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RESEARCH IN RADIOBIOLOGY
Annual Report of Work in Progress
on the Chronic Toxicity Program

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

Respectfully Submitted by:

Thomas F. Dougherty, Director

September 30, 1964

Contract No. AT (11-1)-119

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AVAILABILITY OF PREVIOUS REPORTS

Copies of our reports may be obtained from the Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C.

<u>Report</u>	<u>Date</u>	<u>Title</u>	<u>Photocopy</u>	<u>Microfilm</u>
C00-119-231	Sep 1964	Research in Radiobiology	\$17.20	\$8.60
C00-119-230	Jul 1964	Safety Manual	3.10	1.55
C00-119-229	Mar 1964	Research in Radiobiology	13.00	5.84
C00-228	Sep 1963	Research in Radiobiology	13.00	5.84
C00-227	Mar 1963	Research in Radiobiology	15.50	7.22
C00-226	Sep 1962	Research in Radiobiology	10.50	4.31
C00-225	Mar 1962	Research in Radiobiology	10.50	4.28
C00-224	Sep 1961	Research in Radiobiology	9.10	3.32
C00-223	Mar 1961	Research in Radiobiology	6.30	3.00
C00-222	Sep 1960	Research in Radiobiology	22.80	7.20
C00-221	Aug 1960	Interim Report of Sr ⁹⁰	3.30	2.40
C00-220	Mar 1960	Research in Radiobiology	34.80	9.90
C00-219	Sep 1959	Semi-Annual Report	10.80	3.90
C00-218	Mar 1959	Annual Report	34.80	9.90
AECU-4112	Feb 1959	Radioactive Fallout	3.30	2.40
C00-217	Sep 1958	Semi-Annual Report	28.80	8.40
C00-216	Mar 1958	Escape of Radon & Thoron	9.30	6.30
C00-215	Mar 1958	Annual Report	28.80	8.40
AECU-3583	Sep 1957	Semi-Annual Report	19.80	6.30
AECU-3522	Mar 1957	Annual Report	27.30	8.10
TID-16459	Sep 1956	Semi-Annual Report	5.60	2.05
TID-16458	Mar 1956	Annual Report	10.50	4.40
AECU-3109	Sep 1955	Semi-Annual Report	12.30	4.50
AECU-3418	Mar 1955	Annual Report	9.30	3.60
TID-7639	Consultants Meeting		19.75	9.40

Many of the above reports are also available from this laboratory.

GROUP LEADERS AND SECTIONS

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

Section	Group Leader
Director	Prof. Thomas F. Dougherty
Biochemistry	Dr. David L. Berliner
Bone	Dr. Webster S. S. Jee
Chemistry	Dr. Betsy J. Stover
Clinic & Pathology	Dr. Glenn N. Taylor
Hematology	Dr. Jean H. Dougherty
Physics	Dr. Charles W. Mays
Radiology	Dr. William R. Christensen

CURRENT CENSUS OF THE BEAGLE COLONY

September 30, 1964

Assignment	Number of Dogs
Plutonium, toxicity	36
Plutonium, test	9
Radium, toxicity (Ra ²²⁶)	36
Radium, test (Ra ²²⁶)	7
Quickradium, test (Ra ²²⁴)	10
Radiothorium, toxicity	54
Radiothorium, test	0
Mesothorium, toxicity	50
Mesothorium, test	0
Strontium, toxicity	70
Strontium, test	1
Controls, aging	44
Controls, x-ray	5
Unassigned (Male 31, Female 39)	70
Total	392

INJECTION TABLES

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

Dogs are put into the toxicity study groups of six or seven of the same sex (five or six dose levels plus one control). Litter mates are used whenever possible. Each animal receives the designated dose of one radionuclide in a single I.V. injection. The animals are injected 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 five radionuclides, Ra^{226} , Pu^{239} , Ra^{228} , Th^{228} , and Sr^{90} . The current injection program is limited to Ra^{226} and Pu^{239} at lower dose levels.

The five dose levels designated by integers are those specified at the early meetings of the consultants, and those designated by non-integers have been added by the laboratory staff. Since those dose levels were originally specified as "retained" doses, the actual injected doses are four times the desired "retained" doses of Ra^{226} , Ra^{228} (Mesothorium), and Sr^{90} , and 1.11 times the desired "retained" doses of Pu^{239} and Th^{228} (Radiothorium).*

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

average Ra^{226}	retention = 0.25 after 271 days
average Pu^{239}	retention = 0.90 after 6 days
average Ra^{228}	retention = 0.25 after 214 days
average Th^{228}	retention = 0.90 after 6 days
average Sr^{90}	retention = 0.25 after 135 days

the radionuclides except Sr^{90} , in which case they are greater by a factor of 10. Dose level 1 is the basis of the scheme, and is 10 times the maximum permissible Ra^{226} in man. $\text{Level 1} = 10 \times \frac{0.1 \mu\text{C Ra}^{226}}{70 \text{ kg man}} = 0.0143$ "retained" $\mu\text{C/kg}$. All other dose 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 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 = Ra^{226} , P = Pu^{239} , M = Ra^{228} , T = Th^{228} , S = Sr^{90} , Q = Ra^{224} , J = Sr^{85} , and A = Aging Control.

"A" following the regular dog number means that the dog is a replacement. "H" following the regular dog number means that the dog received its dose in more than one injection. "B", "C", or "D" denotes assignment to serial sacrifice schedule. Any of the above letters denoting a radionuclide may follow the final number, in which case the letter indicates that two radionuclides were given. The dose level refers to the radionuclide appearing first in the identifying code.

Example: M1R5 is a male animal in the first radium group at the highest dose level.

Although M1R5, M1R4, M1R3, M1R2, M1R1, and M1R0 constitute a group and were injected at the same time, the tables are arranged according to dose level to facilitate comparison of all the R5 animals, all the R4 animals, etc.

The average radiation doses to the skeleton in rads for Pu^{239} , Ra^{228} and Th^{228} were calculated from equations and graphs in our Progress Report C00-218, March 1959, pp. 121-145. Ra^{226} and Sr^{90} doses in this Report are completely revised: They are based on the individual retentions observed in each dog.

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 eminent or when life could no longer be humanely prolonged.

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TABLE I. TOXICITY ANIMALS (SEPT. 30, 1964)

A. RADIUM-226*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
M01R0.0	558	8.03		20 04 53	3116	
M02R0.0	487	14.6		16 11 53	3675	
F03R0.0	601	11.4		10 03 54	2139	
M04R0.0	461	11.0		7 04 54	3829	
M05R0.0	460	6.57		22 06 54	3753	
F06R0.0	483	8.43		27 7 54	3182	
M07R0.0	511	11.0		24 8 54	3360	
F08R0.0	638	8.21		21 12 54	3361	
F09R0.0	700	11.7		11 4 55	1550	
M10R0.0	522	10.9		27 07 55	3353	
F11R0.0	544	10.2		20 12 55	3207	
F12R0.0	501	8.68		17 01 56	3179	
M13R0.0	515	12.3		4 3 64	210	
M13R0.2	529	9.77	.00577	4 3 64	210	
M13R0.5	529	11.0	.0171	4 3 64	210	
M01R1.0	471	8.48	.0618	20 04 53	4181	
M02R1.0	627	10.0	.0876	16 11 53	3971	
F03R1.0	706	8.68	.0576	10 03 54	3857	
M04R1.0	414	8.60	.0642	7 04 54	2038	108
M05R1.0	490	11.7	.0436	22 06 54	3753	
F06R1.0	483	7.23	.0584	27 07 54	3718	
M07R1.0	511	11.4	.0651	24 8 54	3544	167
F08R1.0	861	8.98	.0559	21 12 54	2988	93
F09R1.0	781	9.88	.0521	11 04 55	3460	
M10R1.0	523	11.5	.0573	27 07 55	3353	
F11R1.0	511	11.2	.0522	20 12 55	3207	
F12R1.0	501	9.71	.0444	17 01 56	3179	
M13R1.0	529	11.7	.0527	4 3 64	210	
M01R1.7	523	9.98	.137	17 01 56	3179	
M02R1.7	598	7.85	.163	30 11 56	1273	117
M02R1.7A	493	12.0	.222	6 3 63	574	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M01R0.0	SEMINOMA, LYMPHOSARCOMA
M02R0.0	TRANSITIONAL CELL CARCINOMA
F03R0.0	STATUS EPILEPTICUS
M04R0.0	
M05R0.0	
F06R0.0	STATUS EPILEPTICUS
M07R0.0	STATUS EPILEPTICUS, NEPHRITIS
F08R0.0	PANCREATIC ADENOCARCINOMA
F09R0.0	AORTIC BODY TUMOR
M10R0.0	
F11R0.0	
F12R0.0	
M13R0.0	

M13R0.2

M13R0.5

M01R1.0	
M02R1.0	
F03R1.0	
M04R1.0	TRAUMA
M05R1.0	
F06R1.0	
M07R1.0	STATUS EPILEPTICUS
F08R1.0	LYMPHOSARCOMA
F09R1.0	
M10R1.0	
F11R1.0	
F12R1.0	
M13R1.0	

M01R1.7
M02R1.7
M02R1.7A

LYMPHOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED		DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64 DEATH	
F03R1.7	473	13.1	.165	20	12	55	3207	
M04R1.7	514	6.20	.163	20	12	55	3207	
M05R1.7	511	10.1	.151	20	12	55	3207	
F06R1.7	491	7.9	.152	20	12	55	3207	
M07R1.7	598	7.17	.163	30	11	56	2861	
F08R1.7	491	9.50	.154	20	12	55	2577	378
F09R1.7	598	7.55	.168	30	11	56	2861	
M10R1.7	590	9.57	.167	30	11	56	557	109
M10R1.7A	545	10.6	.183	7	01	59	2093	
F11R1.7	598	8.17	.165	30	11	56	2861	
F12R1.7	590	8.95	.167	30	11	56	2399	210
M01R2.0	471	8.74	.382	20	04	53	3440	860
M02R2.0	592	8.21	.387	16	11	53	3775	626
F03R2.0	541	8.53	.347	10	03	54	3857	
M04R2.0	414	10.5	.361	7	04	54	325	191
M04R2.0A	420	10.6	.306	11	04	55	3460	
M05R2.0	461	11.5	.267	22	06	54	3753	
F06R2.0	486	10.6	.360	27	07	54	3718	
M07R2.0	514	11.1	.413	24	8	54	3425	923
F08R2.0	572	6.95	.331	21	12	54	3571	
F09R2.0	592	9.38	.317	11	04	55	3460	
M10R2.0	523	9.95	.345	27	07	55	3353	
F11R2.0	495	9.30	.310	20	12	55	3207	
F12R2.0	497	10.3	.281	17	1	56	2948	743
M01R3.0	473	8.91	1.20	20	04	53	2850	2398
M02R3.0	470	9.02	1.21	16	11	53	2226	1730
F03R3.0	386	7.74	1.11	10	03	54	2497	2326
M04R3.0	412	11.7	1.16	7	04	54	1917	2365
M05R3.0	461	13.0	.846	22	06	54	2955	2320
F06R3.0	486	9.75	1.14	27	07	54	1932	2250
M07R3.0	514	12.3	1.29	24	08	54	2099	3033
F08R3.0	542	7.76	1.03	21	12	54	2612	1919
F09R3.0	551	8.02	.987	11	04	55	2487	1841
M10R3.0	525	10.1	1.06	27	07	55	1737	2340
F11R3.0	495	12.9	.938	20	12	55	1610	1335
F12R3.0	497	11.4	.883	17	1	56	1897	1641
M01R4.0	471	9.08	3.51	20	04	53	1606	6589

DOG NUMBER	COMMENTS ON DEAD DOGS
F03R1.7	
M04R1.7	
M05R1.7	
F06R1.7	
M07R1.7	
F08R1.7	DRUG ALLERGY
F09R1.7	
M10R1.7	TRAUMA
M10R1.7A	
F11R1.7	
F12R1.7	NOT DETERMINED
M01R2.0	HEMANGIOSARCOMA
M02R2.0	OSTEOSARCOMA
F03R2.0	
M04R2.0	PERFORATED ILEUM
M04R2.0A	
M05R2.0	
F06R2.0	
M07R2.0	OSTEOSARCOMA, CUSHING SYNDROME
F08R2.0	
F09R2.0	
M10R2.0	
F11R2.0	
F12R2.0	MAMMARY ADENOCARCINOMA
M01R3.0	OSTEOSARCOMA
M02R3.0	OSTEOSARCOMA
F03R3.0	OSTEOSARCOMA
M04R3.0	OSTEOSARCOMA
M05R3.0	OSTEOSARCOMA
F06R3.0	OSTEOSARCOMA
M07R3.0	OSTEOSARCOMA
F08R3.0	OSTEOSARCOMA
F09R3.0	OSTEOSARCOMA
M10R3.0	OSTEOSARCOMA
F11R3.0	PYOMETRITIS + SECONDARY PERITONITIS
F12R3.0	OSTEOSARCOMA
M01R4.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64	DEATH	
M02R4.0	470	9.53	3.55	16	11	53		1884	6158
F03R4.0	384	8.65	3.33	10	03	54		490	2216
F03R4.0A	598	7.20	3.10	30	11	56		1614	3864
M04R4.0	408	8.83	3.47	7	04	54		1518	6073
M05R4.0	461	13.2	2.42	22	06	54		1659	4515
F06R4.0	486	8.55	3.44	27	07	54		1939	7146
M07R4.0	453	9.55	3.88	24	8	54		1647	5851
F08R4.0	474	8.94	3.14	21	12	54		1324	4626
F09R4.0	542	8.53	3.02	11	04	55		1471	4105
M10R4.0	527	10.8	3.28	27	07	55		1553	7592
F11R4.0	491	10.4	2.84	20	12	55		1469	5285
F12R4.0	496	9.61	2.81	17	01	56		1435	3886
M01R5.0	473	9.87	10.5	20	04	53		908	14982
M02R5.0	470	8.85	10.8	16	11	53		1380	18093
F03R5.0	380	7.82	10.1	10	03	54		481	7177
M04R5.0	408	8.90	10.6	7	04	54		1091	16453
M05R5.0	458	10.9	10.1	22	6	54		1220	15463
F06R5.0	486	9.66	10.2	27	07	54		1015	15438
M07R5.0	453	8.85	11.9	24	08	54		1288	16740
F08R5.0	474	7.76	9.68	21	12	54		968	11592
F09R5.0	420	9.16	9.48	11	04	55		1288	16015
M10R5.0	527	10.7	10.2	27	07	55		825	11120

DOG
NUMBER

COMMENTS ON DEAD DOGS

M02R 4.0
F03R 4.0
F03R 4.0A
M04R 4.0
M05R 4.0
F06R 4.0
M07R 4.0
F08R 4.0
F09R 4.0
M10R 4.0
F11R 4.0
F12R 4.0

OSTEOSARCOMA
CANINE DISTEMPER
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA

M01R 5.0
M02R 5.0
F03R 5.0
M04R 5.0
M05R 5.0
F06R 5.0
M07R 5.0
F08R 5.0
F09R 5.0
M10R 5.0

OSTEOSARCOMA
OSTEOSARCOMA
CANINE DISTEMPER
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA + ANEMIA
OSTEOSARCOMA + FRACTURED MANDIBLE

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ c / KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
M01P0.0	443	9.70		1 12 52		4003
F02P0.0	424	6.36		2 03 53		2755
M03P0.0	515	10.8		1 06 53	4139	
M04P0.0	426	10.7		16 09 53	4032	
F05P0.0	620	9.75		14 10 53	4004	
F06P0.0	410	5.59		12 05 54	3794	
F07P0.0	515	6.90		25 10 54	3628	
M08P0.0	585	10.9		15 03 55	3487	
F09P0.0	658	11.0		22 11 55		3032
F10P0.0	658	11.0		22 11 55	3235	
M11P0.0	602	10.3		24 04 56	3081	
M12P0.0	630	10.9		29 05 56	3046	
F13P0.0	517	9.47		4 3 64	210	
F14P0.0	452	9.89		12 5 64	14	
M31P0.0B	452	11.8		12 5 64	141	
M31P0.0C	452	12.6		12 5 64	141	
M32P0.0B	452	11.2		12 5 64	141	
F13P0.1	515	9.46	.00068	4 3 64	210	
F14P0.1	452	10.3	.00055	12 5 64	141	
M31P0.1B	517	12.2	.00068	4 3 64	210	
F13P0.2	517	9.44	.00206	4 3 64	210	
F14P0.2	516	7.44	.00173	12 5 64	141	
M31P0.2B	515	10.7	.00185	4 3 64	210	
F31P0.2C	452	11.9	.00169	12 5 64	141	
F31P0.2D	429	9.35	.00186	12 5 64	141	
F13P0.5	517	9.93	.00540	4 3 64	210	
F14P0.5	516	9.98	.00493	12 5 64	141	
M31P0.5B	515	10.5	.00549	4 3 64	210	
M01P1.0	442	9.41	.0150	1 12 52	4321	
F02P1.0	422	6.85	.0163	2 03 53	4230	
M03P1.0	515	8.00	.0165	1 06 53	4139	

DOG

NUMBER

COMMENTS ON DEAD DOGS

M01P0.0

SPLenic RUPTURE, METASTATIC SEMINOMA
ANESTHETIC ACCIDENT

F02P0.0

M03P0.0

M04P0.0

F05P0.0

F06P0.0

F07P0.0

M08P0.0

F09P0.0

PULMONARY EMBOLISM, NEPHRITIS

F10P0.0

M11P0.0

M12P0.0

F13P0.0

F14P0.0

M31P0.0B

M31P0.0C

M32P0.0B

F13P0.1

F14P0.1

M31P0.1B

F13P0.2

F14P0.2

M31P0.2B

F31P0.2C

F31P0.2D

F13P0.5

F14P0.5

M31P0.5B

M01P1.0

F02P1.0

M03P1.0

DOG NUMBER	AT INJECTION		INJECTED (μ c / KG)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M04P1.0	608	9.97	.0139	16	09	53	4032	
F05P1.0	620	8.80	.0142	14	10	53	1539	30
F05P1.0A	472	11.0	.0168	3	09	58	2219	
F06P1.0	410	7.38	.0140	12	05	54	3794	
F07P1.0	510	6.36	.0167	25	10	54	3628	
M08P1.0	453	10.6	.0172	15	3	55	3367	83
F09P1.0	556	7.87	.0168	9	09	55	2257	60
F10P1.0	641	12.00	.0152	22	11	55	3235	
M11P1.0	602	8.90	.0157	24	04	56	3081	
M12P1.0	629	9.67	.0167	29	5	56	2374	60
M13P1.0	504	12.7	.0153	3	09	58	2219	
M01P1.7	657	8.72	.0475	26	06	56	3018	
F02P1.7	527	8.62	.0431	22	11	55	3235	
M03P1.7	642	8.63	.0495	26	06	56	3018	
M04P1.7	673	8.37	.0484	10	10	56	2912	
F05P1.7	642	11.6	.0493	26	6	56	2659	190
F06P1.7	642	10.3	.0459	26	06	56	2221	170
F07P1.7	756	9.73	.0481	10	10	56	2912	
F08P1.7	673	13.6	.0479	10	10	56	2912	
F09P1.7	756	9.72	.0485	10	10	56	2500	180
F10P1.7	739	10.6	.0495	10	10	56	467	40
F10P1.7A	472	8.07	.0457	3	09	58	2219	
M11P1.7	599	11.6	.0486	24	4	56	2777	200
M12P1.7	673	9.41	.0491	10	10	56	2912	
M13P1.7	504	10.60	.0473	3	09	58	2219	
M01P2.0	442	7.61	.0853	1	12	52	2985	370
F02P2.0	422	7.73	.112	2	03	53	2780	460
M03P2.0	485	10.5	.0940	1	06	53	3185	420
M04P2.0	608	9.84	.0862	16	09	53	2948	380
F05P2.0	594	8.12	.0846	14	10	53	2423	310
F06P2.0	417	7.54	.0902	12	05	54	2947	370
F07P2.0	485	8.40	.0996	25	10	54	2093	310
M08P2.0	406	9.73	.0957	15	03	55	1761	250
F09P2.0	552	9.72	.101	9	09	55	2014	300
F10P2.0	551	7.94	.0968	22	11	55	2912	410
M11P2.0	599	10.3	.0961	24	04	56	1617	230
M12P2.0	622	9.98	.100	29	05	56	2284	370

DOG
NUMBER

COMMENTS ON DEAD DOGS

M04P1.0
F05P1.0
F05P1.0A
F06P1.0
F07P1.0
M08P1.0
F09P1.0
F10P1.0
M11P1.0
M12P1.0
M13P1.0

COLITIS, ENTERITIS + SECONDARY HEPATIC NECROSIS

OSTEOSARCOMA
OSTEOSARCOMA

CHRONIC PANCREATITIS

M01P1.7
F02P1.7
M03P1.7
M04P1.7
F05P1.7
F06P1.7
F07P1.7
F08P1.7
F09P1.7
F10P1.7
F10P1.7A
M11P1.7
M12P1.7
M13P1.7

OSTEOSARCOMA
OSTEOSARCOMA

OSTEOSARCOMA
ACUTE ENTERITIS

BILE DUCT CARCINOMA

M01P2.0
F02P2.0
M03P2.0
M04P2.0
F05P2.0
F06P2.0
F07P2.0
M08P2.0
F09P2.0
F10P2.0
M11P2.0
M12P2.0

OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
SQUAMOUS CELL CARCINOMA
ASPIRATION PNEUMONIA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64	DEATH	
M01P 3.0	417	8.00	.261	1	12	52		1476	580
F02P 3.0	422	6.85	.312	2	03	53		1947	900
M03P 3.0	485	8.74	.291	1	06	53		1604	700
M04P 3.0	608	8.51	.292	16	09	53		1950	840
F05P 3.0	650	8.22	.288	14	10	53		1504	630
F06P 3.0	415	8.38	.282	12	05	54		1617	670
F07P 3.0	485	9.00	.314	25	10	54		1627	750
M08P 3.0	406	9.73	.300	15	03	55		1771	780
F09P 3.0	552	7.67	.300	9	09	55		1894	840
F10P 3.0	533	8.94	.298	22	11	55		1547	700
M11P 3.0	599	10.5	.309	24	04	56		1198	550
M12P 3.0	622	10.2	.308	29	05	56		1659	760
M01P 4.0	442	7.61	.823	1	12	52		1724	2100
F02P 4.0	567	8.65	1.03	2	03	53		1556	2380
M03P 4.0	485	9.36	.929	1	06	53		1198	1680
M04P 4.0	566	8.74	.974	16	09	53		1066	1560
F05P 4.0	650	7.05	.872	14	10	53		1245	1650
F06P 4.0	420	9.26	.811	12	05	54		1357	1660
F07P 4.0	485	8.45	.963	25	10	54		1198	1730
M08P 4.0	651	9.22	.887	15	03	55		1157	1560
F09P 4.0	552	8.58	.960	9	09	55		1343	1920
F10P 4.0	527	6.48	.868	22	11	55		1241	1660
M11P 4.0	596	9.56	.927	24	04	56		1288	1790
M12P 4.0	598	11.4	.838	29	05	56		1463	1840
M01P 5.0	417	8.86	2.67	1	12	52		1324	5370
F02P 5.0	1150	8.75	3.30	2	03	53		1576	7830
M03P 5.0	515	8.10	3.00	1	06	53		499	2340
M04P 5.0	566	9.18	3.17	16	09	53		1562	7380
F05P 5.0	691	8.77	2.77	14	10	53		2059	8690
F06P 5.0	407	7.90	2.57	12	05	54		1194	4620
F07P 5.0	482	8.33	2.99	25	10	54		1491	6630
M08P 5.0	497	9.55	2.69	15	03	55		1192	4840
F09P 5.0	552	9.45	2.73	9	09	55		1145	4750

DOG
NUMBER

COMMENTS ON DEAD DOGS

M01P3.0	OSTEOSARCOMA
F02P3.0	OSTEOSARCOMA
M03P3.0	OSTEOSARCOMA
M04P3.0	OSTEOSARCOMA
F05P3.0	OSTEOSARCOMA
F06P3.0	OSTEOSARCOMA
F07P3.0	OSTEOSARCOMA
M08P3.0	OSTEOSARCOMA
F09P3.0	OSTEOSARCOMA
F10P3.0	OSTEOSARCOMA
M11P3.0	OSTEOSARCOMA
M12P3.0	OSTEOSARCOMA

M01P4.0	OSTEOSARCOMA
F02P4.0	OSTEOSARCOMA
M03P4.0	OSTEOSARCOMA
M04P4.0	OSTEOSARCOMA
F05P4.0	OSTEOSARCOMA
F06P4.0	OSTEOSARCOMA
F07P4.0	OSTEOSARCOMA
M08P4.0	OSTEOSARCOMA
F09P4.0	OSTEOSARCOMA
F10P4.0	OSTEOSARCOMA
M11P4.0	OSTEOSARCOMA
M12P4.0	OSTEOSARCOMA

M01P5.0	OSTEOSARCOMA
F02P5.0	OSTEOSARCOMA + FRACTURED MANDIBLE
M03P5.0	LIVER DEGENERATION + ASCITES
M04P5.0	OSTEOSARCOMA
F05P5.0	OSTEOSARCOMA, LIVER DEGENERATION + HEPATIC HEMORRHAGE
F06P5.0	OSTEOSARCOMA
F07P5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
M08P5.0	GINGIVITIS
F09P5.0	OSTEOSARCOMA, EPISTAXIS + CIRCULATORY COLLAPSE

