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Annual Report of Work in Progress
on the Chronic Toxicity Program

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

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Respectfully Submitted by

THOMAS F. DOUGHERTY, Director

MARCH 31, 1964

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

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

Respectfully Submitted by:
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TABLE OF CONTENTS

	Page
Availability of Previous Reports	iii
Group Leaders and Sections	iiii
Current Census of the Beagle Colony	1
Preface to Injection Tables	2
Injection Tables	
I. Toxicity Animals	
A. Radium-226	6
B. Plutonium-239	12
C. Radium-228 (Mesothorium)	18
D. Thorium-228 (Radiothorium)	24
E. Strontium-90	30
II. Test Animals	
A. Radium-226	36
B. Plutonium-239	42
C. Radium-228 (Mesothorium)	46
D. Thorium-228 (Radiothorium)	48
E. Strontium-90	50
F. Radium-224	54
G. Ancillary	56
Radiation Induced Turbinate Atrophy	59
Considerations in Radium Therapy for Melanoma	74
The Effects of Ionizing Radiations on Endocrine Cells. III. Restoration of Testosterone Production with a Reduced Nicotinamide Adenine Dinucleotide Phosphate Generat- ing System after Localized Irradiation to the Heads of Rats	80
Serum Transaminase Levels in Beagle Dogs Burdened with Pu ²³⁹	93
Quantitative Studies of Trabecular Bone Remodeling	111
Quantitative Autoradiographic Dose Determinations of Pu ²³⁹ in Bones	127
Distribution of Ra ²²⁶ Between Blood Cells and Plasma	133
Sr ⁸⁹ Therapy of a Canine Osteosarcoma	152

Sr ⁹⁰ + Y ⁹⁰ Bremsstrahlung Efficiencies in Mammals of Various Sizes	170
Human Total Body Counter Calibration	186
Determination of Localized Alpha-Dose IV in Soft Tissue Near Radioactive Bone	199
Additions to Bibliography (April 1, 1963 to March 31, 1964)	206

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>
C00-119-229*	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	Jun 1954	Consultants Meeting	19.75	9.40

*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

Dr. Ludvik Peric-Golia, Consultant in Pathology.

CURRENT CENSUS OF THE BEAGLE COLONY

April 30, 1964

Assignment	Number of Dogs
Plutonium, toxicity	33
Plutonium, test	4
Radium, toxicity (Ra^{226})	37
Radium, test (Ra^{226})	10
Quickradium, test (Ra^{224})	11
Radiothorium, toxicity	59
Radiothorium, test	0
Mesothorium, toxicity	54
Mesothorium, test	0
Strontium, toxicity	71
Strontium, test	1
Controls, aging	41
Controls, X-ray	5
Unassigned (Male 26, Female 38)	64
Total	390

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). * The desired "retained" activities are the same for all 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. Level 1 = $10 \times \frac{0.1 \text{ } \mu\text{C Ra}^{226}}{70 \text{ kg man}} = 0.0143 \text{ "retained" } \mu\text{C/kg}$. 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}

*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 330 days
average Pu^{239}	retention = 0.90 after 6 days
average Ra^{228}	retention = 0.25 after 235 days
average Th^{228}	retention = 0.90 after 6 days
average Sr^{90}	retention = 0.25 after 150 days

M = Ra²²⁸, T = Th²²⁸, S = Sr⁹⁰, Q = Ra²²⁴, J = Sr⁸⁵, 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 were calculated for the interval, day of injection to day of death, by means of the equations and graphs which were summarized in the Radiobiology Annual Progress Report for March 31, 1959 (C00-218), pp. 121-145. These dose calculations will be revised as our radioactivity measurements are extended.

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

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 (COO-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.

TABLE I. TOXICITY ANIMALS (MAR. 31. 1964)

A. RADIUM-226

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	3646	
M05R0.0	460	6.57		22	06	54	3570	
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	3170	
F11R0.0	544	10.2		20	12	55	3024	
F12R0.0	501	8.68		17	01	56	2996	
M13R0.0	515	12.3		4	3	64	27	
M13R0.2	529	9.77	.00577	4	3	64	27	
M13R0.5	529	11.0	.0171	4	3	64	27	
M01R1.0	471	8.48	.0618	20	04	53	3998	
M02R1.0	627	10.0	.0876	16	11	53	3788	
F03R1.0	706	8.68	.0576	10	03	54	3674	
M04R1.0	414	8.60	.0642	7	04	54	2038	130
M05R1.0	490	11.7	.0436	22	06	54	3570	
F06R1.0	483	7.23	.0584	27	07	54	3535	
M07R1.0	511	11.4	.0651	24	08	54	3507	
F08R1.0	861	8.98	.0559	21	12	54	2988	160
F09R1.0	781	9.88	.0521	11	04	55	3277	
M10R1.0	523	11.5	.0573	27	07	55	3170	
F11R1.0	511	11.2	.0522	20	12	55	3024	
F12R1.0	501	9.71	.0444	17	01	56	2996	
M13R1.0	529	11.7	.0527	4	3	64	27	
M01R1.7	523	9.98	.137	17	01	56	2996	
M02R1.7	598	7.85	.163	30	11	56	1273	220
M02R1.7A	493	12.0	.222	6	3	63	391	

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
F08R1.0 LYMPHOSARCOMA
F09R1.0
M10R1.0
F11R1.0
F12R1.0
M13R1.0

M01R1.7
M02R1.7 LYMPHOSARCOMA
M02R1.7A

DOG NUMBER	AT INJECTION		INJECTED (μ c /KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/64	DEATH	
F03R1.7	473	13.1	.165	20	12	55	3024		
M04R1.7	514	6.20	.163	20	12	55	3024		
M05R1.7	511	10.1	.151	20	12	55	3024		
F06R1.7	491	7.9	.152	20	12	55	3024		
M07R1.7	598	7.17	.163	30	11	56	2678		
F08R1.7	491	9.50	.154	20	12	55		2577	390
F09R1.7	598	7.55	.168	30	11	56	2678		
M10R1.7	590	9.57	.167	30	11	56		557	110
M10R1.7A	545	10.6	.183	7	01	59	1910		
F11R1.7	598	8.17	.165	30	11	56	2678		
F12R1.7	590	8.95	.167	30	11	56		2399	390
M01R2.0	471	8.74	.382	20	04	53		3440	1240
M02R2.0	592	8.21	.387	16	11	53		3775	1370
F03R2.0	541	8.53	.347	10	03	54	3674		
M04R2.0	414	10.5	.361	7	04	54		325	150
M04R2.0A	420	10.6	.306	11	04	55	3277		
M05R2.0	461	11.5	.267	22	06	54	3570		
F06R2.0	486	10.6	.360	27	07	54	3535		
M07R2.0	514	11.1	.413	24	8	54		3425	1330
F08R2.0	572	6.95	.331	21	12	54	3388		
F09R2.0	592	9.38	.317	11	04	55	3277		
M10R2.0	523	9.95	.345	27	07	55	3170		
F11R2.0	495	9.30	.310	20	12	55	3024		
F12R2.0	497	10.3	.281	17	1	56		2948	800
M01R3.0	473	8.91	1.20	20	04	53		2850	3290
M02R3.0	470	9.02	1.21	16	11	53		2226	2680
F03R3.0	386	7.74	1.11	10	03	54		2497	2710
M04R3.0	412	11.7	1.16	7	04	54		1917	2240
M05R3.0	461	13.0	.846	22	06	54		2955	2280
F06R3.0	486	9.75	1.14	27	07	54		1932	2220
M07R3.0	514	12.3	1.29	24	08	54		2099	2700
F08R3.0	542	7.76	1.03	21	12	54		2612	2620
F09R3.0	551	8.02	.987	11	04	55		2487	2440
M10R3.0	525	10.1	1.06	27	07	55		1737	1870
F11R3.0	495	12.9	.938	20	12	55		1610	1550
F12R3.0	497	11.4	.883	17	1	56		1897	1790
M01R4.0	471	9.08	3.51	20	04	53		1606	5790

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 31/3/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR.		
M02R4.0	470	9.53	3.55	16	11	53	1884	6750
F03R4.0	384	8.65	3.33	10	03	54	490	1920
F03R4.0A	598	7.20	3.10	30	11	56	1614	5160
M04R4.0	408	8.83	3.47	7	04	54	1518	5420
M05R4.0	461	13.2	2.42	22	06	54	1659	4110
F06R4.0	486	8.55	3.44	27	07	54	1939	6700
M07R4.0	453	9.55	3.88	24	8	54	1647	6530
F08R4.0	474	8.94	3.14	21	12	54	1324	4390
F09R4.0	542	8.53	3.02	11	04	55	1471	4620
M10R4.0	527	10.8	3.28	27	07	55	1553	5250
F11R4.0	491	10.4	2.84	20	12	55	1469	4300
F12R4.0	496	9.61	2.81	17	01	56	1435	4170
M01R5.0	473	9.87	10.5	20	04	53	908	10400
M02R5.0	470	8.85	10.8	16	11	53	1380	15500
F03R5.0	380	7.82	10.1	10	03	54	481	5720
M04R5.0	408	8.90	10.6	7	04	54	1091	12400
M05R5.0	458	10.9	10.1	22	6	54	1220	13100
F06R5.0	486	9.66	10.2	27	07	54	1015	11200
M07R5.0	453	8.85	11.9	24	08	54	1288	16200
F08R5.0	474	7.76	9.68	21	12	54	968	10200
F09R5.0	420	9.16	9.48	11	04	55	1288	12900
M10R5.0	527	10.7	10.2	27	07	55	825	9300

DOG
NUMBER

COMMENTS ON DEAD DOGS

M02R4.0	OSTEOSARCOMA
F03R4.0	CANINE DISTEMPER
F03R4.0A	OSTEOSARCOMA
M04R4.0	OSTEOSARCOMA
M05R4.0	OSTEOSARCOMA
F06R4.0	OSTEOSARCOMA
M07R4.0	OSTEOSARCOMA
F08R4.0	OSTEOSARCOMA
F09R4.0	OSTEOSARCOMA
M10R4.0	OSTEOSARCOMA
F11R4.0	OSTEOSARCOMA
F12R4.0	OSTEOSARCOMA
.....	
M01R5.0	OSTEOSARCOMA
M02R5.0	OSTEOSARCOMA
F03R5.0	CANINE DISTEMPER
M04R5.0	OSTEOSARCOMA
M05R5.0	OSTEOSARCOMA
F06R5.0	OSTEOSARCOMA
M07R5.0	OSTEOSARCOMA
F08R5.0	OSTEOSARCOMA
F09R5.0	OSTEOSARCOMA + ANEMIA
M10R5.0	OSTEOSARCOMA + FRACTURED MANDIBLE

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{C/KG}$)	DATE INJECTED			DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	3956	
M04P0.0	426	10.7		16	09	53	3849	
F05P0.0	620	9.75		14	10	53	3821	
F06P0.0	410	5.59		12	05	54	3611	
F07P0.0	515	6.90		25	10	54	3445	
M08P0.0	585	10.9		15	03	55	3304	
F09P0.0	658	11.0		22	11	55	3032	
F10P0.0	658	11.0		22	11	55	3052	
M11P0.0	602	10.3		24	04	56	2898	
M12P0.0	630	10.9		29	05	56	2863	
F13P0.0	517	9.47		4	3	64	27	
F13P0.1	515	9.46	.00068	4	3	64	27	
M31P0.1B	517	12.2	.00068	4	3	64	27	
F13P0.2	517	9.44	.00206	4	3	64	27	
M31P0.2B	515	10.7	.00185	4	3	64	27	
F13P0.5	517	9.93	.00540	4	3	64	27	
M31P0.5B	515	10.5	.00549	4	3	64	27	
M01P1.0	442	9.41	.0150	1	12	52	4138	
F02P1.0	422	6.85	.0163	2	03	53	4047	
M03P1.0	515	8.00	.0165	1	06	53	3956	
M04P1.0	608	9.97	.0139	16	09	53	3849	
F05P1.0	620	8.80	.0142	14	10	53	1539	30
F05P1.0A	472	11.0	.0168	3	09	58	2036	
F06P1.0	410	7.38	.0140	12	05	54	3611	
F07P1.0	510	6.36	.0167	25	10	54	3445	
M08P1.0	453	10.6	.0172	15	03	55	3304	
F09P1.0	556	7.87	.0168	9	09	55	2257	60
F10P1.0	641	12.00	.0152	22	11	55	3052	
M11P1.0	602	8.90	.0157	24	04	56	2898	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M01P0.0
F02P0.0
M03P0.0
M04P0.0
F05P0.0
F06P0.0
F07P0.0
M08P0.0
F09P0.0
F10P0.0
M11P0.0
M12P0.0
F13P0.0

SPLENIC RUPTURE, METASTATIC SEMINOMA
ANESTHETIC ACCIDENT

PULMONARY EMBOLISM, NEPHRITIS

F13P0.1
M31P0.1B

F13P0.2
M31P0.2B

F13P0.5
M31P0.5B

M01P1.0
F02P1.0
M03P1.0
M04P1.0
F05P1.0
F05P1.0A
F06P1.0
F07P1.0
M08P1.0
F09P1.0
F10P1.0
M11P1.0

COLITIS, ENTERITIS + SECONDARY HEPATIC NECROSIS

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	31/3/64	DEATH	
M12P1.0	629	9.67	.0167	29	5	56		2374	60
M13P1.0	504	12.7	.0153	3	09	58	2036		
M01P1.7	657	8.72	.0475	26	06	56	2835		
F02P1.7	527	8.62	.0431	22	11	55	3052		
M03P1.7	642	8.63	.0495	26	06	56	2835		
M04P1.7	673	8.37	.0484	10	10	56	2729		
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	2729		
F08P1.7	673	13.6	.0479	10	10	56	2729		
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	2036		
M11P1.7	599	11.6	.0486	24	4	56		2777	200
M12P1.7	673	9.41	.0491	10	10	56	2729		
M13P1.7	504	10.60	.0473	3	09	58	2036		
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
M01P3.0	417	8.00	.261	1	12	52		1476	580
F02P3.0	422	6.85	.312	2	03	53		1947	900
M03P3.0	485	8.74	.291	1	06	53		1604	700
M04P3.0	608	8.51	.292	16	09	53		1950	840
F05P3.0	650	8.22	.288	14	10	53		1504	630
F06P3.0	415	8.38	.282	12	05	54		1617	670

DOG
NUMBER

COMMENTS ON DEAD DOGS

M12P1.0
M13P1.0

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

M01P3.0
F02P3.0
M03P3.0
M04P3.0
F05P3.0
F06P3.0

OSTEOSARCOMA
OSTEOSARCOMA
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	31/3/64	DEATH	
F07P3.0	485	9.00	.314	25	10	54		1627	750
M08P3.0	406	9.73	.300	15	03	55		1771	780
F09P3.0	552	7.67	.300	9	09	55		1894	840
F10P3.0	533	8.94	.298	22	11	55		1547	700
M11P3.0	599	10.5	.309	24	04	56		1198	550
M12P3.0	622	10.2	.308	29	05	56		1659	760
M01P4.0	442	7.61	.823	1	12	52		1724	2100
F02P4.0	567	8.65	1.03	2	03	53		1556	2380
M03P4.0	485	9.36	.929	1	06	53		1198	1680
M04P4.0	566	8.74	.974	16	09	53		1066	1560
F05P4.0	650	7.05	.872	14	10	53		1245	1650
F06P4.0	420	9.26	.811	12	05	54		1357	1660
F07P4.0	485	8.45	.963	25	10	54		1198	1730
M08P4.0	651	9.22	.887	15	03	55		1157	1560
F09P4.0	552	8.58	.960	9	09	55		1343	1920
F10P4.0	527	6.48	.868	22	11	55		1241	1660
M11P4.0	596	9.56	.927	24	04	56		1288	1790
M12P4.0	598	11.4	.838	29	05	56		1463	1840
M01P5.0	417	8.86	2.67	1	12	52		1324	5370
F02P5.0	1150	8.75	3.30	2	03	53		1576	7830
M03P5.0	515	8.10	3.00	1	06	53		499	2340
M04P5.0	566	9.18	3.17	16	09	53		1562	7380
F05P5.0	691	8.77	2.77	14	10	53		2059	8690
F06P5.0	407	7.90	2.57	12	05	54		1194	4620
F07P5.0	482	8.33	2.99	25	10	54		1491	6630
M08P5.0	497	9.55	2.69	15	03	55		1192	4840
F09P5.0	552	9.45	2.73	9	09	55		1145	4750

DOG,
NUMBER

COMMENTS ON DEAD DOGS

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 31/3/64 DEATH	DOSE TO SKELETON (RADS)
F01M0.0	732	7.33		4 1 54	3451	
F02M0.0	545	6.94		29 11 54	3410	
M03M0.0	579	13.0		13 03 56	2940	
M04M0.0	601	10.3		15 01 57	2632	
F05M0.0	671	11.2		5 03 57	2583	
M06M0.0	492	7.56		23 04 57	2534	
F07M0.0	395	8.71		4 6 57	1414	
F07M0.0A	594	10.9		15 1 63	441	
F08M0.0	654	11.6		9 03 60	1483	
M09M0.0	575	12.4		13 04 60	1448	
M10M0.0	581	13.3		17 7 62	623	
F11M0.0	475	9.31		18 9 62	560	
M12M0.0	695	10.0		22 12 60	1195	
F01M0.5	492	9.47	.0192	17 7 62	623	
F02M0.5	492	9.15	.0189	17 7 62	623	
M03M0.5	493	10.8	.0222	18 9 62	560	
M04M0.5	475	12.8	.0223	18 9 62	560	
F05M0.5	534	7.83	.0188	23 10 62	525	
M06M0.5	510	10.3	.0183	23 10 62	525	
F07M0.5	492	8.87	.0190	17 7 62	623	
F08M0.5	654	12.6	.0191	9 03 60	1483	
M09M0.5	485	11.9	.0192	13 04 60	1448	
M10M0.5	492	10.6	.0192	17 7 62	623	
F11M0.5	505	7.82	.0224	18 9 62	560	
M12M0.5	510	10.6	.0177	23 10 62	525	
F01M1.0	718	7.75	.0561	4 01 54	2952	350
F01M1.0A	590	8.07	.0558	23 10 62	525	
F02M1.0	459	8.25	.0593	29 11 54	3410	
M03M1.0	575	13.8	.0574	13 03 56	2940	
M04M1.0	601	9.90	.0599	15 01 57	2632	
F05M1.0	658	8.80	.0588	5 03 57	2583	
M06M1.0	521	10.6	.0545	23 04 57	2534	
F07M1.0	534	9.89	.0550	4 6 57	2159	
F08M1.0	654	12.4	.0572	9 03 60	1483	
M09M1.0	485	10.1	.0582	13 04 60	1448	
M10M1.0	492	9.43	.0657	17 7 62	623	
F11M1.0	505	8.91	.0671	18 9 62	560	

DOG
NUMBER

COMMENTS ON DEAD DOGS

F01M0.0

PURULENT MENINGOENCEPHALITIS

F02M0.0

M03M0.0

M04M0.0

F05M0.0

M06M0.0

F07M0.0

STATUS EPILEPTICUS

F07M0.0A

F08M0.0

M09M0.0

M10M0.0

F11M0.0

M12M0.0

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

HEMANGIOSARCOMA

F01M1.0A

F02M1.0

M03M1.0

M04M1.0

F05M1.0

M06M1.0

F07M1.0

HEMANGIOSARCOMA

F08M1.0

M09M1.0

M10M1.0

F11M1.0

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
M12M1.0	528	9.27	.0538	23 10 62	525	
F01M1.7	510	7.52	.168	23 10 62	525	
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	2632	
F05M1.7	658	12.8	.170	5 03 57	2583	
M06M1.7	521	10.0	.168	23 04 57	2534	
F07M1.7	534	10.2	.160	4 06 57	2492	
F08M1.7	654	10.8	.171	9 03 60	1483	
M09M1.7	485	12.6	.172	13 04 60	1448	
M10M1.7	492	10.1	.153	17 7 62	623	
F11M1.7	505	10.7	.199	18 9 62	560	
M12M1.7	524	9.28	.167	23 10 62	525	
F01M2.0	676	7.60	.334	4 01 54		1780
F02M2.0	517	8.25	.355	29 11 54		965
M03M2.0	576	11.0	.342	13 03 56		619
M04M2.0	601	9.88	.351	15 1 57		2282
F05M2.0	509	8.30	.355	5 03 57	2583	
M06M2.0	502	12.4	.357	23 04 57	2534	
F07M2.0	534	10.1	.326	4 6 57		2239
F08M2.0	654	12.4	.348	9 03 60	1483	
M09M2.0	630	9.99	.347	13 4 60		1254
M10M2.0	430	11.2	.341	17 7 62	623	
F11M2.0	505	7.03	.411	18 9 62	560	
M12M2.0	524	9.47	.332	23 10 62	525	
F01M3.0	519	10.4	1.04	4 01 54		918
F02M3.0	460	6.70	1.12	29 11 54		1856
M03M3.0	579	10.4	.921	13 3 56		1185
M04M3.0	601	10.2	1.14	15 01 57		1176
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
F08M3.0	633	11.8	1.07	9 3 60		1447
M09M3.0	630	9.83	1.08	13 04 60	1448	

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

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

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

[illegible]

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED ($\mu\text{c/KG}$)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
M10M3.0	581	10.4	1.10	17 7 62	623	
F11M3.0	499	11.0	1.30	18 9 62	560	
M12M3.0	510	12.9	1.05	23 10 62	525	
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 group 1

= 0.03 for group 2, 3

= 0.006 for groups 4, 5, 6, 7, 8,
9, 10, 11, 12, M3M4A, F1M0.5,
F2M0.5, M3M0.5, F1M1A, and
F1M1.7.

