MASTER

JUL 16 1965

COO-119-232

Research in Radiobiology

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

of the

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

Respectfully Submitted by

THOMAS F. DOUGHERTY, Director

APPROVED FOR PUBLIC RELEASE

MARCH 31, 1965

CONTRACT NO. AT (11-1)-119

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

C00-119-232

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:

Thomas F. Dougherty, Director

March 31, 1965

Contract No. AT (11-1)-119

TABLE OF CONTENTS

	Page
Availability of Previous Reports	3
Group Leaders and Sections	4
Preface to Injection Tables	5
Injection Tables	
I. Toxicity Animals	10
A. Radium-226	10
B. Plutonium-239	16
C. Radium-228 (Mesothorium)	24
D. Thorium-228 (Radiothorium)	30
E. Strontium-90	36
II. Test Animals	42
A. Radium-226	42
B. Plutonium-239	48
C. Radium-228 (Mesothorium)	52
D. Thorium-228 (Radiothorium)	54
E. Strontium-90	56
F. Radium-224 (Quickradium)	60
G. Ancillary	62
Current Census of the Beagle Colony	
The Use of Therapy in Experimental Animals	65
Dental Eruption Pattern of the Beagle	68
Thorium-228 Induced Fractures in Beagles	74
The Correlation of Bone Strength with Radiation Dose From Internal Emitters and Bone Resorption	88
Studies of Trabecular Bone in Beagles and Humans	99

Bioassay of the Influence of Cortisol in Bones of Growing Rabbits	122
The Effects of Irradiation on Endocrine Cells V. A Comparison Between the Effects of Inanition and Irradiation on Androgen Production by Murine Testicular Tissue	153
A Comparison of Two Methods of Analyzing the Hematological Data From Strontium-90 Injected Beagles	165
Pilor Study of the Effect of Cortisol on Na_3CaDTPA Enhanced Excretion of Pu^{239} in the Beagle	188
Effect of EDTA on the Excretion of Sr^{90} and Y^{90}	200
The Determination of Half-Periods of Dog Serum Proteins Using S^{35} -L-Methionine	203
Additions to Bibliography	219

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>
C00-119-232*	Mar 1965	Research in Radiobiology	\$22.20	\$11.10
C00-119-231*	Sep 1964	Research in Radiobiology	17.20	8.60
C00-119-230*	Jul 1964	Safety Manual	3.10	1.55
C00-119-229*	Mar 1964	Research in Radiobiology	13.00	5.84
C00-228*	Sep 1963	Research in Radiobiology	13.00	5.84
C00-227*	Mar 1963	Research in Radiobiology	15.50	7.22
C00-226*	Sep 1962	Research in Radiobiology	10.50	4.31
C00-225	Mar 1962	Research in Radiobiology	10.50	4.28
C00-224*	Sep 1961	Research in Radiobiology	9.10	3.32
C00-223*	Mar 1961	Research in Radiobiology	6.30	3.00
C00-222*	Sep 1960	Research in Radiobiology	22.80	7.20
C00-221*	Aug 1960	Interim Report of Sr ⁹⁰	3.30	2.40
C00-220*	Mar 1960	Research in Radiobiology	34.80	9.90
C00-219*	Sep 1959	Semi-Annual Report	10.80	3.90
C00-218*	Mar 1959	Annual Report	34.80	9.90
AECU-4112	Feb 1959	Radioactive Fallout	3.30	2.40
C00-217*	Sep 1958	Semi-Annual Report	28.80	8.40
C00-216*	Mar 1958	Escape of Radon & Thoron	9.30	6.30
C00-215*	Mar 1958	Annual Report	28.80	8.40
AECU-3583	Sep 1957	Semi-Annual Report	19.80	6.30
AECU-3522	Mar 1957	Annual Report	27.30	8.10
TID-16459	Sep 1956	Semi-Annual Report	5.60	2.05
TID-16458	Mar 1956	Annual Report	10.50	4.40
AECU-3109	Sep 1955	Semi-Annual Report	12.30	4.50
AECU-3418	Mar 1955	Annual Report	9.30	3.60
TID-7639	Consultants Meeting		19.75	9.40

*

Also available on request 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

INJECTION TABLES

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

Dogs are put into the toxicity study groups of six or seven of the same sex (five or six dose levels plus one control). Litter mates are used whenever possible. Each animal receives the designated dose of one radionuclide in a single I.V. injection. The animals are injected at approximately 17 months of age. At this age the skeleton is mature with all epiphyses fused except those of the ribs. Twelve such groups have been injected for each of the five radionuclides, Ra^{226} , Pu^{239} , Ra^{228} , Th^{228} , and Sr^{90} . The current injection program is limited to Ra^{226} and Pu^{239} at lower dose levels.

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

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

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

the radionuclides except Sr^{90} , in which case they are greater by a factor of 10. Dose level 1 is the basis of the scheme, and is 10 times the maximum permissible Ra^{226} in man. Level 1 = $10 \times \frac{0.1 \mu\text{C Ra}^{226}}{70 \text{ kg man}} = 0.0143$ "retained" $\mu\text{C/kg}$. All other dose levels are simple multiples of level 1 as shown below.

Level 0.1	is	1/27	of level 1
Level 0.2	is	1/9	of level 1
Level 0.5	is	1/3	of level 1
Level 1.5	is	2 times	level 1
Level 1.7	is	3 times	level 1
Level 2	is	6 times	level 1
Level 3	is	18 times	level 1
Level 4	is	54 times	level 1
Level 5	is	162 times	level 1

The numbering system for the dogs has been built around the injection program and serves as a code to describe each dog's place in the experiment. The first letter tells the sex of toxicity animals (M = male, F = female). When the first letter is T, the dog is a test animal. M, F, or T is followed by a number which denotes chronological order of groups in the case of toxicity dogs and of individual test dogs.

Next comes a code letter for the radionuclide: R = Ra^{226} , P = Pu^{239} , M = Ra^{228} , T = Th^{228} , S = Sr^{90} , Q = Ra^{224} , J = Sr^{85} , and A = Aging Control.

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

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

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

The conditions listed in the injection tables under "Comments on Dead Dogs" present the lesions or factors that had the most prominent effect on the clinical status of the animal. For example, multiple rib fractures, which seldom produced symptoms, are not listed, even though their incidence was usually much higher than the crippling fractures involving the limb bones or mandible. The hematological changes have been omitted unless they were extreme. Increased rate of tooth loss, hepatic changes, eye lesions, and many other factors in the various syndromes have not been included because of space limitations. Over the years many soft tissue tumors have been removed surgically; these tumors were the subject of a separate report, Research in Radiobiology, September 30, 1963 (C00-228), pp. 95-108. In many instances, the conditions that have been listed were the reasons for sacrifice of the animal but they were not the immediate cause of death. Most of the animals were euthanized when death appeared eminent or when life could no longer be humanely prolonged.

DOSIMETRY REVISIONS

A number of new values appear for the dose in rads to the skeleton at death. Ra^{226} , Ra^{228} , and Sr^{90} doses are calculated for each dog using his individually observed retention values: Pu^{239} and Th^{228} doses are from our average skeletal retention equations.

For our standard beagle, the following equations were used for the effective* skeletal retention at (t) days after injection:

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

(5-level only)

$$\text{Ra}^{226} = 0.251e^{-0.982t} + 0.211e^{-0.269t} + 0.210e^{-0.0155t} + 0.177e^{-0.00204t} + 0.151e^{-0.000150t}$$

(lower levels)

$$\text{Rn}^{222}/\text{Ra}^{226} = 0.075 \left[1 - e^{-0.181t} \right] t^{0.158}$$

(all levels)

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

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

(pure at t = 0)

84.4% retention of in vivo produced Th^{228} and daughters.

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

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

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

$$\text{Sr}^{90} = 0.402e^{-0.748t} + 0.259e^{-0.102t} + 0.181e^{-0.00469t} + 0.158e^{-0.000302t}$$

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

We hope to present our detailed retention data and dosimetric analysis in a future report.

Ra^{228} doses deserve special comment. The dose from "pure" Ra^{228} and its in vivo produced daughters is based on our best evaluation of 5.77 ± 0.02 yr for the Ra^{228} half-period. The tabulated total doses include the contributions from Th^{228} contamination in the injection solutions. For example, Th^{228} contaminations of 0.6%, 3%, and 15%, respectively, account for 2.8%, 13% and 42% of the total dose in rads at 1000 days. If injected Th^{228} is 4 times more toxic rad-for-rad than is in vivo produced Th^{228} , these injected Th^{228} contaminations would account for 10%, 37% and 74% of the total biological damage at 1000 days. Therefore, it may be desirable to use only results from the slightly contaminated (0.6% Th^{228}) dogs in evaluation of Ra^{228} toxicity.

TABLE I. TOXICITY ANIMALS (MARCH 31, 1965)

A. RADIUM-226

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO. YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
M01R0.0	558	8.03		20 04 53	3116	
M02R0.0	487	14.6		16 11 53	3675	
F03R0.0	601	11.4		10 03 54	2139	
M04R0.0	461	11.0		7 04 54	4011	
M05R0.0	460	6.57		22 06 54	3935	
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	3535	
F11R0.0	544	10.2		20 12 55	3389	
F12R0.0	501	8.68		17 01 56	3361	
M13R0.0	515	12.3		4 3 64	392	
F14R0.0	536	10.8		23 10 64	159	
M15R0.0	564	12.8		4 2 65	55	
F31R0.0C	536	9.88		23 10 64	159	
F31R0.0B	536	10.6		23 10 64	159	
M13R0.2	529	9.77	.00577	4 3 64	392	
F14R0.2	460	8.10	.00836	23 10 64	159	
M15R0.2	504	10.8	.00873	4 2 65	55	
M13R0.5	529	11.0	.0171	4 3 64	392	
F14R0.5	510	9.75	.0220	23 10 64	159	
M15R0.5	490	10.4	.0263	4 2 65	55	
M01R1.0	471	8.48	.0618	20 04 53	4363	
M02R1.0	627	10.0	.0876	16 11 53	4054	237
F03R1.0	706	8.68	.0576	10 03 54	3850	151
M04R1.0	414	8.60	.0642	7 04 54	2038	108
M05R1.0	490	11.7	.0436	22 06 54	3780	112
F06R1.0	483	7.23	.0584	27 07 54	3900	
M07R1.0	511	11.4	.0651	24 8 54	3544	167
F08R1.0	861	8.98	.0559	21 12 54	2988	93
F09R1.0	781	9.88	.0521	11 04 55	3642	
M10R1.0	523	11.5	.0573	27 07 55	3535	
F11R1.0	511	11.2	.0522	20 12 55	3389	

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	
F14R0.0	
M15R0.0	
F31R0.0C	
F31R0.0B	

M13R0.2
F14R0.2
M15R0.2

M13R0.5
F14R0.5
M15R0.5

M01R1.0	
M02R1.0	SEMINOMA
F03R1.0	MAMMARY GLAND CARCINOMA
M04R1.0	TRAUMA
M05R1.0	TRANSITIONAL CELL CARCINOMA, HYDRONEPHROSIS
F06R1.0	
M07R1.0	STATUS EPILEPTICUS
F08R1.0	LYMPHOSARCOMA
F09R1.0	
M10R1.0	
F11R1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
F12R1.0	501	9.71	.0444	17 01 56	3361	
M13R1.0	529	11.7	.0527	4 3 64	392	
F14R1.0	510	10.5	.0701	23 10 64	159	
M15R1.0	490	8.88	.0797	4 2 65	55	
M01R1.7	523	9.98	.137	17 01 56	3361	
M02R1.7	598	7.85	.163	30 11 56	1273	116
M02R1.7A	493	12.0	.222	6 3 63	756	
F03R1.7	473	13.1	.165	20 12 55	3267	385
M04R1.7	514	6.20	.163	20 12 55	3389	
M05R1.7	511	10.1	.151	20 12 55	3389	
F06R1.7	491	7.9	.152	20 12 55	3389	
M07R1.7	598	7.17	.163	30 11 56	3043	
F08R1.7	491	9.50	.154	20 12 55	2577	378
F09R1.7	598	7.55	.168	30 11 56	3043	
M10R1.7	590	9.57	.167	30 11 56	557	109
M10R1.7A	545	10.6	.183	7 01 59	2275	
F11R1.7	598	8.17	.165	30 11 56	3043	
F12R1.7	590	8.95	.167	30 11 56	2399	210
M01R2.0	471	8.74	.382	20 04 53	3440	860
M02R2.0	592	8.21	.387	16 11 53	3775	625
F03R2.0	541	8.53	.347	10 03 54	4039	
M04R2.0	414	10.5	.361	7 04 54	325	189
M04R2.0A	420	10.6	.306	11 04 55	3642	
M05R2.0	461	11.5	.267	22 06 54	3935	
F06R2.0	486	10.6	.360	27 07 54	3900	
M07R2.0	514	11.1	.413	24 8 54	3425	922
F08R2.0	572	6.95	.331	21 12 54	3753	
F09R2.0	592	9.38	.317	11 04 55	3642	
M10R2.0	523	9.95	.345	27 07 55	3535	
F11R2.0	495	9.30	.310	20 12 55	3297	728
F12R2.0	497	10.3	.281	17 1 56	2948	742
M01R3.0	473	8.91	1.20	20 04 53	2850	2395
M02R3.0	470	9.02	1.21	16 11 53	2226	1727
F03R3.0	386	7.74	1.11	10 03 54	2497	2323

DOG
NUMBER

COMMENTS ON DEAD DOGS

F12R1.0
M13R1.0
F14R1.0
M15R1.0

M01R1.7
M02R1.7 LYMPHOSARCOMA
M02R1.7A
F03R1.7 MAMMARY GLAND CARCINOMA
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 OSTEOSARCOMA
F12R2.0 MAMMARY ADENOCARCINOMA

M01R3.0 OSTEOSARCOMA
M02R3.0 OSTEOSARCOMA
F03R3.0 OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/65	DEATH	
M04R3.0	412	11.7	1.16	7	04	54		1917	2361
M05R3.0	461	13.0	.846	22	06	54		2955	2317
F06R3.0	486	9.75	1.14	27	07	54		1932	2246
M07R3.0	514	12.3	1.29	24	08	54		2099	3029
F08R3.0	542	7.76	1.03	21	12	54		2612	1916
F09R3.0	551	8.02	.987	11	04	55		2487	1839
M10R3.0	525	10.1	1.06	27	07	55		1737	2336
F11R3.0	495	12.9	.938	20	12	55		1610	1333
F12R3.0	497	11.4	.883	17	1	56		1897	1639
M01R4.0	471	9.08	3.51	20	04	53		1606	6575
M02R4.0	470	9.53	3.55	16	11	53		1884	6150
F03R4.0	384	8.65	3.33	10	03	54		490	2208
F03R4.0A	598	7.20	3.10	30	11	56		1614	3855
M04R4.0	408	8.83	3.47	7	04	54		1518	6063
M05R4.0	461	13.2	2.42	22	06	54		1659	4505
F06R4.0	486	8.55	3.44	27	07	54		1939	7133
M07R4.0	453	9.55	3.88	24	8	54		1647	5844
F08R4.0	474	8.94	3.14	21	12	54		1324	4615
F09R4.0	542	8.53	3.02	11	04	55		1471	4095
M10R4.0	527	10.8	3.28	27	07	55		1553	7582
F11R4.0	491	10.4	2.84	20	12	55		1469	5273
F12R4.0	496	9.61	2.81	17	01	56		1435	3877
M01R5.0	473	9.87	10.5	20	04	53		908	14943
M02R5.0	470	8.85	10.8	16	11	53		1380	18071
F03R5.0	380	7.82	10.1	10	03	54		481	7147
M04R5.0	408	8.90	10.6	7	04	54		1091	16417
M05R5.0	458	10.9	10.1	22	6	54		1220	15433
F06R5.0	486	9.66	10.2	27	07	54		1015	15414
M07R5.0	453	8.85	11.9	24	08	54		1288	16708
F08R5.0	474	7.76	9.68	21	12	54		968	11564
F09R5.0	420	9.16	9.48	11	04	55		1288	15941
M10R5.0	527	10.7	10.2	27	07	55		825	11179

DOG
NUMBER

COMMENTS ON DEAD DOGS

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
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 (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 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	4321	
M04P0.0	426	10.7		16	09	53	4214	
F05P0.0	620	9.75		14	10	53	4088	
F06P0.0	410	5.59		12	05	54	3976	
F07P0.0	515	6.90		25	10	54	3810	
M08P0.0	585	10.9		15	03	55	3669	
F09P0.0	658	11.0		22	11	55	3032	
F10P0.0	658	11.0		22	11	55	3417	
M11P0.0	602	10.3		24	04	56	3263	
M12P0.0	630	10.9		29	05	56	3228	
F13P0.0	517	9.47		4	3	64	392	
F14P0.0	452	9.89		12	5	64	14	
M15P0.0	527	12.1		23	10	64	159	
M31P0.0B	452	11.8		12	5	64	323	
M31P0.0C	452	12.6		12	5	64	323	
M32P0.0B	452	11.2		12	5	64	323	
F13P0.1	515	9.46	.00068	4	3	64	392	
F14P0.1	452	10.3	.00055	12	5	64	323	
M15P0.1	536	9.67	.00071	23	10	64	159	
M31P0.1B	517	12.2	.00068	4	3	64	392	
F13P0.2	517	9.44	.00206	4	3	64	392	
F14P0.2	516	7.44	.00173	12	5	64	323	
M15P0.2	505	10.9	.00201	23	10	64	159	
M31P0.2B	515	10.7	.00185	4	3	64	392	
F31P0.2C	452	11.9	.00169	12	5	64	323	
F31P0.2D	429	9.35	.00186	12	5	64	323	
F32P0.2C	494	10.1	.00183	4	2	65	55	
F32P0.2D	490	8.04	.00193	4	2	65	55	
F13P0.5	517	9.93	.00540	4	3	64	392	
F14P0.5	516	9.98	.00493	12	5	64	323	
M15P0.5	505	8.41	.00627	23	10	64	159	
M31P0.5B	515	10.5	.00549	4	3	64	392	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M01P0.0	SPLENIC RUPTURE, METASTATIC SEMINOMA
F02P0.0	ANESTHETIC ACCIDENT
M03P0.0	
M04P0.0	
F05P0.0	ADRENAL CORTICAL CARCINOMA
F06P0.0	
F07P0.0	
M08P0.0	
F09P0.0	PULMONARY EMBOLISM, NEPHRITIS
F10P0.0	
M11P0.0	
M12P0.0	
F13P0.0	
F14P0.0	
M15P0.0	
M31P0.0B	
M31P0.0C	
M32P0.0B	

F13P0.1
F14P0.1
M15P0.1
M31P0.1B

F13P0.2
F14P0.2
M15P0.2
F31P0.2C
M31P0.2B
F31P0.2D
F32P0.2C
F32P0.2D

F13P0.5
F14P0.5
M15P0.5
M31P0.5B

DOG NUMBER	AT INJECTION		INJECTED (mc/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F32P0.5C	494	8.44	.00571	4	2	65	55	
M01P1.0	442	9.41	.0150	1	12	52	4503	
F02P1.0	422	6.85	.0163	2	03	53	4412	
M03P1.0	515	8.00	.0165	1	06	53	4292	102
M04P1.0	608	9.97	.0139	16	09	53	4214	
F05P1.0	620	8.80	.0142	14	10	53	1539	30
F05P1.0A	472	11.0	.0168	3	09	58	2401	
F06P1.0	410	7.38	.0140	12	05	54	3976	
F07P1.0	510	6.36	.0167	25	10	54	3810	
M08P1.0	453	10.6	.0172	15	3	55	3367	83
F09P1.0	556	7.87	.0168	9	09	55	2257	60
F10P1.0	641	12.00	.0152	22	11	55	3417	
M11P1.0	602	8.90	.0157	24	04	56	3263	
M12P1.0	629	9.67	.0167	29	5	56	2374	60
M13P1.0	504	12.7	.0153	3	09	58	2401	
M01P1.7	657	8.72	.0475	26	06	56	3025	210
F02P1.7	527	8.62	.0431	22	11	55	3417	
M03P1.7	642	8.63	.0495	26	06	56	3200	
M04P1.7	673	8.37	.0484	10	10	56	3094	
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	3094	
F08P1.7	673	13.6	.0479	10	10	56	3094	
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	2401	
M11P1.7	599	11.6	.0486	24	4	56	2777	200
M12P1.7	673	9.41	.0491	10	10	56	2973	213
M13P1.7	504	10.60	.0473	3	09	58	2401	
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

DOG
NUMBER

COMMENTS ON DEAD DOGS

F32P0.5C

M01P1.0	
F02P1.0	
M03P1.0	OSTEOSARCOMA
M04P1.0	
F05P1.0	COLITIS, ENTERITIS + SECONDARY HEPATIC NECROSIS
F05P1.0A	
F06P1.0	
F07P1.0	
M08P1.0	OSTEOSARCOMA
F09P1.0	OSTEOSARCOMA
F10P1.0	
M11P1.0	
M12P1.0	CHRONIC PANCREATITIS
M13P1.0	

M01P1.7	OSTEOSARCOMA
F02P1.7	
M03P1.7	
M04P1.7	
F05P1.7	OSTEOSARCOMA
F06P1.7	OSTEOSARCOMA
F07P1.7	
F08P1.7	
F09P1.7	OSTEOSARCOMA
F10P1.7	ACUTE ENTERITIS
F10P1.7A	
M11P1.7	BILE DUCT CARCINOMA
M12P1.7	LEUKEMIA
M13P1.7	

M01P2.0	OSTEOSARCOMA
F02P2.0	OSTEOSARCOMA
M03P2.0	OSTEOSARCOMA
M04P2.0	OSTEOSARCOMA
F05P2.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
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
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

DOG NUMBER	COMMENTS ON DEAD DOGS
---------------	-----------------------

F06P2.0	OSTEOSARCOMA
F07P2.0	SQUAMOUS CELL CARCINOMA
M08P2.0	ASPIRATION PNEUMONIA
F09P2.0	OSTEOSARCOMA
F10P2.0	OSTEOSARCOMA
M11P2.0	OSTEOSARCOMA
M12P2.0	OSTEOSARCOMA

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

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

M01P5.0	OSTEOSARCOMA
F02P5.0	OSTEOSARCOMA + FRACTURED MANDIBLE
M03P5.0	LIVER DEGENERATION + ASCITES

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/65	DEATH	
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

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 31/3/65 DEATH	DOSE TO SKELETON (RADS)
F01M0.0	732	7.33		4 1 54	3451	
F02M0.0	545	6.94		29 11 54	3775	
M03M0.0	579	13.0		13 03 56	3305	
M04M0.0	601	10.3		15 01 57	2997	
F05M0.0	671	11.2		5 03 57	2948	
M06M0.0	492	7.56		23 04 57	2899	
F07M0.0	395	8.71		4 6 57	1414	
F07M0.0A	594	10.9		15 1 63	806	
F08M0.0	654	11.6		9 03 60	1848	
M09M0.0	575	12.4		13 04 60	1813	
M10M0.0	581	13.3		17 7 62	988	
F11M0.0	475	9.31		18 9 62	925	
M12M0.0	695	10.0		22 12 60	1560	
F01M0.5	492	9.47	.0173	17 7 62	988	
F02M0.5	492	9.15	.0173	17 7 62	988	
M03M0.5	493	10.8	.0199	18 9 62	925	
M04M0.5	475	12.8	.0199	18 9 62	925	
F05M0.5	534	7.83	.0172	23 10 62	890	
M06M0.5	510	10.3	.0171	23 10 62	890	
F07M0.5	492	8.87	.0172	17 7 62	988	
F08M0.5	654	12.6	.0159	9 03 60	1848	
M09M0.5	485	11.9	.0170	13 04 60	1813	
M10M0.5	492	10.6	.0174	17 7 62	988	
F11M0.5	505	7.82	.0202	18 9 62	925	
M12M0.5	510	10.6	.0165	23 10 62	890	
F01M1.0	718	7.75	.0463	4 01 54	2952	196
F01M1.0A	590	8.07	.0512	23 10 62	890	
F02M1.0	459	8.25	.0324	29 11 54	3775	
M03M1.0	575	13.8	.0589	13 03 56	3157	306
M04M1.0	601	9.90	.0481	15 01 57	2997	
F05M1.0	658	8.80	.0490	5 03 57	2948	
M06M1.0	521	10.6	.0468	23 04 57	2899	
F07M1.0	534	9.89	.0489	4 6 57	2159	149
F08M1.0	654	12.4	.0491	9 03 60	1848	
M09M1.0	485	10.1	.0504	13 04 60	1813	
M10M1.0	492	9.43	.0501	17 7 62	988	
F11M1.0	505	8.91	.0613	18 9 62	925	

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	OSTEOSARCOMA
M04M1.0	
F05M1.0	
M06M1.0	
F07M1.0	HEMANGIOSARCOMA
F08M1.0	
M09M1.0	
M10M1.0	
F11M1.0	

DOG NUMBER	AT INJECTION		INJECTED (MC/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M12M1.0	528	9.27	.0498	23	10	62	890	
F01M1.7	510	7.52	.151	23	10	62	890	
F02M1.7	560	9.90	.183	13	03	56	2383	733
M03M1.7	576	11.0	.180	13	3	56	2709	723
M04M1.7	601	8.94	.143	15	01	57	2864	424
F05M1.7	658	12.8	.141	5	03	57	2948	
M06M1.7	521	10.0	.144	23	04	57	2899	
F07M1.7	534	10.2	.146	4	6	57	2646	600
F08M1.7	654	10.8	.148	9	03	60	1848	
M09M1.7	485	12.6	.149	13	04	60	1813	
M10M1.7	492	10.1	.124	17	7	62	988	
F11M1.7	505	10.7	.179	18	9	62	925	
M12M1.7	524	9.28	.153	23	10	62	890	
F01M2.0	676	7.60	.276	4	01	54	1780	870
F02M2.0	517	8.25	.194	29	11	54	965	198
M03M2.0	576	11.0	.358	13	03	56	619	355
M04M2.0	601	9.88	.282	15	1	57	2282	1033
F05M2.0	509	8.30	.295	5	3	57	2688	928
M06M2.0	502	12.4	.306	23	4	57	2674	1382
F07M2.0	534	10.1	.298	4	6	57	2239	1064
F08M2.0	654	12.4	.300	9	03	60	1848	
M09M2.0	630	9.99	.302	13	4	60	1254	561
M10M2.0	430	11.2	.311	17	7	62	988	
F11M2.0	505	7.03	.381	18	9	62	925	
M12M2.0	524	9.47	.306	23	10	62	890	
F01M3.0	519	10.4	.858	4	01	54	918	1833
F02M3.0	460	6.70	.612	29	11	54	1856	2075
M03M3.0	579	10.4	.965	13	3	56	1185	2464
M04M3.0	601	10.2	.916	15	01	57	1176	1592
F05M3.0	531	8.51	.940	5	03	57	1869	2148
M06M3.0	502	9.09	.953	23	04	57	1421	1906
F07M3.0	534	9.94	.907	4	6	57	1463	3145
F08M3.0	633	11.8	.950	9	3	60	1447	2158
M09M3.0	630	9.83	.918	13	4	60	1570	2277

DOG
NUMBER

COMMENTS ON DEAD DOGS

M12M1.0

F01M1.7
F02M1.7 OSTEOSARCOMA
M03M1.7 OSTEOSARCOMA
M04M1.7 CARCINOMA SMALL INTESTINE
F05M1.7
M06M1.7
F07M1.7 OSTEOSARCOMA
F08M1.7
M09M1.7
M10M1.7
F11M1.7
M12M1.7

F01M2.0 OSTEOSARCOMA
F02M2.0 INTESTINAL HEMORRHAGE
M03M2.0 PNEUMONIA
M04M2.0 OSTEOSARCOMA
F05M2.0 OSTEOSARCOMA
M06M2.0 OSTEOSARCOMA
F07M2.0 CHRONIC PANCREATITIS
F08M2.0
M09M2.0 RETICULUM CELL SARCOMA OF VERTEBRA
M10M2.0
F11M2.0
M12M2.0

F01M3.0 OSTEOSARCOMA
F02M3.0 OSTEOSARCOMA
M03M3.0 OSTEOSARCOMA
M04M3.0 OSTEOSARCOMA
F05M3.0 OSTEOSARCOMA
M06M3.0 OSTEOSARCOMA
F07M3.0 OSTEOSARCOMA
F08M3.0 OSTEOSARCOMA
M09M3.0 OSTEOSARCOMA

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{c}/\text{KG}$)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M10M3.0	581	10.4	1.00	17	7	62	988	
F11M3.0	499	11.0	1.19	18	9	62	925	
M12M3.0	510	12.9	.987	23	10	62	890	
F01M4.0	510	7.56	2.60	4	01	54	841	5614
F02M4.0	460	6.95	1.86	29	11	54	778	2272
M03M4.0	579	9.65	3.37	13	03	56	418	1795
M03M4.0A	494	7.34	2.64	4	06	57	1063	5604
M04M4.0	609	7.84	2.47	15	1	57	896	2680
F05M4.0	509	9.63	2.67	5	03	57	1064	4358
M06M4.0	502	9.49	2.66	23	04	57	1121	4784
F07M4.0	544	8.40	2.67	4	06	57	1253	4636
F01M5.0	494	7.77	8.11	4	01	54	232	3854
F02M5.0	460	7.35	5.46	29	11	54	780	7666
M03M5.0	579	8.87	10.4	13	3	56	688	14151
M04M5.0	482	7.29	7.89	15	01	57	561	5662
F05M5.0	658	11.1	8.48	5	3	57	770	9559
M06M5.0	580	7.53	8.67	23	4	57	792	6761
F07M5.0	494	7.35	8.92	4	06	57	966	18094

* ($\mu\text{c Th}^{228}/\mu\text{c Ra}^{228}$) injected = 0.15 for F1M1.0, 2.0, 3.0, 4.0, 5.0.

= 0.03 for F2M1.0, 1.7, 2.0, 3.0, 4.0, 5.0
M3M1.0, 1.7, 2.0, 3.0, 4.0, 5.0.

= 0.006 for groups 4, 5, 6, 7, 8, 9, 10,
11, 12, and dogs F1M0.5, F2M0.5,
M3M0.5, F1M1A, F1M1.7 and M3M4.0A.

