 days, orthodontic stimulation was begun. In the unstimulated state, alveolar bone bordering the PDL in the area is a resorbing surface characterized by a basophilic scalloped margin with the presence of occasional osteoclasts. The dark basophilic margin becomes an arrest line following this stimulus, serving as a marker separating original alveolar bone from new bone formed in response to the stimulation. Sacrifices were conducted at 8, 22, 27, 36, and 48 hours post-stimulation. All animals received $1 \mu\text{Ci}$ tritiated thymidine/g body weight intraperitoneally 1 hour pre-sacrifice. The maxillas were placed in 2.5% phosphate-buffered glutaraldehyde for 48 hours, then decalcified in 14% EDTA at pH 7.4 for 2 weeks. Tissues were embedded in modified methyl methacrylate,⁽³⁾ sectioned at 3 microns on a motorized Jung microtome, and processed for detailed autoradiography, using Kodak's NTB liquid emulsion.⁽⁴⁾ After 3 weeks exposure in a light tight can in a cold, dry atmosphere, they were developed in Kodak's D-19 developer solution, and stained in hematoxylin and eosin. The labeling index was determined for all fibroblasts in the PDL, from the alveolar crest to the tooth apex, as well as for three regions: 1) fibroblasts within 50μ of alveolar bone; 2) fibroblasts within 50μ of tooth structure; and 3) all other PDL fibroblasts.

RESULTS

The amount of ^{239}Pu on the alveolar bone surface increased from 1-9

days following injection then decreased by 15 days. After 15 days, the amount of ^{239}Pu decreased from 8 to 22 hours in stimulated and unstimulated PDL's. After 22 hours, the number of tracks on the stimulated side continued to decrease, while there was no additional loss on the unstimulated side (Table 1).

The labeling index of the whole PDL of stimulated control animals reached a high point of 11% at both 22 and 27 hours post stimulation. The labeling index of the same site in ^{239}Pu -injected animals reached 12% at the same time. The labeling index did not change in the unstimulated side at any time (Tables 2 and 3).

However, regional analysis showed that the fibroblasts within 50 μ of alveolar bone reached a labeling index of 22% at 22 hours in control animals, while in Pu-injected animals, the labeling index was 10%. At 27 hours, the control labeling index was 8%, while that in the Pu-injected rats remained at 10%. The fibroblasts in the middle of the PDL reached 10% labeling index at 22 hours and 16% at 27 hours in control animals. In this area, in Pu-injected animals, the labeling index was 15% at both 22 and 27 hours. The fibroblasts next to the tooth showed little difference between control and Pu-injected rats (Tables 2 and 3).

The number of osteoblasts per 1100 μ length of alveolar bone in stimulated control animals increased from 5 to 12 after 48 hours of stimulation, while in ^{239}Pu -injected animals, stimulated PDL's the number increased only from 4 to 8. The number of osteoclasts per 1100 μ length of alveolar bone decreased from 1 to 0 after 48 hours of stimulation, while the number of osteoclasts rose from 3 to 4 in Pu-injected animals (Tables 4 and 5).

DISCUSSION

The amount of ^{239}Pu deposited along the alveolar bone surface increases for 9 days after injection. This is probably due to the uptake of translocated ^{239}Pu from other organs over the 9 day period. It is possible that a peak amount of ^{239}Pu is present between 9 and 15 days, and that a loss of activity has begun and continues beyond 15 days with or without PDL stimulation. The continued loss of ^{239}Pu in the stimulated side reflects the continued presence and activity of osteoclasts through the duration of the stimulus.

Regional differences in the labeling index of PDL fibroblasts showed that the cells next to the alveolar bone within range of the alpha particle responded more slowly and in smaller total numbers to a stimulus, while the cells lying just outside this range responded more quickly than controls. The alpha irradiation is able to make cells in a resting phase (G_0), unable to respond to a stimulus. The response of the cells in the middle shows the ability of the total population of the PDL to "compensate" for injury to an individual portion.

The osteoblasts' and osteoclasts' counts showed that in control animals, the stimulated PDL shifts from bone resorption to bone formation, while in ^{239}Pu -treated stimulated PDL's, the osteoblasts and osteoclasts increase simultaneously. The effect of plutonium was to change the balance of osteoblasts and osteoclasts toward osteoclasts, a confirmation of previous findings.⁽⁵⁾

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TABLE 1

TRACK COUNTS ALONG ALVEOLAR
BONE SURFACE* (Mean $\pm$ S.D.)

DAYS POST-INJECTION					
1	3	5	9	15	
167.0	361.8	326.8	582.5	530.3	
± 15.0	± 34.6	± 27.4	± 28.2	± 29.1	
DOSE RATE (rads/day)					
4.4	9.5	8.6	15.3	14.0	
HOURS POST-STIMULATION (STIMULATED SIDE)					
8	16	22	27	36	48
470.0	380.3	240.5	228.8	194.3	172.5
± 34.8	± 24.1	± 32.1	± 46.3	± 25.6	± 27.3
DOSE RATE (rads/day)					
12.4	10.0	6.4	6.0	5.1	4.5
HOURS POST-STIMULATION (<u>NON</u> -STIMULATED SIDE)					
8	16	22	27	36	48
477.8	425.8	334.0	313.8	332.5	323.0
± 31.9	± 30.7	± 36.5	± 28.9	± 35.2	± 25.9
DOSE RATE (rads/day)					
12.6	11.2	8.8	8.3	8.8	8.5

*Length of bone surface counted is 1100 μ

TABLE 2

LABELING INDEX OF PDL FIBROBLASTS IN
CONTROL FEMALE RATS (Mean $\pm$ S.D.)

	NON- STIMULATED	STIMULATED HOURS POST-STIMULATION				
		8	22	27	36	48
TOTAL % LABEL	2.7 ± 0.3	6.0	11.1 ± 0.5	11.3 ± 0.7	5.0 ± 1.4	3.2
% LABEL NEXT TO BONE	3.1 ± 0.5	3.2	22.1 ± 1.4	7.9 ± 0.3	4.9 ± 1.6	4.5
% LABEL IN MIDDLE	2.7 ± 0.6	8.8	9.9 ± 1.6	15.2 ± 1.7	5.4 ± 1.5	3.1
% LABEL NEXT TO ROOT	2.5 ± 0.2	3.4	6.3 ± 0.9	3.6 ± 0.2	4.2 ± 0.7	1.4

TABLE 3

LABELING INDEX OF PDL FIBROBLASTS IN Pu-INJECTED
FEMALE RATS (Mean $\pm$ S.D.)

	NON- STIMULATED	STIMULATED HOURS POST-STIMULATION				
		8	22	27	36	48
TOTAL % LABEL	3.3 ± 0.9	3.1 ± 0.2	12.7 ± 0.4	12.1 ± 0.8	3.2 ± 0.1	3.5 ± 0.8
% LABEL NEXT TO BONE	5.4 ± 3.0	2.3 ± 0.4	10.4 ± 1.5	10.1 ± 0.5	2.6 ± 0.2	6.1 ± 0.9
% LABEL IN MIDDLE	3.0 ± 0.3	4.3 ± 0.1	15.8 ± 0.5	15.2 ± 0.9	3.6 ± 0.3	2.7 ± 0.6
% LABEL NEXT TO ROOT	2.2 ± 1.1	2.1 ± 0.2	6.6 ± 0.8	4.1 ± 1.2	3.4 ± 0.1	2.4 ± 1.5

TABLE 4

OSTEOBLASTS IN THE PDL OF
FEMALE RATS (Mean $\pm$ S.D.)

	NON- STIMULATED	STIMULATED HOURS POST-STIMULATION				
		8	22	27	36	48
Pu-INJECTED	4.1 ± 1.3	5.5 ± 0.6	4.0 ± 1.8	8.0 ± 1.6	6.0 ± 0.8	8.3 ± 1.0
CONTROL	5.7 ± 1.2	4.0	6.0 ± 0.8	8.5 ± 1.9	12.3 ± 2.2	12.0

TABLE 5

OSTEOCLASTS IN THE PDL OF
FEMALE RATS (Mean $\pm$ S.D.)

	NON- STIMULATED	STIMULATED HOURS POST-STIMULATION				
		8	22	27	36	48
Pu-INJECTED	2.9 ± 1.4	1.8 ± 1.0	1.5 ± 0.6	5.3 ± 0.5	5.0 ± 0.8	4.0 ± 0.8
CONTROL	1.3 ± 0.8	0.5	1.3 ± 0.5	2.0 ± 0.8	0.5 ± 0.6	0.0

HEMATOLOGIC CHANGES FOLLOWING ^{239}Pu INJECTION IN ADULT ST. BERNARDS AND IMMATURE BEAGLES

Jean H. Dougherty and Lowell A. Woodbury

ABSTRACT: The hematologic changes following a single intravenous injection of ^{239}Pu in young adult St. Bernards and 3 month old beagles are reported for the first year following injection. The amounts of ^{239}Pu injected ranged from 0.005 to 0.3 $\mu\text{Ci/kg}$ in the 12 St. Bernards and from 0.3 to 3.0 $\mu\text{Ci/kg}$ in the 20 beagle pups. The dose levels selected were comparable to those in the adult beagles injected in our laboratory. There were no significant alterations in erythrocytes in any of the dose levels in either the St. Bernards or beagle pups. A dose-dependent leukopenia was found in St. Bernards receiving the two highest dose levels (0.1 and 0.3 $\mu\text{Ci/kg}$) which was sustained over the first year. The pattern and degree of cell depression was quite similar to that found in adult beagles. The beagle pups given the highest dose level (0.3 $\mu\text{Ci/kg}$) had a leukopenia and lymphopenia which was not as marked as found in adult beagles receiving the same amount of ^{239}Pu . The increase in skeletal mass of the pups during growth, a more rapid burial of ^{239}Pu within bone volume, and perhaps, a greater capacity of the immature hematopoietic system to recover may be possible reasons for this difference in hematologic toxicity of the injected plutonium.

INTRODUCTION

A pilot study of bone sarcoma induction in adult St. Bernards⁽¹⁾ and bone versus liver damage in immature beagles⁽²⁾ following injection of ^{239}Pu has been underway for several years. As part of the study, serial hematological examinations were made on the dogs at frequent intervals. So far 12 of a contemplated 18 St. Bernards have been given a single intravenous injection of ^{239}Pu at four dose levels (three dogs per dose level) on a basis comparable to those used in the adult beagle studies. These are the 0.5, 1.0, 2.0, and 3.0 dose levels which range from 0.005 to 0.3 $\mu\text{Ci/kg}$ of injected plutonium. There have been 18 three-month old beagle pups injected at three dose levels of ^{239}Pu ; 5 dogs at the 0.0 level, 3 at the 3.0 level (0.3 $\mu\text{Ci/kg}$) and 12 at the 5.0 level (3.0 $\mu\text{Ci/kg}$).

The hematological studies on these dogs have been made in the same manner as in the adult beagles injected with ^{239}Pu in order to evaluate the relative toxicity of plutonium to the hematopoietic system of the young beagles and St. Bernards.