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	OSTEOSARCOMA + CRIPPLING FRACTURE
F02M4.0	OSTEOSARCOMA
M03M4.0	STRANGULATED INGUINAL HERNIA
M03M4.0A	OSTEOSARCOMA, NEPHRITIS, ULCERATIVE GINGIVITIS + PNEUMONIA
M04M4.0	FRACTURED MANDIBLE + ULCERATIVE GINGIVITIS
F05M4.0	OSTEOSARCOMA
M06M4.0	OSTEOSARCOMA
F07M4.0	OSTEOSARCOMA

F01M5.0	NEPHRITIS + SEVERE ANEMIA
F02M5.0	CRIPPLING FRACTURES
M03M5.0	ULCERATIVE GINGIVITIS
M04M5.0	CRIPPLING FRACTURE
F05M5.0	ULCERATIVE GINGIVITIS
M06M5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
F07M5.0	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	31/3/64	DEATH	
M01T0.0	493	8.24		8	02	54	3704		
M02T0.0	488	7.28		28	09	54	3472		
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	1995		
F06T0.0	489	9.64		10	1	61		171	
F06T0.0A	688	8.61		15	12	60	1202		
M07T0.0	517	10.5		7	2	61	1148		
M08T0.0	533	10.8		24	5	61	1042		
F09T0.0	569	8.28		29	6	61	1006		
F10T0.0	536	10.4		28	7	61	977		
F11T0.0	530	9.45		4	6	63	301		
F12T0.0	492	9.09		9	7	63	266		
M01T0.2	682	11.4	.00164	27	03	62	735		
M02T0.2	682	10.4	.00166	27	03	62	735		
F03T0.2	478	9.86	.00163	27	03	62	735		
M04T0.2	478	10.0	.00166	27	03	62	735		
M05T0.2	625	13.8	.00162	9	02	60		889	10
M05T0.2A	530	13.4	.00173	4	6	63	301		
F06T0.2	489	8.85	.00176	10	1	61	1176		
M07T0.2	532	10.5	.00159	7	2	61	1148		
M08T0.2	494	13.9	.00189	24	5	61	1042		
F09T0.2	569	7.82	.00171	29	6	61	1006		
F10T0.2	508	10.5	.00170	28	7	61	977		
F11T0.2	530	9.76	.00171	4	6	63	301		
F12T0.2	492	7.37	.00190	9	7	63	266		
M01T0.5	699	14.3	.00496	7	9	56	2762		
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	1995		
F06T0.5	489	9.66	.00510	10	1	61	1176		
M07T0.5	532	9.11	.00491	7	2	61	1148		
M08T0.5	533	9.53	.00562	24	5	61	1042		
F09T0.5	569	8.62	.00529	29	06	61	1006		
F10T0.5	508	10.2	.00510	28	7	61	977		
F11T0.5	530	7.78	.00518	4	6	63	301		

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 31/3/64 DEATH	DOSE TO SKELETON (RADS)
F12T0.5	492	9.94	.00567	9 7 63	266	
M01T1.0	493	9.36	.0146	8 02 54	3172	120
M02T1.0	699	9.27	.0146	7 09 56	2762	
F03T1.0	723	8.84	.0145	7 09 56	2762	
M04T1.0	699	8.27	.0146	7 09 56	2762	
M05T1.0	513	11.9	.0146	14 10 58	1995	
F06T1.0	489	8.81	.0150	10 1 61	1176	
M07T1.0	532	9.18	.0147	7 2 61	1148	
M08T1.0	533	8.69	.0166	24 5 61	1042	
F09T1.0	527	10.0	.0160	29 6 61	1006	
F10T1.0	508	10.2	.0150	28 7 61	977	
F11T1.0	521	7.55	.0154	4 6 63	301	
F12T1.0	472	9.96	.0167	9 7 63	266	
M01T1.5	699	7.95	.0289	7 09 56	2762	
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 02 60	1512	
F06T1.5	489	8.14	.0292	10 1 61	1176	
M07T1.5	517	8.83	.0292	7 2 61	384	80
M07T1.5A	521	9.08	.0311	4 6 63	301	
M08T1.5	494	11.6	.0324	24 5 61	1042	
F09T1.5	527	8.80	.0306	29 6 61	1006	
F10T1.5	508	11.6	.0295	28 7 61	977	
F11T1.5	518	11.4	.0305	4 6 63	301	
F12T1.5	465	7.56	.0329	9 7 63	266	
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
COMA OF UNKNOWN ETIOLOGY
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		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M08T2.0	533	10.7	.0981	24	5	61	1042	
F09T2.0	527	8.09	.0979	29	6	61	1006	
F10T2.0	508	10.7	.0919	28	7	61	977	
F11T2.0	518	10.8	.0904	4	6	63	301	
F12T2.0	464	8.92	.100	9	7	63	266	
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	1006	
F10T3.0	508	9.26	.280	28	7	61	971	1470
F11T3.0	518	10.3	.290	4	6	63	301	
F12T3.0	459	11.5	.320	9	7	63	266	
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

M01T3.0	OSTEOSARCOMA + SEVERE ANEMIA
M02T3.0	OSTEOSARCOMA + TRAUMA
F03T3.0	OSTEOSARCOMA
M04T3.0	OSTEOSARCOMA
M05T3.0	OSTEOSARCOMA
F06T3.0	OSTEOSARCOMA
M07T3.0	OSTEOSARCOMA
M08T3.0	OSTEOSARCOMA
F09T3.0	
F10T3.0	OSTEOSARCOMA
F11T3.0	
F12T3.0	

M01T4.0	OSTEOSARCOMA + CRIPPLING FRACTURE
M02T4.0	OSTEOSARCOMA, CRIPPLING FRACTURE + NEPHRITIS
M03T4.0	ULCERATIVE GINGIVITIS + NEPHRITIS
M04T4.0	ULCERATIVE GINGIVITIS

M01T5.0	NEPHRITIS
M02T5.0	PANCYTOPENIA

E. STRONTIUM-90

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F01S0.0	502	8.48		18	01	55	3360	
M02S0.0	600	11.1		14	02	56	2968	
M03S0.0	493	9.03		11	09	57	2393	
F04S0.0	520	8.19		15	10	57	2359	
M05S0.0	542	10.6		19	11	57	2324	
M06S0.0	466	9.68		27	05	58	2135	
F07S0.0A	462	9.46		7	01	59	1910	
F08S0.0	483	9.29		19	05	59	1778	
F09S0.0	549	12.4		11	08	59		708
F09S0.0A	535	11.2		4	6	63	301	
M10S0.0	522	13.9		29	09	59	1645	
F11S0.0	541	9.60		3	11	59	1610	
M12S0.0	605	8.99		6	01	60	1546	
F01S1.0	1524	6.84	.573	18	01	55		308
F01S1.0A	521	9.38	.588	14	02	56	2968	20
M02S1.0	567	8.81	.606	14	02	56	2968	
M03S1.0	493	10.9	.572	11	09	57	2393	
F04S1.0	525	8.96	.560	15	10	57	2359	
M05S1.0	555	10.2	.532	19	11	57	2324	
M06S1.0	466	9.56	.581	27	05	58	2135	
F07S1.0	524	9.94	.517	11	11	58	1967	
F08S1.0	483	10.8	.697	19	05	59	1778	
F09S1.0	549	11.6	.534	11	08	59	1694	
M10S1.0	522	11.5	.558	29	09	59	1645	
F11S1.0	543	10.3	.550	3	11	59	1610	
M12S1.0	607	13.7	.559	6	01	60	1546	
F01S1.7	526	7.41	1.78	14	02	56	2968	
M02S1.7	567	11.6	1.84	14	02	56	2968	
M03S1.7	493	9.19	1.69	11	09	57	2393	
F04S1.7	522	9.60	1.68	15	10	57	2359	
M05S1.7	560	9.85	1.60	19	11	57		1715
M05S1.7A	493	11.4	1.78	6	3	63	391	210
M06S1.7	466	10.6	1.72	27	05	58	2135	
F07S1.7	488	10.2	1.60	11	11	58	1967	
F08S1.7	472	8.47	2.03	19	05	59	1778	
F09S1.7	549	10.0	1.62	11	08	59	1694	
M10S1.7	519	13.6	1.66	29	09	59	1645	

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

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE	DAYS SINCE	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		INJECTED D MO YR	INJECTION 31/3/64 DEATH	
F11S1.7	543	11.0	1.68	3 11 59	1610	
M12S1.7	607	11.9	1.68	6 01 60	1546	
F01S2.0	502	5.59	3.70	18 1 55	3269	670
M02S2.0	567	8.97	3.42	14 02 56	2968	
M03S2.0	494	7.82	3.39	11 09 57	2393	
F04S2.0	522	9.68	3.41	15 10 57	2359	
M05S2.0	560	8.72	3.24	19 11 57	2324	
M06S2.0	466	9.19	3.50	27 05 58	2135	
F07S2.0	488	11.2	3.19	11 11 58	1967	
F08S2.0	465	9.49	4.14	19 05 59	1778	
F09S2.0	473	14.1	3.28	11 08 59	1694	
M10S2.0	508	10.7	3.34	29 09 59	1645	
F11S2.0	543	10.4	3.41	3 11 59	1610	
M12S2.0	607	11.6	3.49	6 01 60	1546	
F01S3.0	468	7.36	11.6	18 01 55	3360	
M02S3.0	565	9.62	11.6	14 02 56	2968	
M03S3.0	494	11.4	10.8	11 09 57	2393	
F04S3.0	527	9.17	10.6	15 10 57	2359	
M05S3.0	557	8.90	10.1	19 11 57	2324	
M06S3.0	466	9.44	10.9	27 05 58	2135	
F07S3.0	486	9.80	10.1	11 11 58	1967	
F08S3.0	465	12.5	12.9	19 05 59	1778	
F09S3.0	468	10.0	10.1	11 08 59	1694	
M10S3.0	519	12.5	10.3	29 09 59	1645	
F11S3.0	541	9.00	10.8	3 11 59	1610	
M12S3.0	605	8.43	10.2	6 01 60	1546	
F01S4.0	468	8.74	33.3	18 01 55	3360	
M02S4.0	567	11.2	32.6	14 02 56	2093	4730
M03S4.0	593	9.83	32.1	11 09 57	2393	
F04S4.0	528	8.24	32.1	15 10 57	2359	
M05S4.0	562	9.65	30.6	19 11 57	2324	
M06S4.0	504	16.0	32.7	3 09 58	2036	
F07S4.0	478	10.9	30.9	11 11 58	1967	
F08S4.0	465	10.9	40.6	19 05 59	1778	

DOG NUMBER	COMMENTS ON DEAD DOGS
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F11S1.7	
M12S1.7	

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

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	SQUAMOUS CELL CARCINOMA -GINGIVA-
M02S4.0	
M03S4.0	
F04S4.0	
M05S4.0	
M06S4.0	
F07S4.0	
F08S4.0	

DOG NUMBER	AT INJECTION			DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)	INJECTED (μ c/KG)	D	MO	YR	31/3/64	DEATH	
F09S4.0	468	9.56	30.6	11	08	59	1694		
M10S4.0	517	8.20	31.3	29	09	59	1645		
F11S4.0	542	8.86	32.7	3	11	59	1610		
M12S4.0	605	10.9	32.3	6	01	60	1546		
F01S5.0	434	9.38	103.	18	01	55	960		8340
M02S5.0	551	12.2	102.	14	02	56	255		3060
M02S5.0A	545	11.4	96.6	7	1	59	1740		12560
M03S5.0	507	10.3	102.	10	15	57	2256		15300
F04S5.0	528	11.4	105.	15	10	57	1448		12390
M05S5.0	621	8.53	95.2	19	11	57	1285		9710
M06S5.0	504	9.33	98.8	3	9	58	35		600
M06S5.0A	462	11.2	94.2	7	01	59	1021		8480
F07S5.0	478	10.2	92.7	11	11	58	1129		8900
F08S5.0	535	11.2	90.5	7	01	59	1469		11160
F09S5.0	459	8.82	93.5	11	08	59	1694		
M10S5.0	517	8.55	95.9	29	09	59	990		8490
F11S5.0	542	8.97	102.	3	11	59	1610		
M12S5.0	606	12.5	99.2	6	01	60	1165		9340

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

F01S5.0	OSTEOSARCOMA
M02S5.0	STRANGULATED INGUINAL HERNIA
M02S5.0A	OSTEOSARCOMA
M03S5.0	OSTEOSARCOMA
F04S5.0	OSTEOSARCOMA
M05S5.0	SEVERE ANEMIA, AUTOAGGLUTINATION, INFARCTION, SPLENOMEGALY
M06S5.0	INTESTINAL HEMORRHAGE
M06S5.0A	OSTEOSARCOMA, INFARCTION + THROMBOCYTOPENIA
F07S5.0	STATUS EPILEPTICUS
F08S5.0	OSTEOSARCOMA
F09S5.0	
M10S5.0	SEVERE ANEMIA + THROMBOCYTOPENIA
F11S5.0	
M12S5.0	HEMANGIOSARCOMA

* TABLE II. TEST ANIMALS (MAR. 31. 1964)

A. RADIUM-226*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D. MO. YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
T01R5.0	995	11.1	10.3	1 12 52	1074	11800
T02R5.0	919	8.40	4.39	12 01 53	1368	6270
T03R5.0	1467	8.29	4.76	12 01 53	428	2430
T04R5.0	459	10.0	10.6	6 07 53	1	30
T05R5.0	126	6.14	11.7	6 10 53	1	
T06R5.0	126	6.14	11.4	6 10 53	1	
T07R5.0	126	6.14	11.8	6 10 53	1	
T08R5.0	290	5.52	1.92	10 05 55	58	170
T09R5.0	2276	10.4	1.94	10 05 55	58	170
T10R5.0	43	1.02	1.98	10 05 55	49	
T11R5.0	43	1.58	1.91	10 05 55	49	
T12R5.0	397	12.3	9.72	9 05 56	225	3100
T13R5.0	397	7.59	9.76	9 05 56	188	2430
T14R4.0	674	8.12	3.17	11 07 56	72	340
T15R4.0	672	9.03	3.11	11 07 56	2127	6570
T16R5.0	604	12.4	9.68	11 07 57	12	230
T17R5.0H	383	11.4		28 10 58	1147	11410
T18R5.0H	383	10.5		28 10 58	1226	13140
T19R5.0H	383	9.67		28 10 58	1219	14260
T20R5.0H	383	11.2		28 10 58	1330	13500
T21R5.0H	381	11.1		28 10 58	386	3400
T22R5.0H	381	12.0		28 10 58	587	5250
T23R4.0H	384	9.44		25 11 58	1471	6120
T24R4.0H	384	10.9		25 11 58	1505	5540
T25R4.0H	379	11.3		25 11 58	1309	4370
T26R4.0H	379	11.1		25 11 58	1640	6080
T27R4.0H	372	11.7		25 11 58	1414	4620
T28R3.0H	372	11.5		25 11 58	387	360
T29R5.0	474	13.5	10.4	3 03 59	216	2920

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
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T29R 5.0	NEPHRITIS
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DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
T30R5.0	474	11.5	10.4	3 03 59	178	2550
T31R5.0	471	10.5	10.4	3 03 59	303	3910
T32R3.0	471	11.4	1.13	3 03 59	1855	
T33R3.0	471	10.6	1.15	3 3 59	1822	2120
T34R3.0	470	15.7	1.12	3 3 59	1737	1970
T35R3.0J	670	9.44	.951	5 5 59	8	20
T36R4.0	695	10.2	2.99	22 12 60	1154	3650
T37R4.0	695	9.53	3.00	22 12 60	1195	
T38R4.0	695	10.1	3.02	22 12 60	1195	
T40R1.0	899	13.0	.0483	3 4 62	7	
T41R1.0	899	12.7	.0487	3 4 62	63	10
T42R1.7	967	14.0	.146	4 4 62	7	
T43R1.7	963	13.2	.145	4 4 62	64	10
T44R3.0	938	11.1	.937	4 4 62	68	100
T45R3.0	939	13.6	.941	5 4 62	7	10
T46R3.0	810	12.5	.928	5 4 62	69	100
T47R6.0	99	5.27	29.4	11 6 62	4	270
T48R6.0	2842	11.2	25.1	27 12 62	49	1950
T49R5.0	485	10.6	7.54	2 5 63	5	80
T50R5.0	485	13.7	7.46	2 5 63	15	210
T51R5.0	418	13.3	8.48	8 5 63	92	1110
T52R5.0	418	10.7	8.57	8 5 63	15	240

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 AGE (DAYS)	WEIGHT (KG)	INJECTED ($\mu\text{c/KG}$)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
T53R5.0	418	12.0	8.50	8 5 63	33	480
T54R5.0	417	11.4	8.76	22 5 63	5	90
T55R5.0	417	11.6	8.61	22 5 63	33	490
T56R5.0	417	11.6	8.61	22 5 63	90	1130
T57R4.0	501	12.1	2.72	15 8 63	14	70
T58R4.0	496	11.7	2.41	15 8 63	61	220
T59R4.0	496	9.64	2.57	15 8 63	63	250
T60R4.0	490	12.1	2.33	15 8 63	117	380
T61R4.0	490	9.48	2.70	15 8 63	229	
T62R4.0	490	8.63	2.68	15 8 63	229	
T63R3.0	559	8.72	.899	29 1 64	36	50
T64R3.0	551	8.42	.919	29 1 64	62	
T65R3.0	551	11.6	.922	29 1 64	62	
T66R3.0	549	10.1	.904	29 1 64	62	
T67R3.0	549	12.7	.898	29 1 64	62	
T68R3.0	549	12.1	.917	29 1 64	62	
T69R3.0	499	8.84	.919	29 1 64	62	
T70R3.0	499	14.2	.922	29 1 64	62	

*R5H, R4H, R3H, 6 injections, 20; 6.7, 2.2 μc each, respectively, given at 8 week intervals. The injection to death interval shown is the interval from 1st injection to death; for dose calculations the interval from midpoint of injections to death is used.

T35R3J received 99 μc Sr^{85} .

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T53R5.0	SPECIAL STUDY
T54R5.0	SPECIAL STUDY
T55R5.0	SPECIAL STUDY
T56R5.0	SPECIAL STUDY

T57R4.0	SPECIAL STUDY
T58R4.0	SPECIAL STUDY
T59R4.0	SPECIAL STUDY
T60R4.0	SPECIAL STUDY
T61R4.0	
T62R4.0	

T63R3.0	SPECIAL STUDY
T64R3.0	
T65R3.0	
T66R3.0	
T67R3.0	
T68R3.0	
T69R3.0	
T70R3.0	

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
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	1202	
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

T00P5.0	SPECIAL STUDY
T01P5.0	SPECIAL STUDY
T02P5.0	SPECIAL STUDY
T03P5.0	SPECIAL STUDY
T04P5.0	SPECIAL STUDY
T05P5.0	SPECIAL STUDY
T06P5.0	SPECIAL STUDY
T07P5.0	SPECIAL STUDY
T08P5.0	SPECIAL STUDY
T09P5.0	SPECIAL STUDY
T10P5.0	SPECIAL STUDY
T11P5.0	SPECIAL STUDY
T12P5.0	SPECIAL STUDY
T13P5.0	SPECIAL STUDY
T14P5.0	SPECIAL STUDY
T15P5.0	SPECIAL STUDY
T16P5.0	SPECIAL STUDY
T17P5.0	SPECIAL STUDY
T18P5.0	SPECIAL STUDY
T19P5.0	
T20P5.0	LIVER DEGENERATION, ASCITES + THROMBOCYTOPENIA
T21P5.0	TOXIC NEPHRITIS + LIVER DEGENERATION
T23P1.0	SPECIAL STUDY
T24P1.0	SPECIAL STUDY
T25P1.0	SPECIAL STUDY
T26P1.0	SPECIAL STUDY
T27P3.0	SPECIAL STUDY
T28P1.0	SPECIAL STUDY
T29P3.0	SPECIAL STUDY
T30P1.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ c/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/64 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 31/3/64 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 FRACTURE

D. THORIUM-228 (RADIOTHORIUM) *

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED		DAYS SINCE INJECTION 31/3/64 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 Th²³² 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 AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
T01S0.0	151	7.71		5 03 54	2	
T02S5.0	149	6.85	148.	5 03 54	11	
T03S5.0	144	6.19	148.	5 03 54	18	
T04S5.0	151	7.05	148.	5 03 54	41	
T05S5.0	144	5.25	148.	5 03 54	116	
T06S5.0	155	7.01	87.0	16 03 54		
T07S5.0	155	6.74	87.0	16 03 54	2	
T08S0.0	243	7.00				
T08S H	67			27 09 55	66	
T09S H	67			27 09 55	66	
T10S H	67			27 09 55	132	
T11S H	67			27 09 55	132	
T12S3.0	593	10.6	10.5	11 09 57	5	10
T13S4.0	324	10.5	19.1	8 07 60	8	40
T14S5.0	542	10.0	96.1	7 11 61	9	210
T15S5.0	595	9.43	98.4	7 11 61	30	570
T16S2.0	604	9.71	3.27	8 11 61	9	10
T17S6.0	670	7.18	295.	19 1 62	14	580
T18S6.0	670	5.94	302.	19 1 62	802	
T19S6.0	670	5.43	284.	19 1 62	24	550
T20S4.0J	440	8.54	28.9	2 10 63	13	80

12054.0J	SPECIAL STUDY
11756.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
11856.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
11956.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
11652.0	SPECIAL STUDY
11455.0	SPECIAL STUDY
11555.0	SPECIAL STUDY
11354.0	BREMSSSTRAHLUNG: PHANTOM SAM MCGEE
11253.0	BREMSSSTRAHLUNG: PHANTOM
1095 H	SPECIAL STUDY
1095 H	SPECIAL STUDY
1105 H	SPECIAL STUDY
1115 H	SPECIAL STUDY
10255.0	SPECIAL STUDY
10355.0	SPECIAL STUDY
10455.0	SPECIAL STUDY
10555.0	SPECIAL STUDY
10655.0	SPECIAL STUDY
10755.0	SPECIAL STUDY
10850.0	SPECIAL STUDY
10150.0	SPECIAL STUDY
DOG NUMBER	COMMENTS ON DEAD DOGS

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED ($\mu\text{c}/\text{KG}$)	DATE INJECTED D. MO YR	DAYS SINCE INJECTION 31/3/64 DEATH	DOSE TO SKELETON (RADS)
T21S2.5J	363	7.20	8.3	2 10 63	13	140

* SH, 10 injections, 1 μc each, given at weekly intervals.

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

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 31/3/64. DEATH	DOSE TO SKELETON (RADS)
T01Q3.0J	460	9.55	.875	26 3 63	-	
T02Q4.0	466	12.0	2.91	27 3 63	370	
T03Q4.0	466	13.1	2.91	27 3 63	370	
T04Q5.0	480	9.55	9.71	24 4 63	342	
T05Q5.0	455	9.67	9.59	24 4 63	342	
T06Q6.0	455	8.29	24.6	17 10 63	13	980
T07Q5.0	465	11.8	9.83	6 11 63	146	
T08Q5.0	475	9.77	9.91	6 11 63	16	400
T09Q4.0	503	9.80	2.96	4 12 63	118	
T10Q4.0	503	10.3	2.96	4 12 63	118	
T11Q3.0	495	9.10	1.02	4 12 63	118	
T12Q3.0	495	13.5	1.02	4 12 63	118	
T13Q3.0	495	11.3	1.02	4 12 63	118	
T14Q3.0	438	10.3	1.00	4 12 63	118	

T01Q3.0J received 18.0 μ C Sr⁸⁵.