DOG
NUMBER

COMMENTS ON DEAD DOGS

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 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
M01T0.0	493	8.24		8	02	54	4069	
M02T0.0	488	7.28		28	09	54	3837	
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	2360	
F06T0.0	489	9.64		10	1	61		171
F06T0.0A	688	8.61		15	12	60	1567	
M07T0.0	517	10.5		7	2	61		1412
M08T0.0	533	10.8		24	5	61	1407	
F09T0.0	569	8.28		29	6	61	1371	
F10T0.0	536	10.4		28	7	61	1342	
F11T0.0	530	9.45		4	6	63	666	
F12T0.0	492	9.09		9	7	63	631	
M01T0.2	682	11.4	.00164	27	03	62	1100	
M02T0.2	682	10.4	.00166	27	03	62	1100	
F03T0.2	478	9.86	.00163	27	03	62	1100	
M04T0.2	478	10.0	.00166	27	03	62	1100	
M05T0.2	625	13.8	.00162	9	02	60		889
M05T0.2A	530	13.4	.00173	4	6	63	666	10
F06T0.2	489	8.85	.00176	10	1	61	1541	
M07T0.2	532	10.5	.00159	7	2	61	1513	
M08T0.2	494	13.9	.00189	24	5	61	1407	
F09T0.2	569	7.82	.00171	29	6	61	1371	
F10T0.2	508	10.5	.00170	28	7	61	1342	
F11T0.2	530	9.76	.00171	4	6	63	666	
F12T0.2	492	7.37	.00190	9	7	63	631	
M01T0.5	699	14.3	.00496	7	9	56	3127	
M02T0.5	455	10.5	.00490	28	09	54	1976	41
F03T0.5	659	8.59	.00485	6	6	55	3032	44
M04T0.5	516	8.58	.00540	18	10	55	2159	46
M05T0.5	513	8.46	.00522	14	10	58	2360	
F06T0.5	489	9.66	.00510	10	1	61	1541	
M07T0.5	532	9.11	.00491	7	2	61	1513	
M08T0.5	533	9.53	.00562	24	5	61	1407	
F09T0.5	569	8.62	.00529	29	06	61	1371	
F10T0.5	508	10.2	.00510	28	7	61	1342	
F11T0.5	530	7.78	.00518	4	6	63	666	

DOG
NUMBER

COMMENTS ON DEAD DOGS

M01T0.0
M02T0.0
F03T0.0 BRAIN HEMORRHAGE
M04T0.0 LYMPHOSARCOMA
M05T0.0
F06T0.0 TRAUMA
F06T0.0A
M07T0.0 BRAIN HEMORRHAGE
M08T0.0
F09T0.0
F10T0.0
F11T0.0
F12T0.0

M01T0.2
M02T0.2
F03T0.2
M04T0.2
M05T0.2 STRANGULATION ON VOMITUS + GRAND MAL
M05T0.2A
F06T0.2
M07T0.2
M08T0.2
F09T0.2
F10T0.2
F11T0.2
F12T0.2

M01T0.5
M02T0.5 STRANGULATION ON VOMITUS + GRAND MAL
F03T0.5 PYOMETRITIS + SECONDARY PERITONITIS
M04T0.5 STATUS EPILEPTICUS + PNEUMONIA
M05T0.5
F06T0.5
M07T0.5
M08T0.5
F09T0.5
F10T0.5
F11T0.5

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/65	DEATH	
F12T0.5	492	9.94	.00567	9	7	63	631		
M01T1.0	493	9.36	.0146	8	02	54		3172	132
M02T1.0	699	9.27	.0146	7	09	56	3127		
F03T1.0	723	8.84	.0145	7	09	56	3127		
M04T1.0	699	8.27	.0146	7	09	56	3127		
M05T1.0	513	11.9	.0146	14	10	58	2360		
F06T1.0	489	8.81	.0150	10	1	61	1541		
M07T1.0	532	9.18	.0147	7	2	61	1513		
M08T1.0	533	8.69	.0166	24	5	61	1407		
F09T1.0	527	10.0	.0160	29	6	61	1371		
F10T1.0	508	10.2	.0150	28	7	61	1342		
F11T1.0	521	7.55	.0154	4	6	63	666		
F12T1.0	472	9.96	.0167	9	7	63	631		
M01T1.5	699	7.95	.0289	7	9	56		2894	258
M02T1.5	458	10.0	.0293	28	09	54		2576	257
F03T1.5	609	10.3	.0303	6	06	55		1921	249
M04T1.5	591	8.59	.0299	18	10	55		2309	257
M05T1.5	598	9.65	.0286	9	2	60		1624	223
F06T1.5	489	8.14	.0292	10	1	61	1541		
M07T1.5	517	8.83	.0292	7	2	61		384	95
M07T1.5A	521	9.08	.0311	4	6	63	666		
M08T1.5	494	11.6	.0324	24	5	61	1407		
F09T1.5	527	8.80	.0306	29	6	61	1371		
F10T1.5	508	11.6	.0296	28	7	61	1342		
F11T1.5	518	11.4	.0305	4	6	63	666		
F12T1.5	465	7.56	.0329	9	7	63	631		
M01T2.0	491	10.2	.0976	8	02	54		1282	693
M02T2.0	483	9.16	.0875	28	09	54		1234	611
F03T2.0	474	7.87	.0908	6	06	55		1541	695
M04T2.0	553	13.0	.0900	18	10	55		78	70
M04T2.0A	650	10.6	.0899	7	09	56		1222	625
M05T2.0	598	9.12	.0848	9	02	60		1085	556
F06T2.0	451	8.65	.0879	10	1	61		1108	583
M07T2.0	517	8.85	.0881	7	2	61		1015	558

DOG
NUMBER

COMMENTS ON DEAD DOGS

F12T0.5

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

M01T1.5 OSTEOSARCOMA
M02T1.5 OSTEOSARCOMA
F03T1.5 COMA OF UNKNOWN ETIOLOGY
M04T1.5 OSTEOSARCOMA
M05T1.5 OSTEOSARCOMA
F06T1.5
M07T1.5 LEP TOSPIROSIS
M07T1.5A
M08T1.5
F09T1.5
F10T1.5
F11T1.5
F12T1.5

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

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (MC/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65	DEATH	DOSE TO SKELETON (RADS)
M08T2.0	533	10.7	.0981	24 5 61		1078	641
F09T2.0	527	8.09	.0979	29 6 61		1209	677
F10T2.0	508	10.7	.0919	28 7 61		1022	584
F11T2.0	518	10.8	.0904	4 6 63	666		
F12T2.0	464	8.92	.100	9 7 63	631		
M01T3.0	314	9.15	.301	8 02 54		988	1879
M02T3.0	458	11.9	.301	28 09 54		859	1736
F03T3.0	471	12.0	.272	6 06 55		547	1164
M04T3.0	606	9.69	.285	18 10 55		801	1575
M05T3.0	571	10.7	.269	9 02 60		890	1584
F06T3.0	451	8.83	.282	10 1 61		1156	1909
M07T3.0	427	9.90	.266	7 2 61		861	1536
M08T3.0	494	10.1	.313	24 5 61		685	1566
F09T3.0	511	11.5	.298	29 6 61		1062	1933
F10T3.0	508	9.26	.280	28 7 61		971	1732
F11T3.0	518	10.3	.290	4 6 63	666		
F12T3.0	459	11.5	.320	9 7 63	631		
M01T4.0	480	8.32	.882	8 02 54		645	4237
M02T4.0	458	8.32	.916	28 09 54		833	5185
F03T4.0	461	7.25	.800	6 06 55		763	4290
M04T4.0	606	8.81	.835	18 10 55		793	4587
M01T5.0	480	9.48	2.76	8 02 54		212	5457
M02T5.0	483	8.22	2.63	28 09 54		97	2535

DOG
NUMBER

COMMENTS ON DEAD DOGS

M08T2.0 = OSTEOSARCOMA
F09T2.0 = OSTEOSARCOMA
F10T2.0 = OSTEOSARCOMA
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 = OSTEOSARCOMA
F10T3.0 = OSTEOSARCOMA
F11T3.0
F12T3.0

M01T4.0 = OSTEOSARCOMA + CRIPPLING FRACTURE
M02T4.0 = OSTEOSARCOMA, CRIPPLING FRACTURE + NEPHRITIS
F03T4.0 = 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 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F01S0.0	502	8.48		18	01	55	3725	
M02S0.0	600	11.1		14	02	56	3333	
M03S0.0	493	9.03		11	09	57	2758	
F04S0.0	520	8.19		15	10	57	2724	
M05S0.0	542	10.6		19	11	57	2689	
M06S0.0	466	9.68		27	05	58	2500	
F07S0.0A	462	9.46		7	01	59	2275	
F08S0.0	483	9.29		19	05	59	2143	
F09S0.0	549	12.4		11	08	59		708
F09S0.0A	535	11.2		4	6	63	666	
M10S0.0	522	13.9		29	09	59	2010	
F11S0.0	541	9.60		3	11	59	1975	
M12S0.0	605	8.99		6	01	60	1911	
F01S1.0	1524	6.84	.573	18	01	55		308
F01S1.0A	521	9.38	.588	14	02	56	3333	21
M02S1.0	567	8.81	.606	14	02	56	3333	
M03S1.0	493	10.9	.572	11	09	57	2758	
F04S1.0	525	8.96	.560	15	10	57	2724	
M05S1.0	555	10.2	.532	19	11	57	2689	
M06S1.0	466	9.56	.581	27	05	58	2500	
F07S1.0	524	9.94	.517	11	11	58	2332	
F08S1.0	483	10.8	.697	19	05	59	2143	
F09S1.0	549	11.6	.534	11	08	59	2059	
M10S1.0	522	11.5	.558	29	09	59	2010	
F11S1.0	543	10.3	.550	3	11	59	1975	
M12S1.0	607	13.7	.559	6	01	60	1911	
F01S1.7	526	7.41	1.78	14	02	56	3333	
M02S1.7	567	11.6	1.84	14	02	56	3333	
M03S1.7	493	9.19	1.69	11	09	57	2758	
F04S1.7	522	9.60	1.68	15	10	57	2724	
M05S1.7	560	9.85	1.60	19	11	57		1715
M05S1.7A	493	11.4	1.78	6	3	63	756	161
M06S1.7	466	10.6	1.72	27	05	58	2500	
F07S1.7	488	10.2	1.60	11	11	58	2332	
F08S1.7	472	8.47	2.03	19	5	59		1973
F09S1.7	549	10.0	1.62	11	08	59	2059	215
M10S1.7	519	13.6	1.66	29	09	59	2010	

DOG
NUMBER

COMMENTS ON DEAD DOGS

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

TRAUMA

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

SACRIFICED -IMPROPER INJECTION AGE-

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

COMA OF UNKNOWN ETIOLOGY

STATUS EPILEPTICUS, CHRONIC PANCREATITIS

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
F11S1.7	543	11.0	1.68	3	11	59	1975	
M12S1.7	607	11.9	1.68	6	01	60	1911	
F01S2.0	502	5.59	3.70	18	1	55	3269	480
M02S2.0	567	8.97	3.42	14	02	56	3333	
M03S2.0	494	7.82	3.39	11	09	57	2758	
F04S2.0	522	9.68	3.41	15	10	57	2724	
M05S2.0	560	8.72	3.24	19	11	57	2689	
M06S2.0	466	9.19	3.50	27	05	58	2500	
F07S2.0	488	11.2	3.19	11	11	58	2332	
F08S2.0	465	9.49	4.14	19	05	59	2143	
F09S2.0	473	14.1	3.28	11	08	59	2059	
M10S2.0	508	10.7	3.34	29	09	59	2010	
F11S2.0	543	10.4	3.41	3	11	59	1975	
M12S2.0	607	11.6	3.49	6	01	60	1911	
F01S3.0	468	7.36	11.6	18	01	55	3725	
M02S3.0	565	9.62	11.6	14	02	56	3333	
M03S3.0	494	11.4	10.8	11	09	57	2758	
F04S3.0	527	9.17	10.6	15	10	57	2724	
M05S3.0	557	8.90	10.1	19	11	57	2689	
M06S3.0	466	9.44	10.9	27	05	58	2500	
F07S3.0	486	9.80	10.1	11	11	58	2332	
F08S3.0	465	12.5	12.9	19	05	59	2143	
F09S3.0	468	10.0	10.1	11	08	59	2059	
M10S3.0	519	12.5	10.3	29	09	59	2010	
F11S3.0	541	9.00	10.8	3	11	59	1975	
M12S3.0	605	8.43	10.2	6	01	60	1911	
F01S4.0	468	8.74	33.3	18	01	55	3682	6832
M02S4.0	567	11.2	32.6	14	02	56	2093	5806
M03S4.0	593	9.83	32.1	11	09	57	2758	
F04S4.0	528	8.24	32.1	15	10	57	2724	
M05S4.0	562	9.65	30.6	19	11	57	2689	
M06S4.0	504	16.0	32.7	3	09	58	2401	
F07S4.0	478	10.9	30.9	11	11	58	2332	
F08S4.0	465	10.9	40.6	19	05	59	2143	

DOG
NUMBER

COMMENTS ON DEAD DOGS

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 NOT DETERMINED (NO OSTEOSARCOMA)
M02S4.0 SQUAMOUS CELL CARCINOMA -GINGIVA-
M03S4.0
F04S4.0
M05S4.0
M06S4.0
F07S4.0
F08S4.0

DOG NUMBER	AT INJECTION			DATE		DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	INJECTED			
				D	MO YR		
F09S4.0	468	9.56	30.6	11	08 59	2059	
M10S4.0	517	8.20	31.3	29	09 59	2010	
F11S4.0	542	8.86	32.7	3	11 59	1975	
M12S4.0	605	10.9	32.3	6	01 60	1911	
F01S5.0	434	9.38	103.	18	01 55	960	8127
M02S5.0	551	12.2	102.	14	02 56	255	3202
M02S5.0A	545	11.4	96.6	7	1 59	1740	12007
M03S5.0	507	10.3	102.	15	10 57	2256	16297
F04S5.0	528	11.4	105.	15	10 57	1448	9281
M05S5.0	621	8.53	95.2	19	11 57	1285	9930
M06S5.0	504	9.33	98.8	3	9 58	35	647
M06S5.0A	462	11.2	94.2	7	01 59	1021	11616
F07S5.0	478	10.2	92.7	11	11 58	1129	10822
F08S5.0	535	11.2	90.5	7	01 59	1469	10941
F09S5.0	459	8.82	93.5	11	08 59	1982	13569
M10S5.0	517	8.55	95.9	29	09 59	990	7744
F11S5.0	542	8.97	102.	3	11 59	1667	9956
M12S5.0	606	12.5	99.2	6	01 60	1165	8327

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	SQUAMOUS CELL CARCINOMA ARISING FROM FRONTAL SINUS
M10S5.0	SEVERE ANEMIA + THROMBOCYTOPENIA
F11S5.0	HEMANGIOSARCOMA (LEFT MANDIBLE)
M12S5.0	HEMANGIOSARCOMA (RIB)

TABLE II. TEST ANIMALS (MARCH 31, 1965)

A. RADIUM-226*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
T01R5.0	995	11.1	10.3	1 12 52	1074	9469
T02R5.0	919	8.40	4.39	12 01 53	1368	4724
T03R5.0	1467	8.29	4.76	12 01 53	428	1316
T04R5.0	459	10.0	10.6	6 07 53	1	24
T05R5.0	126	6.14	11.7	6 10 53	1	25
T06R5.0	126	6.14	11.4	6 10 53	1	24
T07R5.0	126	6.14	11.8	6 10 53	1	25
T08R5.0	290	5.52	1.92	10 05 55	58	488
T09R5.0	2276	10.4	1.94	10 05 55	58	585
T10R5.0	43	1.02	1.98	10 05 55	49	293
T11R5.0	43	1.58	1.91	10 05 55	49	363
T12R5.0	397	12.3	9.72	9 05 56	225	2707
T13R5.0	397	7.59	9.76	9 05 56	188	2319
T14R4.0	674	8.12	3.17	11 07 56	72	380
T15R4.0	672	9.03	3.11	11 07 56	2127	5117
T16R5.0	604	12.4	9.68	11 07 57	12	183
T17R5.0H	383	12.2	9.87	28 10 58	1147	13655
T18R5.0H	383	11.1	10.8	28 10 58	1226	12628
T19R5.0H	383	10.67	11.3	28 10 58	1219	11580
T20R5.0H	383	11.4	10.6	28 10 58	1330	12937
T21R5.0H	381	11.8	10.1	28 10 58	386	3710
T22R5.0H	381	11.9	10.1	28 10 58	587	5911
T23R4.0H	384	9.50	4.05	25 11 58	1471	4340
T24R4.0H	384	11.9	3.24	25 11 58	1505	5593
T25R4.0H	379	11.3	3.42	25 11 58	1309	4672
T26R4.0H	379	11.0	3.48	25 11 58	1780	4719
T27R4.0H	372	11.5	3.34	25 11 58	1414	3382
T28R3.0H	372	11.7	1.11	25 11 58	387	357
T29R5.0	474	13.5	10.4	3 03 59	216	3404

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01R5.0 - OSTEOSARCOMA
T02R5.0 OSTEOSARCOMA
T03R5.0 SPECIAL STUDY
T04R5.0 SPECIAL STUDY
T05R5.0 SPECIAL STUDY
T06R5.0 SPECIAL STUDY
T07R5.0 SPECIAL STUDY
T08R5.0 SPECIAL STUDY
T09R5.0 SPECIAL STUDY
T10R5.0 SPECIAL STUDY
T11R5.0 SPECIAL STUDY
T12R5.0 SPECIAL STUDY
T13R5.0 SPECIAL STUDY

T14R4.0 SPECIAL STUDY
T15R4.0 OSTEOSARCOMA

T16R5.0 SPECIAL STUDY
T17R5.0H OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T18R5.0H OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T19R5.0H OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T20R5.0H OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T21R5.0H NEPHRITIS
T22R5.0H CRIPPLING FRACTURES

T23R4.0H OSTEOSARCOMA
T24R4.0H OSTEOSARCOMA
T25R4.0H OSTEOSARCOMA
T26R4.0H OSTEOSARCOMA
T27R4.0H OSTEOSARCOMA

T28R3.0H SPECIAL STUDY

T29R5.0 NEPHRITIS

DOG NUMBER	AT INJECTION		INJECTED (μ c/KG)	DATE INJECTED			DAYS SINCE INJECTION		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR	31/3/65	DEATH	
T30R5.0	474	11.5	10.4	3	03	59		178	2851
T31R5.0	471	10.5	10.4	3	03	59		303	4758
T32R3.0	471	11.4	1.13	3	03	59	2220		
T33R3.0	471	10.6	1.15	3	3	59		1822	1921
T34R3.0	470	15.7	1.12	3	3	59		1737	1603
T35R3.0J	670	9.44	.951	5	5	59		8	14
T36R4.0	695	10.2	2.99	22	12	60		1154	3647
T37R4.0	695	9.53	3.00	22	12	60	1560		
T38R4.0	695	10.1	3.02	22	12	60		1503	3657
T40R1.0	899	13.0	.0483	3	4	62		7	1
T41R1.0	899	12.7	.0487	3	4	62		63	3
T42R1.7	967	14.0	.146	4	4	62		7	2
T43R1.7	963	13.2	.145	4	4	62		64	11
T44R3.0	938	11.1	.937	4	4	62		68	71
T45R3.0	939	13.6	.941	5	4	62		7	12
T46R3.0	810	12.5	.928	5	4	62		69	103
T47R6.0	99	5.27	29.4	11	6	62		4	352
T48R6.0	2842	11.2	25.1	27	12	62		49	1597
T49R5.0	485	10.6	7.54	2	5	63		5	111
T50R5.0	485	13.7	7.46	2	5	63		15	274
T51R5.0	418	13.3	8.48	8	5	63		92	1689
T52R5.0	418	10.7	8.57	8	5	63		15	258

DOG NUMBER	COMMENTS ON DEAD DOGS
T30R5.0	NEPHRITIS
T31R5.0	NEPHRITIS
T32R3.0	
T33R3.0	OSTEOSARCOMA, NEPHRITIS
T34R3.0	OSTEOSARCOMA
T35R3.0J	SPECIAL STUDY
T36R4.0	OSTEOSARCOMA
T37R4.0	
T38R4.0	OSTEOSARCOMA
T40R1.0	SPECIAL STUDY
T41R1.0	SPECIAL STUDY
T42R1.7	SPECIAL STUDY
T43R1.7	SPECIAL STUDY
T44R3.0	SPECIAL STUDY
T45R3.0	SPECIAL STUDY
T46R3.0	SPECIAL STUDY
T47R6.0	SPECIAL STUDY
T48R6.0	LEUKOPENIA, PNEUMONIA + SPECIAL MELANOMA STUDY
T49R5.0	SPECIAL STUDY
T50R5.0	SPECIAL STUDY
T51R5.0	SPECIAL STUDY
T52R5.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION		INJECTED (μ C/KG)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 - DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T53R5.0	418	12.0	8.50	8	5	63	33	611
T54R5.0	417	11.4	8.76	22	5	63	5	88
T55R5.0	417	11.6	8.61	22	5	63	33	534
T56R5.0	417	11.6	8.61	22	5	63	90	1461
T57R4.0	501	12.1	2.72	15	8	63	14	54
T58R4.0	496	11.7	2.41	15	8	63	61	286
T59R4.0	496	9.64	2.57	15	8	63	63	272
T60R4.0	490	12.1	2.33	15	8	63	117	415
T61R4.0	490	9.48	2.70	15	8	63	371	1567
T62R4.0	490	8.63	2.68	15	8	63	460	1586
T63R3.0	559	8.72	.899	29	1	64	36	51
T64R3.0	551	8.42	.919	29	1	64	63	73
T65R3.0	551	11.6	.922	29	1	64	70	99
T66R3.0	549	10.1	.904	29	1	64	132	140
T67R3.0	549	12.7	.898	29	1	64	134	168
T68R3.0	549	12.1	.917	29	1	64	427	
T69R3.0	499	8.84	.919	29	1	64	427	
T70R3.0	499	14.2	.922	29	1	64	427	

*The multiple injection dogs were male beagles born in Davis, California but injected in our laboratory. Each was injected 6 times over a 280 day period with 56 days between each injection. Each Ra^{226} injection was 20.0 μ C for the dogs T17R5H - T22R5H; 6.41 μ C for T23R4H - T27R4H; and 2.16 μ C for T28R3H. Tabulated for each dog are his age at 1st injection, his average weight during the injection period, total μ C/average weight, the date of 1st injection, the time from 1st injection to death, and sum of the skeletal doses computed from each injection to death.

T35R3J also received 99 μ C 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	SPECIAL STUDY
T62R4.0	SPECIAL STUDY

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

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
T00P5.0	647	11.4	3.05	24 06 52	1	10
T01P5.0	581	12.7	3.04	13 10 52	29	160
T02P5.0	914	11.9	6.85	15 09 52	44	520
T03P5.0	942	9.65	3.22	13 10 52	610	3040
T04P5.0	1016	8.78	3.02	13 10 52	365	1750
T05P5.0	474	10.4	2.69	14 12 54	400	1720
T06P5.0	527	6.16	2.73	14 12 54	406	1750
T07P5.0	475	7.40	2.68	14 12 54	777	3230
T08P5.0	527	8.32	2.67	14 12 54	863	3540
T09P5.0	551	10.3	2.80	22 11 55	15	80
T10P5.0	534	11.9	2.74	23 11 55	15	70
T11P5.0	516	12.1	2.76	22 11 55	28	140
T12P5.0	487	9.23	2.74	23 11 55	28	140
T13P5.0	587	8.27	3.16	24 04 56	3	20
T14P5.0	587	9.38	2.43	24 04 56	7	30
T15P5.0	737	8.32	2.79	15 10 56	1	10
T16P5.0	673	10.7	2.85	10 10 56	92	440
T17P5.0	739	11.1	3.01	12 02 57	210	990
T18P5.0	739	8.16	2.83	12 02 57	217	960
T19P5.0	688	8.86	2.91	15 12 60	1400	6129
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	3
T24P1.0	559	13.1	.0172	28 7 61	97	3
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	1

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	OSTEOSARCOMA, BLOOD DYSCRASIA, LIVER DEGENERATION
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 (MC/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 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	4
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
T41P5.0	543	8.50	3.01	30 11 64	121	
T42P5.0	510	11.4	2.40	10 2 65	13	57

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

T41P5.0

T42P5.0 SPECIAL STUDY

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{c/KG}$)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T01M4.5	529	9.13	4.23	8	09	54	314	1598
T02M4.5	463	8.93	4.27	8	09	54	755	5097
T03M5.0	579	9.15	10.6	13	03	56	700	15867

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01M4.5 CANINE DISTEMPER
T02M4.5 SPECIAL STUDY

T03M5.0 ULCERATIVE GINGIVITIS, SEVERE ANEMIA + CRIPPLING FRACTURE

D. THORIUM-228 (RADIOTHORIUM)

DOG NUMBER	AT INJECTION		INJECTED ($\mu\text{c/KG}$)	DATE INJECTED			DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)		D	MO	YR		
T01T5.0	490	9.30	4.88	1	12	53	23	1161
T02T5.0	501	8.48	2.56	8	02	54	77	1979
T03T4.0	429	10.4	.870	8	02	54	820	4878
T04T5.0	455	8.92	2.59	28	09	54	113	2883
T05T5.0	455	10.1	2.32	28	09	54	65	1524
T06T4.0	591	7.01	.884	18	10	55	651	4273
T07T3.0	591	9.23	.298	18	10	55	910	1777
T08T3.0	606	9.23	.293	14	10	58	1043	1883
T09T3.0	447	11.0	.285	4	02	59	1	3
T10T3.0	447	14.2	.289	4	02	59	8	24
T11T3.0	500	8.62	.335	16	6	59	22	76
T12T3.0	514	10.6	.302	7	7	59	22	69
T13T3.0	754	13.1	.298	28	7	59	22	68

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01T5.0 DIED, SPECIAL STUDY
T02T5.0 SPECIAL STUDY

T03T4.0 CRIPPLING FRACTURES + NEPHRITIS

T04T5.0 THROMBOCYTOPENIA + PURPURA
T05T5.0 NEPHRITIS, THROMBOCYTOPENIA + PURPURA

T06T4.0 CRIPPLING FRACTURES

T07T3.0 SPECIAL STUDY
T08T3.0 OSTEOSARCOMA
T09T3.0 SPECIAL STUDY
T10T3.0 SPECIAL STUDY
T11T3.0 SPECIAL STUDY
T12T3.0 SPECIAL STUDY
T13T3.0 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 31/3/65 DEATH	DOSE TO SKELETON (RADS)
T01S0.0	151	7.71		5 03 54	2	
T02S5.0	149	6.85	148.	5 03 54	11	626
T03S5.0	144	6.19	148.	5 03 54	18	957
T04S5.0	151	7.05	148.	5 03 54	41	1945
T05S5.0	144	5.25	148.	5 03 54	116	4863
T06S5.0	155	7.01	87.0	16 03 54	1 hr	2
T07S5.0	155	6.74	87.0	16 03 54	2	84
T08S0.0	243	7.00				
T08S2.0H	67	3.69	2.74	27 09 55	66	31
T09S2.0H	67	2.79	3.62	27 09 55	66	40
T10S2.0H	67	3.11	3.25	27 09 55	132	93
T11S2.0H	67	3.85	2.62	27 09 55	132	75
T12S3.0	593	10.6	10.5	11 09 57	5	15
T13S4.0	324	10.5	19.1	8 07 60	8	50
T14S5.0	542	10.0	96.1	7 11 61	9	175
T15S5.0	595	9.43	98.4	7 11 61	30	423
T16S2.0	604	9.71	3.27	8 11 61	9	6
T17S6.0	670	7.18	295.	19 1 62	14	689
T18S6.0	670	5.94	302.	19 1 62	1167	

DOG NUMBER	COMMENTS ON DEAD DOGS.
---------------	------------------------

T01S0.0	SPECIAL STUDY
---------	---------------

T02S5.0	SPECIAL STUDY
---------	---------------

T03S5.0	SPECIAL STUDY
---------	---------------

T04S5.0	SPECIAL STUDY
---------	---------------

T05S5.0	SPECIAL STUDY
---------	---------------

T06S5.0	SPECIAL STUDY
---------	---------------

T07S5.0	SPECIAL STUDY
---------	---------------

T08S .0	SPECIAL STUDY
---------	---------------

T08S2.0H	SPECIAL STUDY
----------	---------------

T09S2.0H	SPECIAL STUDY
----------	---------------

T10S2.0H	SPECIAL STUDY
----------	---------------

T11S2.0H	SPECIAL STUDY
----------	---------------

T12S3.0	BREMSSTRAHLUNG PHANTOM
---------	------------------------

T13S4.0	BREMSSTRAHLUNG PHANTOM SAM MCGEE
---------	----------------------------------

T14S5.0	SPECIAL STUDY
---------	---------------

T15S5.0	SPECIAL STUDY
---------	---------------

T16S2.0	SPECIAL STUDY
---------	---------------

T17S6.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
---------	--

T18S6.0	
---------	--

DOG NUMBER	AT INJECTION			DATE			DAYS SINCE		DOSE TO SKELETON (RADS)
	AGE (DAYS)	WEIGHT (KG)	INJECTED ($\mu\text{C}/\text{KG}$)	INJECTED D MO YR	31/3/65	DEATH			
T19S6.0	670	5.43	284.	19	1	62	24		637
T20S4.0J	440	8.54	28.9	2	10	63	13		78
T21S2.5J	363	7.20	8.3	2	10	63	13		143

* T08 ... 11S2.0H were given 10 injections, 1 μC each at weekly intervals. Age is at first injection, wt. is average during the injection period, $\mu\text{C}/\text{kg}$ is total Sr^{90} /average weight, date is at first injection, days are from first injection to death, and dose is computed from mid-injection to death.

T20S4.0J received 0.5 μC Sr^{85} .

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

DOG
NUMBER

COMMENTS ON DEAD DOGS

T19S6.0 LEUKOPENIA, THROMBOCYTOPENIA + PURPURA

T20S4.0J SPECIAL STUDY

T21S2.5J SPECIAL STUDY

F. RADIUM-224

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μ C/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
T01Q3.0J	460	9.55	.875	26 3 63	4 hr	
T02Q4.0	466	12.0	2.91	27 3 63	735	
T03Q4.0	466	13.1	2.91	27 3 63	735	
T04Q5.0	480	9.55	9.71	24 4 63	707	
T05Q5.0	455	9.67	9.59	24 4 63	707	
T06Q6.0	455	8.29	24.6	17 10 63	13	980
T07Q5.0	465	11.8	9.83	6 11 63	511	
T08Q5.0	475	9.77	9.91	6 11 63	16	400
T09Q4.0	503	9.80	2.96	4 12 63	483	
T10Q4.0	503	10.3	2.96	4 12 63	262	133
T11Q3.0	495	9.10	1.02	4 12 63	483	
T12Q3.0	495	13.5	1.02	4 12 63	483	
T13Q3.0	495	11.3	1.02	4 12 63	483	
T14Q3.0	438	10.3	1.00	4 12 63	483	

T01Q3.0J received 18.0 μ C Sr^{85} .