This report covers the beagle pups for the first year following injection and the St. Bernards for the first 9 to 12 months after injection.

METHODS

Three control blood counts were made on the dogs during the month prior to injection. The red cell picture is delineated by determination of volume of packed red cells (VPRC) and hemoglobin (Hgb), and the white cells are analyzed by total leukocyte counts (WBC) and absolute numbers of segmented polymorphonuclears (PMNs), lymphocytes, monocytes, and eosinophils.

For the pups, the post-injection values are related to pre-injection means as well as to the means of the 0-level dogs at 3 month intervals for the first year. Since no 0-level St. Bernards were injected because of kennel space limitations at this time, the post-injection changes can only be analyzed by relating these values to pre-injection mean values.

One of the problems in analyzing the serial blood counts on the dogs is that the successive observations on any particular dog may have correlated errors. There are several methods available for analysis of such data and for our purposes the following two methods were chosen.

1. Comparison of control and injected beagle pups.

For the pups, blood counts were available at the same time intervals for 0-level (control), 3- and 5-levels of plutonium injection. There was considerable variation among the pre-injection values for the various dogs

and inspection of the post-injection values suggested that dogs with high pre-injection values of the blood counts tended to have higher post-injection values of the counts. A similar pattern appeared to hold for the low pre- and post-injection blood counts. There were insufficient animals in all groups to allow an extensive analysis of this possible correlation but preliminary tests using relative rankings of the WBC values confirmed its existence, at least for the WBCs. To compensate, in part, for the correlation, the average of the pre-injection values for each animal was subtracted from the post-injection values for the different types of blood cells and for the Hgb and VPRC measurements. The resulting value is the change in the blood count over or under the pre-injection value. This change was used as the unit for statistical analysis. In general, the change had a somewhat smaller variability than the original observation and was therefore used in preference to the absolute values.

The method of analysis used was to compare the change of the various blood cell values of the 3- and the 5-level dogs with the change of the control (0-level) dogs at each time interval. Since two comparisons are made with the same control, the ordinary t-test is not valid so Dunnett's modified t-test for comparing several treatments with a control was employed.^(3,4) Since it was known a priori that the effect of the injected plutonium would be to reduce the various blood cell counts it was possible to specify that only drops in counts would be tested and thereby use a one-sided t-test which doubles the sensitivity of the analysis. The significant results of these tests are marked with arrows in the graphs of mean absolute values for each dose level, at months post-injection where significant differences are found. (See Methods Figs. 5-7).

This method of comparison of the various dose levels of plutonium with a control value could not be used with the St. Bernard dogs as no control group existed and so recourse had to be made to another method.

2. Average cell value analysis made on St. Bernards and beagle pups.

The problem of correlated errors can also be avoided by combining all of the measurements of each individual dog into a single figure for each blood value and conducting the analysis on these combined values. The method selected for use in this study was a time weighted average of the cell counts over a given time. The time analyzed was 12 months for the pups and 9 months for the St. Bernards since dogs given the lower dose levels were injected later than the higher levels.

This average is obtained, for each dog, by multiplying the average cell count for a given period by the length of that period of time and summing over all periods. The result is in units of cell-months and is equivalent to the area under the cell count vs. time curve of a particular dog. This area may be used as the variable for purposes of analysis or it may be divided by the number of months over which the area was measured to give the time-weighted average cell count for that period. The average was chosen for the purpose of analysis as its meaning is more readily comprehended. The area under the cell count vs. time curve was determined for each dog using the trapezoidal rule and this was divided by the length of time over which the area was measured to give the time-weighted average for each dog.

This method provides a new technique for reporting serial hematological measurements. It is particularly useful when the data samples are small and unequal and when there are missing values due to clinical illness of the animals or other factors.

RESULTS

St. Bernards.

Erythrocytes. The red cell picture is represented by mean values for VPRC and Hgb (Fig. 1). It is apparent from the graph that there are no significant depressions of either VPRC or Hgb values for the various dose levels over the first year as compared to pre-injection values. Analysis of the time weighted averages for the first 9 months for both the VPRC and the Hgb showed no significant relation (P greater than 0.2) to the amount of ^{239}Pu that had been injected. There is, thus, no evidence that the injected plutonium had any effect in the first 9 months on the VPRC or the Hgb.

Leukocytes. The responses of the various leukocytes are shown in Figs. 2 and 3. There is an abrupt post-injection depression of the WBC with little recovery to the pre-injection values during the first 12 months. This fall is dose-dependent and is mainly due to the depression in the segmented PMNs (see PMN curve, Fig. 2). For the 3-level dogs at 1 month the cell count is about one-half of the pre-injection values, whereas in the 2-level dogs the depression develops more slowly but becomes about the same as the 3-level dogs at 12 months.

There is less depression of lymphocyte values in the 2- and 3-level dogs, however, most of the mean post-injection counts are below the pre-injection values. The monocyte means are well below the pre-injection mean for the 2- and 3-level dogs throughout the first year. There is no apparent depression of eosinophils except possibly at 9 months where the 2- and 3-level dogs combined have a significantly lower eosinophil count (P less than 0.01) than the 0.5- and 1-level dogs combined.

There seems to be no effect of the plutonium injections on any of the leukocyte types at the 0.5- and 1-levels.

Figure 1. St. Bernards. Mean absolute values of VPRC and hemoglobin versus time post-injection. In all figures, values plotted to the left of the zero time point are pre-injection measurements, and the value plotted at the zero time point is the mean of the pre-injection values.

Figure 2. St. Bernards. Mean absolute values for WBC, segmented PMNs, and Lymphocytes.

Figure 3. St. Bernards. Mean absolute values for monocytes and eosinophils.

Figure 4. St. Bernards. Time-weighted average of cell counts (WBC, PMNs, lymph, and mono.) vs. injected dose of ^{239}Pu . Black dots are the averages of the time-weighted average for each dog at that dose level. Vertical lines terminated by a bar indicate one standard deviation above and below the mean.

The results of the statistical analysis of the time-weighted average of the cell counts is shown in Fig. 4. It will be noted that in the graphs there is a general tendency for the time-weighted average to decrease with increasing amounts of injected plutonium. This decrease was highly significant (P less than 0.001) for the WBC, PMNs, and monocytes. For the lymphocytes, the drop was not statistically significant but was suggestive as the P value was between 0.1 and 0.05. The drop for the eosinophils was non-significant (P greater than 0.2) so they were not included in the graph.

The general conclusion is that there is a firm dose-response relation between the time-weighted average of most leukocyte types and the dose of plutonium-239 injected. The analysis of the time-weighted averages confirms the results obtained by observation of the absolute value curves.

Beagle Pups.

Erythrocytes. The absolute values of the VPRC and of hemoglobin are shown in Fig. 5. As these puppies mature the mean values of both the VPRC and the hemoglobin increase (see Table 1).

Table 1. Age-Related Changes of VPRC and HGB in Non-Irradiated Dogs

	3 month beagle (pre-injection mean)*	15 month beagle (12 mo. post-sham injection)	Adult beagle (15-17 mo.)	Adult St. Bernard (17-18 mo.)
VPRC	38	47	52	48
Hgb	12.1	15.5	16.6	15.8

*Pre-injection values combined for pups at all dose levels.

There seems to be no consistent differences among the various dose levels for either the VPRC or for the hemoglobin values as observed by these curves

Figure 5. Beagle pups. Absolute values for VPRC and Hgb for the first year post-injection. Points marked by an arrow are significantly different from the 0-level values at that time. ? notes questionable biological significance.

(Fig. 5). Statistical analysis of the changes from pre-injection values showed a significant drop from the 0-level values of the VPRC at 4 months and of hemoglobin at 9 months in the 5-level dogs. Little, if any, significance should be attached to these single points on the two curves. (Fifteen t-tests were made on each of the curves and all were non-significant except the one point. There is a probability of about 50 percent that out of 15 t-tests where all were not significant that one or more would show significance by pure chance.)

Leukocytes. Figs. 6 and 7 summarize the absolute values of WBC, Seg. PMNs, lymphocytes, monocytes, and eosinophils. All of the different types of white cells show somewhat lower values during the first month after injection. This was evident even in sham injected 0-level pups. During this first month all dogs are kept in metabolism cages and it was thought that the immobilization might be a factor in this decrease affecting particularly lymphocyte values. A subsequent study in which 4 pups were put in metabolism cages and 4 were kept in runs substantiates this conjecture. However, the counts in 0-level dogs did not show as great a drop as the injected dogs and after the caged period of the 0-level dogs, the PMNs, monocytes, and eosinophils were back to pre-injection levels. The lymphocytes on all dogs seem to become slightly lower as the pups mature. From an observation of the mean values of 5-level pups, there is a depression of all cell types with no recovery of the pmns and lymphocytes. There also appears to be a slight drop in PMNs in 3-level dogs.

Analysis of the time-weighted average of each leukocyte type showed significant decreases in the average with increasing doses of injected plutonium for the WBC, PMNs, lymphocytes, monocytes, and eosinophils. These

Figure 6. Beagle pups. Absolute values for WBC, segmented PMNs, and lymphocytes.

Figure 7. Beagle pups. Absolute values for monocytes and eosinophils.

Figure 8. Beagle pups. Time-weighted average cell count vs. injected dose of ^{239}Pu . See Fig. 4 for explanation of black dots and vertical lines. The standard deviations were too small to be shown for eosinophils.

results are shown in Fig. 8. The general significance of these findings is that a firm dose-response exists between the time-weighted average of the various leukocyte types and the amount of injected plutonium.

DISCUSSION

The pre-injection leukocyte values of the St. Bernards are very similar to those of the adult beagles.⁽⁵⁾ The red cell values (VPRC and Hgb) are lower for the St. Bernards (see Table 1). This may be a species difference or may be due to differences in nutrition and immunization of the St. Bernards which were acquired as adults.

The blood cell values of the beagle pups are similar to beagles of the same age reported for the colony at Davis, California.⁽⁶⁾ These investigators also found that the PMNs were variable in number but without any definite trend. The lymphocytes decreased in the Davis pups from $\sim 4500/\text{mm}^3$ at 3 months to $\sim 3,000$ at 15 months of age. The pre-injection means of the 20 Utah pups was 3,920 and the mean of the five 0-level dogs at 15 months was 3,440. The pre-injection mean of segmented pmns of the pups at 3 months of age was 5,880, and in the controls at 15 months was almost the same, i.e., 5540. Neither monocytes nor lymphocytes changed appreciably over this period. The rise in VPRC and Hgb from 3 to 15 months (Table 1) was similar to that found in the Davis pups.

There have been several reports covering the acute and chronic changes after ^{239}Pu injection into young adult beagles (7,8,9,10). An acute depression in the WBC which was maximal at 2-3 weeks was found for all granular leukocytes down through the 2-level dogs. The fall in lymphocytes was significant at the 4 and 5 level.

At comparable dose levels, i.e., the 2- and 3-levels, the findings in

the St. Bernards are similar to the beagles as to time and percentage change. As with the beagles, there were no significant alterations in leukocytes below the 2-level. There is perhaps less change in eosinophil values of the St. Bernards compared to the beagles.