DOG NUMBER	COMMENTS ON DEAD DOGS
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T01Q3.0J	SPECIAL STUDY
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T02Q4.0	
T03Q4.0	

T04Q5.0	
T05Q5.0	

T06Q6.0	PURPURA HEMORRHAGICA
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T07Q5.0	
T08Q5.0	PURPURA HEMORRHAGICA

T09Q4.0	
T10Q4.0	

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 31/3/64 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	
F14A					3777	
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
F14A	OBTURATING EMBOLISM OF PORTAL VEIN
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

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RADIATION INDUCED TURBINATE ATROPHY

Glenn N. Taylor, Webster S. S. Jee and Walter Angus

Abstract: Radiation induced atrophy of the maxilloturbinate in beagles is induced by Ra^{226} , Ra^{228} , Th^{228} , or Pu^{239} . Thus far, involution following strontium treatment has not been observed. In spite of the extreme involution that sometimes occurs, clinically discernible symptoms were not seen.

Introduction

In the beagle a single intravenous injection of Ra^{226} , Ra^{228} , Th^{228} , or Pu^{239} induced varying degrees of turbinate atrophy at relatively long (years) latent periods. The severity of the change varied with the radionuclide, the dose level, and the latent period. The involution was most apparent in the maxilloturbinate. This atrophy is poorly visualized radiographically, but constitutes a clearly apparent lesion during post mortem examination. Some of the gross and microscopic features of this condition will be presented

Methods

Observations were limited to autopsy cases which were sacrificed or died from other diseases - usually osteosarcoma. All of the animals examined in this study, were treated with a single intravenous injection of the radionuclide.

The turbinate region was exposed by a midsagittal section through the skull and subsequent removal of the nasal septum. The maxilloturbinate was then excised along the maxilloturbinate crest and decalcified in sulfo-salicylic acid. Five transverse blocks representing the various regions were then prepared by standard paraffin techniques and stained with hematoxylin and eosin for histological examination.

Results

The maxilloturbinate consists of a series of primary leaflets that arise from the lateral wall of the nasal cavity. These divide into secondary and tertiary leaflets, with each leaflet oriented parallel to the long axis of the nose and extending from the distal aspect of the ethmoid region to a point near the external nares (Fig. 1). The respective leaflets are composed of a mucous membrane overlying a thin bony lamina that is continuous with the inner aspect of the maxillary bone.

The first grossly discernible lesion appeared as a thickening or beading of the medial margins of the leaflets. This usually began first in the posterior half of the turbinates and appeared to be secondary to localized bone loss and clumping of the overlying soft tissues following removal of the internal bony support (Figs. 2-3). In the most advanced stages of involution, near complete loss of the maxilloturbinate occurred. Examples of representative stages are illustrated in Figs. 1-4.

Microscopically, the dominant lesion was bone necrosis and subsequent bone loss (Figs. 6-7). Peritrabecular fibrosis was present at the higher dose levels. Typical osteoclastic activity was not observed with the bone removal, but the indented surfaces of some of the trabeculae indicated that this was probably the mechanism involved (Fig. 6). Inflammation of the turbinate bone was not seen. However, chronic inflammatory changes with infiltration by eosinophils and plasma cells in the overlying lamina propria and an increase in the goblet cells of the epithelial layer were seen. But, this was not a consistent finding and was also observed in some of the controls. The absence of clinically apparent rhinitis further indicated that the inflammatory factor was not marked and probably did not have an acute

phase at any stage. Increased fibrosis was present in the lamina propria in most advanced cases. In some of the more glandular areas contraction of the residual soft tissues simulated pseudoadenomas, or in the more vascular areas - pseudohemangiomas.

It was not possible to determine the latent periods of these atrophic changes since most of the serially sacrificed animals were relatively short term. Thus, the post injection ranges given in Table I are maximum values and preclude any precise dose-time relationship. However, these data did indicate that relatively long latent periods (years) were required even at the highest dose levels.

The relative toxicity as given in Table I somewhat coincides with the fracture incidence for the respective nuclides (1-3). An obvious exception was Pu²³⁹. Here in marked contrast to the fracture incidence, turbinate atrophy was a significant feature at injected dose levels down to 0.05 $\mu\text{c}/\text{kg}$.

Discussion

Previous descriptions of turbinate atrophy in the dog were not found in a literature survey. However, such atrophy is part of a relatively common infectious disease syndrome in swine. It was also induced artificially in this species by an irritant applied topically within the nasal cavity (4). The bone involution in swine appeared to be secondary to inflammatory changes involving the mucous membrane of the nasal cavity (5). Thus, its pathogenesis differs fundamentally from the cases herein presented where the primary lesion occurred in the osseous tissue as radiation induced bone necrosis and the variable soft tissue inflammatory response followed as a secondary change. One similarity was the absence of observed

osteoclastic activity in the regions of bone resorption.

Dosimetric studies in the turbinate region have not been completed and will be presented in a subsequent report.

Table I - Incidence and Post Injection Interval of Turbinate Atrophy

RADIUM-226						
Injected Dose Level ($\mu\text{c}/\text{kg}$)	No. Dogs Examined	Range of Post- Injection In- terval (days)	Number of Dogs at Respective Stage of Atrophy			
			0	+	++	+++
0	5	1550-3360	5	-	-	-
0.06	1	2988	1	-	-	-
0.16	2	2399-2577	2	-	-	-
0.33	2	3324-3440	2	-	-	-
1.00	11	1737-2955	4	4	3	-
3.00	13	1309-2127	-	1	2	10
10.00*	3	1219-1330	-	-	-	3

*Four serially sacrificed animals ranging from 33 to 92 days post injection were negative.

RADIUM-228						
0	2	1414-3451	2	-	-	-
0.06	2	2159-2952	2	-	-	-
0.16	1	2383	1	-	-	-
0.33	2	1254-2239	-	2	-	-
1.00	3	1176-1856	-	-	3	-

THORIUM-228						
0	1	2592	1	-	-	-
0.005	2	1976-2159	2	-	-	-
0.015	1	3172	1	-	-	-
0.03	3	1921-2576	2	1	-	-
0.09	3	1085-1222	2	1	-	-
0.30	4	685-1156	-	-	2	2

Table I (Con't)

PLUTONIUM-239

Injected Dose Level ($\mu\text{c}/\text{kg}$)	No. Dogs Examined	Range of Post- Injection In- terval (days)	Number of Dogs at Respective Stage of Atrophy			
			0	+	++	+++
0	1	2755	1	-	-	-
0.015*	1	2257	1	-	-	-
0.05	4	2221-2777	1	3	-	-
0.09	10	1617-3185	1	5	4	-
0.30**	3	1547-1895	-	-	-	3
0.90	3	1241-1462	1	-	1	1
3.00	2	474-939	1	1	-	-

*Twelve serially sacrificed dogs ranging from 5 to 746 days post injection were negative.

**Five serially sacrificed dogs ranging from 40 to 755 days post injection were negative.

STRONTIUM-90

33.0	1	2093	1	-	-	-
100.0	8	990-2256	8	-	-	-

Fig. 1

Fig. 2

Figure 1 - Midsagittal section of a normal beagle skull showing the ethmoidal labyrinth (E), the nasal turbinate (N), and the maxilloturbinate (M) with its well developed leaflet structure. (T42R1.7 - 7 days post injection). (X 1.9).

Figure 2 - Midsagittal section of a beagle skull illustrating early but definite involution of the maxilloturbinate (arrow). Clumping and beading of the inner margins of the posterior aspect are clearly seen. (M1P2 - 2985 days post injection). (X 1.9).

Fig. 3

Fig. 4

Figure 3 - Midsagittal section of beagle skull illustrating marked beading and distortion of the maxilloturbinate (arrow). (F8R3 - 2612 days post injection). (X 1.9).

Figure 4 - Midsagittal section of beagle skull with nearly complete loss of the leaflet structure of the maxilloturbinate. Only small remnants remain. (F6T3 - 810 days post injection). (X 1.9).

Fig. 5

Fig. 6

Figure 5 - Transverse section through part of a normal leaflet in the mid-region of the maxilloturbinate of a beagle showing bony lamina (L) with viable osteocytes (O) and bone-lining cells (P); highly vascular lamina propria with relatively loose connective tissue and moderate number of mononuclear cells (M); and overlying epithelium with relatively few goblet cells (G). (MAC4 - 3345 days age). (X 320).

Figure 6 - Transverse section of a leaflet in mid-maxilloturbinate region of a beagle illustrating radiation-induced bone necrosis as evidenced by the empty lacunae and the partial loss of the bone-lining cells; early bone resorption with indentations along the margin suggesting osteoclastic activity; increased fibrosis of the lamina propria; and increased numbers of goblet cells. (F8M3 - 1447 days post injection). (X 320).

Fig. 7

Figure 7 - Transverse section of a markedly involuted leaflet in mid-maxillo-turbinate region illustrating nearly complete resorption of the bony lamina; a small spicule of residual necrotic bone (arrow), marked fibrosis of lamina propria; and a high percentage of goblet cells in the overlying epithelium (same dog as Figure 4). (X 320).

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CONSIDERATIONS IN RADIUM THERAPY FOR MELANOMA

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Abstract: If treatment of human melanomas with injected Ra is to be investigated, the uptake of a tracer injection of Ra by the melanoma should be established before subjecting the patient to a series of therapeutic Ra injections. Present data are too scanty to establish whether this treatment will benefit the human. Thus far, the only success has been in one dog.

Introduction

Following the discovery that injected Ra²²⁶ was taken up in a canine malignant melanoma in sufficient concentration to cause its involution (1,2), we and others (3,4) have tried or are considering this treatment for human melanomas. Preliminary results in humans have not been encouraging (4,5), and for this reason it seems appropriate to (a) present our data on an additional human case, (b) summarize the melanoma/skeletal radium concentrations observed by us in various mammals, and (c) give provisional suggestions on how to investigate future cases.

A. Results in Melanoma Patient D. M.

Patient D. M. is a 42 year old, 70 kg., Japanese-American. His melanoma originated behind his right ear and during the past 14 months there have been six attempts to remove its recurrent metastases surgically. Grossly, this melanoma appeared moderately pigmented, with considerable variation among the pigmentation of individual cells. An I.V.

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tracer injection of 19.5 μc Ra^{226} in saline was followed 5 days later by biopsies of a metastasis and nearby soft tissue. Samples were sealed against radon escape in glass vials and were evaluated for radium concentration by γ -ray counting. Rather than delay analysis until radon activity equaled that of radium (about 1 month would have been required), 2 radon activity evaluations separated by a few days were made and the radium activity was computed from the equation:

$$\text{Ra}^{226} = \frac{A_t - A_o e^{-\lambda t}}{1 - e^{-\lambda t}} \quad (1)$$

where A_o = Rn^{222} activity at initial count.
 A_t = Rn^{222} activity at final count.
 t = time between initial and final count.
 λ = decay constant of $\text{Rn}^{222} = 0.181 \text{ day}^{-1}$.

The observed exhaled radon + body radon 5 days after Ra^{226} injection (just prior to biopsy), indicated a retention of 2.85 μc Ra^{226} . A skeletal concentration of 184 μc Ra^{226}/g was computed, assuming that 1/2 of the retained radium was in a 7 kg. skeleton. This and observed concentrations in biopsy tissue were:

Tissue	μc Ra^{226}/g Wet Tissue
Skeleton	184
Melanoma	16.2
Skin	11.1
Subcutaneous	6.6

Autoradiographically, the small amount of radium in the melanoma was observed to be concentrated preferentially in pigmented cells

(melanocytes and/or melanophores). However, the low uptake in the melanoma relative to that in the skeleton did not justify additional Ra²²⁶ injection at a therapeutic level.

B. Summary of Melanoma/Skeletal Radium Concentrations

We have obtained the following results in individual animals to date:

Mammal	Melanoma	Pigmentation	Days After Ra Inj.	($\frac{\text{pc/g Melanoma}}{\text{pc/g Skeleton}}$)
Beagle (T48R6)	Spon.	High	49	1
Man (D. M.)	Spon.	Med.	5	0.09
Man (M. M.)	Spon.	Low	5	"Low"
Hamster (Syrian Golden)	M-3	Med.	3	0.024
Mouse (C57 Black)	B-16	High	3	0.010
Mouse (C57 Black)	B-16	High	3	0.005
Mouse (C57 Black)	B-16	High	8	0.006

We do not know the reason for the differences in radium uptake among the melanomas of various species, but it may be related to the kinds of melanin, in addition to its abundance. Radium has been observed to concentrate in a synthetic melanin (6).

C. Suggestions for Future Trials

The following suggestions reflect our present thinking, which may prove unsubstantiated. It is possible that most human melanomas will not concentrate sufficient radium to warrant this therapy regardless of whether the radium is given alone or in combination with other agents. Additional trials are needed to form reliable conclusions. Therefore, these suggestions should be regarded only as interim guides.

1. Before testing, evaluate pigmentation on melanoma tissue section. Lightly pigmented melanomas might not concentrate sufficient radium to

justify further investigations.

2. To evaluate radium uptake, inject a tracer of about 20 μc Ra^{226} I.V. Biopsy the melanoma several days later. Seal the sample against radon leak and determine its radium concentration (see equation 1). Determine the skeletal concentration directly (bone sample) or indirectly (total body retention).

3. Determine the melanoma/skeletal radium concentration ratio. This ratio probably should be about 0.5 or greater to justify therapeutic injections of Ra. (The ratio of 1 in a dog was associated with the involution of its melanoma). Detail autoradiograms are desirable to determine if radium is in the melanocytes.

4. If the tracer uptake is sufficiently high, give a series of therapeutic injections of about 200 μc Ra^{226} each, spaced at intervals of about 2 weeks until either (a) the melanoma regresses, or (b) cellular elements of the blood become depressed undesirably. At present we consider Ra^{226} probably more suitable for therapy than Ra^{224} . The retention of the decay-products of both Ra^{226} and Ra^{224} in the tumor is probably small. On the other hand, while skeletal retention of the decay-products of Ra^{226} is low (7), retention of the decay-products of Ra^{224} in the skeleton is high (8). In addition, the response of the human to large doses of Ra^{226} is well known, whereas that from Ra^{224} and its decay-products is unknown. Severe acute effects were not reported in 32 adult humans following a series of injections totaling up to 450 μc Ra^{226} (9), although bone cancers have appeared in some persons many years after radium injection (10).

D. Future Investigations

We are most anxious to continue our study of radium therapy of canine

melanomas, since our only success was in a dog. We are currently paying the shipper \$100, plus shipping charges from anywhere in the U. S. A. to Glenn N. Taylor (D.V.M.), Radiobiology Division, Anatomy Department, University of Utah, Salt Lake City, Utah, (Phone 322-7211-Area Code 801), for each living dog whose malignant melanoma is verified histologically. The dogs will receive excellent veterinary care during and following our attempts to cure them of cancer.

We, also, will serve as an information relay among interested persons for new data on Ra treatment of melanoma. Data should include (a) tissue section of the melanoma, (b) radium isotope and activity injected, (c) radium concentrations in the melanoma, other soft tissues, and the skeleton, (d) therapy dosage, if given, (e) results, if applicable, and (f) the name of the investigator and his laboratory. Results will be shared among persons indicating their interest, with the restriction that no data is to be quoted in the published literature without permission of the investigator.

We are currently investigating several chemical agents for their possible effect in enhancing radium uptake in melanoma tissue.

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THE EFFECTS OF IONIZING RADIATIONS ON ENDOCRINE CELLS.
III. RESTORATION OF TESTOSTERONE PRODUCTION WITH A
REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE
GENERATING SYSTEM AFTER LOCALIZED IRRADIATION TO HEADS OF RATS.

LeGrande C. Ellis and David L. Berliner

Abstract: Male rats (Sprague-Dawley strain) were given 1500r x-irradiation to their heads when 12 weeks of age. Twenty days after irradiation, the animals were euthanized and testicular tissue was removed from both the control animals and the irradiated animals. These tissues were incubated with 5-pregnenolone-7 α -H³ and progesterone-4-C¹⁴, and tissues from each group of animals were incubated with a NADPH-G-S and without a NADPH-G-S. In the irradiated group of animals, less testosterone was produced in vitro than for control animals. Similarly, there was less tritium in this testosterone fraction, and the tritium/carbon-14 ratio for testosterone was also diminished. The addition of a NADPH-G-S to incubations of testicular tissue from irradiated animals restored the diminished testosterone production, the altered tritium/carbon-14 ratios, and the tritium content of testosterone to levels equal to that for similarly treated control animals. These alterations in the biotransformations of steroids by testicular tissue from irradiated rats was attributed to the pituitary's secretion of gonadotropins.

These data also show that the biotransformation of steroids by testicular tissue may be altered even though the histologic or outward appearance of these cells would indicate that they are normal.

Introduction

A detailed study of the effects of x-irradiation on blood corticosteroid levels of the Rhesus monkey was reported in 1955 (1). These workers observed a diphasic response of the blood corticosteroids after whole-body irradiation. This same year, other workers (2,3) showed that the biotransformation of steroids by endocrine tissues was altered after in vitro irradiation of perfused calf adrenal glands. With data obtained through indirect methods of measuring androgen production by testes from x- and microwave irradiated rats, Gunn et al. suggested that other steroid producing tissues might be affected by ionizing radiation (4,5). These workers observed a triphasic response of androgen production by testes from

these animals. Other recent investigations indicate that the biotransformation of steroids by murine testicular tissue also responds triphasicly to whole-body irradiation (6), and that testosterone production is diminished 20 days after localized irradiation of the testes (7). Furthermore, this diminished testosterone production was restored to a level equal to that of similarly treated control animals by the addition of a NADPH-G-S³ (7). Similar observations have been reported for in vitro corticosteroid production by adrenal tissue from dogs previously burdened with internally deposited radionuclides (8).

There are reports in the literature which indicate that the content of gonadotropins in the pituitary is altered after irradiation (9-12). Moreover, it has been suggested that the pituitary and its gonadotropins may be a factor responsible for the alteration of the biotransformation of steroids by testicular tissue from irradiated mice (6). Therefore, the purpose of this investigation is to ascertain if localized irradiation to heads of rats does affect the biotransformation of steroids by testicular tissue and to ascertain if this defect could be partially restored by the addition of a NADPH-G-S.

Materials and Methods

Eight male rats (Sprague-Dawley strain) were irradiated with 1500r of direct radiation to their heads when 12 weeks of age. A 250 kv x-ray machine was used with a setting of 15 ma, with a filter comprised of 0.5 mm Cu and 1 mm Al, and with a distance from x-ray tube to center of head of 50 cm. Rats were immobilized with Nembutal^R (Abbott) with a dose of 5 mg/100 grams body weight. The dose rate for irradiation was measured in air with a Victoreen thimble-type ionization chamber and was corrected for

standard temperature and pressure. All rats were irradiated simultaneously in a special rotating compartment designed so that the rats were placed radially with their heads in the center. The bodies of the rats were shielded caudally from the neck region to the tail. A covering of two 1/8" sheets of lead was used to absorb the x-rays directed towards the body region. A collar of plastic, covered with the lead, was used around the necks to prevent scattering of x-rays back to the body region. The floor, comprised of 3/4" plywood, was mounted on plastic spacers to reduce backscattering of radiations reflected off the metal gearbox located below the compartment. A Victoreen ionization chamber that was placed between two rats during the actual process registered 16.2r.

Prior to and after irradiation, rats were housed in the rat colony and were given laboratory chow and water ad libitum. Control animals were sham-irradiated as indicated above. At 20 days post-irradiation, both groups of animals were exsanguinated, and the testes were removed and prepared for incubation as described elsewhere (6). Testicular tissue from both groups of rats was incubated with 5-pregnenolone- 7α -H³ and progesterone-4-C¹⁴ as described elsewhere (6). In addition, both irradiated and control tissues were incubated with and without a NADPH generating system consisting of 2.5 micromoles NADPH and 2.5 micromoles glucose-6-phosphate (Sigma Chemical Co.).

Sections of testes from both groups of animals were removed for histologic examination. The tissues were fixed in Zenker-formol and sectioned according to standard paraffin techniques. Four irradiated and four sham-irradiated animals were used for assay purposes. Each treatment group was replicated four times. A testis was taken from each rat for incubation without a NADPH-G-S and a testis was taken from each rat for incubation with

a NADPH-G-S. Extraction, separation, characterization, and identification of steroids were carried out as previously described in the literature (6). The per cent conversion of substrates into androgens (androstenedione and testosterone) and other intermediates were calculated as previously described (7). High-polar steroid intermediates (i.e., compounds more polar than testosterone) which were not fully characterized were estimated by the method of triangulation (7,13).

The data for the four experimental groups were analyzed according to the method of analysis of variance model I (14). Rejection of the null hypothesis that the four means are equal was made at the 0.010 level of significance. Within sample mean squares were used as estimates of population variances in calculating standard errors for sample means. Confidence limits (95%) were set on each of the four means. If there were no overlappings of sample means at this specified confidence limit, significance was ascertained.

Results

Irradiation to the heads of rats resulted in a decrease of body weight from 353 grams for control animals to 286 for the irradiated group. Average testicular weights of 3.44 grams and 3.32 grams were recorded for the control and irradiated rats, respectively. This difference in weight did not approach significance at the 0.500 level with a t-test (14). Histological examination of the testes failed to demonstrate any apparent change in either the germinal elements or the interstitial elements of this organ. The data concerning the conversion of 5-pregnenolone- 7α -H³ and progesterone-4-C¹⁴ into androgens and immediate precursors were calculated on a per mg of tissue and a per rat basis. No essential difference was observed between

the two methods of calculation; therefore, only the calculations on a per rat basis are reported in Table I. The total conversion of 5-pregnenolone- $7\alpha\text{-H}^3$ and progesterone- 4-C^{14} into progesterone, 17α -hydroxyprogesterone, androstenedione, testosterone, the high-polar steroid intermediates, and other steroid metabolites, which were not isolated or characterized, appears in Table II. Statistical comparisons of the data (Fig. 1 and Table II) are summarized in Table III. These data (Table II) show that irradiation to the heads of rats with 1500r x-rays did not affect the total biotransformation of 5-pregnenolone- $7\alpha\text{-H}^3$ and progesterone- 4-C^{14} into other metabolites. Similarly, this treatment did not diminish androstenedione production. However, the in vitro production of testosterone by testicular tissue from these rats was diminished after irradiation. The numerical difference in total androgen production did appear to approach significance on the basis of the statistical methods used in this investigation. The data (Table I) indicate that irradiation affected the direct conversion of 5-pregnenolone- $7\alpha\text{-H}^3$ into testosterone, which was pointed out by the marked reduction of the tritium/carbon-14 ratio for testosterone in the irradiated group. Of additional interest is the fact that the tritium/carbon-14 ratios for testosterone are greater than the corresponding ratios for androstenedione in all four observation groups.