C. RADIUM-228 (MESOTHORIUM) *

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ c /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
F01M0.0	732	7.33		4 1 54	3451	
F02M0.0	545	6.94		29 11 54	3593	
M03M0.0	579	13.0		13 03 56	3123	
M04M0.0	601	10.3		15 01 57	2815	
F05M0.0	671	11.2		5 03 57	2766	
M06M0.0	492	7.56		23 04 57	2717	
F07M0.0	395	8.71		4 6 57	1414	
F07M0.0A	594	10.9		15 1 63	624	
F08M0.0	654	11.6		9 03 60	1666	
M09M0.0	575	12.4		13 04 60	1631	
M10M0.0	581	13.3		17 7 62	806	
F11M0.0	475	9.31		18 9 62	743	
M12M0.0	695	10.0		22 12 60	1378	
F01M0.5	492	9.47	.0192	17 7 62	806	
F02M0.5	492	9.15	.0189	17 7 62	806	
M03M0.5	493	10.8	.0222	18 9 62	743	
M04M0.5	475	12.8	.0223	18 9 62	743	
F05M0.5	534	7.83	.0188	23 10 62	708	
M06M0.5	510	10.3	.0183	23 10 62	708	
F07M0.5	492	8.87	.0190	17 7 62	806	
F08M0.5	654	12.6	.0191	9 03 60	1666	
M09M0.5	485	11.9	.0192	13 04 60	1631	
M10M0.5	492	10.6	.0192	17 7 62	806	
F11M0.5	505	7.82	.0224	18 9 62	743	
M12M0.5	510	10.6	.0177	23 10 62	708	
F01M1.0	718	7.75	.0561	4 01 54	2952	350
F01M1.0A	590	8.07	.0558	23 10 62	708	
F02M1.0	459	8.25	.0593	29 11 54	3593	
M03M1.0	575	13.8	.0574	13 03 56	3123	
M04M1.0	601	9.90	.0599	15 01 57	2815	
F05M1.0	658	8.80	.0588	5 03 57	2766	
M06M1.0	521	10.6	.0545	23 04 57	2717	
F07M1.0	534	9.89	.0550	4 6 57	2159	
F08M1.0	654	12.4	.0572	9 03 60	1666	
M09M1.0	485	10.1	.0582	13 04 60	1631	
M10M1.0	492	9.43	.0657	17 7 62	806	
F11M1.0	505	8.91	.0671	18 9 62	743	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F01M0.0
F02M0.0
M03M0.0
M04M0.0
F05M0.0
M06M0.0
F07M0.0
F07M0.0A
F08M0.0
M09M0.0
M10M0.0
F11M0.0
M12M0.0

PURULENT MENINGOENCEPHALITIS

STATUS EPILEPTICUS

F01M0.5
F02M0.5
M03M0.5
M04M0.5
F05M0.5
M06M0.5
F07M0.5
F08M0.5
M09M0.5
M10M0.5
F11M0.5
M12M0.5

F01M1.0
F01M1.0A
F02M1.0
M03M1.0
M04M1.0
F05M1.0
M06M1.0
F07M1.0
F08M1.0
M09M1.0
M10M1.0
F11M1.0

HEMANGIOSARCOMA

HEMANGIOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO. YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
M12M1.0	528	9.27	.0538	23 10 62	708	
F01M1.7	510	7.52	.168	23 10 62	708	
F02M1.7	560	9.90	.173	13 03 56		2383
M03M1.7	576	11.0	.172	13 3 56		2709
M04M1.7	601	8.94	.178	15 01 57	2815	
F05M1.7	658	12.8	.170	5 03 57	2766	
M06M1.7	521	10.0	.168	23 04 57	2717	
F07M1.7	534	10.2	.160	4 6 57		2646
F08M1.7	654	10.8	.171	9 03 60	1666	
M09M1.7	485	12.6	.172	13 04 60	1631	
M10M1.7	492	10.1	.153	17 7 62	806	
F11M1.7	505	10.7	.199	18 9 62	743	
M12M1.7	524	9.28	.167	23 10 62	708	
F01M2.0	676	7.60	.334	4 01 54	1780	1350
F02M2.0	517	8.25	.355	29 11 54	965	540
M03M2.0	576	11.0	.342	13 03 56	619	280
M04M2.0	601	9.88	.351	15 1 57	2282	
F05M2.0	509	8.30	.355	5 3 57	2688	
M06M2.0	502	12.4	.357	23 4 57	2674	
F07M2.0	534	10.1	.326	4 6 57	2239	
F08M2.0	654	12.4	.348	9 03 60	1666	
M09M2.0	630	9.99	.347	13 4 60	1254	
M10M2.0	430	11.2	.341	17 7 62	806	
F11M2.0	505	7.03	.411	18 9 62	743	
M12M2.0	524	9.47	.332	23 10 62	708	
F01M3.0	519	10.4	1.04	4 01 54	918	2110
F02M3.0	460	6.70	1.12	29 11 54	1856	3810
M03M3.0	579	10.4	.921	13 3 56	1185	1820
M04M3.0	601	10.2	1.14	15 01 57	1176	2100
F05M3.0	531	8.51	1.13	5 03 57	1869	
M06M3.0	502	9.09	1.11	23 04 57	1421	
F07M3.0	534	9.94	.993	4 6 57	1463	2040
F08M3.0	633	11.8	1.07	9 3 60	1447	
M09M3.0	630	9.83	1.08	13 4 60	1570	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M12M1.0

F01M1.7

F02M1.7

M03M1.7

M04M1.7

F05M1.7

M06M1.7

F07M1.7

F08M1.7

M09M1.7

M10M1.7

F11M1.7

M12M1.7

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

F01M2.0

F02M2.0

M03M2.0

M04M2.0

F05M2.0

M06M2.0

F07M2.0

F08M2.0

M09M2.0

M10M2.0

F11M2.0

M12M2.0

OSTEOSARCOMA

INTESTINAL HEMORRHAGE

PNEUMONIA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

CHRONIC PANCREATITIS

RETICULUM CELL SARCOMA OF VERTEBRA

F01M3.0

F02M3.0

M03M3.0

M04M3.0

F05M3.0

M06M3.0

F07M3.0

F08M3.0

M09M3.0

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μc / KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64	DEATH	
M10M3.0	581	10.4	1.10	17	7	62	806		
F11M3.0	499	11.0	1.30	18	9	62	743		
M12M3.0	510	12.9	1.05	23	10	62	708		
F01M4.0	510	7.56	3.15	4	01	54	841		5800
F02M4.0	460	6.95	3.41	29	11	54	778		3820
M03M4.0	579	9.65	3.22	13	03	56	418		1640
M03M4.0A	494	7.34	2.89	4	06	57	1063		4650
M04M4.0	609	7.84	3.08	15	1	57	896		3940
F05M4.0	509	9.63	3.21	5	03	57	1064		5450
M06M4.0	502	9.49	3.10	23	04	57	1121		5300
F07M4.0	544	8.40	2.92	4	06	57	1253		
F01M5.0	494	7.77	9.83	4	01	54	232		4130
F02M5.0	460	7.35	10.0	29	11	54	780		11200
M03M5.0	579	8.87	9.93	13	3	56	688		9430
M04M5.0	482	7.29	9.82	15	01	57	561		6570
F05M5.0	658	11.1	10.2	5	3	57	770		10500
M06M5.0	580	7.53	10.1	23	4	57	792		10900
F07M5.0	494	7.35	9.77	4	06	57	966		14200

* ($\mu\text{c Th}^{228} / \mu\text{c Ra}^{228}$) injected = 0.15 for FlM1, 2, 3, 4, 5

= 0.03 for group 2, 3

= 0.006 for groups 4, 5, 6, 7, 8,
9, 10, 11, 12, and dogs
M3M4A, FlM0.5, F2M0.5,
M3M0.5, FlM1A, and FlM1.7.

Ra^{228} injected $\mu\text{c/kg}$ and dose to skeleton values will soon be revised in accordance with the redetermined half-period of Ra^{228} (see C00-222 and C00-225).

DOG

NUMBER

COMMENTS ON DEAD DOGS

M10M3.0

F11M3.0

M12M3.0

F01M4.0

F02M4.0

M03M4.0

M03M4.0A

M04M4.0

F05M4.0

M06M4.0

F07M4.0

OSTEOSARCOMA + CRIPPLING FRACTURE

OSTEOSARCOMA

STRANGULATED INGUINAL HERNIA

OSTEOSARCOMA, NEPHRITIS, ULCERATIVE GINGIVITIS + PNEUMONIA

FRACTURED MANDIBLE + ULCERATIVE GINGIVITIS

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

F01M5.0

F02M5.0

M03M5.0

M04M5.0

F05M5.0

M06M5.0

F07M5.0

NEPHRITIS + SEVERE ANEMIA

CRIPPLING FRACTURES

ULCERATIVE GINGIVITIS

CRIPPLING FRACTURE

ULCERATIVE GINGIVITIS

OSTEOSARCOMA + CRIPPLING FRACTURE

ULCERATIVE GINGIVITIS, MYOCARDIAL INFARCTION + GLAUCOMA

D. THORIUM-228 (RADIOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED (μ c /KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64	DEATH	
M01T0.0	493	8.24		8	02	54	3887		
M02T0.0	488	7.28		28	09	54	3655		
F03T0.0	797	11.6		6	06	55		2592	
M04T0.0	591	8.10		18	10	55		3072	
M05T0.0	458	10.4		14	10	58	2178		
F06T0.0	489	9.64		10	1	61		171	
F06T0.0A	688	8.61		15	12	60	1385		
M07T0.0	517	10.5		7	2	61	1331		
M08T0.0	533	10.8		24	5	61	1225		
F09T0.0	569	8.28		29	6	61	1189		
F10T0.0	536	10.4		28	7	61	1160		
F11T0.0	530	9.45		4	6	63	484		
F12T0.0	492	9.09		9	7	63	449		
M01T0.2	682	11.4	.00164	27	03	62	918		
M02T0.2	682	10.4	.00166	27	03	62	918		
F03T0.2	478	9.86	.00163	27	03	62	918		
M04T0.2	478	10.0	.00166	27	03	62	918		
M05T0.2	625	13.8	.00162	9	02	60		889	10
M05T0.2A	530	13.4	.00173	4	6	63	484		
F06T0.2	489	8.85	.00176	10	1	61	1359		
M07T0.2	532	10.5	.00159	7	2	61	1331		
M08T0.2	494	13.9	.00189	24	5	61	1225		
F09T0.2	569	7.82	.00171	29	6	61	1189		
F10T0.2	508	10.5	.00170	28	7	61	1160		
F11T0.2	530	9.76	.00171	4	6	63	484		
F12T0.2	492	7.37	.00190	9	7	63	449		
M01T0.5	699	14.3	.00496	7	9	56	2945		
M02T0.5	455	10.5	.00490	28	09	54		1976	40
F03T0.5	659	8.59	.00485	6	6	55		3032	40
M04T0.5	516	8.58	.00540	18	10	55		2159	40
M05T0.5	513	8.46	.00522	14	10	58	2178		
F06T0.5	489	9.66	.00510	10	1	61	1359		
M07T0.5	532	9.11	.00491	7	2	61	1331		
M08T0.5	533	9.53	.00562	24	5	61	1225		
F09T0.5	569	8.62	.00529	29	06	61	1189		
F10T0.5	508	10.2	.00510	28	7	61	1160		
F11T0.5	530	7.78	.00518	4	6	63	484		

DOG
NUMBER

COMMENTS ON DEAD DOGS

M01T0.0
M02T0.0
F03T0.0
M04T0.0
M05T0.0
F06T0.0
F06T0.0A
M07T0.0
M08T0.0
F09T0.0
F10T0.0
F11T0.0
F12T0.0

BRAIN HEMORRHAGE
LYMPHOSARCOMA

TRAUMA

M01T0.2
M02T0.2
F03T0.2
M04T0.2
M05T0.2
M05T0.2A
F06T0.2
M07T0.2
M08T0.2
F09T0.2
F10T0.2
F11T0.2
F12T0.2

STRANGULATION ON VOMITUS + GRAND MAL

M01T0.5
M02T0.5
F03T0.5
M04T0.5
M05T0.5
F06T0.5
M07T0.5
M08T0.5
F09T0.5
F10T0.5
F11T0.5

STRANGULATION ON VOMITUS + GRAND MAL
PYOMETRITIS + SECONDARY PERITONITIS
STATUS EPILEPTICUS + PNEUMONIA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
F12T0.5	492	9.94	.00567	9 7 63	449	
M01T1.0	493	9.36	.0146	8 02 54	3172	120
M02T1.0	699	9.27	.0146	7 09 56	2945	
F03T1.0	723	8.84	.0145	7 09 56	2945	
M04T1.0	699	8.27	.0146	7 09 56	2945	
M05T1.0	513	11.9	.0146	14 10 58	2178	
F06T1.0	489	8.81	.0150	10 1 61	1359	
M07T1.0	532	9.18	.0147	7 2 61	1331	
M08T1.0	533	8.69	.0166	24 5 61	1225	
F09T1.0	527	10.0	.0160	29 6 61	1189	
F10T1.0	508	10.2	.0150	28 7 61	1160	
F11T1.0	521	7.55	.0154	4 6 63	484	
F12T1.0	472	9.96	.0167	9 7 63	449	
M01T1.5	699	7.95	.0289	7 9 56	2894	231
M02T1.5	458	10.0	.0293	28 09 54	2576	230
F03T1.5	609	10.3	.0303	6 06 55	1921	220
M04T1.5	591	8.59	.0299	18 10 55	2309	230
M05T1.5	598	9.65	.0286	9 2 60	1624	194
F06T1.5	489	8.14	.0292	10 1 61	1359	
M07T1.5	517	8.83	.0292	7 2 61	384	80
M07T1.5A	521	9.08	.0311	4 6 63	484	
M08T1.5	494	11.6	.0324	24 5 61	1225	
F09T1.5	527	8.80	.0306	29 6 61	1189	
F10T1.5	508	11.6	.0296	28 7 61	1160	
F11T1.5	518	11.4	.0305	4 6 63	484	
F12T1.5	465	7.56	.0329	9 7 63	449	
M01T2.0	491	10.2	.0976	8 02 54	1282	580
M02T2.0	483	9.16	.0875	28 09 54	1234	540
F03T2.0	474	7.87	.0908	6 06 55	1541	610
M04T2.0	553	13.0	.0900	18 10 55	78	60
M04T2.0A	650	10.6	.0899	7 09 56	1222	540
M05T2.0	598	9.12	.0848	9 02 60	1085	480
F06T2.0	451	8.65	.0879	10 1 61	1108	500
M07T2.0	517	8.85	.0881	7 2 61	1015	480

DOG
NUMBER

COMMENTS ON DEAD DOGS

F12T0.5

M01T1.0
M02T1.0
F03T1.0
M04T1.0
M05T1.0
F06T1.0
M07T1.0
M08T1.0
F09T1.0
F10T1.0
F11T1.0
F12T1.0

OSTEOSARCOMA

M01T1.5
M02T1.5
F03T1.5
M04T1.5
M05T1.5
F06T1.5
M07T1.5
M07T1.5A
M08T1.5
F09T1.5
F10T1.5
F11T1.5
F12T1.5

OSTEOSARCOMA
OSTEOSARCOMA
COMA OF UNKNOWN ETIOLOGY
OSTEOSARCOMA
OSTEOSARCOMA

LEPTOSPIROSIS

M01T2.0
M02T2.0
F03T2.0
M04T2.0
M04T2.0A
M05T2.0
F06T2.0
M07T2.0

OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
TRAUMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
M08T2.0	533	10.7	.0981	24 5 61	1078	548
F09T2.0	527	8.09	.0979	29 6 61	1189	
F10T2.0	508	10.7	.0919	28 7 61	1022	498
F11T2.0	518	10.8	.0904	4 6 63	484	
F12T2.0	464	8.92	.100	9 7 63	449	
M01T3.0	314	9.15	.301	8 02 54	988	1590
M02T3.0	458	11.9	.301	28 09 54	859	1400
F03T3.0	471	12.0	.272	6 06 55	547	1080
M04T3.0	606	9.69	.285	18 10 55	801	1390
M05T3.0	571	10.7	.269	9 02 60	890	1340
F06T3.0	451	8.83	.282	10 1 61	1156	1640
M07T3.0	427	9.90	.266	7 2 61	861	1300
M08T3.0	494	10.1	.313	24 5 61	685	1320
F09T3.0	511	11.5	.298	29 6 61	1062	1651
F10T3.0	508	9.26	.280	28 7 61	971	1470
F11T3.0	518	10.3	.290	4 6 63	484	
F12T3.0	459	11.5	.320	9 7 63	449	
M01T4.0	480	8.32	.882	8 02 54	645	3400
M02T4.0	458	8.32	.916	28 09 54	833	4330
F03T4.0	461	7.25	.800	6 06 55	763	4250
M04T4.0	606	8.81	.835	18 10 55	793	4110
M01T5.0	480	9.48	2.76	8 02 54	212	4810
M02T5.0	483	8.22	2.63	28 09 54	97	2080

DOG
NUMBER

COMMENTS ON DEAD DOGS

M08T2.0
F09T2.0
F10T2.0
F11T2.0
F12T2.0

OSTEOSARCOMA

OSTEOSARCOMA

M01T3.0
M02T3.0
F03T3.0
M04T3.0
M05T3.0
F06T3.0
M07T3.0
M08T3.0
F09T3.0
F10T3.0
F11T3.0
F12T3.0

OSTEOSARCOMA + SEVERE ANEMIA

OSTEOSARCOMA + TRAUMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

OSTEOSARCOMA

M01T4.0
M02T4.0
F03T4.0
M04T4.0

OSTEOSARCOMA + CRIPPLING FRACTURE

OSTEOSARCOMA, CRIPPLING FRACTURE + NEPHRITIS

ULCERATIVE GINGIVITIS + NEPHRITIS

ULCERATIVE GINGIVITIS

M01T5.0
M02T5.0

NEPHRITIS

PANCYTOPENIA

E. STRONTIUM-90

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64	DEATH	
F01S0.0	502	8.48		18	01	55		3543	
M02S0.0	600	11.1		14	02	56		3151	
M03S0.0	493	9.03		11	09	57		2576	
F04S0.0	520	8.19		15	10	57		2542	
M05S0.0	542	10.6		19	11	57		2507	
M06S0.0	466	9.68		27	05	58		2318	
F07S0.0A	462	9.46		7	01	59		2093	
F08S0.0	483	9.29		19	05	59		1961	
F09S0.0	549	12.4		11	08	59			708
F09S0.0A	535	11.2		4	6	63		484	
M10S0.0	522	13.9		29	09	59		1828	
F11S0.0	541	9.60		3	11	59		1793	
M12S0.0	605	8.99		6	01	60		1729	
F01S1.0	1524	6.84	.573	18	01	55			308
F01S1.0A	521	9.38	.588	14	02	56		3151	
M02S1.0	567	8.81	.606	14	02	56		3151	
M03S1.0	493	10.9	.572	11	09	57		2576	
F04S1.0	525	8.96	.560	15	10	57		2542	
M05S1.0	555	10.2	.532	19	11	57		2507	
M06S1.0	466	9.56	.581	27	05	58		2318	
F07S1.0	524	9.94	.517	11	11	58		2150	
F08S1.0	483	10.8	.697	19	05	59		1961	
F09S1.0	549	11.6	.534	11	08	59		1877	
M10S1.0	522	11.5	.558	29	09	59		1828	
F11S1.0	543	10.3	.550	3	11	59		1793	
M12S1.0	607	13.7	.559	6	01	60		1729	
F01S1.7	526	7.41	1.78	14	02	56		3151	
M02S1.7	567	11.6	1.84	14	02	56		3151	
M03S1.7	493	9.19	1.69	11	09	57		2576	
F04S1.7	522	9.60	1.68	15	10	57		2542	
M05S1.7	560	9.85	1.60	19	11	57			1715
M05S1.7A	493	11.4	1.78	6	3	63		574	
M06S1.7	466	10.6	1.72	27	05	58		2318	
F07S1.7	488	10.2	1.60	11	11	58		2150	
F08S1.7	472	8.47	2.03	19	5	59			1973
F09S1.7	549	10.0	1.62	11	08	59		1877	
M10S1.7	519	13.6	1.66	29	09	59		1828	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F01S0.0
M02S0.0
M03S0.0
F04S0.0
M05S0.0
M06S0.0
F07S0.0A
F08S0.0
F09S0.0
F09S0.0A
M10S0.0
F11S0.0
M12S0.0

TRAUMA

F01S1.0
F01S1.0A
M02S1.0
M03S1.0
F04S1.0
M05S1.0
M06S1.0
F07S1.0
F08S1.0
F09S1.0
M10S1.0
F11S1.0
M12S1.0

SACRIFICED -IMPROPER INJECTION AGE-

F01S1.7
M02S1.7
M03S1.7
F04S1.7
M05S1.7
M05S1.7A
M06S1.7
F07S1.7
F08S1.7
F09S1.7
M10S1.7

COMA OF UNKNOWN ETIOLOGY

STATUS EPILEPTICUS, CHRONIC PANCREATITIS

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
F11S1.7	543	11.0	1.68	3 11 59	1793	
M12S1.7	607	11.9	1.68	6 01 60	1729	
F01S2.0	502	5.59	3.70	18 1 55	3269	451
M02S2.0	567	8.97	3.42	14 02 56	3151	
M03S2.0	494	7.82	3.39	11 09 57	2576	
F04S2.0	522	9.68	3.41	15 10 57	2542	
M05S2.0	560	8.72	3.24	19 11 57	2507	
M06S2.0	466	9.19	3.50	27 05 58	2318	
F07S2.0	488	11.2	3.19	11 11 58	2150	
F08S2.0	465	9.49	4.14	19 05 59	1961	
F09S2.0	473	14.1	3.28	11 08 59	1877	
M10S2.0	508	10.7	3.34	29 09 59	1828	
F11S2.0	543	10.4	3.41	3 11 59	1793	
M12S2.0	607	11.6	3.49	6 01 60	1729	
F01S3.0	468	7.36	11.6	18 01 55	3543	
M02S3.0	565	9.62	11.6	14 02 56	3151	
M03S3.0	494	11.4	10.8	11 09 57	2576	
F04S3.0	527	9.17	10.6	15 10 57	2542	
M05S3.0	557	8.90	10.1	19 11 57	2507	
M06S3.0	466	9.44	10.9	27 05 58	2318	
F07S3.0	486	9.80	10.1	11 11 58	2150	
F08S3.0	465	12.5	12.9	19 05 59	1961	
F09S3.0	468	10.0	10.1	11 08 59	1877	
M10S3.0	519	12.5	10.3	29 09 59	1828	
F11S3.0	541	9.00	10.8	3 11 59	1793	
M12S3.0	605	8.43	10.2	6 01 60	1729	
F01S4.0	468	8.74	33.3	18 01 55	3543	
M02S4.0	567	11.2	32.6	14 02 56	2093	5464
M03S4.0	593	9.83	32.1	11 09 57	2576	
F04S4.0	528	8.24	32.1	15 10 57	2542	
M05S4.0	562	9.65	30.6	19 11 57	2507	
M06S4.0	504	16.0	32.7	3 09 58	2219	
F07S4.0	478	10.9	30.9	11 11 58	2150	
F08S4.0	465	10.9	40.6	19 05 59	1961	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F11S1.7
M12S1.7

F01S2.0
M02S2.0
M03S2.0
F04S2.0
M05S2.0
M06S2.0
F07S2.0
F08S2.0
F09S2.0
M10S2.0
F11S2.0
M12S2.0

BACTERIAL PNEUMONIA

F01S3.0
M02S3.0
M03S3.0
F04S3.0
M05S3.0
M06S3.0
F07S3.0
F08S3.0
F09S3.0
M10S3.0
F11S3.0
M12S3.0

F01S4.0
M02S4.0
M03S4.0
F04S4.0
M05S4.0
M06S4.0
F07S4.0
F08S4.0

SQUAMOUS CELL CARCINOMA --GINGIVA--

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F09S4.0	468	9.56	30.6	11	08	59	1877	
M10S4.0	517	8.20	31.3	29	09	59	1828	
F11S4.0	542	8.86	32.7	3	11	59	1793	
M12S4.0	605	10.9	32.3	6	01	60	1729	
F01S5.0	434	9.38	103.	18	01	55	960	7700
M02S5.0	551	12.2	102.	14	02	56	255	3070
M02S5.0A	545	11.4	96.6	7	1	59	1740	11361
M03S5.0	507	10.3	102.	15	10	57	2256	15329
F04S5.0	528	11.4	105.	15	10	57	1448	8758
M05S5.0	621	8.53	95.2	19	11	57	1285	9381
M06S5.0	504	9.33	98.8	3	9	58	35	630
M06S5.0A	462	11.2	94.2	7	01	59	1021	11000
F07S5.0	478	10.2	92.7	11	11	58	1129	10236
F08S5.0	535	11.2	90.5	7	01	59	1469	10323
F09S5.0	459	8.82	93.5	11	08	59	1877	
M10S5.0	517	8.55	95.9	29	09	59	990	7335
F11S5.0	542	8.97	102.	3	11	59	1667	9385
M12S5.0	606	12.5	99.2	6	01	60	1165	7874