The absence of any changes in erythrocyte values in St. Bernards at any of the injection levels is in keeping with findings on adult beagles. Only in the 5-level adults was an early transient depression of red cells seen, and at the 4- and 5-levels, and in an occasional 3-level dog as the animals became terminal.

It is difficult to relate hematological changes at the 3- and 5-level in the pups to those of adult beagles receiving the same amount in $\mu\text{Ci/kg}$ at time of injection. There is not only a different rate of bone turnover in the pups but their increase in weight and bone mass are also factors tending to reduce the ^{239}Pu burden with time as expressed in $\mu\text{Ci/kg}$. It has been estimated by Dr. Charles W. Mays of our laboratory that during the first year following injection, the $\mu\text{Ci/kg}$ concentration is reduced by a factor of about 2 due to increase in skeletal mass of the pups. In addition, one should expect a more rapid burial of bone-surface deposited ^{239}Pu due to more rapid bone remodelling and apposition rates. Also, a relatively immature hematopoietic system may react differently to plutonium -- the rate of recovery from damage may be faster.

A comparison of the degree of depression of the various blood values of the 5-level pups versus 5-level adults shows that in the pups the PMNs dropped only 2/3 of the amount that the adults dropped, and for the lymphocytes the drop was 3/4 of that of the adults. This is also the case when comparing 3-level pups to 3-level adults. The explanation for the lesser

toxicity of the ^{239}Pu to the pups is not understood at this time and must await autoradiographic and distribution studies as well as serial sacrifice animals.

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MEASUREMENT OF PLASMA CORTICOSTERONE (CPD.B) LEVELS
IN MAN BY RADIOIMMUNOASSAY*

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ABSTRACT: A method that utilizes radioimmunoassay (RIA) has been developed for measuring plasma cpd B levels in man. Antisera were obtained by immunizing rabbits with cpd B-21-succinyl-BSA. For RIA an antiserum titer of 1:4000 was used for standard curves ranging from 0-1000 pg. A logit-log transformation of the standard curve was linear from 25-100 pg. The antiserum was heterogeneous for cpd B antibodies by Scatchard analysis. The antiserum was highly specific for cpd B with only the following steroids having cross-reactions greater than 1%: cortisol (3%), testosterone (10%), and progesterone (44%). Cpd B was separated from these steroids by paper chromatography using a Bush B₃ system. Plasma blanks obtained from 5 adrenalectomized patients ranged between 34 and 51 ng%. Recovery of radioactive cpd B through the entire method was $67.6 \pm 5.2\%$. Plasma values were corrected for procedural losses but not for blanks. The recovery of known amounts of unlabeled cpd B added to plasma averaged $103 \pm 11\%$. Precision was determined from replicate measurements on the same plasma pools. Coefficients of variation for intra-assay precision were 19% at a level of 52 ng% and 9% at 1143 ng%. For inter-assay precision the CV's were 13% at 44 ng% and 5% at 501 ng%. Rat plasma cpd B levels measured by fluorometry agreed well with values determined by radioimmunoassay ($r = .97$). Plasma cpd B levels were measured in 15 normal

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males and 15 normal females at 8AM in the upright position. The values for males (396 ± 228 ng%) were significantly different than that obtained for females (655 ± 271 ng%) ($p < .01$). Diurnal studies in 2 normal subjects demonstrated that B and F were secreted in a parallel, episodic fashion over a 24 hour period. Dexamethasone administration suppressed plasma Cpd B levels to the range observed in adrenalectomized patients. A 6 hour intravenous administration of 25U of ACTH caused a rise in plasma Cpd B levels in normal subjects that exceeded the increase in cortisol (1290% increase for Cpd B v.s. 290% for F). Two Cushing's patients with hyperplasia were hyperresponsive to ACTH and one Cushing's with adrenal adenoma was hyporesponsive. Conclusions: These observations indicate that Cpd B secretion is ACTH dependent and suggest that plasma Cpd B measurements may be clinically useful especially in adrenal suppression and stimulation tests.

SIMULTANEOUS MEASUREMENT OF MULTIPLE PLASMA STEROIDS BY
RADIOIMMUNOASSAY DEMONSTRATING EPISODIC SECRETION

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ABSTRACT: A method for measuring cortisol, corticosterone, testosterone, progesterone, and 17-OH progesterone simultaneously in 2 ml of plasma has been developed especially for episodic secretion studies in which many frequent assays are required. Steroids were extracted from plasma, separated into 4 fractions by paper chromatography, and individual steroids in each fraction were measured by radioimmunoassay. The individual steroid radioimmunoassays were demonstrated to be sensitive, specific, accurate, and precise.

Baseline 8 AM plasma steroid levels were measured in normal males and menstruating females both before and after ovulation. The values obtained were in good agreement with those previously reported by other investigators using different methods.

In order to establish the normal diurnal variation in both sexes, plasma steroid levels were measured at 20 minute intervals throughout the day in a normal man and a preovulatory woman. When plasma steroid levels were plotted against time frequent intermittent peaks that were consistent with episodic secretion were observed. As a result of this study progesterone, Corticosterone peaks testosterone, and 17-OH progesterone have been added to the list of hormones that are secreted episodically. Corticosterone peaks were well synchronized with cortisol peaks, but those for progesterone, testosterone, and 17-OH progesterone were not. In the male 17-OH progesterone and testosterone peaks correlated well. It was concluded from these observations that corticosterone is secreted directly into the bloodstream by the adrenal under ACTH control, and that the other steroids are secreted from non-adrenal sources under other controls. The synchrony between plasma testosterone and 17-OH progesterone levels in the male suggests a common origin, presumably the testes.

The original demonstration that cortisol is secreted episodically⁽¹⁾ has been confirmed and extended to other hormones.⁽²⁻⁵⁾ These observations have had a great impact on our understanding of the physiology of hormone secretion. Episodic secretion studies are difficult to perform because multiple assays are required. To facilitate these studies a method that utilizes paper chromatography and radioimmunoassay has been developed for simultaneously measuring cortisol, 17-OH progesterone, progesterone, corticosterone, and testosterone in 2 ml of plasma. This method has been used to measure baseline 8 AM plasma steroid levels in normal subjects and to study the normal circadian variation in a normal man and a preovulatory woman.

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MATERIALS AND METHODS

The grade, source, and preparation of all materials, reagents, chemicals, and solvents used in these radioimmunoassays have been given in a previous publication.⁽⁶⁾

All radioactive steroids were tritium-labeled and obtained from New England Nuclear with a specific activity of 30 Ci/mM or more which allowed for the use of not more than 25 µg of tracer steroid in the radioimmunoassays.

Extraction of plasma and paper chromatography of extracts.

Two ml of plasma were extracted with 5 ml of benzene followed by 35 ml of methylene dichloride. The methylene dichloride extract was added to the dried benzene extract, washed with 2 ml of water, and dried. All organic solvents were removed by evaporation under air jets. The plasma extracts were chromatographed on methanol washed Whatman #1 cellulose paper using a Bush B₃ solvent system (petroleum ether 33%, benzene 17%, methanol 40%, and water 10%) with development until the solvent front reached 45 cm (average running time = 2 1/2 hrs.). External radioactive steroid reference standards were run on separate strips and corresponding sections of the unknown strips were cut for specific steroids as follows: Fraction 1 (-0.5 to 6.0 cm from the origin) contained cortisol; fraction 2 (7.5 to 15 cm), corticosterone; fraction 3 (25 to 32 cm), testosterone and 17-OH progesterone; fraction 4 (35 to 42 cm), progesterone. Each paper fraction was eluted with methanol and the dried eluates were analyzed for their respective steroid content by radioimmunoassay.

Preparation of steroid antisera.

Highly specific antisera were prepared for each steroid by immunizing

rabbits with conjugates of the steroid to bovine serum albumin. The synthesis of the steroid conjugates, the dose and route of administration of the antigen, and the injection schedule have been reported in detail previously.⁽⁶⁾ The steroid conjugates that were used for immunization include cortisol-21-hemisuccinate-BSA, progesterone-11-hemisuccinate-BSA, corticosterone-21-hemisuccinate-BSA, testosterone-3-carboxymethyloxime-BSA, and for 17-OH progesterone radioimmunoassay, 11-deoxycortisol-21-hemisuccinate-BSA. Antisera titers ranged from 1:4000 to 1:22,500.

Procedure for radioimmunoassay.

The procedure for radioimmunoassay was essentially the same for all steroids. Standards and unknowns were incubated overnight at 4°C with appropriate dilutions of antisera in .01M phosphate buffer (pH 7.8) that contained 25 µg or less of tracer steroid 0.14M NaCl, .01M EDTA, 0.1% sodium azide, and rabbit gamma globulin (0.25 mgm/ml). Appropriate amounts of goat anti-rabbit gamma globulin serum, determined from equivalence titration curves, were used to separate free from antibody bound steroids. The free radioactivity in the supernatant was measured using a Packard liquid scintillation spectrometer. Standard curves were constructed in the usual fashion and unknown values were obtained directly from the standard curves. The total excursion of the standard curves varied from 0 to 200 to 0 to 600 µg. Steroid concentrations in plasma were calculated from the aliquots and corrected for procedural losses by the use of radioactive recovery controls.

Studies in normal subjects.

The normal subjects in this study were medical students and employees who were healthy by history. All subjects for the 8 AM baseline steroid levels were fasting and upright in position. An indwelling intravenous

catheter was used for blood sampling in the episodic secretion studies. The subjects slept well during the nocturnal phase of the study, occasionally arousing briefly but remaining supine. Sleep was not monitored by electroencephalography. There were no dietary restrictions.

RESULTS

Evaluation of the multisteroid radioimmunoassay.

Specificity - The specificity of this multisteroid radioimmunoassay depends upon three factors: (1) the specificity of the antisera, (2) the concentration of these steroids in blood, and (3) the removal of interfering steroids by paper chromatography. The percent cross-reaction between the antisera for each radioimmunoassay and other steroids in the same chromatographic fraction and other major blood steroids is given in Table 1. The method

Table 1. Steroid cross-reactions with antisera used for radioimmunoassays

Chrom. Fraction	Steroids Tested	Radioimmunoassay				
		F	B	17OHP	T	P
I	Cortisol	100%	3%	1.6%	< 0.01%	0.5%
	Cortisone	7	0.2			
	Aldosterone		1			
II	Corticosterone	4	100	< 0.01	< 0.01	0.8
	11-Deoxycortisol	35	0.8			
	Estradiol	< 0.01	< 0.001		< 0.01	
III	17-OH Progesterone	34	0.2	100	0.01	0.5
	Testosterone	0.3	10	0.7	100	0.5
	11-Deoxycorticosterone	2	26	5	0.03	3
	Dehydroepiandrosterone	0.01	< 0.001	< 0.2	0.02	< 0.02
IV	Progesterone	3	44	10	0.02	100
	Δ^4 -Androstenedione			0.4	1.8	< 0.1

Table 2. Plasma blanks: Steroid values in oophorectomized, adrenalectomized women

Patient	Plasma Steroid Level				
	F μg/dl	B mμg/dl	T mμg/dl	17OHP mμg/dl	P mμg/dl
D. S.	0.1	55	2	0	14
D. W.	0.4	39	7	0	15
P. E.	18*	35	5	12	20

*On cortisol replacement therapy

of Abraham et al. was used to calculate percent cross-reaction.⁽⁹⁾ Steroid cross-reactions within chromatographic fractions did not exceed 7% and were usually less than 1%.