The addition of the NADPH-G-S to incubations of testicular tissue from both the irradiated and control groups of animals resulted in similar tritium/carbon-14 ratios. The tritium content of the testosterone fraction of the irradiated group was also restored to a level equal to that for similarly treated control animals by the addition of the NADPH-G-S. Similarly, testosterone production was restored to the same level as that for similarly

treated control testicular tissue (Table III), and the numerical difference for total androgen production was markedly reduced (Fig. 1). There does appear to be an effect of irradiation on the high-polar steroid fraction (Fig. 1 and Table III) and this effect was not corrected by the addition of a NADPH-G-S.

Discussion

The data from this investigation imply that the in vitro biotransformation of steroids by testicular tissue was altered 20 days after localized irradiation to the heads of rats with 1500r of x-rays. Thus, it would appear that the triphasic response of the biotransformation of steroids observed following whole-body irradiation (6) may be due to a local effect of ionizing radiations on testicular tissue (7) and an effect due to irradiation of the head, which in this experiment may be attributed to the pituitary and its ability to secrete gonadotropins. In this respect, it would appear that localized irradiation to the testes of rats has a greater effect on the biotransformation of steroids observed for this tissue 20 days after treatment than does localized irradiation to the head. The fact that the diminished testosterone production, the altered tritium/carbon-14 ratio for testosterone, and the tritium content of testosterone were restored to levels equal to that for similarly treated control animals, by the addition of a NADPH-G-S, suggests that the altered biotransformation of steroids noticed in this investigation may be due to a lack of an efficient NADPH-G-S. In this respect, both NADH and NADPH are known to be necessary for steroid biotransformation (15,16). As additional support for this concept, irradiation has been shown to lower the amount of oxidized and reduced forms of NAD and NADP in various tissues after whole-body irradiation (17-22). More-

over, Maass (22) has postulated that the cellular synthesis of NAD and NADP is altered after irradiation. However, since the animals in this investigation were irradiated only to the head and part of the neck, the observed effects would appear to be mediated by some humoral factor. Hall and Eik-Nes (23) have postulated that the pituitary gonadotropins, as one of their mechanisms of action, could affect the synthesis or availability of NADPH in testicular tissue. Moreover, Benjamin and Yost (24) have shown that blood levels of TSH and ACTH increased rapidly 24 hours after ionizing radiations. Furthermore, these workers found that this effect was due to direct irradiation of the heads of these animals as this effect could be prevented by shielding the heads of these animals. Witschi et al. (9) some time ago postulated that pituitary gonadotropins may participate in the syndrome of events noticed in the testes after irradiation. These workers observed an increase in the level of circulating gonadotropins in parabiotic joined rats of which one rat was irradiated prior to its union with a non-irradiated partner. Other recent investigations indicate that a decreased content of gonadotropins in the pituitary occurs after irradiation in rabbits (10) and rats (11). Still other workers (12) have observed decreased ventral prostate weights in bone marrow protected rats after irradiation. Therefore, it is highly possible that the diminished biotransformation of 5-pregnenolone- 7α -H³ and progesterone-4-C¹⁴ into testosterone observed in this investigation could be due to an alteration of the pituitary's ability to secrete gonadotropins. These hormones, in turn, could affect the biotransformation of steroids by testicular tissues from these irradiated rats.

The data from this investigation also indicate that the biotransforma-

Table I. Average Quantities of Androgens and Their Immediate Precursors Produced on a Per Rat Basis by Rat Testes Incubated with 5-Pregnenolone-7 α -H³ and Progesterone-4-C¹⁴.

Steroids Isolated	m μ Moles Pregnenolone- 7 α -H ³	m μ Moles Progesterone -4-C ¹⁴	Total m μ Moles	H ³ /C ¹⁴ Ratios
<u>Control</u>				
Progesterone	1.05	2.10	3.15 $\pm$ 0.26 ^a	0.50
17 α -Hydroxyprogesterone	1.25	3.56	4.81 $\pm$ 0.14	0.35
Androstenedione	1.05	2.10	3.15 $\pm$ 0.33	0.50
Testosterone	1.29	1.17	2.46 $\pm$ 0.24	0.10
Total	4.64	8.93	13.57	
<u>Control + NADPH + G-6-P</u>				
Progesterone	0.27	0.31	0.58 $\pm$ 0.02	0.86
17 α -Hydroxyprogesterone	0.26	0.15	0.41 $\pm$ 0.11	1.78
Androstenedione	2.31	4.37	6.68 $\pm$ 0.06	0.53
Testosterone	1.12	2.22	3.34 $\pm$ 0.14	0.50
Total	3.96	7.05	11.01	
<u>Irradiated</u>				
Progesterone	1.04	2.59	3.63 $\pm$ 0.22	0.40
17 α -Hydroxyprogesterone	1.14	3.94	5.08 $\pm$ 0.23	0.29
Androstenedione	0.68	2.10	2.78 $\pm$ 0.15	0.32
Testosterone	0.61	0.91	1.52 $\pm$ 0.08	0.67
Total	3.47	9.54	13.01	
<u>Irradiated + NADPH + G-6-P</u>				
Progesterone	0.30	0.46	0.76 $\pm$ 0.04	0.65
17 α -Hydroxyprogesterone	0.16	0.11	0.27 $\pm$ 0.02	1.41
Androstenedione	2.48	4.49	6.97 $\pm$ 0.22	0.55
Testosterone	1.13	2.00	3.13 $\pm$ 0.16	0.56
Total	4.07	7.06	11.13	

^a Standard error of mean.

Table II. Average Per Cent Conversions of Pregnenolone- 7α -H³ and Progesterone-4-C¹⁴ into Steroid Intermediates and Androgens by Rat Testes.

Treatment	Pregnenolone - 7α -H ³	Progesterone -4-C ¹⁴	Both
Control	68.5 $\pm$ 0.6 ^a	80.5 $\pm$ 1.9	74.5 $\pm$ 1.9
Control + NADPH + G-6-P	94.5 $\pm$ 0.5	94.9 $\pm$ 0.4	94.7 $\pm$ 0.4
Irradiated	64.6 $\pm$ 1.5	75.9 $\pm$ 1.3	70.2 $\pm$ 1.3
Irradiated + NADPH + G-6-P	93.5 $\pm$ 0.3	91.6 $\pm$ 0.4	92.6 $\pm$ 0.3

^aStandard error of means.

Table III. Statistical Comparisons of Data in Figure 1 and Table II.

Comparison	Statistical Probability
<u>Androstenedione</u>	
Control vs. irradiated	$P \gg 0.050$
Control + NADPH-G-S vs. irradiated + NADPH-G-S	$P \gg 0.050$
<u>Testosterone</u>	
Control vs. irradiated	$P < 0.050$
Control vs. NADPH-G-S vs. irradiated + NADPH-G-S	$P \gg 0.050$
<u>Total Androgens</u>	
Control vs. irradiated	Approaches significance at 95% level
Control + NADPH-G-S vs. irradiated + NADPH-G-S	$P \gg 0.050$
<u>High-polar Compounds</u>	
Control vs. irradiated	Approaches significance at 95% level
Control + NADPH-G-S vs irradiated + NADPH-G-S	$P < 0.050$
<u>% Conversion Data (Tritium and carbon-14)</u>	
Control vs. irradiated	$P \gg 0.050$
Control + NADPH-G-S vs. irradiated + NADPH-G-S	$P \gg 0.050$

Figure 1. Steroid intermediates formed by testes from rats irradiated to their heads as expressed on a per rat basis.

tion of steroids by testicular tissue after irradiation may be altered even though histologically these cells appear to be normal.

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SERUM TRANSAMINASE LEVELS
IN BEAGLE DOGS BURDENED WITH PU²³⁹*

Walter Stevens and David L. Berliner

Abstract: Serum glutamic oxaloacetic and glutamic pyruvic transaminase were measured in dogs injected with Pu²³⁹ in dose levels ranging from 0.0157 $\mu\text{c/kg}$ (P1.0) to 2.91 $\mu\text{c/kg}$ (P5.0). A significant difference in SGPT between males and females of the control group was demonstrated. There was no significant difference between the SGOT values of the males and females. There is a correlation between the injected dose of Pu²³⁹ and the per cent of SGOT and SGPT measurements that are elevated. The P5.0 animals showed elevated transaminase values over a period of months. The increase in SGOT and SGPT in plutonium burdened dogs is attributed to the significant deposition of the Pu²³⁹ in the livers of these animals.

Introduction:

The normal range of activity of transaminase in human serum was first described in 1953 (1). Since then the clinical implications of alterations in serum transaminase activity in disease of the heart (2,3), liver (3,4,5), and skeletal muscle (6) have been well documented.

The distribution of transaminase enzymes in the organs of dogs varies; glutamic oxaloacetic (GOT) is highest in the heart, and glutamic pyruvic (GPT) is highest in the liver (1,4,9). The large amounts present in organs such as heart, liver, skeletal muscle, and kidney as compared to normal serum levels suggest that increases in serum levels are associated with tissue injury. This association has proven to be well founded (5,6).

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In liver diseases such as acute toxic hepatitis, infections and viral hepatitis, large increases are found in serum transaminase (4). In infectious and viral hepatitis SGPT usually exceeds SGOT (5). Serum glutamic pyruvic transaminase activities can play an important part in the detection and diagnosis of active liver cell damage and necrosis before any other abnormality is shown by other liver function tests (7).

The collection and analysis of sufficient data on the serum transaminase levels in Pu²³⁹ burdened dogs will allow one to detect early necrotic changes induced by the deposition of Pu²³⁹ in the liver of these dogs.

Methods:

The dogs used in this study are members of a colony of beagles used to study the chronic effects of internally deposited radionuclides (8). The dogs on which the serum transaminases were measured have been previously injected with single intravenous doses of Pu²³⁹. Doses ranged from 0.0157 μ c of Pu²³⁹/kg (one-level plutonium dog, P1.0) to 2.91 μ c of Pu²³⁹/kg (five level plutonium, P5.0).

The serum transaminases were measured, using a colorimetric*method. This method measures the amount of oxaloacetate or pyruvate formed in one hour. One unit of SGPT or SGOT transaminase will form 4.82×10^{-4} μ Moles of glutamate per minute at pH 7.5 at 25°C.

The blood is drawn from the external jugular vein and immediately placed in an ice bath. The serum is separated from the cells in a refrigerated centrifuge. All measurements are made within four hours of the time the blood is drawn. All glassware used is ion-free.

*Sigma Chemical Co. Bulletin 505

Results:

Control Animals. The control group was comprised of 47 non-injected male and female animals which include the P.O. animals. The ages of the animals at the time of measurement ranged from 439 days to 4318 days with a mean age of 2520 days. A total of 111 measurements for each of the serum transaminases were used to compute means and standard deviations for the control groups.

Table I lists the values for the controls as a single master control group and for males and females separately. The mean for the master control group (males plus females) is: SGOT - 18 ± 6.5 units, SGPT 25 ± 10.5 units. The SGOT mean and standard deviation for males is 17.6

TABLE I
Control Beagles

	SGOT	P	SGPT	P	n
Master Control Group (Males & Females)	18 ± 6.5	-	25 ± 10.5	-	111
Males	17.6 ± 6.4	N.S. ^a	28.7 ± 11.1	0.01 ^b	50
Females	18.4 ± 5.95		22.1 ± 8.95		61

^a Non-significant

^b Highly significant

± 6.4 and for females is 18.4 ± 5.95 . There was no statistical difference between these groups. The SGPT mean and standard deviation for males is

28.7 $\pm$ 11.1 units and for females 22.1 $\pm$ 8.95 units. The difference between the male and female groups is highly significant (P 0.01). No correlation between age and SGOT or SGPT was found for the master control group. Ninety-nine per cent of the control dogs had a SGOT value of less than 35 units and 99% of the control dogs had SGPT values of less than 52 units. Serum transaminase values that exceed these limits are considered to be elevated.

Pu²³⁹ burdened animals. Serum transaminases were measured on 37 separate Pu²³⁹ burdened animals. A total of 372 separate measurements have been made on these dogs. Unfortunately, these animals have not been followed from the time they were injected. Burden times usually exceed 5 years. The dogs were injected with a single intravenous dose of Pu²³⁹ ranging from 0.0157 μ c Pu²³⁹/kg to 2.91 μ c Pu²³⁹/kg and now have burden times of 12 to 121 months. There are 11 dogs in the P0.0 group, 19 dogs in the P1.0 group, and 13 dogs in the P1.7 group. There are not enough surviving dogs in the P2.0, P3.0, P4.0 and P5.0 to evaluate as groups.

The data in Fig. 1 illustrates the relationship between the dose of Pu²³⁹ and the per cent of the SGOT measurements that were elevated. Four per cent of the SGOT measurements were elevated in the P0.0 group, 13% in the P1.0 group and 22% in the P1.7 group. None of the SGOT values exceeded 100 units.

Figure 2 illustrates the per cent of the SGPT values that are elevated as compared to the injected dose. The P0.0 group has 2% of its SGPT measurements elevated, P1.0 group has 31% elevated, and the P1.7 group has 46% elevated. Not all of the dogs in the P1.0 and P1.7

Figure 1. The percent of the total SGOT measurements that were elevated in the three Pu²³⁹ dose levels.

Figure 2. The percent of the total SGPT measurements that were elevated in the three Pu²³⁹ dose levels.

SERUM GLUTAMIC OXALOACETIC TRANSAMINASE UNITS

SERUM GLUTAMIC PYRUVIC TRANSAMINASE UNITS

Figure 3. The increase in SGOT values above normal in two Pu²³⁹ bearing dogs at various times past injection.

Figure 4. The increase in SGPT values above normal in two Pu²³⁹ bearing dogs at various times past injection.

group had elevated SGPT values, but some of the Pl.0 and Pl.7 dogs had as many as six elevated SGPT values. The highest SGPT value in the Pl.0 group was 265 units, and only three measurements exceeded 100 units. In the Pl.7 group the highest value was 311 units and 11 measurements were in excess of 100 units.

Two five level plutonium dogs (T19P5 & T21P5) were alive at the beginning of this experiment. T21P5 died in July and was studied for a period of 7 months. T19P5 is still alive and has been examined periodically for the last 11 months.

The data in Fig. 3 shows the variation observed in SGOT in both T19P5 and T21P5. Both dogs when first measured at 22 months post injection were within the normal range. T21P5 showed significantly elevated values at 24, 28, and 29 months post injection. T19P5 had significantly elevated SGOT values at 28, 31, 33 and 35 months post injection. The other values for both dogs are only slightly elevated.

Serum glutamic pyruvic transaminase values for T21P5 and T19P5 over the same time intervals are illustrated in Fig. 4. The increases in SGPT are more marked than those in SGOT. Both dogs when first measured had significantly elevated SGPT, and T19P5 showed a very high value of 600 units at 28 months post injection. A subsequent decrease followed with a low occurring at 32 months post injection; then, at 35 months post injection it increased again to 560 units. T21P5 showed an initial decrease and then a steady increase to 390 units at 29 months post injection. T21P5 died 2 months later.

Discussion

The clinical value of serum transaminase in diagnosing cardiac and liver disease in humans is well established (2,4). Recently, these techniques have been applied to the diagnosis of early necrotic changes in dog liver (7,9), and dogs have been found to respond in the same manner as humans. Serum transaminase levels particularly SGPT, can be used to assess early active liver damage before any abnormality is shown by other liver function tests (7). In general, the extent of the rise reflects the severity and extent of hepatocellular damage.

Various workers have shown increases in serum transaminase following external irradiation to the whole animal (10-14) and to particular organs (15).

Increases in SGOT and SGPT were found in dogs with injected doses ranging from 0.016 $\mu\text{c Pu}^{239}/\text{kg}$ (P1) to 2.91 $\mu\text{c Pu}^{239}/\text{kg}$ (P5). As the injected dose increases, there is a corresponding increase in the percent of the animals' SGOT and SGPT measurements which are elevated. This is undoubtedly due to the increased hepatocellular necrosis caused by the increased radionuclide burden. The SGPT values found in the P5 dogs are the highest found in any of the plutonium toxicity animals.

The fact that a large fraction of the plutonium burdened animals have elevated transaminase can be attributed to the distribution of the radionuclide in the animal. In the plutonium animals 15 to 30% of the injected dose deposits in the liver (16), which is at least 2 to 3 times the level found in livers of dogs burdened with other bone-seeking radionuclides (8). Cochran et al. (17) have estimated that initially

Figure 5. Detailed autoradiograph showing initial uniform distribution of Pu^{239} in liver of P-5 animal sacrificed 24 hours post-injection (400 X).

Figure 6. Contact autoradiograph showing non-uniform distribution of Pu^{239} in liver of a P-5 dog sacrificed 1576 days post-injection (80 X).

Figure 7. Detailed autoradiograph showing non-uniform distribution of Pu^{239} in liver of a P-5 sacrificed 1576 days post injection (400 X).

100% of the hepatic cells are exposed to alpha-particle radiation due to the diffuse pattern of distribution of the plutonium isotope (Fig. 5). However, after one year the distribution pattern changes with the Pu^{239} redepositing in localized areas, "hotspots" in sinusoidal reticuloendothelial cells, and in the condensed reticulum (Figures 6, 7).

Since the Pu^{239} appears to deposit mainly in the sinusoidal reticuloendothelial cells (RES), the increase observed could be related to the phenomena reported by Fisher and Fisher (18). They found that by blocking or stimulating the RES, increases in serum transaminase were observed. These increases in serum transaminase were thought to reflect parenchymal cell damage. Overt hepatic cell necrosis was not evident upon histological examination (18).

These Pu^{239} depositions in the reticuloendothelial cells could also alter liver function since the reticuloendothelial system is intimately related to certain physiological processes such as steroid biotransformation (19). These biotransformations permit the parenchymal cell to conjugate the steroids to form water soluble compounds. The authors have shown recently that external whole-body x-irradiation will decrease the conjugation of steroids (20).

The elevated transaminase values, particularly SGPT in the Pu^{239} dogs indicate that at the time of measurement there was a gradual hepatic necrosis taking place. The greatest degree of necrosis occurs in the five-level animals. Some of the Pl.7 dogs have SGPT values approaching those found in the P5 dogs. This necrosis is substantiated by the

presence of regeneration nodules seen at autopsy in the plutonium burdened dogs. These regeneration nodules do not contain appreciable amounts of plutonium (17). Periods of cellular death and necrosis followed by regeneration nodule formation could produce an irregular transaminase pattern as is seen in the P5 dogs. Due to the death and necrosis of the hepatic cells and reticuloendothelial cells, the diffusely distributed Pu^{239} would be released, translocated and concentrated in another part of the liver, initiating a new wave of cellular destruction. This is well illustrated in Figs. 3 & 4 where we have large peaks of SGOT and SGPT activity followed by drops toward normal values. In the Pl.0 and Pl.7 dogs these extremes are not as marked, indicating a more gradual destructive process.

The amount of individual variation found within each group must be attributed to normal biological variation and to the general state of health of the animal, which varies depending on the age of the dog.

The increase in serum transaminases previously reported by the authors (21), has recently been confirmed by other investigators (22), following the injection of Pu^{239} into dogs. The increase in SGPT occurred prior to any morphological manifestations.

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QUANTITATIVE STUDIES OF TRABECULAR BONE REMODELING

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Abstract: The variation in bone surface occupied by osteoid seams in lumbar vertebral bodies with age has been characterized for 26 dogs. Of the 26 dogs, 20 were normal and 6 were partially or totally immobilized at the time of autopsy. The per cent of bone surface occupied by osteoid seams is used as an index of osteoblastic activity. This osteoblastic activity is found to be about 10% at the age of 600 days, dropping to a low of approximately 5% at the age of 2000 - 3000 days, and rising abruptly at age 3500 days to approximately 20% of the bone surface. The distribution of the osteoid seams is plotted to characterize the pattern of remodeling. Tentatively, it is found that the remodeling occurring was ranked in this order: first, the metaphysis; second, the diaphysis; and third, the epiphysis. Also, the frequency distribution of dorsal to ventral osteoid seams is 1.0, while the ratio of superior to inferior osteoid seams is 0.85.

Introduction

No extensive study to characterize the rate of internal reconstruction or remodeling of adult spongy bone exists. Such information is quite useful as an index of osteoblastic activity as well as a baseline for the study of the effect of aging on the rate of spongy bone remodeling. It is the purpose of this study to establish a baseline of internal reconstruction in spongy bone of adult Beagles in the hope of comparing these studies with spongy bone from animals injected with bone seeking radio-nuclides. We also hope to be able to characterize the changes in remodeling rates with age.

Methods

Twenty-nine young adult Beagles were used. They ranged in age from 660 days to 3777 days. The present study involves only the observations on the 2nd lumbar vertebra from these animals. At autopsy the 2nd lumbar vertebra is removed and sagittally sectioned. One-half of the vertebral

body is placed in absolute acetone, and the other half in Zenker-formol solution. The bone placed in acetone is dehydrated and defatted in changes of absolute acetone and absolute ether. It is then embedded in a mixture of ethyl and butyl methacrylate. The block is sectioned using the mechanized Jung microtome, resulting in an 8 μ thick section (1). The sections are subsequently stained with a May-Grünwald-Giemsa stain identical with that used for blood films. This stain brings out the osteoid seams (preosseous bone) (Fig. 1). The sections are then magnified 80 times by projection and the outline of the bone surfaces is traced on paper (Fig. 2). The areas occupied by the osteoid seams are plotted on these tracings. The lengths and widths of the osteoid seams are measured under the microscope and recorded on the tracing of the vertebral body. The total perimeter of the trabecular or bone surface as well as the osteoid seam perimeter is measured using a map measuring device. The ratio of osteoid seams to total trabecular surface is calculated. This value is expressed in per cent of bone surface occupied by osteoid seams (Table I).