DOG
NUMBER

COMMENTS ON DEAD DOGS

T01Q3.0J SPECIAL STUDY

T02Q4.0
T03Q4.0

T04Q5.0
T05Q5.0

T06Q6.0 = PURPURA HEMORRHAGICA

T07Q5.0
T08Q5.0 = PURPURA HEMORRHAGICA

T09Q4.0
T10Q4.0 STATUS EPILEPTICUS

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

G. ANCILLARY

DOG NUMBER	AT INJECTION AGE WEIGHT (DAYS) (KG)	INJECTED (MC/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/65 DEATH	DOSE TO SKELETON (RADS)
F01A0.0	.			1383	
F02A0.0	.			2492	
M03A0.0	.			1451	
M04A0.0	.			3345	
M05A0.0	.			4713	
M06A0.0	.			5266	
M07A0.0	.			3896	
M08A0.0	.			3746	
F09A0.0	.			3719	
F10A0.0	.			2605	
F11A0.0	.			4198	
F12A0.0	.			4219	
F13A0.0	.			4527	
F14A0.0	.			3777	
F16A0.0	.			4415	
F17A0.0	.			2145	
F19A0.0	.			4166	
F20A0.0	.			2464	
M23A0.0	.			1741	
M24A0.0	.			3074	
M26A0.0	.			4133	
M27A0.0	.			2130	
M28A0.0	.			3114	
F32A0.0	.			1990	
F33A0.0	.			3282	
F34A0.0	.			2584	
M35A0.0	.			529	
M36A0.0	.			1971	
M37A0.0	.			4089	
M42A0.0	.			1265	
F43A0.0	.			3881	
F47A0.0	.			1732	
M50A0.0	.			2264	
F51A0.0	.			1089	
F52A0.0	.			509	
F54A0.0	.			3190	
M56A0.0	.			701	
M58A0.0	.			767	
M59A0.0	.			567	
M73A0.0	.			5695	
M86A0.0	.			499	

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

DOG:

NUMBER -

COMMENTS ON DEAD DOGS

F01A0.0	SPECIAL STUDY
F02A0.0	SPECIAL STUDY
M03A0.0	SPECIAL STUDY
M04A0.0	NOT DETERMINED
M05A0.0	TRANSITIONAL CELL CARCINOMA, NEPHRITIS, PNEUMONIA
M06A0.0	BRAIN HEMORRHAGE
M07A0.0	LYMPHOSARCOMA
M08A0.0	PROGRESSIVE PARALYSIS, CAUSE UNKNOWN
F09A0.0	VAGINAL FIBROMA
F10A0.0	SPECIAL STUDY
F11A0.0	MAMMARY CARCINOMA
F12A0.0	SEVERE OSTEOARTHRITIS
F13A0.0	SPECIAL STUDY
F14A0.0	OBTURATING EMBOLISM OF PORTAL VEIN
F16A0.0	SPECIAL STUDY
F17A0.0	TRAUMA
F19A0.0	MAMMARY GLAND CARCINOMA
F20A0.0	SPECIAL STUDY
M23A0.0	OBTURATING PULMONARY EMBOLISM
M24A0.0	SPECIAL STUDY
M26A0.0	SEMINOMA
M27A0.0	SPECIAL STUDY
M28A0.0	SPECIAL STUDY
F32A0.0	LYMPHOSARCOMA
F33A0.0	OBTURATING PULMONARY EMBOLISM
F34A0.0	SPECIAL STUDY
M35A0.0	SPECIAL STUDY
M36A0.0	SPECIAL STUDY
M37A0.0	SPECIAL STUDY
M42A0.0	STATUS EPILEPTICUS
F43A0.0	SPECIAL STUDY
F47A0.0	SPECIAL STUDY
M50A0.0	SPECIAL STUDY
F51A0.0	SPECIAL STUDY
F52A0.0	SPECIAL STUDY
F54A0.0	SPECIAL STUDY
M56A0.0	VOLVULUS + PERITONITIS
M58A0.0	SPECIAL STUDY
M59A0.0	SPECIAL STUDY
M73A0.0	DEGENERATION OF ADRENAL GLAND + DIABETES MELLITUS
M86A0.0	SPECIAL STUDY

CURRENT CENSUS OF THE BEAGLE COLONY

March 31, 1965

Assignment	Number of Dogs
Plutonium, toxicity (Pu-239)	47
Plutonium, test (Pu-239)	1
Radium, toxicity (Ra-226)	41
Radium, test (Ra-226)	5
Quickradium, test (Ra-224)	10
Radiothorium, toxicity (Th-228)	52
Radiothorium, test (Th-228)	0
Mesothorium, toxicity (Ra-228)	48
Mesothorium, test (Ra-228)	0
Strontium, toxicity (Sr-90)	68
Strontium, test (Sr-90)	1
Controls, aging	48
Controls, x-ray	5
Unassigned (Male - 44, Female - 45)	89
	<hr/>
	415

THE USE OF THERAPY IN EXPERIMENTAL ANIMALS

G. N. Taylor

Abstract: The use of prophylactic treatment and therapy in our large beagle colony is on much the same basis as would be expected in a human or veterinary practice and has been a significant factor in minimizing losses and variation from non-radiation induced factors. It has been indispensable in achieving the maximum life span in a high percentage of our animals. Also, it has been a significant feature in providing the most humane care for our colony.

Prophylactic and therapeutic care constitute one of the important aspects in the management of the large beagle colony at this laboratory. Such treatment has proved to be compatible with the objectives of our studies and has reduced the losses and variations that arise from naturally occurring diseases and accidental factors. Longevity is an important item in our investigation and the costliness of premature death from extraneous causes is obvious--especially in animals that have been observed for five to ten years. An additional consideration in our use of therapy is the manner in which it has facilitated humane treatment. It is very unlikely that such therapy and prophylaxis have influenced the pathogenesis of the radiation induced syndrome.

The prophylactic phase of our colony management has many facets, but two of the most important are isolation and immunization. A relatively isolated environment has been achieved by strict control of human and outside animal contact--especially dogs, the inclusion of a beagle breeding colony within the confines of our laboratory has been fundamental in this regard by removing the hazard of introducing outside animals, which in some instances can be analogous to the "Trojan horse". It has also provided us with a more uniform colony of known genotype,

age and clinical history. The most serious gap in our environmental isolation and the most difficult to control is the presence of vermin. However, thus far, these agents have been kept to low numbers by the frequent application of high pressure steam, window and door screens, etc., and a high degree of cleanliness within the kennel. Insecticides, etc., are not used in the proximity of the colony.

Our immunization procedures are directed toward canine distemper, infectious canine hepatitis, and two strains of leptospirosis, three of the more common canine diseases. The respective antigens are given three times during the first year and annually, thereafter. Passive immunization is given if a severe challenge is suspected. Losses from these conditions are rare.

Closely related to the prophylactic procedures in our colony management is the provision of therapy. The indications include infections--usually non-contagious, trauma, neoplasia, senility, etc. Many of these are non-radiation induced and constitute fortuitous variables. However, their significance can oftentimes be markedly reduced by treatment and with a few exceptions it is the policy at this laboratory to employ such therapy. It can be argued that this in itself constitutes a variable, but we like to think that our treatments are a little less severe than the disease; that a brief course of antibiotics as the alternative to a serious infection is the lesser of two evils. Based on this assumption, we treat most disease syndromes in much the same manner as they would be managed in a private veterinary hospital, including the use of well established drugs and surgical procedures.

One of the most important indications for surgery has been the removal of soft tissue tumors--the incidence of which increases abruptly

beyond six to seven years of age. We do not feel that this obscures any pertinent endpoints since the procedures can oftentimes be performed with few consequences and since our principal interest is in the incidence, the type of tumor, and its latent period--not its growth pattern, etc. We gain this information as long as the excised tissues are evaluated histologically.

A drug that is employed routinely, not therapeutically but for restraint purposes, is sodium pentothal*. It is used primarily in conjunction with radiology and whole body counting procedures. Serious compromises in certain aspects of our program would be necessary if this or a similar general anesthetic could not be used. Clinical, hematological, and blood chemistry studies have not indicated any measurable residual effects from this agent.

Notable exceptions in our use of therapy are bone tumors and radiation induced fractures. These are not treated except for the provision of individual housing, supportive therapy, and analgesics. Radiation induced blood dyscrasias are treated only in certain instances. Bone and/or marrow biopsies are not made. Radiation therapy is never used.

In summary it is the experience of this laboratory that prophylaxis and therapy are desirable if not indispensable factors in the management of our large canine colony--especially in those animals which are to be studied for long periods. Such treatment is part of our overall attempt to provide an environment and care such that the maximum number of animals will die of radiation induced disease or senile changes and not from naturally occurring factors.

*Pentothal sodium, Abbott Laboratories, North Chicago, Illinois.

DENTAL ERUPTION PATTERN OF THE BEAGLE

L. Shabestari, G. N. Taylor and W. Angus

Abstract: Eruption of the deciduous teeth began at ~ three weeks and was completed by the end of the fifth week. The canines appeared first and were succeeded by the incisors and then the molars.

Exfoliation of the temporary teeth began at ~ 115 days and was invariably preceded by eruption of upper permanent premolar 1. The last tooth of the permanent dentition to appear was the third molar - erupting at ~ 175 days.

INTRODUCTION

Most studies which present the dental eruption pattern of the dog are a composite of observations taken from several breeds^(1,2). It is the purpose of this paper to present the eruption time as observed in a single breed, the beagle.

METHODS

Thirty-three healthy purebred beagles, raised at this laboratory, were used in this study. Observations were made triweekly from birth until the eruption of the permanent dentition.

Eruption was taken as the time of gingival perforation and tooth loss as the time of natural exfoliation. Deciduous teeth were never extracted.

Identification of teeth was according to the following dental formulae^(3,4):

<u>Deciduous</u>	<u>Permanent</u>
$I \frac{3}{3} C \frac{1}{1} M \frac{3}{3}$	$I \frac{3}{3} C \frac{1}{1} PM \frac{4}{4} M \frac{2}{3}$

The first premolar was considered to erupt without a deciduous precursor⁽⁵⁾ (Figs. 1 and 2).

RESULTS

The eruption patterns are summarized in Tables 1 and 2. These data indicate the following.

During the third week of life firm swellings were palpable beneath the gums on the occlusal surface. The canines, generally the upper, were the first to appear, followed by the corner, intermediate, and central incisors. The upper arcade erupted first.

As the last of the incisors appeared, the cheek teeth erupted - deciduous molar 2 first, followed by deciduous molar 3 and deciduous molar 1. Unlike the canines and incisors the lower cheek teeth erupted before the upper counterpart. By the end of the 5th week a full set of deciduous teeth was present.

Little change occurred during the next two months. However, the space between the teeth widened as the length of the jaws increased.

The exfoliation and replacement of the deciduous teeth was preceded by the eruption of permanent upper premolar 1. This was a constant feature. Shortly thereafter the incisors were lost - first the central, followed by the intermediate and corner incisors. The order was opposite to the sequence in which they erupted. Permanent incisors appeared a day or two after their precursors were lost.

Molar 1 and lower premolar 1 were the next teeth to appear. Deciduous molars were then exfoliated - generally the upper before the lower. The permanent teeth appeared in a few days following exfoliation. However, permanent premolar 2 was occasionally visible medial to deciduous molar 1 for several days before the latter was lost.

The permanent canines erupted about the same time as the cheek teeth. Exfoliation of the lower deciduous canine revealed its underlying

permanent successor. However, the upper deciduous canine remained posterior to the permanent tooth for several days and occasionally for several weeks. The time of its loss was quite variable.

Permanent molar 2 erupted about 2 weeks after the first molar. The last tooth to appear was molar 3 which generally erupted about three weeks after the other dentition was present.

Table 1. Age in days at eruption (T) and of exfoliation (L) of deciduous teeth. Standard deviations ($\pm$) are shown in parentheses.

		Upper		Lower	
		left	right	left	right
Incisors	1	T 25 (± 3)	25 (± 3)	30 (± 4)	30 (± 4)
	L 116 (± 7)	115 (± 6)	118 (± 8)	118 (± 8)	
	2	T 24 (± 3)	24 (± 3)	29 (± 4)	29 (± 4)
	L 124 (± 7)	124 (± 6)	123 (± 7)	123 (± 7)	
	3	T 23 (± 3)	23 (± 3)	27 (± 4)	27 (± 4)
	L 132 (± 8)	132 (± 10)	130 (± 6)	130 (± 6)	
Canines	T 22 (± 3)	22 (± 3)	23 (± 3)	24 (± 3)	
	L 155 (± 12)	158 (± 15)	145 (± 6)	145 (± 7)	
Molars	1	T 36 (± 6)	36 (± 6)	31 (± 6)	31 (± 6)
	L 148 (± 12)	148 (± 12)	153 (± 8)	153 (± 8)	
	2	T 30 (± 5)	30 (± 5)	28 (± 7)	28 (± 7)
	L 146 (± 9)	146 (± 9)	153 (± 9)	153 (± 8)	
	3	T 35 (± 5)	35 (± 5)	29 (± 6)	29 (± 6)
	L 138 (± 6)	138 (± 6)	157 (± 9)	157 (± 9)	

Table 2. Age in days at eruption of permanent dentition.
Standard deviations (+) are shown in parentheses.

		Upper		Lower	
		left	right	left	right
Incisors	1	120 (+8)	120 (+8)	118 (+7)	118 (+7)
	2	124 (+6)	125 (+5)	125 (+6)	125 (+6)
	3	132 (+7)	133 (+6)	135 (+7)	135 (+7)
Canines		147 (+8)	147 (+8)	145 (+6)	145 (+6)
Premolars	1	111 (+10)	111 (+10)	129 (+11)	129 (+11)
	2	144 (+10)	144 (+10)	138 (+11)	138 (+10)
	3	153 (+9)	153 (+10)	155 (+7)	156 (+8)
	4	136 (+8)	137 (+8)	155 (+9)	155 (+9)
	1	136 (+8)	136 (+8)	130 (+6)	130 (+7)
	2	155 (+6)	155 (+6)	149 (+8)	149 (+8)
	3			174 (+14)	174 (+14)

REFERENCES

1. E. M. Crawford, Preliminary study of dentition in dogs, *Veterinary Medicine*, 32:212-217 (1937).
2. L. Arnall, Some aspects of dental development in the dog: Calcification of crown and root in the deciduous dentition, *J. Small Animal Practice*, 1:169-173 (1961).
3. M. E. Miller, Guide to the Dissection of the Dog, ed. 2, Ithaca, New York, Edward Brothers, Inc., pp. 32-34 (1952).
4. S. Sisson and J. D. Grossman, The Anatomy of the Domestic Animals, W. B. Saunders Co., Philadelphia and London, pp. 972 (1938).
5. L. E. St. Clair and N. D. Jones, Observations on the cheek teeth of the dog, *J.A.V.M.A.* 130:275-279 (1957).

Fig. 1. Radiograph of purebred beagle showing complete deciduous dentition of the upper arcade and the underlying non-erupted permanent incisors (a), canine (b), premolars (c-f) and molars (g-h). 83 days age.

Fig. 2. Radiograph of purebred beagle showing deciduous incisors, canines, and molar teeth of lower arcade and the underlying non-erupted permanent incisors (a), canine (b), premolars (c-f) and molars (g,h,i). Same dog as Fig. 1.

THORIUM-228 INDUCED FRACTURES IN BEAGLES

G. N. Taylor, W. S. S. Jee, W. R. Christensen,
C. E. Rehfeld and N. Nebeker

Abstract: Significant numbers of pathological fractures were induced in beagles by a single intravenous injection of $\sim 0.3 \mu\text{c/kg}$ or $\sim 0.9 \mu\text{c/kg}$ Th^{228} . Fractures were not observed at the next higher level, $2.7 \mu\text{c/kg}$ because of early death from radiation induced nephritis and/or blood dyscrasia. Only one fracture occurred at the $\sim 0.1 \mu\text{c/kg}$ level in nine dogs observed and no fractures were observed below this dose. This lowest level at which a fracture occurred was approximately 6 times higher than the dose which has induced osteosarcomas, thus far.

INTRODUCTION

Five of the most obvious lesions induced by Th^{228} in the dog are osteosarcoma⁽¹⁾, radiation induced nephritis⁽²⁾, blood dyscrasia⁽³⁾, ulcerative gingivitis⁽⁴⁾, and pathological fractures. It is the purpose of this paper to summarize the latter category according to the incidence and anatomical distribution of such fractures.

METHODS

All of the animals used in this study were purebred beagles that were born and reared on this project. The cause of death in most instances was osteosarcoma, but notable exceptions occurred in the 2 highest levels (see Injection Tables that preface this report).

The fracture incidence was determined from post mortem radiographs of the defleshed skeleton. These terminal films facilitated fracture recognition. Also, the total number of fractures was a maximum value for the respective dogs. Evaluation of some healed fractures that were known to have occurred several years previously indicated recognition was still unmistakable at autopsy. In vivo radiographs spaced at approximately 6 month intervals were used to determine the earliest fracture.

The percentages illustrated in the Tables and Figures were calculated by dividing the total number of fractures occurring in a specific bone by the total number of these bones present in the given dose level being considered and multiplying by 100. For example, if 7 dogs were observed at a given dose level the total number of bones used in computing the percentage of ilial fractures would be 14. Twenty-six ribs per dog was used in computing the percentage rib fractures.

Incomplete or fatigue-like fractures were omitted. Those shown for the thoracic vertebrae were limited entirely to the dorsal spinous processes, and those of the lumbar vertebrae involved only the transverse processes.

The photomicrographs are of celloidin sections, prepared from decalcified bone, and stained with hematoxylin and eosin.

At the time of injection all epiphyses except those of the ribs were closed. In many cases additional growth occurred at the costochondral region following treatment with the radionuclide.

Administration of the radionuclide was via a single intravenous injection prepared according to methods described elsewhere⁽⁵⁾. The word "dose" as used in this study refers to this injected μC dose.

RESULTS AND DISCUSSION

A significant number of pathological fractures occurred in 2 of the doses studied - 0.3 $\mu\text{C}/\text{kg}$ and 0.9 $\mu\text{C}/\text{kg}$ (Fig. 1 and Table 1). Short survival times resultant to radiation induced blood dyscrasia, nephritis, and other less clearly apparent lesions precluded fractures at higher levels. Only one fracture was observed below these doses - a rib fracture in a dog injected with 0.0879 $\mu\text{C}/\text{kg}$.

Individual variation in the incidence and distribution was marked.

The total number of fractures per animal varied from 4 to 36 at the 0.9 $\mu\text{C}/\text{kg}$ dose, and 0 to 19 at the 0.3 $\mu\text{C}/\text{kg}$ dose. Three of the 10 dogs observed at this latter level did not develop any fractures.

In the rib cage one important cause of such variation was the different degree of rib growth that occurred after injection of the radionuclide. As noted under METHODS, the costochondral epiphysis in many of the animals had not closed by the time of radionuclide treatment - a factor that was influenced by individual growth patterns, etc., and especially by the nonuniform ages at injection (Table 2). The junction between the pre-injection and post-injection bone in this area was highly susceptible to fracture and the incidence was in direct relationship to the amount of post-injection bone growth. Such fractures have been referred to as "hot line" fractures (6).

The growth potential that oftentimes existed in the ribs at the time of injection also accounted for an interesting dose effect. This is shown in Table 3, which categorizes the rib fractures according to the quarter of the rib in which they occurred. Fractures induced by the 0.9 $\mu\text{C}/\text{kg}$ treatment were limited to the proximal three-fourths. Those produced by the 0.3 $\mu\text{C}/\text{kg}$ dose occurred in all regions, but dominantly as "hot line" fractures in the distal quarter. This dose effect was the result of radiation induced modification of rib growth in the costochondral region. No apparent change was induced by the 0.09 $\mu\text{C}/\text{kg}$ dose (Fig. 2). Elongation continued following the 0.3 $\mu\text{C}/\text{kg}$ dose, but a very prominent "hot line" was formed (Fig. 3) and remodeling in the post-injection bone was abnormal. The remodeling defect was evidenced, in part, by the presence of cartilaginous cores in many of the trabeculae - a feature which was most extreme in the region of the "hot line" (Fig. 4).

The "hot line" was further characterized by a high percentage of necrotic bone and a fibrous marrow. This zone was the most susceptible fracture site in the rib cage. An abrupt transition into viable marrow and viable bone occurred in the post-injection bone immediately distal to this "line", denoting that the uptake of thorium daughters (7,8) was below the amount required to destroy these types of tissue. The next higher dose, 0.9 $\mu\text{c}/\text{kg}$, completely prevented post-injection growth in this region and "hot line" fractures did not occur, although they were numerous in the proximal three-fourths (Fig. 5).

Individual variation plus the relatively small number of dogs observed prevented precise conclusions relative to specific bones, etc. Nevertheless, the data indicated the following more general factors:

- 1) Thorium-228 induced fractures were distributed over a wide range of anatomical sites in both the appendicular and axial skeleton.
- 2) The threshold above which fractures were induced was approximately 0.09 $\mu\text{c}/\text{kg}$.
- 3) The high number of long bone fractures induced by the 0.9 $\mu\text{c}/\text{kg}$ dose was one of the extreme aspects of this dose level, and was a cause for euthanasia in several instances.
- 4) The tendency toward fracture repair was low.
- 5) Thorium-228 induced fractures produced a minimal inflammatory response. The slight amount of pain associated with them was especially obvious - a feature in common with radium (Ra^{226} and Ra^{228}) and Pu^{239} induced fractures (9-11).
- 6) In comparison to 4 other bone-seeking radionuclides studied at this laboratory (Ra^{226} , Ra^{228} , Pu^{239} , and Sr^{90}) Th^{228}

fractures most nearly resembled those of Ra^{228} except for the unique "hot line" fractures produced by the $0.3 \mu\text{c/kg}$ dose which were similar to those of Pu^{239} . Except for the "hot line" rib fractures, differentiation between Th^{228} and Ra^{228} fractures based on the anatomical distribution, healing incidence and general morphological features of the fractures would not be possible.

- 7) The dose which produced only 1 fracture in 9 dogs, $.09 \mu\text{c/kg}$, was approximately 6 times higher than that at which osteosarcomas have occurred thus far. Since many of the lower level animals are still living, this span may become even greater. Thus the occurrence of even a single pathological fracture was a very ominous sign.
- 8) The earliest fracture was observed within the following post-injection intervals (days):

Dose Level	Axial Skeleton	Appendicular Skeleton
$\sim 0.9 \mu\text{c/kg}$	--- to 294 (rib)	395 to 477 (ulna)
$\sim 0.3 \mu\text{c/kg}$	197 to 366 (spinous process)	386 to 591
$\sim 0.1 \mu\text{c/kg}^*$	972 to 1108 (rib)	(none)

*Only one fracture occurred at this level in nine dogs observed.

ACKNOWLEDGEMENTS

The authors wish to thank P. Petermann and W. Angus for their technical assistance.

REFERENCES

1. T. F. Dougherty, Incidence of bone cancer in internally irradiated dogs, Some Aspects of Internal Irradiation, Pergamon Press, Oxford, pp, 47-61 (1962).

2. T. H. Cochran, Histopathological findings, University of Utah, Radiobiology Laboratory Report, AECU-3522, pp. 73-84 (Mar 1957).
3. J. H. Dougherty, Some hematological responses to internal irradiation in the beagle, Some Aspects of Internal Irradiation, Pergamon Press, Oxford (1962).
4. G. N. Taylor, W. R. Christensen, W. S. S. Jee, and C. E. Rehfeld, Gingival ulceration in beagles induced by Ra-226, Ra-228 or Th-228, J. Dental Res., 43:35-43 (1964).
5. T. F. Dougherty, B. J. Stover, J. H. Dougherty, W. S. S. Jee, C. W. Mays, C. E. Rehfeld, W. R. Christensen, and H. C. Goldthorpe, Studies of biological effects of Ra^{226} , Pu^{239} , Ra^{228} (MsTh), Th^{228} (RdTh), and Sr^{90} in adult beagles, Radiation Res., 17:625-681 (1962).
6. W. S. S. Jee and J. S. Arnold, The toxicity of plutonium deposited in skeletal tissues of beagles (I), Lab. Invest. 10:797-825 (1961).
7. B. J. Stover, D. R. Atherton, N. Keller, and D. Buster, Metabolism of the Th-228 decay series in adult beagle dogs, Radiation Res. 12: 657-671 (1960).
8. C. W. Mays, R. Halden, and M. A. Van Dilla, Thoron exhalation in radiothorium-burdened beagles, Radiation Res. 9:439-444 (1958).
9. G. N. Taylor, W. R. Christensen, W. S. S. Jee, C. E. Rehfeld and W. Fisher, Anatomical distribution of fractures in beagles injected with Pu-239, Health Physics, 8:609-613 (1962).
10. G. N. Taylor, W. R. Christensen, W. S. S. Jee, C. E. Rehfeld and P. Petermann, Intercomparison of pathological fractures in beagles injected with Ra-226, Ra-228, Pu-239 or Sr-90, Health Physics (in press).
11. C. E. Rehfeld, B. J. Stover, G. N. Taylor and C. W. Mays, Fracture incidence in beagles receiving single injections of radium or plutonium, Some Aspects of Internal Irradiation, Pergamon Press, Oxford (1962).

Table 1. Incidence and distribution of healed (H) and unhealed (U) fractures following a single intravenous injection of Th^{228} . The values indicate the fractures per site that occurred in the number of dogs indicated in parentheses.

		Thorium ~ 0.1 $\mu\text{c}/\text{kg}$ (9 Dogs)		Thorium ~ 0.3 $\mu\text{c}/\text{kg}$ (10 Dogs)		Thorium ~ 0.9 $\mu\text{c}/\text{kg}$ (4 Dogs)	
		%		%		%	
		U	H	U	H	U	H
<u>Axial Skeleton</u>							
Mandibulae		-	-	-	-	-	-
Head (except Mand.)		-	-	-	-	-	-
Cervical Vertebrae		-	-	-	-	-	-
Thoracic Vertebrae							
	1	-	-	1	-	1	-
	2	-	-	1	-	-	-
	3	-	-	3	-	-	-
	4	-	-	3	-	-	-
	5	-	-	2	-	1	-
	6	-	-	-	1	1	-
	7	-	-	-	-	1	-
	8	-	-	-	-	1	10
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-
	11	-	-	-	-	-	-
	12	-	-	-	-	-	-
	13	-	-	-	-	-	-
Ribs							
	1	1	-	4	-	3	-
	2	-	-	3	1	3	-
	3	-	-	1	2	2	-
	4	-	-	3	3	3	-
	5	-	-	3	4	3	-
	6	-	-	4	2	4	-
	7	-	-	3	1	6	1
	8	-	-	3	4	7	-
	9	-	-	6	6	7	-
	10	-	-	5	3	4	-
	11	-	-	2	1	5	-
	12	-	-	3	-	5	-
	13	-	-	-	-	2	52
Lumbar Vertebrae							
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	6	-	-	2	-	-	-
	7	-	-	2	-	3	-
Sacral Vertebrae		-	-	-	-	-	-
Coccygeal Vertebrae		-	-	-	-	-	-

Table 1. Continued -

	Thorium ~ 0.1 $\mu\text{c/kg}$ (9 Dogs)				Thorium ~ 0.3 $\mu\text{c/kg}$ (10 Dogs)				Thorium ~ 0.9 $\mu\text{c/kg}$ (4 Dogs)			
			%				%				%	
	U	H	U	H	U	H	U	H	U	H	U	H
<u>Appendicular Skeleton</u>												
Scapulae	-	-	-	-	-	-	-	-	1	2	13.0	25
Humeri	-	-	-	-	-	2	-	10	-	-	-	-
Radii	-	-	-	-	-	-	-	-	4	-	50	-
Ulnae	-	-	-	-	-	-	-	-	3	-	38	-
Carpi	-	-	-	-	-	-	-	-	-	-	-	-
Metacarpi	-	-	-	-	-	-	-	-	-	-	-	-
Iliac	-	-	-	-	2	3	10	15	1	2	13	25
Pubes	-	-	-	-	-	-	-	-	-	1	-	13
Ischia	-	-	-	-	-	-	-	-	-	-	-	-
Femora	-	-	-	-	-	-	-	-	3	-	38	-
Tibiae	-	-	-	-	1	-	5	-	1	-	13	-
Fibulae	-	-	-	-	-	-	-	-	1	1	13	13
Metatarsi	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Days age and days post-injection of dogs presented in this study.

Injection Dose	Age at Injection (days)		Days Post Injection	
	Average	Range	Average	Range
Thorium-228				
Level 2	513	451 - 598	1041	1015 - 1541
3	481	314 - 606	882	547 - 1156
4	501	458 - 606	758	645 - 833
5	468	455 - 483	122	65 - 212

[illegible]

~ .1 $\mu\text{C/kg}$ Th-228
(9 Dogs)

[illegible]

Fig. 1. Fracture distribution in beagles treated with a single intravenous injection of Th^{228} .

Fig. 2. Microphotograph of costochondral region of rib showing essentially normal structure. 0.089 $\mu\text{c/kg}$, 1222 days post-injection. H & E. X17.

Fig. 3. Radiograph showing "hot line" fractures, both healed and unhealed, and "hot lines" (arrows) in some of the unfractured ribs. The bone distal to the radio-opaque line represents growth that occurred after the radionuclide treatment. $0.3 \mu\text{c/kg}$. 988 days post-injection.

Fig. 4. Microphotograph of distal aspect of rib showing pre-injection bone with fibrous marrow (PR), post-injection bone containing viable marrow (PI), and the costal cartilage (C). The post-injection bone is characterized by a thinner cortex and cartilaginous cores in many of the trabeculae. The "hot line", the most likely fracture site, is indicated (F). Dog received 0.272 $\mu\text{c/kg Th}^{228}$. 547 days post-injection. H & E. X 17.

Fig 5. Microphotograph of distal aspect of rib showing pre-injection bone devoid of marrow (PR), costal cartilage (C), and absence of post-injection bone growth. Cartilaginous cores are present in many trabeculae at the tip of the rib. $0.835 \mu\text{c/kg Th}^{228}$. 793 days post-injection. H & E. X 17.

THE CORRELATION OF BONE STRENGTH WITH RADIATION DOSE FROM INTERNAL EMITTERS AND BONE RESORPTION

David H. Taysum, Webster S. S. Jee and David O. Clarke*

INTRODUCTION

Investigations of the physical properties of beagle tibiae that contain radionuclides incorporated during life have indicated thus far only tendencies toward weakening with increasing radiation dose^(1,2).

The physical properties were determined by using regularly shaped specimens subjected to pure tensile forces. The measurements made were of force per unit area (stress) and increase in length per unit length (strain).

The specimens were tested to failure, but only the values of the proportional limit were used. The proportional limit is also named the elastic limit.

MATERIALS AND METHODS

The amount of bone actually present was determined by taking the specimens previously tested⁽²⁾ so that the relationship of area and strength could be determined. A cross section was obtained as close to the break area as was possible. This is recognized as being an approximation since the obviously weakest place was where the break occurred and the only course remaining was to examine the site nearest the point of failure. This cross section was ground to approximately 100 μ thickness and was radiographed using 10 K.V.P. x-rays.

The resulting radiograph of a 0.050 inch diameter specimen was enlarged photographically to fifteen inches. The unequivocally radiolucent areas were carefully cut out and weighed. The weights were translated,

*David O. Clark, University of Rochester, Rochester, New York

by appropriate conversion factors, into areas. The percent of bone not present in the cross section was determined for twenty-nine controls and thirty-two irradiated specimens.

To evaluate the effect of the area of bone not present, the percent of bone missing, or porosity, was plotted against: (a) stress, (b) radiation dose, and (c) age, for control and irradiated. A plot of force versus area (micrometer measurement) is also provided.