Plasma blanks - Non-steroidal materials might also react with the anti-sera and interfere with the radioimmunoassays. To test this possibility plasmas from three adrenalectomized, oophorectomized women were assayed for each steroid (Table 2). These values represent plasma blanks for the method. In this study the plasma steroid values were not corrected for plasma blanks.

Sensitivity - With standards the radioimmunoassays could distinguish between 0 and 10 μg or less for each steroid at the 95% confidence limit level. However, the values obtained on steroid-free plasma from adrenalectomized, oophorectomized patients represents the sensitivity limit of the radioimmunoassay in actual practice (Table 2).

Recovery - The average recovery of radioactive steroids added to plasma varied from 61.5 to 75.1% with the standard deviation ranging from 4.4 to 5.6% (Table 3).

Accuracy - To test the accuracy of the method known amounts of unlabeled

Table 3. Accuracy: Recovery of known amounts of steroids added to plasma

Steroid RIA	Radioactive Steroids			Non-Radioactive Steroids*			
	n	% Recovery (mean)	SD	n	Added	Analyzed (mean)	SD
Cortisol	24	72.5	5.6	10	25.0	25.4	1.0
				10	2.5	2.7	0.3
Corticosterone	25	67.6	5.2	4	1000	967	122
				4	500	565	37
				4	200	199	28
Testosterone	9	61.5	4.4	9	1000	1112	97
				6	500	483	100
				8	50	50	12
17-OH Progesterone	27	75.1	4.8	5	100	125	6.1
Progesterone	24	73.3	4.5	12	2000	1971	67
				10	500	532	31
				10	200	225	24

*Cortisol values in $\mu\text{g/dl}$; all other steroids in mg/dl

steroids were added to plasma and analyzed. As shown in Table 3 the recovery of added steroids was essentially quantitative and consistent.

Precision - Within assay precision was determined from replicate measurements in the same assay on plasma pools with high, intermediate, and low levels of steroids within the normal range. Between assay precision was determined from different assays on the same plasma pools. In Table 4 the precision is expressed in terms of the coefficient of variation at each plasma steroid concentration.

Normal 8 AM plasma steroid levels by multisteroid radioimmunoassay.

Normal 8 AM plasma levels of the five steroids were measured simultaneously

Table 4. Precision of multisteroid radioimmunoassay

Steroid RIA	Within Assay			Between Assay		
	n	Plasma Conc.	CV*	n	Plasma Conc.	CV
Cortisol	20	25 µg/dl	5%	9	1 µg/dl	14%
	10	3 "	10			
Corticosterone	10	1143 µg/dl	9			
	10	501 "	8	5	501 µg/dl	5%
	10	52 "	19	7	44 "	13
Testosterone	10	1217 "	8	10	1236 "	9
	10	26 "	13	10	7 "	17
17-OH Progesterone	9	77 "	18	8	90 "	10
Progesterone	10	1012 "	6	6	9 "	32
	10	460 "	10			
	6	46 "	21			

*Coefficient of variation

in 15 normal males and 15 normal females (Table 5). Corticosterone levels were significantly higher in females than in males and higher prior to ovulation. The other steroid levels differed as expected according to sex and the phase of menstruation. In general the values obtained agree well with those reported by other investigators using different methods.⁽¹⁰⁻¹⁶⁾

Circadian rhythm studies in normal subjects.

Plasma levels of the five steroids were measured simultaneously at intervals of 20 minutes throughout most of the day in a 20 year old female during the follicular phase of her menstrual cycle, and in a normal 26 year old male (Fig. 1 and 2). Except for testosterone and 17-OH progesterone in the female all plasma steroid levels fluctuated widely over short periods of time throughout

Table 5. Normal 8 AM plasma steroid levels measured by multisteroid radioimmunoassay*

Steroid**	Males n=15	Females	
		Follicular n=9	Luteal n=6
Cortisol	14 ± 5 µg/dl (4 - 24)	14 ± 5 µg/dl (8 - 20)	13 ± 3 µg/dl (8 - 17)
Corticosterone	396 ± 228 µg/dl (82 - 886)	706 ± 268 µg/dl (215 - 1094)	579 ± 281 µg/dl (212 - 1026)
Progesterone	28 ± 15 µg/dl (12 - 74)	50 ± 34 µg/dl (18 - 116)	1315 ± 359 µg/dl (788 - 1692)
17-OH Progesterone	97 ± 35 µg/dl (40 - 175)	31 ± 9 µg/dl (17 - 43)	238 ± 70 µg/dl (182 - 326)
Testosterone	557 ± 227 µg/dl (275 - 1025)	36 ± 12 µg/dl (25 - 65)	44 ± 27 µg/dl (26 - 98)

*Ad lib diet; upright posture, age 19-50

**Steroid values given as mean ± 1 SD with range in brackets

the day. A plot of the steroid values against time revealed frequent intermittent peaks that were consistent with episodic secretion. Corticosterone peaks were consistently well synchronized with cortisol peaks, but the other steroid peaks were not. In the male testosterone and 17-OH progesterone peaks correlated well, but in the female the levels were fairly constant with only a few uncorrelated minor peaks.

DISCUSSION

A reliable method has been developed for measuring the concentration of multiple steroids simultaneously on 2 ml or less of plasma. This method is

especially useful for episodic secretion studies where frequent analyses over long periods of time are desired. By comparing the concentrations of multiple steroids measured simultaneously at frequent intervals, it is theoretically possible to obtain a better understanding of the physiology of hormone secretion and the mechanisms that control hormonal circadian rhythms. It has been demonstrated that studies of this type are valuable in elucidating the role of hormones in the pathogenesis of congenital adrenal hyperplasia⁽¹⁷⁾ and should prove to be useful in other diseases.

In this study the circadian variation in these plasma steroids has been established in a normal adult male and female for future reference in pathological studies. The growing list of hormones that are secreted episodically has been extended to include progesterone, corticosterone, testosterone, and 17-OH progesterone.

It has been proposed that a hormone is secreted by the adrenal cortex under ACTH control if it is secreted episodically in synchrony with cortisol.⁽⁵⁾ In this study only corticosterone met those criteria completely. Other steroids were secreted episodically but their secretory peaks were not consistently correlated with cortisol. In the male and the preovulatory female it might be expected that progesterone would be secreted primarily by the adrenal cortex. Our results do not support this concept. The observation that major progesterone peaks were consistently out of phase with cortisol suggests a non-adrenal origin, presumably the ovarian follicle in the preovulatory female and the testes in the male.

Since 17-OH progesterone is an important precursor in cortisol biosynthesis, it could conceivably be secreted by the adrenal cortex. Our data do not provide convincing evidence for this possibility. In the preovulatory

woman the 17-OH progesterone levels were low (but measurable), and fairly constant throughout the day. In the male the plasma 17-OH progesterone levels were considerably higher and intermittent secretory peaks were observed that usually did not correlate with cortisol peaks. However, at 2020 hours the highest secretory peak observed for 17-OH progesterone coincided nicely with the highest peak for cortisol and corticosterone. This observation raises the possibility that 17-OH progesterone may be secreted by the adrenal cortex at times when cortisol production is very high. At all other times the 17-OH progesterone peaks were out of phase with cortisol but were fairly well synchronized with testosterone. This finding is consistent with previous reports that 17-OH progesterone is secreted primarily by the testes. (18)

Testosterone has been identified in adrenal cortical extracts and adrenal vein blood, but there is no good evidence from this investigation or other studies that testosterone is secreted by the adrenal cortex in amounts sufficient to affect blood levels. In the preovulatory female the testosterone levels were low and constant throughout the day except for two minor peaks at 0640 and 0840. These two peaks occurred at times when cortisol was being maximally produced and could possibly represent adrenal secretion. However, none of the other cortisol secretory peaks during the rest of the day were associated with testosterone peaks. In the male plasma testosterone levels were much higher than in the female and secretory peaks were nearly completely dissociated from cortisol peaks. Presumably, plasma testosterone levels in the male reflect testicular secretion.

What mechanisms control episodic secretion is unknown. Presumably a control center or "biological clock" in the central nervous system is responsible for the basic circadian rhythm. Whether negative feedback mechanisms

have a normal physiological function in controlling this rhythm has been questioned.⁽²⁾

Although there appears to be a basic circadian rhythm, it is impossible to predict exactly when secretory peaks will occur. This may account in part for the variability in normal ranges within and between laboratories even when blood samples are collected at the same time. Factors other than time may have an important influence, such as stress, exercise, sleep, position, food ingestion, and perhaps others. To ascertain which of these factors are important and how to control them for collecting blood samples under standardized conditions is important. Until this is done, the clinician must exercise caution in the use of a single plasma determination in clinical diagnosis. To compensate for episodic secretion some investigators have successfully used effusion pumps to withdraw blood at a slow constant rate over a set period.^(19,20)

Trivial names and abbreviations used in this paper: 4-pregnen-11 β , 17 α , 21-triol-3,20-dione, cortisol or F; 4-pregnen-17 α , 21-diol-3,11,20-trione, cortisone or E; 4-pregnen-11 β , 21-diol-3,18,20 trione, aldosterone or Aldo; 4-pregnen-11 β , 21-diol-3,20-dione, corticosterone or B; 4-pregnen-17 α , 21-diol-3,20-dione, 11-desoxycortisol or S; 1,3,5 (10)-estratrien-3, 17 β -diol, estradiol or E₂; 4-pregnen-17 α -ol-3,20-dione, 17 α -hydroxyprogesterone or 17OHP; 4-androsten-17 β ol-3-one, testosterone or T; 4-pregnen-21-ol-3,20 dione, desoxycorticosterone or DOC; 5-androsten-3 β -ol-17-one, dehydroepiandrosterone or DHEA; 4-pregnen-3,20-dione, progesterone or P; 4-androsten-3, 17-dione, androstenedione or Δ^4 .

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Figure 1. Diurnal variation in plasma Testosterone, Progesterone, 17-OH Progesterone, Corticosterone, and Cortisol levels in a 20 year old normal menstruating preovulatory woman.

Figure 2. Diurnal variation in plasma Testosterone, Progesterone, 17-OH Progesterone, Corticosterone, and Cortisol levels in a 26 year old normal male.

RADIOIMMUNOASSAY OF HUMAN PLASMA CORTICOSTERONE: METHOD,
MEASUREMENT OF EPISODIC SECRETION AND
ADRENAL SUPPRESSION AND STIMULATION

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INTRODUCTION

Although corticosterone is essential for life in rats and certain other animals, it is not absolutely required by man. Whether it plays an important role in normal physiological processes or disease in man has not been established. We have developed a reliable radioimmunoassay for plasma corticosterone. This method requires only small samples and could be of value in clinical studies on corticosterone. The assay has been applied to studies in normal subjects on 8 a.m. levels, diurnal variations, ACTH stimulation and dexamethasone suppression (1). Corticosterone production has been shown to be both episodic and ACTH dependent.