DOG
NUMBER

COMMENTS ON DEAD DOGS

F09S4.0
M10S4.0
F11S4.0
M12S4.0

F01S5.0
M02S5.0
M02S5.0A
M03S5.0
F04S5.0
M05S5.0
M06S5.0
M06S5.0A
F07S5.0
F08S5.0
F09S5.0
M10S5.0
F11S5.0
M12S5.0

OSTEOSARCOMA
STRANGULATED INGUINAL HERNIA
OSTEOSARCOMA
OSTEOSARCOMA
OSTEOSARCOMA
SEVERE ANEMIA, AUTOAGGLUTINATION, INFARCTION, SPLENOMEGALY
INTESTINAL HEMORRHAGE
OSTEOSARCOMA, INFARCTION + THROMBOCYTOPENIA
STATUS EPILEPTICUS
OSTEOSARCOMA

SEVERE ANEMIA + THROMBOCYTOPENIA
HEMANGIOSARCOMA
HEMANGIOSARCOMA

TABLE II. TEST ANIMALS (SEPT. 30. 1964)

A. RADIUM-226

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS.)
T01R 5.0	995	11.1	10.3	1 12 52	1074	9490
T02R 5.0	919	8.40	4.39	12 01 53	1368	4733
T03R 5.0	1467	8.29	4.76	12 01 53	428	1320
T04R 5.0	459	10.0	10.6	6 07 53	1	53
T05R 5.0	126	6.14	11.7	6 10 53	1	58
T06R 5.0	126	6.14	11.4	6 10 53	1	57
T07R 5.0	126	6.14	11.8	6 10 53	1	59
T08R 5.0	290	5.52	1.92	10 05 55	58	501
T09R 5.0	2276	10.4	1.94	10 05 55	58	602
T10R 5.0	43	1.02	1.98	10 05 55	49	300
T11R 5.0	43	1.58	1.91	10 05 55	49	371
T12R 5.0	397	12.3	9.72	9 05 56	225	2722
T13R 5.0	397	7.59	9.76	9 05 56	188	2341
T14R 4.0	674	8.12	3.17	11 07 56	72	390
T15R 4.0	672	9.03	3.11	11 07 56	2127	5123
T16R 5.0	604	12.4	9.68	11 07 57	12	208
T17R 5.0H	383	12.2		28 10 58	1147	13706
T18R 5.0H	383	11.1		28 10 58	1226	12645
T19R 5.0H	383	10.67		28 10 58	1219	11595
T20R 5.0H	383	11.4		28 10 58	1330	12955
T21R 5.0H	381	11.8		28 10 58	386	3729
T22R 5.0H	381	11.9		28 10 58	587	5930
T23R 4.0H	384	9.50		25 11 58	1471	4347
T24R 4.0H	384	11.9		25 11 58	1505	5602
T25R 4.0H	379	11.3		25 11 58	1309	4681
T26R 4.0H	379	11.0		25 11 58	1780	4726
T27R 4.0H	372	11.5		25 11 58	1414	3388
T28R 3.0H	372	11.7		25 11 58	387	359
T29R 5.0	474	13.5	10.4	3 03 59	216	3434

DOG
NUMBER COMMENTS ON DEAD DOGS

T01R 5.0	OSTEOSARCOMA
T02R 5.0	OSTEOSARCOMA
T03R 5.0	SPECIAL STUDY
T04R 5.0	SPECIAL STUDY
T05R 5.0	SPECIAL STUDY
T06R 5.0	SPECIAL STUDY
T07R 5.0	SPECIAL STUDY
T08R 5.0	SPECIAL STUDY
T09R 5.0	SPECIAL STUDY
T10R 5.0	SPECIAL STUDY
T11R 5.0	SPECIAL STUDY
T12R 5.0	SPECIAL STUDY
T13R 5.0	SPECIAL STUDY
T14R 4.0	SPECIAL STUDY
T15R 4.0	OSTEOSARCOMA
T16R 5.0	SPECIAL STUDY
T17R 5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T18R 5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T19R 5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T20R 5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T21R 5.0H	NEPHRITIS
T22R 5.0H	CRIPPLING FRACTURES
T23R 4.0H	OSTEOSARCOMA
T24R 4.0H	OSTEOSARCOMA
T25R 4.0H	OSTEOSARCOMA
T26R 4.0H	OSTEOSARCOMA
T27R 4.0H	OSTEOSARCOMA
T28R 3.0H	SPECIAL STUDY
T29R 5.0	NEPHRITIS

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	30/9/64	DEATH	
T30R 5.0	474	11.5	10.4	3	03	59		178	2879
T31R 5.0	471	10.5	10.4	3	03	59		303	4788
T32R 3.0	471	11.4	1.13	3	03	59	2038		
T33R 3.0	471	10.6	1.15	3	3	59		1822	1924
T34R 3.0	470	15.7	1.12	3	3	59		1737	1606
T35R 3.0J	670	9.44	.951	5	5	59		8	16
T36R 4.0	695	10.2	2.99	22	12	60		1154	3654
T37R 4.0	695	9.53	3.00	22	12	60		1378	
T38R 4.0	695	10.1	3.02	22	12	60		1378	
T40R 1.0	899	13.0	.0483	3	4	62		7	1
T41R 1.0	899	12.7	.0487	3	4	62		63	3
T42R 1.7	967	14.0	.146	4	4	62		7	2
T43R 1.7	963	13.2	.145	4	4	62		64	12
T44R 3.0	938	11.1	.937	4	4	62		68	72
T45R 3.0	939	13.6	.941	5	4	62		7	14
T46R 3.0	810	12.5	.928	5	4	62		69	105
T47R 6.0	99	5.27	29.4	11	6	62		4	440
T48R 6.0	2842	11.2	25.1	27	12	62		49	1633
T49R 5.0	485	10.6	7.54	2	5	63		5	133
T50R 5.0	485	13.7	7.46	2	5	63		15	305
T51R 5.0	418	13.3	8.48	8	5	63		92	1721
T52R 5.0	418	10.7	8.57	8	5	63		15	286

DOG
NUMBER

COMMENTS ON DEAD DOGS

T30R5.0
T31R5.0

NEPHRITIS
NEPHRITIS

T32R3.0
T33R3.0
T34R3.0
T35R3.0J

OSTEOSARCOMA, NEPHRITIS
OSTEOSARCOMA
SPECIAL STUDY

T36R4.0
T37R4.0
T38R4.0

OSTEOSARCOMA

T40R1.0
T41R1.0

SPECIAL STUDY
SPECIAL STUDY

T42R1.7
T43R1.7

SPECIAL STUDY
SPECIAL STUDY

T44R3.0
T45R3.0
T46R3.0

SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY

T47R6.0
T48R6.0

SPECIAL STUDY
LEUKOPENIA, PNEUMONIA + SPECIAL MELANOMA STUDY

T49R5.0
T50R5.0
T51R5.0
T52R5.0

SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{C}/\text{KG}$)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D.	MO.	YR		
T53R 5.0	418	12.0	8.50	8	5	63	33	632
T54R 5.0	417	11.4	8.76	22	5	63	5	105
T55R 5.0	417	11.6	8.61	22	5	63	33	552
T56R 5.0	417	11.6	8.61	22	5	63	90	1491
T57R 4.0	501	12.1	2.72	15	8	63	14	60
T58R 4.0	496	11.7	2.41	15	8	63	61	292
T59R 4.0	496	9.64	2.57	15	8	63	63	277
T60R 4.0	490	12.1	2.33	15	8	63	117	419
T61R 4.0	490	9.48	2.70	15	8	63	371	1574
T62R 4.0	490	8.63	2.68	15	8	63	412	
T63R 3.0	559	8.72	.899	29	1	64	36	52
T64R 3.0	551	8.42	.919	29	1	64	63	75
T65R 3.0	551	11.6	.922	29	1	64	70	92
T66R 3.0	549	10.1	.904	29	1	64	132	142
T67R 3.0	549	12.7	.898	29	1	64	134	171
T68R 3.0	549	12.1	.917	29	1	64	245	
T69R 3.0	499	8.84	.919	29	1	64	245	
T70R 3.0	499	14.2	.922	29	1	64	245	

* 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 Ra^{226} injection was 20.0 μC for the dogs T17R5H - T22R5H; 6.41 μC for T23R4H - T27R4H; and 2.16 μC for T28R3H. Tabulated for each dog are his age at 1st injection, his average weight during the injection period, 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 μC Sr^{85} .

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T53R 5.0	SPECIAL STUDY
T54R 5.0	SPECIAL STUDY
T55R 5.0	SPECIAL STUDY
T56R 5.0	SPECIAL STUDY

T57R 4.0	SPECIAL STUDY
T58R 4.0	SPECIAL STUDY
T59R 4.0	SPECIAL STUDY
T60R 4.0	SPECIAL STUDY
T61R 4.0	SPECIAL STUDY
T62R 4.0	

T63R 3.0	SPECIAL STUDY
T64R 3.0	SPECIAL STUDY
T65R 3.0	SPECIAL STUDY
T66R 3.0	SPECIAL STUDY
T67R 3.0	SPECIAL STUDY
T68R 3.0	
T69R 3.0	
T70R 3.0	

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
T00P5.0	647	11.4	3.05	24 06 52	1	10
T01P5.0	581	12.7	3.04	13 10 52	29	160
T02P5.0	914	11.9	6.85	15 09 52	44	520
T03P5.0	942	9.65	3.22	13 10 52	610	3040
T04P5.0	1016	8.78	3.02	13 10 52	365	1750
T05P5.0	474	10.4	2.69	14 12 54	400	1720
T06P5.0	527	6.16	2.73	14 12 54	406	1750
T07P5.0	475	7.40	2.68	14 12 54	777	3230
T08P5.0	527	8.32	2.67	14 12 54	863	3540
T09P5.0	551	10.3	2.80	22 11 55	15	80
T10P5.0	534	11.9	2.74	23 11 55	15	70
T11P5.0	516	12.1	2.76	22 11 55	28	140
T12P5.0	487	9.23	2.74	23 11 55	28	140
T13P5.0	587	8.27	3.16	24 04 56	3	20
T14P5.0	587	9.38	2.43	24 04 56	7	30
T15P5.0	737	8.32	2.79	15 10 56	1	10
T16P5.0	673	10.7	2.85	10 10 56	92	440
T17P5.0	739	11.1	3.01	12 02 57	210	990
T18P5.0	739	8.16	2.83	12 02 57	217	960
T19P5.0	688	8.86	2.91	15 12 60	1385	
T20P5.0	688	13.0	2.68	15 12 60	474	1970
T21P5.0	688	10.3	2.72	15 12 60	939	3940
T23P1.0	1485	13.1	.0172	28 7 61	96	
T24P1.0	559	13.1	.0172	28 7 61	97	
T25P1.0	559	13.8	.0167	28 7 61	467	10
T26P1.0	556	12.0	.0160	28 7 61	647	20
T27P3.0	556	11.5	.332	28 7 61	755	390
T28P1.0	552	10.5	.0150	9 8 61	559	10
T29P3.0	552	12.1	.296	9 8 61	560	260
T30P1.0	548	12.4	.0148	9 8 61	35	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T00P 5.0	SPECIAL STUDY
T01P 5.0	SPECIAL STUDY
T02P 5.0	SPECIAL STUDY
T03P 5.0	SPECIAL STUDY
T04P 5.0	SPECIAL STUDY
T05P 5.0	SPECIAL STUDY
T06P 5.0	SPECIAL STUDY
T07P 5.0	SPECIAL STUDY
T08P 5.0	SPECIAL STUDY
T09P 5.0	SPECIAL STUDY
T10P 5.0	SPECIAL STUDY
T11P 5.0	SPECIAL STUDY
T12P 5.0	SPECIAL STUDY
T13P 5.0	SPECIAL STUDY
T14P 5.0	SPECIAL STUDY
T15P 5.0	SPECIAL STUDY
T16P 5.0	SPECIAL STUDY
T17P 5.0	SPECIAL STUDY
T18P 5.0	SPECIAL STUDY
T19P 5.0	

T20P 5.0
T21P 5.0

LIVER DEGENERATION, ASCITES + THROMBOCYTOPENIA
TOXIC NEPHRITIS + LIVER DEGENERATION

T23P 1.0
T24P 1.0
T25P 1.0
T26P 1.0

SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY

T27P 3.0

SPECIAL STUDY

T28P 1.0

SPECIAL STUDY

T29P 3.0

SPECIAL STUDY

T30P 1.0

SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
T31P3.0	519	13.0	.305	9 8 61	40	20
T32P1.0	520	8.47	.0162	9 8 61	274	10
T33P1.0	550	10.7	.0153	15 9 61	375	10
T34P1.0	550	9.68	.0154	15 9 61	746	20
T35P3.0	550	11.9	.303	15 9 61	362	180
T36P1.0	544	10.4	.0158	15 9 61	5	
T37P1.0	542	8.59	.0148	15 9 61	186	
T38P3.0	489	7.96	.304	15 9 61	187	90
T39P1.0	1534	10.7	.0151	15 9 61	376	10
T40P1.0	1534	9.92	.0177	15 9 61	769	20

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

DOG
NUMBER -

COMMENTS ON DEAD DOGS

T31P3.0 SPECIAL STUDY

T32P1.0 SPECIAL STUDY
T33P1.0 SPECIAL STUDY
T34P1.0 SPECIAL STUDY

T35P3.0 SPECIAL STUDY

T36P1.0 SPECIAL STUDY
T37P1.0 SPECIAL STUDY

T38P3.0 SPECIAL STUDY

T39P1.0 SPECIAL STUDY
T40P1.0 SPECIAL STUDY

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{C/KG}$)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T01M4.5	529	9.13	5.55	8	09	54	314	1830
T02M4.5	463	8.93	5.60	8	09	54	755	6110
T03M5.0	579	9.15	10.1	13	03	56	700	18400

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

Ra^{228} injected $\mu\text{C/kg}$ and dose to skeleton values will soon be revised in accordance with the redetermined half-period of Ra^{228} (see C00-222 and C00-225).

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01M4.5
T02M4.5

CANINE DISTEMPER
SPECIAL STUDY

T03M5.0

ULCERATIVE GINGIVITIS, SEVERE ANEMIA + CRIPPLING FRACTUR

D. THORIUM-228 (RADIOTHORIUM) *

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{C/KG}$)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T01T5.0	490	9.30	4.88	1	12	53	23	1300
T02T5.0	501	8.48	2.56	8	02	54	77	1620
T03T4.0	429	10.4	.870	8	02	54	820	4050
T04T5.0	455	8.92	2.59	28	09	54	113	2330
T05T5.0	455	10.1	2.32	28	09	54	65	1210
T06T4.0	591	7.01	.884	18	10	55	651	3570
T07T3.0	591	9.23	.298	18	10	55	910	1550
T08T3.0	606	9.23	.293	14	10	58	1043	1610
T09T3.0	447	11.0	.285	4	02	59	1	
T10T3.0	447	14.2	.289	4	02	59	8	20
T11T3.0	500	8.62	.335	16	6	59	22	60
T12T3.0	514	10.6	.302	7	7	59	22	60
T13T3.0	754	13.1	.298	28	7	59	22	60

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01T5.0
T02T5.0

DIED, SPECIAL STUDY
SPECIAL STUDY

T03T4.0

CRIPPLING FRACTURES + NEPHRITIS

T04T5.0
T05T5.0

THROMBOCYTOPENIA + PURPURA
NEPHRITIS, THROMBOCYTOPENIA + PURPURA

T06T4.0

CRIPPLING FRACTURES

T07T3.0
T08T3.0
T09T3.0
T10T3.0
T11T3.0
T12T3.0
T13T3.0

SPECIAL STUDY
OSTEOSARCOMA
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY
SPECIAL STUDY

E. STRONTIUM-90*

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO.	YR		
T01S0.0	151	7.71		5	03	54	2	
T02S5.0	149	6.85	148.	5	03	54	11	626
T03S5.0	144	6.19	148.	5	03	54	18	958
T04S5.0	151	7.05	148.	5	03	54	41	1945
T05S5.0	144	5.25	148.	5	03	54	116	4863
T06S5.0	155	7.01	87.0	16	03	54		2
T07S5.0	155	6.74	87.0	16	03	54	2	84
T08S0.0	243	7.00						
T08S2.0H	67	3.69	2.74	27	09	55	66	30
T09S2.0H	67	2.79	3.62	27	09	55	66	40
T10S2.0H	67	3.11	3.25	27	09	55	132	69
T11S2.0H	67	3.85	2.62	27	09	55	132	55
T12S3.0	593	10.6	10.5	11	09	57	5	15
T13S4.0	324	10.5	19.1	8	07	60	8	49
T14S5.0	542	10.0	96.1	7	11	61	9	172
T15S5.0	595	9.43	98.4	7	11	61	30	413
T16S2.0	604	9.71	3.27	8	11	61	9	6
T17S6.0	670	7.18	295.	19	1	62	14	676
T18S6.0	670	5.94	302.	19	1	62	985	

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01S0.0 SPECIAL STUDY

T02S5.0 SPECIAL STUDY
T03S5.0 SPECIAL STUDY
T04S5.0 SPECIAL STUDY
T05S5.0 SPECIAL STUDY
T06S5.0 SPECIAL STUDY
T07S5.0 SPECIAL STUDY

T08S .0 SPECIAL STUDY

T08S2.0H SPECIAL STUDY
T09S2.0H SPECIAL STUDY
T10S2.0H SPECIAL STUDY
T11S2.0H SPECIAL STUDY

T12S3.0 BREMSSTRAHLUNG PHANTOM

T13S4.0 BREMSSTRAHLUNG PHANTOM SAM MCGEE

T14S5.0 SPECIAL STUDY
T15S5.0 SPECIAL STUDY

T16S2.0 SPECIAL STUDY

T17S6.0 LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
T18S6.0

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{C}/\text{KG}$)	DATE INJECTED			DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T19S6.0	670	5.43	284.	19	1	62	24	622
T20S4.0J	440	8.54	28.9	2	10	63	13	77
T21S2.5J	363	7.20	8.3	2	10	63	13	140

*T08 ... 11S2.0H were given 10 injections, 1 μC each, given at weekly intervals, age shown is that at first injection which is date shown. Dose is based on weight at mid point of injection interval.

T20S4.0J received 0.5 μC Sr^{85} ...

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

DOG
NUMBER -

COMMENTS ON DEAD DOGS

T19S6.0

LEUKOPENIA, THROMBOCYTOPENIA + PURPURA

T20S4.0J

SPECIAL STUDY

T21S2.5J

SPECIAL STUDY

F. RADIUM-224

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64 DEATH	DOSE TO SKELETON (RADS)
T01Q3.0J	460	9.55	.875	26 3 63	-	
T02Q4.0	466	12.0	2.91	27 3 63	553	
T03Q4.0	466	13.1	2.91	27 3 63	553	
T04Q5.0	480	9.55	9.71	24 4 63	525	
T05Q5.0	455	9.67	9.59	24 4 63	525	
T06Q6.0	455	8.29	24.6	17 10 63	13	980
T07Q5.0	465	11.8	9.83	6 11 63	329	
T08Q5.0	475	9.77	9.91	6 11 63	16	400
T09Q4.0	503	9.80	2.96	4 12 63	301	
T10Q4.0	503	10.3	2.96	4 12 63	262	133
T11Q3.0	495	9.10	1.02	4 12 63	301	
T12Q3.0	495	13.5	1.02	4 12 63	301	
T13Q3.0	495	11.3	1.02	4 12 63	301	
T14Q3.0	438	10.3	1.00	4 12 63	301	

T01Q3.0J received 18.0 μ C Sr⁸⁵.

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01Q3.0J

SPECIAL STUDY

T02Q4.0
T03Q4.0

T04Q5.0
T05Q5.0

T06Q6.0

PURPURA HEMORRHAGICA

T07Q5.0
T08Q5.0

PURPURA HEMORRHAGICA

T09Q4.0
T10Q4.0

STATUS EPILEPTICUS

T11Q3.0
T12Q3.0
T13Q3.0
T14Q3.0

G. ANCILLARY*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 30/9/64	DEATH	DOSE TO SKELETON (RADS)
F01A							1383
F02A							2492
M03A							1451
M04A							3345
M05A							4713
M06A							5266
M07A							3896
M08A							3746
F09A							3719
F10A							2605
F11A							4198
F12A							4219
F13A							4527
F14A							3777
F16A							4415
F17A							2145
F20A							2464
M23A							1741
M24A							3074
M27A							2130
M28A							3114
F32A							1990
F33A							3282
F34A							2584
M35A							529
M36A							1971
M37A							4089
M42A							1265
F43A							3881
F47A							1732
M50A							2264
F51A							1089
F52A							509
F54A							3190
M56A							701
M58A							767
M59A							567
M73A							5695
M86A							499

* Time interval shown here is the animal's age.

DOG
NUMBER

COMMENTS ON DEAD DOGS

F01A	SPECIAL STUDY
F02A	SPECIAL STUDY
M03A	SPECIAL STUDY
M04A	NOT DETERMINED
M05A	
M06A	BRAIN HEMORRHAGE
M07A	LYMPHOSARCOMA
M08A	PROGRESSIVE PARALYSIS, CAUSE UNKNOWN
F09A	VAGINAL FIBROMA
F10A	SPECIAL STUDY
F11A	MAMMARY CARCINOMA
F12A	SEVERE OSTEOARTHRITIS
F13A	SPECIAL STUDY
F14A	OBTURATING EMBOLISM OF PORTAL VEIN
F16A	SPECIAL STUDY
F17A	TRAUMA
F20A	SPECIAL STUDY
M23A	OBTURATING PULMONARY EMBOLISM
M24A	SPECIAL STUDY
M27A	SPECIAL STUDY
M28A	SPECIAL STUDY
F32A	LYMPHOSARCOMA
F33A	OBTURATING PULMONARY EMBOLISM
F34A	SPECIAL STUDY
M35A	SPECIAL STUDY
M36A	SPECIAL STUDY
M37A	SPECIAL STUDY
M42A	STATUS EPILEPTICUS
F43A	SPECIAL STUDY
F47A	SPECIAL STUDY
M50A	SPECIAL STUDY
F51A	SPECIAL STUDY
F52A	SPECIAL STUDY
F54A	SPECIAL STUDY
M56A	VOLVULUS + PERITONITIS
M58A	SPECIAL STUDY
M59A	SPECIAL STUDY
M73A	DEGENERATION OF ADRENAL GLAND + DIABETES MELLITUS
M86A	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
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REPEATED USE OF ANESTHESIA IN AN EXPERIMENTAL DOG COLONY

G. N. Taylor, C. E. Rehfeld,[†] and J. Wrathal

Abstract: Extensive use of thiopental sodium anesthesia over a ten year period, primarily for restraint purposes, has proven to be a safe procedure in beagles. Two anesthetic deaths resulted from 8,083 anesthetics given to 702 dogs at a mean interval of approximately 100 days. Residual effects were not detected. The age classes ranged from 3 months to 14 years and included various degrees of health.

Some of the techniques used in evaluating the beagles at this laboratory are best performed on the anesthetized animal. The quality of radiographs is markedly improved, and the hazard to the x-ray technician is eliminated (Fig. 1). The precision of whole-body counting is significantly enhanced. Some of our whole-body counters could not be used on the unanesthetized animal (Figs. 2 & 3). Other procedures such as dental work, etc., are facilitated.

This laboratory has used thiopental sodium^{*} anesthesia in conjunction with these techniques for over 10 years and it is the purpose of this study to indicate the degree of safety that we have experienced with this drug.

METHODS

Approximately 95% of the cases listed in Table I were anesthetized to facilitate whole-body counting or radiography. In most instances thiopental sodium without any preanesthetic medication was the anesthetic

^{*}Pentothal sodium, Abbott Laboratories, North Chicago, Illinois.

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used. Ether* or methoxyflurane** was used in the relatively small number of exceptions--usually for surgical procedures. However, thiopental sodium was also used in these cases to facilitate induction and intubation. Morphine and/or atropine premedication was generally used in conjunction with the volatile anesthetics.

The desired depth of thiopental sodium anesthesia was maintained by taping the syringe, with the needle inserted into the cephalic vein, to the foreleg and injecting additional amounts as needed (Fig. 1). The duration varied from a few minutes to several hours--averaging approximately 30 to 45 minutes (not including recovery phase). In most instances, especially when used for restraint purposes, the depth was light and did not exceed the top of plane one of the surgical stage as adjudged by skeletal muscle tone (muscles of mastication), pupil size, palpebral and pedal reflexes (1,2).

During the recovery period the dogs were caged in a controlled-temperature room and observed at 10 minute intervals until such time as they were able to raise their heads. Following this stage of recovery, which averaged approximately 80 minutes, the frequent observations were stopped, but cage confinement was continued until completely coordinate. The dog was then returned to its regular run or a separate hospital run if surgery had been performed.

RESULTS

Table 1 summarizes 8,083 anesthetics administered from 1951 through 1964. These cases represent beagles of varying degrees of health ranging

*Ether, Mallinckrodt Chemical Works, St. Louis, Missouri.

**Penthrane, Abbott Laboratories, North Chicago, Illinois.

from excellent to very poor. Many exhibited radiation induced blood dyscrasias, skeletal fractures, osteosarcomas, liver lesions, kidney disease, and miscellaneous conditions. In some cases a combination of some of these conditions existed. The age groups extend from 3 months to 14 years, and senile changes were obvious in a significant per cent of the older animals.