RESULTS

In the initial study⁽²⁾, the diameter of the test specimen was determined with a micrometer. The area calculated on the basis of this measurement was used as the denominator of stress. No correction was made for the fraction of the area that was not bone such as: (a) resorption areas, (b) Haversian canals, (c) Volkmann canals, and (d) nutrient vessel channels, (e) osteocytes, and (f) lacunae. The data of this study when plotted exhibited a great deal of scatter. The scatter was not confined to measurements between different bones alone, for specimens from the same tibia showed wide variation in strength and energy absorbing capacity.

The variation in properties appeared to be as great in control specimens as it was in irradiated specimens. It was felt that part of this variation must be due to variations in the amount of bone actually present.

A graph of force (Fig. 1) versus total area (micrometer measurement) reveals an apparent difference between control and irradiated. This graph is actually a representation of Hooke's law wherein the deflection to the proportional limit is proportional to the applied force and hence zero force should correspond to zero area.

Extension of the least squares fit lines so far from the mean of the data points is done to allow inferences to be made and not to imply that the intercept values are anything but approximations. The means of percent missing and stress of controls and irradiated are compared in Table I.

TABLE I

	Average Percent Bone Missing	Stress lbs/in ² x 10 ³
Controls	4.16	18.30
Irradiated	5.18	16.86
Differences and Significance of the Differences.	1.02P = [0.05 to 0.10]	1.44P = [0.05 to 0.10]
<u>Irradiated</u> Control	124%	92%

Table I shows the following:

a. Specimens from irradiated animals are significantly more porous (radiolucent) than are appropriate controls. The order of magnitude for the tibial compacta examined is approximately a 24% increase in porosity for irradiated versus control.

b. Specimens from irradiated animals are significantly weaker than are control specimens. The order of magnitude of the difference is approximately an 8% decrease in strength for the irradiated bone. The decrease in strength by 8% to 10% is not believed to be acceptable as an explanation for the

spontaneous fractures which some of the animals of the colony have exhibited.

A plot of percent missing (Fig. 2) versus stress reveals a difference between control and irradiated. Healthy control bones show increasing strength with decreasing percent missing ($P < .01$), compared with irradiated which show much greater loss of strength without the corresponding magnitude of percent missing. ($P < 0.01$) This indicates again that removing the obvious radiolucent areas may not have removed all of the area that was non-functional as stress and energy absorbing bone.

A plot of percent missing versus radiation dose (Fig. 3) shows an increase in percent missing with increasing dose. ($P < 0.01$) Thus radiation causes an increase in the percent missing which in turn is reflected in a loss of structural quality. Controls show insignificant change.

A plot of percent missing versus age (Fig. 4) confirms the previous plot and shows that there is no significant increase ($P > 0.70$) in percent missing with age for controls over the times used in this study. For irradiated bones, the seeming increase in % missing with age ($P \approx 0.50$) is ascribed to the increase in cumulative dose with time after injection.

Table II is supplied to show total average skeletal dose, age, range of doses and range of ages.

SUMMARY

Radiation dose delivered to the animal by the incorporation, during life, of radionuclides in bone causes an increase in the porosity of bone with subsequent weakening being manifested.

REFERENCES

1. H. Hollingshaus and C. W. Mays, Changes in mechanical strength of bone due to internally deposited radioelements, Radiobiology Laboratory Annual Progress Report COO-215, 158-179 (1958).
2. D. H. Taysum, F. G. Evans, W. M. Hammer, W. S. S. Jee, C. E. Rehfeld and L. W. Blake, Radionuclides and Bone Strength in Some Aspects of Internal Irradiation, 145-158, Pergamon Press, 1962.

All of the observed loss of strength is not believed to be attributable to porosity since there are indications that some of the bone substance may not contribute to strength.

TABLE II

Values for dogs which were regarded as controls because of their low skeletal dose from internal emitters.

Dog	Rads to Skeleton	Days Since Injection	Age At Death (days)	% Bone Missing	Stress ($\frac{10^3 \text{ lbs}}{\text{in}^2}$)	
↑ Controls	FAC43	0	-	3881	4.52	18.07
		0	-	3881	4.56	18.46
	FAC51	0	-	1089	4.98	14.43
		0	-	1089	2.27	20.00
		0	-	1089	3.13	24.56
		0	-	1089	2.76	17.95
	FAC52	0	-	509	7.69	15.55
		0	-	509	6.48	11.32
		0	-	509	4.78	12.65
		0	-	509	5.04	19.60
		0	-	509	3.76	21.98
		0	-	509	4.58	17.77
	T9T3	3	1	448	3.69	16.40
		3	1	448	3.81	22.10
		3	1	448	3.35	17.90
		3	1	448	4.03	9.31
		3	1	448	4.04	17.60
		3	1	448	3.53	19.30
	T15P5	10	1	738	3.49	19.88
		10	1	738	4.21	19.68
		10	1	738	4.27	20.10
		10	1	738	4.09	22.89
F1S1	21	308	1832	5.83	21.70	
T10T3	24	8	455	2.48	17.70	
	24	8	455	3.49	19.40	
	24	8	455	2.48	22.00	
	24	8	455	6.19	17.00	
	24	8	455	4.06	17.00	
M10R1.7	109	557	1147	3.09	18.67	
↓						

TABLE II (Cont'd)
VALUES FOR SIGNIFICANTLY IRRADIATED DOGS

Dog	Rads to Skeleton	Days Since Injection	Age At Death (days)	% Bone Missing	Stress ($\frac{10^3 \text{ lbs}}{\text{in}^2}$)
M6S5	647	35	539	3.67	15.78
	647	35	539	3.32	19.48
	647	35	539	5.02	14.28
F6P3	670	1617	2032	6.38	15.09
	670	1617	2032	3.96	13.77
	670	1617	2032	2.63	21.27
	670	1617	2032	4.09	22.44
F1M2	870	1780	2456	2.65	21.42
M8P4	1560	1157	1808	8.92	16.38
	1560	1157	1808	3.56	16.03
M3M4	1795	418	997	6.64	16.80
	1795	418	997	4.94	12.70
	1795	418	997	3.66	20.00
	1795	418	997	4.72	11.90
F2M4	2272	778	1238	3.99	17.10
	2272	778	1238	6.06	12.70
	2272	778	1238	2.05	18.40
	2272	778	1238	4.82	17.30
M4M4	2680	896	1505	2.27	21.70
	2680	896	1505	2.74	20.90
	2680	896	1505	1.69	17.70
F8R4	4615	1324	1798	4.49	17.45
	4615	1324	1798	5.39	16.66
F9P5	4750	1145	1697	5.21	13.88
	4750	1145	1697	4.58	15.95
	4750	1145	1697	8.78	22.14
	4750	1145	1697	5.97	16.88
M8P5	4840	1192	1689	15.89	11.78
	4840	1192	1689	11.55	12.33
T2M4.5	5097	755	1218	5.98	15.00
	5097	755	1218	5.92	17.80
	5097	755	1218	4.47	16.70

ACKNOWLEDGEMENTS

The assistance of Renate Tegge in preparing the ground bone specimens and x-ray plates is gratefully recognized.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

STUDIES OF TRABECULAR BONE IN BEAGLES AND HUMANS

W. S. S. Jee, R. G. Bromley, N. L. Dockum, M. Lowe, R. Burggraaf,
R. Mical, Kendra Dedekind and J. S. Arnold*

Abstract: The relationship between the percent of osteoid seams, the percent of osteoid seams in the central portion of the vertebral body, the percent of bone and the total bone surface perimeter within the periosteal envelope of lumbar vertebral bodies with age was studied. Specimens included 72 human and 40 Beagle lumbar vertebral bodies. In the human, the percent of centrally located osteoid seams was 12% at 2 years, 3% at ages 28 to 38, 2 to 14% at ages 40 to 50, and approximately 5% thereafter. In the Beagle the distribution of osteoid seams with age resembled that for the human. The depression of osteoid seams occurred at 28 to 38 years in human and 4.1 to 5.4 years in Beagles. The percent of osteoid seams in the entire vertebral body of humans paralleled that of the central portion, but was generally higher. In humans the percent of bone within the periosteal envelope decreased slower with age than it does in the dog (human slope = -0.0987 vs. dog slope = -0.810). In humans the total bone perimeter was small in the young, increased rapidly to age 40 years, then decreased gradually with age. In the dog the total bone surface perimeter remained almost constant over its life span.

INTRODUCTION

This is a part of a continuing program to study the alteration in cancellous bone with age (1). These changes in spongy bone with age must be characterized before the toxic effects of very low doses of internally deposited bone seeking radionuclides upon the skeleton can be evaluated.

The present report describes a study which characterize the changes in bone, bone surface and osteoid seams in the lumbar vertebral body as a function of time.

MATERIALS AND METHODS

The raw material for these studies included human lumbar vertebrae collected from 72 routine autopsies at the Providence Hospital in Portland, Oregon, by Drs. J. S. Arnold and T. H. Cochran, and from 40 dogs

*Sumerlin Memorial Pathology Laboratory, Donald N. Sharp Memorial Community Hospital, San Diego, California.

from the control colony of Beagles at the Division of Radiobiology, University of Utah. Various causes were responsible for death. Most of the subjects and all the dogs were thoroughly autopsied, providing evaluations of the measurements of the vertebral specimens (1).

Preparation of Human Material:

The frozen human second vertebral body was sawed longitudinally in the sagittal plane into slabs of 5 to 7 mm. A band saw with a skip tooth blade was used, producing a clean cut surface with no packing of the saw blade or detectable homogenization of the adjacent tissue. Cutting the tissue in the frozen state prevents the forcing of bone chips into adjacent tissue. The still frozen bone slabs were placed in cold absolute acetone, defatted and dehydrated by five changes of absolute acetone ether and ten changes of diethyl ether. The specimens were dried and embedded in a mixture of 45% ethyl and 55% butyl methacrylate with 0.9% catalyst. The undecalcified embedded specimens were sectioned at 8 microns by a heavy duty Jung, model "K" microtome. The sections were adhered to gelatinized microscope slides using 70% ethanol as a wetting agent. The methacrylate was then removed by daily changes of chloroform.

Undecalcified sections were stained by the Von Kossa mineralized bone method and counterstained with a mixture of eosin and safranin for osteoid seams and cytological features (1). Fig. 1 illustrates typical human second lumbar vertebrae from a 2.5 and a 53 year old person and compares percent of bone, total osteoid seams and central osteoid seams.

Mineralized bone stained brown or black, while osteoid seams appeared as red smooth strips with sharp borders along the edge of the brown staining trabeculae. Cellular elements appeared pink with purplish nuclei.

Preparation of Dog Bones:

The preparation and handling of the dog bones was slightly different from that of human bones. Details of the procedure have been described in a previous communication (1). These sections were stained with May-Grünwald-Giemsa and Von Kossa's stains.

Four different quantitative studies were conducted on lumbar vertebral bodies: (1) measurement of the percent osteoid seams in the entire second lumbar vertebral body; (2) measurement of the percent osteoid seams in the central portion of the vertebral body; (3) measurement of the percent bone in the periosteal envelope; and (4) measurement of the bone perimeter within the periosteal envelope.

Quantitative Methods:

The stained lumbar vertebral section consisting of cortical and trabecular bone were projected a standardized distance on to uniformly thick tracing paper after which the bone surfaces were traced. Utilizing a microscope properly calibrated with the tracing for known distances, the measured osteoid seam lengths and widths were recorded in the proper position on the tracing. Bone surface perimeter was measured by a mechanical map measuring device calibrated in centimeters. The percent of endosteal surface covered by osteoid seams was calculated by dividing the total seam length in microns by the total exposed bone surface perimeter in microns. The second method of calculating the percent of osteoid seams and bone perimeters involving sampling surface measurements using the Zeiss integrating eyepiece (2,3). The osteoid seam percent was determined microscopically by counting the number of intersections by the horizontal lines of a calibrated ocular micrometer with osteoid seams and dividing it by the number of intersections with trabecular surfaces

(bone perimeter). The bone perimeter in microns was calculated by the equation $\frac{\pi}{2} \times H \times a$. The number of intersections (hits) equals "H". The distance between horizontal lines in microns is represented by "a".

Histological sections were sampled in the following manner. Every other square was counted and the number of hits were multiplied by 2 to represent the entire vertebral section. The sampling was thus uniformly distributed to represent the variation in quantity of seams in different regions. In small sections every field was counted.

The percent of total area composed of bone was measured from a second tracing of the original projected stained slide. The marrow area was cut out and separated from the bone and the representative paper fraction weighed on an analytical balance. The percent of bone was calculated from the weight of bone fraction divided by the combined weight of bone and marrow fractions (Method I).

The above procedure was too tedious and consequently a second method (Method II) was used in which the percent osteoid seams of bone was calculated by projecting stained sections of vertebrae on a grid divided into 2 cm squares. The total number of grid intersections lying within the periosteum was counted. This number was divided into the number of intersections lying on mineralized bone. A mechanical rotating slide holder was used to rotate the slide and counts were repeated at six equally varying positions through a 90 degree arc. The six results were then averaged at the 95% confidence limits determined by the range.

Comparison of Methods:

Table I lists the percent bone, osteoid seams, bone perimeter and the calculated percent osteoid seams for the two methods. The percent bone is in reasonable agreement with a tendency in method II (microscopic

sampling) to obtain higher figures. Sampling by method II (microscopic) of bone surface occupied by osteoid seams and bone perimeter in comparison to the tracing method (Method I) was found to be more variable with the greatest variability in small samples. When the percent of seams is less than 5% the microscopic sampling method was found to be consistently higher.

Percent Bone Within Periosteal Envelope of Human Second Lumbar Vertebral Body:

The percent bone in the periosteal envelope from 58 cases is plotted as a function of age in Fig. 2 revealing a progressive loss of bone as a function of age. The females appeared to have less bone than males. The 2-year-olds contain 25% bone which decreases progressively to 15% at age 80 years. The slope of the line has been found by the least squares method to be $-.0986$. When the slope of the entire population is extrapolated, it appears that bone reaches zero bone at about 235 years.

Individuals containing a high percent of bone in the periosteal envelope (greater than 22% after age 40) are cases dying from heart failure, aortic aneurysm, and acute vascular and pancreatic disturbances. Conversely, individuals with low percent of bone values (values less than 13% after age 40) represent cases of cirrhosis of the liver, chronic pulmonary disease, meningitis with adrenal involvement, simultaneous kidney and vascular disease, and a case of multiple myeloma.

Total Bone Perimeter (bone surfaces) in Human Lumbar Vertebral Bodies:

The total bone perimeter from 62 cases is plotted as a function of age and sex in Fig. 3. It is small at ages 2 and 3 months, increases rapidly up to the age of 49 years and decreases until age 80 years. At age 3 the bone perimeter is approximately $450,000 \mu$, $760,000 \mu$ at age 8,

1,300,000 μ at age 12, peaking at 1,700,000 μ at age 40 then decreasing to an average of 1,000,000 μ at age 80. In Fig. 4 the bone perimeter values are normalized to the smallest periosteal envelope to correct for variations in size. Surprisingly, the bone perimeter values decrease gradually over 9 decades. At age 2 the normalized bone perimeter is about 500,000 μ and decreases to about 200,000 μ at age 90.

Percent of Osteoid Seams in Human Vertebral Bodies:

The percent of bone surface occupied by osteoid seams in human lumbar vertebral bodies is plotted in Figs. 5, 6, 7 and 8. Figs. 5 and 6 are osteoid seam counts restricted to the central portion of the vertebral body (trabecular bone only) from 29 acute cases and 61 acute and chronic cases, respectively. Figs. 7 and 8 are seam counts of the entire vertebral body from 24 acute cases and 72 combined acute and chronic cases, respectively.

The plot of the centrally located osteoid seams from the 29 acute cases shows 12% at 2 years, 3% seams at ages 28 to 38, and varying 2 to 14% at ages 40 and 50 and approximately 5% thereafter (Fig. 5). When all 61 cases were plotted, the low value for percent of seams at age 28 to 38 was still apparent except for a male with a chronic kidney disease who had a 14% seam count (Fig. 6). After 50 years, the osteoid seam values are more variable ranging as high as 20%. In both plots (Figs. 5 and 6), the males consistently exhibited a higher percent of osteoid seams than the females.

The percent of seam values of centrally located seams (trabecular bone) were consistently lower than the values for osteoid seams which included trabecular and cortical bone from 24 acute and 58 combined acute and chronic cases. The latter values showed a greater percent of seam

spread (Figs. 7 and 8). Osteoid seam values as high as 33% were plotted for individuals with kidney diseases in Fig. 8. At any rate, the low osteoid seam values remained apparent between ages 28 to 38 years.

High osteoid seam values were observed in cases with chronic diabetes, asthma, myocardial infarcts, kidney disease, and some vascular diseases (Figs. 6 and 8). Low osteoid seam counts were noted in cases of partial paralysis, cirrhosis of the liver, irradiation therapy coupled with the loss of one testis and a case of multiple myeloma.

In the four liver cases plotted for central and total osteoid seams, it was noted that two cases of cirrhosis of the liver had the lowest percent of osteoid seams. One exhibited 0.8% and the other 0.65% central osteoid seams, respectively (Fig. 6). Two other cases, one involving centrolobular necrosis of the liver showed 2.3% seams while a case with hepatic cysts registered a high 13% seams.

The cases with a history of kidney disease showed an extremely high percent of osteoid seams. Generally, individuals inflicted with some vascular disease (arteriosclerosis) and some form of kidney disease exhibited the highest percent of osteoid seams, namely 22% for centrally located areas and 33% for entire vertebral bodies (Figs. 6 and 8).

Percent of Bone in Lumbar Vertebral Bodies of Adult Beagles:

The young Beagle (500 days or 1.37 years of age) possesses 45% bone in its lumbar vertebral body as opposed to 22% in 30 year-old humans (Fig. 9). This value of 45% gradually decreases to 30% over a span of 4500 days (12.3 years). This is a 15% loss of bone over a span of 12 years. Man loses the same 15% over a span of 90 years. On extrapolation the percent of bone reaches zero in the dog at 54.5 years. The slope of line is calculated to be $-.8097$. Female Beagles tend to possess less bone than the male.

Total Bone Perimeter in Lumbar Vertebral Bodies of Adult Beagles:

The total bone perimeter of young adult Beagles averaged around 500,000 μ between 250-5700 days of age and showed very little variation (Fig. 10). Over a span of 5700 days (15.6 years) there was no loss of bone perimeter in the dog.

Percent of Osteoid Seams in Adult Beagles:

Fig. 11 shows the variation in frequency of osteoid seams with age and sex in which the percent of bone surface occupied by osteoid seams is plotted on the ordinate against the age in days on the abscissa. The plot indicates that between ages 500-800 days, the percent of osteoid seams is about 10%, dropping to approximately 7% at 1000-1500 days and diminishing to less than 5% between 1500-2000 days. Subsequently, between the ages of 2500-3500 days, the osteoid seam values show a rapid rise, followed by a decline at from 3500-5700 days. The shape of the slope resembles the human plot except that the low value of percent of osteoid seams occurs at 1500-2000 days or from 4.1 to 5.4 years as compared to 28-38 years for the human. The same percent increase in osteoid seams is observed at 3000 days (8.2 years) for dogs as compared to age 50-60 for humans.

TABLE I
Comparison

No.	% Bone I*	% Bone II**	Microns Seams I*	Microns Perimeter I*	Microns Seams II**	Microns Perimeter II**	% Seams I*	% Seams II**
107	16.3	23.8			(Not Computed)			
108	(Not Computed)		196,172	571,900	196,000	625,000	34.3	31.5
121	(Not Computed)		44,165	1,372,707	47,600	920,000	3.2	5.2
139	15.4	16.0	165,413	1,082,041	139,000	970,000	15.3	14.4
165	(Not Computed)		9,415	260,000	11,000	223,000	3.6	4.9
173	13.1	11.2	6,592	1,380,000	17,500	1,040,000	.5	1.7
178	14.8	16.8	16,859	839,942	16,500	680,000	2.0	2.4

* Method I, tracing method

** Method II, microscopic sampling

DISCUSSION

Although the study is incomplete, there are a few observations which warrant some discussion. These include some comments on the technique in measuring the percent of bone in the lumbar vertebral body and the comparison of percent of bone and osteoid seams in Beagles and man as a function of age.

Studies describing changes in cancellous bone with age have been reported. Arnold (4) showed excellent illustrations of cleaned vertebral trabecular bone from a normal and osteoporotic individuals. In the osteoporotic, the trabeculae were very much thinner and are delicate with cross bridges of bone greatly reduced. Recently, Johnson (5) reported similar findings that there was a progressive regression of cancellous bone from heavy bars to "delicate filigrees that preceded

complete removal of portions with reinforcement of remaining portions with age". There are studies by Trotter, et al. (5), and Arnold (4) which quantified the changes in trabecular bone with age. Trotter, et al., described a progressive decrease in bone densities ($\frac{\text{weight}}{\text{volume}}$) in cervical, thoracic, lumbar and sacral vertebrae in whites and negroes as a function of age. Arnold described the progressive loss of ash content (gm/cc) and a slight increase in density (gm/cc) of vertebral medullary tissue (trabecular bone only) as a function of age (4). To our knowledge the present study was the first attempt to quantitate the percent of bone in a lumbar vertebral body from a histological section. This method, with further refinement, may be quite useful in determining loss of bone with disease or radiation. Although the method cannot be applied to the living, the present study could contribute findings which gives us a better understanding of impressions gained from the living.

The comparison of the percent of bone with age in man and Beagle showed marked differences. Human beings started with less bone (23.2%) and lost it at a slower rate (regression coefficient [slope] = -0.0987) while the beagle began with more bone (44.1%) and lost it faster (regression coefficient = -0.810). The slope of decrease in bone for man when extrapolated reached zero at 235 years, while the Beagle reached it at 54.5 years. This is a factor of 4.33 difference between dog and man. There is a safety factor of 3.33 ($\frac{235}{70} = 3.33$) for man with a normal life span of 70 years, while the Beagle has a safety factor of 3.63 ($\frac{54.5}{15} = 3.63$) for a life span of 15 years. These numbers are surprisingly close. In reality, the Beagle lumbar vertebral body contains greater percentage of bone at age 15 than human lumbar vertebral centra ever contained in their life span.

It was reassuring to find that in both man and Beagle there was a depression at young adulthood in the percent of trabecular bone surface occupied by osteoid seams. The depression occurred between 28 to 38 years in man and 4 to 6 years in Beagles. This finding is similar to depressions observed at age 35 in the compacta of human ribs by Frost (7), and at 21 to 30 years in the compacta of mandibles by Sissons et al. (8), and Manson (9).

The present data substantiated the study of human mandibles by Manson and Lucas (10). They reported that bone surfaces involved in deposition of new bone dropped to 2% at age 30 to 40 and rose to 5% at the fifth decade. The changes in percent of osteoid seams in human lumbar vertebral bodies were much more variable than in the mandible or femur. For example, Manson reported that the percent of osteoid seam never exceeds 5% of the bone surfaces while in the lumbar vertebral bodies osteoid seams exceeding 5% were not uncommon after age 40 (9).

It is difficult to explain the observed increase of osteoid seams after the fifth decade. The fact that more seams were observed in older bones does not mean more bone was being formed. It could mean that bone apposition and/or mineralization of osteoid were taking place more slowly.

Finally the authors feel that it is not appropriate at this time to discuss the relationship of incidence of osteoid seams and percent bone to disease even though there were obvious differences reported for certain diseases.

REFERENCES

1. W. S. S. Jee, R. S. Mical, N. L. Dockum, R. Tegge, M. Lowe and B. Burggraaf, Quantitative studies of trabecular bone remodelling, Research in Radiobiology, COO-119-229, p. 111-126 (1964).
2. A. Hennig, A critical survey of volume and surface measurement in microscopy. Zeiss Werkzeitschrift, No. 30, 78-87 (1958).
3. K. Michel, Zeiss integrating eyepieces, Carl Zeiss Oberkochen/Württ 40-195 e.
4. J. S. Arnold, Quantitation of mineralization of bone as an organ and tissue in osteoporosis, Clin. Orthopaedic, 17:167-175 (1965).
5. L. C. Johnson, Morphologic Analysis in Pathology; The kinetics of disease and general biology of bone in Bone Biodynamics, (ed. H. M. Frost) p. 543-654, Little Brown & Co., Boston, Mass. (1964).
6. M. Trotter, G. E. Broman and R. R. Peterson, Densities of bones of white and negro skeletons, J. of Bone and Joint Surgery 42A:50-58 (1960).
7. H. M. Frost, Bone Remodeling Dynamics, Charles C. Thomas, Springfield, Illinois (1963).
8. H. A. Sissons, J. Jowsey and L. Stewart, The microradiographic appearance of normal bone tissue at various ages in X-ray Microscopy and X-ray Microanalysis (ed. by A. Engstrom, V. Cosslett and H. Pattee), p. 206-215, Elsevier Publishing Co., Amsterdam (1960).
9. J. D. Manson, Age changes in bone activity in the mandible in Bone and Tooth, Proceedings of the First European Symposium, p. 343-349, Macmillian Co., New York (1964).
10. J. D. Manson and R. B. Lucas, A microradiographic study of age changes in the human mandible. Arch. Oral Biol, 7:761-769 (1962).

CASE # 177
 2.5 years old
 23.7 % BONE
 21.7 % TOTAL OSTEOID SEAMS
 12.9 % CENTRAL OSTEOID SEAMS

CASE # 173
 53 years old
 16.2 % BONE
 4.3 % TOTAL OSTEOID SEAMS
 2.3 % CENTRAL OSTEOID SEAMS

Figure 1. Comparative projection tracings of the second lumbar vertebrae from a 2.5 year old child and a 53 year old man showing per cent of bone, total osteoid seams and central osteoid seams.
 (X4)

Figure 2. Percent bone in human lumbar vertebral bodies as a function of age and sex. There is a progressive loss of bone as a function of age (slope = -0.0987).

Figure 3. Bone surface perimeter in human lumbar vertebral bodies as a function of age and sex. It is small at early ages, increases rapidly to 40 years and decreases until age 80 years.

Figure 4. Bone surface perimeter in human lumbar vertebral bodies as a function of age and sex (normalized data). Data normalized to smallest periosteal envelope to correct for variations in vertebrae size. Bone perimeter values decrease gradually over nine decades.

Figure 5. Osteoid seams on trabeculae of human lumbar vertebral bodies (central portion) as a function of age and sex (acute cases).

Figure 6. Osteoid seams on trabeculae of human lumbar vertebral bodies (central portion) as a function of age and sex (acute and chronic cases). Males exhibited higher percent osteoid seams than females.

Figure 7. Osteoid seams in human lumbar vertebral bodies as a function of age and sex (acute cases).

Figure 8. Osteoid seams in human lumbar vertebral bodies as a function of age and sex (acute and chronic cases). Individuals with kidney diseases have high osteoid seam values.

Figure 9. Percent of bone in Beagle lumbar vertebral bodies as a function of age and sex. There is a general decrease in the percent of bone with age (slope = -0.810).

Figure 10. Bone surface perimeter in Beagle lumbar vertebral bodies as a function of age and sex. Very little variation in the amount bone surface is evident as a result of age.

Figure 11. Osteoid seams in Beagle lumbar vertebral bodies as a function of age and sex (acute and chronic cases).

BIOASSAY OF THE INFLUENCE OF CORTISOL IN BONES OF GROWING RABBITS

W. S. S. Jee, E. L. Blackwood*, Renate Tegge, N. L. Dockum,
R. Haslam and F. A. Kincl**

Abstract: Microradiographs of distal femurs, femoral shafts and calvaria from growing rabbits fed a normal or rachitogenic diet plus 0, 1, 5, 15 mg cortisol/kg/day for 15 days were analyzed for epiphyseal plate thickness, presence of growth arrest plate, regression of metaphyseal trabeculae, thickness of metaphyseal trabeculae, and porosities of femoral shafts and calvaria (bone atrophy). Rabbits fed a normal diet plus 1, 5, 15 mg of cortisol exhibited epiphyseal plate of 0.73, 0.47 and 0.37 times as thick as their controls, while rabbits fed a deficient diet plus 1 and 5 mg of cortisol exhibited epiphyseal plate 0.76 times as thick as their controls. Growth arrest plates appeared in rabbits fed a normal or deficient diet plus 5 and 15 mg of cortisol. Regression of metaphyseal trabeculae occurred only in rabbits fed a normal diet and treated with 5 and 15 mg of cortisol. Porosity values of femoral cortices differed at all dose levels, while porosity values of calvaria differed only in animals fed normal diets plus 5 and 15 mg of cortisol. The dose response relationships between porosities of femoral cortices and graded doses of cortisol exhibited slope values of 1.25 for rabbits fed normal diets and a value of 2.22 for rabbits on deficient diets. There was an advantage in using the rachitogenic diet in the femoral porosity analysis, but the use of the rachitogenic diet was a distinct disadvantage in the analyses of epiphyseal plate thickness, length of metaphysis and calvarium porosity.

INTRODUCTION

One of the principle undesirable side effects of cortisol is a marked loss in mass of bone (osteoporosis or bone atrophy) accompanied by fractures. Since cortisol and related synthetic glucocorticoids are used prominently for a wide spectrum of diseases, it would be valuable to develop a bioassay technique which would quantify the bone resorption

* NIDR predoctoral fellow (NIH Training Grant # 1T1DE-151-01).

** Syntex Laboratories, Palo Alto, California.

induced by these corticoids in the hope that an effective anti-inflammatory drug can be developed without the untoward side effect upon bone. A better understanding of the dose response of bone tissues to the osteolytic effect of various glucocorticoids will be very informative in determining whether these drugs would be useful as agents in the removal of bone seeking radionuclides from the skeleton.

The present communication describes methods for evaluating the effects of cortisol on the elongation of a long bone (growth) and bone atrophy in compact and spongy bones using contact microradiography and quantitative histology. The study compares the response of three dose levels of cortisol in rabbits fed a normal or a rachitogenic diet. Rabbits fed a rachitogenic diet are used because of a previous finding by Storey which showed that bone resorption induced by cortisone was accelerated by a decreased dietary uptake of calcium and phosphorus (1).

MATERIALS AND METHODS

I. Diets and Treatment:

Seventy-two, 6 to 8 week old New Zealand white rabbits weighing about 1000 g were divided into 8 experimental groups (Table I). The rabbits were fed either a normal or a rachitogenic diet No. 2, U.S.P.* and injected subcutaneously in the inner thigh with 0, 1, 5, or 15 mg of cortisol per kg daily for 15 days. The animals were euthanized on day 16. The femur, tibia, and skull were removed, defleshed, and bone sections 5 mm thick were sawed and fixed in acetone. The adrenals and thymus were also removed and weighed. Only 2 out of the 8 animals survived the rachitogenic diet with 15 mg/kg of cortisol daily.

*Yellow corn meal, 76%; wheat gluten, 20%; CaCO_3 , 3%; and NaCl 1%.

TABLE I
Treatment of Rabbits

Diet	Treatment mg Cortisol/kg/day	Number of Rabbits
Normal	None	14
Normal	1	8
Normal	5	12
Normal	15	7
Rachitogenic	None	14
Rachitogenic	1	6
Rachitogenic	5	9
Rachitogenic	15	2
Total		72

II. Quantitative Histology:

1. Porosity studies of mid-femoral shaft and calvarium. Cross sections were taken from the mid-diaphysis (shaft) of the femur, and from a frontal section of the right half of the skull (calvarium) at the point of fusion of the zygomatic arch and temporal bone. The bone section of the skull included the temporal and parietal bones with squamosal suture. The sections were fixed in acetone, defatted in ether and embedded in bioplastic. They were then sawed and ground to a thickness of 100 microns and contact microradiographed (Figs. 1 and 2).