MATERIALS AND METHODS

Corticosterone-1,2-³H with a specific activity of 44 Ci/m Mole was obtained from New England Nuclear and shown to be more than 97% pure by paper chromatography in our laboratory. It was diluted to a concentration of 50 μ Ci/ml in benzene-ethanol, 9:1 (v/v) and stored at 4 C. Unlabelled corticosterone and other steroids were obtained from Mann Research Laboratory or Sigma Chemical Company. These were used without

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further purification. Succinic anhydride (Eastman Organic Chemicals) was crystallized prior to use. 1-Ethyl-3-(3-dimethyl aminopropyl)-carbodiimide hydrochloride (Ott Chemical Co.), o-(carboxymethyl) hydroxylamine hemihydrochloride (Aldrich Chemical Co.), crystalline bovine serum albumin (BSA) and rabbit gamma globulin (Miles Labs), goat anti-rabbit serum (Antibodies Incorporated), and completed Freund's adjuvant (Difco) were also used in this study.

Preparation of antigen and immunization.

The succinate derivative of corticosterone was prepared by the method of Erlanger (2), and twice recrystallized in acetone. The melting point of the corticosterone succinate was 185°C. The derivative was conjugated to BSA as described previously (3). The number of steroid residues conjugated per mole of BSA varied from 7.1 to 17.0 in different batches.

The procedure for immunization of rabbits and the injection schedule has been reported previously (3), with the exception that 2 mg of steroid-protein conjugate was used per dose in this study. The Scatchard plot revealed that the antiserum was non-homogeneous for corticosterone antibody. The antiserum used was a pool of 4 bleedings from one animal and the dilution was 1:4000.

Plasma extraction and paper chromatography.

Corticosterone-1,2-³H (3.7×10^{-3} μ Ci) was added to 5 ml of plasma for control studies on steroid recovery through the extraction and paper chromatography procedure. The plasma was first extracted with 10 ml of benzene and then with 35 ml of dichloromethane. The extracts were com-

bined, dried and chromatographed on Whatman #1 paper in the Bush B₃ solvent system (4). The corticosterone zones of the developed chromatograms were eluted with 8 ml of methanol, dried and redissolved in 1 ml of methanol. The extraction recovery was determined by counting an aliquot of the eluate from control chromatograms. The assay for plasma samples was done on various aliquots of the methanolic eluate. The use of aliquots of varying sizes produces an internal check on the precision of each determination. Cortisol was measured for comparative purposes by radioimmunoassay of the appropriate zone of the chromatogram (5).

Assay procedure.

Standard curves were run with appropriate paper blanks. The maximum range of the standard curve was from 5 to 1000 pg. One hundred μ l of each standard or blank and three aliquots of each plasma extract (20, 50 and 100 μ l) were incubated overnight at 4°C with antiserum in 0.1 M phosphate buffer (pH 7.8) containing 15 pg of tracer steroid, 0.14 M NaCl, 0.01 M EDTA, 0.1% sodium azide and rabbit gamma globulin (0.25 mg/ml). The gamma globulin fraction of goat antirabbit serum was used to separate free from antibody bound steroids. Details of the incubation procedure have been reported previously (5). Supernatant unbound radioactivity was counted in a Packard liquid scintillation spectrometer. Unknown values were obtained from the standard curves and corrected for procedural losses using the extraction control data.

RESULTS

Standard curve.

The means and one standard deviation were determined for 8 standard

curves run on different occasions. A logit-log transformation of the data as described by Rodbard (6) gave a linear relationship between 25 and 1000 pg of corticosterone standard. The sensitivity of the curve was such that 15 pg could be distinguished from zero at the 95% level of confidence. This amount is equivalent to a plasma corticosterone concentration of 1.5 ng/dl.

Specificity.

The specificity of this radioimmunoassay depends on three factors: 1) the specificity of the antiserum; 2) the concentrations of interfering steroids in blood, and 3) partial removal of interfering steroids and other non-steroidal substances from plasma extracts by chromatography. The specificity of the antiserum was evaluated by measuring the cross reactivity of major steroids which are known to occur in the circulation (Table 1) and could interfere with the assay. The percentage cross-reaction of a potentially interfering steroid is the amount that will displace half the trace ^3H -corticosterone as described by Abraham (7). Plasma steroid extracts were fractionated using Bush B_3 system chromatography. The corticosterone fraction obtained after chromatography and elution also contained small quantities of 11-deoxycortisol and 17β -estradiol. These two steroids cross-reacted with the corticosterone antiserum 0.8 and 0.001%, respectively (Table 1). The steroids with the greatest affinity for the antiserum were progesterone (44.3%), testosterone (10%) and cortisol (2.8%). All of these compounds, which are potential sources of major interference, were eliminated by chromatography. No other major circulating corticosteroid exhibited a cross reaction greater than 1% (Table 1) and all of these compounds were also separated by chromatographic fractionation of plasma extracts.

Plasma blanks.

Plasma blanks were run on samples obtained from 5 adrenalectomized patients and one patient with Addison's disease. The corticosterone values ranged from 29-42 ng/dl. Plasma samples obtained from 10 adrenalectomized rats had corticosterone levels between 40 and 120 ng/dl (Table 2). The plasma blanks define the sensitivity limit of the assay in actual practice.

Recovery.

The recovery of tritiated corticosterone through extraction and paper chromatography of plasma samples varied from 58.5 to 77.9%, with a mean of $67.6 \pm 5.2\%$ ($n = 25$). For studies on the accuracy of recovery with this method, three pools of plasma obtained from dexamethasone suppressed subjects were fortified with unlabeled corticosterone in concentrations of 200, 500 and 1000 ng/dl. The unfortified plasma contained 45 ng/dl. The average recoveries for these pools were essentially quantitative (Table 2). The coefficients of variation for corticosterone recovery from fortified plasma varied between 5 and 12%.

Within assay precision.

Three plasma pools with low (0.05 ng/dl), medium (0.5 ng/dl) and high (1.1 ng/dl) levels of corticosterone were used for the determinations of intra-assay precision. Ten replicate determinations were done on each pool and the average and one standard deviation for each group is given in Table 2. The coefficients of variation ranged from 8 to 19%.

Between assay precision.

Inter-assay precision was determined by measuring corticosterone levels on different occasions in two plasma pools. One pool was ob-

tained from a normal man whose adrenal output was suppressed with dexamethasone. The other pool was from a normal male subject. The range of variation between assays was 5 to 13%.

Comparison with fluorometric determination.

Plasma for this study was obtained from rats. High level samples were from rats that had been subjected to ether stress for 10 min. Identical samples were assayed for corticosterone content by both radioimmunoassay and fluorometric analyses. The corticosterone values ($\mu\text{g/dl}$ plasma) determined on each identical pair of samples by the two methods were plotted against each other (Fig. 1). A linear relationship obtained ($r = 0.97$) demonstrating close agreement for the two methods.

Normal subjects.

Plasma corticosterone levels were measured at 8 a.m. in 15 normal men and 15 women ranging in age from 22 to 45 years. All of the subjects had normal salt intake and normal physical activity prior to sampling. The plasma corticosterone values in men and women were significantly different ($p < .01$). In male subjects, the corticosterone levels were 395.7 ± 227.5 ng/dl plasma, while in females they were 655.3 ± 271.0 ng/dl plasma (Table 2). When follicular phase and luteal phase female subjects from the same group were compared, follicular plasma corticosterone values appeared to be higher, but the difference was not statistically significant with the number of samples available (Table 2).

Parallel episodic secretion of corticosterone and cortisol.

Plasma levels of corticosterone and cortisol were measured simultaneously at 20 min intervals for one day in two normal subjects. One subject (E.H.) was a 26 year old male and the other (B.B.) was a 20 year

old female in the follicular phase of her menstrual cycle. The plasma levels of the two steroids were plotted against time for each subject (Fig. 2,3). The plasma levels of the two steroids exhibited episodic peaks throughout the 24 hour period. Both subjects had large peaks of secretion between 2 and 10 a.m. Lesser secretory peaks appeared in both subjects during the afternoon, and the levels rose again in the evening. The plasma corticosterone and cortisol peaks were well synchronized.

Cortisol and corticosterone secretion for a 24 hour period was estimated by the method of Hellman et al. (8). For this purpose, half lives of each of these two steroids were estimated from the disappearance of the steroid secreted in the major morning (7-9 a.m.) peak. Subject E.H. (male) secreted 15.2 mg of cortisol and 0.97 mg of corticosterone in the 24 hour period studied. Subject B.B. (female) produced 10.4 mg of cortisol and 2.02 mg of corticosterone over the same time period.

ACTH stimulation of plasma corticosterone and cortisol levels.

Seven normal subjects were used in this study. Adrenal activity was first suppressed by administering 1 mg of dexamethasone at midnight. A plasma sample was taken the following morning at 8 a.m. Each subject was then given an infusion of 25 units of ACTH. Plasma samples were taken at 5 and 6 hr after the infusion was begun. The data are shown in Fig. 4. For comparison, normal values for 8 a.m. plasma corticosterone and cortisol are included in Fig. 4. Since we obtained a statistically significant difference between 8 a.m. normal male and female corticosterone levels (Table 1) these values were graphed separately. Cor-

corticosterone levels responded to ACTH stimulation with the largest increment ($5.33 \pm 1.19 \mu\text{g/dl}$) over both dexamethasone suppressed plasma values and the 8 a.m. normal range. The absolute value for ACTH stimulated cortisol levels ($44.6 \pm 8.6 \mu\text{g/dl}$) is, of course, higher. However, the increment in corticosterone due to adrenal stimulation is 2-3 times greater than 8 a.m. normal values or dexamethasone suppressed values than the corresponding increase in plasma cortisol.

Dexamethasone suppression of plasma corticosterone and cortisol levels.

Eleven patients with no known adrenal disorder were given 1 mg of dexamethasone at midnight. Blood samples were drawn at 8 a.m. and plasma corticosterone and cortisol were measured. Plasma levels of both steroids were markedly suppressed. The corticosterone level was $0.046 \pm 0.016 \mu\text{g/dl}$. This compares well with the average of $0.057 \mu\text{g/dl}$ determined on the 7 subjects reported in Fig. 4. The plasma cortisol level was suppressed to $1.06 \pm 1 \mu\text{g/dl}$ of plasma.

DISCUSSION

The question of whether variations in plasma corticosterone levels are of physiologic significance requires a sensitive method for measuring this steroid. The method reported here is sensitive enough to allow corticosterone determinations to be done on as little as 2 ml of plasma even though a paper chromatographic step is used to eliminate interfering steroids. After cross-reacting steroids are removed, the assay is sensitive to levels of corticosterone that are well below those found in subjects with Addison's disease (Table 2).

The data obtained by radioimmunoassay on rat plasma correlate well

with fluorometric determinations on identical samples. Similar radioimmunoassay data for plasma corticosterone have been reported in mice (9).