Anesthesia was not used in certain instances where the risk appeared great.

The number of anesthetics given to a single dog ranged from 4 to 89. Two animals, one male and one female, were anesthetized 89 times at one month intervals. This sequence of anesthesia was begun at 6 months of age. Both of these dogs, now 8-1/2 years old, are presently asymptomatic.

In spite of the varied age and health status and the relatively large number of anesthetics given, only 2 anesthetic accident deaths resulted from the administration of 8,083 anesthetics. Also, frequent clinical, hematological and blood chemistry examinations have not indicated any residual affects resultant to thiopental sodium anesthesia - even in the two dogs anesthetized 89 times (3,4)*. This relatively low mortality is attributed to the light, but nevertheless adequate depth of anesthesia generally used, to the very low toxicity of thiopental sodium, and to strict adherence to acceptable techniques.

DISCUSSION

The use of anesthesia in experimental animals is certainly open to criticism, and it has been used with some reluctance at this laboratory.

*This observed safety is in agreement with many other studies (5-9).

However, in our program, elimination of this form of restraint would greatly curtail or preclude some important aspects of our study. The matter then becomes one of deciding if the biological price, if any, that we might be paying for such usage justifies the benefits.

TABLE 1

Summary of Anesthetics Used in Beagle Colony, University of Utah
Radiobiology Laboratory, 1951 Through 1964.
Thiopental Sodium was Used in Most Instances.

Nuclide	Total # Anesthetics Given*	Total # Dogs Anesthetized	Average # Anesthetics Per Dog	Maximum # Anesthetics For Any Single Dog	Mean Interval Between Anesthetics (Days)
Plutonium	1506	139	11	32	151
Radium ²²⁶	2280	153	15	41	113
Mesothorium	1047	92	11	33	129
Strontium	1401	99	14	37	131
Radiothorium	1082	107	10	32	117
Radium ²²⁴	31	13	2	4	146
X-Ray Controls	318	6	53	89	33
Aging Controls	388	80	5	13	361
Unassigned	20	6	3	8	44
Others	10	7	1	4	19
Total	8083	702	12		

* Does not include the anesthesia given just prior to euthanasia.

Our experience, extending over the past ten years, indicates the use of anesthesia to the extent covered in this paper is justified and is compatible with the objectives of our experiment. We are not aware of other studies that have demonstrated irreversible changes within the limits of our usage.

* * * *

The authors wish to thank L. Shabestari and L. Brewster for their technical assistance.

Fig. 1. Anesthetized beagle positioned for radiography. The placement of the syringe with needle in the cephalic vein and the endotracheal catheter is shown. The bags used to "fix" the legs are filled with small lead shot.

Fig. 2. Anesthetized beagle positioned in plastic holder for whole-body gamma counting. The impracticality of this procedure in an unanesthetized animal is obvious. Note television camera used to monitor dog during counting interval. (Photo courtesy of Dr. C. W. Mays).

Fig. 3. Anesthetized beagle being inserted into liquid scintillator counter. Again, this procedure would not be practical in the unanesthetized animal. (Photo courtesy of Dr. C. W. Mays).

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LOW NATURAL INCIDENCE OF OSTEOSARCOMAS IN BEAGLES

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ABSTRACT: The occurrence of osteosarcomas in non-irradiated beagles is extremely low, as it also is in man. Based on a literature search and inquiries to veterinary colleges, the fraction of deaths with natural osteosarcomas for beagles in an environment favoring long life is estimated at 1 to 100 osteosarcomas per 100,000 deaths.

To date in our basic program, 192 radioactive beagles have died. Osteosarcomas were diagnosed in 135 (or 70%). Is the natural incidence of this bone cancer so low in the beagle that every one of these osteosarcomas can be ascribed to internal irradiation? The following study gives an answer of "yes".

Osteosarcomas are an infrequent form of cancer in the dog. Mulligan⁽¹⁾ lists only 5 osteosarcomas out of 1000 consecutive canine neoplasms, of which about 450 were malignant. Cotchin⁽²⁾ found 26 osteosarcomas in examining 820 canine neoplasms.

Canine osteosarcomas are especially uncommon among the smaller breeds⁽²⁻⁴⁾. The following osteosarcoma frequencies were computed (Table 1), using the tumor and admission data of Brodey et al.⁽³⁾ and the weight classifications of Davis⁽⁵⁾, assuming that the weight distributions for mixed and unspecified breeds were similar to those for the listed breeds.

TABLE I

OSTEOSARCOMA FREQUENCY BY DOG WEIGHT

(Computed from tumor and admission data of Brodey
and weight classifications of Davis.)

Weight (lbs.)	Approx. Admissions at U. Penn. Vet. School	Number Osteosarcomas	(<u>Admissions</u> <u>Osteosarcoma</u>)
5 - 15	3269	0	∞
15 - 30	24072	6	4012
30 - 80	22548	92	245
Over 100	861	32	27
Total	50750	130	390

Having found no natural beagle osteosarcomas in the veterinary literature⁽¹⁻⁴⁾, we sent inquiries to 26 veterinary colleges requesting (a) their approximate number of beagle admissions, (b) the number of beagles with osteosarcomas, and (c) the name of anyone believed to know of a beagle osteosarcoma. The 20 replies as of 30 October 1964 revealed only 2 beagle osteosarcomas: one at Texas A. & M. reported by Dr. J. A. Bowen, and the other at Michigan State University reported by Drs. W. O. Brinker, W. A. Priester, and R. A. Tjalma.

The respondents estimated their summed yearly rate of beagle admissions at over 4,000 per year. Yearly registrations of the American Kennel Club⁽⁶⁾ indicate that the average size of the registered beagle population during the past 15 years is close to its present value*.

*In 1963 there were 49,769 beagle registrations; from 1959 thru 1963 an average of 53,583 beagle registrations per year; and from 1949 thru 1963 an average of 50,593 beagle registrations per year⁽⁶⁾.

Assuming these veterinary colleges have kept complete records during the last 5-15 years, the corresponding total number of their beagle admissions is then about 20,000 for a 5 year period, or 60,000 for a 15 year period. The resulting ratio of 10,000-30,000 beagle admissions per beagle osteosarcoma indicates that the beagle is much less susceptible to osteosarcomas than the average dog (Table I).

Using these data, upper and lower limits can be computed for the incidence of osteosarcomas in beagles allowed to live out their natural lifetimes.

FOR AN UPPER LIMIT ASSUME:

- (a) 100% will die with neoplasms,
- (b) the general dog population has 26 osteosarcomas/820 neoplasms⁽²⁾,
- (c) the beagle is 390/10,000 (or 1/26) as susceptible to osteosarcomas as is the average dog (Table I and our Veterinary College survey).

Upper limit on incidence of beagle osteosarcomas

$$\begin{aligned} &= \left[\frac{100 \text{ neoplasms}}{100 \text{ deaths}} \right] \left[\frac{26 \text{ dog osteosar.}}{820 \text{ dog neopla.}} \right] \left[\frac{390 \text{ dog admission/dog osteosar.}}{10,000 \text{ beagle adm/beagle osteosar.}} \right] \\ &= \left[\frac{1 \text{ beagle osteosarcomas}}{1,000 \text{ beagle deaths}} \right] \text{ or } 10^{-3} \end{aligned}$$

FOR A LOWER LIMIT ASSUME:

- (a) 25% will die with neoplasms*
- (b) the general dog population has 5 osteosarcomas/1000 neoplasms⁽¹⁾,

*Including those groups of beagles showing severe radiation effects (such as osteosarcomas), our colony has had 48 deaths of which 12 (or 25%) have been with non-skeletal cancers. Cancers of the soft tissues are expected to become a more prominent form of death as our animals grow older.

(c) the beagle is 390/30,000 (or 1/77) as susceptible to osteosarcomas as is the average dog.

$$\begin{aligned} & \text{Lower limit on incidence of beagle osteosarcomas} \\ &= \left[\frac{25 \text{ neoplasms}}{100 \text{ deaths}} \right] \left[\frac{5 \text{ dog osteosar.}}{1000 \text{ dog neopla.}} \right] \left[\frac{390 \text{ dog admission/dog osteosar.}}{30,000 \text{ beagle adm./beagle osteosar.}} \right] \\ &= \left[\frac{1 \text{ beagle osteosarcoma}}{100,000 \text{ beagle deaths}} \right] \text{ or } 10^{-5} \end{aligned}$$

DISCUSSION

We have computed upper and lower limits to emphasize that the data available do not permit exact calculation of the true incidence. Limitations exist in any questionnaire survey, and there are uncertainties in how beagles compare with the general canine population in age distribution, age at osteosarcoma diagnosis, and reasons for admission to veterinary colleges. Taken at face value, these limits indicate that roughly 1,000-100,000 beagles of long lifespan would be required to produce a single spontaneous osteosarcoma.

At the Long Term Effects session of the 1964 Radiation Research Society meeting we asked investigators studying beagles (at Davis, California; the Argonne National Laboratory; Fort Collins, Colorado; Rochester, New York; Richland, Washington; and Albuquerque, New Mexico) if they were aware of even 1 osteosarcoma in a non-irradiated beagle inside or outside of their colonies. No cases were indicated, nor have we observed any osteosarcomas among our control beagles. This adds weight to the conclusion that naturally-occurring osteosarcomas are extremely rare in beagles.

This low incidence of natural osteosarcomas makes the beagle an ideal research animal for studying the effects of skeletal irradiation.

We will eventually have about 200 low-level radioactive dogs on our project whose skeletal dose-rates are probably too small to shorten group lifespans appreciably. The low probability of $\left[\begin{matrix} 200 \text{ dogs} \\ (10^{-5} \text{ to } 10^{-3})/\text{dog} \end{matrix} \right] = (0.002 \text{ to } 0.2)$ that a single osteosarcoma would arise spontaneously in a group this size gives confidence that each osteosarcoma which develops can be ascribed to irradiation. In the same number of heavily irradiated dogs, the chance occurrence of a spontaneous osteosarcoma is even lower due to the shortened life-span available for such effects to appear.

It is interesting that the osteosarcoma/death ratio in man* of about 2×10^{-4} falls within the range of 10^{-5} to 10^{-3} calculated for the beagle. Relative biological effectiveness values among radio-nuclides in producing osteosarcomas in the beagle can be extrapolated to man with added reliance.

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*In 1962 there were 1750 reported "bone" cancers in a total of 1,757,000 deaths in the U. S. A.⁽⁷⁾ However, not all "bone" cancers reported on death certificates are osteosarcomas. A study by Auerbach⁽⁸⁾ revealed only 35 osteosarcomas in 166 "death certificate reported" bone cancers.

$$\left[\frac{1750 \text{ rept. bone ca.}}{1,757,000 \text{ deaths}} \right] \left[\frac{35 \text{ osteosarcomas}}{166 \text{ rept. bone ca.}} \right] = \frac{2 \text{ human osteosarcomas}}{10,000 \text{ human deaths}}$$

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THE EFFECTS OF IONIZING RADIATIONS ON ENDOCRINE CELLS
IV. INCREASED PRODUCTION OF $17\alpha,20\alpha$ -DIHYDROXYPROGESTERONE BY
RAT TESTES AFTER IRRADIATION

David L. Berliner¹ and LeGrande C. Ellis²

Abstract: Rat testicular mince was incubated with progesterone-4- C^{14} . From the incubation mixture, $17\alpha,20\alpha$ -dihydroxyprogesterone was isolated and characterized as one of the main steroids of the high-polar steroid fraction. The biosynthesis of this steroid has been shown to be altered after various modes of irradiation. When $17\alpha,20\alpha$ -dihydroxyprogesterone was incubated with mouse testicular tissue, only small amounts of this steroid were biotransformed into 17α -hydroxyprogesterone, androstenedione and testosterone. These data indicate that an accumulation of a non-androgenic steroid occurs after direct irradiation to the testes.

Previous investigations on the biotransformation of 5-pregnenolone³ and progesterone into androgens by mouse (1) and rat (2,3) testes have shown that there are considerable amounts of these two substrates that are biotransformed into steroids with a polarity greater than testosterone. Furthermore, the amount of 5-pregnenolone and progesterone being converted into these so-designated "high-polar" steroids was altered following either localized irradiation to the testes (2) or irradiation to the heads (3) of rats. This paper describes the biosynthesis, isolation, and characterization of one of these high-polar steroid compounds, and the subsequent biotransformation of this compound into testosterone and its precursors by murine testicular tissue. The relationship between the biosynthesis of this high-polar steroid with irradiation and aging is also discussed in this paper.

MATERIALS AND METHODS

A large quantity of $17\alpha,20\alpha$ -dihydroxyprogesterone was biosynthesized according to the following procedure. Twenty-six male rats (Sprague-

Dawley strain) were euthenized when 14 weeks of age by light ether anesthesia and cervical dislocation. The testes from these animals were quickly removed and chilled in ice-cold phosphate buffer, pH 7.4. After being trimmed and weighed, the testes were finely minced with razor blades. Progesterone-4-C¹⁴ (New England Nuclear Co.) was purified by paper chromatography (4) and adjusted to a specific activity of 10 µc/10 mg with unlabeled progesterone. The µc (10 mg) of this mixture in 99 ml of methanol and 1 ml of propylene glycol were transferred to a four liter Erlenmeyer flask. The methanol was blown off with a stream of nitrogen and 77.5 grams of testicular mince, 800 ml of phosphate buffer (pH 7.4), and 100 µ moles each of NADPH and glucose-6-phosphate (Sigma Chemical Co.) were transferred to the flask. The contents of the flask were incubated for three hours under atmospheric air at 37.5° C in a constant temperature bath (American Instrument Co.). At this time, the reaction was stopped, and the steroids were extracted and isolated as described elsewhere (1,5). Characterization and identification of 17α, 20α-dihydroxyprogesterone were accomplished through paper chromatography and derivative formation (acetylation with acetic anhydride and oxidation with chromic acid) (5). Steroids on the paper chromatograms were visualized with an ultraviolet scanner and an electronic (Geiger-Mueller) scanner (6).

In order to ascertain if 17α,20α-dihydroxyprogesterone could be bio-transformed into androgens, 92,952 CPM of this steroid were incubated with 0.533 grams of testicular mince from CBA mice (14 weeks of age) in 10 ml of phosphate buffer, pH 7.4. This mixture was incubated 2 1/2 hours at 37.5° C under atmospheric air. No NADPH-generating system was

used with this incubation. Testosterone and its immediate precursors were isolated and characterized as previously described in the literature (1).

RESULTS

A large quantity of progesterone was separated from the steroid mixture after chromatographing the steroid extract in the hexane: formamide system. Similarly, androstenedione, testosterone, and 17α -hydroxyprogesterone were separated from the steroid extract after chromatographing it in the hexane-benzene: formamide system. Steroids from this second chromatogram with an R_f less than 0.30 were eluted from the chromatogram and rechromatographed in the benzene: formamide system. A large quantity of ultraviolet positive, radioactive labeled steroid with an R_f of 0.29 was observed. This compound was identified as $17\alpha,20\alpha$ -dihydroxyprogesterone on the basis of the following characteristics: a) the presence of Δ^4 -3-keto group as demonstrated by an ultraviolet spot on the chromatogram and confirmed by an absorption maximum of 240 $m\mu$ as observed with a Ratio Recording Spectrometer (Beckman Inst. Co.), b) the presence of only one acetyltable group, which disappeared when an aliquot of the steroid was oxidized prior to acetylation, c) no change in mobility when the steroid acetate was subjected to mild oxidation with chromic acid, d) the products formed after mild oxidation of the free steroid with chromic acid were 17α -hydroxyprogesterone and androstenedione, and e) the inversion of polarity of this compound with respect to its isomer $17\alpha,20\beta$ -dihydroxyprogesterone upon acetylation which is typical for 20α - and 20β -hydroxylated steroids (5).

When the original material having an R_f 0.29 in the benzene: formide

system was incubated with mouse testicular tissue, the following steroids were isolated (Table I): 17α -hydroxyprogesterone, androstenedione, and testosterone.

The amounts of high-polar compounds formed after direct irradiation of the testes and heads of rats are recorded in Table II along with their respective probability statements.

DISCUSSION

The isolation and characterization of $17\alpha,20\alpha$ -dihydroxyprogesterone from incubations of rat testicular tissue show that this tissue is capable of reducing the 20-ketone of some steroids. Both 20α - and 20β -reductases have been previously observed in human, fetal testicular tissue (7), and avian testicular tissue (8). Dominguez et al. (9) reported the isolation of the 20β -isomers of progesterone in normal rat testes. Only a small amount of $17\alpha,20\beta$ -dihydroxyprogesterone was observed in this investigation, but 12.6% of the progesterone in the original incubation mixture was converted into $17\alpha,20\alpha$ -dihydroxyprogesterone.

Although the physiologic role of 20α -reductase is not known for the testes, the presence of this enzyme has been reported in malignant tumor tissue (9). Moreover, in avian testes (10) there appear to be four 20 -reductase enzymes (8), which appear to be located in the germinal elements of the testis. These workers (10) observed a small increase in the activity of both 20α - and 20β -reductase enzymes, which was associated with gonadotrophin injections. However, there was a more marked increase in the activity of these two enzymes concomitant with an increase in the germinal elements during the normal cycling of the English Sparrow. Other data indicate that there is an increase in 20 -reductase activity

associated with aging (L. R. Axelrod - personal communication).

The data from this investigation show that $17\alpha,20\alpha$ -dihydroxyprogesterone is one of the main steroids of the high-polar steroid fraction; and, therefore, fluctuates after irradiation (2,3). When rat testes were directly irradiated with 1500 r of x-rays, there was a decrease in the ability of this tissue to biotransform steroids into androgens 20 days after treatment (2). There was, however, an increase in the amount of $17\alpha,20\alpha$ -dihydroxyprogesterone produced per mg of tissue used in the incubations (Table II). On the other hand, the amount of this compound produced by testicular tissue from head-irradiated rats was decreased (Table II) by this treatment. The difference in response noted in these two investigations is probably due to the difference in the mode of administration of the irradiation. It would appear from these data that the in vitro biosynthesis of $17\alpha,20\alpha$ -dihydroxyprogesterone is related to the physiologic state of the testis.

The data from this investigation (Table I) show that $17\alpha,20\alpha$ -dihydroxyprogesterone can be reversibly biotransformed into 17α -hydroxyprogesterone, androstenedione and testosterone. The probable sequence for these biotransformations appears in Fig. 1. It would appear from these data (Table I) that side-chain cleavage of $17\alpha,20\alpha$ -dihydroxyprogesterone into androstenedione represents the minor of two possible routes for the biotransformation of this high-polar compound into androgens. The slow rate of conversion of $17\alpha,20\alpha$ -dihydroxyprogesterone into androgens undoubtedly reflects the low biological activity of 20-hydroxylated derivatives of 17α -hydroxyprogesterone (11) and progesterone (12).

TABLE I

The Conversion of $17\alpha,20\alpha$ -Dihydroxyprogesterone
into Testosterone by Mouse Testes

Steroid Isolated	Counts Per Minute	Percent Conversion
$17\alpha,20\alpha$ -dihydroxyprogesterone	92,952	
17α -hydroxyprogesterone	2,150	2.30%
Androstenedione	250	0.26%
Testosterone	34	0.03%

TABLE II

Amounts of $17\alpha,20\alpha$ -Dihydroxyprogesterone Formed
by Rat Testicular Tissue Following Various Modes
of Irradiation

Experimental Group		
<u>Localized irradiation of testes</u>		
Control	$13.45 \pm 1.5^*$	P 0.05**
Irradiated (1500 r)	28.25 ± 4.70	
<u>Head irradiated rats</u>		
Control	14.16 ± 0.60	P 0.025
Irradiated	11.20 ± 0.10	

* Standard error of mean.

** Statistical comparisons were made by using a "t" test.

Fig. 1. Proposed pathway for the synthesis of $17\alpha, 20\alpha$ -dihydroxyprogesterone and the subsequent conversion of this compound to androgens.

FOOTNOTES

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³In this paper, the following abbreviations are used: 5-pregnenolone, 5-pregnen-3 β -ol-20-one; progesterone, 4-pregnene-3,20,-dione; 17 α -hydroxyprogesterone, 4-pregnen-17 α -ol-3,20-dione; androstenedione, 4-androstene-3,17-dione; testosterone, 4-androsten-17 β -ol-3-one; 17 α ,20 α -dihydroxyprogesterone, 4-pregnene-17 α ,20 α -diol-3-one; 17 α ,20 β -dihydroxyprogesterone, 4-pregnene-17 α ,20 β -diol-3-one; NAD and NADH, oxidized and reduced forms of nicotinamide adenine dinucleotide; and NADP and NADPH, oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate.

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SERUM LACTIC DEHYDROGENASE LEVELS
IN ADULT BEAGLE DOGS

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Abstract: Serum lactic dehydrogenase (LDH) determinations were performed on 101 beagles from a colony used in the study of long range effects of internally deposited radionuclides. Statistical analysis of results revealed no significant differences among groups separated by radioisotope injected, number of days elapsed since nuclide injected, and disease state. Sera from moderately hemolyzed blood samples were significantly higher in LDH level than sera from nonhemolyzed or slightly hemolyzed blood samples. Dogs who received high doses of radionuclides had blood LDH levels significantly higher than those of control dogs. It is concluded that serum LDH is not a consistent indicator of pathologic states in the adult beagle dog.

Innumerable reports have been made concerning the correlation, or lack of it, between lactic dehydrogenase (LDH) activity in serum, plasma, or tissue homogenates, and presence of neoplastic disease. Interpretation of reports has been complicated by the use of myriad methods of assay, and testing of many different species. Thus, comparisons between LDH catalyzing pyruvate to lactate (1, 2, 3, 4), and LDH catalyzing the reverse reaction (5), the many modifications of these procedures, and the differences in the various isozymes (6, 7, 8) of LDH must all be considered. Moreover, not only are the normal values for these procedures not comparable, they differ according to species tested for both average values and those found during disease states.

In our laboratory, as well as in others, studies on the long term effects of internally deposited radionuclides are being pursued in a large colony of beagle dogs (9). Since a very important long term effect of radiation is the formation of tumors, a method of detecting the early presence of neoplastic tissue is desirable. It was our purpose, in this study to establish a norm for LDH in dog serum, and to determine whether

or not LDH level is a good indicator of the presence, growth, and extent of neoplasia.

METHODS

Serum was obtained from routine blood letting of colony dogs. The blood was slowly withdrawn through a large gauge needle and slowly emptied into test tubes, standing in ice, care being taken to avoid hemolyzing the erythrocytes. The blood was then spun in a refrigerated centrifuge and the serum removed. At no time between drawing and testing, was the serum allowed to rise above 5°C . LDH level was determined by the method of Snodgrass (5) at $22.6 \pm 0.3^{\circ}\text{C}$. The temperature was tested inside the cuvette before and after the 3 minute spectrophotometer reading interval.

In general the serum was tested within a few hours of the time it was drawn, although in a few cases, as much as 24 hours elapsed before the LDH level was determined. Samples to be kept longer than 3 hours were frozen. Repeat determinations over 24 hour periods on 15 different frozen samples showed a mean LDH of 30 units, with an average variation of 4 units and a maximum variation of 10 units (one sample only). The procedure was tested against Versatol E¹, and consistently gave results 20% higher than the values reported in the brochure accompanying the standard.

RESULTS

A total of 101 different animals were tested for serum lactic dehydrogenase. Many dogs were tested two and sometimes three separate times,

¹General Diagnostics Division of Warner Chilcott.

but in these cases, only the first determination was used in figuring statistical significance of group differences.

The grand mean for all animals tested was 22.1 units LDH activity.

All of the dogs tested were part of a colony used in the study of internally deposited radionuclides. The radioisotopes included Th^{228} , Ra^{224} , Ra^{226} , Ra^{228} , Sr^{90} and Pu^{239} , all of which had been injected intravenously as a single dose of varying levels of radioactivity (9). Table I is a summary of the LDH values of the dogs, using isotope as the factor of variation. Although the mean values ranged from 17.5 ± 4.5 to 34.0 ± 6.1 , an analysis of variance revealed that there were no significant differences among the groups ($F = 1.3$).

Time elapsed since radioisotope injection was considered as another possible parameter of variation. Since all animals were injected at approximately 500 days of age, this not only correlated with length of time in contact with radionuclide, but also with the dog's actual age. Dogs were separated into four groups, by days post-injection: (a) 0-500, (b) 501-2000, (c) 2001-3000, (d) more than 3000. Table II summarizes this analysis. Pre-injection dogs were not included in this analysis, since group (a) was a good control group of comparable age. Although the older dogs have an average LDH level well below the others, the sample size is small and the standard deviation large. The difference is not significant ($F = 0.66$).