In order to characterize the pattern of bone remodeling the lumbar vertebral bodies are divided into 27 units (Fig. 3). In each of these units the per cent of bone surface occupied by osteoid seams is calculated. Each unit is then ranked from 1 to 27 depending on the magnitude of the per cent of bone surface occupied by osteoid seams. For instance, the seam subunit #1 in Fig. 4 has 31.9% of its bone surface occupied by osteoid seams. It is graded #1. Conversely, subunit #15 contains seams on 2.7% of its bone surface and is graded #25 in rank with the other 26 units. Also, the frequency distribution of osteoid seams located on the dorsal, ventral, superior and inferior bone surface is determined. These

TABLE I
Vertebral Osteoid Seam Data

Animal #	Animal Age	Total Seam Length μ	Total Perimeter in μ	% Seam Lengths Perimeter
678	427	89,031	562,870	15.8
731	485	208,382	616,518	34.0
732	485	31,150	503,732	6.2
F ₆ T ₀	660	43,060	416,550	10.3
MAC56	701	60,097	529,586	11.3
MAC58	767	62,717	604,500	10.4
FAC51	1,089	16,858	483,954	3.70
F ₉ S ₀	1,257	24,148	498,600	4.80
MAC42	1,265	17,830	726,763	2.56
FAC1	1,383	37,040	487,564	7.6
MAC3	1,451	32,850	486,824	6.73
MAC71	1,472	26,064	505,400	5.16
FAC47	1,705	4,560	460,112	0.99
FAC17	2,145	37,600	513,412	7.0
F ₉ R ₀	2,250	3,480	522,732	0.66
MAC50	2,264	30,412	731,293	4.1
FAC2	2,492	5,730	530,100	1.1
FAC34	2,584	21,473	488,870	4.38
FAC10	2,605	24,630	859,012	2.86
MAC24	3,074	34,505	572,928	5.98
MAC28	3,114	73,670	589,820	12.52
F ₂ P ₀	3,179	14,010	388,388	3.62
FAC33	3,282	8,655	454,003	1.91
MAC4	3,345	105,147	578,604	18.2
F ₆ R ₀	3,665	49,317	439,898	11.2
M ₁ R ₀	3,673	10,545	604,302	1.75
FAC9	3,719	14,795	641,508	2.31
MAC8	3,746	2,505	628,040	0.4
FAC14	3,777	131,005	547,120	23.9

distributions are expressed as the ratio of dorsal to ventral and superior to inferior osteoid seams. This is also analyzed to determine some pattern in the remodeling process.

Results

The results of our findings are summarized in Table I. It tabulates the animal code, animal age, total osteoid seam length (microns), total perimeter of bone surface (microns), and per cent of bone surface (perimeter) occupied by osteoid seams ($\% \frac{\text{seam lengths}}{\text{perimeter}}$). The data in which the per cent of bone surface occupied by osteoid seams ($\% \frac{\text{osteoid seam surface}}{\text{endosteal surface}}$) is shown in Fig. 6. Fig 6 also shows the variation in frequency of osteoid seams with age in which the per cent of bone surface occupied by osteoid seams ($\% \frac{\text{osteoid seam surface}}{\text{endosteal surface}}$) is plotted on the ordinate against the age (days) on the abscissa. The open circles represent the 20 animals which have been evaluated clinically as normal. The closed circles represent six animals which were partially or totally paralyzed at the time of autopsy. The plot shows that at between ages 500 - 800 days, the per cent of bone surfaces occupied by osteoid seams is about 10%. Between the ages of 1000 - 1500 days this value drops to approximately 5% and diminishes to less than 5% between the ages of 2000 - 3000 days. Subsequently, between the ages of 3000 - 3500 days, the bone surface occupied by osteoid seams shows a rapid rise. At 3777 days the bone surface occupied by osteoid seams is 23.9%. Although it is much too early to form a definite conclusion, it is quite apparent that the six partially or totally paralyzed animals show a marked diminution in the per cent of bone surfaces occupied by osteoid seams. The osteoid seams from the four immobilized dogs measured at approximately

3500 days show an obvious depression in incidence of osteoid seams. For example, one animal (MAC8), sacrificed at 3746 days has a per cent of bone surface occupied by osteoid seams of 0.4%; while a comparable animal (FAC 14) sacrificed at 3777 days has osteoid seams over perimeter of 23.9%.

Fig. 5 is a vertebral body broken into 27 subunits with each subunit graded for its relative frequency on bone surface occupied by osteoid seams. This same technique is used for 29 animals. Tentatively, the findings indicate that the metaphyseal area represented by subunits 4 - 9 and 19 - 24 have the highest frequency of bone surface occupied by osteoid seams. The diaphyseal area represented by subunits 10 - 18 is second in rank; and finally, the epiphyseal area represented by 1 - 3 and 25 - 27 has the lowest incidence.

In our attempt to characterize the frequency distribution of the ratios of osteoid seams on dorsal, ventral, superior and inferior surfaces, we have tentatively concluded they are somewhat startling. Fig. 7 (top graph) shows a plot of the frequency distribution of the ratio of dorsally to ventrally situated osteoid seams. This ratio of dorsally located to ventrally located seams turns out to be a mean of 1.03 ranging from 0.5 to 4.2. Surprisingly (Fig. 7, bottom graph), shows the ratio of superiorly to inferiorly located osteoid seam averages 0.85 and ranges between 0.17 and 4.3.

Discussion

Our attempt to plot the variations in bone surface occupied by osteoid seams in lumbar vertebral bodies was surprisingly successful. We were quite amazed to find a depression in the per cent of bone surface occupied by osteoid seams at 2000 - 3000 days. This is somewhat similar to the

depression observed in incidence of osteoid seams in a study by Frost (2). Frost has used the incidence of osteoid seams as an expression of the index of osteoblastic activity. Induced osteoid seams are a secretory product of osteoblasts. Therefore, it can be assumed that the incidence of osteoid seams can be related to osteoblastic activity. The findings expressed in Fig. 6 closely coincide with the findings of Frost (2) on human ribs. Frost shows a depression of incidence of osteoid seams at age 35, and a marked rise at age 50 which drops again at age 60. The observed depression of per cent of bone surface occupied by osteoid seams at 2000 - 3000 days in adult Beagles resembles what appears to be a similar physiological phenomenon occurring in man at age 35. It is most astonishing that the dog exhibits the same changing pattern in the rates of bone remodeling as a function of age observed in man. This is most encouraging since this is the first index of bone metabolism which is similar in man and Beagle.

The present study is quite useful in predicting the per cent of bone surface occupied by hotspots of alkaline earth radionuclides. It will quantify the per cent of bone surface occupied by hotspots as a function of age. Each osteoid seam represents a site in which a hotspot will localize should an adult dog be contaminated with an alkaline earth radionuclide. The osteoid seam is a site of new bone growth. New bone growth is the site of hotspot localization. In a dog injected at age 600 days we find that 10% of the bone surface of a vertebral body will be occupied by hotspots. The remaining 90% of the bone surface is occupied by the diffuse component containing much less radioactivity. Conversely, in a Beagle at 3700 days of age, the hotspots occupy 20% of the bone surface

and the remaining 80% of the bone surface is utilized by the diffuse component. If the animal is partially or totally immobilized at the time of the contamination with an alkaline earth radionuclide, very little of the radionuclide will be deposited as hotspots on bone surfaces.

Finally, this study has shown that it is possible to characterize the index of osteoblastic activity (per cent of bone surface occupied by osteoid seams) with age in adult Beagles. Such information will be quite useful in our endeavor to characterize the effects of age and bone seeking radionuclides on the skeleton.

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DISTRIBUTION OF OSTEOID SEAMS IN 2nd LUMBAR VERTEBRAL BODY OF 3114 DAYS OLD BEAGLE

Figure 1 - Eight micron thick trabecula of a typical 2nd lumbar vertebral body from a 3114 day old Beagle showing an osteoid seam-pre-osseous bone, (see arrows). May-Grünwald-Giemsa.

Figure 2 - Diagram of a typical 2nd lumbar vertebral body from a 3114 day old Beagle showing the distribution of osteoid seams and the tracing of bone surfaces. The dark lines on bone surfaces represent the location of the osteoid seams. The ratio of osteoid seam surfaces (microns), divided by the endosteal seam surfaces (microns), is 12.52% in this particular specimen.

DISTRIBUTION OF OSTEOID SEAMS IN 2nd. L.V.B. OF 3114 DAYS OLD BEAGLES

OSTEOID SEAMS (μ)
ENDOSTEAL SURFACE (μ) 12.52 PER CENT

□ PER CENT OF OSTEOID SEAM/ENDOSTEAL SURFACE PER SUBUNIT

△ RANK (HIGHEST TO LOWEST) SEAM DISTRIBUTION

Figure 3 - Outline of a typical 2nd lumbar vertebral body showing the 27 subunits.

Figure 4 - Outline of the 2nd lumbar vertebral body shown in Fig. 2, in which the vertebral body has been divided into 27 subunits. Each subunit represents the per cent of osteoid seam per unit of endosteal surface and is calculated concurrently with each subunit and ranked from the highest to the lowest seam distribution.

Figure 5 - Profile of a typical vertebral body with 27 subunits showing the distribution of osteoid seams in the 2nd lumbar vertebral bodies of 29 Beagles with an age range between 427 to 3777 days. The circle with the number in it represents the number of the subunit. The square in each subunit represents the number of bones out of the 29 animals which lack seams in that particular subunit. The triangle represents the rank from highest to lowest frequency of seam distribution.

Figure 6 - Plot of the variation with age of osteoid seam surface per total endosteal surface, expressed in per cent, in the lumbar vertebral body. The open circles represent normal Beagles. The closed circles indicate Beagles which were partially or totally immobilized at time of autopsy.

FREQUENCY DISTRIBUTIONS OF RATIOS OF OSTEIOD SEAMS

Figure 7 - Upper plot shows the ratio of the frequency distribution of dorsal to ventral osteoid seams and has a mean of 1.03. Lower plot shows the ratio of superior to inferior osteoid seams with a mean of 0.85.

QUANTITATIVE AUTORADIOGRAPHIC DOSE DETERMINATIONS
OF Pu²³⁹ IN BONES

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M. Lowe, C. W. Mays and W. S. S. Jee

Abstract: Quantitative autoradiographic dose determinations of Pu²³⁹ revealed that: the surface deposition of plutonium is non-uniform and varies from bone to bone as well as from one anatomical site to another. Vertebral surface deposits of Pu²³⁹ are higher than deposits found in other bones. Specific dose-rates to given areas may be as high as 11 to 48 times greater than skeletal retention data. Mosaic trabeculae, consisting of viable and non-viable bone, appear in the femoral epiphysis and metaphysis after 186 days in animals receiving 0.3 μC of Pu²³⁹/kg of animal weight. The 0.015 μC Pu²³⁹/kg of weight dose resulted in no gross histopathological changes at one year, but in ten years post administration two cases of osteogenic sarcoma appeared.

Introduction

The present report is a summary of the data relative to the quantitative autoradiographic dose-rate determinations of Pu²³⁹ in bones using an alpha track counting method. It is one phase of a continuous study to attempt to relate radiation dose to resulting damage in the Bone and Autoradiography Group of the Division of Radiobiology, Department of Anatomy, College of Medicine, University of Utah.

The need for a specific reproducible method to correlate quantitatively the specific absorbed dose-rate measured in rads per day to a restricted area of bone surface and cellular elements of the marrow within the 35.4 μ range of alpha particles from Pu²³⁹ was satisfied. The absorbed dose is the amount of energy imparted to matter per unit mass of irradiated material at a specific site of interest. The dose is measured in rads.

The data reported here concerns measurements obtained from experiments on young adult Beagles injected at 489 to 559 days of age with 0.3

and 0.015 μc of Pu^{239} /kg of body weight. The animals were serially sacrificed from 5 to 362 days after the administration of Pu^{239} . This quantitative alpha track study measured the maximum concentrations of alpha tracks from which the average doses to cells over the alpha particle range were calculated. The study included vertebral trabecular and femoral periosteal, endosteal, haversian, epiphyseal and metaphyseal trabecular surfaces.

An early report from our laboratory on animals injected with a higher dose of Pu^{239} (3 μc /kg) determined the concentrations of Pu^{239} in the thoracic vertebrae of Beagles by microdensitometric methods utilizing a Leitz Panphot microscope, a MacBeth-ansco densitometer and a Brown recorder (1). The present study, however, could not gainfully use such a method because of the demand for a prolonged exposure time before a suitable alpha track density that was readable by the microdensitometer appeared on the nuclear track emulsion. For instance, a three year autoradiographic exposure would be necessary for the bones of the 0.015 μc /kg series.

Methods

A mechanized Jung microtome was used to cut sections of undecalcified bone from ethyl-butyl methacrylate blocks. The NTB gel emulsion was painted on the sections (2). After exposures of 180 days for the 0.015 μc /kg animals, and 26 days for the 0.3 μc /kg animals, the autoradiograms were developed in D-19 at 18 degrees C., short stopped for one minute and then fixed. The autoradiographic processing, subsequent decalcification staining takes about one to two months (3).

Four persons individually counted the alpha tracks in specific areas of the highest alpha track concentrations on each of the autoradiograms surveyed. Four separate lumbar vertebrae and six distal femurs from Beagles which had received 0.3 and 0.015 μC of Pu^{239} /kg of animal weight and sacrificed at 5 to 362 days were used. The Pu^{239} was administered as a single intravenous injection in the $^{+4}$ citrate form.

Each of the autoradiograms was divided into small squares which were numbered. The area or areas (where more than one anatomical area occurred) were searched for a specific site containing the greatest number of tracks per counting area. This was usually done for an area 120 μ in length and 40 μ in width which was standardized by calibrating the ocular micrometer net with a stage micrometer for each of the microscopes used. The square that each of the persons counting reported as having the highest count was rechecked and evaluated to positively identify the specific area having the highest alpha track count. Confirmation of the count and dose rate of a given deposit was made by Dr. W. S. S. Jee.

The tracks counted in the specific areas were converted to rad/day by a formula developed by Dr. Charles W. Mays (4) of this laboratory. This formula took into consideration the energy of the alpha particle (5.14 Mev), the alpha particle range in soft tissue (35.4 μ), and the density of the soft tissue (1 g/cm^3). The formula used to compute

the dose-rate from a surface deposit of Pu^{239} is:

$$\text{Dose rate in rads/day} = 290 \frac{T}{L} \frac{(\text{Number of tracks per day})}{(\text{Length of scan in microns})}$$

For example: if $T = \frac{200 \text{ tracks}}{10 \text{ days}} = 20 \text{ tracks per day}$

and 100 μ of bone surface was scanned,

$$\text{Dose rate} = 290 \frac{20}{100} = 58 \text{ rads/day}$$

Investigators have known and it has been previously cited and reaffirmed in this study that the surface deposition of plutonium is non-uniform and varies from bone to bone as well as from one anatomical site to another within the same bone. For instance, the ratio of activities on vertebral trabecular surface deposits to femoral metaphyseal trabecular deposits at sites of maximum concentration is 1.5 when 0.015 μc of Pu^{239} is administered. This is an example of dose-rate variation to similar sites in different bones.

An example of variation in dose-rates within the same bone is found in the distal femur. In femurs from animals receiving 0.015 μc of Pu^{239} /kg of body weight the ratio of the dose-rates of surface deposited Pu^{239} in various sites of the distal femur was 3.0: 2.6: 1.5: 1.2: 1.0 for metaphyseal trabecular, endosteal epiphyseal trabecular, haversian and periosteal surfaces, respectively. This data appears in Fig. 1. The dose-rates to the 35.4 μ soft tissue layer lining mineral surfaces from different sites are compared with the uniform skeletal dose-rate and the ratio of the two is listed in Table I.

TABLE I
Dose-Rates (Rads/Day) to Distal Femora and Lumbar Vertebrae
(0.015 μc Pu^{239} /kg dogs)

Anatomical Site	Maximum Rads/Day	Uniform Skeletal Dose-Rate (Rads/Day)	Ratio
Periosteal surface	.25	.0237	11
Haversian surface	.31	.0237	13
Epiphyseal trabecular surface	.39	.0237	17
Endosteal surface	.65	.0237	27
Metaphyseal trabecular surface	.75	.0237	32
Lumbar vertebral trabecular surface	1.15	.0237	48

The value of this technique involving quantitative autoradiography and microscopic observation of a single area is that at a dose level of 0.3 μc of Pu^{239} /kg of body weight, for example, a mosaic bone formation was found while at the lower dose level of 0.015 μc of Pu^{239} /kg of body weight no gross histopathological changes in bone from dogs sacrificed at early dates were observed. However, in the chronic toxicity studies two dogs have developed osteogenic sarcoma ten years post injection.

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FIGURE 1

DISTRIBUTION OF Ra²²⁶ BETWEEN BLOOD CELLS AND PLASMA

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B. J. Stover and C. W. Mays

Abstract: The concentration of Ra²²⁶ in blood cells, c_c , has been determined by the dilution or washing method and was found to be significant with respect to the plasma concentration, c_p . At 5 days after intravenous injection the average for 6 beagles was $c_c/c_p = 0.205 \pm 0.021$. In a single human study, c_p decreased more rapidly than c_c so that c_c/c_p increased with time. At 0.28 day $c_c/c_p = 0.1$ and increased to 0.6 ± 0.1 at 8 days. The transfer of Ra²²⁶ between cells and plasma is consistent with a diffusion process.

Introduction

In our studies of Ra²²⁶ in adult beagles measurements of circulating Ra²²⁶ were limited to blood plasma since circulating calcium is usually measured only in plasma (1). However, Aub et al. reported in 1938 that in humans with chronic radium exposure, "there seems to be slightly less radium in the red blood cells than in the serum" (2). Also radium has been reported to be almost equally distributed between cells and plasma in rat blood (3).

Blood plasma free of cells is readily obtained by centrifugation. The cellular fraction contains an unknown amount of plasma so that Ra²²⁶ in the cells cannot be determined accurately simply by measuring Ra²²⁶ in the cellular fraction. Thus, a dilution method was used to determine the amount of Ra²²⁶ associated with the cells and the weight of the cells, both of which must be known accurately for a valid concentration determination. The principle of the method is the analytical chemist's theory of washing a precipitate.

Methods

Blood (heparinized) was obtained from 6 young adult beagles 5 days after a single intravenous injection of $3.3 \mu\text{C Ra}^{226}/\text{kg}$ (4). Each blood

sample was weighed, centrifuged, and separated into plasma and cell fractions, each of which was also weighed. A weighed amount of isotonic saline was added to the cell fraction and it was shaken gently so that the plasma which had remained in the cell fraction would be diluted by the saline. The cell saline suspension was then centrifuged, separated, and each portion was weighed. Four washings were done. Lack of discoloration of the saline solution was taken as evidence of minimal cell destruction. Finally, Ra^{226} was measured in each wash solution, the plasma and the washed cells. Calcium was determined by a semi-micro complexometric method.

A second study was made using beagle blood drawn 2 days after intravenous injection of $1.1 \mu\text{C Ra}^{226}/\text{kg}$. The blood was divided into 2 nearly equal portions. One part was washed 3 times with saline; the second was washed 3 times with plasma which was obtained from the same dog 1 day before injection and refrigerated until used.

In the course of the dog studies an unusual opportunity arose to measure the distribution of Ra^{226} between cells and plasma in a human subject as a function of time. Blood was obtained from an adult male at times from 0.008 to 19 days after intravenous injection of $19.5 \mu\text{C Ra}^{226}$ (5).

Results

The weight of the washed cell fraction is high by the amount of the last wash left in it. The amount of Ra^{226} measured in it needs a small correction for the wash left and a correction for any Ra^{226} washed off the cells. Then the actual concentration in cells can be obtained.

Let W = weight of blood
 W_o = weight of plasma removed
 W_n = weight of cell fraction after last wash and separation
 x_o = weight of plasma left in cell fraction after first separation
 W_i = weight of saline added in i th wash
 x_i = weight of saline left in cell fraction after i th wash and separation
 $n-1$ = number of saline washes

Then

$$W_i + x_{i-1} - x_i = \text{weight of saline removed after } \underline{i\text{th}} \text{ wash,} \quad (1)$$

$$x_{n-1} = \text{weight of } (n-1)\underline{\text{st}} \text{ wash left in cell fraction,}$$

$$W_c = W_n - x_{n-1} = \text{actual weight of cells, and} \quad (2)$$

$$W_p' = W_o + x_o = \text{apparent weight of plasma.} \quad (3)$$

A small mechanical loss occurs during the separations. For the whole procedure the fractional loss L , is given by

$$L = \frac{W - (W_p' + W_c)}{W} =$$

$$\frac{1}{W} \left[W - \left\{ W_o + W_n + \sum_{i=1}^{n-1} (W_i + x_{i-1} - x_i) - \sum_{i=1}^{n-1} W_i \right\} \right] \quad (4)$$

Since plasma is more sticky and viscous than the saline washes, we assume that the loss is essentially all plasma. Then we have

$$W_p = W_o + x_o + L W = \text{actual weight of plasma.} \quad (5)$$

To determine the n quantities $x_0 - - - x_{n-1}$ we have $n-1$ of the necessary equations in the weight relations, Eqs (1).

Let c_p = concentration of Ra^{226} in plasma,
 c_i = concentration of Ra^{226} in i th wash,
 c_n = concentration of Ra^{226} in cell fraction after last wash and separation.

If the x_0 grams of plasma are uniformly diluted in the W_1 grams of saline and no Ra^{226} is washed off the cells, then

$$c_1 = c_p x_0 / (W_1 + x_0) \quad (6)$$

For successive washings we have

$$c_i = c_{i-1} x_{i-1} / (W_i + x_{i-1}) \quad (7)$$

(Note that if all the x 's and all the W 's are equal

$$c_{n-1} = c_p \left[x / (W+x) \right]^{n-1}.$$

Eqs. (6) and (7) provide a second set of $n-1$ relations to determine the n x 's giving a total of $2n-2$. Thus, if $n > 2$, we can choose the n relations involving the more accurate measurements and reserve the remaining $n-2$ relations to test one or more of the assumptions.

The best of the data are the weights, which have fractional errors of the order of 10^{-4} . The better concentration data are those for the plasma and the first wash since the count rates are higher. The fractional standard deviations for the concentration measurements ranged from less than

0.02 for plasma to about 0.2 for the fourth wash. Accordingly Eq. (6) was used to calculate x_0 and the remaining x 's were obtained from x_0 and Eqs. (1).

Eqs. (7) were used to test the assumptions that the dilutions were uniform and that no Ra^{226} was washed off the cells. The mean and standard deviation of the mean (i.e. standard error) of the 6 values of the ratio of observed to calculated (Eqs. (7)) concentrations are given in Table I which also includes the range of dilution factors.