To quantify the bone resorption activity of the steroid upon the femoral diaphysis, the microradiographs of the shaft were projected on paper at a magnification of 90. The outlines of the endosteal and periosteal bone surfaces and bone resorption holes and canals larger than 3 mm were traced and the area occupied by the ring of cortical bone (assuming no bone resorption) was cut out and weighed. The holes and

canals with diameter larger than 3 mm (on the tracing) were considered to be resorption spaces and were removed as porosities. The areas of porosity were then cut out and the resulting bone ring was weighed again. The percent porosity was determined by dividing the weight of the bone ring without holes with the weight of the bone ring with holes and multiplying by a 100.

The microradiographs of the calvaria (1.77 mm of the temporal and 4.4 mm of the parietal bones measured from the squamosal suture) were projected on paper at a magnification of 62. Next the outlines of the periosteal and endosteal bone surfaces of the inner and outer tables and the trabeculae in the diploic spaces along with resorption spaces and canals with diameters larger than 2 mm were traced. The calvarium consisting of inner and outer tables and its diploic spaces was cut out and weighed. Then the areas occupied by the marrow in the diploic spaces and the holes in the compacta (resorption spaces and canals) were cut out and the paper representing the remaining calvarium was weighed again. The percent porosity was determined from the weights of the calvarium with and without holes and marrow in the same manner as with the femoral shaft.

2. Distal Femur Analyses. The distal femurs were sawed in the sagittal plane and fixed in acetone. One half was processed, as outlined above, for contact microradiography, while the other half was decalcified in 10% sulfosalicylic acid, doubled embedded in celloidin and paraffin, and sectioned at 8 μ , and stained with hematoxylin and eosin.

The microradiographs of the distal femur were analyzed for epiphyseal plate thickness, presence and thickness of growth arrest plate, length of metaphysis, and thickness of metaphyseal trabeculae at

various intervals from the epiphyseal plate.

The qualitative and quantitative analyses of the hematoxylin and eosin stained distal femurs will be documented in another report.

A. Distal femoral epiphyseal plate thickness. The distal femoral epiphyseal plate was seen as a radiolucent region (black) between the bony plates of the epiphysis and the fine trabeculae of the metaphysis in the microradiographs of distal femur in figures 3 and 4. Some 12 or more measurements at 120 μ intervals were made across the plate to obtain a mean thickness.

B. Presence and thickness of growth arrest plate. The growth arrest plate was a transverse stratum of bone at the junction of the metaphysis and epiphyseal growth plate (Figs. 3 and 4). The mean width of the growth arrest plate was determined by twelve or more measurements at 120 μ intervals.

C. Length of distal femoral metaphysis. A line was drawn traversing the metaphyseal spongiosa connecting a point where the epiphyseal plate dips proximally (Fig. 3) and a point located between the anterior and posterior walls of the distal femoral shaft some 12 mm proximal to the epiphyseal plate. The length of the metaphysis under this line was measured microscopically.

D. Metaphyseal trabecular thickness at 520 μ intervals from the distal femoral epiphyseal plate. The mean thickness of metaphyseal trabeculae which traverse the 520 μ wide lines drawn at 520 μ intervals from the epiphyseal growth plate were measured along the line utilized to determine the width of the metaphysis described above (Fig. 4). Metaphyseal trabeculae thicknesses were measured beginning 1040 μ from the epiphyseal plate to avoid the huge error involved in measuring the

very thin trabeculae at 520 μ in the control specimen.

RESULTS

I. Effect of cortisol on body, adrenal and thymus weights:

Body, adrenal, and thymus weights are listed in Table II. The rabbits injected daily with 0 and 1 mg of cortisol/kg and fed a normal diet gained weight normally (+396 and +353 g, respectively). The rabbits injected with 5 mg of cortisol/kg showed some weight gain (+235 g) while the 15 mg of cortisol/kg showed a very slight weight loss (-22 g). In contrast, the animals fed a rachitogenic diet and injected with 0, 1, and 5 mg of cortisol/kg all showed consistent dose dependent weight losses (-86, -130 and -230 g, respectively).

TABLE II

Effect of Subcutaneously Administered Cortisol
to Immature Male Rabbits on Body, Adrenal and Thymus Weights

Diet	No. Rabbits	Treatment-mg Cortisol/kg	Body Wt. -- Initial	(g+S.E.) Final	Tissue Wt. -- Adrenals	(mg+S.E.) Thymus
Normal	14	0	1020 $\pm$ 70	1416 $\pm$ 38	101 $\pm$ 4.9	2074 $\pm$ 211
Normal	8	1	1049 $\pm$ 64	1402 $\pm$ 39	66.9 $\pm$ 5.1	987 $\pm$ 84
Normal	12	5	988 $\pm$ 56	1224 $\pm$ 64	70.6 $\pm$ 7.6	262 $\pm$ 62
Normal	7	15	1044 $\pm$ 36	1020 $\pm$ 38	53.6 $\pm$ 2.7	94 $\pm$ 13

Rachitogenic	12	0	975 $\pm$ 61	958 $\pm$ 32	79.7 $\pm$ 5.4	318 $\pm$ 33
Rachitogenic	6	1	1005 $\pm$ 60	875 $\pm$ 51	66.3 $\pm$ 5.3	136 $\pm$ 34
Rachitogenic	8	5	1015 $\pm$ 39	847 $\pm$ 30	51.4 $\pm$ 3.4	93 $\pm$ 12
Total			1024 $\pm$ 36			

Terminal adrenal and thymus weights are also listed in Table II. The cortisol treatment produces adrenal atrophy and thymic involution. The rachitogenic diet alone results in some 20% reduction in adrenal weight and some 90% decrease in thymus weight. There is less involution of the thymus when a rachitogenic diet and cortisol treatment are

employed compared to the same dose level on the normal diet, but the final weights are significantly lower.

II. Porosity study of mid-femoral shafts:

Microradiographs of the femoral mid-diaphysis of a control and a rabbit treated with 15 mg cortisol/kg/day are shown in Figure 1 and the results of the porosity measurement from various treatments are listed in Table III.

TABLE III
Statistical Data on Femoral Shafts Porosities
Induced with Graded Doses of Cortisol

Diet	No. Rabbits	Treatment mg Cortisol/kg/day	% Porosity Mean & S.D.	P Value	Ratio Treated/Control
Normal	14	0	3.73 $\pm$ 1.25		1.0
Normal	8	1	5.51 $\pm$ 1.65	<.01	1.44
Normal	10	5	10.78 $\pm$ 1.48	<.001	2.85
Normal	5	15	22.80 $\pm$ 2.00	<.001	6.03

Rachitogenic	14	0	1.99 $\pm$ 1.25		1.0
Rachitogenic	8	1	5.84 $\pm$ 1.65	<.001	2.93
Rachitogenic	9	5	13.61 $\pm$ 1.56	<.001	6.84

Table III shows that the femoral shaft porosities are all statistically different from their respective controls. The ratios of treated to control porosities for rabbits on normal diets are 1.44, 2.85 and 6.03 for 1, 5 and 15 mg of cortisol/kg, respectively. Higher ratios of 2.93 and 6.84 are calculated for the 1 and 5 mg of cortisol/kg dose levels on rachitogenic diet. The dose response line for the normal diet is +1.25 as compared to a slope of +2.2 for the rachitogenic diet (Fig. 5).

The data indicate the combined rachitogenic and cortisol treatment is more effective for differentiating femoral shaft porosities. This is substantiated by the steeper dose response line for rachitogenic

diet and higher ratios of treated to control porosity values for identical dose levels for rabbits fed a rachitogenic diet. The steeper slopes and higher ratios are due to the porosity suppressing effect of the rachitogenic diet alone and the enhanced bone resorption observed with 5 mg of cortisol and a rachitogenic diet. In Table III, the femoral shaft porosity of the controls on rachitogenic diet is 1.99 ± 1.25 in contrast to 3.73 ± 1.25 for the controls on normal diet, while the 5 mg of cortisol and rachitogenic diet group shows an increased porosity value of $13.61 \pm 1.56\%$ to $10.75 \pm 1.48\%$ of its counterpart on a normal diet (statistically different $p < .001$).

TABLE IV
Statistical Data on Calvaria Porosities
Induced by Graded Doses of Cortisol

Diet	No. Rabbits	Treatment mg Cortisol/kg/day	% Porosity Mean & S.D.	P Value	Ratio Treated/Control
Normal	7	0	39.22 ± 6.41	---	1.0
Normal	8	1	38.33 ± 4.66	---	0.97
Normal	8	5	50.22 ± 11.0	<.025	1.51
Normal	4	15	65.58 ± 6.91	<.001	1.68

Rachitogenic	8	0	42.07 ± 5.16	---	---
Rachitogenic	8	1	47.40 ± 8.31	<.10	1.13
Rachitogenic	7	5	49.43 ± 9.16	<.10	1.17
Rachitogenic	2	15	53.63 ± 1.59	<.001	1.27

III. Porosity studies of calvaria:

The statistical data for the calvaria porosities are listed in Table IV. Only the 5 and 15 mg cortisol groups fed normal diets and the 15 mg cortisol group fed a rachitogenic diet show statistically different porosity values from their controls. Surprisingly, the porosity depressing effect of the rachitogenic diet alone and the enhanced

bone resorption from the combined cortisol and rachitogenic diet treatments are not apparent. On the contrary, the majority of the ratios of rachitogenic diet treated to rachitogenic diet control calvaria porosities are lower and the slope of the dose response line is less steep than for their counterparts fed a normal diet. The slopes are +1.83 for normal diet and +0.63 for rachitogenic diet, respectively. (Fig. 6).

The calculated ratios of treated to control calvaria porosity never exceeded 1.68 (Table IV). This is in marked contrast to calculated ratios in excess of 6 for the femoral shaft porosities (Table III).

IV. Influence of cortisol on the distal femur:

1. Epiphyseal Plate Thickness. There is common agreement that cortisol produces an adverse effect upon the growth apparatus resulting in retarded growth in young growing animals (2,3,4,5,6,7). In a short span of two weeks, the influence on the epiphyseal growth plate is detected by a measurement of the width. Table V lists the statistical data on distal femoral epiphyseal plate thicknesses. Rabbits treated with 1, 5, or 15 mg of cortisol/kg/day and fed a normal diet all have significantly decreased ($p < .001$) epiphyseal plate thickness compared to the controls. They are 75, 47 and 37% as thick as their control and their calculated ratios of epiphyseal plate thickness of controls to treated are 1.37, 2.13, and 2.58 for the 1, 5, and 15 mg cortisol group, respectively.

In the rabbits fed the rachitogenic diet alone, the epiphyseal plate thicknesses are reduced to 63% of their normal thickness which markedly reduced the usefulness of rabbits on rachitogenic diet to determine the effect of cortisol on the epiphyseal growth plate. Both the 1 and 5 mg cortisol groups show calculated control to treated

epiphyseal plate thickness ratios of 0.76 and plate thicknesses of about 220 microns. These thicknesses are significantly different from the control at the $p < .025$ level. Figure 6 depicts the type of plot obtained from the dose response to epiphyseal plate thickness data on control and rachitogenic diets.

TABLE V
Statistical Data on Distal Femoral Epiphyseal Plate Thickness
Induced with Graded Doses of Cortisol

Diet	No. Rabbits	Treatment-mg Cortisol/kg/day	Thickness (microns) Mean & S.D.	P Values	Ratio Treated/Control
Normal	8	0	459 $\pm$ 51	---	1.0
Normal	8	1	336 $\pm$ 48	<.001	0.73
Normal	8	5	216 $\pm$ 93	<.001	0.47
Normal	6	15	178 $\pm$ 26	<.001	0.37

Rachitogenic	7	0	288 $\pm$ 57	---	1.0
Rachitogenic	7	1	218 $\pm$ 40	<.025	0.76
Rachitogenic	8	5	219 $\pm$ 49	<.025	0.76

A least squares fit of the points results in a dose response line with slopes of -0.155 for the normal diet and -.026 for the rachitogenic diet. It may well be best to fit the point with lines of two slopes. One line between 0 and 1 or 5 mg of cortisol and another at 5 mg to 15 mg of cortisol.

2. Presence and Thickness of Growth Arrest Plate. The formation of a growth arrest plate (a transverse stratum of bone at the epiphyseal-metaphyseal junction) beneath the epiphyseal growth plate represents another form of disturbance to the growth plate apparatus similar to closure of epiphyses. Bone apposition occurs at the calcified transverse cartilage bars where normally such matrix is dissolved in growing animals. This apposition of bone upon transverse as well as longitudinal calcified

cartilaginous matrix cores results in a growth arrest plate. Histologically the arrest plate is made up of calcified cartilage and lamellar bone.

There is an apparent threshold level for the formation of a growth arrest plate. Only the animals treated with 5 and 15 mg of cortisol fed a normal diet, and 5 mg of cortisol fed a rachitogenic diet show growth arrest plates (Table VI). All these plates are about 160 microns in thickness, individually ranging between 152.5 ± 23.3 to 170.5 ± 40.2 microns.

TABLE VI
Statistical Data on Formation of Growth Arrest Plate
Induced with Graded Doses of Cortisol

Diet	No. Rabbits	Treatment mg Cortisol/kg/day	Growth Arrest Plate Thickness Mean & S.D. (microns)
Normal	8	0	0
Normal	8	1	0
Normal	7	5	152.5 ± 23.3
Normal	6	15	170.5 ± 40.2

Rachitogenic	8	0	0
Rachitogenic	6	1	0
Rachitogenic	8	5	163.6 ± 30.1

3. Effect Upon Width of Metaphyseal Trabeculae.

A. Effect on length of metaphyses. Sissons and Hadfield noted that the influence of cortisone on the spongy bones of human and rabbits includes the loss of trabeculae in the metaphysis (7), and since bone atrophy (osteoporosis) was an uncontested effect upon the cortical bone from treatment of cortisol in these same animals, regression or atrophy of metaphyseal trabeculae was anticipated, but our measurements prove

to be surprising. In the group fed a normal diet, the rabbits treated with 1 mg of cortisol show no bone atrophy, while rabbits fed 5 (p <.075) and 15 (p <.10) mg of cortisol show significant reductions in the width of the metaphysis (Table VII). The treated groups fed a rachitogenic diet show no decrease in metaphyseal trabeculae. In fact, there is a trend toward a significantly wider metaphyseal space in rabbits treated with 1 mg of cortisol daily (p <.075).

TABLE VII
Statistical Data on Length of Metaphysis
After Graded Doses of Cortisol

Diet	No. Rabbits	Treatment-mg Cortisol/kg/day	Trabecular Space (mm) Mean & S.D.	P Values	Ratio Treated/Control
Normal	8	0	4.22 $\pm$ 1.21	---	---
Normal	8	1	4.44 $\pm$ 1.13	---	1.05
Normal	7	5	3.24 $\pm$ 1.15	<.075	0.77
Normal	6	15	3.43 $\pm$ 0.19	<.10	0.81

Rachitogenic	8	0	3.27 $\pm$ 0.61	---	---
Rachitogenic	6	1	4.03 $\pm$ 1.08	<.075	1.23
Rachitogenic	8	5	3.51 $\pm$ 0.97	---	1.07

B. Width of metaphyseal trabeculae at various distances from epiphyseal growth plate. Since only a slight regression in metaphyseal width occurred in rabbits treated with 5 and 15 mg of cortisol/kg daily, there exists the possibility that the steroid may produce partial resorption of the metaphyseal trabeculae resulting in thinned trabeculae, instead of total loss of trabeculation. Thus, the thickness of metaphyseal trabeculae at 520 μ intervals from the epiphyseal plate are measured and listed in Table VIII.

The metaphysis consists of a series of progressively thicker trabeculae beginning from beneath the epiphyseal plate (Fig. 3). In

Table VIII, the metaphyseal trabeculae are listed as being $23.2 \pm 3.4 \mu$ thick at 1040 μ and are progressively increased in thickness to $70.2 \pm 17.2 \mu$ at 4680 μ from the plate. At distances greater than 3640 μ , the trabeculae are quite variable in thickness and comparisons with the treated groups are difficult.

Changes in metaphyseal trabecular thickness can best be seen in Table IX in which increase in trabecular thickness is denoted by an $\uparrow$, decrease in thickness by $\downarrow$, no change by 0, significant changes by * ($p < .20$) and statistically significant changes by ** ($p < .05$). Thus in the 1 mg cortisol group, at 3640 μ from the plate, the trabeculae are statistically thinner than the trabeculae from controls.

In general the rabbits treated with 1 mg of cortisol and fed a normal diet show slightly thickened trabeculae at 1040 μ and from 2080 to 4680 μ from the plate a rather consistent thinning of trabeculae (Table IX). The same dual response occurred with the 5 and 15 mg groups. It differs only in the thicker trabeculae up to 2080 μ from the plate and the thinner trabeculae are restricted to between 2600 to 3640 μ . These values indicate that the spongiosa located proximal to the epiphyseal plate have undergone more bone apposition than bone resorption, while the more distal portion of the spongiosa have undergone more bone resorption than bone apposition subsequent to cortisol treatment. The implication of these findings will be discussed later.

The twofold response is not apparent with cortisol treatment along with a rachitogenic diet (Table IX). Thicker trabeculae are found in the metaphyseal spongiosa up to 2600 μ from the epiphyseal plate. Thinned trabeculae occur only at 3120 μ from the plate in the 5 mg of cortisol group, which indicates that the combined cortisol and rachitogenic diet treatment inhibits osteoclastic activity.

TABLE VIII

Statistical Data on the Metaphyseal Trabecular Thickness
at 520 μ Interval from the Distal Femoral Epiphyseal Plate
After Various Doses of Cortisol

Diet	Normal				Rachitogenic		
Cortisol	0	1	5	15	0	1	5
Distance							
Mean	23.2	26.5	29.8	31.5	30.1	29.5	39.2
S.D.	± 3.4	± 3.6	± 16.7	± 17.7	± 3.4	± 5.2	± 9.2
1040 μ							
No.	6	6	5	5	7	6	8
P Value	---	<.10	---	<.20	---	---	<.01
Mean	36.5	35.3	30.2	30.8	31.25	40.68	39.7
S.D.	± 3.8	± 5.0	± 27.9	± 16.0	± 14.0	± 8.5	± 7.7
1560 μ							
No.	6	6	5	5	7	6	8
P Value	---	---	---	---	---	<.10	<.10
Mean	38.7	31.8	48.1	44.9	42.0	41.1	44.9
S.D.	± 5.8	± 4.6	± 11.3	± 3.6	± 6.8	± 9.1	± 14.7
2080 μ							
No.	6	6	5	5	7	6	8
P Value	---	<.05	<.10	<.05	---	---	---
Mean	49.9	39.4	40.8	31.6	39.9	54.7	45.8
S.D.	± 8.7	± 10.5	± 16.8	± 16.9	± 8.2	± 15.1	± 11.2
2600 μ							
No.	5	6	4	5	7	6	8
P Value	---	<.10	---	<.05	---	<.05	<.20
Mean	48.2	48.3	35.8	38.8	60.5	59.2	47.0
S.D.	± 2.9	± 14.2	± 18.0	± 15.1	± 18.6	± 8.9	± 8.9
3120 μ							
No.	5	6	2	4	7	5	6
P Value	---	---	<.20	<.20	---	---	<.10

TABLE VIII (Con't.)

Statistical Data on the Metaphyseal Trabecular Thickness
at 520 μ Interval from the Distal Femoral Epiphyseal Plate
After Various Doses of Cortisol

Diet	Normal				Rachitogenic		
Cortisol	0	1	5	15	0	1	5
Distance							
Mean	63.4	52.7	42.3	39.5	49.0	56.6	47.1
S.D.	± 17.7	± 4.2	± 13.8	---	± 27.4	± 13.7	± 12.3
3640 μ							
No.	4	5	2	1	4	4	5
P Value	---	<.001	<.10	<.20	---	---	---
Mean	65.0	55.4	47.7	52.0	48.8	67.2	57.1
S.D.	± 32.2	± 7.3	---	---	---	± 16.9	± 22.0
4160 μ							
No.	4	3	2	1	1	3	3
P Value	---	---	---	---	---	---	---
Mean	70.2	42.9	39.0	55.0	---	85.0	55.0
S.D.	± 17.2	± 24.6	---	---	---	± 29.1	---
4680 μ							
No.	2	3	1	1	---	3	1
P Value	---	<.20	---	---	---	---	---
Mean	52.0	55.0	---	---	---	10.0	52.0
S.D.	---	---	---	---	---	---	---
5200 μ							
No.	2	1	---	---	---	---	---
P Value	---	---	---	---	---	---	---

TABLE IX
Differences in Metaphyseal Trabecular Thickness from Their Controls
at Varying Intervals from the Distal Femoral Epiphyseal Plate
After Various Doses of Cortisol

Distance From Plate (μ)	Normal Diet Plus			Rachitogenic Diet	
	1 mg/kg	5 mg/kg	15 mg/kg	1 mg/kg	5 mg/kg
1040	↑*	0	↑*	0	↑*
1560	0	0	0	↑*	↑*
2080	↓**	↑*	↑**	↑*	↑*
2600	↓*	0	↓**	↑**	↑*
3120	0	↓*	↓*	0	↓*
3640	↓**	↓*	↓*	0	0
4160	0	0	0	0	0
4680	↓*	0	0	Y	Y
5200	0	N	N	Y	Y

↑ Trabeculae thicker than control.

↓ Trabeculae thinner than control.

* p < .20

** p < .05

0 No difference in trabecular thickness.

N No values.

Y No control values to compare with.

V. Comparison of rabbits fed control and rachitogenic diets:

Table X lists the statistical data of the principal parameters. All the ratios of rachitogenic to normal diet are less than one except for calvaria porosity. Only the epiphyseal plate thickness is statistically different. It is interesting to note the diametrically opposed responses of femoral shaft and calvaria to the rachitogenic diet alone.

The effect of cortisol and rachitogenic diet on survival is not listed. There is a 40% and a 90% mortality in animals treated with 5

or 15 mg of cortisol/kg daily and a rachitogenic diet, respectively.

TABLE X

Statistical Data on Measurements of the Properties of Distal Femurs,
Mid-Diaphysis of Femurs, and Calvaria of Animals
Fed Normal and Rachitogenic Diets

Parameters	Control Diet	Rachitogenic Diet	Ratio Rachitogenic/Normal
Femoral shaft porosity	3.78 $\pm$ 1.25%	1.99 $\pm$ 1.25%	0.53
Calvaria porosity	39.22 $\pm$ 6.41%	42.07 $\pm$ 5.16%	1.09
Epiphyseal plate thickness (femur)	459 $\pm$ 51 μ^*	288 $\pm$ 57 μ	0.63
Femoral metaphysis	4.22 $\pm$ 1.21mm	3.27 $\pm$ 0.61mm	0.78

*
p < .001

VI. Summary of the influence of cortisol on growing rabbits:

Table XI lists the significant effects at the 95% confidence level of the various parameters in each treatment group. The + represents significant differences from its diet control and 0 represents an insignificant response. Except for weight losses and survival, the table lists no apparent qualitative differences for the influence of cortisol upon bone tissues in rabbits fed normal or rachitogenic diets; however, cortisol in animals fed a normal diet induces a greater quantitative response in bone than in the comparable group fed a rachitogenic diet.

TABLE XI
Significant Differences ($p < .05$) in the Influence of
Cortisol Upon Growing Bone

Parameter	Normal Diet mg Cortisol/kg daily			Rachitogenic Diets mg Cortisol/kg daily	
	1	5	15	1	5
1. Loss of body weight	0	0	+	+	++
2. Adrenal atrophy	+	++	+	+	+
3. Thymic involution	+	++	+	+	+
4. Inc. femoral cortex porosity	+	+	+	+	++
5. Inc. calvarium porosity	0	++	+	0	+
6. Dec. epi. plate thickness	+	++	+	+	+
7. Growth arrest plate	0	+	+	0	+
8. Regression of femoral metaphysis	0	0*	0	0	0
9. Thinner metaphyseal trabeculae	0	0*	+	0	0

*Magnitude of response greater than comparable treatment group fed a different diet.

DISCUSSION

One of the aims of this report was to establish a relatively rapid quantitative histological bioassay for the influence of cortisol on the skeleton in growing rabbits. By employing contact microradiography, it was feasible to establish various test systems in the skeleton which proved quite useful and scientifically stimulating. Decalcification and embedding adjacent areas in celloidin and paraffin was also performed with the hope of defining the cellular population related to

these bony changes; however, these techniques were far too time consuming. Any future attempts to determine the influence of various glucocorticoids upon the growing bones of rabbits will be restricted to measurements of femoral cortex porosity, the epiphyseal plate thickness, presence of growth arrest plate, length of femoral metaphysis and metaphyseal trabeculae thickness at various distances from the epiphyseal plate. With these six endpoints, it was possible to evaluate the osteoporotic influence upon cortical and spongy bones (femoral shaft porosity, length of distal femoral metaphysis and thickness of metaphyseal trabeculae) and the influence on the longitudinal growth of long bones (the epiphyseal plate thickness and presence of growth arrest plate).

Another aim was to test whether animals fed a decreased calcium and phosphorus diet resulted in an increased osteolytic effect of cortisol. This was based on the finding of Storey who observed in weanling rabbits that a diet deficient in calcium and phosphorus will enhance the osteolytic effect of cortisol upon the calvarium (1).

The combined effect of the rachitogenic diet plus cortisol enhanced the osteolytic effect of cortisol upon the femoral shafts, but not in the calvaria. Animals treated with 5 mg of cortisol exhibited a statistical increase in the osteolytic effect of cortisol upon the femoral shaft when combined with the rachitogenic diet. For instance, the rachitogenic diet resulted in a greater porosity with the treatment of 5 mg cortisol daily compared to the same dose with a normal diet. In general, the slope of the dose response line of rachitogenic diet plus cortisol was steeper than the slope of the dose response line of normal diet plus cortisol. Moreover, the increased osteolytic effect of the rachitogenic diet plus 1 mg of cortisol was due to the depression of

its control. In other words, the rachitogenic diet alone resulted in less porosity. On the contrary, the calvarium porosity studies showed that the slope of the dose response line to cortisol plus the rachitogenic diet was much less steep than the slope of the dose response line to cortisol and a normal diet. In summary, the study showed that there was some advantage in using the rachitogenic diet in the femoral shafts porosity analysis while with the calvaria there was a distinct disadvantage in using a rachitogenic diet. What little advantage is gained by the use of a rachitogenic diet in the analysis of the femoral shaft porosity was lost by the fact that animals treated with a rachitogenic diet plus 5 or 15 mg of cortisol have very poor survival rates. There was also an obvious disadvantage in using the combined treatment of the rachitogenic diet and cortisol for the measurements of epiphyseal plate thickness, length of metaphysis, adrenal atrophy and thymic involution.

The effect of glucocorticoids (cortisone) in inhibiting the growth of developing animals was described long ago by Wells and Kendall (2). More detailed studies have been done on rats, guinea pigs, and rabbits by other authors (4,5,6,7). In fact, Cavallero, Bertazzoli, Rossi, and Sala briefly noted histologic changes of narrowing of the epiphyseal plate (5). Follis also described changes in all the above species receiving cortisone (3,4). More recently Sissions and Hadfield measured the thickness of the epiphyseal plate in histological sections of animals treated with various doses of cortisone (7). There was general agreement that a reduction in hypertrophic cartilage and cessation of proliferation of cells of the cartilage columns existed (7). Our next report will deal with quantifying the cellular population in the decalcified histological sections and it would be interesting to

observe whether the findings will support past investigators.

The growth arrest plate appeared to be identical to the plate formed in the process of the closure of the epiphyseal plate. The manner by which the growth arrest plate was formed cannot be elucidated by the present material. Nevertheless, observations from decalcified sections suggest that the following events could contribute to the formation of a plate. Cortisol could conceivably depress the osteoclasts and chondrocytes ability to destroy calcified cartilage or limit the supply of new osteoclasts and chondrocytes. The suppression of these events can result in the persistence of calcified cartilaginous matrix. Also, cortisol does not seem to alter the function of the existing population of osteoclasts and osteoblasts in the primary spongiosa and the large number of osteoblasts and the small number of osteoclasts in this region will result in a net gain of bone. The apposition of more bone and the persistence of more calcified cartilage adds up to a growth arrest plate.

The lack of pronounced osteolytic effect on the distal femoral metaphysis was most surprising. Sissons and Hadfield have reported an osteolytic sequence in which the entire metaphysis was wiped out in 24 days with 10 mg of cortisone daily (7). Contrary to the above finding, the femoral metaphysis was slightly reduced and it was not statistically significant. Indeed, the metaphysis consisting of spongy bone is an exciting site of action of cortisone. Follis has reported a lack of resorption of metaphyseal bone in mice, guinea pigs and rabbits (5) while Sissons and Hadfield showed inconsistent findings in the metaphyseal regions of rats in two series of experiments. In an earlier experiment they reported the loss of trabeculation and in the second experimental run there was a thickening of metaphyseal trabeculae (7).

Under the present experimental conditions, the persistence of the femoral metaphysis may be due to such variables as the duration of treatment, dose, strain of rabbits and/or the use of cortisol instead of cortisone.

The lack of statistically significant reduction in femoral metaphysis was coupled with the observations of thickened trabeculae in the primary spongiosa and thinner trabeculae in the secondary spongiosa. In an attempt to explain the above observations it must be kept in mind that it is our belief cortisol suppresses the transformation of both osteoblasts and osteoclasts from mesenchymal or progenitor cells and that existing cells at the time of cortisol injection will survive and continue to function. In the primary spongiosa there are many more osteoblasts than osteoclasts, and conversely there are more osteoclasts than osteoblasts in the secondary spongiosa. Therefore, the result is, if the source of new osteoblasts and osteoclasts were dried up or slowed down and the elongation of the bone was stopped, a metaphysis in frozen animation (the spongiosa existing at day 0 still remains at day 16); then, the existing osteoblast and osteoclast population could produce thicker trabeculae in the primary spongiosa due to the dominant population of osteoblasts and thinned trabeculae in the secondary spongiosa due to the dominant population of osteoclasts. A preliminary study of the primary spongiosa of the proximal tibial metaphysis tend to support the above sequence. There was a significant decrease in the number of osteoclasts with 5 and 15 mg of cortisol and a statistically significant increase in the thickness of the trabeculae.