A comparison between animals which were clinically well and showed no abnormalities by chemical or hematological laboratory procedures and those which had tumors or other abnormalities was undertaken. Reference to Table III shows the animals to be divided into 5 groups: (a) normal, (b) those with a clinical diagnosis of tumor, probably benign, (c) those with

TABLE I
LDH Values on Radioisotope Burdened Dogs

Isotope	n	Mean LDH Units Per Dog	S. D.	F
Control	35	19.7	3.3	
Th ²²⁸	14	23.9	5.2	
Ra ²²⁴	7	24.9	7.4	
Ra ²²⁶	19	17.5	4.5	
Ra ²²⁸	10	31.6	6.2	
Sr ⁹⁰	10	34.0	6.1	
Pu ²³⁹	15	21.6	5.0	
Total	110	22.8		1.3

TABLE II
LDH Values on Dogs at Various Intervals After Injection

Days PI	n	Mean LDH Units Per Dog	S. D.	F
0-500	28	24.1	4.1	
501-2000	18	26.8	5.1	
2001-3000	16	25.0	5.4	
Over 3000	8	14.2	7.6	
Total	70	23.9		0.66

histologically diagnosed benign tumors, (d) those with histologically identified malignant tumors, and (e) those with abnormal hematological or blood chemical changes, or clinical disease other than tumor. There is tremendous individual variation between animals in the same group, as reflected in the large standard deviations. Perusal of the data reveals no trend toward higher or lower LDH values in any group, and this is born out by the analysis of variance, which indicates that the sick and well dogs comprise approximately the same population.

Owing to the technical problems involved in bleeding large numbers of dogs daily, plus the extreme fragility of the beagle red blood cell, the study included a small percentage of hemolyzed sera. All samples, therefore, were visually compared to a color chart and classified as 0 through 5+ hemolysis. Five sets of samples were analyzed for oxyhemoglobin in a Beckman DU spectrophotometer at 540m μ to establish the approximate range and relative accuracy of the visual method. Sera classified between 0 and 2+ by the visual method varied from 0.010 gm % Hb to 0.018 gm %, with the great majority falling between 0.016 and 0.018 gm %. Sera classified as 3+ measured at 0.038 to 0.042 gm % hemoglobin. Bloods hemolyzed to a greater extent than 3+ were discarded without being assayed for LDH. Table IV presents the statistical analysis of the LDH determinations using degree of hemolysis as the factor of variation. The F ratio of 4.3 is significant, indicating that the means are from different populations. Individual t-tests, using the 0 hemolysis group as the control, indicate that the 3+ group is significantly higher in serum LDH at the 0.02 - 0.05 level ($t = 2.35$).

Because of the high mortality among dogs receiving large doses of radioactivity, only 0, 1, 2, and 3 level (9) dogs were available for LDH

TABLE III
LDH Values on Healthy and Sick Dogs

Group	n	Mean LDH Units Per Dog	S. D.	F
Normal (a)	57	20.6	2.6	
Clinically Benign Tumor (b)	6	30.0	7.9	
Histologically Benign Tumor (c)	4	14.0	9.7	
Malignant Tumor (d)	8	30.8	6.8	
Abnormal (e)	31	21.8	3.5	
Total	106	22.0		0.91

TABLE IV
LDH Values on Hemolyzed Sera

Degree Hemolysis	n	Mean LDH Units Per Dog	S. D.	F
0	41	16.4	2.9	
1+	24	25.9	3.7	
2+	47	22.6	2.7	
3+	5	45.2	8.2	
Total	117	22.1		4.3

assay. Small sample sizes made analysis by isotope impossible, but t-tests were performed comparing 0-level (placebo injection) to each of the 1, 2, and 3 level groups. Level 3 animals received approximately 3 times the dose ($\mu\text{C/kg}$) given 2-level animals, and 20 times that given 1-level animals. The numbers of values from each isotope group were roughly the same for each dose level. Reference to Table V reveals that the 3-level beagles had significantly higher serum LDH values than the control group. Table VI is a compilation of all the individual LDH determinations.

DISCUSSION

The mean LDH value of 22.1 units for 117 dogs tested, is considerably lower than the average value of 59 units obtained by Snodgrass et al. (5), using human serum. Interspecies differences are well known in all enzyme systems, and this result merely reinforces the need to establish norms for the species being considered, as well as for the laboratory in which the work is being done.

The results of the testing of animals receiving different radio-nuclides were not remarkable. These animals had been initially chosen for injection because of comparability of age, lineage, and health. Most of the high level animals in all groups succumb to osteosarcoma, and although the number of osteosarcomas, as well as cases of hepatic necrosis, is largest in the Pu^{239} dogs (9, 10), this was not reflected in any alteration of serum LDH. However, it must be remembered that our samples were definitely weighted to the low dose side and that many of the high level animals died before this study was initiated.

Because animal age is found to effect some enzyme levels, and most radioisotope effects manifest themselves mainly after long term exposure,

TABLE V
t-test Analysis of Serum LDH
in Dogs Which Received Different Doses of Radionuclides

Group	n	Mean LDH Units Per Dog	t	P
0-level	14	15.7		
1-level	12	20.8	0.68	0.5
2-level	8	16.5	0.15	0.8-0.9
3-level	8	56.0	2.90	< 0.01

TABLE VI
Individual LDH Values on All Beagles Tested

Dog	Days PI	Units LDH	Dog	Days PI	Units LDH
FAC44	3300*	16	M8T2	1078	10
45	3301*	14	M3S0	2596	14
76	1484*	16	1	2596	20
MAC61	2218*	12	1.7	2596	8
FAC77	1469*	8	2	2596	40
MAC84	745*	24	3	2596	74
FAC83	745*	14	4	2596	26
15	4386*	14	M5S1.7A	371	96
62	2170*	22	M6S0	2171	10
13	4526*	22	1	2171	10
M2T1	2560	12	1.7	2171	8
F12T0	225	8	2	2171	10
0.5	225	14	3	2171	48
3	225	54	T63R3	26	22
F6T3	1156	98	64	26	56
F3T0.2	727	8	65	26	20
M4	727	4	66	26	16
1	727	10	67	26	14
2	727	2	68	26	18
F10T3	971	36	69	26	22
M8T0	1076	26	70	26	24
0.2	1076	16	T65R3	77	20
0.5	1076	48	66	77	24
1	1076	10	67	77	24
1.5	1076	12	68	77	10

* Age at time of testing.

(TABLE VI Con't.)

Dog	Days PI	Units LDH	Dog	Days PI	Units LDH
T69R3	77	20	F13P0.1	40	16
70	77	10	0.2	40	16
F8R0	3355	22	0.5	40	4
T32R3	1835	14	T19P5	1217	22
M2R1.7A	371	28	F14P0	- 1	10
2	3775	14	0.1	- 1	10
F3R1	3666	8	0.2	- 1	70
2	3666	4	0.5	- 1	20
M13R0	33	12	F31P0B	- 1	20
0.2	33	14	0C	- 1	74
0.5	33	6	0B ₁	- 1	8
1	33	12	0.2	- 1	22
M4R0	3661	10	0.2B	- 1	16
F9R1	3292	8	T64R3	- 1	30
2	3292	20	65	- 1	16
M1R1	4018	12	66	- 1	16
F7M0A	399	14	67	- 1	18
F8M3	1464	28	68	- 1	20
F5M0	2563	16	69	- 1	16
1	2575	10	70	- 1	80
2	2575	20			
M3M0	2934	12			
1	2934	16			
M9M0	1468	14			
0.5	1468	12			
1	1468	36			
3	1468	96			
M6M0	2561	22			
1	2561	72			
1.7	2561	12			
2	2561	14			
F7M0	461	20			
T14Q3	103	18			
11	103	24			
10Q4	103	30			
12Q3	103	42			
9Q4	103	24			
7Q5	168	20			
13Q3	140	116			
12	140	14			
F2P1	4027	24			
T19P5	1196	20			
M8P0	3298	20			
1	3298	24			
M31P0.1B	40	20			
0.2B	40	26			
0.5B	40	14			

the analysis of data as a function of days elapsed since injection was considered as having possible pertinence. Although the data show that this factor is not of significance in this population, contending factors may have interacted, to obliterate a difference of LDH level with age. For example, the enzyme level in older dogs may diminish, while prolonged exposure to internally deposited radionuclides may give rise to conditions leading to elevated LDH. The latter supposition would tend to be discounted, however, on the basis of the analysis of the tumor bearing animals, since these did not show a statistically significant difference in enzyme level either. The dogs which were 3000 days or longer from injection did show a trend toward lower LDH values, with an average value 10 units below the next lower group. Comparison of youngest and oldest dogs confirmed that statistically, no difference exists, ($t = 1.22$, $P = 0.2 - 0.3$); nevertheless in view of the large standard deviations, the trend invites speculation.

The failure to discover any correlation between disease state and LDH level is interesting. Most studies, such as those of Bierman et al. (11), Zimmerman et al. (12), West and Zimmerman (4,13), Hsieh and Blumenthal (14), and Nishio et al. (15), present data as number of tests showing elevated LDH compared to total number of tests run. In all cases the percentages vary tremendously, and it is impossible to determine whether or not the differences are significant in any one experiment. In at least some of these, however (12, 13), there is illustrated some kind of correlation with malignancy, metastatic malignancy, or liver damage. The failure of this study to show any such correlation may be due to too small a sample size. It is more likely, however, that lactic dehydrogenase

activity in serum is not a reliable indicator of pathological states in the dog. Perusal of individual determinations shows several values over 100 units, in which no other abnormality could be found in the animal.

The data from the hemolysis studies indicate what could be logically deduced, namely that elevation of serum LDH level will occur if there is rupture of cells which contain the enzyme in large concentration (2). This, however, presents an interesting contrast with the work of Zimmerman et al. (16), who found normal LDH levels in patients suffering from hemolytic anemia. The mechanisms of hemolysis are, of course, markedly dissimilar, demonstrating that in vivo some metabolic process either removes the LDH from the serum or lowers its level in the weakened erythrocyte.

The main positive data to emerge from this study are the most puzzling. The fact that dogs receiving high levels of internal radiation have high serum LDH levels does not correlate with the normal LDH values found in animals afflicted with the diseases most commonly associated with high doses of the radionuclides in question. Again, the paucity of data from tumor bearing and cirrhotic beagles may be the cause of the confusion. Large amounts of internally deposited radionuclide may either cause a metabolic change reflected in upswing of serum LDH activity or the elevated enzyme level may be indicating early, undetected pathologic states in animals which will eventually develop full blown disease. The nonspecificity of response of serum LDH level in disease states makes interpretation difficult.

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CHEMICAL STATE OF Pu IV IN BEAGLE BLOOD

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Abstract: The method of gel-filtration was used to study in vitro the binding and the distribution of Pu IV in dog plasma. It was found that the main portion of Pu was bound by albumin. Only a small amount of plutonium was associated with high molecular weight proteins. The medium weight globulins of type 7S and 4-5S were essentially free of plutonium.

Studies on the Pu content of plasma after intravenous injection of Pu²³⁹ have been published previously by this laboratory⁽¹⁾. Approximately 85% of the injected Pu²³⁹ is circulating in the plasma at one minute. Between one minute and ten hours, the concentration dropped by a factor of about 2 and decreased from then until three weeks after injection by a factor of 250. When compared to similar treatments using the alkaline earth elements in the place of Pu²³⁹, the plasma concentration of these elements decreased at a rate of more than ten times faster than that of plutonium⁽²⁾. Hence, the binding of Pu²³⁹ to the plasma constituents must be appreciably stronger.

The present investigations are an attempt to determine the binding sites of Pu in plasma and specifically to plasma proteins. All studies were carried out with in vitro tagged plasma proteins and isolated dog proteins.

Both heparinized and unheparinized blood from healthy dogs of the colony was incubated with Pu IV in the citrate buffer which is used for injection solutions. Plasma and blood cells were separated by centrifugation. The blood cells were washed three times with saline solution and analyzed for their Pu content. The uptake was very small, less than 0.5%, evaluated for the amount of cells contained in 1 ml of whole blood.

These cells were ruptured and membranes separated from proteins by high speed centrifugation. The Pu was found to be associated with the cell membranes. No Pu had penetrated into the cells after one hour of incubation.

The plasma was subjected to ultrafiltration by the Toribara method (3). The ratio of the plutonium concentration in the clear ultrafiltrate to the concentration in the original plasma was only $6.774 \times 10^{-3} \pm 2.65 \times 10^{-3}$. No attempt, however, was made to make any prediction of the relative quantities of Pu associated with proteins and the relative quantity of Pu in ionic form since the cellophane bags (Visking Nojax casing) used were found to retain an appreciable quantity of Pu²³⁹ under the physiological conditions applied. The largest fraction of the activity, however, was bound by the non-filterable constituents.

In order to decide whether Pu was not taken up by the cellular fraction of the blood because of an unfavorable equilibrium produced by the fast uptake and tight binding of the metal ion by proteins, or because of the inability of plutonium to penetrate the membranes, blood cells were freed from plasma by repeated washings with either saline solution or a physiological phosphate buffer. The plasma contamination of the blood cells after four successive washings was less than one tenth of one percent. Cells, washed with saline solution, were incubated with Pu IV citrate for times of 0-24 hours, washed again with saline solution and analyzed for Pu. The uptake was very small (less than 1%) and did not increase significantly with time. Cells washed with physiological phosphate buffer were incubated with Pu IV citrate for times of 0-96 hours. The Pu uptake in this case was higher and increased during

Fig. 1. The uptake of Pu^{239} by isolated red blood cells in physiological phosphate buffer over a period of 96 hours. Hemolysis begins to occur at between 18 and 24 hours.

Fig. 2. Protein elution pattern (solid line) of fresh dog plasma after chromatography on Sephadex columns. The dotted line indicates the relative plutonium content of these proteins.

the first 18 hours after incubation. The Pu content, however, declined during the time from 18-96 hours (Fig. 1). This decrease in activity was accompanied by a progressive hemolysis. As a result, it is found possible to tag red blood cells with Pu²³⁹ if a proper buffer is chosen. Hemolysis, however, accompanied by a release of protein into the surrounding medium tends to reverse this reaction, which in turn is a sign of only weak bonding by the cells, possibly only the cell membranes.

Because of the high charge of the plutonium ion, Pu will detach from its protein-binding sites if a sufficient voltage gradient is applied. Therefore, electrophoretic methods have to be excluded from determinations of its binding sites as well as ion exchange chromatography. Gel-filtration methods, however, based primarily on molecular sieving, provided an excellent means for the separation of plasma proteins under mild conditions. Dextran gels of low cross linkage (Sephadex G-100 and G-200) were used in all experiments. The unit column size was 2 by 50 cm. Whenever more than one unit was used, the columns were linked by small Teflon tubing. Fractions of approximately 4 ml were collected in a Packard fraction collector Model 234. The protein content of these fractions was determined spectrophotometrically by reading in a Beckman D.U. spectrophotometer at a wave length of 280 mμ, and the corresponding Pu concentration was measured by a liquid scintillation counting technique. A 4% NaCl - 0.1M Tris solution of pH 7.5 served as the elutriant throughout the experiment. Depending upon the cross linkage of the resin, fresh plasma was separated into 3 main peaks. The protein composition of the different segments of each peak was determined electrophoretically. Peak one contained the fast moving macroglobulins and high molecular

Fig. 3. The protein elution pattern of NaN_3 stabilized day serum on Sephadex columns. The serum was chromatographed at 0.2, and 7 days. The broken lines indicate the relative Pu^{239} content at these time intervals.

Fig. 4. The protein elution pattern of rapidly degraded dog plasma from Sephadex columns. The broken line shows the relative Pu^{239} content associated with the protein peaks.

weight lipoproteins (19S-type). Results compare closely to those reported by Flodin⁽⁴⁾. These were followed by globulins (7S and 4-5S-type). The third peak consisted mostly of albumin and some globulin (molecular weight from 66,000 - 150,000).

In order to get an idea of the relative binding affinities for the various proteins, dried bovine albumin and α , β , γ -dog globulins were dissolved in physiological phosphate buffer, incubated with Pu IV citrate and subjected to gel filtration on a highly cross-linked gel (Sephadex G-25) which permitted the separation of protein bound Pu and nonprotein bound Pu. The α - and β - globulins showed the highest affinity, followed by albumin and γ -globulin. No other meaning, however, should be attached to this experiment except that basically all proteins are capable of interacting with plutonium. The degree of hydration, the homogeneity, the spacing in the protein crystal, and the manner in which groups in side chains are surrounded by water dipoles and peptide linkages in the main chain are exposed, are certainly different in the living system. The distribution of Pu will depend to a high degree on those conditions and a certain selectivity is to be expected.

Plasma was incubated for one hour with Pu²³⁹ and applied to a triple column (Sephadex G100-G200-G100). The corresponding spectra showed that some Pu was associated with the 19S type (high mol. wt.) proteins. Virtually no Pu was contained in the large second peak and the main portion of Pu appeared on the left shoulder of the third peak which contained largely albumin and some globulin. After a steep decline, the activity rose again slightly on the descending limb of the final protein peak (Fig. 2).

Fig. 5. Electrophoretic pattern of the proteins from each of the three peaks separated by gel filtration. The dotted line represents the spectrum of whole plasma.

Pu^{239} tagged and NaN_3 stabilized serum was chromatographed on a G200-G200 column at the following times: 0, 2 days, 7 days. Although there was no significant change in the protein elution pattern, except for the appearance of a small fourth peak at a considerable distance to the right of the third peak, the plutonium content in peaks number one and three became much smaller. The plutonium lost in these peaks appeared in the fourth peak which comprises low molecular weight peptides (Fig. 3).

Fig. 4 shows unstabilized and rapidly decomposing plasma of initially high Pu content. The activity to the right of the third peak in this case did not decline to the usual low level but remained relatively high and ended in a strong peak at the region of low molecular weight peptides.

Fig. 5 illustrates the electrophoretic behavior of the proteins found in peaks one, two and three. The proteins of peak one are high molecular weight globulins (macroglobulins). The proteins of peak two are globulins (α , β & γ) with a molecular weight of 1.5×10^5 - 2.0×10^5 . Peak three, which binds the largest amount of plutonium, was composed primarily of albumin MW 66,000 and a low molecular weight globulin, but it was not determined whether it was a β or γ globulin.

All of these experiments were in vitro studies and not too much significance should be assigned to them in interpreting living systems, but they provided the necessary experimental basis for subsequent in vivo investigation. For instance copper is at first bound by albumin and after a period of one day is almost quantitatively found in the ceruloplasma⁽⁵⁾, so plutonium will probably redistribute itself also. Some kind of redistribution is to be expected from the change in the slope of the plasma concentration curve after one day post injection. The future investiga-

tions will be an attempt to elucidate these changes in the living animal and possibly relate these changes to turnover times of the individual plasma protein constituents.

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METABOLISM OF THE Th^{228} DECAY SERIES IN
ADULT BEAGLE DOGS II. Ra^{224} (ThX),
 Pb^{212} (ThB); AND Bi^{212} (ThC)

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Abstract: Retention and distribution of Ra^{224} , Pb^{212} , and Bi^{212} in beagles have been studied through three years after the intravenous injection of Th^{228} , the parent nuclide, with the decay products in equilibrium. Initially Ra^{224} in plasma decreased more rapidly than Th^{228} in plasma. Pb^{212} was associated with blood cells. Later the concentrations of Ra^{224} in plasma and Pb^{212} in cells were respectively 24 and 480 times that of Th^{228} in plasma. Bi^{212} in plasma was about twice Ra^{224} , and in cells it was about 0.9 Pb^{212} .

At 30 days cumulative excretion of Ra^{224} was 35% compared with 11% for Th^{228} ; 27% of the Ra^{224} atoms formed in vivo were excreted. The Ra^{224} excretion rate decreased with time and at 100 and 1000 days, respectively, 12 and 7% of the atoms formed in vivo were excreted. The fecal excretion rate was greater than and decreased more slowly than the urinary rate. In the highest dose level studied Ra^{224} excretion rates decreased more rapidly than those of lower levels. At 777 days cumulative urinary excretion of Pb^{212} was 0.4%, which was about 0.6% of the Pb^{212} atoms formed in vivo. Cumulative urinary excretion of Bi^{212} ranged from 0.45 to 0.91%.

In the liver the activity of Pb^{212} was slightly higher than that of Ra^{224} which was about 30% higher than that of Th^{228} . All 3 decrease at about the same rate, and this rate is about the same as observed for the 3 in blood. In the spleen Ra^{224} activity was again slightly higher than Th^{228} activity but Pb^{212} activity was only half that of Th^{228} . The rates of decrease were about the same as for liver and blood.

In the kidneys it was also possible to measure Bi^{212} . The mean Bi^{212} activity measured was 17% greater than that of Th^{228} which was 3.5 times that of Ra^{224} and Pb^{212} , the means of the latter two being equal. There was considerable variation in all four sets of data and the rates of biological decrease could not be determined. Some of the Ra^{224} and Bi^{212} in kidneys and urine must come from extra-vascular sites. Pb^{212} in kidneys and urine comes mainly from Tn^{220} decay in blood and kidneys, but other extravascular sites are not excluded.

In one humerus and the third lumbar vertebra there was a progressive loss of decay products; the activity of Th^{228} was greater than that of Ra^{224} which exceeded Pb^{212} .

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Neither the Ra^{224} nor the Pb^{212} data showed a significant rate of biological decrease in either bone. Also the activities of Ra^{224} and of Pb^{212} relative to that of Th^{228} did not show a significant change with time.

In summary, the biological rates of decrease of Ra^{224} and Pb^{212} were found to be about the same as those of Th^{228} . The biological rate constants for the 3 nuclides in bone were found to be of the order of 0.1 of the physical decay constant. For blood, excreta, and soft tissues the biological rate constants were found to be the same order of magnitude as the physical decay constant. Average radiation dose rates to bone, liver, and kidneys have been estimated, and the problem of evaluating the loci of decay of Tn^{220} and Po^{216} has been discussed.

INTRODUCTION

The studies reported herein are a part of the long term studies of the biological effects of Th^{228} and other radionuclides in adult beagles (1). Previously we have reported the metabolism in these beagles of the parent nuclide, Th^{228} , over a period of 3.5 years following injection (2). Earlier, Van Dilla et al. (3) reported the translocation of Ra^{224} during the first few months after injection, and Mays et al. (4) reported the exhalation of Tn^{220} through 1000 days. In this communication we endeavor to complete the picture with a 3.5 year study of Ra^{224} , Pb^{212} and Bi^{212} in blood, excreta, and selected bones and soft tissues. These data with those for Th^{228} (2) and Tn^{220} (4) are then used to estimate average radiation dose rates to several tissues.

METHODS

Each dog was given a single intravenous injection of Th^{228} with its decay products in equilibrium in young adulthood (1). The nearly carrier free Th^{228} injection solution has been described before (2). Ra^{224} and Pb^{212} in the various blood, excreta, and tissue samples were determined by repeated counting in a well-type liquid scintillation gamma

detector until equilibrium with Th^{228} was established. Then Th^{228} was measured in these same samples as has been reported (2). Ra^{224} and Pb^{212} activities were obtained by resolving the plots of count rate vs. time after sample collection or death. It was also possible by this method to determine Bi^{212} (ThC) in the kidneys and special urine samples.

The exception to the above was the determination of Ra^{224} in plasma at times greater than 2 to 3 weeks after injection. By then the gamma activity was so low that alpha measurements were necessary. The plasma was ashed rapidly with nitric acid, and then an aliquot of the solution was placed on a stainless steel disc. Using a conventional 2π proportional counter the alpha activity was measured repeatedly until equilibrium was established.

Determination of Ra^{224} , Pb^{212} , and Bi^{212} as described above is valid only if the gammas detected can be identified with a specific nuclide (or nuclides). Gammas occur throughout the Th^{228} decay series. All gammas occurring prior to Pb^{212} are of low energy except one of 0.241 Mev which occurs in 3.7% of the Ra^{224} disintegrations (5). However, gammas of energy of 0.239 Mev or 0.300 Mev occur in about 77% of the Pb^{212} disintegrations. Thus it is sufficient to compare the detector response to Pb^{212} gammas with the response to the gammas from Bi^{212} and its decay products.

For this comparison Pb^{212} was obtained by bubbling air through a Th^{228} solution in a small container to carry Tn^{220} into a large flask. The flow rate and the volumes were chosen so that a large part of the Tn^{220} decayed in the flask. Pb^{212} was washed out and diluted to a known volume with 6M CH_3COOH . Sufficient time was allowed for Pb^{212} and Bi^{212}

to attain transient equilibrium. Pb^{212} and Bi^{212} were separated by precipitating PbCrO_4 ; isotopic carriers were used for both nuclides.

PbCrO_4 was dissolved in 4M HNO_3 .

Aliquots of the unseparated Pb^{212} Bi^{212} solution and of the separated Pb^{212} and Bi^{212} solutions were counted serially in the liquid scintillator. The growth and decay of alpha activity in an aliquot of the Pb^{212} was also measured. The three decay curves were then resolved.

By comparison with the unseparated sample, loss during the separation was negligible; the fractional recovery was 0.99 ± 0.03 . The degree of separation was determined from the components of the alpha curve and the 10.6 hour component of the gamma curve for the Bi^{212} solution. The fraction of Pb^{212} left in the Bi^{212} solution was only 0.02 while the fraction of Bi^{212} carried with the PbCrO_4 was 0.19. These results are consistent with the fact no special procedures, such as reprecipitation, were done since the short Bi^{212} half-period imposed a time limit. From these yields and the remaining gamma curve components it was determined that the efficiency of the detector for Pb^{212} gammas was not significant with respect to (0-1% of) that for the gammas from Bi^{212} and decay products. Without this important result the growth and decay data obtained from samples from dogs given Th^{228} could not be meaningfully analyzed.