The dilution factor for the first wash is

$$x_0 / (W_1 + x_0) = c_1 / c_p$$

that for the second is

$$\left\{ x_0 / (W_1 + x_0) \right\} \left\{ x_1 / (W_2 + x_1 - x_2) \right\} = c_2 / c_p, \text{ etc.}$$

The t-test was used to determine the significance of the difference between the ratio and unity, and the p values appear in the last column of Table I. The observed concentration in the second wash was equal to that calculated by Eq. (7), which shows that the dilution was uniform. The increasing ratios for the third and fourth washes show that Ra^{226} was being lost from the cells in those washes. This loss correlates with an increasing concentration gradient, for the differences between average cell concentration and average wash concentration (both referred to plasma), $(\overline{c_c} / c_p) - (\overline{c_i(\text{obs})} / c_p)$, were 0.067, 0.168, 0.191, and 0.197 in that order. Thus, most of the loss occurs in the later washes after much of the

Table I

Comparison of Observed and Calculated Concentrations
in the Wash Solutions

Wash	No. Samples	Ra^{226}		
		$c \text{ (calc)} / c_p^a$	$c \text{ (obs)} / c \text{ (calc)}^b$	p^c
1	6	0.131-0.153	unity by definition	-
2	6	0.032-0.049	1.00 ± 0.04	> 0.9
3	6	0.0074-0.0145	1.53 ± 0.12	< 0.001
4	6	0.0016-0.0038	3.66 ± 0.53	< 0.001
Ca				
1	5	0.106-0.129	unity	-
2	5	0.021-0.038	0.90 ± 0.07	~ 0.9
3	3	0.0044-0.0106	1.46 ± 0.49	~ 0.4
4	5	0.0009-0.0025	5.87 ± 1.26	~ 0.02

a Range of values

b Error given is the standard deviation of the mean

c Chance that ratio is unity, by t-test

initial residual plasma Ra^{226} has been removed. The wash loss, a' , can be calculated as the difference between the total Ra^{226} found in the washes plus that in the x_{n-1} grams of the last wash left in the cells and the Ra^{226} in the plasma left in the cell fraction after the first separation.

$$a' = \sum_{i=1}^{n-1} \left\{ c_i(\text{obs}) (W_i + x_{i-1} - x_i) \right\} + c_{n-1}(\text{obs}) x_{n-1} - c_p x_o \quad (8)$$

It can also be calculated as the sum of the difference between observed

and calculated concentrations of the washes times the appropriate weight.

$$a = \sum_{i=1}^{n-1} \left\{ (c_i(\text{obs}) - c_i(\text{calc})) (W_i + x_{i-1} - x_i) \right\} + (c_{n-1}(\text{obs}) - c_{n-1}(\text{calc})) x_{n-1} \quad (9)$$

These two methods of calculating the wash loss are equivalent.

We now have the information necessary to calculate the concentration of Ra^{226} in blood cells, c_c . It is equal to the amount of Ra^{226} in the washed cell fraction plus the wash loss, Eq. (9), minus that in the final wash left in the cells all divided by the cell weight, Eq. (2). Thus, we have

$$c_c = (c_n W_n + a - c_{n-1} x_{n-1}) / W_c \quad (10)$$

The mean values, with standard deviations and ranges of the following quantities are given in Table II: 1. The concentration of Ra^{226} in cells relative to that in plasma; 2. The relative cell concentration if wash loss is neglected, (c'_c / c_p); 3. The fractional mechanical loss of plasma; 4. The wash loss relative to the total Ra^{226} in cells, i.e. $a / c_c W_c$; and 5. The contribution of plasma left after the first separation to the total Ra^{226} in the washings, which is

$$b = \frac{c_p x_o}{\sum_{i=1}^{n-1} c_i(\text{obs}) (W_i + x_{i-1} - x_i) + c_{n-1}(\text{obs}) x_{n-1}} \quad (11)$$

The fractional plasma and cell weights, W_p / W and W_c / W , are given in Table II, and the average volume of packed red cells (VPRC) obtained 6 days prior is also included (6). A numerical example is given in Appendix I.

Table II
Concentration of Ra^{226} in Beagle Blood Cells
Relative to that in Plasma and Related Quantities at 5 Days

Quantity	Eqs.	Mean $\pm$ Standard Dev.	Range
c_c / c_p	10	0.205 ± 0.021	0.178-0.234
c_c' / c_p^a		0.193 ± 0.018	0.170-0.202
L	4	0.018 ± 0.003	0.014-0.022
$a / c_c W_c$	2, 9, 10	0.054 ± 0.031	0.003-0.094
b	11	0.956 ± 0.026	0.917-1.00
W_p / W	5	0.538 ± 0.015	0.522-0.563
W_c / W	2	0.461 ± 0.013	0.437-0.474
VPRC (Pre-Inj.)		51.7 ± 2.0	49.5-055.0
a Wash loss neglected			

A limited parallel study of calcium was also made, unfortunately as an afterthought. The ratios of observed to calculated concentrations of

calcium in the washes are also given in Table I. The Ra^{226} and Ca ratios were compared by the t-test and did not differ significantly, i.e. all p values > 0.1 . Just how meaningful this is is difficult to assess in view of the high standard errors for the calcium ratios. However, there is good correlation between Ra^{226} and Ca results for a given dog, e.g. the lowest values of the ratio for Ra^{226} and Ca were obtained on the same blood sample. No determinations of calcium in the cell fractions could be made with the method we were using because of interference by iron. The plasma and cell weight fractions, Eqs. (5) and (2), calculated from the calcium dilution data are $W_p / W = 0.525 \pm 0.014$ and $W_c / W = 0.480 \pm 0.012$ which compare well with the values in Table II obtained from the Ra^{226} data.

A very limited test of the effect of washing the cells with a foreign fluid, isotonic saline, was made by dividing a sample of cells into roughly equal portions and washing one with saline and one with plasma. If the two washing fluids are equivalent in their action, i.e. simply serve as diluting media and do not remove Ra^{226} from or add it to the cells by chemical effects, then $c_i(\text{obs}) / c_i(\text{calc})$ for the second and third washes and c_c should be the same for the saline and the plasma series. $c_2(\text{obs}) / c_2(\text{calc})$ was 1.1 ± 0.1 for both and $c_3(\text{obs}) / c_3(\text{calc})$ was 1.2 ± 0.25 for saline and 1.7 ± 0.35 for plasma. The large errors result from low count rates. The results do suggest that the use of saline does not vastly affect the determination of Ra^{226} in cells. Unfortunately, c_c was not determined.

The human blood cells were washed once with saline with the exception of the sample obtained at 0.96 days which was washed 4 times. The dilution factors and observed to calculated concentration ratios for the 0.96 day

sample are given in Table III. The errors shown are derived from the fractional standard deviations of the count rates. The count rates were quite

Table III

Comparison of Calculated and Observed Concentrations
in the Washings of Human Blood Cells

Wash	$c(\text{calc}) / c_p$	$c(\text{obs}) / c(\text{calc})$
1	0.269	unity
2	0.105	0.915 ± 0.083
3	0.0387	1.37 ± 0.20
4	0.0138	1.77 ± 0.76

low for the latter washes. The ratio of cell to plasma concentration and related quantities are given in Table IV. Since the whole blood was not

Table IV

Concentration of Ra^{226} in Human Blood Cells
Relative to that in Plasma at 0.96 Day

Quantity	Eqs.
$c_c / c_p = 0.154$	10
$c'_c / c_p = 0.142^a$	
L not determined	4
$a / c_c W_c = 0.080$	2, 9, 10
$b = 0.949$	11
$W_c / W = 0.437^b$	2

a Wash loss neglected

b Calculated assuming $L = 0$

weighed, L could not be determined. If $L = 0.02$, as in the case of the dogs, then W_c / W is about 2% high. Similarly, L was not determined for the samples washed once.

When the cells were washed once, sufficient information was obtained to calculate c_c and W_c . As shown in Fig. 1, c_c decreases more slowly than c_p . Hence, c_c / c_p increases with time over the period studied, as shown in Table V which also includes W_c / W derived from the dilution data. The standard deviations shown are derived from the standard deviations of the count rates.

Table V
Increase in Cell to Plasma Ra^{226} Concentration
Ratio with Time in a Human

Days	c_c / c_p	W_c / W^a
0.008 ^b	0.002 $\pm$ 0.004	0.462 $\pm$ 0.002
0.022 ^b	0.019 $\pm$ 0.003	0.472 $\pm$ 0.002
0.044 ^b	0.086 $\pm$ 0.009	0.470 $\pm$ 0.004
0.281	0.10 $\pm$ ----- ^c	----- ^c
0.960	0.154 $\pm$ 0.006	0.437 $\pm$ 0.006
1.97	0.202 $\pm$ 0.015	0.479 $\pm$ 0.013
2.97	0.205 $\pm$ 0.020	0.471 $\pm$ 0.017
5.13	0.293 $\pm$ 0.040	0.391 $\pm$ 0.025
8.10	0.63 $\pm$ 0.13	0.37 $\pm$ 0.06
19.2	1 ^d	

a Calculated assuming $L = 0$

b Delay in initial centrifugation

c Not determined, $W_c / W = 0.47$ used to correct c_p for significant hemolysis of this sample

d Calculated assuming $0.4 \leq W_c / W \leq 0.5$

Discussion

The sticky problem of determining Ra^{226} in blood cells can be successfully solved by applying a simple washing technique completely and carefully. The data of Table I demonstrate that the residual plasma in the cell fractions can be uniformly diluted with saline. Repeated washings result in some loss of Ra^{226} from the cells, but the loss can be measured and an appropriate correction applied. For 4 washings the average correction was only 5% (Table II). The fact that 96% of the Ra^{226} found in the washings could be accounted for by the amount of plasma left further supports the smallness of the wash loss. As noted above, the loss correlates with the concentration gradient between cells and washing fluid. Of the 6 dogs (Tables I & II) the smallest loss was observed when the gradient was the least.

The limited calcium data of Table I also suggest a diffusion of calcium out of cells when the concentration gradient is high. Kahn has reported the uptake of calcium by blood cells (7). When cells were washed with buffer solutions containing higher than plasma concentrations of calcium, calcium went into or onto the cells and also blocked the influx of potassium. He also demonstrated transfer of calcium between cells and plasma of "normal" calcium concentration by measuring the rate of incorporation of Ca^{45} in cells at 37° and 25° C. The rate studies showed two processes, and the second slower one was shown to be diffusion. His data, as are ours, are incomplete in that calcium in cells was not determined. Kahn cites a literature value of $c_p - c_c = 2.5 \text{ mM Ca} / \text{L plasma} - 1 \text{ mM Ca} / \text{L cells}$ for the concentration gradient. In the literature Ca values can be found ranging from $c_c / c_p = 0$ to 2 with c_c / c_p about 0.1 reported

frequently. Thus, our results and Kahn's suggest that both calcium and Ra^{226} move between cells and plasma at least in part by diffusion.

The accurate determination of the weight fractions can be used to determine trapped plasma in VPRC measurements. Dougherty and Parkinson (8) found that plasma specific gravity = 1.025 ± 0.001 and red cell specific gravity = 1.092 ± 0.004 for beagles of this colony. Using these results and the weight fractions from Table II we obtain for the mean volume fractions

$$V_c / V = 0.446 \quad (12)$$

$$V_p / V = 0.554$$

Although VPRC mean given in Table II was obtained 1 day prior to injection, we will use it and the first of Eqs. (12) to calculate the fraction of trapped plasma, which is $(0.517 - 0.446) / 0.517 = 0.137$. This result is obviously high and is presented only as an example of how Eqs. (12) could be used if we had measured the VPRC at the proper time.

The data of Table V illustrate two limitations of the method, both of which can be easily overcome. First, it was not possible to determine cell concentration by the single wash method in the first sample listed in Table V, but W_c / W could be determined accurately. The concentration of the plasma was so high that after just one dilution the correction $c_1 \times_1$ for the amount of plasma Ra^{226} left in the cell fraction was overwhelming. Had the cells been washed more times to achieve a greater dilution of the residual plasma Ra^{226} , c_c could have been determined.

The second limitation is illustrated by the late measurements when c_p is low and c_l is even lower and cannot be determined with the required accuracy. The correction term $c_l x_l$ is relatively small in this case and the greater problem is that the corrected weight W_c cannot be determined accurately. This problem could easily be solved by adding a radionuclide such as Sr^{85} (or even albumin tagged with I^{131}) to the system and determine W_c by dilution of the carefully chosen amount of Sr^{85} . Prior studies of the rate and degree of incorporation of Sr^{85} onto or into cells in vitro would obviously be necessary. (Use of I^{131} -albumin would require a precise determination of the weight of material added). Neither Sr^{85} or I^{131} would interfere in the analysis for Ra^{226} by de-emanation methods. Thus, c_c could be determined if W_c was accurately measured by Sr^{85} dilution and an estimate of $c_l x_l$ was obtained from a less accurate determination of Ra^{226} in the wash, c_l , and the accurate value of x_l from Sr^{85} dilution. This method would be well suited to low level measurements such as those made by Lucas, et al. (9).

In our studies c_l could not be measured for the 19 day sample and c_c / c_p was estimated by making the rather reasonable assumption that $0.4 \leq W_c / W \leq 0.5$.

Finally it should be noted that our comparison of plasma and saline washing fluids was rather limited and that in the case of repeated washings the saline could alter the cell surface. Also, we used heparinized blood but we have made no effort to assess the effect, if any, of heparin on the distribution of Ra^{226} in blood.

In summary, we have made a few measurements on a dynamic system. The conclusion to be drawn is that in the blood Ra^{226} is associated with both

plasma and cells, and the distribution between the two components depends on various factors. The principal goal of the above studies was solution of a technical problem, correcting for entrapment of plasma in the cell fraction. This led to the following observations related to the transfer mechanism. In the human study c_c was greater when c_p was high. In the dog studies the wash loss correlated with increases in concentration gradient. These results, at least superficially, are consistent with a diffusion mechanism. A definitive determination of the relative roles of diffusion and active transport in the transfer of Ra^{226} and calcium between plasma and cells would require the following:

- 1) In vitro studies of the rate and extent of incorporation at several temperatures.
- 2) Information on the difference in potential between cells and suspending medium (e.g. plasma, saline, physiological buffer solution) so that flows could be related to electrochemical activity gradients rather than concentration gradients.
- 3) Information on effect of heparin, (SO_4 groups bind Ra^{226} or $Ca?$), effect of saline or buffer solution on integrity of cell, effect of substituting for blood serum.

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Figure 1. Radium concentrations in plasma (c_p) and in red cells (c_c) in a 42 year old man at various days after injection. The fractional deviation is shown when in excess of 0.05.

Appendix I

Numerical Example. Blood from Dog T57R4
5 Days After Injection. 4 Saline Washes.

Data:

i	W_i	$W_i + x_{i-1} - x_i$	c_i (obs)	$\pm \sigma_{c_i}$
0	10.709		2.09	$\pm 0.036 (=c_p \pm \sigma_{c_p})$
1	14.816	12.914	0.320	± 0.0076
2	9.747	10.162	0.0940	± 0.0038
3	9.782	10.406	0.0332	± 0.0030
4	9.793	9.701	0.0128	± 0.0028
5	15.136		0.313	± 0.0042

$$W = 25.234$$

Calculations:

$$\text{Eq. (4)} \quad L = \frac{25.234 - (69.028 - 44.139)}{25.234} = 0.014$$

$$\begin{aligned} \text{From Eq. (6)} \quad x_0 &= c_1 W_1 / (c_p - c_1) = 0.320 \times 14.816 / (2.09 - 0.320) \\ &= 2.68 \end{aligned}$$

$$\text{Eqs. (1)} \quad W_1 + x_0 - x_1 = 12.914 = 14.816 + 2.68 - x_1, \quad x_1 = 4.58$$

$$W_2 + x_1 - x_2 = 10.162 = 9.747 + 4.58 - x_2, \quad x_2 = 4.16$$

$$W_3 + x_2 - x_3 = 10.406 = 9.782 + 4.16 - x_3, \quad x_3 = 3.44$$

$$W_4 + x_3 - x_4 = 9.701 = 9.793 + 3.44 - x_4, x_4 = 3.53$$

$$\text{Eq. (5)} \quad W_p = 10.709 + 2.68 + 0.34 = 13.73$$

$$\text{Eq. (2)} \quad W_c = 15.136 - 3.53 = 11.61$$

$$\text{Eq. (7)} \quad c_2(\text{calc}) = 0.320 \times 4.58 / (9.747 + 4.58) = 0.102$$

$$\text{Similarly } c_3(\text{calc}) = 0.0303 \text{ and } c_4(\text{calc}) = 0.0079$$

$$\text{Eq. (8)} \quad a' = 5.55 + 0.045 - 5.60 = 0.00$$

$$\text{Eq. (9)} \quad a = -0.080 + 0.030 + 0.048 + 0.017 = 0.015$$

$$\text{Eq. (10)} \quad c_c = (4.74 - 0.045 + 0.015) / 11.61 = 0.405$$

$$c_c / c_p = 0.405 / 2.09 = 0.194$$

$$a / c_c W_c = 0.015 / 4.71 = 0.003$$

$$\text{Eq. (11)} \quad b = 5.60 / (5.55 + 0.045) = 1.00$$

SR⁸⁹ THERAPY OF A CANINE OSTEOSARCOMA

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Jean H. Dougherty, and D. H. Taysum

Abstract: Sr⁸⁹ was used in the attempted treatment of a moderately calcified osteosarcoma in a beagle. Pre-treatment volume doubling time of the tumor was about 20 days. Six weekly injections were given the animal totaling 8.7 mc Sr⁸⁹. The growth of the tumor appeared to have been arrested temporarily beginning two weeks after the first injection until about six weeks after the final injection. Thereafter, the tumor enlarged with a volume doubling time of about 50 days for the remaining five weeks of the animal's life. A Sr⁸⁹ assay of selected tissues at death showed that the concentration was proportional to both the degree of calcification and to the rate of growth. The average radiation dose to the tumor was about 10,500 rads at death, whereas that to the skeleton was 1,000 rads. Regrowth of the tumor appeared to be most rapid in the lightly mineralized periphery where the Sr⁸⁹ content and local dose were lower.

Introduction

The regression of a malignant melanoma associated with its high uptake of injected Ra²²⁶ (1,2) suggested the treatment of an osteosarcoma with injections of radioactive isotope of an alkaline earth element. This same treatment was proposed previously by Pecher over 20 years ago (3). Unfortunately, Pecher died during World War II, and his studies were not continued by others. However, he did report encouraging preliminary results following the injection of Sr⁸⁹ into a man with what was termed "osteoblastic metastases of prostate carcinoma generalized to the skeleton". Quoting from the final paragraph of ref. (3), "Under a total dose of 8.0 millicuries of radiostrontium (September 1940, December 1940, and January 1941) the pain has almost disappeared. Roentgenologically, the progress of the bone tumors has been interrupted (Since January 1941, as

compared to April 1941). The serum phosphatase activity has dropped progressively from 42 to 6 in six months (Bodansky units, normal: 2 to 5 units), and the general condition of the patient is definitely improved."

A search of the literature failed to discover any more recent attempts to treat calcifying cancer with injected radioactive strontium. Considerable experience, however, has been gained using P^{32} in the treatment of metastatic carcinoma of the prostate (4). P^{32} has the disadvantage of depositing in soft tissue in addition to bone. Also, injected P^{32} delivers a relatively high dose to the bone marrow.

Alkaline earth elements (Ca, Sr, Ba, and Ra) selectively concentrate in areas of rapid mineral accretion, such as in those osteosarcomas which are heavily calcified. The outermost boundaries of such tumors are frequently only slightly calcified. If a nuclide which emits relatively high energy β -particles were used in this treatment, the lightly calcified, rapidly growing area at the tumor boundary might be irradiated by these long range particles originating from deposits of the nuclide in the more heavily calcified areas. Promising beta emitters are listed in Table I. From the standpoint of high average β -energy, cost and availability, Ca^{47} and Sr^{91} can be eliminated. Disadvantages of Sr^{90} include the permanent contamination of the total skeleton, even if the tumor were destroyed, because of its long physical half-period of 28 years. In addition, the injected Y^{90} would probably not concentrate in the rapidly calcifying tissue of an osteosarcoma to the same extent as Sr^{90} (8). The Y^{90} not taken up by the tumor would deliver a disproportionately high dose to bone marrow and other tissue. Sr^{89} and Ba^{140} appear to be the

TABLE I

PROSPECTIVE β -EMITTERS FOR OSTEOSARCOMA THERAPY

Element	Suitable Isotope	Half Period	Average β -Energy*	Cost per mc From Oak Ridge (7)
Calcium	Ca ⁴⁷	4.5 da	0.33	\$200.00
	Sc ⁴⁷ (dau)	3.4 da	0.16	
			0.25 Avg.	
Strontium	Sr ⁸⁹	50.4 da	0.55	\$ 5.00
	Sr ⁹⁰	28 yr.	0.20	\$ 0.10
	Y ⁹⁰ (dau.)	64 hr.	0.93	
			0.56 Avg.	
	Sr ⁹¹	9.7 hr.	0.54	Unavailable
	Y ⁹¹ (dau.)	59 da.	0.58	
			0.56 Avg.	
Barium	Ba ¹⁴⁰	13 da.	0.28	\$ 2.80
	La ¹⁴⁰ (dau.)	40 hr.	0.62	
			0.45 Avg.	
Radium	none	(no β -emitter without α -emitting daughters)		

*Calculated from Ref. 5 except for Sr⁹⁰ + Y⁹⁰, the value for which was taken from Ref. 6.

nuclides of choice. However, the very recently separated Sr fraction of fresh fission products would have most of its activity due to Sr⁹¹ and 3 hr Sr⁹². These would deliver a high dose to the tumor and other skeletal tissue in a relatively short time. Sr⁸⁹ and Sr⁹⁰ would also be present, but the activities would be less.