A further discussion of the three sites chosen for quantitative analyses of the osteolytic effect of cortisol could be of value in an

attempt to clarify the differential responses of these areas of the skeleton. Briefly the three sites consist of the distal femoral metaphysis made up entirely of spongy bone, the femoral shaft made up entirely of compact bone and the calvarium made up with a mixture of cortical and spongy bone. Moreover, the study showed that the femoral shaft was more readily resorbed than the calvarium, which in turn was more easily resorbed than the femoral metaphysis. The differential osteolytic capacity of these sites to cortisol may be related to the bone surfaces available for resorption. Most bone histologists accept the fact that bone lined with osteoid is resistant to osteoclastic activity. In the femoral shaft, there are very few surfaces lined with osteoid, while in the metaphysis and especially in the primary spongiosa nearly every bone surface is lined with osteoid. The decrease in bone surface for bone resorption coupled with a decrease in the number of osteoclasts will result in the retardation of the normal osteolytic sequences in the metaphysis. Conversely, the femoral shaft consisted of primary haversian systems with very few bone surfaces lined with osteoid and with increased doses of cortisol, the number of osteoclasts increase. Why there was an increase in the number of osteoclasts in the compacta and a concomitant decrease in osteoclasts in the metaphysis is most puzzling?

The calvarium is a site made up of both compact and spongy bone. It consists of predominantly cortical bone and few trabeculae in the diploic areas. The inner and outer tables of cortical bone contain very irregular endosteal bone surfaces which make an analysis of this bone almost impossible. It may be more valid to say that the present analytical procedure needs refinement to detect porosity in the calvarium rather than the conclusion that the calvarium is less sensitive than the

femoral shaft to the osteolytic effect of cortisol.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Fred Goldthorpe and Miss Susan Dickman for their invaluable technical assistance.

REFERENCES

1. E. Storey, Cortisone-induced bone resorption in the rabbit, *Endocrinology* 68:533-542 (1961).
2. B. B. Wells and E. C. Kendall, The influence of corticosterone and C_{17} hydroxydehydrocorticosterone (compound E) on somatic growth, *Proc. Mayo Clin.* 15:324-328 (1940).
3. R. H. Follis, Effect of cortisone on growing bones of the rat. *Proc. Soc. Exp. Biol.* 76:722-723 (1951).
4. R. H. Follis, Non-effect of cortisone on growing bones of mice, guinea pigs and rabbits. *Proc. Soc. Exp. Biol.* 78:723-724 (1951).
5. C. Cavallero, C. Bertazzoli, L. Rossi and G. Sala. Studio sperimentale sugli effetti del cortisone. II. Effetti morfologici generali sul ratto normale. *Sperimentale*, 101:209-225 (1951).
6. H. A. Sissons and G. J. Hadfield, The influence of cortisone on the repair of experimental fractures in the rabbit. *Brit. J. Surg.* 39: 172-178 (1951).
7. H. A. Sissons and G. J. Hadfield. The influence of cortisone on the structure and growth of bone. *J. Anatomy*, 89:69-78 (1955).

Fig. 1. Contact microradiographs of 100 μ thick bone section of mid-femoral shaft. The radio-opaque regions are white and the radiolucent regions are black. Note the increased numbers of radiolucent areas in the treated specimen (15 mg of cortisol/kg/day). This is an extreme case (X 15).

Fig. 2. Contact microradiographs of a portion of calvaria showing massive resorption in a rabbit injected with 15 mg of cortisol/kg/day. Note in the control bone the inner (inferior) and outer (superior) tables of compact bone and the few interconnecting spicules of bone in the diploic spaces (X 18).

Fig. 3. Representative contact microradiographs of longitudinal sections of the distal femur. Note the radiolucent region between the epiphysis and the spongy bone of the metaphysis in both the control and the treated specimens. Observe the obvious decrease in thickness of the epiphyseal growth plate in the treated animal (5 mg of cortisol/kg/day) (X 6).

Fig. 4. Higher magnifications of Fig. 3. Note the thinned epiphyseal plate and the obvious growth arrest plate in the treated rabbit. The horizontal white lines are 520 μ wide and are drawn at 520 μ intervals from the epiphyseal plate. The individual trabecular widths were determined at these sites (X 24).

Fig. 5. Linear dose-response plot of femoral shafts porosities in rabbits fed control or rachitogenic diets. The % porosity is plotted on the ordinate and the dose in mg cortisol per kilogram on the abscissa. Note the steeper slope of the dose response line from rabbits fed a rachitogenic diet. The I represents the standard deviation of the mean.

Fig. 6. Linear dose response plot of calvaria porosities in rabbits fed normal or rachitogenic diets. Note the steeper slope of the dose response plot from rabbits fed a normal diet. The I represents the standard deviation of the mean.

Fig. 7. Linear dose response plot of distal femoral epiphyseal thickness in rabbits fed control or rachitogenic diets. Although the lines are not plotted, a least squares fit of the points results in a dose response line with slopes of -0.155 for the normal diet and $-.026$ for the rachitogenic diet. The I represents the standard deviation of the mean.

THE EFFECTS OF IRRADIATION ON ENDOCRINE CELLS
V. A COMPARISON BETWEEN THE EFFECTS OF
INANITION AND IRRADIATION ON ANDROGEN
PRODUCTION BY MURINE TESTICULAR TISSUE

David L. Berliner¹ and LeGrande C. Ellis²

Abstract: Male rats (Holtzman strain) were individually fed 7.5 grams of Laboratory Chow daily for 20 days. Control animals were fed the same ration, and water was given ad libitum to both groups of rats. Treatment was initiated when the animals were 15 weeks of age. Testicular tissue from both groups of animals was incubated with 5-pregnenolone-7 α -H³ and progesterone-4-C¹⁴. Incubations from both groups of animals were made with and without added cofactors. After 20 days of treatment, body weights, testicular weights, and seminal vesicle weights were diminished. Also, the in vitro biosynthesis of androgens was markedly diminished as a result of this treatment. The effects of inanition on androgen biosynthesis differed from that observed previously for irradiation in that the effects were not reversed by the addition of a NADPH-G-S. The reduction in androgen biosynthesis observed in this investigation was attributed to a lack of enzymes required for the biotransformation of 5-pregnenolone and progesterone into androgens. Evidence was observed for a diminished desmolase (i.e., side-chain splitting) activity. The diminished enzyme activity of the testis was attributed to a lack of gonadotrophins. Thus, it would appear that inanition does diminish androgen production by the testis, but the effect differs from that observed for irradiation.

INTRODUCTION

Recent investigations have shown that the biotransformation of steroids by murine testicular tissue is altered after various modes of irradiation (1-3). It has also been shown (4) that death resulting from localized irradiation to the heads of rats can be prevented by introducing feed into the stomachs of these animals via a gastronomy tube. Other workers (5) indicate that vitamin B deficiency or partial inanition results in a diminished androgen synthesis in male rats as indicated by prostatic and seminal vesicle weights. Head irradiated

and total-body irradiated animals may undergo body weight losses due to a self-imposed restricted feed intake due to lesions of the oral cavity (4,6) or the alimentary canal. Therefore, it was deemed advisable to ascertain if inanition will alter the biotransformation of steroids by rat testicular tissue, and to ascertain if this response differs from that observed after irradiation.

MATERIALS AND METHODS

Five male rats (Holtzman strain - 15 weeks of age) were individually fed 7.5 grams of Laboratory Chow (Ralston Purina Co.) daily for 20 days. Four additional rats were fed the same ration ad libitum, and both groups received water ad libitum. At the end of the treatment, both groups of rats were euthanized by ether anesthesia, and the testes were removed, chilled, trimmed, minced, and incubated with 5-pregnenolone-7 α -H³ and progesterone-4-C¹⁴ as described elsewhere (1). Testicular mince from each group of rats was incubated without added cofactor, with 2.5 μ moles of NADPH, and with 2.5 μ moles of NADPH plus 2.5 μ moles of glucose-5-phosphate. In this manner, each treatment was replicated four times with each rat donating 0.5 gm of tissue for each of the three incubations mentioned above.

The steroids were extracted, separated by chromatography and derivative formation, and characterized as previously described in the literature (1,7). Radioactive samples were counted in a liquid scintillation spectrometer (Packard Inst. Co.), and the data were calculated with a digital computer (IBM Model-7040) (1). Statistical comparisons of the data for groups of two only (i.e., body weights, etc.) were made by using a "t" test (8). The remaining data were analyzed according to the method of analysis of variance Model I (8). If the

"F" value calculated from the data were significant, a multiple range test or "Q" test was employed (8) to find where the differences between means were. The 95% level of significance was used throughout the entire procedure. High-polar steroid compounds were estimated by the method of triangulation (9).

RESULTS

Restriction of the feed intake of mature, male rats to 7.5 grams of feed per day for 20 days diminished body weight by approximately one-third. A similar decrease in testicular weight and seminal vesicle

TABLE I. Carcass Weights and Organ Weights of Control and Feed Deprived Rats

Measurement	Control (gm)	Inanition (gm)
Body weight ⁺	327 $\pm$ 6.6*	199 $\pm$ 3.4
Testicular weight ¹	3.26 $\pm$ 0.18	2.72 $\pm$ 0.16
Seminal vesicle weight ²	1.20 $\pm$ 0.08	0.29 $\pm$ 0.20
Testicular weight (gm/100gm body wt.)	1.00 $\pm$ 0.06	1.37 $\pm$ 0.10

* Mean $\pm$ standard error of mean

⁺P < 0.001

¹P < 0.05

²P < 0.001

³P < 0.001

weight was observed for the animals on the restricted feed intake. The decrease in testicular weight was not proportional to the loss in body weight as can be seen in Table I, when testicular weight was expressed on a grams/100 grams of body weight basis. As can be seen in Fig. 1

and Table I, androstenedione and total androgen production was diminished for the treated animals. The addition of either NADPH or a NADPH-G-S increased the androstenedione, testosterone, and total androgen production for both the control and the treated animals. However,

Fig. 1.

there was a greater response to the addition of NADPH or NADPH-G-S by testicular tissue from control animals than for similar tissue from treated rats. It is interesting to note that there was no difference in testosterone production between the control and fasted animals when

NADPH was added to the incubation mixture. There was, however, a difference in testosterone production between the two groups when the NADPH-G-S was added to the incubation mixture.

The addition of either the NADPH or the NADPH-G-S to the incubation mixture increased the biotransformation of progesterone and 17-hydroxyprogesterone into androgens for the control animals. Similarly,

TABLE II. Statistical Comparisons of the Data in Figure 1 for Control Versus Inanition

Comparison	Statistical Probability
No cofactor containing groups	
Androstenedione	P < 0.05
Testosterone	P > 0.05
Total Androgens	P < 0.05
NADPH containing groups	
Androstenedione	P < 0.05
Testosterone	P > 0.05
Total Androgens	P < 0.05
NADPH + glucose-6-phosphate containing groups	
Androstenedione	P < 0.05
Testosterone	P < 0.05
Total Androgens	P < 0.05

an increase in the biotransformation of progesterone and 17 α -hydroxyprogesterone into androgens was observed for the treated animals. There was, however, less 17 α -hydroxyprogesterone biotransformed into androgens by the treated animals as compared to the control group indicative of a diminished side-chain splitting activity. The biotransformation of 5-pregnenolone-7 α -H³ and progesterone-4-C¹⁴ into

high-polar compounds (i.e., compounds with a polarity greater than testosterone) was significantly less ($P < 0.001$) for the treated animals than for the control animals (Fig. 1).

DISCUSSION

The data from this investigation show that restricting the feed intake of rats to 7.5 grams per day for 20 days reduces body weight, testicular weight, and seminal vesicle weight. Androgen production is also reduced as indicated by the diminished seminal vesicle weights of the treated rats and the in vitro biotransformation of 5-pregnenolone- 7α -H³ and progesterone-4-C¹⁴ into androstenedione and total androgens (androstenedione and testosterone). Androstenedione and testosterone were combined into a total androgen fraction since it has been shown that in murine species androstenedione is almost as effective an androgen as testosterone (10). In addition, both of the above compounds are considered to be true secretory products of the mammalian testis (11). Thus, these data show that restriction of feed intake of rats resulted in a diminished biosynthesis by testicular tissue. However, the alteration in steroid biotransformations observed in this investigation differs from that observed for direct irradiation of testes (2) or that observed for direct irradiation to the heads of rats (3). In the two investigations above, the addition of a NADPH-G-S to the incubation mixture reversed most of the defects noticed following irradiation. These data (Fig. 1) show the opposite effect. Instead of reversing the effects of restricting feed intake on androgen biosynthesis, these effects were amplified. In the previous investigations (2,3) the effects of irradiation on androgen biosynthesis by testicular tissue was attributed to the lack of an efficient NADPH-G-S. The data from

this investigation support this conclusion and indicate that in feed-restricted animals, the defect is a lack of steroid biotransforming enzymes and not a diminished cofactor content. The data (Table I) indicate that desmolase (i.e., the enzyme responsible for side-chain splitting of 17α -hydroxyprogesterone to androstenedione) activity is diminished following inanition as indicated by the incubations containing the added cofactors. Letham (12), in his excellent review of the subject, has pointed out that acute and chronic starvation results in a gonadal hypofunction due to diminished circulating levels of gonadotrophins. Furthermore, the endocrine response of the testis to inanition has been termed "psuedohypophysectomy" due to the similarity of the response of the animal to these two treatments (12). Thus, these data show that inanition can diminish androgen production by rat testicular tissue as judged by a diminished accessory organ weight and the diminished in vitro biotransformation of 5-pregnenolone and progesterone into androgens. However, this response differs from that observed for irradiation in that it is not reversed by the addition of a NADPH-G-S.

TABLE III. Average Quantities of 5-Pregnenolone-7 α -H³ and Progesterone-4-C¹⁴
 Biotransformed into Androgens and Their Immediate Precursors
 by Testicular Tissue as Expressed on a Per Rat Basis

Steroid Isolated	M μ Moles 5-Pregnenolone- 7 α -H ³	M μ Moles Progesterone- 4-C ¹⁴	Total	H ³ /C ¹⁴ Ratio
No cofactor containing group				
Control				
Progesterone	1.13	3.11	4.24	0.36
17 α -hydroxyprogesterone	0.86	2.93	3.79	0.29
Androstenedione	0.76	2.38	3.14	0.32
Testosterone	0.31	0.85	1.16	0.36
Inanition				
Progesterone	1.03	3.34	4.37	0.31
17 α -hydroxyprogesterone	0.82	2.80	3.62	0.29
Androstenedione	0.52	1.73	2.25	0.30
Testosterone	0.37	0.05	1.32	0.39
NADPH containing group				
Control				
Progesterone	0.19	0.27	0.46	0.70
17 α -hydroxyprogesterone	0.53	0.27	0.80	1.96
Androstenedione	3.43	6.69	10.12	0.51
Testosterone	0.91	1.50	2.40	0.61
Inanition				
Progesterone	0.44	0.32	0.76	1.38
17 α -hydroxyprogesterone	1.42	1.47	2.89	0.97
Androstenedione	1.77	4.24	6.01	0.42
Testosterone	0.76	1.60	2.36	0.48

TABLE III. Continued...

Steroid Isolated	Mμ Moles 5-Pregnenolone- 7α-H ³	Mμ Moles Progesterone- 4-C ¹⁴	Total	H ³ /C ¹⁴ Ratio
NADPH + glucose-6-phosphate containing group				
Control				
Progesterone	0.32	0.57	0.89	0.56
17α-hydroxyprogesterone	0.18	0.13	0.31	1.38
Androstenedione	2.91	5.63	8.54	0.52
Testosterone	1.69	2.68	4.37	0.63
Inanition				
Progesterone	0.29	0.42	0.71	0.69
17α-hyddoxyprogesterone	0.57	0.50	1.07	1.14
Androstenedione	1.53	3.17	4.70	0.48
Testosterone	0.98	1.77	2.75	0.55

FOOTNOTES

1. U. S. Public Health Service Research Career Development Awardee.
(1-K6-CA-22,57-01).
2. U. S. Public Health Fellow (Training Grant # 5 T1 GM-958-03).
Present address: Department of Zoology, Utah State University,
Logan, Utah.
3. In this paper, the following abbreviations are used: 5-pregnenolone, 5-pregnen-3 β -ol-20-one; progesterone, 4-pregnene-3,20-dione; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NADPH-G-S, reduced nicotinamide adenine dinucleotide phosphate-generating system; 17 α -hydroxyprogesterone, 4-pregnen-17 α -ol-3,20-dione; androstenedione, 4-androstene-3,20-dione; testosterone, 4-androsten-17 β -ol-3-one.

REFERENCES

1. L. C. Ellis and D. L. Berliner, The effects of ionizing radiations on endocrine cells. I. Steroid biotransformations and androgen production by testes from irradiated mice. Radiation Res. 20, 549-563 (1963).
2. D. L. Berliner, L. C. Ellis, and G. N. Taylor, The effects of ionizing radiations on endocrine cells. II. Restoration of androgen production with a reduced nicotinamide adenine dinucleotide phosphate-generating system following irradiation of rat testes. Radiation Res. 22, 345-356 (1964).
3. L. C. Ellis and D. L. Berliner, The effects of ionizing radiations on endocrine cells. III. Restoration of testosterone production with a reduced nicotinamide adenine dinucleotide-phosphate generating system after localized irradiation to heads of rats. Radiation Res. (In Press - 1964).
4. R. Goepp and F. Fitch, Prevention of death in mice after lethal irradiation of the head. Radiation Res. 19, 670-675 (1963).
5. C. R. Moore and L. T. Samuels, The action of testis hormone in correcting changes induced in the rat prostate and seminal vesicles by vitamin B deficiency or partial inanition. Am. J. Physiol. 96, 278-288 (1931).
6. R. Goepp and F. Fitch, Pathological study of oral radiation death in mice. Radiation Res. 16, 833-845 (1962).
7. D. L. Berliner, The microchemical identification of steroids from biological media. In Advances in Tracer Methodology, (Ed. S. Rothchild) Vol. II, Plenum Press, New York (In Press - 1964).
8. G. W. Snedecor, Statistical Methods. Iowa State College Press, Iowa, (1959).

9. D. L. Berliner, O. V. Dominguez, and G. Westenskow, Determination of carbon-14 steroids on paper chromatograms. Anal. Chem. 29, 1797-1800 (1953).
10. R. I. Dorfman and R. A. Shipley, Androgens. pp. 116-128, John Wiley and Sons, New York (1956).
11. C. D. West, V. P. Hollander, T. H. Kritchevsky, and K. Dobriner, The isolation and identification of testosterone, Δ^4 -androstenedione-3, 17, and 7-ketocholesterol from spermatoc vein blood. J. Clin. Endocrinol. Metab. 12, 915-916 (1952).
12. J. H. Letham, Nutrition and accessory sex organs. J. Natl. Cancer Inst. Mono. 12, 201-210 (1963).

A COMPARISON OF TWO METHODS OF ANALYZING THE HEMATOLOGICAL DATA FROM STRONTIUM-90 INJECTED BEAGLES

Jean H. Dougherty, Leon S. Rosenblatt*, and Peter J. Mikolaj

Abstract: Selected hematological data from the strontium injected beagles were subjected to two methods of statistical analysis, a randomized block and a one-way analysis of variance. The experimental dogs were compared both to strontium 0-level dogs and to pooled 0-level dogs from all radionuclides. Comparable results were obtained with either control group in terms of significant differences. The randomized block design analysis of variance was found to be the more sensitive type of analysis and will be the method of choice for subsequent analyses of hematological changes following internal irradiation.

INTRODUCTION

Two statistical methods have been used in an attempt to get meaningful significance values for the relationship of the various hematological traits of control (0-level) dogs to those injected with the different dose levels of the various radionuclides as well as the relationships between the dose levels. The small number of animals at each dose level as well as the fact that the 12 groups of each radionuclide were injected over a long period of time and some dose levels were not added until much later than others in their group are a few of the complicating factors that must be considered in any type of analysis. The strontium dogs were chosen for the preliminary analysis since this is the only radionuclide in which all dose levels in a group were injected at the same time (with the exception of F1S1.7 and a few replacement dogs - i.e., F1S1A, M5S1.7A, etc.). Thus, with this radionuclide the effect of time as a variable could be ruled out. This was

*Biological Frontiers Institute, Livermore, California.

found to be important since time trends have been found in many of the hematological traits⁽¹⁾.

The first strontium analyses were made by Drs. Leon S. Rosenblatt and Fred T. Shultz of Biological Frontiers Institute of Livermore, California, several years ago (1962). They selected the following hematological traits for their preliminary analysis: volume of packed red cells (VPRC), segmented polymorphonuclear neutrophils (pmns) and lymphocytes and compared the mean values from the seven dose levels at six month intervals (from 6 through 48 months post-injection). Analyses were not run beyond 48 months since there were not sufficient dogs on the experiment beyond this time. The data was analyzed by a randomized block design analysis of variance.

During the past year, using the IBM 7040-1401 computer system at the University of Utah Computer Center, we have reanalyzed the same traits from the strontium dogs using a one-way analysis of variance and covering the same time intervals after injection.

METHODS

I. Analyses by Biological Frontiers Institute. Two types of analyses were made: A. Strontium dogs at each dose level (0, 1, 1.7, 2, 3, 4, 5) were tested against the strontium 0-level dogs, and B. Strontium dogs at each level were tested against the 0-level dogs of all radionuclides. This pooled group of 0-level controls will be called the "master controls". They were used to increase the sensitivity of the analysis by providing a larger sample size at the 6 month intervals. Since the 0-level dogs have been introduced into the experiment from 1952-1963, the counts were corrected for the time trends mentioned previously. These correction factors were derived from regression equations

utilizing the yearly means of pre-injection counts of all dogs over this ten year period. The details of the method are given in a previous semi-annual report⁽²⁾.

Analysis A. Strontium Dogs vs. Strontium 0-Level Controls. A series of analyses of variance using the randomized block design were made. The techniques employed were described in an earlier semi-annual report⁽³⁾ and will not be given in detail here. In general, where there were missing values they were estimated by a least squares estimation. The standard errors obtained were utilized in making a series of Duncan's multiple range tests⁽⁴⁾ so that each dose level could be tested against every other dose level. The experimental design of the toxicity experiment permitted these pairwise comparisons of the different dose levels. The number of dogs at each time interval is given at the top of Table 1a. The results of the analysis are seen in Tables 2a (VPRC), 2b (pmns), and 2c (lymphocytes). The means for each dose level are included in the top half of the tables. Significance levels (.05, .01, and .001) are inserted only where significant differences were found. A dash indicates that there were insufficient data available (see Table 1). For these analyses no calendar time correction was necessary and none was made. For this reason the sets of means found in Tables 2 and 3 (see below) for the same traits are not identical.

Table 1a

Number of Dogs in Randomized Block Analysis of Variance

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	10	12	12	10	7	6	6	5
1.0	12	12	12	11	8	7	6	5
1.7	12	11	12	11	8	7	6	5
2.0	11	12	12	11	8	7	6	5
3.0	12	12	12	10	8	7	6	5
4.0	12	12	12	11	8	7	5	5
5.0	13	12	12	11	8	4	2	-
Master* Control	44	46	45	43	38	39	38	35

* Master Control used in Analysis B.

Table 1b

Number of Dogs in One-Way Analysis of Variance

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	12	13	13	10	11	11	11	11
1.0	12	11	12	11	11	12	12	12
1.7	12	12	13	12	12	12	12	12
2.0	11	12	12	12	12	12	12	12
3.0	12	12	12	12	12	12	11	12
4.0	12	12	12	12	12	11	12	12
5.0	13	12	12	12	11	9	6	4
Master* Control	55	58	57	53	51	53	52	48

* Master Control used in place of 0.0 level for Analyses 3 and 4.

Results of Duncan's Multiple Range Test for VPRC

[illegible]

Table 2b (Analysis A)

Results of Duncan's Multiple Range Test for Pmns ($\times 10^3$)

Dose Level	Means at 6 Monthly Intervals							
	6	12	18	24	30	36	42	48
0.0	7.13	5.73	6.26	5.11	5.48	5.00	4.05	3.94
1.0	6.60	6.01	5.39	4.78	4.98	6.86	4.43	4.66
2.0	6.65	5.69	5.52	5.39	5.10	5.61	4.42	5.02
3.0	5.12	5.11	4.69	5.52	4.79	4.81	3.80	3.40
4.0	3.97	3.96	3.45	3.73	3.80	4.71	4.47	5.22
5.0	3.21	2.83	3.04	2.67	3.51	3.34	4.63	--

Comparison	Significance Levels (Blank indicates $P > .05$)							
0.0 vs 1.0								
0.0 vs 2.0								
0.0 vs 3.0	.05		.01					
0.0 vs 4.0	.001	.01	.001	.05				
0.0 vs 5.0	.001	.001	.001	.001	.05			
1.0 vs 2.0								
1.0 vs 3.0								
1.0 vs 4.0	.01	.01	.001					
1.0 vs 5.0	.001	.001	.001	.001		.05		
2.0 vs 3.0								
2.0 vs 4.0	.01	.01	.001	.01				
2.0 vs 5.0	.001	.001	.001	.001				
3.0 vs 4.0	.05	.001	.05	.01				
3.0 vs 5.0			.01	.001				
4.0 vs 5.0				.05				

Table 2c (Analysis A)

Results of Duncan's Multiple Range Tests for Lymphocytes ($\times 10^3$)

Dose Level	Means at 6 Monthly Intervals							
	6	12	18	24	30	36	42	48
0.0	3.67	3.68	3.52	3.10	3.70	3.17	3.32	2.97
1.0	3.60	3.31	3.18	2.94	2.90	2.39	2.43	1.99
2.0	3.34	3.12	3.35	3.02	2.94	2.61	3.02	2.94
3.0	2.78	3.10	2.95	2.98	2.82	2.51	2.76	2.88
4.0	2.88	3.03	2.42	2.50	2.63	2.58	2.89	2.96
5.0	2.16	2.07	1.95	2.29	2.46	2.13	1.90	--
Comparison	Significance Levels (Blank indicates $P > .05$)							
0.0 vs 1.0						.05	.05	.01
0.0 vs 2.0								
0.0 vs 3.0	.05				.05			
0.0 vs 4.0			.01		.05			
0.0 vs 5.0	.001	.001	.001	.05	.01	.01	.01	---
1.0 vs 2.0								.01
1.0 vs 3.0	.05							.01
1.0 vs 4.0			.05					.01
1.0 vs 5.0	.001	.001	.001					---
2.0 vs 3.0								
2.0 vs 4.0			.01					
2.0 vs 5.0	.01	.01	.001	.05			.01	---
3.0 vs 4.0								
3.0 vs 5.0		.01	.01				.05	---
4.0 vs 5.0		.01					.05	---

Analysis B. Strontium Dogs vs. Master Controls. The comparisons of strontium dogs to master controls were made by a series of individual t-tests, testing each strontium dose level against the master controls (see Table 1a for number of master controls). Since the master control data was corrected for time trends, the data on the strontium dogs was also corrected for calendar year. The assumption was made that the same factor or factors producing the time trends on the control dogs were operating on the experimental ones and in the same manner. No pairwise comparisons were made (i.e., between levels) on the corrected data. Tables 3a, 3b, and 3c give the means of the corrected data and the results of the t-tests. The latter are given in terms of probability values. Asterisks indicate that a significant difference has been observed. It may be seen from these tables that the strontium 0-level controls are not significantly different from the master controls (not containing the strontium 0-level controls). In fact, the means are very nearly alike. This is an absolutely necessary condition before one can legitimately test the various injection levels against the master controls. Whether this will hold true for the 0-level of all radionuclides has not as yet been ascertained.

Table 3a VPRC (Analysis B)

Strontium Dogs vs. "Master Controls"

Dose Level	Means at 6 Monthly Intervals							
	6	12	18	24	30	36	42	48
Master Control	49.7	48.8	49.4	48.7	48.7	48.7	47.3	46.2
0.0	50.1	47.2	49.6	49.8	51.0	49.5	48.3	46.1
1.0	50.6	47.1	49.3	47.0	48.5	48.0	48.8	48.6
1.7	50.5	46.4	46.5	47.6	47.9	48.0	48.5	48.0
2.0	47.5	46.7	47.6	48.4	48.5	47.6	48.5	51.2
3.0	49.7	49.9	50.0	48.6	48.1	46.9	47.0	49.8
4.0	49.5	47.5	46.8	46.5	45.9	44.0	42.0	47.4
5.0	47.5	47.8	47.7	47.7	47.2	43.0	43.0	--

Ranges of Significance (Probability Values) from t-test of Individual Dose Levels Against "Master Controls"

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	.80-.90	.20-.30	.80-.90	.50-.60	.20-.30	.60-.70	.50-.60	.90 ⁺
1.0	.50-.60	.20-.30	.90 ⁺	.30-.40	.90 ⁺	.60-.70	.30-.40	.20-.30
1.7	.60-.70	.05-.10	.05-.10	.50-.60	.60-.70	.70-.80	.40-.50	.90 ⁺
2.0	.10-.20	.05-.10	.20-.30	.80-.90	.90 ⁺	.50-.60	.40-.50	.01-.02 [*]
3.0	.90 ⁺	.30-.40	.60-.70	.90 ⁺	.70-.80	.30-.40	.80-.90	.20-.30
4.0	.90 ⁺	.30-.40	.10-.20	.20-.30	.10-.20	.001-.01 [*]	.001-.01 [*]	.50-.60
5.0	.10-.20	.30-.40	.30-.40	.50-.60	.30-.40	.01-.02 [*]	.05-.10	---

Table 3b Pmns (Analysis B)
Strontium Dogs vs. "Master Controls"

Dose Level	Means at 6 Monthly Intervals							
	6	12	18	24	30	36	42	48
Master Control	8.58	8.58	7.58	7.54	7.30	7.34	7.21	7.16
0.0	8.74	7.19	7.85	6.53	6.97	6.80	5.80	5.74
1.0	8.08	7.28	7.14	6.53	6.40	8.30	6.35	6.50
1.7	7.81	7.26	7.21	6.88	7.00	8.07	6.68	7.44
2.0	8.10	7.16	7.12	7.04	6.74	7.31	6.17	6.82
3.0	6.47	6.57	6.28	7.20	6.42	6.51	5.55	5.20
4.0	5.32	5.42	5.04	5.39	5.45	6.43	6.16	7.02
5.0	4.48	4.38	4.43	4.35	4.85	4.92	7.20	--

Ranges of Significance (Probability Values) from t-test
of Individual Dose Levels Against "Master Controls"

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	.80-.90	.10-.20	.10-.20	.10-.20	.70-.80	.50-.60	.10-.20	.05-.10
1.0	.50-.60	.10-.20	.30-.40	.10-.20	.20-.30	.30-.40	.30-.40	.30-.40
1.7	.20-.30	.10-.20	.40-.50	.30-.40	.70-.80	.40-.50	.50-.60	.70-.80
2.0	.50-.60	.10-.20	.30-.40	.40-.50	.40-.50	.90 ⁺	.20-.30	.60-.70
3.0	.001-.01*	.01-.02*	.01-.02*	.60-.70	.30-.40	.30-.40	.05-.10	.001-.01*
4.0	.001*	.001*	.001*	.001-.01*	.02-.05*	.30-.40	.30-.40	.80-.90
5.0	.001*	.001*	.001*	.001*	.001-.01*	.02-.05*	.90 ⁺	---

Table 3c Lymphocytes (Analysis B)
Strontium Dogs vs. "Master Controls"

Dose Level	Means at 6 Monthly Intervals							
	6	12	18	24	30	36	42	48
Master Control	4.40	4.37	4.40	4.20	4.25	4.26	4.30	4.30
0.0	4.53	4.48	4.38	4.01	4.75	3.97	4.27	3.95
1.0	4.36	4.06	3.98	3.82	3.72	3.36	3.39	3.65
1.7	4.38	4.49	4.17	4.20	4.65	4.35	4.42	4.55
2.0	4.12	3.91	4.21	3.92	3.83	3.53	3.72	3.92
3.0	3.51	3.89	3.80	3.92	3.71	3.43	3.97	3.86
4.0	3.62	3.57	3.28	3.40	3.52	3.50	3.84	3.94
5.0	2.87	2.90	2.88	3.00	3.25	3.18	3.25	--

Ranges of Significance (Probability Values) from t-test
of Individual Dose Levels Against "Master Controls"

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	.70-.80	.70-.80	.90 ⁺	.50-.60	.05-.10	.40-.50	.90 ⁺	.30-.40
1.0	.90 ⁺	.30-.40	.20-.30	.20-.30	.05-.10	.02-.05*	.02-.05*	.10-.20
1.7	.90 ⁺	.70-.80	.40-.50	.90 ⁺	.10-.20	.80-.90	.70-.80	.30-.40
2.0	.40-.50	.20-.30	.50-.60	.30-.40	.10-.20	.05-.10	.20-.30	.30-.40
3.0	.01-.02*	.10-.20	.05-.10	.30-.40	.05-.10	.02-.05*	.40-.50	.20-.30
4.0	.02-.05*	.02-.05*	.001*	.01-.02*	.01-.02*	.05-.10	.30-.40	.30-.40
5.0	.001*	.001*	.001*	.001*	.001-.01*	.02-.05*	.10-.20	---

RESULTS OF ANALYSES A AND B

The two methods give almost identical results - i.e., using master controls or 0-level controls. There are a few cases where the t-test indicated significance at the .05 level of probability whereas the Duncan test did not and vice versa. These differences are due to random variables operating on the data. The time intervals studied here are too short to be concerned about whether a value at any one time interval or dose level is significant. One must look at the entire picture. A significant test scattered here and there across the face of the table does not mean very much. However, many significant values at any one dose level or over a long period of time do have "biological" as well as "statistical" significance.