The 8 a.m. normal values measured by this radioimmunoassay in humans are within the range (0.2-1.04 $\mu\text{g/dl}$ plasma) determined by double isotope assay (10). Other investigators have also reported plasma corticosterone values that range from 0.1 to 2.2 $\mu\text{g/dl}$ of plasma (11-13).

The unexpected result observed in 8 a.m. normal subject's plasma corticosterone was the sex difference (Table 1). This difference was statistically significant ($p < 0.01$). A similar result has been reported previously for adrenal vein corticosterone. Pincus and Romanoff measured corticosterone levels in the adrenal venous blood of a mammary carcinoma patient and one with prostatic carcinoma. In the female subject, the corticosterone level was 33 mg/l while in the male subject it was 2.4 mg/l (14).

Although the reason for a sex difference is not known, a possible explanation may lie in the differences in human adrenal biosynthetic pathways to corticosterone and cortisol. Progesterone appears to be the main precursor for corticosterone in human adrenal tissue. In vitro incubations of ^{14}C -progesterone with adrenal tissue from patients with Cushing's syndrome and mammary carcinoma produced more ^{14}C -corticosterone than ^{14}C -cortisol (15). Incubations of ^{14}C -progesterone and ^3H -pregnenolone with human hyperplastic adrenal tissue have demonstrated that progesterone is the major precursor of corticosterone while pregnenolone is the major precursor of cortisol (16,17). Since progesterone is the main precursor of corticosterone in humans, the greater availability of pre-

cursor in females may account for the sex difference. The sex difference in 8 a.m. plasma corticosterone levels may be influenced by episodic adrenal secretion; however, in the present study, the ACTH response and the 24 hour secretory rate were both higher in females also. Recently, a sex difference in plasma cortisone levels has been detected by radioimmunoassay (18).

The results obtained in the diurnal study indicate that corticosterone is secreted in an episodic fashion parallel to cortisol. Corticosterone secretion is also stimulated by exogenous ACTH and suppressed by dexamethasone administration. These data indicate that corticosterone secretion is ACTH dependent. Since the response of plasma corticosterone to exogenous ACTH is greater than the response of cortisol, corticosterone may be a useful index for ACTH tests.

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TABLE 1

CROSS-REACTIONS WITH CORTICOSTERONE ANTISERUM

Corticosterone	100%
Progesterone	44.3%
11-Deoxycorticosterone	26.1%
Testosterone	10%
11-Dehydrocorticosterone	5%
Cortisol	2.8%
21-Deoxycortisol	1.5%*
Aldosterone	1%
11-Deoxycortisol	0.8%*
Cortisone	0.2%
17 α -Hydroxyprogesterone	0.2%
Dehydroepiandrosterone	<0.001%
17 β -Estradiol	<0.001%*

*These steroids remain in the corticosterone chromatographic fraction.

TABLE 2

BASELINE DATA FOR PLASMA CORTICOSTERONE RADIOIMMUNOASSAY

	Mean $\pm$ S.D.	Range	n	Coefficient of variation
Plasma blanks:				
Adrenalectomized/Addisonian patients	37 $\pm$ 4.7 ng/dl	29-42	6	
Adrenalectomized rats	73 $\pm$ 25 ng/dl	40-120	10	
Recovery:				
Radioactivity	67.6 $\pm$ 5.2%	58.5-77.9	25	
Plasma fortified with non- radioactive corticosterone to:				
241 mg/dl	240 $\pm$ 28 ng/dl	216-277	5	5%
541 ng/dl	606 $\pm$ 37 ng/dl	556-631	5	6%
1041 ng/dl	1008 $\pm$ 122 ng/dl	930-1185	5	12%
Within assay precision:				
Low level	52 $\pm$ 10.2 ng/dl	40-71	10	19%
Medium level	501 $\pm$ 37 ng/dl	423-559	10	8%
High level	1143 $\pm$ 107 ng/dl	969-1286	10	9%
Between assay precision:				
Low level	44 $\pm$ 5.8 ng/dl	34-52	7	13%
Medium level	501 $\pm$ 23.7 ng/dl	485-543	5	5%
8 a.m. Normal Subjects:				
Males	395.6 $\pm$ 227.5 ng/dl		15	p < .01
Females	655.3 $\pm$ 271 ng/dl		15

Fig. 1. Fluorometric determinations of rat plasma corticosterone compared with this radioimmunoassay ($r = 0.97$).

Fig. 2. Diurnal plasma levels of plasma corticosterone and cortisol in a normal 26 year old male subject. Both steroids were measured by radioimmunoassay.

Fig. 3. Diurnal variations of plasma corticosterone and cortisol in a normal 20 year old female subject.

Fig. 4. The response of plasma corticosterone and cortisol to ACTH. Normal values at 8 a.m., dexamethasone suppressed (1 mg. @ 2400) and ACTH stimulated (25 units @ 0800) are given for individual normal subjects.

DOSE-RESPONSE RELATIONSHIPS FOR THE INDUCTION OF BONE
SARCOMAS BY β AND α -EMITTERS

and

THE EFFECT OF DOSE-RATE ON DELAYED SOMATIC EFFECTS
FROM LOW LET AND α -RADIATION

(A summary prepared for the NAS-NRC Low Dose
Study Group meeting of 25 July 1973)

Charles W. Mays and Ray D. Lloyd

Abstract: The dose-response relationship for the induction of bone sarcomas by long-lived β -emitters is strongly sigmoid. For α -emitters, the relationship appears linear for rodents, but may be sigmoid for man and dog. In 10 intercomparisons of life shortening and the induction of neoplasms from low LET radiation, the effect per rad at low dose-rates averaged 0.2 of that at high dose-rates. In contrast, for α -irradiation from ^{224}Ra , the incidence of bone sarcomas from a given dose increased as the dose-rate was lowered.

A. DOSE-RESPONSE RELATIONSHIPS FOR LONG-LIVED β -EMITTERS. ⁽¹⁾

In all known cases (mice, rats, beagles, and pigs) the dose-response relationships for the induction of bone sarcomas by skeletally-deposited ^{90}Sr or ^{45}Ca are highly sigmoid. The incidence at low doses is far below that predicted from linear non-threshold relationships fitted through all of the data up to the region of peak incidence. The strongly sigmoid nature of these dose response curves is ascribed primarily to good recovery from low LET radiation when the dose-rates are sufficiently low.

B. DOSE-RESPONSE RELATIONSHIPS FOR α -EMITTERS. ⁽²⁾

In only 1 of 5 rodent experiments with skeletally-deposited α -emitters could a linear dose-response relationship be rejected, and in this one ex-

periment a shortening of average lifespan from fatal infectious diseases may have prevented the appearance of bone sarcomas at the lowest dose-levels. At present, the weight of evidence supports a linear relationship for incidence vs. dose in the induction of bone sarcomas by low doses of α -irradiation in rodents.

In contrast, when α -particles have induced bone sarcomas in humans, present data are fitted better by sigmoid relationships than by linear relationships, both for short-lived ^{224}Ra ⁽²⁻⁴⁾ and for long-lived ^{226}Ra and ^{228}Ra . ^(2,5-7) Could it be that the dose-response for α -particles is different for the higher mammals than for rodents?

Because of this possible difference, results in beagles are of special interest. In all 4 of our long-term experiments with ^{239}Pu , ^{228}Th , ^{228}Ra and ^{226}Ra , our present α -emitter results ⁽⁸⁾ support sigmoid relationships for bone sarcoma induction in accord with present results in humans. But, it must be pointed out that both the human and beagle studies are still in progress. Additional follow-up is especially needed at dose-levels just below those at which cancers have already been induced. The following four on-going studies are of particular importance in evaluation of the risk from α -induced bone sarcomas:

(1) Low level studies of ^{239}Pu in beagles. ⁽⁸⁾ At the University of Utah, over 100 beagles have been injected with ^{239}Pu at levels giving average skeletal doses ranging from 3 to 53 rads at 10 years after injection. Additional low-level beagles are currently being injected with ^{239}Pu . (The lowest average skeletal dose at death is currently 60 rads among our ^{239}Pu -injected beagles that have died with bone sarcomas).

(2) Follow-up of patients injected with ^{224}Ra . Drs. Otto Hug and Fritz Schales have recently started tracing 1000 to 2000 adult German patients injected with ^{224}Ra for the treatment of ankylosing spondylitis. These patients are in addition to those in the Spiess series.^(3,4) The average skeletal dose in the Hug & Schales series of 56 rads is just below the lowest dose of 90 rads in a ^{224}Ra case developing a bone sarcoma. But it will be several more decades before the lifetime risk from low doses of ^{224}Ra can be established firmly, since the human life-span is so long. It is relevant to note that ^{224}Ra decays to a large extent on bone surfaces, somewhat similarly to ^{239}Pu .

(3) Follow-up of patients injected with Thorotrast. Several thousand patients that have been injected with Thorotrast (colloidal thorium dioxide) are being followed up in Germany, Portugal, Denmark and Japan. Rowland & Rundo⁽⁹⁾ have calculated that the average skeletal dose from ^{224}Ra translocating from other parts of the body into the skeleton should be about 25 rads in 25 years for a typical injection of about 25 ml of Thorotrast.

(4) Follow-up of the American radium cases.⁽⁵⁻⁷⁾ Over 1000 dial painters and other radium cases are being studied in the USA. This group is the most thoroughly studied of any humans irradiated with internal emitters. The long follow-up time, averaging about 50 years, makes these subjects particularly valuable for prediction of the total life-span risk to humans.

The question as to whether or not a fundamental difference exists between the shapes of the dose-response curves for higher mammals and rodents seems unlikely to be resolved if future studies do not continue to include large mammals.

C. DOSE-RATE EFFECT FOR RADIATIONS OF LOW LET. (10)

X-rays, γ -rays and β -particles interact with matter so as to transfer nearly all of their energy to electrons. These moving electrons produce a relatively sparse distribution of ionizations and excitations along their paths, and therefore, x-rays, γ -rays and β -particles are known as radiations of low LET (linear energy transfer). The cell culture work of Elkind and Sutton⁽¹¹⁾ showed quite conclusively that considerable repair of the damage from low LET radiation was possible if sufficient time was allowed between successive doses. The implication from the "Elkind effect" is that in general, the residual damage from a given dose of low LET radiation should decrease as the dose-rate is decreased, due to increased available time for biological repair between successive local radiation events.

For the past 2 years, we have been searching the radiobiological literature for intercomparisons that can be made on data from intact animals on the effect per rad at low dose-rates (usually below 0.1 rad/min) vs. that at high dose-rate (usually above 1 rad/min). The following 10 comparisons shown in Table 1 involve the important delayed somatic effects of lifespan shortening and the induction of neoplasms.