A more uniform radiation dose throughout the tumor volume could be obtained from a gamma-emitting isotope of an alkaline earth element. Such an emitter would be especially effective if its gamma-ray energy were in

TABLE II
PROSPECTIVE GAMMA-RAY EMITTERS FOR OSTEOSARCOMA THERAPY

Suitable Isotope	Half-Period	γ -Ray Energy kev, in order of decreasing abundance	Relative γ -ray abundance (8)	Cost per mc from Oak Ridge (7)
Sr ⁸⁵	64 da	513	100	\$ 50.00
Sr ⁹¹	9.7 hr	551	59	unavailable
		1025	30	
		748	27	
		645	15	
		1413	5	
Ba ¹³¹	12 da	93	3	\$ 60.00
		496	360	
		122	200	
		214	150	
		372	100	
		620	33	
		1030	11	
Ba ¹³³	7.2 yr	920	7	\$100.00
		820	2	
		360	74	
		81	32	
Ba ¹⁴⁰	13 da	292	26	\$ 2.80
		70	6	
La ¹⁴⁰	40 hr	537	?	
		30	?	
		1596	100	
		490	50	
		815	45	
		328	40	

the range 15-50 kev so that only a relatively small fraction would be absorbed outside the tumor (9). Sr⁸⁵, Ba¹³¹, Ba¹³³ and Ba¹⁴⁰ are the only suitable nuclides available (Table II). As can be seen from the table, however, the principal gamma energy of none of these nuclides is in the most desirable energy range. Perhaps the optimum treatment of bony tumors

with radioisotopes of alkaline earth elements would be a beta-gamma emitter such as Ba^{140} , or the combination of a beta emitter and a gamma emitter such as Sr^{89} and Ba^{131} .

The therapist strives to maximize the radiation dose delivered to the tumor and minimize the dose to the rest of the body. Marinelli and Goldschmidt (11) introduced the concept that for effective cancer therapy from an internal emitter, the concentration of the nuclide in tumor tissue should be at least 10 to 100 times the average concentration in healthy tissue. The rationale of this concept relates to the ratio of 10 to 1 between the radiation dose of about 5000 rads required to sterilize a typical cancer, and the human $\text{LD}_{50/30}$ total-body radiation dose of about 500 rads.

Materials and Methods

The presence of an osteosarcoma on the distal right femur was demonstrated radiographically in test dog T34R3, 1459 days after the injection of $1.12 \mu\text{C Ra}^{226}/\text{kg}$. The tumor appeared to be moderately calcified. Its volume at this time was less than 1 cm^3 , but had increased in size to about 30 cm^3 after another 118 days. A series of x-ray films made during the next 41 days established the volume doubling time of the tumor to be about 20 days. Tumor volumes were calculated from the effective diameter of the tumor measured on radiographs made at weekly intervals during the entire period of the experiment (Fig. 1).

Treatment consisted of one injection of Sr^{89} solution at weekly intervals for six weeks. The injection schedule and injected activities are summarized in Table III.

Sr⁸⁹ THERAPY FOR OSTEOSARCOMA OF DOG T34R3

1000 RADS IN 2 WEEKS

10,000 RADS IN 15 WEEKS

Figure 1. Radiographs of the right femur of T34R3 (a) soon after the start of Sr⁸⁹ therapy and (b) shortly before death.

TABLE III
T34R3 Sr⁸⁹ INJECTION SCHEDULE

Days After Ra ²²⁶ Injection	ml Sr ⁸⁹ Solution Injected	Injection Site	Injected μ c Sr ⁸⁹	μ c Sr ⁹⁰ in Injection Solution
1618	5 (Solution A)	Right Femoral Artery (Tumor Leg).	1570	132
1625	5 (Solution A)	Right Femoral Artery	1426	132
1632	5 (Solution A)	Right Cephalic Vein (Foreleg)	1295	132
1639	10 (Solution A)	Right Femoral Artery	2352	264
1646	5 (Solution B)	Right Femoral Artery	1068	32
1653	5 (Solution B)	Right Femoral Artery	970	32

A total body count was made before and after each injection and at frequent intervals during the remainder of the animal's life. Both the dog and the 8 x 4 inch NaI (Tl) crystal were shielded from background during counting by six inches of steel. The crystal was further shielded by two inches of Pb below, above, and on three sides. The anesthetized animal was placed for counting inside a plastic box of wall thickness 0.5 cm which positioned his midline in an arc 33 cm from the face of the crystal. This counting arrangement is discussed in reference 12. The right hind (tumor) leg was nearer the detector while in this position.

The bremsstrahlung spectrum of Sr⁸⁹ in the dog was superposed upon the radon-daughters' γ -ray spectrum due to the previously injected Ra²²⁶.

Each time the animal was counted, one count was made with the right hind (tumor) leg covered with 2 inches of Pb. The Bi^{214} (RaC) γ -ray peak at 1.76 Mev was used to indicate the fraction of the total skeleton which was covered by Pb. The Sr^{89} in the total skeleton could then be determined by dividing the Sr^{89} activity in the unshielded portion of the skeleton by (Bi^{214} in unshielded portion/ Bi^{214} in total dog). Thus the Sr^{89} in the tumor could be readily calculated (Table IV).

Total excreta collections were made for the first nine weeks following the initial injection. Comparison was made of the computed Sr^{89} retention in the dog by bremsstrahlung counting of the excreta and the retention measured by total-body bremsstrahlung counting. The results obtained by these two methods were consistent, assuming a bremsstrahlung efficiency (relative x-ray photon output per μc) of 1.30 in the skeleton, 1.15 in the tumor, and 1.00 in the Sr^{89} injection duplicate dissolved in a waterfilled, one-gallon plastic bottle.

Average radiation doses to the tumor volume and the total skeleton (Table V) were calculated using: the tumor volumes obtained from the weekly radiographs and 1.2 gm/cm^3 as the average density of the tumor; 1570 grams as the weight of the skeleton (one-tenth of the total-body weight of the animal at 470 days of age when the Ra^{226} injection was given); 0.55 Mev as the average β -particle energy for Sr^{89} (calculated from Ref. 5); 1.13 Mev as the total β -energy per Sr^{90} - Y^{90} - Zr^{90} disintegration chain for the $\text{Sr}^{90} + \text{Y}^{90}$ contamination in the Sr^{89} solution (6); 50.4 days as the physical half-period of Sr^{89} (13); and skeletal retention and tumor retention values measured by total-body counting.

When the resumption of tumor growth became apparent, the decision was made to attempt the surgical removal of the periphery of the tumor in an effort to determine whether the heavily irradiated interior was still actively proliferating. It was thought that this measure would prolong the animal's life. Therefore, at about thirteen weeks after the initial Sr^{89} injection, a large fraction (about $2/3$ of the volume) of the tumor was removed. Death occurred one day after surgery. A total-body count was made on the animal before and after the operation. All of the tumor tissue which was removed was also counted. This intercomparison provided a check on both the total activity and the fraction of retained Sr^{89} in the tumor. The values obtained by counting the intact animal were verified by this procedure.

Tumor samples of varying degrees of calcification and radiographically determined varying growth rates were evaluated for their Sr^{89} activity. The Ca content of each sample was determined by ashing, using 0.39 as the Ca fraction of the ashed weight (14).

Results and Discussion

Table IV summarizes the total-body counting results. It can be seen from the final column of this table that an average of more than one-half the retained Sr^{89} was deposited in the tumor. Calculated radiation doses to the tumor and the total skeleton are shown in Table V. The tumor consistently received more than 10 times greater an average dose than did the total skeleton.

The marked hematological changes produced by the general skeletal irradiation are demonstrated in Figure 2. The white cells and platelets in particular exhibited the most serious decrease.

Figure 2. Hematological response of T34R3 to Sr^{89} therapy.

TABLE IV

SUMMARY OF T34R3 TOTAL-BODY COUNTING

(All data reported refer to Sr^{89} injected at least one week prior to counting)

Days after Ra^{226} Injection	Fraction of one 5 ml Sr^{89} injec- tion in skeleton	Fraction of one 5 ml Sr^{89} injec- tion in tumor	Per cent of retained Sr^{89} in tumor
1625	0.135	0.128	48.6
1632	0.286	0.340	54.3
1639	0.365	0.490	57.3
1646	0.647	0.749	53.6
1653	0.645	0.866	57.3
1660	0.742	0.860	53.7
1667	0.631	0.770	55.0
1674	0.623	0.669	51.8
1690	0.542	0.698	56.3
1693	0.476	0.668	58.4
1737	0.464	0.664	58.9
1739 (After surgery)	0.464	0.275	37.2

Table V should be compared with Figure 3, a plot of the change in tumor volume with time. That the striking change in the tumor growth rate which occurred following the initiation of Sr^{89} therapy was produced by the treatment tends to be substantiated by a comparison of the enlargement rates of similar tumors. Most osteosarcomas in other beagles which we have studied have tended to maintain constant doubling times throughout the entire period of observation (Fig 4).

Figure 3. Changes in the volume of the tumor on the distal right femur of T34R3 showing the inhibition of growth rate following Sr⁸⁹ therapy.

TABLE V
CALCULATED RADIATION DOSE TO T34R3 AS A RESULT OF Sr^{89} THERAPY

Days After Ra^{226} Injection	Cumulative Radiation Dose From Sr^{89} and $\text{Sr}^{90} + \text{Y}^{90}$		
	Rads to Tumor	Rads to Skeleton	Tumor Skeleton
1625	324	30	10.8
1632	1022	89	11.5
1639	1892	157	12.0
1646	3063	270	11.4
1653	4227	372	11.4
1660	5282	478	11.0
1667	6132	561	10.9
1674	6799	637	10.7
1681	7423	702	10.6
1688	8003	759	10.5
1695	8521	812	10.5
1702	8984	859	10.5
1710	9392	901	10.4
1717	9736	940	10.4
1724	10039	976	10.3
1731	10301	1009	10.2
1738	10525	1040	10.1

Table VI and Table VII indicate that the deposition of Sr^{89} was affected by both the degree of calcification and the growth rate of calcified tissues. By the time of death, the Sr^{89} concentration in the tumor had been diluted by its growth following Sr^{89} therapy.

Figure 4. A comparison of the growth rates of osteosarcomas in several other beagles with the one in T34R3.

TABLE VI
Sr⁸⁹ Concentration in T34R3
Individual Bones at Death

Bone	Per cent one 5 ml Sr ⁸⁹ dose/kg*	$\mu\text{C Rn}$ kg	Sr ⁸⁹ * Rn
R. Femur (with about 1/3 of tumor volume)	123.1	0.32	384.7
L. Femur	32.5	0.68	47.8
R. Tibia+Fibula	27.9	0.69	40.4
L. Tibia+Fibula	21.9	0.80	27.4
R. Humerus	26.3	0.71	37.0
L. Humerus	32.8	0.73	44.9
3rd Lumbar Vertebra	40.0	0.62	64.5
Average of above 6 Normal Bones	30.2	0.70	43.1

*Assuming a relative bremsstrahlung efficiency of 1.30.

TABLE VII
Sr⁸⁹ Concentration and Ca
Content of Some Selected Tissue of T34R3 at Death

Sample	Ca content % wet weight	Per cent of one 5 ml Sr ⁸⁹ injection/kg**
R. Patella	12.7	75
Very Bony Tumor	6.1	247
Moderately Calcified Tumor	4.0	174
Old, Slowly Growing Tumor Bud	3.1	45
Cartillagenous Tumor	2.3	87
Lung Metastases (Each about 1 mm in diameter)	1.3	7
New, Rapidly Growing Tumor Bud	0.7	3
Liver Metastasis (About 1 cm in diameter)	0.4	0
Normal Lung Tissue	0.3	0
Tumor Removed at Surgery	-	124
Tumor Remaining with Femur	-	237
Total Tumor	-	141

**Assuming a relative bremsstrahlung efficiency of 1.15.

The greater uptake of Sr^{89} by an osteosarcoma as compared with normal bone was clearly demonstrated by this experiment. A marked decrease in the growth rate of this tumor occurred. The effects of the Sr^{89} on the tumor were only temporary, but the animal's life was lengthened by about 100 days. Although amputation continues to be the treatment of choice for such a tumor appearing on a limb, radiation therapy using an internal emitter such as Sr^{89} may prove useful for calcified tumors in anatomical locations where surgery is not feasible. It would be especially useful if a practical method of preventing or decreasing the deposition of activity in healthy skeletal tissue would be employed. The experimental results suggest that the failure to achieve a cure was primarily due to the fact that some portions of the tumor received much less than the average tumor dose. Stanford Cade claimed that osteosarcomas can be cured by uniform radiation doses of 8,000 to 10,000 rads (15). Successful treatments have been reported by others using radiation doses in the same range (16, 17).

Closed-circuit perfusion presents another method of extending this treatment. The deposition of a radionuclide from the blood is proportional to the average plasma concentration in the zone of deposition (2); therefore, an alternative to the administration of a greater amount of activity for increased deposition would be to extend the period of time during which the plasma concentration is relatively high. This can be accomplished by circulating radionuclide-labelled blood through a body member isolated from the general circulation using a heart-lung machine. For parts of the body, such as the limbs, which are readily adaptable to closed circuit perfusion, amputation can also

be performed. However, the lung appears to be an organ which might be treated by this method. A "lung" machine could be used in conjunction with the patient's heart for the general circulation, and a "heart" machine could circulate Sr^{89} labelled blood through the lung. Since mineralized metastases from bony tumors are not uncommon in the lung, such a treatment might be used (following the removal of the primary tumor) to destroy these metastases. Non-radioactive blood could be used to flush out the readily exchangeable Sr^{89} before the lung is re-connected to the general circulation. However, as was seen in this animal, metastases may also occur outside the lung.

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Sr⁹⁰ + Y⁹⁰ BREMSSTRAHLUNG EFFICIENCIES
IN MAMMALS OF VARIOUS SIZES

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

Abstract: Measurements of total-body bremsstrahlung efficiency (x-ray photon output/ μ c relative to that in water) have been made in the intact mouse, rat, rabbit and dog sacrificed two weeks after the injection of Sr⁹⁰ + Sr⁸⁵. Efficiencies were determined by a comparison of the bremsstrahlung output from Sr⁹⁰ + Y⁹⁰ and the gamma-ray emission of Sr⁸⁵. Results were checked by a β -assay of the ashed animals. Bremsstrahlung efficiencies averaged 1.10 in a 0.04 kg mouse, 1.14 in a 0.13 kg rat, 1.23 in a 2.6 kg rabbit, and 1.32 in an 8.5 kg beagle. Extrapolating to a 70 kg human, a relative bremsstrahlung efficiency of about 1.4 is predicted.

Introduction

Sr⁹⁰ can be detected in the skeletons of animals including humans by the bremsstrahlung x-rays which are associated with the β -decay of Sr⁹⁰ and its daughter Y⁹⁰ (1,2,3,4). Of interest to the investigator is the quantitation of body burdens in accident cases, industrial exposures, experimental patients, and laboratory animals. Accurate measurement of Sr⁹⁰ can be useful in the management of accidental exposures so that the decision of whether or how to treat them can be intelligently made (5,6,7,8). Metabolic data which is obtained by the external counting of Sr⁹⁰ in experimental subjects and animals can only be as accurate as the total-body counting results.

In addition to the factors of counting geometry and sample self-absorption which must be considered in total-body gamma-ray counting, the investigator doing total-body bremsstrahlung counting is confronted with the difficulty of determining the relative x-ray photon output per μ c Sr⁹⁰ from sample to sample. This variable, which is known as the bremsstrahlung efficiency, must be introduced as a consequence of the way in which brems-

strahlung is produced in conjunction with β -particle emission.

The total bremsstrahlung output of a $\text{Sr}^{90} + \text{Y}^{90}$ source is composed of x-ray photons produced as internal bremsstrahlung and external bremsstrahlung. Photons which appear when β -particles are emitted from a nucleus are known as internal bremsstrahlung. The number of photons produced in this way from a $\text{Sr}^{90} + \text{Y}^{90}$ source of given activity is a constant, since the intensity is determined by the atomic number of the emitter. When the emitted β -particles are stopped in a thick absorber, external bremsstrahlung can be produced. The photon intensity resulting from the absorption of emitted β -particles emerging from a $\text{Sr}^{90} + \text{Y}^{90}$ source is directly proportional to the atomic number of the absorbing material (9). Therefore, the medium by which the $\text{Sr}^{90} + \text{Y}^{90}$ is surrounded, influences the total bremsstrahlung output of the source. The higher atomic number of the atoms in bone increases the photon output/ $\mu\text{c Sr}^{90}$ deposited in the skeleton as compared with that in water or another medium of lower atomic number.

It has been shown (10) that the bremsstrahlung efficiency of individual beagle bones labelled with Sr^{90} increases with increasing effective atomic number (Figure 1). However, the fraction of the total emitted β -particle energy which is absorbed in bone can vary for Sr^{90} -labelled skeletons which differ greatly in size (11). The total-body bremsstrahlung output can therefore be influenced by still another factor, bone diameter, at least for relatively small skeletons.

Materials and Methods

In order to determine experimentally the effect of skeletal size on total bremsstrahlung output, bremsstrahlung efficiencies were measured in a series of mammals differing in body weight by nearly three orders of

Figure 1. The total bremsstrahlung output of $\text{Sr}^{90} + \text{Y}^{90}$ in bone increases with the increasing atomic number of the intact bone.

magnitude. Species represented were the mouse, rat, rabbit, and dog. Adult animals were injected with a solution containing Sr^{90} and Sr^{85} two weeks prior to their sacrifice (Table I). Carcasses were preserved by

Table I

Table of Injected Activities

Animal	Days of Age At Injection	Body Weight kg.	Injected $\mu\text{c } \text{Sr}^{90}$	Injected $\mu\text{c } \text{Sr}^{85}$
Mouse	151	0.038	26	0.05
Rat	83	0.130	26	0.05
Rabbit	570	2.600	247	0.474
Beagle	440	8.540	247	0.474

freezing for an additional two weeks until the Y^{90} and Sr^{90} activities were equal. The photon spectrum of each animal between 50 and 1000 kev was then compared to that from a duplicate of its injected dose in an appropriate-sized water-filled polyethylene phantom. An 8 x 4 inch NaI(Tl) crystal and a 400 channel pulse-height analyser were used for these measurements.

The gamma-ray emission rate of Sr^{85} at 513 kev is independent of its environment, while the photon emission rate at all energies up to 2240 kev of $\text{Sr}^{90} + \text{Y}^{90}$ is influenced by the atomic number of the material in which it is deposited. Therefore, the bremsstrahlung efficiency of $\text{Sr}^{90} + \text{Y}^{90}$ in each animal could be obtained by comparing the Sr^{85} gamma-ray output and the bremsstrahlung output in the animal and in its injection duplicate. The apparent $\text{Sr}^{90}/\text{Sr}^{85}$ ratio in the animal divided by the apparent $\text{Sr}^{90}/\text{Sr}^{85}$ ratio in the water-filled phantom yielded the bremsstrahlung efficiency in the animal relative to that in water. Figures 2, 3, and 4 show examples of

Figure 2. Bremsstrahlung x-ray spectrum of an aqueous solution of $\text{Sr}^{90} + \text{Y}^{90}$ as detected by the 8 x 4 inch NaI crystal used in these studies.

- 175 -

Figure 3. Gamma-ray spectrum of Sr^{85} .

Figure 4. The photon spectrum of the $Sr^{85} + Sr^{90} + Y^{90}$ injection solution used in the studies. Indicated are the portions of the spectrum employed in the calculation of the Sr^{85} γ -ray contribution to the total counting rate at all energies.

the photon spectra of Sr^{90} , Sr^{85} , and the $\text{Sr}^{90} + \text{Sr}^{85}$ injection solution.

The distribution of Sr^{90} in the skeleton of each animal was assumed to be duplicated by the Sr^{85} . Counting geometry was therefore identical for the two isotopes. Photon absorption in the animal and in the other materials between it and the detector would be the same for the 513 kev gamma-ray of Sr^{85} and the bremsstrahlung photons of similar energy. The corrected counting rate for each isotope relative to the injection duplicate was obtained by a two-channel analysis using the Sr^{85} peak (band A) and a portion of the bremsstrahlung spectrum on each side of it (band B) as shown in Figure 4.* Two additional standards for each animal identical in configuration to the injection duplicate were necessary. One contained only $\text{Sr}^{90} + \text{Y}^{90}$ and the other contained only Sr^{85} . Once the Sr^{85} contribution to the total photon output of the sample at all energies had been established by the method of simultaneous equations, it could be subtracted from the total. The residual counts were due to the $\text{Sr}^{90} + \text{Y}^{90}$ bremsstrahlung between 50 and 1000 kev.

Some difficulty was encountered with the natural and fallout activity in the larger animals, especially the rabbit. The gamma-ray peak at about 750 kev due to $\text{Zr}^{95} + \text{Nb}^{95}$, the peak at 662 kev from $\text{Cs}^{137} + \text{Ba}^{137\text{m}}$, and that at about 500 kev due to $\text{Ru}^{103} + \text{Rh}^{103}$ and $\text{Ru}^{106} + \text{Rh}^{106}$ interfered with the accurate estimation of the bremsstrahlung/ Sr^{85} ratio in the animals. One solution to this difficulty would be to inject more strontium

*The method of determining the activities of each of a mixture of emitters by the solution of simultaneous equations is discussed in detail in reference 1.

activity and to increase the administered Sr^{85} in relation to the Sr^{90} . This procedure is recommended to an investigator who wishes to employ this technique in the future. However, after the animals in the study had been sacrificed, the optimum time to correct the difficulty by this method was past. Corrections were therefore used.

The total-body spectrum of the beagle and that of the rabbit had been recorded before strontium injection. The delay of about one month until counting for the experiment was done constituted a substantial fraction of one half-period for both Ru^{103} and Zr^{95} . Therefore, the in vivo activities of these two nuclides were different from those in the sacrificed animals. Also, an unforeseen complication was that the rabbit's diet during the three weeks between pre-injection counting and sacrifice had a much different $\text{Cs}^{137}/\text{K}^{40}$ ratio than the diet which it had been fed by the supplier before it was obtained for the experiment. In addition to the changed $\text{Cs}^{137}/\text{K}^{40}$ ratio in the flesh, the considerable amount of food material remaining in the GI tract at death provided another source of difficulty. Because of these factors, a correction based on the pre-injection count of the rabbit was not strictly appropriate. The best determination of the apparent $\text{Sr}^{90}/\text{Sr}^{85}$ ratio in the rabbit skeleton was made on the eviscerated, skinned, and defleshed animal submerged in distilled water in a one-liter capacity polyethylene bottle.

As a check on the accuracy of the method of determining bremsstrahlung efficiency using Sr^{85} , the rat, mouse, and rabbit were ashed and dissolved in nitric acid. The resulting solutions were assayed for Sr^{90} by β -counting. The apparent $\mu\text{c Sr}^{90}$ determined in each animal by bremsstrahlung counting against a water-filled Sr^{90} standard was divided by the $\mu\text{c Sr}^{90}$ obtained

from the β -assay. The quotient in each case was the bremsstrahlung efficiency in the animal relative to that in water. These values were compared to those obtained using the bremsstrahlung/gamma-ray ratio.