In general, for VPRC the two higher dose levels show no significance until the 36th month. However, there are very few dogs at the 5.0-level at this time. The 2.0-level is higher at 48 months compared to the controls. This is probably a sampling error which is to be expected.

The pmn comparisons indicate highly significant differences during the first 18 months for the three highest levels, with "statistical" recovery (as opposed to "biological" recovery) by 24, 36, and 42 months for levels 3.0, 4.0 and 5.0, respectively. The lymphocytes (Tables 2c and 3c) behave similarly with significant decreases from the controls at the 4 and 5-level. The 3-level was affected but not to the same extent as the higher levels. The reason why the 1.0-level dogs are different from controls after 36 months is not known.

II. Analysis C Performed at the University of Utah Computer Center. A one-way analysis of variance was performed using dose level

as the independent variable. If the computed F value was significant when compared to the reference F value at the .01 and .05 levels of probability, (except in analysis 4 where only the .01-level was used) the data was then further analyzed according to the distribution of studentized range⁽⁵⁾ to determine which dose levels were responsible for the significant differences.

This procedure was followed for four different analyses in order to evaluate which one would provide the most efficient method. The one-way analysis of variance, assuming a completely randomized design, was chosen since the problem under consideration was one involving the detection of differences among a colony of irradiated beagles. For purposes of comparison, a small error mean square would indicate an efficient method. Analysis C was subdivided as follows:

Analysis 1. The uncorrected values for each dose level of the strontium dogs were tested against the strontium control (0-level) measurements.

Analysis 2. Correction factors were added to individual measurements of both experimental and control dogs of Analysis 1 and the results were computed.

In view of the earlier study (Analysis B of this report) which utilized the master controls, two further analyses were made using the master controls.

Analysis 3. The master control values (including the strontium 0-level dogs) were tested against the uncorrected experimental dog measurements.

Analysis 4. The master control data was tested against the time-trend corrected values for the 1.0 through 5.0 dose levels.

RESULTS OF ANALYSIS C

The results of Analyses 1 and 4 are presented in Tables 4 and 5

(a, b, c), respectively. The number of dogs at the different dose levels from 6 through 48 months post-injection are found in Table 1b.

Table 4a (Analysis 1)

Dose Level	Mean VPRC							
	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	52.8	50.3	52.8	54.2	53.3	52.3	52.4	50.8
1.0	53.6	51.5	52.7	52.0	53.6	52.3	53.0	51.1
1.7	53.7	50.2	50.2	51.6	51.8	52.1	53.4	51.5
2.0	50.6	49.8	50.9	51.9	52.0	51.8	51.6	52.1
3.0	52.7	53.1	53.3	52.6	52.8	51.3	52.4	52.9
4.0	52.4	50.7	50.2	50.7	51.0	50.7	51.2	52.4
5.0	50.8	51.0	51.3	51.9	52.2	51.6	50.5	51.3

No significant differences in VPRC.

Since additional groups of strontium dogs were added to the project subsequent to 1962, and dogs had also been on experiment for a longer time, the sample sizes at the 6 month time intervals are somewhat larger than those from the earlier analyses. In particular, the 1962 analyses did not have any 5.0-level dogs at 48 months while the later analyses did. The results from all four analyses were found to be quite similar. The data from Analyses 1 and 4 were chosen as being representative of the results obtained from all the tests. The first part of each table contains the mean values for the VPRC, pmns and lymphocytes while the remainder of the table shows where significant differences occur. A blank indicates that the probability of a difference is greater than .05 in Table 4 and .01 in Table 5.

The VPRC showed no significant differences except for a few

scattered instances in Analysis 3. Although the mean values for pmns and lymphocytes were different (Tables 4 and 5) depending on whether the correction factors and/or the master controls were used, the results in terms of significant differences were quite similar. The combination of master control values (corrected) compared to uncorrected experimental data from Analysis 3 produced a few additional significant differences between the master controls and the injected dogs. However, the remainder of the comparisons - i.e., the pair-wise tests of the various combinations of levels from all four analyses, were very much alike.

The results of Analysis C indicate that the application of correction factors to the individual measurements from both experimental and control dogs tends to increase the mean values for pmns and lymphocytes while the VPRC values decrease. The relative position of these values in relation to dose level, however, remains unchanged.

Table 4b (Analysis 1)
Mean Values of Pmns (X10³)

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	7.08	5.73	6.23	4.73	5.02	5.23	4.85	4.74
1.0	6.73	5.93	5.53	4.83	5.12	4.82	4.52	4.43
1.7	6.23	5.83	5.60	5.25	5.31	5.70	4.65	5.11
2.0	6.69	5.69	5.53	5.44	5.16	5.05	4.42	5.22
3.0	5.13	5.11	4.69	5.45	4.98	4.70	4.61	4.48
4.0	3.98	3.96	3.45	3.66	4.05	3.54	4.47	4.42
5.0	3.04	2.88	2.80	2.58	2.94	3.02	3.68	3.55
Comparison	Blank Indicates P > .05							
0.0 vs 1.0			.01					
0.0 vs 1.7	.01		.01	.05				
0.0 vs 2.0			.01	.01				
0.0 vs 3.0	.01	.01	.01	.01				
0.0 vs 4.0	.01	.01	.01	.01	.01	.01		
0.0 vs 5.0	.01	.01	.01	.01	.01	.01		
1.0 vs 1.7				.05		.01		
1.0 vs 2.0				.01				
1.0 vs 3.0	.01	.01	.01	.01				
1.0 vs 4.0	.01	.01	.01	.01	.01	.01		
1.0 vs 5.0	.01	.01	.01	.01	.01	.01		
1.7 vs 2.0						.05		
1.7 vs 3.0	.01	.01	.01			.01		
1.7 vs 4.0	.01	.01	.01	.01	.01	.01		
1.7 vs 5.0	.01	.01	.01	.01	.01	.01		
2.0 vs 3.0	.01	.01	.01					
2.0 vs 4.0	.01	.01	.01	.01	.01	.01		
2.0 vs 5.0	.01	.01	.01	.01	.01	.01		
3.0 vs 4.0	.01	.01	.01	.01	.01	.01		
3.0 vs 5.0	.01	.01	.01	.01	.01	.01		
4.0 vs 5.0	.01	.01	.01	.01	.01	.05		

Table 4c (Analysis 1)
Mean Values of Lymphocytes ($\times 10^3$)

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
0.0	3.65	3.60	3.53	3.22	3.17	3.08	3.09	2.56
1.0	3.62	3.28	3.11	3.06	2.86	2.82	2.71	2.55
1.7	3.97	3.58	3.31	3.31	3.38	3.12	2.95	2.85
2.0	3.35	3.12	3.35	2.99	2.83	2.59	2.57	2.75
3.0	2.78	3.10	2.95	3.01	2.69	2.52	2.97	2.73
4.0	2.88	2.78	2.42	2.53	2.41	2.35	2.55	2.36
5.0	2.12	2.08	2.00	2.09	2.19	2.04	1.89	1.69
Comparison	Blank Indicates P > .05							
0.0 vs 1.0		.01	.01		.01	.05		
0.0 vs 1.7	.05				.05			
0.0 vs 2.0	.05	.01			.01	.05	.05	
0.0 vs 3.0	.01	.01	.01		.01	.05		
0.0 vs 4.0	.01	.01	.01	.05	.01	.05	.05	
0.0 vs 5.0	.01	.01	.01	.05	.01	.05	.05	
1.0 vs 1.7	.05	.05			.01	.05		
1.0 vs 2.0	.05							
1.0 vs 3.0	.01					.05		
1.0 vs 4.0	.01	.01	.01	.05	.01	.05		
1.0 vs 5.0	.01	.01	.01	.05	.01	.05	.05	
1.7 vs 2.0	.01	.01			.01	.05		
1.7 vs 3.0	.01	.01	.01		.01	.05		
1.7 vs 4.0	.01	.01	.01	.05	.01	.05		
1.7 vs 5.0	.01	.01	.01	.05	.01	.05	.05	
2.0 vs 3.0	.01		.01					
2.0 vs 4.0	.01	.05	.01	.05	.01			
2.0 vs 5.0	.01	.01	.01	.05	.01	.05	.05	
3.0 vs 4.0		.05	.01	.05	.01			
3.0 vs 5.0	.01	.01	.01	.05	.01	.05	.05	
4.0 vs 5.0	.01	.01	.01	.05	.05	.05	.05	

Table 5a (Analysis 4)

Mean VPRC

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
Master Control	50.2	48.4	49.6	49.4	48.0	48.7	48.1	47.2
1.0	51.4	49.1	50.1	49.3	50.6	49.2	49.7	47.6
1.7	52.5	48.3	48.3	50.0	48.8	49.4	50.8	48.7
2.0	48.4	47.5	48.4	49.2	49.1	48.8	48.3	48.6
3.0	50.5	50.8	50.8	49.9	49.9	48.3	49.1	49.5
4.0	50.3	48.4	47.6	48.0	48.1	47.6	47.9	49.0
5.0	48.7	48.6	48.7	49.1	49.2	48.3	47.0	47.6

No significant differences in VPRC.

Table 5b (Analysis 4)

Mean Values for Pmns (X10³)

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
Master Control	7.92	7.89	7.11	7.01	6.63	6.81	6.54	6.46
1.0	7.44	6.71	6.37	5.70	6.11	5.83	5.60	5.57
1.7	6.87	6.47	6.50	6.09	6.34	6.41	5.54	6.14
2.0	7.43	6.44	6.35	6.32	6.11	6.05	5.49	6.35
3.0	5.82	5.86	5.51	6.32	5.92	5.70	5.67	5.61
4.0	4.68	4.71	4.28	4.53	5.01	4.55	5.55	5.55
5.0	3.75	3.67	3.66	3.49	3.91	4.09	4.83	4.77
Comparison	Blank Indicates P > .01							
M.C.* vs 1.0		.01	.01	.01		.01		
M.C.* vs 1.7	.01	.01	.01	.01				
M.C.* vs 2.0		.01	.01					
M.C.* vs 3.0	.01	.01	.01	.01		.01		
M.C.* vs 4.0	.01	.01	.01	.01	.01	.01		
M.C.* vs 5.0	.01	.01	.01	.01	.01	.01		
1.0 vs 1.7								
1.0 vs 2.0								
1.0 vs 3.0	.01		.01					
1.0 vs 4.0	.01	.01	.01	.01	.01	.01		
1.0 vs 5.0	.01	.01	.01	.01	.01	.01		
1.7 vs 2.0								
1.7 vs 3.0	.01		.01					
1.7 vs 4.0	.01	.01	.01	.01	.01	.01		
1.7 vs 5.0	.01	.01	.01	.01	.01	.01		
2.0 vs 3.0	.01		.01					
2.0 vs 4.0	.01	.01	.01	.01	.01	.01		
2.0 vs 5.0	.01	.01	.01	.01	.01	.01		
3.0 vs 4.0	.01	.01	.01	.01	.01	.01		
3.0 vs 5.0	.01	.01	.01	.01	.01	.01		
4.0 vs 5.0	.01	.01	.01	.01	.01			

* M.C. refers to Master Control.

Table 5c (Analysis 4)
Mean Values for Lymphocytes ($\times 10^3$)

Dose Level	Months Post-Injection							
	6	12	18	24	30	36	42	48
Master Control	4.22	4.15	4.18	3.99	3.87	3.98	3.98	3.91
1.0	4.16	3.88	3.75	3.73	3.62	3.60	3.55	3.43
1.7	4.65	4.10	3.89	3.88	4.13	3.79	3.61	3.73
2.0	3.91	3.69	3.98	3.66	3.55	3.36	3.40	3.62
3.0	3.31	3.67	3.58	3.68	3.42	3.29	3.79	3.60
4.0	3.42	3.35	3.06	3.21	3.15	3.13	3.38	3.23
5.0	2.67	2.68	2.66	2.80	2.94	2.86	2.77	2.62
Comparison	Blank Indicates P > .01							
M.C.* vs 1.0			.01					
M.C.* vs 1.7	.01							
M.C.* vs 2.0		.01			.01	.01		
M.C.* vs 3.0	.01	.01	.01		.01	.01		
M.C.* vs 4.0	.01	.01	.01	.01	.01	.01		
M.C.* vs 5.0	.01	.01	.01	.01	.01	.01		.01
1.0 vs 1.7	.01				.01			
1.0 vs 2.0								
1.0 vs 3.0	.01							
1.0 vs 4.0	.01	.01	.01	.01	.01	.01		
1.0 vs 5.0	.01	.01	.01	.01	.01	.01		
1.7 vs 2.0	.01				.01	.01		
1.7 vs 3.0	.01				.01	.01		
1.7 vs 4.0	.01	.01	.01	.01	.01	.01		
1.7 vs 5.0	.01	.01	.01	.01	.01	.01		.01
2.0 vs 3.0	.01		.01					
2.0 vs 4.0	.01		.01	.01	.01			
2.0 vs 5.0	.01	.01	.01	.01	.01	.01		.01
3.0 vs 4.0			.01	.01	.01			
3.0 vs 5.0	.01	.01	.01	.01	.01	.01		.01
4.0 vs 5.0		.01	.01	.01	.01			

* M.C. refers to Master Control.

DISCUSSION OF ANALYSES A AND C

Investigation into the variance components of the four one-way analyses suggested that all of these methods were unsuitable in terms of minimizing the error mean squares. The values of this component for the periods up to 24 months post-injection are given in Table 6.

Table 6
Error Mean Squares for Strontium Data

	Pmns				Lymphocytes			
	Mo. Post-Injection				Mo. Post-Injection			
	6	12	18	24	6	12	18	24
Analysis A	3.95	1.92	1.26	1.45	0.78	0.58	0.60	0.58
Analysis 1	3.77	2.15	1.65	1.53	0.98	0.92	0.76	0.74
Analysis 2	3.81	1.95	1.60	1.52	0.95	0.80	0.75	0.55
Analysis 3	3.82	4.43	1.93	3.05	1.05	0.98	0.82	0.67
Analysis 4	3.86	4.42	1.91	3.05	1.05	0.94	0.83	0.67

Although the sample sizes differed, for purposes of comparison, the numbers of dogs were nearly the same from Analyses 1 and 2 of the one-way analysis and Analysis A of the randomized block design. It was found in the 1962 analysis at 18 months post-injection for the pmns and 6, 12, and 18 months post-injection for the lymphocytes that the test for blocks indicated this component was highly significant. At these same intervals, the error mean squares from the randomized block design are seen to be much lower than those obtained from the four one-way analyses (Table 6). Thus, although block (group to group) differences may not be found at all the time intervals, when they are present, use of the one-way analysis of variance does not allow the elimination of this source of variation. Because the underlying experimental design

appears amenable to the randomized block design and after observing the smaller error mean squares obtained from the earlier analysis as compared to the ones more recently found, it is apparent that the former method will provide greater sensitivity in the detection of significant differences.

GENERAL SUMMARY

After comparing the results of the randomized block to the one-way analysis of variance, it appears that the former will provide more meaningful significance values. Perhaps the reason for this is that the experimental design of this project is essentially that of randomized blocks. One of the reasons this design was used was because of space problems in housing the dogs. It was anticipated that the several groups (blocks) of dogs entering the experiment would not only be different from each other initially but would also experience different sets of environments. Initial differences would be due to (1) environmental conditions under which the pups were raised and differences in immunization procedures, (2) sex differences - each group consisted of dogs of the same sex, and (3) genetic differences - differences in studs and also each group usually contained some sets of sibs. In addition, after injection each group would be expected to share a more or less common environment which would not necessarily be the same as that experienced by dogs in another group. This is particularly true since it took several years to enter all groups for any given radionuclide into the project, i.e., in the case of strontium dogs which were entered from January 1955 to January 1960.

Statistically, the use of blocks permitted the removal of block to block (or group to group) variation when present and thus reduced the

error. The greater number of significant differences found when using the one-way analysis of variance are in part, no doubt, the result of variability due to block. Eliminating this possible source of variation by use of the randomized block design will make the comparisons between the various combinations of dose levels within a radionuclide more precise. Since this is the more sensitive method of analysis it will be the method of choice for subsequent analyses of hematological changes following internal irradiation.

REFERENCES

1. Dougherty, J. H. and Rosenblatt, L. S., Changes in the hemogram of the beagle with age. J. of Gerontology 20, 131-138 (1965).
2. Dougherty, J. H., Rosenblatt, L. S. and Shultz, F. T., Analysis of the effects of age on the hemogram of the beagle. Radiobiology Laboratory, University of Utah Annual Report C00-228, 79-93 (September 1963).
3. Dougherty, J. H., Rosenblatt, L. S. and Welch, Q. B., A preliminary analysis of the dose-time relationship of selected hematological values. Radiobiology Laboratory, University of Utah Annual Report C00-224, 46-56 (September 1961).
4. Harter, H. L., Critical values for Duncan's new multiple range test. Biometrics 16, 671-685 (1960).
5. Pearson, E. S. and Hartley, H. O., Biometrika Tables for Statisticians. Vol. 1, 176-177. Published for the Biometrika Trustees at the University Press, Cambridge (1954).

PILOT STUDY OF THE EFFECT OF CORTISOL
ON Na_3CaDTPA ENHANCED EXCRETION OF Pu^{239} IN THE BEAGLE

D. R. Atherton, T. F. Dougherty, F. W. Bruenger,
G. N. Taylor and B. J. Stover

Abstract: Two massive doses of cortisol were given to mobilize Pu^{239} to make it available for chelation by Na_3CaDTPA and subsequent excretion. No practically significant increase in excretion was observed, and there was no significant removal of Pu^{239} from the dog's liver.

INTRODUCTION

In general chelating agents significantly increase the urinary excretion of various radionuclides, only if the chelate is administered soon after exposure when a large fraction of the radionuclide is still in the blood. As the blood level decreases, the chelate treatment becomes less effective. Further, as time increases the radionuclide often becomes more intimately trapped in the tissues as a consequence of both normal and radiation induced changes occurring in the tissues, and the effect of this is to decrease the chance of the radionuclide's returning to the blood.

In principle, a solution to this problem exists. It is the administration of an agent which stimulates biological activity of the tissue or tissues involved so that some of the intimately trapped radionuclide is released and available to the blood. Then follows administration of a chelate to bind the mobilized radionuclide and carry it to the kidneys to be excreted.

Following intravenous injection of PuIV citrate most of the Pu^{239} is retained in bone and liver. Cortisol acts on bone and the reticulo-endothelial system. The chelating agent, trisodium calcium diethylenetriaminepentaacetate, Na_3CaDTPA , enhances excretion of Pu^{239} . Thus we have a test system.

EXPERIMENTAL METHODS

Dog T21P5 was injected with $2.72 \mu\text{C Pu}^{239}/\text{kg}$ when it was 688 days old. The dog was used for calorimetric and blood protein measurements for about a year and a half. Following this the dog was available for other studies.

Expt. I. At 557 days the dog was put in a metabolism cage, and urine and feces were collected and analyzed for Pu^{239} for a 14 day period to determine the pretreatment excretion rates.

On the 15th day $0.133 \text{ g Na}_3\text{CaDTPA}$ ($\sim 0.0142/\text{kg}$) in 50 ml isotonic saline pH ~ 7 was given subcutaneously. The same treatment was given each of the next three days (16, 17, 18), then no treatment for three days, followed by four more days of treatment. The dog received $8 \times 0.133 = 1.06 \text{ g Na}_3\text{CaDTPA}$ in 11 days. Urine and feces were collected and analyzed for 18 days at which time the dog was returned to its kennel.

Expt. II. At 760 days after injection the dog was returned to a metabolism cage, and a 14 day study of pretreatment urinary and fecal excretion rates was done as before. Beginning on the 15th day the same Na_3CaDTPA treatment schedule described above was followed. In addition, on the first day of both four day Na_3CaDTPA treatment periods, 618 mg cortisol (50 mg/kg) were given intramuscularly one hour prior to the Na_3CaDTPA . The dog thus received 1.24 g cortisol and 1.06 g Na_3CaDTPA . Urine and feces were collected and analyzed for 28 days.

At autopsy (939 days after injection) known fractions of liver, kidneys, and spleen were obtained and analyzed for Pu^{239} .

The analytical method for Pu^{239} has been reported (1).

RESULTS

Excretion rate measurements.

The effects of the treatments of Expts. I and II on Pu^{239} excretion rates are best compared in relation to the 14 day baseline values for each experiment. The mean urinary, $\bar{U}$, fecal, $\bar{F}$, and total $\bar{U} + \bar{F} = \bar{X}$, excretion rates for the two 14 day periods are given in Table I, which also includes $\bar{F}/\bar{U}$. The fractional standard deviations of these means are of the order of 0.1. In Table I are given the calculated values derived from our extensive studies of Pu^{239} excretion by the beagle (2). During the first 14 day control period observed Pu^{239} excretion by T21P5 was consistent with results of extensive long term studies. During the second control period urinary excretion was lower than expected.

TABLE I

Comparison of T21P5 excretion rates
(% injected Pu^{239} excreted per day)
with expected rates (2)

t	T21P5				Calculated			
	$\bar{U}$	$\bar{F}$	$\bar{X}$	$\bar{F}/\bar{U}$	U	F	X	F/U
564	0.0042	0.0077	0.0119	1.8	0.0045	0.0059	0.0104	1.3
767	0.0017	0.0061	0.0078	3.6	0.0040	0.0049	0.0089	1.2

The results of the Na_3CaDTPA treatment (Expt. I) are summarized in Fig. 1. Both urinary and fecal excretion increased relative to the 14 day pretreatment means, and the relative increases are shown in Fig. 1. The highest daily urinary excretion was about 60 times the pretreatment urinary excretion, while the highest fecal increase was about 11. Even

RELATIVE EXCRETION OF Pu^{239} FOLLOWING Na_3CaDTPA TREATMENT.

FIGURE 1

Relative increase in daily urinary and fecal excretion of Pu^{239} with Na_3CaDTPA treatment. (Expt. I).

though these factors are impressive, the excretion rates are so low that much greater relative increases are needed to remove Pu^{239} significantly as shown in Table II.

TABLE II
% Injected Pu^{239} removed by Na_3CaDTPA treatment
(Expt. I) in 18 days

	$\sum U$	$\sum F$	$\sum X$	$\frac{\sum F}{\sum U}$
Excreted with treatment	2.30	0.78	3.08	0.34
Expected without treatment	0.08	0.14	0.22	1.8
Net removal	2.22	0.64	2.86	-
Relative increase	30	6	14	-

The results of the combined Na_3CaDTPA plus cortisol treatment (Expt. II) appear in Fig. 2. Again the results are presented as relative increases over the immediate pretreatment means. Greater relative increases in urinary excretion were observed with the highest being about 120, and the highest fecal increase was 9, about the same as in Expt. I. However, since the pretreatment urinary rate was considerably lower for Expt. II, the relative increase in urinary plus fecal excretion for 18 days was the same in Expts. I and II. The 18 and 28 day excretion totals are given in Table III. Comparison of Tables II and III shows that, while less Pu^{239} was removed in Expt. II, the relative increases in urinary plus fecal excretion were the same for Expts. I and II.

RELATIVE EXCRETION OF Pu^{239} FOLLOWING CORTISOL- $\text{Na}_3\text{Ca DTPA}$ TREATMENT.

FIGURE 2

Relative increase in daily urinary and fecal excretion of Pu^{239} with Cortisol- $\text{Na}_3\text{Ca DTPA}$ treatment. (Expt. II).

TABLE III

% Injected Pu^{239} removed by Na_3CaDTPA
and cortisol treatment (Expt. II)

	$\sum U$	$\sum F$	$\sum X$	$\frac{\sum F}{\sum U}$
18 day totals				
Excreted with treatment	1.47	0.45	1.92	0.31
Expected without treatment	0.03	0.11	0.14	3.6
Net removal	1.44	0.34	1.78	-
Relative increase	48	4	14	-
28 day totals				
Excreted with treatment	1.58	0.51	2.09	0.32
Expected without treatment	0.05	0.17	0.22	3.6
Net removal	1.53	0.34	1.87	-
Relative increase	33	3	10	-

Following the large doses of cortisol there was an increase in the amount of urine per day as shown in Fig. 3. In the course of the Pu^{239} analyses a large increase in urinary excretion of chloride was noted.

Tissue Analyses

The liver weighed 371 g and contained 9.26% of the injected Pu^{239} . The expected amount for a 5-level dog at 939 days after injection is 10.0% (3). The difference, 10.0 - 9.26, is not significant, $0.9 > p > 0.8$ * Thus the net effect of the treatment of Expts. I and II was no reduction in Pu^{239} in the liver.

*Important note: The 95% confidence limits for the constants in the equation for liver retention of Pu^{239} in 5-level dogs in Ref. (3), $L = 29.8e^{-0.00116t}$, are in error. They should be
 $b = 0.0016 \pm 0.0029$
 $\log a = 1.474 \pm 0.117$

DAILY VOLUME OF URINE FOLLOWING CORTISOL - Na_3Ca DTPA TREATMENT.

FIGURE 3

Increase in volume of urine voided daily with Cortisol- Na_3Ca DTPA treatment. Relative increase is seen on the left and absolute volume voided is seen on the right. (Expt. II).

The spleen weighed 43 g and contained 0.46% of the injected Pu^{239} . From our early studies the expected amount for 939 days is 0.17% (2). Better comparison can be made with the group of eight 5-level dogs which had a mean spleen content of $(0.19 \pm 0.12)\%$ Pu^{239} at 1443 ± 302 days after injection. The difference, $0.46 - 0.19$, is on the borderline of significance, $p \sim 0.05$. Thus the treatments had little or no effect on the amount of Pu^{239} in the spleen.

The kidneys weighed 68 g and contained 0.34% of the injected Pu^{239} . The early studies give 0.15% as the expected amount (2). The mean for the eight 5-level dogs was $(0.14 \pm 0.006)\%$. This difference, $0.34 - 0.14$, is significant, $p \sim 0.01$. Thus the treatments increased slightly the amount of Pu^{239} in the kidneys. A considerable increase in the inorganic residue after ashing the kidneys was also noted. This was presumed to be calcium and phosphate.

Clinical Symptoms and Post Mortem Findings

Mild anorexia, polydipsia and polyuria were associated with these treatments. The latter two symptoms were unquestionably cortisone induced. All were transitory and apparent recovery occurred shortly after the treatments were completed.

The terminal illness began four months after the final hydrocortisone and Na_3CaDTPA treatment and lasted approximately two weeks. This was characterized by nearly complete anorexia, high body temperature, cardiac arrhythmia with multiple premature beats, proteinuria and melena.

The post mortem findings included marked liver changes that were typical of those induced by Pu^{239} ; severe vegetative valvular endocarditis, involving both the aortic and pulmonary valves; massive kidney infarcts, many of which were calcified; myocardial infarcts; focal zones of myo-

carditis; splenic infarcts; stomach ulceration; and enteritis. Except for the plutonium induced liver changes the most significant post mortem lesions appeared to be a consequence of the valvular disease and were not clearly related to the hydrocortisone and Na_3CaDTPA treatment.

DISCUSSION

A number of studies have been made of the use of DTPA to enhance excretion of plutonium. Ballou, (4) for example, removed in 4 days a total of 76% of the Pu^{239} injected into rats by treating the animals once with DTPA 1 hour following injection. If treatment was delayed for 18 days, the same treatment with DTPA resulted in a 4 day excretion total of only 7% of the plutonium injected. Smith et al. in experiments with pigs (5) delayed DTPA treatment until 60 days after injection of Pu^{239} . These studies, which involved treatment with DTPA on 5 consecutive days resulted in 12 day totals of 11 and 19% removal of the injected plutonium.

When dog T21P5 became available, it seemed a good opportunity to study the effect of DTPA in a larger animal at a fairly long (1 1/2 years) time after injection.

To be effective in practice, a treatment program should remove essentially all of the radionuclide. At 564 days the calculated fractional retention is 0.79 (2), and so the 8 doses of Na_3CaDTPA resulted in the removal of only 3.6% of the Pu^{239} in the dog. At 767 days retention is 0.77 so that only 2.3% was removed in the 1st 18 days of the Na_3CaDTPA plus cortisol experiment. However, prior to the studies it was pointed out by W. S. S. Jee that a 5-level dog might prove to be a poor choice because of the peritrabecular fibrosis which occurs at this time at this relatively high dose level (6). Thus, it is possible that more Pu^{239}

could be removed from a lower level dog.

The results of Expt. I are quite comparable with Norwood's (7) study of a human exposure case. At 30 weeks after exposure Na_3CaDTPA initially increased urinary excretion by a factor of 55 and also resulted in an average fecal increase of 4. Comparable numbers for T21P5 were 60 and 6, respectively. Since the baseline excretion rates in Norwood's case were 0.001 - 0.002% of the estimated Pu^{239} in the subject, no effective removal was achieved in this case either.

Norwood (7) found that the effectiveness of Na_3CaDTPA in removing Pu^{239} from the human decreases with time. The histograms (Fig. 1 and 2) presented here appear to indicate a similar tendency. Extrapolation of a line determined by the two peaks shows that the treatment would be completely ineffective in less than 100 days. Considering this together with the fact that the pretreatment urinary excretion for Expt. II was considerably less than expected, suggests that treatment with Na_3CaDTPA at long times after deposition of plutonium remove only a very limited amount, most of which ultimately would have been excreted. That is, the effect may be more an acceleration of excretion than an increase.