Blood cells and plasma were separated by centrifugation and then counted without further treatment, except for the late plasmas described above. Continuous excretion studies were made for the first 22 days after injection for two dogs and 30 days for two dogs. The urine and feces were ashed as rapidly as possible, and then 50 ml aliquots were counted serially to determine Ra^{224} . Later 96 hour collections were

made. An aliquot of the urine was sealed in a No. 2 can. A little distilled water was added to the feces, then they were homogenized in a blender, and an aliquot was sealed in a can. Phenol was used as a preservative in both cases. Ra^{224} was then determined by counting in a larger but very similar well-type counter.

The samples obtained at autopsy were the left humerus, the third lumbar vertebra, one kidney, and known fractions of the liver and spleen. These samples were put in 50 ml centrifuge tubes, covered with formalin, and sealed. The serial counting was begun one to three hours after death of the dog and continued until equilibrium was evident.

RESULTS AND DISCUSSION

In presenting the results, the activity of Ra^{224} in a specific sample is given as the percent of the Ra^{224} activity which would be in equilibrium with that amount of Th^{228} which had been injected and allowed to decay to the time of sample collection. Percent Ra^{224} is defined by Eq. (1) and is

$$\% \text{Ra}^{224} = \frac{\mu\text{c Ra}^{224} \text{ in sample} \times 100}{\mu\text{c Th}^{228} \text{ injected} \times e^{-\lambda_{\text{Th}^{228}} t}} \quad (1)$$

Percent Pb^{212} and percent Bi^{212} are defined in the same way. These definitions separate biological and radioactive decay factors and also permit direct comparison of the activities of the decay products with that of the series parent, Th^{228} . The Th^{228} data were present similarly and in reference (2)

$$\begin{aligned} \% \text{Th}^{228} &= \% \text{Th}^{228} \text{ injected} \times e^{-\lambda_{\text{Th}^{228}} t} \\ &= \frac{\mu\text{c Th}^{228} \text{ in sample} \times 100}{\mu\text{c Th}^{228} \text{ injected} \times e^{-\lambda_{\text{Th}^{228}} t}} \end{aligned} \quad (2)$$

Thus, if the ratio of Ra^{224} activity to Th^{228} activity is desired at $t = T$, Eqs. (1) and (2) yield

$$\frac{\% \text{Ra}^{224} (T)}{\% \text{Th}^{228} (T)} = \frac{\mu\text{c Ra}^{224} \text{ in sample}}{\mu\text{c Th}^{228} \text{ in sample}} \quad (3)$$

Analogous comparisons of Pb^{212} and Bi^{212} activities relative to that of Th^{228} can be made.

Single exponentials, $y = ae^{-bt}$ were calculated for the various sets of data by applying the classical least squares method to the transformed equation $\log y = \log a - bt \log e$. The 90% symmetrical confidence limits for the parameters in the equations which follow are given in Table I. The t-test was used to determine whether b differed significantly from zero, and the resulting value of p is given in the last column of Table I. The single exponential was chosen arbitrarily to facilitate dose rate calculations which include the exponential decay factor.

Blood. The concentrations of Ra^{224} in blood plasma and of Pb^{212} in blood cells were measured through 1234 days after injection of Th^{228} in equilibrium with its decay products. From 2 minutes through 64 days blood from four 5-level ($2.8 \mu\text{c Th}^{228}/\text{kg}$ injected) and two 4-level ($0.90 \mu\text{c}/\text{kg}$) dogs was measured. The average and the range of $\% \text{Ra}^{224}/\text{g plasma}$

and of % Pb²¹²/g cells in the 6 dogs are shown in Figs. 1 and 2, respectively. The measurements which were made from 77 to 1234 days are also

TABLE I
Equation Constants^a with 90% Symmetrical
Confidence Limits

Eq. No.	Quantity and Nuclide		n	log a	b x 10 ³	p
4	P	Ra ²²⁴	31	-3.253 ± 0.078	1.31 ± 0.34	<0.01
6	C	Pb ²¹²	32	-1.958 ± 0.088	0.97 ± 0.21	<0.01
17	U (all)	Ra ²²⁴	67	-0.184 ± 0.071	0.92 ± 0.26	<0.01
18	U (no 4)	Ra ²²⁴	53	-0.130 ± 0.056	0.91 ± 0.19	<0.01
19	U (4)	Ra ²²⁴	14	-0.194 ± 0.122	1.91 ± 0.53	<0.01
20	F (all)	Ra ²²⁴	65	+0.056 ± 0.114	0.53 ± 0.15	<0.01
21	F (no 4)	Ra ²²⁴	51	+0.062 ± 0.062	0.48 ± 0.15	<0.01
22	F (4)	Ra ²²⁴	14	+0.135 ± 0.098	1.24 ± 0.42	<0.01
23	X (all)	Ra ²²⁴	65	+0.266 ± 0.068	0.75 ± 0.24	<0.01
24	X (no 4)	Ra ²²⁴	51	+0.287 ± 0.076	0.72 ± 0.26	<0.01
25	X (4)	Ra ²²⁴	14	+0.305 ± 0.079	1.43 ± 0.34	<0.01
49	L	Ra ²²⁴	14	+0.719 ± 0.125	1.05 ± 0.43	<0.01
51	L	Pb ²¹²	14	+0.736 ± 0.180	1.03 ± 0.63	<0.02
52	S	Ra ²²⁴	11	-0.470 ± 0.184	1.10 ± 0.73	<0.05
54	S	Pb ²¹²	11	-0.800 ± 0.223	0.91 ± 0.88	<0.10

^aCalculated by classical method of least squares.

shown in Figs. 1 and 2 where each point represents a single measurement.

Fig. 1. $\% \text{Ra}^{224}/\text{g plasma}$. Dotted curves in sections A and B show expected contributions of injected Ra^{224} .

Fig. 2. % Pb^{212} /g blood cells. Dotted curves in sections A and B show expected contribution of injected Pb^{212} .

The samples were obtained mainly from 3- (0.30 $\mu\text{C/kg}$) and 4-level dogs with a few from 2- (0.096 $\mu\text{C/kg}$) and 5-level dogs, and many samples were obtained shortly before euthanasia.

In blood Th^{228} is essentially all in the plasma in non-diffusible form. From 2 minutes to 4 hours the concentration decreases by a factor of 3. At 3 weeks the concentration is only 10^{-4} of that at 2 minutes. Thereafter concentration decreases at a slower rate. Initially Ra^{224} in plasma drops much more rapidly. At 2 and 15 minutes the Ra^{224} to Th^{228} concentration ratios are about 0.4 and 0.1, respectively. At 72 minutes the ratio for 2 dogs remained near 0.1 but was too low to measure for the other 4. This was the case for all 6 dogs at 4 hours, and for 3 of them at 8 hours when the other 3 averaged nearly 0.2. The Ra^{224} to Th^{228} ratio then increases as the injected Ra^{224} disappears and Ra^{224} formed in vivo predominates. Values for 1 day and 1 week are 0.5 and 1.1, respectively. The dotted curve in Fig. 1 shows how the concentration of injected Ra^{224} would decrease. It was calculated from data for Ra^{226} in beagle plasma (6) to which appropriate Ra^{224} decay factors had been applied.

The data for % Ra^{224} /g plasma from 63 to 1234 days can be described by a single exponential which is

$$P(\text{Ra}^{224}) = 5.6 \times 10^{-4} e^{-0.0013t} \quad (4)$$

For this period $P(\text{Th}^{228})$ shows the same time dependence (2). Thus,

$$\frac{P(Ra^{224})}{P(Th^{228})} = \frac{5.6 \times 10^{-4} e^{-0.0013t}}{2.3 \times 10^{-5} e^{-0.0013t}} \approx 24 \quad (5)$$

and average Ra^{224} activity in plasma is about 24 times that of Th^{228} .

In beagle blood Pb^{212} is predominantly (> 98%) in the cellular fraction (7). In these studies determination of Pb^{212} in plasma and Ra^{224} in cells was not feasible, either because of the predominance of the other nuclide or because of ashing of some plasmas.

At first Pb^{212} in blood decreased rapidly and at 2 minutes the average concentration was about the same as that of Ra^{224} . Very soon some of the Pb^{212} returned to the blood and the peak concentration was more than twice the two minute value. The dotted line in Fig. 2 shows how injected Pb^{212} would decrease (7). The peak was reached shortly after 15 minutes and persisted through 4 hours (Fig. 2) at which time the concentration of Pb^{212} in cells was about twice that of Th^{228} in plasma. Thereafter, the activity of Pb^{212} in blood remained greater than that of Ra^{224} or Th^{228} . The data for % Pb^{212} /g cells from 63 to 1282 days have been described by a single exponential which is

$$C(Pb^{212}) = 0.0110 e^{-0.00097t} \quad (6)$$

The coefficients of t in Eqs. (4) and (6) do not differ significantly, by the t -test. Thus, within the limits of the measurements, the concentrations of Pb^{212} in cells and Ra^{224} and Th^{228} in plasma decrease at the same rate. From 2 months to 3.5 years the following relation holds:

$$C(\text{Pb}^{212}) \cong 20 \text{ } P(\text{Ra}^{224}) \cong 480 \text{ } P(\text{Th}^{228}) \quad (7)$$

Measurement of Bi^{212} in plasma and cells was difficult since centrifugation and separation required an appreciable part of a Bi^{212} half-period. However, the concentration of Bi^{212} in plasma was measured at 15 times ranging from a few minutes to 1 year after injection. The results are given in Table II which also includes the Ra^{224} concentration

TABLE II
Activity of Bi^{212} in Plasma Compared
with that of Ra^{224}

Dog	(days) ^t	% Bi^{212} /g	% Ra^{224} /g	$\text{Bi}^{212}/\text{Ra}^{224}$
T4T5	0.0016	0.051	0.063	0.8
T5T5	0.0026	0.030	0.042	0.7
T5T5	0.0104	0.026	0.0072	3.6
T5T5	0.0513	0.004	<0.004	>1.0
T4T5	1.00	1.0×10^{-3}	2.4×10^{-3}	0.5
T4T5	2.00	1.2×10^{-3}	2.0×10^{-3}	0.6
T5T5	6.99	1.0×10^{-3}	1.0×10^{-3}	1.0
T5T5	21	1.3×10^{-3}	0.8×10^{-3}	1.6
T4T5	21	1.7×10^{-3}	0.9×10^{-3}	1.9
T4T5	63	0.93×10^{-3}	0.49×10^{-3}	1.9
T5T5	65	1.1×10^{-3}	0.51×10^{-3}	2.2
M2T5	97	0.92×10^{-3}	0.63×10^{-3}	1.5
M2T4	105	1.0×10^{-3}	0.4×10^{-3}	2.5
T4T5	113	0.67×10^{-3}	0.74×10^{-3}	0.9
M1T4	364	1.0×10^{-3}	0.6×10^{-3}	1.7

in the same samples. From 21 days to 1 year the average Bi^{212} to Ra^{224} ratio is 1.8. Thus we estimate the long term plasma contraction of Bi^{212} as

$$P(\text{Bi}^{212}) \sim 2 P(\text{Ra}^{224}) \sim 0.1 C(\text{Pb}^{212}) \quad (8)$$

The source of the plasma Bi^{212} could be either the blood cells or extravascular sites or both. Early counts obtained on the cell samples did not yield a definitive answer. In connection with this problem it should be noted that there will always be an early increase in the count rate, i.e. Bi^{212} activity, in these cell samples as soon as dC/dt becomes small compared with the Pb^{212} decay rate. The reason is that, when Pb^{212} is at a steady state in the blood, the activities of Pb^{212} and Bi^{212} will be related by conditions of secular equilibrium. When the blood is removed from the dog the steady state no longer exists and Pb^{212} disappears according to its decay constant, and Bi^{212} activity increases until transient equilibrium conditions are attained. Then, using A to denote activity, we have

$$A_{\text{Bi}^{212}} = \frac{\lambda_{\text{Bi}^{212}}}{\lambda_{\text{Bi}^{212}} - \lambda_{\text{Pb}^{212}}} A_{\text{Pb}^{212}} = 1.11 A_{\text{Pb}^{212}} \quad (9)$$

Thus, if Bi^{212} in plasma came from cells, the anticipated increase in cell count rates would be small and of about the same magnitude is the increase resulting from a change from secular to transient equilibrium conditions.

It was usually 30 to 40 minutes after the blood was drawn when the first count was made, and usually an early increase was noted. However, the data obtained sufficed only to show that most of the Bi^{212} formed in the cells also decays there. Thus the blood cells are a mobile alpha source and irradiate capillary walls and other cells which may come in range.

Excretion. The results of the early continuous excretion studies are shown in Figs. 3 and 4 in which the means and ranges are plotted. The injected doses for the dogs studied 22 days were 2.59 and 2.32 $\mu\text{C}/\text{kg}$, (5-level) and those for the 30 day study were 0.884 (4-level) and 0.298 (3-level) $\mu\text{C}/\text{kg}$. The dogs given the two highest doses exhibited fever, loss of appetite, etc., as early as 9 days (1), and this appears to have affected fecal excretion of Ra^{224} .

The cumulative urinary excretion rose to an apparent steady state value of 15% with a range of only 2-3% (Fig. 3). Much greater variation was seen in the cumulative fecal excretion (Fig. 3). It rose rapidly to about 20%, then at about 10 days the two 5-level dogs began to show a decrease which accounts for the dip in mean values from 12-22 days. Urinary plus fecal excretion (Curve A, Fig. 4) has a similar dip. (Note that cumulative excretion can decrease when the daily excretion of Ra^{224} is less than the daily decrease by decay of the total Ra^{224} outside the dog). Curve B is the mean cumulative excretion of Th^{228} by the same dogs over the same intervals. For comparison, the ranges for 10 and 22 days are shown. By 10 days the Th^{228} excretion rate was fairly low and there was no important effect on cumulative Th^{228} excretion from 10 to 22 days for the 5-level dogs. Curve C shows the cumulative excretion

Fig. 3. Cumulative urinary and fecal excretion of Ra^{224} . 0-22 days, mean and range for 4 beagles, 22-30 days, mean and range for 2 beagles.

Fig. 4. A. Cumulative excretion of Ra^{224} . 0-22 days, mean and range for 4 beagles, 22-30 days, mean and range for 2 beagles. B. Mean cumulative excretion of Th^{228} by same dogs. C. Calculated cumulative excretion of injected Ra^{224} .

of injected Ra^{224} calculated from Ra^{226} data and the Ra^{224} decay factor. Thus, at a month after injection of Th^{228} , about 35% of the Ra^{224} is decaying outside the dog but only 11% of the parent Th^{228} decays outside, (if the dog is not suffering serious early effects). The fractional retention of Ra^{224} being formed in vivo at this time is $f = R(\text{Ra}^{224}) / R(\text{Th}^{228}) = 0.65/0.89 = 0.73$, i.e. 1 out of 4 Ra^{224} atoms formed in the dog is excreted.

Sixty-five measurements of daily urinary and fecal excretion of Ra^{224} were made during the period 100 to 1260 days after injection. Dogs at dose levels 4 ($0.90 \mu\text{C Th}^{228}/\text{kg}$), 3 ($0.30 \mu\text{C Th}^{228}/\text{kg}$), 2 ($0.096 \mu\text{C Th}^{228}/\text{kg}$), 1.5 ($0.032 \mu\text{C Th}^{228}/\text{kg}$), and 1 ($0.016 \mu\text{C Th}^{228}/\text{kg}$) were included. The latest 4- and 3-level measurements were at 832 and 956 days, respectively, which times are close to maximum survival times for these levels. The latest 1-level measurements were at 430 days.

In both urinary and fecal samples the activity of Ra^{224} was considerably greater than that of Th^{228} . After the first day the gamma count rate varies with time as does the Ra^{224} activity which is

$$A_2 = A_1^0 (e^{-\lambda_1 t} - e^{-\lambda_2 t}) + A_2^0 e^{-\lambda_2 t} \quad (10)$$

since $\lambda_2/(\lambda_2 - \lambda_1) = 1$. (Subscripts 1 and 2 denote Th^{228} and Ra^{224} , respectively). Rearranging Eq. (10) gives

$$A_2 = A_1^0 e^{-\lambda_1 t} + (A_2^0 - A_1^0) e^{-\lambda_2 t} \quad (11)$$

Further, the counting is done using a Th^{228} reference standard which has

the effect of dividing by $e^{-\lambda_1 t}$, i.e.

$$\begin{aligned} A_2 e^{\lambda_1 t} &= A_1^0 + (A_2^0 - A_1^0) e^{-(\lambda_2 - \lambda_1)t} \\ &= A_1^0 + (A_2^0 - A_1^0) e^{-\lambda_2 t} \end{aligned} \quad (12)$$

since $\lambda_2 \gg \lambda_1$.

If C is the gamma count rate of the sample relative to that of the Th^{228} reference standard, then we have

$$C = a + b e^{-\lambda_2 t} \quad (13)$$

From Eqs. (12) and (13),

$$A_1^0 = k a \quad (14)$$

and

$$A_2^0 - A_1^0 = \frac{k b}{\lambda_3 / (\lambda_3 - \lambda_2)} \quad (15)$$

where k is a constant, and, because of the transient equilibrium relation between Ra^{224} and Pb^{212} , denoted by subscript 3, b must be reduced as shown. In principle then we can determine A_1^0 and A_2^0 from the graph of C vs. t . In practice, however, the excretion of Th^{228} was too low to be determined precisely in this way, so that, subsequent to gamma counting, the samples were ashed and Th^{228} was determined, ultimately, by α -counting (2). The component a proved to be a measure of contamination which included Th^{228} and any traces of gamma emitters present in the

sample intrinsically or from cages, containers, etc. Thus, determination of a gave no direct information, but it was necessary to have a to obtain b accurately.

Hence, from gamma counting we obtain $A_2^0 - A_1^0$, the amount by which Ra^{224} activity exceeds Th^{228} activity. The procedure in working up these data has been to fit equations to $A_2^0 - A_1^0$ to obtain, for example,

$$U'(Ra^{224}) = U(Ra^{224}) - U(Th^{228}) = ae^{-bt} \quad (16)$$

Then $U(Th^{228})$ from Ref. (2) is added to Eq. (14) to obtain $U(Ra^{224})$.

The results are given in the three graphs of Fig. 5. The 4-level dogs exhibited marked skeletal and kidney damage (1) and this was reflected in decreased excretion of Ra^{224} . A similar effect on urinary excretion of Th^{228} by 4-level dogs has been reported (2). Equations for the 9 curves shown in Fig. 5 are as follows:

$$U' = 0.656 e^{-0.00092t} \quad (\text{all levels}) \quad (17)$$

$$U' = 0.742 e^{-0.00091t} \quad (\text{all but 4}) \quad (18)$$

$$U' = 0.640 e^{-0.00191t} \quad (4 \text{ only}) \quad (19)$$

$$F' = 1.14 e^{-0.00053t} \quad (\text{all levels}) \quad (20)$$

$$F' = 1.15 e^{-0.00048t} \quad (\text{all but 4}) \quad (21)$$

$$F' = 1.36 e^{-0.00124t} \quad (4 \text{ only}) \quad (22)$$

$$X' = 1.85 e^{-0.00075t} \quad (\text{all levels}) \quad (23)$$

$$X' = 1.94 e^{-0.00072t} \quad (\text{all but 4}) \quad (24)$$

$$X' = 2.02 e^{-0.00143t} \quad (4 \text{ only}) \quad (25)$$

Fig. 5. $(\% Ra^{224} - \% Th^{228})$ excreted per day. A. Urinary, B. Fecal, and C. Urinary plus fecal excretion. On each graph curve 1 was calculated using all points, curve 2 using level-1, level-1.5, level-2, and level-3 points and curve 3 using level-4 points only.

Units are % Ra^{224} /day and days. The 90% symmetrical confidence limits for each log a and each b appear in Table I. The limits for log a may at first be misleading. For example, consider Eq. (20), F(all), for which $\log a = 0.0564 \pm 0.1139$. The limits on $a = 1.14$ are the antilogs of $0.0564 - 0.1139$ and $0.0564 + 0.1139$ which are 0.88 and 1.48, respectively.

The coefficients of t in the exponentials (the b's) of Eqs. (17-25) were compared using the t-test. In all three cases (U, F, and X) the values for the "all" and "all but 4" groups did not differ, but those for the "all" and "4 only" groups did differ, $p < 0.01$.

During this period, 100-1260 days, excretion of Th^{228} is much lower, and the urinary rate decreases twice as fast while the total rate decreases about 1-1/2 times as fast (2). The equations for the "all" group are

$$U(\text{Th}^{228}) = 0.019 e^{-0.0018t} \quad (26)$$

and

$$X(\text{Th}^{228}) = 0.021 e^{-0.0011t} \quad (27)$$

The Th^{228} data were worked up in the dark ages of log tables and desk calculators, and for this reason a separate equation for $F(\text{Th}^{228})$ was not obtained.

Combining Eqs. (17) and (26) in the manner of Eq. (16) we obtain for the daily urinary excretion for all levels

$$U(\text{Ra}^{224}) = 0.656 e^{-0.00092t} + 0.019 e^{-0.0018t} \quad (28)$$

$$\approx 0.675 e^{-0.00092t}$$

for the period of interest. Similarly the total daily rate from Eqs. (23) and (27) is

$$X(\text{Ra}^{224}) = 1.85 e^{-0.00075t} + 0.021 e^{-0.0011t} \quad (29)$$

$$\approx 1.87 e^{-0.00075t}$$

The important question to be answered from the excretion studies is what fraction of the Ra^{224} formed in the dog is decaying outside the dog from 100 to 1260 days. This result is easily computed from Eq. (29) for X since the rate of physical decay of Ra^{224} ($\lambda = 0.191\text{d}^{-1}$) is much greater than the rates at which the daily excretion of Ra^{224} and Th^{228} change. These are

$$d\ln X(\text{Ra}^{224})/dt = -0.00075 \quad (30)$$

and

$$d\ln X(\text{Th}^{228})/dt = -0.0011 \quad (31)$$

Therefore, these changes can be neglected in summing to obtain cumulative excretion. On a given day T in the period 100 to 1260 days, the excretion for that day is $X(T) = 1.87 e^{-0.00075T}$. On the $T-1$ st day $X(T-1)$ was excreted and by T it has been reduced by decay by $e^{-\lambda}$ so that $X(T-1) e^{-\lambda} = 1.87 e^{-0.00075(T-1)} e^{-0.191} = 1.87 e^{-0.00075T} e^{-0.191 + 0.00075}$

$\cong X(T) e^{-\lambda}$ (since $0.00075 \ll 0.191$) gives the Ra^{224} outside the dog as a result of the T-1st day's excretion. Similarly the T-2nd days excretion contributes $X(T) e^{-2\lambda}$. The total Ra^{224} which is formed in the dog but excreted to decay outside the dog, $\sum X$, is thus given by

$$\begin{aligned} \sum X(T) &= X(T) + X(T) e^{-\lambda} + X(T) e^{-2\lambda} + - - - - \\ &= X(T) (1 + e^{-\lambda} + e^{-2\lambda} + - - - -) \\ &= X(T) / (1 - e^{-\lambda}) = 5.75 X(T) \end{aligned} \quad (32)$$

The biological retention of Ra^{224} is given by

$$\begin{aligned} R_e(Ra^{224}) &= R_e(Th^{228}) - 5.75 X(Ra^{224}) \\ &= 19.1 e^{-0.0011t} + 67.9 - 10.8 e^{-0.00075t} \end{aligned} \quad (33)$$

and the actual retention is

$$\begin{aligned} R'_e(Ra^{224}) &= R_e(Ra^{224}) e^{-\lambda_{Th^{228}} t} = \\ 19.1 e^{-0.0021t} + 67.9 e^{-0.0010t} - 10.8 e^{-0.00175t} \end{aligned} \quad (34)$$

where $R_e(Th^{228})$ was obtained from excretion data (2). If we use the probably better estimate of Th^{228} retention derived from analyses of selected bones and soft tissues plus 9 carcasses (2), we have

$$R(Ra^{224}) = 18 e^{-0.0010t} + 69 e^{-0.00014t} - 10.8 e^{-0.00075t} \quad (35)$$

and

$$R'_e(Ra^{224}) = 18 e^{-0.0020t} + 69 e^{-0.00114t} - 10.8 e^{-0.00175t} \quad (36)$$

For times greater than about a year Eq. (35) appears to be better than Eq. (33). Earlier they are about equivalent.

From Eq. (35) we calculate the fractional retention of Ra^{224} formed in vivo to be

$$f = \frac{R(Ra^{224})}{R(Th^{228})} = 1 - \frac{10.8 e^{-0.00075t}}{18 e^{-0.0010t} + 69 e^{-0.00014t}} \quad (37)$$

$$= 1 - \frac{1}{1.67 e^{-0.00025t} + 6.39 e^{+0.00061t}}$$

Thus, f approaches unity as t increases. At 100, 500, and 1000 days Eq. (37) gives $f = 0.88, 0.90,$ and 0.92 , respectively. In the early studies $f \approx 0.73$ at 30 days. This observed value is lower than would be calculated from Eq. (37) derived from data obtained at later times, which is illustrative of the difference in the nature of the Th^{228} deposition at short and long times.