Tentatively, and subject to revision when indicated by future data, a best estimate of the overall effectiveness of low vs. high dose-rates from sparsely-ionizing radiation might be taken as 0.2 for the summed delayed somatic effects of life shortening and the induction of combined neoplasms, with reasonable bounds of 0.1 to 0.5 for this overall effectiveness factor. The individual effectiveness factors vary with end-

Table 1.
 $\frac{\text{Effectiveness at LOWER Dose-Rate}}{\text{Effectiveness at HIGHER Dose-Rate}}$

Life Shortening	{ Beagles (Andersen ⁽¹²⁾ ; Casarett ⁽¹³⁾)	0.08
	{ RF male mice (Upton et al) ⁽¹⁴⁾	0.07
	{ RF female mice (Upton et al) ⁽¹⁴⁾	0.45
Leukemia	{ RF male mice (Upton et al) ⁽¹⁴⁾	0.14
	{ RF female mice (Upton et al) ⁽¹⁴⁾	0.26
	{ CBA & C57Bl mice (R.H. Mole) ⁽¹⁵⁾	0.15
	{ LAF ₁ female mice (Grahn et al) ⁽¹⁶⁾	0.2
Bone sarcomas in CFl female mice (Finkel) ^(17,18)		0.05
Mammary tumors in S.D. female rats (Shellabarger) ⁽¹⁹⁾		0.68
Thyroid tumors in Lister & Long-Evans rats (Doniach) ⁽²⁰⁾		0.1
Average $\pm$ S.D.		0.2 $\pm$ 0.2

point and species (for example, 0.05 for bone sarcomas in CFl mice vs. 0.68 for mammary tumors in Sprague-Dawley rats). In addition, the effectiveness factor may be influenced by the total dose, dose-rate, and other parameters.

D. PROTRACTION EFFECT FOR α -IRRADIATION FROM ^{224}Ra .

As correctly predicted by Dr. Walter A. Müller (Institute für Strahlungsforschung, Neuherberg, Germany), the incidence of bone sarcomas from a given total dose of ^{224}Ra increases as the irradiation period is increased and the dose-rate is consequently decreased. This effect has been seen both in humans⁽⁴⁾ and mice.⁽²¹⁾ Plausible reasons for the higher incidence when the α -irradiation is protracted, rather than abrupt

are: (a) increased numbers of irradiated cells, (b) less subsequent killing of "pre-malignant" cells, and (c) prolonged stimulus for cell division.

CONCLUSION

Careful study of irradiated animals and humans has not only provided useful guidance for radiation protection, but has contributed toward a better understanding of the processes by which malignancies can be induced.

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CANCER INDUCTION IN MAN FROM INTERNAL RADIOACTIVITY*

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ABSTRACT: The indicated malignancies have been induced in exposed persons by the following radionuclides and their decay products:

<u>RADIONUCLIDE</u>	<u>EXPOSED PERSONS</u>	<u>INDUCED MALIGNANCIES</u>
^{222}Rn	Uranium miners	Lung carcinomas
^{226}Ra	Dial painters	Bone sar. & sinus carc.
^{232}Th	Thorotrast cases	Liver cancers
^{224}Ra	German patients	Bone sarcomas
^{32}P	Polycythemia patients	Leukemia
^{131}I	Marshall islanders	Thyroid tumors

Each of these examples will be discussed in turn.

URANIUM MINERS

The inhalation of the noble gas ^{222}Rn and its radioactive decay products has been inducing lung cancers in underground miners for some 400 years (Agricola, 1597). It is still causing more cancers per year that are definitely attributable to irradiation than any other source of internally-deposited radioactivity in man. Since 1964, about 10 deaths each year have occurred from lung cancer in the American uranium miners alone (Lundin et al., 1971, Appendix D).

* Invited paper presented at the Symposium "Internal Emitters in Man" Argonne National Laboratory, 25 January 1973, and in press, Health Physics.

In the primitive Erzgebirge (Ore Mountains) of Central Europe, extending from Schneeberg (Snow Mountain) Germany to Joachimsthal (Joachim's Valley) Czechoslovakia, the underground miners for hundreds of years have been dying of a fatal lung disease locally called "Bergkrankheit" (mountain illness). Only within the last century was Bergkrankheit correctly identified as lung cancer (Härtling and Hesse, 1879). During the short period from 1875 through 1912 there were 276 deaths due to lung cancer in the Schneeberg miners alone (Arnstein, 1913). In an excellent review of lung cancer and uranium mining, Seltzer (1965) quotes data which indicate that for these miners the fraction of deaths due to lung cancer was about 25% from 1875 through 1912, but about 50% after the First World War. Certainly some of the apparent increase was simply due to better medical diagnosis, but one cannot help but remember that it was about the time of the First World War that cigarette smoking became widespread. However, no information is available on the smoking habits of European miners.

In American uranium miners who smoke cigarettes, the incidence of lung cancer is 10 times higher than in non-smoking uranium miners (Lundin et al., 1971, pg. 85). The message is simple. To paraphrase the well-known slogan on alcohol and gasoline, "Don't mix cigarette smoke with radioactivity."

Except for the persistent efforts of Duncan Holaday and his colleagues to promote adequate mine ventilation (Holaday et al., 1957; Holaday, 1969), the tragedy of Schneeberg and Joachimsthal would have been repeated on an even larger scale in the U.S.A. with over 1000 uranium miners dying from radiation-induced lung cancer. From 1950 to April 1973, there have been 235 known cases of respiratory cancer among the American Uranium miners (Archer et al., 1973).

RADIUM DIAL PAINTERS

Ingestion of radium by the American dial painters began about the time of the First World War, and by 1924 the first death from a radium-induced bone sarcoma was recorded (Martland, 1931). The early investigations of Martland are notable not only for his careful analysis leading to the inescapable conclusion that these bone cancers were radiation-induced, but in the objective way he handled this emotion-laden controversy --- and won!

Until recently there were two major studies of the American radium subjects; one at MIT (Evans et al., 1972) and the other at Argonne (Finkel et al., 1969). These have been the most extensive studies on the effects of internally-deposited radioactivity in man that have ever been attempted. Realizing the enormous value to humanity of a continued follow-up beyond his own retirement time, Evans urged that a Center for Human Radiobiology be organized (Evans et al., 1969). An orderly transition has been made, and the American radium cases are now being followed actively at the Argonne Center for Human Radiobiology (Rowland et al., 1971). Robley Evans will always be esteemed, not only as the man who first organized a systematic study of the dial painters some 40 years ago, but also as the visionary who made it possible for this unfinished work to be carried on to a successful completion.

As of 1971, 54 cases of bone sarcoma and 20 cases of head sinus carcinoma had been identified in the American radium subjects of known dosage (Keane, 1971; Rowland et al., 1971). In addition, a few brain tumors (Finkel et al., 1969) and a few leukemias (Finkel et al., 1969; Loutit, 1970) may have been radiation-induced among these subjects.

THOROTRAST

Thorotrast (colloidal thorium dioxide) was first injected into humans as an x-ray contrast medium in 1928. Because it can cause cancer, this radioactive material is no longer approved for use in humans. When injected into the blood stream, Thorotrast deposits in the liver, spleen, and marrow where it is retained almost indefinitely. In humans, Thorotrast has produced 3 distinct types of liver cancer: hemangioendotheliomas, hepatic carcinomas, and bile duct carcinomas. It also has been associated with an increased incidence of leukemia and lung cancer. The question is unresolved as to how much of the carcinogenic effect of Thorotrast is due to its radioactivity and how much is due to its chemical properties. This question needs to be resolved before the Thorotrast results can be applied to estimation of the risk to the livers of plutonium workers. The effects of Thorotrast are presented in detail in the proceedings of a symposium by the New York Academy of Sciences (Swarm and contributing authors, 1967).

GERMAN ^{224}Ra PATIENTS

In 1964 I had a German-born technician named Ursula Leschke. While doing library research for some other purpose long since forgotten, she ran across an obscure abstract about radiation-induced bone tumors in children who had been injected with ^{224}Ra (Spiess et al., 1962). Thus, it is possible that I was the first American to recognize the implication of these studies. I obtained a reprint from Professor Spiess and showed it to Dr. R. E. Rowland. Rowland became very excited and had it translated into English by Dr. Elmar Willip at the Argonne National Laboratory. A few years later in 1967, I was able to persuade Heinz Spiess to come to Sun Valley, Idaho and share his findings with scientists from all over the world at an international symposium

on Delayed Effects of Bone-Seeking Radionuclides. His fascinating story was a bombshell --- completely new to nearly everyone at the meeting, with the possible exception of Robin Mole (who seems to learn of everything before anyone else), Rowland, and myself. During parts of 1969, 1970, and 1972 I had the good fortune to work directly with Spiess in Germany. The 1970 trip was especially eventful, but not just for scientific reasons. For it was in England on my way to Germany that I met the beautiful Irish girl with the French name (Desirée) who is now my wife! So let me now summarize what we now know about the effects of ^{224}Ra in humans.

Shortly after the Second World War a series of about 2000 German patients, both children and adults, were given repeated injections of short-lived ^{224}Ra ($T_{1/2} = 3.62$ days) mainly for the intended treatment of tuberculosis and ankylosing spondylitis (Spiess et al., 1962; Spiess, 1967; Spiess and Mays, 1970). By 1950 Spiess (the "Harrison Martland" of Germany) had proved decisively that ^{224}Ra was completely ineffective in the treatment of tuberculosis, and consequently, it is no longer being used for this purpose. However, at the present time about 100 new spondylitic patients are being injected with ^{224}Ra each year in Germany, because of the belief by some physicians that ^{224}Ra treatments may partially alleviate this disease.

In the Spiess series, 53 bone sarcomas have occurred, and it is likely that all, or nearly all, were radiation-induced due to the low natural incidence of bone sarcoma. Males and females seem equally sensitive to the induction of bone sarcoma, and the sensitivity appears unaffected by the presence or the absence of bone disease at the time of treatment. To my knowledge the Spiess series provides the only possibility of directly comparing the effects of bone-seeking radionuclides in children vs. adults. For equal skeletal

doses and equal protraction of the injection series, children seem only slightly more sensitive than adults to bone sarcoma induction (Spiess and Mays, 1973).

An interesting finding with α -emitting ^{224}Ra was a greater incidence of bone sarcoma from a given total dose when the span of the injections was increased in humans (Spiess and Mays, 1973). The increased incidence with increasing protraction of α -particle irradiation had been predicted previously by some very sharp reasoning on the part of Dr. Walter Müller, and this effect has now been confirmed experimentally in mice at the important radiobiology studies led by Prof. Otto Hug at Neuherberg (Luz et al, 1972). The observed increase in sarcoma incidence for protracted α -particle irradiation from ^{224}Ra may be due to (1) increased numbers of irradiated cells, (2) less killing of "pre-malignant" cells, (3) prolonged stimulus for cell division, and (4) little repair of local damage from α -particles. In contrast, large recovery usually occurs from low LET irradiation at low dose-rates (Brown et al, 1968; Russell, 1972; Mays and Lloyd, 1972) and consequently the induction of tumors from a given dose of x-rays, γ -rays, or β -particles usually, but not always, drops drastically as the dose-rate is lowered and the irradiation period is thereby lengthened (Upton et al, 1970; Grahn et al, 1972; Mole, 1959; Finkel and Biskis, 1959 and 1968; Doniach, 1963; Shellabarger, 1972).