The failure of cortisol and Na_3CaDTPA to act together to remove Pu^{239} may have resulted from having inadequate chelating agent at the sites of cortisol action. Pu^{239} released at these sites would have a number of substances competing for it and could be redeposited before it could react with the Na_3CaDTPA .

REFERENCES

1. F. W. Bruenger, B. J. Stover, and D. R. Atherton, Determination of Plutonium in Biological Material by Solvent Extraction with Primary Amines. *Anal. Chem.* 35, 1671 - 1673 (1963).
2. B. J. Stover, D. R. Atherton, and N. Keller, Metabolism of Pu^{239} in Adult Beagle Dogs. *Radiation Research* 10, 130 - 147 (1959).
3. B. J. Stover, D. R. Atherton, F. W. Bruenger, and D. S. Buster, Further Studies of the Metabolism of Pu^{239} in Adult Beagles. *Health Physics* 8, 589 - 597 (1962).
4. J. E. Ballou, Removal of Deposited Plutonium by Triethylenetetramine-Hexaacetic Acid. *Nature* 193, 4822, 1303 - 1304 (1962).
5. Victor H. Smith, John E. Ballou, William J. Clarke and Roy C. Thompson, Effectiveness of DTPA in Removing Plutonium from the Pig, *Proc. Soc. Expt. Biol. & Med.* 107, 120 - 123 (1961).
6. T. F. Dougherty, B. J. Stover, J. H. Dougherty, W. S. S. Jee, C. W. Mays, C. E. Rehfeld, W. R. Christensen, and H. C. Goldthorpe, Studies of the Biological effects of Ra^{226} , Pu^{239} , Ra^{228} (MsTh_2), Th^{228} (RdTh), and Sr^{90} in Adult Beagles. *Radiation Research*, 17:4, (1962).
7. W. Daggett Norwood, Long-Term Administration of DTPA for Plutonium Elimination, *J. of Occupational Health* 4, 3, 130 - 132 (1962).

EFFECT OF EDTA ON THE EXCRETION OF Sr^{90} AND Y^{90}

D. R. Atherton

Abstract: Administration of $\text{Ca Na}_2 \text{EDTA}$ to four dogs which had been injected with Sr^{90} 0.7 years previously failed to enhance the excretion of Sr^{90} but did increase the urinary excretion of Y^{90} by a factor of about 5.

INTRODUCTION

The effect of EDTA on the excretion of thorium, radium, and lead in dogs was reported previously^{(1) (2)}. From these studies and the work of Foreman⁽³⁾, it seemed unlikely that EDTA would effect an enhancement of the urinary excretion of strontium but it did seem likely that the excretion rate of yttrium would be increased. In our studies dogs are injected with Sr^{90} which decays to 2.64 d. Y^{90} and the two nuclides are quite close to being in equilibrium in the animal⁽⁴⁾. If Y^{90} formed in vivo moves to the blood and then is redeposited, it could be chelated by EDTA while in the blood and subsequently excreted.

EXPERIMENTAL

Four beagles, F4S4, M5S4, F4S5 and M5S5, were given Sr^{90} intravenously in a sodium citrate-citric acid buffer solution, 0.08M in citrate, at pH~3.5. The Sr^{90} doses were 32.1, 30.6, 105 and 95.2 $\mu\text{c}/\text{kg}$, respectively.

On the 273rd day following injection M5S4 and M5S5 were placed in metabolism cages, 24 hour urine specimens were collected and analysed for its Sr^{90} and Y^{90} content. After collection of the urine specimen the animals were given 0.2 g EDTA in 50 ml physiologic saline subcutaneously after first preparing the area to be injected with procaine. Urine on each dog was collected for 24 hours as a single sample following the EDTA treatment.

On the 301st day following injection the same procedure as outlined above was followed with F4S4 and F4S5 except that the dose of EDTA was 0.1 g.

Analyses were done by serially counting 1.0 ml aliquots of the urine specimen in a plastic scintillator-well counter⁽⁵⁾ which responds to the high energy (2.2 mev) β from the decay of Y^{90} but not to the low energy (0.61 mev) β of Sr^{90} . The change in count rate with time together with a final steady state count rate obtained when $Sr^{90} - Y^{90}$ equilibrium is achieved provided the information with which the relative amounts of each nuclide was determined.

RESULTS AND DISCUSSION

The experiment provided the data summarized in Table 1 and contained no surprises. That the urinary excretion of Sr^{90} appears to have increased with the EDTA treatment is hardly to be considered as significant because the day to day variation is of the same magnitude as the tabulated increase. The increase in the urinary excretion of Y^{90} with the EDTA treatment results in the urinary elimination of about equal amounts of Y^{90} and Sr^{90} .

Once again the ineffectiveness of EDTA as a biological decontaminating agent for the alkaline earth elements has been shown. There is no question that this chelating agent would be more effective in the removal of Y^{90} if given earlier.

These results tend to support the earlier observation of Arnold et al⁽⁴⁾ that Y^{90} produced in vivo from long term skeletal deposits of Sr^{90} in young dogs does not escape from the local areas of bone in which it is produced. If translocation did occur the yttrium concentration in the blood would be increased and, therefore, available for chelation by EDTA with subsequent excretion in the urine.

TABLE 1

Urinary Excretion of Sr^{90} and Y^{90} as
Influenced by Administration of EDTA

	EDTA Dose (g.)	Control %/day $\times 10^3$ Sr^{90} Y^{90}		$\frac{\text{Sr}^{90} \text{ Control}}{\text{Y}^{90} \text{ Control}}$	$\text{Sr}^{90} \frac{\text{EDTA}}{\text{Control}}$	$\text{Y}^{90} \frac{\text{EDTA}}{\text{Control}}$
F4S4	0.1	13.	3.9	3.3	1.2	4.1
F4S5	0.1	6.0	1.2	5.0	1.0	5.2
M5S4	0.2	4.8	1.3	3.7	1.5	5.6
M5S5	0.2	4.4	1.7	2.6	1.5	5.4

REFERENCES

1. B. J. Stover, D. R. Atherton, E. F. Holt and D. S. Buster, Radiobiology Laboratory Annual Progress Report, COO-215, pp. 127-131, March 1958.
2. Ann G. Ruhmann, David R. Atherton and Betsy J. Stover, Effect of Chelating Agents on Excretion and Blood Level of Pb^{212} Formed in vivo, Rad. Res. 16, 584 (1962).
3. H. Foreman, Chelating Agents, Ind. Med. & Surg., 24, 287-393 (1955).
4. J. S. Arnold, B. J. Stover and M. A. Van Dilla, Failure of Y^{90} to escape from skeletally-fixed Sr^{90} , Proc. Soc. Exptl. Biol. Med., 90, 260-263 (1955).
5. F. W. Bruenger, D. R. Atherton and Betsy J. Stover, Determination of Sr^{90} and Th^{228} in Biological Materials, Health Physics 9, 232-235, (1963).

THE DETERMINATION OF HALF-PERIODS OF DOG SERUM PROTEINS USING S^{35} -L-METHIONINE

F. W. Bruenger, W. Stevens, and B. J. Stover

Abstract: The measurement of the half-periods of serum proteins is of interest due to the relationships between turnover of these proteins and the binding of heavy radionuclides by them. In order to measure these half-periods we used S^{35} -L-Methionine injected I.V. into a young healthy beagle dog. Blood samples were taken from 2 hours through 42 days after injection. Total S^{35} activity of the serum and of the protein-bound S^{35} was measured. The protein fractions were separated by two methods, electrophoresis and gel-filtration. The half-period of each protein fraction was then determined by measuring its S^{35} activity over the period of 42 days. Single exponentials to describe the decrease of S^{35} were computed using the method of least squares.

INTRODUCTION

A study of the half-lives of canine plasma proteins is an important part of a more extensive investigation of the binding of $PuIV$ to plasma proteins in the dog. The task of determining meaningful turnover times of plasma proteins is a very difficult one. It has been pursued by a number of scientists for more than two decades, and a variety of methods has been applied⁽¹⁻⁹⁾.

Until now none of these methods gave entirely satisfactory results. Administration of exogenously labeled proteins generally results in apparent half-lives which are short compared with those from studies using endogenously labeled proteins. Limitations of the exogenous labeling methods are 1) there is no assurance that the protein being labeled by I^{131} or various other radioactive moieties will not suffer any chemical changes during the labeling procedure, and 2) the introduction of heavy or strongly negative groups into the protein will alter its stereo-specificity (structurally or electronically) and will

certainly have some influence on its breakdown pattern. Endogenously labeled proteins on the other hand, suffer from the fact that the label can be recycled and thus produce turnover times which are too long. The present study was carried out with endogenously labeled protein using a tracer dose of S^{35} -L-methionine as the labeling agent.

MATERIALS AND METHODS

An adult beagle dog, 480 days old, from our colony was kept in a metabolism cage for 14 days. The dog was not fed for 24 hours prior to the injection of approximately 3 mC of S^{35} -L-methionine. The specific activity of this preparation was 53.7 mC/mM, (3 mC = 26.7 mg). Thirty minutes before injection, the dog was given two grams of purified equine albumin suspended in water by stomach tube. The equine albumin contains all the necessary amino acids but little or no methionine⁽¹⁰⁾. Canine albumin is reported to contain 4 moles of methionine per mole of albumin⁽¹¹⁾.

It was assumed that 24 hours without food was sufficiently long to deplete the supply of amino acids required by the liver for synthesis of the blood proteins. The dog could then increase all its amino acids except L-methionine by metabolizing the equine albumin. This would result in a deficiency of L-methionine molecules relative to the other amino acids. Then, when the labeled S^{35} -L-methionine is given, there will be a relatively greater fraction of S^{35} -L-methionine available for protein synthesis. Incorporation of S^{35} -L-methionine will be increased since the chance that a labeled molecule is used is proportional to the fraction of that kind of molecule that is labeled.

The S^{35} -L-methionine was diluted to 10 ml with isotonic saline solution and injected into the femoral vein.

Beginning at two hours after injection blood samples of 12 ml each were drawn for analysis. Collection of blood samples continued at increasing intervals for 42 days. The blood was allowed to clot and then the serum was separated by centrifugation. This procedure left approximately 5 ml of serum which were divided as follows:

2 x 100 λ for total S^{35} -activity

2 x 500 λ for protein-bound S^{35} -activity

2 ml for Gel-Filtration

Remaining serum was used for electrophoresis, (and duplication if necessary). Protein bound S^{35} was separated from the total serum S^{35} by passing a known aliquot of serum through a small column, packed with the highly crosslinked resin "Sephadex G-25". A solution of 4% NaCl + 0.1 M Tris having a pH $\approx$ 7.4 was used as the elutriant. The elution volume was kept slightly larger than the void volume so that all components with a molecular weight $<25,000$ stayed on the column and components with a molecular weight $>25,000$ were eluted. Aliquots of this eluate containing the protein were then counted.

The S^{35} content of the various protein fractions was determined by two different procedures. The first was the conventional fractionation of protein constituents by electrophoresis and subsequent analysis of the S^{35} radioactivity. The second was the determination of the S^{35} -radioactivity following separation of the serum proteins according to their molecular weights and sizes as described originally by Flodin⁽¹²⁾.

Electrophoresis was carried out on cellulose acetate membrane (Beckman Microzone). Each sample was applied sixteen times and, after development, the separated protein fractions were cut out and counted in a (Packard) liquid scintillation spectrometer.

Gel-filtration was performed according to the following procedure. After addition of sucrose to 2 ml of serum, the samples were layered on top of a system of two columns (20 x 500 mm) filled with a suspension of Sephadex G-100 and G-200 in the same buffer as described for Sephadex G-25. The columns were kept at a temperature of between 0° - 5° C and the proteins were eluted in 3 ml portions using a (Packard) fraction collector. The protein content of these fractions was determined spectrophotometrically at a wavelength of 280 mμ. The elution pattern was the same as described earlier⁽¹³⁾. One ml aliquots were transferred to glass vials and counted. A standard curve relating absorbance and protein content on an arbitrary scale was evaluated for the concentration range of proteins encountered in this experiment. This curve was used in evaluation of the data. Appropriate corrections were applied for S³⁵ decay and for depletion of the total amount of S³⁵ in the dog from the drawing of a significant amount of blood.

RESULTS

Throughout the experiment, the S³⁵ content of the whole serum was slightly higher than that of the protein bound S³⁵. Material having a molecular weight >25,000 was considered protein; a molecular weight <25,000 was considered non-protein material. When plotted on a semi-logarithmic scale, the slopes of the resulting two lines differed only slightly, giving half-periods of 19.6 ± 0.6 days for whole serum and 18.4 ± 0.8 days for protein bound S³⁵. The difference in half-periods, however, was not significant ($0.3 > p > 0.2$).

Of the protein fractions separated electrophoretically albumin had the longest half-period and the α-globulin fraction the shortest. The β₁-globulin fraction half-period was also short and not significantly

Fig. 1. Change of S^{35} activity in whole serum and in the protein fraction of serum during the time from 0-45 days after administration of S^{35} -L-methionine.

different from that of the α -globulins. The half-period for the β_2 -globulins was slightly longer than that of the β_1 -globulins. The half-period for the γ -globulins was significantly longer than those of the other globulins. The half-periods and their standard deviations are given in Table I and the respective p-values for the significance of the differences of half-periods are given in Table II.

Table I
Half-Periods and 1 Day Activities of Protein Fractions
Separated by Microzone Electrophoresis

	Total cpm at $\Delta T = 1$ Day	^a $t_{1/2}$ days	$t_{1/2}$ days	% of Total Protein
Albumin	1.716×10^3	23.3	4.0	54.01
α -Globulin	1.292×10^3	12.8	1.4	16.78
β_1 -Globulin	0.747×10^3	13.5	1.5	12.51
β_2 -Globulin	0.727×10^3	15.6	0.9	10.45
γ -Globulin	0.537×10^3	19.5	2.2	6.25

^a
n = 14 in all cases.

Table II
p Values for Significance of Difference
of Half-Periods Given in Table I

	α -Globulin	β_1 -Globulin	β_2 -Globulin	γ -Globulin
Albumin	< 0.01	< 0.01	0.02-0.01	0.4 - 0.3
α -Globulin	-	0.8-.07	0.2 - 0.1	0.02-0.01
β_1 -Globulin		-	0.3 - 0.2	0.05-0.02
β_2 -Globulin			-	0.05-0.02

In the gel-filtration procedure protein fractions were eluted in three peaks as described earlier⁽¹³⁾. Specific activities* were

*These specific activities are defined as cpm/ml of eluate.

Fig. 2. Decrease in the radioactivity of the various S^{35} labeled protein fractions and the sums of these fractions as determined by microzone electrophoresis.

evaluated for the fraction having the highest optical density of each of the three peaks and for the fractions on either side of the one with the highest optical density. These fractions were denoted as: "Ante-", "Maximum" and "Post-". Results are presented in Table III.

Table III
Half-Periods for Protein Fractions Separated by Gel-Filtration

	n	$t_{1/2}$ days	$\sigma_{t_{1/2}}$ days
Peak I - Macroglobulins and Lipoproteins			
Ante	11	22.7	6.9
Maximum	15	22.5	5.4
Post	16	17.1	2.4
Peak II - α -, β -, and γ -Globulins			
Ante	17	14.7	1.8
Maximum	16	14.3	1.2
Post	15	13.0	1.9
Peak III - Albumin and some α -, β -Globulin			
Ante	13	23.7	2.3
Maximum	15	23.5	2.2
Post	16	22.9	2.4

Peak I is the highest MW fraction and contains macroglobulins and lipoproteins. Peak II consists of α -, β -, and γ -globulins, while Peak III contains the albumin plus some α - and β -globulins. In each peak the Ante-, Maximum, and Post- components had half-periods which did not differ significantly. Thus, within the limits of the measurements each peak was homogeneous with respect to turn-over times. Peaks I and III had about the same half-periods, but both differed from that of II (I vs. II, $0.05 > p > 0.02$ and II vs. III, $p < 0.01$). The half-period of II was close to those of the α - and β -globulins separated by electrophoresis, and the half-period of III agrees well with that of

Fig. 3. Elimination of the S^{35} label from serum proteins of high molecular weight as determined by gel-filtration.

Fig. 4. Elimination of the S^{35} label from serum proteins of medium molecular weight (globulins) as determined by gel-filtration.

Fig. 5. Elimination of the S^{35} label from the gel-filtration fraction containing mostly albumin.

albumin (see Table I). The curve for the Ante- component of III showed an early increase in S^{35} activity. During the first few days the following equation holds:

$$y = 7707 (e^{-0.0293t} - e^{-2.65t})$$

The half-period for the building-up of S^{35} in III Ante- was 6.3 hours. There were not sufficient data to calculate the growth in III Maximum and III Post, but early points available were consistent with a similar growth rate.

Early points for the components of I and II showed that the rate of incorporation of S^{35} into these fractions was considerably more rapid than that of III. There were not enough early data for the fractions separated electrophoretically to permit observation of growth of S^{35} activity in them.

DISCUSSION

It is difficult to evaluate results in terms of absolute half-lives of the different protein constituents due to the possible reutilization of labeled amino acids. Recycling of S^{35} compounds is well established, but the extent of the recycling varies greatly (1,2,5,8). A variety of data is available for humans, rats, rabbits, and some data are available for unspecified breeds of dogs⁽⁴⁾. They all differ by a wide margin, but the general pattern of half-periods is consistent with our findings.

Reutilization of labeled amino acids depends on many factors. It has not yet been established whether the protein molecule has to break down to amino acids before its building blocks can be recycled. It is impossible to decrease reentry significantly by adding large

quantities of unlabeled amino acids of the same kind as the one bearing the label⁽⁵⁾. This would not provide material competing with the label in the synthesis of new protein since excess amino acids are cleared from the blood stream very rapidly. Also, Margen and Tarver point out that sites of degradation and synthesis may be close to the same pool and hence a high specific activity precursor pool might exist from degraded protein⁽²⁾.

The 24 hour period of fasting apparently stimulated the incorporation of S^{35} -L-methionine into newly formed plasma proteins since approximately 5% of the labeled amino acid circulated in protein bound form in the serum two hours after administration. The "faster than exponential drop-off"* reported by others⁽⁶⁾ during the first day after introduction of labeled proteins did not show up in either the curve representing the S^{35} content of the whole serum or the curve representing the protein bound S^{35} . The first three data points were slightly higher but this was not significant. If a quasi steady state is produced by balancing losses of S^{35} into the interstitial protein pool against synthesis of new labeled proteins, the S^{35} concentration of whole serum will be slightly higher than that of the total serum proteins but both concentrations should decrease at the same rate. This, in fact, was the case. This difference then is the maximum amount of S^{35} -amino acids, i.e., L-methionine and L-cystine (since methionine may be converted to cystine, as shown by du Vigneaud⁽¹⁴⁾ and also by Tarver⁽¹⁵⁾ for rats), that would be available for reentry.

* i.e., this type of data is more adequately represented by a sum of two or more exponential terms whose slopes are greatly different.

Since the α - and β -globulins attained their maximum activity faster than albumin did, the degree of labeling of α - and β -globulins was significantly greater[±] than the one for albumin (see Table I). This means also that the globulins were probably more uniformly labeled than albumin because the albumin activity was still increasing at the time when the S^{35} -L-methionine supply was already greatly diminished. The γ -globulin showed a different behavior which is consistent with the observed half-values because shorter half-lives require higher rates of formation. The γ -globulins, however, exhibited a considerably longer half-life than other globulins.

The analysis of gel-filtration data was more complex than the interpretation of electrophoretic data. The gel-filtration method, however, was included to correlate the data presented here with the work on the plasma protein binding of PuIV which is currently underway at this laboratory. Gel-filtration separated serum constituents according to their molecular size and not to their electrophoretic mobilities. This caused a considerable degree of overlapping of constituents which were clearly separated by electrophoresis and sometimes a distinct separation resulted where electrophoretic mobilities were equal.

Peak I contained a variety of high molecular weight proteins⁽¹²⁾ as α_{2m} -, β_{2m} -globulins, 19 S γ -globulin, anti-A γ -globulins, 19 S cold agglutinin and α -, β -lipoprotein. This peak exhibited an unexpectedly long half-life for S^{35} .

Half-lives for Peak II were those expected in a mixture of all globulins. The low value in the "Post" fraction was again consistent with the fact that electrophoretic patterns show an increasing quantity of α -globulin and transferrin (β -globulin) in this area.

Peak III activities were dominated by the high quantities of albumin present. Although some fast moving α -globulins and some transferrin were present in this peak, S^{35} half-lives were identical with those obtained by microzone electrophoresis.

ACKNOWLEDGEMENT

The authors would like to thank Dr. D. L. Berliner for his help and encouragement.

REFERENCES

1. McFarlane, A. S., Use of labeled plasma proteins in the study of nutritional problems, Progress in Biophys. 7, 115 (1957).
2. Margen, S. H. and Tarver, H., The preparation of labeled albumin for turnover studies, Atomlight, No. 39, July 1964.
3. Choen, S., Holloway, R. C., Mathews, C., and McFarlane, A. S., Distribution and elimination of I^{131} and C^{14} -labeled plasma proteins in the rabbit. Biochem. J. 62, 145-154 (1956).
4. McFarlane, A. S., Labeling of plasma proteins with radioactive iodine, Biochem. J. 62, 135-43 (1956).
5. Bronsky, D., Hyman, S., and Armstrong, H., The persistence in the blood of the radioactive label of albumins, gamma globulins and globulins of intermediate mobility. J. Lab. and Clin. Med., Sept. 1957 (348-357).
6. Penn, N. W., Mandeles, S., and Anker, H. S., On the kinetics of turnover serum albumin. Biochim. et Biophys. Acta 26:349-360 (1957).
7. Fink, R. M., Enns, Plasma protein metabolism, Normal and Associated with Shock, J. Exp. Med. 80, 455-475 (1944).
8. Margen, Sh., and Tarver, Comparative studies on the turnover of serum albumin in normal human subjects, J. Clin. Invest. 35, 1161 (1956).

9. Niklas, A. and Maurer, W., Neubildung von Serum-Eiweiss-Fraktionen Nach Gabe von S^{35} -L-Methionin. Biochem 323, 89-103 (1952).
10. Sorm, F., Comparative studies in the field of protein microstructure. Symp. on Protein Structure 79 (1957), Publ. by G. Wiley & Sons, Inc.
11. Allerton, S. E., Elwyn, D., Edsall, G. T. and Spahr, P. F., Isolation and amino acid composition of dog plasma albumin. J. Biol. Chem. 237, No. 1, 85-88 (1962).
12. Flodin, P., and Willander, G., Fractionation of human serum proteins by gel-filtration, Biochim. Bioph. Acta 63, 403-410 (1962).
13. Stevens, W., Bruenger, F. W., and Stover, B. J., In vitro studies of the interactions of PuIV with blood proteins. Radiation Research (In press).
14. du Vigneaud, V., Audrieth, L. F., and Loring, H. S., The reduction of cystine in liquid ammonia by metallic sodium, J. Am. Chem. Soc. 52, 4500 (1930).
15. Tarver, H., and Schmidt, C. L. A., The conversion of methionine to cystine: Experiments with radioactive sulfur, J. Biol. Chem. 130, 67 (1939).

ADDITIONS TO BIBLIOGRAPHY
(April 1, 1964 to March 31, 1965)

Books

1. Henry Eyring, Douglas Henderson, Betsy Jones Stover and Edward M. Eyring: Statistical Mechanics and Dynamics, John Wiley & Sons Inc., New York (1964).

Publications

1. Jean H. Dougherty and Leon S. Rosenblatt: Changes in the hemogram of the Beagle with age, J. of Gerontology 20:131-138 (1965).
2. David R. Atherton, Betsy J. Stover and Charles W. Mays: Soft tissue retention of ^{226}Ra in the Beagle, Health Physics J. 11, 101-108 (1965).
3. G. N. Taylor, W. R. Christensen, W. S. S. Jee, C. E. Rehfeld and P. Petermann, Intercomparison of pathological fractures in Beagles injected with Ra^{226} , Ra^{228} , Pu^{239} and Sr^{90} , Health Physics J. (in press) 1965.
4. Gottlieb L. Schneebeli and Thomas F. Dougherty: A time-lapse cinematography procedure for the study of steroid hormone effects on murine megakaryocytes in vitro. The Microscope and Crystal Front 14:8, 319-331, (1965).
5. N. L. Dockum: Autoradiographic localization of Zn-65 , The Microscope and Crystal Front 14:4, 137-141 (1964).
6. W. S. S. Jee: The influence of reduced local vascularity on the rate of internal reconstruction of adult long bone cortex, In Bone Biodynamics, Ed. by H. M. Frost, Little Brown and Co., Boston, 259-277 (1964).
7. W. S. S. Jee: A critical survey of the analysis of the microscopic distribution of some bone-seeking radionuclides and assessment of absorbed dose, In Assessment of Radioactivity in Man, Vol. I, International Atomic Energy Agency, Vienna, 369-393 (1964).
8. L. C. Ellis and D. L. Berliner: Alteration of steroid biotransformation by murine testicular tissue x-irradiation, 5^a Reunion Anual. Soc. Mex. Nutricion y Endocrinologia, 99, (1964).
9. W. S. S. Jee, E. L. Blackwood, N. L. Dockum, D. L. Berliner and F. A. Kincl: Bio assay of cortisol in growing bones, Proceedings of 5^a Reunion Anual. Sociedad Mexicana de Nutricion y Endocrinologia, 157-168, (1964).

10. A. J. Gallegos, D. H. Hill, R. J. Williams and D. L. Berliner: Formacion de fibroblastos resistentes a esteroides, 5^a Reunion Anual. Soc. Mex. Nutricion y Endocrinologia, 169 (1964).
11. T. F. Dougherty, M. L. Berliner, G. L. Schneebeli and D. L. Berliner: Hormonal control of lymphatic structure and function. Leukopoiesis in Health and Disease, Ann. N. Y. Acad. Sci. 113:825, (1964).
12. D. L. Berliner: Biotransformation of corticosteroids as related to inflammation, Ann. N. Y. Acad. Sci. 116:1078 (1964).
13. D. L. Berliner: The microchemical identification of steroids from biological media, in Advances in Tracer Methodology, Vol. II, Plenum Press, New York (1964).
14. D. L. Berliner, G. Taylor and L. C. Ellis: The effects of ionizing radiation on endocrine cells. II. Restoration of androgen production with a reduced nicotinamide adenine dinucleotide phosphate-generating system of irradiation of rat testes, Rad. Res. 22:345, (1964).
15. L. C. Ellis and D. L. Berliner: The effects of ionizing radiation on endocrine cells. III. Restoration of androgen production with a reduced nicotinamide adenine dinucleotide phosphate-generating system after localized irradiation to heads of rats, Rad. Res. 23:156, (1964).
16. D. L. Berliner and L. C. Ellis, The effects of irradiation on endocrine cells IV. Increased production of 17 α , 20 α -Dihydroxyprogesterone in rat testes after irradiation, Rad. Res. 24, 368-373 (1965).
17. D. L. Berliner and L. C. Ellis: The effects of irradiation on endocrine cells. V. A comparison between the effects of inanition and irradiation on androgen production by murine testicular tissue, Rad. Res. 24:572-578 (1965).
18. W. Stevens and D. L. Berliner: Serum transaminase levels in Beagle dogs burdened with Pu²³⁹, Rad. Res. 23:420 (1964).
19. A. G. Ruhmann and D. L. Berliner: Effect of steroids on growth of mouse fibroblasts in vitro, Endocrinology (In press).
20. Walter Stevens, Carolyn Collesides and Thomas F. Dougherty: Effects of cortisol on the incorporation of thymidine-2-¹⁴C into nucleic acids of lymphatic tissue from adrenalectomized CBA mice. Endocrinology (In press).
21. Truls Brinck-Johnsen and Thomas F. Dougherty: Studies on the effect of cortisol and ACTH on incorporation of adenine-8-¹⁴C into the lymphatic tissue nucleic acids. Acta Endocrinologia (In press).

22. Martha L. Berliner and T. F. Dougherty: Interconversion of cortisol and cortisone by normal and leukemic murine lymphocytes. *Extrait de Acta Union Internationale Contre Le Cancer*, XX:4-5, 1133-1136 (1964).
23. G. F. Leong, D. M. Cazes, M. L. Berliner and D. L. Berliner: Biliary excretion of dihydro-11-keto progesterone-4-C¹⁴ by isolated perfused liver, *Am. J. Physiol.* 207:1030 (1964).
24. D. L. Berliner: Studies of the mechanisms by which cells become resistant to corticosteroids, in Hormone Related Tumors, American Cancer Society (In press).
25. Robert C. Pendleton, Charles W. Mays, Ray D. Lloyd and Norman V. Hancock: Fallout plutonium-239 and zirconium-95 in the lungs of deer, *Nature* 202:4933, 715-716 (1964).
26. R. C. Pendleton, R. D. Lloyd, C. W. Mays and B. W. Church: Trophic level effect on the accumulation of caesium-137 in cougars feeding on mule deer, *Nature* 204:4959, 708-709 (1964).
27. R. J. Della Rosa, M. Goldman, A. C. Andersen, C. W. Mays and B. J. Stover: Absorption and retention of ingested strontium and calcium in beagles as a function of age, *Nature* 205:4967, 197-198 (1965).
28. Robert C. Pendleton, R. D. Lloyd, C. W. Mays, D. R. Atherton and N. V. Chamberlain: Radium contamination at the Jordan River Delta of the Great Salt Lake, *Journal water pollution control federation*, 36:7, 925-927 (1964).

Abstracts

1. Walter Stevens, Jr. and Thomas F. Dougherty: The effect of cortisol on the incorporation of thymidine-C¹⁴ into nucleic acids of lymphatic tissue. *Fed. Proc.* 23:2, (March-April 1964).
2. R. K. Haslam, W. S. S. Jee, N. Dockum and K. A. Sears: Microdensitometric analysis of the quantitative microscopic distribution of radium-226 in dental tissues and bone of adult beagles. *J. Dental Res.* 43:763 (1964).
3. E. L. Blackwood, W. S. S. Jee, R. K. Haslam and F. Kincl: Bioassay of cortisol activity in growing bones. *Anat. Rec.* 151:325 (1965).
4. N. L. Dockum, R. G. Bromley, M. Lowe, R. Mical and J. S. Arnold: Quantitative studies on trabecular bones of dogs and humans. *Anat. Rec.* 151:344 (1965).
5. R. D. Kingston, D. H. Taysum and K. Dedekind: Frequency distribution of recently formed haversian systems in mandibular bone of the beagle. *Anat. Rec.* 151:372 (1965).

6. D. L. Berliner and W. Stevens: A comparison of serum transaminase levels in dogs burdened with Pu^{239} , Ra^{228} , Ra^{226} , Ra^{224} , Th^{228} , and Sr^{90} , Rad. Res. 25:1,175 (1965).
7. Ray D. Lloyd and Charles W. Mays: Knothole I: a new well-type γ -ray detector, Rad. Res, 25:1,212 (1965).