If we use the model of Van Dilla et al., which assumes that Ra^{224} formed in vivo will, on reaching the blood, be excreted and redistributed as though it had been injected intravenously, (3), we can calculate from f the fraction of Ra^{224} which undergoes translocation. First we must calculate $R(Ra^{224})/R(Th^{228}) = f^*$ for the case in which every atom of Ra^{224} formed in vivo goes to the blood. This model also assumes that a Ra^{224} atom goes to blood shortly, i.e. $\ll 3.64$ days after its formation.

Let A_1^0 = activity of Th^{228} injected at $t = 0$. At $t > 0$ the activity of Th^{228} in the dog is

$$A_1(\text{dog}) = A_1^0 e^{-\lambda_1 t} R_1(t) \quad (38)$$

where $R_1(t) = R(\text{Th}^{228})$ above. The number of atoms of Ra^{224} formed in vivo during the interval x to $x + dx$ is

$$A_1(\text{dog}) dx = A_1^0 e^{-\lambda_1 x} R_1(x) dx$$

At some time later these will be reduced by decay and excretion to

$$A_1^0 e^{-\lambda_1 x} R_1(x) e^{-\lambda_2 (t-x)} R_2(t-x) dx$$

Multiplying by λ_2 and integrating from $x = 0$ to $x = t$ gives the activity of Ra^{224} in the dog at t :

$$A_2(\text{dog}) = \lambda_2 \int_0^t A_1^0 e^{-\lambda_1 x} R_1(x) e^{-\lambda_2 (t-x)} R_2(t-x) dx \quad (39)$$

as noted above both the rate of decay and the rate of decrease of $R(\text{Th}^{228})$ after a few weeks are small compared with the rate of decay of Ra^{224} so that we introduce no significant error by taking $A_1(\text{dog})$ outside the integral sign. For $R_2(t)$ we use the equation for Ra^{226} retention in beagles (6):

$$R = 0.79t^{-0.20} \quad (40)$$

These lead to the relation

$$\left(\frac{A_2}{A_1}\right)_{\text{dog}} = \frac{R(\text{Ra}^{224})}{R(\text{Th}^{228})} = f^* = \quad (41)$$

$$\lambda_2^{0.79} \int_0^t e^{-\lambda_2(t-x)} (t-x)^{-0.20} dx$$

$$= \lambda_2^{0.79} \int_0^t e^{-\lambda_2 z} z^{-0.20} dz$$

Where $z = t-x$. Again because of the relatively rapid rate of decay we can let $t \rightarrow \infty$ so that

$$f^* = \frac{\lambda_2^{0.79}}{\lambda_2^{(-0.20 + 1)}} \Gamma(-0.20 + 1) =$$

$$0.79 (0.191)^{0.20} \Gamma(0.80) =$$

(42)

$$\frac{0.79 (0.191)^{0.20}}{0.80} \Gamma(1.81) =$$

$$\frac{0.79 \times 0.931}{0.80 \times 1.39} = 0.66$$

Thus, if all Ra^{224} atoms formed in the dog moved to the blood about 1/3 of them would be excreted since

$$1 - f^* = 0.34 \quad (43)$$

The observed values of $1-f$ are less than 0.34 which means that within

the limits of the model used, only part of the Ra^{224} translocates. The fraction which translocates $F_t(\text{Ra}^{224})$ is given by

$$F_t(\text{Ra}^{224}) = \frac{1 - f}{1 - f^*} \quad (44)$$

$F_t(\text{Ra}^{224})$ calculated from excretion data, i.e. Eqs. (37) and (44), decreases slowly with time as shown in Fig. 6. Using Eq. (25) the same calculation has been made for 4-level dogs only. Since $F_t(\text{Ra}^{224})$ for 4-level dogs decreases more rapidly than that calculated for all the dogs, radiation damage (probably skeletal and renal) is a factor in the decrease of translocation of Ra^{224} .

Mays et al. (4) obtained relations for percent thoron exhalation (relative to in vivo-produced thoron) for dose levels 4, 3, and 2 and lower. Choosing their 3-level equation as representative of all the levels and converting to our units we obtain

$$\begin{aligned} \% \text{Tn}^{220} \text{ exhaled} &= 8t^{-0.17} (0.93t^{-0.023} - 0.47t^{-0.31}) \\ &= 7.4t^{-0.19} - 3.8t^{-0.31} \approx 2.5e^{-0.0003t} \end{aligned} \quad (45)$$

The approximation to the exponential was obtained graphically and is quite good for $t = 200$ to 1200 days. Thus, for thoron retention we have

$$\begin{aligned} R(\text{Tn}^{220}) &= R(\text{Ra}^{224}) - 2.5e^{-0.0003t} \\ &\approx 0.98 R(\text{Ra}^{224}) \end{aligned} \quad (46)$$

Fig. 6. Calculated (Eq. (42)) fraction Ra^{224} formed in the dog which translocates. Upper curved derived from all the excretion data, lower from 4-level only. At 20-30 days $F_T(\text{Ra}^{224}) = 0.79$

An extensive study of the excretion of Pb^{212} comparable with the Ra^{224} studies would have required such enormous amounts of technical time that it could not be justified. Because of the 10.6 hour half period it would be necessary to collect excreta every few hours around the clock in order to obtain an accurate value for Pb^{212} in the sample at the time of excretion. Then properly accumulating results from these samples would give the % Pb^{212} formed in the dog which decayed outside the dog.

A further complication in determining urinary excretion of Pb^{212} is that in a freshly voided sample the activity of Bi^{212} is high relative to both Pb^{212} and Ra^{224} . Thus the counting schedule must be planned to obtain sufficient measurements after several Bi^{212} half-periods but within the first 1 to 2 Pb^{212} half-periods.

We do have findings from excretion studies that indicate most of the Pb^{212} formed in the dog decays there. When beagles were given intravenous injections of Pb^{212} , the biological excretion at 24 hours was only 4%, and urinary excretion of 5 times fecal excretion (7).

In addition, two short series of freshly voided urines from a 3-level Th^{228} dog were counted to determine the relative activities of Ra^{224} , Pb^{212} , and Bi^{212} .

The count rate data in these two studies were resolved using a Gauss-Siedel iterative procedure of least squares analysis on an IBM 7090 computer. This method has been described by Richmond et al. (8).

In the first series dog T8T3, housed in a metabolism cage, was given a bowl of milk at the beginning of the work day, and then the cage was checked at 30-minute intervals for urine. When the first urine was

found it was sealed in an ampoule and serial counting was begun 15 to 20 minutes after collection. A sample was obtained on each of three successive mornings. The activities of Ra^{224} , Pb^{212} , and Bi^{212} were all considerably greater than that of Th^{228} and, for reasons given above, the results are expressed as

$$\begin{aligned} U'(\text{Ra}^{224}, \text{Pb}^{212}, \text{or Bi}^{212}) = \\ U(\text{Ra}^{224}, \text{Pb}^{212}, \text{or Bi}^{212}) - U(\text{Th}^{228}). \end{aligned}$$

Further, it was assumed that the urine was excreted at the mid-point of the 30 minutes between cage checks and the activity of Bi^{212} was corrected for 15 minutes decay. The results are given in Table III and clearly show that some Pb^{212} and Bi^{212} formed in the dog decays outside the dog.

TABLE III
Activities of Pb^{212} and Bi^{212} Relative
to Ra^{224} in Freshly Voided Urine

Days After Injection	$U'(\text{Pb}^{212})/U'(\text{Ra}^{224})$	$U'(\text{Bi}^{212})/U'(\text{Ra}^{224})$
671	1.72	18.0
672	1.04	9.2
673	1.10	11.2

In the second series, to obtain a more quantitative relation between Pb^{212} , Bi^{212} , and Ra^{224} urinary excretion, samples were collected over a 24 hour interval. Dog T8T3 was given water and milk at 0830 hours 777

days after injection and urine was collected at 2 hour intervals for 12 hours and at the end of 24 hours. The activities of the 3 nuclides in each sample at time of collection are given in Table IV. Also, given are the cumulative activities for each time. At 678 days the measured daily urinary excretion of Ra^{224} by this dog was

$$U'(\text{Ra}^{224}) = 0.252$$

TABLE IV

Cumulative Urinary Excretion of Ra^{224} , Pb^{212} , and Bi^{212} From 0830 Hours 777 Days After Injection to 0830 778 Days

t Hours	Ra^{224}		Pb^{212}		Bi^{212}	
	Sample	Cumulative	Sample	Cumulative	Sample	Cumulative
0	0	0	0	0	0	0
2	0.011	0.011	0.022	0.022	0.27	0.27
4	0.020	0.031	0.030	0.049	0.30	0.37
6	0.036	0.066	0.032	0.075	0.36	0.45
8	0.031	0.096	0.059	0.124	0.42	0.54
10	0.032	0.126	0.063	0.172	0.39	0.53
12	0.038	0.162	0.087	0.242	0.78	0.91
24	0.081	0.228	0.117	0.227	--	--

The cumulative value from the seven samples spanning a day is about the same, 0.228. Thus apparently there was no marked effect of the extra liquid given the dog at the beginning of the period.

The cumulative values shown for Pb^{212} are slightly high because some Pb^{212} grew in from Ra^{224} after the sample was voided but before

collection time. If we assume that the urine was voided at the mid-points of the collection intervals, and correct accordingly, the 24 hour cumulative value is 0.201, which does not differ much from 0.227. For estimating % Pb^{212} formed in the dog which decays outside, this correction is small enough to be ignored. Summing as in Eq. (32) gives

$$\sum U'(\text{Pb}^{212}) = \frac{0.227}{1 - e^{-\lambda_{\text{Pb}^{212}}}} \approx 0.4 \quad (47)$$

Fecal excretion was not measured, but from the intravenous Pb^{212} studies it is expected to be less than the urinary excretion. However, the urinary to fecal ratio is probably lower in these dogs where Pb^{212} is being formed continuously in the blood. These limited findings show that a small amount of Pb^{212} is excreted, and we estimate Pb^{212} retention to be

$$R(\text{Pb}^{212}) \approx 0.99 R(\text{Tn}^{220}) = 0.97 R(\text{Ra}^{224}) \quad (48)$$

The cumulative Bi^{212} excretion from 6 to 12 hours is the steady state value for % Bi^{212} formed in the dog but decaying outside, since 6 hours is 6 half-periods for Bi^{212} . Again the correction for Bi^{212} growth from Pb^{212} after voiding has not been made. Since the activity of Bi^{212} is about 10 times that of Pb^{212} , the correction to the mid-point of the 2 hour interval would be about 5%, which is negligible. Fecal excretion is assumed negligible because of the short half-period. Thus,

$$\sum X'(\text{Bi}^{212}) \sim 0.45 \text{ to } 0.91 \quad (49)$$

and we estimate retention as

$$R(\text{Bi}^{212}) \sim 0.99 R(\text{Pb}^{212}) = 0.96 R(\text{Ra}^{224}) \quad (50)$$

In Table V appear retention estimates (both biological, R , and actual, R'), for the Th^{228} series at 100 and 1000 days. In comparison, whole body γ -counting data gave an average of

$$R(\text{Bi}^{212}) = 0.52$$

from 10 to 1800 days(1).

TABLE V
Estimated Retention of Th^{228} Series
at 100 and 1000 Days

Nuclide	t = 100		t = 1000	
	R	R'	R	R'
Th^{228}	85.0	77.0	66.6	24.4
Ra^{224}	75.0	68.0	61.5	22.6
Tn^{220}	73.5	66.5	60.3	22.1
Pb^{212}	72.6	65.6	59.6	21.8
Bi^{212}	72.0	65.2	59.0	21.6

Liver. The activities of Ra^{224} and Pb^{212} were determined in 14 of the 22 liver samples in which Th^{228} was measured (2). The results are given both in Fig. 7 and Table VI. Included in the latter are some of the important clinical observations made at time of euthanasia. The clinical status of the dogs is perhaps the most serious limitation of the data.

Fig. 7. Activities of Ra^{224} and Pb^{212} in liver.

TABLE VI
Activities of Th^{228} , Ra^{224} , and Pb^{212}
in Liver

Dog	Days After Injection	% Th^{228}	% Ra^{224}	% Pb^{212}	Comments (1)
T5T5	65	6.4	7.2	8.2	thrombocytopenia
M4T2	78	5.3	4.4	6.4	trauma, shock
M2T5	97	5.7	5.7	5.7	pancytopenia
T4T5	113	4.3	5.6	4.7	thrombocytopenia
M1T5	212	2.6	3.1	3.4	nephritis
F3T3	547	2.2	3.0	3.1	osteosarcoma
M1T4	645	0.68	1.2	1.0	crippling fractures
T6T4	651	3.6	2.5	2.4	crippling fractures
F3T4	763	1.7	3.1	4.1	ulcerative gingivitis
M4T4	793	0.96	2.3	2.1	ulcerative gingivitis
M4T3	801	1.4	1.7	1.8	osteosarcoma
M2T4	833	1.1	2.5	1.9	osteosarcoma
M1T3	988	1.5	1.8	2.6	osteosarcoma
M1T2 ^a	1282	3.9	2.0	2.5	osteosarcoma

^aFractional counting errors high.

The three nuclides, Th^{228} , Ra^{224} , and Pb^{212} were in (or close to) equilibrium in only one case. In 10 samples Ra^{224} activity exceeded that of Th^{228} . It was lower for M4T2, T6T4, and M1T2. M4T2 died in shock the day after severe injury in a dog fight. Thus, in a few cases some of the Ra^{224} produced in the liver is moved out, while in most of the dogs some Ra^{224} formed elsewhere in the animal is moved into the liver. The equation for Ra^{224} in liver is

$$L(\text{Ra}^{224}) = 5.2e^{-0.0010t} \quad (51)$$

which shows the same time dependence as that for Th^{228} (2),

$$L(\text{Th}^{228}) = 4.0e^{-0.00094t} \quad (52)$$

Pb^{212} activity exceeded that of Th^{228} in 11 samples and Ra^{224} in 8. The equation for Pb^{212} in liver is

$$L(\text{Pb}^{212}) = 5.4e^{-0.0010t} \quad (53)$$

Eqs. (4) and (6) for Ra^{224} in plasma and Pb^{212} in blood also show the same time dependence. This simplifies answering the question of the effect of residual blood on the determination of Ra^{224} and Pb^{212} in liver. The 14 livers weighed 335 ± 94 g, so that the concentration of Ra^{224} in plasma relative to liver is only

$$\frac{5.6 \times 10^{-4}}{5.2/335} = 0.036$$

and the effect is negligible. However, the concentration of Pb^{212} in blood cells relative to liver is

$$\frac{0.011}{5.4/335} = 0.68$$

Thus, some of the Pb^{212} measured may be associated with blood cells, and it is likely that some of the variation observed in Pb^{212} results from differences in degree of exsanguination of the dog.

Spleen. The activities of Ra^{224} and Pb^{212} were determined in 11 of the spleens in which Th^{228} was measured. The results appear both in

Fig. 8 and Table VII. Ra^{224} activity was less than Th^{228} activity in 3 spleens, about the same in 3, and higher in 5. The equation is essentially the same as that for Th^{228} . They are

$$S(\text{Ra}^{224}) = 0.34e^{-0.0011t} \quad (54)$$

and (2)

$$S(\text{Th}^{228}) = 0.32e^{-0.0010t} \quad (55)$$

The average weight of the spleens was 29.5 ± 11.0 g with a range of 10.7 to 47.0 g (!), so that Ra^{224} concentration in plasma relative to that in spleen is

$$\frac{5.6 \times 10^{-4}}{0.34/29.5} = 0.053$$

In all cases the activity of Pb^{212} was less than that of Ra^{224} which means that either or both Tn^{220} and Pb^{212} are being moved out of the spleen. The Pb^{212} activity was less than that of Th^{228} in all but 2 spleens. The data can be described approximately by the equation

$$S(\text{Pb}^{212}) = 0.16e^{-0.00091t} \quad (56)$$

In the dog the spleen is a storage site for red blood cells (9), and this is probably a factor in the great range of values observed for both weight of and activity of Pb^{212} in the spleen. The concentration of Pb^{212} in the spleen ranged from 0.28 to 1.5 times that in blood cells (Table VII). Further, the bone marrow of all these dogs had received considerable irradiation and it is likely that they had to draw on their

Fig. 8. Activities of Ra^{224} and Pb^{212} in spleen.

TABLE VII
Activities of Th^{228} , Ra^{224} , Pb^{212} and Bi^{212}
in Spleen and Kidneys

Dog	Days After Injection	Spleen			$\text{Pb}^{212}/\text{g spleen}$ $\text{Pb}^{212}/\text{g b. cells}$	Kidneys			
		Th^{228}	Ra^{224}	Pb^{212}		Th^{228}	Ra^{224}	Pb^{212}	Bi^{212}
T5T5	65	0.27	0.38	0.15	0.44	1.09	0.29	0.34	2.13
M4T2	78	0.39	0.39	0.32	--	2.00	0.59	0.54	1.07
M2T5	97	0.38	0.27	0.18	0.41	0.98	0.36	0.30	1.36
T4T5	113	0.20	0.28	0.07	0.79	0.98	0.24	0.21	--
M1T5	212	--	--	--	--	1.06	0.30	0.08	--
F3T3	547	0.30	0.21	0.06	0.60	1.03	0.35	0.48	2.48
M1T4	645	0.05	0.07	0.05	0.38	0.29	0.10	0.13	0.58
T6T4	651	0.13	0.11	0.06	0.28	0.82	0.20	--	0.58
F3T4	763	0.11	0.11	0.05	1.5	1.69	0.35	0.16	--
M4T4	793	0.17	0.27	0.09	0.48	0.74	0.16	--	0.29
M4T3	801	0.17	0.17	0.11	0.65	0.73	0.19	0.42	1.43
M2T4	833	0.10	0.18	0.12	0.71	0.64	0.15	0.10	0.22
M1T3	988	0.06	--	--	--	0.82	0.36	0.37	0.51

splenic reserves (10). This suggests that at earlier times and/or lower dose levels the % Pb^{212} in spleen would be higher.

Kidneys. The activity of Ra^{224} was determined in the kidneys of 13 dogs, that of Pb^{212} in 11, and that of Bi^{212} in 10. The results appear in Table VII and Fig. 9. When descending exponentials were calculated for each of the 3 sets of data the coefficients of t in the exponential did not differ significantly from zero. A similar result was obtained for Th^{228} (2). The mean values from Table VII are

$$K(\text{Th}^{228}) = 0.99 \pm 0.44 \quad (57)$$

$$K(\text{Ra}^{224}) = 0.28 \pm 0.13 \quad (58)$$

$$K(\text{Pb}^{212}) = 0.28 \pm 0.16 \quad (59)$$

$$K(\text{Bi}^{212}) = 1.16 \pm 0.76 \quad (60)$$

Activities of Ra^{224} , Pb^{212} , and Bi^{212} , in Kidneys

Fig. 9. The activities of Ra^{224} , Pb^{212} , and Bi^{212} in kidneys.

The activity of Ra^{224} was less than that of Th^{228} in all cases including M1T5 which suffered severe kidney damage. The average of the ratios of Ra^{224} to Th^{228} activity was 0.29 ± 0.07 , while the ratio of the averages was 0.28 ± 0.18 . Thus there is more consistency in the activity of Ra^{224} relative to the activity of Th^{228} and more variation in % Ra^{224} and % Th^{228} .

From Eqs. (57) and (58) and the cumulative urinary excretion of Ra^{224} formed in the dog, Eqs. (28) and (32) it can be shown that the kidneys are clearing Ra^{224} from the blood. If Ra^{224} were not being moved to the kidneys from other parts of the dog then $\Sigma U + K(\text{Ra}^{224})$ would be equal to 0.99 ± 0.44 , Eq. (57), i.e. Th^{228} decaying in the kidneys would be the sole source. However, at $t = 100$ days $\Sigma U + K(\text{Ra}^{224}) = 3.7$ and $3.7 - 0.99 = 2.7\%$ Ra^{224} is removed from other parts of the animal. At 500 days, $\Sigma U + K(\text{Ra}^{224}) = 2.7$, still well in excess of 0.99 ± 0.44 .

Of the 4 nuclides, Pb^{212} was the most difficult to determine. Success depended on having sufficient points during the short period which begins when Bi^{212} has decayed to transient equilibrium with Pb^{212} and ends when Pb^{212} reaches transient equilibrium with Ra^{224} . In all 11 kidneys the activity of Pb^{212} was less than that of Th^{228} , and in 6 it was less than that of Ra^{224} and higher in 5, with the mean values about the same, Eqs. (58) and (59).

Next we relate some of the Pb^{212} findings. At 778 days 0.4% Pb^{212} (0.23% Pb^{212}/d) was decaying outside dog T8T3 via urinary excretion. Since the activities of Ra^{224} and Pb^{212} in the kidneys are about the same, Pb^{212} is being brought to the kidneys by the blood. At 784 days

T8T3 had 0.0065% Pb^{212} /g blood cells (compare with the 784 day value of 0.0051 from Eq. (6)). Thus, the effective clearance is

$$\frac{0.23\% \text{ Pb}^{212}/\text{d}}{0.0065\% \text{ Pb}^{212}/\text{g blood cells}} = \frac{35 \text{ g cells}}{\text{day}} \quad (61)$$

From the studies in which beagle blood cells were tagged with Pb^{212} in vitro and reinjected (7), we calculate (using V.P.R.C. = 0.49) a clearance of 31 g blood cells/day.

Dog T8T3 weighed 7.5 kg, and using 43 ml cells/kg dog (10) and 1.09 g cells/ml cells (11) we calculate

$$0.0065 \times 1.09 \times 43.0 \times 7.50 = 2.3\% \text{ Pb}^{212} \quad (62)$$

for the steady state activity of Pb^{212} associated with circulating blood cells.

Since Bi^{212} has a short half period (1 hour), its activity at time of death can be determined only if counting is begun soon after death. Also, the determination is facilitated when the activity of Bi^{212} is high relative to that of Pb^{212} . This injects some bias into the results. Counting of the 10 kidneys for which there are Bi^{212} entries in Table VII was begun at a mean time of 1.3 hours after death (range 0.8 to 2.2 hours). The times for the other 3 were 1.6, 2.5, and 2.0 hours. It is likely that the Bi^{212} activity in each of these 3 was greater than Pb^{212} but less than the average, Eq. (60), so that the average given is slightly high for the set of 13 kidneys. In 4 of 10 kidneys Bi^{212} activity

was lower than that of Th^{228} and it was higher in 6. In all cases where determination was possible the activity of Bi^{212} exceeded that of Ra^{224} and of Pb^{212} . Thus Bi^{212} is being moved to the kidneys from other parts of the animal.

Dog T8T3 provides suggestive evidence that the combined activity of Bi^{212} in kidneys and urine is greater than that expected from Bi^{212} formed in the kidney and Bi^{212} formed in or on and escaping from blood cells. The measured steady state activity of Bi^{212} in urine was 0.45 - 0.91% Bi^{212} , Eq. (49). Using this value plus the average kidney Bi^{212} activity of Eq. (60) we estimate the total in urine and kidneys at 1.6 to 2.1% Bi^{212} . By Eq. (59) we expect formation of Bi^{212} in kidneys to be about 0.3%. Associated with the blood cells is 2.3% Pb^{212} , Eq. (62). One early count was obtained from this sample (at 42 mins. after this blood was drawn). From this one point the calculated Bi^{212} activity in blood cells was 1.03 that of Pb^{212} . Hence little or no Bi^{212} was moving out of the cells so that the cells were not a significant source of the Bi^{212} in kidneys and urine. Thus we conclude that Bi^{212} is moved to the kidneys and urine from other (not blood cells) sites in the dog by way of the plasma.

The following related study gives further support to this conclusion. A 7 year old female beagle, F54A, was anesthetized with nembutol, a urinary catheter was introduced, and the dog was given an intravenous injection of 11.1 μC Pb^{212} with Bi^{212} in transient equilibrium in citrate buffer pH $\approx$ 3.5, 0.08M total citrate. Blood was drawn at 3.4 min. and at hourly intervals for 6 hours. Urine was obtained, via the catheter, at hourly intervals for 6 hours. The anesthesia was maintained for the

duration of the experiment. At 1 hour after injection 100 ml normal saline was given subcutaneously. After the final urine was withdrawn, the bladder was washed with 10 ml saline which was then added to the urine sample. Pb^{212} and Bi^{212} activities were measured in the various plasma, blood cell, and urine samples and the results appear in Table VIII.

TABLE VIII

Pb^{212} and Bi^{212} in Blood Cells and Plasma and in Urine
Obtained by Catheterization Following Intravenous Injection
of Pb^{212} and Bi^{212} in Transient Equilibrium

t (min)	Plasma		Cells		
	% Pb^{212} /g	% Bi^{212} /g	% Pb^{212} /g	% Bi^{212} /g	Bi^{212} / Pb^{212}
3.4	0.012	0.069	0.089	0.020	0.22
61.1	0.002	0.009	0.113	0.041	0.36
118	≤ 0.001	0.006	0.119	0.072	0.60
179	≤ 0.001	0.005	0.118	0.092	0.78
243	≤ 0.001	0.003	0.119	0.091	0.77
305	≤ 0.001	0.004	0.115	0.099	0.86
365	≤ 0.001	0.004	0.114	0.109	0.93

Urine						
t (min)	% Pb^{212}	Cum.	% Bi^{212}	Cum.	$\frac{\text{Cum. \% } Bi^{212}}{\text{\% } Pb^{212} \text{ /g Cells}}$	
		% Pb^{212}		% Bi^{212}	$\frac{\text{\% } Bi^{212}}{\text{\% } Pb^{212}}$	
59	0.51	0.51	2.25	2.25	25	4.4
116	0.67	1.18	2.44	3.68	32	3.6
178	0.76	1.94	2.37	4.30	36	3.1
240	0.35	2.29	1.33	3.59	30	3.8
300	0.14	2.43	0.71	2.62	23	5.0
360	0.19	2.62	0.85	2.25	20	4.6

Before considering the results it should be noted that the study has 3 limitations with respect to using the results to help understand

the migrations of the Th^{228} decay products. First is the unknown effect of the 6 hour anesthesia; second the dog was 7.1 years old whereas the Th^{228} dogs were roughly 1.5 to 5 years (T8T3 was 3.8 years at 778 days after injection); third, the dog was purchased from an outside kennel 3 weeks prior to the experiment and there was no medical record available to indicate whether there had been diseases which could have led to impairment of kidney function.