The dog was not ashed. However, a beagle which lived about the same number of days after Sr^{90} injection had been ashed for a previous study, and the bremsstrahlung efficiency had been determined bone by bone (10). The weighted average of the values for this animal was used as a check on the value obtained for the beagle in the present experiment.

Results and Discussion

The measured bremsstrahlung efficiencies are presented in Table II

Table II

Measured Bremsstrahlung Efficiencies

Animal	Percent Retention Of Injected Strontium	Relative Bremsstrahlung Efficiency		Average Bremsstrahlung Efficiency
		Determined by $\left(\frac{\text{Sr}^{90} \text{ Brems.}}{\text{Sr}^{90}-\beta}\right)$	Determined by $\left(\frac{\text{Sr}^{90} \text{ Brems.}}{\text{Sr}^{85} \text{ gamma}}\right)$	
Mouse	16%	1.10	1.10	1.10
Rat	16%	1.18	1.11	1.14
Rabbit	4%	1.19	1.26	1.23
Beagle	36%	(1.33)*	1.32	1.32

* Determined from data given in Reference 10.

and in Figure 5. It can be seen that the efficiencies tend to increase with increasing body weight.

The data in Table III are based on the measured escape of $\text{Sr}^{90} + \text{Y}^{90}$ β -particle energy from the defleshed bones of mice, rats, rabbits, and

Figure 5. Relative bremsstrahlung efficiencies determined in this experiment and extrapolated to the human.

beagles and on the calculated escape from intact skeletons. The values listed for the calculated efficiencies were computed from the fraction of β -energy absorbed in the skeleton normalizing to a bremsstrahlung efficiency of 1.32 for a beagle (Figure 5). Figure 6 is a plot of the computed and observed bremsstrahlung efficiencies vs. body weights for the four species

Table III

Theoretical Bremsstrahlung Efficiency
Calculated from Skeletal Absorption of Y^{90} β -Energy
(see Parmley, et al., in Some Aspects of Internal Irradiation, 1962)

Animal	Body Wt. (kg)	Skeletal Absorption of Y^{90} β -Energy	Calculated Bremss. Efficiency	Observed Bremss. Efficiency
Mouse	0.038	26%	1.11	1.10
Rat	0.130	35%	1.15	1.14
Rabbit	2.600	63%	1.27	1.23
Dog	8.540	75%	1.32*	1.32
Human	70.	87%	1.37	1.40**

* Normalized to observed efficiency in dog.

** Extrapolated from observed animal data.

involved in this study. Comparison of the observed and theoretical efficiencies (Table III and Figure 6) indicates that they are in reasonably good agreement. Extrapolation to a 70 kg human by both methods indicates that a relative bremsstrahlung efficiency of about 1.4 is to be expected.

The bremsstrahlung efficiencies reported herein were determined in animals which had been sacrificed soon after Sr^{90} injection. Figure 7 indicates that the efficiency increases with increasing time after injection.

Figure 6. A comparison of the calculated bremsstrahlung efficiencies of Sr^{90} + Y^{90} in various mammals with their observed values.

BREMSSTRAHLUNG EFFICIENCY OF $\text{Sr}^{90} + \text{Y}^{90}$ IN BONE

Figure 7. These data suggest that the relative bremsstrahlung efficiency of a bone increases with increasing time after Sr^{90} injection.

This has been substantiated by data of Goldman and Della Rosa, University of California at Davis, (12) for whole beagle skeletons. Such an effect might be expected as a consequence of the process of bone growth. As bone remodeling proceeds, strontium is removed from areas of most rapid turnover such as trabecular bone (where injected strontium is initially deposited in a relatively high concentration) faster than from cortical bone where the initial concentration is lower. Strontium concentration may become highest in dense cortical bone as time after Sr^{90} injection increases. The effective atomic number of this bone is higher than that of trabecular bone, and the photon output/ μc Sr^{90} would therefore be increased.

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HUMAN TOTAL BODY COUNTER CALIBRATION*

R. D. Lloyd

Introduction

The response of a detector used as a total-body counter to the photon radiation emitted by the human body may be calibrated by several methods. Two of these methods have been defined as "rigorous" (1).

The first of these is to administer a known amount of the emitter of interest to a human subject. After a suitable time interval the subject is counted. In this way the detector response per unit activity for that nuclide distributed in the human body for the particular counting arrangement can be established.

The second rigorous method requires two total-body counts of a subject separated by an interval during which a sizable fraction of the body burden is excreted and for whom the intake during this time is negligible or known. The excreted activity is accurately determined, and the observed difference between the two decay corrected total-body counts represents the response due to the activity excreted. This method is of great usefulness in accident cases where the initial burden of an emitter is unknown and for a subject in whom the distribution of the nuclide within the body does not greatly change between the two counts.

Eisenbud (2) used a third method of calibrating a human counter for thyroid I¹³¹ which can also be described as rigorous. Cadavers were

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counted intact. The thyroids were then removed and counted separately using a more favorable standard geometry.

Calibration of the human total-body counter at this laboratory for K^{40} and Cs^{137} using healthy volunteers was accomplished using the first method described. The response of this counter to thyroid I^{131} was also calibrated, but the method of Eisenbud was not used, since the volunteers wished to remain both healthy and intact.

Calibration for Cs^{137} using Cs^{132}

As if for the exclusive convenience of total-body counter calibrators concerned with Cs^{137} , nature has provided another isotope of cesium which has a gamma-ray energy nearly identical to Cs^{137} insuring equal gamma-ray self absorption, a short (6.5 day) physical half-period, and a high gamma-ray abundance. This allows small activities to be administered to subjects for calibration purposes with an associated radiation exposure which is low (less than 2 mrem/ μ c compared to a maximum permissible weekly dose of 100 mrem for radiation workers). It has been assumed that Cs^{132} and Cs^{137} are metabolized in virtually an identical fashion. Therefore, these two isotopes would be similarly distributed in the body.

Cs^{132} was used to calibrate the total-body counter for Cs^{137} during January, 1963. The counting arrangement for humans (Figure 1) consisted of an 8 x 4 inch NaI(Tl) crystal suspended over the subject who from head to knees was bent in an approximation of an arc. The distance from both the seat and back rest of the subject positioner to the center of the crystal face was 50 cm. Subject and detector were housed in a steel chamber of wall thickness six inches. Data were recorded by a 400 channel pulse-height analyser.

Ten healthy adult volunteers (6 males and 4 females) of different but representative body builds were used in the Cs^{132} study. Fasted subjects were given $0.1 \mu\text{C}$ Cs^{132} Cl orally. After a delay of about two hours and prior to subsequent excretion, each subject was counted against an ingestion duplicate in a 10 ml glass ampoule housed in an 8 cm diameter x 11 cm long lucite cylinder. This cylinder was placed in the 9 cm diameter axial tunnel of a "human phantom" consisting of a polyethylene cylinder 21 cm in diameter x 40 cm long. The human phantom was positioned for counting along the back rest of the human positioner in the place which would have been occupied by a subject's torso. The center of the ampoule was 20 cm above the seat. Twenty minute counting times were used. The background subtracted by the analyser consisted of a 20 minute pre- Cs^{132} ingestion gross count which had been made on the subject and stored on magnetic tape.

In order to determine the change in counting rate as the ingested Cs^{132} became distributed throughout the body, two of the subjects were counted immediately after ingestion. Counting was repeated at suitable intervals out to more than an additional 50 hours (Fig. 2). Total excreta collections were made, and the excreta were assayed for Cs^{132} . The ratio of the subject's count rate to that of the ingestion duplicate in the human phantom was corrected for excretion and automatically for radioactive decay, as the Cs^{132} in the subject and standard decayed at identical rates. Only a tiny fraction (less than 2%) of the ingested Cs^{132} was excreted during the 50 hour interval. The relative counting rates of both subjects decreased rapidly during the period immediately after ingestion. By two hours post ingestion the change in counting rate with time had become much less rapid. The average equilibrium ratio for the two subjects followed out to

50 hours was 0.89, and the 2 hour relative counting rates of all the other subjects were corrected to an assumed equilibrium by using this ratio.

It was suspected that the chief difference in counting rate per μC Cs^{132} in different subjects would be due to the differences in gamma-ray self absorption for thick and thin persons. Subject height was believed to exert a secondary influence. Therefore, the "effective diameter" of each subject was computed assuming that the human body consists of a cylinder of unit density. The weight in grams (assumed to equal the volume in cm^3) was divided by the height in cm. The diameter of the circle having the resulting area was then computed and this value used as the effective diameter of the subject. Table I lists these data for the ten calibration subjects.

A plot was made of the ratio of the counting rate in the subjects to that of an equal activity in the human phantom, versus the computed effective diameters (Fig. 3). The resulting linear function could then be used to calculate the relative subject-to-standard response for persons of effective diameters between these limits. It is of course assumed that this relationship is also valid for Cs^{137} , since the Cs^{137} standard used in routine counting is similar in size and shape to the Cs^{132} ingestion duplicate, and it is counted inside the human phantom in an identical fashion.

Calibration for K^{40} using K^{42}

The gamma-ray energy of K^{42} is close to that of K^{40} . As with Cs^{132} , the short half-period of K^{42} (12.4 hr.) allows a moderate activity to be administered to a human subject with a resulting radiation exposure which is reasonably low (less than 1 mrem/ μC).

Calibration of the total-body counter for K^{40} using K^{42} was accomplished using techniques identical to those described in the preceding section where possible. An unavoidable difference was that subject RP (see Table I) was not available at this time (October 1963), and another volunteer BC of approximately the same body build had to be substituted. Since the K^{40} standard (508 g dry KCl) used in routine human counting is contained in a cylinder 9 cm in diameter and 17.5 cm long, the K^{42} ingestion duplicate was

Table I
Body Configuration of Subjects
Used in
 Cs^{132} and K^{42} Calibration

Subject	Sex	Height, cm	For Cs^{132} Study		For K^{42} Study	
			Weight, kg	Effective Diameter, cm	Weight, kg	Effective Diameter, cm
TE	F	163	45.0	18.8	45.4	18.9
DT	M	184	57.3	20.0	53.6	19.3
RM	F	168	62.3	21.7	59.1	21.3
RL	M	170	67.7	22.6	65.9	22.3
AR	F	160	65.9	22.9	65.9	22.9
CM	M	178	78.6	23.8	77.3	23.5
PW	M	184	84.1	24.2	82.3	23.8
DB	F	161	76.4	24.6	78.2	24.9
DA	M	174	91.8	26.0	92.7	26.0
BC	M	187	-	-	102.3	26.4
RP	M	176	104.1	27.5	-	-

distributed in a plastic bottle of similar dimensions containing distilled water. This bottle could then be centered in the human phantom for counting so that the geometry would be identical to the K^{40} standard. Ingestion

doses for this study consisted of $1 \mu\text{C K}^{42}$. It was found that the one day post-ingestion count corrected for radioactive decay and excretion was virtually the same as the 8 hour post ingestion count, similarly corrected, in the two subjects studied for the extended period. However, the corrected two hour post-ingestion count had a somewhat greater relative value (Fig. 4). Therefore, all the two hour counts were multiplied by 0.93, the average equilibrium
2 hour ratio for the two subjects followed.

Fig. 5 is a plot of the results of this study. Effective diameters were computed as previously described. The observation that the average subject to standard ratio is much nearer to 1.0 in Fig. 5 than in Fig. 3 is probably explained by the relative standard-to-detector distances involved. A substantial fraction of the K^{42} standard was several cm closer to the crystal than the center of the human phantom where the "point" Cs^{132} standard was located. Since the relative count rates of point sources at different distances from a point detector vary inversely as the squares of the distances, such as effect might be expected. The gamma-ray energies of Cs^{132} and K^{42} differ by more than a factor of 2. Therefore the self-absorption K^{42} γ -radiation in the subjects and the phantom would be less than that of Cs^{132} .

The linear function graphed in Fig. 5 was assumed to be valid for K^{40} as well as for K^{42} .

Calibration for I^{131}

Unlike the procedures for Cs^{132} and K^{42} previously described, direct administration of I^{131} was not attempted for calibration purposes. During the summer of 1962, testing of nuclear devices at the Nevada Test Site resulted in the deposition of fresh fission products over the state of Utah.

I^{131} was subsequently discovered in the state's milk supply (3,4). A total of 23 Utah residents were counted at this time to determine their thyroid I^{131} burdens. The detector was the 8 x 4 inch NaI crystal surrounded by 2 inches of Pb except for a 4 x 4 inch opening. Each subject's neck was positioned for counting over this opening with the thyroid gland centered (4). The standard used for these studies was an Abbot I^{131} Radiocap in the thyroid position of a sugar-filled phantom. After an interval of about two weeks, a total of 53 subjects were total-body counted. Ten of these individuals had also been thyroid counted. The same I^{131} standard was used as for thyroid counting. It was placed at the thyroid position corresponding to that of each individual, as the bulk of total-body I^{131} is located in the thyroid (5).

The I^{131} intake by four of the people who were both thyroid and total-body counted before the first count and between the two counts could be determined with some accuracy. The I^{131} concentration in their milk supply was measured several times during the time that I^{131} was found in the milk, and their daily milk consumption was recorded.

The percent I^{131} retention was computed for each of these individuals for the two counts (4). Assuming that their relative iodine uptakes did not greatly change during this period, the two values should have been equal. The averages for these persons for total body measurement of I^{131} uptake did in fact equal the average I^{131} uptake measured by thyroid counting. It was therefore concluded that thyroid I^{131} burdens as measured by the total-body counter could be directly compared to those determined by thyroid counting.

I^{131} uptakes reported by Wellman of the U.S.P.H.S. for the ten individuals in his study averaged about 15% (6). The counting facility utilized was that of Eisenbud at New York University. A value of 20% is obtained by integrating over constant continuous exposure Lushbaugh's single-intake I^{131} retention equation determined in 26 normal subjects (7). Comar et al. found a 24 hour uptake of 20% in eleven healthy adults (8). The average I^{131} uptake for the 24 individuals measured in our study of 17% as reported in reference (4) is in fairly good agreement with these values. It appears that a provisional calibration of our total-body counter for thyroid I^{131} has been made.

Comparison of Results with Los Alamos Scientific Laboratory

Within one week of having his Cs^{137} and K^{40} body burdens evaluated at Los Alamos by both a liquid and crystal human counter, (January, 1963) subject GDW was also measured using the counter described herein. Using the calibration corrections obtained from these studies, his body burden of Cs^{137} and K were determined to be 9702 pc and 186 grams respectively. The two Los Alamos values were 9900 pc Cs^{137} and 205 grams K (Humco II), and 7900 pc Cs^{137} and 188 grams K (NaI), which are in fairly good agreement with our values.

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Figure 1. Subject position during human total-body counting.

Figure 2. Change in relative counting efficiency with time after Cs^{132} ingestion.

Figure 3. Effect of body build on relative counting efficiency for Cs^{132} .

Figure 4. Change in relative counting efficiency with time after K^{42} ingestion.

Figure 5. Effect of body build on relative counting efficiency for K^{42} .

DETERMINATION OF LOCALIZED ALPHA-DOSE IV
IN SOFT TISSUE NEAR RADIOACTIVE BONE

Charles W. Mays and Alden B. Tueller

Abstract: Dose-rates are calculated to the soft tissues close to flat radioactive bone mineral containing Pu^{239} , Th^{228} or Ra^{226} on its surface or throughout its volume. The dose-rates are evaluated at various distances from the contaminated bone.

In Reference (3) the average dose-rates received by the soft tissues lying between 0 and 10 microns from flat radioactive bone surfaces were calculated. The arbitrary selection of this region was motivated (a) by the evidence of Marshall and Finkel that the sensitive sites for osteosarcoma induction appear to be in the cells adjoining or nearby the microscopic bone surfaces (4), and (b) because the dose-rates are maximum at the bone surfaces.

Recently, Maureen Owen has pointed out the possible importance of certain cells (which she calls pre-osteoblasts) located more than 1 cell-layer from bone mineral (5). Although Th^{228} and Pu^{239} both deposit on bone surfaces (6), the mean skeletal dose-rate from Th^{228} + daughters is 2-3 times more effective than that from Pu^{239} in inducing osteosarcomas in our beagles (7). Could the greater effectiveness of Th^{228} + daughters be due, at least in part, to the longer range of their α -particles in soft tissue*?

*Computed ranges (3) are: $\text{Pu}^{239} = 35.4$ microns;

$\text{Th}^{228} = 38.1 \mu$, $\text{Ra}^{224} = 41.1 \mu$, $\text{Tn}^{220} = 48.1 \mu$, $\text{Po}^{216} = 54.3 \mu$, $\left[\begin{array}{l} \text{Bi}^{212}=45.5 \mu \\ \text{Po}^{212}=82.1 \mu \end{array} \right]$
 $\text{Ra}^{226} = 31.6 \mu$, $\text{Rn}^{222} = 39.1 \mu$, $\text{Po}^{218} = 44.8 \mu$, $\text{Po}^{214} = 66.3 \mu$.

Because of these questions, we have evaluated the dose-rates at distances varying from the bone surface out to the maximum ranges of the α -particles in soft tissue.

Ranges and energies were taken from Reference (3) and uniform energy loss along the alpha track is used for simplicity.

DOSE FROM A CONTAMINATED PLANE

The dose-rate to the soft tissue at a distance (X) from a uniform α -emitting plane is (page 179 Reference 1):

$$\text{Dose-rate from plane} = \frac{-D E}{2 \rho R} \ln \left(\frac{X}{R} \right) \quad (1)$$

where D = disintegration rate per unit area of contaminated surface.

E = average α -energy per disintegration.

ρ = density of soft tissue (taken as 1 g/cm³).

R = range of the α -particles in soft tissue and distance (X) does not exceed range (R).

Dose-rates from surface deposits of Pu²³⁹, and from [Th²²⁸ + 90% Ra²²⁴ + 80% subsequent daughters], are shown in Figure 1.

These values are illustrative since daughter retentions change with time.

The average dose-rate to the soft tissue between distance (X₁) and (X₂) from a uniform α -emitting plane is found by integrating Equation 1 and is:

$$\text{Av. dose-rate from plane} = \frac{D E}{2 \rho R} \left[\frac{1}{X_2 - X_1} \right] \left(X_2 \left(1 - \ln \frac{X_2}{R} \right) - X_1 \left(1 - \ln \frac{X_1}{R} \right) \right) \quad (2)$$

Average dose-rates within 10 micron bands are tabulated in Table 1 for surface deposits of Pu²³⁹, and for Th²²⁸ and its α -emitting daughters.

Daughter dose-rates should be multiplied by their respective fractional retentions.

Figure 1. Local α dose-rate near a radioactive bone surface.

TABLE 1

Av. Dose-Rate in Rads/Day From a Surface
Deposit of 1 Nanocurie (10^{-3}uc)/ cm^2

Nuclide	0-10 μ	10-20 μ	20-30 μ	30-40 μ	40-50 μ	50-60 μ	60-70 μ	70-80 μ
Pu ²³⁹	84.2	32.6	13.2	1.6				
Th ²²⁸	84.8	34.5	15.5	3.4				
Ra ²²⁴	85.2	36.3	17.8	5.8	0.1			
Tn ²²⁰	85.9	39.6	22.1	10.7	2.4			
Po ²¹⁶	85.9	41.7	25.0	14.1	6.1	0.1		
Bi ²¹² x.36	30.8	13.8	7.4	3.3	0.4			
Po ²¹² x.64	54.4	30.1	21.0	15.0	10.6	7.0	4.1	1.6

DOSE FROM A CONTAMINATED VOLUME

The dose-rate to the soft tissue at a distance (X) from a flat, thick slab of bone, uniformly contaminated with an α -emitter is (page 204 Reference 2):

$$\text{Dose-rate from Volume} = \frac{NE R'}{2PR} \left[1 - \frac{X}{R} + \frac{X}{R} \ln \left(\frac{X}{R} \right) \right] \quad (3)$$

where N = disintegration rate per unit volume of bone.

E = average α -energy per disintegration.

P = density of soft tissue (taken as 1 g/cm³).

R' = range of α -particles in bone.

R = range of α -particles in soft tissue and distance (X) does not exceed range (R).

Dose-rates from volume deposits of Pu²³⁹, from [Th²²⁸ + 90% Ra²²⁴ + 80% subsequent daughters], and from [Ra²²⁶ + 25% (Rn²²² + Po²¹⁸ + Po²¹⁴)] are shown in Figure 2. These values are illustrative since daughter retentions change with time.

The average dose-rate to the soft tissue between distances (X₁) and (X₂) from a uniform α -emitting volume is found by integrating Equation 3 and is:

$$\text{Av. dose-rate from volume} = \frac{NE R'}{2PR} \left[\frac{1}{X_2 - X_1} \right] \left[X_2 \left(1 - \frac{3X_2}{4R} + \frac{X_2}{2R} \ln \frac{X_2}{R} \right) - X_1 \left(1 - \frac{3X_1}{4R} + \frac{X_1}{2R} \ln \frac{X_1}{R} \right) \right] \quad (4)$$

Figure 2. Local α dose-rate near a radioactive bone volume.

Average dose-rates within 10 micron bands are tabulated in Table 2 for volume deposits of Pu^{239} , for Th^{228} and daughters, and for Ra^{226} and daughters. Daughter dose-rates should be multiplied by their respective fractional retentions. $\text{Rn}^{222}/\text{Ra}^{226} \approx 0.057t^{0.2}$ at (t) days after radium deposition (8).

TABLE 2
Av. Dose-Rate in Rads/Day From a Volume
Deposit of 1 $\mu\text{c/g}$ Bone

Nuclide	0-10 μ	10-20 μ	20-30 μ	30-40 μ	40-50 μ	50-60 μ	60-70 μ	70-80 μ
Pu^{239}	113.1	40.9	9.8	0.4				
Th^{228}	122.3	48.1	14.0	1.3				
Ra^{224}	132.1	56.6	19.1	3.0	0.0			
Tn^{220}	155.8	73.5	32.6	9.9	0.9			
Po^{216}	172.6	91.3	45.4	18.2	4.2	0.1		
$\text{Bi}^{212} \times .36$	52.6	24.4	9.8	2.5	0.1			
$\text{Po}^{216} \times .64$	158.3	103.5	68.0	42.9	25.0	12.7	4.9	0.9
Ra^{226}	100.0	31.1	5.0	0.0				
Rn^{222}	125.6	50.7	15.6	1.7				
Po^{218}	144.1	66.1	26.0	6.2	0.2			
Po^{214}	206.3	122.5	71.4	37.9	16.5	4.6	0.3	

We hope that these dose-rate calculations will aid in the correlation of the biological changes produced by α -emitters in the skeleton.

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