No bone sarcomas have yet been reported in any ^{224}Ra patient below an average skeletal dose of 90 rads. But this does not prove the existence of a threshold because the Spiess series contains only 170 adults with average skeletal doses below 90 rads for whom the injection span is also known. A simple linear model fit through all of the Spiess adult data (0.9% incidence/100 rads) predicts only 0.8 cases in the 170 adults whose skeletal doses ranged from 7 to 89 rads and averaged 54 rads. If 0.8 cases were the correct expectation, there would be a 45% chance of observing no cases (binomial distribution). Thus, there are too few low-dose patients in the Spiess series to reject either the linear or threshold possibilities.

Fate has provided perhaps the only opportunity that will ever exist to evaluate the bone sarcoma risk from an α -emitter to an adequate number of humans in the critical region just below the lowest dose where the observed incidence has been appreciable. A new study directed by Professor Otto Hug and being run by Dr. Fritz Schales should be able to trace 1000 to 2000 ankylosing spondylitis patients that are not included in the Spiess series. These adult patients typically received a total of 280 μCi ^{224}Ra in 10 fractions with a week between each injection. The calculated average skeletal dose is 56 rads. The following 20-year risk estimates are based on various dose-response models fit to the Spiess data:

TABLE 1. Predicted 20-year risk from current treatment
(56 rads av. skel. dose to spondylitic adults)

Dose-Response Model	$\left[\frac{\text{Predicted bone sarcomas}}{1000 \text{ patients}} \right]$
Linear	5
[Linear]x[Injection span factor]	3
[Dose Squared]x[Exponential]	2
Threshold above 56 rads	0

For 1000 patients at 20 years after injection, the linear model predicts 5 cases, whereas the threshold model predicts none. It should be possible to reject at least one of these extremes. So far, no bone sarcomas have been found in patients of the new series, but only a few hundred of these new patients have now been examined, and many were injected with ^{224}Ra only during the past few years.

In addition to providing fundamental knowledge on the dose-response relationship for cancer induction by α -emitters in humans, the German studies hav

application, not only in evaluating the risk from ^{224}Ra treatment of ankylosing spondylitis, but also in estimation of the hazard to workers in the plutonium industry. ^{224}Ra resembles ^{239}Pu in that within the skeleton, large fractions of the disintegrations from both radionuclides occur on bone surfaces.

POLYCYTHEMIA VERA PATIENTS

Poly (many) + cyt (cell) + hemia (blood) + vera (true) is a blood disease of unknown cause that is characterized by an over-production of red cells. If untreated, most patients die within a few years from blood clots, hemorrhage, and related effects. In the late 1930's it was demonstrated that the over-production of red cells could be controlled for a considerable time by repeated injections of radioactive ^{32}P which concentrates in the skeleton (Lawrence et al., 1953). This extended the median lifespan of polycythemic patients to about 12 years after diagnosis. It was also observed that about 10 to 20% of the polycythemic patients treated with ^{32}P developed leukemia. This raised the question of whether these leukemias were induced by ^{32}P , or whether the leukemias were a natural polycythemic transition completed during the added survival time made available by therapy. A detailed analysis (Modan and Lilienfeld, 1965) indicated that the vast majority of leukemias in the ^{32}P -treated patients were radiation-induced and could not be accounted for by increased survival time alone. This conclusion of Modan and Lilienfeld becomes even clearer if their data are adjusted for the decreasing number of patients at risk each year after diagnosis. This I have done using the appropriate survival statistics of Kaplan and Meier (1958). The corrected cumulative incidences are presented in Fig. 1.

From Table 22 of Modan and Lilienfeld, the average total injected activity per patient appears to be about 20 millicuries of ^{32}P . Assuming a 20% uptake in the 10 kg total skeleton of standard man, an effective retention half-time of 14.1 days, and an average energy of 0.69 MeV per β -particle from ^{32}P (ICRP, 1960), the calculated average skeletal dose to these patients is about 300 rads.

In the 10-year interval between 4 and 14 years after diagnosis, the corrected cumulative incidence rose to 20% in the ^{32}P -treated patients, and to 2% in the non-irradiated patients. Thus, a corrected cumulative incidence of $20\% - 2\% = 18\%$ seems to have been radiation-induced. Assuming a linear dose-response relationship, the indicated efficiency of the radiation for inducing leukemia in polycythemic patients seems to be about:

$$\frac{18 \text{ leukemias/10 years}}{(100 \text{ persons})(300 \text{ rads})} = \frac{60 \text{ leukemias/yr}}{10^6 \text{ person rads}}$$

This is 60 times higher than the efficiency of leukemia induction of 1 leukemia per year/ 10^6 person rads observed in the Nagasaki A-bomb survivors 5 to 21 years after exposure to a virtually pure γ -ray dose in which the neutron component was negligible (Ishimaru et al., 1971).

It is clear that polycythemia vera, a disease characterized by overproduction of blood cells, renders these patients unusually susceptible to radiation-induced leukemia. Although ^{32}P may give the best survival of any therapy now available, hopefully other methods of treatment may be found that give even longer survival than ^{32}P therapy and can avoid its high leukemic risk.

MARSHALL ISLANDERS

On 1 March 1954, the 15-megaton thermonuclear device "Bravo" was detonated at Bikini Atoll, vaporizing the small island on which the explosion occurred. Fission accounted for about 2/3 of the total yield. Powdered coral from the former island, mixed with highly radioactive fission products, fell back into the Pacific ocean in a massive plume extending hundreds of miles down wind. But not all of the radioactivity fell into the ocean. Part fell on Rongelap and other inhabited islands about 100 miles distant from the detonation.

Based on γ -ray measurements on Rongelap and iodine measurements in pooled urine samples, Ralph James and John Gofman estimated the total thyroid dose to a 3 to 4 year old child from internal plus external radiation to average between 700 and 1400 rads (James, 1964). Because no correction was made for incomplete collection of urine, the true dose may be somewhat higher than the mid-range. Perhaps 1200 rads might tentatively be regarded as a reasonable average for Rongelap children exposed when under 10 years of age. The Rongelap adults received lower thyroid doses, estimated at about 350 rads.

As of 1969, or 15 years after irradiation, thyroid tumors had developed in 15 of the 19 Rongelap children exposed at less than 10 years of age, and in 3 of the 34 Rongelap islanders who were more than 10 years old at exposure (Conard et al., 1970). Most of these tumors were benign adenomatous thyroid nodules, but 3 were malignant thyroid carcinomas. More carcinomas might have developed if the adenomas had not been removed surgically by partial or total thyroidectomy soon after detection, and if the Rongelap islanders had not been given supplemental thyroid hormone starting in 1965. Due to the low natural incidence of thyroid disease in unirradiated islanders, there seems

little doubt that most of these thyroid tumors were radiation-induced, and that additional tumors may occur.

Utah may have narrowly missed becoming "Rongelap revisited." On 19 May 1953, the 32-kiloton nuclear test "Harry" exploded in the Nevada desert. The fallout streamed some 100 miles down wind directly into the farming community of St. George, Utah. Although the fission yield was only about 1/300 of that for the Bravo explosion which rained fallout upon Rongelap islanders, an ecological factor present in Utah but absent in Rongelap almost made up for the difference in yield. St. George fallout fell upon pasture lands. Cows eating forage contaminated with fresh fallout absorb radioactive iodine through their digestive system, and transfer much of the iodine to their milk. Infants drinking this milk absorb the iodine and concentrate it in their thyroid glands. Knapp (1964) has estimated that the thyroid dose to a 1-year old child drinking 1 liter of milk per day from cows grazing on pasture contaminated with fresh fallout is 66 to 240 times higher from fallout ^{131}I than from external fallout gamma radiation, and Hollister (1963) working independently has substantiated Knapp's values. There were no cows on Rongelap, and the potential exposure was reduced further by evacuating the islanders 2 days after the explosion.

Tamplin and Fisher (1966) have estimated the average thyroid dose to St. George children under 5 years of age. Their values ranged from 30 to 240 rads with a "best estimate" of 120 rads. Thus, it is possible that the dose to the St. George children was 1/10 of that causing a 79% incidence of thyroid tumors in the Rongelap children. If the children near St. George got 1/10 of the thyroid dose received by the Rongelap children, and if the dose-response relationship was linear, then radiation-induced thyroid tumors

would be predicted in 7.9% of the St. George children, or in 110 of the 1378 children near St. George who were followed up 15 years later (Weiss et al., 1971).

A total of 18 cases of benign thyroid tumors were observed among 1378 children near St. George, of which about 13 ± 5 cases were expected naturally, based on the observation of 20 thyroid tumors among 2140 non-irradiated children near Safford, Arizona. Thus, perhaps 0 to 10 cases at St. George were radiation-induced, which is much less than the linearly-predicted 110 cases. This discrepancy between observation and linear prediction suggests that (a) the dose-response relationship for the induction of thyroid tumors may be non-linear, (b) the actual doses may differ from the best estimates, or (c) other factors may be important.

If the actual thyroid doses to the Rongelap children averaged 1400 rads (the upper estimate by James and Gofman), while the actual thyroid doses to the St. George children averaged 30 rads (the lower estimate by Tamplin and Fisher), then on the linear model 23 cases of radiation-induced thyroid tumors would be predicted for the St. George children. Conversely, if the actual doses were 700 rads at Rongelap and 240 rads at St. George, then 373 cases would be predicted for St. George, based on the linear assumption. These upper and lower linear predictions are both well above the actual 0 to 10 cases attributable to irradiation.

While a difference in susceptibility to thyroid tumor induction is entirely possible, the higher intake of stable iodine by the Rongelap islanders might have reduced their susceptibility to thyroid disease, relative to that of an inland population with less dietary iodine. On the other hand, a large part of the dose at Rongelap was from short-lived isotopes of iodine inhaled

from air and imbibed in drinking water, whereas, these short-lived isotopes were less important at St. George due to the time delay between fallout arrival and milk consumption.

The observed incidences at St. George vs. Rongelap are fitted well by a variety of dose-response relationships including dose squared and dose cubed models, without the necessity of assuming dosimetric errors or differences in susceptibility. Yet, because these complications may exist, additional evidence would be very desirable. Did St. George slay the "invincible" dragon of linearity? Perhaps not. But St. George may have bent the dragon's straight tail a little.

CONCLUSION

The induction of malignancy in humans by 6 internally-deposited radioactivities has been surveyed. Virtually all of the induced malignancies arose within the irradiated tissues. Very likely other radionuclides will add to the list. Which will be unlucky number seven? Will it be plutonium?

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CORRECTED CUMULATIVE INCIDENCE OF LEUKEMIA IN PATIENTS WITH POLYCYTHEMIA VERA
(Data from B. Modan and A.M. Lilienfeld, *Medicine* 44, pp 305-344, 1965)

Fig. 1. Corrected cumulative incidence of leukemia in patients with polycythemia vera. Correction is made for the decreasing number of patients at risk at each year after diagnosis using the method of Kaplan and Meier (1958). The final corrected cumulative incidence is 20% in the ³²P-treated patients, but only 2% in the non-irradiated patients suggesting that about 90% of the leukemias in the ³²P-treated group were radiation-induced, with the other 10% due to other factors.

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