A measured aliquot of the injection standard was counted alternately with the samples and the data were analyzed in terms of ratios of sample to standard count rates. This method removes the Pb^{212} decay factor and also effects the decay constant of Bi^{212} insofar as determining its growth and decay in sets of samples is concerned. The advantage of the method is that the biological rates can be examined more easily if the effect of physical decay is removed.

Let subscripts 1 and 2 denote Pb^{212} and Bi^{212} , and s and S the sample and standard count rates, respectively. The activities in transient equilibrium are given by

$$A_1 = A_1^0 e^{-\lambda_1 t} \quad (63)$$

$$A_2 = \frac{\lambda_2}{\lambda_2 - \lambda_1} A_1^0 (e^{-\lambda_1 t} - e^{-\lambda_2 t}) + A_2^0 e^{-\lambda_2 t} \quad (64)$$

$$A_2^0 = \frac{\lambda_2}{\lambda_2 - \lambda_1} A_1^0 = 1.11 A_1^0 \quad (65)$$

Remembering that the detector essentially measures only Bi^{212} the

standard count rate is

$$S = c \left\{ 1.11 A_1^0 (e^{-\lambda_1 t} - e^{-\lambda_2 t}) \right\} + A_2^0 e^{-\lambda_2 t} = c A_2^0 e^{-\lambda_1 t} \quad (66)$$

where c is a constant including detection efficiency and dilution correction. The count rate of a sample collected at $t = T$ is, for $t \geq T$,

$$s = c' \left\{ x 1.11 A_1^T (e^{-\lambda_1 (t-T)} - e^{-\lambda_2 (t-T)}) \right\} + y A_2^T e^{-\lambda_2 (t-T)} \quad (67)$$

where x and y are fractions of the Pb^{212} and Bi^{212} at $t = T$. The sample to standard count rate ratio is then

$$\begin{aligned} \frac{s}{S} &= \frac{c' A_2^T (x e^{-\lambda_1 (t-T)} + (y-x) e^{-\lambda_2 (t-T)})}{c A_2^0 e^{-\lambda_1 t}} \\ &= \frac{c'}{c} \left\{ x + (y-x) e^{-(\lambda_2 - \lambda_1) (t-T)} \right\} \end{aligned} \quad (68)$$

since $A_2^0 e^{-\lambda_1 t} = A_2^T e^{-\lambda_1 (t-T)}$. Thus we have

$$\% Pb^{212} = \left(\frac{c'}{c} x \right) 100 \quad (69)$$

and

$$\% Bi^{212} = \left(\frac{c'}{c} y \right) 100 \quad (70)$$

Note that c'/c is just a ratio of dilution corrections.

As seen in Table VIII, Pb^{212} concentration in blood cells reached a

peak at about 2 hours, very much the same as in Ref. (7) and Fig. 2 whereas plasma concentration decreased rapidly to less than 1% that of cells. % Bi²¹²/g in plasma decreased too but was always higher than the % Pb²¹²/g plasma. The concentration of Bi²¹² in cells increased throughout the course of the experiment. If Bi²¹² is associated with cells only if it is formed in cells, the ratio of activities is

$$\frac{\% \text{ Bi}^{212}}{\% \text{ Pb}^{212}} = a(1 - e^{-(\lambda_{\text{Bi}^{212}} - \lambda_{\text{Pb}^{212}})t}) \quad (71)$$

where a is fractional retention of Bi²¹² by cells. If no Bi²¹² formed in cells is lost from cells, then a = 1. Eq. (71) with a = 1 is shown as the solid curve in Fig. 10. The least squares principle was used to calculate the asymptote from the observed Bi²¹²/Pb²¹² ratios (3.4 min. point excluded) and yielded

$$a = 0.916 \quad (72)$$

Thus 90% or more of the Bi²¹² formed in cells decays there.

From the peak concentration of Pb²¹² in cells the estimated total blood cell mass is (2/3) 100%/0.12%/g = 555 g cells (7). The blood cell volume estimated from dog weight is 13 kg/.43 ml/kg = 560 ml cells (10).

The activities of Pb²¹² and Bi²¹² in each urine sample at time of collection are given in Table VIII. Since the Pb²¹² decay factor has been taken out, the cumulative Pb²¹² is obtained by simply adding and Bi²¹² is accumulated using $e^{-(\lambda_{\text{Bi}^{212}} - \lambda_{\text{Pb}^{212}})t}$. At 6 hours the cumulative urinary excretion of Pb²¹² is 2.6% and the clearance is roughly

Fig. 10. $\text{Bi}^{212}/\text{Pb}^{212}$ in blood cells after intravenous injection of Pb^{212} and Bi^{212} in transient equilibrium. Solid curve is growth curve for no loss of Bi^{212} from cells and broken curve is for 8.4% loss.

1 - 2 g cells/hour.

The 5 and 6 hour values for cumulative Bi^{212} urinary excretion are essentially steady state values. They are 2.6 and 2.2% Bi^{212} , respectively, and are equal to the activities of Bi^{212} being produced in 23 and 20 g blood cells. Thus the steady state urinary Bi^{212} is about $(23/550) \cdot 100 = 4\%$ of the Bi^{212} formed in blood cells and about 1/2 of the Bi^{212} lost from these cells. If the cells lose Bi^{212} only to the kidneys, then the effective transit time from cells through the kidneys to the bladder is about 1 hour, i.e. 1 Bi^{212} half-period.

The last column in Table VIII gives the ratios, % $\text{Bi}^{212}/\% \text{Pb}^{212}$, for each urine sample.

Next we apply these results to the Th^{228} decay product studies. First these results show at least 90% of the Bi^{212} formed in blood cells decays there. Hence, blood cells are not the principal source of the relatively high activities of Bi^{212} found in kidneys of dogs given Th^{228} . Second, the $\text{Bi}^{212}/\text{Pb}^{212}$ activity ratio in urine was considerably lower than in the urine samples from T8T3 described above. Since these urines were obtained by catheter a higher ratio would be expected because there was a shorter period for Bi^{212} decay between the time the urine left the kidneys and sample collection time.

Both of these factors provide additional evidence that some Bi^{212} formed at sites other than blood cells and kidneys is being moved to the kidneys. We have no indication of which sites are being depleted, although it does seem likely that they would be highly vascular.

Humerus and Third Lumbar Vertebra. The activities of Ra^{224} and Pb^{212} in 15 humeri, of Ra^{224} in 13 and Pb^{212} in 12 third lumbar vertebrae

have been measured. The results are shown in Fig. 11. In all of the humeri both Ra^{224} and Pb^{212} activities were less than that of Th^{228} , and Pb^{212} activity was less than that of Ra^{224} except in one case in which they were nearly equal. In one vertebra the activities of Th^{228} , Ra^{224} , and Pb^{212} were equal, in two others Pb^{212} and Ra^{224} activities were

Fig. 11. The activities of Ra^{224} and Pb^{212} in one humerus and the third lumbar vertebra.

equal. In the others the activity of Pb^{212} was less than that of Ra^{224} which was less than that of Th^{228} .

The data were analyzed for a change with time, as described above, but there was no significant change for either nuclide in either bone. The sums of the activities in the two bones, H + V, and the several activity ratios in each bone, H and V, and the sum, H + V, also did not show a significant change with time. The means and standard deviations of these various quantities are given in Table IX, which also includes the previously reported measurements of Th^{228} (2).

TABLE IX
Activities of Ra^{224} and Pb^{212} in One Humerus (H) and the Third Lumbar Vertebra (V)

Quantity ^a	n	$\bar{H}$	n	$\bar{V}$	n	$\overline{(H + V)}$
% Ra^{224}	15	2.67 ± 0.25	13	0.972 ± 0.179	13	3.64 ± 0.32
$\text{Ra}^{224}/\text{Th}^{228}$	15	0.904 ± 0.052	13	0.892 ± 0.074	13	0.895 ± 0.054
% Pb^{212}	15	2.55 ± 0.32	12	0.940 ± 0.193	12	3.53 ± 0.38
$\text{Pb}^{212}/\text{Th}^{228}$	15	0.862 ± 0.076	12	0.854 ± 0.098	12	0.866 ± 0.070
$\text{Pb}^{212}/\text{Ra}^{224}$	15	0.954 ± 0.058	12	0.965 ± 0.032	12	0.967 ± 0.029
% Th^{228} ^b	21	2.86 ± 0.45	20	$1.2e^{-0.00025t}$	20	$4.2e^{-0.00014t}$

a Mean and standard deviation are given.

b From Ref. (2).

These two bones contained 6.1% of the Th^{228} in the skeleton and this was used to calculate the equation for Th^{228} in the skeleton:

$$B(\text{Th}^{228}) = 69e^{-0.00014t} \quad (73)$$

If we assume that the $\text{Ra}^{224}/\text{Th}^{228}$ ratio in H + V is representative of the entire skeleton, then these two bones contain 6.1% of the Ra^{224} in the skeleton, and the equation for total Ra^{224} in bone is

$$B(\text{Ra}^{224}) = 0.895 B(\text{Th}^{228}) = 62e^{-0.00014t} \quad (74)$$

The latter assumption seems valid since the major part of the Ra^{224} does not translocate at times greater than 100 days. The counter argument is that % $\text{Ra}^{224}/\text{bone}$ did not decrease with time by the above mentioned criteria. Similarly we estimate the total Pb^{212} in the skeleton to be

$$\begin{aligned} B(\text{Pb}^{212}) &= 0.967 B(\text{Ra}^{224}) = 0.866 B(\text{Th}^{228}) \\ &= 60e^{-0.00014t} \end{aligned} \quad (75)$$

Balancing the Books. From excretion studies we have determined the % Ra^{224} which decays in the dog, and from measurement of selected bones and soft tissues we have determined the % Ra^{224} which decays at these sites. Are the results from the two kinds of measurements compatible? Fig. 12A. provides a graphical comparison. At the top is plotted the retention equation, Eq. (35). The bottom curve is the estimate of Ra^{224} in bone, Eq. (74), and above it is the sum of the Ra^{224} in bone, liver, spleen, and kidneys, Eqs. (74, 52, 54, and 58). The total Ra^{224} in plasma is small compared with this sum (about 0.25% at 100 days and about

0.08% at 1000 days) and was not included. There is one more site about which we have some information; it is the intestinal tract. If we guess

Fig. 12. A. Comparison of the retention of Ra^{224} , R , with the Ra^{224} in bone, B , with the total Ra^{224} in bone, liver, spleen, and kidneys, $B + L + S + K$, and with that total plus the estimated Ra^{224} in the intestinal tract, $2 F'$. B. Comparison of the retention of Pb^{212} , R , with the Pb^{212} in bone, B , and with the total Pb^{212} in bone, liver, spleen, kidneys, and blood cells, $B + L + S + K + 430C$.

that the intestinal tract contains twice the daily fecal excretion of Ra^{224} and add $2 F'$, from Eq. (20) to the above sum we obtain the broken curve of Fig. 12A. This curve is just a little lower than the retention curve, which is appropriate for at least two reasons. First, there undoubtedly is some Ra^{224} in the other soft tissues which were not measured, and, second, retention derived from excretion is more likely to be high than low.

In Fig. 12B. a similar comparison of the Pb^{212} results has been made. The top curve is the retention equation, Eq. (48). The bottom curve is the estimate of Pb^{212} in bone, Eq. (75). The middle curve is the sum of the Pb^{212} in bone, liver, spleen, kidneys, and blood cells, from Eqs. (75, 53, 56, 59 and 6). The total weight of blood cells used is

$$\frac{43 \text{ ml}}{\text{kg}} \times 9.2 \text{ kg} \times \frac{1.098}{\text{ml}} = 430 \text{ g cells.}$$

Again the sum curve is seen to differ appropriately from the retention curve. Thus the results of the two kinds of measurements are consistent.

Some Estimates of Average Radiation Dose Rates. The short-lived nuclide Tn^{220} presents a problem in estimating radiation dose rates since its half-period is too short to permit its measurement in tissue samples obtained at autopsy. From Ra^{224} retention, Eq. (36), we know how much Tn^{220} is formed in the dog and from the Tn^{220} exhalation studies (4) we know how much Tn^{220} decays in the dog, but we lack precise information on how much Tn^{220} decays in tissues of interest.

Let us consider bone. The biological rate of decrease of Th^{228} in

bone is slow with a half-period of about 14 years, Eq. (73). Throughout the period studied the activity of Ra^{224} was less than that of Th^{228} showing that some Ra^{224} in bone moves out to decay elsewhere. Also the biological rate of decrease of Ra^{224} in bone is at least as slow as that of Th^{228} , and may be slower, Table IX. The activity of Pb^{212} in bone was less than that of Ra^{224} . This means that either some Tn^{220} or some Pb^{212} or some of both nuclides move out of bone to decay elsewhere. There is the further possibility that some of the Pb^{212} found in bone was formed elsewhere in the dog and then deposited in bone. If bone were the only site of formation of Tn^{220} we could readily distinguish among these possibilities, but since there is appreciable Ra^{224} in soft tissues this is not possible.

However, we can estimate Tn^{220} activity in bone from several different sets of assumptions and make comparisons.

First we reject the possibility that Tn^{220} activity in bone is greater than that of Ra^{224} . This possibility would require a transfer of Tn^{220} from soft tissues to bone which is incompatible with our experience with Ra^{224} formed in vivo and also with Rn^{222} formed in vivo.

Second we examine the possibility that the activities of Tn^{220} and Ra^{224} in bone are equal, i.e.

$$B_{\max}(\text{Tn}^{220}) = B(\text{Ra}^{224}) = 62e^{-0.00014t} \quad (76)$$

Eq. (76) provides an upper limit for Tn^{220} in bone and it states that there is no net transfer of Tn^{220} from bone to blood and/or soft tissues, and, hence that the soft tissues are the source of the Tn^{220} which decays

in blood and which is exhaled. Intuitively we expect that Tn^{220} would move more rapidly from a highly vascular soft tissue than from bone. If the transit time from soft tissue to blood is close to the Tn^{220} half-period and the transit time from bone to blood is appreciably greater, then soft tissues will be depleted of Tn^{220} and bone will not.

Experimentally we know that there is ample Ra^{224} in soft tissues to supply the Tn^{220} which decays in blood or is exhaled. An additional clue comes from our studies of Pb^{212} associated with the blood cells. The principal source of this Pb^{212} is undoubtedly the Tn^{220} which decays in the blood with transfer of Pb^{212} from bone and soft tissues being a minor source. The biological rate of decrease of Pb^{212} in blood cells, from Eq. (4), must be the same as that rate for its source. Comparison of Eqs. (4, 52, 54 and 74) shows that the biological rate of decrease of Pb^{212} in blood cells is essentially the same as the rate at which Ra^{224} in liver and spleen decreases, but it is about 7 times greater than the rate at which Ra^{224} in bone decreases. From the requirement of equivalent rates it follows that soft tissues are the source of most of the Tn^{220} going to the blood.

The third possibility to examine is that all the Tn^{220} which decays in blood or is exhaled comes from bone and none comes from soft tissues. While this seems highly unlikely, it is worth considering since it provides a method of setting a lower limit on the activity of Tn^{220} in bone.

Assume that Pb^{212} formed from Tn^{220} decay in the blood behaves as Pb^{212} given intravenously, i.e. that 52% decays in the blood (7). Also assume Tn^{220} decay in blood is the only source of the Pb^{212} associated with blood cells. Then we can calculate an upper limit for how much

Tn^{220} (and Po^{214}) decays in blood. It is

$$\text{Max. \% Tn}^{220} \text{ decaying in blood} = 430\text{C}/0.52 = 9.1e^{-0.00097t} \quad (77)$$

The minimum activity of Tn^{220} in bone is then the Tn^{220} formed in bone diminished by the Tn^{220} which decays in blood and which is exhaled.

Using the estimate of Eq. (45) for the latter together with Eqs. (74) and (76) we have

$$B_{\min}(\text{Tn}^{220}) = 62e^{-0.00014t} - 9.1e^{-0.00097t} - 2.5e^{-0.0003t} \quad (78)$$

Next we calculate maximum and minimum average radiation dose rates to bone using Eqs. (73, 74, 75, 76, and 78) for Th^{228} , Ra^{224} , Pb^{212} , and $\text{Tn}^{220}_{\max.}$ and $\text{Tn}^{220}_{\min.}$, respectively. Early counts on bone samples showed that there was no great difference between Pb^{212} and Bi^{212} activities. The studies of Bi^{212} in blood, kidneys, and urine presented above showed that 1 to 1.5% Bi^{212} moves to kidneys and urine from other sites. We assume that this small amount of Bi^{212} does not come from bone, and that the activities of Pb^{212} , Bi^{212} , Po^{212} , and Tl^{208} in bone are equal. Then we have, assuming skeletal weight = 0.1 body weight and using average alpha, beta, and recoil energies (1),

$$\begin{aligned} \frac{dD_{\max}}{dt} &= \left\{ 0.69 \times (5.50 + 0.62 \times 19.07 + 0.60 \times 8.70) \right\} \times \\ &\quad e^{-0.00014t} e^{-\lambda_{\text{Th}^{228}} t} \times I \times 0.512 \\ &= 10.6 e^{-0.00114t} I \text{ rads/day} \end{aligned} \quad (79)$$

where $\lambda_{Th}^{228} = 0.00100$, $I = \mu c \text{ Th}^{228}$ injected/kg, and 0.512 is the product of the necessary conversion factors. The minimum estimate is

$$\begin{aligned} \frac{dD_{min}}{dt} &= \frac{dD_{max}}{dt} - \left\{ 0.091e^{-0.00097t} + 0.025e^{-0.0003t} \right\} \times \\ &\quad 13.3 e^{-\lambda_{Th}^{228}t} \times I \times 0.512 \quad (80) \\ &= 10.6e^{-0.00114t} - 0.62e^{-0.00197t} - 0.17e^{-0.0013t} I \text{ rads/day} \end{aligned}$$

Integrating Eqs. (79) and (80) gives the maximum and minimum average radiation dose to bone:

$$D_{max} = 9300 (1 - e^{-0.00114t}) I \text{ rads} \quad (81)$$

and

$$D_{min} = D_{max} - 315 (1 - e^{-0.00197t}) - 130 (1 - e^{-0.0013t}) I \text{ rads} \quad (82)$$

The actual dose rate is most certainly greater than the calculated minimum, Eq. (80), for the two assumptions, 1) that soft tissues retain all Tn^{220} produced there but bone does not, and 2) that no Pb^{212} leaves bone even though Ra^{224} is known to leave bone and Tn^{220} is assumed to do so, are quite extreme.

The maximum calculated dose rate is based on more realistic assumptions which are consistent with the short half-period of Tn^{220} and the correlation between the biological rate of decrease of Pb^{212} in blood cells and that for Ra^{224} in liver and spleen. Eqs. (79) and (81) appear to be the equations of choice. Also, the difference between the two is small, at 100 and 1000 days, dD_{min}/dt is 0.931 and 0.961 dD_{max}/dt ,

respectively. Thus, any overestimation of bone dose rate by Eq. (79) is small and not significant with respect to other uncertainties in these studies.

Although the Tn^{220} problem does not have a large effect on the calculation of average radiation dose rate to bone, we are completely lacking in information on its effect on local bone dose rates.

Another problem for which we lack information is the effect of the decay of Tn^{220} and Po^{216} in blood on the observed toxicity of Th^{228} . Since we have only measured the sum of the effects of Tn^{220} and Pb^{212} migrations and cannot separate them, we can only set an upper limit for the energy dissipated. From Eq. (77) it is

$$\begin{aligned} \text{Max. rate of energy release from } \text{Tn}^{220} \text{ and } \text{Po}^{216} \text{ decay in blood} = \\ 6200 \text{ I W } e^{-0.00197t} \text{ ergs/day} \end{aligned} \quad (83)$$

where W is the dog weight at injection. This energy is in addition to that which results from decay of Pb^{212} plus decay products in or on blood cells. It is given by

$$\begin{aligned} \text{Rate of energy release from } \text{Pb}^{212} \text{ and decay products on or in blood cells} = \\ 1950 \text{ I W } e^{-0.00197t} \text{ ergs/day} \end{aligned} \quad (84)$$

We lack specific information on the sites which receive the energy, Eqs. (83) and (84). Some possible consequences are damage to capillary walls

with ensuing decrease in effectiveness of the vascular system, and damage of critical cells in tissues which fall within range.

Estimation of even average radiation dose rates to soft tissues is severely limited by the Tn^{220} problem. As an example, consider the liver. Using Eqs. (52), (51), and (53) and the average weight at injection of 9.04 kg for these 14 dogs, (the average liver weight given above is 335 g) we calculate the dose rate for the two cases of complete retention and complete loss of Tn^{220} .

$$\text{If } L(\text{Tn}^{220}) = L(\text{Ra}^{224}), \text{ then} \quad (85)$$

$$\frac{dD_{\max}}{dt} = 2.32 \text{ I } e^{-0.0020t}$$

and if

$$\frac{dD_{\min}}{dt} = 1.37 \text{ I } e^{-0.0020t} \quad (86)$$

Here the minimum is only 0.59 the maximum. Also some part of the energy given in Eqs. (83) and (84) results in some localized irradiation of the liver.

It is interesting to note the higher liver dose rate, Eq. (85), does not differ greatly during the first year from that for dogs given about $2.7 \text{ } \mu\text{C Pu}^{239}/\text{kg}$ (5-level), (12). For these dogs,

$$\frac{dD(\text{Pu}^{239})}{dt} = 2.46 \text{ I } e^{-0.00116t} \quad (87)$$

For this group of 22 dogs, mean weight at injection = 9.58 kg, and mean

liver weight = 310 g.

Since kidney damage has been observed in 4- and 5-level Th^{228} dogs (1), and since the urinary excretion of both Th^{228} (2) and Ra^{224} (see above) decreases more rapidly in the case of 4-level dogs than for lower level dogs, it is appropriate to estimate some average radiation dose rates to the kidneys. Of the 13 dogs listed in Table VII, kidney measurements are complete for 8 of them. The maximum and minimum dose rates, assuming complete retention and complete loss of Tn^{220} , respectively, can be calculated easily from Eqs. (57, 58, 59, and 60). However, the weight of kidneys of the 8 dogs ranged from 32.1 g to 79.1 g, a factor of 2.5. For this reason it seems more appropriate to tabulate individual values for the 8 dogs, and these appear in Table X. Since the activity of Ra^{224} is less than that of either Th^{228} or Bi^{212} , the maximum and minimum calculations do not differ as much as do those for the liver.

TABLE X
Average Radiation Dose Rates to Kidneys
at Time of Death

Dog	Days After Injection	Wt. of Kidneys (gs)	I = $\mu\text{CTh}^{228}/\text{kg}$ Injected	dD_{max}/dt (rads/day)	dD_{min}/dt (rads/day)
T5T5	65	59.0	2.32	$0.24\text{IW}^a = 5.6$	$0.21\text{IW} = 4.9$
M4T2	78	79.1	0.090	$0.19\text{IW} = 0.22$	$0.14\text{IW} = 0.16$
M2T5	97	61.0	2.63	$0.18\text{IW} = 3.9$	$0.14\text{IW} = 3.0$
F3T3	547	61.5	0.272	$0.16\text{IW} = 0.52$	$0.14\text{IW} = 0.46$
M1T4	645	32.5	0.882	$0.069\text{IW} = 0.51$	$0.058\text{IW} = 0.43$
M4T3	801	46.0	0.285	$0.098\text{IW} = 0.27$	$0.086\text{IW} = 0.24$
M2T4	833	32.1	0.916	$0.057\text{IW} = 0.43$	$0.043\text{IW} = 0.32$
M1T3	988	75.8	0.301	$0.060\text{IW} = 0.16$	$0.048\text{IW} = 0.13$

a W = weight at injection

Further, in connection with the dose level effect on urinary excretion of Th^{228} and Ra^{224} , two factors should be noted, 1) there did not appear to be a dose level effect on urinary volume; 2) measurements of Th^{228} and Ra^{224} in plasma were too limited to permit examination for a dose level effect, so that we cannot differentiate between decreased kidney ability to excrete Th^{228} and Ra^{224} and decreases in the Th^{228} and Ra^{224} brought to the kidneys by the blood. Fecal excretion was depressed, too.

A final comment is that, while the elusiveness of Tn^{220} and the limited extent of the Pb^{212} and Bi^{212} measurements impose some limitations on the results, the most important limitation would seem to be that many of the measurements were made on higher level terminally ill dogs.

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